# FISH DISEASES

## Volume 2

*Editors*
Jorge C. Eiras
Helmut Segner
Thomas Wahli
B.G. Kapoor

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*Editors*

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# Preface

Diseases are a major threat to both wild and farmed fish. Pathogen-induced alterations in viability and growth of wild fish stocks can have implications on diversity and ecological status of aquatic ecosystems, as fish are main components of aquatic communities, and they can directly affect the exploitation of wild and farmed fish as a protein source for human consumption. With fish farming having developed strongly during the last decades, the number of farmed species is steadily increasing and the search for suitable species for farming is still continuing, especially in the tropical countries. At the same time wild fish stocks available for fishing are declining, and this gives added importance to fish protein production from farming, be it fish for daily consumption or for luxury food.

Fish diseases are critical to the success of the fish farming industry since disease-related economic losses can be devastating. Biological and environmental factors associated with fish farming promote and favour the action of pathogens. As a matter of fact, disease outbreaks occur more often in farmed than in wild fish.

Even in wild populations, fish diseases can be a major factor influencing abundance and distribution of wild fish. Disease-related reduction of reproduction and/or age-specific survival can strongly affect wild stocks as it has been demonstrated for several species in distinct geographical locations. Furthermore, wild fish populations can act as reservoirs for infectious agents. It is also important to stress that a number of pathogens, affecting wild or farmed fish, may be significant as zoonotic aetiological agents.

Therefore, knowledge of fish diseases is of great relevance, both from a scientific as well as an applied point of view. Detailed knowledge of the

pathogens, their interaction with the fish host as well as the response of the host is a pre-requisite for disease diagnostics as well as disease prevention and treatment. Further, since infection and disease processes in fish depend largely on environmental conditions, the study of fish diseases has to carefully consider the living "milieu" as pathogenic factor in its own right or as a factor modulating pathogen-host interaction and disease manifestation.

The present two-volume book on Fish Diseases aims to present comprehensive and up-to-date information on the infectious and non-infectious diseases of fish. All chapters are written by internationally recognized researchers with long experience in the particular field. It is the hope of the Editors and the Publisher that the book will be useful for fish farmers, managers, researchers and graduate students interested in fish diseases.

**The Editors**

# Contents

*Preface* v
*List of Contributors* ix

VOLUME 1

1. **General Fish Histopathology** 1
   *Renate Reimschuessel*
2. **Diseases Caused by Virus: Viral Diseases in Salmonids** 41
   *S.M. Bergmann and D. Fichtner*
3. **Virus Diseases of Cyprinids** 87
   *P.F. Dixon*
4. **Viral Diseases of Cultured Marine Fish** 185
   *G. Bovo and D. Florio*
5. **Bacterial Diseases of Fish** 239
   *Øivind Bergh*
6. **Fungal Diseases of Fish** 279
   *D.J. Alderman*
7. **Microsporidia** 351
   *Jiří Lom*
8. **Amoeboid Protists as Parasites of Fish** 397
   *Iva Dyková*
9. **Diseases Caused by Flagellates** 421
   *Pilar Alvarez-Pellitero*

10. Diseases Caused by Apicomplexans 517
*Dieter Steinhagen and Angela J. Davies*

11. Diseases Caused by Ciliophora 569
*Angelo Colorni*

VOLUME 2

12. Myxozoan Diseases 613
*Stephen W. Feist*

13. Diseases Caused by Monogenea 683
*Ian D. Whittington and Leslie A. Chisholm*

14. Digeneans as Enemies of Fishes 817
*Stephen A. Bullard and Robin M. Overstreet*

15. Diseases Caused by Cestoda 977
*Joanna Danuta Borucinska*

16. Acanthocephala 1025
*Horst Taraschewski*

17. Phylum Nematoda 1063
*Anindo Choudhury and Rebecca A. Cole*

18. Diseases Caused by Crustacea 1115
*Wojciech Piasecki and Annemarié Avenant-Oldewage*

19. Non-infectious Diseases: Nutritional Factors 1201
*Delbert M. Gatlin III*

20. Environmental Diseases of Intensively Reared Fish 1225
*David J. Speare*

21. A Guide for Aquaculture Veterinarians: What should be done at a Farm Visit 1255
*H.J. Schlotfeldt*

Index 1269

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CHAPTER

# 1

# General Fish Histopathology

**Renate Reimschuessel**

Histopathology is an important tool for diagnosing fish diseases (Sindermann *et al.*, 1980; Johnson and Bergman, 1984; Meyers and Hendricks, 1985; Hinton *et al.*, 1987; Noga, 1996). Occasionally, gross observations are sufficient to make a correct diagnosis, but usually more information is necessary to determine the etiology of clinical disease or mortality. It is important to have a systematic approach to necropsy examination, sampling tissues and evaluating histopathology (Reimschuessel, 1993, 1999). This chapter will describe the basic histopathological changes found in diseased or injured tissues. Specific diseases will be covered in detail in subsequent chapters.

Before beginning to 'read slides', there are a number of factors to consider. The clinical history and gross necropsy results usually contain information which helps to interpret histological findings as incidental or part of an overall disease process (Reimschuessel *et al.*, 1988; Stoskopf, 1993; Wildgoose and Lewbart, 2001). For example, the state of the animal just before necropsy affects the microscopic condition of organs such as

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gills and intestines which deteriorate rapidly following death. Therefore, in a moribund fish, gill or intestinal degeneration would be significant, while in a fish that died an hour prior to necropsy, such findings are expected post-mortem changes. Complete descriptions of gross lesions, especially the size, location and extent are also quite helpful for interpretation. For example, since even small nodules may not fit entirely into the tissue cassette, samples from a 2-cm and a 6-cm diameter nodule may look the same on the slide. The impact on the animal of these differently sized lesions, however, could be quite different. Using a necropsy worksheet (Fig. 1.1) during the gross post-mortem examination helps ensure consistent observations. One has to record data such as species, weight, length, general body condition, and descriptions of external lesions and samples taken for wet mounts and cultures. One also needs to examine internal organs for color, size, texture and any notable alterations from the normal appearance. Lists such as those found on the worksheet help ensure all organs are examined and those sampled for histopathology are identified. In general, it is best to include all tissues when submitting histopathology, as there are often microscopic changes present in grossly normal organs, and those changes may be key to a correct diagnosis.

Finally, it is vital that all tissue samples are handled appropriately. Lift samples gently without compressing or stretching, rapidly preserve tissues in an adequate volume of an appropriate fixative and note any special processing such as decalcification. Tissues that are crushed or inadequately preserved, either due to delay or insufficient fixative volume, will show signs of deterioration that could either obscure true lesions, or be misinterpreted as anti-mortem effects.

Many histopathological lesions in fish are similar to those described in mammalian pathology references (Ferguson, 2006; Harshbarger and Clark, 1990; Cotran *et al.*, 1999; Roberts, 2001). Most pathologic lesions can be categorized into 6 main groups:

1. Degenerative Changes
2. Growth Changes
3. Hemodynamic Changes
4. Infectious Changes
5. Inflammatory Changes
6. Miscellaneous Changes

Date_____Initials_____ACCESSION #_____

SAMPLES (Check box if taken – describe lesions):

☐ Blood Sample pcv_____tp_____ Study #_____

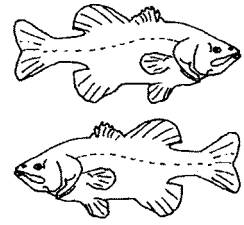
☐ Culture Surface Lesions _____ Species_____

☐ Skin Scrape _____ Tank _____

☐ Gill Biopsy _____ Batch Letter _____

General Body Condition Checklist: Check if examined and normal. Describe abnormalities.

| |
|---|
| ☐ 1. Weight (grams) |
| ☐ 2. Length (cm) |
| ☐ 3. Body shape |
| ☐ 4. Fins |
| ☐ 5. Eyes |
| ☐ 6. Nares |
| ☐ 7. Oral Cavity |
| ☐ 8.Anus/Urogenital Pore |



Indicate location of lesions on the picture

Organ Evaluation and Sampling Checklist – check if organ was examind and normal. Check Histology box if tissues were taken*

| Describe abnormal findings. | Histology taken |
|---|---|
| ☐ 1. Sample Surface Lesions | ☐ |
| ☐ 2. Operculum | ☐ |
| ☐ 3. Gills | ☐ |
| ☐ 4. Eyes | ☐ |
| ☐ 5. Open body cavities | |
| ☐ 6. Heart | ☐ |
| ☐ 7. Abdominal Body Block | ☐ |
| ☐ 8. Liver | ☐ |
| ☐ 9. Spleen | ☐ |
| ☐ 10. Gonads | ☐ |
| ☐ 11. Swimbladder | ☐ |
| ☐ 12. Cranial and Caudal Kidneys | ☐☐ |
| ☐ 13. Muscle, Vertebra, Spinal Cord | ☐☐☐ |
| ☐ 14. Skin, Lateral Line, Nares | ☐☐☐ |
| ☐ 15. Brain | ☐ |
| ☐ 16. Stomach and Intestine | ☐ |

*If fish is small and body was sampled whole or in sections check here. ☐

**Fig. 1.1** An example of a gross necropsy checklist. External examination list is printed on the front, internal examination list is on the back of the sheet.

Although they are separated to define and characterize the changes, these categories are often interlinked. For example, degenerative changes usually arise following some type of adverse event. Those events could be external trauma, vascular disturbances, infections, nutritional deficiencies or toxicant exposure. Infectious diseases can directly cause degenerative changes, or they can cause inflammation which also induces degenerative changes. Neoplasms can outgrow their blood supply and also compress surrounding tissue, both of which can induce degenerative effects, either directly or indirectly via inflammation. Nevertheless, for descriptive purposes, these categories help organize the different lesions encountered when evaluating microscopic pathology. A consistent approach to the terminology used to describe lesions, such as the codes defined in Reimschuessel *et al.* (1992), can facilitate organizing data when conducting large survey studies. Recent advances in database programs make it easier to enter and sort data from these types of investigations, but those programs also require using consistent terms.

Individual changes in each of the general categories outlined in Table 1.1 are described in alphabetical order and photomicrographs of selected lesions are included to demonstrate the appearance of these

**Table 1.1** A listing of the different types of pathologic changes that typically occur in fish tissues.

| *Degenerative Changes* | *Growth Changes* | *Hemodynamic Changes* | *Inflammatory Changes* |
|---|---|---|---|
| Apoptosis | Atrophy | Congestion | Abscess |
| Caseous | Dysplasia | Dilatation | Acute Inflammation |
| Autolysis | Fibrosis | Edema | Chronic-active Inflammation |
| Calcification | Foci of Cell Alteration | Embolism | |
| Dystrophic | Clear Cell | Hemorrhage | Chronic Inflammation |
| Metastatic | Acidophilic | Telangiectasia | Granulomatous |
| Dilatation | Basophilic | Thrombus | Giant Cells |
| Erosion | Granulation Tissue | | |
| Fatty Change | Giant Cell | *Infectious Changes* | *Miscellaneous Changes* |
| Hyaline | Hyperplasia | Bacterial | Cyst |
| Necrosis | Hypertrophy | Fungal | Pigment Accumulation |
| Coagulation Necrosis | Metaplasia | Viral | Ectopic Thyroid |
| Liquefactive Necrosis | Neoplasia | Parasitic | Basophilic Tubules |
| Caseous Necrosis | Benign | | Macrophage Aggregates |
| Ulceration | Malignant | | |
| Vacuolar Degeneration | Regeneration/Repair Thickened | | |

alterations. It is important to note that these examples are selected from a variety of fish species whose normal organ histology can vary significantly. In some cases, what is normal for one species would be abnormal in another fish species, e.g., shark livers cells normally accumulate large quantities of lipid, while such accumulation in the typical bony fish would be pathological. Familiarity with normal histology is, thus, essential. Unfortunately there are few fish histology references in print (Grizzle and Rogers, 1976; Groman, 1982; Hibiya, 1982; Yasutake and Wales, 1983; Amin *et al.*, 1992) but a number of websites have images available (US Fish and Wildlife website, Yonkos *et al.*, 1999). Photomicrographs are of tissues stained with hematoxylin and eosin, unless another stain is specified.

## DEGENERATIVE CHANGES

Cell injury, regardless of the inducing agent, can result in either reversible injury or cell death. The adverse events causing degenerative changes are primarily infectious agents, inadequate oxygen or blood supply, inflammation, and physical agents such as trauma, toxicants and radiation.

### Apoptosis

For every cell, there is 'a time to live and a time to die'. Apoptosis is a form of programmed cell death, or cell suicide. It can occur in the form of a normal developmental process such as tail resorption in tadpoles as they become frogs. It can also be induced by other factors such as viral infection, cytotoxic T lymphocytes, DNA damage, radiation and some chemicals. Cells undergoing apoptosis appear shrunken, the cytoplasm is eosinophilic and the nuclear material has condensed chromatin (dark and fragmented). Remnants of such cells can be seen in phagocytic cells (Fig. 1.2A).

### Autolysis

This is the post-mortem degeneration of cells by lytic enzymes. This can occur rapidly after death, especially in gill and intestine. The tissue sections often look pale and poorly defined, with incomplete cell boundaries. In early stages this appears similar to coagulation necrosis due to ischemia (no blood supply) (Fig. 1.2B-C).

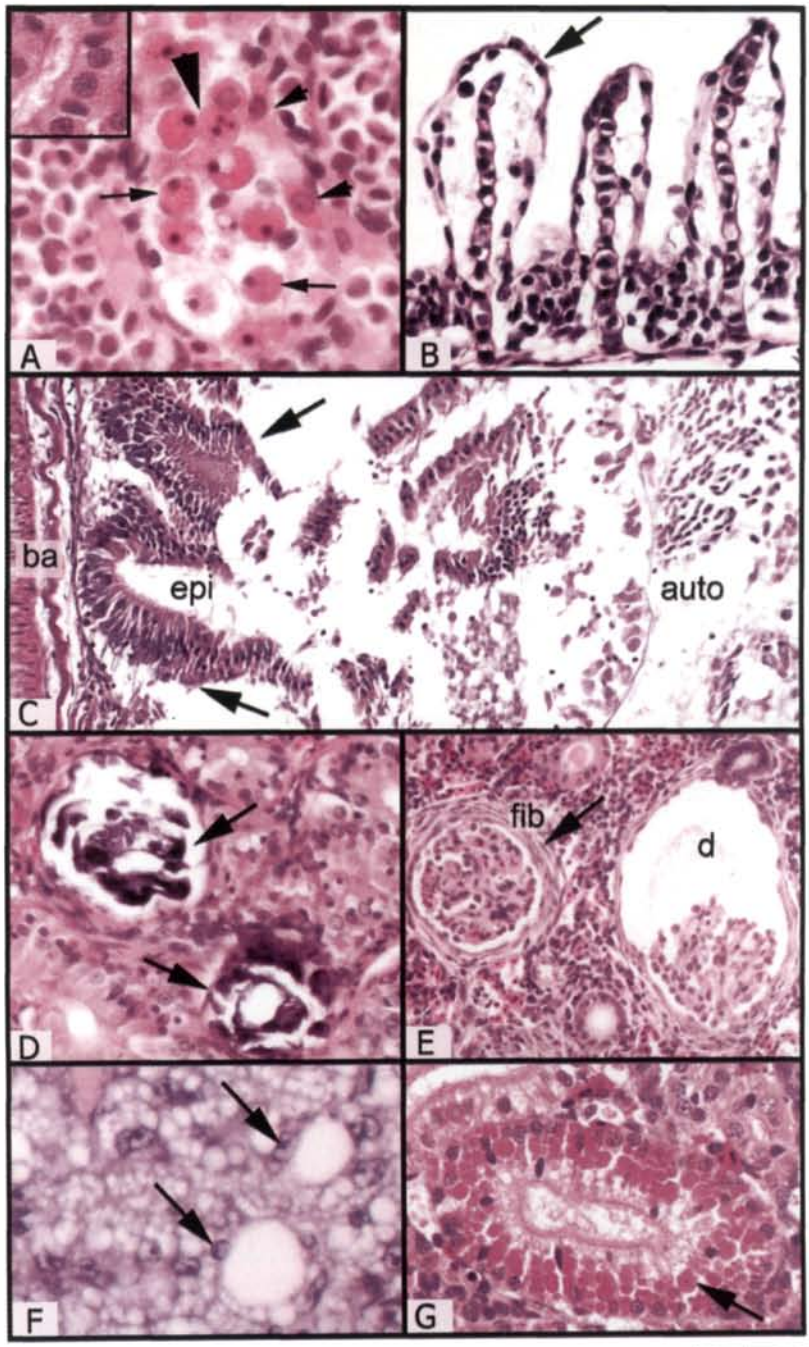



*Fig. 1.2 Contd.*

**Fig. 1.2** **A. Apoptosis:** Toadfish, kidney. Renal proximal tubule cells following gentamicin administration. Gentamicin induces apoptosis in mammalian and fish proximal tubule cells. Apoptotic cells (thin arrows) have detached from the basement membrane and are located in the tubule lumen. These cells have a bright eosinophilic cytoplasm and very dark compacted nuclei (pyknosis). One cell (large arrowhead) has a fragmented nucleus (karyorrhexis). Two cells (small arrowheads) with more normal appearing nuclei are still attached to the basement membrane. The renal tubular epithelium is normally comprised of cuboidal cells with large centrally located nuclei (inset left corner). The many basophilic cells surrounding the tubule remnant are hematopoietic cells.

**B. Autolysis:** Trout, gill. Early autolysis, 1-2 hours postmortem. The epithelium (arrow) has lifted off the lamellae leaving large clear spaces containing scattered desquamated cells. This change is frequently reported following toxicant exposure, but may readily be produced as an artifact if the gills are not placed in fixative immediately following death. Autolytic changes in gills occur within minutes after death thus these organs should be one of the first removed for histopathology.

**C. Autolysis:** Northern pufferfish, intestines. The epithelium (epi-arrows), normal at the base (ba), has sloughed off the basement membrane and fragmented within the lumen. All the tissue on the right part of the slide is autolytic (auto). The intestines contain many proteolytic enzymes which destroy the epithelial cells rapidly following death. Condition of the intestinal epithelium can be used to assess (generally) the length of time after death before the tissues were placed into fixative. Early autolysis resembles coagulative necrosis, the difference being that necrosis is cell death occurring prior to the death of the animal, autolysis is cell death occurring after the animal died.

**D. Calcification:** Tilapia, kidney. Two large foci of calcified material (arrows) located in the posterior kidney. Tubules may contain calcified material in their lumens (renal calculi) or they may become entirely calcified as is the case in this figure.

**E. Dilatation:** Goldfish, kidney. Bowman's space is greatly dilated (d) in this glomerulus, while the other glomerulus has a fibrotic capsule (fib-arrow).

**F. Fatty change:** Mummichog, liver. Fat may accumulate to varying degrees in fish hepatocytes, but extreme accumulation is seen in two hepatocytes here. The lipid has distended the cell, shoving the nucleus (arrows) to the side creating the classical "signet ring" appearance.

**G. Hyaline:** Goldfish, kidney. The proximal tubular epithelium contains many eosinophilic granules or "hyaline droplets" (arrow identifies one droplet). In proximal tubules this is usually the result of protein resorption from the luminal fluid, indicating possible glomerular dysfunction.

## Calcification

### *Dystrophic Calcification*

This occurs in dead or degenerating tissues with subsequent calcium deposition. The causes of calcium precipitation within the dead tissue is unknown but is not related to the calcium content of the blood. Change in alkalinity has been suggested to favor precipitation of calcium within the degenerating and/or dead tissues (Fig. 1.2D).

### *Metastatic Calcification*

Metastatic calcification is the precipitation of calcium salts in tissues as a result of persistently high serum calcium levels. This process is due to deranged calcium metabolism, not due to cell injury.

## Dilatation

Dilatation is the expansion of an organ, vessel, ductule, or tubule (Fig. 1.2E).

## Erosion

Erosion is the sloughing of epithelium from a surface, skin or mucus membrane, in which the basement membrane remains intact. These types of lesions usually can heal within a few days by migration and division of the remaining epithelial cells.

## Fatty Change

Here, intracytoplasmic accumulations known as fatty metamorphosis or fatty degeneration are in the form of fine or large fat droplets. In H&E sections that have been treated with xylene during the embedding process, fat appears as clear spaces within cells, although small remnants of fat may appear as slightly grey inclusions. Frozen sections stained with vital dyes such as Sudan Black or Oil Red-O are needed to demonstrate fat in tissues. Fat accumulation may be so extensive that the nucleus is pushed to one side of the cell, causing the cell to appear like a signet ring. Fatty change can result from increased fatty acid synthesis, decreased mitochondrial oxidation of fatty acids, increased triglyceride production or impaired lipoprotein release. Most commonly found in liver but fatty change can also occur in heart or kidney (Fig. 1.2F).

## Hyaline

Hyaline is a term used to describe any pink, homogeneous, structureless material. It can be either intracellular or extracellular. Injury to certain cells can cause a hyaline change of varying degree in the cytoplasm. Hyaline droplets (numerous, closely grouped, eosinophilic structures) within the cytoplasm in renal tubular epithelium are reabsorbed protein passed into the filtrate by injured or abnormally permeable glomeruli. Other intracytoplasmic hyaline inclusions can be immunoglobulin, viral proteins and prekeratin filaments. Extracellular deposits can be precipitated plasma proteins, basement membrane material and amyloid. It is important to recognize that the term hyaline is used to describe an alteration which results from different pathologic conditions (Figs. 1.2G, 1.8B).

## Necrosis

Necrosis is the non-programmed death of cells or tissues while the organism as a whole is still alive. Causes of necrosis include toxins—both chemical and biological—lack of proper innervation or blood supply, inflammation and physical injuries. Oxygen radicals are one mechanism by which inflammation, chemicals or ionizing radiation can cause cell injury leading to necrosis. There are three major types of necrosis, namely:

### *Coagulation Necrosis*

Here the dead cells are still somewhat recognizable and the organ structure is moderately maintained. Cells may take on an eosinophilic appearance and nuclei become compact (pyknotic) or undergo fragmentation (karyorrhexis) or lysis (karyolysis). Infarcts usually cause coagulation necrosis (Fig. 1.3A-D).

### *Liquefactive Necrosis*

In liquefactive necrosis, the cells have lost their structure and the tissue architecture is totally disrupted. Liquefactive necrosis is often associated with inflammation, although in the brain an infarct will cause liquefaction of the affected tissue without inflammation (Fig. 1.4A-C).

### *Caseous Necrosis*

Caseous necrosis is a combination of coagulation and liquefactive necrosis encountered primarily in mycobacterial granulomata. The name arises

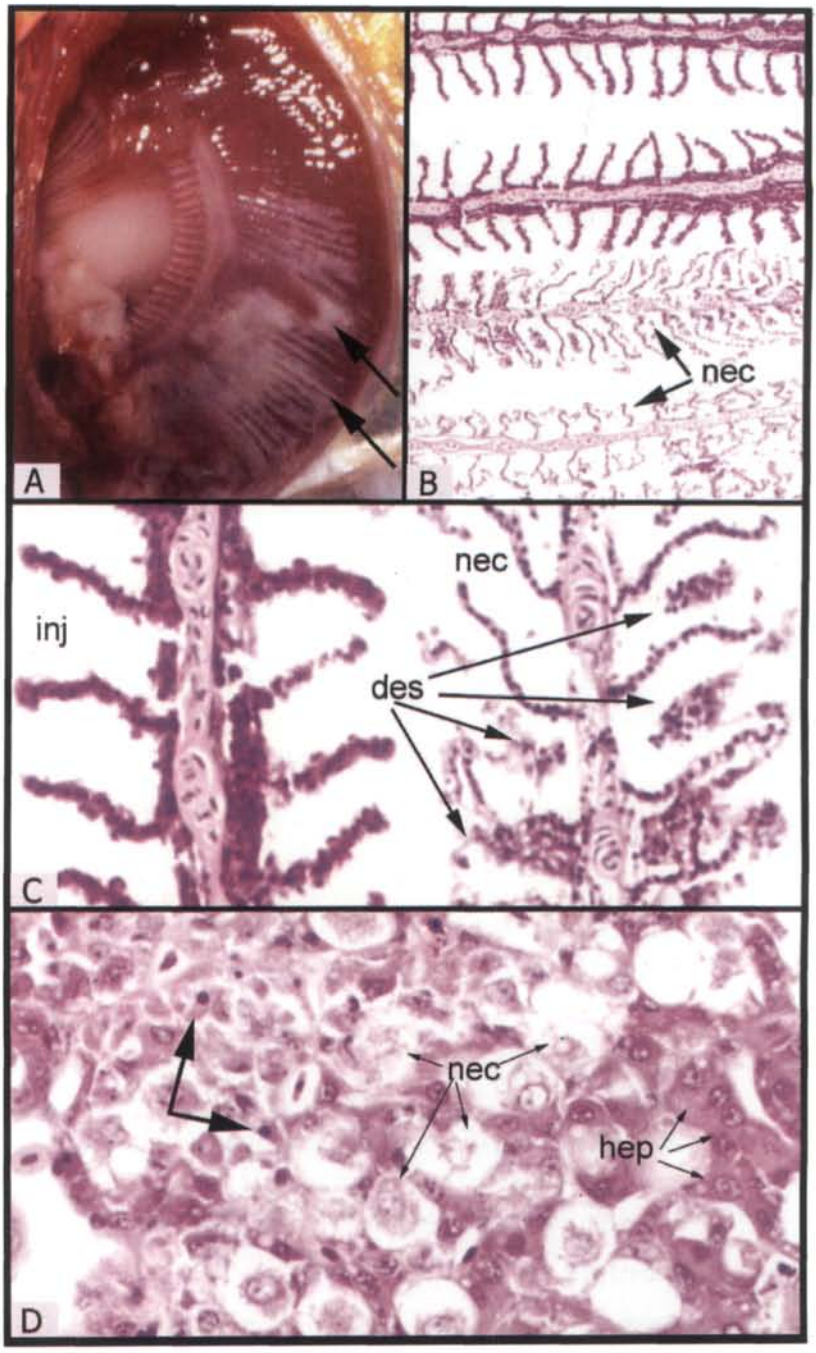



*Fig. 1.3 Contd.*

from the gross, cheesy appearance of the whitish gray material found within tubercles. Microscopically, there is a center of amorphous granular debris surrounded by a wall of inflammatory cells (epithelioid macrophagès) and fibroblasts (Fig. 1.4D, E).

## Ulceration

An ulcer is the loss of epithelium from a surface, skin or mucus membrane, in which the basement membrane has been breached. Typically, inflammation and hemorrhage are present in adjacent tissue. Over time these can exhibit healing which includes re-population of the epithelium and also deposition of fibrous connective tissue to breach larger defects (Fig. 1.4A-C).

## Vacuolar Degeneration

This is also called hydropic swelling, characterized by swollen cells with granular appearing cytoplasm and a normally located nucleus. When large vacuoles are present, the term vacuolar degeneration is often used. Cells can appear pale and vacuolated or reticulated. The clear vacuoles may be small and multiple or confluent. Vacuoles arise following cell injury which causes altered ATP output, impairing membrane permeability and active transport. Increased cytoplasmic sodium ions increase the water content,

**Fig. 1.3** **A. Necrosis-Coagulation:** Goldfish, gill—following extended formalin treatment. Necrotic gill tissue (arrows) is white, compared to the normal red gill. These lesions were the result of a thrombus in a gill artery resulting in an infarct.

**B. Necrosis-Coagulation:** Goldfish, gill—following extended formalin treatment. The two lower filaments are necrotic (nec-arrows). Cells have sloughed from the lamellar epithelium and base. Cells remaining on the filament stain pale, but the gill structure remains generally identifiable, a hallmark of coagulation necrosis.

**C. Necrosis-Coagulation:** Goldfish, gill—following extended formalin treatment. Higher magnification of 1.3B. Desquamated (des-arrows) cells are located between remnants of lamellae on the necrotic filament (nec). The epithelium of the other filament (inj) has a ragged appearance, the result of direct cell injury due to the prolonged formalin treatment. That injury would have been sublethal and could have been repaired if the animal had survived.

**D. Necrosis-Coagulation:** Trout, liver. Many very pale, enlarged necrotic hepatocytes are located in this figure (nec-arrows). The nuclei of these cells are undergoing karyolysis (nuclear disintegration or lysis). Other necrotic cells (large arrows) have very condensed nuclei (pyknotic) and a compact, intensely eosinophilic cytoplasm. Normal hepatocytes are present on the right (hep-arrows).

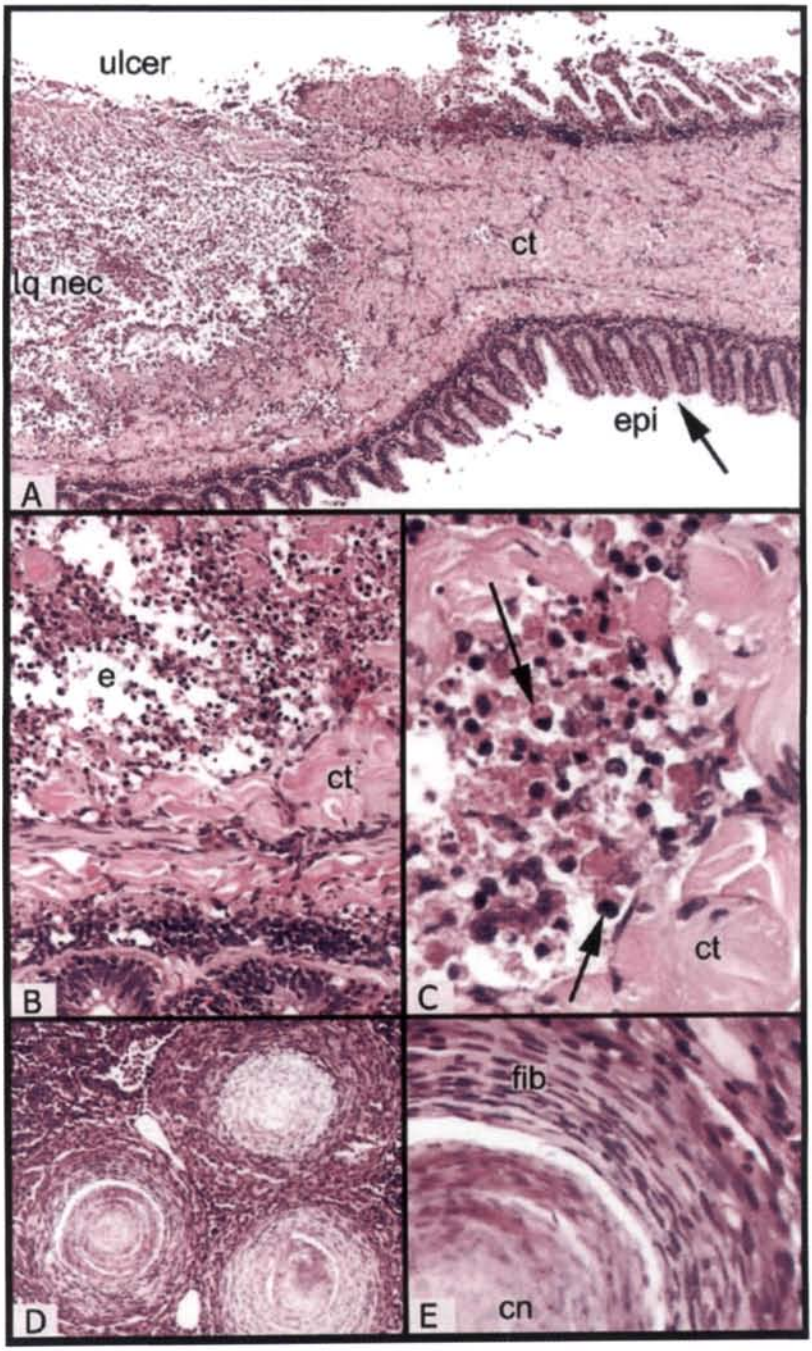



*Fig. 1.4 Contd.*

distending endoplasmic reticulum or mitochondria, and the entire cell swells. The condition is reversible. In skin epithelium, this condition may be referred to as 'spongiosis' (Fig. 1.5A, B).

## GROWTH CHANGES

These changes are primary alterations in proliferation, organization or size of cells and tissues.

**Atrophy** – reduced cell size by loss of cell substance. When numerous cells are involved the entire organ diminishes in size and is atrophic.

**Dysplasia** – is an alteration in adult cells characterized by variation in their size, shape and organization. This term usually refers to epithelial cells that have undergone irregular, atypical proliferative changes in response to chronic irritation or inflammation (Fig. 1.6E).

**Fibrosis** – abnormal formation or accumulation of fibrous tissue, predominantly collagen and fibroblasts, which can occur in various organs and tissues. Fibrosis is usually the result of tissue injury and chronic inflammation where normal structures have been destroyed. These areas are sometimes grossly apparent as scars. (Figs. 1.2E, 1.4D, E, 1.5C, 1.6E)

### Foci of Cell Alteration

These are considered pre-neoplastic lesions, most commonly in the liver. Cells take on a different appearance from cells in the surrounding tissue.

**Fig. 1.4** **A. Liquefactive necrosis:** Sandbar shark, spiral valve. Liquefactive necrosis destroys the normal architecture of the tissue due to enzymatic digestion. This spiral valve has normal epithelium on one side (epi-arrow) and is ulcerated on the other side (ulcer). This focus of liquefactive necrosis (lq nec) has distended the spiral valve and replaced the connective tissue (ct).

**B. Liquefactive necrosis:** Sandbar shark, spiral valve. Higher magnification of 1.4A. There is a prominent inflammatory infiltrate with loss of normal tissue structures. The clear area surrounding the cellular debris is edema (e) bordered by connective tissue (ct).

**C. Liquefactive necrosis:** Sandbar shark, spiral valve. Higher magnification of 1.4B. Necrotic debris and many heterophils [also called granulocytes or neutrophils] (arrows) containing bright eosinophilic granules at the margin of the lesion's normal connective tissue stroma (ct).

**D. Caseous necrosis:** Goldfish, spleen. Three large granulomata from a mycobacterial infection. The central region contains a necrotic core which is surrounded by epithelioid macrophages and a fibrous capsule.

**E. Caseous necrosis:** Goldfish, spleen. Higher magnification of 1.4D. The core of caseous necrotic (cn) material is surrounded by a prominent fibrous capsule (fib).

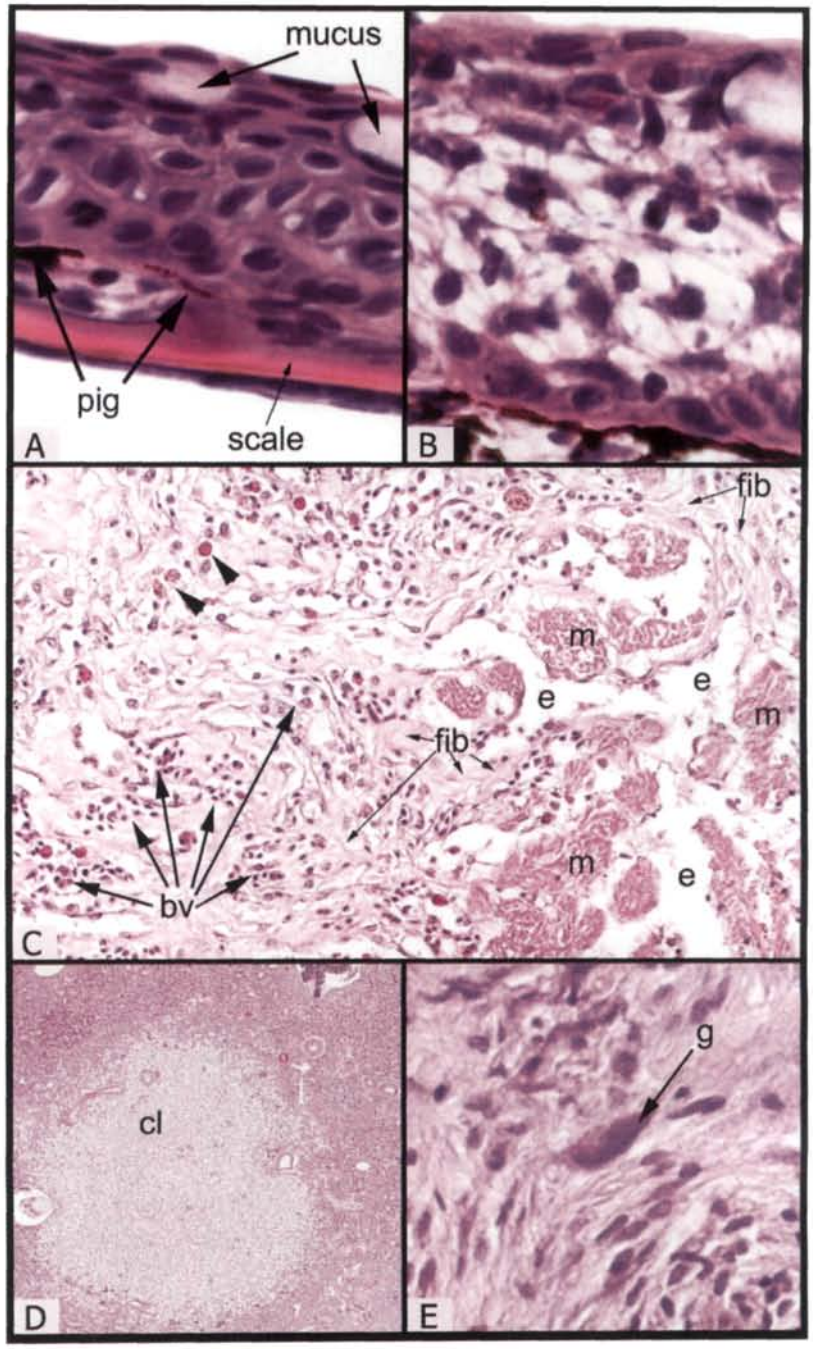



*Fig. 1.5 Contd.*

For example, a discrete population of hepatocytes may take on a clear, pink (eosinophilic) or bluish (basophilic) appearance compared to the surrounding hepatocytes. Other parameters such as size and shape are similar to the unaltered surrounding cells. Such lesions have been demonstrated in surveys of fish found in sites contaminated by polyaromatic hydrocarbons (Fig. 1.5D).

## Granulation Tissue

This tissue develops during wound healing. Once the necrotic debris has been cleared away by an inflammatory response, fibroblasts and capillaries migrate into the lesion. Macrophages are commonly found among the many capillaries and fibrous tissue. On gross examination, granulation tissue looks pinkish and bleeds readily due to the presence of many capillaries. Over time most of the capillaries are resorbed and replaced by moderately vascularized connective tissue, i.e., a scar (Fig. 1.5C).

## Giant Cells

Giant cells or extremely large parenchymal cells may be found in anaplastic neoplasms (see neoplasia). These cells may be large individual cells or multinucleated cells that have formed either by nuclear division without cytokinesis or from cellular fusion (Fig. 1.5E).

**Fig. 1.5** **A. Normal skin:** Trout, skin. Epithelial cells in the epidermis have a moderate amount of pink cytoplasm. Two mucus cells are located at the surface (mucus-arrows), and a black pigment cell (pig-arrows) is present near the bright eosinophilic scale (scale-arrow).

**B. Vacuolar degeneration:** Trout, skin. The cells with vacuolar degeneration are distended and their cytoplasm has a clear or ground glass appearance. The nuclei look relatively normal. The enlarged cells make the epidermis thicker than the normal.

**C. Granulation tissue:** Hogfish, heart. Granulation tissue contains many capillaries (bv-arrows) and scattered mononuclear cells (arrowheads), consisting of macrophages and in this case also eosinophilic granulocytic cells. During the repair phase, fibroblasts begin to lay down collagen fibers (fib-arrows). Edema (e) is present surrounding remaining myocytes (m).

**D. Foci of cell alteration:** Flounder, liver. Foci of alteration can be eosinophilic, basophilic, or in this case, clear cell foci (cl). Such lesions are considered to be pre-neoplastic.

**E. Giant cells:** Goldfish, skin-fibrosarcoma. Neoplasms may contain very large "giant" cells (g). This fibrosarcoma contains spindle shaped cells that vary greatly in size, nuclear staining intensity and nuclear size. Such heterogeneity in morphology is called "anaplasia" and is a characteristic of more aggressive neoplasms.

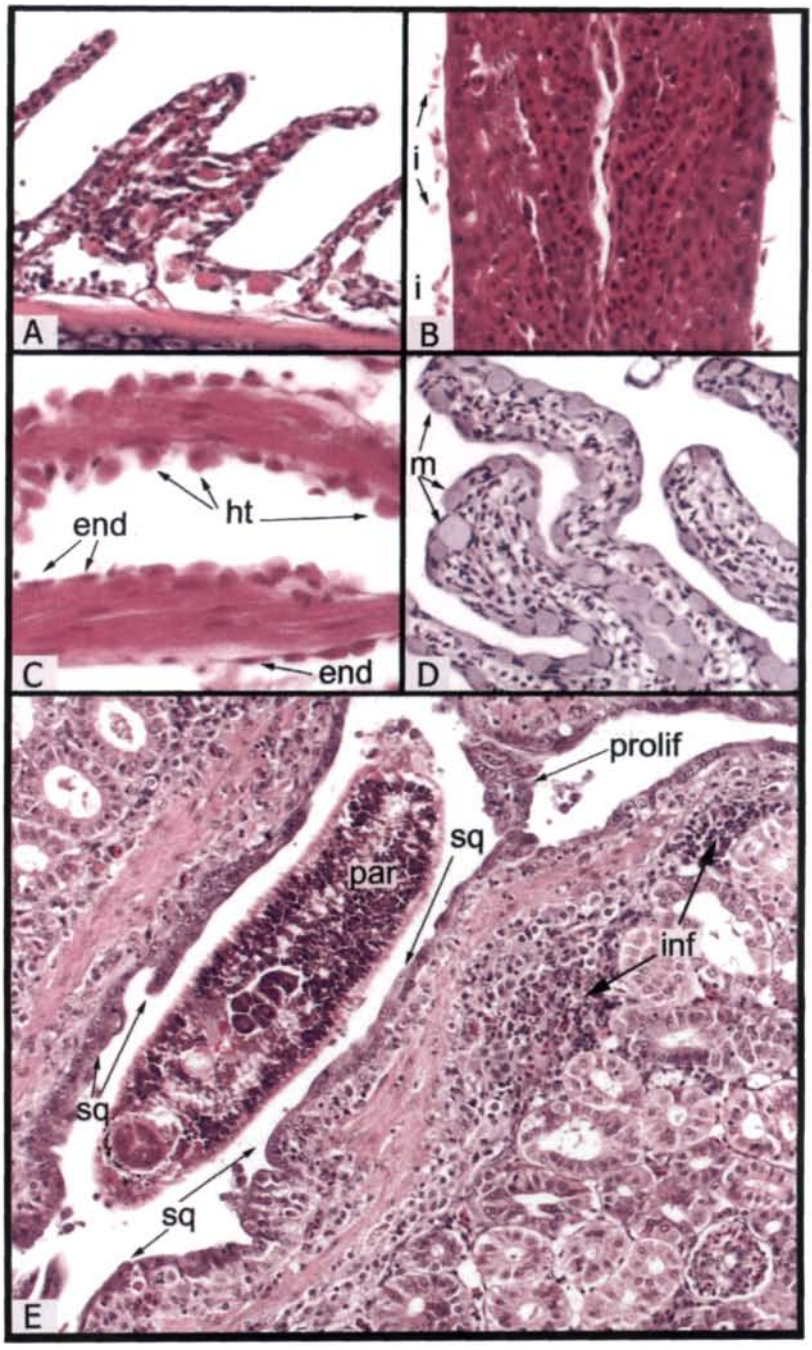



*Fig. 1.6 Contd.*

## Hyperplasia

Hyperplasia is an increase in the number of cells in an organ or tissue. The organ may also have increased in volume. Hyperplasia is frequently encountered in gills and is a non-specific response. Gills become hyperplasic due to poor water quality, toxicant exposures, nutritional deficiencies and parasitic infections. Hyperplasia may be a normal physiological response, such as pancreatic islet cell hyperplasia during spawning (Fig. 1.6A, B, D).

## Hypertrophy

Hypertrophy is an increase in the size of cells which may increase the size of the organ. It is also used to describe the increase in the size of an organ. The atrial phagocytic endothelium (part of the fishes reticulo-endothelial system) frequently responds to infections by becoming hypertrophic. Gill epithelium becomes hypertrophic in response to waterborne irritants and parasitism (Fig. 1.6C, D).

Loss of – This loss refers to the loss of cells or tissues normally present, e.g., chronic trauma in aquaculture can cause loss of fins.

**Fig. 1.6** **A. Hyperplasia:** Northern pufferfish, gill. Epithelial proliferation between two lamellae has produced a "doublet" or fusion of two lamellae.

**B. Hyperplasia:** Channel catfish, gill. A gill parasite, *Ichthyoboda* sp. (i), which looks like small exfoliated cells, has induced massive respiratory epithelial cell hyperplasia (proliferation) resulting in lamellar fusion along the entire filament. The increased cell mass reduced oxygen perfusion to the capillaries and the fish demonstrated respiratory distress by gulping at the surface of the water.

**C. Hypertrophy:** Hogfish, heart, atrium. Many of the atrial phagocytic endothelial cells are enlarged (hypertrophic – ht). Several cells have their normal flattened shape (end). The atrial endothelial cells can phagocytize circulating substances such as iron or bacteria. They are often hypertrophic, as seen here, during infections.

**D. Hypertrophy and hyperplasia:** Green moray eel, gill. Mucus cells (m-arrows), normally located at the base of lamellae, are enlarged and have proliferated, covering the entire surface of these lamellae.

**E. Metaplasia:** Largemouth bass, ureter. Most of the columnar epithelium has become squamous (sq-arrows) in response to the large monogenean parasite (par) in the ureter lumen. One section of epithelium has proliferated (prolif-arrow), an example of dysplasia following metaplasia. There is a chronic inflammatory infiltrate (inf-arrows) present adjacent to the fibrous tissue surrounding the ureter.

## Metaplasia

Metaplasia is a reversible change in which one adult cell type—epithelial or mesenchymal—is replaced by another adult cell type. Metaplasia often occurs following chronic injury induced by physical factors (e.g., parasitism, rough surfaces), or by toxic substances (e.g., squamous metaplasia of the cuboidal ciliated epithelium in a smoker's bronchi) (Fig. 1.6E).

## Neoplasia

Neoplasia is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues, and persists in the same excessive manner after cessation of the stimuli which evoked the change. Growth activity usually predominates over function although the latter is not necessarily lacking. Neoplasms have been reported in almost all organs of fish. Some neoplasms are associated with environmental contaminants.

### *Benign Neoplasms*

Benign neoplasms are those that tend to grow slowly and expansively. They can be well tolerated by the organism and do not necessarily interfere with the organism's overall health or shorten its life expectancy unless they are in some vital spot or interfere with an important organ. They are usually composed of well-differentiated mature cells characteristic of the tissue of origin. Example: Papilloma – a benign epithelial tumor which grows outward from an epithelial surface, sometimes pedunculated. The neoplastic epithelial cells resemble the epithelium of the original tissue and are situated externally around a branching core of connective tissue containing blood vessels. The neoplastic cells do not penetrate the basement membrane and invade the underlying tissues or form metastases (Fig. 1.7A, B).

### *Malignant Neoplasms*

Malignant neoplasms are generally more rapidly growing than benign lesions, infiltrating and extending into normal structures. These neoplasms tend to interfere with the organism's health and, at least in mammals, will eventually cause death. Malignant neoplasms are usually composed of cells less differentiated (more of a primitive embryonic-type

of cell) than the normal tissue of origin. Cells are anaplastic, i.e., vary in size, staining intensity, and nuclear to cytoplasmic ratio. Multiple nuclei may be present and mitotic figures are often encountered (Fig. 1.7C-F).

### Regeneration/Repair

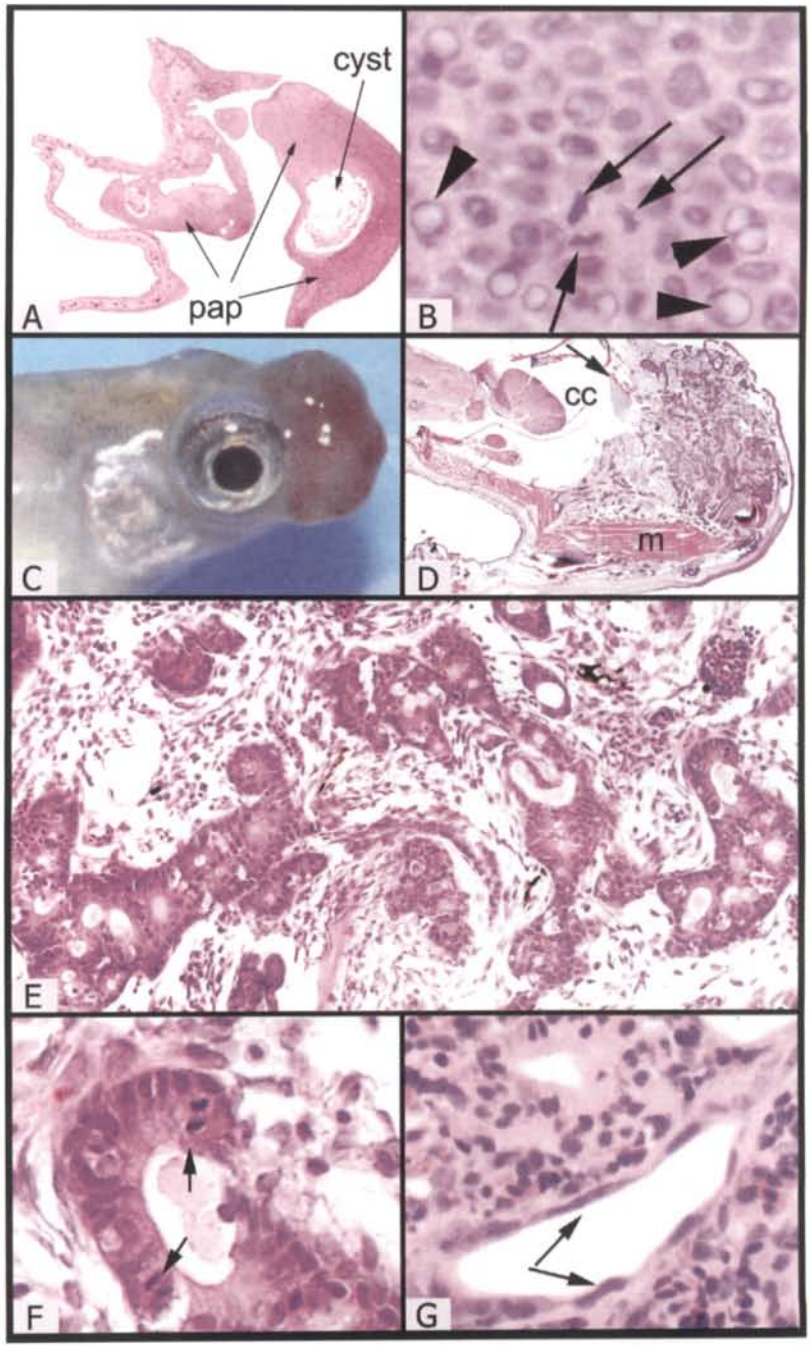
This refers to repopulation of an injured tissue by cells of the same type of cells that were present before, with the same functional characteristics. Fish can regenerate scales and fins following extensive injury. They are also capable of producing new nephrons, unlike mammals that can only regenerate tubules within an existing nephron. In a wound where the basement membrane has not been breached, regeneration can be accomplished by cell division of labile cells. Labile cells are capable of division and fill any defects by division and migration. In more extensive injuries which breach the basement membrane, the defect is replaced with scar tissue comprised primarily fibroblasts and collagen after the damaged tissue has been cleared by inflammatory cells. There are several stages of wound healing, the initial injury, which usually involves vascular changes with hemorrhage and edema, followed by an acute inflammatory response. During tissue repair chronic inflammatory cells enter the lesion, phagocytize debris while capillaries and fibroblasts grow in to fill the tissue defect (Figs. 1.7G, 1.8A).

### Thickened

This is a non-specific term referring to an increase in the size of a structure or organ without identifying the reason for this increase. When the increase in size of an organ is due to cell proliferation, the tissue's 'thickening' results from hyperplasia. Thickening of a tissue can also be due to intercellular edema or intracellular fluid accumulation making the cells themselves enlarge. Extracellular accumulation or deposition of substances such as amyloid can also thicken structures (Figs. 1.6A, B, 1.8B).

## HEMODYNAMIC CHANGES

Vascular system injury usually results in either flow obstruction or leakage. Hypoxia, excess pressure and/or fluid accumulation can induce additional degenerative effects in surrounding tissue.

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## Congestion

Congestion is an excess of blood within vessels in a given region. Congestion can result from too much blood being delivered by the arteries (active) or too little being drained by the veins (passive) (Fig. 1.8C-E).

## Dilatation

Dilation is the expansion of an organ, vessel, ductule, or tubule (in this case refers to blood vessels). Most of the time this change accompanies congestion. Dilation of capillaries is one of the first responses to trauma. This is followed by changes in permeability allowing fluid into tissues,

**Fig. 1.7** **A. Neoplasia, benign:** Gar, skin. A large papilloma (pap-arrows) is located at the end of this section of fin, with normal fin located on the left side. At this magnification the neoplasm looks like a section of very thickened epithelium. There is a large cyst (cyst-arrow) located within the largest section of neoplasm.

**B. Neoplasia, benign:** Gar, skin. Higher magnification of the papilloma in 1.7A. The neoplasm comprised fairly uniform epithelial cells with large nuclei and a moderate amount of cytoplasm. Some of the nuclei contain clear vacuoles, which may be viral inclusions. Several mitotic figures (arrow) are present in the section.

**C. Neoplasia, malignant:** Medaka, skin. Gross appearance of a pinkish growth over the maxillary region of this fish.

**D. Neoplasia, malignant:** Medaka, skin. Low magnification of the lesion in 1.7C. The neoplasm (almost all of the dark basophilic tissue in this section) has an overlying epithelium, but extends through most of the head tissues. Neoplastic cells border the cartilage and bone (arrow) of the skull but have not invaded into the cranial cavity (cc). Some neoplastic cells have infiltrated around and into the eosinophilic jaw muscle (m).

**E. Neoplasia, malignant:** Medaka, skin. Higher magnification of the neoplasm in 1.7D. The neoplasm, an adenocarcinoma, potentially arising from the nasal epithelium, is quite disorganized. Neoplastic cells form small glandular foci, but also form streaming extensions into the surrounding connective tissue. Scattered melanin pigment cells (black) and inflammatory cells are located throughout the stroma.

**F. Neoplasia, malignant:** Medaka, skin. Higher magnification of the neoplasm in 1.7D, E. An incomplete gland formed by neoplastic cells contains some amorphous pale eosinophilic material in its lumen. Two mitotic figures (arrows) are present in this focus. Surrounding cells include some inflammatory mononuclear cells on the right, and some irregular shaped neoplastic cells on top and to the left of the gland.

**G. Regeneration/Repair:** Goldfish, kidney. Regenerative epithelium (arrows) in a proximal tubule 1 week following toxicant induced cell death. Cells from the uninjured sections of the nephron flatten and migrate to the injured section to cover the basement membrane. Once the defect is covered, the cells divide and re-establish their cuboidal shape.

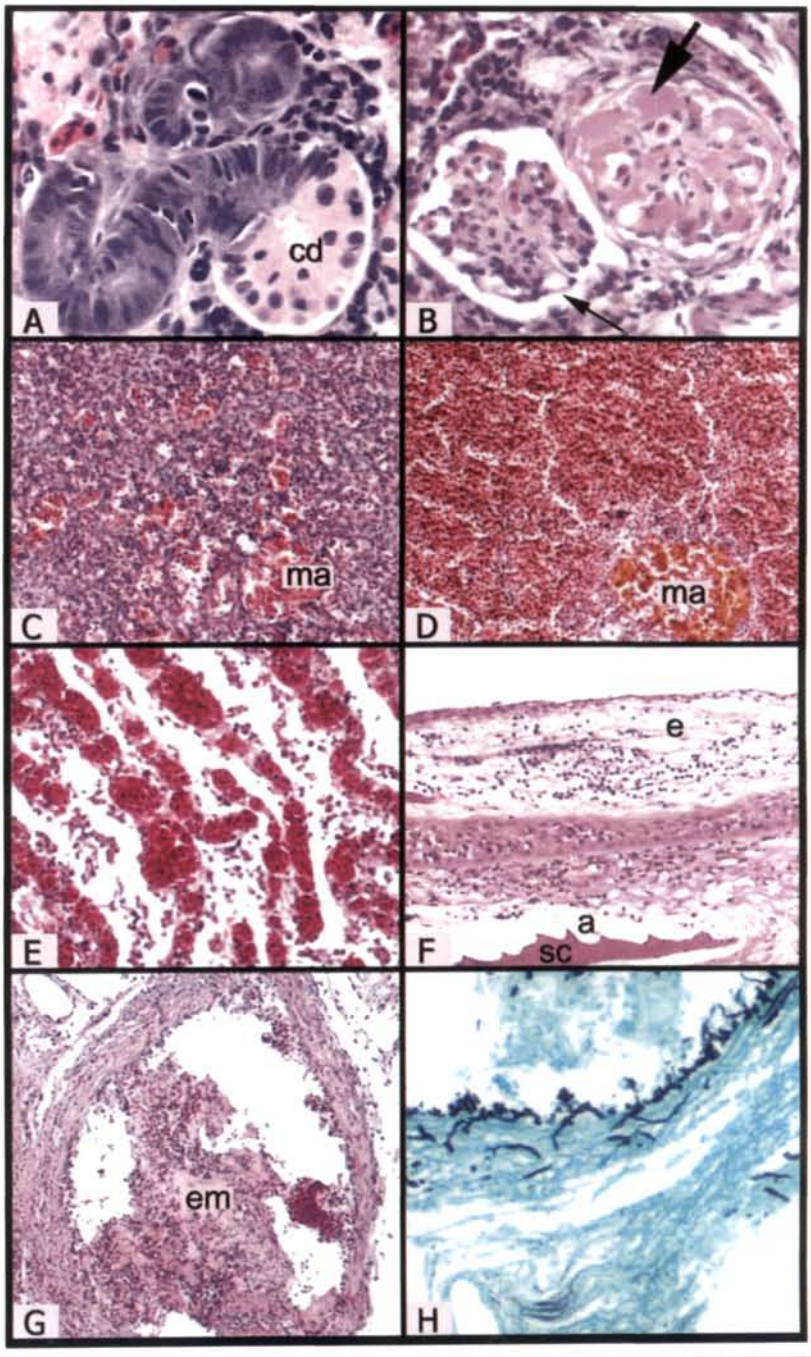



*Fig. 1.8 Contd.*

resulting in edema and migration of inflammatory cells from vessels into the affected area (Fig. 1.8E).

## Edema

Edema is an extracellular fluid which looks like clear spaces in the stroma, separating parenchymal cells from each other. Edema is frequently found in tissues that have sustained injury, often with concurrent inflammation (Figs. 1.4A, 1.5C, 1.8F).

**Fig. 1.8** **A. Regeneration/Repair:** Goldfish, kidney. New nephron (basophilic tubule) penetrating into a pre-existing pale eosinophilic collecting duct (cd). New nephrons develop throughout the life of fish as the kidneys continually grow with the rest of the animal. The presence of large numbers of new nephrons in an adult fish indicates that the kidney has sustained some type of injury and is attempting to replace missing tubules.

**B. Thickened:** Largemouth bass, kidney. The glomerular capillary walls are very thickened in the right glomerulus. Compare the thickened capillary loop (large arrow) with the more normal loop in the left glomerulus (small arrow).

**C. Congestion:** Northern puffer, spleen. Normal spleen for comparison with 1.8D, same magnification. Small macrophage aggregates (ma) are located throughout the section.

**D. Congestion:** Northern puffer, spleen. This spleen has generalized vascular congestion which makes it look very red at low magnification (same magnification as 1.8C). A larger macrophage aggregate (ma) is located in the lower right corner.

**E. Dilatation/Congestion:** Cichlid, colon. The blood vessels are dilated and packed red blood cells. Grossly, such tissue will appear red, but the cells are all contained within blood vessels (compare with hemorrhage 1.9A).

**F. Edema:** Goldfish, skin. The dermis is about 4 times as thick as it should be due to fluid accumulation, edema (e), between the layers of connective tissue. In sections, edema looks like clear spaces and must be differentiated from spaces created during tissue processing. In this case, there are inflammatory cells located within the lesion, along with wisps of connective tissue. Space artifacts created by tearing or shrinkage after hydration and dehydration usually have nothing inside them, such as the artifact (a) surrounding the scale (sc).

**G. Embolism:** Doctorfish, gill-artery. An embolism is foreign matter that has been trapped in a blood vessel, this can be an infectious organism, gas, or even a blood clot which has broken loose and floated to another vessel. The embolism here is a thrombus containing many fungal hyphae. The primary lesion was a skin bite wound that developed a fungal infection which subsequently became systemic. Fungal embolic thrombi were located in multiple areas, including peritoneum, liver and gill arteries.

**H. Embolism:** Doctorfish, gill-artery. Higher magnification of 1.8G. The fungal hyphae (black structures) can be seen invading the vessel wall. (Grocott's methenamine silver stain).

## Embolism

Embolism is a detached intravascular solid, liquid or gaseous mass that is carried by the blood to a site distant from its point of origin. A thrombus can also be an embolus if it has broken off from one site and becomes lodged in a secondary site. Hyper-oxygenation of the fish's water can lead to gas emboli (gas bubble disease) (Figs. 1.8G, H, 1.9E).

## Hemorrhage

In this situation, blood escapes from the cardiovascular system, usually from vessel rupture. Extravasation of erythrocytes due to their passage through apparently unruptured venular and capillary walls is known as diapedesis. Hemorrhage is frequently seen in traumatic injuries but can also be found in organs affected by toxicants (Fig. 1.9A, D).

## Telangiectasia

In mammalian histology, this term refers to microdilations capillaries. In fish, however, this term has been used to describe gill hemorrhage following rupture of the lamellar capillaries and pillar cells. This can be artificially induced by killing the fish with a sharp blow to the head which creates a burst of pressure in the ventral aorta and rupture of the gill capillaries. Rupture of gill capillaries can also occur following toxicant exposure (Fig. 1.9D).

## Thrombus

Thrombus is a clotted mass of blood clot formed from the constituents of the blood within the vessels or the heart during life, often in areas of chronic endothelial injury. A thrombus usually appears pinkish adhering to the vessel wall. It may contain laminated 'lines of Zahn', which are alternate layers of platelets, fibrin and blood cells. Post-mortem blood clots, not thrombi, appear as clumps of red cells within the blood vessels. Those red cells may only be present on one side (the down side) due to gravity separating the cells from the serum. Over a period of time, in a surviving host, the thrombi become organized. Inflammatory cells enter the phagocytize necrotic debris and induce fibrinolysis with proteolytic enzymes. Fibroblasts and capillaries proliferate and invade the thrombus from the vessel wall, causing the thrombus to retract from the vessel walls

and become recanalized. Some disease states can induce widespread thrombus formation, termed 'disseminated intravascular coagulation' (DIC). Thrombi form in and occlude many blood vessels throughout the body. This causes ischemia in the tissues fed by those vessels, but also uses up the clotting factors, paradoxically causing the animal to bleed easily. It is usually a terminal condition (Figs. 1.8G, 1.9B, C).

## INFECTIOUS CHANGES

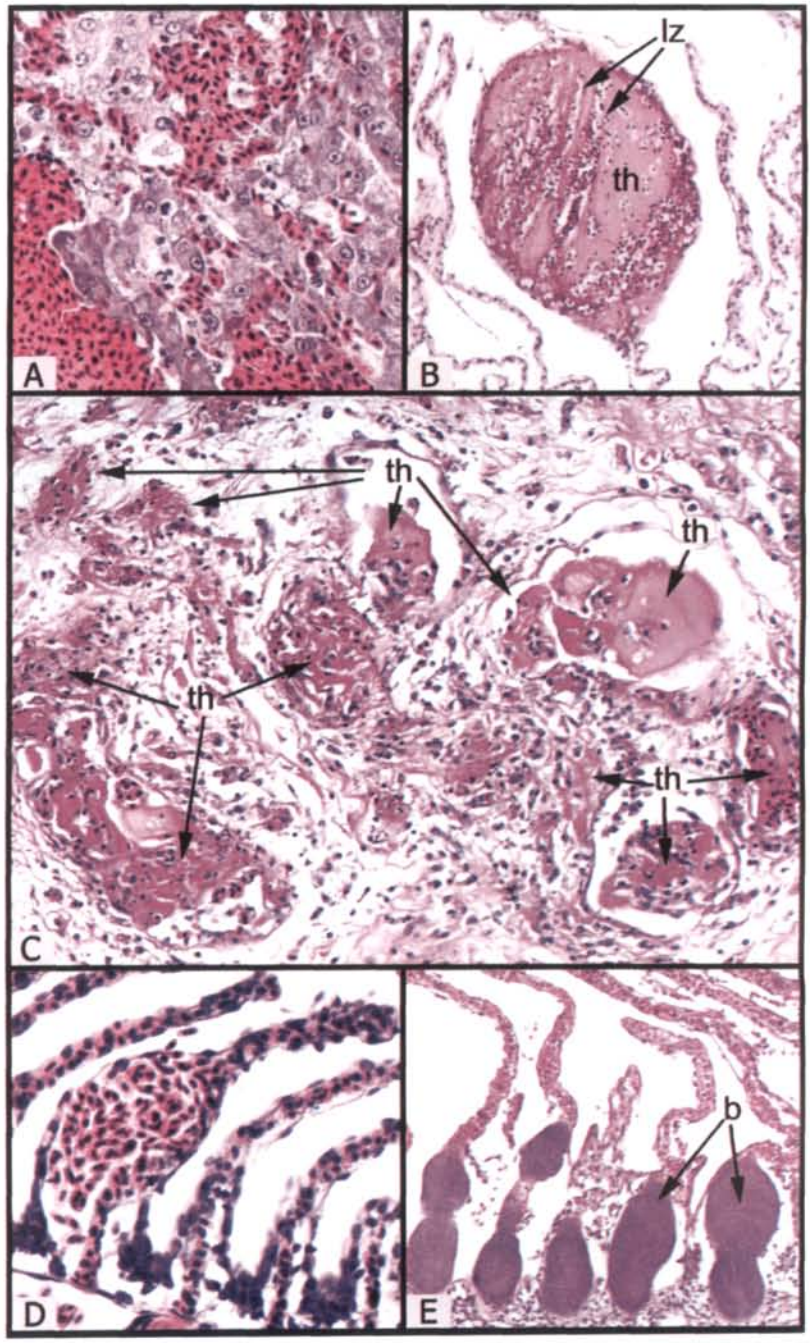
Pathogens can cause a variety of lesions. The organisms may injure or kill host cells by directly invading the cells. Pathogens may also produce toxins, (secreted into extracellular fluid or internal toxins released after the pathogen dies). Those toxins can damage or lyse host cells and may also be chemotactic for host inflammatory cells. The host inflammatory cells may release substances that both injure the pathogen and/or the host cells and promotes chemotaxis for more inflammatory cells. Host responses to persistent pathogens include chronic inflammation along with an attempt to contain the pathogen by surrounding it with fibrous tissue. Specific pathogens will be covered in depth in other chapters.

### Bacterial

Bacteria in tissues can originate from a local invasion, for example, via a skin ulcer, or they have come from a fumigant systemic infection that colonized multiple organs. Bacteria are often associated with liquefactive necrosis and an inflammatory infiltrate, but sometimes bacteria are just present in tissues without associated pathology. Mycobacterium typically induce granuloma formation. Bacteria usually appear bluish on routine H&E stains. Gram and Acid Fast stains can help categorize the bacterial pathogens (Figs. 1.9E, 1.10A, B, 1.13D-F).

### Fungal

Fungal infections are often secondary to another disease or environmental problem. In fish, the organisms tend to elicit strong inflammatory responses and often granulomata. The organisms may appear brownish, but are often difficult to discern without the use of special stains such as Grocott's methenamine silver (GMS) or Periodic Acid Schiffs (PAS) stains (Figs. 1.8G, H, 1.10C, D).

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## Viral

Few viral infections can be diagnosed via histopathology. A notable exception is *Lymphocystis*, an iridovirus which causes characteristic and massive cellular hypertrophy. Other viruses may cause intranuclear or intracytoplasmic vacuoles or inclusion bodies. Additional techniques such as electron microscopy, immunostaining or culture are usually needed to confirm a diagnosis (Fig. 1.10F, G).

## Parasitism

Parasites are organisms that live on or in a host, deriving benefit from that host and sometimes injuring the host. Parasites can be Metazoan, Mesozoan or Protozoans. In the metazoan kingdom, parasites include the Arthropoda, Platyhelminthes (cestodes, trematodes), Mollusca (glochidia-larval forms) and Aschelminthes (nematodes) phyla. Parasites from the kingdom Protista consist primarily of unicellular organisms, although some are colonial. Protozoans more frequently observed in tissue sections include Sarcomastigophora (i.e., flagellates and amoeba), Apicomplexa (i.e., coccidians such as *Eimeria*), Microspora (i.e., *Glugea*), Myxozoa (i.e., *Myxobolus*), Ciliophora (i.e., *Uronema*), and the new phylum Neomonada in the subkingdom Neozoa which contains the

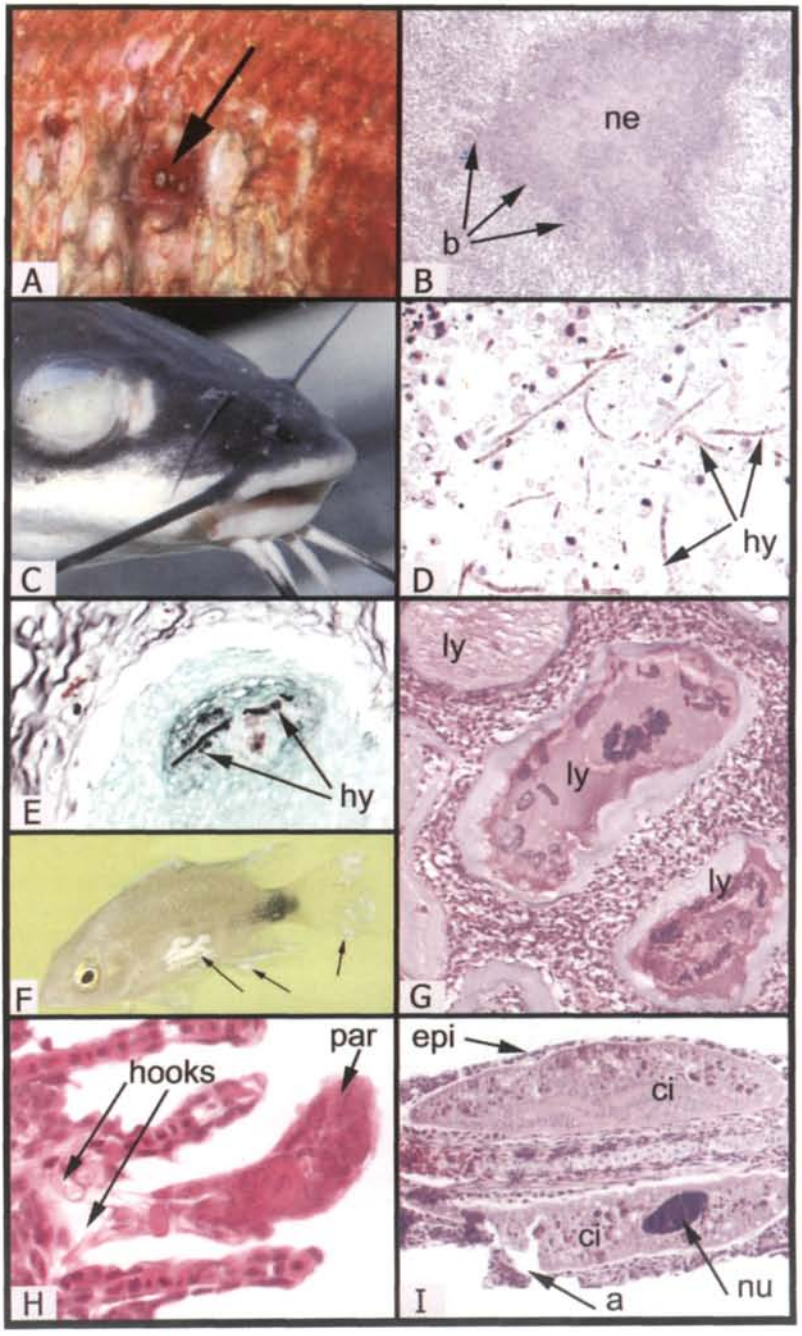
**Fig. 1.9** **A. Hemorrhage:** Alewife, liver. Red blood cells are located between cords of hepatocytes due to fresh hemorrhage. This appearance is typical of a fresh blood clot.

**B. Thrombus:** Pinecone fish, gill. This thrombus is the result of a past gill hemorrhage (such as the one in 1.9D). The clotted blood has organized into a thrombus. White blood cells are present at the margins of an amorphous eosinophilic material. Within the thrombus are layers of platelets and cells forming "Lines of Zahn" (lz-arrows). Over time, large thrombi may become re-canalized by ingrowing capillaries.

**C. Thrombus:** African cichlid, peritoneum. This section shows multiple thrombi (th-arrows) located in the peritoneal fascia, the result of disseminated intravascular coagulation (DIC). Paradoxically, animals with DIC frequently present with hemorrhage because their clotting factors have been exhausted by the extensive intravascular coagulation.

**D. Telangiectasia:** Trout, gill. The gill hemorrhage seen in this section is often called telangiectasia, but it really is rupture of pillar cells and hemorrhage. True telangiectasia is dilatation of the microvasculature.

**E. Bacterial infection:** Wolffish, gill. Bacteria (b-arrows) are lodged in the gill vasculature. The localization of bacterial aggregates in multiple organs is characteristic of *Aeromonas salmonicida* and *Chlamydia/Rickettsia* infections. There is rarely any inflammation or necrosis in the adjacent tissues.

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mesomycetozoans (Rosette agent). Parasites can induce epithelial cell injury and responses such as hyperplasia and metaplasia. They can also promote inflammatory responses which then cause additional pathology in the tissues (Figs. 1.6B, E, 1.10H, I, 1.11A, B, C-H).

## INFLAMMATORY CHANGES

Inflammation is one of the body's main defenses against pathogens. It is also vital for repair of injuries that cause cell death. The host inflammatory response clears away non-viable material or, if this is not possible, sets up a barrier to allow the damaged tissues to heal. Many factors can initiate the cascade of events that result in an inflammatory response. Dead or injured cells release substances that are chemotactic for inflammatory cells. There

**Fig. 1.10** **A. Bacterial infection:** Squirrelfish, skin. Gross appearance of an ulcer produced by a systemic *Edwardiella tarda* infection. Although the opening through the skin is small (arrow), the actual lesion extends rather deeply, below the pale, discolored scales.

**B. Bacterial infection:** Squirrelfish, spleen. *Edwardiella tarda* infection with bacterial colonies (b-arrows) located at the periphery of the lesion and a central necrotic (ne) core. Over time surviving fish may wall off these lesions producing granulomata.

**C. Fungal infection:** Channel catfish, eye. Gross appearance of a superficial fungal infection of the eye.

**D. Fungal infection:** Atlantic menhaden, skin. Fungal hyphae appear brownish (hy-arrows) in this wet mount of an external fungal infection stained with Hemacolor.

**E. Fungal infection:** Angelfish, skin. Fungal hyphae (hy-arrows) appear black in this biopsy specimen of a small raised white skin lesion, which looked similar to the lesions seen in 1.10F. (Grocott's methenamine silver stain).

**F. Viral infection:** Cichlid, fins. *Lymphocystis* viral infection (arrows) appears as a white, nodular material on the fins. This infection is often induced by stress but is self limiting and will heal if the animals are put in a less stressful environment.

**G. Viral infection:** Goldfish, skin. The infected skin cells (ly) containing the *Lymphocystis* virus are extremely hypertrophic. Compare these enormous cells with the surrounding small epithelial cells whose nuclei look pinpoint size at this magnification.

**H. Parasitic infection:** Goldfish, gill. This monogenean trematode (par-arrow) has prominent haptor hooks for attachment (hooks-arrows).

**I. Parasitic infection:** Koi, gill. Two ciliate parasites (ci), *Ichthyophthirius*, in the gill. This stage of the parasite is located under the epithelium (epi-arrow) making it quite difficult to treat with topical or bath treatments. The large, basophilic structure in the bottom parasite is the nucleus (nu-arrow). Some of the parasite and host epithelium has been torn off of the section, an artifact (a-arrow) produced while cutting the section.

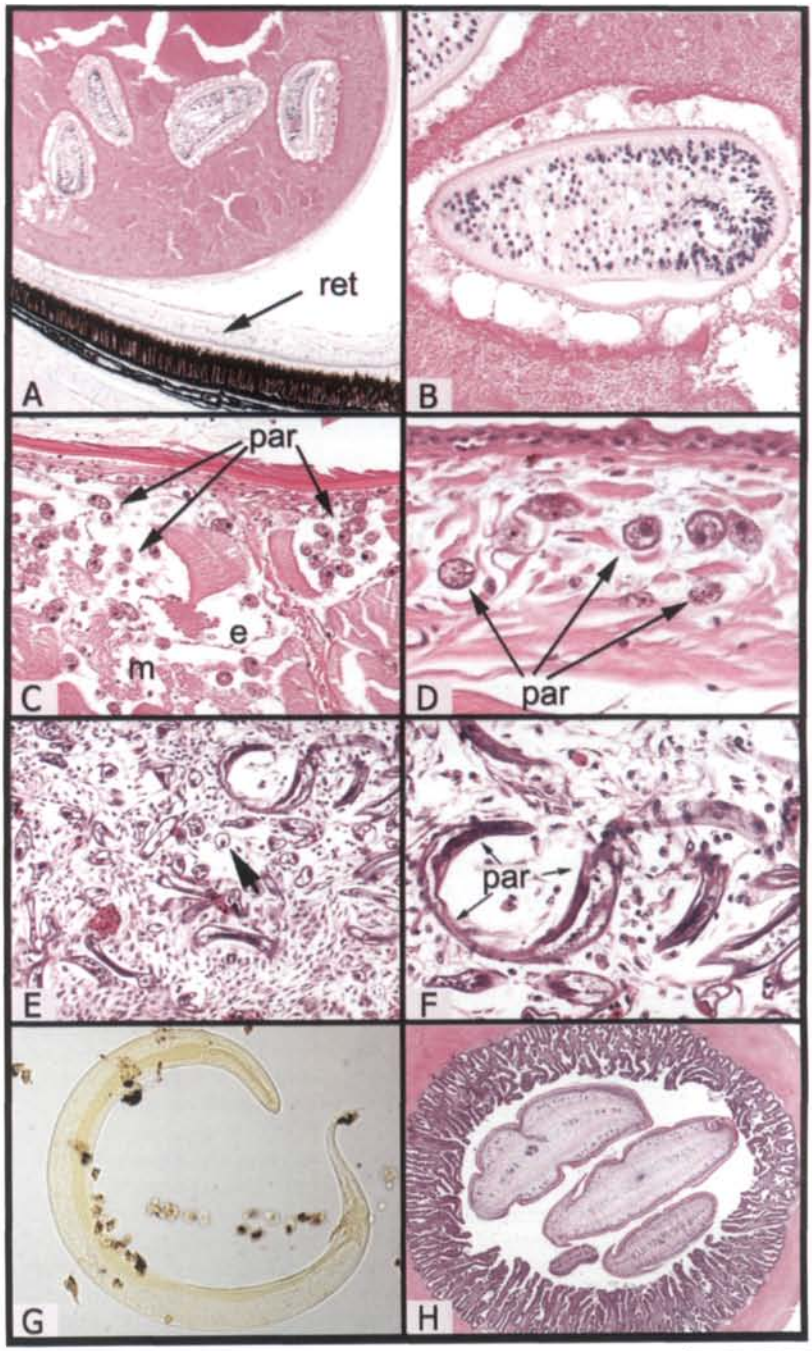


*Fig. 1.11 Contd.*

are also a number of substances (such as vasoactive amines, plasma proteases, complement, the kinin system, and the clotting system proteins) that, when activated by breaks in the vascular system, trigger the inflammatory cells to respond. Antibodies to pathogens also activate the host immune cells and induce migration toward the affected area.

The initial inflammatory response is composed primarily of polymorphonuclear cells (also called PMNs, neutrophils and granulocytes, or in sharks, heterophils). These cells arrive at an injured or infected site, responding to chemotactic substances and or alterations in blood flow and vessel permeability. PMNs phagocytize smaller pathogens and also release hydrolytic enzymes from their granules into the affected tissue. These

**Fig. 1.11** **A. Parasitic infection:** Brown bullhead catfish, lens. Section of a lens containing trematode (digene) metacercaria in the lens. The parasites' migration within the lens may cause the lens to herniate. The herniated portion of the lens containing the parasites is often located outside of the fish's visual field, perhaps providing a survival advantage to the host. The retina (ret-arrow) is located at the bottom of the section.

**B. Parasitic infection:** Brown bullhead catfish, lens. Higher magnification of 1.11A showing a trematode (digene) in the lens. Typically there is minimal inflammation but there is usually focal cortical liquefaction surrounding the parasites, as seen here.

**C. Parasitic infection:** Pipefish, skin. Many ciliated protozoan parasites (par-arrows) are located throughout the subcutis and muscle. These parasites have caused muscle (m) necrosis with fragmentation and edema (e).

**D. Parasitic infection:** Pipefish, skin. Higher magnification of the ciliated protozoan (par-arrows) from the fish in 1.11C, but the dermal connective tissue of this section.

**E. Parasitic infection:** Brown shark, uterus. Larval nematodes located throughout the edematous tissue. In cross-section (arrowhead) the parasite has a prominent cuticle with a deeply basophilic core substance, often located eccentrically, surrounded by a clear space. Many of the larvae are cut tangentially, but one has the "C" shape similar to the parasite in the wet mount 1.11G.

**F. Parasitic infection:** Brown shark, uterus. Higher magnification of the "C" shaped larval nematode (par-arrows) in 1.11E. A few eosinophilic heterophils are located in the edematous tissue around the parasites.

**G. Parasitic infection:** Brown shark, uterus. Wet mount made with the formalin fixed tissues of the fish in 1.11E, F. The larval nematode has a buccal cavity and intestinal tract (darker gold). Exfoliated uterine epithelial cells are adhering to the surface of the parasite (unstained).

**H. Parasitic infection:** Boxfish, intestine. A very large cestode parasite is present in the lumen of the intestine. These sections may all be cut through a single, folded parasite. Some small basophilic foci (calcareous corpuscles or mineral concretions that help identify this as a cestode) are located in the parenchyma along the parasite's tegument but are difficult to resolve at this magnification.

enzymes kill bacteria, but can also damage the host cells. The substances released into the extracellular space by the PMNs, pathogens and host cells are powerful mediators of the inflammatory reaction, amplifying the response by producing greater chemotactic signals and vascular changes.

Over time, as the response becomes more chronic, mononuclear cells enter the lesion and begin to clean up the material. These cells are primarily macrophages, lymphocytes and plasma cells. These cells are recruited to the injured area by the factors described above. Macrophages engulf foreign material and necrotic debris, eventually clearing the lesion. As this process proceeds, capillaries and fibroblasts proliferate. If the tissue contains labile (cells capable of division) parenchymal cells, then those will also begin to proliferate and repopulate the site. Otherwise, fibroblasts fill the defect by laying down collagen producing a scar. During this process, the macrophage numbers decline. If the injurious agent is not eliminated (such as Mycobacteria or continual trauma), the macrophages remain at the site. Granulomata form as the body attempts to contain or wall off the offending agent.

## Abscess

Abscess is a localized collection of pus usually caused by pyogenic bacteria. It is composed of PMNs in a space created by either separation of pre existing cellular elements or by liquefactive necrosis of the surrounding tissues. As it develops, an outside zone of fibroblast proliferation eventually walls off the abscess, limiting further spread (Fig. 1.3A-C).

## Acute Inflammation

This stage has a rapid course (usually up to 48 hours) and is not proliferative. Pathologic features include changes in vascular flow, changes in vascular permeability, and leukocytic exudation consisting mainly of PMNs and monocytes. This phase of inflammation is rather fast and not frequently observed in fish necropsy tissues since the lesions usually progress to a chronic stage before the animal dies. More frequently PMNs are found in peripheral blood smears. PMNs have variably shaped nuclei—some highly segmented and a variety of intracytoplasmic granules. Shark heterophils have eosinophilic granules (large and small), but these are not the same as the eosinophilic granulocytic cells of teleosts or the eosinophils of mammals (Fig. 1.12A-D).

## Chronic-active Inflammation

The transition from acute to chronic inflammation is often difficult to pinpoint. Characteristics of acute and chronic inflammation can occur and it is referred to as chronic-active inflammation. Chronic-active inflammation can occur when the inciting stimulus is persistent, invoking cellular infiltrations characteristic to both acute and chronic inflammatory processes. Cell types include some PMNs, mononuclear cells such as macrophages, and sometimes eosinophilic granulocytic cells. Shark heterophils are found in both acute and chronic-active responses. Eosinophilic granulocytic cells (EGC) are can be present in chronic or chronic-active inflammation, sometimes associated with parasitic infections (Figs. 1.5C, 1.12E, F).

## Chronic Inflammation

Persistent inflammatory stimuli lead to chronic inflammation. Such stimuli include large necrotic foci (from trauma or hypoxia), continuing injury from an infectious process, or immune mediated diseases. The predominant cell types associated with chronic inflammation are lymphocytes, plasma cells, and macrophages. Chronic inflammation often results in proliferation of surrounding connective tissue (Figs. 1.5C, 1.12 G, H, 1.13A-C).

## Granulomatous Inflammation

This is a form of chronic inflammation characterized by focal accumulations of mononuclear cells, mainly macrophages (histiocytes-tissue macrophages) and epithelioid (those macrophages that have taken on an almost epithelial appearance) surrounded by fibroblasts. Lymphocytes, plasma cells, and giant cells may be associated with the granuloma. A fibrous capsule of varying thickness surrounds the inflammatory epithelioid cells with a central core of necrotic debris. Granulomatous lesions can occur as a result of certain bacteria, *Mycobacterium* sp. and *Flavobacterium* sp., fungus, *Nocardia* sp., reactions to foreign bodies (i.e., cholesterol, silica), and parasites. Species identification of parasites within granulomata is often difficult, but Chitwood and Lichtenfels (1973) have described a number of parasites in section (Figs. 1.4D, E, 1.12H).

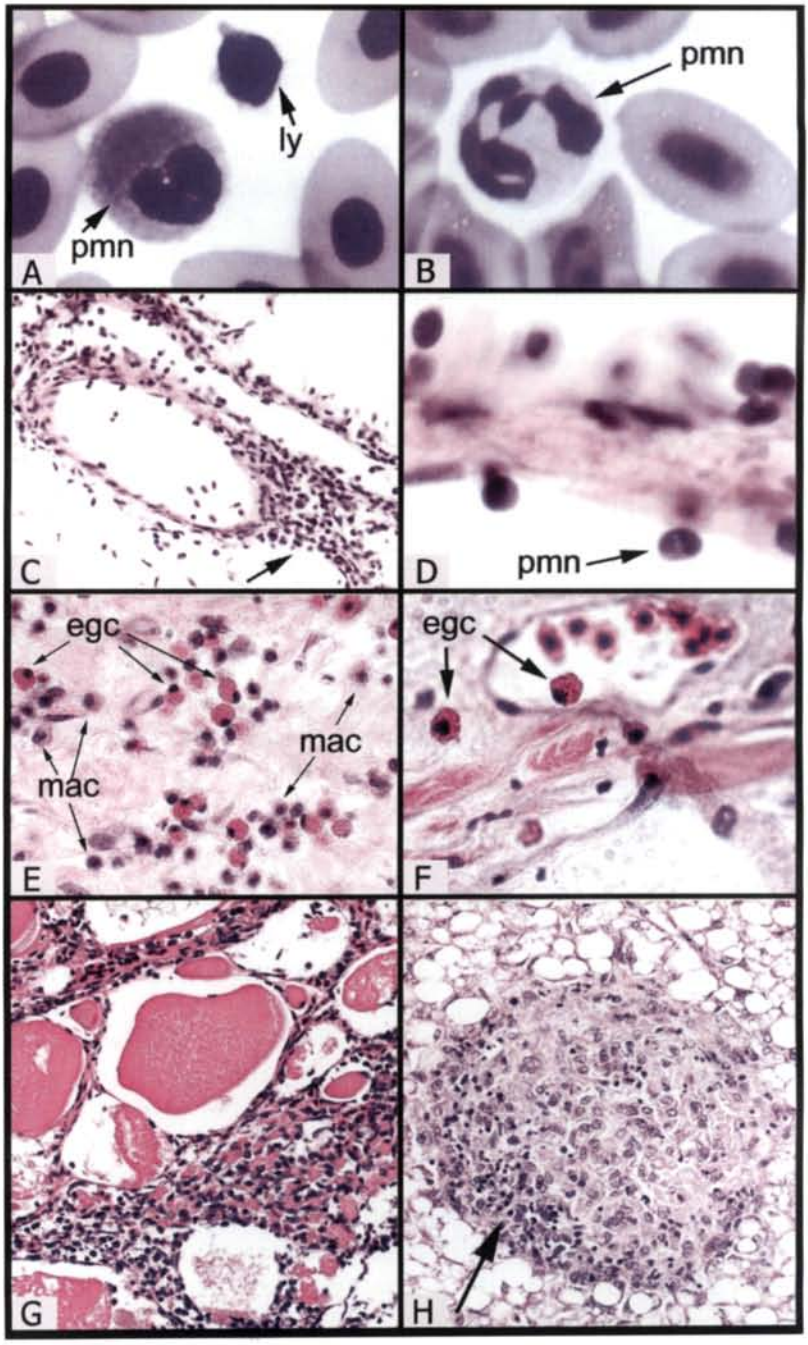


*Fig. 1.12 Contd.*

## Giant Cells

Giant cells are large multinucleated cells usually formed by fusion of macrophages, rarely nuclear division. The nuclei are sometimes arranged around the periphery in a horseshoe pattern forming the Langhans' type giant cell. Giant cells also are formed in the presence of indigestible material or foreign bodies. They are seen most commonly in mycobacterial and fungal infections (Fig. 1.13C).

**Fig. 1.12** **A. Inflammation—polymorphonuclear cell:** Koi, blood. The morphology of fish granulocytes or polymorphonuclear (PMN) cells, as their name implies, varies between different fish species. Koi PMNs are usually only bent (pmn-arrow), as seen here. They can have a variety of cytoplasmic granules. Shark granulocytes (called heterophils) have eosinophilic granules, but they are different cells from the teleost eosinophilic granulocytic cells (EGC) found in tissues, both of which are different from mammalian blood eosinophils. Present also is a small lymphocyte (ly-arrow) with typically scant cytoplasm. Multiple nucleated oval red blood cells surround the two white blood cells (Hemacolor stain).

**B. Inflammation—polymorphonuclear cell:** Atlantic salmon, blood. This polymorphonuclear cell (pmn-arrow) has multiple nuclear segments and is surrounded by red cells (Hemacolor stain).

**C. Acute inflammation:** Trout, peritoneal blood vessel. A number of cells adhere to the endothelium within the blood vessel. Inflammatory cells follow chemotactic signals, migrating through the vessel wall to the surrounding tissues. Such migration creates "perivascular cuffing," the cluster (arrow) of inflammatory cells surrounding the vessel.

**D. Acute inflammation:** Trout, peritoneal blood vessel. Higher magnification of 1.12C. Two cells, one a polymorphonuclear cell (pmn-arrow), adhere to the vascular endothelium. PMN's are not frequently found in tissue sections, as the lesions usually have progressed to a chronic stage before the fish dies or a biopsy specimen is taken.

**E. Chronic active inflammation:** Squirrel fish, eye. Eosinophilic granulocytic cells (egc-arrows) migrate through retroorbital connective tissue. Present also are numerous macrophages (mac-arrows). EGCs are frequently encountered in fish tissues, and are not only associated with parasitic infections.

**F. Chronic active inflammation:** Squirrel fish, eye. Eosinophilic granulocytic cells (egc-arrows) located inside and outside a small blood vessel.

**G. Chronic inflammation:** Trout, muscle. Macrophage and lymphocyte infiltrate between myocytes, some of which are undergoing fragmentation and necrosis.

**H. Chronic inflammation:** Killifish, liver. Early granuloma composed of epithelioid macrophages and remnants of other mononuclear cells (arrow). Over time this type of nodule will develop a fibrous capsule.

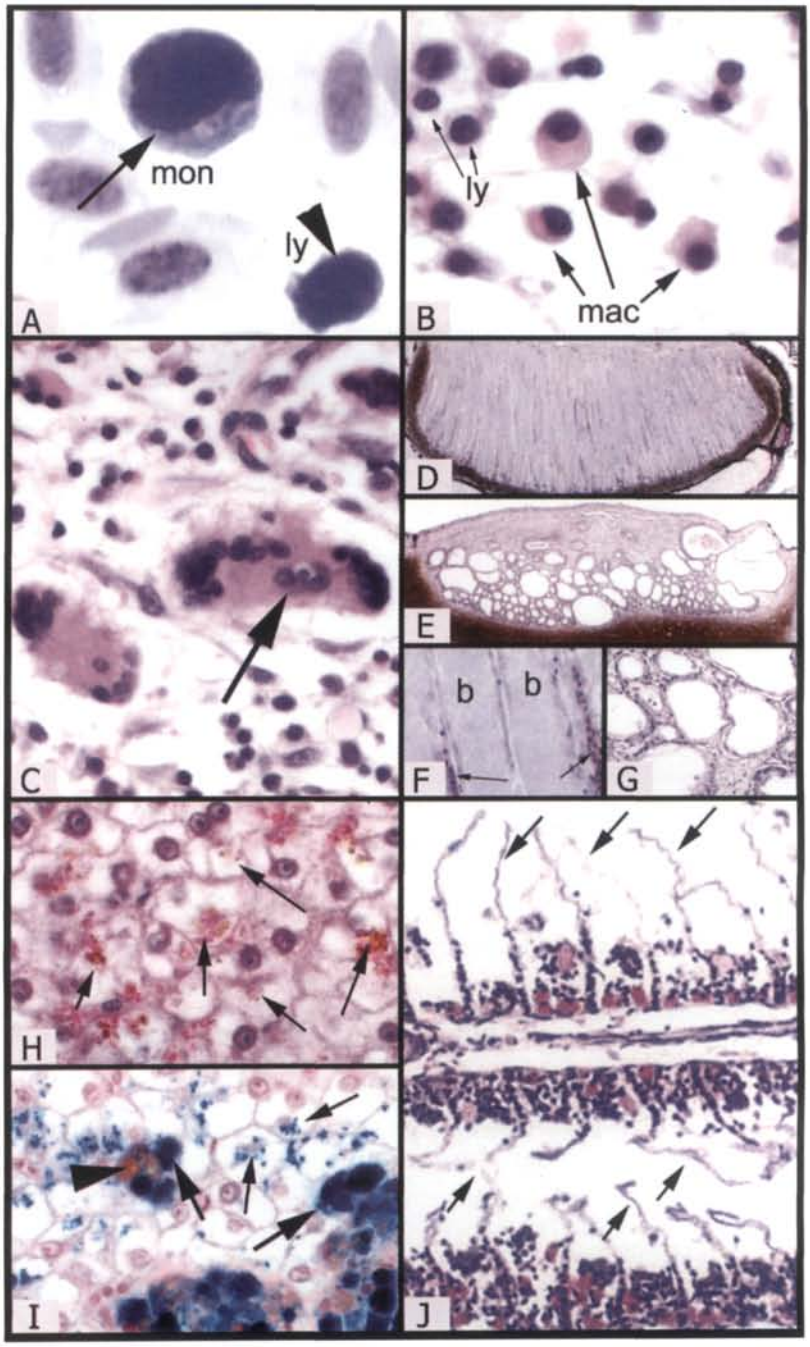


*Fig. 1.13 Contd.*

**Fig. 1.13** **A. Monocyte inflammation:** Atlantic salmon, blood. These cells (mon-arrow) have an unsegmented nucleus and the cytoplasm is devoid of granules. A lymphocyte (ly-arrowhead) is present as are multiple nucleated oval red blood cells (Hemacolor stain).

**B. Chronic inflammation:** Green moray eel, dermis. Prominent macrophages (mac-arrows) and lymphocytes (ly-arrows) are seen in this section of dermis.

**C. Chronic giant cell inflammation:** Green moray eel, dermis. Two multinucleated giant cells surrounded by other mononuclear cells. These cells are created by the fusion of multiple macrophages, often surrounding an organism or foreign body that cannot be destroyed or phagocytized. The arrow points to a cluster of gram positive bacteria inside a vacuole within one of these giant cells. This type of cell is frequently encountered in mycobacterial infections.

**D. Cyst:** Flashlight fish, light organ-normal. This is the normal appearance of these organs. The tissue has a reflective basal layer underlying the parallel cylinders which contain symbiotic bioluminescent bacteria. These structures have small capillaries (1.13F arrows) which run parallel to, and between the cylinders of bacteria, possibly providing nutrients to the bacteria.

**E. Cyst:** Flashlight fish, light organ-cystic. This light organ did not glow for several months before the animal's death. Present are cystic structures, empty cavities with an epithelial lining. These are quite different from the normal cylindrical structures that hold the bacteria. The microenvironment of these cysts may not be able to sustain the symbiotic bacteria.

**F. Cyst:** Flashlight fish, light organ-normal. Higher magnification of 1.13D. The bacteria (b) are the hazy blue-grey material located in the cylindrical structures. The arrows point to the capillaries that parallel those structures.

**G. Cyst:** Flashlight fish, light organ-cystic. Higher magnification of 1.13E. The cysts are devoid of the bacteria, and are surrounded by fibrous connective tissue.

**H. Pigment accumulation:** Red catfish-liver. The arrows indicate golden granules of iron pigment which has accumulated in the cytoplasm of these hepatocytes.

**I. Pigment accumulation:** Red catfish-liver. Special stain showing the iron pigment as blue. Small inclusions (thin arrows) are present in the hepatocyte cytoplasm. Large accumulations (larger arrows) are present in macrophages (within macrophage aggregates). Present also in multiple macrophages is the pinkish-orange aging pigment, ceroid/lipofuscin (arrowhead).

**J. Artifact:** Many artifacts can occur during the process of making slides. It is important to handle tissue appropriately when harvesting, preserving, and processing them. The pathologist must decide whether the finding is a true lesions or the result of processing. This photomicrograph shows normal gills that were blotted on a paper towel before placing into fixative. So, to paraphrase an old adage "Pathologist Beware."

## MISCELLANEOUS/POINTS/NOTES

### Cyst

A cyst is a cavity that may contain a fluid or a semisolid material and is surrounded by a definite wall. Cysts are typically lined by epithelial cells. They can arise due to duct or tubule blockage, parasites, tumors, developmental errors, or hemorrhage (hematoma) that has become encapsulated (Fig. 1.13D-G).

### Pigment Accumulation

Pigment accumulation may occur in or around cells. Frequently encountered pigments include melanin (black), lipofuscin/ceroid (yellow brown) an aging pigment, and hemosiderin (yellow brown), a breakdown product of blood. Bilirubin (green-brown) may occur in hepatocytes from bile stasis. An excess of iron (yellow gold) may be found as a result of hemolysis or increased iron uptake. Melanin is often found in fish macrophages, especially in renal macrophage aggregates (Fig. 1.13H, I).

### Ectopic Thyroid

Thyroid tissue in bony fish is generally not encapsulated and capable of widespread ectopic growth in various organs. Ectopic thyroid is not considered a lesion but should be recorded for reference when observed.

### Basophilic Tubules

These renal tubules have been described as developing immature tubules. They are extremely basophilic and contain large nuclei with frequent mitotic figures. They have been noted in young, rapidly growing fish as well as fish that have been moved from freshwater to seawater or those that have experienced tubular injury. Large numbers of basophilic developing tubules in adult fish kidneys may indicate previous renal damage (Fig. 1.8A).

### Macrophage Aggregates

Also known as melanomacrophage centers, macrophage aggregates are commonly associated with organs in teleost fishes, primarily the spleen, kidney, and liver. Macrophage aggregates can vary in size and number, depending on age, sex, disease, and dietary imbalances (Figs. 1.8D, 1.13I).

## References


Amin, A.B., L. Mortenssen and T. Poppe. 1992. *Histology Atlas, Normal Structure of Salmonids. International edition.* Akvapatologisk Laboratorium AS, Bodo.

Cotran, R.S., V. Kumar and S.L. Robbins. 1999. *Robbins Pathologic Basis of Disease.* 6th Edition. W.B. Saunders, Philadelphia.

Ferguson, H.W. 2006. *Systemic Pathology of Fish: A Text and Atlas of Normal Tissues in Teleosts and their Responses in Disease.* H.W. Ferguson (ed.). Scotian press, http://www.scotianpress.com/main.html

Grizzle, J.M. and W.A. Rogers. 1976. *Anatomy and Histology of the Channel Catfish.* Auburn University, Auburn.

Groman, D.B. 1982. *Histology of the Striped Bass.* American Fisheries Society Monograph No. 3, American Fisheries Society, Bethesda.

Harshbarger, J.C. and J.B. Clark. 1990. Epizootiology of neoplasms in bony fish of North-America. *Science of the Total Environment* 94: 1-32.

Hibiya, T. 1982. *An Atlas of Fish Histology: Normal and Pathological Features.* Gustav Fisher Verlag, Stuttgart.

Hinton, D.E., R.C. Lantz, J.A. Hampton, P.R. McCuskey and R.S. McCuskey. 1987. Normal versus abnormal structure: considerations in morphologic responses of teleosts to pollutants. *Environmental Health Perspectives* 71: 139-146.

Johnson, R.D. and H.L. Bergman. 1984. Use of histopathology in aquatic toxicology: a critique. In: *Contaminant Effects on Fisheries,* V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.). John Wiley & Sons, New York, pp. 19-36.

Meyers, T.R. and J.D. Hendricks. 1985. Histopathology. In: *Fundamentals of Aquatic Toxicology: Methods and Applications,* G.M. Rand and S.R. Petrocelli (eds.). Hemisphere Publishing Corporation, New York, pp. 283-331.

Noga E.J. 1996. *Fish Disease: Diagnosis and Treatment.* C.V. Mosby, St. Louis.

Reimschuessel, R. 1993. Postmortem Examination. In: *Fish Medicine,* M.K. Stoskopf (ed.). W.B. Saunders, Philadelphia, pp. 160-165.

Reimschuessel, R. 1999. Necropsy techniques in aquarium fish. In: *Kirk's Current Veterinary Therapy,* J.D. Bonagura (ed.). W.B. Saunders, Philadelphia, pp. 1198-1203.

Reimschuessel, R., E.B. May, R.O. Bennett and M.M. Lipsky. 1988. Necropsy examination of fish. In: *Veterinary Clinics of North America: Tropical Fish Medicine,* M.K Stoskopf (ed.). W.B. Saunders, Philadelphia, Vol. 18, pp. 427-433.

Reimschuessel, R., R.O. Bennett and M.M. Lipsky. 1992. A histological lesion classification system. *Journal of Aquatic Animal Health* 4: 135-143.

Roberts, R.J. (ed.). 2001. *Fish Pathology.* W.B. Saunders, London.

Sindermann, C.J., F.B. Bang, N.O. Christensen, V. Dethlefsen, J.C. Harshbarger, J.R. Mitchell and M.F. Mulcahy. 1980. The role and value of pathobiology in pollution effects monitoring programs. *Rapports et procès-verbaux des réunions, commission internationale pour l'exploration scientifique de la mer méditerranée* 179: 135-151.

Stoskopf, M.K. 1993. Clinical examination and procedures. In: *Fish Medicine,* M.K. Stoskopf (ed.). W.B. Saunders, Philadelphia, pp. 62-78.

US Fish and Wildlife website, *Trout Histology Image Collection* (http://training.fws.gov/BART/fish/histo1.html). Accessed 01-06-06.

Wildgoose, W.H. and G.A. Lewbart. 2001. Therapeutics. In: *Manual of Ornamental Fish*, W.H. Wildgoose (ed.). 2nd Edition. Manson Publishing, Gloucester, pp. 237-258.

Yasutake, W.T. and J.H. Wales. 1983. *Micrsocpic Anatomy of Salmonids: An Atlas*. United States Fish and Wildlife Service Resource Publication 150, Washington, D.C.

Yonkos, L.T., D.J. Fisher, R. Reimschuessel and A.S. Kane. 2000. *Digital Atlas of Fathead Minnow Normal Histology*. An online publication of the University of Maryland Aquatic Pathobiology Center (http://aquaticpath.umd.edu/fhm). Accessed 01-06-06.

CHAPTER

# 2

# Diseases Caused by Virus: Viral Diseases in Salmonids

**S.M. Bergmann[1] and D. Fichtner[2]**

## INTRODUCTION

Viruses are amongst the smallest of pathogens, and the majority of animal viruses range in size from approximately 20 to 400 nm in diameter or length, although some bacilliform or filamentous viruses grow even longer. The majority of viruses can only be visualized by an electron microscope, although the largest viruses can just be seen with powerful light microscopes. Viruses comprise a nucleic acid core surrounded by a protein coat, which may or may not be surrounded by—or incorporate—a lipid-containing envelope. Proteins may project from the surface of the virus/envelope, and may appear as spikes or knobs under the electron microscope. Viruses can have various morphologies from roughly spherical, icosahedral or brick-shaped to long filaments. Animal rhabdoviruses are commonly bullet-shaped. The nucleic acid is either DNA or RNA (never both), which can be double- or single-stranded, and

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there may be just one or several molecules of nucleic acid in the virus. This apparently simple structure of viruses belies the complex strategies for replication in their hosts. One of the important features of viruses from a diagnostician's viewpoint is that they will only replicate in living cells. This means that unless the virus will replicate in cell cultures (living cells cultivated in a nutrient medium *in vitro*), it can be a long, slow process—firstly to identify a virus as the cause of a 'new' disease, and then to be able to diagnose the condition on a routine basis. This feature of viruses has hampered work on virus diseases in the past, but newer molecular diagnostic methods that are being developed or that are already available will be invaluable in future diagnostic investigations.

## DISEASES OF SALMONIDS

### Viral Haemorrhagic Septicaemia

Viral haemorrhagic septicaemia (VHS) is a disease of farmed fish, especially rainbow trout (Wolf, 1988; Smail, 2000; Fig. 2.1), turbot (Schlotfeldt *et al.*, 1991), Japanese flounder (Isshiki *et al.*, 2001) as well as several wild marine species (Meyers *et al.*, 1994, 1999; Traxler *et al.*, 1999; Hedrick *et al.*, 2003) caused by viral haemorrhagic septicaemia virus (VHSV, synonym: Egtved virus), a virus belonging to the genus *Novirhabdovirus* within the family Rhabdoviridae (Walker *et al.*, 2000).

### Hosts

The number of VHSV-susceptible host species is steadily increasing and includes, among others, rainbow trout and other species of trout, Atlantic salmon, inhabitants of freshwater streams, rivers and lakes, and several farmed and wild marine fish species but also non-salmonid fish such as pike and eel (Bergmann *et al.*, 2003). The number of wild marine species from which VHSV has been isolated is still growing (see Table 2.1) and has led some to conclude that VHSV is endemic in marine or anadromous fish species. The reservoirs of VHSV are clinically infected fish as well as covert carriers among cultured and wild fish. Virulent virus is shed primarily in the urine and faeces, whereas kidney, spleen, and heart are the sites where virus exists permanently. Survivors of epizootics become probably lifelong carriers. The finding that a broad range of wild fish species, in both, the marine and freshwater environments can carry the virus provides strong evidence that viral populations can be maintained in the wild and act as a source of infection for farmed fish. Conversely, naïve, wild fish are susceptible to infection with virus derived from farmed fish

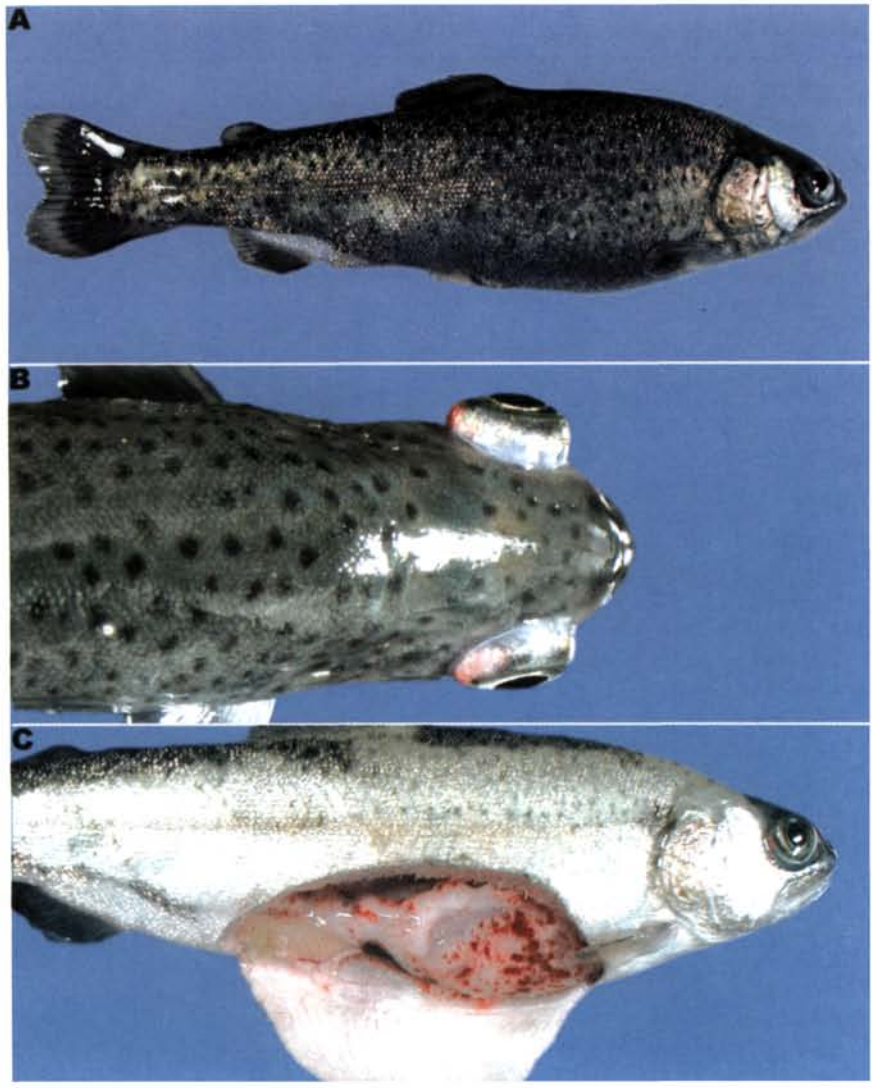


**Fig. 2.1** VHS diseased rainbow trout with darkening of skin (A), exophthalmia (B) and generalised petechial haemorrhaging in the muscle tissue and internal organs (C).

(Jorgensen, 1982; Enzmann and Konrad, 1985). Thus, the bi-directional transfer of virus between farmed and wild fish populations plays a significant role in maintaining viral populations (Meyers *et al.*, 1994) and, once established at a location, for example, in a farm stock and therefore in the water catchments system, the disease becomes endemic due to the ever-presence of carrier fish.

**Table 2.1** Hosts for viral haemorrhagic septicaemia virus (Modified according to OIE, Anonymous, 2006).

| *Common name* | *Scientific name* |
|---|---|
| Atlantic salmon | *Salmo salar* |
| Atlantic cod | *Gadus morhua* |
| Atlantic herring | *Clupea harengus* |
| Black cod | *Anaplopoma fimbria* |
| Blue whiting | *Micromesistius poutassou* |
| Brook trout | *Salvelinus fontinalis* |
| Brown trout | *Salmo trutta* |
| Chinook salmon | *Oncorhynchus tshawytscha* |
| Coho salmon | *O. kisutch* |
| Common dab | *Limanda limanda* |
| English sole | *Paraphrys vetulus* |
| European eel | *Anguilla anguilla* |
| Flounder | *Platichthys flesus* |
| Golden trout | *Salmo aguabonita* |
| Grayling | *Thymallus thymallus* |
| Greenland halibut | *Reinhardtius hippoglossoides* |
| Haddock | *Melanogrammus aeglefinus* |
| Japanese flounder | *Paralichthys olivaceus* |
| Lake trout | *Salvelinus namaycush* |
| Lesser argentine | *Argentina sphyraena* |
| Norway pout | *Trisopterus esmarkii* |
| Pacific cod | *Gadus macrocephalus* |
| Pacific hake | *Merluccius productus* |
| Pacific herring | *Clupea harengus pallasi* |
| Pacific mackerel | *Scomber japonicus* |
| Pacific sandlance | *Ammodytes hexapterus* |
| Pike | *Esox lucius* |
| Pilchard | *Sardinops sagax* |
| Plaice | *Pleuronectes platessa* |
| Poor cod | *Trisopterus minutus* |
| Rainbow trout | *Oncorhynchus mykiss* |
| Rockling | *Rhinonemus cimbrius* |
| Sea bass | *Dicentrarchus labrax* |
| Shiner perch | *Cymatogaster aggregata* |
| Smelt | *Thaleichthys pacificus* |
| Sprat | *Sprattus sprattus* |
| Surf smelt | *Hypomesus pretiosus pretiosus* |
| Three-spined stickleback | *Gasterosteus aculeatus* |
| Turbot | *Scophthalmus maximus* |
| Sand goby | *Pomatoschistus minutus* |
| Walleye pollock | *Theragra chalcogramma* |
| Whitefish | *Coregonus* sp. |
| Whiting | *Merlangius merlangius* |

Several factors influence susceptibility to VHSV. Among each fish species, there is individual variability in susceptibility, and the age of the fish appears to be of some importance—the younger the fish, the higher the susceptibility. All ages of fish are susceptible to infection, but younger fish are more susceptible than older fish, probably due to the development of at least some immunity in older fish following prior exposure. In highly susceptible fish stocks, however, overt infection is seen in all sizes of fish.

## Disease Symptoms

Clearly visible clinical signs of VHS are recognizable in the acute phase of the infection. Fish display a dark skin colour, exophthalmia, pale gills, and haemorrhages in fins, eyes and skin. Abnormal swimming behaviour and the development of ascites is possible. In fry, very often, 100% mortality can occur. In the chronic phase of the VHSV infection, often no external symptoms are visible. In addition, following the acute phase, the disease can occur in a subclinical, chronic state in which affected fish do not show any external signs. Due to the tropism of the virus for the brain, probably as a result of endothelium destruction, VHS can also occur in a nervous form, characterized by severe abnormal swimming behaviour such as flashing or spiralling.

Knowledge of the mechanism of virus transmission has come mainly from studies on European freshwater VHSV isolates that have shown transmission to be horizontal through contact with other infected fish or contaminated water, fomites, etc. Virus is shed from infected fish via the urine (Neukirch, 1985) and reproductive fluids and can also be transferred by piscivorous birds (Peters and Neukirch, 1986). Transmission occurs in the temperature range 1–12°C and the incubation time is 1–2 weeks at the higher temperatures and 3–4 weeks at the lower temperatures within this range. During and immediately following an epizootic (which can involve mass mortality) in the wild (Meyers *et al.*, 1999) or farmed populations in freshwater (Meier and Wahli, 1988) or marine environments (Isshiki *et al.*, 2001; Hedrick *et al.*, 2003), virus can be isolated readily in cell culture. Kidney and spleen tissues yield the highest viral titres.

### *Clinical Signs and Methods*

Visible signs are not VHS-specific but typical for systemic infection and also depend on the stage of infection and the age of the animals:

*Acute phase*: rapidly occurring mortality, lethargy, darkening of skin, exophthalmia, anaemia (pale gills), haemorrhages at the base of fins, gills, eyes and skin, abnormal swimming such as flashing and spiralling, distended abdomen due to oedema in peritoneal cavity.

***Chronic phase:*** no visible external signs.

***Nervous form:*** due to the tropism of the virus for the brain, the nervous form is characterized by severe abnormal swimming behaviour such as constant flashing and spiralling.

***Latent infections:*** no external signs occur.

*Anatomical pathology*: the kidney and liver are markedly affected. The kidney is dark red in the acute phase, but can show severe necrosis in moribund fish. The liver is pale and mottled. There can be generalized petechial haemorrhaging in the skin, muscle tissue and internal organs. The gastrointestinal tract is devoid of food.

*Microscopic pathology*: the kidney, liver and spleen show extensive focal necrosis and degeneration—cytoplasmic vacuoles, pyknosis, karyolysis and lymphocytic invasion. While the skeletal muscle does not appear to be a site of infection, erythrocytes can accumulate in the skeletal muscle bundles and fibres without causing damage to the muscle *per se* (Smail, 2000).

*Electron microscopy/cytopathology*: VHSV is a typical bullet-shaped rhabdovirus, 60–75 nm in diameter and 180–240 nm in length. Ultrastructural aspects of the development of viral infection in cell culture have been described by Baroni *et al.* (1982) and Granzow *et al.* (1997).

## Disease Aetiology

The aetiological agent of VHS is a rhabdovirus (Egtved virus) belonging to the genus *Novirhabdovirus* within the family Rhabdoviridae. The bullet-shaped virions have a size of 70 × 180 nm. The negative-sense, single-stranded RNA genome (approx. 11,000 nucleotides) is surrounded by an envelope with glycoprotein projections. These antigens represent surface antigen that can induce neutralizing antibodies. The genome encodes six structural proteins (N, P, M, G, NV and L proteins).

Three serotypes were distinguished with monoclonal and polyclonal antibodies by neutralization test (Olesen *et al.*, 1993):

Type 1 – e.g., strain F1 (Denmark)

Type 2 – e.g., Hededam isolate (Denmark)

Type 3 – e.g., strain 23/75 (France)

The antibody induced neutralizing activity is based on epitopes located on the envelope glycoprotein. By means of serology it is not possible to differentiate between freshwater and marine VHSV isolates. Therefore, gene grouping is of major importance for the differentiation of these VHSV isolates or strains. Four genotypes (I-IV, Einer-Jensen *et al.*, 2004; Snow *et al.*, 2004,) have been identified so far, as shown in Table 2.2. While rainbow trout infected by marine isolates showed a low or no mortality (Winton *et al.*, 1989; Dixon *et al.*, 1997; Follett *et al.*, 1997), most of the freshwater isolates induced high mortality in fry after immersion or i.p. infection. Marine isolates can be very virulent to farmed or free living marine species such as turbot (Schlotfeldt *et al.*, 1991; King *et al.*, 2001b), Pacific herring (Kocan *et al.*, 1997; Meyers *et al.*, 1999), cod (Snow *et al.*, 2000) or Japanese flounder (Isshiki *et al.*, 2001). Being an enveloped virus, VHSV is sensitive to UVC at 200-280 nm that can reduce the infectious virus titre to three logs (Oye and Rimstad, 2001). This can be used in hatcheries for treatment of the influx water. Furthermore, VHSV is sensitive to a broad range of disinfectants (Wolf, 1988; Smail, 1999). The disinfection success also depends on the use of saltwater instead of buffers for dilution of the virus (Kurita *et al.*, 2002).

**Table 2.2** Viral haemorrhagic septicaemia virus genotypes.

| *Genotype* | *Distribution* |
|---|---|
| Ia | Isolates from farmed rainbow trout in continental Europe |
| Ib | Isolates from marine hosts in the Baltic Sea, Skagerrak and Kattegat, related to Ia |
| II | Isolates from marine hosts in the Baltic Sea, unlike Ib, with no strong link to Ia |
| III | Isolates from around the British Isles |
| IV (a, b) | Isolates from the Pacific Northwest IVa and Japan, Great Lake area IVb |

In carrier (clinically healthy) fish (Meier *et al.*, 1994), detection of VHSV is most difficult. While VHSV will grow in a range of fish cell lines, the BF-2 (Olesen and Jorgensen, 1992) or RTG-2 cell line appear to be highly sensitive to infection by European freshwater strains. However, susceptibility of a cell line to infection will depend on a range of parameters including cell line lineage and viral strain differences (Isshiki *et al.*, 2001; Hedrick *et al.*, 2003).

## Disease Pathogenesis

Disease generally occurs at temperatures between 4°C and 14°C. Temperatures above 15°C seem to be inhibitory to virus growth (Clark and

Soriano, 1974). At water temperatures between 15°C and 18°C, the disease generally occurs with a modest mortality. At higher temperatures than 18°C, disease rarely occurs (Wolf, 1988). Low water temperatures (1–5°C) generally result in an extended disease course with low daily mortality but high-cumulated mortality. VHS outbreaks occur during all seasons, but are most common in spring and autumn when water temperatures fluctuate. Details are reviewed by Wolf (1988) and Smail (1999). As with other viral diseases of fish, predisposing factors include stress and fish age. VHSV can remain covert unless a stress event triggers an outbreak of the disease (Horlyck *et al.*, 1984). Severity of disease is inversely proportional to fish age; young fish (e.g., rainbow trout fry) are more susceptible to disease and associated mortality than older fish (e.g., fingerlings and growers). Other still not investigated factors will also influence the disease event.

## Diagnostics

### *Agent Detection and Identification Methods*

The standard methods for VHSV detection are based on direct identification methods such as the isolation in cell culture followed by identification using indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA) or nucleic acid-based methods (e.g., reverse-transcription polymerase chain reaction (RT-PCR). Due to its low sensitivity, the direct immunological demonstration of VHSV antigen in infected fish tissues can only be used when VHS infection is suspected (clinical signs, epidemiological data and/or suspicious histopathology). Nucleic acid detection technology using direct identification of the VHSV genome in fish tissue is under evaluation. These extremely sensitive techniques can also be used for confirmation of overt infection in fish, but have not yet been validated properly for use in surveillance programmes with the aim to obtain an approved VHS-free status.

### *Direct Detection Methods*

#### Histopathology

The kidney and liver are target organs, and examination of histological sections from diseased fish reveals degeneration and necrosis of haematopoietic tissues of the kidney (and the spleen) and focal degeneration and necrosis of the liver. Sections of the skeletal muscle may

show many foci of red blood cells, while the muscle fibres remain undamaged.

## Direct Agent Isolation and Identification

Isolation of VHS virus in a number of different permanent growing cell cultures has been described (Olesen and Jorgensen, 1992; Lorenzen *et al.*, 1999). This method is used as 'Gold' Standard also for surveillance programmes. The disadvantage is that there is always a detection limit, which depends on the cell line used as well as on the type of virus and the age/size of the fish. While in the case of fingerlings the whole body can be used for virus detection, suitable samples from larger fish are kidney, spleen, heart and/or encephalon and ovarian fluid from broodfish. BF-2 and RTG-2 are the recommended cell lines, but EPC or FHM cells can also be used. The latter generally show a lower susceptibility to VHSV (Olesen and Jorgensen, 1992; Lorenzen *et al.*, 1999). Infected cells are known to be used in antibody-based antigen detection methods: virus identification by neutralization test, indirect or direct IFAT (Fichtner *et al.*, 1998, 2000), ELISA or immunohistochemical (IHC) methods (Mourton *et al.*, 1992). IHC can also be used for very quick identification of virus obtained from imprints of the target organs kidney and liver (Evensen *et al.*, 1994). All antibody tests for the identification of the virus based on antibodies are influenced by their sensitivity and specificity to different VHSV sero- or genotypes. In contrast, RT-PCR is not influenced by the VHSV sero- or genotype. This method is used for confirmation of other results and is becoming more common due to its rapidity and sensitivity (Miller *et al.*, 1998). Further, sequencing of RT-PCR products allows the researcher to gain essential epidemiological data (Stone *et al.*, 1997; Snow *et al.*, 2004). For all detection and identification methods, a proper validation for their use in surveillance programmes, especially for diagnostic sensitivity and specificity, is necessary.

## Indirect Identification (Serology)

The serological examination of virus infections of fish is not accepted so far as routine diagnostic method (LaPatra, 1996; Lorenzen and LaPatra, 1999). The reason is the limited knowledge on the immunological processes that take place in the fish during all phases of a VHSV infection. Therefore, an assessment of the antibody level is difficult to use in routine diagnostics.

## Disease Treatment and Prophylaxis

In the absence of anti-viral agents and approved vaccines, control methods for VHS currently consist of official health surveillance schemes in combination with control policy measures. Methods that have been successful in reducing the number of infected farms in an endemic area and in preventing re-infection, such as stamping-out and fallowing procedures, have been reviewed by Olesen (1998). Methods to reduce the impact of the disease rely on the prevention of contact between virus and host species (hatchery disinfection, use of specific-pathogen free [SPF] stock, use of spring or bore water or resistance breeding) as well as on the reduction of physiological stressors.

While immune mechanisms in fish are not fully understood (Lorenzen and LaPatra, 1999), immune resistance to VHSV due to prior exposure of fish to virus has well been established (Wolf, 1988). However, although research on vaccine development for VHS has been ongoing for three or more decades, a commercial vaccine is not yet available.

## Epidemiology/Geography/Distribution

The carrier status of freshwater fish species for VHSV is well known (Jorgensen, 1982; Enzmann and Konrad, 1985). One way to assess the prevalence of VHSV carriers is the detection of VHSV antibodies. Antibodies were found in 6–67% of the investigated sera (Enzmann *et al.*, 1992; Meyers *et al.*, 1999). Recently, VHSV was discovered by cell culture isolation in a wide range of marine species in coastal waters of continental North America (Mortensen *et al.*, 1999; Hedrick *et al.*, 2003), Japan (Takano, 2000), U.K. (King *et al.*, 2001a) and Europe (Brudeseth and Evensen, 2002). Based on these investigations, a real prevalence between 0.02 – 4.7% was detected. In the 1980s, VHS was restricted to fresh water species, but a number of additional fish were found to be infected by VHSV such as brown trout and pike (Jorgensen, 1980; Meier and Jorgensen, 1980), whereas the U.K. and Ireland were regarded as free of this virus. In the late 1980s, VHSV was detected and isolated in Pacific salmon off the Pacific North American coast (Brunson *et al.*, 1989; Hopper, 1989; Winton *et al.*, 1989), which resulted in extensive investigations in a large number of farmed and wild fish species along the Pacific and Atlantic North American coast (Meyers *et al.*, 1994, 1999; Traxler *et al.*, 1999; Dopazo *et al.*, 2002; Hedrick *et al.*, 2003), the waters around the U.K. (Dixon *et al.*, 1997; Smail, 2000; King *et al.*, 2001a), the

Baltic Sea, Skagerrak and Kattegat (Mortensen *et al.*, 1999), and in the waters around Japan (Takano *et al.*, 2000).

In 2004, four main genotypes of VHSV and several subgroups that correlate with the geographical distribution were demonstrated (Table 2.2). The ability to differentiate between these four genotypes will be necessary for future disease management (Einer-Jensen *et al.*, 2004; Snow *et al.*, 2004).

## Infectious Haematopoietic Necrosis

Infectious haematopoietic necrosis (IHN) is a disease affecting several species of salmonid and other fish species caused by a rhabdovirus, the infectious haematopoietic necrosis virus (IHNV).

## Hosts

Susceptible species include: rainbow or steelhead trout (*Oncorhynchus mykiss*), Pacific salmon including chinook (*O. tshawytscha*), sockeye salmon (*O. nerka*), chum salmon (*O. keta*), masou salmon (*O. masou*), and coho salmon (*O. kisutch*) and Atlantic salmon (*Salmo salar*) but also the European eel (*Anguilla anguilla*) kept in farms (Bergmann *et al.*, 2003).

## Disease Symptoms

The disease is typically characterized by gross signs that include lethargy interspersed with bouts of frenzied, abnormal activity, darkening of the skin, pale gills, ascites, distended abdomen, exophthalmia, and internal and external petechial haemorrhages. Internally, the fish appear anaemic and lack food in the gut. Liver, kidney and spleen are mostly pale. Histopathologic findings reveal degenerative necrosis in haematopoietic tissues, kidney, spleen, liver, pancreas and digestive tract. The blood of affected fry shows reduced haematocrit, leucopoenia, degeneration of leukocytes and thrombocytes and large amounts of cellular debris.

Infection with IHNV often leads to mortality due to osmotic imbalance, and occurs within a clinical context of oedema and haemorrhages. Virus multiplication in endothelial cells of blood capillaries, haematopoietic tissues, and kidney cells underlies the clinical signs. High levels of virus are shed from infected juvenile fish. The most important environmental factor affecting the progress of IHN is the water temperature. Clinical disease occurs between 8°C and 15°C under natural

conditions but also at 23°C, when the virus is adapted to a non-salmonid species such as eel.

Depending on the fish species, the rearing conditions, the temperature, and, to some extent, the virus strain, outbreaks of IHN may range from explosive to chronic. Losses in acute outbreaks will exceed several per cent of the population per day and cumulative mortality may reach 90–95% or more. In chronic cases, losses are protracted and fish can be observed in various stages of the disease in the pond. The disease severity is exacerbated by co-infection with certain bacterial pathogens, such as *Flavobacterium psychrophilum.*

## Disease Aetiology

The IHNV contains a non-segmented, negative-sense, single-stranded RNA genome of approximately 11,000 nucleotides that encodes six proteins. Taxonomically, the virus is placed into the *Novirhabdovirus* genus of the family Rhabdoviridae.

Isolates of IHNV show genetic differences that appear to be most strongly related to geographical location and infected fish species. The genogroups, based on the mid glycoprotein gene (G) sequence, are termed as Upper (U), Middle (M) and Low (L) (Kurath *et al.*, 2003). On the basis of antigenic studies conducted with neutralizing polyclonal rabbit antisera, IHNV isolates seem to form a single serogroup (Engelking *et al.*, 1991). However, monoclonal antibodies have revealed a number of neutralizing epitopes on the glycoprotein (Winton *et al.*, 1988; Ristow and Arnzen, 1991; Huang *et al.*, 1994), as well as the existence of a non-neutralizing group epitope represented by the nucleoprotein (Ristow and Arnzen de Avila, 1989). Variations in the virulence and host preference of IHNV strains have been recorded in both, natural cases of disease and experimental infections (LaPatra *et al.*, 1993a, b; Bergmann *et al.*, 2003).

IHNV is heat, acid and ether labile. The virus is stable in fresh water for at least one month, especially if organic material is present. It is completely inactivated by common disinfectants and by drying out (Wolf, 1988).

## Diagnosis

The 'Gold Standard' for detection of IHNV is the isolation of the virus in cell culture followed by its immunological or molecular identification. While the other diagnostic methods listed below can be used for

confirmation of the identity of virus isolated in cell culture or for confirmation of overt infections in fish, they are not approved for use as primary surveillance methods for obtaining or maintaining an approved IHN-free status. Due to substantial variation in the strength and duration of the serological responses of fish to virus infections, the detection of fish antibodies to viruses has, thus far, not been accepted as a routine diagnostic method for assessing the viral status of fish populations. In the future, validation of serological techniques for diagnosis of fish virus infections could render the use of fish serology more widely acceptable for diagnostic purposes.

## Clinical Signs and Methods

IHN occurs among several species of salmonids with fry being the most highly susceptible stage. Older fish are typically more resistant to clinical disease. Under natural conditions, most clinical cases of IHN are seen in fry when the water temperature is between 8 and 15°C. During outbreaks, fish are typically lethargic with bouts of frenzied, abnormal activity such as spiral swimming and flashing. Affected fish exhibit darkening of the skin, pale gills, ascites, distended abdomen, exophthalmia, and internal and external petechial haemorrhages. A trailing faecal cast is observed in some species. Spinal deformities are present among some of the surviving fish.

Internally, fish appear anaemic and lack food in the gut. Ascitic fluid and petechia are observed in the organs of the body cavity. The liver, kidney and spleen are pale.

*Histopathological* findings reveal degenerative necrosis in haematopoietic tissues, kidney, spleen, liver, pancreas and digestive tract. The blood of affected fry shows reduced haematocrit, leucopenia, degeneration of leukocytes and thrombocytes, and large amounts of cellular debris. The cellular debris, termed necrobiotic bodies, can be seen in stained tissue imprints from the anterior kidney and has diagnostic value. As with other haemorrhagic viremias of fish, in severe cases, blood chemistry is altered .

*Electron microscopy* of virus-infected cells reveals bullet-shaped virions of approximately 150–190 nm in length and 65–75 nm in width (Wolf, 1988). The virions are visible at the cell surface or within vacuoles or intracellular spaces after budding through cellular membranes. The virion possesses an outer envelope containing host lipids and the viral

glycoprotein spikes that react with immunogold staining to decorate the virion surface.

### Direct Agent Isolation and Identification

The traditional procedure for detection of IHNV is based on virus isolation in cell culture. The most suitable cell line is the EPC cell (Fijan *et al.*, 1983). Confirmatory identification may be achieved by use of immunological (neutralization, indirect fluorescent antibody test or enzyme-linked immunosorbent assay), or molecular (DNA probe or polymerase chain reaction) methods (Dixon and Hill, 1984; LaPatra *et al.*, 1989; Arakawa *et al.*, 1990; Arnzen *et al.*, 1991; Deering *et al.*, 1991; Jorgensen *et al.*, 1991; Winton and Einer-Jensen, 2002). The identification of the agent is similar to the detection and identification of VHSV.

### Indirect Identification (Serology)

Serology of virus infection of fish is not accepted so far as a routine diagnostic method. Reasons are the limited knowledge of the immunological processes that take place in the fish during all phases of an IHNV infection. Therefore, an assessment of the antibody level is difficult to use in routine diagnostics.

### Used Diagnostic Methods to Declare Freedom from IHN

The prescribed method for *targeted surveillance* to declare freedom from IHN is the isolation of virus in cell culture. For this purpose, the most susceptible stages of the most susceptible species should be examined using the most sensitive diagnostic methods. Reproductive fluids and tissues collected from adult fish of a susceptible species at spawning should be included in at least one of the sampling periods each year.

## Disease Treatment/Prophylaxis

Control methods for IHN currently rely on the avoidance of exposure to the virus through the implementation of strict control policies and sound hygiene practices (Winton, 1991). The thorough disinfection of fertilized eggs and the incubation of eggs and rearing of fry and fingerlings on virus-free water supplies in premises completely separated from those harbouring possible virus carriers and free from possible contact with

inanimate objects, are critical to prevent the occurrence of IHNV in a defined fish production site.

At present, vaccination of salmonids against IHN is at an experimental stage; several new vaccine preparations, however, have shown substantial promise in both, laboratory and field trials (Winton, 1997). Both, autogenous, killed vaccines and a novel DNA vaccine have proven commercially useful in Atlantic salmon net-pen aquaculture on the west coast of North America or for larger salmonids in captive propagation where a vaccine can be delivered economically by injection. However, application of the available IHN vaccines to millions of very small fish will require additional research on novel methods for mass delivery.

## Epidemiology/Geography

Historically, the geographical range of IHN was limited to the coastal area of the northwestern part of North America, but the disease has spread to continental Europe and the Far East via the importation of infected fish and/or eggs. Once IHNV has been introduced in a farmed stock or in a watershed, the disease may become established among the carrier fish.

Among individuals of each fish species, there is a high degree of variation in susceptibility to IHNV. The age of the fish is extremely important: the younger the fish, the more susceptible it is to the disease (Bergmann *et al.*, 2003). As with viral haemorrhagic septicaemia virus, a good overall fish health condition seems to decrease the susceptibility to overt IHN, while co-infections with bacterial diseases (e.g., bacterial coldwater disease, infections with *Aeromonas salmonicida* strains), handling and other types of stress frequently cause subclinical infections to become overt. Fish become increasingly immunologically resistant to infection with age until spawning, when they once again become highly susceptible and may shed large amounts of virus in sexual products. Survivors of IHN demonstrate a strong protective humoral immunity with the synthesis of circulating antibodies to the virus.

## Epizootic Haematopoietic Necrosis

Epizootic haematopoietic necrosis (EHN) is a systemic acute clinical or subclinical infection of finfish with epizootic haematopoietic necrosis virus (EHNV), an iridovirus within the Ranavirus group.

## Hosts

Only two species are susceptible to natural EHNV infections, red fin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) (Langdon *et al.*, 1986, 1988; Langdon and Humphrey, 1987; Langdon, 1989). However, many other finfish species are infectable by EHNV experimentally, e.g., Macquarie perch (*Macquaria australasica*), silver perch (*Bidyanus bidyanus*), mosquito fish (*Gambusia affinis*) and mountain galaxias (*Galaxias olidus*) died after immersion. Murray cod (*Maccullochella peeli*), golden perch (*Macquaria ambigua*), Australian bass (*Macquaria novemaculeata*), Macquarie perch, silver perch and Atlantic salmon (*Salmo salar*) were susceptible after intraperitoneal injection of EHNV. Cyprinid species seem to be resistant (Langdon, 1989). Based on these results, EHNV can be classified as a pathogen that can seriously infect and kill a large number of fish species.

In general, all ages and sizes of rainbow trout and red fin perch can be affected by EHNV.

## Disease Symptoms

Clinically, moribund rainbow trout were dark in colour, showed anorexia and sometimes ataxia. Gross lesions were abdominal distension, swelling of the spleen and kidney, and, rarely, pale foci in the liver. Microscopical lesions included focal to extensive, acute renal haematopoietic necrosis; multiple tiny foci of acute hepatocellular necrosis; focal to extensive, acute splenic necrosis; mild branchial hyperplasia with occasional focal necrosis; congestion, oedema and necrosis in the wall of the swimbladder; and focal acute necrotizing myocarditis, necrotic cells and debris being prominent within the cardiac lumen (and within blood vessels throughout the body generally). Round to oval basophilic intracytoplasmic inclusions were commonly present in hepatocytes surrounding necrotic foci and were sometimes seen in cells in the interstitium of the kidney or in the spleen. Less common lesions included focal necrosis of the pancreas, thyroid, pseudobranch, thymus and gastrointestinal crypt epithelium.

Intercurrent infections were not observed in red fin perch; gross lesions included multiple pale foci in the liver, haemorrhages at the bases of the fins, swelling of the spleen and kidney and focal haemorrhages in the gills (Reddacliff and Whittington, 1996).

In rainbow trout, a disease outbreak is generally difficult to identify due to very low mortality rates. Natural outbreaks are normally connected

to poor husbandry, overcrowding, insufficient water exchange and/or inappropriate food. The resulting skin damage can be a route of virus entry. Water temperatures between 11 and 20°C seem to be important for possible outbreaks of the disease (Whittington *et al.*, 1994, 1999). At higher temperatures, the incubation time is much shorter (3–10 days) than at 8–10°C (14–32 days) (Whittington and Reddacliff, 1995).

In *red fin perch*, the disease is recognized by spectacular epizootic mortality in fish of any age, affecting a very large population. Fingerlings, juveniles and adults are affected. When the disease is first recognized in an area, there is a dramatic population decline (Langdon *et al.*, 1986; Langdon and Humphrey, 1987; Whittington *et al.*, 1996). Natural epizootics of EHNV affecting juvenile and adult red fin perch occur mostly in summer time (Langdon *et al.*, 1986; Langdon and Humphrey, 1987; Whittington *et al.*, 1996).

## Disease Aetiology

EHNV is a member of the genus *Ranavirus* within the family Iridoviridae with the type species Frog virus 3 (FV3) (Chinchar *et al.*, 2005). Other members are Bohle virus (BIV), European catfish virus (ECV), European sheatfish virus (ESV) and Santee-Cooper ranavirus as well as many other tentatively systemic iridoviruses. Ranaviruses have been isolated from healthy or diseased frogs, salamanders, fish and reptiles in America, Europe and Australia (Wolf *et al.*, 1968; Fijan *et al.*, 1991; Speare and Smith, 1992; Drury *et al.*, 1995; Zupanovic *et al.*, 1998; Chinchar, 2002; Hyatt *et al.*, 2002). Ranaviruses consist of a large (150–180 nm) icosahedral virion, a double-stranded DNA genome of 150–170 kb, and replicate in nucleus and cytoplasm with cytoplasmic assembly (Chinchar *et al.*, 2005). They possess common antigens, the major ones of which can be detected by ELISA and IFAT, but no effective neutralizing antibodies have been produced to assist identification or detection.

Since the recognition of the disease in Australia in 1986, similar systemic necrotizing iridovirus syndromes have been reported in farmed fish, e.g. catfish (*Ictalurus melas*) in France (ECV) (Pozet *et al.*, 1992), sheatfish (*Silurus glanis*) in Germany (ESV) (Ahne *et al.*, 1989a, 1990), turbot (*Scophthalmus maximus*) in Denmark (Bloch and Larsen, 1993) and others in Finland (Tapiovaara *et al.*, 1998; Ariel *et al.*, 1999).

EHNV and ECV/ESV are distinct viruses that can be differentiated using genomic analysis (Mao *et al.*, 1996, 1997; Ahne *et al.*, 1998; Hyatt

*et al.*, 2000; Marsh *et al.*, 2002). This enables epidemiological separation of disease events in finfish in Australia (EHNV) and Europe (ECV/ESV) and its differentiation of these from ranaviral occurrences in frogs and toads (e.g. FV3 and BIV).

Survival outside the host (i.e., in the natural environment): EHNV is extremely resistant to drying and can survive for months in water. It can persist in frozen fish tissues for more than 2 years (Langdon, 1989) and frozen fish carcasses for at least one year (Whittington *et al.*, 1996). For these reasons, it is presumed that EHNV would persist for months to years on a fish farm in water and sediment and on plants and equipment.

Stability of the agent: EHNV is susceptible to 70% ethanol, 200 mg/litre sodium hypochlorite or heating to 60°C for 15 minutes (Langdon, 1989).

## Disease Pathogenesis

Disease symptoms occur in fingerlings and juvenile fish of both, rainbow trout and red fin perch. Target organs are liver, kidney, spleen and other parenchymal tissues. EHNV has not been detected in gonadal tissue or ovarian fluid, and these tissues are therefore not suitable for surveillance of broodstock. Once infected, the fish remain lifelong carriers. EHNV has spread between rainbow trout farms by transfer of infected fingerlings and probably transport water (Langdon *et al.*, 1988; Whittington *et al.*, 1994, 1999). It is assumed that consignments of fish contain a low proportion of individuals with progressive subclinical or clinical infection, rather than carrier fish. The low prevalence of infection in rainbow trout means that active infection can easily go unrecognized in a population and can be spread by fish trade. Data does not exist on the possible vertical transmission of EHNV on or within ova, and disinfection protocols for ova have not been evaluated. EHNV has not yet been isolated from ovarian tissues or from broodstock, therefore milt and ovarian fluids may not be suitable samples for testing for exclusion of EHNV.

## Diagnosis

External signs are not specific for EHN. Fish are simply found dead. Moribund fish may have loss of equilibrium, flared operculae and may be dark in colour. There may be signals of poor husbandry practices, such as overcrowding or suboptimal water quality manifesting as lesions in skin, fin and gill (Reddacliff and Whittington, 1996) which can also fail to appear.

Internal changes in a small number of fish may be enlargement of internal organs such as kidney, liver or spleen. Sometimes focal white to yellow lesions in the liver can be observed corresponding to necrotic areas (Reddacliff and Whittington, 1996).

In light microscopy, necrotic foci are visible in liver, head kidney and spleen which also can be found in heart, pancreas, gastrointestinal tract, gill and pseudobranch (Reddacliff and Whittington, 1996). Basophilic intracytoplasmic inclusion bodies may be seen in liver and kidney.

Cytoplasmic inclusions, a sign of virus assembly, are mostly detected in cells of necrotic areas of kidney, liver or spleen by electron microscopy. In the cytoplasm in aggregates with a paracrystalline structure non-enveloped icosahedral viruses are apparent. Complete virions with electron-dense cores bud from the infected cells through the plasma membrane. The nuclei of infected cells and tissues are often dislocated and pushed to the cell margin (Eaton *et al.*, 1991; Hyatt, 1991).

## Direct Agent Detection and Identification Methods

Cell cultivation of isolated virus is the gold standard. EHNV grows well in permanent cell lines such as BF-2 (bluegill fry cell line; ATCC CCL 91), FHM (fathead minnow; ATCC CCL 42), EPC (*epithelioma papulosum cyprini* [Fijan *et al.*, 1983]) and CHSE-214 (Chinook salmon embryo cell line; ATCC CRL 1681) at temperatures ranging between 15 and 22°C (Crane *et al.*, 2005). Isolation and growth on BF-2 cell at 22°C is the method preferred by the OIE reference laboratory.

The identification of viruses in cell culture is carried out by immunostaining (Reddacliff and Whittington, 1996), ELISA (Hyatt *et al.*, 1991; Whittington and Steiner, 1993; Whittington *et al.*, 1994, Whittington and Reddacliff, 1995), immunoelectron microscopy (Hyatt, 1991; Hyatt *et al.*, 1991; Hengstberger *et al.*, 1993; Reddacliff and Whittington, 1996), PCR (Marsh *et al.*, 2002) or some other methods. Therefore, the OIE reference laboratory suggests cell culture isolation and antigen ELISA for identification and confirmation as well as molecular methods (PCR and sequencing).

## Disease Treatment/Prophylaxis

Currently, there is no vaccine available. General good husbandry practices are necessary. Disease control in rainbow trout is limited to reducing stocking rates and increasing water quality.

## Epidemiology/Geography?

EHNV and by the virus's caused disease are found only in Australia. Experimental infections with EHNV have been published in Macquarie perch, mosquito fish, silver perch, mountain galaxias, Murray cod, and Atlantic salmon but no natural infections have been observed in any of these species till date.

## Infectious Salmon Anaemia

Infectious salmon anaemia (ISA) is a disease of Atlantic salmon (*Salmo salar*) only (Thorud and Djupvik, 1988) and is caused by the infectious salmon anaemia virus, an orthomyxovirus (ISAV) (Falk *et al.*, 1997; Mjaaland *et al.*, 1997; Krossoy *et al.*, 1999) (Fig. 2.2). ISA primarily affects fish either held in seawater or exposed to seawater. However, indications of disease outbreaks in fish held in freshwater have also been reported (Nylund *et al.*, 1998).

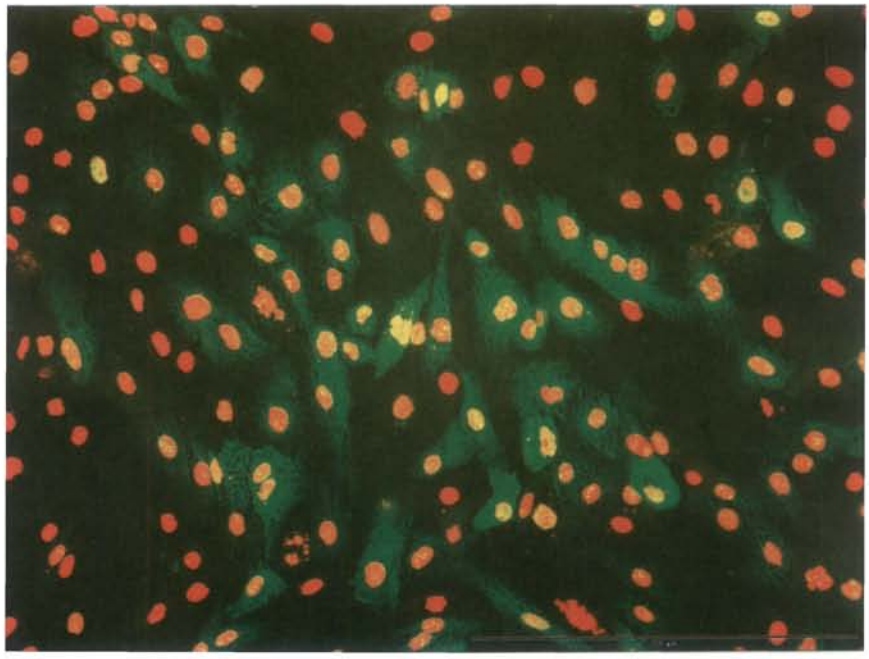

**Fig. 2.2** ISAV isolated in SHK-1 cells identified by indirect fluorescent antibody test assay with monoclonal antibodies (positive reactions green, cell nuclei red).

## Hosts

Natural outbreaks of ISA have been recorded in farmed Atlantic salmon only, while subclinically infected feral Atlantic salmon, brown trout and sea trout (*S. trutta*) have been identified by RT-PCR (Raynard *et al.*, 2001). ISAV has also been detected in two marine species, pollack (*Pollachius pollachius*) and cod (*Gadus morhua*), but only in fish collected in the vicinity of cages with Atlantic salmon exhibiting ISA (MacLean *et al.*, 2003). Following experimental infection, replication of ISAV has been demonstrated in several fish species, including brown trout, sea trout, rainbow trout (*O. mykiss*), Arctic char (*Salvelinus alpinus*) and herring (*Clupea harengus*) (Nylund *et al.*, 1995, 1997, 2002). Attempts have been made to induce infection or disease in pollack (*P. pollachius*), but with negative results (Snow *et al.*, 2002).

## Disease Symptoms

The disease may appear as a systemic and lethal condition characterized by severe anaemia and haemorrhages in several organs. The most prominent signs observed are pale gills, exophthalmia, distended abdomen, and petechia in the eye chamber; skin haemorrhages in the abdomen and scale oedema can occur. The major findings on post-mortem examination are circulatory disturbances in several organs caused by endothelial injury in peripheral blood vessels. These clinical manifestations of ISA are found in four vital organs: liver, kidney, gut or gill. The liver manifestation is characterized by dark livers caused by haemorrhagic liver necrosis. The kidney manifestation is characterized by moderately swollen kidneys with interstitial haemorrhaging and tubular necrosis. The gut manifestation is characterized by dark red guts caused by haemorrhaging within the intestinal wall but not into the gut lumen. In addition to being pale, the gill manifestation includes, in some cases, accumulation of blood, especially in the central venous sinus of the gill filaments. Lesions in the liver and gut are easily visible, while lesions in the gills and kidney are less obvious. In some ISA outbreaks, one of the organ manifestations can dominate, while in other outbreaks all of the manifestations can be found. In individual fish, all manifestations may be found to some extent. Outbreaks dominated by either the liver or kidney manifestation appear to be most common. Haematocrit values of $<10$ in end stages may be observed. Diseased Atlantic salmon presenting with anaemia and circulatory disturbances should be examined for ISAV

infection as detailed below, if there is no other, obvious cause to explain their condition. Morbidity and mortality during an outbreak of ISA may vary significantly within and between different net pens in a seawater fish farm, and between different fish farms. Morbidity and mortality within a net pen may start at very low levels. Typically, daily mortality in affected net pens may initially range from 0.5 to 1%, but may increase with time. Without intervention, mortality increases and seems to peak in early summer and winter. The range of cumulative mortality during an outbreak is from insignificant to moderate, but in severe cases, cumulative mortality exceeding 90% may be experienced. Initially, an outbreak of ISA may be limited to one or two net pens over an extended period and the spread to other net pens may take months. In outbreaks where affected smolts have been infected in well boats during transport, a simultaneous outbreak may occur. Although natural outbreaks of ISA have been recorded in farmed Atlantic salmon only, subclinically infected feral Atlantic salmon, brown trout (*S. trutta fario)* and sea trout (*S. trutta trutta*) have been identified (Raynard *et al.*, 2001). ISAV has also been detected in two marine species, pollack (*Pollachius pollachius*) and cod (*Gadus morhua*) (MacLean *et al.*, 2003).

## Disease Aetiology

ISAV is an enveloped virus, 100–130 nm in diameter, with a genome consisting of eight single-stranded RNA segments of negative polarity, showing haemagglutination, receptor-destruction and fusion activity (Dannevig *et al.*, 1995; Falk *et al.*, 1997; Mjaaland *et al.*, 1997; Eliassen *et al.*, 2000; Falk *et al.*, 2004).

The morphological, physiochemical and genetic properties of ISAV are similar to those of the other members of the family Orthomyxoviridae (Rimstad and Mjaaland, 2002). ISAV has recently been classified as the type species of the new genus *Isavirus* within this virus family. The nucleotide sequences of all eight genome segments have been described (Clouthier *et al.*, 2002). Four major structural proteins have been identified, including a 68 kDa nucleoprotein, a 22 kDa matrix protein, a 42 kDa haemagglutinin-esterase protein and a 50 kDa surface glycoprotein with putative fusion activity, coded by genome segments 3, 8, 6 and 5, respectively (Falk *et al.*, 2004). Sequence analysis of various gene segments has revealed differences between isolates within as well as between defined geographical areas (Blake *et al.*, 1999; Devold *et al.*, 2001;

Kibenge *et al.*, 2001; Krossoy *et al.*, 2001). Analysis of the haemagglutinin-esterase gene has demonstrated the presence of two major groups of ISAV isolates, one European and one North American group. The European group may further be divided into three major subgroups as suggested by Nylund *et al.* (2003). A small, (hypervariable) highly polymorphic region (HPR) of the haemagglutinin gene has been identified (Krossoy *et al.*, 2001), and all European ISAV isolates may be genotyped according to variations in this region. However, there is no direct correlation between phylogenetic groups and HPR groups.

## Disease Pathogenesis

*Susceptible stages of the host:* In Atlantic salmon, disease outbreaks are mainly reported in seawater cages; only a few cases have been reported in the freshwater stage, including one case in yolk sac fry (Nylund *et al.*, 1998). Furthermore, ISA has been experimentally induced in both, Atlantic salmon fry and parr kept in freshwater. Genetics may also play an important role in the susceptibility of Atlantic salmon to ISA, as a functional association between disease resistance and major histocompatibility (MHC) class I and II polymorphism has been demonstrated (Grimholt *et al.*, 2003).

*Target organs and infected tissue:* Endothelial cells in many organs (heart, liver, kidney, spleen and others).

*Persistent infection in lifelong carriers:* Persistent infection in lifelong carriers has not been documented in Atlantic salmon, but on the farm level, infection may persist in the population by continuous infection of new individuals that do not cause any recognizable disease problems. Experimental infection of rainbow trout and brown trout indicates that persistent infection in these species is possible (Nylund and Jakobsen, 1995; Nylund *et al.*, 1995).

*Vectors:* Passive transfer of ISA by salmon lice (*Lepeophtheirus salmonis*) has been demonstrated under experimental conditions (Nylund *et al.*, 1993).

*Transmission mechanisms:* The disease is spread horizontally by water-borne transmission as shown by experimental infection studies. There is no strong evidence for vertical transmission through infected gonadal products. It has been suggested that the spread of disease over long distances is caused by transportation of smolt, either infected prior to shipping or by well boats contaminated with ISAV. Contamination of well

boats may be either due to previous transport of infected fish or to intake water from areas with farms harbouring diseased fish.

## Diagnosis

The diagnosis of ISA was initally based on clinical and pathological findings only (Evensen *et al.*, 1991). Following isolation of the causative agent in susceptible cell cultures (SHK-1, AK, TO), a number of direct methods for detection of virus and confirmation of the diagnosis have been established. These include isolation of the virus in cell culture followed by immunological identification (Dannevig *et al.*, 1995; Falk *et al.*, 1998), immunological demonstration of ISAV antigen in tissues (Falk *et al.*, 1998) and RT-PCR techniques (Mjaaland *et al.*, 1997; Devold *et al.*, 2000) or a real-time PCR. Differential diagnoses are: other anaemic and haemorrhagic conditions, and winter ulcer and septicaemias caused by infections with *Moritella viscosa.*

## Disease Treatment/Prophylaxis

Vaccination against ISA has been carried out in North America during the past 5 years, but the currently available vaccines do not seem to offer complete protection. The vaccines, which are inactivated whole virus vaccines, do not lead to virus clearance in immunized fish, which may thus become virus carriers (Kibenge *et al.*, 2003).

The incidence of ISA may be greatly reduced by implementation of legislatory measures or husbandry practices regarding the movement of fish, mandatory health control, transport and slaughterhouse regulations. Specific measures including restrictions on affected, suspected and neighbouring farms, enforced sanitary slaughtering, generation segregation ('all in/all out') as well as disinfection of offal and wastewater from fish slaughterhouses and fish processing plants may also contribute to reducing the incidence of the disease.

## Epidemiology/Geography

Epidemiological studies (Vagsholm *et al.*, 1994; Jarp and Karlsen, 1997) have shown that the risk of ISA transmission is closely linked to husbandry practices in aquaculture and horizontal transmission. Geographical proximity (<5 km) to farms with ISA outbreaks or slaughterhouses/processing plants releasing contaminated water,

numerous smolt deliveries and the use of well boats, and sharing staff and equipment are all considered to be significant risk factors.

In a net pen containing diseased fish, the prevalence can vary extensively, while in adjacent net pens ISAV may be difficult to detect, even by the most sensitive methods. Therefore, for diagnostic investigations, it is important to sample from net pens containing diseased fish.

Initially reported in Norway in the mid-1980s, ISA has since then been reported in Canada (New Brunswick in 1996 and Nova Scotia in 2000), the United Kingdom (Scotland in 1998 and later in the Shetland Islands), the Faroe Islands (2000) and the U.S.A. (Maine in 2001) (Rodger *et al.*, 1998; Lovely *et al.*, 1999; Bouchard *et al.*, 2001). The causal virus has been isolated from Coho salmon from Chile (Kibenge *et al.*, 2001) and from rainbow trout in Ireland in 2002.

## Economic Importance

ISA is regarded as an important disease in the countries where it has been reported. Most countries have, therefore, initiated, general legislatory measures and/or control programmes to combat the disease. The economic and production impact on the farming industry lies mainly in losses caused by the disease itself, and to the implementation of governmental measures such as contingency plans and control programmes.

## Infectious Pancreatic Necrosis

Infectious pancreatic necrosis (IPN) is a highly contagious viral disease of young salmonid fish kept under intensive rearing conditions (Wolf *et al.*, 1960; Hill, 1982; Wolf, 1988). The disease is induced by the IPN virus (IPNV). There exist at least nine serotypes of the causative birnavirus.

## Hosts

The disease most characteristically occurs in rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), several Pacific salmon species (*Oncorhynchus* spp.) but also danio zebra fish (*Brachydanio rerio*) and yellowtail (*Seriola lalandi*).

Other species known to be susceptible to variant strains of birnavirus are amago salmon (*O. rhodurus*), Arctic char (*Salvelinus alpinus*), Atlantic menhadden (*Brevoortia tyrannus*), carangids (Carangidae), cichlids (Cichlidae), common scallop (*Pecten maximus*), cutthroat trout (*Salmo clarki*), Danube salmon (*Salmo hucho*), drums/croakers (Sciaenidae), eels (*Anguilla* spp.), grayling (*Thymallus thymallus*), halibut (*Hippoglossus stenolepis*), herrings/sardines (Clupeidae), Japanese amberjack (*Seriola quinqueradiata*), lake trout (*Salvelinus namaycush*), lampreys (Petromyzontidae), left-eye flounders (Bothidae), loach (*Misgurnus anguillicaudatus*), loaches (Cobitidae), masou salmon (*O. masou*), perches (Percidae), pikes (Esocidae), silversides (Atherinidae), soles (Soleidae), Southwest European nase (*Chondrostoma toxostoma*), striped snakehead (*Channa striatus*), suckers (Catostomidae), summer flounder (*Paralichthys dentatus*), white seabass (Moronidae), whitefish (Coregonidae), carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), red fin perch (*Perca fluviatilis*), southern flounder (*Paralichthys lethostigma*, estuarine), halibut (*Hippoglossus hippoglossus*) and yellow fin bream (*Acanthopagrus australis*). There exist additionally asymptomatic carriers of birnaviruses, e.g., coalfish (*Pollachius virens*), discus fish (*Symphysodon discus*), heron (*Ardea cinerea*), loach (Cobitidae), minnow (*Phoxinus phoxinus*), noble crayfish (*Astacus astacus*), river lamprey (*Lampetra fluviatilis*), shore crab (*Carcinus maenas*), Spanish barbel (*Barbus graellsi*) and white suckers (*Catostomas commersoni*). Other fish susceptible to and/or infectable with birnaviruses can also pose as carriers.

## Disease Symptoms

Animals may show one or more of the signs listed below, but disease may also be present in the absence of any signs. Most of the signs are not very pathognomonic to IPNV, e.g., sudden mortality starting in fry and "fast growing" individuals, infected fish are found at the bottom, sometimes showing corkscrew movements (spiralling) or a swollen belly; exophthalmia or a dark skin colour is observed. The gut can be empty or filled with clear mucus. Microscopically, necrotic areas are found in pancreas, oesophagus or stomach. Petechial bleedings are not very common.

## Disease Aetiology

IPNV consists of a bi-segmented double-stranded RNA and belongs to the family Birnaviridae (Dobos and Roberts, 1982). IPNV strains display

wide antigenic diversity (Dorson and Torchy, 1981; Okamoto *et al.*, 1983; Melby *et al.*, 1994; Hill and Way, 1995). They fall into two serogroups that do not cross-react in serum neutralization tests (Underwood *et al.*, 1977; Olesen *et al.*, 1988; Ahne *et al.*, 1989) using polyclonal rabbit antisera. Most of the strains belong to serogroup A which is divided into at least nine serotypes (Hill and Way, 1995). Strains of different origin also show differences in virulence (Hill, 1982, 1992; McAllister and Owens, 1995).

Many virus types affect also marine finfish and shellfish, but do not cause diseases.

## Disease Pathogenesis

On the farm level, mortality can vary between 10 and 90%, depending on the water temperature, animal, age and size of the fish, virus strain and rearing conditions.

Transmission of the IPNV is possible horizontally and vertically via the fertilized egg. The virus is shed into the water via urine, reproductive fluid and/or faeces. Contaminated nets, basins, equipment, fish-eating birds and mammals or humans as well as asymptomatic carrier fish can also be a source of infection.

## Diagnosis

*The gold standard* for IPNV detection is the isolation of virus in cell cultures (Agius *et al.*, 1982). Identification will be routinely done by iIFA (OIE, 2006), serum neutralization (Hill and Way, 1995) or ELISA (Dixon and Hill, 1983). The diagnosis of clinical outbreaks is based on histology (McKnight and Roberts, 1976) followed by virus identification in infected tissues (Evensen and Rimstad, 1990), by virus isolation in cell cultures and as confirmation ELISA or iIFA.

Serological methods such as antibody ELISA or neutralization test using fish sera are not accepted as a routine method because of insufficient knowledge on the immune response in fish.

Clinically infected fish tissue is used for direct virus detection by iIFA, ELISA or Co-agglutination test (Taksdal and Thorud, 1999) or immunohistochemistry.

## Disease Treatment/Prophylaxis

Currently, there neither is a vaccine nor an effective treatment after infection with IPNV. Control measures focus on the implementation of control policies and of hygiene practices in salmonid husbandry to avoid the introduction of infected eggs, fish and equipment.

## Epidemiology/Geography

The disease has a wide geographical distribution; it occurs in most major salmonid-farming countries of North and South America, Europe and Asia. Oceania is free of the disease (OIE, 2006).

## Recent Development and Outlook

As for other fish diseases, new methods for IPNV detection such as RT-PCR (Taksdal *et al.*, 2001), Multiplex RT-PCR (Williams *et al.*, 1999) or real time PCR (Watanabe *et al.*, 2006) is under evaluation as a tool for diagnostics.

## Sleeping Disease

Sleeping disease (SD), an economically important danger to farmed freshwater rainbow trout (*Oncorhynchus mykiss* (Walbaum)), is caused by an atypical *Alphavirus* (Villoing *et al.*, 2000).

## Hosts

SD is currently a matter of concern for salmonid fish farms in some parts of the world.

## Disease Symptoms

Rainbow trout affected by SD typically lie on their sides at the bottom and show characteristic histological lesions of pancreas, heart and skeletal muscle (Boucher *et al.*, 1994; Boucher and Baudin Laurencin, 1994). SD is confirmed when typical lesions of cardiac necrosis, pancreatic atrophy and skeletal muscle degeneration (Boucher and Baudin Laurencin, 1996) have been observed on histological examination. The mortality in affected fish population ranges from 3 to 22%.

## Disease Aetiology

SD is a serious disease of rainbow trout caused by sleeping disease virus (SDV).

The virus nature of this disease agent was recognized by Boucher and Baudin Laurencin (1996) and SDV was characterized as an *Alphavirus* within the family Togaviridae (Villoing *et al.*, 2000). SDV is closely related to SPDV, the virus responsible for *Salmon Pancreas Disease* (SPD) (Desvignes *et al.*, 2002; Weston *et al.*, 2002), a disease of sea-reared salmon (*Salmo salar*). SPD, first described by Munro *et al.* (1984) in Scotland, produces pathological changes in the pancreas, heart and skeletal muscles of the affected salmon (Nelson *et al.*, 1995). The disease has also been reported in the USA (Kent and Elston 1987), France and Spain (Raynard *et al.* 1992), Norway (Poppe *et al.* 1989), and Ireland (Nelson *et al.*, 1995). Both viruses, SDV and SPDV are classified as atypical *Alphaviruses* (Weston *et al.*, 1999; Villoing *et al.*, 2000; Weston *et al.*, 2002) with a similarity of 91.1% over their complete genome. When compared to the non-structural proteins of the other, typical *Alphaviruses*, the identity is 41.7 to 43.6% only (Villoing *et al.*, 2000). SDV and SPDV exhibit 95% amino acid identity over their non-structural proteins and induce cross-protection in fish (Boucher and Baudin Laurencin 1996). For the first time, Villoing *et al.* (2000) characterized and assigned the viral agent (SDV) responsible for SD in rainbow trout to the alphavirus subfamily. The finding that SDV replicated in cell culture at low temperature substantiated the temperature dependence of SDV growth in fish. Natural outbreaks of SD and experimental disease transmission to rainbow trout appeared almost exclusively at 10°C. Several lines of evidence suggest that SDV belongs to the Togaviridae, e.g., electron microscopy, which revealed numerous enveloped viral particles of roughly 60 nm in diameter, and studies on the viral genome, which demonstrated the presence of a 12-kb genomic RNA.

## Disease Pathogenesis

The most characteristic sign of the disease is the unusual behaviour of the fish, which stay on their side at the bottom of the tank as if they were sleeping. Histological observations of diseased fish showed a chronological appearance of lesions in the pancreas, in the heart, and in the muscle during the last stage of the disease (Boucher *et al.*, 1994, 1995).

Transmission of SD obviously occurs through contact with contaminated tissue from fish affected with SD (Boucher *et al.*, 1994) but also with meat fluid of fish carcasses. Although other *Alphaviruses* are usually transmitted by arthropods (Chamberlain, 1980), it is unlikely that a sangivorous parasite serves as reservoir for SDV, since direct transmission from fish to fish has been experimentally demonstrated by cohabitation experiments (Boucher, 1995).

Characteristic pathological lesions were observed in infected fish as early as 7 dpi. Lesions were first detected in exocrine pancreas and subsequently observed in heart and skeletal muscles. Neutralizing antibodies to SDV were detected in infected fish from 14 to 70 dpi. Infected fish displayed typical signs of SD 1 month pi and the mortality can reach 18% within 44 days. Kerbart Boscher *et al.* (2006) experimentally reproduced all the pathognomonic features of natural outbreaks of SD in 1-year-old rainbow trout.

## Diagnosis

### *Agent Detection and Identification Methods*

SDV was detected in serum, kidney and brain of infected fish from 4 to 21 days post-infection (dpi) (Kerbart Boscher *et al.*, 2006). However, all attempts to isolate a viral agent on commonly available fish cell lines by inoculating organ homogenates from diseased fish remained unsuccessful until recently (Castric *et al.*, 1997). Isolation of SDV in cell culture was first successful when plasma of infected fish was directly inoculated into salmonid cell lines (CHSE-214 and RTG-2). The CPE induced in SDV-infected cells is incomplete 10 days p.i. and is characterized by a low multiplication rate. The lack of obvious CPE at a low multiplicity of infection may explain why during the past 10 years no virus has been recovered from SD outbreaks (Villoing *et al.*, 2000). This was illustrated during the isolation of S49P when a transient CPE was first observed at third passage in a minority of samples (Castric *et al.*, 1997). SDV infections result in a sequential pathology, initially affecting the pancreas, then the heart and finally the skeletal muscle 3–5 weeks post-infection (Boucher and Baudin Laurencin, 1996). As clinical signs are predominantly associated with the skeletal muscle pathology, viral titres in the tissues of fish sampled at this time are likely to be low, further hampering efforts at isolation. Another factor to be considered is the

suggestion by Villoing *et al.* (2000) that the optimal replication temperature for SDV in RTG-2 cells is 10°C. In some laboratories the examination of cell cultures for evidence of CPE was supplemented by immunostaining (Rowley *et al.*, 1998).

### *Direct Detection Methods*

Virus can be isolated in freshly prepared cell culture by inoculation of pooled, ground tissue material, e.g., spleen, heart, pyloric caeca/pancreas and brain (Anon. 1997; Graham *et al.*, 2003). Also, plasma or serum obtained from clinically infected fish can be used. After 7–10 days of incubation at 10 to 15°C, a CPE may occur. From the following passage, an iIFA using a mab (Welsh *et al.*, 2000) may be carried out. Ground tissue can also be used for RT-PCR investigations (Villoing *et al.*, 2000; Weston *et al.*, 2002).

### *Indirect Identification (Serology)*

SD can be confirmed when typical lesions of cardiac necrosis, pancreatic atrophy and skeletal muscle degeneration (Boucher and Baudin Laurencin, 1996) have been observed on histological examination, as also through the detection of virus-specific antibodies (Graham *et al.*, 2003) using an immunoperoxidase based virus neutralization assay (Graham *et al.*, 2006).

## Disease Treatment and Prophylaxis

Until now there is no treatment. Only strict hygienic measures, proper diagnosis of SDV in relayed fish and control of the human behaviour with respect to other farms that may be infected have been recommended.

## Epidemiology/Geography/Distribution

SD has been observed in farmed rainbow trout in France, especially in Brittany, for several years (Boucher and Baudin Laurencin, 1994; Boucher *et al.*, 1994). More recently, SD has also been observed in Italy (Bovo, pers. comm.), the U.K. (Branson, 2002; Graham *et al.*, 2003) and in Germany (Bergmann *et al.*, 2008, in press).

## References

Agius, C., H. Mangunwiryo, R.H. Johnson and D.A. Smail. 1982. A more sensitive technique for isolating infectious pancreatic necrosis virus from asymptomatic carrier rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 5: 285-292.

Ahne, W., H.J. Schlotfeldt and I. Thomsen. 1989. Fish viruses: Isolation of an icosahedral cytoplasmic deoxyribovirus from sheatfish (*Silurus glanis*). *Journal of Veterinary Medicine* B36: 333-336.

Ahne, W., M. Ogawa and H.J. Schlotfeldt. 1990. Fish viruses: transmission and pathogenicity of an icosahedral cytoplasmic deoxyribovirus isolated from sheatfish *Silurus glanis*. *Journal of Veterinary Medicine* B37: 187-190.

Ahne, W., P.E.V. Jorgensen, N.J. Olesen, T. Fischer-Scherl and R. Hoffmann. 1989. Aquatic Birnaviruses: Virus of the serogroup II isolated from an IPN outbreak in brook trout (*Salvelinus fontinalis*). *Bulletin of the European Association of Fish Pathologists* 9: 14(16).

Ahne, W., M. Bearzotti, M. Bremont and S. Essbauer. 1998. Comparison of European systemic piscine and amphibian iridoviruses with epizootic haematopoietic necrosis virus and frog virus 3. *Journal of Veterinary Medicine* B45: 373-383.

Anonymous. 1997. General processing of organs/fluid samples for virological examination. In: *Diagnostic Manual for Aquatic Animal Diseases*. 2nd Edition, pp. 9-10. Office International des Epizooties, Paris.

Anonymous. 2006. *Manual of Diagnostic Tests for Aquatic Animals*. 5th Edition, OIE, World Organization for Animal Health, Paris.

Arakawa, C.K., R.E. Deering, K.H. Higman, K.H. Oshima, P.J. O'Hara and J.R. Winton. 1990. Polymerase chain reaction (PCR) amplification of a nucleoprotein gene sequence of infectious hematopoietic necrosis virus. *Diseases of Aquatic Organisms* 8: 165-170.

Ariel, E., H. Tapiovaara and N.J. Olesen. 1999. Comparison of pike perch (*Stizostedion lucioperca*), cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) iridovirus isolates with reference to other piscine and amphibian iridovirus isolates. *European Association of Fish Pathologists*, VIII. *International Conference on Diseases of Fish and Shellfish*, 20-24 September, Rhodes, Greece.

Arnzen, J.M., S.S. Ristow, C.P. Hesson and J. Lientz. 1991. Rapid fluorescent antibody tests for infectious hematopoietic necrosis virus (IHNV) utilizing monoclonal antibodies to the nucleoprotein and glycoprotein. *Journal of Aquatic Animal Health* 3: 109-113.

Baroni, A., R. Galesso and G. Bovo. 1982. Ultrastructural observations on the virus of haemorrhagic septicaemia in culture. *Journal of Fish Diseases* 5: 439-444.

Bergmann, S.M., D. Fichtner, H.F. Skall, H.J. Schlotfeldt and N.J. Olesen. 2003. Age- and weight-dependent susceptibility of rainbow trout *Oncorhynchus mykiss* to isolates of infectious haematopoietic necrosis virus (IHNV) of varying virulence. *Diseases of Aquatic Organisms* 55: 205-210.

Bergmann, S.M., D. Fichtner, R. Riebe and J. Castric. 2008. First isolation and identification of sleeping disease virus (SDV) in Germany. (In Press).

Blake, S., D. Bouchard, W. Keleher, M. Opitz and B.L. Nicholson. 1999. Genomic relationship of the North American isolate of infectious salmon anemia virus (ISAV) to the Norwegian strain of ISAV. *Diseases of Aquatic Organisms* 35: 139-144.

Bloch, B. and J.L. Larsen. 1993. An iridovirus-like agent associated with systemic infection in cultured turbot *Scophthalmus maximus* fry in Denmark. *Diseases of Aquatic Organisms* 15: 235-240.

Bouchard, D.A., K. Brockway, C. Giray, W. Keleher and P.L. Merrill. 2001. First report of infectious salmon anemia (ISA) in the United States. *Bulletin of the European Association of Fish Pathologists* 21: 86-88.

Boucher, P. 1995. Etiologie et pathogénie de la maladie du pancréas et de la maladie du sommeil chez les salmonidés d'élevage. Ph.D. Thesis. Université de Rennes I, Rennes, France.

Boucher, P. and F. Baudin Laurencin. 1996. Sleeping disease and pancreas disease: comparative histopathology and acquired cross-protection. *Journal of Fish Diseases* 19: 303-310.

Boucher, P. and F. Baudin Laurencin. 1994. Sleeping disease (SD) of salmonids. *Bulletin of the European Association of Fish Pathologists* 14: 179-180.

Boucher, P., J. Castric and F. Baudin Laurencin. 1994. Observation of virus-like particles in rainbow trout *Oncorhynchus mykiss* infected with sleeping disease virulent material. *Bulletin of the European Association of Fish Pathologists* 14: 215-216.

Branson, E. 2002. Sleeping disease of trout. *Veterinary Record* 150: 759-760.

Brudeseth, B.E. and O. Evensen. 2002. Occurrence of viral haemorrhagic septicaemia virus (VHSV) in wild marine fish species in the coastal regions of Norway. *Diseases of Aquatic Organisms* 52: 21-28.

Brunson, R., R. True and J. Yancey. 1989. VHS virus isolated at Makah national fish hatchery. *American Fisheries Society Fish Health Newsletter* 17: 3-4.

Cannon, R.M. and R.T. Roe. 1982. *Livestock Disease Surveys: A Field Manual for Veterinarians*. Australian Government Publishing Service, Canberra.

Castric, J. and P. De Kinkelin. 1984. Experimental study of the susceptibility of two marine fish species, sea bass *(Dicentrarchus labrax)* and turbot *(Scophthalmus maximus)*, to viral haemorrhagic septicaemia. *Aquaculture* 14: 203-212.

Castric, J., F. Baudin Laurencin, M. Brémont, J. Jeffroy, A. Le Ven and M. Béarzotti. 1997. Isolation of the virus responsible for sleeping-disease in experimentally infected rainbow trout *Oncorhynchus mykiss*. *Bulletin of the European Association of Fish Pathologists* 17: 27-30.

Chamberlain, R.W. 1980. Epidemiology of arthropod-borne togaviruses: the role of arthropods as hosts and vectors and of vertebrate hosts in natural transmission cycles. In: *The Togaviruses: Biology, Structure, Replication*, R.W. Schlesinger (ed.). Academic Press, Orlando, pp. 175-227.

Chinchar, G., S. Essbauer, J.G. He, A. Hyatt, T. Miyazaki, V. Seligy and T. Williams. 2005. Family Iridoviridae. In: *Virus Taxonomy. Classification and Nomeclature of Viruses*, Eighth Report of the International Committee on the Taxonomy of Viruses, C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball (eds.). Academic Press, San Diego, pp. 145-161.

Chinchar, V.G. 2002. Ranaviruses (family Iridoviridae): Emerging cold-blooded killers—Brief review. *Archives of Virology* 147: 447-470.

Clark, H.F. and E.Z. Soriano. 1974. Fish rhabdovirus replication in non-piscine cell culture: new system for the study of rhabdovirus-cell interaction in which the virus and cell have different temperature optima. *Infection and Immunity* 10: 180-188.

Clouthier, S.C., T. Rector, N.E. Brown and E.D. Anderson. 2002. Genomic organization of infectious salmon anaemia virus. *Journal of General Virology* 83: 421-428.

Crane, M.S.J., J. Young and L. Williams. 2005. Epizootic haematopoietic necrosis virus (EHNV): growth in fish cell lines at different temperatures. *Bulletin of the European Association of Fish Pathologists* 25: 228-231.

Dannevig, B.H., K. Falk and E. Namork. 1995. Isolation of the causal virus of infectious salmon anemia (ISA) in a long-term cell line from Atlantic salmon head kidney. *Journal of General Virology* 76: 1353-1359.

Deering, R.E., C.K. Arakawa, K.H. Oshima, P.J. O'Hara, M.L. Landolt and J.R. Winton. 1991. Development of a biotinylated DNA probe for detection and identification of infectious hematopoietic necrosis virus. *Diseases of Aquatic Organisms* 11: 57-65.

Desvignes, L., C. Quentel, F. le V.A. Lamour. 2002. Pathogenesis and immune response in Atlantic salmon (*Salmo salar* L.) parr experimentally infected with salmon pancreas disease virus (SPDV). *Fish and Shellfish Immunology* 12: 77-95.

Devold, M., B. Krossoy, V. Aspehaug and A. Nylund. 2000. Use of RT-PCR for diagnosis of infectious salmon anaemia virus (ISAV) in carrier sea trout *Salmo trutta* after experimental infection. *Diseases of Aquatic Organisms* 40: 9-18.

Devold, M., K. Falk, B. Dale, B. Krossoy, E. Biering, V. Aspehaug, F. Nilsen and A. Nylund. 2001. Strain variation, based on the hemagglutinin gene, in Norwegian ISA virus isolates collected from 1987 to 2001: Indications of recombination. *Diseases of Aquatic Organisms* 47: 119-128.

Dixon, P.F. and B.J. Hill. 1983. Rapid detection of infectious pancreatic necrosis virus (IPNV) by the enzyme-linked immunosorbent assay. *Journal of General Virology* 64: 321-330.

Dixon, P.F. and B.J. Hill. 1984. Rapid detection of fish rhabdoviruses by the enzyme-linked immunosorbent assay (ELISA). *Aquaculture* 42: 1-12.

Dixon, P.F., S. Feist, E. Kehoe, L. Parry, D.M. Stone and K. Way. 1997. Isolation of viral haemorrhagic septicaemia virus from Atlantic herring *Clupea harengus* from the English Channel. *Diseases of Aquatic Organisms* 30: 81-89.

Dobos, P. and T.E. Roberts. 1982. The molecular biology of infectious pancreatic necrosis virus: A review. *Canadian Journal of Infectious Diseases and Medical Microbiology* 29: 377-384.

Dopazo, C.P., I. Bandin, C. Lopez-Vazquez, J. Lamas, M. Noya and J.L. Barja. 2002. Isolation of viral hemorrhagic septicemia virus from Greenland halibut *Reinhardtius hippoglossoides* caught at the Flemish Cap. *Diseases of Aquatic Organisms* 50: 171-179.

Dorson, M. and C. Torchy. 1981. The influence of fish age and water temperature on mortalities of rainbow trout *Salmo gairdneri* Richardson, caused by a European strain of infectious pancreatic necrosis virus. *Journal of Fish Diseases* 4: 213-221.

Drolet, B.S., P.P. Chiou, J. Heidel and J.C. Leong. 1995. Detection of truncated virus particles in a persistent RNA virus infection *in vivo*. *Journal of Virology* 69: 2140-2147.

Drury, S.E.N., R.E. Gough and A.A. Cunningham. 1995. Isolation of an iridovirus-like agent from common frogs (*Rana temporaria*). *Veterinary Record* 137: 72-73.

Eaton, B.T., A.D. Hyatt and S. Hengstberger. 1991. Epizootic haematopoietic necrosis virus: purification and classification. *Journal of Fish Diseases* 14: 157-169.

Einer-Jensen, K., P. Ahrens, R. Forsberg and N. Lørenzen. 2004. Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. *Journal of General Virology* 85: 1167-1179.

Eliassen, T.M., M.K. Froystad, B.H. Dannevig, M. Jankowska, A. Brech, K. Falk, K. Romoren and T. Gjoen. 2000. Initial events in infectious salmon anemia virus infection: Evidence for the requirement of a low-pH step. *Journal of Virology* 74: 218-227.

Engelking, H.M., J.B. Harry and J.C. Leong. 1991. Comparison of representative strains of infectious hematopoietic necrosis virus by serological neutralization and cross-protection assays. *Applied and Environmental Microbiology* 57: 1372-1378.

Enzmann, P.-J. and M. Konrad. 1985. Inapparent infections of brown trout with VHS-virus. *Bulletin of the European Association of Fish Pathologists* 5: 81-83.

Enzmann, P.-J., M. Konrad and J. Rapp. 1992. Epizootiological studies on viral haemorrhagic septicaemia in brown trout *Salmo trutta fario*. *Diseases of Aquatic Organisms* 12: 143-146.

Evensen, O. and E. Rimstad. 1990. Immunohistochemical identification of infectious pancreatic virus in paraffin-embedded tissues of Atlantic salmon (*Salmo salar*). *Journal of Veterinary Diagnostic Investigation* 2: 288-293.

Evensen, O., K.E. Thorud and Y.A. Olsen. 1991. A morphological study of the gross and light microscopic lesions of infectious anaemia in Atlantic salmon (*Salmo salar*). *Research in Veterinary Science* 51: 215-222.

Evensen, O., W. Meier, T. Wahli, N.J. Olesen, P.E.V. Jorgensen and T. Hastein. 1994. Comparison of immunohistochemistry and virus cultivation for detection of viral haemorrhagic septicaemia virus in experimentally infected rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 20: 101-109.

Falk, K., E. Namork, E. Rimstad, S. Mjaaland and B.H. Dannevig. 1997. Characterization of infectious salmon anemia virus, an orthomyxo-like virus isolated from Atlantic salmon (*Salmo salar* L). *Journal of Virology* 71: 9016-9023.

Falk, K., E. Namork and B.H. Dannevig. 1998. Characterization and application of a monoclonal antibody against infectious salmon anaemia virus. *Diseases of Aquatic Organisms* 34: 77-85.

Falk, K., V. Aspehaug, R. Vlasak and C. Endresen. 2004. Identification and characterization of viral structural proteins of infectious salmon anemia virus. *Journal of Virology* 78: 3063-3071.

Fichtner, D., S. Bergmann, P.-J. Enzmann and H. Granzow. 1998. Characterisation of Viral Haemorrhagic Septicaemia (VHS) virus-isolates. *Bulletin of the European Association of Fish Pathologists* 18: 56-61.

Fichtner, D., S. Bergmann, P.-J. Enzmann, H. Granzow, H. Schütze, D. Mock and J.-W. Schäfer. 2000. Isolation and characterisation of a variant strain of Infectious Haematopoietic Necrosis (IHN) virus. *Bulletin of the European Association of Fish Pathologists* 20: 135-142.

Fijan, N., D. Sulimanovic, M. Bearzotti, D. Muzinic, L.O.Z. Zwillenberg, S. Chilmonczyk, J.F. Vautherot and P. De Kinkelin. 1983. Some properties of the *epithelioma papulosum cyprini* (EPC) cell line from carp *Cyprinus carpio*. *Annals of Virology Institute Pasteur* 134: 207-220.

Fijan, N., Z. Matasin, Z. Petrinec, I. Valpotic and L.O. Zwillenberg. 1991. Isolation of an iridovirus-like agent from the green frog (*Rana esculenta* L.). *Veterinarski Arhiv* 61: 151-158.

Follett, J.E., T.R. Meyers, T.O. Burton and J.L. Geesin. 1997. Comparative susceptibilities of salmonid species in Alaska to infectious hematopoietic necrosis virus (IHNV) and North American viral hemorrhagic septicemia virus (VHSV). *Journal of Aquatic Animal Health* 9: 34-40.

Graham, D.A., H.M. Rowley, I.W. Walker, J.H. Weston, E.J. Branson and D. Todd. 2003. First isolation of sleeping disease virus from rainbow trout, *Oncorhynchus mykiss* (Walbaum), in the United Kingdom. *Journal of Fish Diseases* 26: 691-694.

Graham, D.A., H.M. Rowley, D. Todd, V. Jewhurst and K. Parker. 2006. Serological survey for sleeping disease in trout in Northern Ireland. *Veterinary Record* 2002; 151: 187.

Granzow, H., F. Weiland, D. Fichtner and P.-J. Enzmann. 1997. Studies of the ultrastructure and morphogenesis of fish pathogenic viruses grown in cell culture. *Journal of Fish Diseases* 20: 1-10.

Grimholt, U., S. Larsen, R. Nordmo, P. Midtlyng, S. Kjoeglum, A. Storset, S. Saebo and R.J. Stet. 2003. MHC polymorphism and disease resistance in Atlantic salmon *(Salmo salar)*; facing pathogens with single expressed major histocompatibility class I and class II loci. *Immunogenetics* 55: 210-219.

Hedrick, R.P., W.N. Batts, S. Yun, G.S. Traxler, J. Kaufman and J.R. Winton. 2003. Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus. *Diseases of Aquatic Organisms* 55: 211-220.

Hengstberger, S.G., A.D. Hyatt, R. Speare and B.E.H. Coupar. 1993. Comparison of epizootic haematopoietic necrosis and Bohle iridoviruses, recently isolated Australian iridoviruses. *Diseases of Aquatic Organisms* 15: 93-107.

Hill, B.J. 1982. Infectious pancreatic necrosis and its virulence. In: *Microbial Diseases of Fish* (Special Publication of the Society for General Microbiology), R.J. Roberts (ed.). Academic Press, London, pp. 91-114.

Hill, B.J. 1992. Impact of viral diseases of salmonid fish in the European Community. In: *Salmonid Diseases*, T. Kimura (ed.). Hokkaido University Press, Sapporo, Japan, pp. 48-59.

Hill, B.J. and K. Way. 1995. Serological classification of infectious pancreatic necrosis (IPN) virus and other aquatic birnaviruses. *Annual Review of Fish Diseases* 5: 55-77.

Hopper, K. 1989. The first isolation of VHSV from Chinook salmon at Glenwood Springs, Orcas Island, Washington. *American Fisheries Society Fish Health Newsletter* 17: 1.

Horlyck, V., S. Mellergard, I. Dalsgaard and P.E.V. Jorgensen. 1984. Occurrence of VHS in Danish maricultured rainbow trout. *Bulletin of the European Association of Fish Pathologists* 4: 11-13.

Huang, C., M.-S. Chien, M. Landolt and J.R. Winton. 1994. Characterization of the infectious hematopoietic necrosis virus glycoprotein using neutralizing monoclonal antibodies. *Diseases of Aquatic Organisms* 18: 29-35.

Hyatt, A.D. 1991. Immunogold labelling techniques, In: *Electron Microscopy in Biology: A Practical Approach*, R. Harris (ed.). IRL Press, Oxford, pp. 59-81.

Hyatt, A.D., B.T. Eaton, S. Hengstberger and G. Russel. 1991. Epizootic hematopoietic necrosis virus: Detection by ELISA, immuno-histochemistry and electron microscopy. *Journal of Fish Diseases* 14: 605-617.

Hyatt, A.D., A.R. Gould, Z. Zupanovich, A.A. Cunningham, S. Hengstberger, R.J. Whittington, J. Kattenbelt and B.E.H. Coupar. 2000. Characterisation of Piscine and Amphibian Iridoviruses. *Archives of Virology* 145: 301-331.

Hyatt, A.D., M. Williamson, B.E.H. Coupar, D. Middleton, S.G. Hengstberger, A.R. Gould, P. Selleck, T.G. Wise, J. Kattenbelt, A.A. Cunningham and J. Lee. 2002. First identification of a ranavirus from green pythons (*Chondropython viridis*). *Journal of Wildlife Diseases* 38: 239-252.

Isshiki, T., T. Nishizawa, T. Kobayashi, T. Nagano and T. Miyazaki. 2001. An outbreak of VHSV (viral hemorrhagic septicemia virus) infection in farmed Japanese flounder *Paralichthys olivaceus* in Japan. *Diseases of Aquatic Organisms* 47: 87-99.

Jarp, J. and E. Karlsen. 1997. Infectious salmon anaemia (ISA) risk factors in sea-cultured Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 28: 79-86.

Jorgensen, P.E.V. 1980. Egtved virus: The susceptibility of brown trout and rainbow trout to eight virus isolates and the significance of the findings for VHS control. In: *Fish Diseases*, W. Ahne (ed.). Third COPRAQ-Session. Springer-Verlag, Berlin, pp. 3-7.

Jorgensen, P.E.V. 1982. Egtved virus: occurrence of inapparent infections with virulent virus in free-living rainbow trout, *Salmo gairdneri* Richardson, at low temperatures. *Journal of Fish Diseases* 5: 251-255.

Jorgensen, P.E.V., N.J. Olesen, N. Lorenzen, J.R. Winton and S.S. Ristow. 1991. Infectious hematopoietic necrosis (IHN) and viral hemorrhagic septicemia (VHS): detection of trout antibodies to the causative viruses by means of plaque neutralization, immunofluorescence, and enzyme-linked immunosorbent assay. *Journal of Aquatic Animal Health* 3: 100-108.

Kent, M.L. and R.A. Elston. 1987. Pancreas disease in pen-reared Atlantic Salmon in North America. *Bulletin of the European Association of Fish Pathologists* 7: 29-31.

Kerbart Boscher, S., M. McLoughlin, A. Le Ven, J. Cabon, M. Baud and J. Castric. 2006. Experimental transmission of sleeping disease in one-year-old rainbow trout, *Oncorhynchus mykiss* (Walbaum), induced by sleeping disease virus. *Journal of Fish Diseases* 29: 263-273.

Kibenge, F.S.B., M.J.T. Kibenge, T. Joseph and J. McDougal. 2003. The development of infectious salmon anemia virus vaccines in Canada. In: *International Response to Infectious Salmon Anemia: Prevention, Control and Eradication*. Technical Bulletin 1902, O. Miller and R.C. Cipriano (eds.). Proceedings of a Symposium, 3-4 September 2002, New Orleans, Louisiana, USA. US Department of Agriculture, Animal and Plant Health Inspection Service; US Department of the Interior, US Geological Survey; U.S. Department of Commerce, National Marine Fisheries Service, Washington DC, USA, pp. 39-49.

Kibenge, F.S.B., O.N. Garate, G. Johnson, R. Arriagada, M.J.T. Kibenge and D. Wadowska. 2001. Isolation and identification of infectious salmon anaemia virus (ISAV) from Coho salmon in Chile. *Diseases of Aquatic Organisms* 45: 9-18.

Kibenge, F.S.B., M.J.T. Kibenge, P.K. McKenna, P. Stothard, R. Marshall, R.R. Cusack and S. McGeachy. 2001. Antigenic variation among isolates of infectious salmon anaemia virus correlates with genetic variation of the viral haemagglutinin gene. *Journal of General Virology* 82: 2869-2879.

Kibenge, M.T., B. Opazo, A.H. Rojas and F.S. Kibenge. 2002. Serological evidence of infectious salmon anaemia virus (ISAV) infection in farmed fishes, using an indirect enzyme-linked immunosorbent assay (ELISA). *Diseases of Aquatic Organisms* 51: 1-11.

King, J.A., M. Snow, D.A. Smail and R.S. Raynard. 2001. Distribution of viral haemorrhagic septicaemia virus in wild fish species of the North Sea, north east Atlantic Ocean and Irish Sea. *Diseases of Aquatic Organisms* 47: 81-86.

King, J.A., M. Snow, H.F. Skall and R.S. Raynard. 2001. Experimental susceptibility of Atlantic salmon *Salmo salar* and turbot *Scophthalmus maximus* to European freshwater and marine isolates of viral haemorrhagic septicaemia virus. *Diseases of Aquatic Organisms* 47: 25-31.

Kocan, R., M. Bradley, N. Elder, T.R. Meyers, W.N. Batts and J.R. Winton. 1997. North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory-reared Pacific herring. *Journal of Aquatic Animal Health* 9: 279-290.

Krossoy, B., I. Hordvik, F. Nilsen, A. Nylund and C. Endresen. 1999. The putative polymerase sequence of infectious salmon anemia virus suggests a new genus within the Orthomyxoviridae. *Journal of Virology* 73: 2136-2142.

Krossoy, B., F. Nilsen, K. Falk, C. Endresen and A. Nylund. 2001. Phylogenetic analysis of infectious salmon anaemia virus isolates from Norway, Canada and Scotland. *Diseases of Aquatic Organisms* 44: 1-6.

Kurath, G., K.A. Garver, R.M. Troyer, E.J. Emmeneggger, K. Einer-Jensen and E.D. Anderson. 2003. Phylogeography of infectious haematopoietic necrosis virus in North America. *Journal of General Virology* 84: 803-814.

Kurita, J., Y. Iida, K. Nakajima and K. Inouye. 2002. Virucidal effects of various disinfectants on viral hemorrhagic septicemia virus (VHSV) isolated from Japanese flounder. *Fish Pathology* 37: 175-181.

Langdon, J.S. 1989. Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in red fin perch *Perca fluviatilis* L., and 11 other teleosts. *Journal of Fish Diseases* 12: 295-310.

Langdon, J.S. and J.D. Humphrey. 1987. Epizootic Hematopoietic Necrosis a new viral disease in redfin perch *Perca fluviatilis* L. in Australia. *Journal of Fish Diseases* 10: 289-298.

Langdon, J.S., J.D. Humphrey and L.M. Williams. 1988. Outbreaks of an EHNV-like iridovirus in cultured rainbow trout, *Salmo gairdneri* Richardson, in Australia. *Journal of Fish Diseases* 11: 93-96.

Langdon, J.S., J.D. Humphrey, L.M. Williams, A.D. Hyatt and H.A. Westbury. 1986. First virus isolation from Australian fish: an iridovirus-like pathogen from red fin perch, *Perca fluviatilis* L. *Journal of Fish Diseases* 9: 263-268.

LaPatra, S.E. 1996. The use of serological techniques for virus surveillance and certification of finfish. *Annual Review of Fish Diseases* 6: 15-28.

LaPatra, S.E., K.A. Roberti, J.S. Rohovec and J.L. Fryer. 1989. Fluorescent antibody test for the rapid diagnosis of infectious hematopoietic necrosis virus. *Journal of Aquatic Animal Health* 1: 29-36.

LaPatra, S.E., J.L. Fryer and J.S. Rohovec. 1993a. Virulence comparison of different electropherotypes of infectious hematopoietic necrosis virus. *Diseases of Aquatic Organisms* 16: 115-120.

LaPatra, S.E., T. Turner, K.A. Lauda, G.R. Jones and S. Walker. 1993b. Characterization of the humoral response of rainbow trout to infectious hematopoietic necrosis virus. *Journal of Aquatic Animal Health* 5: 165-171.

Lorenzen, N. and S. LaPatra. 1999. Immunity to rhabdoviruses in rainbow trout: the antibody response. *Fish and Shellfish Immunology* 9: 345-360.

Lorenzen, E., B. Carstensen and N.J. Olesen. 1999. Inter-laboratory comparison of cell lines for susceptibility to three viruses: VHSV, IHNV and IPNV. *Diseases of Aquatic Organisms* 37: 81-88.

Lovdal, T. and O. Enger. 2002. Detection of infectious salmon anemia virus in sea water by nested RT-PCR. *Diseases of Aquatic Organisms* 49: 123-128.

Lovely, J.E., B.H. Dannevig, K. Falk, L. Hutchin, A.M. MacKinnon, K.J. Melville, E. Rimstad and S.G. Griffiths. 1999. First identification of infectious salmon anaemia virus in North America with haemorrhagic kidney syndrome. *Diseases of Aquatic Organisms* 35: 145-148.

MacLean, S.A., D.A. Bouchard and S.K. Ellis. 2003. Survey of non-salmonid marine fishes for detection of infectious salmon anaemia virus and other salmonid pathogens. In: *International Response to Infectious Salmon Anemia: Prevention, Control and Eradication*. Technical Bulletin 1902, O. Miller and R.C. Cipriano (eds.). *Proceedings of a Symposium*, 3-4 September 2002, New Orleans, Louisiana, USA. US Department of Agriculture, Animal and Plant Health Inspection Service; US Department of the Interior, US Geological Survey; U.S. Department of Commerce, National Marine Fisheries Service, Washington DC, USA, pp. 135-143.

Mao, J., T.N. Tham, G.A. Gentry, A. Aubertin and V.G. Chinchar. 1996. Cloning, sequence analysis, and expression of the major capsid protein of the iridovirus frog virus 3. *Journal of Virology* 216: 431-436.

Mao, J., R.P. Hedrick and V.G. Chinchar. 1997. Molecular characterisation, sequence analysis and taxonomic position of newly isolated fish iridoviruses. *Journal of Virology* 229: 212-220.

Marsh, I.B., R.J. Whittington, B. O'Rourke, A.D. Hyatt and O. Chisholm. 2002. Rapid differentiation of Australian, European and American ranaviruses based on variation in major capsid protein gene sequence. *Molecular Cell Probes* 16: 137-151.

McAllister, P.E. and W.J. Owens. 1995. Assessment of the virulence of fish and molluscan isolates of infectious pancreatic necrosis virus for salmonid fish by challenge of brook trout, *Salvelinus fontinalis* (Mitchill). *Journal of Fish Diseases* 18: 97-103.

McKnight, I.J. and R.J. Roberts. 1976. The pathology of infectious pancreatic necrosis. I. The sequential histopathology of the naturally occurring condition. *British Veterinary Journal* 132: 76-85.

Meier, W. and P.E.V. Jorgensen. 1980. Isolation of VHS virus from pike fry *(Esox lucius)* with hemorrhagic symptoms. In: *Fish Diseases*, W. Ahne (ed.). Third COPRAQ-Session. Springer-Verlag, Berlin, pp. 8-17.

Meier, W. and T. Wahli. 1988. Viral haemorrhagic septicaemia (VHS) in grayling, *Thymallus thymallus* L. *Journal of Fish Diseases* 11: 481-487.

Meier, W., M. Schmitt and T. Wahli. 1994. Viral hemorrhagic septicemia (VHS) of nonsalmonids. *Annual Review of Fish Diseases* 4: 359-373.

Melby, H.P., P. Caswell-Reno and K. Falk. 1994. Antigenic analysis of Norwegian aquatic birnavirus isolates using monoclonal antibodies with special reference to fish species, age and health status. *Journal of Fish Diseases* 17: 85-91.

Meyers, T.R., S. Short, K. Lipson, W.N. Batts, J.R. Winton, J. Wilcock and E. Brown. 1994. Association of viral hemorrhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA. *Diseases of Aquatic Organisms* 19: 27-37.

Meyers, T.R., S. Short and K. Lipson. 1999. Isolation of the North American strain of viral hemorrhagic septicemia virus (VHSV) associated with epizootic mortality in two new host species of Alaskan marine fish. *Diseases of Aquatic Organisms* 38: 81-86.

Miller, T.A., J. Rapp, U. Wastlhuber, R.W. Hoffmann and P.-J. Enzmann. 1998. Rapid and sensitive reverse transcriptase-polymerase chain reaction based detection and differential diagnosis of fish pathogenic rhabdoviruses in organ samples and cultured cells. *Diseases of Aquatic Organisms* 34: 13-20.

Mjaaland, S., E. Rimstad, K. Falk and B.H. Dannevig. 1997. Genomic characterisation of the virus causing infectious salmon anemia in Atlantic salmon *(Salmo salar* L.): An orthomyxo-like virus in a teleost. *Journal of Virology* 71: 7681-7686.

Mortensen, H.F., O.E. Heuer, N. Lorenzen, L. Otte and N.J. Olesen. 1999. Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea, Kattegat, Skagerrak and the North Sea. *Virus Research* 63: 95-106.

Mourton, C., B. Romestand, P. De Kinkelin, J. Jeffroy, R. Le Gouvello and B. Pau. 1992. Highly sensitive immunoassay for direct diagnosis of viral hemorrhagic septicaemia which uses anti-nucleocapsid monoclonal antibodies. *Journal of Clinical Microbiology* 30: 2338-2345.

Munir, K. and F.S. Kibenge. 2004. Detection of infectious salmon anaemia virus by real-time RT-PCR. *Journal of Virological Methods* 117: 37-47.

Munro, A.L.S., A.E. Ellis, A.H. McVicar, H.A. McLay and E.A. Needham. 1984. An exocrine pancreas disease of farmed Atlantic salmon in Scotland. *Helgoländer Meeresuntersuchungen* 37: 571-586.

Murphy, T.M., H.D. Rodger, E.M. Drinan, F. Gannon, P. Kruse and W. Korting. 1992. The sequential pathology of pancreas disease in Atlantic salmon farms in Ireland. *Journal of Fish Diseases* 15: 401-408.

Nelson, R.T., M.F. McLoughlin, H.M. Rowley, M.A. Platten and J.I. McCormick. 1995. Isolation of a toga-like virus from farmed Atlantic salmon *Salmo salar* with pancreas disease. *Diseases of Aquatic Organisms* 22: 25-32.

Neukirch, M. 1985. Uptake, multiplication, and excretion of viral haemorrhagic septicaemia virus in rainbow trout *(Salmo gairdneri)*. In: *Fish and Shellfish Pathology*, A.E. Ellis (ed.). Academic Press, London, pp. 295-300.

Nylund, A. and P. Jakobsen. 1995. Sea trout as a carrier of infectious salmon anaemia virus. *Journal of Fish Biology* 47: 174-176.

Nylund, A., C. Wallace and T. Hovland. 1993. The possible role of *Lepeophtheirus salmonis* (Krøyer) in the transmission of infectious salmon anaemia. In: *Pathogens of Wild and Farmed Fish: Sea Lice*, G. Boxhall and D. Defaye (eds.). Ellis Horwood, London, pp. 367-373.

Nylund, A., S. Alexandersen, B.J. Rolland and P. Jakobsen. 1995. Infectious salmon anaemia virus (ISAV) in brown trout. *Journal of Aquatic Animal Health* 7: 236-240.

Nylund, A., A.M. Kvenseth, B. Krossoy and K. Hodneland. 1997. Replication of the infectious salmon anaemia virus (ISAV) in rainbow trout *Oncorhynchus mykiss*, (Wallbaum), 1792. *Journal of Fish Diseases* 20: 275-279.

Nylund, A., B. Krossoy, M. Devold, V. Asphaug, N.O. Steine and T. Hovland. 1998. Outbreak of ISA during first feeding of salmon fry *(Salmo salar)*. *Bulletin of the European Association of Fish Pathologists* 19: 71-74.

Nylund, A., M. Devold, J. Mullins and H. Plarre. 2002. Herring *(Clupea harengus)*: A host for infectious salmon anemia virus (ISAV). *Bulletin of the European Association of Fish Pathologists* 22: 311-318.

Nylund, A., M. Devold, H. Plarre, E. Isdal and M. Aarseth. 2003. Emergence and maintenance of infectious salmon anaemia virus (ISAV) in Europe: a new hypothesis. *Diseases of Aquatic Organisms* 56: 11-24.

Okamoto, N., T. Sano, R.P. Hedrick and J.L. Fryer. 1983. Antigenic relationships of selected strains of infectious pancreatic necrosis virus and European eel virus. *Journal of Fish Diseases* 6: 19-25.

Olesen, N.J. 1998. Sanitation of viral haemorrhagic septicaemia (VHS). *Journal of Applied Ichthyology* 14: 173-177.

Olesen, N.J. and P.E.V. Jorgensen. 1992. Comparative susceptibility of three fish cell lines to Egtved virus, the virus of viral haemorrhagic septicaemia (VHS). *Diseases of Aquatic Organisms* 12: 235-237.

Olesen, N.J., N. Lorenzen and P.E.V. Jorgensen. 1993. Serological differences among isolates of viral haemorrhagic septicaemia virus detected by neutralizing monoclonal and polyclonal antibodies. *Diseases of Aquatic Organisms* 16: 163-170.

Olesen, N.J., P.E.V. Jorgensen, B. Bloch and S. Mellergaard. 1988. Isolation of an IPN-like virus belonging to the serogroup II of the aquatic birnaviruses from dab (*Limanda limanda*). *Journal of Fish Diseases* 11: 449-451.

Oye, A.K. and E. Rimstad. 2001. Inactivation of infectious salmon anaemia virus, viral haemorrhagic septicaemia virus and infectious pancreatic necrosis virus in water using UVC irradiation. *Diseases of Aquatic Organisms* 48: 1-5.

Parry, L. and P.F. Dixon. 1997. Stability of nine viral haemorrhagic septicaemia virus (VHSV) isolates in seawater. *Bulletin of the European Association of Fish Pathologists* 17: 31-36.

Peters, F. and M. Neukirch. 1986. Transmission of some fish pathogenic viruses by the heron, *Ardea cinerea*. *Journal of Fish Diseases* 9: 539-544.

Poppe, T., E. Rimstad and B. Hyllseth. 1989. Pancreas disease in Atlantic salmon (*Salmo salar*) post-smolt infected with infectious pancreatic necrosis virus (IPNV). *Bulletin of the European Association of Fish Pathologists* 9: 83-85.

Pozet, F., M. Morand, A. Moussa, C. Torhy and P. De Kinkelin. 1992. Isolation and preliminary characterization of a pathogenic icosahedral deoxyribovirus from the catfish (*Ictalurus melas*). *Diseases of Aquatic Organisms* 14: 35-42.

Rasmussen, C.J. 1965. A biological study of the Egtved disease (INuL). *Annals of the New York Academy of Sciences* 126: 427-460.

Raynard, R.S., G. Houghton and A.L.S. Munro. 1992. *Pancreas disease of Atlantic salmon*: Proceedings of a European Commission Workshop. Scottish Office Aquaculture Report No. 1. The Scottish Office Aquaculture and Fisheries, Aberdeen, 2-4.

Raynard, R.S., A.G. Murray and A. Gregory. 2001. Infectious salmon anaemia virus in wild fish from Scotland. *Diseases of Aquatic Organisms* 46: 93-100.

Reddacliff, L.A. and R.J. Whittington. 1996. Pathology of epizootic haematopoeitic necrosis virus (EHNV) infection in rainbow trout (*Oncorhynchus mykiss* Walbaum) and redfin perch (*Perca fluviatilis* L.). *Journal of Comparative Pathology* 15: 103-115.

Rimstad, E. and S. Mjaaland. 2002. Infectious salmon anaemia virus. An orthomyxovirus causing en emerging infection in Atlantic salmon. *APMIS* 110: 273-282.

Ristow, S.S. and J.M. Arnzen. 1989. Development of monoclonal antibodies that recognize a type 2 specific and a common epitope on the nucleoprotein of infectious hematopoietic necrosis virus. *Journal of Aquatic Animal Health* 1: 119-125.

Ristow, S.S. and J.M. Arnzen De Avila. 1991. Monoclonal antibodies to the glycoprotein and nucleoprotein of infectious hematopoietic necrosis virus (IHNV) reveal differences among isolates of the virus by fluorescence, neutralization and electrophoresis. *Diseases of Aquatic Organisms* 11: 105-115.

Rodger, H.D., T. Turnbull, F. Muir, S. Millar and R. Richards. 1998. Infectious salmon anaemia (ISA) in the United Kingdom. *Bulletin of the European Association of Fish Pathologists* 18: 115-116.

Rowley, H.M., C.E. Doherty, M.F. McLoughlin and M.D. Welsh. 1998. Isolation of salmon pancreas disease virus (SPDV) from farmed Atlantic salmon, *Salmo salar* L., in Scotland. *Journal of Fish Diseases* 21: 469-471.

Schlotfeldt, H.-J., W. Ahne, P.E.V. Jorgensen and W. Glende. 1991. Occurrence of viral haemorrhagic septicaemia in turbot *(Scophthalmus maximus)*—A natural outbreak. *Bulletin of the European Association of Fish Pathologists* 11: 105-107.

Simon, R.C. and W.B. Schill. 1984. Tables of sample size requirements for detection of fish infected by pathogens: three confidence levels for different infection prevalence and various population sizes. *Journal of Fish Diseases* 7: 515-520.

Smail, D.A. 1999. Viral Haemorrhagic Septicaemia. In: *Fish Diseases and Disorders*, P.T.K. Woo and D.W. Bruno (eds.). CABI Publishing, Wallingford, Oxon, Vol. 3, pp. 123-148.

Smail, D.A. 2000. Isolation and identification of viral haemorrhagic septicaemia (VHS) viruses from cod *Gadus morhua* with the ulcus syndrome and from haddock *Melanogrammus aeglefinus* having skin haemorrhages in the North Sea. *Diseases of Aquatic Organisms* 41: 231-235.

Snow, M. and C.O. Cunningham. 2000. Virulence and nucleotide sequence analysis of marine viral haemorrhagic septicaemia virus following *in vivo* passage in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 42: 17-26.

Snow, M., C.O. Cunningham and I.R. Bricknell. 2000. Susceptibility of juvenile Atlantic cod *Gadus morhua* to viral haemorrhagic septicaemia virus isolated from wild-caught Atlantic cod. *Diseases of Aquatic Organisms* 41: 225-229.

Snow, M., R. Raynard, D.W. Bruno, A.P. Van Nieuwstadt, N.J. Olesen, T. Lovold and C. Wallace. 2002. Investigation into the susceptibility of saithe *Pollachius virens* to infectious salmon anaemia virus (ISAV) and their potential role as a vector for viral transmission. *Diseases of Aquatic Organisms* 50: 13-18.

Snow, M., N. Bain, J. Black, V. Taupin, C.O. Cunningham, J.A. King, H.F. Skall and R.S. Raynard 2004. Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). *Diseases of Aquatic Organisms* 61: 11-21.

Speare, R. and J.R. Smith. 1992. An iridovirus-like agent isolated from the ornate burrowing frog *Limnodynastes ornatus* in northern Australia. *Diseases of Aquatic Organisms* 14: 51-57.

Steiner, K.A., R.J. Whittington, R.K. Petersen, C. Hornitzky and H. Garnett. 1991. Purification of epizootic haematopoietic necrosis virus and its detection using ELISA. *Journal of Virological Methods* 33: 199-209.

Stone, D.M., K. Way and P.F. Dixon. 1997. Nucleotide sequence of the glycoprotein gene of viral haemorrhagic septicaemia (VHS) viruses from different geographical areas: A link between VHS in farmed fish species and viruses isolated from North Sea cod (*Gadus morhua* L.). *Journal of General Virology* 78: 1319-1326.

Takano, R., T. Nishizawa, M. Arimoto and K. Muroga. 2000. Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild Japanese flounder, *Paralichthys olivaceus*. *Bulletin of the European Association of Fish Pathologists* 20: 186-192.

Taksdal, T. and K.E. Thorud. 1999. Evaluation of a rapid co-agglutination (COA) test for the detection of infectious pancreatic necrosis virus (IPNV) in tissue samples of Atlantic salmon (*Salmo salar*). *Journal of Fish Diseases* 22: 117-124.

Taksdal, T., B.H. Dannevig and E. Rimstad. 2001. Detection of infectious pancreatic necrosis (IPN)-virus in experimentally infected Atlantic salmon parr by RT-PCR and cell culture isolation. *Bulletin of the European Association of Fish Pathologists* 21: 214-219.

Tapiovaara, H., N.J. Olesen, J. Linden, C.-H. Rimaila-Parnanen Von Bonsdorff. 1998. Isolation of an iridovirus from pikeperch (*Stizostedion lucioperca*). *Diseases of Aquatic Organisms* 32: 185-193.

Thorud, K.E. and H.O. Djupvik. 1988. Infectious salmon anaemia in Atlantic salmon (*Salmo salar* L.). *Bulletin of the European Association of Fish Pathologists* 8: 109-111.

Traxler, G.S., D. Kieser and J. Richard. 1999. Mass mortality of pilchard and herring associated with viral hemorrhagic septicemia virus in British Columbia Canada. *American Fisheries Society Fish Health Newsletter* 27: 3-4.

Underwood, B.O., C.J. Smale, F. Brown and B.J. Hill. 1977. Relationship of a virus from *Tellina tenuis* to infectious pancreatic necrosis virus. *Journal of General Virology* 36: 93-109.

Vagsholm, I., H.O. Djupvik, F.V. Willumsen, A.M. Tveit and K. Tangen. 1994. Infectious salmon anaemia (ISA) epidemiology in Norway. *Preventive Veterinary Medicine* 19: 277-290.

Villoing, S., J. Castric, J. Jeffroy, A. Le Ven, R. Thiery and M. Bremont. 2000a. An RT-PCR-based method for the diagnosis of the sleeping disease virus in experimentally and naturally infected salmonids. *Diseases of Aquatic Organisms* 40: 19-27.

Villoing, S., M. Bearzotti, S. Chilmonczyk, J. Castric and M. Bremont. 2000b. Rainbow trout sleeping disease virus is an atypical alphavirus. *Journal of Virology* 74: 173-183.

Walker, P.J., A. Benmansour, R. Dietzgen, R.-X. Fang, A.O. Jackson, G. Kurath, J.C. Leong, S. Nadin-Davies, R.B. Tesh and N. Tordo. 2000. Family Rhabdoviridae. In: *Virus Taxonomy*, Seventh Report of the International Committee on Taxonomy of Viruses, M.H.V. van Regenmortel, C.M. Fauquet, D.H.L. Bishop, E.B. Carstens, M.K. Estes, S.M. Lemon, J. Maniloff, M.A. Mayo, D.J. McGeoch, C.R. Pringle and R.B. Wickner (eds.). Academic Press, San Diego, USA.

Watanabe, K., M. Karlsen, M. Devold, E. Isdal, A. Litlabø and A. Nylund. 2006. Virus-like particles associated with heart and skeletal muscle inflammation (HSMI). *Diseases of Aquatic Organisms* 70: 183-192.

Welsh, M., J. Weston, B. Borghmans, D. Mackie, H. Rowley, R. Nelson, M. McLoughlin and D. Todd. 2000. Biochemical characterisation of salmon pancreas disease virus. *Journal of General Virology* 81: 813-820.

Weston, J.H., M.D. Welsh, M.F. McLoughlin and D. Todd. 1999. Salmon pancreas disease virus, an alphavirus infecting farmed Atlantic salmon *Salmo salar* L. *Virology* 256: 188-195.

Weston, J.H., S. Villoing, M. Bremont, J. Castric, M. Pfeffer, V. Jewhurst, M. McLoughlin, O. Rodseth, K.E. Christie, J. Koumans and D. Todd. 2002. Comparison of two aquatic alphaviruses, salmon pancreas disease virus and sleeping disease virus, by using genome sequence analysis, monoclonal reactivity, and cross-infection. *Journal of Virology* 76: 6155-6163.

Whittington, R.J. 1992. Evaluation of a simple method for improving the precision of an ELISA detecting antibody in serum. *Journal of Immunological Methods* 148: 57-64.

Whittington, R.J. and G.L. Reddacliff. 1995. Influence of environmental temperature on experimental infection of red fin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) with epizootic haematopoietic necrosis virus, an Australian iridovirus. *Australian Veterinary Journal* 72: 421-424.

Whittington, R.J. and K.A. Steiner. 1993. Epizootic haematopoietic necrosis virus (EHNV): improved ELISA for detection in fish tissues and cell cultures and an efficient method for release of antigen from tissues. *Journal of Virological Methods* 43: 205-220.

Whittington, R.J., A. Philbey, G.L. Reddacliff and A.R. Macgown. 1994. Epidemiology of epizootic haematopoietic necrosis virus (EHNV) infection in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum): findings based on virus isolation, antigen capture ELISA and serology. *Journal of Fish Diseases* 17: 205-218.

Whittington, R.J., C. Kearns, A.D. Hyatt, S. Hengstberger and T. Rutzou. 1996. Spread of epizootic haematopoietic necrosis virus (EHNV) in red fin perch (*Perca fluviatilis*) in southern Australia. *Australian Veterinary Journal* 73: 112-114.

Whittington, R.J., L.A. Reddacliff, I. Marsh, C. Kearns, Z. Zupanovic and R.B. Callinan. 1999. Further observations on the epidemiology and spread of epizootic haematopoietic necrosis virus (EHNV) in farmed rainbow trout *Oncorhynchus mykiss*

in southeastern Australia and a recommended sampling strategy for surveillance. *Diseases of Aquatic Organisms* 35: 125-130.

Williams, K., S. Blake, A. Sweeney, J.T. Singer and B.L. Nicholson. 1999. Multiplex reverse transcriptase PCR assay for simultaneous detection of three fish viruses. *Journal of Clinical Microbiology* 12: 4139-4141.

Winton, J.R. 1991. Recent advances in the detection and control of infectious hematopoietic necrosis virus (IHNV) in aquaculture. *Annual Review of Fish Diseases* 1: 83-93.

Winton, J.R. 1997. Immunization with viral antigens: Infectious haematopoietic necrosis. *Developmental Biology* 90: 211-220.

Winton, J.R. and K. Einer-Jensen. 2002. Molecular diagnosis of infectious hematopoietic necrosis and viral hemorrhagic septicemia. In: *Molecular Diagnosis of Salmonid Diseases*, C.O. Cunningham (ed.). Kluwer Academic Publishers, Dordrecht, pp. 49-79.

Winton, J.R., C.K. Arakawa, C.N. Lannan and J.L. Fryer. 1988. Neutralizing monoclonal antibodies recognize antigenic variants among isolates of infectious hematopoietic necrosis virus. *Diseases of Aquatic Organisms* 4: 199-204.

Winton, J.R., W.N. Batts, T. Nishizawa and C.M. Stehr. 1989. Characterization of the first North American isolates of viral hemorrhagic septicemia virus. *American Fisheries Society Fish Health Newsletter* 17: 2-3.

Wolf, K. 1988. *Fish Viruses and Fish Viral Diseases*. Cornell University Press, Ithaca, pp. 217-249.

Wolf, K. 1988. *Fish Viruses and Fish Viral Diseases*. Cornell University Press, Ithaca, 476 pp.

Wolf, K. 1988. Infectious hematopoietic necrosis. In: *Fish Viruses and Fish Viral Diseases*. Cornell University Press, Ithaca, pp. 83-114.

Wolf, K., S.F. Snieszko, C.E. Dunbar and E. Pyle. 1960. Virus nature of infectious pancreatic necrosis virus in trout. *Proceedings of the Society for Experimental Biology and Medicine* 104: 105-108.

Wolf, K., G.L. Bullock, C.E. Dunbar and M.C. Quimby. 1968. Tadpole edema virus: A viscerotrophic pathogen for anuran amphibians. *Journal of Infectious Diseases* 118: 253-262.

Zupanovic, Z., C. Musso, G. Lopez, C.L. Louriero, A.D. Hyatt, S. Hengstberger and A.J. Robinson. 1998. Isolation and characterisation of iridoviruses from the giant toad *Bufo marinus* in Venezuela. *Diseases of Aquatic Organisms* 33: 1-9.

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CHAPTER

3

# Virus Diseases of Cyprinids

**P.F. Dixon**

## INTRODUCTION

Virus diseases of cyprinids are amongst the most well-known disease conditions affecting cyprinids as they impact cultured food fish species, warm-water and cold-water ornamental species, and sports fish. The so-called 'carp pox' disease, which causes raised areas (epidermal hyperplasia) on the skin of affected fish, is familiar to many anglers and hobbyists, although they do not necessarily realize that it is a virus disease.

The following sections will describe the important virus diseases of cyprinids, and other reports of virus isolations from—or observations of—viruses in cyprinids will also be listed. However, cyprinids only experimentally susceptible to viruses that occur in no other cyprinid will not be discussed. This chapter will concentrate on disease aspects, and will not include details of pure virological interest, e.g., replication strategy or virus properties unless it is necessary for the understanding of the disease, its identification or control.

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## Spring Viraemia of Carp

Spring viraemia of carp (SVC) is an acute, haemorrhagic disease of several carp and other cyprinid species. It is caused by a rhabdovirus, SVC virus (SVCV). In early literature, disease(s) with the names such as infectious ascites, haemorrhagic septicaemia, infectious dropsy and rubella were described, which were eventually grouped into two types of disease: an acute form (SVC) caused by a virus; and a chronic form, termed carp erythrodermatitis, primarily caused by *Aeromonas salmonicida*. Fijan (1972) unravelled the aetiology of the disease. For this reason, some of the literature describing the disease may not be accurate in relation to the disease currently recognized as SVC, as the observations or experiments may not have been on SVC or SVCV.

It has been very difficult to distinguish SVCV from certain other rhabdoviruses such as pike fry rhabdovirus (PFRV) by some serological tests, but nucleotide sequence analysis (Stone *et al.*, 2003) provides evidence that SVCV was distinct from PFRV and a large number of other serologically related virus isolates (see below). Many of those isolates are clustered together in a Genogroup called the tench rhabdovirus group (TenRV). Those viruses will be discussed in the section on the Tench Rhabdovirus Group.

## Hosts

The hosts of SVCV are predominantly cyprinids (Table 3.1). Two cyprinids, golden shiner (*Notemigonis crysoleucas*) and zebra fish (*Danio rerio*), are experimentally susceptible to the virus (Goodwin, 2002; Sanders *et al.*, 2003). The virus has also been isolated from two species of penaeid shrimp in Hawaii, *Litopenaeus (Penaeus) stylirostris* and *L. (Penaeus) vannamei* (Lu *et al.*, 1991). That virus was originally called rhabdovirus of penaeid shrimp, but it is serologically related to SVCV; the nucleotide sequence of its glycoprotein (G) gene is practically identical to that of SVCV, and the two viruses should be considered to be the same (Lu and Loh, 1994; Lu *et al.*, 1994; Johnson *et al.*, 1999). A virus that cross-reacts with SVCV antiserum in the fluorescent antibody test (FAT) has been isolated from the black bullhead (*Ameirus* [*Ictalurus*] *melas*) (Selli *et al.*, 2002), but the identity of the virus is uncertain because of the non-specificity of many SVCV antisera in that test.

The reported case of rainbow trout (Shelkunov, pers. comm.; Stone *et al.*, 2003) concerned survivors of a bath infection with viral

Table 3.1 Fish species from which SVCV has been isolated.

| *Species* | *Common name* | *Reference* |
|---|---|---|
| **1. Cyprinids** | | |
| *Aristichthys nobilis* | Bighead carp | Shchelkunov and Shchelkunova (1989) |
| *Carassius auratus* | Goldfish | Jørgensen *et al.* (1989) |
| *C. carassius* | Crucian carp | Kölbl (1975) |
| *Ctenopharyngodon idella* | Grass carp, white amur | Shchelkunov and Shchelkunova (1989) |
| *Cyprinus carpio* | Common carp | Fijan *et al.* (1971) |
| *Hypophthalmichthys molitrix* | Silver carp | Roudikov (1980) |
| *Leuciscus idus* | Orfe, ide | Dixon *et al.* (1994) |
| *Tinca tinca* | Tench | Dixon *et al.* (1994) |
| *Rutilus rutilus* | Roach | Dixon (1997) |
| **2. Non-cyprinids** | | |
| *Esox lucius* | Pike | Koutná *et al.* (2003) |
| *Oncorhynchus mykiss* | Rainbow trout | I. Shchelkunov (pers. comm.); Stone *et al.* (2003) |
| *Silurus glanis* | Sheatfish, European catfish, wels | Fijan *et al.* (1984) |

haemorrhagic septicaemia virus (VHSV), and 2 years later, when they were 3 years old (500-800 g), they were injected with prednisolone and thermally stressed. Virus was isolated from internal organs, fins, skin, gills and brain, which was shown by reverse transcription polymerase chain reaction (RT-PCR) to be SVCV; subsequent studies revealed it to be a mixture of two SVCV isolates belonging to different SVCV sub Genogroups. The virus was thought to have cross-infected the rainbow trout from carp infected with SVCV, that were held at the same experimental facility. If confirmed, this raises serious questions regarding the ability of SVCV to cross the species barrier and raise the profile of SVCV as a threat to rainbow trout, and possibly other salmonid species.

## Disease Symptoms

The symptoms of the disease are somewhat non-specific, but the relatively consistent features are abdominal distension (dropsy) and haemorrhages in the skin. There may be exophthalmia and the gills may be pale; the eyes and gills may have petechial haemorrhages. The vent may be swollen and inflamed. Affected fish may be darker in colour, become lethargic and then exhibit erratic swimming or loss of balance. Internally, the abdomen is

SVCV has been isolated from diseased 1-year-old rainbow trout held on a fish farm. The virus was experimentally pathogenic for carp (Svetlana *et al.*, 2006).

usually filled with an excess of fluid which may contain blood. Viscera are often oedematous, and internal organs and musculature often exhibit petechial haemorrhages, as does the wall of the swimbladder (one of the early names of the disease was swimbladder inflammation) (Fijan, 1972; Bachmann and Ahne, 1973, 1999; Roudikov, 1980; Jeremic *et al.*, 2004).

The histopathological picture reflects the clinical signs, with pathology observed in the liver, pancreas, intestine, spleen, kidney, heart, muscle, and air bladder. Oedema, inflammation, haemorrhage and necrosis are the most common features (Negele, 1977; Roudikov, 1980; Osadchaya and Rudenko, 1981; Fijan, 1999).

## Disease Aetiology

A number of different laboratories demonstrated the viral aetiology of SVC in the 1970s, but the difficulty in communicating results—particularly between western and eastern Europe and the states of the former USSR—meant that a lot of work was duplicated (Osadchaya, 1964; Fijan *et al.*, 1971; Bachmann and Ahne, 1973; Roudikov, 1980). The virus isolated by Fijan *et al.* (1971) was called *Rhabdovirus carpio*, and a rhabdovirus was isolated from fish exhibiting swimbladder inflammation (Bachmann and Ahne, 1973), which at the time was still considered to be a separate disease from SVC. The biological properties of the two viruses were similar (Bachmann and Ahne, 1974), and serological comparisons showed them to be identical (Kinkelin and Le Berre, 1974; Hill *et al.*, 1975). The virus is classified as a tentative species in the *Vesiculovirus* genus of the virus family *Rhabdoviridae*, and the name approved by the International Committee on Taxonomy of Viruses is spring viraemia of carp virus (SVCV) (Fauquet *et al.*, 2005). That name will be used here, not *Rhabdovirus carpio*.

The virus has the typical bullet shape of rhabdoviruses (Fig 3.1). Negatively stained particles are approximately 60-90 nm wide by 90-180 nm long (Fijan, 1972; Bachmann and Ahne, 1973; Cohen and Lenoir, 1974; Hill *et al.*, 1975; Tesarcik *et al.*, 1977b). The virion comprises a nucleocapsid surrounded by an envelope. The nucleocapsid comprises a linear, single-stranded molecule of RNA and the following viral proteins: nucleocapsid (N), phosphoprotein (P) (formerly called non-structural, NS protein) and large (L) protein; the envelope comprises host lipid and virus glycoprotein (G) and interfaces with the nucleocapsid via the matrix (M) protein (Lenoir, 1973; Sokol *et al.*, 1974; Hill *et al.*, 1975; Lenoir and Kinkelin, 1975; Deuter and Enzmann, 1986; Lu and Loh, 1994). The

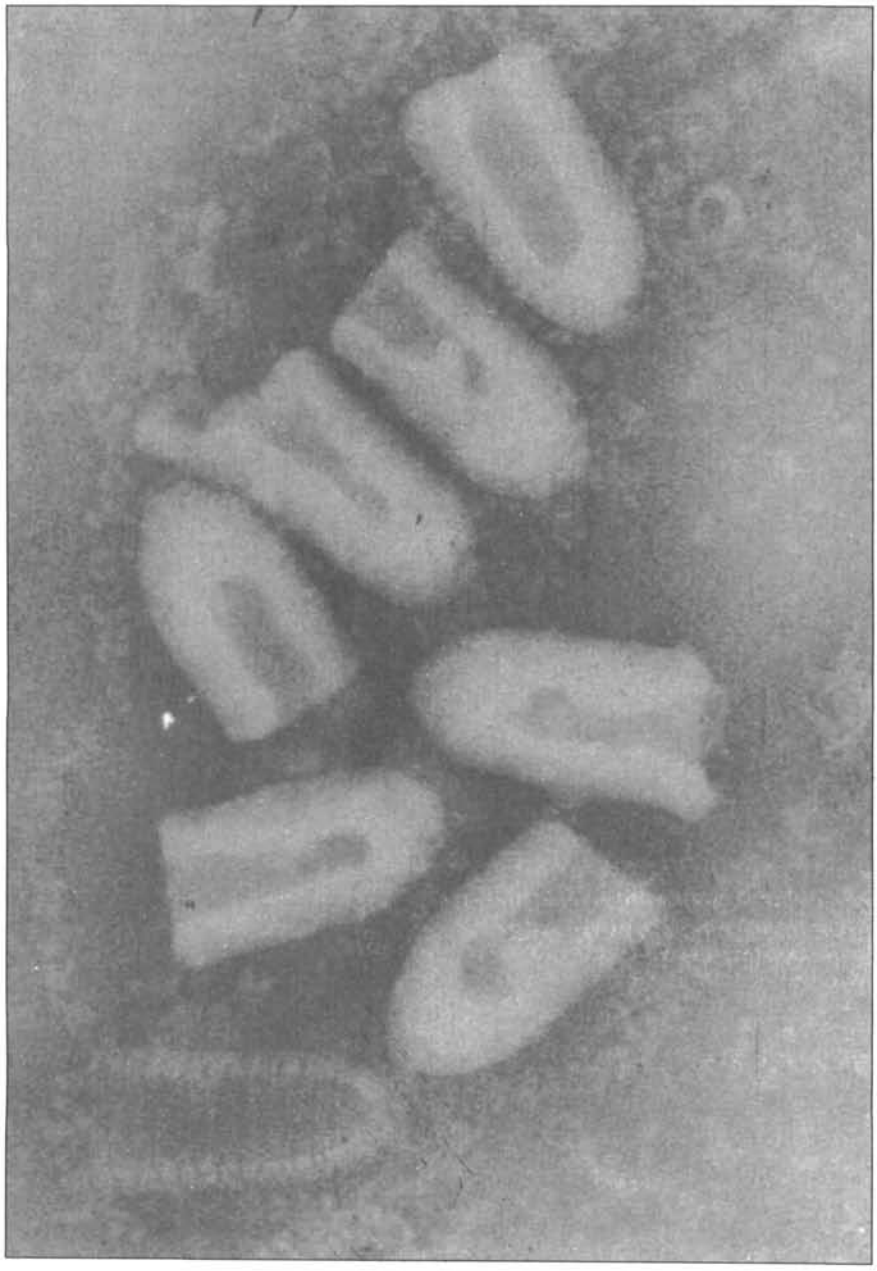

**Fig. 3.1** Spring viraemia of carp virus, negative stain.

genome has been sequenced by Hoffmann *et al.* (2002) and contains five open reading frames coding for the five structural proteins, in the gene order from 3′ to 5′: N, P, M, G and L. Nucleotide sequence analyses and the deduced amino acid sequences of the genome showed high homology with vesicular stomatitis virus, the type species of the *Vesiculovirus* genus (Björklund *et al.*, 1995, 1996; Hoffmann *et al.*, 2002).

Stone *et al.* (2003) compared the partial nucleotide sequence of the G-gene of SVCV, PFRV and other putative fish vesiculovirus isolates. The isolates were divided into four Genogroups: Genogroup I comprised SVCV isolates, Genogroup II comprised a single isolate from grass carp, Genogroup III comprised the reference PFRV isolate, and Genogroup IV comprised unassigned isolates and isolates previously identified as PFRV (Fig. 3.2). The latter Genogroup was called the tench rhabdovirus (TenRV) Genogroup after the host of the earliest isolate assigned to it (Ahne *et al.*, 1982). Further phylogenetic analyses of the SVCV

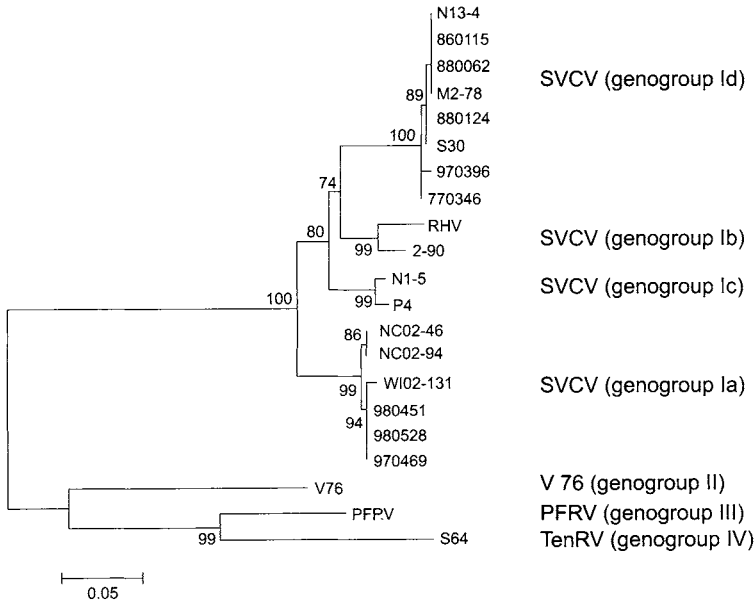


**Fig. 3.2** Genogroups of SVCV, PFRV and other fish vesiculoviruses. Neighbour-joining tree based on a 426 bp (nt 429-855) partial glycoprotein sequence published by Stone *et al.* (2003) and Dikkeboom *et al.* (2004). The tree was generated using MEGA 3.1 (Kumar *et al.*, 2004). Analysis was done on 1000 bootstrapped data sets and values of >70% are shown on the tree. The scale bar represents substitutions per nucleotide site.

Genogroup divided it into four subgroups (a, b, c, and d) which to a certain extent, also differentiated the isolates according to their geographic origins. Subgroup 'a' contained isolates from Asia, subgroup 'b' contained isolates from Moldova and Ukraine, subgroup 'c' contained isolates from Ukraine and Russia, and subgroup 'd' mainly contained isolates from Europe, but also included one isolate from Moldova and one from the Ukraine. Genetic differences between SVCV isolates from Europe and Russia have also been reported (Oreshkova and Koutna cited by Einer-Jensen *et al.*, 2002). A ribonuclease protection assay has been used to differentiate SVCV and PFRV (Ahne *et al.*, 1998).

## Disease Pathogenesis

The disease is temperature dependent, with most field mortalities occurring between approximately 5-18°C (Fijan, 1988; Ahne *et al.*, 2002), although it has occurred in the non-cyprinid sheatfish at 22-23°C (Fijan *et al.*, 1984). It usually occurs, or is most severe in the spring, although it can be expected from November to July (Fijan, 1999). All ages of carp are susceptible under field conditions, although during the aquaculture production cycle the most likely age groups to be affected are 9-12 months and 21-24 months (Fijan, 1988). Under experimental conditions, common carp and grass carp appeared to become more resistant with increasing age (Shchelkunov and Shchelkunova, 1989). The effect of temperature on the outcome of the disease has been investigated (Baudouy *et al.*, 1980a, b; Marcotegui *et al.*, 1992), with the general conclusion that although a rise in temperature was not necessary to cause disease, there were higher mortalities or a faster course of disease in fish that had over wintered compared with those infected at the end of summer. The poor condition of over wintered fish was suggested to be a risk factor. Most reports of the occurrence of SVC have been from culture facilities, but feral carp can be affected. In an outbreak in a lake in the USA, feral carp ranging in size from 46-90 cm were affected, and the larger fish had an average weight of 6 kg (Dikkeboom *et al.*, 2004); it was estimated that the mortalities represented 20% of the carp population in the lake. Under experimental conditions, the virulence of different isolates can vary (Shchelkunov and Shchelkunova, 1989), and wild carp were found to be more susceptible than farmed carp (Hill, 1977).

The portal of entry of the virus appears to be the gill, a viraemia ensues and the virus rapidly spreads to the liver, kidney, spleen and alimentary tract. The virus can be detected in faeces and is shed into the water

(Ahne, 1978a, b; Baudouy *et al.*, 1980a). High titres of virus were measured in organ pools by Ahne (1978b), and Fijan *et al.* (1971) recorded high titres of virus in the liver and kidney of infected fish, but much lower titres in spleen, gills and brain.

Fish mount an immune response following infection with SVCV, and as is typical for fish, the response is influenced by water temperature. Carp of 1-2 kg injected with SVCV at 13-14°C responded slowly and the serum had lower neutralizing titres compared with fish injected at 25°C (Fijan *et al.*, 1977). In a similar study, Ahne (1980a) reported that mortalities in carp infected at 10-12°C reached 90% within 30 days following infection, with no detectable neutralizing antibody produced, whereas carp infected at 20-22°C did not die from the disease, and antibody was detected 30 days post infection. Carp infected at 20°C produced antibody more rapidly than carp infected at 10°C or 15°C, but the highest antibody titres were detected at about the same time post infection at all three temperatures (Ahne, 1980a, 1986a). No neutralizing antibody was detected in 25-30 g carp 30 days post-infection with SVCV at 13°C (Ahne, 1978b). Neutralizing antibody was detected 2.5 and 4 months post intraperitoneal (IP) injection of 80 g carp with three different concentrations of SVCV (Baudouy, 1978). Interferon was produced within 24 hours post-infection of 80 g carp with SVCV at 20°C. It was detected at 4 days but not at 14 days post-infection (Baudouy, 1978; Kinkelin *et al.*, 1982).

## Diagnostics

The clinical signs of SVC are very non-specific, and whilst they may be used as grounds for suspicion of the disease, diagnostic tests that confirm the presence of the virus (or antigen or nucleic acid) must be carried out. The virus grows in a number of established fish, reptilian, mammalian and avian cell lines, as also in primary fish cell cultures at 20-23°C (Bachmann and Ahne, 1974; Clark and Soriano, 1974). The World Organization for Animal Health (OIE) recommends the use of *Epithelioma papulosum cyprini* (EPC) or fathead minnow (FHM) cell lines (OIE, 2003a, b) to diagnose the disease. Homogenates of viscera and encephalon are inoculated onto the cell lines and incubated at 20°C. SVCV produces a lytic cytopathic effect (CPE). Isolation of virus from a gill biopsy taken during the clinical stage of disease was proposed as a non-lethal method for screening carp for SVCV (Baudouy *et al.*, 1980a). Kinkelin and Le Berre (1974) reported that no interference effects were seen when the

virus was serially passaged without dilution, or was infected at high multiplicity, but that has not been the experience in this laboratory. Interference effects have been observed under similar conditions, and passage at high dilution is recommended.

Confirmation of the virus is by seroneutralization or other serological tests, or by the RT-PCR. Serological tests applied to extracts of clinically diseased fish can be used as a rapid presumptive diagnostic method. Serological tests that have been used to identify SVCV, either directly in fish tissues or in cell cultures, include immunoperoxidase staining (Faisal and Ahne, 1980, 1983, 1984), the FAT (Kölbl, 1980; Faisal and Ahne, 1983, 1984; Kocis *et al.*, 1983; Wizigmann *et al.*, 1983) and the enzyme-linked immunosorbent assay (ELISA) (Way, 1991; Rodák *et al.*, 1993). Virus neutralization was enhanced by the addition of guinea pig complement (Clerx *et al.*, 1978). Several RT-PCR procedures for the identification or detection of SVCV have been described (Oreshkova *et al.*, 1999; Gao *et al.*, 2002; Koutná *et al.*, 2003; Stone *et al.*, 2003). The RT-PCR described by Stone *et al.* (2003) uses degenerate primers in order to detect isolates in the four known SVCV subgroups, but it will also detect other cyprinid vesiculoviruses, such as TenRV. Therefore, the nucleotide sequence of the RT-PCR amplicon must be determined in order to confirm the identity of SVCV (OIE, 2003b). This would be best practice for all other RT-PCR procedures described for SVCV. The virus has also been detected in cell cultures and infected fish tissues by using biotinilated DNA probes for M gene or G gene sequences (Oreshkova *et al.*, 1995, 1999). However, the M gene probe was not specific and also hybridized with cell culture grown viral haemorrhagic septicaemia virus (VHSV) and an eel rhabdovirus (Oreshkova *et al.*, 1995).

Care must be taken with interpreting the results of serological tests for SVCV, as there are possibly different serotypes of the virus, and other putative fish vesiculoviruses such as TenRV and PFRV cross react in some tests. Bachmann and Ahne (1974) suggested that one isolate from carp may represent a serological sub-type of SVCV. Likewise, Ahne (1986b) isolated a virus from carp that could not be identified as either SVCV or PFRV by virus neutralization, and he suggested that the isolate was either a type intermediate between SVCV and PFRV, or a sub-type of SVCV. Dikkeboom *et al.* (2004) reported that an SVCV isolate from the USA did not react in an unspecified immunocytochemical test with an antiserum prepared against a European isolate. The US isolate was not neutralized

to the same degree as a European isolate, but it cross-reacted strongly in an ELISA that used an antiserum produced against a European isolate. The FAT cannot differentiate SVCV from PFRV (Jørgensen *et al.*, 1989; Ahne *et al.*, 1998). Both those viruses and TenRV also cross-react in the ELISA, but they can be distinguished by comparing the absorbance values obtained in reciprocal tests (Jørgensen *et al.*, 1989; Rowley *et al.*, 2001; Way *et al.*, 2003). Commercial test kits for the identification of SVCV have been compared. A FAT based on a monoclonal antibody (MAb) against SVCV detected all four SVCV isolates tested, but it also detected one of three PFRV isolates tested (Ariel and Olesen, 2001). A similar MAb-based FAT detected most, but not all, SVCV subgroup 'd' isolates tested, but did not detect isolates from the other three SVCV subgroups (Dixon and Longshaw, 2005). The same authors reported that an ELISA based on a polyclonal antiserum did not differentiate between isolates from the four cyprinid vesiculovirus Genogroups.

Whilst SVCV is readily isolated from clinically infected fish, it is unusual to isolate SVCV from fish surviving SVCV infection. However, Békési and Csontos (1985) isolated the virus from 3 of 491 (0.61%) ovarian fluid samples but not from seminal fluid samples. Liu *et al.* (2004) isolated SVCV from pools of kidney, brain and spleen from common (10-25 cm body size) and koi (25-40 cm body size) carp exhibiting no external or internal signs of disease. The fish were from two separate sites in China out of 65 ornamental fish farms surveyed.

Detection of antibody against SVCV in fish has been used as an alternative to virus isolation as a means of identifying fish that are survivors of SVC, particularly in disease surveys of aquaculture sites (Kölbl and Kainz, 1977; Dangschat, 1979; Wizigmann *et al.*, 1980, 1983; Hoppe and Wernery, 1985). The antibody has been detected by immunofluorescence (Litvinenko and Temnikhanov, 1989); indirect haemagglutination (Sulimanovic, 1973), virus neutralization (Baudouy, 1978) and ELISA (Rodák *et al.*, 1986; Kretzschmar and Dresenkamp, 1987; Dixon *et al.*, 1994). Dixon *et al.* (1994) developed a competitive assay to detect the antibody, and showed that it was more sensitive and detected a greater number of positive fish than virus neutralization. The assay has been used to determine the spread of SVCV-exposed fish from Cedar Lake, USA to the Mississippi following a disease outbreak (PF Dixon and S Marquenski, unpubl. data).

## Disease Treatment/Prophylaxis

Currently, there are no commercially available treatments or vaccines to control or prevent the disease. Good biosecurity and management practices such as use of footbaths, disinfection of equipment, etc., are, therefore, essential for trying to control or prevent the disease. The virus can be inactivated by iodine (Ahne and Held, 1980), and by a variety of chemical and physical treatments (Ahne, 1976, 1982b, c).

The disease is reportable to the OIE, and in some countries it is controlled under national legislation. That may include movement controls, control of imports of susceptible species, or controls of imports from countries where the disease is known to be present. In some countries, it may not be possible to eradicate the disease either nationally or locally (Jeremic *et al.*, 2004).

Research has been undertaken to investigate the use of single-stranded (ss) and double-stranded (ds) RNA, the latter of which is an interferon inducer, as a prophylactic measure against SVC (Masycheva *et al.*, 1995; Alikin *et al.*, 1996). Injection of ds RNA was effective in *in vivo* experiments, but delivery by a bath treatment and injection of ss RNA were not. The compound methisoprinol inhibited the replication of SVCV (Siwicki *et al.*, 2003), and the authors proposed assessing the efficacy of the compound under carp culture conditions.

There have been many reports on the apparent success of vaccination against SVCV (reviewed by Fijan, 1984, 1988, 1999; Dixon, 1997), but no vaccine has been commercially licensed as yet. Although a commercial inactivated vaccine was produced in the former Czechoslovakia and used in large-scale field trials in the 1970s and 1980s (Tesarcik *et al.*, 1978, 1982, 1984; Tesarcik and Macura, 1981; Macura *et al.*, 1983), it is no longer available. Likewise, an attenuated vaccine underwent field trials in Austria (Kölbl, 1990) but despite improving the survival of vaccinated carp, the vaccine was not marketed. Research is being carried out into DNA vaccination of carp against SVCV (Russell, 1996), and in an initial trial a relative protection of 48% was obtained (Kanellos *et al.*, 2006).

There are no published data confirming that strains of carp resistant to SVCV have been developed (Ahne *et al.*, 2002).

## Epidemiology/Geographic Distribution

For many years, the known geographic range of SVC was European countries and countries which were part of the former Soviet Union:

Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, France, Germany, Great Britain, Hungary, Italy, Poland, Rumania, Serbia, Slovakia, Spain, Switzerland, Yugoslavia, Belarus, Georgia, Lithuania, Moldova, Russian Federation and Ukraine (Fijan *et al.*, 1971; Bachmann and Ahne, 1973; Baudouy, 1975; Kölbl, 1975; Békési and Szabó, 1977, 1979; Tesarcik *et al.*, 1977a, b; Bucke and Finlay, 1979; Ghittino *et al.*, 1980; Roudikov, 1980; Osadchaya and Rudenko, 1981; Kocis *et al.*, 1983; Marcotegui *et al.*, 1992; Fijan, 1999; Antychowicz, 2000; Bernet and Wahli, 2002). However, the virus has also been isolated from common and koi carp in the USA (Goodwin, 2002; Dikkeboom *et al.*, 2004) and China (Liu *et al.*, 2004), and from goldfish in Brazil (Alexandrino *et al.*, 1998). Viruses from the USA and China were confirmed as SVCV by nucleotide sequence comparisons, and the virus from Brazil was confirmed using a monoclonal antibody-based test kit for SVCV (L. Romano, pers. comm.) that is specific for SVCV (Dixon and Longshaw, 2005).

The disease is usually seen under aquaculture conditions, but it has also been recorded in feral carp (Munoz *et al.*, 1994; Dikkeboom *et al.*, 2004). In aquaculture, SVC is most likely to be observed in carp that are 9-12 or 21-24 months old (Fijan, 1988), but all ages of carp are susceptible to the virus. Although the virus has been isolated from ovarian fluid (Békési and Csontos, 1985), there is no evidence of vertical transmission of the virus. The virus has been detected in faeces of infected fish during the clinical infection, and for several weeks post-clinical disease, and is excreted into the rearing water (Ahne, 1978a; Pfeil-Putzien, 1978; Pfeil-Putzien and Baath, 1978; Baudouy *et al.*, 1980a, b, c; Shchelkunov and Shchelkunova, 1989). At 10°C, the SVCV survived for at least 35 days in river water, and for at least 42 days in pond sludge. At 10 or 20°C the virus survived for at least 4 days. The virus survived for at least 4 days in pond sludge stored outdoors and exposed to a temperature variation of 15-30°C. Virus that was dried and stored in the open and exposed to a temperature variation of 11-27°C survived for at least 14 days (Ahne, 1982a, b).

Under experimental conditions, the virus can be transmitted by bath infections, or by cohabitation of infected and naïve fish. The gills appear to be the main portal of entry of the virus (Hill, 1977; Ahne, 1978a, b, 1986a; Baudouy *et al.*, 1980a, b, c; Hattenberger-Baudouy *et al.*, 1987; Shchelkunov and Shchelkunova, 1989). There are no data on whether virus shedding by survivors of SVC occurs continuously, or whether virus

shedding ceases, but it can recur following a particular stimulus, e.g., a stressful event.

The virus can also be transmitted by ectoparasites such as the carp louse, *Argulus foliaceus*, and the leech, *Piscicola geometra* (Ahne, 1978a, 1985a; Pfeil-Putzien, 1978; Pfeil-Putzien and Baath, 1978). The virus may be transmitted by piscivorous birds such as the heron (Peters and Neukirch, 1986), and the virus has also been transmitted from carp to pike by predation under experimental conditions (Ahne, 1985b). A further possible mode of transmission is by the movement of baitfish (Goodwin *et al.*, 2004), but to date data do not exist that show this occurrence. The main mode of transmission of the virus from one area to another is via movements of infected fish. This is of particular concern considering the fact that the virus is often found in goldfish and koi carp, which are transported worldwide for the ornamental fish trade (Bernet and Wahli, 2002; Stone *et al.*, 2003; author's unpublished data).

## Economic Importance

There are no up-to-date figures for the cost of SVC to the carp farming industry, but it has long been considered a major source of economic loss in those countries in which it occurs, from direct losses of fish, and disinfection and restocking costs (Fijan, 1988, 1999). A further economic impact may be on the ornamental fish trade, particularly on the koi carp and goldfish sectors. Following the outbreak of SVC in the USA, the Animal and Plant Health Inspection Service authorized $11.7 million to implement a SVC control and indemnity programme (Martin, 2003).

## Recent Developments and Outlook

The preferred method for identification of SVCV is by using the RT-PCR—either directly of infected fish tissues, or following isolation of the virus in cell culture. The RT-PCR amplicons should then be sequenced to confirm the identity of the virus. The latter step cannot always be done in many laboratories, which often delays making a definitive diagnosis. This laboratory has developed a macro array that allows identification of cyprinid viruses to one of the four Genogroups, and in addition will also sub-type SVCV isolates (D. Stone, unpubl. data). That technology will be made available to diagnostic laboratories.

Trials of possible DNA vaccines have demonstrated that this approach may be valuable in protecting carp against SVCV

(Kanellos *et al.*, 2006). A relative percentage survival of 48% was achieved, but further work is necessary to increase the survival rate if DNA vaccination against SVCV is to be promoted as a protection against the disease.

## Tench Rhabdovirus Group

It has been very difficult to distinguish SVCV from certain other rhabdoviruses such as PFRV by some serological tests. Nucleotide sequence analysis of those viruses was undertaken in an attempt to compare those viruses in an alternative way (Stone *et al.*, 2003). The results of the analysis showed that SVCV could be distinguished from PFRV, but more significantly that many isolates previously classified as PFRV by serology, were quite distinct from PFRV by nucleotide sequence analysis. In fact, there are very few 'true' PFRV isolates, and the majority of them were re-classified in a new Genogroup, the Tench rhabdovirus (TenRV) Genogroup. This section provides details of those isolates.

## Hosts

The virus has been isolated from a number of cyprinid and non-cyprinid hosts (Table 3.2).

Common carp are susceptible to infection under experimental conditions (Haenen and Davidse, 1993). TenRV was initially identified as PFRV by serology, and it is possible that a number of other fish species that

**Table 3.2** Fish species from which TenRV has been isolated.

| *Species* | *Common name* | *Reference* |
|---|---|---|
| **1. Cyprinids** | | |
| *Abramis brama* | Bream | Rowley *et al.* (2001) |
| *Blicca bjoerkna* | White bream | Ahne *et al.* (1982) |
| *Carassius carassius* | Crucian carp | Stone *et al.* (2003) |
| *Ctenopharyngodon idella* | Grass carp, white amur | Stone *et al.* (2003) |
| *Leuciscus idus* | Orfe, ide | Stone *et al.* (2003) |
| *Pseudorasbora parva* | False harlequin | Ahne and Thomsen (1986) |
| *Rutilus rutilus* | Roach | Haenen and Davidse (1989) |
| *Tinca tinca* | Tench | Ahne *et al.* (1982) |
| **2. Non-cyprinids** | | |
| *Salmo trutta* | Brown trout | Adair and McLoughlin (1986) |
| *Oncorhynchus mykiss* | Rainbow trout | Rowley *et al.* (2001) |

have been reported to be hosts of PFRV based on FAT or virus neutralization (Ahne *et al.*, 1998; Jørgensen *et al.*, 1989) may, in fact, not be hosts of PFRV, but hosts of TenRV.

## Disease Symptoms

Few data exist on the clinical signs and histopathology of the disease. Affected bream from Northern Ireland were in poor condition and had haemorrhages at the bases of the pectoral fins and on the sides of the body (Rowley *et al.*, 2001). Internally, there was ascites, but no other obvious signs except for the fact that there was very little body fat. Histopathology revealed congestion of the internal organs, but no other specific changes. A case report from England records fin erosion, skin ulcers, gill necrosis, petechial haemorrhages and haemorrhages at the fin bases; some of the haemorrhages could have been a result of netting or handling (Way *et al.*, 2003). Internal signs included ascites, pale or discoloured liver, and haemorrhages in the gut, liver, muscle and adipose tissue. Many of the fish had exterior fungal infections, parasite infestations and bacterial pathogens were isolated, but these were most likely secondary infections. It is difficult to judge what pathological signs are caused by the virus infection, and what are caused by the secondary infections. Histological examination showed a range of pathology, but hepatocellular necrosis was a consistent feature in different species (Way *et al.*, 2003). Haemorrhages at the fin bases, in the skin, and in internal organs have been reported in other cases (Ahne *et al.*, 1982), and exophthalmia, abdominal distension and irregular swimming have also been reported (Adair and McLoughlin, 1986). Many of the fish that have been diagnosed as being infected with TenRV at our laboratory (Stone *et al.*, 2003) have presented with clinical signs that were similar to those of SVCV, particularly haemorrhages of the skin and fin bases.

Some or all of the following pathological signs were observed in roach, common carp and grass carp experimentally infected with a TenRV isolate from roach: abnormal behaviour, exophthalmia, haemorrhages on the fins and in the muscle, and distended abdomen (Haenen and Davidse, 1989). Internally, haemorrhages were observed in the kidney, and there was degeneration of the kidney and muscles.

The virus has also been isolated from fish with no clinical signs (Adair and McLoughlin, 1986; Ahne and Thomsen, 1986; Haenen and Davidse, 1989; Rowley *et al.*, 2001).

## Disease Aetiology

The disease is caused by a rhabdovirus, approximately 110-155 × 75-80 nm (Ahne *et al.*, 1982; Adair and McLoughlin, 1986; Haenen and Davidse, 1989; Rowley *et al.*, 2001). There are few other data on the causative virus other than that it is sensitive to chloroform treatment, as would be expected of a rhabdovirus (Ahne and Thomsen, 1986). The relationship between TenRV and SVCV has been discussed in the section on SVCV earlier in this chapter.

## Disease Pathogenesis

The disease has caused high losses in bream (approximately 20,000 juveniles in one outbreak) and tench (Rowley *et al.*, 2001; Way *et al.*, 2003). However, as noted above, the virus has been isolated on several occasions from different species showing no signs of disease. Temperature data for the disease are sparse, but it has occurred in the spring when the water temperature was 11-13.5°C (Rowley *et al.*, 2001), and the virus has been isolated from diseased or clinically healthy fish in February in both England and The Netherlands, when the water temperatures are likely to be low (Haenen and Davidse, 1989; Way *et al.*, 2003). Successful experimental transmission has been carried out at 14-20°C (Haenen and Davidse, 1993), 14 or 15°C (Way *et al.*, 2003) and 18°C (Rowley *et al.*, 2001). Under experimental conditions, an isolate from roach killed 85.2% of exposed roach, 75.3% of exposed ide, 45.4% of exposed common carp, 18.3% of exposed grass carp, but no exposed rainbow trout or pike died (other than by cannibalism in the latter species) (Haenen and Davidse, 1993). Brown trout and rainbow trout were refractory to infection with an isolate from brown trout (Adair and McLoughlin, 1986), and rainbow trout, tench, grass carp, and pike were refractory to infection with isolates from tench and white bream at 12-13°C (Ahne *et al.*, 1982).

## Diagnostics

The virus has been isolated from pools of internal organs (usually combinations of liver, kidney, spleen and brain) in EPC (Ahne *et al.*, 1982; Ahne and Thomsen, 1986), FHM (Ahne *et al.*, 1982; Ahne and Thomsen, 1986), rainbow trout gonad (RTG-2) cells (Haenen and Davidse, 1989) and bluegill fry (BF-2) cells (Adair and McLoughlin, 1986). Chinook salmon embryo (CHSE-214) cells were refractory to the

virus (Rowley *et al.*, 2001). Many of the isolates were initially identified as PFRV by serology, usually virus neutralization tests (Ahne *et al.*, 1982; Adair and McLoughlin, 1986; Ahne and Thomsen, 1986; Haenen and Davidse, 1993). Reciprocal ELISA comparisons using antisera against SVCV and PFRVV showed that the TenRV isolates were different from both those viruses (Rowley *et al.*, 2001; Way *et al.*, 2003), although they too would have been classified as PFRV by virus neutralization tests. Several TenRV isolates cross-reacted in a commercial test kit for SVCV that used a polyclonal antiserum, but not in a SVCV test kit that used a monoclonal antibody (Dixon and Longshaw, 2005).

The isolates can be identified by RT-PCR followed by nucleotide sequencing of the amplicon (Stone *et al.*, 2003), and currently this is the only definitive way of distinguishing TenRV isolates from isolates in Genogroups I, II and III.

Antibody in fish that reacts with TenRV has been detected using a competitive ELISA (K. Way *et al.*, unpubl. data), based on that described for detecting fish antibody against SVCV (Dixon *et al.*, 1994). That assay has been used to detect populations of coarse fish that may have been exposed to TenRV.

## Disease Treatment/Prophylaxis

There are no treatments or vaccines available, and currently it is unlikely that specific vaccines will be produced against the virus. Should vaccines against SVCV become available—and there was a need to vaccinate fish against TenRV—it would be of interest to determine whether the SVCV vaccine would cross-protect. Good husbandry and biosecurity should help protect against, or lessen the impact of the disease.

## Epidemiology/Geographic Distribution

The virus has been isolated from fish from England (Way *et al.*, 2003), Germany (Ahne *et al.*, 1982; Ahne and Thomsen, 1986), Northern Ireland (Adair and McLoughlin, 1986; Rowley *et al.*, 2001) and The Netherlands (Haenen and Davidse, 1989).

Very few epidemiological data have been reported. However, it is likely that populations of fish may harbour the virus without showing any clinical signs (Adair and McLoughlin, 1986; Ahne and Thomsen, 1986; Haenen and Davidse, 1989; Rowley *et al.*, 2001), and only exhibit clinical disease under particular conditions. This was borne out in two studies in

which virus isolated from high mortalities in wild fish produced only low mortality in experimental transmission trials, which included the original hosts of the viruses (Rowley *et al.*, 2001; Way *et al.*, 2003). The mortalities in wild fish in one of the reports followed a decrease in water quality (Rowley *et al.*, 2001). The authors also speculated that as the virus had been recorded in Northern Ireland in 1984, but had not been associated with disease until 1998 and 1999, factors that could induce disease had been absent in most years. Mortalities in several fish species occurred following transport and handling (Way *et al.*, 2003). No virus was isolated during follow-up studies in the following year.

## Economic Importance

As most of the reports of disease caused by TenRV have been in wild fish, it is difficult to place a value on the losses. However, the disease was identified at two UK Environment Agency fish farms that provide coarse fish for re-stocking rivers and lakes, and up to 1.5 million fish were destroyed in order to prevent the spread of the disease (Penson, 2004). One estimate of the cost of disinfection was £250,000 ($427,000) (Penson, 2004), but the total losses could be much more on including the loss of production and re-stocking costs.

## Recent Developments and Outlook

A rhabdovirus was isolated from a single moribund 3-year-old grass carp in Germany (Ahne, 1975). There were haemorrhages on the ventral surface and at the fin bases, necrosis of fins, serous ascetic fluid, the stomach was inflamed, the spleen swollen, pale liver and petechial haemorrhages on the swim-bladder wall. The virus was virulent for young grass carp (0.1-0.17 g), causing 100% mortality in 9 days at 23°C following bath infection and was later shown to be indistinguishable from PFRV in virus neutralization studies (Clerx *et al.*, 1978; Jørgensen *et al.*, 1989). However, nucleotide sequence analysis of this isolate has shown it to be a member of the TenRV Genogroup (unpubl. data).

The macro array described in the section on SVCV will also distinguish TenRV isolates from other cyprinid vesiculoviruses.

## Koi Herpesvirus

Over recent years, koi herpesvirus (KHV) disease has become the most important virus disease of cyprinids as it occurs in ornamental fish, food

fish and wild fish, and has a very wide geographic distribution. It has had a major economic impact, and there has been rapid development of diagnostic methods to detect the causative virus, and of vaccines to control it.

## Hosts

Koi herpesvirus disease occurs in C. *carpio*, the common carp and its ornamental variety, koi carp (fancy carp). It first came to prominence as the cause of mortality in koi carp (Bretzinger *et al.*, 1999; Hedrick *et al.*, 1999, 2000, 2006; Walster, 1999; Gilad *et al.*, 2001), but it also affects cultured common carp (Hedrick *et al.*, 2000; Mires, 2001; Perelberg *et al.*, 2003; Sano *et al.*, 2004a; Schlotfeldt, 2004; Sunarto *et al.*, 2004) and feral common carp (Denham, 2003; Terhune *et al.*, 2004). Koi or common carp are often raised in polyculture with other fish species, but no signs of disease or mortalities have been observed in those other fish, either under normal polyculture conditions or following experimental cohabitation with infected fish, or exposure to the virus. Refractory species include goldfish (Bretzinger *et al.*, 1999; Goodwin, 2000; Hedrick *et al.*, 2000; Perelberg *et al.*, 2003; Takashima *et al.*, 2005), sturgeon (*Acipenser* sp.) (Bretzinger *et al.*, 1999), Nile tilapia (*Oreochromis niloticus*) (Perelberg *et al.*, 2003; Sunarto *et al.*, 2004), silver perch (*Bidyanus bidyanus*), silver carp (Perelberg *et al.*, 2003; Takashima *et al.*, 2005), grass carp (Goodwin, 2000; Perelberg *et al.*, 2003; Schlotfeldt, 2004), golden shiners, fathead minnows (*Pimephales promelas*) (Goodwin, 2000), tench, bighead carp (Schlotfeldt, 2004), giant gouramy (*Osphronemus goramy*), Indonesian catfish (species unknown), Siam catfish (*Pseudomystus siamensis*) (Sunarto *et al.*, 2004); channel catfish (*Ictalurus punctatus*), lake smelt (*Hypomesus nipponensis*), ice fish (*Salangichthys microdon*), goby (*Tridentiger kuroiwae brevispinis*) and freshwater prawn (*Macrobrachium nipponense*) (Takashima *et al.*, 2005). Hybrids of goldfish and common carp are resistant to mortality following IP injection with the virus, but 4/10 fish were positive for viral DNA by the PCR at 25 days post infection (Hedrick *et al.*, 2006).

However, Bergmann *et al.* (2005) reported experimental transmission of KHV to goldfish and crucian carp. They cohabited goldfish and crucian carp with KHV-diseased koi carp from Thailand, and 15 days following the start of co-habitation, some goldfish died. The dead goldfish had swollen abdomens and a pronounced lateral line, and were positive for KHV DNA by PCR. Goldfish that had been co-habited with koi carp infected with a

'European' KHV isolate survived the co-habitation period. Surviving goldfish were able to transmit the disease to naïve common carp. The crucian carp survived the co-habitation, and showed no signs of disease and also appeared to be able to transmit the virus to naïve fish. Grass carp may also act as carriers of the virus (S. Bergmann, pers. comm., and also see Hedrick *et al.* (2006)).

## Disease Symptoms

External signs of the disease are variable, but the most consistent pathology is seen in the gills (Fig. 3.3). They are usually discoloured, necrotic, inflamed and mucus may trail from them. There may be erosion of primary lamellae, fusion of secondary lamellae, and the tips of the primary and secondary lamellae may be swollen (Bretzinger *et al.*, 1999; Walster, 1999; Hedrick *et al.*, 2000; Gray *et al.*, 2002; Denham, 2003; Perelberg *et al.*, 2003; Sano *et al.*, 2004a, b; Tu *et al.*, 2004a, b; Sunarto *et al.*, 2004; Hutoran *et al.*, 2005). Enophthalmia (sunken eyes) is another typical feature of the disease. There may be a pale discolouration or

**Fig. 3.3** Gill of koi carp affected by KHV. Areas of necrotic tissue can be seen.

reddening of the skin, which may also have a rough texture, focal or total loss of epidermis and over- or under-production of mucus. The fins may have haemorrhages at their bases, and there may be fin erosion. The fish may be lethargic but with occasional periods of hyperactivity, disorientated, anorexic and they may show rapid respiration (Bretzinger *et al.*, 1999; Walster, 1999; Hedrick *et al.*, 2000; Denham, 2003; Perelberg *et al.*, 2003; Sano *et al.*, 2004a, b). Internal signs also vary in occurrence, but there may be adhesions with or without abnormal colouration of internal organs (lighter or darker). The kidney or liver may be enlarged, and they may also exhibit petechial haemorrhages (Bretzinger *et al.*, 1999; Walster, 1999; Hedrick *et al.*, 2000; Perelberg *et al.*, 2003).

Many diseased fish are also infested with ectoparasites such as *Argulus* sp., *Chilodonella* sp., *Cryptobia* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., *Ichthyobodo* sp., *Ichthyophthirius* sp., *Trichodina* sp. and gill monogeneans, as well as numerous species of bacteria (Bretzinger *et al.*, 1999; Walster, 1999; Hedrick *et al.*, 2000; Perelberg *et al.*, 2003; Sano *et al.*, 2004a, b; Terhune *et al.*, 2004). Those organisms are likely to be secondary invaders (Pikarsky *et al.*, 2004), and it has been suggested that *Argulus* sp. may possibly transmit the disease (Walster, 2000).

The histopathology of the disease is rather non-specific and variable, but inflammation and necrosis of gill tissues is a consistent feature. Gills also exhibit hyperplasia and hypertrophy of branchial epithelium, and fusion of secondary lamellae and adhesion of gill filaments can be seen. Necrosis, ranging from small areas of necrotic epithelial cells of secondary lamellae to complete loss of the lamellae is observed. Branchial epithelial cells and leucocytes may have prominent nuclear swelling, margination of chromatin to give a 'signet ring' appearance (Fig. 3.4), and pale diffuse eosinophilic intranuclear inclusions have been observed (Bretzinger *et al.*, 1999; Walster, 1999; Hedrick *et al.*, 2000; Perelberg *et al.*, 2003; Sano *et al.*, 2004a, b; Pikarsky *et al.*, 2004; Sunarto *et al.*, 2004). Inflammation, necrosis and nuclear inclusions have been observed (individually or together) in other organs, particularly the kidney, but also in the spleen, pancreas, liver, brain, gut and oral epithelium (Bretzinger *et al.*, 1999; Walster, 1999; Hedrick *et al.*, 2000; Perelberg *et al.*, 2003; Pikarsky *et al.*, 2004; Sano *et al.*, 2004a, b).

## Disease Aetiology

There have been different opinions as to the classification of the aetiological agent of the disease. In their paper first describing the disease

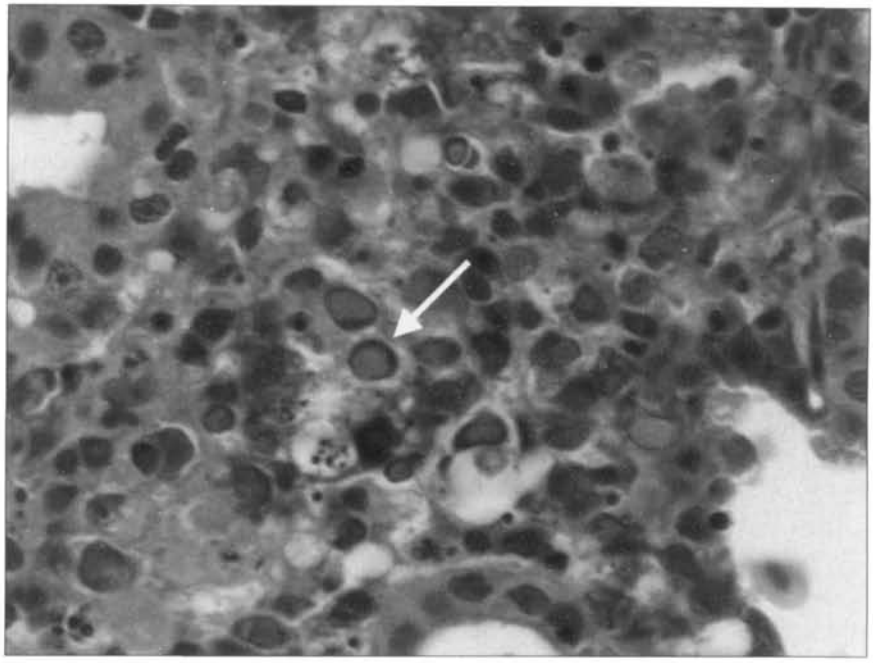

**Fig. 3.4** Liver from KHV-infected koi carp showing signet-ring appearance of nuclei of infected cells (arrowed). Haematoxylin and eosin stain.

and its aetiology, Hedrick *et al.* (2000) depicted the causative virus as a herpesvirus, naming it koi herpesvirus and hence the name of the disease koi herpesvirus disease. However, Ronen *et al.* (2003) and Hutoran *et al.* (2005) did not concur as the DNA genome was larger than any other herpesvirus and it shared some nucleotide sequences with other viruses besides herpesviruses. They named the causative agent of the disease carp nephritis and gill necrosis virus. Waltzek *et al.* (2005) provided further evidence to support the classification of the virus as a herpesvirus, and named it cyprinid herpesvirus 3 (CyHV-3) following the nomenclature of other cyprinid herpesviruses: CyHV-1 (carp pox virus, herpesviral epidermal hyperplasia) and CyHV-2 (goldfish haematopoietic necrosis virus).

Herpesvirus assembly is still a subject of dispute but, commonly, nucleocapsids (nucleoprotein core surrounded by a proteinaceous capsid) are predominantly seen in the nucleus from where they migrate to the cytoplasm and become surrounded by a proteinaceous tegument. They then become enveloped (surrounded by a membrane) by budding into

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However, Kortet and colleagues have extensively studied epidermal hyperplasia in roach populations in Finland. Eight out of 11 populations studied exhibited epidermal hyperplasia, and the highest prevalence was 62% (Kortet *et al.*, 2002). The peak prevalence was during spawning time (April/May) when severe disease occurred, with a second, lower, peak in August. The highest prevalence was in polluted areas. There was a higher prevalence in males compared to females (Kortet *et al.*, 2002; Korkea-aho *et al.*, 2006). There was a negative correlation between the number of frontal breeding tubercles (a feature of males in breeding condition) and the intensity of epidermal hyperplasia (Vainikka *et al.*, 2004), but a positive correlation between testosterone (which may have an immunosuppressive effect) and elaborate breeding tubercles and disease (Kortet *et al.*, 2005). However, there was no correlation between cortisol levels, which increase during stress and can also have an immunosuppressive effect, and disease intensity in roach (Vainikka *et al.*, 2004). Any correlation between fish length or age and prevalence of epidermal hyperplasia varied between locations. In some areas there was no correlation but in others there was a positive correlation (Kortet *et al.*, 2002, 2004; Korkea-aho *et al.*, 2006). There was no correlation between epidermal hyperplasia and condition factor, nor the dominance ranking of males (Kortet *et al.*, 2004) and no correlation between severity of disease and disease prevalence (Korkea-aho *et al.*, 2006) or mortality (Kortet *et al.*, 2003).

The disease can be transmitted by cohabitation or by rubbing lesion tissue against scarified epithelium (Sonstegard and Sonstegard, 1978; N. Fijan, pers. comm. in Wolf, 1988; Jiang, 1995), which may mimic natural routes of infection. Large numbers of *Argulus* sp. have been seen on affected carp, and it may be a disease vector (Timur, 1991). Poor environmental conditions, excessive stocking density, etc., may be factors in the disease (Prost, 1977; Jiang, 1995). The disease is commonly seen in the early months of the year during winter or spring (Hines *et al.*, 1974; Steinhagen *et al.*, 1992; Barnes *et al.*, 1993; Jiang, 1995; Kortet *et al.*, 2002). This is at a time when many fish may be in poor condition following over-wintering, and, depending on age and species, may be preparing for spawning.

The disease in cyprinids has been reported from Bulgaria (Konstantinov, 1977), Finland (Halvorsen, 1980), Germany (Schubert, 1964), Hungary (Békési *et al.*, 1979), Norway (Halvorsen, 1980), Poland (Kocylowski, 1965; Miaczynski, 1965), the United Kingdom (Mawdesley-

Thomas and Bucke, 1967), the former Yugoslavia (Fijan *et al.*, 1983), Israel (Hines *et al.*, 1974), Turkey (Timur, 1991), former states of the USSR and Asia (Mcallister *et al.*, 1985), China (Jiang *et al.*, 1991b), Korea (Egusa, 1992) and the USA (Sonstegard and Sonstegard, 1978).

### Economic Importance

The appearance of diseased food fish or ornamental fish makes them unsaleable, and any mortality of older fish has economic consequences for the fish producer or retailer.

### Recent Developments and Outlook

The potential for CyHV-1 to cause cross reactions in assays to detect fish antibody against KHV, and the increased likelihood of isolating CyHV-1 when KF-1 cells are used in diagnostic tests will raise awareness of CyHV-1 for those 'nuisance' effects. Diagnosticians will need to take that into account when investigating KHV and potentially other diseases of carp. However, if CyHV-1 can be isolated in KF-1 cells from species other than carp, there is the potential for comparative studies on those isolates and a greater understanding of herpesviruses of cyprinids.

## HERPESVIRAL HAEMATOPOIETIC NECROSIS (HVHN)

### Hosts

The only known host is the goldfish. The common carp is refractory to experimental infection (Jung and Miyazaki, 1995). Hybrids of goldfish and common carp were resistant to mortality when injected IP with the virus, but 6/10 fish were positive for viral DNA when tested by the PCR 25 days post infection (Hedrick *et al.*, 2006).

### Disease Symptoms

There are no specific external signs of disease, but the fish become lethargic and stay on the pond bottom, and anorexia has been observed. Internally, there was ascites, the gills, liver and kidney were pale, the spleen was enlarged with white nodular lesions, and the intestines were devoid of food (Jung and Miyazaki, 1995; Groff *et al.*, 1998; Chang *et al.*, 1999; Stephens *et al.*, 2004; Goodwin *et al.*, 2006a). The most notable histopathology is usually seen in the kidney and spleen (Jung and

Miyazaki, 1995; Groff *et al.*, 1998; Chang *et al.*, 1999; Stephens *et al.*, 2004; Goodwin *et al.*, 2006a). There was necrosis of the haematopoietic cells in the kidney and in the pulp and sheathed arteries in the spleen, sometimes accompanied by haemorrhages. Slight necrosis of renal tubular epithelium and glomeruli was observed. Necrotic foci in the lamina propria and submucosa of the intestine have been reported in some cases (Jung and Miyazaki, 1995), but not in others (Groff *et al.*, 1998). Pancreatic necrosis was observed in some fish (Jung and Miyazaki, 1995). Hypertrophy and hyperplasia of the branchial epithelium with or without fusion of adjacent secondary lamellae has been reported (Groff *et al.*, 1998; Stephens *et al.*, 2004). The nuclei enlarged and margination of the chromatin has been observed in gill epithelia, and in cells in the liver, spleen, kidney and thymus (Groff *et al.*, 1998; Chang *et al.*, 1999; Stephens *et al.*, 2004; Goodwin *et al.*, 2006a).

## Disease Aetiology

Virus particles with the typical morphology of herpesviruses were observed by electron microscopy. Nucleocapsids were 95-117 nm diameter, and sometimes formed crystalline arrays; enveloped virus was 170-225 nm diameter (Jung and Miyazaki, 1995; Groff *et al.*, 1998; Chang *et al.*, 1999; Stephens *et al.*, 2004; Goodwin *et al.*, 2006a). The virus was termed goldfish haematopoietic necrosis virus (GFHNV) by Jung and Miyazaki (1995). The virus is listed as an unassigned virus in the family Herpesviridae by the ICTV, under the name Cyprinid herpesvirus 2 (CyHV-2) (Fauquet *et al.*, 2005).

Goodwin *et al.* (2006a) reported that the nucleotide sequences of the amplicons obtained after PCR on material obtained from several cases in the USA that occurred in different states between 2002 and 2004, and from the case reported by Groff *et al.* (1998) were 100% homologous, and only slightly different from the sequences obtained by Waltzek *et al.* (2005). The latter authors have compared the sequences of four genes of CyHV-1, CyHV-2 and KHV (CyHV-3, proposed) and found that all three viruses were closely related, but distinct.

## Disease Pathogenesis

The disease has been observed in all ages of fish, and can produce almost 100% mortality (Jung and Miyazaki, 1995; Groff *et al.*, 1998; Chang *et al.*, 1999). It occurs at water temperatures between 15-25°C (Jung and

Miyazaki, 1995; Groff *et al.*, 1998; Chang *et al.*, 1999; Stephens *et al.*, 2004) and has been observed both in the spring and autumn (Jung and Miyazaki, 1995). The disease regresses above 25°C. Mortalities started 3-6 days after IP injection, and reached 100% within 13 days in groups of goldfish injected with a high titre of virus. Goldfish may develop immunity to the virus as survivors of one infection did not succumb to infection the following year during a second outbreak of disease (Goodwin *et al.*, 2006a).

## Diagnostics

The virus has been isolated from pools of spleen and kidney on EPC, FHM and tilapia ovary (TO-2) cells at 20°C. Eel kidney (EK-1), CHSE-214 and RTG-2 cells were refractory to the virus. The highest titres were obtained in FHM cells, but the virus could not be grown beyond the fourth passage (Jung and Miyazaki, 1995). However, Groff *et al.* (1998) were unable to isolate the virus from pools of kidney in EPC, FHM and CHSE-214 cells at 20°C, and Goodwin *et al.* (2006a) did not isolate the virus from kidney, spleen and gills in KF-1, FHM or EPC cells. Cell cultures were obtained from several tissues from goldfish, including goldfish fin (GFF) (Li and Fukuda, 2003). The cells supported the replication of the virus at 20°C, albeit at low titre, and the virus was passaged at least 8 times. Another cell line of goldfish origin, goldfish fin (GF-1) has also been used to propagate the virus, but only low amounts of virus were produced (Waltzek *et al.*, 2005). The virus has been isolated from extracts of gill tissue in KF-1 cells incubated at 20°C at this laboratory (K. Jeffery, D. Stone and K. Way, unpubl. data). The virus was identified by the PCR, and confirmed by comparison of sequence data with published sequences (Waltzek *et al.*, 2005). No virus was isolated when pools of brain, kidney and spleen from the same groups of fish were inoculated onto BF-2 cells at 15°C or EPC cells at 15 and 20°C. A less extensive viral CPE was observed at the first subculture onto KF-1 cells, but no CPE was evident at the second subculture onto that cell line.

A PCR using degenerate primers based on the conserved regions of the DNA polymerase gene has been used to identify the virus (Goodwin *et al.*, 2006a). The PCR was refined and a real time PCR was developed that could detect the presence of viral DNA in clinical cases of disease, and also in apparently healthy fish (Goodwin *et al.*, 2006b).

## Disease Treatment/Prophylaxis

It has been suggested that raising the temperature and adding sodium chloride to the water may reduce the severity of the disease, but if that is the case, the elevated temperatures must be maintained (Goodwin *et al.*, 2006a).

## Epidemiology/Geographic Distribution

The disease occurs in Japan (Jung and Miyazaki, 1995), the USA (Groff *et al.*, 1998), Taiwan (Chang *et al.*, 1999), Australia (Stephens *et al.*, 2004), Germany (Bergmann, 2003) and the UK (K. Way, unpubl. data).

The disease has occurred following the introduction of new broodfish into a facility (Chang *et al.*, 1999), or purchase (and presumed handling and transport of the fish) (Stephens *et al.*, 2004; Goodwin *et al.*, 2006a), but in other cases, the source of the infection was unknown. Viral DNA has been detected in goldfish broodfish, and investigations are in hand to determine whether the virus is vertically transmitted (Goodwin *et al.*, 2006b).

## Economic Importance

The disease has a high mortality rate, e.g., 90% mortality of 2,000,000 fry in Taiwan (Chang *et al.*, 1999), 100% mortality of all ages of goldfish in Japan (Jung and Miyazaki, 1995) which has a severe effect on production.

## Recent Developments and Outlook

Awareness of this disease is increasing, particularly amongst goldfish breeders. They in turn are notifying diagnostic laboratories of mortality episodes that previously would have gone unrecorded, and it is likely that the geographic range of the disease will expand. This disease may be recognized as having serious economic consequences for the goldfish industry.

## Grass Carp Haemorrhage Disease

Grass carp haemorrhage disease (GCHD), caused by grass carp reovirus (GCRV), is an economically important disease of grass carp and other species, which for many years had been identified only in China. Recently, the virus has been identified in grass carp and other species in the USA

(McEntire *et al.*, 2003). Moreover, the virus was shown to be practically identical to another virus in the USA that had been known as golden shiner virus (GSV), (Attoui *et al.*, 2002; McEntire *et al.*, 2003). The two diseases will be reviewed separately here, although in addition to being caused by the same virus, it can be seen that there are some common features to both diseases.

## Hosts

The disease affects yearling and younger grass carp (Section on Virus Study, 1978; Chen and Jiang, 1984), black carp (*Mylopharyngodon piceus*) (Di and Yu, 1986; Zhai *et al.*, 1987), topmouth gudgeon (stone moroko) (*Pseudorasbora parva*) (Chen *et al.*, 1985 cited by Wolf, 1988; Wang *et al.*, 1994). The rare minnow (*Gobiocypris rarus*) (Wang *et al.*, 1993, 1994), bighead carp and goldfish (Ding *et al.*, 1991) were all susceptible to experimental infection which led to mortality in all species. Ding *et al.* (1991) also experimentally infected the minnow (*Hemiculter bleekeri*), which resulted in the death of 1 of 11 fish, and reported that there were no mortalities in Wuchang bream (blunt snout bream) (*Megalobrama amblycephala*), silver carp, common carp and oriental weatherfish (Asian pond loach) (*Misgurnus anguillicaudatus*) following experimental infection. However, those authors tested all the experimentally infected fish for GCRV antigen by the ELISA, but only detected it in the silver carp and minnow. The reason why no virus antigen was detected in the bighead carp and goldfish is not apparent, unless the mortalities observed following experimental infection were not caused by GCRV. The silver carp and minnow are potential asymptomatic carriers of the virus. In the USA, the virus has been isolated from diseased cultured golden shiners and fathead minnows as well as asymptomatic wild creek chubs (*Semotilus atromaculatus*) (McEntire *et al.*, 2003; Goodwin *et al.*, 2006c).

## Disease Symptoms

As its name suggests, one of the more obvious and consistent signs of disease is haemorrhages. They can occur at the fin bases and on the opercula, and internally in the oral cavity, liver, kidney, spleen, intestine and in the musculature (Section of Virus Study, 1978; Chen *et al.*, 1985; Zheng *et al.*, 1986; Zhai *et al.*, 1987). The fish may also exhibit exophthalmia. It has been reported that two isolates of the virus cause differing pathologies (Guo and Jiang, 1993). One isolate caused

hyperaemia and haemorrhages in the muscles and oral cavity, whereas the other isolate caused hyperaemia and haemorrhages in the liver and spleen, as well as degeneration and necrosis in the liver. Abnormally red dorsal musculature was visible through the dermis of diseased fathead minnows (Goodwin *et al.*, 2006c).

Histological examination reveals haemorrhages and necrosis in most internal organs. The endothelia of small blood vessels of grass carp were enlarged and microthrombi formed. There was hyaline degeneration of kidney tubules, vacuolar degeneration in the liver and congestion of the intestines. The numbers of erythrocytes and leucocytes decreased (Zheng *et al.*, 1986). Epithelial cells of the kidney tubules of the black carp enlarged, and some became vacuolated with atrophied nuclei, and cellular debris accumulated in the lumen (Di and Yu, 1986). Electron microscopy revealed abnormal morphology of, and a decrease in, rough and smooth endoplasmic reticulum, Golgi bodies and mitochondria (Di and Yu, 1986; Zheng *et al.*, 1991). The histopathology of experimentally infected rare minnow was similar (Wang *et al.*, 1995a), but there was also hypertrophy of the gills and fusion of secondary lamellae.

Tissue damage causes a change in the blood levels of several enzymes and serum proteins (Zhu *et al.*, 1986, 1987; Cai, 1992; Zhu and Xie, 1993; Zeng *et al.*, 1995).

## Disease Aetiology

It is now considered that GCHD is caused by an aquareovirus, but a virus 22-30 nm diameter—a putative picornavirus—has been observed by electron microscopy in grass carp with GCHD (Mao *et al.*, 1988; Mao and Shao, 1989; Shao *et al.*, 1990). Both viruses were isolated from fish tissues, and each was used to infect different groups of fish. The two viruses each induced a haemorrhagic disease, but the picorna-like virus produced haemorrhages in the musculature whilst the aquareovirus produced haemorrhages in the intestine (Mao *et al.*, 1989; Shao *et al.*, 1990). There is no further information on the picorna-like virus.

Grass carp reovirus is an aquareovirus, having the typical reovirus double capsid layer and a diameter of 65-70 nm (Chen and Jiang, 1983, 1984; Chen *et al.*, 1985; Jiang and Ahne, 1989; Zeng and He, 1992). Eleven structural polypeptides are resolved on polyacrylamide gels, ranging in molecular mass between 27 and 130 kDa (Wang *et al.*, 1990, 1995b; Chen *et al.*, 1992a; Ke *et al.*, 1992). Antisera have been produced

against the individual polypeptides, and used in the ELISA; antisera against four of the polypeptides neutralized the virus (Wang *et al.*, 1995b). The virus genome comprises 11 segments of double-stranded RNA, often grouped in small, medium and large classes (Jiang and Ahne, 1989; Ke *et al.*, 1990; Wang *et al.*, 1990; Zeng and Zuo, 1991; Rangel *et al.*, 1999; Jiang, 2004). The virus genome has been sequenced (Attoui *et al.*, 2002), and the sequence and function of many of the individual segments determined (Tian *et al.*, 1999; Fang *et al.*, 2000, 2003; Qiu *et al.*, 2001a, b, c, d; Qiu and Luongo, 2003).

The virus genus *Aquareovirus* has been divided into six different groups (A, B, C, D, E and F) by RNA-RNA blot hybridization (Lupiani *et al.*, 1993; Subramanian *et al.*, 1997). The divisions were originally called Genogroups (Lupiani *et al.*, 1993), but the ICTV lists these as species (*Aquareovirus* A [ARV-A] to *Aquareovirus* F [ARV-F]) (Fauquet *et al.*, 2005), and each species comprises one or more strains. Rangel *et al.* (1999) investigated the relationship of GCRV to members of the other *Aquareovirus* species/genogroups by RNA-RNA blot hybridization and found the virus to be different from all those they compared and suggested that GCRV be assigned to a new genogroup, G. However, Attoui *et al.* (2002) compared the gene sequences of genome segments of GCRV and GSV and reported that they had 90.56-97.68% identity. Their genome sizes differed by only 1 bp, and their genomes had identical electropherotypes on agarose gels. Those authors arrived at the conclusion that the two viruses were two isolates of the species, ARV-C, with GSV as the sole member. It has been reported that there are nucleotide sequence differences between isolates of GCRV (Li *et al.*, 1997b), and potentially the different results of Rangel *et al.* (1999) and Attoui *et al.* (2002) could have been because they were analyzing different variants of GCRV. Seng *et al.* (2005a, b) confirmed the findings of Attoui *et al.* (2002) by reporting that genome segments 6 and 10 of GCRV and GSV shared 94.1% and 90.6% nucleotide sequence identity and 99.1% and 95.7% amino acid sequence identity, respectively. McEntire *et al.* (2003) isolated aquareoviruses from grass carp and golden shiners in the USA and compared their isolates, and reference GSV by RT-PCR using primers based on sequences of GCRV. All three isolates produced amplicons in the RT-PCR, and nucleotide sequence comparisons of the amplicons suggested to the authors that GCRV and GSV were isolates of the same *Aquareovirus* species, ARV-C, in agreement with Attoui *et al.* (2002); that relationship has been accepted by the ICTV (Fauquet *et al.*,

2005). McEntire *et al.* (2003) hypothesized that GCRV was present in grass carp that were imported into the USA in 1963 from Taiwan and Malaysia, and thence infected golden shiners, and so GSV in reality is GCRV. Hedrick *et al.* (1989) isolated an aquareovirus from grass carp that had been imported into the USA, and it was serologically identified as GSV—it is likely, therefore, that this virus was actually GCRV.

## Disease Pathogenesis

The disease occurs between 25-33°C (Section of Virus Study, 1978; Chen *et al.*, 1985, 1988; Zhai *et al.*, 1987; Jiang, 2004), and the concomitant decrease in dissolved oxygen contributes to the severity of the disease. Increased algal growth and an increase in total nitrogen and nitrite at the elevated temperatures also exacerbates the disease (Chen *et al.*, 1988; Wang and Hu, 1989). The disease usually occurs in fingerlings in late June to the end of September, and in 2-year-old fish in early May-June (Chen *et al.*, 1985). Wang *et al.* (1993) considered the gills to be important in the infection process.

The course of disease has been studied in experimentally infected rare minnows (Wang *et al.*, 1994). The disease can occur at temperatures between 22-32°C in 1-6 months old fish. At 28 and 32°C, mortalities first occurred at 6 days post-infection and peaked at 6-7 days post-infection, and the disease caused over 90% mortality. The disease duration increased at lower temperatures, but the mortality decreased, and there was no mortality at 18°C.

Virus neutralizing antibody is produced against the virus, and the highest titres are produced between 19-27°C (Yang and Zuo, 1990a, 1991, 1997b, a). The titres declined at temperatures above 30-32°C, and were not detected below 10°C. Interferon is produced by infected fish, and was detected 1 day following experimental infection, peaking at 3 days post-infection (Shao *et al.*, 1999). Interferon production was influenced by factors such as titre of inoculum and nutritional status of the fish, and the levels detected increased at the temperature increased. The Mx gene in grass carp is upregulated by GCRV infection (Wang *et al.*, 2003).

## Diagnostics

Diagnosis of GCRV by clinical signs alone is unreliable, and virus isolation in cell culture followed by identification, or direct identification in fish tissues is necessary. The virus has been isolated or grown in the following

cell lines: grass carp lip (ZC-7901) (Zhang and Yang, 1981), grass carp ovary (GCO), grass carp fin (GCF) (Chen *et al.*, 1985), C. *idella* kidney (CIK) (Zuo *et al.*, 1986), crucian carp blastula embryo (CAB-80), herbivorous bream caudal fins (BCC), loach haploid gastrula embryo (PHG), grass carp gastrula embryo (GCE), grass carp caudal fin (GCTF-2), grass carp kidney (GCK-84) (Deng *et al.*, 1985), grass carp snout (PSF) (Li *et al.*, 1988), grass carp embryo (CP-80) (Ye *et al.*, 1992), EPC (Zhai *et al.*, 1993; McEntire *et al.*, 2003), CHSE-214 (Rangel *et al.*, 1999) and RTG-2 (McEntire *et al.*, 2003).

Jiang and Ahne (1989) tested the susceptibility of several fish, reptile and mammal cell lines, but no cytopathic effect was observed, even following multiple passages. However, virus was observed in some cells by electron microscopy. The virus is now grown in some of the cell lines tested, and the grass carp kidney (CK) cell line, which was refractory on initial testing, is now recommended for isolation of the virus (Jiang, 2004). The virus grows between 20-35°C in GCO and GCF cell lines, but the optimum temperatures were between 25-30°C (Chen *et al.*, 1985); a temperature of 25°C is recommended for isolation of the virus in CK cells (Jiang, 2004).

The virus has been identified in cell culture harvests or in fish tissues by the ELISA (Min *et al.*, 1986; Ding *et al.*, 1991; Jiang *et al.*, 1994), dot ELISA on nitrocellulose membranes (Shao *et al.*, 1996), co-agglutination with antibody-sensitized staphylococci (CoA) (Yang *et al.*, 1991), counter-current electrophoresis (Min *et al.*, 1986) and immunoelectron microscopy (Chen and Jiang, 1983, 1984; Chen *et al.*, 1985; Jiang and Ahne, 1989), but the latter method is not recommended for routine use. The ELISA, dot ELISA and CoA are more rapid and sensitive than the other serological methods, and of these three methods, the CoA is the most rapid, but the dot ELISA is the most sensitive. Identification of GCRV by neutralization with specific antibody does not appear to be used routinely.

There may be different serotypes or biotypes of the virus. Isolates differing in RNA profile, antigenicity, *in vitro* properties, and virulence for grass carp have been identified which, in some cases, are related to the geographic origins of the isolates (Jiang and Ahne, 1989; Ke *et al.*, 1990; Guo and Jiang, 1993; Jiang *et al.*, 1994; Li *et al.*, 1997b).

The virus has also been detected in infected cell cultures and infected fish by RT-PCR (Li *et al.*, 1996, 1997a, b; Wang *et al.*, 1997; McEntire

*et al.*, 2003; Jiang, 2004). Seng *et al.* (2004) designed a RT-PCR using degenerate primers that not only detected GCRV but also aquareoviruses such as striped bass reovirus and chum salmon reovirus (CSV) that are a different *Aquareovirus* species. Some RT-PCR methods were very specific, and did not detect all GCRV isolates tested (Li *et al.*, 1997b; Jiang, 2004), suggesting that either the primer pairs were designed against non-conserved gene sequences, or that there may be different genotypes of the virus. The latter is most likely, as the RT-PCR described by Li *et al.* (1997b) detected only one of two viruses tested, and the two viruses differed in electrophoretic mobility of their RNA and in their cell line specificity.

## Disease Treatment/Prophylaxis

Reducing the stocking density of fingerling grass carp in late summer or autumn, and altering the ratio of grass carp, silver carp and bighead carp in polyculture systems has been suggested as means of lessening the impact of the disease (Chen *et al.*, 1994a). Hemoglobin in fish exposed to high nitrite levels in ponds can be converted to methemoglobin, which increases their susceptibility to GCHD. The addition of calcium hydroxide to ponds followed by sodium hypochlorite can reduce the nitrite levels (Wang and Hu, 1989).

There has been extensive work on producing vaccines against GCRV, and they have been available in China for many years. The vaccines are either inactivated or attenuated virus; inactivated vaccines have reduced the mortality rate of grass carp fingerlings from 70 to 30% (Jiang, 1995). It has been suggested that GCRV subunits obtained by treating the virus with a non-ionic detergent, NP-40, could be used as vaccine (Fang *et al.*, 1997), as rabbit antisera produced against the subunits neutralized the virus. However, to date, there have been no reports of that approach.

Formalin is usually used for inactivation of the virus (Chen *et al.*, 1985; Yang and Zuo, 1990b). In addition to cell-culture-derived virus, virus from crude harvests of tissues from infected fish have been inactivated for use as a vaccine (Chen *et al.*, 1985, 1994b; H. Gao cited by Nie and Pan, 1985; Zhang *et al.*, 1990; Jiang, 2004). Inactivated vaccine has been reported to be stable for almost a year at 4°C (Chen *et al.*, 1985), but for only 8 days at 28°C (Yang and Zuo, 1993). Freund's complete adjuvant increased the efficacy of the vaccine, and aluminium hydroxide did not increase protection, but did increase the titre of neutralizing antibody in the fish. A booster immunization increased the

efficacy of the vaccine and also increased the titre of neutralizing antibody (Yang and Zuo, 1993). There were also differences in efficacy of the inactivated vaccines according to the virus strain used (Yang *et al.*, 1989). Neutralizing antibody was detected 5 days post-vaccination at 28°C, and levels peaked 20 days post-vaccination, and then stayed at approximately the same level. The protection lasted for 8 months, and neutralizing antibody was still present after 13 months (Yang and Zeng, 1993). High levels of protection and high levels of virus neutralizing antibody were obtained at average temperatures of approximately 19°C or 27°C, but at an average temperature of 32°C, both levels of protection and virus neutralizing antibody titres were lower (Yang and Zuo, 1991, 1997a, b). No protection occurred and no virus-neutralizing antibody was detected following vaccination below 10°C.

Attenuation of virus has been achieved by multiple passage through cell cultures (Xu *et al.*, 1994; Jiang, 1995), and the attenuated virus did not regain virulence following 10 passages through fish. Attenuation was also achieved by passaging the virus in cell cultures that contained an extract of Eucalyptus (Xu *et al.*, 1998), and the vaccine was 100% effective. Large-scale production of vaccine virus has been achieved by the use of roller bottle cultures (Yang *et al.*, 1992; Zeng *et al.*, 1998) or microcarrier suspension culture (Chen *et al.*, 1992b; Ye *et al.*, 1992). Two viruses were selected as vaccine strains because of their rapid growth to high titres in cell culture and efficacy at protecting the fish following vaccination (Luo *et al.*, 1993).

Methods of vaccinating fry by immersion have been investigated. X. Yang (pers. comm. to Jiang, 1995) used a hyperosmotic method (a bath in a 3% sodium chloride solution followed by immersion in vaccine), but the protection rate over immersion in vaccine only was just 5-10%. An improvement in both efficacy and duration of protection following immersion in a bath of inactivated vaccine was obtained when the bath contained an extract of henbane (*Hyoscamus* sp.) (Zhang *et al.*, 1990). Henbane and an inactivated vaccine were also added to the pelleted feed that was given daily to grass carp fingerlings (Chen *et al.*, 1994b). The treated grass carp had both a higher survival rate and weight gain compared with non-treated fish.

Vaccination against GCRV is widely practised in China (Jiang, 1995, 2004), but Wang *et al.* (2000) reported that it was unsuccessful. The latter authors are attempting to produce antiviral peptides to control the

disease. Initial work has identified some peptides with antiviral activity *in vitro*, but they were only able to reduce the viral titre, rather than inhibit virus growth completely. Two drugs, coded 87-05 and 88-01 (Kelieao-Yufukang), had antiviral activity against GCRV (Zhu *et al.*, 1993). The two drugs had an effect individually, but had a synergistic effect when combined together. Attempts have been made to genetically modify grass carp to produce strains that are resistant to GCHD, but with limited success (Yu *et al.*, 1996; Zhong *et al.*, 2002) in grass carp.

### Epidemiology/Geographic Distribution

The disease occurs in China, Vietnam (Jiang, 2004) and the virus has been isolated in the USA (McEntire *et al.*, 2003; Goodwin *et al.*, 2006c). Although the *virus* has been isolated in the USA, it is difficult to say from data presented whether the *disease* did actually occur there. One of the isolations of the virus in the USA was from diseased grass carp at an aquaculture site experiencing significant mortalities, but apart from lethargic swimming, no other disease information was included in the report. If it is accepted that the disease in golden shiners is just the manifestation of GCHD in another host species, the disease as well as the virus can be said to occur in the USA. In China, the disease occurs in several provinces, particularly those along the Yangtze River (Chen *et al.*, 1985; Zhai *et al.*, 1987; Ke *et al.*, 1990; Jiang, 2004), and also along the Min Jiang (Min River) and Huang He (Yellow River) (Ke *et al.*, 1990).

### Economic Importance

The grass carp is one of the major aquaculture species in China, representing 20% of the total freshwater fishery production (Jiang, 2004) and GCHD has had a significant effect on production, but no figures are available to quantify the losses in economic terms. According to Jiang (1995), only 30% of grass carp fingerlings survive to market size because of the disease.

## GOLDEN SHINER VIRUS

### Hosts

The primary host for the disease is the golden shiner (Plumb *et al.*, 1979), but the virus has also been isolated from apparently healthy grass carp (Hedrick *et al.*, 1989), moribund grass carp (McEntire *et al.*, 2003), and

recently from moribund fathead minnow and creek chubs (McEntire *et al.*, 2003; Goodwin *et al.*, 2006c). All the above species except for the creek chub were in culture; the virus has never been isolated from wild golden shiners (Goodwin *et al.*, 2004).

## Disease Symptoms

The virus was first isolated from golden shiners that were listless, swam near the surface but dived when disturbed only to return to the surface shortly afterwards (Plumb *et al.*, 1979). Petechial haemorrhages were observed in the cornea, on the ventral body surface, in visceral fat and in intestinal mucosa. Petechial haemorrhages in the dorsal musculature gave the back a reddish colour. Petechial haemorrhages have also been observed at the bases of fins (McEntire *et al.*, 2003). In one case, gross internal lesions were not observed, even though there were external signs of disease (McEntire *et al.*, 2003).

## Disease Aetiology

In thin sections, the virus was 70 nm diameter, replicated in cytoplasm and contained dsRNA (Plumb *et al.*, 1979). Size estimates for negative stained virus were 75 nm diameter (Winton *et al.*, 1987) or 50-55 nm diameter (Goodwin *et al.*, 2006c), and the virus had the characteristic morphology of an aquareovirus. The virus had 11 segments of ds RNA, confirming that GSV was an aquareovirus (Winton *et al.*, 1987; Varner and Lewis, 1991; Lupiani *et al.*, 1993). Two different estimates of the molecular masses of the RNA segments of GSV were similar (Winton *et al.*, 1987; Varner and Lewis, 1991), but they differed from an estimate of the molecular mass of a GCHD isolate (Wang *et al.*, 1990).

The virus was serologically related by virus neutralization to aquareoviruses isolated from tench and chub (Ahne and Kölbl, 1987, 1989), CSV and 13P2 viruses (Brady and Plumb, 1988) and catfish reovirus (CRV) (Amend *et al.*, 1984; Brady and Plumb, 1988). However, in cross-neutralization tests with GSV, CSV and CRV, there was only a very distant serological relationship between the viruses, which were considered as different serotypes (Hedrick *et al.*, 1984). Likewise, using cross-neutralization tests and cross-immunodot tests Dopazo *et al.* (1996) arrived at the conclusion that GSV, CRV, turbot reovirus, striped bass virus and Simpson coho salmon virus belonged to separate serogroups.

Lupiani *et al.* (1993) compared 19 aquareovirus isolates, including GSV by RNA-RNA blot hybridization, and were able to divide the isolates between 5 Genogroups, A to E. GSV was unrelated to any of the other isolates and was the sole member of Genogroup C. These Genogroups are now recognized as *Aquareovirus* species (see section on GCHD). Comparative studies have shown that GSV and GCHD are the same virus (Attoui *et al.*, 2002; McEntire *et al.*, 2003). It was hypothesized that GCHD was imported into the USA with grass carp and the virus subsequently infected golden shiners, and so the name GCHD should take preference over GSV (McEntire *et al.*, 2003). (See the section on GCHD for more details.)

## Disease Pathogenesis

Low mortality rates are usually associated with the infection, although high losses may occur when infected fish are held in crowded holding tanks (Plumb *et al.*, 1979). Those authors also reported that GSV was isolated from a golden shiner mortality event in which approximately 75% of 12000 fish held in a dealer's tank died. However, Plumb (1981) discussed a similar mortality in tank-held golden shiners (which may have been the same case), but reported that a severe bacterial infection in the fish may have affected the mortality rate. There is anecdotal evidence that larger fish may be more susceptible to infection with GSV (Plumb, 1981). A positive correlation between crowding and increased incidence of infection (but not mortality) was shown by laboratory studies (Schwedler and Plumb, 1982a)

GSV injected by the IP route into golden shiners maintained at 21-22°C killed 3/5 fish between 1 and 2 weeks post-infection. However, no mortality occurred, nor were clinical signs observed in a second trial conducted at 28°C, but the virus was re-isolated (Plumb *et al.*, 1979). This finding was not substantiated in a later, similar, experiment in which there was 100% mortality in some groups of IP injected fish maintained at 28°C (Brady and Plumb, 1991).

The GSV isolate from grass carp was used to challenge alevin grass carp and golden shiners by bath exposure at 25°C (Hedrick *et al.*, 1989). There were no mortalities, and although the virus was re-isolated from both species 7 days post-infection, it was isolated from only 1/4 golden shiners 28 days post infection. The virus from grass carp was also injected by the IP route into juvenile grass carp held at 25°C. Again, there were no

mortalities or apparent disease, but virus was recovered from 12/16 fish three months post-infection.

Higher mortalities at lower temperatures were reported by McEntire *et al.* (2003). Ten-month-old fish in ponds were dying at a rate of 100-1000 fish per day at 15°C over a 2-3-week period and 1-year-old fish showed a similar pattern of mortality at 20-24°C. It was postulated that either the disease had not been recognized under those conditions before, or the mortality was caused by another factor, and the virus was isolated from sub-clinical infections.

## Diagnostics

The virus has been isolated from pools of viscera in FHM cells at 30°C (Plumb *et al.*, 1979), pooled spleen and kidney in EPC cells (Hedrick *et al.*, 1989), homogenates of whole fish in EPC and FHM cells at 25°C (Goodwin *et al.*, 2006c) and from homogenates of whole fish in EPC cells at 20°C (McEntire *et al.*, 2003). GSV grew in FHM cells between 20-30°C, with an optimum of 30°C, but it does not grow in those cells at 15 or 35°C (Schwedler and Plumb, 1982b). The isolated virus also grows in CHSE-214 cells between 20°C and 30°C (Hedrick *et al.*, 1984; Winton *et al.*, 1987; Varner and Lewis, 1991), in BB cells at 28-30°C (Winton *et al.*, 1987), and in RTG-2 cells at 20°C (McEntire *et al.*, 2003). The virus has been propagated in cultures of *Sarotherodon mossambicus* (TmB), in which it produced higher titres compared with FHM cells (Lewis and Marks, 1985). The virus did not grow in channel catfish ovary (CCO) cells nor redfish dorsal fin (RFDF) cells (Plumb *et al.*, 1979; Varner and Lewis, 1991). Plumb *et al.* (1979) reported that the virus did not grow in RTG-2 or BB cells, but other groups were able to propagate the virus in those cell lines (cited above).

## Disease Treatment/Prophylaxis

The virus is not affected by pH 3 or pH 10 at 25°C for two hours, nor by a temperature of 50°C for 30 min (Plumb *et al.*, 1979).

Some states of the USA have banned the movement of live baitfish, but frozen wild-caught bait is often not subject to the same restrictions (Goodwin *et al.*, 2004). Biosecurity measures such as disinfection of eggs, growing eggs in hatcheries in well water under controlled conditions, and stocking fish into ponds containing well water are employed in the bait industry (Goodwin *et al.*, 2004).

## Epidemiology/Geographic Distribution

The disease generally occurs above 25°C (Schwedler and Plumb, 1982b) or within the range 27-32°C (Plumb *et al.*, 1979), but it has been observed at 15°C and 20-24°C (McEntire *et al.*, 2003). In *in vitro* experiments, the virus was stable for at least six months at 4°C. Virus persisted in culture medium for 148 days at 20°C, but was inactivated within 7 days at 30°C (Plumb *et al.*, 1979). The virus was inactivated within 15 days when held in pond water at 4, 20, 25 and 30°C (Brady *et al.*, 1993).

The virus is widely distributed in cultured golden shiners in the southern US (Winton, 1989). It has only been isolated from sick cultured fish, but never during a health screen of a farm (Goodwin *et al.*, 2004). Movements of baitfish, particularly frozen fish, are a likely means of transferring the virus within the USA.

## Economic Importance

The golden shiner baitfish industry in the USA is worth many millions of dollars (Goodwin *et al.*, 2004). Whilst GSV can cause high mortalities under certain conditions, such as high stocking density, under good husbandry conditions the virus is of low pathogenicity, and does not appear to be a constraint on the industry.

## Aquabirnaviruses in Cyprinids

A number of aquabirnaviruses have been isolated from cyprinids, but in the main they do not appear to cause disease in the hosts from which they were isolated. However, many of the viruses are serologically related to the aquabirnaviruses causing infectious pancreatic necrosis (IPN) and the cyprinid hosts may be of importance as reservoirs of the causative virus, IPN virus (IPNV).

## Hosts

The cyprinid hosts from which aquabirnaviruses have been isolated are listed in Table 3.4.

The black carp isolate came from a cell-line derived from the swimbladder of that species, and it was presumed that the donor fish was an aquabirnavirus carrier.

Table 3.4 Cyprinid host from which aquabirnaviruses have been isolated.

| *Species* | *Common name* | *Reference* |
|---|---|---|
| *Abramis brama* | Bream | Hill (1982) |
| *Barbus barbus* | Barbel | Ahne (1980b) |
| *B. graellsi* | Barb | Ortega *et al.* (1993) |
| *B. schwanenfeldi* | Schwanenfeld's barb | Ahne (1984) |
| *Blicca bjoerkna* | White bream, silver bream | W. Ahne, pers. comm. in Hill (1982)<br>Ahne (1984) |
| *Brachydanio rerio* | Zebrafish | Ahne (1982a) |
| *Carassius auratus* | Goldfish | Adair and Ferguson (1981) |
| *C. carassius* | Crucian carp | Hill (1982) |
| *Chondrostoma nasus* | Nase | W. Ahne, pers. comm. in Hill (1982)<br>Ahne (1984) |
| *Cyprinus carpio* | Common carp | Ahne (1980b) |
| *Gobio gobio* | Gudgeon | W. Ahne, pers. comm. in Hill (1982)<br>Ahne (1984) |
| *Hampala dispar* | Eye-spot barb | Wattanavijarn *et al.* (1988) |
| *Leuciscus souffia* | Varione, bludgeon | G. Bovo, pers. comm.; Hill and Way (1995) |
| *Mylopharyngodon piceus* | Black carp | Chen *et al.* (1993) |
| *Phoxinus phoxinus* | Minnow | Munro *et al.* (1976) |
| *Rutilus rutilus* | Roach | Hill (1982) |
| *Scardinius erythrophthalmus* | Rudd | W. Ahne, pers. comm. in Hill (1982)<br>Ahne (1984) |

## Disease Symptoms

The majority of the fish showed no signs of disease, and none of them showed signs of IPN. The bream were in 'poor condition' but no histological changes associated with IPN were observed (Adair and Ferguson, 1981), and the eye-spot barb were part of an investigation into the epizootic ulcerative syndrome (Wattanavijarn *et al.*, 1988).

## Disease Aetiology

The isolates from the different species were neutralized by aquabirnavirus antisera, although they were not all of the same serotype. The serotypes were A2 (Sp) (Munro *et al.*, 1976; Ahne, 1982a; Wattanavijarn *et al.*, 1988; Ortega *et al.*, 1993), A3 (Ab) (Adair and Ferguson, 1981; Chen *et al.*, 1993), A4 (He) (Ahne, 1980b) and B1 (TV-1) (Hill, 1982).

## Diagnostics

The viruses were isolated in cell culture using methods as described for IPNV using cell lines such as BF-2, RTG-2 and EPC. The eyespot barb isolate was isolated in an unpublished *Ophicephalus lucius* cell line, but it also grew in RTG-2 and EPC cells (Wattanavijarn *et al.*, 1988).

## Epidemiology/Geographic Distribution

The viruses have been isolated from fish in, or imported from, France (Nougayrede, 1988), Germany (Ahne, 1980b), Italy (G. Bovo, pers. comm.), Lao PDR (Wattanavijarn *et al.*, 1988), Spain (Ortega *et al.*, 1993), Taiwan (Chen *et al.*, 1993), the UK (Munro *et al.*, 1976; Adair and Ferguson, 1981; Hill, 1982) and 'the Far East' (Adair and Ferguson, 1981).

In some cases, the fish were collected from waters in close proximity to areas where IPNV-positive rainbow trout farms were located (Munro *et al.*, 1976; Ortega *et al.*, 1993), or in an area possibly stocked with IPNV-positive rainbow trout (Ortega *et al.*, 1993). However, the bream were in a river from which IPNV had never been recorded and there were no fish farms in the area (Adair and Ferguson, 1981), and the zebra fish were from a laboratory population (Ahne, 1982a). There are no details of whether the other species were from IPNV-positive or negative areas.

Experimental infections of rainbow trout using isolates from some cyprinid hosts have been attempted. UK isolates from carp, roach, bream, and crucian carp were pathogenic for rainbow trout fry, producing clinical signs of IPN (Hill, 1982). Different isolates had different virulence, but no further details were given. Isolates from barbel and carp were not virulent for rainbow trout, Danube salmon or carp (Ahne, 1980b). However, those isolates were of the A4 serotype, and IPNV A4 was found to be avirulent for rainbow trout fry in later experiments (Ahne *et al.*, 1989). Subsequently, Ahne *et al.* (1989) reported that an isolate from carp produced a low mortality (8.3%) and clinical signs of IPN in rainbow trout fry by bath exposure, and that isolates from goldfish and barbel were avirulent for that species. Conversely, when sexually mature male rainbow trout were injected with the carp isolate, and their sperm was used to fertilise rainbow trout eggs, there was 95.7% mortality in the progeny fry—similar to that produced by the positive control virulent IPNV serotype A2. Carp fingerlings did not show clinical signs of disease following IP

injection with an IPNV isolate from rainbow trout, but the virus appeared to replicate in internal organs over the 16-day observation period following infection (Daud and Agius, 1987).

## Economic Importance

The aquabirnaviruses do not appear to be of importance for the cyprinid hosts themselves, but the cyprinids may be of high importance as reservoirs of IPNV, with the potential to transmit the virus to susceptible species. The importance of cyprinids as reservoirs is increased if, as appears to be the case, they do not become clinically diseased nor suffer losses and are purely carriers of the virus.

## Carp Edema (Koi Sleepy Disease)

The disease called carp edema has occurred in koi carp in Japan since the mid-1970s, and was shown to be caused by a virus morphologically resembling a pox virus. It should be noted that the virus is a potential member of the virus family Poxviridae, and is not related to the virus causing carp pox, which is a member of the virus family Herpesviridae. A disease called koi sleepy disease was reported later, which was similar to carp edema in most clinical signs, histopathology and electron microscopy. Furthermore, a PCR developed for carp edema virus (CEV) detected the virus causing koi sleepy disease. These two conditions will, therefore, be considered to be caused by the same virus, unless contrary evidence is presented.

## Hosts

The disease has only been reported from koi carp.

## Disease Symptoms

The predominant clinical sign of carp edema disease is a distended abdomen, whereas that has not been described as feature of koi with sleepy disease. Fish with the latter disease often have sunken eyes and may have haemorrhages on the skin. In both conditions, there is usually hyperplasia of the gill, fusion of gill lamellae, and the tips of the lamellae may become club-shaped (Ono *et al.*, 1986, 1993; Oyamatsu *et al.*, 1997a; Miyazaki *et al.*, 2005). Fish with edema tend to rise to the surface of the water, and

may gather at the walls of ponds, whereas those with sleepy disease lie at the bottom of ponds. The differences in the clinical picture may be related to the age of the fish and their culture conditions (Miyazaki *et al.*, 2005). Mortalities can be between 80-100%, and often occur within a few days of the onset of disease (Ono *et al.*, 1986; Oyamatsu *et al.*, 1997a). Mortalities started 3-6 days following experimental bath infection, and over the subsequent 8-16 days there was 72-98% mortality (Oyamatsu *et al.*, 1997a).

Histological changes in the gills were thickening of the gill lamellae following a proliferation of epithelial cells. Adhesion of the lamellae occurred, which spread to all gill filaments in severe cases (Ono *et al.*, 1986; Oyamatsu *et al.*, 1997a; Miyazaki *et al.*, 2005). Virus-like particles were seen in the cytoplasm of epithelial cells of the secondary gill lamellae (Ono *et al.*, 1986, 1993; Oyamatsu *et al.*, 1997a; Miyazaki *et al.*, 2005). It was occasionally seen in skin, but never in other tissues (Oyamatsu *et al.*, 1997a). Pathological changes such as cloudy swelling and mitochondrial degeneration were also seen in the liver, kidney, spleen, musculature and heart of fish affected by koi sleepy disease (Miyazaki *et al.*, 2005).

## Disease Aetiology/Pathogenesis

The disease appears to be caused by a large spherical to oval virus, 250-280 nm diameter. It has a roughly spherical- to crescent-shaped core, and the outer envelope has a crenellated or globular appearance, described as mulberry-like (Ono *et al.*, 1986, 1993; Oyamatsu *et al.*, 1997a; Miyazaki *et al.*, 2005). The agent has not been cultured *in vitro*, but transmission trials by bath infection have been conducted with filtered homogenates of infected fish (Ono *et al.*, 1993; Oyamatsu *et al.*, 1997a). The disease was reproduced with identical pathology and histopathology, and the same pox-like viruses were seen by electron microscopy. The virus was serially passaged through the fish 12 times with identical results (Oyamatsu *et al.*, 1997a). Infectivity for the transmission trials was primarily associated with gill tissue, although an extract from eviscerated bodies, or bodies with just gill removed was infectious, but only at low dilutions. Extracts of kidney, spleen, intestine, hepatopancreas and heart were not infectious (Oyamatsu *et al.*, 1997a).

Carp edema occurs in June and July (Ono *et al.*, 1986; Ono *et al.*, 1993; Oyamatsu *et al.*, 1997a), and experimental infections have been

successful at 20-23°C (Oyamatsu *et al.*, 1997a). Sleepy disease occurs between 15° and 25°C (Miyazaki *et al.*, 2005).

## Diagnostics

The virus did not replicate in any of 31 fish cell lines tested, including the commonly used cyprinid and non-cyprinid cell lines routinely used in fish diagnostic laboratories (Ono *et al.*, 1986; Oyamatsu *et al.*, 1997a). However, a nested PCR was developed for diagnosis of carp edema disease (Oyamatsu *et al.*, 1997b), which also produced a product of the expected size when tested against gill tissues from koi with sleepy disease (Miyazaki *et al.*, 2005). In the latter study, gill and skin from around lesions were PCR positive, but liver and kidney tissues were found to be negative. However, in a survey for CEV in fish with sleepy disease by PCR, brain, kidney and spleen were positive in addition to gill tissue (Amita *et al.*, 2002).

## Disease Treatment/Prophylaxis

Koi farmers hold fish in 0.5% salt water after transportation from earth nursery ponds to concrete-lined ponds in the spring. The value of this was tested by taking fish from an earth pond and dividing them into two groups. One group was maintained in freshwater and the second in 0.5% salt water for 25 days. There was 75% mortality in the freshwater group, but no mortality occurred in the salt-water group (Miyazaki *et al.*, 2005). Fish from the latter group did show hyperplasia of gill lamellae. It was thought that nitrite toxicity caused methemoglobinemia in the freshwater fish, and that stress influenced the development of the disease, whereas the salt water reduced the toxicity of nitrite, and the fish in salt water exhibited only slight methemoglobinemia. It was also thought that the salt might have had a direct osmotic effect on the gills that protected them from the disease.

## Epidemiology/Geographic Distribution

The disease has only been reported from Japan. Young koi carp are usually grown in earthen nursery ponds, and then transferred to cement ponds in autumn to overwinter. The disease is usually seen in the cement ponds in spring/summer (Ono *et al.*, 1986) and often in autumn also (Miyazaki *et al.*, 2005).

## Economic Importance

No value has been put on the losses caused by this disease, but they are potentially significant as the disease apparently causes high mortalities in koi carp on a regular basis.

## Miscellaneous Diseases or Observations

There have been several preliminary reports—including single papers—of diseases of cyprinids, viruses isolated from apparently healthy fish or of observations of virus-like particles by electron microscopy. They will be included here for completeness, and listed by the putative causal virus.

### *Aquareoviridae*

#### Tench and Chub

Aquareoviruses were isolated from internal organs of apparently healthy tench and chub (Ahne and Kölbl, 1987, 1989) from Germany. The viruses were isolated in EPC and FHM cells. The viruses grew best in FHM cells, but also replicated to a lesser extent in RTG-2, CHSE-214, CHH1 and BB cells; BF-2 cells were refractory to the viruses. The viruses were 70-75 nm diameter and had 11 segments of ds RNA. An antiserum raised against the tench isolate neutralized both tench and chub isolates, and there was a serological relationship between those isolates and GSV and CSV; catfish reovirus (CRV) did not react with the antiserum. The tench and chub isolates could be distinguished by the electrophoretic mobility of their RNA segments which were different from those of CSV and GRV. The tench and chub isolates were not pathogenic for carp, roach and perch following IP injection, and virus was not re-isolated from internal organs (Ahne and Kölbl, 1989).

#### Common Carp

An aquareovirus, isolate 89/24, was isolated in EPC cells inoculated with a homogenate of internal organs of a common carp affected by epidermal hyperplasia (Jiang *et al.*, 1991a) from Germany. The virus also replicated well in CHSE-214, CIK, grass carp ovary (CO) and rainbow trout liver (R1) cells, to a lesser extent in grass carp kidney (CK), pike gonad (PG), CAR and FHM cells but not in RTG-2 cells. The virus was 65-70 nm diameter and had 11 strands of ds RNA. The virus was different from

GCHD virus by serology, electrophoretic mobility of their RNA segments and by pathogenicity studies. Isolate 89/24 was avirulent for common carp, grass carp, bighead carp and silver carp. The electrophoretic mobilities of the RNA segments of isolate 89/24 were different from those of CSV, CRV, 13P2, GSV, tench aquareovirus and chub aquareovirus.

Sano and Fukuda (1987) reported the isolation of an aquareovirus from koi carp in Japan. The virus grew well in CHSE-214, FHM, EPC, EO-1, EK-1 amongst others, and less well in RTG-2 and BF-2 cells. There was no other information.

### Golden Ide

During investigations into epidermal hyperplasia in the golden ide, *Leuciscus idus melanotus*, in Germany a virus was isolated in FHM and EPC cells at 20°C from extracts of pooled liver, kidney and spleen from one fish (Steinhagen *et al.*, 1992). The virus was unlike the herpesvirus associated with the epidermal hyperplasia, and was subsequently identified as an aquareovirus (Neukirch *et al.*, 1999b), and was called golden ide reovirus (GIRV). The virus was 50-70 nm diameter, and contained 11 segments of ds RNA. The virus was not neutralized by antiserum against CSV, and its electrophoretic profile differed from that of CSV. The virus had a lytic CPE in EPC and FHM cells, and so differed from the majority of isolated aquareoviruses, which produce syncitia in cell culture. The infectivity of the virus in cell culture is enhanced by the addition of trypsin to the cell culture medium (Neukirch and Kruse, 1993; Neukirch, 2000). The virus also grew well in CHSE-214 and BF-2 cells; it also grew but to a lower titre in RTG-2 cells (Neukirch, 2000). Virus transmission trials at 16-18°C and 10-16°C were attempted with golden ide and common carp by IP injection or skin scarification followed by exposure to the virus (Neukirch, 2000). The virus was also IP injected into rainbow trout maintained at 8-12°C. No virus-specific mortality occurred in any of the groups. Virus was re-isolated from both golden ide and carp 10-weeks post-infection at 10-16°C, and from golden ide only at 16-18°C.

## *Coronaviridae*

### Unnamed Virus

Goldfish in a fish experimental station in Mexico began to suffer from a disease that caused a 10% morbidity rate and a 5-6% mortality rate.

Affected fish exhibited haemorrhages and erosions of the fins, oedema at the fin bases, haemorrhages on the skin, lepidorthosis, abdominal distension caused by excess ascetic fluid, prolapse of the anus and loss of equilibrium. Homogenates of liver, spleen, kidney and swimbladder were inoculated onto FHM, BB and RTG-2 cells, primary cultures of carp and trout ovaries and onto established and primary cultures of mammalian cells, but no CPE occurred. Coronavirus-like virus particles were observed in sections of the kidneys (Yáñez *et al.*, 1980).

### Cyprinid coronavirus 1, *Coronavirus cyprini*, carp coronavirus

Similarly to the above, common carp reared in a laboratory in Japan began to die, exhibiting only erythrematous skin on the abdomen (Sano *et al.*, 1988). Homogenates of kidney, spleen and liver produced CPE following inoculation onto FHM cells and incubation at 20°C. The virus was passaged onto other cell lines including BF-2, EPC and RTG-2, but the highest titres were obtained in FHM cells. Negative-stained virus was 60-110 nm diameter, and morphologically resembled a coronavirus. The virus was transmitted to carp fry by bath challenge, and caused a cumulative mortality of 72.5% in 20 days. Affected fry had swollen, haemorrhagic abdomens and swam in a circular manner. By histology there was hepatic and renal necrosis, and necrosis and destruction of the intestinal tunica mucosa. The virus was designated as Cyprinid coronavirus 1, given the Latin name *Coronavirus cyprini* and the vernacular name carp coronavirus (CACV).

### Viraemia-associated Ana-aki-byo

Koi carp and other cyprinids in Japan suffer from dermal ulcerative diseases, given the generic name ana-aki-byo, most of which are caused by bacteria, or bacteria are involved as secondary pathogens. However, a different type of ana-aki-byo was recognized in the late 1990s, which had a virus involvement and was termed viraemia-associated ana-aki-byo (Miyazaki *et al.*, 2000). The disease appears to primarily affect C. *carpio*, and causes ulcerative lesions and haemorrhages on the body; mortality of ulcerated fish also occurred. Scales were separated in the ulcerative lesions and either the dermis or lateral musculature was involved. The heart and kidney were swollen, and the latter organ was discoloured. Histological examination revealed necrosis of dermal connective tissue,

haemorrhages and inflammatory cell infiltration. No bacteria were associated with necrotic tissues, but were observed in ulcers. On culture, no dominant bacterial species was determined. The ulcers penetrated to the lateral musculature in severe cases, but healing ulcers were also observed. Necrosis was seen in the heart, spleen and kidney, and in some fish in the intestine. Pathology was also observed in the liver and brain. Electron microscopy revealed virus-like particles in the cytoplasm of necrotic cells of haematopoietic tissue. The particles were spherical, 100-180 nm diameter and appeared to have spikes on their surface. Virus was isolated from homogenates of dermal lesions, kidney, spleen and heart in EPC cells, but not in FHM, CHSE-214, RTG-2 or EK-1 cells. The virus could be passaged two or three times in EPC cells, but thereafter the infectivity declined. The virus contained RNA and was ether sensitive, i.e., it possessed an envelope. Isolated virus was used to infect common carp by IP or intracutaneous (IC) injection; fish were held at 25°C in water with or without oxytetracycline. Fish receiving virus by the IC route exhibited dermal lesions at the injection site and elsewhere on the body; those fish maintained without oxytetracycline had much more severe ulcers and erosions, with bacterial involvement. Mortalities (2/5 fish) occurred in both the groups. Fish challenged by the IP route did not exhibit dermal lesions, but mortalities ensued (4/8 fish to 4/5 fish) in the different groups. The virus was re-isolated from moribund fish challenged by both routes. In a subsequent study, the disease was transmitted by co-habitation of infected and naïve fish that had surface damage by having a small number of scales removed (Miyazaki *et al.*, 2001). *Aeromonas hydrophila* was isolated from oedematous scale sacs and ascetic fluid, and the authors speculated that some of the tissue damage observed was caused by bacterial toxins. Injection (IP or IC) of carp with *A. hydrophila* alone did not cause any mortality or pathological changes.

## *Iridoviridae*

### Goldfish Virus 1 and 2

Viruses morphologically resembling iridoviruses were isolated on two separate occasions from swimbladder cell cultures derived from apparently healthy goldfish (Berry *et al.*, 1983) in the USA. The first virus, named goldfish virus 1 (GFV-1), was isolated from a juvenile fish from a commercial source and the second virus, GFV-2, was isolated from an adult goldfish from a pond. On both occasions the initial swimbladder cell

cultures exhibited a focal CPE, and medium from the cultures was inoculated onto goldfish fin (CAR) cells. A progressive CPE occurred, resulting in the destruction of the CAR cell monolayers within one week. The two viruses were practically identical in all laboratory studies, and so they could in fact be the same virus. No attempts to infect goldfish or other fish species were reported. As there have been no reports of diseases of goldfish caused by iridoviruses, it must be assumed that the viruses are of no significance for goldfish.

### *Cyprinus carpio* Iridovirus (CCIV)

Gill necrosis of carp and other species has been a serious cause of mortality in carp culture in Eastern Europe and the former Soviet Union for many decades. The cause is still unknown, but water quality and infectious agents such as bacteria and viruses have been suggested as being involved in the disease. Iridoviruses have been isolated from cases of gill necrosis of carp, but whether they are the prime cause of the disease has not been resolved. In fact, one of the authors initially responsible for isolating an iridovirus from cases of gill necrosis and suggesting it may be the cause of the disease later presented evidence to suggest that the virus was not the prime cause of the disease (Shchelkunov and Shchelkunova, 1990). The fish from which viruses were isolated were 1-, 2- and 3-year-old carp and spawners (Popkova and Shchelkunov, 1978; Shchelkunov and Shchelkunova, 1984a). The gills were coated in mucus and oedematous and gill lamellae were discoloured; occasionally the gills were anaemic. Pools of liver kidney and spleen, and gill tissue were homogenized separately and inoculated onto FHM and EPC cells and incubated at 22 and 28°C. Two viruses, 1LZ and 4BZ were isolated in FHM cells from gill tissues from 2/61 samples. The 1LZ isolate could not be passaged more than 10 times whereas the 4BZ isolate was readily maintained in cell culture; it also grew in EPC cells. The virus contained DNA, was icosahedral, 190-210 nm diameter and morphologically resembled an iridovirus. However, the virus was isolated infrequently from cases of gill necrosis. The virus did not always grow to high titre in EPC cells, but grew to a higher titre in MCT and immature carp ovary (ICO) cells (Shchelkunov, 1987).

The results of experimental infections of carp have been inconsistent. Sometimes mortalities or the disease did occur, but they also occurred in control fish, and some of the virus that was re-isolated may have been

residual inoculum (Shchelkunov and Shchelkunova, 1984b, 1990). The virus was not pathogenic for grass carp fry or silver carp × bighead carp hybrid fry by bath immersion or IP injection (Shchelkunov, 1987). Rainbow trout and goldfish were experimentally infected with the virus by various routes (Shchelkunov and Shchelkunova, 1990). The virus was re-isolated from internal organs of goldfish that had been infected by IP injection, but not from rainbow trout. The overall conclusion was that CCIV was not the cause of carp gill necrosis, and it was not a threat to other species.

## ORTHOMYXOVIRIDAE AND PARAMYXOVIRIDAE

### Isolates 1551, 1609 and 1617

During an investigation of gill necrosis and mortalities in koi carp, Neukirch and Kunz (2001) inoculated tissue extracts from diseased fish into CaF-2, CCB, CCG and EPC cells. Paramyxo-like viruses were isolated on two occasions in all four cell lines (isolates 1551 and 1609), and an orthomyo-like virus was isolated on one occasion in CaF-2 and CCB cells (isolate 1617). The paramyxo-like viruses were 160-190 nm diameter with surface projections, and the orthomyo-like virus was 70-80 nm diameter, also with surface projections. All three viruses contained RNA. Neukirch and Kunz (2001) stated that the paramyxo-like virus isolate 1551 was the uncharacterized agent causing a CPE that had been described in an earlier paper (Neukirch *et al.*, 1999a). Isolate 1551 produced a cytopathic effect in EPC, CCG and CCB cells, but not in FHM or CHSE cells. The virus was injected by the IP route into 2-year-old koi carp that were maintained at 19-21°C. There were no mortalities, and the main pathology, gill hyperplasia, that occurred in both test and control fish was most likely caused by an infection with *Dactylogyrus* sp.

### Isolates D 54/98, D 4/99, D11/99, D 30/99 and D 49/99

The CPE caused by the paramyxo-like viruses in the above reports was massive syncitia formation, and such a CPE was also observed in EPC cells by Body *et al.* (2000) during their investigations into mortalities in koi carp. The cells had been inoculated with extracts of gill, spleen and kidney and incubated at 14°C or 21°C. The CPE occurred after 2-3 weeks at the latter temperature, and after one month at the former temperature; it only occurred in cell cultures inoculated with gill tissue. The virus could be

passaged onto FHM but not RTG-2 cells. The virus infectivity was removed by treatment with chloroform, and that property, along with its morphology, 150-200 nm with surface spikes, led the authors to suggest that the could be a paramyxovirus.

Such a CPE has been observed in this laboratory in EPC, FHM and KF-1 cells inoculated with liver, spleen and kidney extracts of koi or common carp and incubated at 20°C (Fig. 3.7). In many cases SVCV or KHV were also isolated, but in others only this CPE was observed. Our experimental infections of carp with this agent have produced contradictory results, and its significance for carp remains unknown.

## *Picornaviridae*

### Warm Springs Virus

During a survey of disease in wild fish populations a picorna-like virus was isolated from apparently healthy sandbar shiners (*Notropis scepticus*) in North Carolina, USA (Iwanowicz *et al.*, 2000). Homogenates of liver, kidney, spleen and intestine were inoculated onto EPC and FHM cells that were then incubated at 20°C. The virus was isolated in both cell lines, and was also passaged in RTG-2 cells. It contained single-stranded RNA and negative stained particles were 20-25 nm diameter, suggesting that it was a picornavirus. The virus was named Warm Springs Virus after the location of the laboratory of isolation.

### Unnamed Virus

A putative picornavirus isolated from grass carp has been discussed in the section on GCHD.

## *Rhabdoviridae*

### Viral Haemorrhagic Septicaemia Virus

Viral haemorrhagic septicaemia virus (VHSV) was isolated from the barb (*Barbus graellsi*) in Spain (Basurco and Coll, 1989), but no information on the effect of the virus on the host was provided. The molecular masses of the polypeptides of the virus were identical to those of other Spanish VHSV isolates.

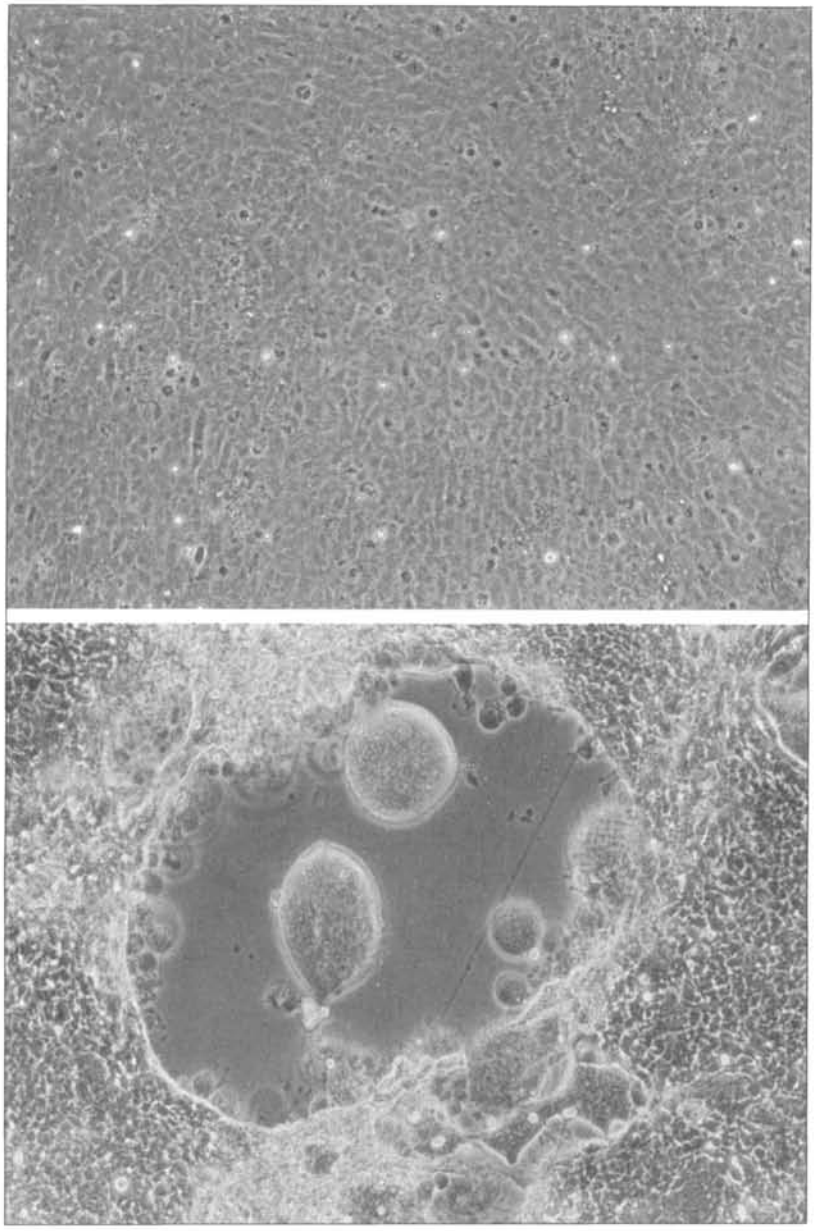

**Fig. 3.7** Cytopathic effect caused by putative myxo-like virus in KF-1 cells, lower picture. Extensive syncitia are produced, and large aggregates of cells round up and detach from the cell monolayer. Control KF-1 cells, upper picture.

## V 76

Stone *et al.* (2003) compared the partial nucleotide sequences of the G gene of SVCV and PFRV and other vesiculovirus isolates, and were able to separate the isolates into four Genogroups. The Genogroups were I (SVCV), II (V 76), III (PFRV) and IV (TenRV), and sections on SVCV and TenRV are included earlier in this Chapter. Genogroup II comprised the isolate V 76 from grass carp. The virus was originally isolated from a single haemorrhagic 2-3-year-old grass carp in Germany (Ahne, 1986b). The virus was isolated from homogenates of kidney, spleen, liver and intestine inoculated onto FHM cells incubated at 20°C. The virus had typical rhabdovirus bullet-shaped morphology, and measured 70 nm × 120-140 nm. Neutralization studies using antisera against SVCV, PFRV and the homologous virus showed that V 76 was not related to SVCV. V 76 shared some common antigens with PFRV, although both viruses could be distinguished with the antisera used. In another study, V 76 was neutralized to the same extent as PFRV (Jørgensen *et al.*, 1989). The virus was virulent for 90 g grass carp at 12°C following IP injection. Mortality started 2 weeks post-infection leading to a 66% cumulative mortality 5 weeks post-infection. The virus was less virulent for 30-35 g common carp infected under the same conditions. In two experiments, there was 10% and 16% cumulative mortality that commenced approximately 4 weeks post-injection.

## Fathead Minnow Rhabdovirus

A bacilliform virus with many of the properties of a rhabdovirus was isolated from fathead minnows from Arkansas, USA (Granzow *et al.*, 2001; Iwanowicz and Goodwin, 2002). The minnows were moribund, and had petechial haemorrhages in the eyes and skin. Chronic mortalities had been occurring in farmed fish for approximately two months. The virus, called fathead minnow rhabdovirus (FHMRV), was isolated from whole fish homogenates in EPC cells at 21°C, and also grew well in RTG-2 and FHM cells. It grew slowly in CCO and BB cells, and only produced small areas of CPE that did not progress in KF-1 cells. BF-2 and CHSE-214 cells were refractory. The CPE appeared as syncitia formation, and occurred rapidly between 15 and 25°C in EPC cells. The virus contained RNA, was sensitive to lipid solvents and the virions were bacilliform, 31-47 nm × 130-185 nm, with occasional bullet-shaped particles being observed. The molecular masses of virion polypeptides were consistent with those of

rhabdoviruses, but an additional polypeptide was present. FHMRV did not react with antisera against infectious haematopoietic necrosis virus (IHNV), VHSV, SVCV, PFRV, IPNV serotype A1 and GSV. The virus was virulent for golden shiners following challenge by IP injection at 17°C and 19°C. Mortalities started to occur at 3 days post-infection at 19°C reaching a cumulative mortality of 85% after 30 days, whereas at 17°C, mortality did not occur until day 13 post-infection, and reached a cumulative mortality of 90% after 30 days. Channel catfish were challenged by IP injection, and rainbow trout, goldfish and golden shiners were challenged by IP injection and concurrent cohabitation with infected fathead minnows. The disease was not transmitted to any of the heterologous species, nor was virus re-isolated from them.

### Crucian Carp Haematopoietic Necrosis Virus

In a study of host-virus reactions *in vitro*, Somamoto *et al.* (2002) used a previously unpublished rhabdovirus called crucian carp haematopoietic necrosis virus (CHNV) isolated in Japan. Note that the Latin name for crucian carp was given as C. *auratus*, which is referred to as goldfish elsewhere in this chapter; crucian carp is used as the English name for C. *carassius*. The virus replicated in 'CFS' and 'CFK' cells (full names not provided). No further details were given, apart from the virus having 'virulence' for the fish.

## Unclassified Viruses, or Virus-like Particles

### Ctenopharyngodon idella *virus Hungary 33/86*

This virus, CIVH 33/86 was isolated from apparently healthy grass carp imported from Hungary into Germany (Ahne *et al.*, 1987). The virus was isolated from homogenates of liver, spleen and kidney inoculated onto CAR, FHM, BF-2, RTG-2, common carp leucocyte (CLC) and PG cells incubated at 20°C, although the CPE was most extensive in the former three cell lines. The CPE was syncitia formation. CHSE-214 and rainbow trout liver (R-1) cells were refractory to the virus. The virus was sensitive to lipid solvents and contained single-stranded RNA. Negative stained virus was rod-shaped, 50-56 × 170-220 nm. The virus was not neutralized by antisera against SVCV, PFRV, IHNV, VHSV or EVX. As commented on by Iwanowicz and Goodwin (2002), this virus bears many similarities to FHMRV in size, morphology, nucleic acid and syncitia production in cell culture. It would be interesting to have more detailed comparisons of

these two viruses. Although isolated in different continents, if the two viruses are related, there may be the same link between imports of the virus into the USA with grass carp, as there was with GCHD/GSV.

## Isolate DF 24/00

Granzow *et al.* (2001) isolated a virus during a monitoring programme for diseases in wild fish in Germany. The virus was isolated in EPC cells inoculated with homogenates of heart, kidney, spleen and swimbladder, but other details were not specified except that they were in accordance with European Union guidelines. The virions were bacilliform, 22-25 × 170-180 nm in thin section, but the size following negative staining varied with the staining method used. The nucleocapsid was bounded by an envelope possessing spikes. The nucleic acid was RNA, and preliminary studies suggested that it was single-stranded and non-segmented. The virus possessed features similar to those in the virus families Coronaviridae, Rhabdoviridae and Baculoviridae, but could not be classified in any of those families.

## Isolate 86-86B

During a survey for viruses in grass carp and golden shiners in California waters, or to be imported there, Hedrick *et al.* (1989) isolated a number of aquareoviruses from spleen and kidney tissues inoculated onto EPC cells. In addition, another virus, 86-86B, was isolated which did not produce the typical aquareovirus syncitial CPE, and it was not neutralized by antisera against GSV, CRV, IPNV or IHNV. The virions were round or hexagonal in profile, 81 nm diameter and preliminary studies suggested that the nucleic acid was not double-stranded RNA. The virus did not replicate in experimentally infected golden shiners.

## Viral Systemic Necrosis of Carp

Investigations into mass mortalities of koi carp in Korea that occurred in 1998 and after have been undertaken by a number of groups. More than 70% of koi carp farms have suffered heavy losses from the disease (Oh *et al.*, 2001). The clinical picture is similar to that of KHV. Mortalities occur at water temperatures of 20-24°C, the mortality rate is 60-95%, and clinical signs include mucus covering the gills and body, gill necrosis and lesions on the body of some fish (Oh *et al.*, 2001; Cho *et al.*, 2005).

Homogenates of gills, liver, spleen, kidney and surface lesions were inoculated onto CHSE-214, FHM, EPC and RTG-2 cells and incubated at 20°C. A CPE was only observed in FHM cells. Examination of infected cells under the electron microscope revealed spherical virus-like particles 70-80 nm diameter. The virus was highly pathogenic for koi and common carp following bath infection at 20°C, producing 100% and 80% cumulative mortality, respectively, by 15 days post infection. Cho *et al.* (2005) reported further work on the disease, and the inference from their paper is that they used the virus described by Oh *et al.* (2001); the former authors named the disease viral systemic necrosis of carp. The virus was shown not to be KHV by the PCR. Vaccines are being developed, and preliminary trials with formalin-killed virus and heat-killed virus have been conducted. Heat-killed virus did not induce protection whereas formalin-killed virus did.

## Unnamed Condition

An enlarged swimbladder from a common carp from Utah, USA had the typical ultrastructure of a fibroma (Inchausty and Heckmann, 1996). Viral inclusions were observed in the swimbladder tissues which were similar to those seen in SVCV-infected cells, and the authors also reported virus-like particles in the cells and suggested that the condition might have been caused by SVCV. The published electron micrographs are at low magnification, and it is very difficult to see the virus-like particles described. However, as the virus-like particles were observed in both the nucleus and cytoplasm, they are unlikely to be a rhabdovirus such as SVCV, which is only known to replicate in the cytoplasm.

## References

Adair, B.M. and H.W. Ferguson. 1981. Isolation of infectious pancreatic necrosis (IPN) virus from non-salmonid fish. *Journal of Fish Diseases* 4: 69-76.

Adair, B.M. and M. McLoughlin. 1986. Isolation of pike fry rhabdovirus from brown trout (*Salmo trutta*). *Bulletin of the European Association of Fish Pathologists* 6: 85-86.

Adkison, M.A., O. Gilad and R.P. Hedrick. 2005. An enzyme linked immunosorbent assay (ELISA) for detection of antibodies to the koi herpesvirus (KHV) in the serum of koi *Cyprinus carpio*. *Fish Pathology* 40: 53-62.

Ahne, W. 1975. A rhabdovirus isolated from grass carp (*Ctenopharyngodon idella* Val.). *Archives of Virology* 48: 181-185.

Ahne, W. 1976. Untersuchungen über die Stabilität des karpfenpathogenen Virusstammes 10/3. *Fisch und Umwelt* 2: 121-127.

Ahne, W. 1978a. Untersuchungsergebnisse über die akute Form der Bauchwassersucht (Frühlingsvirämie) der Karpfen. *Fischwirt* 28: 46-47.

Ahne, W. 1978b. Uptake and multiplication of spring viraemia of carp virus in carp, *Cyprinus carpio* L. *Journal of Fish Diseases* 1: 265-268.

Ahne, W. 1980a. Rhabdovirus carpio-Infektion beim Karpfen (*Cyprinus carpio*): Untersuchungen über Reaktionen des Wirtsorganismus. *Fortschritte in der Veterinärmedizin* 30: 180-183.

Ahne, W. 1980b. Vorkommen des Virus der Infektiösen Pankreasnekrose der Forellen (IPN) bei verschiedenen Fischarten. *Berliner und Münchener Tierärztliche Wochenschrift* 93: 14-16.

Ahne, W. 1982a. Isolation of infectious pancreatic necrosis virus from zebra danio, *Brachydanio rerio*. *Bulletin of the European Association of Fish Pathologists* 2: 8.

Ahne, W. 1982b. Untersuchungen zur Tenazität der Fischviren. *Fortschritte in der Veterinärmedizin* 35: 305-309.

Ahne, W. 1982c. Vergleichende Untersuchungen über die Stabilität von vier fischpathogenen Viren (VHSV, PFR, SVCV, IPNV). *Zentralblatt Fur Veterinarmedizin-Reihe* B29: 457-476.

Ahne, W. 1984. Aktuelle Forschungsergebnisse über das Vorkommen, die Verbreitung und die Stabilität des Virus der Infektiösen Pankreasnekrose der Forellen (IPNV). *Fischer und Teichwirt* 8: 228-229.

Ahne, W. 1985a. *Argulus foliaceus* L. and *Piscicola geometra* L. as mechanical vectors of spring viraemia of carp virus (SVCV). *Journal of Fish Diseases* 8: 241-242.

Ahne, W. 1985b. Viral infection cycles in pike (*Esox lucius* L.). *Journal of Applied Ichthyology* 1: 90-91.

Ahne, W. 1986a. The influence of environmental temperature and infection route on the immune response of carp (*Cyprinus carpio*) to spring viremia of carp virus (SVCV). *Veterinary Immunology and Immunopathology* 12: 383-386.

Ahne, W. 1986b. Unterschiedliche biologische Eigenschaften 4 cyprinidenpathogener Rhabdovirusisolate. *Journal of Veterinary Medicine* B33: 253-259.

Ahne, W. and C. Held. 1980. Untersuchungen über die viruzide Wirkung von Actomar® $K_{30}$ auf fischpathogene Viren. *Tierärztliche Umschau* 35: 308-318.

Ahne, W. and O. Kölbl. 1987. Occurrence of reoviruses in European cyprinid fishes (*Tinca tinca* Lin.; *Leuciscus cephalus* Lin.). *Journal of Applied Ichthyology* 3: 139-141.

Ahne, W. and O. Kölbl. 1989. Some properties of reoviruses isolated from tench (*Tinca tinca*) and chub (*Leuciscus cephalus*). In: *Viruses of Lower Vertebrates*, W. Ahne and E. Kurstak (eds.). Springer-Verlag, Berlin, pp. 250-256.

Ahne, W. and I. Thomsen. 1986. Isolation of pike fry rhabdovirus from *Pseudorasbora parva* (Temminck and Schlegel). *Journal of Fish Diseases* 9: 555-556.

Ahne, W., H. Mahnel and P. Steinhagen. 1982. Isolation of pike fry rhabdovirus from tench, *Tinca tinca* L., and white bream, *Blicca bjoerkna* (L.). *Journal of Fish Diseases* 5: 535-537.

Ahne, W., Y. Jiang and I. Thomsen. 1987. A new virus isolated from cultured grass carp *Ctenopharyngodon idella*. *Diseases of Aquatic Organisms* 3: 181-185.

Ahne, W., R.K. Kelly and H.-J. Schlotfeldt. 1989. Factors affecting the transmission and outbreak of infectious pancreatic necrosis (IPN). In: *Fish Health Protection Strategies*,

K. Lillelund and H. Rosenthal (eds.). Bundesministerium für Forschung und Technologie, Hamburg/Bonn, pp. 19-67.

Ahne, W., G. Kurath and J. Winton. 1998. A ribonuclease protection assay can distinguish spring viremia of carp virus from pike fry rhabdovirus. *Bulletin of the European Association of Fish Pathologists* 18: 220-224.

Ahne, W., H.V. Bjorklund, S. Essbauer, N. Fijan, G. Kurath and J.R. Winton. 2002. Spring viremia of carp (SVC). *Diseases of Aquatic Organisms* 52: 261-272.

Alexandrino, A.C., M.J.T. Ranzani-Paiva and L.A. Romano. 1998. Identificación de viremia primaveral de la carpa (VPC) *Carrassius auratus* en San Pablo, Brasil. *Revista Ceres* 45: 125-137.

Alikin, Y.S., I.S. Shchelkunov, T.I. Shchelkunova, O.A. Kupinskaya, V.I. Masycheva, V.P. Klimenko and V.A. Fadina. 1996. Prophylactic treatment of viral diseases in fish using native RNA linked to soluble and corpuscular carriers. *Journal of Fish Biology* 49: 195-205.

Amend, D.F., T. McDowell and R.P. Hedrick. 1984. Characteristics of a previously unidentified virus from channel catfish (*Ictalurus punctatus*). *Canadian Journal of Fisheries and Aquatic Sciences* 41: 807-811.

Amita, K., M. Oe, H. Matoyama, N. Yamaguchi and H. Fukuda. 2002. A survey of koi herpesvirus and carp edema virus in colorcarp cultured in Niigata Prefecture, Japan. *Fish Pathology* 37: 197-198.

Anonymous. 2005. KHV information from Momotaro koi farm. *Accessed at:* http://www.momotaro-koi.org/english/khv/owabi.html.

Antychowicz, J. 2000. Zwalczanie chorób ryb w krajach Unii Europejskiej. *Medycyna Weterynaryjna* 56: 293-295.

Ariel, E. and N.J. Olesen. 2001. Assessment of a commercial kit collection for diagnosis of the fish viruses: IHNV, IPNV, SVCV and VHSV. *Bulletin of the European Association of Fish Pathologists* 21: 6-11.

Attoui, H., Q. Fang, F.M. Jaafar, J.F. Cantaloube, P. Biagini, P. deMicco and X. deLamballerie. 2002. Common evolutionary origin of aquareoviruses and orthoreoviruses revealed by genome characterization of Golden shiner reovirus, Grass carp reovirus, Striped bass reovirus and golden ide reovirus (genus *Aquareovirus*, family Reoviridae). *Journal of General Virology* 83: 1941-1951.

Bachmann, P.A. and W. Ahne. 1973. Isolation and characterization of agent causing swimbladder inflammation in carp. *Nature (London)* 244: 235-237.

Bachmann, P.A. and W. Ahne. 1974. Biological properties and identification of the agent causing swimbladder inflammation in carp. *Archiv für Gesampte Virusforschung* 44: 261-269.

Barnes, A., A.G. Owen, S.W. Feist and D. Bucke. 1993. An investigation into the occurrence of epidermal hyperplasia and papilloma in barbel (*Barbus barbus* L.) from a river in southern England. *Bulletin of the European Association of Fish Pathologists* 13: 115-118.

Basurco, B. and J.M. Coll. 1989. Spanish isolates and reference strains of viral *Haemorrhagic septicaemia* virus show similar protein size patterns. *Bulletin of the European Association of Fish Pathologists* 9: 92-95.

Baudouy, A.M. 1975. Virémie printanière de la carpe: premiers isolements du virus en France. *Bulletin de l'Office International des Epizooties* 83: 717-722.

Baudouy, A.M. 1978. Relation hôte-virus au cours de la Virémie Printanière de la Carpe. *Comptes Rendus Hebdomadaires Des Seances De l'Academie Des Sciences* D286: 1225-1228.

Baudouy, A.M., M. Danton and G. Merle. 1980a. Experimental infection of susceptible carp fingerlings with spring viremia of carp virus, under wintering environmental conditions. In: *Fish Diseases*. Third COPRAQ-Session, W. Ahne (ed.). Springer-Verlag, Berlin, pp. 23-27.

Baudouy, A.M., M. Danton and G. Merle. 1980b. Virémie printanière de la carpe. Résultats de contaminations expérimentales effectuées au printemps. *Annales de Recherches Vétérinaires* 11: 245-249.

Baudouy, A.M., M. Danton and G. Merle. 1980c. Virémie printanière de la carpe: étude expérimentale de l'infection évoluant à différentes températures. *Annales de l'Institut Pasteur/Virologie* E131: 479-488.

Bercovier, H., Y. Fishman, R. Nahary, S. Sinai, A. Zlotkin, M. Eyngor, O. Gilad, A. Eldar and R.P. Hedrick. 2005. Cloning of the koi herpesvirus (KHV) gene encoding thymidine kinase and its use for a highly sensitive PCR based diagnosis. *BMC Microbiology* 5: 1-9.

Bergmann, S. 2003. An overview of the diseases affecting cyprinids in Germany. In: *Report on the Workshop on Diagnostic Techniques for Fish Diseases, with Special Emphasis on Carp*, p. 20. *Accessed at:* http://www.eu-crlfish.org/wordDocs/workshops/_Toc 50445251.

Bergmann, S.M., P. Lutze, U. Fischer and D. Fichtner. 2005. Possible vectors for koi herpesvirus (KHV) infection. *European Association of Fish Pathologists. 12th International Conference 'Diseases of Fish and Shellfish' (Abstract)* O-001-p. 6.

Bergmann, S.M., J. Kempter, J. Sadowski and D. Fichtner. 2006. First detection, confirmation and isolation of koi herpesvirus (KHV) in cultured common carp (*Cyprinus carpio* L.) in Poland. *Bulletin of the European Association of Fish Pathologists* 26: 97-104.

Bernet, D. and T. Wahli. 2002. First report of SVC infection in koi carp in Switzerland. *Bulletin of the European Association of Fish Pathologists* 22: 229-231.

Berry, E.S., T.B. Shea and J. Gabliks. 1983. Two iridovirus isolates from *Carassius auratus*. *Journal of Fish Diseases* 6: 501-510.

Békési, L. and L. Csontos. 1985. Isolation of spring viraemia of carp virus from asymptomatic broodstock carp, *Cyprinus carpio* L. *Journal of Fish Diseases* 8: 471-472.

Békési, L. and E. Szabó. 1977. Rhabdovirus carpio elso izolalasa hazai pontyallomanyokbol. *Magyár Àllatorvosok Lapja* 32: 369-371.

Békési, L. and E. Szabó. 1979. A rhabdovirus isolated from carps in Hungary. Experimental infection of carps and resistance to the virus. *Acta Microbiologica Academiae Scientarium Hungaricae* 26: 193-197.

Békési, L., F. Rátz and E. Szabó. 1979. Adatok az ún. pontyhimlő (*epithelioma papulosum*) oktánahoz. *Magyár Àllatorvosok Lapja* 34: 101-104.

Bin, C.S. 2005a. Koi herpesvirus disease in Singapore. OIE *Disease Information* 18 No. 39.

Bin, C.S. 2005b. Koi herpesvirus disease in Singapore. Follow-up report No. 1 (Final report). *OIE Disease Information* 18 No. 42.

Björklund, H.V., E.J. Emmenegger and G. Kuràth. 1995. Comparison of the polymerases (L genes) of spring viremia of carp virus and infectious hematopoietic necrosis virus. *Veterinary Research* 26: 394-398.

Björklund, H.V., K.H. Higman and G. Kurath. 1996. The glycoprotein genes and gene junctions of the fish rhabdoviruses spring viremia of carp virus and hirame rhabdovirus—Analysis of relationships with other rhabdoviruses. *Virus Research* 42: 65-80.

Body, A., F. Lieffrig, G. Charlier and A. Collard. 2000. Isolation of virus-like particles from koi (*Cyprinus carpio*) suffering gill necrosis. *Bulletin of the European Association of Fish Pathologists* 20: 87-88.

Brady, Y.J. and J.A. Plumb. 1988. Serological comparisons of golden shiner virus, chum salmon reovirus, reovirus 13P2 and catfish reovirus. *Journal of Fish Diseases* 11: 441-443.

Brady, Y.J. and J.A. Plumb. 1991. Replication of four aquatic reoviruses in experimentally infected golden shiners (*Notemigonus crysoleucas*). *Journal of Wildlife Diseases* 27: 463-466.

Brady, Y.J., J.A. Plumb and J.R. Winton. 1993. Survival in pond water of four reoviruses isolated from aquatic animals. *Journal of Applied Aquaculture* 2: 85-91.

Bretzinger, A., T. Fischer-Scherl, M. Oumouna, R. Hoffmann and U. Truyen. 1999. Mass mortalities in koi carp, *Cyprinus carpio*, associated with gill and skin disease. *Bulletin of the European Association of Fish Pathologists* 19: 182-185.

Bucke, D. and J. Finlay. 1979. Identification of spring viraemia in carp (*Cyprinus carpio* L.) in Great Britain. *Veterinary Record* 104: 69-71.

Cai, W. 1992. Isoenzymatic changes in grass carp, *Ctenopharyngodon idellus* Cuvier & Valenciennes, affected with haemorrhagic disease. *Journal of Fish Diseases* 15: 305-312.

Calle, P.P., T. McNamara and Y. Kress. 1995. Herpesvirus associated cutaneous papillomas in koi carp (*Cyprinus carpio*). *Proceedings of the International Association of Aquatic Animal Medicine* 26: 81-82.

Calle, P.P., T. McNamara and Y. Kress. 1999. Herpesvirus-associated papillomas in koi carp (*Cyprinus carpio*). *Journal of Zoo and Wildlife Medicine* 30: 165-169.

Chang, P.H., S.H. Lee, H.C. Chiang and M.H. Jong. 1999. Epizootic of herpes-like virus infection in goldfish, *Carassius auratus* in Taiwan. *Fish Pathology* 34: 209-210.

Chen, L., Y. Chen and D. Ni. 1994. The rational design of pond fish culture with reference to the principles of yield increase IV. Systematic prevention and cure of infectious diseases of grass carp. *Acta Hydrobiologica Sinica* 18: 1-7.

Chen, M.-M., G.-H. Kou and S.-N. Chen. 1993. Establishment and characterization of a cell line persistently infected with infectious pancreatic necrosis virus. *Bulletin of the Institute of Zoology, Academia Sinica* 32: 265-272.

Chen, Y. and Y. Jiang. 1983. Morphology and its physical and chemical characters of reovirus of grass carp. *Science Bulletin* 18: 1138-1140.

Chen, Y. and Y. Jiang. 1984. Morphological and physico-chemical characterization of the hemorrhagic virus of grass carp. *Kexue Tongbao* 29: 832-835.

Chen, Y., H. Gao, Y. Jiang and Y. Wang. 1985. A study of pathogenesis of haemorrhage of grass carp and its immunization with inactivated vaccine. In: *Proceedings of the Asian Symposium on Freshwater Fish Culture*, Y. Chi (ed.). The China Society of Fisheries, Beijing, pp. 332-342.

Chen, Y., C. Yang and R. Yao. 1988. The relationship between hemorrhagic disease of grass carp (*Ctenopharyngodon idellus*) and ecological-environment. *Acta Ecologica Sinica* 8: 242-248.

Chen, Y., W. Wang, L. Ke and Y. Cai. 1992a. Glycoproteins and structural polypeptide antigenicity of grass carp hemorrhage virus (GCHV). *Chinese Journal of Virology* 8: 57-61.

Chen, Y., J. Ye and Z. Shen. 1994. Effect of henbane and tissue vaccine supplement on the growth-promoting and disease-resistance of grass carp *Ctenopharyngodon idellus*. *Journal of Zhejiang College of Fisheries* 13: 104-109.

Chen, Z.H., Y.L. Chen, Y. Shi, X.P. Yie and G.Z. Yang. 1992b. Microcarrier culture of fish cells and viruses in cell culture bioreactor. *Canadian Journal of Microbiology* 38: 222-225.

Cho, M.-Y., S.-K. Shon and S.I. Park. 2005. The status of viral diseases of carp in Korea: its control and research development. *Bulletin of Fisheries Research Agency* Supplement No. 2: 23-25.

Choi, D.L., S.G. Sohn, J.D. Bang, J.W. Do and M.S. Park. 2004. Ultrastructural identification of a herpes-like virus infection in common carp *Cyprinus carpio* in Korea. *Diseases of Aquatic Organisms* 61: 165-168.

Clark, H.F. and E.Z. Soriano. 1974. Fish rhabdovirus replication in non-piscine cell culture: new system for the study of rhabdovirus-cell interaction in which the virus and cell have different temperature optima. *Infection and Immunity* 10: 180-188.

Clerx, J.P.M., M.C. Horzinek and D.M.E. Osterhaus. 1978. Neutralization and enhancement of infectivity of non-salmonid fish rhabdoviruses by rabbit and pike immune sera. *Journal of General Virology* 40: 297-308.

Cohen, J. and G. Lenoir. 1974. Ultrastructure et morphologie de quatre rhabdovirus de poissons. *Annales de Recherches Vétérinaires* 5: 443-450.

Dangschat, H. 1979. Zur Verbreitung von Infektionskrankheiten bei einheimischen Nutzfischen. *Berliner und Münchener Tierärztliche Wochenschrift* 92: 318-322.

Daud, H.M. and C. Agius. 1987. Experimental infection of common carp, *Cyprinus carpio* L., by infectious pancreatic necrosis. *Journal of Fish Biology* 31 (Supplement A): 257-258.

Deng, C.-X., X.-Q. Yang and H.-Z. Chen. 1985. Susceptibility to grass carp reovirus (GCRV) of several fish cell lines. *Acta Hydrobiologica Sinica* 9: 351-358.

Denham, K. 2003. Koi herpesvirus in wild fish. *Veterinary Record* 153: 507.

Deuter, A. and P.-J. Enzmann. 1986. Comparative biochemical and serological studies on two fish-pathogenic rhabdoviruses (VHS-V and SVC-V). *Journal of Veterinary Medicine* B33: 36-46.

Di, Z.-Y. and H.-X. Yu. 1986. Preliminary observation of histological and ultramicroscopic structure on the kidney (normal and haemorrhagic diseased) of black carp (*Mylopharyngodon piceus*). *Acta Hydrobiologica Sinica* 10: 224-227.

Dikkeboom, A.L., C. Radi, K. Toohey-Kurth, S.V. Marcquenski, M. Engel, A.E. Goodwin, K. Way, D.M. Stone and C.B. Longshaw. 2004. First report of spring viremia of carp virus (SVCV) in wild common carp in North America. *Journal of Aquatic Animal Health* 16: 169-178.

Ding, Q., L. Yu, L. Ke and Y. Cai. 1991. Study on infecting other fishes with grass carp hemorrhage virus. *Virologica Sinica* 6: 371-373.

Dishon, A., A. Perelberg, J. Bishara-Shieban, M. Ilouze, M. Davidovich, S. Werker and M. Kotler. 2005. Detection of carp interstitial nephritis and gill necrosis virus in fish droppings. *Applied and Environmental Microbiology* 71: 7285-7291.

Dixon, P.F. 1997. Immunization with viral antigens: viral diseases of carp and catfish. In: *Fish Vaccinology*, R. Gudding, A. Lillehaug, P.J. Midtlyng and F. Brown (eds.). S. Karger, Basel, pp. 221-232.

Dixon, P.F., A.-M. Hattenberger-Baudouy and K. Way. 1994. Detection of carp antibodies to spring viraemia of carp virus by a competitive immunoassay. *Diseases of Aquatic Organisms* 19: 181-186.

Dixon, P.F. and C.B. Longshaw. 2005. Assessment of commercial test kits for identification of spring viraemia of carp virus. *Diseases of Aquatic Organisms* 67: 25-29.

Dopazo, C.P., I. Bandin, C. Rivas, C. Cepeda and J.L. Barja. 1996. Antigenic differences among aquareoviruses correlate with previously established genogroups. *Diseases of Aquatic Organisms* 26: 159-162.

Egusa, S. 1992. *Infectious Diseases of Fish*. A.A. Balkema, Brookfield.

Einer-Jensen, K., H. Björklund, S. Oreshkova, I. Shchelkunov, T. Vesely and N. Lorenzen. 2002. Detection and typing of fish viruses. *Bulletin of the European Association of Fish Pathologists* 22: 158-165.

Faisal, M. and W. Ahne. 1980. Use of Immunoperoxidase technique for detection of fish virus antigens. In: *Fish Diseases*, W. Ahne (ed.). Third COPRAQ-Session. Springer-Verlag, Berlin, pp. 186-192.

Faisal, M. and W. Ahne. 1983. Quantitativer Nachweis des Spring Viremia of Carp Virus (SVCV)-Antigens mit Hilfe der Immunofluoreszenz-und Immunoperoxidaseplaque-stechnik. *Zentralblatt Fur Veterinarmedizin-Reihe* B30: 466-470.

Faisal, M. and W. Ahne. 1984. Spring viremia of carp virus (SVCV): comparison of immunoperoxidase, fluorescent antibody and cell culture isolation techniques for detection of antigen. *Journal of Fish Diseases* 7: 57-64.

Fang, Q., H. Jiang and L. Ke. 1997. Preparation of grass carp hemorrhage virus antigen subunit. *Virologica Sinica* 12: 77-82.

Fang, Q., H. Attoui, J.F. Cantaloube, P. Biagini, Z. Zhu, P. de Micco and X. de Lamballerie. 2000. Sequence of genome segments 1, 2, and 3 of the grass carp reovirus (Genus *Aquareovirus*, family Reoviridae). *Biochemical and Biophysical Research Communications* 274: 762-766.

Fang, Q., Q.Q. Ding, Y.P. Wang and Z.Y. Zhu. 2003. Expression of grass carp reovirus RNA polymerase gene and purification of its product. *Virologica Sinica* 18: 169-173.

Fauquet, C.M., M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball. 2005. *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*. Academic Press, London.

Fijan, N. 1972. Infectious dropsy in carp—A disease complex. *Symposium of the Zoological Society of London* 30: 39-51.

Fijan, N. 1984. Vaccination of fish in European pond culture: prospects and constraints. In: *Fish, Pathogens and Environment in European Polyculture*, J. Oláh (ed.). Akadémiai Kiadó, Budapest, pp. 233-241.

Fijan, N. 1988. Vaccination against spring viraemia of carp. In: *Fish Vaccination*, A.E. Ellis (ed.). Academic Press, London, pp. 204-215.

Fijan, N. 1999. Spring viraemia of carp and other viral diseases and agents of warm-water fish. In: *Fish Diseases and Disorders 3: Viral, Bacterial and Fungal Infections*, P.T.K. Woo and D.W. Bruno (eds.). CAB International, New York, pp. 177-244.

Fijan, N., Z. Petrinec, D. Sulimanovic and L.O. Zwillenberg. 1971. Isolation of the viral causative agent from the acute form of infectious dropsy of carp. *Veterinarsky Arhiv* 41: 125-138.

Fijan, N., Z. Petrinec, Z. Stancl, M. Dorson and M. Le Berre. 1977. Hyperimmunization of carp with *Rhabdovirus carpio*. *Bulletin de l'Office International des Epizooties* 87: 439-440.

Fijan, N., D. Sulimanovic, M. Bearzotti, D. Muzinic, L.O. Zwillenberg, S. Chilmonczyk, J.F. Vautherot and P. de Kinkelin. 1983. Some properties of the *Epithelioma papulosum cyprini* (EPC) cell line from carp *Cyprinus carpio*. *Annales de l'Institut Pasteur/Virologie* 134: 207-220.

Fijan, N., Z. Matasin, Z. Jeney, J. Oláh and L.O. Zwillenberg. 1984. Isolation of Rhabdovirus carpio from sheatfish (*Silurus glanis*) fry. In: *Fish, Pathogens and Environment in European Polyculture*, J. Oláh (ed.). Akadémiai Kiadó, Budapest, pp. 17-24.

Gao, L., X. Shi, H. Liu and Y. Jiang. 2002. Detection of spring viremia of carp virus (SVCV) gene using reverse-polymerase chain-reaction (RT-PCR). *Acta Hydrobiologica Sinica* 26: 452-456.

Gessner, C. 1558. *Historiae Animalium Liber IIII, qui est Piscium et Aquatilium Animantium Natura*. Tiguri, Zurich.

Ghittino, P., E. Beccaria and A. Ferrari. 1980. Caso di ascite infettiva della carpa modernamente diagnosticato anche in Italia. *Rivista Italiana di Piscicoltura e Ittiopatologia* 15: 1-4.

Gilad, O., S. Yun, K. Andree, M. Adkinson, A. Zlotkin, H. Bercovier, A. Eldar and R.P. Hedrick. 2001. Characteristics of the koi herpesvirus (KHV) and development of a polymerase chain reaction (PCR) assay to detect the virus in koi *Cyprinus carpio koi*. *Fish Health Newsletter* 29: 4.

Gilad, O., S. Yun, K.B. Andree, M.A. Adkison, A. Zlotkin, H. Bercovier, A. Eldar and R.P. Hedrick. 2002. Initial characteristics of koi herpesvirus and development of a polymerase chain reaction assay to detect the virus in koi, *Cyprinus carpio koi*. *Diseases of Aquatic Organisms* 48: 101-108.

Gilad, O., S. Yun, M.A. Adkison, K. Way, N.H. Willits, H. Bercovier and R.P. Hedrick. 2003. Molecular comparison of isolates of an emerging fish pathogen, koi herpesvirus, and the effect of water temperature on mortality of experimentally infected koi. *Journal of General Virology* 84: 2661-2667.

Gilad, O., S. Yun, F.J. Zagmutt-Vergara, C.M. Leutenegger, H. Bercovier and R.P. Hedrick. 2004. Concentrations of a koi herpesvirus (KHV) in tissues of experimentally infected *Cyprinus carpio koi* as assessed by real-time TaqMan PCR. *Diseases of Aquatic Organisms* 60: 179-187.

Goodwin, A. 2000. A new koi virus. *Aquaculture Magazine* March/April: 25-26.

Goodwin, A.E. 2002. First report of spring viremia of carp virus (SVCV) in North America. *Journal of Aquatic Animal Health* 14: 161-164.

Goodwin, A.E., J.E. Peterson, T.R. Meyers and D.J. Money. 2004. Transmission of exotic fish viruses: The relative risks of wild and cultured bait. *Fisheries* 29: 19-23.

Goodwin, A.E., L.H. Khoo, S.E. LaPatra, A. Bonar, D.W. Key, M. Garner, M.V. Lee and L. Hanson. 2006a. Goldfish hematopoietic necrosis herpesvirus (Cyprinid herpesvirus 2) in the USA: Molecular confirmation of isolates from diseased fish. *Journal of Aquatic Animal Health* 18: 11-18.

Goodwin, A.E., G.E. Merry and J. Sadler. 2006b. Detection of the herpesviral hematopoietic necrosis disease agent (Cyprinid herpesvirus 2) in moribund and healthy goldfish: Validation of a quantitative PCR diagnostic method. *Diseases of Aquatic Organisms* 69: 137-142.

Goodwin, A.E., D.K. Nayak and R.S. Bakal. 2006c. Natural infections of wild creek chubs (*Semotilus atromaculatus*) and cultured fathead minnows (*Pimephales promelas*) by Chinese grass carp reovirus (golden shiner virus). *Journal of Aquatic Animal Health* 18: 35-38.

Granzow, H., F. Weiland, D. Fichtner, H. Schütze, A. Karger, E. Mundt, B. Dresenkamp, P. Martin and T.C. Mettenleiter. 2001. Identification and ultrastructural characterization of a novel virus from fish. *Journal of General Virology* 82: 2849-2859.

Gray, W.L., L. Mullis, S.E. LaPatra, J.M. Groff and A. Goodwin. 2002. Detection of koi herpesvirus DNA in tissues of infected fish. *Journal of Fish Diseases* 25: 171-178.

Groff, J.M., S.E. LaPatra, R.J. Munn and J.G. Zinkl. 1998. A viral epizootic in cultured populations of juvenile goldfish due to a putative herpesvirus etiology. *Journal of Veterinary Diagnostic Investigation* 10: 375-378.

Gunimaladevi, I., T. Kono, M.N. Venugopal and M. Sakai. 2004. Detection of koi herpesvirus in common carp, *Cyprinus carpio* L., by loop-mediated isothermal amplification. *Journal of Fish Diseases* 27: 583-589.

Guo, Q. and Y. Jiang. 1993. Histopathological studies of the hemorrhagic disease of grass carp infected by two types of grass carp reovirus. In: *Transactions in Research on Fish Diseases*. Department of Fish Diseases, Institute of Hydrobiology, Chinese Academy of Sciences (eds.). China Ocean Press, Beijing, China, pp. 7-11.

Haempel, O. 1951. Neue Untersuchungen über die Pockenkrankheit der Fische. *Archiv für Fischereiwissenschaft* 3: 55-64.

Haenen, O.L.M. and A. Davidse. 1989. Isolation of pike fry rhabdovirus from roach (*Rutilus rutilus*). *Bulletin of the European Association of Fish Pathologists* 9: 116.

Haenen, O.L.M. and A. Davidse. 1993. Comparative pathogenicity of two strains of pike fry rhabdovirus and spring viremia of carp virus for young roach, common carp, grass carp and rainbow trout. *Diseases of Aquatic Organisms* 15: 87-92.

Haenen, O.L.M., K. Way, S.M. Bergmann and E. Ariel. 2004. The emergence of koi herpesvirus and its significance to European aquaculture. *Bulletin of the European Association of Fish Pathologists* 24: 293-307.

Halvorsen, O. 1980. A roach (*Rutilus rutilus* L.) with pox disease. *Nytt Magazin for Zoologi* 14: 5-6.

Hattenberger-Baudouy, A.-M., M. Danton and G. Merle. 1987. Infection expérimentale de l'alevin de carpe *Cyprinus carpio* L. par le virus de la virémie printanière de la carpe (V.P.C.) en eau chaude. *Bulletin Français de Pêche et Pisciculture* 307: 89-90.

Hedrick, R.P., R. Rosemark, D. Aronstein, J.R. Winton, T. McDowell and D.F. Amend. 1984. Characteristics of a new reovirus from channel catfish (*Ictalurus punctatus*). *Journal of General Virology* 65: 1527-1534.

Hedrick, R.P., J.M. Groff, T. McDowell and W.H. Wingfield. 1989. Characteristics of reoviruses isolated from cyprinid fishes in California, USA. In: *Viruses of Lower Vertebrates*, W. Ahne and E. Kurstak (eds.). Springer-Verlag, Berlin, pp. 241-249.

Hedrick, R.P., J.M. Groff, M.S. Okihiro and T.S. McDowell. 1990. Herpesviruses detected in papillomatous skin growths of koi carp (*Cyprinus carpio*). *Journal of Wildlife Diseases* 26: 578-581.

Hedrick, R.P., O. Gilad, S. Yun and J.V. Spangenberg. 1999. An herpesvirus associated with mass mortality of juvenile and adult koi *Cyprinus carpio*. *Fish Health News* 27: 7.

Hedrick, R.P., O. Gilad, S. Yun, J.V. Spangenberg, G.D. Marty, R.W. Nordhausen, M.J. Kebus, H. Bercovier and A. Eldar. 2000. A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. *Journal of Aquatic Animal Health* 12: 44-57.

Hedrick, R.P., T.B. Waltzek and T.S. McDowell. 2006. Susceptibility of koi carp, common carp, goldfish, and goldfish × common carp hybrids to cyprinid herpesvirus-2 and herpesvirus-3. *Journal of Aquatic Animal Health* 18: 26-34.

Hill, B.J. 1977. Studies on SVC virulence and immunization. *Bulletin de l'Office International des Epizooties* 87: 455-456.

Hill, B.J. 1982. Infectious pancreatic necrosis virus and its virulence. In: *Microbial Diseases of Fish*, R.J. Roberts (ed.). Society for General Microbiology, Reading, pp. 91-114.

Hill, B.J. and K. Way. 1995. Serological classification of infectious pancreatic necrosis (IPN) virus and other aquatic birnaviruses. *Annual Review of Fish Diseases* 5: 55-77.

Hill, B.J., B.O. Underwood, C.J. Smale and F. Brown. 1975. Physico-chemical and serological characterization of five rhabdoviruses infecting fish. *Journal of General Virology* 27: 369-378.

Hines, R.S., G.W. Wohlfarth, R. Moav and G. Hulata. 1974. Genetic differences in susceptibility to two diseases among strains of the common carp. *Aquaculture* 3: 187-197.

Hoffmann, B., H. Schütze and T.C. Mettenleiter. 2002. Determination of the complete genomic sequence and analysis of the gene products of the virus of Spring Viremia of Carp, a fish rhabdovirus. *Virus Research* 84: 89-100.

Hoppe, K. and U. Wernery. 1985. Ergebnisse serologischer Untersuchungen zum Nachweis von Antikörpern gegen die Viren der infektiösen Pankreasnekrose (IPN), der viralen hämorrhagischen Sepikämie (VHS) und der Fruhlingsvirämie (SVC) in Schleswig-Holstein sowie deren rechtliche Auswirkungen. *Deutsche Tierärztliche Wochenschrift* 92: 172-174.

Huat, L.K., S. Kueh and P.Y. Kwang. 2004. Current status of transboundary fish diseases in Singapore: occurrence, surveillance, research and training. In: *Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training*, C.R. Lavilla-Pitogo and K. Nagasawa (eds.). SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, pp. 199-210.

Hutoran, M., A. Ronen, A. Perelberg, M. Ilouze, A. Dishon, I. Bejerano, N. Chen and M. Kotler. 2005. Description of an as yet unclassified DNA virus from diseased *Cyprinus carpio* species. *Journal of Virology* 79: 1983-1991.

Inchausty, V.H. and R.A. Heckmann. 1996. Neoplasm of a viral origin from the swimbladder in carp, *Cyprinus carpio*, Linnaeus, 1758, from Utah Lake, Utah County, Utah. *Journal of Aquariculture and Aquatic Sciences* 8: 7-15.

Ishioka, T., M. Yoshizumi, S. Izumi, K. Suzuki, H. Suzuki, K. Kozawa, M. Arai, K. Nobusawa, Y. Morita, M. Kato, T. Hoshino, T. Iida, K. Kosuge and H. Kimura. 2005. Detection and sequence analysis of DNA polymerase and major envelope protein genes in koi herpesviruses derived from *Cyprinus carpio* in Gunma prefecture, Japan. *Veterinary Microbiology* 110: 27-33.

Iwanowicz, L.R. and A.E. Goodwin. 2002. A new bacilliform fathead minnow rhabdovirus that produces syncytia in tissue culture. *Archives of Virology* 147: 899-915.

Iwanowicz, L.R., A.E. Goodwin and N. Heil. 2000. A small RNA virus isolated from apparently healthy wild sandbar shiners, *Notropis scepticus* (Jordan & Gilbert). *Journal of Fish Diseases* 23: 349-352.

Jeremic, S., J.-D. Dobrila and V. Radosavljevic. 2004. Dissemination of spring viraemia of carp (SVC) in Serbia during the period 1992-2002. *Acta Veterinaria (Beograd)* 54: 289-299.

Jiang, Y. 1995. Advances in fish virology research in China. In: *Diseases in Asian Aquaculture II*, M. Shariff, J.R. Arthur and R.P. Subasinghe (eds.). Fish Health Section, Asian Fisheries Society, Manila, pp. 211-225.

Jiang, Y. 2004. Hemorrhagic disease of grass carp—Disease card. NACA, Bangkok, pp. 1-8. *Accessed at:* www.enaca.org/Health/DiseaseLibrary/Hemorrhagic_Disease.pdf.

Jiang, Y. and W. Ahne. 1989. Some properties of the etiological agent of the hemorrhagic disease of grass carp and black carp. In: *Viruses of Lower Vertebrates*, W. Ahne and E. Kurstak (eds.). Springer-Verlag, Berlin, pp. 227-240.

Jiang, Y., W. Ahne, Y. Li and M. Ogawa. 1991a. Isolation of an aquareovirus from common carp (*Cyprinus carpio*) in the P.R. China. In: *Viruses of Lower Vertebrates*, J.L. Fryer (ed.). Oregon State University, Corvallis, Oregon, pp. 287-292.

Jiang, Y., Y. Li and Z. Li. 1991b. Electron microscopic observation of pathogen of carp-pox disease. *Acta Hydrobiologica Sinica* 15: 193-195.

Jiang, Y., W. Ahne and P.E.V. Jørgensen. 1994. Comparison of the characteristics of four aquareovirus isolated from fish in P.R. China. *International Symposium on Aquatic Animal Health: Program and Abstracts, Davis, California, USA. University of California, School of Veterinary Medicine, 1994* p1. (Abstract).

Johnson, M.C., J.M. Maxwell, P.C. Loh and J.-A.C. Leong. 1999. Molecular characterization of the glycoproteins from two warm water rhabdoviruses: snakehead rhabdovirus (SHRV) and rhabdovirus of penaeid shrimp (RPS)/spring viremia of carp virus (SVCV). *Virus Research* 64: 95-106.

Jørgensen, P.E.V., N.J. Olesen, W. Ahne and N. Lorenzen. 1989. SVCV and PFR viruses: Serological examination of 22 isolates indicates close relationship between the two fish rhabdoviruses. In: *Viruses of Lower Vertebrates*, W. Ahne and E. Kurstak (eds.). Springer-Verlag, Berlin, pp. 349-366.

Jung, S.J. and T. Miyazaki. 1995. Herpesviral haematopoietic necrosis of goldfish, *Carassius auratus* (L). *Journal of Fish Diseases* 18: 211-220.

Kanellos, T., I.D. Sylvester, F. D'Mello, C.R. Howard, A. Mackie, P.F. Dixon, K.-C. Chang, A. Ramstad, P.J. Midtlyng and P.H. Russell. 2006. DNA vaccination can protect *Cyprinus carpio* against spring viraemia of carp virus. *Vaccine* 24: 4927-4933.

Kasai, H., Y. Muto and M. Yoshimizu. 2005. Virucidal effects of ultraviolet, heat treatment and disinfectants against koi herpesvirus (KHV). *Fish Pathology* 40: 137-138.

Ke, L., Q. Fang and Y. Cai. 1990. Characteristics of a novel isolate of grass carp hemorrhagic virus. *Acta Hydrobiologica Sinica* 14: 153-159.

Ke, L., W. Wang, Q. Fang and Y. Cai. 1992. Studies on the *in vitro* translation of grass carp hemmorrhage virus and its proteins. *Chinese Journal of Virology* 8: 169-173.

Kinkelin, P. de and M. Le Berre. 1974. Rhabdovirus des poissons II propriétés *in vitro* du virus de la virémie printanière de la carpe. *Annales de Microbiologie de l'Institut Pasteur* A125: 113-124.

Kinkelin, P. de, M. Dorson and A.M. Hattenberger-Baudouy. 1982. Interferon synthesis in trout and carp after viral infection. *Developmental and Comparative Immunology* Supplement 2: 167-174.

Kocis, J., P. Kosuth and G. Benko. 1983. The diagnosis of spring viremia of carps. *Folia Veterinaria* 27: 67-77.

Kocylowski, B. 1965. The role of virus in septicaemia of carp (*Cyprinus carpio*) and pox of carp. Influence of environment on infection. *Annals of the New York Academy of Sciences* 126: 616-619.

Konstantinov, A. 1977. A case of pox in the fish bleak (*Alburnus alburnus*). *Bulgarian Academy of Sciences, General and Comparative Pathology* 3: 94-96.

Korkea-aho, T., A. Vainikka and J. Taskinen. 2006. Factors affecting the intensity of epidermal papillomatosis in populations of roach, *Rutilus rutilus* (L.), estimated as scale coverage. *Journal of Fish Diseases* 29: 115-122.

Kortet, R., A. Vainikka and J. Taskinen. 2003. Effect of epidermal papillomatosis on survival of the freshwater fish *Rutilus rutilus*. *Diseases of Aquatic Organisms* 57: 163-465.

Koutná, M., T. Vesely, I. Psikal and J. Hulová. 2003. Identification of spring viraemia of carp virus (SVCV) by combined RT-PCR and nested PCR. *Diseases of Aquatic Organisms* 55: 229-235.

Kortet, R., A. Vainikka and J. Taskinen. 2002. Epizootic cutaneous papillomatosis in roach *Rutilus rutilus*: sex and size dependence, seasonal occurrence and between-population differences. *Diseases of Aquatic Organisms* 52: 185-190.

Kortet, R., J. Taskinen, A. Vainikka and H. Ylonen. 2004. Breeding tubercles, papillomatosis and dominance behaviour of male roach (*Rutilus rutilus*) during the spawning period. *Ethology* 110: 591-601.

Kortet, R., A. Vainikka, M.J. Rantala, I. Jokinen and J. Taskinen. 2005. Sexual ornamentation, androgens and papillomatosis in male roach (*Rutilus rutilus*). *Evolutionary Ecology Research* 5: 411-419.

Kölbl, O. 1975. Neue Erkenntnisse über die Bauchwassersucht bei Karpfen. *Österreichs Fischerie* 28: 69-72.

Kölbl, O. 1980. Diagnostic de la virémie printanière de la carpe et essais d'immunisation contre cette maladie. *Bulletin de l'Office International des Epizooties* 92: 1055-1068.

Kölbl, O. 1990. Entwicklung eines Impfstoffes gegen die Fruhjahrsviramie der Karpfen (Spring Viraemia of Carp, SVC); Feldversuchserfahrungen. *Tierärztliche Umschau* 45: 624-649.

Kölbl, O. and E. Kainz. 1977. Die Verbreitung des Erregers der infektiösen Bauchwassersucht bzw. der Fruehlingsviräme in den österreichischen Karpfenteichwirtschaften. *Österreichs Fischerie* 30: 80-83.

Kretzschmar, C. and B. Dresenkamp. 1987. Erste Versuche mit dem Festphasen-ELISA zum serologischen Nachweis der Fruhjahrsviramie der Karpfen im Vergleich zum Serumneutralisationstest. *Monatshefte der Veterinärmedizin* 42: 64-67.

Kumar, S., K. Tamura and M. Nei. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* 5: 150-163.

Latiff, F.A. 2004. Current status of transboundary fish diseases in Malaysia: occurrence, surveillance, research and training. In: *Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training*, C.R. Lavilla-Pitogo and K. Nagasawa (eds.). SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, pp. 131-157.

Lenoir, G. 1973. Structural proteins of spring viremia virus of carp. *Biochemical and Biophysical Research Communications* 51: 895-899.

Lenoir, G. and P. de Kinkelin. 1975. Fish rhabdoviruses: Comparative study of protein structure. *Journal of Virology* 16: 259-262.

Lewis, D.H. and J.E. Marks. 1985. Microcultures of *Sarotherodon mossambicus* (Peters) cells: Their use in detecting fish viruses. *Journal of Fish Diseases* 8: 477-488.

Li, H., S. Xu, Y. Liao and B. Wu. 1988. Establishment of cell line PSF from the fibroblast cell of grass carp snout tissue and studies of its biological characteristics. *Journal of the Chinese Academy of Fishery Sciences* 1: 1-8.

Li, J., T. Wang and R. Lu. 1996. Rapid preparation of dsRNA template in reverse transcription (RT)-polymerase chain reaction (PCR). *Journal of the Ocean University of Qingdao* 26: 495-499.

Li, J., T. Wang, L. Zhou, Y. Yi, H. Liu, R. Lu and H. Chen. 1997a. Direct detection of grass carp hemorrhagic virus in infected fish by reverse transcription (RT)-polymerase chain reaction (PCR). *Journal of Fisheries of China* 21: 175-179.

Li, J., T.H. Wang, Y.L. Yi, H.Q. Liu, R.H. Lu and H.X. Chen. 1997b. A detection method for grass carp hemorrhagic virus (GCHV) based on a reverse transcription polymerase chain reaction. *Diseases of Aquatic Organisms* 29: 7-12.

Li, X. and H. Fukuda. 2003. *In vitro* culture of goldfish cell sensitive to goldfish herpesvirus. *Journal of Shanghai Fisheries University* 12: 12-18.

Litvinenko, V.V. and Y.D. Temnikhanov. 1989. Detection of antibodies against *Rhabdovirus carpio* in the blood serum of fish with the use of luminescent microscopy. *Rybnoe Khozyaistvo (Kiev)* 43: 62-64.

Liu, H., L. Gao, X. Shi, T. Gu, Y. Jiang and H. Chen. 2004. Isolation of spring viraemia of carp virus (SVCV) from cultured koi (*Cyprinus carpio koi*) and common carp (*C. carpio carpio*) in PR China. *Bulletin of the European Association of Fish Pathologists* 24: 194-202.

Lu, Y. and P.C. Loh. 1994. Viral structural proteins and genome analyses of the rhabdovirus of penaeid shrimp (RPS). *Diseases of Aquatic Organisms* 19: 187-192.

Lu, Y., E.C.B.J. Nadala, J.A. Brock and P.C. Loh. 1991. A new virus isolate from infectious hypodermal and hematopoietic necrosis virus (IHHNV)-infected penaeid shrimps. *Journal of Virological Methods* 31: 189-196.

Lu, Y., P.C. Loh and E.C.B. Nadala. 1994. Serological studies of the rhabdovirus of penaeid shrimp (RPS) and Its relationship to three other fish rhabdoviruses. *Journal of Fish Diseases* 17: 303-309.

Luo, Y., G. Yang and X. Ye. 1993. Selection of haemorrhagic virus strains from grass carp (*Ctenopharyngodon idella*). *Oceanologia et Limnologia Sinica* 24: 503-506.

Lupiani, B., F.M. Hetrick and S.K. Samal. 1993. Genetic analysis of aquareoviruses using RNA-RNA blot hybridization. *Virology* 197: 475-479.

Macura, B., J. Tesarcik and J. Rehulka. 1983. Survey of methods of specific immunoprophylaxis of carp spring viremia in Czechoslovakia. *Prace VÚRH Vodnany* 12: 50-56.

Mao, S. and J. Shao. 1989. Studies on the reproduction of pathogenic virus of grass carp hemorrhage in fish cells. *Journal of the Hangzhou University of Natural Sciences and Education* 16: 471-475.

Mao, S., Q. Hang, H. Chen, N. Zhang and G. Yang. 1988. Study on cytopathology of two pathogenic viruses of hemorrhage in grass carp, *Ctenopharynogodon idella*. *Oceanologia et Limnologia Sinica* 19: 435-438.

Mao, S., J. Shao, Q. Hang and N. Zhang. 1989. On pathogen of hemorrhage of grass carp (*Ctenopharyngodon idella* C. et V.). *Journal of Fisheries of China* 13: 1-5.

Marcotegui, M.A., A. Estepa, D. Frías and J.M. Coll. 1992. First report of a rhabdovirus affecting carp in Spain. *Bulletin of the European Association of Fish Pathologists* 12: 50-52.

Martin, R. 2003. OMB approves $11.7 million for SVC program. *Fish Farming News* January/February: 3-4.

Masycheva, V.I., Y.S. Alikin, V.P. Klimenko, V.A. Fadina, I.S. Shchelkunov, T.I. Shchelkunova and O.A. Kupinskaya. 1995. Comparative antiviral effects of dsRNA on lower and higher vertebrates. *Veterinary Research* 26: 536-538.

Mawdesley-Thomas, L.E. and D. Bucke. 1967. Fish pox in the roach (*Rutilus rutilus* L.). *Veterinary Record* 81: 56.

Mcallister, P.E., B.C. Lidgerding, R.L. Herman, L.C. Hoyer and J. Hankins. 1985. Viral diseases of fish: First report of carp pox in golden ide (*Leuciscus idus*) in North America. *Journal of Wildlife Diseases* 21: 199-204.

McEntire, M.E., L.R. Iwanowicz and A.E. Goodwin. 2003. Molecular, physical, and clinical evidence that golden shiner virus and grass carp reovirus are variants of the same virus. *Journal of Aquatic Animal Health* 15: 257-263.

McVeigh, S. 2005. KHV identified in South Africa. *Fish Farming International* 31: 27.

Mettenleiter, T.C. 2002. Herpesvirus assembly and egress. *Journal of Virology* 76: 1537-1547.

Mettenleiter, T.C. 2004. Budding events in herpesvirus morphogenesis. *Virus Research* 106: 167-180.

Miaczynski, T.B. 1965. Viral diseases and diseases of uncertain etiology in fish in Poland. *Annals of the New York Academy of Sciences* 126: 620-628.

Min, S., S. Hong and Y. Cai. 1986. The preparation and application of several antisera against fish reovirus (FRV). *Journal of Fisheries of China* 10: 383-388.

Mires, D. 2001. Forecasted supply and demand for comestible fish in Israel—2001-2005. *Israeli Journal of Aquaculture* 53: 5-14.

Miyazaki, T., H. Okamoto, T. Kageyama and T. Kobayashi 2000. Viremia-associated ana-aki-byo, a new viral disease in common carp *Cyprinus carpio* in Japan. *Diseases of Aquatic Organisms* 39: 183-192.

Miyazaki, T., T. Kageyama, M. Miura and T. Yoshida. 2001. Histopathology of viremia-associated ana-aki-byo in combination with *Aeromonas hydrophila* in common carp *Cyprinus carpio* in Japan. *Diseases of Aquatic Organisms* 44: 109-120.

Miyazaki, T., T. Isshiki and H. Katsuyuki. 2005. Histopathological and electron microscopy on sleepy disease of koi *Cyprinus carpio koi* studies in Japan. *Diseases of Aquatic Organisms* 65: 197-207.

Morita, N. and T. Sano. 1990. Regression effect of carp, *Cyprinus carpio* L., peripheral blood lymphocytes on CHV-induced carp papilloma. *Journal of Fish Diseases* 13: 505-511.

Munoz, M.J., A. Castano, T. Blazquez, M. Vega, G. Carbonell, J.A. Ortiz, M. Carballo and J.V. Tarazona. 1994. Toxicity identification evaluations for the investigation of fish kills: a case study. *Chemosphere* 29: 55-61.

Munro, A.L.S., J. Liversidge and K.G.R. Elson. 1976. The distribution and prevalence of infectious pancreatic necrosis virus in wild fish in Loch Awe. *Proceedings of the Royal Society of Edinburgh* B75: 223-232.

Musa, N., N.K. Leong and A. Sunarto. 2005. Koi herpesvirus (KHV)—An emerging pathogen in koi. *Colloquium on Viruses of Veterinary and Public Health Importance, Bangi, Malaysia*, pp. 146-147.

Negele, R.D. 1977. Histopathological changes in some organs of experimentally infected carp fingerlings with *Rhabdovirus carpio*. *Bulletin de l'Office International des Epizooties* 87: 449-450.

Neukirch, M. 2000. Biological characteristics of golden ide reovirus (GIRV). *Bulletin of the European Association of Fish Pathologists* 20: 70-76.

Neukirch, M. and P. Kruse. 1993. Enhancement of the *in vitro* infectivity of V 834 in the presence of trypsin. *Journal of Fish Diseases* 16: 155-159.

Neukirch, M. and U. Kunz. 2001. Isolation and preliminary characterization of several viruses from koi (*Cyprinus carpio*) suffering gill necrosis and mortality. *Bulletin of the European Association of Fish Pathologists* 21: 125-135.

Neukirch, M., K. Böttcher and S. Bunnajirakul. 1999a. Isolation of a virus from koi with altered gills. *Bulletin of the European Association of Fish Pathologists* 19: 221-224.

Neukirch, M., L. Haas, H. Lehmann and V. von Messeling. 1999b. Preliminary characterization of a reovirus isolated from golden ide *Leuciscus idus melanotus*. *Diseases of Aquatic Organisms* 35: 159-164.

Nie, D.-S. and J.-P. Pan. 1985. Diseases of grass carp (*Ctenopharyngodon idellus* Valenciennes, 1844) in China, a review from 1953 to 1983. *Fish Pathology* 20: 323-330.

Nishizawa, T., M. Yoshimizu, J. Winton and T. Kimura. 1992a. Comparison of structural proteins on fish rhabdoviruses. In: *Proceedings of the OJI International Symposium on Salmonid Diseases*, Hokkaido University Press, Sapporo, Japan, pp. 72-79.

Nougayrede, P. 1988. Enquête sur la répartition des virus des salmonidés dans les salmonicultures de la région Aquitaine et des régions limitrophes. *Bulletin Français de Pêche et Pisciculture*, pp. 134-138.

Oh, M.-J., S.-J. Jung, T.J. Choi, H.-R. Kim, K.V. Rajendran, Y.-J. Kim, M.-A. Park and S.-K. Chun. 2001. A viral disease occurring in common carp *Cyprinus carpio* in Korea. *Fish Pathology* 36: 147-151.

OIE 2003a. *Diagnostic Manual for Aquatic Animal Diseases*. OIE, Paris.

OIE 2003b. *Manual of Diagnostic Tests for Aquatic Animals*. Accessed at: http://www.oie.int/eng/normes/fmanual/A_summry.htm.

Ono, S.-I., A. Nagai and N. Sugai. 1986. A histopathological study on juvenile common carp, *Cyprinus carpio*, showing edema. *Fish Pathology* 21: 167-175.

Ono, S.-I., A. Nagai and N. Sugai. 1993. Studies on the diseased juvenile common carp, (*Cyprinus carpio*), showing edema: On the experimental infection with immersion method. *Journal of the Faculty of Marine Science and Technology, Tokai University* 35: 93-101.

Oreshkova, S.F., N.V. Tikunova, I.S. Shchelkunov and A.A. Ilyichev. 1995. Detection of spring viremia of carp virus by hybridization with biotinylated DNA probes. *Veterinary Research* 26: 533-535.

Oreshkova, S.F., I.S. Shchelkunov, N.V. Tikunova, T.I. Shchelkunova, A.T. Puzyrev and A.A. Ilyichev. 1999. Detection of spring viremia of carp virus isolates by hybridization with non-radioactive probes and amplification by polymerase chain reaction. *Virus Research* 63: 3-10.

Ortega, C., E. Planas, J. Docando, J.L. Muzquiz, J.L. Alonso and M.C. Simon. 1993. Epidemiological risk factors affecting the presentation of viral agents in freshwater aquaculture in north-eastern Spain. *Bulletin of the European Association of Fish Pathologists* 13: 154-156.

Osadchaya, E.F. 1964. Vydelenie citopatogennyh agentov ot karpov pri ostroi formi krasnukhi. *Veterinarija* 41: 29.

Osadchaya, E.F. and A.P. Rudenko. 1981. Patogennost virusov, vydelennyh pri krasnuhe (vesennej viremii) karpov i kliniko-morfologicheskaja harakteristika estestvennogo techenija bolezni i v eksperimente. *Rybnoe Khozyaistvo (Kiev)* 32: 66-71.

Oyamatsu, T., N. Hata, K. Yamada, T. Sano and H. Fukuda. 1997a. An etiological study on mass mortality of cultured common carp juveniles showing edema. *Fish Pathology* 32: 81-88.

Oyamatsu, T., H. Matoyama, K.Y. Yamamoto and H. Fukuda. 1997b. A trial for the detection of carp edema virus by using polymerase reaction. *Suisanzoshoku* 45: 247-251.

Penson, S. 2004. Closed for business until Oct 2006. *Angling Times*, Tuesday December 21: 2-3.

Perelberg, A., M. Smirnov, M. Hutoran, A. Diamant, Y. Bejerano and M. Kotler. 2003. Epidemiological description of a new viral disease afflicting cultured *Cyprinus carpio* in Israel. *Israeli Journal of Aquaculture* 55: 5-12.

Perelberg, A., A. Ronen, M. Hutoran, Y. Smith and M. Kotler. 2005. Protection of cultured *Cyprinus carpio* against a lethal viral disease by an attenuated virus vaccine. *Vaccine* 23: 3396-3403.

Peters, F. and M. Neukirch. 1986. Transmission of some fish pathogenic viruses by the heron, *Ardea cinerea*. *Journal of Fish Diseases* 9: 539-544.

Pfeil-Putzien, C. 1978. Experimentelle Übertragung der Frühjahrsvirämie (spring viraemia) der Karpfen durch Karpfenläuse (*Argulus foliaceus*). *Zentralblatt Für Veterinarmedizin-Reihe* B25: 319-323.

Pfeil-Putzien, C. and C. Baath. 1978. Nachweis einer Rhabdovirus-carpio-Infektion bei Karpfen im Herbst. *Berliner und Münchener Tierärztliche Wochenschrift* 91: 445-447.

Pikarsky, E., A. Ronen, J. Abramowitz, B. Levavi-Sivan, M. Hutoran, Y. Shapira, M. Steinitz, A. Perelberg, D. Soffer and M. Kotler. 2004. Pathogenesis of acute viral disease induced in fish by carp interstitial nephritis and gill necrosis virus. *Journal of Virology* 78: 9544-9551.

Plumb, J.A. 1981. Viral diseases of cultured warmwater fish. In: *Proceedings of Republic of China-United States Cooperative Science Seminar on Fish Diseases (NSC Symposium Series No 3)*, National Science Council, Republic of China, pp. 67-73.

Plumb, J.A., P.R. Bowser, J.M. Grizzle and A.J. Mitchell. 1979. Fish viruses: A double-stranded RNA icosahedral virus from a North American cyprinid. *Journal of the Fisheries Research Board of Canada* 36: 1390-1394.

Popkova, T.I. and I.S. Shchelkunov. 1978. Isolation of a virus from carp diseased with gill necrosis. *Rybnoe Khozyaistvo* 4: 34-38.

Prost, M. 1977. Choroby nowotworowe ryb. *Medycyna Weterynaryjna* 33: 705-711.

Qiu, T. and C.L. Luongo. 2003. Identification of two histidines necessary for reovirus mRNA guanylyltransferase activity. *Virology* 316: 313-324.

Qiu, T., R.H. Lu, J. Zhang and Z.Y. Zhu. 2001a. Complete nucleotide sequence of the S10 genome segment of grass carp reovirus (GCRV). *Diseases of Aquatic Organisms* 44: 69-74.

Qiu, T., R.H. Lu, J. Zhang and Z.Y. Zhu. 2001b. Molecular characterization and expression of the M6 gene of grass carp hemorrhage virus (GCHV), an aquareovirus. *Archives of Virology* 146: 1391-1397.

Qiu, T., R.H. Lu, J. Zhang and Z.Y. Zhu. 2001c. The molecular characterization of RNA segment S9 of grass carp hemorrhage virus (GCHV), an aquareovirus. *Aquaculture* 203: 1-7.

Qiu, T., J. Zhang, R.H. Lu and Z.Y. Zhu. 2001d. Genome segment S8 of grass carp hemorrhage virus encodes a virion protein. *Intervirology* 44: 317-320.

Racz, F., L. Békési and E. Szabo. 1980. Neuere Beitraege zur Aetiologie der Karpfenpocken. *Fisch und Umwelt* 8: 49-58.

Rangel, A.A.C., D.D. Rockemann, F.M. Hetrick and S.K. Samal. 1999. Identification of grass carp haemorrhage virus as a new genogroup of aquareovirus. *Journal of General Virology* 80: 9-2402.

Regidor, S.E., J.D. Albaladejo and J.R. Somga. 2004. Current status of transboundary fish diseases in the Philippines: occurrence, surveillance, research and training. In: *Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training*, C.R. Lavilla-Pitogo and K. Nagasawa (eds.). SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, pp. 171-198.

Rodák, L., Z. Pospisil, J. Tománek, T. Vesely and J. Hampl. 1986. Enzymoimunologická (ELISA) diagnostika jarní virémie kapru. *Veterinarni Medicina (Praha)* 31: 505-512.

Rodák, L., Z. Pospisil, J. Tománek, T. Vesely, T. Obr and L. Valicek. 1993. Enzyme-linked immunosorbent assay (ELISA) for the detection of spring viraemia of carp virus (SVCV) in tissue homogenates of the carp, *Cyprinus carpio* L. *Journal of Fish Diseases* 16: 101-111.

Ronen, A., A. Perelberg, J. Abramowitz, M. Hutoran, S. Tinman, I. Bejerano, M. Steinitz and M. Kotler. 2003. Efficient vaccine against the virus causing a lethal disease in cultured *Cyprinus carpio*. *Vaccine* 21: 4677-4684.

Roudikov, N.I. 1980. La virose printanière des poissons en U.R.S.S. *Bulletin de l'Office International des Epizooties* 92: 1069-1077.

Rowley, H., D.A. Graham, S. Campbell, K. Way, D.M. Stone, W.L. Curran and D.G. Bryson. 2001. Isolation and characterisation of rhabdovirus from wild common bream *Abramis brama*, roach *Rutilus rutilus*, farmed brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* in Northern Ireland. *Diseases of Aquatic Organisms* 48: 7-15.

Russell, P. 1996. Do we have the answer to spring viraemia of carp? *Koi Health Quarterly* Winter 95/6: 5.

Sanders, G.E., W.N. Batts and J.R. Winton. 2003. Susceptibility of zebrafish (*Danio rerio*) to a model pathogen, spring viremia of carp virus. *Comparative Medicine* 53: 514-521.

Sano, T. and H. Fukuda. 1987. Principal microbial diseases of mariculture in Japan. *Aquaculture* 67: 59-69.

Sano, T., H. Fukuda and M. Furukawa. 1985a. Herpesvirus cyprini: Biological and oncogenic properties. *Fish Pathology* 20: 381-388.

Sano, T., H. Fukuda, M. Furukawa, H. Hosoya and Y. Moriya. 1985b. A herpesvirus isolated from carp papilloma in Japan. In: *Fish and Shellfish Pathology*, A.E. Ellis (ed.). Academic Press, London, pp. 307-312.

Sano, T., T. Yamaki and H. Fukuda. 1988. A novel carp coronavirus: characterization and pathogenicity. *International Fish Health Conference, Vancouver, Canada* p. 160.

Sano, T., N. Motita, N. Shima and M. Akimoto. 1990. A preliminary report on pathogenicity and oncogenicity of cyprinid herpesvirus. *Bulletin of the European Association of Fish Pathologists* 10: 11-13.

Sano, N., R. Honda, H. Fukuda and T. Sano. 1991a. Herpesvirus cyprini: Restriction endonuclease cleavage profiles of the viral DNA. *Fish Pathology* 26: 207-208.

Sano, T., N. Morita, N. Shima and M. Akimoto. 1991b. Herpesvirus cyprini: Lethality and oncogenicity. *Journal of Fish Diseases* 14: 533-543.

Sano, N., M. Sano, T. Sano and R. Hondo. 1992. Herpesvirus cyprini: Detection of the viral genome by *in situ* hybridization. *Journal of Fish Diseases* 15: 153-162.

Sano, N., M. Moriwake, R. Hondo and T. Sano. 1993a. Herpesvirus cyprini: A search for viral genome in infected fish by *in situ* hybridization. *Journal of Fish Diseases* 16: 495-499.

Sano, N., M. Moriwake and T. Sano. 1993b. Herpesvirus cyprini: Thermal effects on pathogenicity and oncogenicity. *Fish Pathology* 28: 171-175.

Sano, M., T. Ito, J. Kurita, T. Yanai, N. Watanabe, S. Miwa and T. Iida. 2004a. First detection of koi herpesvirus in cultured common carp *Cyprinus carpio* in Japan. *Fish Pathology* 39: 165-167.

Sano, M., T. Ito, J. Kurita, K. Yuasa, S. Miwa and T. Iida. 2004b. Experience on common carp mass mortality in Japan. In: *Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training*, C.R. Lavilla-Pitogo and K. Nagasawa (eds.). SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, pp. 13-19.

Schlotfeldt, H.F. 2004. Severe losses of common carp in Germany due to Koi Herpesvirus (KHV). *Bulletin of the European Association of Fish Pathologists* 24: 216-217.

Schubert, G. 1964. Elektronenmikroskopische Untersuchungen zur Pockenkrankheit des Karpfens. *Zeitschrift für Naturforschung* B19: 675-682.

Schwedler, T.E. and J.A. Plumb. 1982a. Golden shiner virus: Effects of stocking density on incidence of viral infection. *Progressive Fish-Culturist* 44: 151-152.

Schwedler, T.E. and J.A. Plumb. 1982b. *In vitro* growth kinetics and thermostability of the golden shiner virus. *Journal of Wildlife Diseases* 18: 441-446.

Section of Virus Study. 1978. Studies on the causative agent of hemorrhage of the grass carp (*Ctenopharyngodon idella*). *Acta Hydrobiologica Sinica* 6: 321-330.

Selli, L., A. Manfrin, F. Mutinelli, P. Giacometti, E. Cappellozza and G. Bovo. 2002. Isolamento, da pesce gatto (*Ictalurus melas*), di un agente virale sierologicamente correlato al virus della viremia primaverile della carpa (SVCV). *Bollettino Societa Italiana di Patologia Ittica* 14: 3-13.

Seng, E.K., Q. Fang, T.J. Lam and Y.M. Sin. 2004. Development of a rapid, sensitive and specific diagnostic assay for fish *Aquareovirus* based on RT-PCR. *Journal of Virological Methods* 118: 111-122.

Seng, E.K., Q. Fang, Y.M. Sin and T.J. Lam. 2005a. Molecular cloning, DNA sequence analysis, and expression of cDNA sequence of RNA genomic segment 6 (S6) that encodes a viral outer capsid protein of threadfin aquareovirus (TFV). *Virus Genes* 30: 209-221.

Seng, E.K., Q. Fang, Y.M. Sin and T.J. Lam. 2005b. Molecular characterization of a major outer capsid protein encoded by the Threadfin aquareovirus (TFV) gene segment 10 (S10). *Archives of Virology* 150: 2021-2036.

Shao, J., S. Mao, Y. Shen, N. Zhang and G. Yang. 1990. Studies on the isolation and pathogenicity of two kinds of hemorrhagic virus of grass carp, *Ctenopharynogodon idella*. *Journal of the Hangzhou University of Natural Sciences and Education* 17: 74-79.

Shao, J., L. Xiang, Y. Li and S. Mao. 1996. Studies on the detection of grass carp haemorrhagic virus by enzyme linked immunosorbent assay on nitrocellulose membrance (Dot-ELISA). *Journal of Fisheries of China* 20: 6-12.

Shao, J., L. Xiang and Y. Li. 1999. Studies on the conditions for inducing of interferon from *Ctenopharyngodon idella*. *Journal of Fisheries of China* 23: 122-127.

Shapira, Y., A. Benet-Perlberg, T. Zak, G. Hulata and B. Levavi-Sivan. 2002. Differences in resistance to Koi Herpes Virus and growth rate between strains of carp (*Cyprinus carpio*) and their hybrids. *Israeli Journal of Aquaculture* 54: 62-63.

Shapira, Y., Y. Magen, T. Zak, M. Kotler, G. Hulata and B. Levavi-Sivan. 2005. Differential resistance to koi herpes virus (KHV)/carp interstitial nephritis and gill necrosis virus (CNGV) among common carp (*Cyprinus carpio* L.) strains and crossbreds. *Aquaculture* 245: 1-11.

Shchelkunov, I.S. 1987. Investigation of the carp iridovirus in cell cultures and in different fish species. In: *Proceedings of the All-Union Research Institute of Pond Fisheries*, V.A. Musselius and I.S. Shesterin (eds.). VNIIPRKH, Moscow, Vol. 50, pp. 11-16.

Shchelkunov, I.S. and T.I. Shchelkunova. 1984a. Results of virological studies on gill necrosis. In: *Fish, Pathogens and Environment in European Polyculture*, J. Oláh (ed.). Szarvas, Hungary, pp. 31-43.

Shchelkunov, I.S. and T.I. Shchelkunova. 1984b. Some biological characteristics of carp iridovirus *in vitro*. In: *Proceedings of the All-Union Research Institute of Pond Fisheries*, V.A. Musselius and I.S. Shesterin (eds.). VNIIPRKH, Moscow, Vol. 40, pp. 16-25.

Shchelkunov, I.S. and T.I. Shchelkunova. 1989. *Rhabdovirus carpio* in herbivorous fishes: Isolation, pathology and comparative susceptibility of fishes. In: *Viruses of Lower Vertebrates*, W. Ahne and E. Kurstak (eds.). Springer-Verlag, Berlin, pp. 333-348.

Shchelkunov, I.S. and T.I. Shchelkunova. 1990. Infectivity experiments with *Cyprinus carpio* iridovirus (CCIV), a virus unassociated with carp gill necrosis. *Journal of Fish Diseases* 13: 475-484.

Siwicki, A.K., F. Pozet, M. Morand, B. Kazun, S. Trapkowska and J. Malaczewska. 2003. Influence of methisoprinol on the replication of rhabdoviruses isolated from carp (*Cyprinus carpio*) and catfish (*Ictalurus melas*): *in vitro* study. *Polish Journal of Veterinary Sciences* 6: 47-50.

Sokol, F., H.F. Clark, T.J. Wiktor, M.L. McFalls, D.H.L. Bishop and J.F. Obijeski. 1974. Structural phosphoproteins associated with ten rhabdoviruses. *Journal of General Virology* 24: 433-445.

Soliman, H. and M. El Matbouli. 2005. An inexpensive and rapid diagnostic method of Koi Herpesvirus (KHV) infection by loop-mediated isothermal amplification. *Virology Journal* 2: 83: http://www.virologyj.com/content/2-1/83.

Somamoto, T., T. Nakanishi and N. Okamoto. 2002. Role of specific cell-mediated cytotoxicity in protecting fish from viral infections. *Virology* 297: 120-127.

Sonstegard, R.A. and K.S. Sonstegard. 1978. Herpesvirus-associated epidermal hyperplasia in fish (carp). In: *Proceedings of the International Symposium on Oncogenesis and Herpesviruses III*, G. de-Thé, W. Henle and F. Rapp (eds.). International Agency for Research on Cancer, Scientific Publication 24, Lyons, pp. 863-868.

St-Hilaire, S., N. Beevers, K. Way, R.M. Le Deuff, P. Martin and C. Joiner. 2005. Reactivation of koi herpesvirus infections in common carp *Cyprinus carpio*. *Diseases of Aquatic Organisms* 67: 15-23.

Steinhagen, D., P. Kruse and M. Neukirch. 1992. Virus-associated epidermal hyperplasia in golden ide *Leuciscus idus melanotus*. *Diseases of Aquatic Organisms* 13: 225-229.

Stephens, F.J., S.R. Raidal and B. Jones. 2004. Haematopoietic necrosis in a goldfish (*Carassius auratus*) associated with an agent morphologically similar to herpesvirus. *Australian Veterinary Journal* 82: 167-169.

Stone, D.M., W. Ahne, K.D. Denham, P.F. Dixon, C.T.Y. Liu, A.M. Sheppard, G.R. Taylor and K. Way. 2003. Nucleotide sequence analysis of the glycoprotein gene of putative spring viraemia of carp virus and pike fry rhabdovirus isolates reveals four genogroups. *Diseases of Aquatic Organisms* 53: 203-210.

Subramanian, K., F.M. Hetrick and S.K. Samal. 1997. Identification of a new genogroup of aquareovirus by RNA-RNA hybridization. *Journal of General Virology* 78: 1385-1388.

Sulimanovic, D. 1973. Immunity of carp to *Rhabdovirus carpio* and determination of antibodies by indirect hemagglutination. *Veterinarsky Arhiv* 43: 153-161.

Sunarto, A., A. Rukyani and T. Itami. 2005. Indonesian experience on the outbreak of koi herpesvirus in koi and carp (*Cyprinus carpio*). *Bulletin of Fisheries Research Agency* Supplement No. 2: 15-21.

Sunarto, A., T. Widodo, I. Koesharyani, H. Supriyadi, L. Gardenia, B. Sugianti and D. Rukmono. 2004. Transboundary fish diseases in Indonesia: occurrence, surveillance, research and training. In: *Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training*, C.R. Lavilla-Pitogo and K. Nagasawa (eds.). SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, pp. 91-121.

Svetlana, J., V. Ivetic and V. Radosavljevic. (2006). *Rhabdovirus carpio* as a causative agent of disease in rainbow trout (*Oncorhynchus mykiss*-Walbaum). *Acta Veterinaria* (Beograd) 56: 553-558.

Takashima, Y., N. Watanabe, T. Yanai and T. Nakamura. 2005. The status of koi herpesvirus disease outbreaks in Lake Kasumigaura and Kitaura. *Bulletin of Fisheries Research Agency* Supplement No. 2: 65-71.

Terhune, J.S., J.M. Grizzle, K. Hayden and S.D. McClenahan. 2004. First report of koi herpesvirus in wild common carp in the Western Hemisphere. *Fish Health Newsletter. American Fisheries Society, Fish Health Section* 32: 8-9.

Tesarcik, J. and B. Macura. 1981. Provozní vakcinace kapru proti jarní virémii na závodech Státního rybárství (I.). *Buletin VÚRH Vodnany* 17: 3-11.

Tesarcik, J., B. Macura and L. Dedek. 1977a. Izolace virového agens z prípadu jarní virémie kapru v CSR. *Buletin VÚRH Vodnany* 13: 10-14.

Tesarcik, J., B. Macura, L. Dedek, L. Valicek and B. Smid. 1977b. Isolation and electron microscopy of a rhabdovirus from the acute form of infectious dropsy of carp (spring viraemia of carp). *Zentralblatt Fur Veterinarmedizin-Reihe* B24: 340-343.

Tesarcik, J., B. Macura and J. Rehulka. 1978. Vyhodnocení sirsího klinického pokusu s aplikací biopreparátu proti jarni virémii kapru v roce 1977. *Buletin VÚRH Vodnany* 14: 3-6.

Tesarcik, J., B. Macura, J. Trtek, M. Flora and B. Sana. 1982. Provozní vakcinace kapru proti jarní virémii na závodech Státního rybárství (II.). *Buletin VÚRH Vodnany* 18: 13-19.

Tesarcik, J., B. Macura, J. Rehulka, M. Hrdonka and V. Konasova. 1984. Summarised results of pilot vaccination of carp against spring viremia. *Prace VÚRH Vodnany* 13: 68-74.

Theungphachanh, T. 2004. Current status of transboundary fish diseases in Lao PDR: occurrence, surveillance, research and training. In: *Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training*, C.R. Lavilla-Pitogo and K. Nagasawa (eds.). SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, pp. 123-130.

Tian, J., G. Zou and Q. Fang. 1999. cDNA synthesis, cloning and partial nucleotide sequence of grass carp hemorrhage virus (GCHV). *Virologica Sinica* 14: 87-92.

Timur, G. 1991. A histological study of a carp pox (*Viral epithelioma*) disease in Turkey. *Bulletin of the European Association of Fish Pathologists* 11: 171-173.

Tu, C., S.-Y. Lin and H.-T. Sung. 2004a. Current status of koi herpesvirus disease in Taiwan. In: *Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training*, C.R. Lavilla-Pitogo and K. Nagasawa (eds.). SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, pp. 21-24.

Tu, C., M.C. Weng, J.R. Shiau and S.Y. Lin. 2004b. Detection of koi herpesvirus in koi *Cyprinus carpio* in Taiwan. *Fish Pathology* 39: 109-110.

Vainikka, A., R. Kortet and J. Taskinen. 2004. Epizootic cutaneous papillomatosis, cortisol and male ornamentation during and after breeding in the roach *Rutilus rutilus*. *Diseases of Aquatic Organisms* 60: 189-195.

van Duijn, C. 1973. *Diseases of Fish*. Butterworth, London.

Varner, P.W. and D.H. Lewis. 1991. Characterization of a virus associated with head and lateral line erosion syndrome in marine angelfish. *Journal of Aquatic Animal Health* 3: 198-205.

Walster, C. 1999. Clinical observations of severe mortalities in koi carp, *Cyprinus carpio*, with gill disease. *Fish Veterinary Journal* 3: 54-58.

Walster, C. 2000. Koi carp mortality syndrome: An update. *Fish Veterinary Journal* 5: 72-75.

Walster, C. 2003. A note on koi herpes virus (KHV): Current situation and issues arising. *Fish Veterinary Journal* 7: 77-81.

Waltzek, T.B. and R.P. Hedrick. 2004. Koi herpesvirus update 2004. *California Veterinarian* July-August: 14-16.

Waltzek, T.B., G.O. Kelley, D.M. Stone, K. Way, L. Hanson, H. Fukuda, I. Hirono, T. Aoki, A.J. Davison and R.P. Hedrick. 2005. Koi herpesvirus represents a third cyprinid herpesvirus (CyHV-3) in the family *Herpesviridae*. *Journal of General Virology* 86: 1659-1667.

Wang, B., L.H. Ke, H. Jiang, C.Z. Li and P. Tien. 2000. Selection of a specific peptide from a nona-peptide library for *in vitro* inhibition of grass carp hemorrhage virus replication. *Virus Research* 67: 119-125.

Wang, H. and D. Hu. 1989. Toxicity of nitrite to grass carp (*Ctenopharyngodon idella*) in ponds and its way of prevention. *Journal of Fisheries of China* 13: 207-214.

Wang, T., H. Chen, P. Liu, H. Liu, W. Guo and Y. Yi. 1993. Observations on the ultra-thin sections of the main organs and tissues of hemorrhagic *Gobiocypris rarus* artificially infected by grass carp hemorrhagic virus (GCHV). *Acta Hydrobiologica Sinica* 17: 343-346.

Wang, T., P. Liu, H. Chen, H. Liu, Y. Yi and W. Guo. 1994. Preliminary study on the susceptibility of *Gobiocypris rarus* to hemorrhagic virus of grass carp (GCHV). *Acta Hydrobiologica Sinica* 18: 144-149.

Wang, T., Y. Yi, H. Chen, H. Liu and W. Guo. 1995a. Histopathological observation on hemorrhagic rare minnow (*Gobiocypris rarus*) infected artificially with hemorrhagic virus of grass carp. *Acta Hydrobiologica Sinica* 19: 350-353.

Wang, T., T. Zhao, Q. Fang, H. Jiang and L. Ke. 1995b. Immunogenicity of grass carp hemorrhage virus polypeptides. *Virologica Sinica* 10: 166-168.

Wang, T., J. Li, Y. Yi, L. Zhou, H. Liu, R. Lu and H. Chen. 1997. Detection of hemorrhagic virus of grass carp by reverse transcription-polymerase chain reaction. *Oceanologia et Limnologia Sinica* 28: 1-6.

Wang, W., Y. Chen, L. Ke and Y. Cai. 1990. Fine structure, genome and polypeptides of a grass carp hemorrhage virus (GCHV) isolate from the South Lake in Wuhan Hubei. *Chinese Journal of Virology* 6: 44-49.

Wang, W., J. Bai, H. Lao, X. Ye and J. Luo. 2003. Cloning of cDNA for grass carp Mx protein and its expression in prokaryocyte. *Journal of Fishery Sciences of China* 10: 365-369.

Wattanavijarn, W., C. Torchy, J. Tangtronpiros and P. de Kinkelin. 1988. Isolation of a birnavirus belonging to Sp serotype, from South East Asia fishes. *Bulletin of the European Association of Fish Pathologists* 8: 106-108.

Way, K. 1991. Rapid detection of SVC virus antigen in infected cell cultures and clinically diseased carp by the enzyme-linked immunosorbent assay (ELISA). *Journal of Applied Ichthyology* 7: 95-107.

Way, K., S.J. Bark, C.B. Longshaw, K.D. Denham, P.F. Dixon, S.W. Feist, R. Gardiner, M.J. Gubbins, R.-M. Le Deuff, P.D. Martin, D.M. Stone and G. Taylor. 2003. Isolation of a rhabdovirus during outbreaks of disease in cyprinid fish species at fishery sites in England. *Diseases of Aquatic Organisms* 57: 43-50.

Winton, J.R. 1989. Picornaviruses and reoviruses of fishes. In: *Viruses of Lower Vertebrates*, W. Ahne and E. Kurstak (eds.). Springer-Verlag, Berlin, pp. 216-226.

Winton, J.R., C.N. Lannan, J.L. Fryer, R.P. Hedrick, T.R. Meyers, J.A. Plumb and T. Yamamoto. 1987. Morphological and biochemical properties of four members of a novel group of reoviruses isolated from aquatic animals. *Journal of General Virology* 68: 353-364.

Wizigmann, G., C. Pfeil-Putzien, C. Baath, H. Dangschat and G. Köppel. 1980. Serologische Untersuchungen über das Vorkommen von Antikörpern gegenüber *Rhabdovirus carpio* bei Karpfen in bayerischen Teichwirtschaften. *Fisch und Umwelt* 8: 31-36.

Wizigmann, G., H. Dangschat, C. Baath and C. Pfeil-Putzien. 1983. Untersuchungen über Virusinfektionen bei Sü§wasserfischen in Bayern. *Tierärztliche Umschau* 38: 250-258.

Wolf, K. 1988. *Fish Viruses and Fish Viral Diseases*. Cornell University Press, Ithaca.

Xu, S., H. Li, G. Deng and L. Jiang. 1994. The preparation and immune effect of attenuated live vaccine obtained through cell culture for hemorrhage of grass carp. *Journal of Fisheries of China* 18: 110-117.

Xu, S., H. Li, G. Deng, L. Jiang and Y. Bai. 1998. Study on attenuation of virus of hemorrhage of grass carp, *Ctenopharyngodon idella*. *Journal of Shanghai Fisheries University* 7: 211-216.

Yang, X. and L. Zeng. 1993. The immune response of grass carp immunized with vaccine CFRV. *Journal of Fisheries of China* 17: 312-318.

Yang, X. and W. Zuo. 1990a. Effect of water temperature on the immune response of grass carp. *Journal of the Chinese Academy of Fishery Sciences* 3: 29-34.

Yang, X. and W. Zuo. 1990b. Inactive vaccine for hemorrhage of grass carp: an approach to the optimum method of inactivation of FRV. *Journal of the Chinese Academy of Fishery Sciences* 4: 27-33.

Yang, X. and W. Zuo. 1991. Mechanism of water temperature-dependent immune response in grass carp, *Ctenopharyngodon idella* C. et V. *Journal of the Chinese Academy of Fishery Sciences* 4: 27-33.

Yang, X. and W. Zuo. 1993. Inactivated vaccine obtained through cell culture for hemorrhage of grass carp (*Ctenopharyngodon idella*): The stability of vaccine and influence of adjuvants and booster immunization on the immune response. *Acta Hydrobiologica Sinica* 17: 46-52.

Yang, X. and W. Zuo. 1997a. Relationship between water temperature and immune response of grass carp (*Ctenopharyngodon idella* C. et V.). *Asian Fisheries Science* 10: 169-177.

Yang, X. and W. Zuo. 1997b. Relationship between water temperature and the immune response of grass carp, *Ctenopharyngodon idella* C. et V. *Acta Zooloogica Sinica* 43: 42-48.

Yang, X., Y. Luo and X. Ye. 1991. Rapid serological diagnosis of grass carp haemorrhagic virus by coagglutination test using antibody-sensitized staphylococci protein A. *Journal of Fisheries of China* 15: 27-33.

Yang, X., C. Xia and W. Zuo. 1989. Inactive vaccine for hemorrhage of grass carp: comparison of immunogenicity and immunizing dose between two strains. *Journal of Fisheries of China* 13: 138-144.

Yang, X., C. Xia and W. Zuo. 1992. Inactive vaccine by passage on cell line of grass carp (*Ctenopharyngodon idella* C. et V.): Enlarging culture of cell and virus. *Freshwater Fisheries* 22: 3-7.

Yáñez, D., P. Correa, E. Hernández and J.P. Hernández. 1980. Investigacion preliminar de la posible presencia de enfermedades virales de las carpas en Mexico. *Revista Latinoamericano de Microbiologica* 22: 51-52.

Ye, X., G. Yang, Y. Luo, Y. Chen and Z. Chen. 1992. Large-scale culture of grass carp cell and virus by using bioreactor. *Journal of Fisheries of China* 16: 1-6.

Yoshino, M., H. Watari, T. Kojima and M. Ikedo. 2006. Sensitive and rapid detection of koi herpesvirus by LAMP method. *Fish Pathology* 41: 19-27.

Yu, L., W. Zuo, Y. Fang and W. Zheng. 1996. Cell-engineering grass carp produced by the combination of electric fusion and nuclear transplantation. *Journal of Fisheries of China* 20: 314-318.

Yuasa, K., M. Sano, J. Kurita, T. Ito and T. Iida. 2005. Improvement of a PCR method with the Sph 1-5 primer set for the detection of koi herpesvirus (KHV). *Fish Pathology* 40: 37-39.

Zeng, B., C. Chen and G. Ji. 1995. Studies on the pathology of the grass carp 3. The change of the relative activity of malate dehydrogenase (MDH) isozyme in some

tissues and organs of the hemorrhagic grass carp. *Acta Scientiarum Naturalium Universitatis Sunyatseni* 34: 76-81.

Zeng, L. and W. Zuo. 1991. Genome SDS-PAGE analysis and nucleic acid identification of grass carp hemorrhage virus strain 854. *Freshwater Fisheries* 21: 17-19.

Zeng, L. and L. He. 1992. Studies on the purification and physical-chemical characteristics of the grass carp hemorrhage virus strain 854. *Freshwater Fisheries* 22: 3-5.

Zeng, L., X. Yang, L. He, X. Ai, J. He and W. Zuo. 1998. Batch processing technology of cell cultured killed vaccine against the hemorrhage of grass carp. *Journal of Fishery Sciences of China* 5: 62-67.

Zhai, Z.Y., H.-W. Ko, H.-X. Yu, Z.-N. Gu, X.-Z. Li, S.-G. Cao and M.-Y. Gong. 1987. Viral hemorrhagic disease of black carp (*Mylopharyngodon piceus*). *Zoological Research* 8: 379-386.

Zhai, Z.Y., H.D. Chen, H.Y. Guo and S.Q. Cao. 1993. Isolation and identification of black carp haemorrhagic disease virus. *Fisheries Science and Technology Information* 20: 20-22.

Zhang, N., G. Yang, W. Yi, J. Shen and Z. Cao. 1990. Supplementary effect of henbane on the bathing immunization of summer fingerlings of grass carp (*Ctenopharyngodon idella*). *Journal of Zhejiang College of Fisheries* 9: 79-84.

Zhang, N.C. and G.-Z. Yang. 1981. The establishment of strain ZC-7901 and substrain ZC-7901$S_1$ of grass carp (*Ctenopharyngodon idella*) lip cell. *Acta Biologica Experimentia Sinica* 14: 101-106.

Zheng, D.-C., Q.-Y. Huang, W.-Q. Cai and H.-D. Lu. 1986. Histopathological studies on the hemorrhage disease of grass carp. *Journal of Fisheries of China* 10: 151-159.

Zheng, D.-C, Q. Huang, L. Zhao and X. Zhou. 1991. Electron microscopic observation on the hemorrhage disease of grass carp. *Journal of Fisheries of China* 15: 317-321.

Zhong, J.Y., Y.P. Wang and Z.Y. Zhu. 2002. Introduction of the human lactoferrin gene into grass carp (*Ctenopharyngodon idella*) to increase resistance against GCH virus. *Aquaculture* 214: 93-101.

Zhu, X. and Q. Xie. 1993. Studies on the mechanism resulted in liver functional metabolic disturbance by grass carp hemorrhagic disease virus. *Transactions of Research on Fish Diseases* 1: 1-6.

Zhu, X., L. Jia and M. Zhang. 1986. Haemopathological study of hemorrhagic disease on the grass carp fingerlings in the latency and development period. In: *Proceedings of the Asian Symposium on Freshwater Fish Culture*, Y. Chi (ed.). The China Society of Fisheries, Beijing, pp. 352-359.

Zhu, X., L. Jia and M. Zhang. 1987. Haemopathological study on latent and developing period of hemorrhagic disease of grass carp fingerlings. *Acta Hydrobiologica Sinica* 11: 59-66.

Zhu, X., A. Li and Q. Xie. 1993. Experimental studies on Kelieao-Yufukang against hemorrhagic disease virus of grass carp. *Transactions of Research on Fish Diseases* 1: 20-25.

Zuo, W.-G., H.-X. Qian, Y.-F. Xu, S.-Y. Du and X.-L. Yang. 1986. A cell line derived from the kidney of grass carp (*Ctenopharyngodon idella*). *Journal of Fisheries of China* 10: 11-17.

CHAPTER

4

# Viral Diseases of Cultured Marine Fish

**G. Bovo* and D. Florio**

## DISEASES CAUSED BY NODAVIRUSES

Since the first occurrence of a previously unknown disease, characterized by vacuolating brain lesions associated to nodavirus infection, similar diseases have been reported from more than 40 marine species belonging to different families. According to OIE (Office International des Epizooties) all these epizootics should be regarded as a single disease to be identified with the official name Viral Encephalopathy and Retinopathy (VER).

Affected fish show erratic swimming behaviour and may suffer serious losses according to the species, age and temperature. The disease has a worldwide distribution having been reported in both sub-tropical and cold-temperate regions from Asia, Australia, North America and Europe. The causal agents are small non-enveloped single stranded RNA viruses

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belonging to the family Nodaviridae, genus *Betanodavirus*. Four main genotypes, showing high homology, have preliminarily been established. Horizontal transmission represents the main route of virus spreading but vertical transmission is highly suspected in some species.

The diagnosis may be performed by cultivation of the viruses on cell cultures followed by identification with immunological or molecular methods. Results from serology are still inconsistent and need to be better evaluated.

The wide host range and the detection of the causal agents in wild fish suggest that nodaviruses are widespread in the marine environment representing a permanent threat for the aquaculture industry. The control of the disease is based on the application of strict general hygiene measures and removal of positive brood fish. No commercial vaccine is so far available.

## VIRAL ENCEPHALOPATHY AND RETINOPATHY

Viral Encephalopathy and Retinopathy (VER) (Munday *et al.*, 1992; OIE, 2003a) also known as sea-bass encephalitis (Bellance and Gallet de Saint-Aurin, 1988), Viral Nervous Necrosis (VNN) (Yoshikoshi and Inoue, 1990), turbot encephalomyelitis (Bloch *et al.*, 1991), fish encephalitis (Comps *et al.*, 1996; Delsert *et al.*, 1997) is a neuropathological condition, characterized by vacuolation and necrosis of the central nervous system (CNS) and the retina caused by nodavirus infection. The disease has been reported so far from more than 40 marine species belonging to different families (Glazebrook and Campbell, 1987; Bellance and Gallet de Saint-Aurin, 1988; Mori *et al.*, 1991, 1992; Nakai *et al.*, 1994; Nguyen *et al.*, 1994; Chua *et al.*, 1995; Danayadol *et al.*, 1995; Grotmol *et al.*, 1995; Muroga, 1995; Comps and Raymond, 1996; Comps *et al.*, 1996; Fukuda *et al.*, 1996; Jung *et al.*, 1996; Chi *et al.*, 1997, 2001, 2003; Le Breton *et al.*, 1997; Zafran *et al.*, 2000; Curtis *et al.*, 2001; Kim *et al.*, 2001; Starkey *et al.*, 2001; Lai *et al.*, 2001a; Barker *et al.*, 2002; Maeno *et al.*, 2002; Munday *et al.*, 2002; Oh *et al.*, 2002; Hegde *et al.*, 2003; Athanossopoulou, 2004; Ucko *et al.*, 2004; Zorriehzahra *et al.*, 2005). Most of them are affected as larvae or juveniles showing mass mortalities. VER has a worldwide distribution having been reported to occur in Australia, Asia, North America and Europe where it represents a serious economic threat for the marine aquaculture industry (Le Breton *et al.*, 1997; Munday and Nakai, 1997; Munday *et al.*, 2002).

## Aetiology

The causal agents are small (25-30 nm) non-enveloped icosahedral virions (Fig. 4.1) belonging to the family Nodaviridae, genus *Betanodavirus* (Mori *et al.*, 1992; Comps *et al.*, 1994; Schneemann *et al.*, 2005).

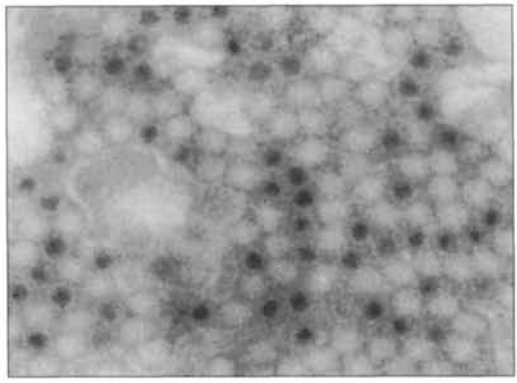

**Fig. 4.1** Viral particles from brain of naturaly infected sea bass (*Dicentrarchus labrax*).

The family includes a second genus: the *Alphanodavirus* which covers viral agents primarily affecting insects; the two genus are clearly distinguished according to their RNA1 and RNA2 sequences (Nishizawa *et al.*, 1995; Delsert *et al.*, 1997; Nagai and Nishizawa, 1999; Nishizawa *et al.*, 1999).

The bi-segmented genome consists of two positive-sense ssRNAs. The RNA1 segment of striped jack nodavirus (SJNNV) (Mr $1.01 \times 10^6$; 3.1 Kb) encodes a non-structural 100-Kda protein (protein 1a). The RNA2 (Mr $0.49 \times 10^6$; 1.4 Kb) encodes a 42-Kda protein (protein 2a). A sgRNA3 segment (Mr about $0.13 \times 10^6$; 0,4 Kb) derived from RNA1 has been detected (Mori *et al.*, 1992; Nagai and Nishizawa, 1999; Sommerset and Nerland, 2004; Schneemann *et al.*, 2005).

Significant RNA2 homology has been described within the betanodavirus genus, both at nucleotide and amino acid level (Nishizawa *et al.*, 1995). Based on the phylogenetic analysis of the variable region (T4) of the coat protein, betanodaviruses have been clustered into four main genotypes which are considered distinct species: striped jack nervous necrosis virus-type (SJNNV); tiger puffer nervous necrosis virus-type (TPNNV); barfin flounder nervous necrosis virus-type (BFNNV) and red

spotted grouper nervous necrosis virus-type (RGNNV) (Nishizawa *et al.*, 1997; Dalla Valle *et al.*, 2001).

Furthermore, several isolates originating from different fish species and/or geographical areas (Glazebrook and Campbell, 1987; Breuil *et al.*, 1991; Chua *et al.*, 1994; Nguyen *et al.*, 1994; Danayadol *et al.*, 1995; Grotmol *et al.*, 1995; Comps *et al.*, 1996; Delsert *et al.*, 1997; Starkey *et al.*, 2001) are tentatively considered to belong to distinct species (Schneemann *et al.*, 2005). In Table 4.1 the current taxonomy of betanodaviruses is reported.

Three main serotypes have been preliminarily identified by testing different virus species with a cross-neutralization assay (Mori *et al.*, 2003).

Since the first appearance of the disease several attempts to isolate and replicate the causal agents *in vitro* were carried out using existing cell lines with unsatisfactory results until SSN-1 cells were inoculated (Frerichs *et al.*, 1996). Afterwards further susceptible cell lines have been established (Chi *et al.*, 1999; Iwamoto *et al.*, 2000; Chang *et al.*, 2001; Lai *et al.*, 2001a, 2003) representing a valid alternative to SSN-1 cell line which is naturally contaminated by a C-type retrovirus (Hart *et al.*, 1996).

**Table 4.1** List of species in the genus *Betanodavirus* (Schneemann *et al.*, 2005).

| | |
|---|---|
| Species in the genus | *Barfin flounder nervous necrosis virus* (BFNNV) |
| | *Red spotted grouper nervous necrosis virus* (RGNNV) |
| | *Striped jack nervous necrosis virus* (SJNNV) |
| | *Tiger puffer nervous necrosis virus* (TPNNV) |
| Tentative species in the genus | Atlantic cod nervous necrosis virus (ACNNV) |
| | Atlantic halibut nodavirus (AHNV) |
| | *Dicentrarchus labrax* encephalitis virus (DlEV) |
| | Dragon grouper nervous necrosis virus (DGNNV) |
| | Greasy grouper nervous necrosis virus (GGNNV) |
| | Grouper nervous necrosis virus (GNNV) |
| | Halibut nervous necrosis virus (HNNV) |
| | Japanese flounder nervous necrosis virus (JFNNV) |
| | *Lates calcarifer* encephalitis virus (LcEV) |
| | *Malabaricus grouper* nervous necrosis virus (MGNNV) |
| | Sea-bass nervous necrosis virus (SBNNV) |
| | *Umbrina cirrosa* nodavirus (UCNV) |

## Clinical Signs

The disease mainly occurs during larval and juvenile stages, but market-size and broodfish may also be affected, particularly European sea-bass (Le Breton *et al.*, 1997; Bovo *et al.*, 1999), sevenband grouper (Fukuda *et al.*, 1996) and Atlantic halibut (Aspehaugh *et al.*, 1999). Infections occurring at larval stages may induce 100% mortalities in affected lots while juveniles and adults are more resistant showing mortalities ranging from 15-60% (Sweetman *et al.*, 1996; Bovo *et al.*, 1999). First symptoms appear between 1 and 35 days after hatching depending on the species affected. In European sea bass first symptoms appear usually at 15-17 days after hatching (Perič, pers. comm.). In general, affected larvae show abnormal swimming behaviour, some of them assuming a vertical position other resting on the surface mainly due to swimbladder hyperinflation.

Spinning motions in all directions, resting on the surface, body flexion and muscular tremors are commonly observed in juveniles while in adult fish abnormal swimming behaviour represents the most common symptom. In European sea-bass (*Dicentrarchus labrax*) affected fish may swim in circles or to the right, some float on their sides or belly up and few fish exhibit a vertical position with the head or caudal peduncle out of water (Bovo *et al.*, 1999), furthermore epithelium necrosis on upper jaw and head area (Fig. 4.2), possibly due to traumatic origin, is frequently observed, particularly in concrete ponds (Bovo *et al.*, 1996; Sweetman *et al.*, 1996; Le Breton *et al.*, 1997)

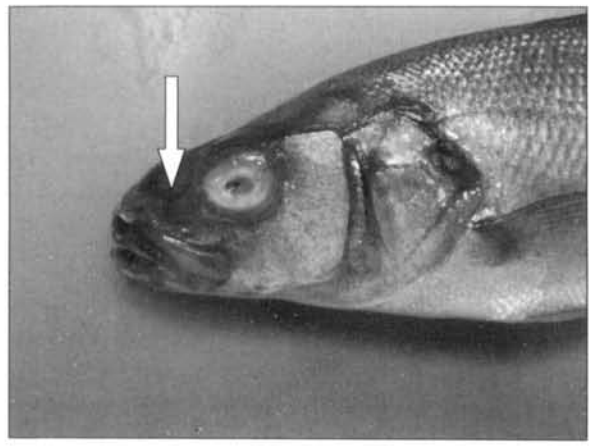

**Fig. 4.2** Extensive necrotic lesions (arrows) in the cephalic area of juvenile sea bass (*Dicentrarchus labrax*) due to traumatic origin.

Flat fish show very little symptoms. Affected specimens may remain for long periods on the tank bottom showing body dorsal flexion with the head and tail elevated. Body tremors are evident and from time to time fish try to swim up but soon afterwards *they fall down giving an impression of falling leaves twirled by the wind* (Perič, pers. comm.).

Skin pigmentation may also be affected; barramundi (*Lates calcarifer*) and halibut (*Hippoglossus hippoglossus*) larvae become paler, whereas groupers (*Epinephelus* spp.), juvenile Atlantic halibut (*Hippoglossus hippoglossus*), European sea-bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*) become darker (Glazebrook *et al.*, 1990; Yoshikoshi and Inoue, 1990; Bloch *et al.*, 1991; Breuil *et al.*, 1991; Mori *et al.*, 1991, 1992; Boonyaratpalin *et al.*, 1996; Grotmol *et al.*, 1997; Munday *et al.*, 2002).

The appearance of clinical symptoms may be significantly affected by temperature (Arimoto *et al.*, 1994; Fukuda *et al.*, 1996; Tanaka *et al.*, 1998). In European sea-bass clinical signs become more evident when temperature is higher than 22-25°C (Sweetman *et al.*, 1996; Bovo *et al.*, 1999) and usually disappear when temperature is less than 18-20°C, but in rare occasions typical symptoms and moderate mortality have been reported to occur at very low temperature (Borghesan *et al.*, 2003). Similar observations have been reported in sevenband grouper (Tanaka *et al.*, 1998).

## Anatomo-pathological Changes and Histopathology

Neither external nor internal lesions have been associated to VER infection nevertheless swimbladder hyperinflation has been often recorded in European sea-bass (*Dicentrarchus labrax*), barramundi (*Lates calcarifer*) and striped jack (*Pseudocaranx dentex*) (Breuil *et al.*, 1991; Munday *et al.*, 2002). Vacuolation and necrosis of brain, spinal cord and granular layers of the retina (Fig. 4.3) are the most common histopathological findings. Brain intracytoplasmic basophilic inclusions have been frequently reported, as well as pyknosis and karyorrhexis (Glazebrook *et al.*, 1990; Yoshikoshi and Inoue, 1990; Breuil *et al.*, 1991). Virus replication may occur in all regions of the brain (Grotmol *et al.*, 1997; Johansen *et al.*, 2002; Munday *et al.*, 2002) and lesions are homogenously distributed in the grey matter.

In general, larvae are more severely affected than adult fish that may show very little lesions even in presence of clear symptoms (Comps *et al.*,

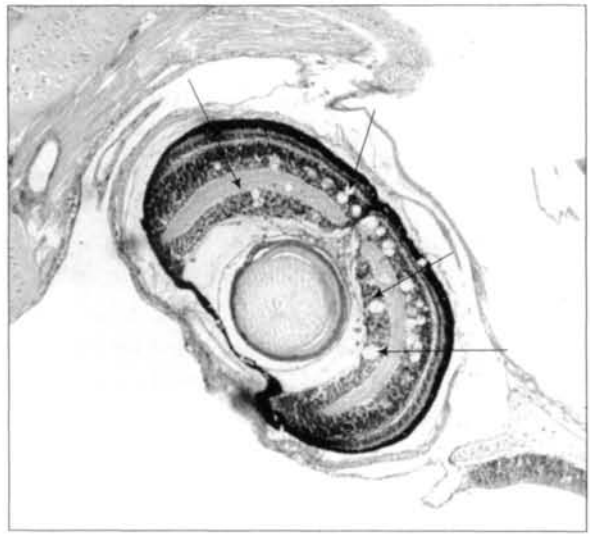

**Fig. 4.3** Vacuolations in the granular layers of the retina (arrows).

1996; Galeotti *et al.*, 1999) while according to Le Breton *et al.* (1997) ocular lesions are more prominent in adult than in larvae.

## Epidemiology, Host Range and Geographical Distribution

Horizontal transmission has been fully demonstrated by immersion in water contaminated with tissue culture virus (Peducasse *et al.*, 1999) or infected tissues (Glazebrook *et al.*, 1990) and by cohabitation (Arimoto *et al.*, 1993). The transmission rate is enhanced by high stocking density, high temperatures and presence of carrier species in the farm or in the wild (Le Breton *et al.*, 1997; Castric *et al.*, 2001; Chi *et al.*, 2003). The high resistance of betanodaviruses to environmental conditions (Frerichs *et al.*, 1996; Frerichs *et al.*, 2000; Maltese and Bovo, 2001; Munday *et al.*, 2002) increases the likelihood of horizontal transmission which is very common in endemic areas when small fish are released from hatcheries into the floating cages or ponds.

According to Chi *et al.* (2000) horizontal transmission may take place also through feeding virus-contaminated biological natural diets like *Artemia* sp. nauplii, the copepod *Tigriopus japonicus* and the shrimp *Acetesinte medius* in which VER genome has been detected by semi-nested PCR (Chi *et al.*, 2003). Mori *et al.* (2005) suspected a similar mechanism when feeding farmed stocks with raw contaminated fish.

Since VER has been frequently detected in larvae and juveniles even in hatcheries supplied with UV-treated water, vertical transmission has been highly suspected in some species (Breuil *et al.*, 1991; Arimoto *et al.*, 1992; Comps *et al.*, 1996; Yoshimizu *et al.*, 1997; Grotmol and Totland, 2000) and is believed to be an important route of infection in farmed fish (Breuil *et al.*, 2002). Sub-clinically infected hosts might represent a dangerous covert infection source and several reports have been recently focused on the possibility of disease transmission from infected broodfish (Castric *et al.*, 2001; Johansen *et al.*, 2002; Chi *et al.*, 2003). The hypothesis of vertical transmission is supported by the detection of viral antigens and viral genome in gonads and eggs by ELISA (Arimoto *et al.*, 1992), immunofluorescence (IFAT) (Nguyen *et al.*, 1997) and RT-PCR (Mushiake *et al.*, 1994; Nishizawa *et al.*, 1996; Nguyen *et al.*, 1997; De Mas *et al.*, 1998; Dalla Valle *et al.*, 2000; Breuil *et al.*, 2002).

Recently, the virus has been identified in eggs and larvae originating from broodfish experimentally infected by i.m. injection (Breuil *et al.*, 2002). These results clearly suggest that infected parents may transmit the infection to their offspring even if it has still to be proved whether the virus is inside or outside the eggs.

Natural VER infection has been reported from several fish species all over the world, as shown in Table 4.2. Furthermore, wolffish (*Anarchicas minor*) and Mozambique tilapia (*Oreochromis mossambicus*) have been experimentally infected (Skliris and Richards, 1999a; Sommer *et al.*, 2004).

Betanodaviruses have been first regarded as pathogens affecting marine fish. However, more recently, several outbreaks have been reported from species reared in freshwater environment: Australian catfish (*Tandanus tandanus*), sleepy cod (*Oxyeleotris lineolatus*) and barramundi (*Lates calcarifer*) (Munday *et al.*, 2002); European eel (*Anguilla anguilla*), Chinese catfish (*Parasilurus asotus*) (Chi *et al.*, 2003) and guppy (*Poecilia reticulata*) (Hegde *et al.*, 2003). These results suggest that salinity is not a limiting parameter but some doubts have been raised about the infection source which, in some occasions, could be traced back to the marine environment. Furthermore, questions about the lack of barriers between different species have been raised following the finding of sea-bass infection by nodavirus strains showing distinct genomes (Thiery *et al.*, 1999a).

Glazebrook and Campbell (1987) reported the first description of brain lesions, possibly associated with betanodavirus infection in

**Table 4.2** Host and geographical occurrence of VER infection.

| *Order* | *Family* | *Species* | *Fish* | *Country* | *Reference* |
|---|---|---|---|---|---|
| Anguilliformes | Anguillidae | *Anguilla anguilla* | European eel | Taiwan | Chi *et al.* (2001) |
| Gadiformes | Gadidae | *Gadus morhua* | Atlantic cod | USA | Johnson *et al.* (2002) |
| | | | | UK | Starkey *et al.* (2001) |
| | | | | Canada | Gagnè *et al.* (2004) |
| | | *Melanogrammus aeglefinus* | Haddock | Canada | Gagnè *et al.* (2004) |
| Perciformes | Latidae | *Lates calcarifer* | Barramundi | Australia | Glazebrook and Campbell (1987) |
| | | | | China | Munday *et al.* (2002) |
| | | | | Indonesia | Zafran Harada *et al.* (1998) |
| | | | | Israel | Munday *et al.* (2002) |
| | | | | Malaysia | Awang (1987) |
| | | | | Philippines | Maeno *et al.* (2004) |
| | | | | Singapore | Chang *et al.* (1997) |
| | | | | Tahiti | Renault *et al.* (1991) |
| | | | | Taiwan | Chi *et al.* (2001) |
| | | | | Israel | Ucko *et al.* (2004) |
| | | | | Thailand | Glazebrook *et al.* (1990) |
| | | | | India | Azad *et al.* (2005) |
| | Lateolabracidae | *Lateolabrax japonicus* | Japanese sea bass | Japan | Jung *et al.* (1996) |
| | Moronidae | *Dicentrarchus labrax* | Sea-bass | Caribbean | Bellance *et al.* (1988) |
| | | | | France | Breuil *et al.* (1991) |
| | | | | Greece | Le Breton *et al.* (1997) |

*(Table 4.2 Contd.)*

(Table 4.2 Contd.)

| | | | | | |
|---|---|---|---|---|---|
| Perciformes | | | | Italy | Bovo *et al.* (1999) |
| | | | | Malta | Skliris *et al.* (2001) |
| | | | | Portugal | Skliris *et al.* (2001) |
| | | | | Spain | Skliris *et al.* (2001) |
| | | | | Israel | Ucko *et al.* (2004) |
| | Serranidae | *Epinephelus akaara* | Red spotted grouper | Japan | Mori *et al.* (1991) |
| | | | | Taiwan | Chi *et al.* (1997) |
| | | *E. awoara* | Yellow grouper | Taiwan | Lai *et al.* (2001b) |
| | | *E. fuscoguttatus* | Brown-marbled grouper | Taiwan | Chi *et al.* (1997) |
| | | *E. coioides* | Orange spotted grouper | Philippines | Maeno *et al.* (2002) |
| | | | | Taiwan | Chi *et al.* (2003) |
| | | *E. malabaricus* | Malabar grouper | Thailand | Danayadol *et al.* (1995) |
| | | *E. marginatus* | Dusky grouper | Mediterranean | Le Breton (1997) |
| | | *E. moara* | Kelp grouper | Japan | Nakai *et al.* (1994) |
| | | *E. septemfasciatus* | Convict grouper | Japan | Fukuda *et al.* (1996) |
| | | | | Korea | Sohn and Park (1998) |
| | | *E. tauvina* | Greasy grouper | Malaysia | Bondad-Reantaso *et al.* (2000) |
| | | | | Philippines | Bondad-Reantaso *et al.* (2000) |
| | | | | Singapore | Chua *et al.* (1995) |
| | | *E. aeneus* | White grouper | Israel | Ucko *et al.* (2004) |
| | | *Chromileptes altivelis* | Humpback grouper | Indonesia | Zafran *et al.* (2000) |
| | | | | Taiwan | Chi *et al.* (2003) |

(Table 4.2 Contd.)

*(Table 4.2 Contd.)*

| | | | | | |
|---|---|---|---|---|---|
| Perciformes | Latridae | *Latris lineata* | Striped trumpeter | Australia | Munday *et al.* (2002) |
| | Carangidae | *Pseudocaranx dentex* | Striped jack | Japan | Mori *et al.* (1992) |
| | | *Seriola dumerili* | Greater amberjack | Japan | Muroga (1995) |
| | | *Trachinotus blochii* | Snubnose pompano | Taiwan | Chi *et al.* (2001) |
| | | *T. falcatus* | Yellow-wax pompano | Taiwan | Chi *et al.* (2003) |
| | Sparidae | *Sparus aurata* | Gilt-head seabream | France | Comps and Raymond (1996) |
| | | | | Italy | Dalla Valle *et al.* (2000) |
| | Sciaenidae | *Sciaenops ocellatus* | Red drum | Korea | Oh *et al.* (2002) |
| | | | | Israel | Ucko *et al.* (2004) |
| | | *Umbrina cirrhosa* | Shi drum | Italy | Pavoletti *et al.* (1998) |
| | | | | France | Comps *et al.* (1996) |
| | | *Atractoscion nobilis* | White weak fish | USA | Curtis *et al.* (2001) |
| | Oplegnathidae | *Oplegnathus fasciatus* | Japanese parrot fish | Japan | Yoshikoshi and Inoue (1990) |
| | | *O. punctatus* | Rock porgy | Japan | Mori *et al.* (1992) |
| | Eleotridae | *Oxyeleotris lineolatus* | Sleepy cod | Australia | Munday *et al.* (2002) |
| | Rachycentridae | *Rachycentron canadum* | Cobia | Taiwan | Chi *et al.* (2001) |
| | Mugilidae | *Mugil cephalus* | Grey mullet | Israel | Ucko *et al.* (2004) |
| | | *Liza auratus* | Golden grey mullet | Iran | Zorriehzahra *et al.* (2005) |
| | Lutjanidae | *Lutjanus erythropterus* | Crimson snapper | Taiwan | Chi *et al.* (2003) |
| Pleuronectiformes | Pleuronectidae | *Verasper moseri* | Barfin flounder | Japan | Muroga (1995) |
| | | *Hippoglossus hippoglossus* | Atlantic halibut | Norway | Grotmol *et al.* (1995) |
| | | *Pseudopleuronectes americanus* | Winter flounder | Canada | Barker *et al.* (2002) |

*(Table 4.2 Contd.)*

*(Table 4.2 Contd.)*

| | | | | | |
|---|---|---|---|---|---|
| Pleuronectiformes | Paralichthydae | *Paralichthys olivaceus* | Japanese flounder | Japan | Nguyen *et al.* (1994) |
| | Scophthalmidae | *Scophthalmus maximus* | Turbot | Norway | Bloch *et al.* (1991) |
| | Soleidae | *Solea solea* | Common sole | United Kingdom | Starkey *et al.* (2001) |
| Tetraodontiformes | Triodontidae | *Takifugu rubripes* | Tiger puffer | Japan | Nakai *et al.* (1994) |
| Siluriformes | Siluridae | *Parasilurus asotus* | Chinese catfish | Taiwan | Chi *et al.* (2003) |
| | Plotosidae | *Tandanus tandanus* | Australian catfish | Australia | Munday *et al.* (2002) |
| Cyprinodontiformes | Poeciliidae | *Poecilia reticulata* | Guppy | Singapore | Hegde *et al.* (2003) |
| Scorpeniformes | Sebastidae | *Sebastes oblongus* | Oblong rockfish | Korea | Kim *et al.* (2001) |
| Acipenseriformes | Acipenseridae | *Acipenser gueldestaedi* | Russian sturgeon | Greece | Athanssopoulou *et al.* (2004) |

barramundi (*Lates calcarifer*) reared in Australia. Later on, Bellance and Gallet de Saint-Aurin (1988) described a similar pathological condition in a population of European sea-bass (*Dicentrarchus labrax*), originating from France and reared in Martinique. Since 1990 several papers have been published on the occurrence of the disease in different species reared in Asia (Yoshikoshi and Inoue, 1990; Mori *et al.*, 1991; Nakai *et al.*, 1994; Nguyen *et al.*, 1994; Chua *et al.*, 1995; Danayadol *et al.*, 1995; Muroga, 1995; Fukuda *et al.*, 1996; Jung *et al.*, 1996; Sohn and Park, 1998; Zafran *et al.*, 1998, 2000; Bondad-Reantaso *et al.*, 2000; Chi *et al.*, 2001, 2003; Lai *et al.*, 2001a; Maeno *et al.*, 2002; Oh *et al.*, 2002; Hegde *et al.*, 2003).

In Norway VER was first described in turbot (*Scophthalmus maximus*) (Bloch *et al.*, 1991) and few years later detected in Atlantic halibut (*Hippoglossus hippoglossus*) larvae and juveniles (Grotmol *et al.*, 1995).

In the Mediterranean region the disease was reported since the early 1990s affecting European sea-bass larvae and juveniles reared in France (Breuil *et al.*, 1991). Until that time VER was not considered to be a serious problem but suddenly, during summer 1995, the disease acquired a high virulence spreading all over the Mediterranean and causing dramatic losses in many European sea bass on-growing farms (Bovo *et al.*, 1996; Sweetman *et al.*, 1996; Le Breton *et al.*, 1997). In some farms Shi drum (*Umbrina cirrosa*) was severely affected too (Pavoletti *et al.*, 1998). During the same period sporadic outbreaks have been detected in wild groupers (Bovo, results unpublished).

More recently, betanodavirus infections have been reported in the U.K. (Starkey *et al.*, 2000, 2001), Israel (Ucko *et al.*, 2004), North America (Curtis *et al.*, 2001; Barker *et al.*, 2002; Gagnè *et al.*, 2004), Iran (Zorriehzahra *et al.*, 2005) and India (Azad *et al.*, 2005).

## Pathogenesis

The most consistent lesions described in clinically infected fish are detected in brain, retina and spinal cord; mortality is therefore supposed to occur as a consequence of nervous tissue lesions.

Le Breton *et al.* (1997) reported that all sizes of reared European sea bass must be considered susceptible to betanodavirus infection but the age, temperature and route of infection may significantly affect the mortality rate in several species (Arimoto *et al.*, 1993; Tanaka *et al.*, 1998; Peducasse *et al.*, 1999, Borghesan *et al.*, 2003).

Peducasse *et al.* (1999) clearly demonstrated the occurrence of horizontal transmission in juvenile European sea-bass following i.p. and i.m. injection as well as by bath exposure, cohabitation and oral route but the portals of entry when infecting by cohabitation and bath exposure was not definitively identified.

In experimentally infected larvae of Atlantic halibut (*Hippoglossus hippoglossus*), the virus was found at the same sampling time in the brain and in the stratified epithelium of the cranial region of the intestine, suggesting that this tissue may play an important role in the entry and first virus replication (Grotmol *et al.*, 1999). In the same study, other tissues than brain and intestine, such as spinal cord, retina, cranial ganglia, liver, olfactory epithelium, yolk sac epithelium gills and pectoral fins were found positive.

In naturally infected striped jack (*Pseudocaranx dentex*), the virus has been detected in degenerated epidermal cells but the portal of entry was doubtful because of the simultaneous presence of large amount of virus in the CNS (Nguyen *et al.*, 1996).

In most studies, both retina and brain were found positive (Peducasse *et al.*, 1999; Húsgaro *et al.*, 2001) and investigations carried out in striped jack and Atlantic halibut have shown that positivity first appears in the brain and later in the retina (Nguyen *et al.*, 1996; Grotmol *et al.*, 1999).

Studies on striped jack (*Pseudocaranx dentex*) broodstock revealed the presence of infection in 13-year-old fish not only in the gonads but also in the liver, stomach and intestine (Nguyen *et al.*, 1997). According to the authors, the stress occurring in broodfish during the spawning season may induce an enhancement of virus replication and the replicated virus can then be shed from gonads and intestine resulting in contamination off eggs and hatched larvae.

In Atlantic halibut endocardial lesions, associated to vacuolating encephalopathy and retinopathy, were observed in juvenile fish suffering extensive losses. According to the authors (Grotmol *et al.*, 1997), the heart lesions may precede the infection of the nervous system.

The detection of viral antigen or its genome from mature gonads reinforces the likelihood of vertical transmission (Arimoto *et al.*, 1992; Mushiake *et al.*, 1994; Dalla Valle *et al.*, 2000).

## Diagnosis

Since the first appearance of the disease, histology and EM were the only available diagnostic tools for several years. More sensitive and specific tests were later on established, particularly when susceptible cell lines became available (Frerichs *et al.*, 1996; Chi *et al.*, 1999; Watanabe and Yoshimizu, 1999; Iwamoto *et al.*, 2000; Chang *et al.*, 2001; Lai *et al.*, 2001a, b, 2003). According to the OIE (2003a) when clinically affected fish are suspected VER infection should be diagnosed by virus isolation on SSN-1 or E-11 cells followed by identification by immunofluorescence (IFAT), immunohistochemistry (IHC) or reverse transcriptase-polymerase chain reaction (RT-PCR). In case of asymptomatic fish, the only suggested diagnostic test is virus isolation on the same cell lines, followed by identification with RT-PCR or IFAT.

According to Frerichs *et al.* (1996) 'a specific cytopathic effect develops initially on SSN-1 as localized areas of rounded, granular, refractile cells which spread through the cell sheet over a further 3-4 days to form a meshwork of degenerating cells before complete degeneration of the monolayer within about 10 days'. Different cytopathic effects (cpe), including rounded, granular, refractile cells and heavy cytoplasmic vacuolations (Fig. 4.4), were reported by Iwamoto *et al.* (1999) after inoculation of SSN-1 cell monolayers with different isolates. Because of significant differences in optimal growth temperature among the four established genotypes, e.g., 25-30°C for RGNNV and SJNNV, 20°C for TPNNV and 15°C for BFNNV genotype, it is very important to select suitable incubation temperatures, according to the virus genotype known or suspected to be present in the geographic area of sampling.

Concerning molecular methods, several papers have been published, particularly in the last decade (Nishizawa *et al.*, 1994; Comps *et al.*, 1996; Nguyen *et al.*, 1997; De Mas *et al.*, 1998; Peducasse *et al.*, 1999; Thiery *et al.*, 1999b; Dalla Valle *et al.*, 2000, 2005; Huang *et al.*, 2001; Iwamoto *et al.*, 2001; Gomez *et al.*, 2004; Johansen *et al.*, 2004a; Starkey *et al.*, 2004). Due to their high sensitivity and specificity, these methods represent a suitable tool for diagnostic and research purposes and their use should be encouraged in addition to the traditional methods.

IHC and IFAT have been applied mostly in pathogenetic studies but their use could be easily applied for diagnostic purposes (Nguyen *et al.*, 1996, 1997; Thiery *et al.*, 1997; Bovo *et al.*, 1998a; Mutinelli *et al.*, 1998; Grotmol *et al.*, 1999; Peducasse *et al.*, 1999; Skliris and Richards, 1999b;

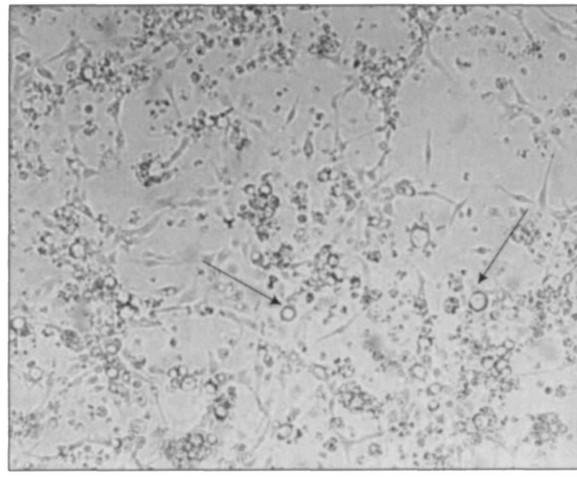

**Fig. 4.4** Extensive cpe in SSN-1 cells. Typical cell vacuolations are evident (arrows).

Totland *et al.*, 1999; Lai *et al.*, 2001b; Johansen *et al.*, 2002, 2004a; Grove *et al.*, 2003; Mladineo, 2003). Furthermore, ELISA tests for detection of viral antigens have been successfully applied (Arimoto *et al.*, 1992; Shieh and Chi, 2005).

Due to the insufficient experience on the immune responses of fish to betanodavirus infections, no serological method has been yet considered as suitable for assessing the health status of farmed populations. Nevertheless, different applications of ELISA to detect specific antibodies in sera in order to assess their health status have been published (Mushiake *et al.*, 1992; Breuil and Romenstand, 1999; Watanabe *et al.*, 2000; Huang *et al.*, 2001; Breuil *et al.*, 2002).

## Control

The lack of complete knowledge of epidemiology and pathogenetic mechanisms makes any specific control measures very difficult; for these reasons a complex approach strategy, based on non-lethal screening of broodstock population at individual level together with eradication measures has been recommended.

Molecular methods based on RT-PCR (Nishizawa *et al.*, 1994; Grotmol *et al.*, 2000) or nested RT-PCR (Dalla Valle *et al.*, 2000) and real-time PCR (Dalla Valle *et al.*, 2005) have been developed and employed to detect viral genome in gonads, blood and seminal fluid.

Dalla Valle *et al.* (2000) simultaneously tested blood; ovary biopsies and sperm obtained from European sea-bass and shi drum brood fish by RT-PCR and nested PCR. The results clearly demonstrate that nested PCR is by far more sensitive and a significantly higher prevalence was detected in brood-fish when nested PCR was applied instead of RT-PCR, particularly when blood, gonads and sperm were tested. Similar results have been obtained by Breuil *et al.* (2002) and Bleie (pers. comm.) who detected 8% positivity in Atlantic halibut by RT-PCR while when the same tissues were processed by real-time PCR the positivity increased to 52%. More recently, a real-time PCR showing a 10 times higher sensitivity than RT-PCR previously described was reported by the same authors (Dalla Valle *et al.*, 2005). These results confirm the high sensitivity and specificity of molecular methods and underline their usefulness for selection of virus free broodstock. The most relevant experience concerns the control of VER in striped jack (*Pseudocaranx dentex*). In Japan, where this species is reared since many years, RT-PCR has been extensively applied to detect and eliminate positive brood fish. Nevertheless, according to Nishizawa *et al.* (1996), the method failed to detect 100% of the positive carriers possibly because of not enough sensitivity. For this very reason, immunological methods have been proposed to support molecular investigations (Breuil *et al.*, 2000; Watanabe *et al.*, 2000).

The potential for vertical transmission partially frustrates every protocol of egg disinfection. Nevertheless, this procedure has been suggested by several authors (Arimoto *et al.*, 1996; Grotmol and Totland, 2000) in order to avoid surface contamination and reduce the rate of infection transmission from broodfish to their offspring. Inlet water could be mixed with nascent ozone and used only once avoiding any water recycling (Munday and Nakai, 1997). According to Grotmol and Totland (2000), it was possible to inactivate the contaminant viral particles present on the egg surface reducing the risk of virus transmission to hatching larvae by treating eggs obtained from experimentally infected halibut with ozone. Nevertheless, the procedure applied was not completely effective (Johansen and Grotmol, pers. comm.) and similar uncertainty also exists as regards cod and turbot eggs.

The use of UV has also been suggested to treat inlet water in hatcheries in order to avoid virus contamination from the environment (Arimoto *et al.*, 1996; Frerichs *et al.*, 2000; Maltese and Bovo, 2001).

Despite the increasing knowledge gained on fish vaccinology and different studies performed on betanodaviruses, in recent years, no

commercial vaccine is so far available (Sommerset *et al.*, 2005a). Significant protection has been induced in juvenile but not in adult turbots vaccinated i.p. with an oil-based recombinant partial capsid protein vaccine obtained from striped jack nervous necrosis virus (Húsgaro *et al.*, 2001). Significant protection, associated to high neutralization antibody titres, has been obtained in sevenband groupers vaccinated i.m. with the recombinant coat protein (Tanaka *et al.*, 2001).

More recently, a DNA and a recombinant vaccine based on the same capsid-encoding region of Atlantic halibut nodavirus (AHNV) were tested on turbot challenged i.m. (100 µl AHNV $1 \times 10^8$ $TCID_{50}$/ml) 10 weeks post vaccination. A significant protection was obtained with the recombinant capsid protein while no protection has been obtained with the DNA vaccine (Sommerset *et al.*, 2005b).

Surprisingly, a DNA vaccine directed against the glycoprotein of viral haemorrhagic septicaemia virus (VHSV) induced an early protection against nodavirus challenge in turbot (Sommerset *et al.*, 2003).

Besides development of an effective vaccine, which will represent the definitive solution for VER control, strict hygiene measures should be adopted in addition to broodstock control and egg disinfection. Ponds, tanks, boots, nets and all facilities used in the farm should be carefully disinfected by suitable products. Low iodophor concentrations (25-100 ppm) have á strong virucidal effect on the pathogen (Arimoto *et al.*, 1996; Frerichs *et al.*, 2000; Maltese and Bovo, 2001). Similar results may be obtained with chlorine (Arimoto *et al.*, 1996; Frerichs *et al.*, 2000) while data concerning the activity of formalin suggests a poor effect of this chemical (Frerichs *et al.*, 2000).

## DISEASES CAUSED BY IRIDOVIRUSES

Iridoviruses include old as well as emerging viruses affecting a wide variety of wild and cultured fish both in fresh and saltwater environment (Wolf, 1988; Hetrick and Hedrick, 1993). According to the International Committee of Virus Taxonomy Iridoviruses are large (120-350 nm in diameter), icosahedral, mostly enveloped DNA viruses, belonging to the family Iridoviridae. The viral genome consists of a single linear dsDNA molecule, 140-303 Kb in size and virions contain up to a maximum of 36 polypeptides. The family Iridoviridae includes five genera *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Lymphocystivirus* and the recently established genus *Megalocytivirus* (Chinchar *et al.*, 2005). Agents affecting the fish are

classified in the genera *Ranavirus* (*Epizootic haematopoietic necrosis virus, European catfish virus* and *Santee-Cooper ranavirus*); *Lymphocystivirus* (*Lymphocystis disease virus*-1) and *Megalocytivirus* (*Infectious spleen* and *kidney necrosis virus*) as reported in Table 4.3.

During the previous decades, several virus strains belonging to the family Iridoviridae have been isolated from various fish species suffering serious losses. Strains associated with high morbidity and mortality and inducing necrotic lesions in the haematopoietic tissue (Hedrick *et al.*, 1992; Inouye *et al.*, 1992; Moody and Owens, 1994; Hedrick and McDowell, 1995; Miyata *et al.*, 1997; Do *et al.*, 2005), have been tentatively classified in the genus *Ranavirus*. The following viruses have been included in this classification: Cod ulcus syndrome iridovirus (Jensen and Larsen, 1979), Cyprinid iridovirus (Berry *et al.*, 1983), Red fin perch iridovirus (EHNV) (Langdon *et al.*, 1986), Eel iridovirus (Nakajima *et al.*, 1998), Sheatfish iridovirus (ESV) (Ahne *et al.*, 1989), Turbot iridovirus (TBIV) (Bloch and Larsen, 1993), catfish irido-like virus (Pozet *et al.*, 1992; Bovo *et al.*, 1993), Large mouth bass iridovirus (LMBV) (Plumb *et al.*, 1996) and pike perch iridovirus (Tapiovaara *et al.*, 1998).

A second group including strains isolated from several fish species in which cell hypertrophy affecting several tissues was the most constant finding, was tentatively classified as a new established genus *Megalocytivirus*: Red seabream iridovirus (RSIV) (Inouye *et al.*, 1992), Grouper sleepy iridovirus (GSDIV) (Chua *et al.*, 1994), Sea-bass iridovirus (SBIV) (Matsuoka *et al.*, 1996), dwarf gourami iridovirus (DGIV) (Sudthongkong *et al.*, 2002), African Lamprey iridovirus (ALIV) (Sudthongkong *et al.*, 2002), grouper iridovirus (GIV) (Chao and Pang, 1997) and the Taiwan grouper iridovirus (TGIV) ( Chou *et al.*, 1998; Chao *et al.*, 2002).

The genus *Lymphocystivirus* includes only one species, *Lymphocystis disease virus*-1 (LCDV-1), characterized by a restricted tropism and lesions confined mainly to the skin and fin tissues (Wolf, 1988; Anders, 1989). Further iridoviral isolates like Lymphocystis disease virus-2 and Dab Lymphocystis disease virus (Darai *et al.*, 1983) have been tentatively ascribed to the same genus, while white sturgeon iridovirus (WSIV) (Hedrick *et al.*, 1990) and erythrocitic necrosis virus (ENV) (Appy *et al.*, 1976) are still unassigned.

Particular attention has been paid to iridoviruses during the recent years because of their growing importance for the aquaculture industry

**Table 4.3** List of fish viral species in the family Iridoviridae isolated from fish (Chinchar *et al.*, 2005).

| *Genus* | *Virus names* | *Virus species* |
|---|---|---|
| *Ranavirus* | Epizootic haematopoietic necrosis virus (EHNV) | *Epizootic haematopoietic necrosis virus* |
| | European catfish virus (ECV)<br>European sheatfish virus (ESV) | *European catfish virus* |
| | Santee-Cooper ranavirus (SCRV)<br>Largemouth bass virus (LMBV)<br>Guppy virus 6 (GV6)<br>Doctor fish virus (DFV) | *Santee-Cooper ranavirus* |
| | Singapore grouper iridovirus (SGIV) | Singapore grouper Iridovirus (tentative) |
| *Lymphocystivirus* | Lymphocystis disease virus-1 (LCDV-1)<br>Flounder lymphocystis disease virus (FLDV)<br>Flounder virus | *Lymphocystis disease virus-1* |
| | Lymphocystis disease virus-2 (LCDV-2) | Lymphocystis disease virus-2 (tentative) |
| | Dab lymphocystis disease virus | Dab Lymphocystis disease virus (tentative) |
| *Megalocytivirus* | Inf. spleen and kidney necrosis iridovirus (ISKNV)<br>Grouper sleepy disease iridovirus (GSDIV)<br>Red Seabream iridovirus (RSIV)<br>Sea bass iridovirus (SBIV)<br>Taiwan grouper iridovirus (TGIV)<br>Dwarf gourami iridovirus (DGIV)<br>African lamprey iridovirus (ALIV) | *Infectious spleen and kidney necrosis virus* |
| Unassigned virus within the family | White sturgeon iridovirus (WSIV)<br>Erythrocytic necrosis virus (ENV) | |

(Chao *et al.*, 2002). Serious epizootics have been reported in several Asian countries particularly from Japan, Korea, Hong Kong, Singapore, Taiwan and Thailand (Inouye *et al.*, 1992; Chua *et al.*, 1994; Kasornchandra and Khongpradit, 1997; Miyata *et al.*, 1997; Jung and Oh, 2000). Furthermore, there is some evidence on their worrying ecological impact in several geographical regions (Cunningham *et al.*, 1996; Daszak *et al.*, 1999; Zhang *et al.*, 1999).

## LYMPHOCYSTIS DISEASE

### Introduction

The name Lymphocystis originates from Woodcock (1904) who believed the skin nodular lesions observed in clinical diseased fish were due to a sporozoan, namely *Lymphocystis johnstonei.*

Lymphocystis disease (LCD) is an old well-known pathological condition (Lowe, 1874) affecting a wide variety of fresh, brackish and marine wild teleosts (Anders and Darai, 1985). Several tropical aquarium fish are also known to be susceptible (Nigrelli and Ruggieri, 1965; Durham and Anderson, 1981). LCD is well documented by exhaustive investigations in Northern Atlantic fish populations (Stich *et al.*, 1977). Furthermore, complete reviews have been published, particularly in the past (McAllister, 1980; Pilcher and Fryer, 1980; Möller and Anders, 1983). The disease is characterized by the appearance on the cutis, tissue of fins and, less frequently, in internal organs of hypertrophied connective cells, found singly or in clusters, macroscopically resembling neoplastic-like lesions. The viral aetiology was established by Walker (1962) and the virus was isolated few years later on BF-2 cells (Wolf *et al.*, 1966).

Lymphocystis viruses induce a chronic benign disease. The dermatotropic infections are self-limiting and the clinical signs usually disappear spontaneously (Nigrelli and Ruggieri, 1965).

In spite of the extensive literature existing on LCD in wild fish, very few reports have been published from aquaculture and they mainly concern marine species (Paperna *et al.*, 1982; Masoero *et al.*, 1986) in which, during the last decades, LCD has caused serious economic problems (Zhang *et al.*, 2004).

### Aetiology

LCD is caused by one or more DNA viral agents, characterized by icosahedral symmetry with a large size range of 130-380 nm in diameter,

belonging to the family Iridoviridae, genus *Lymphocystivirus*. According to Robin and Berthiaume (1981), two different particles may be distinguished by EM; large particles which possibly represent the infectious material (300-350 nm) and smaller non-infectious particles (100-150 nm). The virus is glycerol, ether and heat labile and does not haemagglutinate. Infectivity is maintained after freezing and thawing and the virus may be easily preserved at low temperature (Wolf, 1988). The virus has a strong tropism for fibroblastic tissues where it replicates. BF-2 cells, originating from blue gill fish *Lepomis macrochirus*, was the first cell culture permitting the *in vitro* replication of the virus but later on different cell cultures like GHSBL-1 (Bovo *et al.* 1993), SAF-1 (Perez-Prieto *et al.*, 1999), HINAE (Kasai and Yoshimizu, 2001), GCO and GCK (Zhang *et al.*, 2003) were established. Two genetically distinct strains have been identified so far: LCDV-1 found in flounder (*Platichthys flesus*) and plaice (*Pleuronectes platessa*) and LCDV-2, which occurs mainly in dabs (*Limanda limanda*) (Darai *et al.*, 1983; Anders, 1989; Schnitzler and Darai, 1993; Williams *et al.*, 2000). While there are sufficient data for designation of LCDV-1 as a viral species only preliminary information exist in the literature in order to designate LCDV-2 as a distinct species. The genome of LCDV-1 is 102.7 kbp long (Tidona and Darai, 1997). The LCDV-2 genome consists of 98 kbp. LCDV-1 and LCDV-2 virions are characterized by different polypeptide patterns (Williams *et al.*, 2000). Because LCD has been reported to occur worldwide in at least 125 different fish species in seawater and freshwater (Tidona and Darai, 1999), it has been supposed that some differences in genome structure, gene organization and DNA sequences may exist among strains isolated from different fish species or from different geographic regions (Zhang *et al.*, 2004).

## Pathogenesis and Transmission

The pathogenetic process has been fully described by Wolf and Carlson (1965). Diseased fish seem to represent the most important source of infection while skin wounds may provide an important entry site (Olson, 1958); furthermore leeches and other parasites have been suggested to act as passive vectors (Lawler *et al.*, 1974; Petty and Magnuson, 1974). The virus invades the fibroblastic host cells in two to three days. The result of virus replication in the host is a significant hypertrophy (50,000-100,000x) of the infected cells (Fig. 4.5) that lose their mitosis capacity (Wolf, 1968).

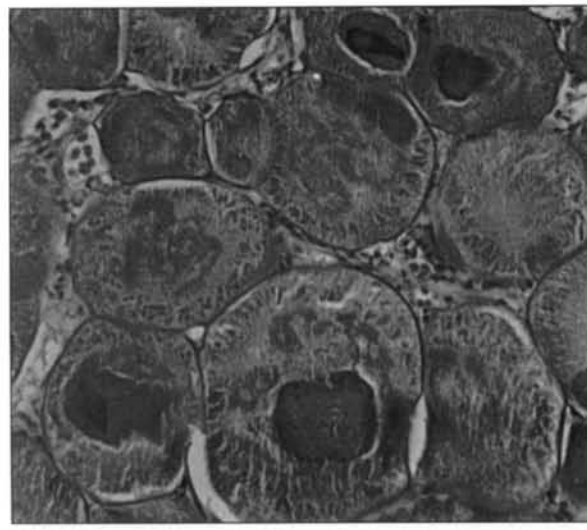

**Fig. 4.5** Hypertrophic cells from skin of seabream (*Sparus aurata*) affected by LCD.

Grossly visible lesions can develop from 1 week up to one year or more after the entry of the virus in the host cells. Following a mature infection cycle the cytoplasm—in which large inclusion bodies are evident—becomes basophilic and the nucleus undergoes degenerative changes releasing a very high amount of virus in the environment. At this stage, a thick hyaline capsule surrounds the hypertrophied fibroblast or lymphocyst.

Horizontal transmission seems to be the most probable route of infection. The spreading of the diseases is enhanced by hatchery conditions where high stocking density and very susceptible fish are present. During an acute outbreak affecting juvenile seabream (*Sparus aurata*) reared in northern Italy, $10^6$ $TCID_{50}$/ml were detected in wastewater (Bovo, results not published). Trash fish used as feed may be a further important source of infection (Leong and Colorni, 2002). Wherever originating, the virus invades the fish possibly through the gills or injured skin. First replication may take place in epithelial cells from which target tissues are reached. Incubation times range from about 10 days at 25°C to longer, depending on species involved, temperature, and other variables.

## Signs and Pathological Changes

Affected fish show whitish to greyish tissue nodules growing in clusters on the skin and fin surface (Fig. 4.6).

**Fig. 4.6** Juvenile seabream (*Sparus aurata*) severely affected by LCD.

In the Mediterranean region, the disease is frequently observed in seabream (*Sparus aurata*) hatcheries. Fish showing heavy infection stop eating for six to eight weeks and become lethargic. Swimming may be impaired by the presence of large nodules on the fins. The infection rate is increased by stress factors, malnutrition, high stocking density or water pollution (Grinwis *et al.*, 1995; Dethlefsen *et al.*, 2000). Infected fish seem to be more susceptible to infection by other microorganisms (Flügel, 1985).

In very severe infections, most of the skin surface and fin may be covered with nodular lesions, giving the fish a disgusting aspect. Lesions consist of hypertrophied dermal fibroblasts or lymphocysts which may aggregate to form masses of individual nodules, cream, pink, or grey in colour that range from 0.3 mm to >2.0 mm in diameter. The nodules may comprise both normal and hypertrophied cells and are commonly detected on the skin surface or on the fins. Hypertrophied cells have also been detected in the oral cavity, gastro-intestinal tract, ovaries, spleen and heart (Schäperclaus, 1992).

Heavy indirect losses may occur due to secondary bacterial infections starting from skin wounds caused by cannibalism on the protruding tissue nodules. When the disease occurs in 5-6 months old specimens, mortality can reach 10-15%. The disease occurs at temperatures ranging from 22 to 27°C (Bovo *et al.*, 1998b).

## Host and Geographic Range

LCD has been reported in a wide host range including more than 125 freshwater and marine species belonging to 34 families and 9 orders (Tidona and Darai, 1999). LCD is quite common in gilt-head seabream aquaculture facilities (Garcia-Rosado *et al.*, 1999). 1-2 months old juveniles are frequently affected. Factors such as high stocking density and external trauma may enhance viral transmission and provoke the appearance of clinical signs (Wolf, 1988; LeDeuff and Renault, 1993).

The first detection of LCDV was reported by Paperna *et al.* (1982) in gilt-head seabream (*Sparus aurata*) reared in Israel, originally imported from the Mediterranean Sea. Later Colorni and Diamant (1995) described LCD in the red drum (*Sciaenops ocellatus*) introduced from Texas, U.S.A. The disease has been described throughout the Mediterranean area including Italy (Masoero *et al.*, 1986), Portugal (Menezes *et al.*, 1987), Spain (Basurco *et al.*, 1990), Greece and Turkey (Moate *et al.*, 1992) and France (LeDeuff and Renault, 1993). In Italy, the disease is mainly found in farmed gilt-head seabream and more rarely in sharpsnout seabream (*Diplodus puntazzo*) (Alborali *et al.*, 1996). Alonso *et al.* (2005) reported the isolation of the virus from cultured sole (*Solea senegalensis*) and wild blackspot (*Pagellus bogaraveo*) in Spain.

LCD has been identified as a major viral disease in Japanese mariculture (Muroga, 1995). In East Asia, it has been reported in Japanese seaperch (*Lateolabrax japonicus*) (Miyazaki and Egusa, 1972; Chen, 1996), yellowtail (*Seriola quinqueradiata*) (Matsusato, 1975), Japanese flounder (*Paralichthys olivaceus*) (Tanaka *et al.*, 1984; Park and Sohn, 1996), red seabream (*Pagrus major*) (Muroga, 1995; Chen, 1996; Park and Sohn, 1996) and rockfish (*Sebastes schlegeli*) (Chun, 1988). In Southeast Asia, only barramundi(*Lates calcarifer*) has been affected (Limsuwan *et al.*, 1983; Chao, 1984). In recent years, LCD has been reported to occur frequently in cultured Japanese flounder (*Paralichthys olivaceus*)in China (Sun *et al.*, 2000; Xu *et al.*, 2000).

## Diagnosis

A presumptive diagnosis of LCD may be easily achieved during the clinical stage when nodules are clearly visible in the skin. Skin or fin biopsies submitted to histopathology may provide a definitive diagnosis of LCD and EM studies may confirm the presence of typical icosahedral virions.

The lymphocysts have irregular, basophilic, intracytoplasmic inclusion bodies and are surrounded by a smooth, eosinophilic capsule.

The causal agent does not replicate very efficiently *in vitro* and very few cell lines are available for diagnostic purposes. Comuzzi and Bovo (1993) succeeded to isolate the virus on GHSBL-1 cells (Gilt-head seabream larvae) inoculated with fresh tissue nodules and observed a clear cpe during five consecutive passages (Fig. 4.7).

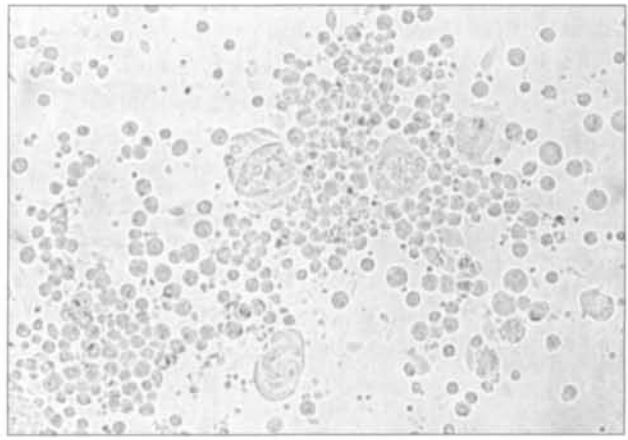

**Fig. 4.7** Extensive cpe caused by LCDV in GHSBL-1 cells.

Perez-Prieto *et al.* (1999) evaluated the susceptibility of the continuous cell line SAF-1 derived from the fins of the gilt-head seabream but with limited success. In fact the cpe was recorded at 3 days post-inoculation affecting 25% of the cells and at day 6 p.i. 50% of the cells displayed cpe. Kasai and Yoshimitzu (2001) described the susceptibility of HINAE cells to Japanese flounder lymphocystis disease virus. Recently, Zhang *et al.* (2003) reported the susceptibility of two grass carp cell lines (GCO, grass carp ovary and GCK, grass carp kidney) to LCDV isolated from cultured Japanese flounder in China and demonstrated, by electron microscopy, the propagation of virus particles in the infected GCO cells.

Furthermore, LCDV infections may be diagnosed by molecular methods like polymerase chain reaction (PCR) (Schnitzler and Darai, 1993; Tidona *et al.*, 1998; Alonso *et al.*, 2005), or by ELISA (Lorenzen and Dixon, 1991).

### Control

No effective therapy or vaccine is so far available. LCD can only be controlled by excluding the causative agent and ensuring that no material from unreliable sources is introduced into the farm. Therefore, it has been suggested to treat inlet hatchery water by UV radiation (Bovo *et al.*, 1998b). When infection appears in the hatchery particular attention must be paid to the treatment of wastewater to ensure that all viral loads leaving the farm are innocuous, thus avoiding the possibility to establish dangerous endemics in nearby waters (Bovo *et al.*, 1998b). An effective but still experimental vaccine has been developed against Japanese flounder lymphocystis disease using an inactivated virus (Yoshimizu and Iwamoto, 2001).

## VIRAL ERYTHROCYTIC NECROSIS

### Introduction

Viral erythrocytic necrosis (VEN) (Haney *et al.*, 1992), first described as piscine erythrocytic necrosis (PEN), is a chronic pathological condition of erythrocytes, occurring in several poikilothermic vertebrates, including fish (Wolf, 1988). The disease was observed since the late 1800s and was first reported in Atlantic cod (*Gadus morhua*) (Laird and Bullock, 1969). Since then, VEN has been observed in several marine and anadromous species. From an economical point of view, VEN is not considered to represent an important risk for aquaculture.

### Aetiology and Pathogenesis

The infectious nature of VEN was demonstrated by experimental transmission (Evelyn and Traxler, 1978; MacMillan and Mulcahy, 1979; Reno *et al.*, 1986). A virus belonging to the family Iridoviridae, unassigned genus, replicating in the erythrocytes of affected fish (Appy *et al.*, 1976) is considered to be the causal agent of the disease. Due to the wide host range and particle size, different fish species are supposed to be infected by distinct agents. In fact, virus particles have the same icosahedral symmetry but different sizes ranging from 150 to 360 nm depending on fish species. The virus has been observed only by EM. Attempts to replicate the virus on different cell lines failed (Evelyn and Traxler, 1978; Pintò *et al.*, 1991) and no virus isolation has been so far reported. The viral agent is likely a DNA virus, which replicates inside the peripheral blood cells

where it produces typical inclusion bodies and induces a reduction of oxygen transfer causing the cell destruction (Reno and Nicholson, 1980). Heavy infections seem to occur only in juvenile fish causing severe haemolytic anaemia characterized by 2-10% haematocrit value (MacMillan and Mulcahy, 1979). In chum salmon, 90% of the peripheral erythrocytes show the presence of inclusion bodies after 1 month's post-experimental infection (MacMillan *et al.*, 1989).

## Clinical Signs

Juvenile fish are more susceptible to experimental disease and may show the presence of blood inclusion bodies 2 to 7 days p.i. depending on fish species (MacMillan and Mulcahy, 1979). No external signs are evident and the disease appears usually as a chronic form. During the infection, typical inclusion bodies (0.8-4 μm) may be found in peripheral erythrocytes (Laird and Bullock, 1969). Pale gills, heart, liver and alimentary tract have been reported following experimental infection in Pacific salmon (Evelyn and Traxler, 1978).

Mortality is normally negligible and depends on species and age of affected fish furthermore it may be influenced by concurrent diseases (Evelyn and Traxler, 1978). Serious epizootics have been reported only in Pacific herring (Meyers *et al.*, 1986).

## Host and Geographic Distribution

VEN infection is widely distributed having been detected in Atlantic Ocean (Reno *et al.*, 1978), North and South Pacific ocean (Evelyn and Traxler, 1978; Rohovec and Amandi, 1981; Meyers *et al.*, 1986; Reyes and Campalans, 1987), Taiwan (Chen *et al.*, 1985), the U.K. and Mediterranean region (Eiras, 1984; Pintò *et al.*, 1989; Eiras and Santos, 1992). Marine and anadromous species like Atlantic cod (Laird and Bullock, 1969; Reno and Nicholson, 1981), Atlantic and Pacific herrings (Reno *et al.*, 1978; Meyers *et al.*, 1986), coho salmon, chum salmon, pink salmon, chinook salmon and steelhead trout have been found naturally susceptible (Evelyn and Traxeler, 1978; Rohovec and Amandi, 1981; Bell and Traxeler, 1985). In the Mediterranean area, the infection has been detected in wild and reared European sea-bass (*Dicentrarchus labrax*) (Pintò *et al.*, 1989). Fish surviving the infection are considered to become carriers and possibly infect young fish.

### Diagnosis

A presumptive diagnosis of VEN is currently dependent on the observation of typical inclusion bodies in Giemsa-stained blood smears. The inclusion bodies are located in the cytoplasm of erythrocytes. They are single for each cell and stain eosinophilic. The observation of irido-like particles by EM may confirm the histological diagnosis. A confirmative method, based on the application of IFAT in blood smears has been reported (Pintò *et al.*, 1991).

### Control

The control of the disease in fish farms relies on the continuous blood monitoring of broodfish in order to avoid any possibility of viral transmission to their offspring.

## RED SEABREAM IRIDOVIRUS DISEASE

Red seabream iridovirus disease (RSIVD) is a serious disease firstly observed in Japan causing significant losses mainly in cultured red seabream (*Pagrus major*) (Inouye *et al.*, 1992; Nakajima and Sorimachi, 1994). The causal agent preliminarily included in the *Ranavirus* genus (Hedrick *et al.*, 1992) has been more recently classified into the newly established genus *Megalocytivirus* as proposed by He *et al.* (2001) and considered as a strain of *Infectious spleen and kidney necrosis virus* (Chinchar *et al.*, 2005). The virion is 200-240 nm in diameter and is inactivated by chloroform and ether treatment. The fully sequenced genome is about 112 Kbp (Kurita *et al.*, 2002). The virus has been replicated in a limited number of cell lines: grunt fin (GF) cells (Clem *et al.*, 1961), KRE-3 (Nakajima and Sorimachi, 1994) following incubation at 20-25°C.

Juvenile red seabream reared in cages are highly susceptible (Matsuoka *et al.*, 1996) but significant mortalities have also been observed in market-size fish (Nakajima *et al.*, 1998). Overt infections have been reported from further cultured marine fish including yellowtail (*Seriola quinqueradiata*), Japanese seaperch (*Lateolabrax* sp.) and Japanese parrotfish (*Oplegnatus fasciatus*) (Matsuoka *et al.*, 1996).

Affected fish are lethargic and exhibit severe anaemia. Haemorrhagic petechiae are found in the gills and the spleen is very often enlarged (Inouye *et al.*, 1992; Jung *et al.*, 1997; Nakajima *et al.*, 1998). Hypertrophied cells staining deeply basophilic with Giemsa are a common

finding in spleen, heart, kidney, liver and gills of infected fish (Inouye *et al.*, 1992; Chao *et al.*, 2004). A similar disease, affecting brown-spotted grouper (*Epinephelus malabaricus*) has been observed in Thailand by Danayadol *et al.* (1996); nevertheless, experimental infection with the same isolate failed to induce the disease in red seabream (Miyata *et al.*, 1997).

Heavy losses associated to RSIV-like disease have been reported in Japan (Matsuoka *et al.*, 1996; Nakajima, 1997; Kawakami and Nakajima, 2002) and several Asian countries including Korea (Jung and Oh, 2000; Sohn *et al.*, 2000; Kim *et al.*, 2002; Do *et al.*, 2005), Taiwan (Chou *et al.*, 1998; Wang *et al.*, 2003), Thailand (Sudthongkong *et al.*, 2002), China (He *et al.*, 2000) in more than 30 species belonging to different taxonomic orders.

Sano *et al.* (2002) tested the susceptibility of estuary cod (*Epinephelus malabaricus*), spangled emperor (*Lethrinus nebulosus*) and cobia (*Rachycentron canadum*) cultured in subtropical Japan. The authors recorded high mortalities in estuary cod, while spangled emperor (*Lethrinus nebulosus*) and cobia (*Rachycentron canadum*) were quite resistant. Gibson-Kueh *et al.* (2004) reported a systemic iridoviral disease associated with high mortality in cultured mullet (*Mugil cephalus*) and in tiger grouper (*Epinephelus fuscoguttatus*). These isolates share common genetic and antigenic patterns with RSIV.

According to the recent report of the International Committee of Virus Taxonomy (Chinchar *et al.*, 2005), the most systemic iridoviruses isolated during the last decade in Asian countries from different hosts appear to be strains of the same viral species, namely, *Infectious spleen and kidney necrosis virus*.

## Diagnosis

A presumptive diagnosis of RSID may be achieved by histopathology studies. According to Inouye *et al.* (1992), typical enlarged cells may be easily observed by light microscopy in most internal organs (kidney, spleen, heart, liver and gills) of clinically affected fish, after H&E or Giemsa staining.

According to OIE (2003b), the diagnosis of RSIVD is based on direct methods through isolation of the causal agent followed by serological identification with monoclonal antibodies or by direct identification of the virus in infected tissues by IFAT (Nakajima and Sorimachi, 1995; Nakajima *et al.*, 1995) or PCR (Oshima *et al.*, 1996, 1998; Kurita *et al.*,

1998). The virus may be easily isolated from kidney and spleen of the infected fish, after inoculation on grunt fin (GF) cells (Clem *et al.*, 1961) incubated at 25°C. Different cell lines, as blue gill fibroblast (BF-2) (Wolf *et al.*, 1966) and KRE-3 (Nakajima and Sorimachi, 1994), which have been demonstrated to be susceptible, may be used as an alternative. The grouper strain replicates in epithelioma papulosum cyprini (EPC) and GF cell lines (Kasornchandra and Khongpradit, 1997).

Caipang *et al.* (2003) first developed a quantitative real-time PCR and later on described the application of a loop-mediated isothermal amplification (LAMP) procedure. This method was at least 10 times more sensitive than conventional PCR in detecting the presence of RSIV (Caipang *et al.*, 2004).

### Control

Control methods currently rely on the application of strict hygiene measures and avoidance of the causal agent from rearing water in hatcheries. A formalin-killed vaccine has proved to be effective in a field trial tested in red seabream (Nakajima *et al.*, 1999). DNA vaccines are under investigation (Marlowe *et al.*, 2005).

## TURBOT IRIDOVIRUS DISEASE

The first report concerning the isolation of an irido-like agent associated with systemic infection in cultured turbot (*Scopthalmus maximus*) fry dates back to the 1990s (Bloch and Larsen, 1993). Affected fish showed reduced feed intake, lethargy, abnormal swimming behaviour, skin darkening particularly on the fin and tail and 70% mortality rate. Muscular spasms preceding the death have been also recorded. EM preparation obtained from gills, liver and kidney revealed the presence of icosahedral irido-like viral particles, 160-185 nm in diameter. More recently, a similar disease characterized by heavy mortalities in juvenile turbot cultured in west coast of Korea was reported (Kim *et al.*, 2005). According to the authors, affected fish were lethargic and showed reduced appetite, exopthalmus, pale body colour, gill and liver anaemia, distended abdomen and enlarged spleen and kidney.

Enlarged cells were found in kidney, spleen, gills, heart, stomach, intestine, liver, pancreas and skin. These findings, which have not been reported previously by Bloch and Larsen (1993), are consistent with those

described in RSIV infection in red seabream (Inouye *et al.*, 1992). EM observations confirmed the presence of hexagonal-shape virions 136-159 nm in diameter, inside the enlarged cells and necrotic areas in several tissues.

## DISEASES CAUSED BY AQUAREOVIRUSES

Aquareoviruses are a group of non-enveloped double-layered capsid RNA viruses belonging to the family Reoviridae, genus *Aquareovirus*, isolated both from freshwater and marine organisms, including finfish, shellfish and *Crustacea* (Hetrick *et al.*, 1992; Hetrick and Hedrick, 1993). The viral genome consists of 11 segments of dsRNA and the virions contain seven structural proteins (VP1-VP7) and five non-structural proteins. Based on whole viral RNA-RNA dot blot hybridization, five genogroups have been established (Lupiani *et al.*, 1993). Different isolates representative of each genogroup were later compared by cross-neutralization and immunodot test procedures. The results showed that the serogroups determined by cross-neutralization correlate with the previously established genogroups (Dopazo *et al.*, 1996). More recently, 6 different species have been officially established as reported in Table 4.4.

Aquareoviruses may replicate in several fish and mammalian cell cultures inducing large syncytia (Winton *et al.*, 1987). Most aquareoviruses have been isolated from apparently healthy fish but, in some occasions, they have been detected in fish suffering moderate (Lupiani *et al.*, 1989) or heavy mortalities (Cusack *et al.*, 2001).

### Turbot Aquareovirus Disease

In 1987, a new syndrome occurred in Spain in reared turbot (*Scopthalmus maximus*). The cumulative mortality at the end of the outbreak lasting for 3 months was 4%. The laboratory investigations revealed the presence of gram-negative bacteria identified as *Vibrio splendidus* and a viral agent preliminarily identified as reo-like virus (Lupiani *et al.*, 1989). As the applied antibiotic therapy was unsuccessful, the new isolated viral agent was considered to be the major responsible for the mortality. Affected fish showed abdomen distension, pale liver and haemorrhages in the anal area and at the fin bases. The stomach was swollen and the intestine empty, filled with mucous; furthermore, a reddish fluid was present in the coelomic cavity. Since the first observation further outbreaks were

**Table 4.4** List of fish virus in the genus *Aquareovirus* (Samal *et al.*, 2005).

| *Species in the genus* | *Virus name* |
|---|---|
| *Aquareovirus A* | Angel fish reovirus (AFRV)<br>Atlantic salmon reovirus AS (ASRV)<br>Atlantic salmon reovirus HBR (HBRV)<br>Atlantic salmon reovirus TS (TSRV)<br>Chinook salmon reovirus DRC (DRCRV)<br>Chum salmon reovirus CS (CSRV)<br>Masou salmon reovirus MS (MSRV)<br>Smelt reovirus (SRV)<br>Striped bass reovirus (SBRV) |
| *Aquareovirus B* | Chinook salmon reovirus B (GRCV)<br>Chinook salmon reovirus ICR (ICRV),<br>Chinook salmon reovirus LBS (LBSV)<br>Chinook salmon reovirus YRC (YRCV),<br>Coho salmon reovirus CSR (CSRV)<br>Coho salmon reovirus ELC (ELCV)<br>Coho salmon reovirus SCS (SCSV) |
| *Aquareovirus C* | Grass carp reovirus (GCRV)<br>Golden shiner reovirus (GSRV) |
| *Aquareovirus D* | Channel catfish reovirus (CCRV) |
| *Aquareovirus E* | Turbot reovirus (TRV) |
| *Aquareovirus F* | Coho salmon reovirus SSR (SSRV)<br>Chum salmon reovirus PSR (PSRV) |
| Tentative species | Chub reovirus (CHRV)<br>Golden ide reovirus (GIRV)<br>Landlocked salmon reovirus (LSRV)<br>Tench reovirus (TNRV) |

recorded in the same geographical area and the same viral agent was repeatedly isolated (Rivas *et al.*, 1995).

Non-enveloped icosahedral particles, 70 nm in diameter, provided with a double capsid layer were detected by EM. According to Samal *et al.* (2005), this agent has been definitively classified in the *Aquareovirus* genus with the name *Aquareovirus E*. The first virus isolation was obtained on CHSE-214 after 2-3 weeks' incubation period. After cell culture adaptation, the virus was easily replicated on CHSE-214 between 4-15°C and on EPC at 15°C but not at 20°C. A clear cpe, consisting of large

syncytia containing 25 or more nuclei, was evident 4-7 days' post-inoculation. In BF-2 cells, the replication of the agent was very slow while in FHM, BB and RTG-2 no cpe was detected during the first passage (Lupiani *et al.*, 1989).

In EPC cells, a temporary cpe appeared but later the overgrowing of new cells restored the normal appearance of the monolayers.

Experimental infection caused only low mortality and the virus was isolated from infected tissues during a 48-day period at very low titres, suggesting that a chronic condition is more likely established and fish may harbour the virus for a long period representing an important source for virus spread. According to Rivas *et al.* (1996), the virus may enter into and replicate in macrophages.

## Seabream Aquareovirus Disease

An aquareovirus has been isolated in Spain from seabream (*Sparus aurata*) fry suffering moderate mortalities (Bandin *et al.*, 1995). No external signs were evident in the affected fish. The virus was isolated on RTM (Rainbow trout mesothelioma) cells after two blind passages.

Virions obtained from supernatant of cell cultures showing extensive cpe appeared as icosahedral particles with an average size of 75 nm. After characterization studies, the strain has been included in the family Reoviridae and the SBA (Seabream Aquareovirus) acronym has been proposed by the authors. No definitive classification has been proposed for this agent that is still unassigned. A similar strain has been isolated on CHSE-214 from seabream affected by winter syndrome in Italy (Bovo *et al.* 1995) (Fig. 4.8).

## Atlantic Halibut Aquareovirus Disease

A severe mortality has been recorded in reared Atlantic halibut (*Hippoglossus hippoglossus*) larvae weighing approximately 1g. The first sign was the decrease of artemia uptake. After a few days, a significant mortality started. Affected fish became dark and lethargic and displayed swollen intestine transparent or white with abdominal distension due to ascitic fluid accumulation. Heart, kidney and liver were pale.

Multifocal liver necrosis, associated with the presence of syncytia and acute necrosis of proximal kidney tubules were the main findings detected by histopathology. No cellular infiltrate was evident. During laboratory

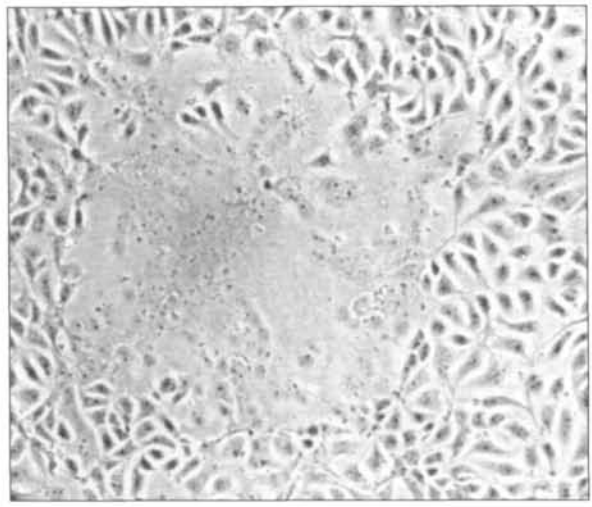

**Fig. 4.8** A large syncytium caused by Aquareovirus infection in CHSE-214 cells.

investigations, a Gram-negative unidentified *Vibrio* was isolated from 2 out of 10 fish. Furthermore, from each fish, a viral agent producing syncytia in CHSE-214 cells was isolated after 48 h of incubation at 15°C, while no cpe was detected on EPC, SHK and GFT cell lines after 21 days.

Spherical, non-enveloped, double capsid viral particles 70 nm in size, morphologically consistent with reoviruses were observed by EM in the hepatocytes.

The disease lasted for 10 weeks giving a cumulative mortality of 58%. The origin of the infection was unclear but vertical transmission and water source were considered mainly because parents were captured in the wild and the inlet water was untreated (Cusak *et al.*, 2001).

## References

Ahne, W., H.J. Schlotfeldt and I. Thomsen. 1989. Fish viruses: isolation of an icosahedral cytoplasmic deoxiribovirus from sheatfish (*Silurus glanis*). *Journal of Veterinary Medicine* B 36: 333-336.

Alborali, L., P. Paternello, A. Lavazza, M. Caggiano and G. Lombardi. 1996. An outbreak of Lymphocystis in *Diplodus puntazzo*. *Bollettino Società Italiana Patologia Ittica* 20: 2-7.

Alonso, M.C., I. Cano, E. Garcia-Rosado, D. Castro, J. Lamas, J.L. Barja and J.J. Borrego. 2005. Isolation of lymphocystis disease virus from sole, *Solea senegalensis* Kaup, and blackspot seabream, *Pagellus bogaraveo* (Brünnich). *Journal of Fish Diseases* 28: 221-228.

Anders, K. 1989. Lymphocystis disease of fish. In: *Viruses of Lower Vertebrates*, W. Ahne and E. Kurstak (eds.). Springer-Verlag, Berlin, pp. 141-160.

Anders, K. and G. Darai. 1985. Genome analysis of fish lymphocystis disease virus. In: *Fish and Shellfish Pathology*, A.E. Ellis (ed.). Academic Press, London, pp. 301-306.

Appy, R.G., M.D.B. Burt and T.J. Morris. 1976. Viral nature of piscine erythrocytic necrosis in the blood of Atlantic cod (*Gadus morhua*). *Journal of the Fisheries Research Board of Canada* 33: 1380-1385.

Arimoto, M., K. Mushiake, Y. Mizuta, T. Nakai, K. Muroga and I. Furusawa. 1992. Detection of striped jack nervous necrosis virus (SJNNV) by enzyme-linked immunosorbent assay (ELISA). *Fish Pathology* 27: 191-195.

Arimoto, M., K. Mori, T. Nakai, K. Muroga and I. Furusawa. 1993. Pathogenicity of the causative agent of viral nervous necrosis disease in striped jack, *Pseudocaranx dentex* (Bloch & Schneider). *Journal of Fish Diseases* 16: 461-469.

Arimoto, M., K. Maruyama and I. Furusawa. 1994. Epizootology of viral nervous necrosis (VNN) in striped jack. *Fish Pathology* 29: 19-24.

Arimoto, M., J. Sato, K. Maruyama, G. Minura and I. Furusawa. 1996. Effect of chemical and physical treatments on the inactivation of striped jack necrosis virus (SJNNV). *Aquaculture*143: 15-22.

Aspehaugh, V., M. Devold and A. Nylund. 1999. The phylogenetic relationship of nervous necrosis virus from halibut (*Hippoglossus hippoglossus*). *Bulletin of the European Association of Fish Pathologists* 19: 196-202.

Athanassopoulou, F., C. Billinis and T. Prapas. 2004. Important disease conditions of newly cultured species in intensive freshwater farms in Greece: First incidence of nodavirus infection in *Acipenser* sp. *Diseases of Aquatic Organisms* 60: 247-252.

Awang, A.B. 1987. Sea bass (*Lates calcarifer*) larvae and fry production in Malaysia. In: *Management of Wild and Cultured Sea Bass/Barramundi* Lates calcarifer, J.W. Copland and D.I. Grey (eds.). ACIAR Press, Canberra, pp. 144-147.

Azad, I.S., M.S. Shekhar, A.R. Thirunavukkarasu, M. Poornima, M. Kailasam, J.J.S. Rajan, S.A. Ali, M. Abraham and P. Ravichandran. 2005. Nodavirus infection causes mortalities in hatchery produced larvae of *Lates calcarifer*: First report from India. *Diseases of Aquatic Organisms* 63: 113-118.

Bandin, I., C. Rivas, M. Noya, J.M. Cutrin, J.L. Barja and C.P. Dopazo. 1995. Isolation of a new aquareovirus from gilt-head seabream cultured in Galicia (N.W. Spain). *Bulletin of the European Association of Fish Pathologists* 15: 157-159.

Barker, D.E., A.M. Mackinnon, L. Boston, M.D.B. Burt, D.K. Cone, D.J. Speare, S. Griffiths, M. Cook, R. Ritchie and G. Olivier. 2002. First report of piscine nodavirus infecting wild winter flounder *Pleuronectes americanus* in Passamaquoddy Bay, New Brunswick, Canada. *Diseases of Aquatic Organisms* 45: 25-31.

Basurco, B., M.A. Marcotegui, A. Rueda, A. Tiana, A. Castellanos, J.V. Tarazona, M.J. Muñoz and J.M. Coll. 1990. First report of Lymphocystis disease in *Sparus aurata* (Linnaeus) in Spain. *Bulletin of European Association of Fish Pathologists* 10: 71-73.

Bell, G.R. and G.S. Traxeler. 1985. First record of viral erythrocytic necrosis and *Ceratomyxa shasta* Noble, 1950 (Myxozoa:Myxosporea) in feral pink salmon (*Oncorhynchus gorbuscha* Walbaum). *Journal of Wildlife Diseases* 21: 169-171.

Bellance, R. and D. Gallet de Saint-Aurin. 1988. L'encephalite virale de loup de mer. *Caraibes Medical* 2: 105-114.

Berry, E.S., T.B. Shea and J. Gabliks. 1983. Two iridovirus isolates from *Carassius auratus* L. *Journal of Fish Diseases* 6: 501-510.

Bloch, B. and J.L. Larsen. 1993. An iridovirus-like agent associated with systemic infection in cultured turbot *Scopthalmus maximus* fry in Denmark. *Diseases of Aquatic Organisms* 15: 235-240.

Bloch, B., K. Gravningen and J.L. Larsen. 1991. Encephalomyelitis among turbot associated with a picornavirus-like agent. *Diseases of Aquatic Organisms* 10: 65-70.

Bondad-Reantaso, M.G., S. Kanchanakhan and S. Chinabut. 2000. Review of grouper diseases and health management strategies for grouper and marine finfish diseases. In: *APE-CFWG02/2000 Workshop. Development of a Regional Research Program on Grouper Virus Trasmission and Vaccine Development*, Bangkok, 18-20 October 2000, pp. 27-60.

Boonyaratpalin, S., K. Supamattaya, J. Kasornchandra and R.W. Hoffman. 1996. Picorna-like virus associated with mortality and spongiosus encephalopathy in grouper *Epinephelus malabaricus*. *Diseases of Aquatic Organisms* 26: 75-80.

Borghesan, F., L. Selli, A. Manfrin, F. Mutinelli, K. Qualtieri, S. Ormelli and G. Bovo. 2003. Winter outbreak of viral encephalo-retinopathy in farmed sea bass (*Dicentrarchus labrax*). *Bollettino Società Italiana Patologia Ittica* 15-23.

Bovo, G., M. Comuzzi, S. De Mas, G. Ceschia, G. Giorgetti, P. Giacometti and E. Cappellozza. 1993. Isolation of an Irido-like viral agent from breeding catfish (*Ictalurus melas*). *Bollettino Società Italiana Patologia Ittica* 11: 3-10.

Bovo, G., F. Borghesan, M. Comuzzi, G. Ceschia and G. Giorgetti. 1995. 'Winter Disease' in reared seabream: Preliminary observations. *Bollettino Società Italiana Patologia Ittica* 17: 2-12.

Bovo, G., F. Borghesan, F. Mutinelli, F. Montesi and M. Comuzzi. 1996. Viral Encephalo-retinopathy of reared sea bass: First detection in Italy. *Bollettino Società Italiana Patologia Ittica* 19: 52-64.

Bovo, G., C. Maltese, R. Rosin, F. Borghesan, F. Mutinelli and F. Montesi. 1998a. Viral encephalo-retinopathy of cultured sea bass: Diagnostic aspects. *Biologia Marina Mediterranea* 5: 1621-1626.

Bovo, G., C. Maltese, F. Borghesan, F. Mutinelli, S. De Mas and F. Montesi. 1998b. Viral diseases affecting marine aquaculture in Italy. *XXXIII International Symposium on New Species for Mediterranean Aquaculture*, Alghero, 22-24th April. pp. 165-173.

Bovo, G., T. Nishizawa, C. Maltese, F. Borghesan, F. Mulinelli, F. Montesi and S. De Mas. 1999. Viral encephalopathy and retinopathy of farmed marine fish species in Italy. *Virus Research* 63: 143-146.

Breuil, G. and B. Romenstand. 1999. A rapid ELISA method for detecting specific antibody level against nodavirus in the serum of the sea bass, *Dicentrarchus labrax* (L.): Application to the screening of spawners in a sea bass hatchery. *Journal of Fish Diseases* 22: 45-52.

Breuil, G., J.R. Bonami, J.F. Pepin and Y. Pichot. 1991. Viral infection (picorna-like virus) associated with mass mortalities in hatchery reared sea bass (*Dicentrarchus labrax*) larvae and juveniles. *Aquaculture* 97: 109-116.

Breuil, G., J.F. Pepin, J. Castric, C. Fauvel and R. Thiery. 2000. Detection of serum antibodies against nodavirus in wild and farmed adult sea bass: Application to the screening of broodstock in sea bass hatcheries. *Bulletin of the European Association of Fish Pathologists* 20: 95-100.

Breuil, G., J.F.P. Pepin, S. Boscher and R. Thiery. 2002. Experimental vertical transmission of nodavirus from broodfish to eggs and larvae of the sea bass, *Dicentrarchus labrax* (L.). *Journal of Fish Diseases* 25: 697-702.

Caipang, C.M.A., I. Hirono and T. Aoki. 2003. Development of real-time PCR assay for the detection and quantification of red seabream iridovirus (RSIV). *Fish Pathology* 38: 1-7.

Caipang, C.M.A., I. Haraguchi, T. Ohira, I. Hirono and T. Aoki. 2004. Rapid detection of a fish iridovirus using loop-mediated isothermal amplification (LAMP). *Journal of Virological Methods* 121: 155-161.

Castric, I., R. Thiery, J. Jeffroy, P. de Kinkelin and J.C. Raymond. 2001. Seabream *Sparus aurata*, an asymptomatic contagious fish host for nodavirus. *Diseases of Aquatic Organisms* 47: 33-38.

Chang, S.F., G.H. Ngoh and S. Kueh. 1997. Detection of viral nervous necrosis nodavirus by reverse transcription polymerase chain reaction in locally farmed marine food fish. *Singapore Veterinary Journal* 21: 39-44.

Chang, S.F., G.H. Ngoh, L.F.S. Kueh, Q.W. Qin, C.L. Chen, T.J. Lam and Y.M. Sin. 2001. Development of a tropical marine fish cell line from Asian seabass (*Lates calcarifer*) for virus isolation. *Aquaculture* 192: 133-145.

Chao, C.B. and V.F. Pang. 1997. An outbreak of an irido-like infection in cultured grouper (*Epinephelus* spp.) in Taiwan. *Journal of Chinese Society of Veterinary Sciences* 23: 411-422.

Chao, C.B., S.C. Yang, H.Y. Tsai, C.Y. Chen, C.S. Lin and H.T. Huang. 2002. A nested PCR for the detection of grouper iridovirus in Taiwan (TGIV) in cultured hybrid grouper, giant seaperch, and largemouth bass. *Journal of Aquatic Animal Health* 14: 104-113.

Chao, C.B., C.Y. Chen, Y.Y. Lai, C.S. Lin and H.T. Huang. 2004. Histological, ultrastructural, and *in situ* hybridization study on enlarged cells in grouper *Epinephelus* hybrids infected by grouper iridovirus in Taiwan (TGIV). *Diseases of Aquatic Organisms* 58: 127-142.

Chao, T.M. 1984. Studies on the transmissibility of lympocystis disease occurring in seabass (*Lates calcarifer* Bloch). *Singapore Journal of Primary Industries* 12: 11-16.

Chen, B.S., 1996. An overview of the disease situation, diagnostic technique, preventives and treatments for cage-cultured, high value marine fishes in China. In: *Aquaculture Health Management Strategies for Marine Fishes*, K.L. Main and C. Rosenfeld (eds.). The Oceanic Institute, Hawaii, pp. 109-116.

Chen, S.N., G.H. Kou, R.P. Hedrick and J.L. Fryer. 1985. The occurrence of viral infections of fish in Taiwan. In: *Fish and Shellfish Pathology*, A.E Ellis (ed.), *First International Conference of the European Association of Fish Pathologists, Plymouth, England*, Sept. 20-23, 1983. Academic Press, London, pp. 313-320.

Chi, S.C., C.F. Lo, G.H. Kou, P.S. Chang, S.E. Peng and S.N. Chen. 1997. Mass mortalities associated with viral nervous necrosis (VNN) disease in two species of hatchery-

reared grouper, *Epinephelus fuscoguttatus* and *Epinephelus akaara* (Temminck & Schlegel). *Journal of Fish Diseases* 20: 185-193.

Chi, S.C., W.W. Hu and B.J. Lo. 1999. Establishment and characterization of a continuous cell line (GF-1) derived from grouper, *Epinephelus coioides* (Hamilton): A cell line susceptible to grouper nervous necrosis virus (GNNV). *Journal of Fish Diseases* 22: 173-182.

Chi, S.C., J. Lo, S.C. Lin, W.W. Wen, G.H. Lo, G.H. Kou and S.N. Chen. 2000. The survey of viral nervous necrosis among cultured grouper in Taiwan. In: *APEC FWG 02/ 2000. Development of a Regional Research Program on Grouper Virus Trasmission and Vaccine Development*, Bangkok, 18-20 October 2000.

Chi, S.C., K.W. Lee and S.J. Hwang. 2001. Investigation of host range of fish nodavirus in Taiwan. In: *Tenth International Conference of the European Association of Fish Pathologists*, September 9-14, 2001, Dublin. Abstract O-049.

Chi, S.C., J.R. Shieh and S.J. Lin. 2003. Genetic and antigenic analysis of betanodaviruses isolated from aquatic organisms in Taiwan. *Diseases of Aquatic Organisms* 55: 221-228.

Chinchar, V.G., S. Essbauer, J.G. He, A. Hyatt, T. Miyazaki, V. Seligy and T. Williams. 2005. Family Iridoviridae. In: *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*, M.H.V. Fauquet, C.M. Mayo, M.A. Maniloff, J. Desselberger and L.A. Ball (eds.). Elsevier, Academic Press, New York, pp. 145-162.

Chou, H.Y., C.C. Hsu and T.Y. Peng. 1998. Isolation and characterization of a pathogenic iridovirus from cultured grouper (*Epinephelus* sp.) in Taiwan. *Fish Pathology* 33: 201-206.

Chua, F.H.C., M.L. Ng, K.L. Ng, J.J. Loo and J.Y. Wee. 1994. Investigation of outbreaks of a novel disease, 'Sleepy Grouper Disease', affecting the brown-spotted grouper (*Epinephelus tauvina* Forskal). *Journal of Fish Diseases* 17: 417-427.

Chua, F.H.C., J.J. Loo and J.Y. Wee. 1995. Mass mortality in juvenile greasy grouper, *Epinephelus tauvina*, associated with vacuolating encephalopathy and retinopathy. In: *Diseases in Asian Aquaculture II*, M. Shariff, J.R. Arthur and R.P. Subasinghe (eds.). Fish Health Section, Asian Fisheries Society, Manila, pp. 235-241.

Chun, S.K., 1988. Studies on lymphocystis disease in *Sebastes schlegeli*. *Journal of Fish Pathology* 1: 72-76.

Clem, L.W., L. Moewus and M.M. Sigel. 1961. Studies with cells from marine fish in tissue culture. *Proceeding Society of Experimental Biology and Medicine* 108: 762-766.

Colorni, A. and A. Diamant. 1995. Splenic and cardiac lymphocystis in the red drum, *Sciaenops ocellatus* (L.). *Journal of Fish Diseases* 18: 467-471.

Comps, M. and J.C. Raymond. 1996. Virus-like particles in the retina of the seabream, *Sparus aurata*. *Bulletin of European Association of Fish Pathologists* 16: 161-163.

Comps, M., J.F. Pepin and J.R. Bonami. 1994. Purification and characterization of two fish encephalitis viruses (FEV) infecting *Lates calcarifer* and *Dicentrarchus labrax*. *Aquaculture* 123: 1-10.

Comps, M., M. Trindade and C. Delsert. 1996. Investigation of fish encephalitis viruses (FEV) expression in marine fishes using DIG-labelled probes. *Aquaculture* 143: 113-121.

Comuzzi, M. and G. Bovo. 1993. Establishment of a fish cell line from gilt-head seabream (*Sparus aurata*). In: *EAFP Sixth International Conference of Diseases of Fish and Shellfish*, EAFP (ed.). 5-10 September 1993, Brest, 130.

Cunningham, A.A., T.E. Langton, P.M. Bennet, J.F. Lewin, S.E. Drury, R.E. Gouch and S.K. Macgregor. 1996. Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). *Philosophical Transactions of the Royal Society of London.* B 351: 1539-1557.

Curtis, P.A., M. Drawbridge, T. Iwamoto, T. Nakai, R.P. Hedrick and A.P. Gendron. 2001. Nodavirus infection of juvenile white sea bass *Atractoscion nobilis*, cultured in southern California: First record of viral nervous necrosis (VNN) in North America. *Journal of Fish Diseases* 24: 263-271.

Cusack, R.R., D.B. Groman, A.M. MacKinnon, F.S.B. Kibenge, D. Wadowska and N. Brown. 2001. Pathology associated with an aquareovirus in captive juvenile Atlantic halibut *Hippoglossus hyippoglossus* and an experimental treatment strategy for a concurrent bacterial infection. *Diseases of Aquatic Organisms* 44: 7-16.

Dalla Valle, L., L. Zanella, P. Patarnello, L. Paolucci, P. Belvedere and L. Colombo. 2000. Development of a sensitive diagnostic assay for fish nervous necrosis virus based on RT-PCR plus nested PCR. *Journal of Fish Diseases* 23: 321-327.

Dalla Valle, L., E. Negrisolo, P. Patarnello, L. Zanella, C. Maltese, G. Bovo and L. Colombo. 2001. Sequence comparison and phylogenetic analysis of fish nodavirus based on the coat protein gene. *Archives of Virology* 146: 1125-1137.

Dalla Valle, L., V. Toffolo, M. Lamprecht, C. Maltese, G. Bovo, P. Belvedere and L. Colombo. 2005. Development of a sensitive and quantitative diagnostic assay for fish nervous necrosis virus based on two-target real-time PCR. *Veterinary Microbiology* 110: 167-179.

Danayadol, Y., S. Direkbusarakom and K. Supamattaya. 1995. Viral nervous necrosis in brownspotted grouper, *Epinephelus malabaricus*, cultured in Thailand. In: *Diseases in Asian Aquaculture II*, M. Shariff, J.R. Arthur and R.P. Subasinghe (eds.). Fish Health Section, Asian Fisheries Society, Manila. pp. 227-233.

Danayadol, Y., S. Direkbusarakom, S. Boonyaratpalin, T. Miyata and M. Miyata. 1996. An outbreak of irido-like infection in brown-spotted grouper (*Epinephelus malabaricus*) cultured in Thailand. *The Aquatic Animal Health Research Institute Newsletter*, 5-6.

Darai, G., K. Anders, H.G. Koch, H. Delius, H. Gelderblom, C. Samalecos and R.M. Flügel. 1983. Analysis of the genome of fish lymphocystis disease virus isolated directly from epidermal tumours of *Pleuronectes*. *Virology* 126: 466-479.

Daszak, P., L. Berger, A.A. Cunningham, A.D. Hyatt, D.E. Green and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* 5: 735-748.

Delsert, C., N. Morin and M. Comps. 1997. A fish encephalitis virus that differs from other nodaviruses by its capsid protein processing. *Archives of Virology* 142: 359-371.

De Mas, S., N. Vicari, D. Pellati, V. Bertazzo, G. Bovo, F. Borghesan, F. Montesi, F. Mutinelli, L. Dalla Valle and E. Tisato. 1998. RT-PCR technique application for sea bass (*Dicentrarchus labrax*) viral encephaloretinopathy diagnosis. *Bollettino Società Italiana Patologia Ittica* 10: 11-23.

Dethlefsen, V., T. Lang and P. Köves. 2000. Regional patterns in prevalence of principal external diseases of dab *Limanda limanda* in the North Sea and adjacent areas 1992-1997. *Diseases of Aquatic Organisms* 42: 119-132.

Do, J.W., S.J. Cha, J.S. Kim, E.J. An, M.S. Park, J.W. Kim, Y.C. Kim, M.A. Park and J.W. Park. 2005. Sequence variation in the gene encoding the major capsid protein of Korean fish iridoviruses. *Archives of Virology* 150: 351-359.

Dopazo, C.P., I. Bandin, C. Rivas, C. Cepeda and J.L. Barja. 1996. Antigenic differences among aquareoviruses correlate with previously established genogroups. *Diseases of Aquatic Organisms* 26: 159-162.

Durham, P.J. and C.D. Anderson. 1981. Lymphocystis disease in imported tropical fish. *New Zealand Veterinary Journal* 29: 88-91.

Eiras, J.C. 1984. Virus infection of marine fish: prevalence of viral erythrocytic necrosis (VEN) in *Mugil cephalus* L., *Blennius pholis* L., and *Platichthys flesus* L. in coastal waters of Portugal. *Bulletin of the European Association of Fish Pathologists* 4: 52-56.

Eiras, J.C. and P.J. Santos. 1992. Presumptive viral erythrocytic necrosis (VEN) in fishes of the Portuguese coast. *Bulletin of European Association of Fish Pathologists* 12: 45-47.

Evelyn, T.P.T. and G.S. Traxler. 1978. Viral Erythrocytic Necrosis: occurrence in Pacific salmon and experimental transmission. *Journal of the Fisheries Research Board of Canada* 35: 903-907.

Flügel, R.M. 1985. Lymphocystis disease virus. *Current Topics in Microbiology and Immunology* 116: 133-150.

Frerichs, G.N., H.D. Rodger and Z. Peric. 1996. Cell culture isolation of piscine neuropathy nodavirus from juvenile sea bass, *Dicentrarchus labrax*. *Journal of General Virology* 77: 2067-2071.

Frerichs, G.N., A. Tweedie, W.G. Starkey and R.H. Richards. 2000. Temperature, pH and electrolyte sensitivity, and heat, UV and disinfection inactivation of sea bass (*Dicentrarchus labrax*) neuropathy virus. *Aquaculture* 185: 13-24.

Fukuda, Y., H.D. Nguyen, M. Furuhashi and T. Nakai. 1996. Mass mortality of cultured sevenband grouper, *Epinephelus septemfasciatus*, associated with viral nervous necrosis. *Fish Pathology* 31: 165-170.

Gagné, N., S.C. Johnson, A.M. Cook-Versloot and G. Olivier. 2004. Molecular detection and characterization of nodavirus in several marine fish species from the northeastern Atlantic. *Diseases of Aquatic Organisms* 62: 181-189.

Galeotti, M., P. Beraldo, P. Patarnello, G. Sarli and D. Volpatti. 1999. Viral encephalopathy-retinopathy (VER-VNN) with absence of cellular vacuolization in juveniles of sea bass (*D. labrax* L.). *Bollettino Società Italiana Patologia Ittica* 27: 45-56.

Garcia-Rosado, E., D. Castro, S. Rodriguez, S.I. Perez-Prieto and J.J. Borrego. 1999. Isolation and characterization of lymphocystis virus (FLDV) from gilt-head seabream (*Sparus aurata* L.) using a new homologous cell line. *Bulletin of the European Association of Fish Pathologists* 19: 53-56.

Gibson-Kueh, S., G.H. Ngoh-Lim, P. Netto, J. Kurita, K. Nakajima and M.L. Ng. 2004. A systemic iridoviral disease in mullet, *Mugil cephalus* L., and tiger grouper,

*Epinephelus fuscoguttatus* Forsskal: A first report and study. *Journal of Fish Diseases* 27: 693-699.

Glazebrook, J.S. and R.S.F. Campbell. 1987. Diseases of barramundi (*Lates calcarifer*) in Australia: A review. In: *Management of Wild and Cultured Sea Bass/Barramundi Lates calcarifer*, J.W. Copland and D.I. Grey (eds.). ACIAR Press, Canberra, pp. 204-206.

Glazebrook, J.S., M.P. Heasman and S.W. de Beer. 1990. Picornalike viral particles associated with mortalities in larval barramundi, *Lates calcarifer* (Bloch). *Journal of Fish Diseases* 13: 245-249.

Gomez, D.K., J. Sato, K. Mushiake, T. Isshiki, Y. Okinaka and T. Nakai. 2004. PCR-based detection of betanodaviruses from cultured and wild marine fish with no clinical signs. *Journal of Fish Diseases* 27: 603-608.

Grinwis, G.C.M., P.W. Wester, A. Kamstra, E.F.J. Van de Brandhof, J.E. Van Dijk, R.J. Leewis, M.A. Vaal, A.D. Vethaak and J.G. Vos. 1995. Laboratory maintenance, topographical anatomy and histology of flounder, *Platichthys flesus*. *National Institute of Public Health and Environmental Protection*, Bilthoven, Netherlands, Report No. 732402 001.

Grotmol, S. and G.K. Totland. 2000. Surface disinfection of Atlantic halibut *Hippoglossus hippoglossus* eggs with ozonated seawater inactivates nodavirus and increases survival of the larvae. *Diseases of Aquatic Organisms* 39: 89-96.

Grotmol, S., G.K. Totland, A. Kvellestad, K. Fjell and A.B. Olsen. 1995. Mass mortality of larval and juvenile hatchery reared halibut (*Hippoglossus hippoglossus* L.) associated with the presence of virus-like particles in the central nervous system and retina. *Bulletin of the European Association of Fish Pathologists* 15: 176-180.

Grotmol, S., G.K. Totland, K. Torud and B.K. Hjeltnes. 1997. Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: A probable cause of mass mortality of cultured larval and juvenile Atlantic halibut *Hippoglossus hippoglossus*. *Diseases of Aquatic Organisms* 29: 85-97.

Grotmol, S., O. Bergh and G.K. Totland. 1999. Transmission of viral encephalopathy and retinopathy (VER) to yolk-sac larvae of the Atlaintic halibut *Hippoglossus hippoglossus*: occurrence of nodavirus in various organs and a possible route of infection. *Diseases of Aquatic Organisms* 36: 95-106.

Grotmol, S., A.H. Nerland, E. Biering, G.K. Totland and T. Nishizawa. 2000. Characterisation of capsid protein gene from a nodavirus strain affecting the Atlantic halibut *Hippoglossus hippoglossus* and design of an optimal reverse-transcriptase polymerase chain reaction (RT-PCR) detection assay. *Diseases of Aquatic Organisms* 39: 79-88.

Grove, S., R. Johansen, B.H. Dannevig, B.H. Reitan and T. Ranheim. 2003. Experimental infection of Atlantic hippoglossus with nodavirus: Tissue distribution and immune response. *Diseases of Aquatic Organisms* 53: 211-221.

Haney, D.C., D.A. Hursh, M.C. Mix and J.R. Winton. 1992. Physiological and haematological changes in chum salmon artificially infected with erythrocytic necrosis virus. *Journal of Aquatic Animal Health* 4: 48-57.

Hart, D., G.N. Frerichs, A. Rambaut and D.E. Onion. 1996. Complete nucleotide sequence and transcriptional analysis of the snakehead fish retrovirus. *Journal of Virology* 70: 3606-3616.

He, J.G., S.P. Wang, K. Zeng, Z.J. Huang and S.M. Chan. 2000. Systemic disease caused by an iridovirus-like agent in cultured mandarinfish, *Siniperca chuatsi* (Basilewsky), in China. *Journal of Fish Diseases* 23: 219-222.

He, J.G., M. Deng, S.P. Weng, Z. Li, S.Y. Zhou, Q.X. Long, X.Z. Wang and S.M. Chan. 2001. Complete genome sequence of the grouper iridovirus and comparison of genomic organization with those of other iridoviruses. *Journal of Virology* 79: 2010-2023.

Hedrick, R.P. and T.S. McDowell. 1995. Properties of iridoviruses from ornamental fish. *Veterinary Research* 26: 423-427.

Hedrick, R.P., J.M. Groff, T.S. McDowell and W.H. Wingfield. 1990. An iridovirus infection of the integument of the white sturgeon *Acipenser transmontanus*. *Diseases of Aquatic Organisms* 8: 39-44.

Hedrick, R.P., T.S. McDowell, W. Ahne, C. Torhy and P. de Kinkelin. 1992. Properties of three iridovirus-like agents associated with systemic infections of fish. *Diseases of Aquatic Organisms* 13: 203-209.

Hegde, A., H.C. Teh, T.J. Lam and Y.M. Sin. 2003. Nodavirus infection in freshwater ornamental fish, guppy *Poecilia reticulata*—comparative characterization and pathogenicity studies. *Archives of Virology* 148: 575-586.

Hetrick, F.M. and R.P. Hedrick. 1993. New viruses described in finfish from 1988-1992. *Annual Review of Fish Diseases* 3: 187-207.

Hetrick, F.M., S.K. Samal, B. Lupiani, C.P. Dopazo, K. Subramanian and S.B. Mohanty. 1992. Members of the family Reoviridae found in aquatic animals. *Proceedings of the OJI International Symposium on Salmonid Diseases*. Hokkaido University Press, Sapporo, Japan, pp. 33-40.

Huang, B., C. Tan, S.F. Chang, B. Munday, J.A. Mathew, G.H. Ngoh and J. Kwang. 2001. Detection of nodavirus in barramundi, *Lates calcarifer* (Bloch), using recombinant coat protein-based ELISA and RT-PCR. *Journal of Fish Diseases* 24: 135-141.

Húsgaro, S., S. Grotmol, B. Hjeltnes, O.M. Rodseth and E. Biering. 2001. Immune response to a recombinant capsid protein of striped jack nervous necrosis virus (SJNNV) in turbot *Scopthalmus maximus* and Atlantic halibut *Hippoglossus hippoglossus*, and evaluation of a vaccine against SJNNV. *Diseases of Aquatic Organisms* 45: 33-44.

Inouye, K., K. Yamano, Y. Maeno, K. Nakajima, M. Matsuoka, Y. Wada and M. Sorimachi. 1992. Iridovirus infection of cultured red seabream *Pagrus major*. *Fish Pathology* 27: 19-27.

Iwamoto, T., K. Mori, M. Arimoto and T. Nakai. 1999. High permissivity of the fish cell line SSN-1 for piscine nodaviruses. *Diseases of Aquatic Organisms* 39: 37-41.

Iwamoto, T., T. Nakai, K. Mori, M. Arimoto and I. Furusawa. 2000. Cloning of the fish cell line SSN-1 for piscine nodaviruses. *Diseases of Aquatic Organisms* 43: 81-89.

Iwamoto, T., K. Mori, M. Arimoto and T. Nakai. 2001. A combined cell-culture and RT-PCR method for rapid detection of piscine nodaviruses. *Journal of Fish Diseases* 24: 231-236.

Jensen, N.J. and J.L. Larsen. 1979. The ulcus syndrome in cod (*Gadus morhua*). Pathological and histopathological study. *Nordisk Veterinaer Medicin* 31: 221-228.

Johansen, R., T. Ranheim, M.K. Hansen, T. Taksdal and G.K. Totland. 2002. Pathological changes in juvenile Atlantic halibut *Hippoglossus hippoglossus* persistently infected with nodavirus. *Diseases of Aquatic Organisms* 50: 161-169.

Johansen, R., I. Sommerset, B. Tørud, K. Korsnes, M. Hjortaas, F. Nilsen, A.H. Nerland and B.H. Dannevig. 2004a. Characterization of nodavirus and viral encephalopathy and retinopathy in farmed turbot, *Scopthalmus maximus* (L.). *Journal of Fish Diseases* 27: 591-601.

Johansen, R., S. Grove, A.K. Svendsen, I. Modahi and B. Dannevig. 2004b. A sequential study of pathological findings in Atlantic halibut, *Hippoglossus hippoglossus* (L.), throughout one year after an acute outbreak of viral encephalopathy and retinopathy. *Journal of Fish Diseases* 27: 327-341.

Johnson, S.C., S.A. Sperker, C.T. Leggiadro, D.B. Groman, S.G. Griffiths, R.J. Ritchie, M.D. Cook and R.R. Cusack. 2002. Identification and characterization of a piscine neuropathy and nodavirus from juvenile Atlantic cod from the Atlantic coast of North America. *Journal of Aquatic Animal Health* 14: 124-133.

Jung, S.J. and M.J. Oh. 2000. Iridovirus-like infection associated with high mortalities of striped beakpearch *Oplegnatus fasciatus* (Temninck et Schlegel), in southern coastal areas of the Korean Peninsula. *Journal of Fish Diseases* 23: 223-226.

Jung, S.J., T. Miyazaki, M. Miyata and T. Oishi. 1996. Histopathological studies on viral nervous necrosis in a new host Japanese sea bass *Lateolabrax japonicus*. *Bulletin of the Faculty of Bioresources*, Mie University, Tsu City, Mie-Pref, Japan 16: 9-16.

Jung, S.J., T. Miyazaki, M. Miyata, Y. Danayadol and S. Tanaka. 1997. Pathogenicity of iridovirus from Japan and Thailand for the red seabream *Pagrus major* in Japan, and histopathology of experimentally infected fish. *Fisheries Science* 63: 735-740.

Kasai, H. and M. Yoshimizu. 2001. Establishment of two Japanese flounder embryo cell lines. *Bulletin of Fisheries Science*. Hokkaido University, Hakodate, Japan 52: 67-70.

Kasornchandra, J. and R. Khongpradit. 1997. Isolation and preliminary characterization of a pathogenic iridovirus agent in nursing grouper, *Epinephelus malabaricus*. In: *Diseases in Asian Aquaculture*, T.W. Flegel and I.H. MacRae (eds.). FHS/AFS Press, Manila, pp. 61-66.

Kawakami, H. and K. Nakajima. 2002. Cultured fish species affected by red seabream iridoviral disease from 1996 to 2000. *Fish Pathology* 37: 45-47.

Kim, S.R., S.J. Jung, Y.J. Kim, J.D. Kim, T.S. Jung, T.J. Choi, M. Yoshimizu and M.J. Oh. 2001. Phylogenic comparison of Viral Nervous Necrosis (VNN) viruses occurring seed production period. *Journal of Korean Fisheries Society* 35: 237-241.

Kim, Y.J., S.J. Jung, T.J. Choi, H.R. Kim, K.V. Rajendran and M.J. Oh. 2002. PCR amplification and sequence analysis of irido-like virus infecting fish in Korea. *Journal of Fish Diseases* 25: 121-124.

Kim, W.S., M.J. Oh, S.J. Jung, Y.J. Kim and S.I. Kitamura. 2005. Characterization of an iridovirus detected from cultured turbot *Scopthalmus maximus* in Korea. *Diseases of Aquatic Organisms* 64: 175-180.

Kurita, J., K. Nakajima, I. Girono and T. Aoki. 1998. Polymerase chain reaction (PCR) amplification of DNA of red seabream iridovirus (RSIV). *Fish Pathology* 33: 17-23.

Kurita, J., K. Nakajima, I. Girono and T. Aoki. 2002. Complete genome sequencing of red seabream *Iridovirus* (RSIV). *Fisheries Science* 68: 113-115.

Lai, Y-S., S. Murali, H.C. Chiu, H.Y. Ju, Y.S. Lin, S.C. Chen, I.C. Guo, K. Fang and C.Y. Chang. 2001a. Propagation of yellow grouper nervous necrosis virus (YGNNV) in a new nodavirus-susceptible cell line from yellow grouper, *Epinephelus awoara* (Temminck & Schlegel), brain tissue. *Journal of Fish Diseases* 24: 299-309.

Lai, Y-S., H.C. Chiu, S. Murali, I.C. Guo, S.C. Chen, K. Fang and C.Y. Chang. 2001b. *In vitro* neutralization by monoclonal antibodies against yellow grouper nervous necrosis virus (YGNNV) and immunolocalization of virus infection in yellow grouper, *Epinephelus awoara* (Temmink & Schlegel). *Journal of Fish Diseases* 24: 237-244.

Lai, Y-S., J.A.C. John, C.H. Lin, I.C. Guo, I.C. Chen, K. Fang, C.H. Lin and C.Y. Chang. 2003. Establishment of cell lines from a tropical grouper, *Epinephelus awoara* (Temmink & Schlegel) and their susceptibility to grouper irido- and nodaviruses. *Journal of Fish Diseases* 26: 31-42.

Laird, M. and W.L. Bullock. 1969. Marine fish haematozoa from New Brunswick and New England. *Journal of the Fisheries Research Board of Canada* 26: 1075-1102.

Langdon, J.S., J.D. Humphrey, L.D. Williams, A.D. Hyatt and H.A. Westbury. 1986. First virus isolation from Australian fish: an iridovirus-like pathogen from red-fin perch, *Perca fluvialis* L. in Australia. *Journal of Fish Diseases* 9: 263-268.

Lawler, A.R., H.D. Howse and D.V. Cook. 1974. Lymphocystis infections in the silver perch, *Bairdiella chrysura*. *Journal of Mississippi Academy of Sciences* 19: 183.

Le Breton, A., L. Grisez, J. Sweetman and F. Ollevier. 1997. Viral nervous necrosis (VNN) associated with mass mortalities in cage-reared sea bass *Dicentrarchus labrax* L. *Journal of Fish Diseases* 20: 145-151.

LeDeuff, R.M. and T. Renault. 1993. Lymphocystis outbreaks in farmed seabream, *Sparus aurata*, first report on French Mediterranean coast. *Bulletin of European Association of Fish Pathologists* 13: 130-133.

Leong, T.S. and A.A. Colorni. 2002. Infectious diseases of warmwater fish in marine and brackish waters. In: *Diseases and Disorders of Finfish in Cage Culture*, P.T.K. Woo, D.W. Bruno and L.H.S. Lim (eds.). CABI Publishing, Wallingford, Oxon, U.K., pp. 193-230.

Limsuwan, C., S. Chinabut and Y. Danayadol. 1983. Lymphocystis disease in seabass (*Lates calcarifer*). National Inland Fisheries Institute, Technical Paper n. 21, 6 pp.

Lorenzen, K and P.F. Dixon. 1991. Prevalence of antibodies to lymphocystis virus in estuarine flounder *Platichthys flesus*. *Diseases of Aquatic Organisms* 11: 99-103.

Lowe, J. 1874. Fauna and flora of Norfolk. Part IV. Fishes. *Transactions of the Norfolk and Norwich Naturalist' Society* (for 1873-1874), pp. 21-56.

Lupiani, B., C.P. Dopazo, A. Ledo, B. Fouz, J.L. Barja, F.M. Hetrick and A.E. Toranzo. 1989. New syndrome of bacterial and viral etiology in cultured turbot *Scopthalmus maximus*. *Journal of Aquatic Animal Health* 1: 197-204.

Lupiani, B., F.M. Hetrick and S.K. Samal. 1993. Genetic analysis of aquareoviruses using RNA-RNA blot hybridization. *Virology* 197: 475-479.

MacMillan, J.R. and D. Mulcahy. 1979. Artificial transmission to and susceptibility of Puget sound fish to viral erythrocytic necrosis (VEN). *Journal of the Fisheries Research Board of Canada* 36: 1097-1101.

MacMillan, J.R., D. Mulcahy and M.L. Landolt. 1989. Cytopathology and coagulopathy associated with viral erythrocytic necrosis in chum salmon (*Oncorhinchus keta*). *Journal of Aquatic Animal Health* 1: 255-262.

Maeno, Y., L.D. de la Peña and E.R. Cruz-Lacierda. 2002. Nodavirus infection in hatchery-reared orange-spotted grouper, *Epinephelus coioides*: First record of viral nervous necrosis (VNN) in the Philippines. *Fish Pathology* 37: 87-89.

Maeno, Y., L.D. de la Peña and E.R. Cruz-Lacierda. 2004. Mass mortalities associated with viral nervous necrosis in hatchery-reared sea bass *Lates calcarifer* in the Philippines. *Japan Agricultural Research Quarterly* 38: 69-73.

Maltese, C. and G. Bovo. 2001. Effects of some chemico-physical treatments on the virus causing encephalo-retinopathy in farmed sea bass (*Dicentrarchus labrax*). *Bollettino Società Italiana Patologia Ittica* 31: 3-16.

Marlowe, C., A. Caipang, I. Hirono and T. Aoki. 2005. Development of DNA vaccine for the control of Red Seabream Iridoviral Disease (RSIDV). In: *Progress in Fish Vaccinology. Developmental Biology*, P.J. Midltlyng (ed.). S.Karger, Basel, 2005, 121, p.316. (Abstract).

Masoero, L., C. Ercolini, M. Caggiano and A. Rosso. 1986. Osservazioni preliminari sulla Linfocisti in una maricoltura intensiva italiana. *Rivista Italiana di Piscicoltura e Ittiopatologia* 2: 70-74.

Matsuoka, S., K. Inouye and K. Nakajima. 1996. Cultured fish species affected by red seabream iridoviral disease from 1991 to 1995. *Fish Pathology* 31: 233-234.

Matsusato, T. 1975. Lymphocystis disease in cultured yellowtail *Seriola quinqueradiata*. *Fish Pathology* 10: 90-93.

McAllister, P.E. 1980. Fish viruses and viral infections. In: *Comprehensive Virology*, H. Fraenkel-Conrat and R.R. Wagner (eds.). Plenum Press, New York, pp. 401-470.

Menezes, J., A. Ramos and T.G. Pereira. 1987. Lymphocystis disease: An outbreak in *Sparus aurata* from Ria Formosa, south coast of Portugal. *Aquaculture* 67: 222-225.

Meyers, T.R., A.K. Hauck, W.D. Blankenbeckler and T. Minicucci. 1986. First report of viral erythrocytic necrosis in Alaska, USA, associated with epizootic mortality in Pacific herring, *Clupea harengus pallasi*. *Journal of Fish Diseases* 9: 479-491.

Miyata, M., K. Matsuno, S.J. Jung, Y. Danayadol and T. Miyazaki. 1997. Genetic similarity of iridoviruses from Japan and Thailand. *Journal of Fish Diseases* 20: 127-134.

Miyazaki, T. and S. Egusa. 1972. A histopathological observation of lymphocystis disease in the sea bass *Lateolabrax japonicus* (Cuvier and Valanciennes). *Fish Pathology* 6: 83-89.

Mladineo, I. 2003. The immunohistochemical study of nodavirus changes in larval, juveniles and adult sea bass tissue. *Journal of Applied Ichthiology* 19: 366-370.

Moate, R.M., J.E. Harris and S. McMahon. 1992. Lymphocystis infections in cultured gilt-head seabream (*Sparus aurata*) in the Aegean Sea. *Bulletin of the European Association of Fish Pathologists* 12: 134-136.

Möller, H. and K. Anders. 1983. *Krankheiten und Parasiten der Meeresfische*. Möller, Kiel.

Moody, N.J.G. and L. Owens. 1994. Experimental demostration of the pathogenicity of a frog virus, Bohle iridovirus, for a fish species, barramundi *Lates calcarifer*. *Diseases of Aquatic Organisms* 18: 95-102.

Mori, K., T. Nakai, M. Nagahara, K. Muroga, T. Mekuchi and T. Kanno. 1991. A viral disease in hatchery-reared larvae and juveniles of redspotted grouper. *Fish Pathology* 26: 209-210.

Mori, K.I., T. Nakai, K. Muroga, M. Arimoto, K. Mushiake and I. Ferusawa. 1992. Properties of a new virus belonging to nodaviridae found in larval striped jack (*Pseudocaranx dentex*) with nervous necrosis. *Virology* 187: 368-371.

Mori, K., T. Mangioku, T. Iwamoto, M. Arimoto, S. Tanaka and T. Nakai. 2003. Serological relationships among genotypic variants of betanodavirus. *Diseases of Aquatic Organisms* 57: 19-26.

Mori, K., T. Sugaya, T. Nishioka, D.K. Gomez, Y. Fujinamy, M. Oka, M. Arimoto, Y. Okinaka and T. Nakai. 2005. Detection of betanodaviruses from feed fish used in marine aquaculture. In: *EAFP 12th International Conference Diseases of Fish and Shellfish*, Sept. 11-16, 2005, Copenhagen, Abstract O-146.

Munday, B.L. and T. Nakai. 1997. Special topic review: Nodaviruses as pathogens in larval and juvenile marine finfish. *World Journal Microbiology and Biotechnology* 13: 375-381.

Munday, B.L., J.S. Langdon, A. Hyatt and J.D. Humphrey. 1992. Mass mortality associated with a viral-induced vacuolating encephalopathy and retinopathy of larval and juvenile barramundi, *Lates calcarifer* Bloch. *Aquaculture* 103: 197-211.

Munday, B.L., J. Kwang and N. Moody. 2002. Betanodavirus infections of teleost fish: A review. *Journal of Fish Diseases* 25: 127-142.

Muroga, K. 1995. Viral and bacterial diseases in larval and juvenile marine fish and shellfish: A review. *Fish Pathology* 30: 71-85.

Mushiake, K., M. Arimoto, T. Furusawa, I. Furusawa, T. Nakai and K. Muroga. 1992. Detection of antibodies against striped jack nervous necrosis virus (SJNNV) from brood stocks of striped jack. *Nippon Suisan Gakkaishi* 58: 2351-2356.

Mushiake, K., T. Nishizawa, T. Nakai, I. Furusawa and K. Muroga. 1994. Control of VNN in striped jack: Selection of spawners based on the detection of SJNNV gene by polymerase chain reaction (PCR). *Fish Pathology* 29: 177-182.

Mutinelli, F., M.A. Bozza, L. Basilicata and G. Bovo. 1998. Histological and immunohistochemical diagnosis of Nodavirus Encephaloretinopathy in farmed sea bass (*Dicentrarchus labrax*). *Bollettino Società Italiana Patologia Ittica* 23: 2-10.

Nagai, T. and T. Nishizawa. 1999. Sequence of the non-structural protein gene encoded by RNA 1 of striped jack nervous necrosis virus. *Journal of General Virology* 80: 3019-3022.

Nakai, T., H.D. Nguyen, T. Nishizawa, K. Muroga, M. Arimoto and K. Ootsuki. 1994. Occurence of viral nervous necrosis in kelp grouper and tiger puffer. *Fish Pathology* 29: 211-212.

Nakajima, K. 1997. Red seabream iridovirus in Japan. Antigen analysis and comparison with other fish iridoviruses. NRIA International Workshop. *New Approach to Viral Diseases of Aquatic Animals, Kyoto*. National Research Institute of Aquaculture, Nansei, Japan, pp. 108-118.

Nakajima, K. and M. Sorimachi. 1994. Biological and physicochemical properties of the iridovirus isolated from cultured red seabream *Pagrus major*. *Fish Pathology* 29: 29-33.

Nakajima, K. and M. Sorimachi. 1995. Production of monoclonal antibodies against red seabream iridovirus. *Fish Pathology* 30: 47-52.

Nakajima, K., Y. Maeno, M. Fukudome, Y. Fukuda, S. Tanaka, S. Matsuoka and M. Sorimachi. 1995. Immunofluorescence test for the rapid diagnosis of red seabream iridovirus infection using monoclonal antibody. *Fish Pathology* 30: 115-119.

Nakajima, K., Y. Maeno, K. Yokoyama, C. Kaji and S. Manabe. 1998. Antigen analysis of red seabream iridovirus and comparison with other fish iridoviruses. *Fish Pathology* 33: 73-78.

Nakajima, K., Y. Maeno, K. Honda, K. Yokoyama, T. Tooriyama and S. Manabe. 1999. Effectiveness of a vaccine against RSIV disease in a field trial test. *Diseases of Aquatic Organisms* 22: 25-32.

Nguyen, H.D., T. Mekuchi, K. Imura, T. Nakai, T. Nishizawa and K. Muroga. 1994. Occurrence of viral nervous necrosis (VNN) in hatchery reared juvenile Japanese flounder *Paralichthys olivaceus*. *Fisheries Science* 60: 551-554.

Nguyen, H.D., T. Nakai and K. Muroga. 1996. Progression of striped jack nervous necrosis virus (SJNNV) infection in naturally and experimentally infected striped jack *Pseudocaranx dentex* larvae. *Diseases of Aquatic Organisms* 24: 99-105.

Nguyen, H.D., K. Mushiake, T. Nakai and K. Muroga. 1997. Tissue distribution of striped jack nervous necrosis virus (SJNNV) in adult striped jack. *Diseases of Aquatic Organisms* 28: 87-91.

Nigrelli, R.F. and G.D. Ruggieri. 1965. Studies on virus diseases of fishes. Spontaneous and experimentally induced cellular hypertrophy (Lymphocystis disease) in fishes of the New York Aquarium, with a report of a new cases and an annotated bibliography (1874-1956). *Zoologica* 50: 83-96.

Nishizawa, T., K. Mori, T. Nakai, I. Furusawa and K. Muroga. 1994. Polymerase chain reaction (PCR) amplification of RNA of striped jack nervous necrosis virus (SJNNV). *Diseases of Aquatic Organisms* 18: 103-107.

Nishizawa, T., K. Mori, M. Furuhashi, T. Nakai, I. Furusawa and K. Muroga. 1995. Comparison of the coat protein genes of five fish nodaviruses, the causative agents of nervous necrosis in marine fish. *Journal of General Virology* 76: 1563-1569.

Nishizawa, T., K. Muroga and M. Arimoto. 1996. Failure of polymerase chain reaction (PCR) method to detect striped jack nervous necrosis virus (SJNNV) in striped jack *Pseudocaranx dentex* selected as spawners. *Journal of Aquatic Animal Health* 8: 332-334.

Nishizawa, T., T. Furuhashi, T. Nagai, T. Nakai and K. Muroga. 1997. Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. *Applied and Environmental Microbiology* 63: 1633-1636.

Nishizawa, T., R. Takano and K. Muroga. 1999. Mapping a neutralizing epitope on the coat protein of striped jack nervous necrosis virus. *Journal of General Virology* 80: 3023-3027.

Oh, M.J., S.J. Jung, S.R. Kim, K.V. Rajendran, H.J. Kim, T.J. Choi, H.R. Kim and J.D. Kim. 2002. A fish nodavirus associated with mass mortality in hatchery reared red drum, *Sciaenops ocellatus*. *Aquaculture* 211: 1-7.

Office International des Epizooties. 2003a. Viral Encephalopathy and retinopathy. In: *Manual of Diagnostic Tests for Aquatic Animals*, OIE (ed.). 4th Edition. Paris, pp. 135-141.

Office International des Epizooties. 2003b. Red seabream iridovirus disease. In: *Manual of Diagnostic Tests for Aquatic Animals*, OIE (ed.). 4th Edition. Paris, France, pp. 207-211.

Olson, D.E. 1958. Statistic of a walleye sport fishery in a Minnesota lake. *Transactions of the American Fisheries Society* 87: 52-72.

Oshima, S., J. Hata, C. Segawa, N. Hurasawa and S. Yamashita. 1996. A method for direct DNA amplification of uncharacterised DNA viruses and for development of a viral polymerase chain reaction assay: Application to the red seabream iridovirus. *Analytical Biochemistry* 242: 15-19.

Oshima, S., J. Hata, N. Hirasawa, T. Ohtaka, I. Hirono, T. Aoki and S. Yamashita. 1998. Rapid diagnosis of red seabream iridovirus infection using the polymerase chain reaction. *Diseases of Aquatic Organisms* 32: 87-90.

Paperna, I., I. Sabnai and A. Colorni. 1982. An outbreak of lymphocystis in *Sparus aurata* L., in the Gulf of Aqaba, Red Sea. *Journal of Fish Diseases* 5: 433-437.

Park, M.A. and S.G. Sohn. 1996. Pratical approaches to marine fish health problems in Korea. In: *Aquaculture Health Management Strategies for Marine Fishes*, K.L. Main and C. Rosenfeld (eds). The Oceanic Institute, Hawaii, pp. 69-80.

Pavoletti, E., M. Prearo, M. Ghittino and C. Ghittino. 1998. Case of Viral Nervous Necrosis (VNN) in shy drum (*Umbrina cirrosa*) with description of clinical symptomatology and anatomo-histopathological features. *Bollettino Società Italiana Patologia Ittica* 23: 24-33.

Peducasse, S., R. Castric, R. Thiery, J. Jeffroy, A. Le Ven and F. Baudin-Laurencin. 1999. Comparative studies of viral encephalopathy and retinopathy in juvenile sea bass *Dicentrarchus labrax* infected in different ways. *Diseases of Aquatic Organisms* 36: 11-20.

Perez-Prieto, S.I., S. Rodriguez-Sant-Jean, E. Garcia-Rosaso, D. Castro, M.C. Alvarez and J.J. Borrego. 1999. Virus susceptibility of the fish cell line SAF-1 derived from gilt-head seabream. *Diseases of Aquatic Organisms* 35: 149-153.

Petty, L.L. and J.J. Magnuson. 1974. Lymphocystis in age 0 bluegills (*Lepomis machrochirus*) relative to heated effluent in Lake Monona, Wisconsin. *Journal of the Fisheries Research Board of Canada* 31: 1189-1193.

Pilcher, K.S. and J.L. Fryer. 1980. The viral diseases of fish. Part I: Diseases of proven viral etiology. *CRC Critical Reviews in Microbiology* 7: 287-363.

Pintò, R.M., P. Alvarez, A. Bosch and J. Jofre. 1989. Occurrence of a viral erythrocytic infection in the Mediterranean Sea Bass (*Dicentrarchus labrax*). *Journal of Fish Diseases* 12: 185-191.

Pintò, R.M., J. Jofre and A. Bosch. 1991. Viral erythrocytic necrosis infection in sea bass: Virus purification and confirmative diagnosis. *Archives of Virology* 120: 83-91.

Plumb, J.A., J.M. Grizzle, H.E. Young, A.D. Noyes and S. Lamprecht. 1996. An iridovirus isolated from wild largemouth bass. *Journal of Aquatic Animal Health* 8: 265-270.

Pozet, F., M. Morand, A. Moussa, C. Torhy and P. De Kinkelin. 1992. Isolation and preliminary characterization of a pathogenic icosahedral deoxiribovirus from catfish (*Ictalurus melas*). *Diseases of Aquatic Organisms* 14: 35-42.

Renault, T., P. Haffner, F. Baudin-Laurencin, G. Breuil and J.R. Bonami. 1991. Mass mortalities in hatchery-reared sea bass (*Lates calcarifer*) larvae associated with the

presence in the brain and retina of virus-like particles. *Bulletin of the European Association of Fish Pathologists* 11: 68-73.

Reno, P.W. and B.L. Nicholson. 1980. Viral Erythrocytic Necrosis (VEN) in Atlantic cod (*Gadus morhua*): *in vitro* studies. *Journal of the Fisheries Research Board of Canada* 37: 2276-2281.

Reno, P.W. and B.L. Nicholson. 1981. Ultrastructure and prevalence of viral erythrocytic necrosis (VEN) virus in Atlantic cod, *Gadus morhua* L., from the northern Atlantic Ocean. *Journal of Fish Diseases* 4: 361-370.

Reno, P.W., M. Philippon-Fried and B.L. Nicholson. 1978. Ultrastructural studies of piscine erythrocytic necrosis (PEN) in Atlantic herring (*Clupea harengus harengus*). *Journal of Fisheries Research Board of Canada* 35: 148-154.

Reno, P.W., K. Kleftis, S.W. Sherburne and B.L. Nicholson. 1986. Experimental infection and pathogenesis of viral erythrocytic necrosis (VEN) in Atlantic cod, *Gadus morhua*. *Canadian Journal of Fisheries and Aquatic Sciences* 43: 945-951.

Reyes, X.P. and M.B. Campalans. 1987. Necrosis eritrocitica viral (VEN) presunta en salmon coho de la Decima region, Chile. *Investigaciones Marinas, Valparaiso* 15: 25-31.

Rivas, C., C. Cepeda, I. Bandin, C.P. Dopazo and J.L. Barja. 1995. Inability of the turbot aquareovirus to replicate in rainbow trout. *Bulletin of the European Association of Fish Pathologists* 15: 20-22.

Rivas, C., I. Bandin, M. Noya, C.P. Dopazo, C. Cepeda and J.L. Barja. 1996. Effect of the turbot aquareovirus on fish macrophages using an *in vitro* model. *Diseases of Aquatic Organisms* 25: 209-216.

Robin, J. and L. Berthiaume. 1981. Purification of lymphocystis disease virus (LDV) grown In tissue culture. Evidences for the presence of two types of viral particles. *Revue Canadienne de Biologie* 40: 323-329.

Rohovec, J.S. and A. Amandi. 1981. Incidence of viral erythrocytic necrosis among hatchery reared salmonids. In: *Disease Diagnosis and Control in North American Marine Aquaculture*, C.J. Sinderman and D.V Lightner (eds.). Elsevier, Amsterdam, pp. 339-340.

Samal, S.K., H. Attoui, F. Mohd Jaafar and P.P.C. Mertens. 2005. Genus *Aquareovirus*. In: *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*, M.H.V. Fauquet, C.M. Mayo, M.A. Maniloff, J. Desselberger and L.A. Ball (eds.). Elsevier, Academic Press, New York, pp. 511-516.

Sano, M., M. Minagawa and K. Nakajima. 2002. Multiplication of red seabream iridovirus (RSIV) in the experimentally infected grouper *Epinephelus malabaricus*. *Fish Pathology* 37: 163-168.

Schäperclaus, W. 1992. Diseases caused by pathogens. In: *Fish Diseases*, W. Schäperclaus, H. Kulow and K. Schreckenbach (eds.). A.A. Balkema Press, Rotterdam, pp. 311-319.

Schneemann, A., L.A. Ball, C. Delsert, J.E. Johnson and T. Nishizawa. 2005. Family Nodaviridae. In: *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*, M.H.V. Fauquet, C.M. Mayo, M.A. Maniloff, J. Desselberger and L.A. Ball (eds.). Elsevier, Academic Press, New York, pp. 865-872.

Schnitzler, P. and G. Darai. 1993. Identification of the gene encoding the major capsid protein from fish lymphocystis disease virus. *Journal of General Virology* 74: 2143-2150.

Shieh, J.R. and S.C. Chi. 2005. Production of monoclonal antibodies against grouper nervous necrosis virus (GNNV) and development of an antigen capture ELISA. *Diseases of Aquatic Organisms* 63: 53-60.

Skliris, G.P. and R.H. Richards. 1999a. Nodavirus isolated from experimentally infected tilapia, *Oreochromis mossambicus* (Peters). *Journal of Fish Diseases* 22: 315-318.

Skliris, G.P. and R.H. Richards. 1999b. Induction of nodavirus disease in sea bass, *Dicentrarchus labrax*, using different infection models. *Virus Research* 63: 85-93.

Skliris, G.P., J.V. Krondiris, D.C. Sideris, A.P. Shinn, W.G. Starkey and R.H. Richards. 2001. Phylogenetic and antigenic characterization of new fish nodavirus isolates from Europe and Asia. *Virus Research* 75: 59-67.

Sohn, S.G. and M.A. Park. 1998. Viral diseases of cultured marine fish and shrimp in Korea. *Fish Pathology* 33: 189-192.

Sohn, S.G., D.L. Choi, J.W. Do, G.Y. Hwang and J.W. Park. 2000. Mass mortalities of cultured striped beakperch, *Oplegnathus fasciatus* by iridoviral infection. *Journal of Fish Pathology* 13: 121-127.

Sommer, A-I., M.A. Strand, E. Rasmussen and S. Mennen. 2004. Susceptibility of spotted wolffish *Anarchicas minor* to experimental infection with nodavirus and pancreatic necrosis virus. *Diseases of Aquatic Organisms* 59: 101-108.

Sommerset, I. and A.H. Nerland. 2004. Complete sequence of RNA 1 and subgenomic RNA 3 of Atlantic halibut nodavirus (AHNV). *Diseases of Aquatic Organisms* 58: 117-125.

Sommerset, I., E. Lorenzen, N. Lorenzen, H. Bleie and A.H. Nerland. 2003. A DNA vaccine directed against a rainbow trout rhabdovirus induces early protection against a nodavirus challenge in turbot. *Vaccine* 21: 4661-4667.

Sommerset, I., B. Krossoy, E. Biering and P. Frost. 2005a. Vaccines for fish in aquaculture. *Expert Review of Vaccines* 4: 89-101.

Sommerset, I., R. Skern, E. Biering, H. Bleie, I.U. Fiksdal, S. Grove and A.H. Nerland. 2005b. Protection against Atlantic halibut nodavirus in turbot is induced by recombinant capsid protein vaccination but not following DANN vaccination. *Fish and Shellfish Immunology* 18: 13-29.

Starkey, W.G., J.H. Ireland, K.F. Muir, A.P. Shinn, R.H. Richards and H.W. Ferguson. 2000. Isolation of nodavirus from Scottish farmed halibut, *Hippoglossus hippoglossus* (L.). *Journal of Fish Diseases* 23: 419-422.

Starkey, W.G., J.H. Ireland, K.F. Muir, M.E. Jenkins, W. Roy, R.H. Richards and H.W. Ferguson. 2001. Nodavirus infection of Atlantic cod, *Dover sole* and halibut in the United Kingdom. In: *Tenth International Conference of the European Association of Fish Pathologists*, Sept. 9-14, 2001, Dublin. Abstract p. 116.

Starkey, W.G., R.M. Millar, M.E. Jenkins, J.H. Ireland, K.F. Muir and R.H. Richards. 2004. Detection of piscine nodaviruses by real-time nucleic acid sequence based amplification (NASBA). *Diseases of Aquatic Organisms* 59: 93-100.

Stich, H.F., A.B. Action, B.P. Dunn, K. Oishi, F. Yamazaki, T. Harada, G. Peters and N. Peters. 1977. Geographic variations in tumor prevalence among marine fish populations. *International Journal of Cancer* 20: 780-791.

Sudthongkong, C., M. Miyata and T. Miyazaki. 2002. Iridovirus disease in two ornamental tropical freshwater fishes: African lampeye and dwarf gurami. *Diseases of Aquatic Organisms* 48: 163-173.

Sun, X., L. Qu and J. Zhang. 2000. Pathogenicity and immunogenicity of lymphocystis virus of Japanese flounder (*Paralichthys olivaceus*). *High Technology Letters* 9: 19-21.

Sweetman, E., J. Sweetman, A. Le Breton and L. Grisez. 1996. Nodavirus: A review of the findings of the XIV/NODA/95 investigation. In: *Seabass and Seabream Culture: Problems and Prospects,* European Aquaculture Society. Verona, Italy, October 16-18. pp. 87-101.

Tanaka, M., M. Yoshimizu, H. Kusakari and T. Kimura. 1984. Lymphocystis disease in *Kurosoi sebastes* Schlegeli and Hirame *Paralichthys olivaceus* in Hokkaido, Japan. *Bulletin of the Japanese Society of Scientific Fisheries* 50: 37-42.

Tanaka, A., H. Aoki and T. Nakai. 1998. Pathogenicity of the nodavirus detected from diseased sevenband grouper *Epinephelus septemfasciatus*. *Fish Pathology* 33: 31-36.

Tanaka, S., K. Mori, M. Arimoto, T. Iwamoto and T. Nakai. 2001. Protective immunity of sevenband grouper, *Epinephelus septemfasciatus* Thunberg, against experimental viral nervous necrosis. *Journal of Fish Diseases* 24: 15-22.

Tapiovaara, H., N.J. Olesen, J. Linden, E. Rimaila-Parnanen and C.H. vonBonsdorff. 1998. Isolation of an iridovirus from pike-perch *Stizostedion lucioperca*. *Diseases of Aquatic Organisms* 32: 185-193.

Thiery, R., S. Peducasse, J. Castric, A. Le Ven and J. Jeffroy. 1997a. Experimental transmission of viral encephalopathy and retinopathy to juvenile sea bass (*Dicentrarchus labrax*). *Bulletin of the European Association of Fish Pathologists* 17: 118-121.

Thiéry, R., C. Arnauld and C. Delsert. 1999a. Two isolates of sea bass, *Dicentrarchus labrax* L., nervous necrosis virus with distinct genomes. *Journal of Fish Diseases* 22: 201-207.

Thiery, R., J.C. Raymond and J. Castric. 1999b. Natural outbreak of viral encephalopathy and retinopathy in juvenile sea bass, *Dicentrarchus labrax:* study by nested reverse transcriptase-polymerase chain reaction. *Virus Research* 63: 11-17.

Tidona, C.A. and G. Darai. 1997. The complete DNA sequence of lymphocystis disease virus. *Virology* 230: 207-216.

Tidona, C.A. and G. Darai. 1999. Lymphocystis disease virus (Iridoviridae). In: *Encyclopedia of Virology*, A. Granoff and R.G. Webster (eds.). Academic Press, New York, pp. 908-911.

Tidona, C.A., P. Schnitzler, R. Kehm and G. Darai. 1998. Is the major capsid protein of iridoviruses a suitable targhet for the study of viral evolution? *Virus Genes* 16: 59-66.

Totland, G.K., S. Grotmol, Y. Morita, T. Nishioka and T. Nakai. 1999. Pathogenicity of nodavirus strains from striped jack *Pseudocaranx dentex* and Atlantic halibut *Hippoglossus hioppoglossus*, studied by waterborne challenge of yolk-sac larvae of both teleost species. *Diseases of Aquatic Organisms* 38: 169-175.

Ucko, M., A. Colorni and A. Diamant. 2004. Nodavirus infections in Israeli mariculture. *Journal of Fish Diseases* 27: 459-469.

Walker, R. 1962. Fine structure of lymphocystis virus of fish. *Virology* 18: 503-505.

Wang, C.S., H.H. Shih, C.C. Ku and S.N. Chen. 2003. Studies on epizootic iridovirus infection among red seabream, *Pagrus major* (Temminck & Schlegel), cultured in Taiwan. *Journal of Fish Diseases* 26: 127-133.

Watanabe, K-I. and M. Yoshimizu. 1999. Susceptibility of fish cell lines against fish nodavirus. *Fish Pathology* 34: 213-214.

Watanabe, K., T. Nishizawa and M. Yoshimizu. 2000. Selection of broodstock candidates of barfin flounder using an ELISA system with recombinant protein of barfin flounder nervous necrosis virus. *Diseases of Aquatic Organisms* 41: 219-223.

Williams, T., G. Chinchar, G. Darai, A. Hyatt, J. Kalmakoff and V. Seligy. 2000. Family Iridoviridae. In: *Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses*, M.H.V van Regenmortel, C.M. Fauquet, D.H.L. Bishop, E.B. Carstens, M.K. Estes, S.M. Lemon, M.A. Mayo, D.J. McGeoch, C.R. Pringle and R.B. Wickner (eds.). Academic Press, New York, San Diego, pp. 167-182.

Winton, J.R., C.N. Lannan, J.L. Fryer, R.P. Hedrick, T.R. Meyers, J.A. Plumb and T. Yamamoto. 1987. Morphological and biochemical properties of four members of a novel group of reoviruses isolated from aquatic animals. *Journal of General Virology* 68: 353-364.

Wolf, K. 1968. Lymphocystis disease of fish. *Fish Diseases Leaflet Bureau of Sport Fisheries Wildlife* No. 13.

Wolf, K. 1988. Viral erythrocytic necrosis. In: *Fish Viruses and Fish Viral Disease*, K. Wolf (ed.). Cornell University Press, New York, pp. 268-291.

Wolf, K. and C.P. Carlson. 1965. Multiplication of lymphocystis virus in the bluegill (*Lepomis macrochirus*). *Annals of the New York Academy of Sciences* 126: 414-419.

Wolf, K., M. Gravell and R.G. Malsberger. 1966. Lymphocystis virus: isolation and propagation in centrarchid fish cell lines. *Science* 151: 1004-1005.

Woodcock, H.M. 1904. Note on a remarkable parasite of plaice and flounders. *Transactions of Liverpool Biological Society* 18: 143-152.

Xu, H.T., C.A. Piao, Z.L. Jiang and W.X. Wang. 2000. Study on the causative agent of lymphocystic disease in cultured flounder, *Paralichthys olivaceus*, in Mainland China. *Chinese Journal of Virology* 16: 223-226.

Yoshikoshi, K. and K. Inoue. 1990. Viral nervous necrosis in hatchery-reared larvae and juveniles of Japanese parrotfish, *Oplegnathus fasciatus* (Temminck & Schlegel). *Journal of Fish Diseases* 13: 69-77.

Yoshimizu, M. and R. Iwamoto. 2001. The development of protective vaccine against Japanese flounder lymphocystis disease using an inactivated virus. *Fish Disease Center News*. Annual Reports of Fish Disease Control FIDIC, pp. 1-14, Tokyo.

Yoshimizu, M., K. Suzuki, T. Nishizawa, J.R. Winton and Y. Ezura. 1997. Antibody screening for the identification of nervous necrosis carriers in flounder broodstock. In: *Proceedings NRIA International Workshop on New Approaches to Viral Diseases of Aquatic Animals*, Kyoto, pp. 124-130.

Zafran, T. Harada, I. Koesharyani, K. Yuasa and K. Hatai. 1998. Indonesian hatchery reared seabass larvae (*Lates calcarifer*) associated with viral nervous necrosis (VNN). *Indonesian Fisheries Research Journal* 4: 19-22.

Zafran Koesharyani, J.F., Johnny, K. Yuasa, T. Harada and K. Hatai. 2000. Viral nervous necrosis in humpback grouper *Chromileptes altivelis* larvae and juveniles. *Fish Pathology* 35: 95-96.

Zhang, Q.Y., Z.Q. Li and J.F. Gui. 1999. Studies on morphogenesis and cellular interaction of *Rana grylio* virus in an infected cell line. *Aquaculture* 175: 185-197.

Zhang, Q.Y., H.M. Rusn, Z.Q. Li, X.P. Yuan and J.F. Gui. 2003. Infection and propagation of Lymphocystis virus isolated from the cultured flounder *Paralichthys olivaceus* in grass carp cell lines. *Diseases of Aquatic Organisms* 57: 27-34.

Zhang, Q.Y., F. Xiao, J. Xie, Z.Q. Li and J.F. Gui. 2004. Complete genome sequence of lymphocystis disease virus isolated from China. *Journal of Virology* 78: 6982-6994.

Zorriehzahra, M.J., T. Nakai, I. Sharifpour, D. Gomes Kaw, C. Shaw-Chi, M. Soltani, H. Hi. Mohd Daud, M. Sharif Roani and A. Saidi. 2005. Mortality of wild golden mullet (*Liza auratus*) in Iranian Waters of the Caspian Sea, associated with Viral Nervous Necrosis-like agent. *Iranian Journal of Fisheries Sciences* 45: 43-58.

CHAPTER

# 5

# Bacterial Diseases of Fish

**Øivind Bergh**[1,2]

## *AEROMONAS SALMONICIDA* SUBSP. *SALMONICIDA*

The disease 'furunculosis' is caused by the Gram-negative bacterium *Aeromonas salmonicida* subspecies *salmonicida*. *Aeromonas salmonicida* has been recognized as a pathogen of fish for over 100 years. Emmerich and Weibel (1894) made the first authentic report of its isolation during a disease outbreak at a Bavarian hatchery (Hiney and Olivier, 1999). The original epizootic spread of furunculosis in both Europe and America and particularly in the British Isles is strongly suggestive of an introduced pathogen (Bakke and Harris, 1998), although today, its distribution is nearly global.

### Clinical Signs and Gross Pathology

The name of the disease 'furunculosis' refers to lesions in the dermis seen as characteristic umbonate ulcers. The organism penetrates into the

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underlying tissues and organs. Munro and Hastings (1993) described the epizootiology and gross pathology as follows: In a population, a continuum of pathologies may be presented from the most acute to the chronic. Acutely dying fish most often show few external signs, whereas in chronic cases one or more of the following symptoms may be present: darkening, lethargy, inappetence, petechiation at fin bases and sometimes gross swellings. The gills are very pale. Internally, the blood vessels surrounding the lower intestine are inflamed and often so are those of the pyloric caeca. The intestine is devoid of food and may have exudates of blood, mucus and cellular debris. The spleen is swollen and cherry red, whereas the liver is grey to greenish. Petechiae are common on all serosal surfaces. The swimbladder may be swollen and cloudy. The kidney is swollen. Haematocrit measurements often show severely depressed red cell numbers.

## Bacterial Characteristics

*Aeromonas salmonicida* subsp. *salmonicida* are characterized by brownish pigment production and by being nonmotile, facultatively anaerobic gram-negative rods. They possess an A-protein layer and autoagglutinate in distilled water. They reduce nitrate, liquefy gelatin, hydrolyse starch and are oxidase positive.

## Bacterial Transmission

The bacterium can be transferred to marine fish, but only at low rates. Investigating a natural outbreak at a fish farm with wrasse, cod and halibut, Hjeltnes *et al.* (1995) demonstrated transmission to one individual corkwing wrasse, *Symphodus melops*. In challenge experiments with yolk sac larvae of halibut and turbot, transmission to turbot was demonstrated, however, the bacterium could only be demonstrated by immunohistochemistry in the gastrointestinal tract (Bergh *et al.*, 1997). No transmission to halibut larvae was found, despite mortality probably caused by bacterial toxins.

The bacterium has been demonstrated to persist in the surroundings of aquaculture farms for a certain period and its hydrophobic nature causes it to accumulate at surface layers or to associate with organic wastes and fish feed (Enger, 1997). To combat infections, the fish farmers used orally administrated antibacterial agents, the organic waste constituted a pathway for the drugs into the marine environment resulting in

accumulation and high concentrations in the sediments (Jacobsen and Berglind, 1988; Samuelsen *et al.*, 1992). As explained elsewhere in this article, residues of the antibacterial agents were also found in the wild fauna in the vicinity of the medicated fish farms, the bacteria in the sediments developed high frequencies of resistance against the drugs (Lunestad, 1992). Additional environmental problems emerged when organic enriched sediments proved to be a refuge in which bacteria pathogenic to fish could survive for at least 18 months (Husevåg, 1994).

## Diagnostics

Apart from the gross pathological signs, diagnosis is confirmed by isolation of the bacterium. Rapid serology-based kits are commercially available.

## Host Register

Descriptions of the introduction and spread of furunculsis in Norway and Iceland provides good evidence of infection and disease occurring in both farmed and wild fish. *Aeromonas salmonicida* subsp. *salmonicida* mainly affects salmonids (Table 5.1). In addition, the bacterium has been isolated from a range of freshwater fish (Bernoth, 1997), which is beyond the scope of the present review. Furunculosis has been demonstrated in turbot, *Scophthalmus maximus* (Nougayrede *et al.*, 1990), cuckoo wrasse, *Labrus bimaculatus* (Treasurer and Cox, 1991), goldsinny wrasse *Ctenolabrus rupestris* (Treasurer and Laidler, 1994). Atlantic cod *Gadus morhua* and coalfish *Polachius virens* (Willumsen, 1990). Except for the two latter, which were incidental isolations, the isolations were reported to be clinical, however it should be emphasized that there is no evidence of frequent disease outbreaks in non-salmonids. The bacterium has almost a worldwide distribution where salmonid fish occur (Hiney and Olivier, 1999).

Furunculosis was first found in Norway in 1964 following the import of rainbow trout from Denmark. The disease was spread to several farms and to wild fish in the river Numedalslågen. In 1985, furunculosis was discovered in marine salmon farms in Norway to which infected salmon smolts from Scotland had been imported (Egidius, 1987a; Johnsen and Jensen, 1994). This resulted in a major epizootic that culminated in the years 1990-1992 (Lillehaug *et al.*, 2003), after which the emergence of oil-based adjuvant vaccines has more or less eliminated the problem in farmed salmon (reviewed by Sommerset *et al.*, 2005).

Table 5.1 Salmonid fish species from which typical *Aeromonas salmonicida* has been isolated (after Bernoth, 1997) (FW= Freshwater, SW = Seawater).

| | | |
|---|---|---|
| Atlantic salmon | *Salmo salar* | FW/SW |
| Amago salmon | *Oncorhynchus rhodurus* | FW |
| Brook trout | *Salvelinus fontinalis* | FW |
| Brown trout | *Salmo trutta m. lacustris* | FW |
| Chinook salmon | *Oncorhynchus tshawytscha* | Not specified |
| Chum salmon | *Oncorhynchus keta* | FW |
| Coho salmon | *Oncorhynchus kisutch* | FW |
| Cutthroat trout | *Salmo clarki* | FW |
| Dolly Varden | *Salvelinus malma* | FW |
| Japanese charr | *Salvelinus leucomaenis* | Not specified |
| Lake trout | *Salvelinus namaycush* | FW |
| Masu salmon | *Oncorhynchuis masou* | FW |
| Pink salmon | *Oncorhynchus gorbuscha* | FW |
| Pollan | *Coregonus pollan* | FW |
| Rainbow trout | *Oncorhynchus mykiss* | FW |
| Sea trout | *Salmo trutta m. trutta* | FW |
| Sockeye salmon | *Oncorhynchus nerka* | Not specified |

## Transfer from Farmed to Wild Fish and *vice versa*

Direct evidence for transmission from farmed to wild fish is scarce, but epizootiological data suggest that such transmission is important. Prior to the epizootic in Norway in 1990-92, the disease was virtually absent in Norway apart from a single outbreak several years before. In 1992, 550 farms were infected, the disease had spread from 22 natural watercourses in 1989 to 72 in 1992. Rapid spread of the disease was associated with several factors, including escapees and natural movement of wild fish in the sea (Johnsen and Jensen, 1994) and, therefore, evidence is provided of spread from wild to farmed fish. Control of the disease following the introduction of efficient vaccines has probably reduced the problem to a degree where it is no longer a major threat to wild salmon. Evidence from marine fish suggests that interactions do not play a major role.

## Impact on Wild Fish and Aquaculture

The disease has been demonstrated in both farmed and wild fish of Atlantic salmon and other salmonids and non-salmonids listed above with

significant impacts in wild fish but especially farmed fish where mortalities could range from 10-20% and up to 50% before the development of effective vaccines.

Ecological consequences are not known. The epidemic losses of wild salmonids in the early twentieth century in the UK were recorded by Mackie *et al.* (1930, 1933, 1935) but these reports do not suggest depression of population numbers on an overall basis. The effective elimination of the problem in aquaculture has probably resulted in a dramatically reduced infection pressure towards wild salmon; the disease is not considered a major problem to wild salmon in Norway today.

The disease is currently well controlled by vaccination of farmed fish (reviewed by Sommerset *et al.*, 2005) Thus, furunculosis is not considered to be a major disease problem to wild salmon today. Infection at very low prevalence is likely to be endemic within some wild salmonid populations of Europe and wild fish with furunculosis are occasionally recorded, typically associated with high temperatures, low water flow in rivers and other stressful conditions.

## *AEROMONAS SALMONICIDA*—ATYPICAL STRAINS

Apart from the 'typical' subspecies *salmonicida*, the species *Aeromonas salmonicida* comprises a large number of relatively diverse strains commonly referred to as atypical *A. salmonicida*. These atypical strains are responsible for several disease problems in both salmonids and non-salmonids. This is a diverse group of bacteria causing a range of different signs in different hosts (reviewed by Gudmundsdóttir, 1998; Wiklund and Dalsgaard, 1998; Hiney and Olivier, 1999). Among the best-known diseases associated with atypical *A. salmonicida* is ulcer disease of flounder, *Platichthys flesus*, but atypical strains are apparently involved in more disease outbreaks than previously believed (Wiklund and Dalsgaard, 1998).

### Clinical Signs and Gross Pathology

The most common clinical signs in salmonids is skin ulceration but a number of other signs have been recognized, some of which are similar to those seen in cases of typical furunculosis (Gudmundsdóttir, 1998; Hiney and Olivier, 1999). In non-salmonids, superficial skin ulcerations are also common.

In recent challenge experiments with cod, pathological changes, including haemorrhage, early stages of granuloma formation and necrotic changes, were seen in several organs (Magnadóttir *et al.*, 2002). Infection appeared to induce non-specific antibody activity against trinitrophenyl (TNP)-haptenated protein and may have activated the complement system. Specific antibody response against atypical *A. salmonicida* was not detected.

## Bacterial Characteristics

All isolates are strongly autoaggregating gram-negative rods. Useful phenotypical criteria for identification are listed by Munro and Hastings (1993). An extracellular A-layer has been detected in different atypical strains, but virulence mechanisms different from those described for typical *A. salmonicida* subsp. *salmonicida*, for example, an extracellular metallo-protease, a different iron utilization mechanism have been described (reviewed by Wiklund and Dalsgaard, 1998).

The classification of atypical *A. salmonicida* is not clear-cut. Two major groups have been identified according to the biochemical and enzymatic profiles, similar to the subspecies *achromogenes* and *nova*, respectively. Studies by Gunnlaugsdóttir and Gudmundsdóttir (1996) and Gudmundsdóttir *et al.* (2003), have demonstrated the diversity of *Aeromonas salmonicida* strains in *in vivo* and *in vitro* studies on extracellular proteases. The subspecies *salmonicida* constitutes a third subgroup. Genomic characterization further supports that *A. salmonicida* can be divided into subgroups (Hiney and Olivier, 1999).

## Diagnostics

Diagnosis is usually based on culturing the pathogen and further identification according to 16S rDNA and/or biochemical characteristics. The identification of members of the subspecies *achromogenes* is straightforward as they grow on normal media such as Tryptone Soya Agar or Brain Heart Infusion Agar, producing a brownish pigment on both media. Samples of ulcers or lesions in addition to kidney tissue should be cultured because some isolates cannot be found in internal organs. Strains belonging to the subspecies *nova* are frequently more difficult to grow, because blood agar is normally required (Hiney and Olivier, 1999). Culture media and growth conditions are further reviewed by Austin and Austin (1999).

## Evidence for Infection and Disease in Wild Fish

Infections in several wild and farmed species have been demonstrated as listed in Tables 5.2 and 5.3.

In Iceland, Atlantic salmon migrating from sea are occasionally caught moribund or dead in rivers with external and internal lesions, which may be difficult to distinguish from those caused by *A. salmonicida* ssp. *salmonicida* strains (Gudmundsdóttir, 1998). Epizootics have never been observed in these rivers as only incidental diseased fish are found.

**Table 5.2** Fish species from which atypical *Aeromonas salmonicida* have been isolated (from Bernoth, 1997, with additional data from Ingilæ *et al.*, 2000).

| | |
|---|---|
| Atlantic halibut | *Hippoglossus hippoglossus* |
| Atlantic cod | *Gadus morhua* |
| Atlantic salmon | *Salmo salar* |
| American plaice | *Hippoglossoides platessoides* |
| Arctic charr | *Salvelinus alpinus* |
| Black rockfish | *Sebastes schlegeli* |
| Brook trout | *Salmo trutta m. lacustris* |
| Chum salmon | *Oncorhynchus keta* |
| Coho salmon | *Oncorhynchus kisutch* |
| Common wolfish | *Anarhichas lupus* |
| Dab | *Limanda limanda* |
| Flounder | *Platichthys flesus* |
| Goldsinny wrasse | *Ctenolabrus rupestris* |
| Greenback flounder | *Rhombosolea tapirina* |
| Greenling | *Hexogrammos otakii* |
| Haddock | *Melanogrammus aeglefinus* |
| Japanese flounder | *Paralichthys olivaceus* |
| Pacific herring | *Clupea harengus pallasi* |
| Plaice | *Pleuronectes platessa* |
| Rainbow trout | *Oncorhynchus mykiss* |
| Sablefish | *Anoploma fimbria* |
| | *Ammodytes lancea* |
| Sand eels | *Hyperoplus lanceolatus* |
| Sea trout | *Salmo trutta m. trutta* |
| Spotted halibut | *Eopsetta grigorjewi* |
| Spotted wolfish | *Anarhichas minor* |
| Sockeye salmon | *Oncorhynchus nerka* |
| Tom cod | *Gadus microgasus* |
| Turbot | *Scophthalmus maximus* |

## Evidence for Infection and Disease in Farmed Fish

Atypical *A. salmonicida* have been isolated from a range of marine fish species (Table 5.2). The number of host species is rapidly increasing (Gudmundsdóttir, 1998; Hiney and Olivier, 1999). Geographical range extends from Northern Europe, South Africa, North America, Japan and Australia (Wiklund and Dalsgaard, 1998).

## Transfer from Farmed to Wild and *vice versa*

Interactions between wrasse, which is used as cleanerfish, and Atlantic salmon has attracted certain interest; however, there is no evidence from field studies (Samuelsen *et al.*, 2003) that atypical *A. salmonicida* is transferred from wrasse to salmon. Carriers of atypical *A. salmonicida* are apparently common in Norwegian populations of corkwing wrasse and outbreaks may be trigged by stress (Samuelsen *et al.*, 2002). Thus, wild caught wrasses, which are introduced to marine salmon cages, could transfer infection to farmed fish but the mechanism by which this occurs is a farm management practice and is not providing evidence that transmission would occur naturally.

Magnadóttir *et al.* (2002) described infection and disease in wild-caught cod ongrown intensively. As with the case for wrasse, this is another example of how introducing wild fish to a farm can transmit disease to farm stock through a management practice. There is no evidence that transmission is occurring from farmed to wild fish although there is evidence that farmed fish may be infected from wild sources of bacteria. Limited information is available about the ecology, spread and survival of atypical strains in water (reviewed by Wiklund and Dalsgaard, 1998), although some cases indicate that transmission have been linked to the transfer of infected fish (Hiney and Olivier, 1999). Infection appears endemic within many wild stocks and also within certain sectors of marine aquaculture.

## Motile Aeromonads

Diseases caused by motile aeromonads are reported from all over the world from freshwater as well as marine environments (Aoki, 1999). A wide variety of farmed and wild freshwater fish species (coregonids, perch, bream, salmonids, cyprinids, eel) but also some marine species have been found to be infected (Bullock *et al.*, 1971). Further, infections in amphibians, reptiles and freshwater prawns (Roberts, 1993) and humans (Janda and Abbott, 1996) have been published.

Different names have been assigned to diseases caused by motile aeromonads: haemorrhagic septicaemia, motile aeromonas septicaemia (Austin and Austin, 1999), redsore disease (Hazen *et al.*, 1978), and red pest (Roberts, 1993). The most obvious symptoms of the disease are cutaneous haemorrhages of fins and trunk, skin ulcerations, fin and tail rot (Roberts, 1993). Internally, hyperaemia of viscera with haemorrhage over mesenteries and within visceral and parietal peritoneum can be found (Roberts, 1993). Although losses due to motile aeromonad infections in farmed fish are reported (Faisal *et al.*, 1989; Sioutas *et al.*, 1991; Nielsen *et al.*, 2001; Wahli *et al.*, 2005), it is not always clear whether the bacteria are the primary cause (Athanassopoulou *et al.*, 2004).

At least 10 different species are recognized from fish. In fish *Aeromonas hydrophila*, *A. sobria* (*A. veronii* subsp. *sobria*), *A. caviae*, and *A. veronii* are frequently isolated from diseased fish. The bacteria are fermentative, gram-negative rods (0.8–1.0 × 1.0–3.5 μm), motile by means of single polar flagella. Different virulence factors have been detected including cytotoxins and proteases, haemolysins, siderophores, S-layer on cell surface, acetylcholinesterase (reviewed by Aoki, 1999).

## *MORITELLA VISCOSA*

*Moritella viscosa* (formerly *Vibrio viscosus*) is associated with the disease 'winter ulcer' in Atlantic salmon *Salmo salar* and has been reported in farming of salmon since the 1980s (Lunder, 1992), predominantly occuring at temperatures lower than 10°C. The disease has been associated with the use of seawater in smolt production, and is considered to be a major animal welfare problem, due to the large ulcerations. The mortality is usually low, but the disease has caused serious problems in farming of salmon in Norway and Iceland. Economic losses are related to loss of fish and lowered quality of fish recovering from the disease.

Pathogenesis of winter ulcer is related to development of lateral skin lesions, affected fish are usually easy to recognize. The size of the lesions might range from barely visible to large, confluent lesions covering the body surface, with degenerative changes in underlying muscle. Changes may also occur in gill pallor and fin rot. The disease is also characterized by diffuse or petechial haemorrhages in the liver, pyloric caeca and perivisceral fat, necrosis in the spleen and kidney (Salte *et al.*, 1994; Lunder *et al.*, 1995; Benediktsdóttir *et al.*, 1998; Bruno *et al.*, 1998).

The disease has been observed in countries around the North Atlantic. Winter ulcer and isolation of *Moritella viscosa* has been reported in farmed Atlantic salmon *Salmo salar* (Lunder, 1992), rainbow trout *Oncorhynchus mykiss* (Benediktsdóttir *et al.*, 2000) and Atlantic cod *Gadus morhua* (Colquhoun *et al.*, 2004). The bacterium has also been isolated from wild rainbow trout, lumpsucker *Cylopterus lumpus* and captive wild plaice *Pleuronectes platessa* (Benediktsdóttir *et al.*, 2000; Lunder *et al.*, 2000). Experimental infection has also been shown in turbot *Scophthalmus maximus* and Atlantic halibut *Hippoglossus hippoglossus* (Gudmundsdóttir *et al.*, 2001; Björnsdóttir *et al.*, 2004).

M. *viscosa* is gram-negative and forms long cells when grown in liquid media and short or elongated curved rods on solid medium. Creamy-yellow colonies are formed on MSA-B and TSA+NaCl, with average colony size of 0.5 mm after 24 hours at 15-22°C. The colonies are viscous and form long threads when lifted from the plate. A prolonged incubation time is often needed when grown in test media; the bacterium requires 1% peptone for optimal growth. The literature is conflicting when it comes to NaCl and temperature optima, as both 1-4% NaCl and 25°C incubation (Lunder *et al.*, 2000), 2-3% NaCl and no growth at 25°C (Benediktsdóttir *et al.*, 2000) have been published. Key biochemical properties to discriminate M. *viscosa* from other pathogenic vibrios are positive lysine decarboxylase reaction and no utilization of citrate, mannitol and sucrose. Diagnostics are based on pathological changes, as described above, examination of tissue from lesions and kidney for presence of M. *viscosa*. Usually, the bacterium has to be identified in a mixture of other bacteria isolated from lesions, pure cultures of M. *viscosa* are identified by colony morphology and biochemical characteristics.

Two distinct subgroups of the bacterium are formed when using the fingerprint method AFLP, reflecting the geographical origins of Norway and Iceland (Benediktsdóttir *et al.*, 2000). In contrast, *V. wodanis* are more heterogeneous and do not form distinct subgroups (Lunder *et al.*, 2000). AFLP is suggested to be of the most reliable fingerprinting identification tools of Vibrionaceae and has been used to examine the diversity of this group (Thompson *et al.*, 2004).

M. *viscosa* is transmitted horizontally. Several other bacterial species have been isolated from lesions in association with M. *viscosa*, suggesting potential interaction. Bacterial species such as *V. wodanis*, *V. logei* and *V. fischeri* have been isolated from winter ulcer lesions, but only M. *viscosa*

has shown to be effective when inoculated in experimental challenge. This observation and the shown protection given when using the bacterium in an inactivated oil-adjuvanted vaccine, suggest M. *viscosa* may have an important role as a primary pathogen (Lunder *et al.*, 1995; Bendiktsdóttir *et al.*, 1998; Bruno *et al.*, 1998; Greger and Goodrich, 1999). However, the possible role of other bacterial species associated with winter ulcer lesions is not fully understood.

Despite the introduction of a vaccine, which seems to have protection in experimental challenge, winter ulcer is causing losses and raises questions concerning animal welfare in affected fish. In addition, several bacterial species are associated with the disease, there might exist a complex interaction among various bacterial species frequently isolated from lesions. As such, possible interactions are poorly understood. Further knowledge may prove important to improve prophylaxis and treatment of the disease.

## *LISTONELLA (VIBRIO) ANGUILLARUM*

*Listonella* (formerly *Vibrio*) *anguillarum* has long been known as a causative agent of disease, regularly causes epizootics in wild and cultured populations of several fish species (Egidius, 1987b, articles cited therein), the oldest known descriptions of the disease dating from 1817. Vibriosis is known in a wide range of fish species. It caused severe problems to salmonid farming before the advent of modern vaccines (reviewed by Lillehaug *et al.*, 2003; Sommerset *et al.*, 2005). It is still frequently causing problems to 'new' aquaculture species such as halibut (reviewed by Bergh *et al.*, 2001) and cod (reviewed by Bricknell *et al.*, 2006; Samuelsen *et al.*, 2006). It should be noted that the bacterium is also associated with diseases in crustaceans and molluscs.

### Clinical Signs and Gross Pathology

Vibriosis is a classical gram-negative haemorrhagic septicaemia. The outstanding feature of clinical vibriosis is the level of anaemia. Typical signs are red spots on the ventral and lateral areas of the fish, swollen and dark skin lesions that ulcerate, releasing a bloody exudate. There are also corneal lesions. In acute and severe epizootics, most fish die without showing external signs.

## Distribution and Hosts

Vibriosis occurs in cultured and wild marine fish and salmonids in salt or brackish waters, particularly in shallow waters during late summer. In a North European perspective, the most important hosts include all cultured fish species, and the bacterium is commonly isolated from fish in all marine environments.

Table 5.3 Some cultured species susceptible to vibriosis from which *L. anguillarum* has been isolated.

| | |
|---|---|
| Atlantic halibut | *Hippoglossus hippoglossus* |
| Atlantic cod | *Gadus morhua* |
| Atlantic salmon | *Salmo salar* |
| Arctic charr | *Salvelinus alpinus* |
| Coho salmon | *Oncorhynchus kisutch* |
| Common wolfish | *Anarhichas lupus* |
| Eel | *Anguilla anguilla* |
| Japanese eel | *Anguilla japonica* |
| Gilthead seabream | *Sparus aurata* |
| Rainbow trout | *Oncorhynchus mykiss* |
| Sea bass | *Sparus aurata* |
| Sharpshout sea bream | *Diplodus puntazzo* |
| Striped bass | *Morone saxatilis* |
| Spotted wolfish | *Anarhichas minor* |
| Turbot | *Scophthalmus maximus* |
| Yellowtail | *Seriola quinqueradiata* |
| Winter flounder | *Pseudopleuronectes americanus* |

In addition, *L. anguillarum* has been isolated from a range of wild species in the vicinity of farms. Of particular interest may be saithe *Polachius virens*, in which several epizootics have been described. Vibriosis caused by *L. anguillarum* has occurred in both wild and farmed populations of salmonids. Smith (1961) described the disease in finnock sea trout (*Salmo trutta*) from the river Dee in Scotland and Håstein and Lindstad (1991) stated that the disease has occurred in several Norwegian salmon rivers up to 10 km from river mouths and in regions where no aquaculture took place.

## Bacterial Characteristics

*Listonella anguillarum* is a polarly flagellated gram-negative curved rod, facultatively anaerobic with a GC content of 43-46%. It grows rapidly at 25-30°C in rich media such as brain-heart, trypticase soy broth, provided the medium is supplemented with NaCl. The bacterium is characterized by the vibroid (comma-shaped) morphology. A list of phenotypical characteristics is provided by Hjeltnes and Roberts (1993). *L. anguillarum* is serologically diverse, up to twenty-three O-antigen serotypes have been described (Pedersen *et al.*, 1999), but only serotypes O1, O2 and to some extent O3 have been associated with mortalities (Toranzo *et al.*, 2005). Serotype O1 is antigenically homeogenous, whereas heterogeneity has been demonstrated within serotypes O2 and O3.

## Bacterial Transmission

*L. anguillarum* may possibly be the most common fish pathogenic bacterium in the world, frequently causing disease in a wide range of hosts. The bacterium is a typical opportunist, being able to survive for a long time outside the host (Hoff, 1989), frequently attacking weakened individuals (Vadstein *et al.*, 2004). Therefore, many wild and cultured fish populations are likely to be endemically infected in many areas of Europe. Different hosts show different susceptibility to the different serotypes. Whereas serotypes O1 and O2 affect a variety of fish, type O3 affects mainly eel and ayu (Toranzo *et al.*, 2005). Serotype O2b has only been detected in strictly marine fish, presently dominates among isolates from cod aquaculture (D. Colquhoun National Veterinary Institute, Oslo, Norway, pers. comm.). In contrast, serotype O2a has been isolated from marine fish as well as salmonids. Genetic differences within and among the serotypes have also been detected, for instance a genetic difference between isolates of serotype O2 from northern and southern Europe, respectively (reviewed by Toranzo *et al.*, 2005). Contact between fish seems to be an important factor for the spread of the pathogen, however the bacterium is widely distributed in wild fish as well as food organisms and is transmitted horizontally. There is evidence of accumulation of *L. anguillarum* in live feed organisms such as *Artemia* spp. and rotifers, *Brachionus plicatilis* (Korsnes *et al.*, 2006). Early life stages are particularly susceptible to *L. anguillarum* and other opportunists (reviewed by Vadstein *et al.*, 2004).

### Diagnostics

*L. anguillarum* can be presumptively diagnosed on basis of standard biochemical tests. However, a serological confirmation employing serotype-specific polyclonal antisera is necessary. Rapid test kits for presumptive identification are available and quite commonly used in the field diagnostics (reviewed by Toranzo *et al.*, 2005).

### Prophylaxis

Vaccination is regarded as necessary in aquaculture (Sommerset *et al.*, 2005), both in terms of reducing mortalities, probably also in terms of reducing challenge pressure to wild stocks, and also to reduce environmental impact of excess use of antibacteria agents (Lillehaug *et al.*, 2003)

## *VIBRIO SALMONICIDA*

Vibriosis results from systemic infections caused by *Listonella* (*Vibrio*) *anguillarum* and notably *Vibrio salmonicida* and caused major problems to the Atlantic salmon industry during the 1980s (Bruno *et al.*, 1986; Egidius 1987). *Vibrio salmonicida*, isolated from salmon and described as a species by Egidius *et al.* (1986) caused a major epizootic culminating in 1987, associated with the use of approximately 50 tonnes of antibacterial agents in salmon aquaculture that year. As with the major furunculosis epizootic three to five years later, the disease was initially counteracted with high quantities of antibacterial agents, but the introduction of vaccines has effectively eliminated the problem (reviewed by Sommerset *et al.*, 2005).

### Clinical Signs, Gross Pathology and Diagnostics

First clinical signs are inappetence and disorganized swimming. Pathology varies with the stage in the disease process, but common external signs are pallor of the gills, haemorrhage on the fin base, redness and swelling, with occasional prolapse at the rectum, and petechial haemorrhage on the ventral abdominal wall. Haemorrhagic ulceration or dark patches on the operculum and dorsum are common findings. Fish may, however, die in the peracute phase without any signs of disease (Hjeltnes and Roberts, 1993). The characteristics of this disease are anaemia and haemorrhages, with a general septicaemia. Large amounts of the characteristically vibrioid cells can be seen in moribund fish. Rapid serological test kits are available.

## Bacterial Characteristics

*V. salmonicida* is a facultatively anaerobic motile rod, possessing several polar flagella. When isolated from fish it shows a high degree of pleomophism (Egidius *et al.*, 1986). *V. salmonicida* is halophilic and requires a minimum of 0.5% NaCl in the media. Useful biochemical characteristics are listed by Hjeltnes and Roberts (1993) and Actis *et al.* (1999).

## Transmission, Distribution and Hosts

The bacterium is transmitted horizontally. Epizootiological studies based on plasmid profiles suggest that the bacterium was transferred from cod to salmon and *vice versa* in fish farms in Northern Norway (Sørum *et al.*, 1990). The pathogenesis does not seem to be plasmid mediated (Wiik *et al.*, 1989). The bacterium appears to be restricted to relatively cold waters; the host range is narrower than is the case for *Listonella anguillarum*. The so-called Hitra disease first appeared in 1977 and occurred for the first time on a large scale in 1979 in fish farms on the island of Hitra in central Norway. Since then, the disease has spread along the coast of Norway, in Scotland (Bruno *et al.*, 1986), the Faroe Islands, Canada (referred in Actis *et al.*, 1999) and Iceland (unpublished data from the Fish Disease Laboratory (FDL) at the Institute for Experimental Pathology at Keldur, University of Iceland). However, the mechanisms of how the disease was spread were not investigated, any potential interactions between farmed and wild populations of fish are not known.

Hitra disease occurs generally in late autumn, winter or early spring, characteristically at lower temperatures than classical vibriosis, hence the term 'coldwater vibriosis'. The bacterium has been isolated from salmon *Salmo salar*, cod *Gadus morhua* and halibut *Hippoglossus hippoglossus* (Bergh *et al.*, 2001; Bricknell *et al.*, 2006; Samuelsen *et al.*, 2006). Whereas it is clearly an important pathogen for salmon, isolations from cod and particularly halibut are scarce and could be secondary isolates (Bergh *et al.*, 2001; Samuelsen *et al.* 2006).

There are no reports of infection in wild fish. However, the bacterium has been isolated from wild caught cod kept in net-pens for ongrowth. Evidence for infection and disease outbreaks is limited to farmed fish, notably salmon.

## Other *Vibrio* spp.

Although *L. anguillarum* is regarded as the dominant species causing vibriosis, several other *Vibrio* species, are known pathogens. Typically, most *Vibrio* spp. are opportunists, causing infections in environments like hatcheries, or invading weakened hosts. Many are abundant in marine environments, associated with fish and other organisms. Many are also coral pathogens or bivalve pathogens. *Vibrio harveyi* is a major pathogen of shrimp (Thompson *et al.*, 2004). *Vibrio tapetis* is a pathogen of many bivalves, causing Brown Ring Disease, but has also been isolated from halibut and corkwing wrasse (Paillard, 2004). Among other *Vibrio* spp. that may cause vibriosis are *V. fischeri, V. ordalii, V. pelagius, V. splendidus,* and *V. logei.* An extensive review of pathogenic *Vibrio* spp. and their rapidly changing taxonomy is given by Thompson *et al.* (2004).

***Vibrio vulnificus*** is a marine bacterial species from tropical and warm brackish waters, which comprises strains that are potential pathogens for humans and aquatic organisms (reviewed by Thompson *et al.* 2004). Biotype 1 is an opportunistic pathogenic for humans and is indole positive. Biotype 2 is subdivided into three different serovars. Serovar E corresponds to the original isolates that gave rise to the subdivision of the species into biotypes. *Vibrio vulnificus* serovar E (formerly biotype 2) is the etiologic agent that is responsible for the main infectious disease affecting farmed eels and is classically considered an obligate eel pathogen (Amaro *et al.*, 1995). This disease is the main cause of losses in brackish water eel culture in Europe. Pathology is similar to that caused by *L. anguillarum* and consists of a bacterial hemorrhagic septicaemia. The bacterium infects healthy eels causing development of red patches or swollen lesions on the trunk or tail (Tison *et al.*, 1982). In the late stages of infection, histopathological changes are observed in the gills and internal organs. The main portals of entry into the eel body are the gills (Marco-Noales *et al.*, 2001). The pathogen can also infect eels through the gastrointestinal tract by contaminated food. It can survive low salinities for a prolonged time. A capsule seems to be essential for waterborne infectivity (Amaro *et al.*, 1995). The spread of the disease is depending on temperature and salinity. Eels can transmit *V. vulnificus,* notably serovar E, to humans.

***Vibrio alginolyticus*** is an abundant organism in marine environments, isolated from many different marine organisms (Carli *et al.*, 1993). It has

been found in finfish, shellfish, and marine sediments and has been suggested as a pathogen for humans (Blake *et al.*, 1980). It has caused outbreaks of vibriosis in grouper (*Epinephelus malabaricus*) (Lee, 1995), juvenile turbot (*Scophthalmus maximus*) (Austin and Austin, 1999), gilthead sea bream (*Sparus aurata*) (Colorni *et al.*, 1981; Balebona *et al.*, 1998) and sea bass (*Dicentrarchus labrax*) (Bakhrouf *et al.*, 1995). This species also has been associated with other *Vibrio* species in high-mortality outbreaks related to abdominal swelling in larvae of several fish species (Kusuda *et al.*, 1986; Sedano *et al.*, 1996). *Vibrio alginolyticus* is a typical opportunist, affecting sea bream after handling, when the skin is damaged (Colorni and Diamant, 1992; Balebona, 1998).

Symptoms are dark skin, septicaemia, haemorrhaging and sometimes ulcers of the skin (Colorni *et al.*, 1981). The main cause of mortality in vibriosis by *V. alginolyticus* is the destruction of critical components of both the circulatory and immune systems (Lee *et al.*, 2003).

### *Yersinia ruckeri*

*Yersinia ruckeri* causes Enteric redmouth disease, ERM, an important disease of salmonid fish and was first described by Ross *et al.* (1966) and Rucker (1966). Signs of ERM-particularly in infected rainbow trout may include haemorrhages in various tissues and organs, particularly around the mouth, in the gills, in the musculature, the peritoneum, the body fat, the viscera and in the low intestine. Clinical infection can also be characterized by a yellow discharge from the vent. Furthermore, severe inflammations of the distal portion of the intestine and vent are frequently observed. *Y. ruckeri* has been isolated from different salmonids (*Salmo* sp., *Salvelinus* sp., *Thymallus* sp., *Coregonus* sp., *Oncorhynchus* sp.), pike (*Esox* sp.), (Davies and Frerichs, 1989), from eel (*Anguilla* sp.) and carp (*Cyprinus carpio*), perch (*Perca* sp.), roach (*Rutilus rutilus*), sturgeon and burbot. Further, it has also been isolated from freshwater invertebrates (Dulinet *et al.*, 1976). It is probably persistant in the environment, and is horizontally transmitted.

### *Tenacibaculum* spp.

*Tenacibaculum maritimum* (formerly *Cytophaga marina* and *Flexibacter marinus*) is an opportunistic bacterial pathogen that causes a disease called 'flexibacteriosis' in marine fish. Other names that have been used for flexibacteriosis are: 'gliding bacterial disease', 'eroded mouth syndrome'

and 'black patch necrosis'. Based on phylogenetic, chemotaxonomic and phenotypic characteristics, it has been proposed that *Flexibacter maritimus* is transferred to the new genus *Tenacibaculum*, as *Tenacibaculum maritimum* (Sukuii *et al.*, 2001). Initially, *T. maritimum* was isolated from marine fish cultured in Japan in 1979 (Hikida *et al.*, 1979). The first report in Europe was published in 1982, in Dover sole, *Solea solea* (Campbell and Buswell, 1982). It is now well documented that flexibacteriosis is widely spread in Europe, the USA and Japan.

Both, adult and juvenile fish can be affected, but the pathological picture is more severe in younger fish (Wakabayashi, 1994). Increased water temperature, various stressors and skin surface condition play an important role in the appearance of the disease (Toranzo *et al.*, 2005). The affected fish have eroded and haemorrhagic mouth, skin ulcers, frayed fins and tail rot (Toranzo *et al.*, 2005). Gills may also be affected. A systemic disease can develop, involving different internal organs. The skin lesions can also act as sites of entry of other pathogens.

Based on phenotypic and biochemical characteristics, *T. maritimum* is considered a homogeneous taxon. Serologically, at least three groups of *T. maritimum* isolates from strictly marine fish could be distinguished (Avendano *et al.*, 2003). These groups are associated with the host origin: group 1 comprises the strains isolated from sole, group 2 the isolates from sea bream and sea bass, group 3 corresponds to the turbot isolates. On molecular level, using randomly amplified polymorphic DNA-PCR, three groups of isolates were distinguished (Avendano-Herrera *et al.*, 2004a, b). The first group comprised all strains isolated from sole (*Solea senegalensis* and *S. solea*) and gilt-head sea bream (*Sparus aurata*), the second comprised the isolates from yellowtail (*Seriola quinqueradiata*), Atlantic salmon (*Salmo salar*) and turbot (*Scophthalmus maximus*), the third group one isolate from *Pagrus major* and one from *Solea solea*.

*T. maritimum* is a bacterium commonly found in sea water (Salati *et al.*, 2005). It is considered an opportunistic pathogen, causing problems only when some conditions, such as increased water temperature, stress, bad skin condition and other stressful conditions, occur. It is an opportunistic pathogen although commonly found in rearing water disease develops only under certain adverse environmental conditions. In many cases, its significance is underestimated because its isolation requires selective culture media, something that is not done on a routine basis. No published studies concerning disease interactions between wild and farmed fish are

available and future epidemiological studies should take into account the opportunistic character of the pathogen.

The closely related species *T. ovolyticum* (formerly *Flexibacter ovolyticus*) has been isolated from Atlantic halibut (*Hippoglossus hippoglossus*) eggs and larvae (Hansen *et al.*, 1992). It possesses the ability to penetrate the eggshell and may cause mortalities to larvae (Bergh *et al.*, 1992).

### *Flavobacterium psychrophilum*

*Flavobacterium psychrophilum* affects all species of salmonids, coho salmon and rainbow trout being the most susceptible for clinical disease. Ayu (*Plecoglossus altivelis*) was also affected. The pathogen is also reported from non-salmonid fish, having been associated with disease in eel (*Anguilla anguilla*) and three species of cyprinids (reviewed by Nematollahi *et al.*, 2003).

*F. psychrophilum* does not grow on the standard diagnostic growth media like blood agar, and special low nutrient growth media are necessary (Nematollahi *et al.*, 2003). Yellow pigmentation of the colonies allows initial recognition. Confirmative biochemical features include enzymatic profile, a strong proteolytic activity and no utilization of carbohydrates or degradation of polysaccharides (Bernardet and Kerouault, 1989). Serological as well as molecular methods have been developed for rapid diagnosis (reviewed by Nematollahi *et al.*, 2003). The genotype could be more related to the original host species than geographical area of isolation (Izumi *et al.*, 2003). Strains can also be classified in high- and low-virulent groups (Dalsgaard, 1993; Madsen *et al.*, 2005). The taxonomy has been extensively revised (Bernardet *et al.*, 1996).

*F. psychrophilum* causes disease signs mostly in low water temperature (3-15°C). Clinical signs of the disease are dependent on the size of the fish. In highly susceptible coho salmon fry the yolk sac can be affected, resulting in skin erosion and high mortality up to 50%. Rainbow trout fry syndrome (RTFS) affects fish in the size of 0.2-2 g, exhibiting anaemia and inflammation of internal organs, most typically the spleen, and sometimes external lesions. Mortality rate can be 10-70%. In juvenile fish erosions of fins, skin ulcers and sometimes necrotic myositis or osteochondritis is seen (reviewed by Nematollahi *et al.*, 2003).

*F. psychrophilum* can be found in the water environment of infected fish farms (Wiklund *et al.*, 2000; Dalsgaard *et al.*, 2001; Madetoja and

Wiklund, 2002). Isolations from wild fish seem to be more prevalent in the environments in contact with fish farm effluents (Dalsgaard *et al.*, 2001; Madetoja and Wiklund, 2002). However, serological and genetic analyses show more diversity in the isolates from the wild fish compared with the isolates from outbreaks in the fish farm (Dalsgaard *et al.*, 2001). The bacterium can occasionally be isolated from inlet water (Madetoja and Wiklund, 2002), but inlet water seems not to be the main route of infection, while isolations from there are only sporadic (Madetoja and Wiklund, 2002; Madsen *et al.*, 2005). The bacterium can be transmitted hoizontally as well as vertically. Contaminated farms harbour the bacterium in water as well as in asymptomatic fish, and clinical disease is suffered in unfavourable conditions, often connected to handling stress like vaccination or grading.

### *Flavobacterium columnare*

'Columnaris disease' or 'saddleback disease', caused by *Flavobacterium columnare*, has been reported globally in many fish species, including commercially important species as channel catfish (*Ictalurus punctatus*) (Hawke and Thune, 1992) and salmonids (Morrison *et al.*, 1981; Bernardet, 1989). It is also commonly found in ornamental fish as cotton-wool disease or mouth fungus (Decostere *et al.*, 1998). The clinical disease causes severe problems for aquaculture in warm climates (Hawke and Thune, 1992; Figueiredo *et al.*, 2005), but considerable mortality is also reported from temperate climates during warm water periods (Morrison *et al.*, 1981; Koski *et al.*, 1993). The bacterium is widely present in the environment. There are several reports of infection by *F. columnare* in wild fish (reviewed by Austin and Austin, 1999).

Columnaris disease affects fish externally, causing necrotic lesions in skin or gills. In salmonid fish, the infection typically starts from the base of the dorsal fin (Morrison *et al.*, 1981). The bacterium seems to invade to inner organs only when the external lesions are well developed (Wakabayashi, 1993). Clinical diseases typically appear when temperatures go above 15°C (Wakabayashi, 1993). Water temperature seems to be the main environmental factor in relation to mortality, but other stress causing factors like low dissolved oxygen level and high levels of ammonia or organic matter may influence the mortality (Austin and Austin, 1999; Decostere *et al.*, 1999b).

*F. columnare* does not grow on the standard diagnostic growth media like blood agar, and special low nutrient growth media are necessary (Austin and Austin, 1999). It grows slowly and other bacteria can inhibit the growth, making the use of selective media (Hawke and Thune, 1992) or dilution methods (Tiirola *et al.*, 2002) advisable. Colony form is easily recognizable, yellow with irregular margins, strongly adhering to solid media (Austin and Austin, 1999). High- and low-pathogenic strains have been detected, the pathogenic potential being connected with the ability to adhere to the gills (Decostere *et al.*, 1999a).

### *Photobacterium damselae* subsp. *piscicida*

*Photobacterium damselae* ssp. *piscicida* (formerly *Pasteurella piscicida*) is the causative agent of 'pasteurellosis', a disease also known as pseudotuberculosis due to the formation of granular lesions in spleen and kidney of the infected fish. The bacterium was originally isolated during mortalities occurring in natural populations of white perch (*Morone americanus*) and striped bass (*M. saxatilis*) in 1963 in Chesapeake Bay, USA (Snieszko *et al.*, 1964). It was estimated that the bacterium killed approximately 50% of the population. Initially, the pathogen was placed in the genus *Pasteurella*, based on the morphological and biochemical characteristics and the term 'pasteurellosis' is still used for reference purposes. On the basis of its 16S rRNA gene, it was placed under the present genus *Photobacterium* (Gauthier *et al.*, 1995; Trüper and De'Clari, 1997). It can affect many fish species, both wild and cultured.

The disease has been considered one of the most serious bacterial pathological conditions in over 20 fish species in Japan, the USA and, from 1990, in Europe too (Toranzo *et al.*, 1991). In the Mediterranean area, the bacterium has been causing great losses in the marine culture, in particular in gilthead seabream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (Kvitt *et al.*, 2002; Toranzo *et al.*, 2005). The mortality in the cultured species ranges from low, in sub-clinical forms, which occur at low temperatures, to as high as 90%, in some batches (Hawke *et al.*, 2003). Usually, the loss is around 10%. In wild populations, extreme cases of estimated 50% mortality have been reported (first isolation in wild population, in Chesapeake Bay, USA) (Snieszko *et al.*, 1964).

Externally, the infected fish appear darker and, in some, cases hemorrhagic areas in the head and gills are present. Internally, the pathological changes depend on whether the disease is in the acute or the

chronic stage. In the acute form, the internal organs show multifocal necrosis. In the chronic form, whitish patches are formed in the infected organs, although their formation depends on the fish species and whether the infection is natural or experimental (Magariños *et al.*, 1996). Sub-clinical infections, where fish become carriers, also occur at low water temperatures (Magariños *et al.*, 2001). Young fish tend to be more susceptible to the pathogen (Toranzo *et al.*, 2005)

Regardless of the geographic origin and source of isolation, this pathogen exhibits homogeneous biochemical and serological characteristics Only at the molecular level, distinct clones can be distinguished: the European, the Japanese and the Israeli ones (Kvitt *et al.*, 2002). Isolation from internal organs of diseased fish is achieved on tryptic soy agar (TSA), brain heart infusion agar (BHIA) and blood agar, all supplemented with 1-2% NaCl, after 2-4 days incubation at 22°C. Presumptive identification is carried out according to morphological features (short gram-negative, non-motile rods with bipolar staining) and using biochemical assays. In terms of antigenic profiles, *P. damselae* ssp. *piscicida* isolates are a very homogenous group. Thus, many immunological techniques, such as ELISA and fluorescent antibody techniques (IFAT) and immunohistochemistry (IHC) have been developed to identify all isolates. Commercial kits using polyclonal anti-*Ph. damselae* ssp. *piscida* antibodies are also used.

## *FRANCISELLA* SP.

It was recently demonstrated that a *Francisella* sp. caused granulomas in skeletal muscle and several organs. The bacterium has been isolated and identified as a *Fransicella* sp. by two independent research teams (Nylund *et al.*, 2006; Olsen *et al.*, 2006). The disease was described as a novel systemic granulomatous inflammatory disease in farmed Atlantic cod, associated with a previously undescribed bacterium belonging to the genus *Francisella*. The bacterium show high similarity to *F. philomiragia* and to some extent to *F. tularensis*, and has a facultative intracellular nature. The bacterium does not grow on standard media. Blood agar supplemented with 0.1% cystein and 1% glucose is commonly used (Nylund *et al.*, 2006).

Challenge experiments have confirmed that the bacterium is the causative agent of the disease. *Francisella* sp. has also been associated with mortality of intensively cultured hybrid striped bass, and *Francisella* sp. was recently isolated from salmon in Chile. Reports from several cod farmers

suggest that this disease problem is highly significant, and that the problem is rapidly increasing, although epizootiological data are still unsystematic and scarce. Data still not published in peer-reviewed journals seem to indicate that the disease is widespread in wild cod stocks and that a wild reservoir is probably of importance (Alfjorden *et al.*, 2006; Nylund and Ottem, 2006). Findings of cod with francisellosis in southern Skagerak—upstream the coastal current relative to all Norwegian cod farms—indicate that the wild reservoir is independent of the cod farms. Monitoring surveys off the west coast of Sweden demonstrated that up to 20% of the one year class of cod caught in fish traps in the area had skin ulcers and other symptoms of francisellosis, and a *Francisella* sp. with high similarity to strains previously isolated from Norwegian cod farms was isolated.

### *Piscirickettsia salmonis*

*Piscirickettsia salmonis* causes an epizootic disease of fish called piscirickettsiosis. It is an intracellular pathogen with increasing frequency in a variety of fish species around the world. In addition, there are reports of its occurrence in freshwater stock and rickettsia-like pathogens may be involved in epizootics affecting other cultured fish species (Bravo, 1994; Almendras *et al.*, 1997). Piscirickettsiosis associated with mortality have been reported from Atlantic salmon farming in Norway and Scotland (Grant *et al.*, 1996; Olsen *et al.*, 1997; Birrell *et al.*, 2003). Hygiene and management policy are the only control methods. As with other intracellular bacterial fish pathogens, no vaccine is currently available. Antibiotics have limited effect.

## Clinical Signs, Gross Pathology

Clinical signs include anorexia, darkening of the skin, lethargy, swimming near the water surface and respiratory distress (Branson and Nieto Diaz Muñoz, 1991; Cvitanich *et al.*, 1991). Skin lesions include perianal and periocular haemorrhage, abdominal petechiae and shallow haemorrhagic ulcers, varying between 0.5 and 1.5 cm in diameter. Characteristic ring-shaped, yellow to cream-coloured subcapsular nodules can be observed throughout the liver of chronically infected fish (Cvitanich *et al.*, 1991). The abdomen is frequently distended. Petechiae on the serosa surfaces of the intestine, pyloric caeca and swim bladder have been observed in Atlantic salmon. Other findings are ascites, diffuse swelling, general pallor

and multifocal pale areas in the kidney and spleen. Bilateral exophthalmia and an ulcerative inflammatory reaction around the mouth have been described.

## Host Distribution and Transmission

All marine cultured salmonids in southern Chile, including Atlantic, chinook and masou salmon and rainbow trout are susceptible (Fryer *et al.*, 1990; Lannan and Fryer, 1993). In Europe *P. salmonis* has been detected in farmed Atlantic salmon (Olsen *et al.*, 1997). Morphologically and serologically similar pathogenic rickettsial organisms have also been reported in British Columbia in farmed chinook, coho, pink and Atlantic salmon in seawater (Brocklebank *et al.*, 1992; Kent, 1992; Jones *et al.*, 1998), in Ireland and Scotland from Atlantic salmon (Rodger and Drinan, 1993; Grant *et al.*, 1996; Birrell *et al.*, 2003). The geographical distribution of *P. salmonis* and related organisms is ubiquitous.

*P. salmonis* also affects European sea bass, *Dicentrarchus labrax* and white sea bass, *Atractoscion nobilis* (Comps *et al.*, 1996; Chen *et al.*, 2000). A piscirickettsia-like organism (PLO) has also been described in cultured tilapia in several countries (Chern and Chao, 1994; Mauel *et al.*, 2003). There are several reports of rickettsia-like organisms associated with diseases of fish and other marine organisms, particularly bivalves, and the problem is probably underdiagnosed. The bacteria can be transmitted vertically as well as horizontally.

## Bacterial Characteristics and Diagnosis

*Piscirickettsia salmonis* is an obligate pathogenic bacterium that causes a serious, systemic infection of salmonids in sea water (Fryer *et al.*, 1992). The organism is an intracellular, gram-negative pathogen that is predominantly coccoid, non-motile, often pleomorphic, non-encapsulated ranging in size from 0.5 to 1.8 μm (Cvitanich *et al.*, 1991; Fryer *et al.*, 1992). *P. salmonis* replicates within membrane-bound cytoplasmic vacuoles in selected fish cell lines and in the cells of tissues throughout infected fish. A specific diagnosis requires isolation of the causative organism. Kidney tissue from affected fish is aseptically removed, homogenised and inoculated on a cell monolayer with an antibiotic free growth media. *P. salmonis* have been cultured in six fish cell lines maintained in buffered Eagles minimum essential medium (MEM) supplemented with 10% foetal bovine serum (Fryer *et al.*, 1990). Four of

these susceptible fish cell lines have been derived from salmonid species, chinook salmon embryo (CHSE 214), chum salmon heart (CHH 1), coho salmon embryo (CSE 119) and rainbow trout gonad (RTG 2). The other cell lines are from warm water fish species (Almendras and Fuentealba 1997). Optimal *in vitro* growth occurs at 15–18°C, but is retarded above 20°C and below 10°C. Immunocytochemical methods are available as fast, alternative diagnostic methods.

### *Renibacterium salmoninarum*

The causative agent of Bacterial Kidney Disease (BKD) is an intracellular, gram-positive bacterium, *Renibacterium salmoninarum*. BKD is widespread and is reported from many countries in Europe, in both North and South America as well as from Asia. Salmonid species of the genus *Oncorhynchus*, *Salmo*, *Salvelinus* are affected as well as Danube salmon *Hucho hucho* (Pfeil-Putzien *et al.*, 1985), grayling *Thymallus thymallus* (Kettler *et al.*, 1986) and whitefish *Coregonus lavaretus* (Rimaila-Päränen, 2002). Clinical BKD has been reported in the non-salmonid fish ayu *Plecoglossus altivelis* (Nagai and Iida, 2002) and Pacific hake *Merluccius productus* (Kent *et al.*, 1998). Pacific salmon as pink (*O. gorbuscha*), sockeye (*O. nerka*) and chinook salmon (*O. tshawytscha*) are regarded as more susceptible than Atlantic salmon or rainbow trout. Vertical transmission from BKD infected females to their eggs is reported and since the bacterium can resist *intra ovum* the infection might be transmitted after surface disinfection (Bruno and Munro, 1986; Evelyn *et al.*, 1986).

There are few reports on clinical disease in wild fish but evidence suggests that the bacterium is widespread in wild populations. The prevalence of *R. salmoninarum* antigens, tested by ELISA, in wild salmonid stocks in Alaska has been reported as up to 90% (Meyers *et al.*, 1993b). Although it is possible that infection with BKD results in mortality in the open sea, the magnitude of this mortality is not possible to estimate, although returning survivors are a potential risk for vertical transmission to future generations. High prevalence of BKD has also been found in wild Arctic charr and brown trout in Iceland. However, as most fish show no signs of disease, the findings indicate that the bacterium is endemic in these populations and ecological factors are suggested to influence the epidemiology of the disease (Jónsdóttir *et al.*, 1998).

The external lesions include a distended abdomen, loss of balance, exophthalmia, petechiae and haemorrhaging around the fins and the

lateral line and superficial blisters of the skin. The most obvious internal lesion is a swollen kidney containing greyish-white nodules of various sizes. Similar nodules might occur in spleen, heart and liver. Petechial haemorrhage of the muscle lining of the peritoneum is described, as well as an accumulation of ascitic fluid in the abdominal cavity. The spleen can be enlarged. Gills and internal organs are pale in colour providing evidence of anaemia (Belding and Merrill, 1935; Earp *et al.*, 1953; Wood and Yasutake, 1956; Fryer and Sanders, 1981; Bruno, 1986). An opaque membrane covering internal organs is described in BKD affected fish living at low temperatures (Smith, 1964; Bruno, 1986). BKD is reported from fresh as well as from marine environments and the highest mortality is recorded at temperatures of 7-12°C, with a decrease in mortality at elevated water temperatures (Fryer and Lannan, 1993).

*R. salmoninarum* is slow growing (Bruno and Munro, 1986; Benediktsdóttir *et al.*, 1991). Several immunological techniques have been used for routine investigations, e.g., immunofluorescence (Bullock and Stuckey, 1975; Bullock *et al.*, 1980) and enzyme-linked immunosorbent assays (ELISA) (Gudmundsdóttir *et al.*, 1993). To an increasing extent, molecular identification has been applied for BKD (Brown *et al.*, 1994). *R. salmoninarum* has a high degree of biochemical, genetic and serological uniformity.

Control programmes with active surveillance are in operation in several countries. The enzootic nature of BKD, particularly in North American regions, makes control of the disease in hatchery-reared salmonids difficult. In restocking programmes, testing and use of specific-pathogen-free broodstock fish is necessary.

## ECOLOGICAL CONSIDERATIONS

Our comprehension of interactions between bacteria at the species level and fish is limited, except maybe for a few fish pathogens. This research area is, however, developing rapidly and several reviews have been published (Gatesoupe, 1999; Hansen and Olafsen, 1999; Ringø and Birkbeck, 1999). Due to limited knowledge on normal flora and normal fish-microbe interactions, it may be adequate to classify bacteria based on a general ecological scheme. A differentiation between opportunistic and non-opportunistic species may be appropriate (Vadstein *et al.*, 2004), as may also the division between parasitic and mutualistic species. The first division has with success been applied to aquaculture, and is theoretically

formalized as the r/K-concept (Andrew and Harris, 1986). Of course, the r/K-concept does not entail an either or but r and K rather represents the two extremes in a continuum. r-selection occur in an un-crowded environment with a high supply of substrate per capita, and r-strategists are considered as opportunistic species. K-selection, on the other hand, occurs in crowded environments with a low supply of nutrients per cell (Salvesen and Vadstein, 2000). Opportunistic pathogens are often characterized as r-strategists, but this group probably contains a large number of non-pathogenic, opportunistic species that may cause some of the experienced problems in the rearing of marine larvae.

At the community level, r- and K-selected species dominate in pioneer (developmental) and mature (climax) communities, respectively. These two types of communities also have distinct differences in characteristics. Pioneer communities will generally be systems with low stability against perturbations and with a low biological control. Matured water inhabited by K-strategists will, on the other hand, be stable systems, with high biological control and high resistance to perturbations below a specific level.

Current intensive production methods used for marine fish tend to increase the carrying capacity of the system and to select opportunistic microbes. This tendency is stronger the more intensive and closed the system is. The reasons for such an unfavourable development are a high load of organic matter, large oscillations in this load, and direct perturbations of the bacterial community. Critical factors that sustain such a development are decimation of bacteria in in-flowing water without controlled re-colonization of the microbial community, high loads of bacteria and organic matter together with feed, internal load of organic matter as faeces from live feed and larvae or as dead larvae. Intensive larval-rearing systems may serve as an example of a system with major negative perturbations, whereas open cages with good water circulation is an example of the opposite. Two major strategies are avialable in order to control bacterial growth in aquaculture systems:

*Non-selective reduction of bacteria:* The methods used for non-selective control can be grouped in two. Some methods are aimed at a non-permanent or non-stable reduction (disinfection and grazer control), whereas others are aimed at a reduction in the carrying capacity of the system (reduction in input and removal of organic matter).

*Selective enhancement of bacteria:* The application of probiotic bacteria in livestock production and for humans has received considerable interest

and to some extent also in aquaculture (Gatesoupe, 1999). Probiotics can be defined as microorganisms that are able to colonize the digestive tract, to maintain or increase the natural gut flora to prevent colonization of pathogenic organisms, and to secure optimal utility of the feed. Their use can improve health and productivity of farmed animals. Probiotics can be administrated both by direct addition of the selected organisms to the tanks, or by incorporation of the organisms into live or formulated feed. The regulation of the composition of the bacteria by selective measures differs from the probiotics concept by the fact that no organisms are added to the system. Instead, a physical, chemical or biological factor is used as a selective force (Vadstein *et al.*, 2004).

## References

Actis, L.A., M.E. Tolmasky and J.H. Crosa. 1999. Vibriosis. In: *Fish Diseases and Disorders*. Viral, Bacterial and Fungal Infections, P.T.K. Woo and D.W. Bruno (eds.). CABI Publishing, Wallingford, Oxon, U.K., Vol. 3, pp. 523-557.

Alfjorden, A., E. Jansson and K.E. Johansson. 2006. A systemic granulomatous inflammatory disease in wild Atlantic cod, *Gadus morhua* associated with a bacterium of the genus *Francisella*. *DIPNET Newsletter Nr. 44*.

Almendras, F.E. and I.C. Fuentealba. 1997. Salmonid rickettsial septicaemia caused by *Piscirickettsia salmonis*: A review. *Diseases of Aquatic Organisms* 29: 137-144.

Almendras, F.E., I.C. Fuentealba, S.R.M. Jones, F. Markham and E. Spangler. 1997. Experimental infection and horizontal transmission of *Piscirickettsia salmonis* in freshwater-raised Atlantic salmon *Salmo salar* L. *Journal of Fish Diseases* 20: 409-418.

Amaro, C. and E.G. Biosca. 1996. *Vibrio vulnificus* biotype 2, pathogenic for eels, is also an opportunistic pathogen for humans. *Applied Environmental Microbiology* 62: 1454-1457.

Amaro, C., E.G. Biosca, B. Fouz, E. Alcaide and C. Esteve. 1995. Evidence that water transmits *Vibrio vulnificus* biotype 2 infections to eels. *Applied Environmental Microbiology* 61: 1113-1137.

Andrew, J.H. and R.F. Harris. 1986. r- and K-selection in microbial ecology. *Advances in Microbial Ecology* 9: 99-147.

Aoki, T. 1999. Motile Aeromonads (*Aeromonas hydrophila*). In: *Fish Diseases and Disorders*, P.T.K. Woo and D.W. Bruno (eds.). First Edition. CABI Publishing, Wallingford, Oxon, U.K., Vol. 3: Viral, Bacterial and Fungal Infections, pp. 427-453.

Austin, B. and D.A. Austin. 1999. Bacterial fish pathogens. *Diseases of Farmed and Wild Fish*. Third Revised Edition, Springer-Verlag, Berlin, Germany.

Athanassopoulou, F., C. Billinisand and T. Prapas. 2004. Important disease conditions of newly cultured species in intensive freshwater farms in Greece: first incidence of nodavirus infection in *Acipenser* sp.. *Diseases of Aquatic Organisms* 60: 247-252.

Avendano-Herrera, R., B. Magarinos, A.E. Toranzo, R. Beaz and J.L. Romalde. 2004a. Species-specific polymerase chain reaction primer sets for the diagnosis of *Tenacibaculum maritimum* infection. *Diseases of Aquatic Organisms* 62: 75-83.

Avendano-Herrera, R., J. Rodriguez, B. Magarinos, J. Romalde and A. Toranzo. 2004b. Intraspecific diversity of the marine fish pathogen *Tenacibaculum maritimum* as determined by randomly amplified polymorphic DNA-PCR. *Journal of Applied Microbiology* 96: 871-877.

Bakke, T.A. and P.D. Harris. 1998. Diseases and parasites in wild Atlantic salmon (*Salmo salar*) populations. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 247-266.

Bakhrouf, A., H. Ben Ouada and R. Oueslati. 1995. Sea bass *Dicentrarchus labrax* vibriosis treatment in a pisciculture area, in Monastir, Tunisia. *Marine Life* 5: 47-54.

Balebona, M.C., M.J. Andreu, A. Bordas, I. Zorilla, M. Moringo and J.J. Borrego. 1998. Pathogenicity of *Vibrio alginolyticus* for cultered gilt-head sea bream (*Sparus aurata* L.). *Applied Environmental Microbiology* 64: 4269-4275.

Benediktsdóttir, E., S. Helgason and S. Gudmundsdóttir. 1991. Incubation time for the cultivation of *Renibacterium salmoninarum* from Atlantic salmon, *Salmo salar* L. broodfish. *Journal of Fish Diseases* 14: 97-102.

Benediktsdóttir, E., S. Helgasson and H. Sigurjonsdóttir. 1998. *Vibrio* sp. isolated from salmonids with shallow skin lesions and reared at low temperature. *Journal of Fish Diseases* 21: 19-28.

Belding, D.L. and B.A. Merrill. 1935. Preliminary report upon a hatchery disease of the salmonidae. *Transactions of the American Fisheries Society* 65: 76-84.

Benediktsdóttir, E., L. Verdonck, C. Sproer, S. Helgason and J. Swings. 2000. Characterization of *Vibrio viscosus* and *Vibrio wodanis* isolated at different geographical locations: a proposal for reclassification of *Vibrio viscosus* as *Moritella viscosa* comb. nov. *International Journal of Systematic Evolutionary Microbiology* 50: 479-488.

Bergh, Ø., G.H. Hansen and R.E. Taxt. 1992. Experimental infection of eggs and yolk sac larvae of halibut, *Hippoglossus hippoglossus* L. *Journal of Fish Diseases* 15: 379-391.

Bergh, Ø., B. Hjeltnes and A.B. Skiftesvik. 1997. Experimental infection of turbot, *Scophthalmus maximus* and halibut *Hippoglossus hippoglossus* yolk sac larvae with *Aeromonas salmonicida* subsp. *salmonicida*. *Diseases of Aquatic Organisms* 29: 13-20.

Bergh, Ø., F. Nilsen and O.B. Samuelsen. 2001. Diseases, prophylaxis and treatment of the Atlantic halibut: A review. *Diseases of Aquatic Organisms* 48: 57-74.

Bernardet, J.F. and B. Kerouault. 1989. Phenotypic and genomic studies of *Cytophaga psychrophila* isolated from diseased rainbow trout (*Oncorhynchus mykiss*) in France. *Applied Environmental Microbiology* 55: 1796-1800.

Bernardet, J.F., P. Segers, M. Vancanneyt, F. Berthe, K. Kersters and P. Vandamme. 1996. Cutting the Gordian knot: Emended classification and description of the genus *Flavobacterium*, emended description of the family Flavobacteriaceae, proposal of *Flavobacterium hydatis* nom. nov. (Basonym, *Cytophaga aquatilis* Strohl and tait 1978). *International Journal of Systematic Bacteriology* 46: 128-148.

Bernoth, E.M. 1997. Furunculosis: the history of the disease and of disease research. In: *Furunculosis: Multidisciplinary Fish Disease Research*, E.M. Bernoth, A.E. Ellis, P.J. Midtlyng, G. Olivier and P. Smith (eds.). Academic Press, London, pp. 1-20.

Birrell, J., S. Mitchell and D.W. Bruno. 2003. *Piscirickettsia salmonis* in farmed Atlantic salmon, *Salmo salar* in Scotland. *Bulletin of European Association of Fish Pathologists* 23: 213-217.

Blake, P., R.E. Weaver and D.J. Hollis. 1980. Diseases of humans (other than cholera) caused by vibrios. *Annual Review of Microbiology* 34: 341-367.

Björnsdóttir, B., S. Gudmundsdóttir, S.H. Bambir, B. Magnadottir and B.K. Gudmundsdóttir. 2004. Experimental infection of turbot, *Scophthalmus maximus* (L.), by *Moritella viscosa*, vaccination effort and vaccine-induced side effects. *Journal of Fish Diseases* 27: 645-655.

Branson, E.J. and D. Nieto Diaz Muñoz. 1991. Description of a new disease condition occurring in farmed coho salmon *Oncorhynchus kisutch* (Walbaum), in South America. *Journal of Fish Diseases* 14: 147-156.

Bravo, S. 1994. Piscirickettsiosis in freshwater. *Bulletin of European Association of Fish Pathologists* 14: 137-138.

Bricknell, I.R., J.E. Bron and T. Bowden. 2006. Diseases of gadoid fish in cultivation: A review. *ICES Journal of Marine Science* 63: 253-266.

Brocklebank, J.R., D.J. Speare, R.D. Armstrong and T. Evelyn. 1992. Septicemia suspected to be caused by a rickettsia-like agent in farmed Atlantic salmon. *Canadian Veterinary Journal* 33: 407-408.

Brown, L.L., G.K. Iwama, T.P.T. Evelyn, W.S. Nelson and R.P. Levine. 1994. Use of the polymerase chain reaction (PCR) to detect DNA from *Renibacterium salmoninarum* within individual salmonid eggs. *Diseases of Aquatic Organisms* 18: 165-171.

Bruno, D.W. and A.L.S. Munro. 1986. Observations on *Renibacterium salmoninarum* and the salmonid egg. *Diseases of Aquatic Organisms* 1: 83-87.

Bruno, D.W. 1986. Histopathology of bacterial kidney disease in laboratory infected rainbow trout *Salmo gairdneri* Richardson, Atlantic salmon *Salmo salar* L., with reference to naturally infected fish. *Journal of Fish Diseases* 9: 523-557.

Bruno, D.W., T.S. Hastings and A.E. Ellis. 1986. Histopathology, bacteriology and experimental transmission of a cold water vibriosis in Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 1: 163-168.

Bruno, D.W., J. Griffiths, J. Petrie and T.S. Hastings. 1998. *Vibrio viscosus* in farmed Atlantic salmon *Salmo salar* in Scotland: Field and experimental observations. *Diseases of Aquatic Organisms* 34: 161-166.

Bullock, G.L. and H.M. Stuckey. 1975. Fluorescent antibody identification and detection of the *Corynebacterium* causing kidney disease of salmonids. *Journal of Fisheries Research Board of Canada* 32: 2224-2227.

Bullock, G.L., B.R. Griffin and H.M. Stuckey. 1980. Detection of *Corynebacterium salmonius* by direct fluorescent antibody test. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 719-721.

Bullock, G.L., D.A. Conroy and S.F. Snieszko. 1971. Septicemic diseases caused by motile Aeromonads and Pseudomonads. In: *Diseases of Fishes*, S.F. Snieszko and H.R. Axelrod (eds.). T.F.H. Publications, Neptune, New Jersey, pp. 21-41.

Campbell, A.C. and J.A. Buswell. 1982. An investigation into the bacterial etiology of 'black patch necrosis' in Dover sole *Solea solea* L. *Journal of Fish Diseases* 5: 495-508.

Carli, A., L. Pane, L. Casareto, S. Bertone and C. Pruzzo. 1993. Occurrence of *Vibrio alginolyticus* in Ligurian coast rosk pools (Tyrrhenian Sea, Italy) and its association with the copepod *Tigriopus fulvus* (Fisher 1860). *Applied Environmental Microbiology* 59: 1960-1962.

Chen, M.F., S. Yun, G.D. Marty, T.S. McDowell, M. House, J.A. Appersen, T.A. Guenther, K.D. Arkush and R.P. Hedrick. 2000. A *Piscirickettsia salmonis*-like bacterium associated with mortality of white seabass *Atractoscion nobilis*. *Diseases of Aquatic Organisms* 43: 117-126.

Chern, R.S. and C.B. Chao. 1994. Outbreaks of a disease caused by rickettsia-like organism in cultured tilapias in Taiwan. *Fish Pathology* 29: 61-71.

Colorni, A. and A. Diamant. 1992. Pathology in mariculture: Specific problems and research in Israel. *Israel Journal of Aquaculture/Bamidgeh* 44: 140-141.

Colorni, A., I. Paperna and H. Gordin. 1981. Bacterial infections in gilt-head sea bream *Sparus aurata* cultured at Elat. *Aquaculture* 23: 257-267.

Colquhoun, D.J., H. Hovland, H. Hellberg, T. Haug and H. Nilsen. 2004. *Moritella viscosa* isolated from farmed Atlantic cod (*Gadus morhua*). *Bulletin of European Association of Fish Pathologists* 24: 109-114.

Comps, M., J.C. Raymond and G.N. Plassiart. 1996. Rickettsia-like organism infecting juvenile sea-bass *Dicentrarchus labrax*. *Bulletin of European Association of Fish Pathologists* 16: 30-33.

Cvitanich, J.D., O. Gárate and C.E. Smith. 1991. The isolation of a rickettsia-like organism causing disease and mortality in Chilean salmonids and its confirmation by Koch's postulate. *Journal of Fish Diseases* 14: 121-145.

Dalsgaard, I. 1993. Virulence mechanisms in *Cytophaga psychrophila* and other *Cytophaga*-like bacteria pathogenic for fish. *Annual Review of Fish Diseases* 3: 127-144.

Dalsgaard, I., L. Madsen, S. Busch and H. Korsholm. 2001. Transfer of bacterial pathogens between wild and farmed fish. *EAFP International Conference*, Dublin 9-14.9. pp. 265.

Davies, R.L. and G.N. Frerichs. 1989. Morphological and biochemical differences among isolates of *Yersinia ruckeri* obtained from wide geographical areas. *Journal of Fish Diseases* 12: 357-365.

Decostere, A., F. Haesebrouck and L. Devriese. 1998. Characterization of four *Flavobacterium columnare* (*Flexibacter columnaris*) strains isolated from tropical fish. *Veterinary Microbiology* 62: 35-45.

Decostere, A., F. Haesebrouck, G. Charlier and R. Ducatelle. 1999a. The association of *Flavobacterium columnare* strains of high and low virulence with gill tissue of black mollies (*Poecilia sphenops*). *Veterinary Microbiology* 67: 287-298.

Decostere, A., F. Haesebroeck, J. Turnbull and G. Charlier. 1999b. Influence of water quality and temperature on adhesion of high and low virulence *Flavobacterium columnare* strains to isolated gill arches. *Journal of Fish Diseases* 22: 1-11.

Dulin, M.P., T. Huddleston, R.E. Larson and G.W. Klontz. 1976. Enteric redmouth disease. Forest. Wildlife and Range Experimental Station Bulletin, University of Idaho, Moscow, ID. 8: 15pp.

Earp, B.J., C.H. Ellis and E.J. Ordal. 1953. Kidney disease in young salmon. State of Washington Department of Fisheries in cooperation with Department of Microbiology, Oceanography and the School of Fisheries of the University of Washington, Seattle.

Egidius, E. 1987a. Import of furunculosis to Norway with Atlantic salmon smolts from Scotland. *ICES Mariculture Committee* CM 1987/F: 8:7.

Egidius, E. 1987b. Vibriosis: Pathogenicity and Pathology: A review. *Aquaculture* 67: 15-28.

Egidius, E., R. Wiik, K. Andersen, K.A. Hoff and B. Hjeltnes. 1986. *Vibrio salmonicida* sp. nov. a new fish pathogen. *International Journal of Systematic Bacteriology* 36: 518-520.

Emmerich, R. and E. Weibel. 1894. Ueber eine durch Bakterien erengte Seuche unter der Forellen. *Arch Hygiene und Bakteriologie* 21: 1-21.

Enger, Ø. 1997. Survival and inactivation of *Aeromonas salmonicida* outside the host—a most superficial way of life. In: *Furunculosis: Multidisciplinary Fish Disease Research*, E.M. Bernoth, A.E. Ellis, P.J. Midtlyng, G. Olivier and P. Smith (eds). Academic Press, London, pp. 59-177.

Evelyn, T.P.T., L. Prosperi-Porta and J.E. Ketcheson. 1986. Experimental intra-ovum infection of salmonid eggs with *Renibacterium salmoninarum* and vertical transmission of the pathogen with such eggs despite their treatment with erythromycin. *Diseases of Aquatic Organisms* 1: 197-202.

Faisal, M., W. Popp and M. Refai. 1989. *Aeromonas hydrophila* related septicemia in the Nile tilapia *Oreochromis niloticus*. *Berliner und Muenchner Tieraerztliche Wochenschrift* 102: 87-93.

Figueiredo, H., P. Klesius, C. Arias, J. Evans, C. Shoemaker, D. Pereira and M. Peixoto. 2005. Isolation and characterization of strains of *Flavobacterium columnare* from Brazil. *Journal of Fish Diseases* 28: 199-204.

Fryer, J.L. and C.N. Lannan. 1993. The history and current status of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in Pacific salmon. *Fisheries Research* 17: 15-33.

Fryer, J.L. and J.E. Sanders. 1981. Bacterial kidney disease of salmonid fish. *Annual Review of Microbiology* 35: 273-298.

Fryer, J.L., C.N. Lannan, L.H. Garcès, J.J. Larenas and P.A. Smith. 1990. Isolation of a rickettsiales-like organism from diseased coho salmon (*Oncorhynchus kisutch*) in Chile. *Fish Pathology* 25: 107-114.

Fryer, J.L., C.N. Lannan, S.J. Giovannoni and N.D. Wood. 1992. *Piscirickettsia salmonis* gen. nov., sp. nov., the causative agent of an epizootic disease in salmonid fishes. *International Journal of Systematic Bacteriology* 42: 120-126.

Gauthier, G., B. Lafay, R. Ruimy, V. Breittmayer, J.L. Nicolas, M. Gauthier and R. Christen. 1995. Small-subunit rRNA sequences and whole DNA relatedness concur for the reassignment of *Pasteurella piscicida* (Sniezsko *et al.*) Janssen and Surgalla to the genus *Photobacterium* as *Photobacterium damsela* subsp. *piscicida* comb. nov. *International Journal of Systematic Bacteriology* 45: 139-144.

Gatesoupe, F.J. 1999. The use of probiotics in aquaculture. *Aquaculture* 180: 147-165.

Grant, A.N., A.G. Brown, D.I. Cox, T.H. Birbeck and A.A. Griffen. 1996. Rickettsia-like organism in farmed salmon. *Veterinary Record* 27: 423.

Greger, E. and T. Goodrich. 1999. Vaccine development for winter ulcer disease, *Vibrio viscosus*, in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 22: 193-199.

Gudmundsdóttir, B.K. 1998. Infections by atypical strains of the bacterium *Aeromonas salmonicida*. Búvísindi Icelandic Agriculture Sciences 12: 61-72.

Gudmundsdóttir, S., E. Benediktsdottir and S. Helgason. 1993. Detection of *Renibacterium salmoninarum* in salmonid kidney samples: A comparison of results using double-sandwich ELISA and isolation on selective medium. *Journal of Fish Diseases* 16: 185-195.

Gudmundsdóttir, B.K., B. Magnadottir and S. Gudmonsdóttir. 2001. Experimental infection of juvenile turbot (*Scophthalmus maximus*), halibut (*Hippoglossus hippolossus*) and cod (*Gadus morhua*) with the bacterium *Moritella viscosa*. *10th International Conference, Diseases of Fish and Shellfish. European Association of Fish Pathologists (EAFP). Abstract Book O-88pp.*

Gudmundsdóttir, B.K., I. Hvanndal, B. Björnsdóttir and U. Wagner. 2003. Analysis of exotoxins produced by atypical isolates of the fish pathogen *Aeromonas salmonicida*, by enzymatic and serological methods. *Journal of Fish Diseases* 26: 15-29.

Gunnlaugsdóttir, B. and B.K. Gudmundsdóttir. 1996. Pathogenicity of atypical *Aeromonas salmonicida* in Atlantic salmon compared with protease production. *Journal of Applied Microbiology* 1997 83: 542-551. *Applied Bacteriology* 80: 105-113.

Hansen, G.H. and J. Olafsen. 1999. Bacterial interactions in early life stages of marine cold water fish. *Microbial Ecology* 38: 1-26.

Hansen, G.H., Ø. Bergh, J. Michaelsen and D. Knappskog. 1992. *Flexibacter ovolyticus* sp. nov., a pathogen of eggs and larvae of Atlantic halibut, *Hippoglossus hippoglossus* L. *International Journal of Systematic Bacteriology* 42: 451-458.

Håstein, T. and T. Lindstad. 1991. Diseases in wild and cultured salmon: possible interaction. *Aquaculture* 98: 277-288.

Hazen, T.C., M.L. Raker, G.W. Esch and C.B. Fliermans. 1978. Ultrastructure of red-sore lesions on largemouth bass (*Micropterus salmoides*) association of the ciliate *Epistylis* sp. and the bacterium *Aeromonas hydrophila*. *Journal of Protozoology* 25: 351-355.

Hawke, P. and R. Thune. 1992. Systemic isolation and antimicrobial susceptibility of *Cytophaga columnaris* from commercially reared channel catfish. *Journal of Aquatic Animal Health* 4: 109-113.

Hawke, J.P., R.L. Thune, R.K. Cooper, E. Judice and M. Kelly-Smith. 2003. Molecular and phenotypic characterization of strains of *Photobacterium damselae* subsp. *piscicida* isolated from hybrid striped bass cultured in Louisiana, USA. *Journal of Aquatic Animal Health* 15: 189-201.

Hikida, M., H. Wakabayashi, S. Egusa and K. Masumura. 1979. *Flexibacter* sp., a gliding bacterium pathogenic to some marine fishes in Japan. *Bulletin of Japanese Society of Scientific Fisheries* 45: 421-428.

Hiney, M. and G. Olivier. 1999. Furunculosis (*Aeromonas salmonicida*). In: *Fish Diseases and Disorders*, P.T.K. Woo and D.W. Bruno (eds). CABI Publishing, Wallingford, Oxon, U.K., Volume 3: Viral, Bacterial and Fungal infections, pp. 341-426.

Hjeltnes, B. and R.J. Roberts. 1993. Vibriosis. In: *Bacterial Diseases of Fish*, V. Inglis, R.J. Roberts and N. Bromage (eds.). Blackwell Science, Oxford, UK, pp. 109-121.

Hjeltnes, B., Ø Bergh, H. Wergeland and J.C. Holm. 1995. Susceptibility of Atlantic cod, *Gadus morhua*, halibut, *Hippoglossus hippoglossus* and wrasse (Labridae) to *Aeromonas salmonicida* subsp. *salmonicida* and the possibility of tramsnission of furunculosis from farmed salmon *Salmo salar* to marine fish. *Diseases of Aquatic Organisms* 23: 25-31.

Hoff, K.A. 1989. Survival of *Vibrio anguillarum* and *Vibrio salmonicida* at different salinities. *Applied Environmental Microbiology* 55: 1775-1786.

Husevåg, B. 1994. *Survival of Aeromonas salmonicida and Vibrio salmonicida in marine fish farm environments*. Doctor Scientarium Thesis. Department of Microbiology, University of Bergen, Norway.

Ingilæ, M., J.A. Arnesen, V. Lund and G. Eggset. 2000. Vaccination of Atlantic halibut *Hippoglossus hippoglossus* L., spotted wolfish *Anarhicas minor* L. against atypical *Aeromonas salmonicida*. *Aquaculture* 183: 31-44.

Izumi, S., F. Aranishi and H. Wakabayashi. 2003. Genotyping of *Flavobacterium psychrophilum* using PCR-RFLP analysis. *Diseases of Aquatic Organisms* 56: 207-214.

Jacobsen, P. and L. Berglind. 1988. Persistence of oxytetracycline in sediments from fish farms. *Aquaculture* 70: 365-370.

Janda, J.M. and S.L. Abbott. 1996. Human pathogens. In: *The Genus Aeromonas*, B. Austin, M. Altwegg, P.J. Gosling and P. Joseph (eds). John Wiley and Sons, Chichester, pp. 149-173.

Johnsen, B.O. and A.J. Jensen. 1994. The spread of furunculosis in salmonids in Norwegian rivers. *Journal of Fish Biology* 45: 47-55.

Jones, S.M., R.J.F. Markham, D.B. Groman and R.R. Cusack. 1998. Virulence and antigenic characteristics of a cultured Rickettsiales-like organism isolated from farmed Atlantic salmon *Salmo salar* in eastern Canada. *Diseases of Aquatic Organisms* 33: 25-31.

Jónsdóttir, H., H. Malmquist, S. Snorrason, G. Gudbergsson and S. Gudmundsdóttir. 1998. Epidemiology of *Renibacterium salmoninarum* in wild Arctic charr and brown trout in Iceland. *Journal of Fish Biology* 53: 322-339.

Kent, M.L. 1992. Diseases of seawater netpen-reared salmonid fishes in the Pacific Northwest. *Canadian Special Publication of Fisheries Aquatic Science* 116: 76pp.

Kent, M.L., G.S. Traxler, D. Kieser, J. Richard, S.C. Dawe, R.W. Shaw, G. Prosperi-Porta, J. Ketcheson and T. Evelyn. 1998. Survey of salmonid pathogens in ocean-caught fishes in British Canada. *Journal of Aquatic Animal Health* 10: 211-219.

Kettler, S., C. Pfeil-Putzien and R. Hoffmann. 1986. Infection of grayling (*Thymallus thymallus*) with the agent of bacterial kidney disease (BKD). *Bulletin of European Association of Fish Pathologists* 6: 69-71.

Korsnes, K., O. Nicolaisen, C.K. Skaar, A.H. Nerland and Ø Bergh. 2006. Bacteria in the gut of juvenile cod *Gadus morhua* fed live feed enriched with four different commercial diets. *ICES Journal of Marine Science* 63: 296-301.

Koski, P., V. Hirvelä-Koski and J. Bernardet. 1993. *Flexibacter columnaris* infection in arctic char (*Salvelinus alpinus* (L.)). First isolation in Finland. *Bulletin of European Association of Fish Pathologists* 13: 66-69.

Kusuda, R., J. Yokoyama and K. Kawai. 1986. Bacteriological study on cause of mass mortalities in cultured black sea bream fry. *Bulletin of the Japanese Society of Scientific Fisheries* 52: 1745-1751.

Kvitt, H., M. Ucko, A. Colorni, C. Batargias, A. Zlotkin and W. Knibb. 2002. *Photobacterium damselae* ssp. *piscicida*: detection by direct amplification of 16S rRNA gene sequences and genotypic variation as determined by amplified fragment length polymorphism (AFLP). *Diseases of Aquatic Organisms* 48: 187-195.

Lannan, C.N. and J.L. Fryer. 1993. *Piscirickettsia salmonis*, a major pathogen of salmonid fish in Chile. *Fish Research* 17: 115-121.

Lee, K.K. 1995. Pathogenesis studies on *Vibrio alginolyticus* in the grouper, *Epinephelus malabaricus* (Bloch et Schneider). *Microbiology and Pathogenesis* 19: 39-48.

Lillehaug, A., B.T. Lunestad and K. Grave. 2003. Epidemiological description of bacterial diseases in Norwegian aquaculture: A description base on antibiotic prescription data for the ten-year period 1991 to 2000. *Diseases of Aquatic Organisms* 53: 115-125.

Lunder, T. 1992. Winter ulcer in Atlantic salmon. A study of pathological changes, transmissibility, bacterial isolates. In: Doctor Scientiarum Thesis. The Norwegian College of Veterinary Medicine, Oslo, Norway.

Lunder, T., O. Evensen, G. Holstad and T. Hastein. 1995. Winter ulcer in the Atlantic salmon *Salmo Salar*—pathological and bacteriological investigations and transmission experiments. *Diseases of Aquatic Organisms* 23: 39-49.

Lunder, T., H. Sorum, G. Holstad, A.G. Steigerwalt, P. Mowinckel and D.J. Brenner. 2000. Phenotypic and genotypic characterization of *Vibrio viscosus* sp. nov. and *Vibrio wodanis* sp. nov. isolated from Atlantic salmon (*Salmo salar*) with 'winter ulcer'. *International Journal of Systematic Evolutionary Microbiology* 50: 427-450.

Lunestad, B.T. 1992. *Environmental effects of antibacterial agents used in aquaculture*. Doctor Scientiarum Thesis, University of Bergen, Norway.

Madetoja, J. and T. Wiklund. 2002. Detection of the fish pathogen *Flavobacterium psychrophilum* in water from fish farms. *Systematic Applied Microbiology* 25: 259-266.

Magnadottir, B., S.H. Bambir, B.K. Gudmundsdóttir, L. Pilstrøm and S. Helgason 2002. Atypical *Aeromonas salmonicida* infection in naturally and experimentally infected cod, *Gadus morhua* L. *Journal of Fish Diseases* 25: 583-597.

Mackie, T.J., J.A. Arkwright, T.E. Pryce-Tannett, J.C. Mottram, W.S. Johnston and W.J.M. Menzies. 1930, 1933, 1935. Interim, Second and Final Reports of the Furunculosis Committee. HMSO, Edinburgh.

Madsen, L., J. Moller and I. Dalsgaard. 2005. *Flavobacterium psychrophilum* in rainbow trout, *Oncorhynchus mykiss* (Walbaum), hatcheries: Studies on broodstock, eggs, fry and environment. *Journal of Fish Diseases* 28: 39-47.

Magariños, B., A.E. Toranzo and J.L. Romalde. 1996. Phenotypic and pathobiological characteristics of *Pasteurella piscicida*. *Annual Review of Fish Diseases* 6: 41-64.

Magariños, B., N. Couso, M. Noya, P. Merino, A.E. Toranzo and J. Lamas. 2001. Effect of temperature on the development of pasteurellosis in carrier gilt-head seabream (*Sparus aurata*). *Aquaculture* 195: 17-21.

Marco-Noales, E., M. Milan, B. Fouz, E. Sanjuan and C. Amaro. 2001. Transmission to Eels, Portals of Entry, Putative Reservoirs of *Vibrio vulnificus* Serovar E (Biotype 2). *Applied Environmental Microbiology* 67: 4717-4725.

Mauel, M.J., D.L. Miller, K. Frazier, A.D. Liggett, L. Styer, D. Montgomery-Brock and J. Brock. 2003. Characterization of a piscirickettsiosis-like disease in Hawaiian tilapia. *Diseases of Aquatic Organisms* 53: 249-255.

Meyers, T.R., S. Short, C. Farrington, K. Lipson, H.J. Geiger and R. Gates. 1993a. Comparison of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody test (FAT) for measuring the prevalences and levels of

*Renibacterium salmoninarum* in wild and hatchery stocks of salmonid fishes in Alaska, USA. *Diseases of Aquatic Organisms* 16: 181-189.

Meyers, T.R., S. Short, C. Farrington, K. Lipson, H.J. Geiger and R. Gates. 1993b. Establishment of a negative-positive threshold optical density value for the enzyme-linked immunosorbent assay (ELISA) to detect soluble antigen of *Renibacterium salmoninarum* in Alaska pacific salmon. *Diseases of Aquatic Organisms* 16: 191-197.

Morrison, C., J. Cornick, G. Shum and B. Zwicker. 1981. Microbiology and histopathology of 'saddleback' disease of underyearling Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 4: 243-258.

Munro, A.L.S. and T.S. Hastings. 1993. Furunculosis. In: *Bacterial Diseases of Fish*, V. Inglis, R.J. Roberts and N. Bromage (eds.). Blackwell Science, Oxford, UK, pp. 123-142.

Nagai, T. and Y. Iida. 2002. Occurrence of bacterial kidney disease in cultured ayu. *Fish Pathology* 37: 77-81.

Nielsen, M.E., L. Hoi, A.S. Schmidt, D. Qian, T. Shimada, J.Y. Shen and J.L. Larsen. 2001. Is *Aeromonas hydrophila* the dominant motile *Aeromonas* species that causes disease outbreaks in aquaculture production in the Zhejiang Province of China? *Diseases of Aquatic Organisms* 46: 23-29.

Nylund, A. and K. Ottem. 2006. Francisellosis a new cod (*Gadus morhua*) disease caused by *Francisella* n.sp. *DIPNET Newsletter* No. 45.

Nylund, A., K. Ottem, K. Watanabe, E. Karlsbakk and B. Krossøy. 2006. *Francisella* sp. (Family Francisellaceae) causing mortality in Norwegian cod (*Gadus morhua*) farming. *Archives of Microbiology* 185: 383-392.

Olsen, A.B., H.P. Melby, L. Speilberg, Ø. Evensen and T. Håstein. 1997. *Piscirickettsia salmonis* infection in Atlantic salmon *Salmo salar* in Norway—epidemiological, pathological and microbiological findings. *Diseases of Aquatic Organisms* 31: 35-48.

Olsen, A.B., J. Mikalsen, M. Rode, A. Alfjorden, E. Hoel, K. Straume-Lie, R. Haldorsen and D. Colquhoun. 2006. A novel systemic granulomatous inflammatory disease in farmed Atlantic cod, *Gadus morhua* L., associated with a bacterium belonging to the genus *Francisella*. *Journal of Fish Diseases* 29: 307-311.

Paillard, C. 2004. A short review of brown ring disease, a vibriosis affecting clams, *Ruditapes phillippinarum* and *Ruditapes decussatus*. *Aquatic Living Resources* 17: 467-475.

Pfeil-Putzien, C., R. Hoffmann and W. Popp. 1985. Preliminary report on the occurrence of bacterial kidney disease in Germany. *Bulletin of European Association of Fish Pathologists* 5: 30-31.

Nematollahi, A., A. Decostere, F. Pasmans and F. Haesebrouck. 2003. *Flavobacterium psychrophilum* infections in salmonid fish. *Journal of Fish Diseases* 26: 563-574.

Nougayrede, P., E. Sochon and A. Vuillaume. 1990. Isolation of *Aeromonas salmonicida* subspecies salmonicida in farmed turbot (*Psetta maxima*) in France. *Bulletin of European Association of Fish Pathologists* 10: 129-140.

Pedersen, K., L. Grisez, R. van Houdth, T. Tiainen, F. Ollevier and J.L. Larsen. 1999. Extended serotyping scheme for *Vibrio anguillarum* with the definition of seven provisional O-serogroups. *Current Microbiology* 38: 183-189.

Ringø, E. and T.H. Birkbeck. 1999. Intestinal microflora of fish larvae and fry. *Aquaculture Research* 30: 73-93.

Rimaila-Päränen, E. 2002. First case of bacterial kidney disease (BKD) in whitefish (*Coregonus lavaretus*) in Finland. *Bulletin of European Association of Fish Pathologists* 22: 403-404.

Roberts, R.J. 1993. Motile aeromonad septicaemia. In: *Bacterial Diseases of Fish*, V. Inglis, R.J. Roberts and N.J. Bromage (eds.). Blackwell Scientific Publications, London, pp. 143-155.

Rodger, H.D. and E.M. Drinan. 1993. Observation of a rickettsia-like organism in Atlantic salmon, *Salmo salar* L. in Ireland. *Journal of Fish Diseases* 16: 361-369.

Ross, A.J., R.R. Rucker and W.H. Ewing. 1966. Description of a bacterium associated with redmouth disease of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Microbiology* 12: 763-770.

Rucker, R.R. 1966. Redmouth disease in rainbow trout (*Salmo gairdneri*). *Bulletin of Office International of Epizooties* 5: 825-830.

Salati, F., C. Cubadda, I. Viale and R. Kusuda. 2005. Immune response of sea bass *Dicentrarchus labrax* to *Tenacibaculum maritimum* antigens. *Fisheries Science* 71: 563-567.

Salte, R., K.A. Rorvik, E. Reed and K. Norberg. 1994. Winter Ulcers of the skin in Atlantic Salmon, *Salmo Salar* L-pathogenesis and possible etiology. *Journal of Fish Diseases* 17: 661-665.

Salvesen, I. and O. Vadstein. 2000. Evaluation of plate count methods for determination of maximum specific growth rate in mixed microbial communities, and its possible application for diversity assessment. *Journal of Applied Bacteriology* 88: 442-448.

Samuelsen, O.B., B.T. Lunestad, B. Husevåg, T. Hølleland and A. Ervik. 1992. Residues of oxolinic acid in wild fauna following medication in fish farms. *Diseases of Aquatic Organisms* 1: 111-119.

Samuelsen, O.B., A. Ervik, L. Torkildsen and Ø. Bergh. 2002. The efficacy of a single intraperitoneal injection of either flumequine or oxytetracycline hydrochloride in prevention of outbreaks of atypical *Aeromonas salmonicida* infection in goldsinny wrasse, *Ctenolabrus rupestris* L. following stress. *Aquaculture International* 10: 257-264.

Samuelsen, O.B., P.G. Kvenseth, J.H. Andreassen, L. Torkildsen, A. Ervik and Ø. Bergh. 2003. The efficacy of a single intraperitoneal injection of oxolinic acid in the treatment of bacterial infections in goldsinny wrasse (*Ctenolabrus rupestris*) and corkwing wrasse (*Symphodus melops*) studied under field and laboratory conditions. *Journal of Veterinary Pharmacology and Therapeutics* 26: 181-186.

Samuelsen, O.B., A.H. Nerland, T. Svåsand, M. Schrøder, T. Jørgensen and Ø. Bergh. 2006. Viral and bactrial diseases of the Atlantic cod, *Gadus morhua*, their prophylaxis and treatment: A review. *Diseases of Aquatic Organisms* 71: 239-254.

Sedano, J., I. Zorrilla, M.A. Morinigo, M.C. Balebona, A. Vidaurreta, M.A. Bordas and J.J. Borrego. 1996. Microbial origin of the abdominal swelling affecting farmed larvae of gilt-head seabream, *Sparus aurata* L. *Aquaculture Research* 27: 323-333.

Sioutas, S., R.W. Hoffmann, C. Pfeil-Putzien and T. Fischer-Scherl. 1991. Carp erythrodermatitis (CE) due to an *Aeromonas hydrophila* infection. Casuistic and experimental results. *Zentrablatt Veterinary Medicine* B38: 186-194.

Smith, I.W. 1961. A disease in finnock due to *Vibrio anguillarum*. *Journal of General Microbiology* 24: 247-252.

Smith, I.W. 1964. *The Occurrence and Pathology of Dee Disease*. Department of Agriculture and Fisheries for Scotland, Edinburgh, pp. 1-13.

Snieszko, S.F., G.L. Bullock, E. Hollis and J.G. Boone. 1964. *Pasteurella* sp. from an epizootic of white perch (*Roccus americanus*) in Chesapeake Bay tidewater areas. *Journal of Bacteriology* 88: 1814-1815.

Sommerset, I., B. Krossøy, E. Biering and P. Frost. 2005. Vaccines for fish in aquaculture. *Experimental Review of Vaccines* 4: 89-101.

Sørum, H., A. Hvaal, M. Heum, F. Daae and R. Wiik. 1990. Plasmid profiling of *Vibrio salmonicida* for epidemiological studies of cold water vibriosis in Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*). *Applied Environmental Microbiology* 56: 1033-1037.

Sukui, M., Y. Nakagawa, S. Harayama and S. Yamamot. 2001. Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: Proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. *International Journal of Systematic Evolutionary Microbiology* 51: 1639-1652.

Tiirola, M., E. Valtonen, P. Rintamäki-Kinnunen and M. Kulomaa. 2002. Diagnosis of flavobacteriosis by direct amplification of rRNA genes. *Diseases of Aquatic Organisms* 51: 93-100.

Tison, D.L., M. Nishibuchi, J.D. Greenwood and R.J. Seidler. 1982. *Vibrio vulnificus* Biogroup 2: New Biogroup Pathogenic for Eels. *Applied and Environmental Microbiology* 44: 640-646.

Thompson, F.L., T. Iida and J. Swings. 2004. Biodiversity of vibrios. *Microbiology and Molecular Biology Reviews* 68: 403-431.

Toranzo, A.E., S. Barreiro, J.F. Casal, A. Figueras, B. Magarinos and J.L. Barja. 1991. Pasteurellosis in cultured gilthead seabream (*Sparus aurata*): first report in Spain. *Aquaculture* 99: 1-15.

Toranzo, A.E., B. Magariños and J.L. Romalde. 2005. A review of the main bacterial fish diseases in mariculture systems. *Aquaculture* 246: 37-61.

Treasurer, J. and D. Cox. 1991. The occurrence of *Aeromonas salmonicida* in wrasse (Labridae) and implications for Atlantic salmon farming. *Bulletin of European Association of Fish Pathologists* 11: 208-210.

Treasurer, J. and L.A. Laidler. 1994. *Aeromonas salmonicida* infection in wrasse (Labridae) used as cleaner fish on Atlantic salmon, *Salmo salar* L. farm. *Journal of Fish Diseases* 17: 155-161.

Trüper, H.G. and L. De'Clari. 1997. Taxonomic note: necessary correction of epithets formed as substantives (nouns) 'in apposition'. *International Journal of Systematic Bacteriology* 47: 908-909.

Vadstein, O., T.A. Mo and Ø. Bergh. 2004. Microbial interactions, prophylaxis and diseases. In: *Culture of Cold-water Marine Fish*, E. Moksness, E. Kjørsvik and Y. Olsen (eds.). Blackwell Scientific Publications, London, pp. 28-72.

Wahli, T., S.E. Burr, D. Pugovkin, O. Mueller and J. Frey. 2005. *Aeromonas sobria*, a causative agent of disease in farmed perch, *Perca fluviatilis* L. *Journal of Fish Diseases* 28: 141-150.

Wakabayashi, H. 1993. Columnaris disease. In: *Bacterial Diseases of Fish*, V. Inglis, R. Roberts and N. Bromage (eds.). Blackwell Scientific Publications, Oxford, pp. 23-39.

Wiik, R., K. Andersen, F. Daae and K. Hoff. 1989. Virulence studies based on plasmid profiles of the fish pathogen *Vibrio salmonicida*. *Applied Environmental Microbiology* 55: 819-825.f

Wiklund, T. and I. Dalsgaard. 1998. Occurrence and significance of atypical *Aeromonas salmonicida* in non-salmonid and salmonid fish species: A review. *Diseases of Aquatic Organisms* 32: 49-64.

Wiklund, T., L. Madsen, M. Bruun and I. Dalsgaard. 2000. Detection of *Flavobacterium psychrophilum* from fish tissue and water samples by PCR amplification. *Journal of Applied Microbiology* 88: 299-307.

Willumsen, B. 1990. *Aeromonas salmonicida* subsp. *salmonicida* isolated from Atlantic cod and coalfish. *Bulletin of European Association of Fish Pathologists* 10: 62-63.

Wood, E.M. and W. Yasutake. 1956. Histopathology of kidney disease in fish. *American Journal of Pathology* 32: 845-857.

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CHAPTER

# 6

# Fungal Diseases of Fish

**D.J. Alderman**

## INTRODUCTION

Over the last 30 years, a number of overviews of fungal diseases of aquatic animals have been published. Some have been restricted to fish (Neish and Hughes, 1980; Egusa, 1992; Bruno and Wood, 1999; Yanong, 2003); while others have covered a wider range of aquatic animals (Alderman, 1982a; Lauckner, 1984). A few have been free-standing reviews (Noga, 1993; Mueller; 1994; Yanong, 2003), whilst others—as this one here—have been included in fish diseases treatises. Thirty years ago, there was a reasonable consensus as to the range of organisms that could be included in such a review. It was widely recognized that there was a taxonomic gulf between the 'Phycomycetes' and the higher fungi (Deuteromycetes, Ascomycetes and Basidiomycetes), but both were regarded as fungi in the wider sense and are still regarded as the province of the mycologist. Although regarded as taxonomic orphans, it was also generally accepted that pathogens such as *Ichthyophonus* and *Branchiomyces* should also be included in such an overview.

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Conventional fungal taxonomy is based heavily upon morphology and full identification often depends on the presence of a range of morphological stages that may not be present in culture. In conventional terms, fungi have the following morphological characteristics. They are eukaryotic and multicellular, many consist of hyphae that grow apically and, although the majority are saprophytic, many are symbiotes that form permanent associations with plants (mycorrhiza) and algae (lichen) and some have developed a parasitic mode of life and are major parasites of plants and animals. In the last ten years or so, advances in molecular taxonomy have given us a better view of the relationships between the various groups of organisms that have conventionally been included as fungi and included in overviews of this type. The fungi *sensu lato* are now regarded as having only morphological and not taxonomic affinities and falling into three separate kingdoms. The first of these, the true fungi, the Eumycota includes the Ascomycetes and Basidiomycetes; the second, the Chromista (Stramenopiles), includes the oomycetes or water moulds as well as diatoms and brown algae. Finally, there is what is now recognized on a molecular basis to be a group of organisms which, while appearing to lie at the base of the Eukaryote tree, seem to be best placed in the Kingdom Protoctista in a clade currently referred to as the DRIPs (Ragan *et al.*, 1996). DRIP includes *Dermocystidium*, the Rosette agent, *Ichthyophonus* and *Psorospermium*. The remaining 'fungal' fish parasite, *Branchiomyces*, has never been validly described; even its morphology in fish is inadequately described and it will not be considered further in this review.

By far the most important of these groups of 'fungal' pathogens of fish are the Chromistan oomycetes, which include the water moulds *Saprolegnia* and *Aphanomyces*. These differ from the Eumycota in numerous ways, including the presence of motile biflagellate zoospores as the primary means of asexual reproduction and the absence of chitin from their cell walls which instead are basically cellulosic. The majority are saprophytic or perthotrophic (necrotrophic), but those specialized in the parasitic mode encompass both aquatic animal and an extensive range of plant pathogens.

This review concentrates primarily upon fungal diseases of fish, but where appropriate, also refers briefly to fungal infections of other aquatic animals, primarily crustaceans, where the same or closely related fungal taxa are also significant pathogens.

## CHROMISTA, OOMYCETES

Although there are a large number of Oomycete genera—several of which have been reported from lesions on fish—the two most important ones by far are *Saprolegnia* and *Aphanomyces*. Both genera belong to the Family Saprolegniaceae and both comprise a large number of species, but only three of these are of major significance, *Saprolegnia parasitica*, *Aphanomyces astaci* and *Aphanomyces invadans*. They are primarily freshwater organisms, although there is some evidence of tolerance of slightly brackish water for *Aphanomyces invadans*. The related family Peronosporaceae contains a number of virulent plant pathogens (e.g., *Phytophthora infestans*) and, indeed, the genus *Aphanomyces* itself contains well known plant pathogenic members (Johnson *et al.*, 2000). It has been suggested that the oomycetes should be reclassified within a taxon, Peronosporomycetidae (Dick, 1995, 1999), but this has not been generally accepted and oomycete will be used here since it is the most frequently used term in studies of fish diseases.

The genera of the Saprolegniaceae are delimited by the morphological and developmental features of the zoosporangia and the type of zoospore formation and release. Zoosporangia comprise the primary character for separating genera which has been retained despite the fact that environmental conditions, particularly temperature, can affect the morphology and development of zoosporangia and the pattern of zoospore release and activity (Salvin, 1941). Use of cultures grown on a standard medium under standard conditions can markedly reduce this problem.

Within each genus, speciation is based principally on the morphology and development of the sexual stages, oogonia and antheridia. Features such as oogonial size, presence and types of ornamentation on oogonial walls, presence of pits in the oogonial walls, number and size of oospores in oogonia, differences in stored lipid-like globules have all been used as diagnostic features; origins of antheridia are also important, whether they are androgynous or diclinous. Since, as will be explained below, sexual stages are largely or completely lacking in the fish (and crustacean) pathogenic members of the Saprolegniales, the detailed morphology of these stages is not discussed here. Readers are referred to the treatises of Seymour (1970) and Johnson *et al.* (2000) or to the overview by Hughes (1994).

It should be emphasized at an early stage that although this review concentrates on the three species mentioned above, there are also reports

of other members of the Saprolegniales and of other *Saprolegnia* spp. and *Aphanomyces* spp. from fish and from crustaceans. Although in many cases these may be reports of opportunistic infections of wounds and skin of immuno-compromised fish or be the result of misidentification, it is unlikely that this is true in every case. Nevertheless, the majority of such case reports are so scattered and lacking in detail that no good assessment of their significance is possible.

*Saprolegnia* is by far the most important invader of fish in temperate climates. Evidence as to whether *Saprolegnia* is a true primary parasite that can infect healthy intact non-immuno compromised fish is equivocal. It presents a major problem to commercial salmonid hatcheries where it invades damaged and unfertilized eggs and then overgrows, smothers and invades viable eggs unless action to control or remove it is taken. Molecular studies have now given a much better understanding of the fish pathogenic species *Saprolegnia parasitica.*

*Aphanomyces* species are both major plant and animal pathogens. *Aphanomyces astaci,* which will be mentioned only briefly, is the classical pathogen causing the disease known as crayfish plague which has brought susceptible European freshwater crayfish populations to the status of endangered species. Details of the spread of this pathogen have been reviewed (Alderman, 1996) and its morphology and virulence have been described (Alderman and Polglase, 1986; Alderman *et al.*, 1987).

Finally, *Aphanomyces invadans* is the aetiologic agent of what is now recognized as a single disease which is known by a range of 'local' names in different parts of the world, including mycotic granulomatosis (MG) in Japan (Egusa, 1992), EUS (epizootic necrosis syndrome) in southeast Asia and the Indian subcontinent (Roberts *et al.*, 1993), Red Spot Disease in Australia (McKenzie and Hall, 1976) and UM (ulcerative mycosis) in estuaries on the east coast of North America (Noga and Dykstra, 1986).

With both EUS and UM, there are interesting similarities between the disputes that arose over the recognition that *Aphanomyces invadans* was the aetiologic agent of UM and EUS and recognition that *Aphanomyces astaci* was the aetiologic agent of crayfish plague. In the case of *Aphanomyces astaci,* although there was strong evidence that a fungus, probably an *Aphanomyces* was responsible, the renowned fish pathologist Hofer, with strong acolyte support, insisted that the cause was bacteriological and vituperation and uncertainty reigned over more than 20 years (Alderman, 1996). Again, in case of both EUS and with UM,

most of the original investigators into the condition were sure that an *Aphanomyces* was involved, a strong lobby for a putative viral aetiology (or failing that, a multiple cause aetiology) developed for EUS and, to an extent, still exists (Baldock *et al.*, 2005). On the other hand, for UM, the proposal is that the ulcerative skin lesions characteristic of MG Atlantic menhaden could be attributed to outbreaks of the putatively toxic zoospore stages of a dinoflagellate, *Pfeisteria piscicida*. Again, the disputes involved were fierce (Burkholder *et al.*, 1992, 1995; Noga *et al.*, 1996; Kiryu *et al.*, 2002). It is interesting to reflect that separated by 100 years, how such remarkably similar battles between research groups supporting *Aphanomyces* aetiologies and vigorously disputing them have ruled over a long period in each case.

The three *Saprolegnia* and *Aphanomyces* species that are characteristically pathogenic to aquatic animals all share another character that renders conventional morphologically based taxonomy difficult to apply and contributes to the confusion. As explained above, species definitions in the Oomycetes is dependent on the morphology of the sexual stages. Although at one time or another, descriptions of sexual reproductive stages for *Saprolegnia parasitica* and *Aphanomyces astaci* have been published, these descriptions are now regarded with scepticism. No sexual stage has been described for *Aphanomyces invadans*. The importance of this is that, in conventional Oomycete taxonomy, whilst it is the morphology of the asexual reproductive bodies, zoosporangia and, in particular the methods of zoospore discharge and presence or suppression of primary zoospores that is the diagnostic character that separates Oomycete genera. Speciation, however, is conventionally dependent on the morphology of the sexual stages, oogonia and antheridia. Where these are absent—as in the three most important aquatic animal pathogens—conventional morphological definitions cannot be applied. The problems that have arisen from this will be considered in greater detail under *Saprolegnia parasitica*.

Nutritionally, Oomycetes live as saprobes, perthotrophs, or parasites or (in some cases) as all three, depending on circumstances. A number of species that one might nominally consider as saprobes can live as perthotrophs given suitable dead cells or tissues on the bodies of living, prospective hosts. There is little question that under the right conditions, some of these organisms can invade the living tissues of the host and, thus, become true parasites.

As indicated above, asexual reproduction in the Saprolegniales is accomplished by means of unicellular, biflagellate zoospores (planonts) produced in (usually) terminal sporangia separated from the hyphal filaments by basal septa (Figs. 6.1, 6.2). Such features of zoosporangium development and mode of spore release are important for distinguishing genera within the Saprolegniaceae.

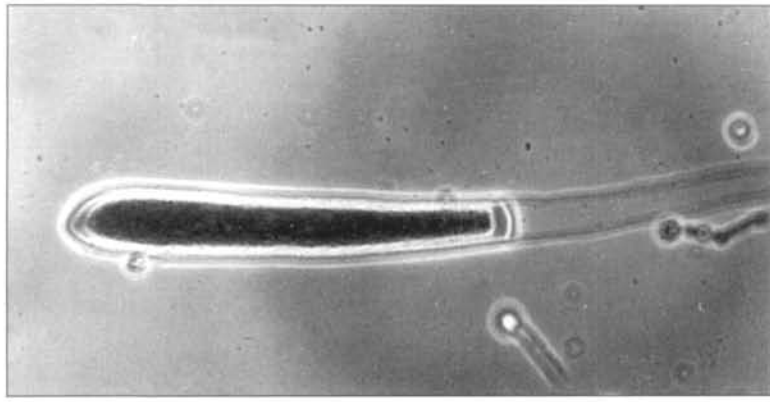

**Fig. 6.1** Mature *Saprolegnia parasitica* zoosporangium.

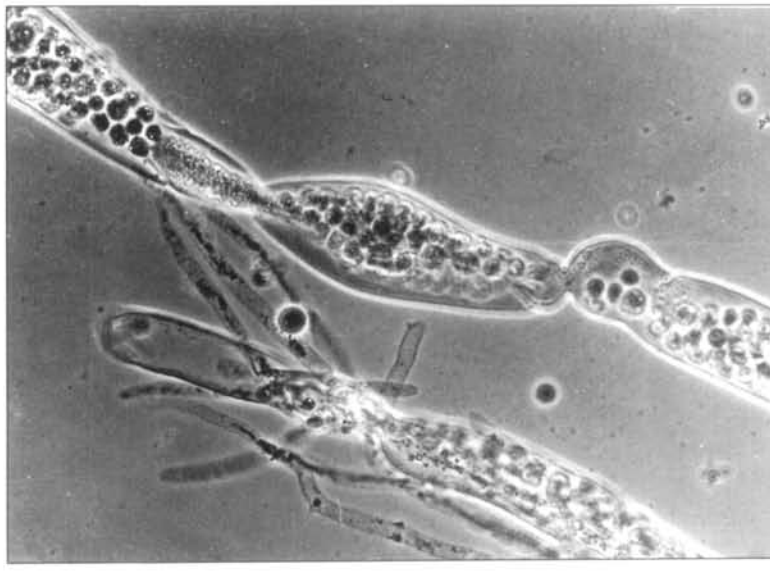

**Fig. 6.2** *Saprolegnia parasitica* zoosporangium during discharge.

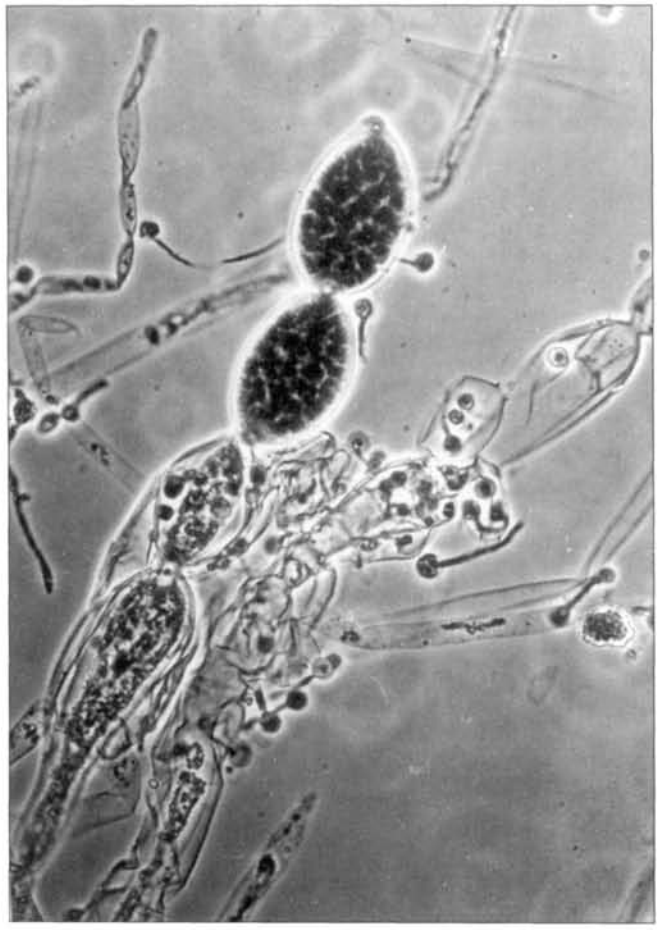

**Fig. 6.3** *Saprolegnia parasitica* zoosporangium discharging, germinating secondary zoospore cysts. Note that the germinating hyphae are much narrower than the mature hyphae.

Zoospores are usually both diplanetic and dimorphic, i.e., there are two different swimming stages involving two different kinds of zoospores: pyriform primary zoospores and reniform secondary zoospores. Each type of zoospore not only has a different shape but also a different point of flagellar insertion. Furthermore, either or both of the two swimming stages may be suppressed to varying degrees in some genera. Both zoospore forms encyst, in some cases immediately, in others after swimming for varying periods of time. Primary zoospore cysts germinate in order to release secondary zoospores. The primary cyst, therefore, acts as a miniature

sporangium and releases the reniform secondary zoospore which is the main motile stage. The secondary zoospore can maintain motility for some time, even days under the right environmental conditions, until it also encysts to produce a secondary zoospore cyst (Fig. 6.3).

In *Saprolegnia*, primary zoospores are discharged, swim, encyst and the cysts discharge secondary zoospores that swim actively. The sporangia are clavate (club shaped) (Fig. 6.4). In the genus *Achlya*, the primary zoospore is suppressed and does not swim but is, instead, discharged as amoeboid cells that form into a clump at mouth of the sporangium, form cysts which subsequently release secondary zoospores. *Achlya* sporangia are, like *Saprolegnia* sporangia, clavate in form. *Aphanomyces* vegetative hyphae are in general narrower than those of *Achlya* and *Saprolegnia* and the zoosporangium is also narrow, no wider than the hypha with only a single row of spores, which like those of *Achlya* discharge as amoeboid aplanonts, encyst at the tip of the zoosporangium and subsequently discharge secondary zoospores. Other combinations of zoosporangium and zoospore discharge types are characteristic of other Saprolegniacean genera (Neish and Hughes, 1980; Hughes, 1994).

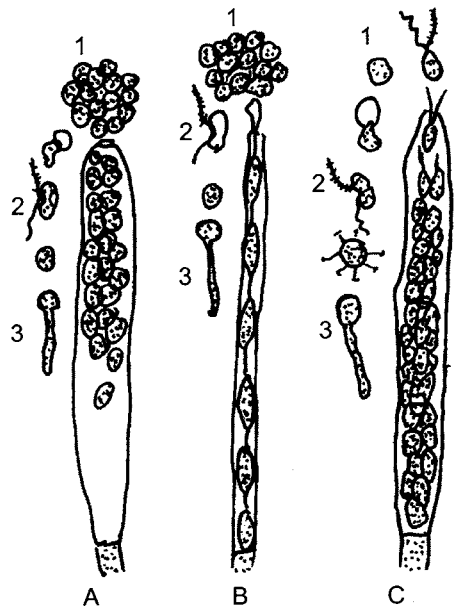


**Fig. 6.4** Oomycete zoosporangia: (A) *Achlya*, (B) *Aphanomyces* (C) *Saprolegnia*. 1 = Primary spore/zoospore, 2 = Secondary zoospore, 3 = Germinating zoospore.

In addition to asexual reproduction by zoospores, gemmae or chlamydospores (asexual aplanospores) formed through modification of hyphal segments may be produced abundantly. These are common in isolates obtained from fish, although most members of the family produce them in culture when growth conditions begin to stale and nutrient availability is reduced. Gemmae may be irregular in shape and may occur in catenulate chains, germinating to produce hyphae, or a hypha bearing a terminal zoosporangium.

## Oomycete Fish Pathogenic Genera and Species

Examination of the existing literature makes it clear that, in addition to the three species referred to above, a range of Oomycete genera have been identified in association with fish and fish eggs. Bruno and Wood (1999) list *Saprolegnia, Achlya, Aphanomyces, Dictyuchus, Isoachlya* and *Thraustotheca*, amongst others, in addition to 15 species of *Saprolegnia*. It is important to be aware that in a majority of these cases, there is little or no evidence to confirm that the organism concerned is pathogenic and not simply a saprophyte growing on damaged tissues. Until the last 20 years, there had been few attempts to satisfy Koch's postulates with allegedly fish pathogenic fungi and, in practice, there is little to distinguish one oomycete vegetative hypha from another in a lesion on a fish. Thus, even where an organism has been grown from a fish skin lesion and identified, there is no proven link between that isolate and the cause of the lesion unless the infection can be reproduced by challenge under controlled circumstances and the pathogen recovered.

## Fish Eggs

Whilst the majority of the literature concentrates on infections in fish, whether fry, fingerling or adult, in salmonid aquaculture at least, the greater problem has lain with infections occurring in eggs, particularly under intensive hatchery conditions. For several weeks, from fertilization, salmonid eggs are extremely sensitive to any physical disturbance which may prove lethal. Inevitably, not all of the eggs are viable and during the incubation period, dead and non-viable eggs will become infested with opportunist saprobes, particularly water moulds such as *Saprolegnia* and related genera. These infestations spread from the non-viable eggs and will overgrow, smother and kill the viable eggs (Fig. 6.5). Such infestations have been traditionally controlled in two ways, firstly by removing

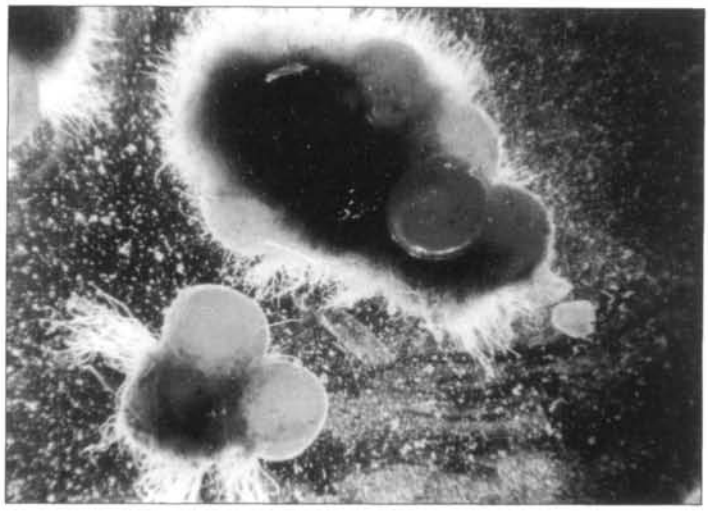

**Fig. 6.5** Salmonid eggs infested with *Saprolegnia parasitica*.

non-viable eggs, preferably as soon as they are seen to be non-viable and, secondly by chemical treatment so as to eliminate the infestation. Where eggs are held in single layers in traditional hatchery trays, removal of non-viable eggs by hand picking is a practical, if labour-expensive method, provided great care is taken to avoid shock damage to the viable eggs nearby. Where systems such as stacked trays and vertical incubators are used, hand picking is impossible and if some form of chemical treatment is not used, the whole incubator may quite easily end up as a mass of dead infested eggs. (For fuller discussion of antifungal treatments, see the appropriate section later in this chapter.) Whilst malachite green was in use, this provided an effective and economic means of controlling egg infestations; today's replacements are less effective.

In general, infestations of fish eggs, particularly in salmonid hatcheries were treated and, thus, controlled so that little or no interest was given to such inevitable infestations. The range of oomycete and other fungi that take advantage of the nutrients provided by the masses of eggs in intensive hatcheries has never really been explored. The majority are undoubtedly Oomycetes, but anyone who has examined an infected mass of eggs will attest to the fact that, although the masses of white oomycete hyphae make up the most grossly visible component, such masses swarm with bacteria, protozoa and a range of unidentifiable fungal hyphae.

## Isolation and Culture

Since most oomycete infections in fish are associated with skin lesions that are open to the external environment, there is contamination with a range of opportunist saprobes. This makes isolation of primary invaders problematical. In culture on artificial media, opportunists may well be expected to outgrow pathogens.

The original method of isolating aquatic fungi for study was by the use of 'baits' such as autoclaved insects including houseflies, various larvae and grubs as well as a variety of seeds placed in water samples where they could become infested. Of the latter, the hemp seed is still used as a suitable substrate for growth for Oomycetes. On such substrates bacterial and protozoan contamination was less of a problem than it became when the use of agar media for aquatic fungi became common.

Once an axenic culture is established, culture and maintenance of oomycetes on agar media is straightforward. Various methods have been used to obtain axenic cultures and rid the culture of contaminating organisms. These include using very small pieces of tissue as inocula ($<2$ mm$^3$), searing of surfaces from which inocula are to be taken with red hot scalpel blades, rinsing of inocula in alcohol, placing inocula inside glass rings so that the hyphae have to 'dive down' through the agar, hopefully leaving behind contaminants and, finally, the inclusion of antibiotics in the isolation medium. Inocula should be from areas deep in a lesion rather than superficial or skin surfaces to reduce the frequency of contaminants. Unquestionably, newcomers to isolating aquatic animal fungal pathogens find considerable difficulty in sorting the pathogenic 'wheat' from the opportunist saprophyte 'chaff'. For both *Saprolegnia parasitica* and *Aphanomyces invadans* (and for that matter with *Aphanomyces astaci*), there is evidence of investigators struggling to culture the pathogen and being mislead when they cultured morphologically similar, usually faster growing, opportunist organisms. Evidence suggests that it is experience and long-term persistence that converts 'difficult to isolate' to 'not difficult when you know how'.

In some cases, it may prove possible to isolate zoospores swimming from zoosporangia growing from a lesion or from excized tissue held in sterile water to which antibiotics have been added. Often, the pathogenic strain rather than any opportunistic contaminating strains may mature and zoosporulate first.

Penicillin and streptomycin were the most commonly used antibiotics for isolation of oomycetes and were incorporated into appropriate media at 150 to 1000 IU $ml^{-1}$ and 250 to 1000 mg $ml^{-1}$, respectively. Other antimicrobial agents, such as chloramphenicol, gentamycin, neomycin, oxolinic acid, potassium tellurite and carbenicillin, have also been used by some workers.

The OIE Manual (2006) lists isolation media for *Aphanomyces astaci* and *A. invadans*; these media are suitable for use with any Oomycetes. The author of this review used the penicillin–streptomycin combination for many years since this was readily available in suitable sterile vial quantities intended for human paraenteral injection that could be added simply to a suitable agar medium post autoclaving before pouring of the plates. That combination is presently not readily available commercially and the streptomycin component was sometimes suspected of reducing growth of isolates. With the introduction of 4-quinolone antibiotics into aquaculture, the opportunity was taken to change to penicillin (still in sterile vials for injection)–oxolinic acid combination. Oxolinic acid (100 mg $l^{-1}$) has the advantage that it is stable during autoclaving so that only the penicillin needs to be added post autoclaving. This combination has now been quite widely accepted.

A range of suitable media for isolation (with antibiotics) and culture of oomycetes have been used by different investigators (Bruno and Wood, 1999). In general, these organisms are not fastidious and any combination of glucose–peptone or yeast extract media may be used. In general, relatively low-nutrient media (e.g., 5 g glucose, 1 g yeast extract or peptone per litre) are to be preferred with an incubation temperature of 15°C for temperate species. In general, natural river water rather than demineralized water gives better results.

The coenocytic nature of the oomycete thallus means that swabbing, smearing or streaking methods of culture and subculture cannot be used reliably; the organisms are best subcultured by transferring small blocks of agar and inverting these colony side down on the new culture medium. Their normal habitats are aquatic or water soil plant interfaces and they are extremely sensitive to desiccation, although thicker walled resistant stages (gemmae) do exist in many species. Agar plates for use with oomycetes should not be dried, but left moist after pouring—these are, after all, water moulds and survival as a Petri dish culture is often little more than 5 or 6 weeks. Tape-sealed plates with reduced evaporation may

survive longer. Maintenance media do not require the antibiotics. Long-term maintenance of cultures is best achieved on agar slopes in screw-capped containers (Universal 30 ml screw tops), either by addition of sterile mineral oil once the colony is established on the slope or by holding these slope cultures at 4°C to 7°C and subculturing at 6- or 12-month intervals. The caps must be tight to prevent evaporation and contamination.

## Induction of Zoosporulation and Sexual Reproduction

Once unifungal cultures have been established, zoosporulation may be induced by transferring a small piece of colony containing agar to sterile water. Natural river or pond water will often give the best results. Zoosporangia may be expected to form within 24 h and zoospores will be discharged shortly after. Time taken will depend on temperature, species and strain (OIE, 2006).

For fish challenge studies (see below), it is necessary to produce large numbers of zoospores. This can be achieved by transferring agar plugs from plate culture to broth culture using the same nutrients, but without the agar. Flasks containing several litres of broth are used (e.g., 1-2 litres of broth in a 5-litre flask). Depending on the number of initial plug inocula used, large amounts of free-floating hyphae will develop quickly. These may then be harvested, washed with several changes of sterile water and resuspended in sterile river water. Zoosporangia will develop and large numbers of zoospores will soon be produced (Alderman and Polglase, 1986; Stueland *et al.*, 2005). An alternative method is to infect sterile hemp seeds by placing them on agar cultures for 2-3 days, placing these in muslin bags and then suspending them in the inflow water of an experimental tank (Pottinger and Day, 1999). This results in a continuous production of zoospores into the inflow water. The time required will depend on species, strain and temperature.

Sexual stages (where present) take time to develop and may need several weeks as agar cultures stale. Old cultures on sterile hemp seeds may also develop sexual stages (Fig. 6.6). The Saprolegniaceae are oogamous, formed by the delimitation from vegetative hyphae of specialized gametangia, the oogonium and antheridium. The contents of the oogonium segment into one or more oospheres. Subsequently, the uninucleate oospheres, devoid of any periplasm, mature into oospores. The species are defined by the morphological details such as origin of the

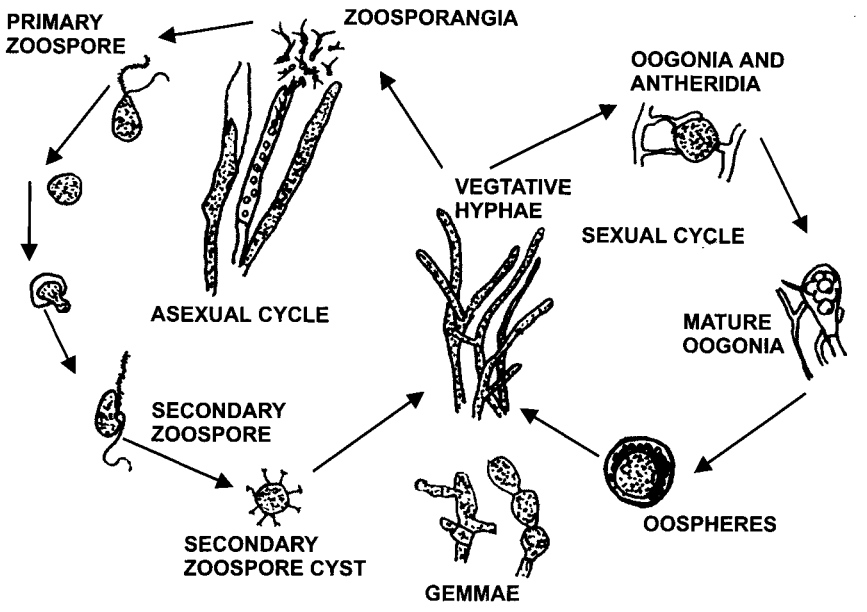


**Fig. 6.6** Generalized oomycete life cycle.

oogonial branches, e.g., lateral, terminal, intercalary or sessile; oogonial shape and nature of the wall, e.g., pitted or unpitted, smooth or ornamented (Johnson *et al.*, 2002). Origin and nature of the antheridial branches, if present, also form part of the species morphological definition. Since sexual structures are absent or suppressed in the fish pathogenic species, no further details of such stages will be considered.

## Histological Methods

A short general comment on application of histological methods to oomycete infections may perhaps best be made at this point. Whilst Eumycete hyphae are generally readily visible using standard histological stains such as haematoxylin and eosin (H&E), oomycete hyphae are much more difficult to differentiate in the tissues. Silver stains such as Grocott methenamine silver (GMS), give a good definition of the infecting hyphae, but the traditional counter stain—light green—gives poor definition of the host tissues. Use of a combination of H&E and GMS stains offers a good definition of both host tissues and Oomycete hyphae and this is recommended for all histological investigation into such lesions (Fig. 6.7).

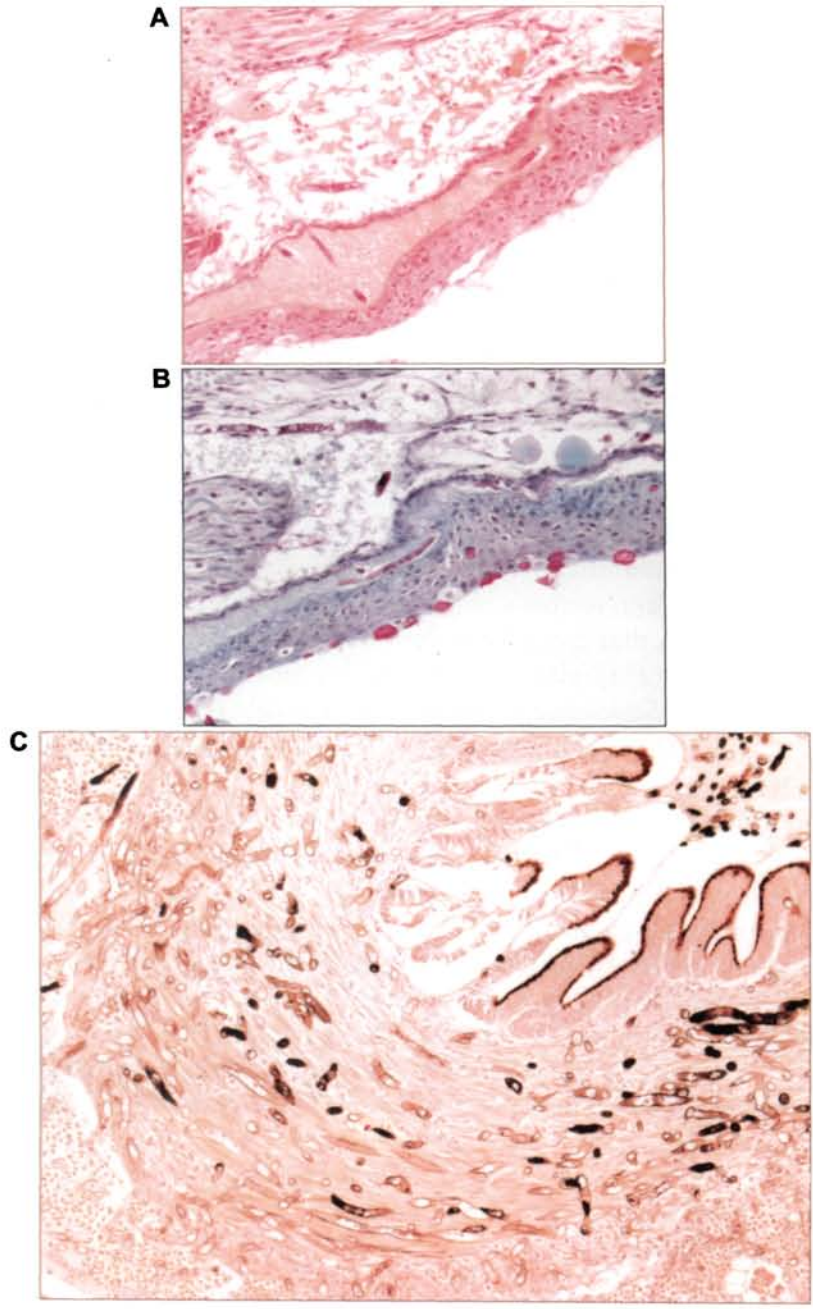


**Fig. 6.7** *Saprolegnia parasitica* in fish tissues. (A) Haematoxylin and eosin, (B) Feulgen, (C) Grocott methenamine silver, haematoxylin and eosin.

## *Saprolegnia*

Examination of the existing literature makes it clear that a range of *Saprolegnia* species has been identified in association with fish and fish eggs. There are a number of conventional taxonomic treatises on the genus, including Coker's original publication (1923), Seymour's discourse on *Saprolegnia* itself (1970) and an extensive treatise on the Saprolegniales published online (Johnson *et al.*, 2002). Although the latter is primarily a taxonomic work, separate review chapters on saprolegniasis in fish and on crayfish plague are included in the treatise. A number of reviews concentrate around the fish disease aspect, including Neish and Hughes (1980), Bruno and Wood (1999) and a symposium volume (Mueller, 1994) on salmon Saprolegniasis. The symposium edited by Mueller (1994) may be thought of as taking place directly due to the loss of malachite green as an antifungal agent resulting in an inability to control saprolegniasis in salmonid culture. There was, thus, a marked increase in interest in *S. parasitica*, both into the somewhat confused nature of its taxonomy and into methods of control. The volume not only contains a range of papers that give an excellent overview of the state of scientific understanding of saprolegniasis in 1992, but also contains an exhaustive bibliography of *Saprolegnia* in salmonids to that date (Hughes *et al.*, 1994).

In salmonids, saprolegniasis is so common, both on fish and especially on eggs in the hatchery that it is relatively rare for a proper identification to be attempted. Also, when it is identified, it is rare to read the details of methods employed to attempt to ensure that the organism isolated is either a primary pathogen or a secondary, but pathogenic invader. A degree of scepticism about the range of *Saprolegnia* species reported from fish lesions perhaps needs to be exercised. This is not to aver that the range of *Saprolegnia* spp. listed in reviews such as Neish and Hughes (1980) and Bruno and Wood (1999) as being isolated from fish lesions have not been correctly identified, but to suggest that in many cases, these may be chance isolations with no relevance to the aetiology of the lesion. Bruno and Wood (1999) have identified reports of 15 different species of *Saprolegnia* from fish. Of the species listed, approximately half are species not accepted as valid species by Johnson *et al.* (1999). There is clearly much confusion on the issue.

## *Saprolegnia parasitica*

### History

As Hughes (1994) pointed out, associations between fishes and Oomycete fungi have been known for many years. Arderon (1748) reported what was clearly an infection in roach, *Rutilus rutilus* and which may well have been the first record of a mycotic disease of any vertebrate animal (Ainsworth, 1976).

The so-called 'salmon disease' devastated salmon stocks in British rivers in the late 1870s and led to the creation of a Royal Commission to enquire into the causes of the disease, being the first to focus attention on these organisms as major pathogens of wild salmonids. The Commission report (Buckland *et al.*, 1880) identified *Saprolegnia* as a major, if not the sole factor, in the disease. For a full discussion of the Royal Commission report see Hughes (1994). Whether the 'salmon disease' of 1880 was the same as that which attracted further attention in the 1970s, namely Ulcerative Dermal Necrosis (UDN), is uncertain, but the precise aetiology of both also remains uncertain (Neish and Hughes, 1980). Whilst probably not the causal agents of the initial UDN skin lesions, it is the subsequent fungal infection of the skin ulcers which leads to the ultimate death of the fish (Fig. 6.8). Both 'salmon disease' and UDN were of interest because of the importance of the salmon sport fish industry with its influential lobbying abilities.

With the development of salmonid aquaculture in the last 50 years, the involvement *Saprolegnia* both in the hatchery and in stressed adult salmonids became recognized as a continuing problem. However, little scientific attention was paid because, as Hughes (1994) pointed out, there was a view (perhaps dating back to the Royal Commission Report) that *Saprolegnia* infections were secondary; the organism was universally present in freshwater and an effective treatment, namely malachite green that had been introduced in 1936 (Foster and Woodbury, 1936), was readily available. To quote Hughes (1994), 'In the days of malachite green, most fisheries biologists thought of saprolegniasis only rarely, if they thought of it at all.'

### Pathology

In fish, Saprolegniosis is primarily seen as a superficial chronic infection, in the form of cotton-wool-like tufts on the skin and gills of infected fish

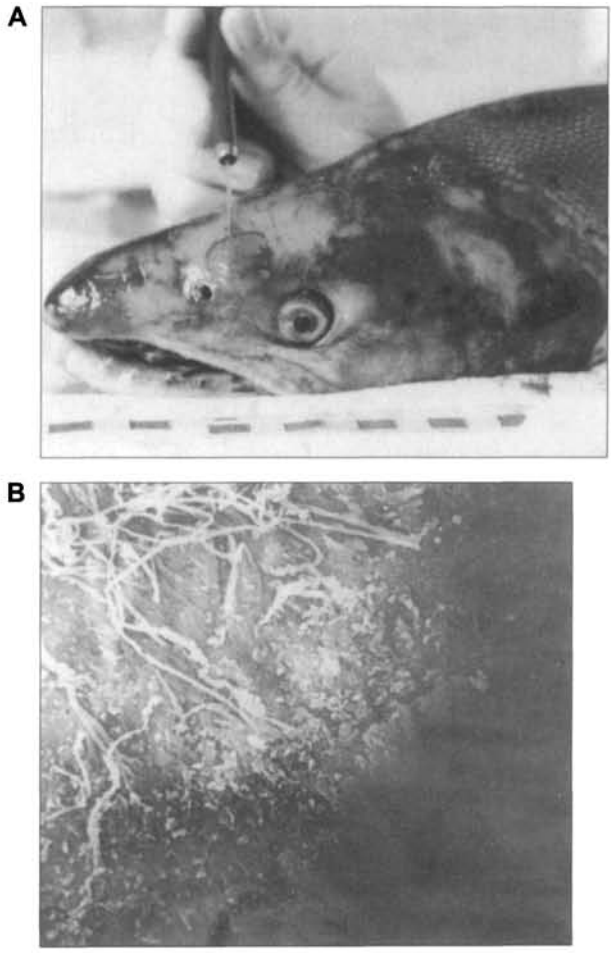


**Fig. 6.8** Ulcerative dermal necrosis (UDN). (A) Lesion, (B) Fungal hyphae on lesion (SEM).

(Fig. 6.9) (Neish and Hughes, 1980). In severe cases, most of the body surface may be affected. Beneath the white hyphal tufts, skin lesions are grey to white in colour, with a characteristic circular shape (Willoughby, 1989). Lesions may develop anywhere on the body surface, but dorsal fins (giving a characteristic saddleback lesion), gills and pectoral fins are frequent primary sites. These are areas particularly susceptible to physical insult. Infections may also develop on lesions caused by concurrent infections by other pathogens (Fig. 6.10) (Neish and Hughes, 1980) or as a result of toxic insult (Figs. 6.11, 6.12).

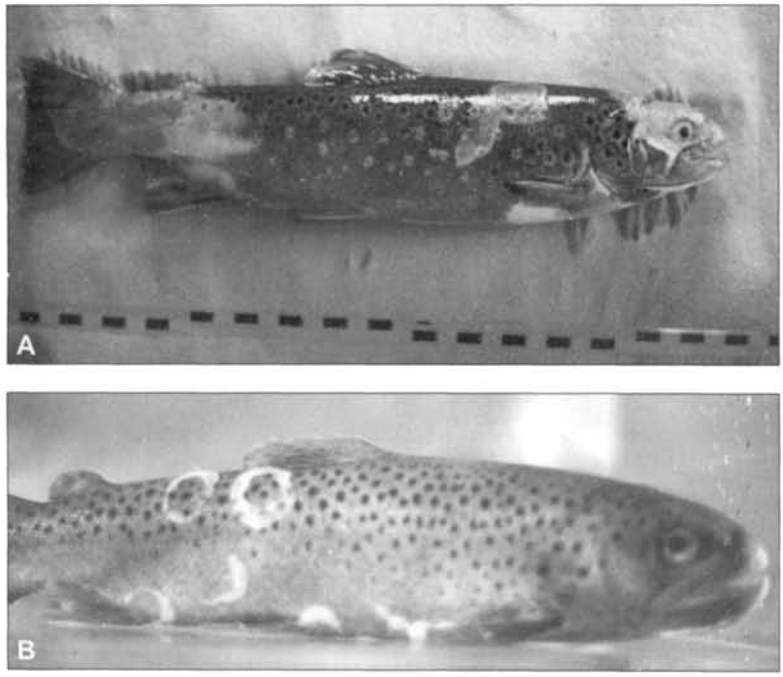


**Fig. 6.9A and B** Classic oomycete lesions on sea trout, *Salmo trutta*.

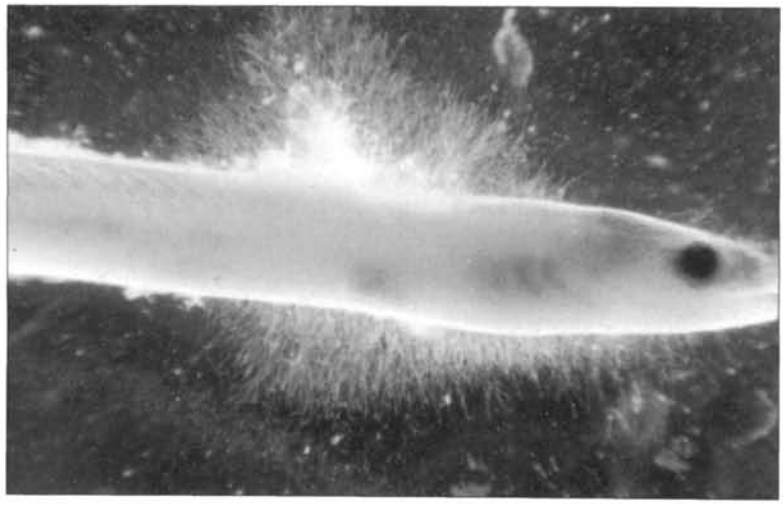

**Fig. 6.10** Secondary oomycete infection of elver.

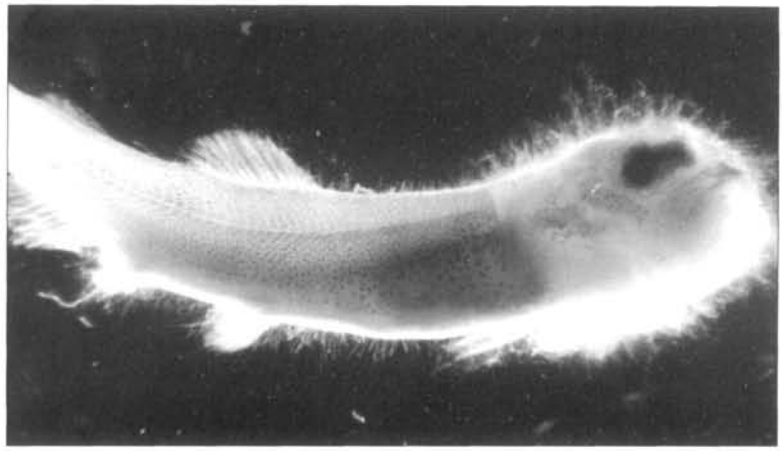

**Fig. 6.11** Rainbow trout fry, Saprolegniaisis.

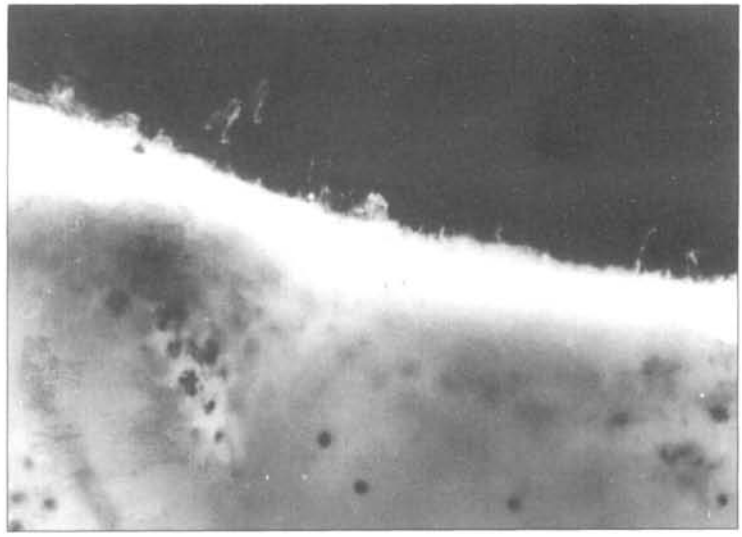

**Fig. 6.12** Rainbow trout fry, generalized *Saprolegnia* infection, secondary to ingestion of toxic substance. The entire body surface is covered by hyphae which originate from gut necrosis. Infestation is with *Saprolegnia diclina* not *S. parasitica*.

In salmonids, during sexual maturity, the skin of male fish appears to be particularly susceptible (White, 1975; Richards and Pickering, 1978), possibly due to differences in the number of goblet cells in the skin of male and female fish (McKay, 1967; Neish, 1977). An increase in corticosteroid

levels in brown trout from 1-4 ng $ml^{-1}$ to 9-10 ng $ml^{-1}$ resulted in a marked increase in the susceptibility of the fish to *Saprolegnia* infection (Pickering and Pottinger, 1985). In nature, increases in cortisol and sex hormones associated with sexual maturity may, thus, increase susceptibility to saprolegniasis (Pickering, 1977). Treatments with a chemosterilant during the later stages of spermatogenesis reduced the prevalence of saprolegniasis in brown trout parr (Pottinger and Pickering, 1985) and in precocious male 1+ Atlantic salmon parr (Murphy, 1981).

Overall, the view has been established that healthy salmonids with intact integument are largely refractory to infection even by aggressive strains of *S. parasitica* and infection. Even with high zoospore challenge under laboratory conditions, it is required that fish be treated in some way in order to render the skin vulnerable, either by physical or chemical stresses.

Once established, such lesions may develop rapidly. Animals become lethargic, losing their equilibrium. Although the lesions are visually dramatic, animals may survive for some time in this lethargic state. It is generally believed that the cause of death is likely to be associated with impaired osmoregulation due to loss of epithelial integrity (Gardner, 1974; Hargens and Perez, 1975). This is reflected in reduction of major ions in serum and hypoproteinemia (Neish and Hughes 1980; Duran *et al.*, 1987; Hatai *et al.*, 1990). Elevated serum enzymes, including lactate dehydrogenase, glutamate-oxyalate transaminase, glutamate-pyruvate transferase, creatine phosphokinase, alkaline phosphatase and acid phosphatase, have been reported in association with saprolegniasis in brown trout (Duran *et al.*, 1987). When the gills are involved, respiratory difficulties may also make a significant contribution (Bruno and Stamps, 1987).

Although *S. parasitica* infections are largely superficial, case reports have been published involving the gut epithelium in brook trout, *Salvelinus fontinalis* (Agersborg, 1933), rainbow trout fingerlings (Davis and Lazar, 1941) and the alimentary tracts of rainbow trout and amago salmon, *Oncorhynchus rhodurus* (Hatai and Egusa, 1977; Miyazaki *et al.*, 1977). In the first two cases, *S. ferax*, and in the second two, *S. diclina*, were identified, which strongly suggests that these were not primary fungal infections. As mentioned above, one such primary cause may be toxic insult.

## Taxonomic Status

The general confusion regarding *Saprolegnia* continues with the definition of *Saprolegnia parasitica* as a species. This has resulted in considerable uncertainty ever since the name was first used by Coker for one of the four new species of *Saprolegnia* in his 1923 monograph of the Saprolegniaceae (Coker, 1923). Coker's new species was described from isolates that had been growing on fish, although the species of fish are not listed. The full description of the species given by Coker is repeated by Hughes (1994) who points out that one of the critical points in the description was 'Sexual reproduction not observed so far in our form and very rarely observed by others (Huxley)'. This flew in the face of conventional taxonomic rules by describing a species 'primarily on the basis of structures or features *it does not have* is quite simply bad taxonomy' (Hughes, 1994). In terms of conventional taxonomy, this may be true, but in the light of recent advances in understanding, it seems clear that Coker was correct.

Neish and Hughes (1980) and Hughes (1994) provide good historical descriptions of Saprolegniasis and of the different interpretations of *Saprolegnia parasitica*, so these aspects are not considered in detail here. However, some background is needed to place the current situation in context. Coker's original description (1923) was amended by Kanouse (1932) from isolates from various fishes and fish eggs. Through manipulations of culture conditions, Kanouse reported having obtained the oogonia and antheridia in *S. parasitica* that were needed for a conventional species description in the Saprolegniaceae. Kanouse (1932), therefore, formally amended the description of *S. parasitica* Coker to incorporate features of the sexual cycle into the formal description of the species. From that point forward, only isolates with those sexual characteristics should—by conventional morphologically based taxonomy—have been referred to as *Saprolegnia parasitica*. There is, however, no evidence that Kanouse was able to make a proper comparison with Coker's original material. In contrast, a few years later, Tiffney (1939a), reported studies of the ability of a sub-culture of Coker's type of *S. parasitica* to infect fish and amphibians. Clearly, Kanouse could have, but apparently did not obtain Coker's original strain. The relationship between *S. parasitica* sensu Kanouse and the original *S. parasitica* sensu Coke is, therefore, uncertain.

The mycotaxonomists' view from Kanouse onwards would be that *S. parasitica* should, henceforth, only be used for isolates for which sexual

stages were present, without any real thought as to what might be done with the preponderance of asexual isolates. Thus, Tiffney (1939b) reported that in 95% of fish his isolates could not be identified by oogonial characters. He concluded his discussion of these by pragmatically suggesting that it would be best to consider *Saprolegnia parasitica* a species of convenience until more data was available. Fish pathologists tended to follow this approach and numerous authors continued to apply the name *S. parasitica* in the original 'Coker' sense, despite some 'scolding' from mycologists (O'Bier, 1960; Scott and O'Bier 1962). Scott and O'Bier (1962) commented, 'It is difficult to understand the persistence of such an error in the literature and the perpetuation of it by well-informed workers', and proposed that a desirable temporary solution would be to place in *S. parasitica* only those isolates that exhibited sexuality *and* possessed the diagnostic features of the taxon. This again ignored the reality of isolates being cultured from infected fish. To refer to such isolates as *Saprolegnia* sp. would have been equally unsatisfactory.

Neish (1976) proposed the rejection of *Saprolegnia parasitica* Coker *as* a *nomen ambiguum* (Article 69 of the International Code of Botanical Nomenclature). He also suggested that all *S. parasitica* isolates with sexual stages could be identified as *Saprolegnia diclina* and that perhaps a valid concept could be that a *Saprolegnia diclina–Saprolegnia parasitica* complex existed with the asexual '*S. parasitica*' strains lying at one extreme. The possibility that Kanouse might have been looking at *S. diclina* and not *S. parasitica* does not seem to have been considered. This view attained some acceptance and was used in a range of publications (Pickering *et al.*, 1979; Beakes, 1983; Wood and Willoughby, 1986; Wood *et al.*, 1988; Cross and Willoughby, 1989). Even so, the problem of the identification of asexual fish isolates remained and came back into prominence in Hatai and Willoughby (1988) who effectively realized that a return to Coker's original concept was needed. 'Isolates were made from six separate lesions and in sub-culture the fungi were all identified as *Saprolegnia*, but no oogonia were obtained. The isolates were confirmed as being pathogenic to fish and were, therefore, regarded as *S. parasitica* Coker (1923).'

Particularly at the ultrastructural level, morphological studies have made it possible to identify different subgroups amongst *Saprolegnia* isolates. In an ultrastructural study of secondary zoospore cyst morphology, Beakes (1983) was able to identify *S. parasitica* from *S. diclina* whilst Fregeneda-Grandes *et al.* (2000) distinguished the fish lesion

isolates from water isolates. Physiological variations such as those of esterase isoenzyme patterns (Beakes and Ford, 1983) and differences in radial growth rate (Willoughby and Copland, 1984; Hatai *et al.*, 1990) have also been used to identify distinct groups of fish lesion isolates.

The development of molecular approaches to taxonomy has begun to clarify matters further. For a start, they have provided the evidence that the Oomycetes are Chromistans and we now better understand the major relationships/lack of relationship between the groups that were once regarded as the Fungi, *sensu lato*. At the species level, the concept of a *Saprolegnia parasitica–Saprolegnia diclina* complex is now discarded and recent work in Scandinavia throws considerable light on the matter of *S. parasitica, sensu* Coker.

Although the *Saprolegnia* in salmonid symposium (Mueller, 1994) took place before the full development of molecular methods, a project related to that symposium produced some of the first applications molecular methods to the problem. Studies conducted by Whisler (1996) have been published electronically by the funding body (Bonneville Power Commission) and have attracted little scientific notice. Of the data presented in Whisler (1996), two stand out. Studying *Saprolegnia* isolates from the Columbia River basin by means of RAPD-PCR (random amplification of polymorphic DNA polymerase chain reaction), he was able to distinguish *S. parasitica* from other *Saprolegnia* species such as *S. diclina, S. ferax* and *S. hypogyna*. A number of populations of *S. parasitica* were identified in the Columbia River basin using this method

Whisler (1996) also reported on pathogenicity studies conducted with *S. parasitica* on adult Chinook salmon. In fish held after a social stress (overcrowding) challenge study, one died and left unobserved became eventually a mycelial mat in which oogonia were found. Whisler (1996) suggested that whilst fresh isolates on artificial media may not yield sexual stages, long incubation on natural substrates may allow the development of sexual stages in *Saprolegnia parasitica*. Whilst Whisler clearly believed this and there is logic in the argument that when natural substrates become exhausted a shift to sexual reproduction might be an appropriate strategy for an organism of this type, it is impossible to exclude contamination. A dead fish will be infested by a huge range of opportunist organisms, including saprophytic *Saprolegnia* that may well produce sexual stages, even if *S. parasitica* is also recoverable from the lesion. The significance of Whisler's report that sexual stages were relatively common

in *S. parasitica* in the Columbia River area compared with results from elsewhere is, therefore, unclear.

Restriction fragment length polymorphisms (RFLPs) have been found to be extremely useful for classification of *Saprolegnia* and can distinguish *S. parasitica* from *S. diclina*. Molina *et al.* (1995) stated 'Correlation of the rDNA data with morphological and ultrastructural features showed that *S. diclina* and *S. parasitica* are not conspecific'. RAPD-PCR was also applied for analysis of the fish pathogenic *Saprolegnia* genome by Diéguez-Uribeondo *et al.* (1996), who demonstrated a group of closely related strains of *S. parasitica* isolated from infected trout in Spain, whilst Bangyeekhun *et al.* (2001) showed the presence of three genetically distinct groups of catfish isolates of *Saprolegnia* sp. in Mississippi.

Developing this approach further, Bangyeekhun *et al.* (2003) investigated 31 isolates of *S. parasitica*, mostly originating from infected salmon or trout, using a combination of RAPD-PCR technique and the presence or absence of repeated zoospore emergence to characterize *Saprolegnia* sp. isolates. Representatives of the previously studied Spanish and Mississippi catfish strains were included. Twenty-six of these isolates originated from Scandinavia (Finland and Sweden), but others, including reference cultures were from the U.K., Spain and the U.S.A. (from catfish) and from a crayfish in Turkey. Some of these strains had been previously been identified as *S. parasitica*. All were shown by RAPD-PCR to be genetically near identical. The authors suggest that these isolates represent a virulent form of the organism that has been widely spread by clonal propagation. In addition to the 26 extremely closely related strains, the Spanish and catfish strains and a U.K. trout reference isolate also grouped with the Scandinavian strains, although less closely (less than 80%).

The widespread distribution of this genotype in Scandinavia combined with the fact that isolates from Turkey, the U.K. and the U.S.A. are also very similar, led the authors to suggest that this represents a stable asexual clone which is well adapted to infecting fish. The range of isolates from outside of Scandinavia included in the study were too limited to draw conclusions more widely, but it seems possible that this clone causes the majority of infections of fish in aquaculture. Of the 31 isolates examined by Bangyeekhun *et al.* (2003), none of the clone isolates formed sexual stages in culture, so that conventional morphological speciation was not possible. None of the Scandinavian isolates was characterized by repeated

zoospore emergence, but the less-related Spanish and Mississippi catfish strains were certainly so.

The results of this range studies support the view that *S. parasitica* sensu Coker should be regarded as a valid species particularly adapted to parasitism of salmonids, despite the lack of sexual stages. Indeed, the views expressed by Neish (1976) and Hughes (1994) that a valid *Saprolegnia* species could not be defined unless the requisite sexual stages could be found have been shown to be untenable. *S. parasitica* is clearly a valid taxon.

Although other species of *Saprolegnia*, particularly *S. diclina*, are reported from fish, they do not seem to be particularly aggressive and are best regarded as secondary opportunist perthotrophs. Indeed, in his challenge studies, Whisler (1999) recorded chance 'non-threatening' lesions were detected in his fish stocks and that such lesions normally regressed and healed, unlike those associated with *S. parasitica*. He found that the species involved in such lesions were *S. diclina* and *S. ferax*. Many authors have noted that the sexual *S. diclina* is morphologically similar to *S. parasitica* and have regarded *Saprolegnia parasitica* as an asexual form of *S. diclina*, referring to it as *S. diclina* Type I (Fregeneda-Grandes *et al.*, 2000). Whilst *Saprolegnia parasitica* might perhaps have originated from a strain of *S. diclina* in the past, molecular studies by Molina *et al.* (1995) have determined a clear separation between the two. Bangyeekhun *et al.* (2003) suggest that the second clade identified in their study (Groups 2 and 3) may represent *S. diclina*. *S. diclina* is, therefore, perhaps best regarded as one of the more frequent opportunists, but not a primary pathogen.

Two characters of *S. parasitica*, as currently understood, have attracted considerable attention. They are the ultrastructure of the wall of the cyst of the secondary zoospore and the ability for repeated zoospore emergence. Secondary zoospore cysts of *S. parasitica* bear hooked hairs on their surfaces (Figs. 6.13-6.15). In particular, Pickering *et al.* (1979) found that the walls of secondary spore cysts of *Saprolegnia* spp. isolated from fish have groups or bundles of long, hooked hairs, whereas saprophytic *Saprolegnia* isolates produced secondary cysts with short, single, hooked hairs on their surfaces. Other authors confirmed the same and it is now regarded as one of the salient characters of fish pathogenic *Saprolegnia*, i.e., of *S. parasitica*. Presence of long, hooked hairs has been speculated to be advantageous to fish parasites (Beakes *et al.*, 1994) in the sense that

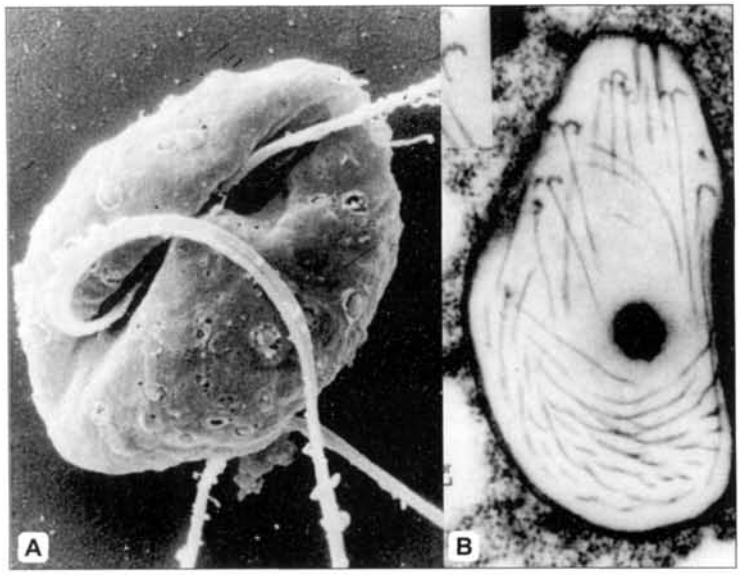


**Fig. 6.13** (A) *Saprolegnia parasitica*, secondary zoospore (SEM), (B) TEM section through secondary zoospore showing hooked hairs within zoospore prior to discharge at encystment (G. Beakes).

they could assist the spore in attaching to the fish surface before it is able to germinate. Stueland *et al.* (2005) examined 17 strains of *Saprolegnia* spp. for morphological and physiological characteristics, and 7 were examined for their pathogenicity to Atlantic salmon. The authors documented considerable variation in pathogenicity to Atlantic salmon parr between the different strains. Their key finding was that a high initial hyphal growth rate of germinating spore cysts, together with the presence of long hooked hairs on the cyst walls, were consistent characteristics of fish pathogenic strains.

The ability of an encysted secondary spore to release a new zoospore generation instead of germinating is termed 'repeated zoospore emergence'. In *Aphanomyces astaci*, Cerenius and Söderhäll (1985) demonstrated repeated zoospore emergence and proposed this to be an adaptation to a parasitic mode of life. The majority of isolates of *Saprolegnia parasitica* in the study of Bangyeekhun *et al.* (2003) and in the trout isolates of *Saprolegnia parasitica* as reported by Diéguez-Uribeondo *et al.* (1996) did not exhibit repeated zoospore emergence.

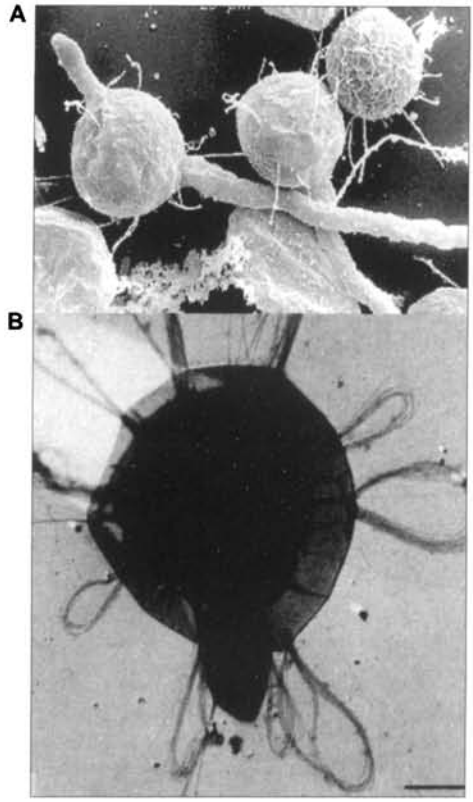


**Fig. 6.14** (A) *Saprolegnia parasitica* secondary zoospore cysts germinating, hooked hairs visible on walls. (B) *Saprolegnia parasitica* encysted secondary zoospore showing long hooked hairs on wall (G. Beakes).

Summarizing, therefore, it has become clear within the previous decade that the view taken by Coker in 1923 has been proved to be correct. *Saprolegnia parasitica* does exist as a definable species, but one that does not fit in the conventional morphological boundaries. It probably exists in the form of a range of strains or clones in different parts of the world. Thus, Diéguez-Uribeondo *et al.* (1996) demonstrated a group of closely related strains of Spanish *S. parasitica* isolated from infected trout, Whisler reported at least 3 populations of *S. parasitica* strains on Pacific salmon within the Columbia River basin and Bangyeekhun *et al.* (2001) showed the presence of 3 genetically distinct groups of catfish isolates of *Saprolegnia* sp. in Mississippi. Bangyeekhun *et al.* (2003), by RAPD

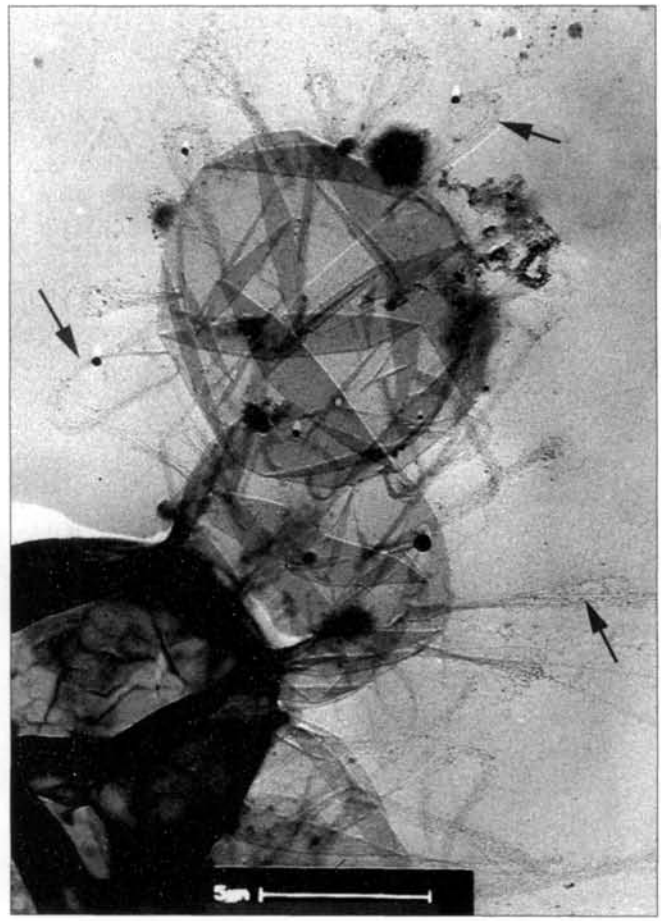

**Fig. 6.15** *Saprolegnia parasitica* secondary zoospore cyst walls, showing long hooked hairs (arrowed) (G. Beakes)..

analysis, showed that the genetic similarities between Finnish and Swedish isolates and Spanish and American isolates was less than 80% and that the genotype variation among *Saprolegnia* sp. isolates in their study suggested a degree of genetic dissimilarity between fish pathogenic *Saprolegnia* from different geographic locations.

Since sexual reproduction is rare at best, such genetic diversity is unsurprising; it is the extent of the similarities that is perhaps more of a surprise. However, here, we must accept the fact that the rapid spread of

aquaculture and sport fishing in a period of little more than 150 years is based on a few salmonid species that have been, and continue to be, transferred around the world. During this time, no practical attempts were made to prevent the spread of an effective pathogen such as *Saprolegnia parasitica* on fish, fish eggs and fishing and aquacultural equipment. Therefore, the spread of a series of specialized and virulent fish pathogenic strains of *Saprolegnia parasitica* is not surprising and, indeed, was probably inevitable.

## Challenge Methods

Infection of healthy salmonids held under good conditions, even with freshly isolated and apparently virulent *S. parasitica*, is recognized to be difficult to achieve with any consistency. Apart from satisfying Koch's postulates to confirm that *S. parasitica* is pathogenic to fish, in order to show that potential fungicides have an effect *in vivo*, reliable challenge methods are needed. Two methods have become standard, the ami-momi method of Hatai and Hoshiai (1993) and the cortisol stress method of Pottinger and Day (1999). Both these methods depend on overcoming the innate resistance of salmonids to infection, the ami-momi by producing standardized skin damage, the cortisol method by inducing an extended period of stress.

The ami-momi method consists of capturing a standard number of fish in a standard net and shaking them in the air for 2 minutes (Hatai and Hoshiai, 1993) before exposing them to a static bath of zoospores. Differences in net type and fish size will obviously have an effect, but the method does allow standardization between sites and studies. Pottinger and Day (1999) induced prolonged stress to produce a reproducible challenge method by injecting fish intraperitoneally with 20 mg hydrocortisone in coconut oil. The coconut oil acts as a depot for the cortisol. Fish are then exposed to *S. parasitica* zoospores from groups of *S. parasitica* infected hemp seeds held in the inflow water (Figs. 6.16-6.18).

Variants of the ami-momi method have been used in a number of studies (Hatai *et al.*, 1990; Whisler, 1994; Howe and Stehly, 1998) as has the cortisol stress method. Figure 6.19 shows the results of one cortisol stress *S. parasitica* challenge study.

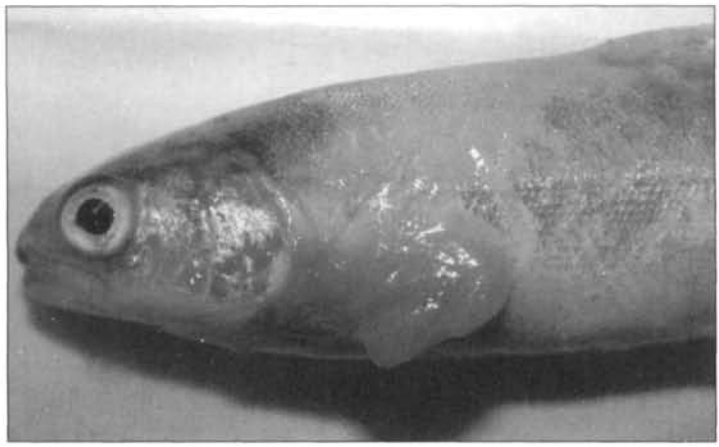

**Fig. 6.16** Rainbow trout Saprolegniasis, cortisol challenge.

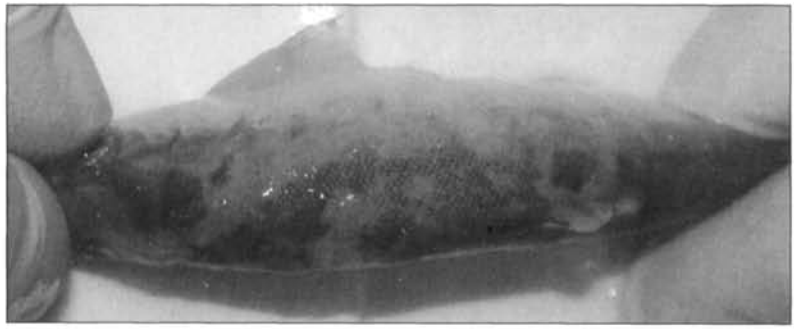

**Fig. 6.17** Rainbow trout Saprolegniasis, cortisol challenge.

## *Aphanomyces invadans*

### History

The disease syndrome, which is formally designated 'Epizootic Ulcerative Syndrome' (EUS) (FAO, 1986), has been associated with major fish mortalities in many countries for more than 30 years. It took most of this time before investigators researching the mortalities occurring in different fish species in separate parts of the world arrived at the conclusion that this might be a single disease and, therefore, a range of different names had been used to describe the syndrome.

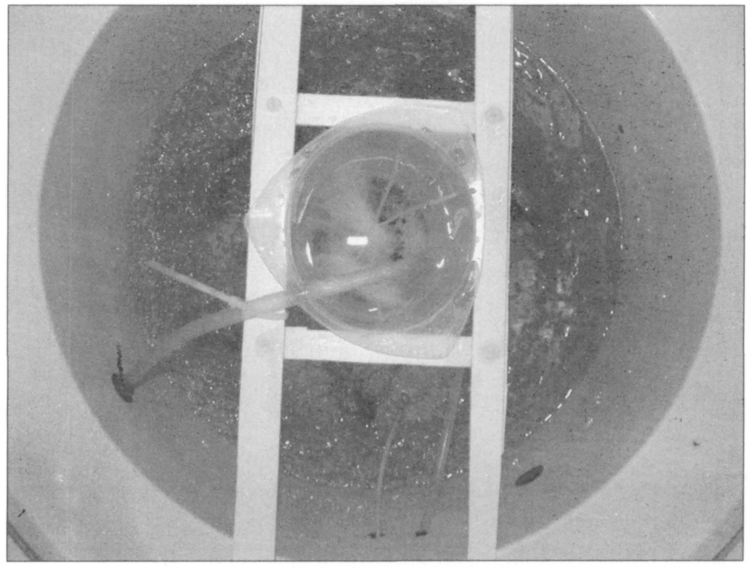

**Fig. 6.18** Rainbow trout Saprolegniasis, cortisol challenge.

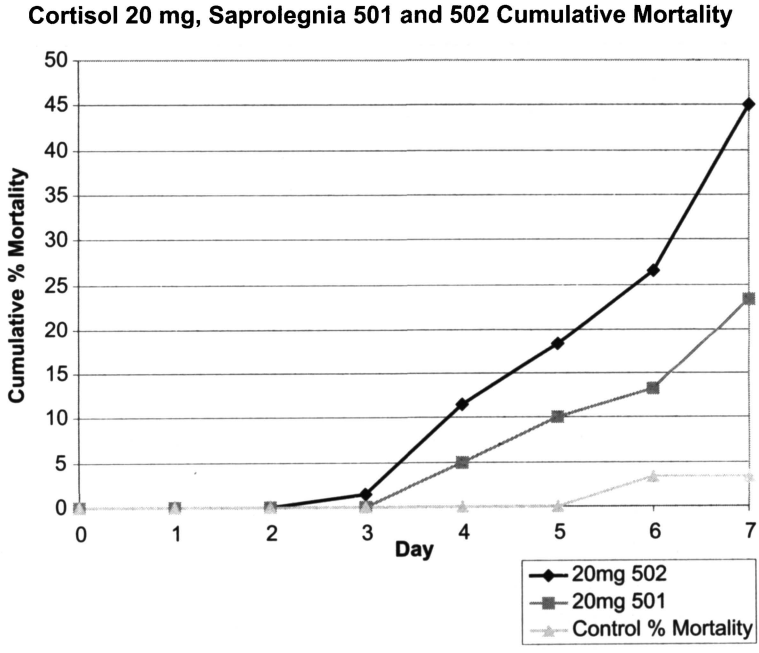


**Fig. 6.19** Rainbow trout Saprolegniasis, cortisol challenge results.

The first fish mortalities that can be linked to EUS were in Japan in 1971, where an outbreak in ayu (*Plecoglossus altivelis*) was recognized to be a fungal disease, probably an *Aphanomyces* infection (Egusa and Masuda, 1971). Similar infections were found in other species and histological examination led to the recognition of fungal aetiology and the syndrome name Mycotic Granulomatosis (MG) was coined (Miyazaki and Egusa, 1972, 1973a, b). Unfortunately, these investigations did not attract any widespread recognition since they were published in Japanese. In Australia, a similar epizootic cutaneous ulcerative syndrome in estuarine mullet *Mugil cephalus* was named Red Spot Disease (RSD) (McKenzie and Hall, 1976). Similar conditions with dermal ulcerations with large-scale mortalities have occurred throughout Southeast and South Asia. Particularly susceptible species include the striped snakehead *Channa striata*, silver barb *Barbodes gonionotus* and swamp eel *Monopterus albus*, (Tonguthai, 1985; Bhaumik *et al.*, 1991; Vishwanath *et al.*, 1997). Some species are susceptible in the colder parts of their range, e.g., rohu *Labeo rohita*. The syndrome was given its present name in 1986 at the Consultation of Experts on Ulcerative Fish Diseases in Bangkok (FAO, 1986). Evidence has been accumulated showing the spread of the disease over a short period of time across S.E. Asia (Fig. 6.20). In the United States, similar ulcerative lesions, designated ulcerative mycosis (UM) (Noga and Dykstra, 1986) have occurred in estuarine fish along the east coast since at least 1978 (Blazer, 2002).

Over time, as understanding of EUS has developed, a number of reviews have been published including Roberts *et al.* (1993), Chinabut (1995), Roberts (1997), Chinabut (1998), Lilley *et al.* (1998) for EUS itself. Egusa (1992) summarized the situation with MG in Japan, while Noga (1993) assessed the findings about UM to the early 1990s. In the light of this information, it became generally accepted that EUS (*sensu lato*) is characterized by the presence of ulcerative, dermal lesions in which invasive fungal hyphae have elicited a granulomatous response. It was also generally accepted that, despite extensive investigations in many affected countries, no naturally occurring, epidemiologically similar, but pathologically distinct cutaneous ulcerative syndrome had been found (Baldock *et al.*, 2005).

However, there remained two key areas of dispute among scientists, both relating to pathogenesis of the dermal ulcers. These differences were first brought into focus by the formal definition in 1994 of EUS as

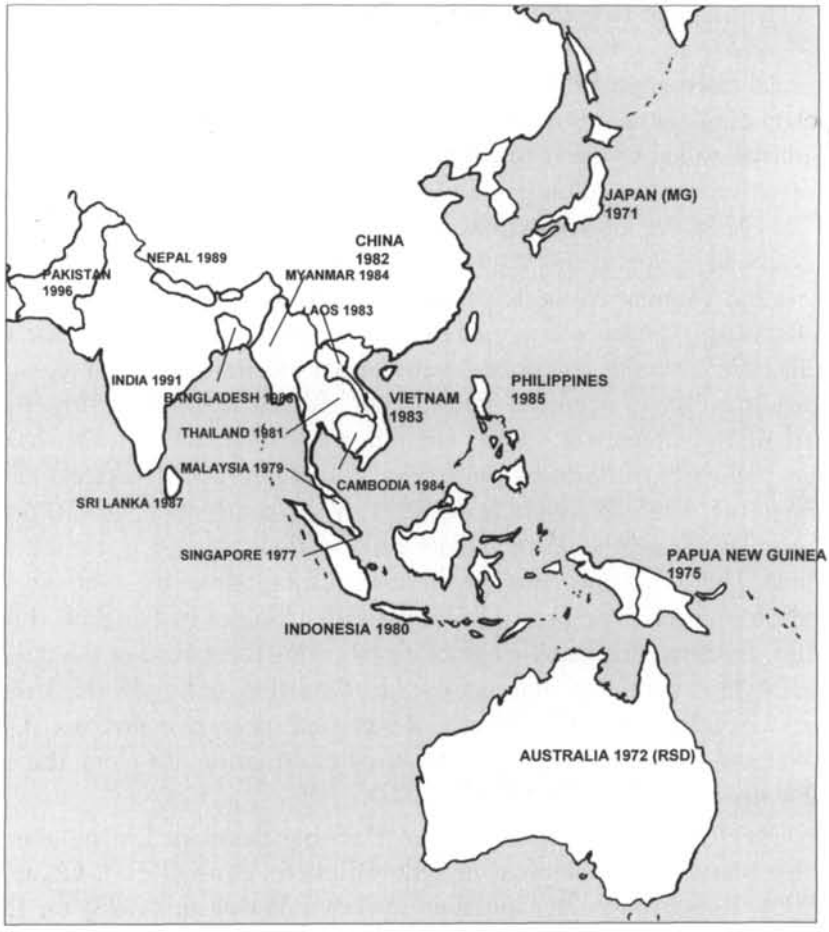


**Fig. 6.20** Spread of EUS in Asia Pacific (redrawn after Lilley, 1997).

'a seasonal epizootic condition of freshwater and estuarine warm water fish of complex infectious aetiology characterized by the presence of invasive *Aphanomyces* infection and necrotizing ulcerative lesions typically leading to a granulomatous response' (Roberts *et al.*, 1994). Some scientists propose that a number of invasive fungal species, not necessarily including *Aphanomyces invadans* in all the cases, are involved in ulcer formation; others believe that only *A. invadans* is consistently present and responsible for the observed tissue destruction. Also disputed is whether or not various viruses or bacteria recovered from EUS cases have essential or

merely opportunist roles in ulcer formation (Lio-Po, 1992, 2001; Baldock *et al.*, 2005).

An overview of the arguments in regard to *Aphanomyces invadans* or polymicrobial infection aetiologies for EUS as outlined by proponents of both has been published recently (Baldock *et al.*, 2005). In summary, the range of reasons for these conflicting views include:

- The difficulty of isolating *Aphanomyces invadans* from infected fish.
- The difficulty of inducing the clinical disease with *Aphanomyces invadans* except by injection of spores.
- The presence of other organisms in infected animals.
- A pre-established attitude that water moulds are not aggressive pathogens.

The presence of many opportunistic organisms in the open ulcers on affected fish complicated attempts to isolate a primary infectious agent. All too often, investigators seem to have allowed themselves to lose focus and be distracted by these difficulties. Contributing to this, until recently, has been a lack of both molecular diagnostic techniques and a readily reproducible model for experimental infection. The situation is not helped when some authors do not give a clear definition of what they believe to be EUS in their studies, since not all ulcers are EUS, while other authors failed to include histopathological data, until recently essential for confirmatory diagnosis of EUS (Lilley *et al.*, 1998; OIE, 2006).

None of the above reasons would be unfamiliar to researchers into saprolegniosis or, even less, for that matter, into crayfish plague. Similar reasons were raised repeatedly by those arguing against a primary 'fungal' aetiology for both of these diseases.

Ulcerative mycosis (UM) was first recognized as a specific syndrome in estuaries in North Carolina in 1984. Since then, occurrences have been recognized from Long Island in the north to Florida in the south (Hargis, 1985; Noga, 1993; Blazer *et al.*, 1999). The characteristic deeply penetrating ulcers on Atlantic menhaden *Brevoortia tyrannus* and other estuarine species have, however, been observed in US coastal waters since 1978 and possibly earlier (Sindermann, 1988). The prevalence in young-of-year Atlantic menhaden can be as high as 90% (Arhenholz *et al.*, 1987). *Aphanomyces*-like fungi were observed in lesions and isolates of *Aphanomyces* spp. were obtained from these lesions. These isolates were, however, not *Aphanomyces invadans*, but were opportunist secondary

invaders of the lesions. As is normal under such circumstances, the opportunists outgrew and displaced the pathogen in isolation attempts.

In the early 1990s, attention was diverted by suggestions that the UM lesions on menhaden were associated with exposure to *Pfiesteria piscicida*, a toxin-producing, bloom-forming, heterotrophic dinoflagellate (Burkholder *et al.*, 1992, 1995; Noga *et al.*, 1996). Burkholder and Glasgow (1997) proposed that *Pfiesteria* toxin or toxins were causes of the lesions, and that *Aphanomyces invadans* was simply an opportunistic invader colonizing these lesions. Continuing failure to isolate *Aphanomyces invadans* and the presence of easily isolated opportunist *Aphanomyces* spp. made it more difficult to refute these theories. Laboratory challenges with *P. piscicida*, however, equally failed to produce the deeply penetrating granulomatous ulcers commonly observed in wild Atlantic menhaden (Noga *et al.*, 1996; Vogelbein *et al.*, 2001). With successful isolation of *Aphanomyces invadans*, laboratory challenges using isolates of *A. invadans* in the absence of *P. piscicida* have now confirmed not only that *P. piscicida* is not the primary agent of UM (Blazer *et al.*, 2002; Kiryu *et al.*, 2002), but that it is not involved in the disease.

## Taxonomic Status

The oomycete, now known as *Aphanomyces invadans*, was first described by Hatai (1980), as *A. pisćicida* from isolates recovered from ayu. Hatai (1980) described this new species, *Aphanomyces piscicida*. Based on the characteristics of the asexual reproductive stages of isolates, no oogonia were observed. *Aphanomyces piscicida* was differentiated from *Aphanomyces* spp. from other aquatic organisms (i.e., *A. laevis* from rainbow trout, *Aphanomyces* sp. of tropical fishes and *A. astaci* of crayfish). Subsequent studies on the disease yielded isolates from dwarf gourami and ornamental fish (Hatai *et al.*, 1994; Rha *et al.*, 1996; Hanjanvanit *et al.*, 1997) that were, in each case, morphologically and culturally consistent with *A. piscicida*. Unfortunately, Hatai (1980) did not include the description in Latin nor did he designate a type specimen, as is obligatory for a valid description of a new fungal species under the International Code for Botanical Nomenclature (ICBN).

One has to assume that when Willoughby *et al.* (1995) described a new species, *Aphanomyces invaderis* as the causative agent for EUS in Southeast Asia, they were unclear about any relationship to *A. piscicida* described 15 years earlier by Hatai (1980). Otherwise, it would have been

more appropriate for them to have validated Hatai's earlier description by providing the missing Latin diagnosis and type specimen. Instead, partly because the taxonomic description by Willoughby *et al.* (1995) did include a Latin diagnosis, when Lilley *et al.* (1997) demonstrated conclusively by RAPD-PCR that *Aphanomyces invadans* and *Aphanomyces piscicida* were indeed conspecific, *Aphanomyces invaderis* became the only valid binomial for the species. Unfortunately, the species name that Willoughby *et al.* (1995) chose also fell foul of the ICBN because it was ungrammatical and has subsequently been changed, as provided under the ICBN, to *A. invadans* (David and Kirk, 1997) (Fig. 6.21). Japanese workers have, however, tended to continue to use *A. piscicida*, which has led to some regrettable and continuing confusion in the existing literature.

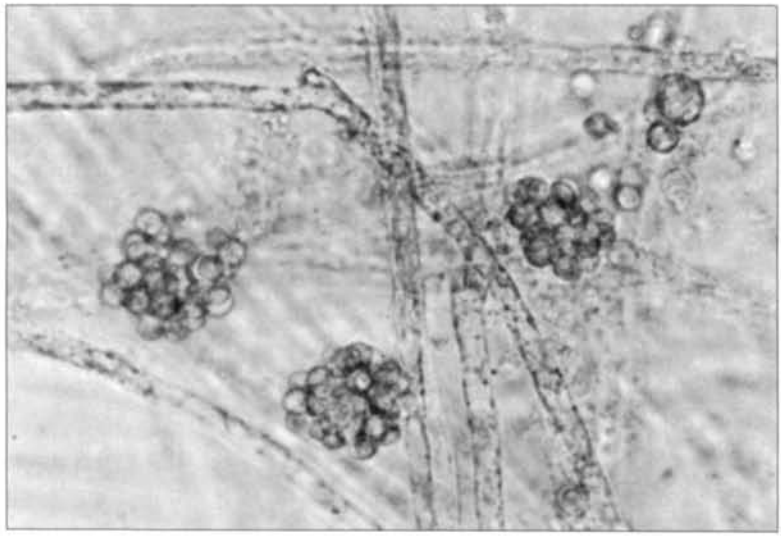

**Fig. 6.21** *Aphanomyces invadans*, encysted primary spores in fresh smear from lesion on infected snakehead (J. Lilley).

Unsurprisingly, a considerable number of investigators have applied molecular methods to the problem of identifying *A. invadans*. Over a period of ten years, these investigations have markedly improved our understanding of *A. invadans* that has developed (Lilley *et al.*, 1997; Blazer, 2002) following studies on EUS and MG isolates to which, more recently, UM isolates have been added. *A. invadans* has now been investigated using a full range of molecular methods, including RAPD-PCR, RFLP,

FISH plus studies on physiology and growth of isolates to give an overall picture of the pathogen.

Lilley *et al.* (1997) used random amplification of polymorphic DNA (RAPD)-PCR to investigate 20 isolates of *A. invadans* from cases of MG and EUS across Southeast Asia. These isolates were compared by RFLP and (RAPD)-PCR. Also included in the study were six saprophytic *Aphanomyces* species from EUS-affected areas, four isolates of the crayfish plague fungus *Aphanomyces astaci*, and two *Aphanomyces* spp. isolates from fish affected by UM on the east coast of the U.S.A. The latter, however, were not the UM pathogen, since it had not yet been isolated from UM lesions at that time.

In a follow-up study, Lilley *et al.* (2003) used RFLP analyses, RAPD and rDNA sequencing to compare 30 isolates of *A. invadans* from across Southeast Asia, Australia and Japan with related taxa. Twenty non-pathogenic *Aphanomyces* (including again two non-pathogenic *Aphanomyces* sp. isolates from the U.S.A.) were included in the study, along with two *Achlya* and two *Saprolegnia*. They were able to confirm that all the Asian *A. invadans* isolates did indeed represent a single fish-pathogenic species, which could readily be differentiated from other species by RFLP analyses of rDNA, sequencing the ITS1 region, and by RAPD analysis. Phadee *et al.* (2004) further developed the species-specific PCR to the point at which *A. invadans* could be reliably detected in tissue samples from EUS lesions and Vandersea *et al.* (2006) introduced an IFAT, which they suggested, might have value for screening environmental samples for sources of *Aphanomyces invadans*.

Lilley *et al.* (1997) reported having felt that their *Aphanomyces invadans* isolates were not only conspecific but that they probably constituted a single clonal genotype. The Lilley *et al.* (2003) results again indicated an extreme lack of genetic diversity between the Asian *A. invadans* isolates from across Asia. The fact that *A. invadans* does not produce sexual structures was suggested as accounting for the genetic homogeneity between all the isolates. The authors proposed that genetic homogeneity of Asian *A. invadans* isolates from diverse geographic locations showed that it had colonized of Asia quite recently, perhaps in one relatively rapid episode.

The investigations carried out by Lilley *et al.* (1997, 2003) included a wide range of Asian strains, but none from cases of UM in the U.S.A. since successful isolation of *A. invadans* was not achieved until 2002

(Blazer *et al.*, 2002; *Kiryu et al.*, 2002). Using the methods established for *A. invadans* in Asia (Willoughby and Roberts, 1994), Blazer *et al.* (2002) successfully isolated *A. invadans* from UM infected menhaden and carried out a range of comparative studies using an earlier non-pathogenic menhaden Aphanomyces isolate and an Asian EUS *A. invadans* isolate. Morphologically, the EUS and UM isolates were found to be identical and by PCR, amplification with *A. invadans*-specific primers, both the Thai EUS isolate and the new N. American isolate produced a diagnostic 98-bp product. Sequencing of the ITS1 region of WIC produced a 202-bp segment (GenBank AF349614) that showed 100% homology with Asian *A. invadans* isolates (GenBank AF349610–AF349613). The American UM strain was shown to be pathogenic to Asian snakehead, producing typical EUS lesions, and was recovered from those lesions. Additionally, Blazer *et al.* (2002) reported *A. invadans* to be present in lesions on a bluegill *Lepomis macrochirus* from a farm pond in Georgia and channel catfish *Ictalurus punctatus* from a farm pond in Louisiana. Kiryu *et al.* (2002) were also able to infect menhaden with the American isolate, producing typical UM lesions.

*Aphanomyces invadans* has, therefore, been demonstrated to be a single species pathogenic to a wide range of fish species in fresh and low salinity estuarine waters across Japan, Australia, Southeast Asia and the East coast of North America. The extreme genetic homogeneity of the Asian isolates, of course, shows interesting similarities with the presence of a widespread *S. parasitica* clone in Scandinavia.

## Pathology

EUS outbreaks have been associated with mass mortality of various species of fish, freshwater or in low-salinity brackish water, in the wild (including rice fields, estuaries, lakes and rivers) and in farms. Early gross clinical signs of infection as described in the OIE Manual of Diagnostic Tests (OIE, 2006) include loss of appetite and the fish become darker in colour. The infected fish may float below the surface of the water and for periods become hyperactive with a very jerky pattern of movement.

The first clinical signs are usually small, pinhead-sized red spot skin lesions on the body surface, head, operculum or caudal peduncle. These small lesions have very little apparent disruption of surrounding tissues. Histopathological examination at this stage reveals hyphae associated with necrosis and inflammation (Vishwanath *et al.*, 1997). The size of the

lesions and extent of the necrosis grows. Granulomata usually form around the hyphae while the lesions are 2-4 cm in diameter. Infected fish usually present dermal ulcers and an associated loss of scales, haemorrhage, oedema developing necrotic open ulcers on the body surface (Fig. 6.22A, B). Aseptate hyphae may be found in fresh squash preparations of such lesions, extending to the skeletal muscle within granulomas (Fig. 6.23) (Callinan *et al.*, 1989; Vishwanath *et al.*, 1997; Lilley *et al.*, 1998).

In some advanced lesions, fungal hyphae invade the abdominal viscera, kidney, liver, or spinal cord in several fish species and this is almost certainly the cause of death (Chinabut, 1990; Wada *et al.*, 1994; Vishwanath *et al.*, 1997, 1998; Lilley *et al.*, 1998). Lesions occur on the flank or dorsum. In species such as snakehead which are highly susceptible, the lesions are more extensive and can lead to complete erosion of the posterior part of the body, or to necrosis of both soft and hard tissues of the cranium, so that the brain can be exposed in the living animal.

In Atlantic menhaden, the gross and microscopic pathology of UM is identical to that described for epizootic ulcerative syndrome (EUS) of Indo-Pacific fish (Egusa, 1992; Fraser *et al.*, 1992; Wada *et al.*, 1994; Lilley *et al.*, 1998). The ulcers are circular, often located near the anus, and deeply penetrating, with peripheral haemorrhage, extensive necrosis, and tissue loss (Noga and Dykstra, 1986; Noga, 1993). Microscopically, these ulcers are characterized by extensive myonecrosis and granulomatous myositis. This condition was initially called ulcerative mycosis (UM) by Noga and Dykstra (1986) because highly invasive, deeply penetrating oomycete hyphae were observed consistently in the menhaden lesions (Noga *et al.*, 1988; Noga, 1993; Blazer *et al.*, 1999; Dykstra and Kane, 2000).

Aseptate hyphae of *Aphanomyces invadans* (12-25 μm in diameter) can be seen in muscle squash preparations taken from the infected area around the lesions. Lesion scrapes generally—as might be expected—also show secondary fungal, bacterial and/or parasitic infections.

Histologically, the aseptate mycelium is the stage found in infected fish. It is relatively wide in fish at 6-27 μm, but considerably narrower in culture (Roberts *et al.*, 1993). Miles *et al.* (2003), who developed a monoclonal antibody (MAb) against *A. invadans*, reported that at the periphery of infected lesions, pinpoint areas of fluorescence were observed around the hyphae, often extending into parts of the sections that still

**Fig. 6.22A and B** EUS lesions on snakehead (J. Lilley).

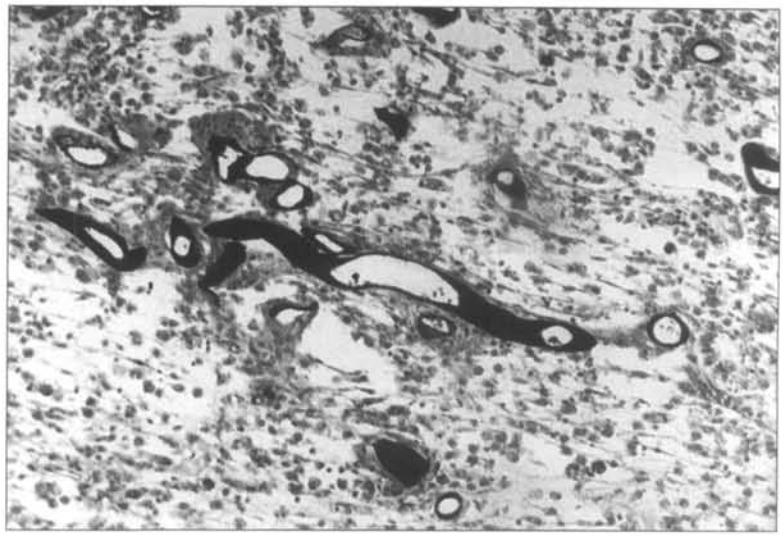

**Fig. 6.23** EUS histology, *A. invadans*, hyphae in muscle with early granulomatous response (Grocott H&E) (J. Lilley).

contained intact muscle fibres. In some cases, developed hyphae were absent or only found in the dermis, and only the sources of pinpoint fluorescence appeared in the musculature. At higher magnifications, the pinpoints appeared filamentous, but no clear images were obtained at sufficient magnification. The authors suggested that these might comprise a hitherto unreported part of the *A. invadans*, but no further reports on these possible stages have been published to date.

EUS occurs mostly during periods of low temperatures and after periods of heavy rainfall (Baldock *et al.*, 2005). These conditions may favour sporulation of *Aphanomyces invadans*, and low temperatures have been shown to delay the inflammatory response of fish to fungal infection (Baldock *et al.*, 2005).

As with Saprolegniasis, the cause of death is unclear, but they appear to be very similar. Cruz-Lacierda and Shariff (1994) reported that levels of blood haemoglobin and serum protein drop during infection, suggesting that the loss of osmotic integrity resulting in potentially fatal sero-dilution may take place through open lesions. Alternatively, opportunistic bacterial infections develop rapidly in the open lesions, which may be the ultimate cause of death (Boonyaratpalin, 1989).

## Isolation and Challenge

Methods for isolation of *Aphanomyces invadans* from lesions on infected animals are as described for Chromistan infections in general (above).

Both with MG and UM, early investigations suggested that *Aphanomyces* spp. were always present in the lesions. With MG in Japan, it seems that the investigators were of the view that *Aphanomyces piscicida (invadans)* was the likely aetiologic agent and isolation into was fairly readily achieved and laboratory infections to confirm its pathogenicity, completed. Although the initial investigators of UM in the U.S.A. were evidently also of the view that an oomycete was central to the disease, what are now recognized to be secondary opportunists were the only isolates to be obtained from infected tissues (Dykstra *et al.*, 1986; Noga and Dykstra, 1986). Experimental transmission studies with those isolates did not confirm fungal aetiology (Noga, 1993) and, therefore, when the alternative aetiology of *Pfiesteria piscicida* was proposed, this alternative cause could not be dismissed immediately.

Despite the various difficulties encountered by the various investigators, *Aphanomyces invadans* has now been isolated from all 'flavours' of EUS lesions (UM, MG, RSD and EUS itself) and lesions have now been successfully reproduced in the laboratory by injection of fish with hyphae (Hatai and Egusa, 1977; Roberts *et al.*, 1993; Blazer *et al.*, 2002; Kiryu *et al.*, 2002) and secondary zoospores (Wada *et al.*, 1996; Lilley and Roberts, 1997; Catap and Munday, 1998; Khan *et al.*, 1998; Kiryu *et al.*, 2002; Johnson *et al.*, 2004), cohabitation with infected fish (Cruz-Lacierda and Shariff, 1995), and bath exposures to secondary zoospores after exposure to acidified water (Callinan *et al.*, 1994). Low salinities (2–6‰) were correlated with the occurrence of UM in Atlantic menhaden (Dykstra *et al.*, 1986), and the associated *Aphanomyces* sp. isolated from the ulcers grew best in low-salinity agars (Blazer *et al.*, 2002). Key factors in outbreaks of EUS in Southeast Asia are warm temperatures (19°C) and low salinity (Chinabut *et al.*, 1995; Lilley *et al.*, 1998), conditions that appear to be necessary to the transmission of both UM and EUS.

Kiryu *et al.* (2002) conducted a range of laboratory challenges of Atlantic menhaden to fulfil Koch's postulates by experimentally reproducing the lesions associated with UM in Atlantic menhaden using three different isolates of *Aphanomyces* by injection and bath challenge routes.

As is the case with developing challenges with *Saprolegnia parasitica*, investigators investigating the infectivity of *A. invadans* found that infectivity was markedly enhanced by providing a portal of entry. Callinan *et al.* (1994) who were the first to produce EUS lesions in fish by exposure to *A. invadans* zoospores only achieved this after physical and chemical abrasion of the fish skin. They were unable to induce EUS lesions in fish without first facilitating entry of the pathogen. Kiryu *et al.* (2002) employed a variant of the ami-momi method (Hatai and Hoshiai, 1993) by injury from net handling or manual removal of scales, producing 100% lesion prevalence in the exposed fish.

Experimental studies suggest that snakehead and Atlantic menhaden are susceptible to infection by *A. invadans* without other predisposing factors. Other fish species have been found to be much less susceptible unless stress or integument damage predisposes. Over 50 species of fish have been confirmed by histological diagnosis to be susceptible, but some important culture species, including tilapia, milkfish and Chinese carp, have been shown to be resistant (Baldock *et al.*, 2005).

## Oomycetes: Conclusions

It has been clear for many years that the oomycetes comprise significant pathogens of fresh and brackish water fish, especially the salmonids. As suggested by Hughes (1994), the availability of malachite green as a cheap and effective fungicide disguised the significance of *Saprolegnia parasitica* as a pathogen for many years. Loss of malachite green, occurring at the same time that *Aphanomyces invadans* was spreading through Asia and was finally recognized as the cause of the longstanding UM problem in US east coast estuaries has now rekindled interest in methods of control and prevention of oomycete infections.

A particularly valuable contribution is being made by the application of the full range of molecular biological methods to the problem. The recognition that both of these major pathogens *S. parasitica* and *A. invadans* exist over wide areas as clones of very limited genetic variability, confirms that sexual stages are suppressed or lost in these species, for which conventional morphologically based speciation is therefore impossible. Work with the closely related pathogen of freshwater crayfish, *Aphanomyces astaci* has shown that this species has been introduced into Europe from N. America a number of times (Lilley *et al.*, 1997) over nearly 150 years and that a number of different strains can be

identified and associated with the different routes and potentially sources of introduction. Possibly, further studies of *Saprolegnia parasitica* over more strains and wider areas will show similar results; movements of salmonids and their eggs to most suitable waters around the world have given plenty of opportunity for this to occur. *Aphanomyces invadans*, at least in Asia, is a different case, we are apparently seeing the effect of a new pathogenic species, first identified in Japan that has spread across Southeast Asia in less than 30 years. Further studies into relationships between EUS and UM strains will be of considerable interest.

The group responsible for what may have been the first reported mycotic disease of a vertebrate (Arderon, 1748) continues to be of interest.

## EUMYCOTA, DEUTEROMYCOTINA

To date, all true fungi reported as fish pathogens are members of the Deuteromycotina (previously the Fungi Imperfecti). This is a large group, not strictly a coherent taxonomic grouping but is, instead, a collection of fungi for which only the vegetative mycelium and the asexual spore stages (conidia) are known. These organisms are, therefore, only known by their anamorph stages. In a few cases, the sexual forms, teleomorphs are known; these are considered as 'perfect' and, thus, the definitive taxa. In many cases, the organisms appear to have lost the ability to reproduce sexually, all for which the sexual stages are known are members of the Ascomycotina.

The Deuteromycetes consist of three groups. These are all morphological classes and consist of the Coelomycetes whose spores are produced in or on special fruiting structures, the Hyphomycetes which are sterile non-sporulating mycelia or bear conidia and the Blastomycetes which are the Ascomycetous and Basidiomycetous yeasts for which sexual stages are yet to be demonstrated. Two morphological subforms are recognized within the Hyphomycetes—those with brown to black coloured hyphae, the Dematiaceous fungi and those with colourless or hyaline hyphae.

A number of different terms have been used in the description of diseases of Deuteromycete aetiology (e.g., phaeohyphomycosis), but whether there is a practical need for these terms is questionable and need not be discussed further.

## COELOMYCETES

Only one species of Coelomycete has been reported as responsible for disease in aquatic animals. *Phoma herbarum*, a fungal plant saprophyte, has been found infecting salmonid fry and fingerlings in the Pacific Northwest of the United States of America, in Alaska and in Southwest England. In culture *P. herbarum* has hyaline septate hyphae that bear pycnidia, spherical spore containing bodies with darkly pigmented walls (Figs. 6.24, 6.25).

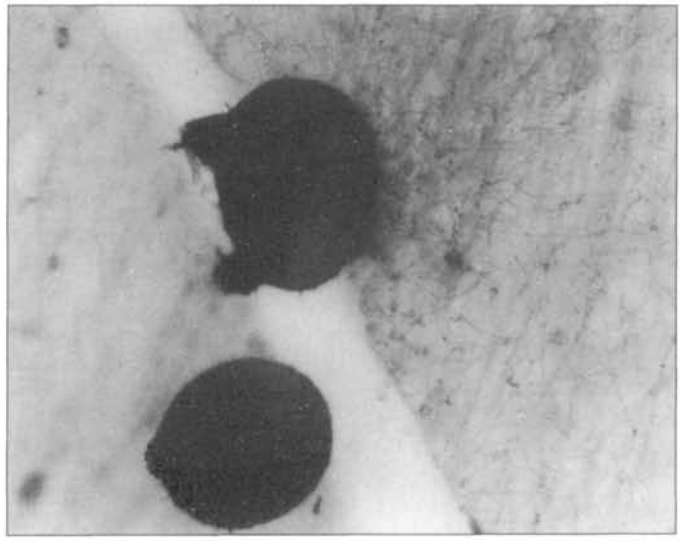

**Fig. 6.24** *Phoma herbarum*, pycnidia in culture.

In one American site, annual losses of 2.6% over 6 years were attributed to this infection. External signs consisted of swollen and haemorrhagic vent and compression of the abdominal area. Caudal fin haemorrhaging was sometimes extensive, with petechiae occurring occasionally on the lateral and ventral body surfaces. Severely affected animals rested on their sides on the bottom but were capable of righting themselves when startled. Histopathological investigation has indicated that the earliest detectable mycelium occurred in the pneumatic duct area of the swimbladder, with the infection becoming systemic thereafter (Figs. 6.26, 6.27). The swimbladder is usually free of fluid, whilst the stomach may contain a watery fluid. Penetration of the swimbladder walls

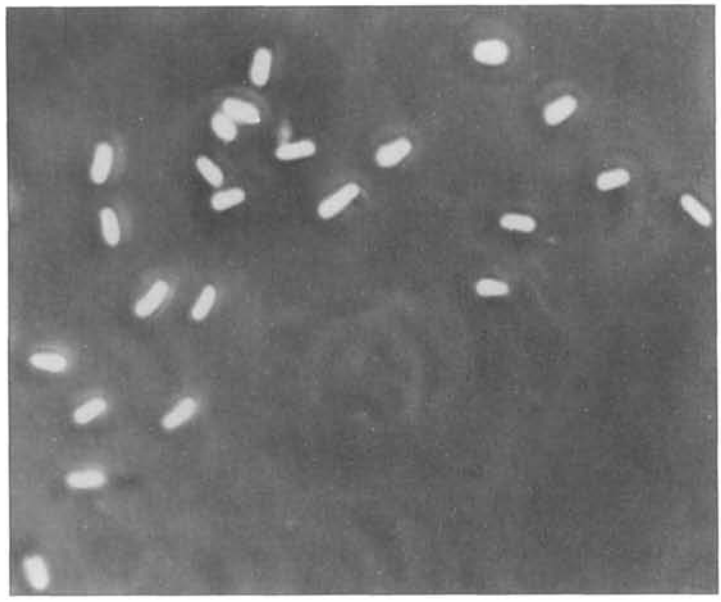

**Fig. 6.25** *Phoma herbarum* spores (conidia).

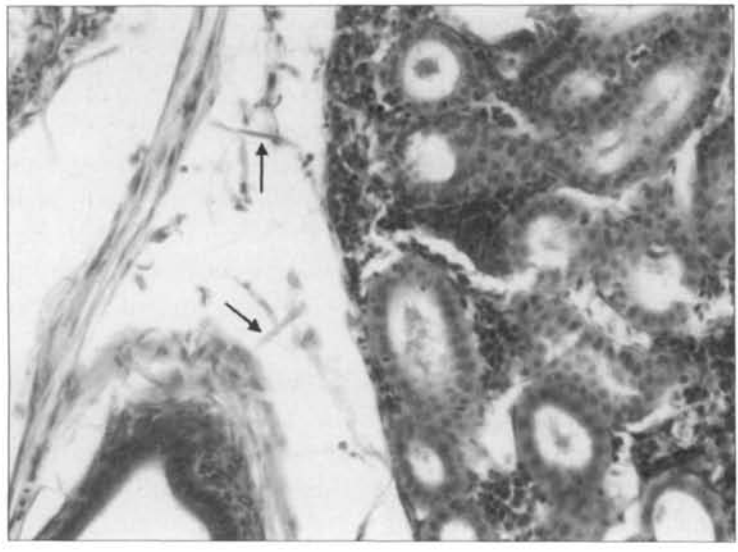

**Fig. 6.26** *Phoma herbarum*, hyphae in kidney and peritoneal cavity (arrows) of rainbow trout.

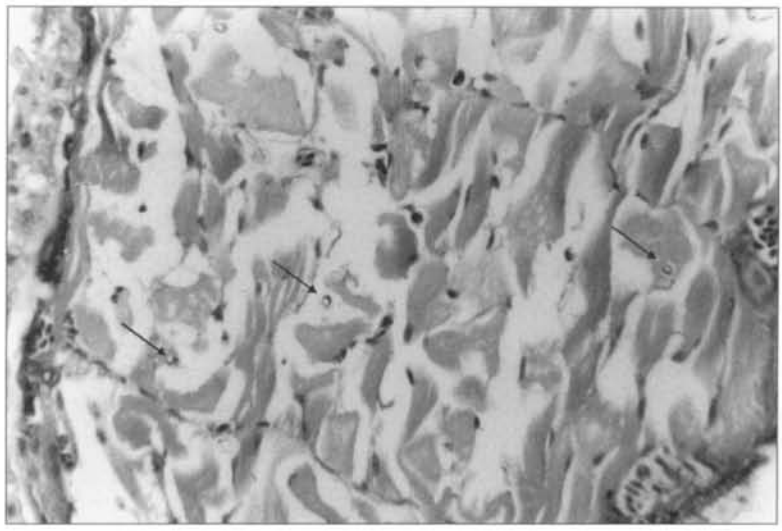

**Fig. 6.27** *Phoma herbarum*, hyphae (arrows) in muscle of rainbow trout (Grocott H&E).

results in extensive inflammatory reaction in the surrounding tissues as the fungus invades the visceral organs. Ross *et al.* (1975) succeeded in producing laboratory infections in chinook salmon fry both by feeding *Phoma* spores mixed with fish food (one infection in 24 animals) and by spraying an aerosol of *P. herbarum* conidia over the surface of a container in which anaesthetized salmon fry were recovering from MS222 anaesthesia (4 in 100 became infected). Alderman (1982a) suggested that the latter route might be typical; the case in SW England was in a hatchery surrounded by deciduous woodland. High concentrations of the aero-aquatic spores of *P. herbarum* growing on leaf detritus may be ingested during swimbladder inflation in fry resulting in infection of the swimbladder.

Hatai *et al.* (1986) reported visceral mycosis and associated losses of 67% due to a *Phoma* infection in fry of ayu *Plecoglossus altivelis* at a Japanese hatchery; fish showed visceral adhesions to the swimbladder, kidney, intestine, liver, pancreas, abdominal wall, and muscle. Histopathological observations and the distribution of fungal hyphae in the visceral organs of infected fish also suggested the primary site of infection to be the swimbladder.

Recently, Burton *et al.* (2004) have reported the association of *P. herbarum* with salmonid mortalities in 20 different Alaskan hatcheries.

Infected fish ranged from fry to smolts and include a variety of species including Chinook salmon *Oncorhynchus tshawytscha*, sockeye salmon *O. nerka*, coho salmon *O. kisutch*, and Arctic Grayling *Thymallus arcticus*. Infections have affected a variety of different organs, including swimbladder, intestine, rectum, spleen, and kidney. Cumulative mortalities were low, but continued until the infected fish were eliminated from the population.

Burton *et al.* (2004) also conducted challenge studies to determine whether *P. herbarum* could be transmitted to juvenile Chinook salmon *O. tshawytscha* by the oral and airborne routes. *P. herbarum* that had been isolated from juvenile Chinook salmon sustaining a low-level mortality in a hatchery was grown in the laboratory. Quantified inocula of fungal conidia were mixed with dry fish food for the feeding studies, sprayed on the surface of the water, and injected into fish. The feed and airborne challenge routes were unsuccessful, but injection resulted in a cumulative mortality of 65% in fingerlings and 56% in fry.

External gross clinical signs of infection included haemorrhage at the anal vent and fungus visible on the skin; internally hyphae were present in the gut and distended gut and swimbladder.

## HYPHOMYCETES

The Hyphomycetes are Deuteromycetes in which the mycelium is either sterile or bears its asexual spores on hyphae which are not formed into a distinct and characteristic fruiting body. A number of genera have been reported associated with infections in fish; these include *Fusarium*, *Ochroconis*, *Exophiala* and *Cladosporium*.

### *Fusarium*

The form genus *Fusarium* comprises a large number of species. It consists of those Hyphomycetes with fusion macroconidia with a foot cell that bears some kind of 'heel'. Microconidia and terminal or intercalary chlamydosopores may or may not be present. The authoritative taxonomic work on this genus is Booth (1971).

One species, *F. culmorium* has been reported as being responsible for the mortality of 200 carp in a new earth pond (Horter, 1960), the infection possibly being derived from a large inoculum of spores present because of layers of beech leaves on the pond bottom. Apart from this, where the

*Fusarium* species has been identified, the species involved appears to have been *F. solani*. This species has been reported to be associated with a wide range of infections in aquatic animals. Summarized by Yanong (2003) in freshwater, these include several fish species from reservoirs in India (Deepa *et al.*, 2000) and desert pupfish kept in an aquarium (Ostland *et al.*, 1987). In the marine environment, Yanong (2003) found reports involving species such as angelfish (French angelfish, *Pomacanthus paru*, grey angelfish, *P. arcuatus*, blue angelfish *Holocanthus bermudensis* and queen angelfish *H. ciliaris*), parrotfish (midnight parrotfish *Scarus coelestinus*, and rainbow parrotfish *S. guacamaia*), hammerhead sharks (bonnethead sharks, *Sphyrna tiburo* and scalloped hammerhead sharks, *S. lewini*). Details of these cases can be found in Yanong (2003); in many cases the animals are aquarium fish and may represent animals damaged in handling or otherwise compromised. Cases reported from the natural or farm environment are rare.

*F. solani* is also well recognized as a significant pathogen for marine crustacean species, especially under conditions of intensive aquaculture. In lobsters (Fisher *et al.*, 1978; Alderman, 1980), *Fusarium* infection takes the form of a chronic localized erosion of the exoskeleton, often accompanied by the penetration of encapsulated fungal mycelium deep into the underlying tissues. In Japan, Egusa and Ueda (1972) reported *F. solani* 'black gill disease' in the Kuruma prawn, *Penaeus japonicus*, characterized by the presence of many black spots in the gills, accompanied in severe cases by necrosis or collapse of the gills.

### *Ochroconis*

Two species of this genus have been reported associated with lesions in fish. *O. tshawytscha* was isolated by Doty and Slater (1946) from young chinook salmon (*Oncorhynchus tshawytscha*) infecting the posterior half of a kidney in a small percentage of the fish from a California hatchery. The investigators could not induce infection and the species has subsequently been isolated from soil (Barron and Bush, 1962; Roy *et al.*, 1962). First reported as a pathogen of frogs (Elkan and Philpot, 1973), a second species *O. humicola* was then identified as inducing a weakly contagious disease of laboratory held juvenile coho salmon (*O. kisutch*) (Ross and Yasutake, 1973). External lesions were not consistently present, and in their absence the only indication of disease was an enlarged abdomen. The body cavity usually contained watery fluid and adhesions frequently occurred between

the various visceral organs, some of which exhibited grey coloured lesions. Histopathologically, the condition was found to be systemic with the fungal mycelium responsible for the development of numerous large lesions throughout the tissues. Subsequently, Ajello *et al.* (1977) reported a series of epizootics in rainbow trout (*O. mykiss*) in a Tennessee fish hatchery between 1969 and 1973 until a new source of water was obtained. Mortalities began shortly after egg hatch (September), peaking in autumn with infections rarely being observed in summer. External signs consisted of vesicular areas on the flank, together with small- to medium-size ulcers, oedema and exophthalmia. Haemorrhages were found throughout the entire body cavity and internal organs were studded with mycelial aggregates. Histology showed abundant dematiaceous septate hyphae in all infected tissues with chronic granulomata in the kidneys displacing normal tissue (Figs. 6.28, 6.29). Bath challenges in suspensions of *O. humicola* mycelium and spores failed to infect, but intra-peritoneal injections succeeded within 3 days, death generally occurring within 7-10 days after inoculation with similar clinical and histopathological features to those of natural infections.

Further cases of fish infections with *O. humicola* were reported by Hatai and Kubota (1989) from masou salmon *O. masou*, in catfish, *Clarias*

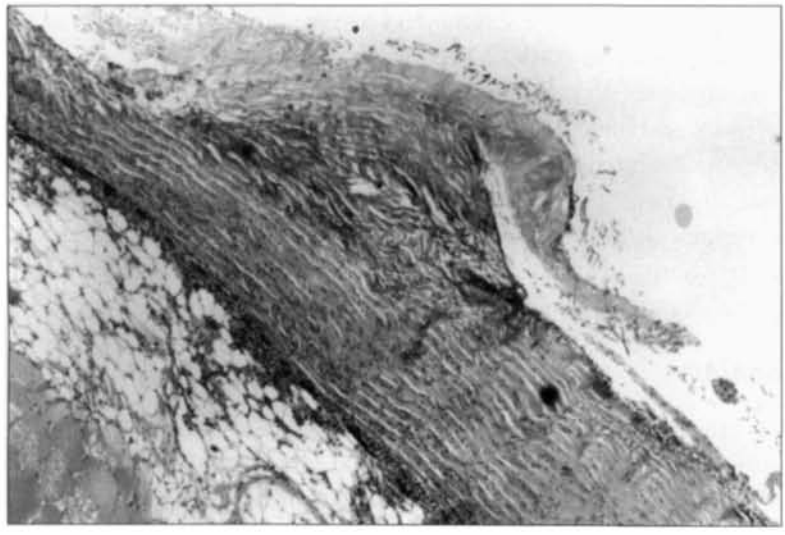

**Fig. 6.28** *Ochroconis humicola*, skin lesion produced by intra-muscular injection.

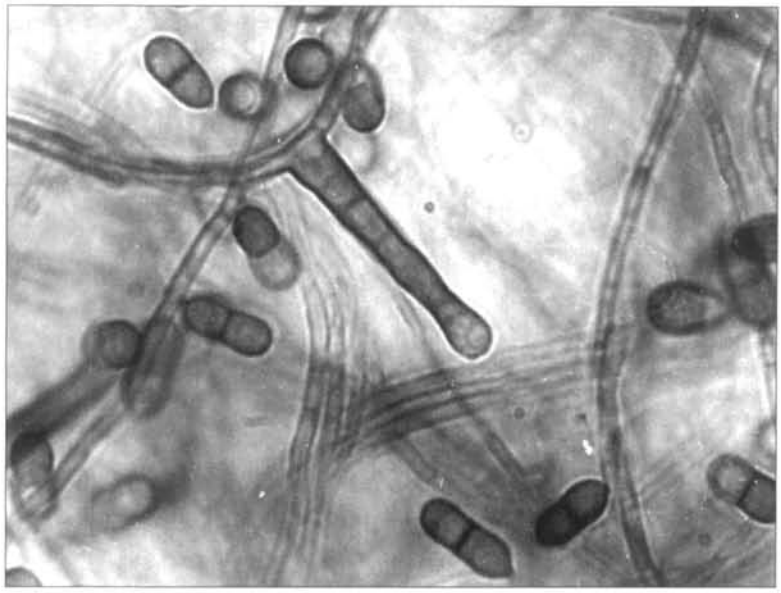

**Fig. 6.29** *Ochroconis humicola* spores (conidia).

*batrachus* by Bhattacharya (1988), in Atlantic salmon, *Salmo salar* (Schaumann and Priebe, 1994) and in cultured marine devil stinger (Wada *et al.*, 1995), red seabream and marbled rockfish (Wada *et al.*, 2005) from Japan. Bowater *et al.* (2003) reported on a single case in barramundi cod (*Cromileptes altivelis*) in Australia. Here, the authors suggested the infection could have been associated with the fact that the fish had been captured by reef divers who had punctured its swimbladder with an hypodermic needle on capture in order to prevent it from floating in the capture tank. Blaylock *et al.* (2001) reported a similar occurrence in red snapper where the fungi concerned were *Cladosporium sphaerospermum* and *Penicillium coryophillum*.

### *Exophiala*

There are a number of reports of members of this genus being found as parasites of fish. *E. salmonis* was reported (Carmichael, 1966) from three widely separated mass mortalities at one hatchery in Alberta, Canada. The years concerned were 1948, 1956 and 1960 and all the outbreaks were in winter. Gross signs were exophthalmia and cranial ulcers, with infection levels up to 40%. Histopathological investigation showed necrotic and granulomatous response with the presence of giant cells in the brain.

A further case of *Exophiala* infection was described by Fijan (1969) amongst channel catfish (*Ictalurus punctatus)* in Alabama. Tissue response was either non-proliferative or proliferative and granulomatous. Isolations were carried out and the pathology was reproduced in healthy fish by means of intraperitoneal injection. The organism was subsequently described as *E. pisciphila* by McGinnis and Ajello (1974).

Richards *et al.* (1978) reported a further mortality due to *E. salmonis*, this time in *Salmo salar* second year smolts in sea cages on the Scottish West Coast. The outbreak was limited to one cage, in which approximately 10% of fish died during a 4-month period. Affected fish had grossly swollen posterior abdomens due to enlargement of the posterior kidney, which itself showed large (circa 3.5 cm) greyish white nodules with lesions in some animals extending into the dorsal musculature. Histopathological investigations showed proliferating mycelium invading the kidney, with a variable degree of involvement of heart, liver, spleen, pancreas and muscle (Fig. 6.30). The tissue reaction was granulomatous and giant cells were present. The authors gave extensive and detailed description of the histopathology of this mycosis. Isolates were made and identified as *E. salmonis*. Since only one cage of fish was infected, the authors suggested

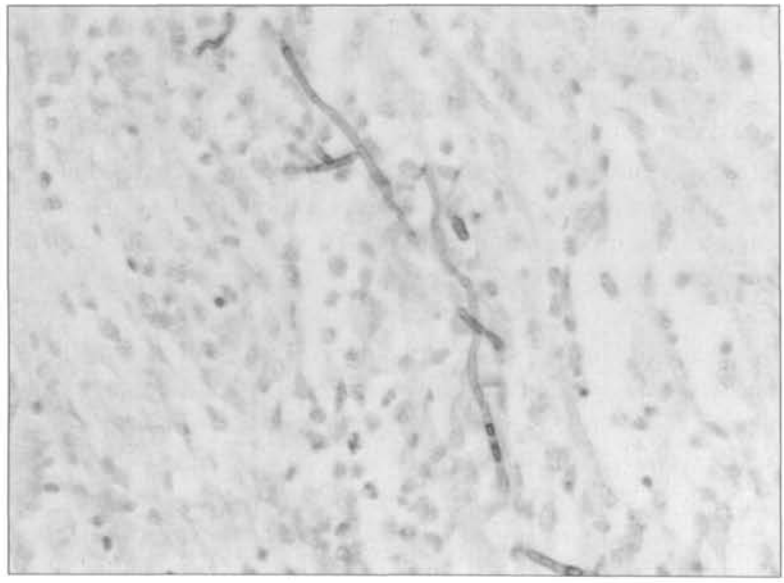

**Fig. 6.30** *Exophiala salmonis* dematiaceous hyphae in muscle (PAS).

that infection might have been as a result of a focal fungal contamination of the artificial diet. De Hoog (1977) reported that *E. salmonis* had also been isolated from *Aurucaria* Wood and that *E. brunnea* Papendorf isolated from soil is synonymous with *E. salmonis*.

Blazer and Wolke (1979) described further cases in a marine aquarium in New England. Five natural infections were found in *Steneotomus versicolor, Gadus morhua, Hippocampus hudsonius, Xanthichthys ringens* and *Amphiprion sebae*. The authors gave detailed descriptions of both the natural infections and experimentally induced ones. Externally, two fish (*X. ringens* and *H. hudsonius*) showed dermal masses which the authors attributed to the fungus. Internally, two animals (*S. versicolor* and *G. morhua*) had internal lesions raised (1 to 5 mm) round yellow to white areas in the liver, kidney, myocardium, swimbladder and spleen. All natural cases except for *G. morhua* were regarded as acute and were non-proliferative. Systemic infections in cod have also been noted in samples collected during fish health monitoring cruises in the English Channel (Alderman, unpublished).

Alderman and Feist (1985), examining the kidneys of rainbow trout *Oncorhynchus mykiss* recovering from Proliferative Kidney Disease, noted unusual lesions consisting of white nodular areas in the body of the kidney. The lesions were discrete and a clean white in colour, in clear contrast to the indistinct off-white to grey-white of the healing PKD lesions. An initial microscopic examination of fresh unstained squash preparations showed the presence in the lesions of septate dematiaceous fungal hyphae.

Histologically, the lesions were well defined and inflammatory in nature, often with necrotic centres, or more fibrous with numerous multinucleate giant cells. Inflammatory lesions exhibited a marked cellular response to the presence of relatively few fungal hyphae, whilst in contrast, necrotic tissue was heavily infiltrated with hyphae.

Isolation of the organism into culture was accomplished and the hyphae, which were identical to those seen in the lesions, grew out into the agar from the inoculum and developed conidia which were identified as 'near *Exophiala jeanselmei*' (Fig. 6.31).

Yanong (2003) lists further *Exophiala* cases, again mainly aquarium associated, including lumpfish (*Cyclopterus lumpus*), Atlantic wolffish (*Anarhichas lupus*), smooth dogfish (*Mustelus canis*) and thorn back ray (*Platyrhinoidis triseriata*).

**Fig. 6.31** *Exophiala salmonis* spores (conidia).

### *Paecilomyces*

This genus has been reported in a number of cases, including in tilapia wasting disease from Puerto Rico (Rand *et al.*, 2000). Externally, the fish were emaciated, and showed eroded fins and occasionally scaleless haemorrhagic lesions. Infections were marked by the presence of numerous small golden to reddish brown granulomas throughout the internal organs.

## DEUTEROMYCETE—CONCLUSIONS

Case reports of infections of fish by Deuteromycete fungi are clearly sporadic and may well reflect only those cases where an appropriate fish health specialist becomes involved. In many cases, lesions may be noted with fungal involvement if histological investigation is undertaken, by which time isolation and identification of the fungus involved is impossible. It is, however, clear that a number of fungal genera have the ability to act either as primary pathogens or, more often, as secondary invaders of wounds. Genera such as *Phoma*, *Fusarium* (also well known in Crustacean infections), *Ochroconis* and *Exophiala* have been found repeatedly. No clear pattern of disease has been associated; in farmed fish infections tend to result in slow chronic mortalities that may have resulted from a specific exposure incident, or occur year on year. Infections in wild

fish may well be more common than reports would suggest. Reports of cases in wild caught aquarium fish would suggest the same, but may be equally associated either with the fact that lesioned fish attract attention or to the possibility that the artificial aquarium conditions may result in greater frequency of infection.

## THERAPY

For over seventy years (Foster and Woodbury, 1936), there existed a simple and effective therapy for most fungal infections of fish. Malachite green was highly effective, simple and cheap to use. Increasing measures to protect consumers meant that 'traditional treatments' such as malachite green could no longer be tolerated unless adequate modern consumer and environmental safety could be demonstrated. The dye group (phenylmethanes) to which malachite green belongs was generally poorly regarded toxicologically and as an old non-patentable product there was no possibility that the investment needed for safety studies would be available.

### Control Methods

#### *Chemical*

For nearly seventy years, control of *S. parasitica* infections of salmonids and salmonid eggs in aquaculture was not a problem; the triarylmethane dye malachite green (Foster and Woodbury, 1936) was a cheap and highly effective therapeutant used throughout the industry. Therefore, the result was that saprolegniasis was itself not a problem in the eyes of fish farmers or fish health scientists. A few laboratories foresaw looming in the future an inevitable tidal wave of regulatory controls on the use of veterinary medicines in animal husbandry and could also see that malachite green would be a regulatory nightmare. Alderman (1982b, 1985), Bailey (1983a, b, 1984) and Bailey and Jeffery (1985) published studies of test methods and results of screens for alternative fungicides but with malachite green still permitted for use there was no commercial incentive for the pharmaceutical industry to become involved. Moreover, it needs to be recalled that both *S. parasitica* and *A. invadans* are not Eumycetes, but are Chromistans. In the last 50 years, the crop protection industry has moved from a broad spectrum to pathogen target-specific fungicides. This meant that the range of possible crop fungicides that might be effective against

oomycetes was limited, even if companies were interested in applying crop protection products to a new market.

The use of malachite green was assumed by users to be a topical disinfectant fungicide and parasiticide and, thus, for a long time there was little consideration paid to the possibility that such topically applied therapeutants might also be absorbed systemically and produce significant internal effects and persistent residues, despite the evident respiratory distress of over-exposed fish. The evidence of Steffens *et al.* (1961), Werth and Boiteaux (1967) and Meyer and Jorgenson (1983) made it clear that the dye would enter cells and could result in adverse effects. Indeed, the lipophilic nature of the carbinol form (Alderman, 1985, 1994) means that this form of malachite green enters the cells and tissues readily and the high octanol/water partition coefficient of the carbinol form of malachite green means that bioaccumulation is inevitable. Further research showed that in addition to the dye and carbinol forms of malachite green, a metabolite, leucomalachite green also existed and that this form persisted even longer in fish tissues than the carbinol form. Detectable residues of leuco-malachite green have been found more than a year after the last treatment.

The problems that have led to the prohibition of use of malachite green in aquaculture did not derive directly from scientific evidence that its residues presented a specific consumer or environmental hazard. Indeed, there was little evidence from good controlled toxicology studies to show that malachite green presented problems, although such evidence existed for structurally related dyes. Instead, the problem arose from the introduction of more stringent consumer protection controls on residues of medicines in food animals in the U.S.A. and in the European Union. Permission to supply or to use anything for a medicinal purpose in a food animal species could only be granted if the supplier was able to satisfy regulators that no adverse effects would result (Alderman, 1999). Control of disease is by definition a medicinal purpose. Proof of absence of risk was required rather than absence of proof of a risk. Malachite green as an old chemical with no patent protection and with a likely cost of millions in dollars or Euros to complete regulatory tests with no certainty of success was clearly doomed. In the EU, the problem was that there was no modern scientific evidence of harm and, therefore, malachite green could not be placed in the prohibited list of the appropriate European law (CEC, 1990), Annex IV of this regulation requires proof of harm. Instead, it has become regarded as an 'unapproved' veterinary residue, which EU member states have to check for using sensitive methodologies in their veterinary

residues monitoring programmes (CEC Directive 96/23). In the U.S.A. the search for scientific evidence to show that malachite green residues were harmful led to the funding of a multi-year toxicology study that failed to demonstrate conclusively that malachite green was indeed harmful (Culp, 2004). By the end of the 1990s, regulatory and monitoring requirements meant that any further use of malachite green, even on fish eggs, became impossible.

Setting aside formalin which is approved for use as an agent to control *Saprolegnia* in the U.S.A. but which is not particularly effective and has clear user hazards, hydrogen peroxide and salt (or seawater flushes where possible) have been shown to have possible applications. Several groups (Waterstrat and Marking, 1995; Schreier *et al.* 1996; Gaikowski *et al.*, 1998; Rach *et al.*, 2005) have reported on the effectiveness of hydrogen peroxide for controlling *Saprolegnia* on developing eggs of rainbow trout and Chinook salmon. However, high doses were necessary; the fish toxicity of hydrogen peroxide increases rapidly with slight increases in water temperature, often making the therapeutic index dangerously narrow. Furthermore, hydrogen peroxide, although excellent in terms of consumer and environmental safety, is a dangerous substance to transport and hold.

The volumes of NaCl required to produce salt dips, baths and flushes make the application of this otherwise cheap and fairly effective method impractical except at the rare seashore-based hatchery sites. Baths of up to 3.5% NaCl (roughly equivalent to 10% seawater) are needed (Bruno and Wood, 1994).

Other possible agents include iodophores which can only be used on eggs and a material as traditional as malachite green, namely copper sulphate. Copper sulphate is of doubtful efficacy (Carballo *et al.*, 1995) against *Saprolegnia* and the use of quantities sufficient to have any fungicidal effect would undoubtedly have significant adverse environmental effects.

Fortunately, the imminent demise of malachite green did—in Europe at least—provide a financial case for the pharmaceutical industry to try to develop a replacement for malachite green. Initial support for regulatory studies by the UK government led to the approval of bronopol (as Pyceze®) for use in the trout industry throughout most of Europe. For eggs, a concentration of 50 mg $l^{-1}$ bronopol should be maintained for 30 minutes in daily treatments from 24 hr post-fertilization until hatching.

Fish should be treated at 20 mg $l^{-1}$ bronopol for 30 minutes per day for up to 14 days consecutively. Two practical points perhaps need to be made. For those used to malachite green, the fact that with bronopol once prepared, the active concentration is invisible in water perhaps causes some initial uncertainty; secondly, it is important to realize that commercial bronopol will not be effective since it will not be in the correct formulation to maintain correct solubility.

As regards *Aphanomyces invadans*, although Lilley *et al.* (1997) and Campbell *et al.* (2001) investigated a range of potential fungicides, little was of practical value and control of EUS once present in natural waters is probably impossible. In outbreaks occurring in small, closed water-bodies, liming water and improving water quality, together with removal of infected fish, can be effective in reducing mortalities.

No studies into the control of Deuteromycete infections of fish have been published. Low frequency of infections reported tend to discourage such investigations.

## Control Methods

### *Biological*

A number of attempts to develop biological control methods against saprolegniosis have been made. Cooper *et al.* (1997) pointed out that barley straw can be used to prevent algal and cyanobacterial growths in ponds and showed some effect against species of *Saprolegnia* including *S. parasitica*. Grazing organisms such as amphipods and isopods have also been proposed as possible means for control fungal outbreaks (Oseid, 1977).

Hatai and Willoughby (1988) reported that *Pseudomonas fluorescens* could strongly inhibit the radial growth of *S. parasitica in vitro*. More recently, Lategan *et al.* (2004a, b) found that *Aeromonas media* had a probiotic effect and could significantly reduce saprolegniosis in eel and silver perch in laboratory and field studies. Current investigations (Lategan *et al.*, 2006) suggest that a 2,3 benzopyrrole produced by *A. media* may play a major role.

To date, none of these approaches has yet yielded a practical application.

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## References

Agersborg, H.P.K. 1933. Salient problems in the artificial rearing of salmonid fishes with special reference to intestinal fungisitosis and the cause of white-spot disease. *Transactions of American Fisheries Society* 63: 240-250.

Ainsworth, G.C. 1976. *An Introduction to the History of Mycology*. Cambridge University Press, London.

Ajello, L., M.R. McGinnis and J. Camper. 1977. An outbreak of phaeohypnomycosis in rainbow trout caused by *Scolecobasidium humicola*. *Mycopathologia* 62: 15-22.

Alderman, D.J. 1980. *Fusarium solani* causing an exoskeletal pathology in cultured lobsters, *Homarus vulgaris*. *Transactions of British Mycological Society* 76: 25-27.

Alderman, D.J. 1982a. Fungal diseases of aquatic animals. In: *Microbial Diseases of Fish*, R.J. Roberts (ed.). *Special Publications of the Society for General Microbiology* 9: 189-242.

Alderman, D.J. 1982b. *In vitro* testing of fisheries chemotherapeutants. *Journal of Fish Diseases* 5: 113-123.

Alderman, D.J. 1985. Malachite green: A review. *Journal of Fish Diseases* 8: 289-298.

Alderman, D.J. 1994. Control of oomycete pathogens in aquaculture. pp. 111-129. In: *Salmon Saprolegniasis*, G.J. Mueller (ed.) Report to Bonneville Power Administration, Contract No. 1990BP02836, Project No. 199006100, 266 (BPA Report DOE/BP-02836-1), 269pp. Available electronically at http://www.efw.bpa.gov/Publications/A02836-1.pdf

Alderman, D.J. 1996. Geographical spread of bacterial and fungal diseases of crustaceans. OIE *International Conference on the Prevention of Diseases of Aquatic Animals through International Trade*. Paris, June 7-9, 1995. Office International des Epizooties, Paris, *OIE Scientific and Technical Review* 15: 603-632.

Alderman, D.J. 1999. Requirements for the approval of veterinary therapeutics or growth enhancers use din fish production in the European Union. In: *Xenobiotics in Fish*, D.J. Smith, W.H. Gingerich and M.G. Beconi-Barker (eds.). *Proceedings of an American Chemical Society Symposium on Xenobiotic Metabolism in Fish*, Dallas, Texas, 1998, pp. 25-38.

Alderman, D.J. and S.W.F. Feist. 1985. *Exophiala* infection of kidney of rainbow trout recovering from proliferative kidney disease. *Transactions of British Mycological Society* 84: 157-158.

Alderman, D.J. and J.L. Polglase. 1986. *Aphanomyces astaci*: Isolation and culture. *Journal of Fish Diseases* 9: 367-379.

Alderman, D.J., J.L. Polglase and M. Frayling. 1987. *Aphanomyces astaci* pathogenicity under laboratory and field conditions. *Journal of Fish Diseases* 10: 385-393.

Arderon, W. 1748. The substance of a letter from Mr. William Arderon F.R.S. to Mr. Henry Baker F.R.S. *Philosophical Transactions of Royal Society of London* B 45: 321-323. [Quoted by Hughes, 1994].

Arhenholz, D.W., J.F. Guthrie and R.M. Clayton. 1987. Observations on ulcerative mycosis infections on Atlantic menhaden (*Brevoortia tyrannus*). NOAA, Technical Memorandum NMFS-SEFC-196. C.

Bailey, T.A. 1983a. Method for in vitro screening of aquatic fungicides. *Journal of Fish Diseases* 6: 91-100.

Bailey, T.A. 1983b. Screening fungicides for use in fish culture: evaluation of the agar plug transfer, cellophane transfer, and agar dilution methods. *Progressive Fish Culturist* 45: 24-27.

Bailey, T.A. 1984. Effects of twenty-five compounds on four species of aquatic fungi (Saprolegniales) pathogenic to fish. *Aquaculture* 38: 97-104.

Bailey, T.A. and M. Jeffrey. 1989. Evaluation of 215 candidate fungicides for use in fish culture. US Fish and Wildlife Service, La Crosse, 9 pp.

Baldock, F.C., V. Blazer, R.B. Callinan, K. Hatai, I. Karunasagar, C.V. Mohan and M.G. Bondad-Reantaso. 2005. Outcomes of a Short Expert Consultation on Epizootic Ulcerative Syndrome (EUS): Re-examination of causal factors, Case definition and nomenclature. In: *Diseases in Asian Aquaculture*, V.P.J. Walker, R.G. Lester and M.G. Bondad-Reantaso (eds.). Fish Health Section, Asian Fisheries Society, Manila, pp. 555-585.

Bangyeekhun, E., S.M.A. Quiniou, J.E. Bly and L. Cerenius. 2001. Characterisation of *Saprolegnia* sp. isolates from channel catfish. *Diseases of Aquatic Organisms* 45: 53-59.

Bangyeekhun, E., P. Pylkkö, P. Vennerström, H. Kuronen and L. Cerenius. 2003. Prevalence of a single fish-pathogenic *Saprolegnia* sp. clone in Finland and Sweden. *Diseases of Aquatic Organisms* 53: 47-53.

Barron, G.L. and L.F. Bush. 1962. Studies on the soil hyphomycete *Scolecobasidium*. *Canadian Journal of Botany* 40: 77-84.

Beakes, G.W. 1983. A comparative account of cyst coat ontogeny in saprophytic and fish lesion (pathogenic) isolates of the *Saprolegnia diclina-parasitica* complex. *Canadian Journal of Botany* 61: 603-625.

Beakes, G.W. and H. Ford. 1983. Esterase isoenzyme variation in the genus *Saprolegnia*, with particular reference to the fish-pathogenic *S. diclina-parasitica* complex. *Journal of General Microbiology* 129: 2605-2619.

Beakes, G.W., S.E. Wood and A.W. Burr. 1994. Features which characterize isolates from Salmon fish lesions: A Review. pp. 33-66. In: *Salmon Saprolegniasis*, G.J. Mueller (ed.). Report to Bonneville Power Administration, Contract No. 1990BP02836, Project No. 199006100, 266 (BPA Report DOE/BP-02836-1), 269pp. Available electronically at http://www.efw.bpa.gov/Publications/A02836-1.pdf

Bhattacharya, U. 1988. *Scolebasidium humicola*, a new fungal fish infection from India. *Environment and Ecology* 6: 532-533.

Bhaumik, U., P.K. Pandit and J.G. Chatterjee. 1991. Impact of epizootic ulcerative syndrome on the fish yield, consumption and trade in West Bengal. *Journal of the Inland Fisheries Society of India* 23: 45-51.

Blaylock, R.B., R.M. Overstreet and M.A. Klich. 2001. Mycoses in red snapper (*Lutjanus eampechanus*) caused by two deuteromycete fungi (*Penicillium corylophilum* and *Cladosporium sphaerospennum*). *Hydrobiologia* 460: 221-228.

Blazer, V.S. and R.E. Wolke. 1979. An *Exophiala*-like fungus as the cause of a systemic mycosis of marine fish. *Journal of Fish Diseases* 2: 145-152.

Blazer, V.S., W.K. Vogelbein, C.L. Densmore, E.B. May, J.H. Lilley and D.E. Zwerner. 1999. *Aphanomyces* as a cause of ulcerative skin lesions of menhaden from Chesapeake Bay tributaries. *Journal of Aquatic Animal Health* 11: 340-349.

Blazer, V.S., J.H. Lilley, W.B. Schill, Y. Kiryu, C.L. Densmore, V. Panyawachira and S. Chinabut. 2002. *Aphanomyces invadans* in Atlantic menhaden along the East Coast of the United States. *Journal of Aquatic Animal Health* 14: 1-10.

Boonyaratpalin, S. 1989. Bacterial pathogens involved in the epizootic ulcerative of fish in Southeast Asia. *Journal of Aquatic Animal Health* 1: 272-276.

Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England.

Bowater, R.O., A. Thomas, R.G. Shivas and J.D. Humphrey. 2003. Deuteromycotic fungi infecting barramundi cod, *Cromileptes altivelis* (Valenciennes), from Australia. *Journal of Fish Diseases* 26: 681-686.

Bruno, D.W. and D.J. Stamps. 1987. Saprolegniasis of Atlantic salmon, *Salmo salar* L., fry. *Journal of Fish Diseases* 10: 513-517.

Bruno, D.W. and B.P. Wood. 1999. *Saprolegnia* and other Oomycetes. In: *Fish Diseases and Disorders*, P.T. Woo and D.W. Bruno (eds.). First Edition. CABI Publishing, Wallingford, Oxon, U.K., Vol. 3, *Viral, Bacterial and Fungal Infections*, 874 pp.

Buckland, F., S. Walpole and A. Young. 1880. *Report on the Disease which has Recently Prevailed Among the Salmon of the Tweed, Eden, and Other Rivers in England and Scotland.* Her Majesty's Stationery Office, London, 125 pp.

Burkholder, J.M., E.J. Noga, C.W. Hobbs, H.B. Glasgow Jr. and S.A. Smith. 1992. New 'phantom' dinoflagellate, causative agent of major estuarine fish kills. *Nature (London)* 358: 407-410.

Burkholder, J.M., H.B. Glasgow, Jr. and C.W. Hobbs. 1995. Fish kills linked to a toxic ambush-predator dinoflagellate: Distribution and environmental conditions. *Marine Ecology Progress Series* 124: 43-61.

Burton, T.O., T.R. Meyers, N.S. Starkey and J.E. Follet. 2004. Experimental transmission of the fungus *Phoma herbarum* to Chinook salmon. *Journal of Aquatic Animal Health* 16: 251-257.

Callinan, R.B. 1994. A comparative review of *Aphanomyces* species associated with epizootic ulcerative syndrome, red spot disease and mycotic granulomatosis. In: *Proceedings of the ODA Regional Seminar on Epizootic Ulcerative Syndrome*, 25–27 January 1994. R.J. Roberts, I. McRae and B. Campbell (eds.), The Aquatic Animal Health Research Institute, Bangkok, pp. 248-252.

Callinan, R.B., G.C. Fraser and J.L. Virgona. 1989. Pathology of red spot disease in sea mullet, *Mugil cephalus* L., from eastern Australia. *Journal of Fish Diseases* 12: 467-479.

Campbell, R.E., J.H. Lilley, Y. Taukhid, V. Panyawachira and S. Kanchanakhan. 2001. *In vitro* screening of novel treatments for *Aphanomyces invadans*. *Aquaculture Research* 32: 223-233.

Carballo, M., M.J. Munoz, M. Cuellar and J.V. Tarazona. 1995. Effects of waterborne copper, cyanide, ammonia, and nitrite on stress parameters and changes in susceptibility to saprolegniosis in rainbow trout *(Oncorhynchus mykiss)*. *Applied and Environmental Microbiology* 61: 2108-2112.

Carmichael, J.W. 1966. Cerebral mycetoma of trout due to *Phialophora*-like fungus. *Sabouraudia* 6: 120-123.

Catap, E.S. and B.L. Munday. 2002. Development of a method for reproducing epizootic ulcerative syndrome using controlled doses of *Aphanomyces invadans* in species with different salinity requirements. *Aquaculture* 209: 35-47.

Chinabut, S. 1998. Epizootic ulcerative syndrome: Information up to 1997. *Fish Pathology* 33: 321-326.

Chinabut, S., R.J. Roberts, G.R. Willoughby and M.D. Pearson. 1995. Histopathology of snakehead, *Channa striatus* (Bloch), experimentally infected with the specific *Aphanomyces* fungus associated with epizootic ulcerative syndrome (EUS) at different temperatures. *Journal of Fish Diseases* 18: 41-47.

Coker, W.C. 1923. *The Saprolegniaceae, with Notes on other Water Molds*. University of North Carolina Press, Chapel Hill, NC. 201 pp. (reprinted in 1969 as Vol. 20 *Bibliotheca Mycologica*, J. Cramer, Lehre, Germany).

Coopera, J.A., J.M. Pillinger and I. Ridge. 1997. Barley straw inhibits growth of some aquatic saprolegniaceous fungi. *Aquaculture* 156: 157-163.

Council of the European Communities. 1990. Council Regulation No. 2377/90/EEC laying down a community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Official Journal of the European Communities* No L224/18.

Council of the European Communities. 1996. Council Directive No. 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC., *Official Journal of the European Communities* No L125/10.

Cross, M.L. and L.G. Willoughby. 1989. Enhanced vulnerability of rainbow trout (*Salmo gairdneri)* to *Saprolegnia* infection following treatment of the fish with an androgen. *Mycology Research* 93: 379-402.

Cruz-Lacierda, E.R. and M. Shariff. 1994. The hematological changes in snakehead (*Ophicephalus striatus*) affected by epizootic ulcerative syndrome. In: *Proceedings of the Third Asian Fisheries Forum*, L.M. Chou, A.D. Munro, T.J. Lam, T.W. Chen, L.K.K. Cheong, J.K. Ding, K.K. Hooi, H.W. Khoo, V.P.E. Phang, K.F. Shim and C.H. Tan (eds.). Asian Fisheries Society, Manila, Philippines, pp. 361-364.

Culp, S.J. 2004. NTP toxicology report on toxicology studies on malachite green chloride and leucomalachite green. US Department of Health and Human Services, National Toxicology Program, NIH Publication 04-04416, 101pp. (http://ntp-server.niehs.nih.gov /ntp/htdocs /ST_rpts /tox071.pdf).

David, J.C. and P.M. Kirk. 1997. Index of Fungi. IMI. CABI Publishing, Wallingford, Oxon, U.K.

Davis, H.S. and E.C. Lazar. 1941. A new fungus disease of trout. *Transactions of American Fisheries Society* 70: 264-271.

De Hoog, G.S. 1977. The black yeasts and allied hyphomycetes. *Studies in Mycology*, Centraalbureau voor Schimmelcultures, Baarn, 15: 1-140.

Deepa, B., G.S. Bisht, R.D. Khulbe and D. Bisht. 2000. *Fusarium*—a new threat to fish population in reservoirs of Kumaun, India. *Current Science* 78: 1241-1245.

Dick, M.W. 1995. Sexual reproduction in the Peronosporomycetes (chromistan fungi). *Canadian Journal of Botany* 73: S714-S724.

Dick, M.W. 1999. Classification of the Peronosporomycetes. In: *Encyclopaedia of Food Microbiology*, R. Robinson, C. Batt and P. Patel (eds.). Academic Press, London, pp. 871-882.

Diéguez-Uribeondo, J., L. Cerenius and K. Söderhäll. 1996. Physiological characterization of *Saprolegnia parasitica* isolates from brown trout. *Aquaculture* 140: 247-257.

Doty, M.S. and D.W. Slater. 1946. A new species of *Heterosporium* pathogenic on young chinook salmon. *American Midland Naturalist* 36: 663-665.

Duran, A., L.B. Rodriguez Aparicio, A. Reglero and J. Perez-Diaz. 1987. Changes in the serum enzymes of *Saprolegnia*-infected brown trout, *Salmo trutta* L. *Journal of Fish Diseases* 10: 505-507.

Dykstra, M.J., E.J. Noga, J.F. Levine, D.W. Moye and J.H. Hawkins. 1986. Characterization of the *Aphanomyces* species involved with ulcerative mycosis (UM) in menhaden. *Mycologia* 78: 664-672.

Egusa, S. 1992. *Infectious Diseases of Fish*. Balkema, Rotterdam.

Egusa, S. and N. Masuda. 1971. A new fungal disease in *Plecoglossus altevelis*. *Fish Pathology* 6: 41-46. (In Japanese).

Egusa, S. and T. Ueda. 1972. A *Fusarium* sp. associated with Black Gill Disease of the Kuruma prawn, *Penaeus japonicus* Bate. *Bulletin of the Japanese Society of Scientific Fisheries* 38: 1253-1260.

Elkan, E. and C.M. Philpot. 1973. Mycotic infections in frogs due to a *Phialophora*-like fungus. *Sabouraudia* 11: 99-105.

Fijan, N. 1969. Systemic mycosis in channel catfish. *Bulletin of the Wildlife Diseases Association* 5: 109-110.

Fisher, W.S., E.H. Nilson, J.F. Steenbergen and D.V. Lightner. 1978. Microbial diseases of cultured lobsters: a review. *Aquaculture* 14: 115-140.

Food and Agricultural Organisation of the United Nations (FAO) 1986. Report of the expert consultation on ulcerative fish diseases in the Asia-Pacific region. (TCP/RAS/4508). Bangkok, August 1986. FAO, Regional Office for Asia and the Pacific, Bangkok.

Foster, F.J. and L. Woodbury. 1936. The use of malachite green as a fish fungicide and antiseptic. *Progressive Fish Culturist* 18: 7-9.

Fraser, G.C., R.B. Callinan and L.M. Calder. 1992. *Aphanomyces* species associated with red spot disease: an ulcerative disease of estuarine fish from eastern Australia. *Journal of Fish Diseases* 15: 173-181.

Fregeneda Grandes, J.M., M. Fernández Díez and J.M. Aller Gancedo. 2000. Ultrastructural analysis of *Saprolegnia* secondary zoospore cyst ornamentation from infected wild brown trout, *Salmo trutta* L., and river water indicates two distinct morphotypes among long spined isolates. *Journal of Fish Diseases* 23: 147-160.

Gaikowski, M.P., J.J Rach, J.J. Olson, R.T. Ramsay and M. Wolgamood. 1998. Toxicity of hydrogen peroxide treatments to rainbow trout eggs. *Journal of Aquatic Animal Health* 10: 241-251.

Gardner, M.L.G. 1974. Impaired osmoregulation in infected salmon, *Salmo salar* L. *Journal of Marine Biological Association, Plymouth* 54: 635-639.

Hargens, A.R. and M. Perez. 1975. Edema in spawning salmon. *Journal of Fisheries Research Board of Canada* 32: 2538-2541.

Hargis, W.H. 1985. Quantitative effects of marine diseases on fish and shellfish populations, *Transactions of North American Wildlife Nature Research Conference* 50: 608-640.

Hatai, K. and S. Egusa. 1977. Studies on visceral mycosis of salmonid fry. II. Characteristics of fungi isolated from the abdominal cavity of amago salmon fry. *Fish Pathology* 11: 187-193.

Hatai, K. and G.I. Hoshiai. 1993. Characteristics of two *Saprolegnia* species isolated from coho salmon with saprolegniosis. *Journal of Aquatic Animal Health* 5: 115-118.

Hatai, K. and S.S. Kubota. 1989. A visceral mycosis in cultured Masu Salmon *(Oncorhynchus masou)* caused by a species of *Ochroconis*. *Journal of Wildlife Diseases* 25: 83-88.

Hatai, K. and L.G. Willoughby. 1988. *Saprolegnia parasitica* from rainbow trout inhibited by a bacterium *Pseudomonas fluorescens*. *Bulletin of European Association of Fish Pathologists* 8: 27-29.

Hatai, K., Y. Fujimaki, S. Egusa and Y. Jo. 1986. A visceral mycosis in ayu fry, *Plecoglossus altivelis* Temminck and Schlegel, caused by a species of *Phoma*. *Journal of Fish Diseases* 9: 111-116.

Hatai, K., L.G. Willoughby and G.W. Beakes. 1990. Some characteristics of *Saprolegnia* obtained from fish hatcheries in Japan. *Mycological Research* 94: 182-190.

Horter, H. 1960. *Fusarium* als erreger einer hautmykose bei karpfen. *Zeitschrift Parastic Kunde* 20: 355-358.

Howe, G.E. and G.R. Stehly. 1998. Experimental infection of rainbow trout with *Saprolegnia parasitica*. *Journal of Aquatic Animal Health* 10: 397-404.

Hughes, G.C., G.J. Mueller and J.J. Choi. 1994. Bibliography *Saprolegnia* in salmon. pp. 211-253. In: *Salmon Saprolegniasis*, G.J. Mueller (ed.). Report to Bonneville Power Administration, Contract No. 1990BP02836, Project No. 199006100, 266 (BPA Report DOE/BP-02836-1), 269pp. Available electronically at http://www.efw.bpa.gov/Publications/A02836-1.pdf

Johnson, R.A., J. Zabrecky, Y. Kiryu and J.D. Shields. 2004. Infection experiments with *Aphanomyces invadans* in four species of estuarine fish. *Journal of Fish Diseases* 27: 287-295.

Johnson, T.W., R.L. Seymour and D.E. Padgett. 2000. *Biology and Systematics of the Saprolegniaceae*. Available on line as part of the University of North Carolina's Ilumina digital library http://dl.uncw.edu. Contents page for this treatise is at: http://dl.uncw.edu/digilib/biology/fungi/taxonomy%20and%20systematics/padgett%20book

Khan, M.H., L. Marshall, K.D. Thompson, R.E. Campbell and J.H. Lilley. 1998. Susceptibility of five fish species (Nile tilapia, rosy barb, rainbow trout, stickleback

and roach) to intramuscular injection with the oomycete fish pathogen, *Aphanomyces invadans*. *Bulletin of the European Association of Fish Pathologists* 18: 192-197.

Kanouse, B.B. 1932. A physiological and morphological study of *Saprolegnia parasitica*. *Mycologia* 24: 431-452.

Kiryu, Y., J.D. Shields, W.K. Vogelbein, E.D. Zwerner, H. Kator and V.S. Blazer. 2002. Induction of skin ulcers in Atlantic menhaden by injection and aqueous exposure to the zoospores of *Aphanomyces invadans*. *Journal of Aquatic Animal Health* 14: 11-24.

Lategan, M.J., F.R. Torpy and L.F. Gibson. 2004a. Biocontrol of saprolegniosis in silver perch *Bidyanus bidyanus* (Mitchell) by *Aeromonas media* strain A199. *Aquaculture* 235: 77-88.

Lategan, M.J., F.R. Torpy and L.F. Gibson. 2004b. Control of saprolegniosis in the eel *Anguilla australis* Richardson by *Aeromonas media* strain A199. *Aquaculture* 240: 19-27.

Lategan, M.J., W. Booth, R. Shimmon and L.F. Gibson. 2006. An inhibitory substance produced by *Aeromonas media* A199, an aquatic probiotic. *Aquaculture* 254: 115-124.

Lauckner, G. 1984. Agents: Fungi. In: *Diseases of Marine Animals*, O. Kinne (ed.). Vol. IV, Part 1, Pisces. Biologische Station Helgoland, Hamburg, Germany, pp. 89-113.

Lilley, J.H. and S. Chinabut. 2000. DNA-based studies on *Aphanomyces invadans*, the fungal pathogen of epizootic ulcerative syndrome (EUS). In: *DNA-based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation of the Detection of Aquatic Pathogens and Diseases*, P. Walker and R. Subasinghe (eds.). Food and Agriculture Organization of the United Nations Fisheries Technical Paper No. 395, FAO, Rome, Italy, pp. 83-87. [http://www.fao.org/DOCREP/005/X4946E/X4946E00.HTM].

Lilley, J.H. and V. Inglis. 1997. Comparative effects of various antibiotics, fungicides and disinfectants on *Aphanomyces invaderis* and other saprolegniaceous fungi. *Aquaculture Research* 28: 461-469.

Lilley, J.H. and R.J. Roberts. 1997. Pathogenicity and culture studies comparing Aphanomyces involved in epizootic ulcerative syndrome (EUS) with other similar fungi. *Journal of Fish Diseases* 20: 101-110.

Lilley, J.H., D. Hart, R.H. Richards, R.J. Roberts, L. Cerenius and K. Söderhäll. 1997. Pan-Asian spread of single fungal clone results in large-scale fish kills. *Veterinary Record* 140: 653-654.

Lilley, J.H., R.B. Callinan, S. Chinabut, S. Kanchanakhan, I.H. MacRae and M.J. Phillips. 1998. *Epizootic Ulcerative Syndrome (EUS) Technical Handbook*. The Aquatic Animal Health Research Institute, Bangkok. 88 pp.

Lilley, J.H., D. Hart, V. Panyawachira, S. Kanchanakhan, S. Chinabut, K. Söderhäll and L. Cerenius. 2003. Molecular characterization of the fish-pathogenic fungus *Aphanomyces invadans*. *Journal of Fish Diseases* 26: 263-275.

Lio-Po, G.D., L.J. Albright and E.V. Alapide-Tendencia. 1992. *Aeromonas hydroplilla* in the epizootic ulcerative syndrome (EUS) of snakehead, *Ophicephalus striatus*, and catfish, *Clarias batrachus*: Quantitative estimation in natural infection and

experimental induction of dermonecrotic lesion. In: *Diseases in Asian Aquaculture* I. M. Shariff, R.P. Subasinghe and J.R. Arthur (eds.). Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp. 461-474.

Lio-Po, G.D., L.J. Albright, G.S. Traxler and E.M. Leano. 2001. Pathogenicity of the epizootic ulcerative syndrome (EUS)-associated rhabdovirus to snakehead *Ophicephalus striatus*. *Fish Pathology* 36: 57-66.

Marking, L.L., J.J. Rach and T.M. Schreier. 1994. Search for Antifungal Agents in Fish Culture. pp. 131-148. In: *Salmon Saprolegniasis*, G.J. Mueller (ed.). Report to Bonneville Power Administration, Contract No. 1990BP02836, Project No. 199006100, 266 (BPA Report DOE/BP-02836-1), 269pp. Available electronically at http://www.efw.bpa.gov/Publications/A02836-1.pdf

McGinnis, M.R. and L. Ajello. 1974. A new species of *Exophiala* isolated from channel catfish. *Mycologia* 66: 518-520.

McKay, D.L. 1967. *Saprolegnia diclina Humphrey as a parasite of the salmonid, Oncorhynchus kisutch*. M.S. Thesis. University of British Columbia, Vancouver, Canada.

McKenzie, R.A. and W.T.K. Hall. 1976. Dermal ulceration of mullet (*Mugil cephalus*). *Australian Veterinary Journal* 52: 230-231.

Meyer, F.P. and T.A. Jorgenson. 1984. Teratological and other effects of malachite green on development in rabbits and rainbow trout. *Transaction of American Fisheries Society* 112: 818-824.

Miles, D.J.C., K.D. Thompson, J.H. Lilley and A. Adams. 2003. Immunofluorescence of the epizootic ulcerative syndrome pathogen, *Aphanomyces invadans*, using a monoclonal antibody. *Diseases of Aquatic Organisms* 55: 77-84.

Miyazaki, T. and S. Egusa. 1972. Studies on mycotic granulomatosis in freshwater fish I. Mycotic granulomatosis in goldfish. *Fish Pathology* 7: 15-25. (In Japanese).

Miyazaki, T. and S. Egusa. 1973a. Studies on mycotic granulomatosis in freshwater fish II. Mycotic granulomatosis prevailed in goldfish. *Fish Pathology* 7: 125-133. (In Japanese).

Miyazaki, T. and S. Egusa. 1973b. Studies on mycotic granulomatosis in freshwater fish III. Bluegill. Mycotic granulomatosis in bluegill. *Fish Pathology* 8: 41-43. (In Japanese).

Miyazaki, T., S.S. Kubota and F. Yashiro. 1977. Studies on visceral mycosis of salmonid fry–I. Histopathology. *Fish Pathology* 11: 183-186.

Molina, F.I., S.C. Jong and G. Ma. 1995. Molecular characterization and identification of *Saprolegnia* by restriction analysis of genes coding for ribosomal RNA. *Antonie van Leeuwenhoek* 68: 65-74.

Mueller, G.J. 1994. Salmon Saprolegniasis. Report to Bonneville Power Administration, Contract No. 1990BP02836, Project No. 199006100, 266 (BPA Report DOE/BP-02836-1), 269pp. Available electronically at http://www.efw.bpa.gov/Publications/A02836-1.pdf

Murphy, T.M. 1981. The use of chemosterilants to lower the frequency of skin fungal infection amongst precocious male 1+ Atlantic salmon parr *Salmo salar* L. *Journal of Fish Diseases* 4: 387-395.

Neish, G.A. 1976. *Observations on the pathology of Saprolegniasis of Pacific salmon and on the identity of the fungi associated with this disease.* Ph.D. Dissertation. University of British Columbia, Vancouver, Canada.

Neish, G.A. and G.C. Hughes. 1980. *Fungal Diseases of Fishes.* T.F.H Publications, Inc., Neptune City, NJ.

Noga, E.J. 1993 Water mould infections in fresh water fish: Recent advances. *Annual Reviews of Fish Diseases* 3: 291-304.

Noga, E.J. and M.J. Dykstra. 1986. Oomycete fungi associated with ulcerative mycosis in menhaden, *Brevoortia tyrannus* (Latrobe). *Journal of Fish Diseases* 9: 47-53.

Noga, E.J., L. Khoo, J.B. Stevens, Z. Fan and J.M. Burkholder. 1996. Novel toxic dinoflagellate causes epidemic disease in estuarine fish. *Marine Pollution Bulletin* 32: 219-224.

O'Bier, A.H. 1960. *A study of the aquatic phycomycetes associated with diseased fish and fish eggs.* Ph.D. Dissertation. Virginia Polytechnic Institute, Blacksburg, VA.

Office International des Epizooties (OIE) 2006. *Manual of Diagnostic Tests for Aquatic Animals* 2006, OIE, Paris, 469 pp.

Oseid, D.M. 1977. Control of fungus growth on fish eggs by *Asellus militaris* and *Gammarus pseudolimnacus. Transactions of the American Fisheries Society* 106: 192-195.

Phadee, P., O. Kurata, K. Hatai, I. Hirono and T. Aoki. 2004. Detection and identification of fish-pathogenic *Aphanomyces piscicida* using Polymerase Chain Reaction (PCR) with species-specific primers. *Journal of Aquatic Animal Health* 16: 220-230.

Pickering, A.D. 1977. Seasonal changes in the epidermis of the brown trout *(Salmo trutta* (L.). *Journal of Fish Biology* 10: 561-566.

Pickering, A.D., L.G. Willoughby and C.B. McGrory. 1979. Fine structure of secondary zoospore cyst cases of isolates from infected fish. *Transactions of British Mycological Society* 72: 427-436.

Pottinger, T.G. and J.G. Day. 1999. A *Saprolegnia parasitica* challenge system for rainbow trout: assessment of Pyceze as an anti-fungal agent for both fish and ova. *Diseases of Aquatic Organisms* 36: 129-141.

Pottinger, T.G. and A.D. Pickering. 1985. The effects of 11-ketotesterone and testosterone on the skin structure of brown trout, *Salmo trutta* L. *General and Comparative Endocrinology* 59: 335-342.

Rach, J.J., S. Redman, D. Bast and M.P. Gaikowski. 2005. Efficacy of hydrogen peroxide versus formalin treatments to control mortality associated with saprolegniasis on lake trout eggs. *North American Journal of Aquaculture* 67: 148-154.

Ragan, M.A., C.L. Goggin, R.A. Cawthorn, L.A. Cerenius, A.V. Jamieson, S.M. Plourde, T.A. Rand, K. Söderhäll and R.R. Gutell. 1996. A novel clade of protistan parasites near the animal–fungal divergence. *Proceedings of the National Academy of Sciences of the United States of America* 93: 11907-11912.

Rand, T.G., L. Bunkley-Williams and E.H. Williams. 2000. A hyphomycete fungus, *Paecilomyces tilacinus,* associated with wasting disease in two species of tilapia from Puerto Rico. *Journal of Aquatic Animal Health* 12: 149-156.

Richards, R.H., A. Holliman and S. Helgasson. 1978. *Exophiala salmonis* infection in Atlantic salmon. *Journal of Fish Diseases* 1: 357-368.

Richards, R.H. and A.D. Pickering. 1978. Frequency and distribution patterns of *Saprolegnia* infection in wild and brown trout *Salmo trutta* L. and char *Salvelinus alpinus* (L.). *Journal of Fish Diseases* 1: 69-82.

Roberts, R.J. 1997. Epizootic ulcerative syndrome (EUS): Progress since 1985. In: *Diseases in Asian Aquaculture* IV, T.W. Flegel and I.H. MacRae (eds.). Fish Health Section, Asian Fisheries Society, Manila, Phillippines, pp. 125-128.

Roberts, R.J., L.G. Willoughby and S. Chinabut. 1993. Mycotic aspects of epizootic ulcerative syndrome (EUS) of Asian fishes. *Journal of Fish Diseases* 16: 169-183.

Roberts, R.J., B. Campbell and I.H. MacRae. 1994. *Proceedings of the Regional Seminar on Epizootic Ulcerative Syndrome, Jan. 1994*. The Aquatic Animal Health Research Institute, Bangkok.

Ross, A.J. and W.T. Yasutake. 1973. *Scolecobasidium humicola*, a fungal pathogen of fish. *Journal of the Fisheries Research Board of Canada* 30: 994-995.

Ross, A.L., W.T. Yasutake and S. Leek. 1975. *Phoma herbarum*, a fungal plant saprophyte as a fish pathogen. *Journal of the Fisheries Research Board of Canada* 32: 1648-1652.

Roy, R.Y., R.S. Dwivadi and R.R. Mishra. 1962. Two new species of *Scolecobasidium* from soil. *Lloydia* 25: 164-166.

Salvin, S.B. 1941. Comparative studies on the primary and secondary zoospores of the Saprolegniaceae. 1. Influence of temperature. *Mycologia* 33: 592-600.

Schaumann, K. and K. Priebe. 1994. *Ochroconis humicola* causing muscular black spot disease of Atlantic salmon (*Salmo salar*). *Canadian Journal of Botany* 72: 1629-1634.

Schreier, T.M., J.J. Rach and G.E. Howe. 1996. Efficacy of formalin, hydrogen peroxide, and sodium chloride on fungal-infected rainbow trout eggs. *Aquaculture* 140: 323-331.

Scott, W.W. and A.H. O'Bier. 1962. Aquatic fungi associated with diseased fish and fish eggs. *Progressive Fish Culturist* 24: 3-15.

Seymour, R.L. 1970. The genus *Saprolegnia*. *Nova Hedwigia* 19: 1-124.

Sindermann, C.J. 1988. Epizootic ulcerative syndromes in coastal/estuarine fish. *NOAA Technical Memorandum* NMFS-F/NEC-54.

Steffens, W., U. Leider, D. Nehring and H.-W. Hattop. 1961. Moglicheiten und Gefahren der Anwendung von Malachitgriin in der Fischerei. *Zeitschrift Fischerei* 10: 745-771.

Stueland, S., K. Hatai and I. Skaar. 2005. Morphological and physiological characteristics of *Saprolegnia* spp. strains pathogenic to Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 28: 445-453.

Tiffney, W.N. 1939a. The host range of *Saprolegnia parasitica*. *Mycologia* 31: 310-321.

Tiffney, W.N. 1939b. The identity of certain species of the Saprolegniaceae parasitic to fish. *Journal of Elisha Mitchell Scientific Society* 55: 134-151.

Tonguthai, K. 1985. A preliminary account of the ulcerative fish diseases in the Indo-Pacific region (a comprehensive study based on Thai experiences). FAO, Bangkok, Thailand. 39 pp.

Vandersea, M.W., R.W. Litaker, B. Yonnish, E. Sosa, J.H. Landsberg, C. Pullinger, P. Moon-Butzin, J. Green, J.A. Morris, H. Kator, E.J. Noga and P.A. Tester. 2006. Molecular assays for detecting *Aphanomyces invadans* in ulcerative mycotic fish lesions. *Applied and Environmental Microbiology* 72: 1551-1557.

Vishwanath, T.S., C.V. Mohan and K.M. Shankar. 1997. Clinical and histopathological characterization of different types of lesions associated with epizootic ulcerative syndrome (EUS). *Journal of Aquaculture in the Tropics* 12: 35-42.

Vishwanath, T.S., C.V. Mohan and K.M. Shankar. 1998. Epizootic ulcerative syndrome (EUS), associated with a fungal pathogen, in Indian fishes: Histopathology—'A cause for invasiveness'. *Aquaculture* 165: 1-9.

Vogelbein, W.K., J.D. Shields, L.W. Haas, K.S. Reece and D.E. Zwerner. 2001. Skin ulcers in estuarine fishes: a comparative pathological evaluation of wild, and laboratory exposed fish. *Environmental Health Perspectives* 109: 687-693.

Wada, S., K. Yuasa, S. Rha, K. Nakamura and K. Hatai. 1994. Histopathology of *Aphanomyces* infection in dwarf gourami *(Colisa lalia)*. *Fish Pathology* 29: 229-237.

Wada, S., S.A. Rha, T. Kondoh, H. Suda, K. Hatai and H. Ishii. 1996. Histopathological comparison between ayu and carp artificially infected with *Aphanomyces piscicida*. *Fish Pathology* 31: 71-80.

Wada, S., C. Hanjavanit, O. Kurata and K. Hatai. 2005. *Ochroconis humicola* infection in red seabream *Pagrus major* and marbled rockfish *Sebastiscus marmoratus* cultured in Japan. *Fisheries Science* 71: 682-684.

Waterstrat, P.R. and L.L. Marking. 1995. Clinical evaluation of formalin, hydrogen peroxide, and sodium chloride for the treatment of *Saprolegnia parasitica* on fall chinook salmon eggs. *Progressive Fish Culturist* 57: 287-291.

Werth, G. and A. Boiteaux. 1967. The toxicology of the triphenylmethane dyestuff, malachite green, as an uncoupler of oxidative phosphorylation *in vivo* and *in vitro*. *Archives Toxicology* 23: 82-103.

Whisler, H.C. 1996 *Identification of Saprolegnia spp. pathogenic in Chinook Salmon*, Final Report, Bonneville Power Administration, DOE/BP-02836-2, 58 pp.

White, D.A. 1975. Ecology of an annual *Saprolegnia* sp. (Phycomycete) outbreak in wild brown trout. *Verhanslungen der Internationalen Vereinigung fuer Theoretische und Angewandte Limnologie* 19: 2456-2460.

Willoughby, L.G. 1989. Continued defence of salmonid fish against Saprolegnia fungus, after its establishment. *Journal of Fish Diseases* 12: 63-67.

Willoughby, L.G. and R.J. Roberts. 1994. Improved methodology for isolation of the *Aphanomyces* fungal pathogen of epizootic ulcerative syndrome (EUS) in Asian fish. *Journal of Fish Diseases* 17: 541-543.

Willoughby, L.G., A.D. Pickering and H.G. Johnson. 1984. Polycell-gel assay of water for spores of Saprolegniaceae (fungi). Especially those of the *Saprolegnia* pathogen of fish. *Hydrobiologia* 113: 237-248.

Willoughby, L.G., R.J. Roberts and S. Chinabut. 1995. *Aphanomyces invaderis* sp. nov., the fungal pathogen of freshwater tropical fish affected by epizootic ulcerative syndrome. *Journal of Fish Diseases* 18: 273-275.

Wood, J.W. 1974. *Diseases of Pacific Salmon—Their Prevention and Treatment*. 2nd Edition. Department of Fisheries, Hatcheries Division, State of Washington.

Wood, S.E. and L.G. Willoughby. 1986. Ecological obsevations on the fungal colonization of fish by Saprolegniaceae in Windermere. *Journal of Applied Ecology* 23: 737-749.

Wood, S.E., L.G. Willoughby and G.W. Beakes. 1988. Experimental studies on uptake and interaction of spores of the complex with external mucus of brown trout (*Salmo trutta*). *Transactions of British Mycological Society* 90: 63-73.

Yanong, R.P.E. 2003. Fungal diseases of fish. *Veterinary Clinics of North America—Exotic Animal Practice* 6: 377-400.

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CHAPTER

# 7

# Microsporidia

**Jiří Lom**

## INTRODUCTION

Microsporidia are strictly intracellular, eukaryotic parasites characterized by unicellular spores containing one sporoplasm and an elaborate extrusion apparatus serving to inject the infective germ, the sporoplasm, through a hollow polar tube into the cell of a new host. The proliferative stages may be multinucleate and possess a very primitive structure; true mitochondria are missing throughout the life cycle. Food uptake is accomplished by membrane transport. Many of the genera parasitic in fish enter a complicated coexistence with their host cell, inducing a special type of hypertrophy and turning it into a structure called xenoma. Early analysis of the SSU rDNA placed microsporidia at the base of the eukaryote phylogenetic tree. Recent research indicates, however, that they are close to fungi or even belong to fungi. They have the smallest genomes among eukaryotes (in two fish microsporidian species examined thus far, it is 6.2 and 19.5 Mbp), prokaryote-like ribosomes and they miss

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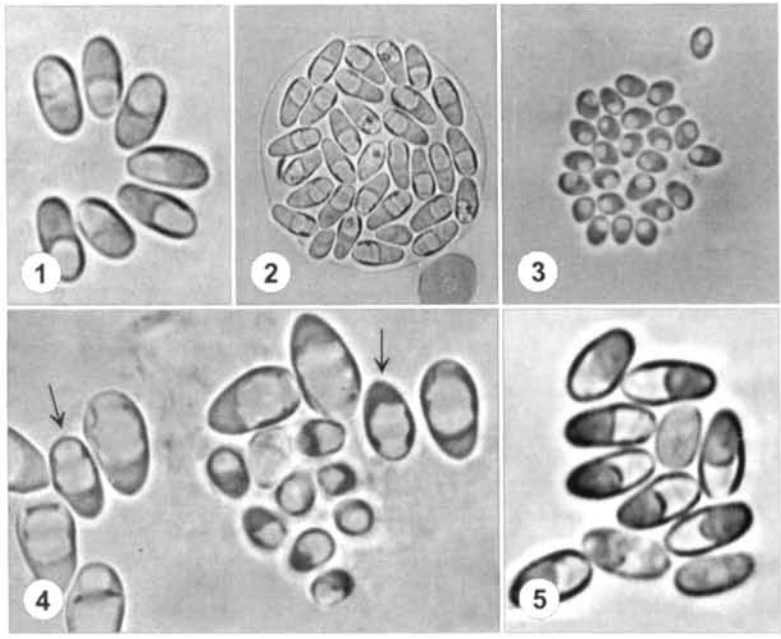
well-developed eukaryotic cell organelles. These features are explained by their highly specialized intracellular lifestyle. They are parasites of almost all-animal phyla, being most abundant in arthropods and fish (and are associated with AIDS-debilitated humans). Microsporidia enjoy increasing attention as biologically extremely intriguing objects, and as deleterious pathogens causing diseases in animals and humans.

As of December 2007, there have been about 100 microsporidian species infecting fish which belong to 16 genera, in addition to 93 findings ranged to the collective group *Microsporidium*, or classified provisionally as unidentified species of one of the genera. The catalogue of microsporidia known to infect fish was published by Lom (2002). Earlier, comprehensive treatises of fish hosts and microsporidian species were presented by Canning and Lom (1986), Lom and Dyková (1992) and Dyková (1995). A recent overall information on Microsporidia can be found in Wittner and Weiss (1999). Ultrastructure and identification have been reviewed by Larsson (1986, 1999). Microsporidia exert detrimental action in stocks of feral commercial fish; one of the first reports on such damage is by Fischtal (1944). They may also reduce growth and fecundity of affected fish, decrease the marketing value of the catch and impair swimming ability, thus, exposing the host fish to higher predation.

At present, the main interest in microsporidia as fish pathogens is directed to prevent the economic losses they cause in aquacultures of both food and ornamental fish. High density of fish in cultures, a certain degree of stress that such cultures involve, and direct transmission of these parasites favour the infection. Cultures of food fishes such as salmonids, *Sparus aurata*, *Anguilla japonica* or *Plecoglossus altivelis* often suffer from microsporidioses and so do ornamental fishes like neon tetras, zebrafish and angelfish. On the other hand, the large supply of infected fishes and their easy availability make the study of the morphology, biology and host parasite relations of these fascinating parasites of fish quite easy. Thus, a great part of facts reported below is based on studies of microsporidia from captive fishes.

## STRUCTURE

Spores of species infecting fish range in size between 2 μm (*Nucleospora*) and about 12 μm (macrospores of *Ovipleistophora mirandellae*), and are usually ellipsoid or egg-shaped (Figs. 7.1–7.5). Observed in light microscope, they are refractile and feature a voluminous, lucent posterior




**Figs. 7.1 to 7.5** Fresh spores of fish microsporidia; magnification × 1.200. **7.1.** *Glugea anomala*. **7.2.** *Ovipleistophora mirandellae*; spores within the thin membrane of sporophorous vesicle. **7.3.** *Kabatana arthuri*. **7.4.** Micro-, macro- and medium- sized (arrows) spores of *Heterosporis schuberti*. **7.5.** *Loma branchialis*.

vacuole. They have an imperforate, resistant and double-layered spore wall up to about 0.3 μm thick, with an outer, thin proteinaceous layer (exospore) and a thick, chitinous inner layer (endospore). An innermost simple cell membrane envelops the spore content.

The intricate extrusion apparatus is constituted by the polar tube, polaroplast and posterior vacuole (Fig. 7.6). In quiescent spores, the polar tube is inserted into the base of a laminar structure, shaped like a mushroom cap, i.e., the anchoring disc (= the polar cap of light microscopy) situated at the anterior apex of the spore. From there, the tube extends obliquely backwards to eventually make a single or double coil along the walls of the posterior part of the spore. The tube has, in its transverse section, ring-like layers around a central core and lies in a membranous sheath, acting as a sleeve through which the tube glides when extruding.

The proximal part of the tube, often wider and more rigid, is surrounded by the polaroplast, an organelle made up of a stack of thin

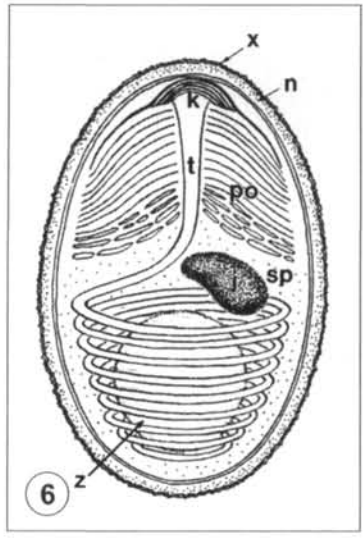



**Fig. 7.6** Diagram of microsporidian spore structure. x—exospore, n—endospore, k—anchoring disc of the polar tube t, po—polaroplast, sp—sporoplasm, i—nucleus, z—posterior vacuole.

membranes posteriorly loosened into layers of vesicles. Posterior vacuole, encircled by polar tube turns, plays an important role in the extrusion mechanism and sometimes contains an inclusion. In fish microsporidia, the posterior vacuole is quite voluminous, occupying often even two thirds (e.g., in *Ovipleistophora ovariae*) of the spore length.

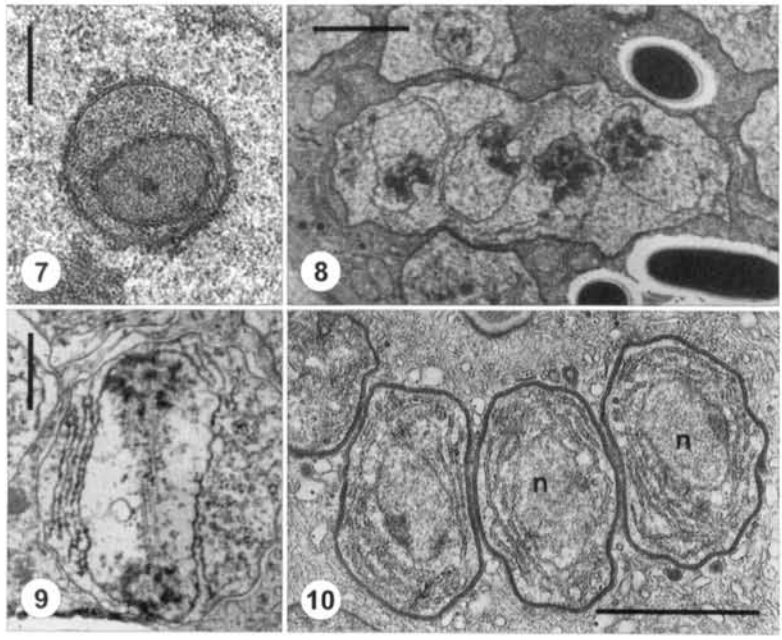
The sporoplasm, the infective germ itself, situated in the biconcave space between the polaroplast and posterior vacuole, only contains some cisternae of endoplasmic reticulum and free ribosomes, often in form of polyribosomes arranged in wide ribbons. There is either a single nucleus or two nuclei, closely apposed with surfaces flattened along the place of contact to form a diplokaryon.

In the digestive tract, the polar tube discharges and injects the sporoplasm into the host cell. This spore hatching is followed by parasite proliferation, merogony, producing a great number of parasites; then the sporogony sets in, the process producing spores.

Microsporidia develop in the cytoplasm of the host cell, exceptionally within its nucleus. Merogony stages—uninucleate (Fig. 7.7) or multinucleate meronts (Fig. 7.8) dividing to produce daughter meronts—are poorly endowed with cell organelles, restricted to rough endoplasmic

reticulum, free ribosomes, various vesicles of undetermined nature and mitosomes, i.e., remnants of the once present mitochondria (Williams and Keeling, 2005). In most fish microsporidia, all life cycle stages have a single, isolated nucleus; a diplokaryon is an exception. Nuclear division is a cryptomitosis, taking place within an intact nuclear envelope (Fig. 7.9). Meronts lie mostly in direct contact with the host cell cytoplasm, sometimes within an envelope of host rough endoplasmic reticulum. An exception is the genus *Heterosporis*, in which there is a thick envelope around all developmental stages, growing along with proliferation of the parasite. As a rule, meronts have a thin plasmalemma, but in *Pleistophora* they have a thick envelope. There may be one or several generations of meronts. Multinucleate meronts may reach up to 30 μm.

Sporogony is initiated by deposition of a dense coat on the surface of plasmalemma of the meront (Fig. 7.10). Grown sporonts are generally multinucleate; in fact, they are sporogonial plasmodia, and may be located within a cavity. This is delimited by a membrane, which is either of host




**Figs. 7.7 to 7.10** Electron micrographs. **7.7** Early meront of *Nucleospora secunda*, bar = 500 nm. **7.8.** Elongate, multinucleate meront of *Loma acerinae*, bar = 2 nm. **7.9.** Cryptomitosis in a sporoblast mother cell of *Loma acerinae*, bar = 1 nm. **7.10.** Sporonts of *Kabatana takedai*, produced by constriction of an elongated meront, bar = 1 mm.

cell origin (i.e., parasitophorous vacuole) or is produced by the parasite (i.e., sporophorous vesicle—SPV). The SPV may be merontogenic if it appears already on late meronts, or sporontogenic if it is only formed on sporonts. Sporogonial plasmodia divide in various ways, by plasmotomy by repeated segmentation, by serial constriction of an elongated plasmodium, or by budding off individual cells which eventually turn directly into the sporoblasts. In other cases, a sporoblast mother cell is produced first and then divides again into two sporoblasts. Sporoblasts mature into spores; a primitive Golgi appears, which is associated with the origin of the polar tube. In some species spores of different sizes are produced in the form of smaller microspores and larger macrospores. Sometimes there may be spores of intermediate sizes.

Exceptional sporogenesis occurs in *Nucleospora*, where the foundations of the extrusion apparatus already appear in the sporogonial plasmodium before its cleavage into sporoblasts (Fig. 7.46).

There exist only few reports on the length of the cycle. In experimental infections with *Kabatana takedai*, the cycle was completed on day 13 (Awakura, 1974; Urawa, 1989). In *Heterosporis anguillarum*, Tsui *et al.* (1988) found that at 25°C merogony was completed after 5 days, while sporogony took 17-25 days.

## LIFE CYCLE

The life cycle starts when the spore is ingested. The most intriguing part of the cycles is spore hatching. It is the most sophisticated infection mechanism in biology, yet to be sufficiently elucidated. In the digestive tract of the fish, under a proper stimulus (this can be artificially simulated by the action of various chemicals such as hydrogen peroxide, various acids or alkalis), water enters the spore and a considerable osmotic pressure builds up inside (Undeen and Frixione, 1990). It has been suggested (Frixione *et al.*, 1997) that aquaporine proteins in the plasma membrane may facilitate the influx of water into the spore. The achieved pressure is associated with the swelling of the polaroplast, especially of the posterior vacuole, which is quite instrumental in the process and contains key peroxisomal enzymes (Findley *et al.*, 2005). The pressure expels the tube explosively through the apical, thinnest points of the spore shell. The tube turns inside out (Lom and Vávra, 1963), and the spore contents, the sporoplasm with the nucleus are ejected instantaneously through the tube into a newly formed membranous sac at the tip. The everting filament,

driven by a great osmotic force, is solid and stiff enough to puncture any host cell in its path and injects the sporoplasm into it, leaving an empty spore shell behind.

The further fate of the sporoplasm in the host was only followed in species of several genera. It has been generally supposed that macrophages (Weissenberg, 1968) or neutrophils (Bekhti and Bouix, 1985; Canning and Lom, 1986; Pekkarinen and Lom, 1999) are the first sites of infection for *Glugea* spp. Consequently, merogonial proliferation may presumably start in the cells that were initially infected. Sanchez *et al.* (2001b) have discovered that *Loma salmonae* migrates from mucosal epithelium to the lamina propria of the intestine before reaching the final destination in the gills. The dividing merogony stages were by then already detected within infected blood cells in the heart, thus, proving the haematogenous spread by the infected blood cells. Transportation and dissemination via blood cells has also been found in *Tetramicra brevifilum* (Matthews and Matthews, 1980). The transport cells were suggested to be intraepithelial lymphocytes, T cells or migratory cells such as monocytes. These cells perhaps phagocytize the parasite in lamina propria of the intestine, or become directly infected by injection of the sporoplasm.

Having reached the final target cell, the parasite may implement one of the two strategies. Some proliferate within the host cell until it is eventually replete with mature spores. The cell may, hereby, be more or less distended, and eventually disintegrates and breaks open unless cells of the host reaction unsheathe it. However, most microsporidia infecting fish stimulate the infected cell to enormous hypertrophy. Such formations with completely changed cell structure are termed xenoparasitic complexes or xenomas and have mostly the size of 0.1–0.3 mm; they may rarely exceed 1 cm in size. The term was coined in the early works of Chatton (1920) and Weissenberg (1949). In the xenomas, along with the cell, also the nucleus is hypertrophic (Fig. 7.11). It may be located in the centre of the cell, branched into numerous projections, or it may be segmented into a number of fragments which sometimes constitute a peripheral network. The cytoplasm shows a variety of cell structures not seen in the original cell. The plasmalemma of the cell may bear fine microvilli or is covered by a thick wall of various structure, laminar or granular. It may also be covered with host collagen fibrils and fibroblasts.

Along with the parasite, which undergoes merogony and sporogony inside it, the xenoma forms a physiologically and morphologically integrated whole with its own development in the host at the expense of

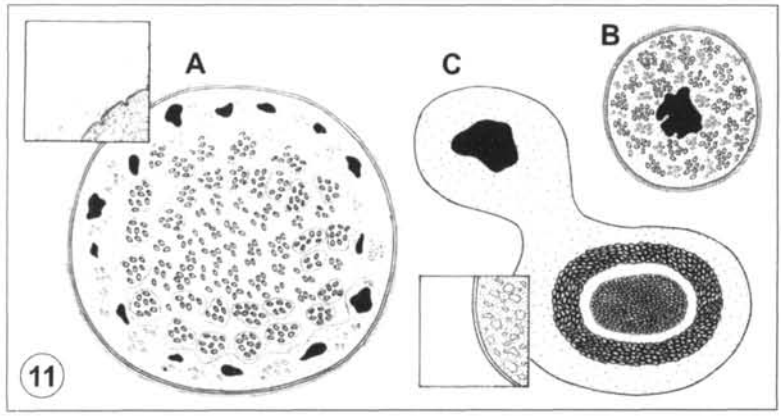



**Fig. 7.11** Diagrams of various types of xenomas produced by fish microsporidia. A: *Glugea anomala*—developmental stages of the parasite are restricted to the periphery, as well as the fragments of the hypertrophic cell nucleus; SPV and spores are found in the centre of xenoma. Xenoma wall covering the plasma membrane has a laminated structure (inset). B: *Loma acerinae*—there is a single central hypertrophic nucleus, developmental stages are mixed throughout the xenoma cytoplasm with groups of mature spores. C: *Spraguea lophii*—the parasite develops in one part of the cell only, however, the whole neurone and its nucleus are hypertrophic. The parasite mass consists of a central agglomeration of slender *Nosema*-type spores, surrounded by a layer of large *Nosemoides*-type spores. The xenoma wall is a simple cell membrane (inset). (Modified from Lom and Dyková, 1992).

which it grows. It has been generally accepted that xenoma offers optimal conditions for the growth of the parasite, and simultaneously protects it against the immune system of the host. At the same time, the isolated parasite's free spread in the host organism is supposed to be made impossible. These assumptions may be rather inaccurate, since it is also known that spores may discharge their polar tubes and sporoplasms across the xenoma wall and thus infect further cells of the host (e.g., in *Loma acerinae*—Lom and Pekkarinen, 1999, in *L. myrophis*—Matos *et al.*, 2003), and because as soon as the xenoma has terminated the growth and turned into a bag full of spores, it is attacked by the host's cell reaction (see below).

In *Loma salmonae*, merogony already starts in the transport cells. The journey of the cell with already dividing cells or merozoites ends in the gills in the vascular space between the pillar cells (Rodríguez-Tovar *et al.*, 2003b). Then, either the pillar cell phagocytizes the parasite from the leukocyte and converts into a xenoma, or the extensions of the pillar cell retract to make space for the leukocyte, which turns into a xenoma itself.

Another possibility is that the leukocyte hosting the merozoites transmigrates through extravascular spaces and succeeds in reaching the connective tissue and forms xenomas in the gill filament. Other leukocytes stay confined between the endothelium and basement membrane after having exited from the blood vessel (Rodríguez-Tovar *et al.*, 2003b).

To what extent the infected transport cell may eventually turn into the xenoma in microsporidian species-forming xenomas remains to be known.

The as yet proven final target cells of fish microsporidia that form xenomas are macrophages (also acting as transport cells), neutrophil leukocytes, pillar cells in the gills, myocytes, ganglion cells, and most probably also connective tissue cells.

The period for which a fish remains infected is for most species not safely known. For *Loma salmonae* it is >80 days at 10°C (Shaw and Kent, 1999), in infections of internal organs the infection persists as long as the spores are not destroyed by host defence reaction, which in some, e.g., *Spraguea lophii*, may be quite a long period.

## TRANSMISSION

In water environment, spores may stay viable for months—various authors state between 100 days and one year. New infections are, as a rule, contracted perorally, by way of spore ingestion. However, not always does the peroral infection succeed and the reasons are not obvious; one of the factors required might be a stress situation rendering the immune system inefficient. In heavy infections, infectious spores may be discharged through the skin ruptures or may be liberated from the infected gills. In intestinal infections, they may be released with faeces, or they get into the water after the death of the host from disintegrated tissues. Such horizontal transmission is, thus, realized without physical contact with the infectious fish. Intermediate hosts are yet to be proven. Crustaceans may accumulate viable spores in their digestive tract and act as transport or paratenic hosts and were reported to be important for transmission especially in *Glugea* infections.

Trans-ovarial transmission has been suggested in some species (e.g., *Pleistophora hyphessobryconis*—Lom and Dyková, 1992, *Loma salmonae*—Docker *et al.*, 1997) but has not yet been safely proven, although Summerfelt (1972) postulated trans-ovarial transmission in

*Ovipleistophora ovariae* by demonstrating the microsporidian in blastulas. Auto-infection, i.e., hatching of spores inside the host in which they developed, may be a natural process and has been documented in xenoma-forming microsporidians (e.g., Rodríguez-Tovar *et al.*, 2003a; Lom and Dyková, 2005), which happens both by discharging sporoplasm through the xenoma wall and by spread of spores from ruptured xenomas. It has been equally suspected in *Pleistophora* infections (Nigrelli, 1946) to explain massive infections rather than suppose migration of merozoite stages. Experimentally, microsporidia can easily be transmitted intraperitoneally, intramuscularly, intravascularly or by anal instillation (Shaw and Kent, 1999). In some cases, fish resisted to various methods of experimental infection (e.g., *Kabatana takedai*—Fujiyama *et al.*, 2002), being, however, susceptible to 'infectious' river water; the authors suggested that an unknown infectious stage was present. Lee *et al.* (2004) proved an exceptional way of transmission: spores of *Glugea plecoglossi* could infect *Oncorhynchus mykiss* through the skin at places of skin abrasion. The released sporoplasms were found passing from epidermis to muscle layer even after six hours.

The development of infection may be temperature dependent, e.g., in *Glugea stephani,* the parasite is absent and cannot thrive in waters at temperatures below 15°C (Olson, 1981). Under suitable temperature conditions, *G. stephani* can be cycled experimentally in *Pseudopleuronectes americanus* (Cali *et al.*, 1986). The development of *Loma salmonae* in *Oncorhynchus mykiss* proceeds quicker at higher (19°C) temperature and hence, also, the fish are faster cleared of the infection (Becker and Speare, 2004a).

## EFFECT ON THE HOST

Microsporidian infection can damage the host in many ways. It can destroy fish cells and tissues, inflict retarded growth, decrease the fecundity and induce parasitic castration, can disfigure, cripple, debilitate fishes and, finally, cause their death. Macroscopic lesions due to microsporidian infection are often quite conspicuous when inspecting fish surface or while dissecting the infected fish (Figs. 7.12–7.15).

Cell and tissue reactions to microsporidia vary, depending on whether the parasite forms xenomas or whether its spread throughout the tissue is not limited by these formations.

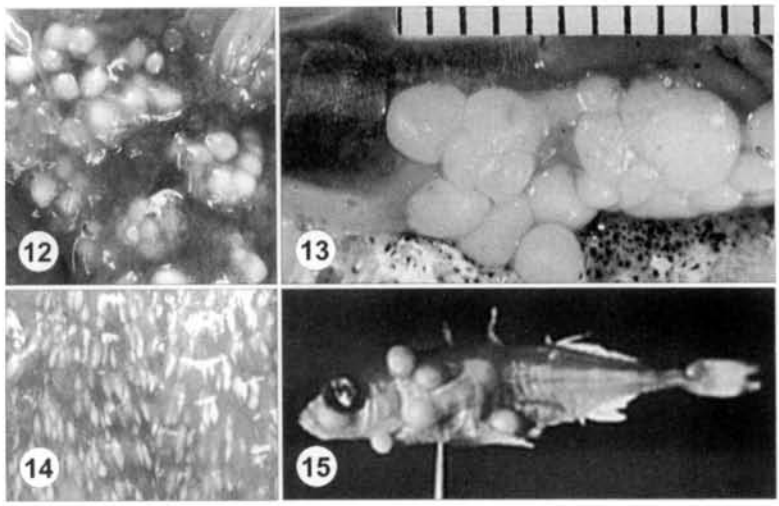



**Fig. 7.12** Oocytes of *Abramis brama* infected with *Ovipleistophora mirandellae*, × 2.
**Fig. 7.13** Xenomas of *Glugea hertwigi* seen in opened body cavity of *Osmerus mordax*, with a mm scale.
**Fig. 7.14** Foci of *Kabatana takedai* in muscles of *Oncorhynchus mykiss*, × 3.
**Fig. 7.15** Stickleback *Gasterosteus aculeatus* with subcutaneous xenomas of *Glugea anomala*. (Modified from Lom and Dyková, 1992.)

Tissue reaction to xenomas differs according to the stage of xenoma development (Dyková and Lom, 1978, 1980), i.e., we can observe the sequence of a weakly reactive stage, a productive stage and granuloma involution.

The weakly reactive stage is associated with young xenomas, which still lack a mature thick wall, being covered with a thin cell membrane and uniformly filled with meronts. At this stage, a noticeable cell reaction is absent. The surrounding host tissue is injured by pressure atrophy only. This may promote proliferation of the connective tissue, which forms a layer investing the xenoma.

The productive stage is elicited by a fully developed, large xenoma encased with a thick wall in which spore production proceeds, and later, in which nothing is left of the original host cell cytoplasm and the xenoma is replete with spores. Mature xenomas excite proliferative inflammation, the beginning of which is associated with changes in the xenoma wall. They may differ according to the structure of the wall. In *Glugea*-type wall swelling sets on and later fibroblasts appear wedged between the stacks of lamellae constituting the wall, which results in complete destruction of

the wall. Then the xenoma content is invaded by newly formed granulation tissue constituted by proliferating fibroblasts, histiocytes and newly formed capillaries. Thus, the xenoma is gradually turned into a granuloma, with a diminishing spore mass inside it (Figs. 7.16–7.20). PAS positive material and calcium deposits indicate dystrophic changes within the centre of the xenoma. In some xenoma infections, in addition to fibroblasts, a capsule composed of eosinophilic granular cells was reported to form around the xenoma (Reimschuessel *et al.*, 1987).

The granuloma involution stage occurs when the spore mass is eliminated by phagocytosis. The granulation tissue matures, the granuloma gradually diminishes and a gradual separation of the tissue lesion takes place.

From its first stage the tissue reaction to xenomas is directed toward the isolation and destruction of the parasite and, quite often, a complete repair is achieved.

This is the general picture seen in xenoma infections, but there may be some differences. In *Spraguea lophii*, there is also exudative proliferative inflammation around the xenoma situated within the ganglion cell and in *Ichthyosporidium giganteum*, the epitheloid cells are typical of the proliferative inflammation stage.

Tissue reactions to microsporidia, which do not elicit xenoma formation, have been studied mainly in muscle-infecting *Pleistophora* species. The cell contents of the infected cell are gradually replaced by the proliferating parasite until, eventually, it is replaced completely and the cell is destroyed. There is no remarkable cell hypertrophy and only a minor cell infiltration between the myosepta. The parasite may proliferate in different ways.

The parasite may multiply in one mass until the volume of the muscle fibre is completely filled by closely packed spores still within the sarcolemma. Only then the productive stage of inflammation is reached, the fibre is enveloped by connective tissue and granulomas arise. They are often of giant size and later subject to necrotic changes. No regeneration of destroyed muscle fibre takes place. This is the case of, e.g., *Pleistophora macrozoarcidis* (Fig. 7.21). Rarely, the presence of numerous foci of spores, cell infiltration and associated granulomas may be manifested as liquefaction of the skeletal muscle, as reported by Pulsford and Matthews (1991) in *Trisopterus esmarkii* infected by an unidentified *Pleistophora* species.

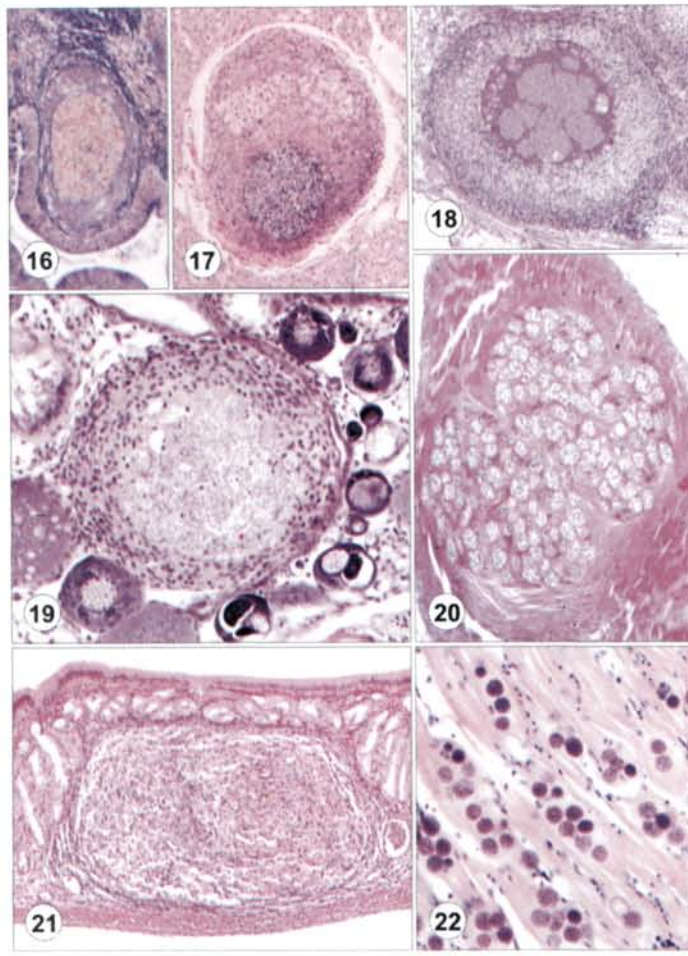



**Fig. 7.16** Proliferating granulation tissue in *Loma acerinae* xenoma stained by Masson's trichrome, × 120.

**Fig. 7.17** *Tetramicra brevifilum* xenoma in the liver of *Scophthalmus maximus*, transformed into a granuloma. H&E, × 150.

**Fig. 7.18** Xenoma of *Spraguea lophii* in a neuron of *Lophius piscatorius*, partly transformed into a granuloma. H&E, × 220.

**Fig. 7.19** Granuloma in the ovary of *Nothobranchius rubripinnis* replacing a *Glugea anomala* xenoma. H&E, × 250.

**Fig. 7.20** Lesion with stages of *Pleistophora macrozoarcidis* in the muscle bundle of *Macrozoarces americanus*, H&E, × 350.

**Fig. 7.21** Granulomatous lesion replacing *Glugea anomala* xenoma in the glandular part of the stomach wall in *Gasterosteus aculeatus*. H&E, × 220.

**Fig. 7.22** Islets with sporophorous vesicles of *Pleistophora hyphessobryconis* in muscle fibers of neon tetra. H&E, × 200. (From Lom and Dyková, 1992, with the permission of *Folia Parasitologica*.)

In *P. hyphessobryconis*, the microsporidian forms isolated islets in the sarcoplasm (Fig. 7.22), each parasite group being surrounded by a 'halo' of disintegrated sarcoplasm. These islets proliferate, but the muscle fibre is destroyed well before all its volume is replaced by the parasite. Granulomas with phagocytized spores from the destroyed muscle are found outside the muscle.

## DIAGNOSTICS

The oldest and most simple method is the direct detection of parasites. External symptoms like whitish cyst-like structures on the surface or, upon dissection, inside the fish suggest microsporidian infection, which can be confirmed by microscopical proof of spores. When fresh, their large posterior vacuole differentiates them easily from possibly similar structures like yeast or fat droplets. Spores or developmental stages can also be detected by systematic inspection of fresh tissue mounts or stained histological sections.

For staining of fixed spores in smears or sections, several methods can be used. Periodic acid-Schiff reaction (PAS) for detection of polysaccharides reveals the characteristic purple-red granule, the polar cap, at the anterior end of the spore. The spores are easily distinguished by Giemsa stain, toluidine blue stain and especially by tissue Gram stain. The spores are Gram positive, at variance with other protozoan cysts or spores, and even single spores become easily visible in smears or sections against an unstained background. Brown and Hopps modification (Luna, 1968) demonstrates them deep violet-purple. For various other methods see Weber and Schwartz (1999). In some cases, the inspection of ultra thin section in the electron microscope is helpful, e.g., in *Nucleospora* infections.

Especially convenient is the use of fluorescent dyes. These are chemofluorescent optical-brightening agents, actually chitin stains requiring observation in an ultraviolet microscope. Using the correct wavelength, the chitinous wall of mature microsporidian spores fluoresces brightly, facilitating the detection of spores (Lee *et al.*, 2004). However, small fungal spores may also fluoresce. There are many optical brighteners, e.g., Calcofluor White 2MR (American Cyanamid Corp., Princeton, N.J.), Fungi-Fluor Kit (Polysciences, Inc., Warrington, Pa.) or Uvitex 2B or 213 (Ciba Geigy, Basel, Switzerland) (see Garcia, 2002). Calcofluor White was also used for detection of *Nucleospora salmonis* (Guzmán *et al.*, 2001).

Recently, more sophisticated molecular methods of detection include the rapid *in situ* hybridization technique (ISH), which have the advantage of being able to reveal the parasite even immediately after its entry into the fish tissue. The ISH protocols with DIG-labelled oligonucleotide probes are designed from SSU rRNA of the given microsporidian species. The method is more suitable to detect the presporogenic developmental stages rather than mature spores, so that combination with fluorescent dyes is recommended (Lee *et al.*, 2003). The process of ISH can be completed within 2 hours and is, thus, quite convenient to use.

## PREVENTION AND TREATMENT

Removal and disinfection of spores can limit the spread of infection, wherever it is applicable. In lakes, this can be done by drying boats and tools used in fishing and by proper disposal of infected fish (burial, incineration). In aquaria, proper disinfection of the contents and instruments is quite feasible.

In salmonid hatcheries—and perhaps also elsewhere—ultraviolet light control of horizontal transmission can be implemented. Incoming water (2 l $min^{-2}$) in the circulation system exposed to UV light at an estimated 283 500 $\mu W\ s^{-1}\ cm^{-2}$ was made safe of infectious spores (Becker and Speare, 2004b).

Very few chemicals have been used to control fish microsporidioses, and none of these has yet been introduced as a routine procedure. The most widely used drug is fumagillin, originally used to treat *Nosema apis* in honeybees, recently used against human microsporidioses and also applied against myxosporidioses (available from AG Scientific, Biochemical Manufacturers and from Fermentek, Israel). It was reported to be effective against *Heterosporis anguillarum* in eels (Kano *et al.*, 1982), against *Nucleospora salmonis* in *Oncorhynchus tshawytscha* (Hedrick *et al.*, 1991) and against *Loma salmonae* in the same host (Kent and Dawe, 1994). The chemical is administered with the feed; usually the drug, soluble in alcohol, is mixed with alcohol, sprayed on the feed, which is then coated with oil. The dose recommended generally for salmonids is 3 to 10 mg of fumagillin per kg of fish, but Shaw and Kent (1999) were successful in controlling the infection with only 2 or 4 mg of fumagillin per kg of fish per day.

An analog of fumagillin, TNP-470 (Takeda Chemical Industries Ltd.) administered orally at 1.0 or 0.1 mg of drug per kg of fish per day for six

weeks reduced markedly the infections of salmons with *Loma salmonae* and *Nucleospora salmonis* (Shaw and Kent, 1999).

There are a few isolated reports on other drugs. Nagel and Summerfelt (1977) succeeded to reduce infection of *Notemigonus chrysoleucas* with *Ovipleistophora ovariae* using oral treatment at 2.2 g of nitrofurazon per kg feed. Awakura and Kurahashi (1967) reported inhibition of merogony in *Kabatana takedai* by amprolium administered at 0.06% of body weight for up to 48 days. However, there were some toxic side effects.

Attempts with experimental vaccination met with partial success. *Oncorhynchus mykiss* juveniles exposed to low-virulence variant of *Loma salmonae*, when later challenged with *L. salmonae* spores of 'normal' virulence, resulted in xenoma number in gill filaments 14x lower than that observed in naive controls (Sanchez *et al.*, 2001a).

## *IN VITRO* CULTURE

This approach, although still in its infancy, is the most promising in what concerns possible discoveries on the life cycle and methods of chemical treatment. *Nucleospora salmonis* is the first fish microsporidian which was maintained *in vitro*, in lymphocyte cultures (Wongtavatchai *et al.*, 1995). Another one is a *Glugea* sp. (in fact, a *Microgemma* sp.?) from the liver of *Hyperoplus lanceolatus*, which was grown on mosquito *Aedes albopictus* larval cells, but failed to grow on salmon embryo cells (Lores *et al.*, 2003).

## DIVERSITY AND CLASSIFICATION

Fifteen genera (plus the collective group *Microsporidium*) with a total of 95 named and 93 provisionally classified species were described from fish. Ten named genera elicit xenoma formation in fish (E—species live in estuarine, F—in freshwater, M—in marine hosts): *Amazonspora* Azevedo and Matos, 2003 (E); *Glugea* Thélohan, 1891 (E, F, M); *Ichthyosporidium* Caullery and Mesnil, 1905 (M); *Loma* Morrison and Sprague, 1881 (M, F); *Microfilum* Faye, Toguebaye and Bouix, 1991 (M); *Microgemma* Ralphs and Matthews, 1986 (E, M); *Neonosemoides* Faye, Toguebaye and Bouix, 1996 (E); *Pseudoloma* Matthews, Brown, Larison, Bishop-Stewart and Kent, 2001 (F); *Spraguea* Vávra and Sprague, 1976 (M); *Tetramicra* Matthews and Matthews, 1980 (M). Assignment of three species from marine fish into the genus *Nosemoides* Vinckier, 1975 is obviously erroneous. Species of five genera do not elicit xenomas: *Heterosporis* Schubert, 1969 (F); *Kabatana* Lom, Dyková and Tonguthai, 2000 (F);

*Nucleospora* Hedrick, Groff and Baxa, 1991 (F); *Ovipleistophora* Pekkarinen, Lom and Nilsen, 2002 (F) and *Pleistophora* Gurley, 1893 (F, M). Collective group *Microsporidium* Balbiani, 1884 includes temporarily assigned xenoma- and non-xenoma forming species.

In this overview, microsporidian pathogens of fish are listed in groups complying with those in the phylogenetic tree of Lom and Nilsen (2003), set up according to the results of SSU rDNA sequence comparison. Identical groups are found in the microsporidian tree devised by Vossbrinck and Debrunner-Vossbrinck (2005). Only some of these groups fit into taxonomical family ranks of Sprague *et al.* (1992). In general, the kinship of microsporidian genera resulting from molecular analysis is in strong disagreement with taxonomic arrangement based on morphology.

**Group 1** (Family Pleistophoridae Doflein, 1901)

*Pleistophora, Heterosporis, Ovipleistophora.*

**Group 2** (Family Glugeidae Thélohan, 1892)

*Glugea*

**Group 3** (clade unidentifiable with previous classifications)

*Loma, Ichthyosporidium, Pseudoloma*

**Group 4** embraces the family Spragueidae Weissenberg, 1976, Tetramicridae Matthews and Matthews, 1980 and the genus *Kabatana*.

*Spraguea, Tetramicra, Microgemma*

**Group 5** (Family Enterocytozoonidae Cali and Owen, 1990)

*Nucleospora*

Species of other microsporidian genera from fish were not subjected to molecular analysis and thus they cannot be arranged into any phylogenetic tree.

## Group 1

Genus *Pleistophora* Gurley, 1893 includes species forming 6 to 200 spores within a firm SPV wall. SPVs with mature spores measure from 20 to 40 μm. Some species produce micro- and microspores, depending on the number of last divisions of the sporont. Meronts are multinucleate, generally rounded. All stages have isolated nuclei. The genus does not form xenomas; typically, the parasite spreads through myocyte cytoplasm. Eventually, muscle bundles may be replete with mature spores, appearing

like whitish parasite foci. There are 25 named species and 7 described as 'sp'.

*P. typicalis* Gurley, 1893 (type species of the genus), a rather common parasite, forms whitish spindle-shaped infection foci in skeletal muscles of *Myoxocephalus scorpius* from the Atlantic coasts of Europe. Each focus is a muscle bundle replete with SPVs containing mature spores (Fig. 7.23). Rare macrospores, 8 per SPV, average 7.5 × 3 μm in size; microspores, averaging 4.4 × 2.3 μm in size, are many per SPV averaging 23 μm. The extent of pathogenicity has not yet been studied in this species.

*P. ehrenbaumi* Reichenow, 1929 infects the musculature of wolf fishes, species promising for aquaculture, *Anarhichas lupus* and *A. minor* in the North Sea. Spores of two sizes are produced, micro- (3 × 1.5 μm) and macrospores (7.5 × 3.5 μm, both sizes refer to fixed spores). There are 4, 8, 16 or more spores per SPV. The foci of infection in the muscles may have different size. Mostly they are small, needle-like formations, but they can reach an enormous size. In heavy infections are then the muscle fibres replaced by a mass of SPVs, the infected fish exhibit giant tumour-like swellings of up to 15 × 8 cm beneath the skin (Egidius and Soleim, 1986). Such fishes are emaciated, suggesting a fatal prognosis. Meyer (1952) claimed the prevalence in Iceland waters to be 10%.

*P. hippoglossoides* Bosanquet, 1910 infects flatfish in the North Sea—*Drepanopsetta hippoglossoides*, *Hippoglossoides platessoides*, and *Solea solea*. The parasite is found in muscles of the trunk, body cavity and of fins. Heavily infected specimen display in their muscles whitish nodules up to 10 mm in size showing externally and projecting into the body cavity (Kabata, 1959). Elongate-ovoid or pyriform spores average 4.7 × 2.7 μm. Gross symptoms in infected fish make them unfit for human consumption.

*P. hyphessobryconis* Schäperclaus, 1941 is a pest of freshwater aquarium fish, distributed the world over, known mainly from neon and glowlight tetras, *Paracheirodon inessi* and *Hemigrammus erythrozonus*, but also infecting some 16 other hosts species, including goldfish. The parasite was obviously originally imported with ornamental fish from the upper Amazon River. There are 20 to 130 spores per SPV; spores are monomorphic, and the size averaging 6 × 4 μm. The parasite infects skeletal muscles (Fig. 7.24). In heavy infections, the parasite may spread to other body organs. In infected tetras the vivid colours fade or are lost altogether, areas of heavily infected muscle show through the skin as white patches. Each parasite within the muscle fibre is encircled by destroyed

sarcoplasm appearing as a palely stainable halo. Eventually, complete muscles are disintegrated and contain, in addition to host phagocytes, a mass of free spores and SPVs. A predilection site for free spores is subcutaneous tissue from where they penetrate the skin. This is the way in which the spores are released from fish still alive. Heavy infections are lethal; the symptomatic phase may last up to several weeks.

*P. macrozoarcidis* Nigrelli, 1946, infects the commercially important species *Macrozoarces americanus* in western North Atlantic. The parasite forms rare sausage-shaped macrospores up to 8 μm in length and ovoid microspores averaging 5.5 × 3.5 μm. Early infection shows as minute whitish cylinders about 0.5 mm long—advanced infection may appear as tumour-like masses—up to 8 cm, often recognizable as bulges on the body surface, full of mature spores. Eventually, the most seriously infected muscle fibers become hyalinized and destroyed, and what is left is granular cell debris full of spores' remnants. These infections had a bad prognosis for the host. The prevalence of infection, increasing with the age of the fish, reached 38% in some localities; this explains that in the forties of the past century this parasites contributed of the demise of the use of the host, the ocean pout, as commercially important fish (Nigrelli, 1946; Sheehy *et al.*, 1974).

Genus *Heterosporis* Schubert, 1969, includes species with merogony and sporogony proceeding within a firm envelope, the sporophorocyst wall, which grows around the parasite stages as they develop. Spores originate within a SPV, there are micro- and macrospores, the latter attain in size 7.8 to 8 μm and display a very large posterior vacuole. Four named species and two described as 'sp'.

The type species is *H. finki* Schubert, 1969, described from the ornamental fish *Pterophyllum scalare*. It was described as infecting connective cells of the oesophagus where it—at variance with other species of the genus—induces formation of small xenomas up to 0.3 μm in size with several enlarged nuclei around the sporophorocyst. Spores are ovoid, in most SPVs they occur as macrospores averaging 8 × 2.5 μm, some SPVs contain microspores averaging 3 × 1. 5 μm. Infection in myocytes is common, however, and there the nuclei are not markedly hypertrophic. The sporophorocysts, up to 50 μm, may pervade large segments of musculature. Serious infections cause emaciation; the muscles are reduced to creamy texture. Necrotic regions about 5 mm large appear on the surface of the body (Michel *et al.*, 1989).

*H. schuberti* Lom *et al.* (1989), is a parasite of ornamental fish *Pseudocrenilabrus multicolor* and *Ancistrus cirrhosus*. Infected myocytes are hypertrophied. Young meronts with one to three nuclei are rounded cells already within a thick sporophorocyst wall (Fig. 7.25). As the sporophorocyst grows—it may reach the size of 120 μm (Fig. 7.26)—the sarcoplasm of the myocytes in contact is affected—the myofibrils become radially oriented around its wall. Some SPVs harbour up to 27 broadly ovoid microspores averaging 4.2 × 2.9 μm (Fig. 7.4). The rest are spores ranging from medium-sized to macrospores, altogether averaging 7.1 × 3.9 μm. In macrospores, the posterior vacuole occupies 2/3 of the spore length and there are only 4 to 15 macrospores per SPV (Lom *et al.*, 1989). Severe infections of muscle tissue have been repeatedly reported from both hosts and the infection inflicted up to 95% mortalities in the latter host.

*H. anguillarum* (Hoshina, 1951) infects skeletal muscles of cultured Japanese eels, *Anguilla japonica*. Elongate ovoid macrospores with a huge posterior vacuole occupying 2/3 of the spore average 7.8 × 4.5 μm, microspores 3.5 × 2.4 μm (Lom *et al.*, 2002). Symptoms of the disease are small whitish spots under the skin and sometimes external swellings. The complete development from infection to mature spores takes 22 to 30 days at 25°C. Heavy infections may cause deformities and muscle liquefaction (T´sui and Wang, 1988) and may inflict considerable mortalities, less severe cases cause growth retardation or loss of market value.

*Heterosporis* sp. of Sutherland *et al.* (2003), infects myocytes of *Perca flavescens*, *Stizostedion vitreum* and *Esox lucius* in the lakes in Wisconsin and Minnesota (U.S.A.) with prevalence usually less than 15–30%. Experimental infection succeeded in six further hosts including *Perca fluviatilis*, *Cyprinus carpio*, *Carassius auratus*, *Ictalurus punctatus* or *Oncorhynchus mykiss*. SPVs in sporophorocysts contain 8 or 16 mature spores. Infected flesh is milky white in colour and granular in texture. Infection appears to originate just behind the head and spreads posteriorly. The appearance of the parasite has serious implications for baitfish harvest or for wild fish as forage for commercial fish (Sutherland *et al.*, 2003).

Genus *Ovipleistophora* Pekkarinen *et al.* (2002), has globular meront stages encased with a wide layer of tiny vesicles. Inside this layer a thick envelope (Fig. 7.27) envelops the meronts. This enveloping layer eventually breaks open to release cells, which develop into sporogonial plasmodia also having a thick envelope, the SPV wall. Inside it, polysporoblastic sporogony proceeds resulting in formation of a large

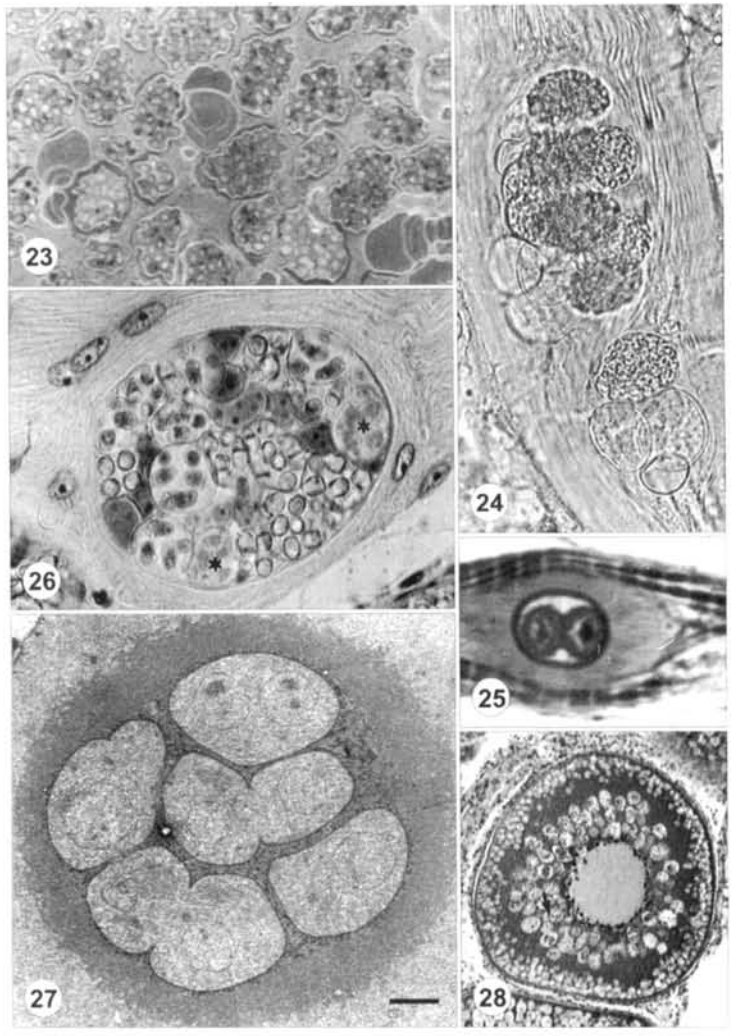



**Fig. 7.23** A mass of SPVs and dividing sporonts of *Pleistophora typicalis* in a degenerate muscle fibre of *Myoxocephalus scorpius*. × 500.

**Fig. 7.24** *P. hyphessobryconis*, agglomeration of SPVs and sporonts within a muscle fibre of goldfish. × 400.

**Figs. 7.25, 7.26** *Heterosporis schuberti* in stained tissue section. **7.25.** An initial stage, a meront dividing within a thick sporophorocyst envelope wedged in an area of altered cytoplasm, × 1900. **7.26.** A large sporophorocyst with mostly sporogony stages and SPVs with spores embedded in a muscle fibre, × 800.

**Figs. 7.27, 7.28** *Ovipleistophora mirandellae*. **7.27.** A group of meronts within the thick envelope from an oocyte of *Gymnocephalus cernuus*, electron micrograph, bar = 2 mm. **7.28.** Developing SPVs in an oocyte of *Blicca bjoerkna*, × 190.

**Figs. 7.23 to 7.26 and Fig. 7.28** (Modified from Lom and Dyková, 1992).

number of spores. As a rule, there are both micro- and macrospores. They are typically parasites of fish oocytes (Pekkarinen and Lom, 2002). There exist only two species.

*Ovipleistophora mirandellae* (Vaney and Conte, 1901) Pekkarinen *et al.*, 2002 (syn. *P. elegans* Auerbach, 1910, *P. longifilis* Schuberg, 1910, *P. oolytica* Weiser, 1949) infects gonads of freshwater fishes. It is more or less common in oocytes of European cyprinids *Abramis brama* (Fig. 7.12), *Alburnus alburnus*, *Gobio gobio*, *Leuciscus cephalus*, rarely in *Gymnocephalus cernuus*, *Esox lucius* and *Hucho hucho*. It was also found in the connective tissue in testis of *Rutilius rutilus* and species of the genus *Barbus*. There are SPVs with microspores (av. 3 × 1.5 μm), middle-sized spores (av. 4.8 × 2.7 μm) (about 100 spores per SPV) and macrospores, which average 9.7 × 4.2 μm; there are up to 60 spores per SPV (Fig. 7.2). Up to 10 to 20% follicles are infected, the oocytes being replete with parasite stages (Fig. 7.28). Oocytes are probably infected before the zona radiata is formed. The oocytes or their remnants are encircled by an inflammatory reaction of the proliferative granulomatous type, containing many phagocytized spores in the process of digestion. Such infections may result in reduced fecundity. In testes, the parasites thrive in the supporting connective tissue among the seminiferous tubules and they also develop in epithelial cells of seminiferous canaliculi and induce their hypertrophy, signalled already by Schuberg (1910). This obliterates the lumen of the canaliculi, which eventually fuse together while the surrounding tissue degenerates. Hypertrophic cells ultimately break down.

*Ovipleistophora ovariae* (Summerfelt, 1964) is a common parasite in ovaries of baitfish (*Notemigonus crysoleucas* and *Pimephales promelas*) in Midwestern and southern states and California in the USA, with overall prevalence of about 45%, reaching sometimes 100%. The prevalence varies with the year's season, being higher in May and June, the spawning time. The SPVs contain about 12 ovoid spores averaging 8.4 × 4.2 μm. Infected ovary appears mottled with white spots where the destroyed oocytes are clustered. Summerfelt and Warner (1970) described the pathogenesis. In the infected ovary, there is an influx of phagocytes that destroy the oocyte, atretic follicles are invaded by fibroblasts, and infected oocytes develop a stroma of connective tissue and increase in size. Although mortalities were not observed, the infection results in parasitic castration reducing the fecundity by over 40%, thus, affecting the bait fish

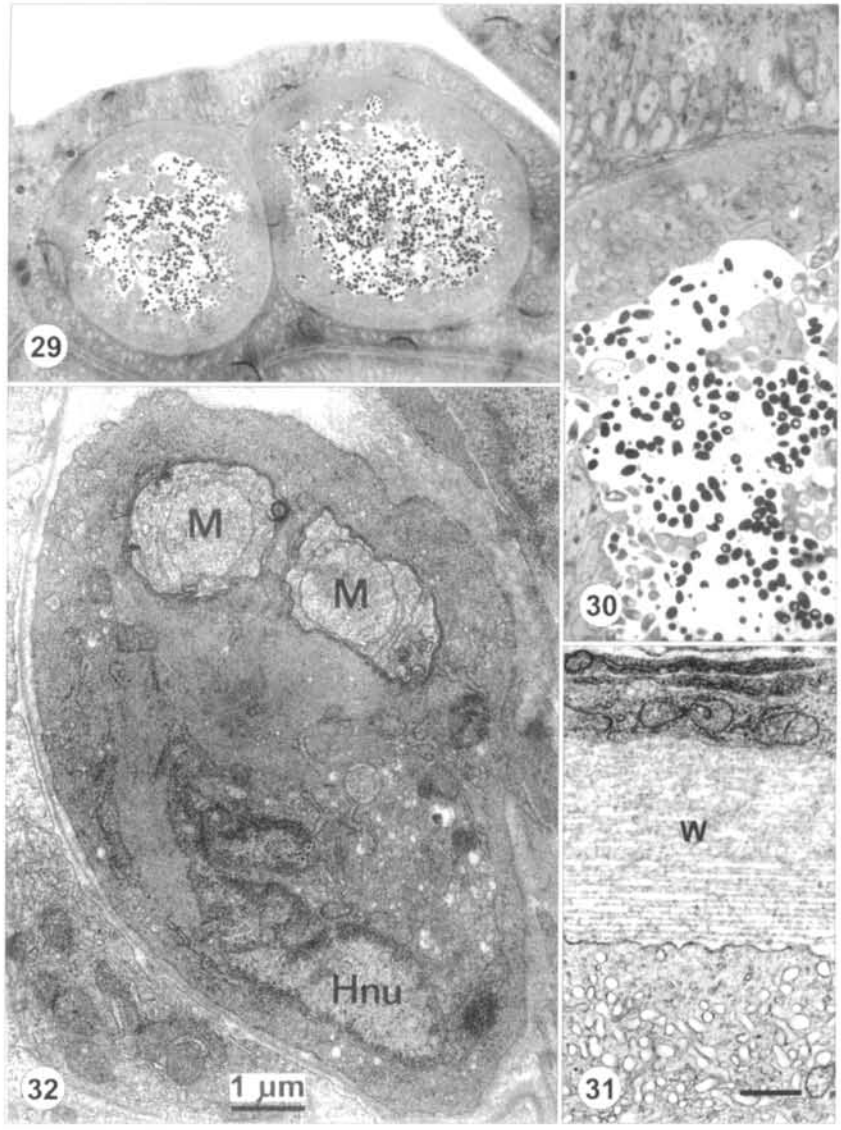
industry. Interestingly, infected fish are greater than the healthy ones, since nutrients used for egg production were used for faster growth.

## Group 2

Genus *Glugea* Thélohan, 1891. The species are characterized by a xenoma wall of laminar structure, formed by sloughed-off layers of the cell coat (Fig. 7.31). In mature xenomas, the developmental stages are confined to their periphery, while mature spores concentrate in the centre (Figs. 7.29, 7.30). Developmental stages are cylindrical or oval multinucleate meronts, enveloped by a cisterna of host endoplasmic reticulum. Sporogonial plasmodia develop inside SPVs with a membrane-like wall. In sporogony, plasmodia undergo multiple fission to produce up to 20 uninucleate sporoblast mother cells, which divide to produce two sporoblasts maturing into spores. Xenomas, from a fraction of mm up to 14 mm in size have widely branched host cell nuclei. There are 31 named species and 13 described as 'sp'.

G. *anomala* (Moniez, 1887) Gurley, 1893 (type species of the genus) is a parasite of little economic importance, but it became an important model for the research on fish microsporidians. It is a common, well-known parasite of freshwater and euryhaline sticklebacks *Gasterosteus aculeatus* and *Pungitius pungitius* in Eurasia and North America. The spores (Fig. 7.1) average 4.5 × 2.3 μm. The xenomas, located in connective tissues of almost all body organs, may reach the size of up to 3–4 mm. They may bulge from the surface; internal xenomas may distort the body wall. However, even fish heavily infected with large xenomas seem to tolerate the parasite well (Canning and Lom, 1986).

G. *atherinae* Berrebi, 1979, infects connective tissue of various body organs in the euryhaline host *Atherina boyeri* in brackish lagoons on the French Mediterranean coast with prevalence up to 21%. Xenomas of two size classes were reported—plentiful small ones, up to 0.2 mm in diameter, and large ones, up to 14 mm in size that count no more than four per one fish. Elongate ovoid spores average 5.7 × 2.9 μm. Intestinal obstruction, pressure atrophy may be a cause of concern in stocks of this commercially important smelt. Fish become infected as yearlings—symptoms are a conspicuously swollen body wall—and again in their second year of life. Mathieu-Daude *et al.* (1992) suggest that this species is a junior synonym of G. *anomala*.




**Figs. 7.29, 7.30** Maturing xenomas of *Glugea anomala* beneath the intestinal epithelium of a stickleback; semithin sections, toludine blue, × 250. **7.29.** Section of two xenomas. **7.30.** Periphery of xenoma with developmental stages of the parasite and fragments of host cell nucleus, × 800.

**Fig. 7.31** Laminar structure of *Glugea* xenoma wall; bar = 1 mm.

**Fig. 7.32** A very early xenoma of *Loma acerinae* in what probably was a neutrophil leucocyte, showing two meronts (M) and lobose host cell nucleus (Hnu).

G. *cepedianae* (Putz *et al.*, 1965) Canning and Lom, 1986, infects up to 65% of young shad, *Dorosoma cepedianum* in Ohio, USA. Xenomas of the size of up to 1 cm are located in the body cavity, sometimes protruding from the side of the fish; spores average 4.9 × 2.8 μm. Heavy infections are supposed to inflict losses in the infected shad stocks. Price (1986) studied prevalence.

G. *hertwigi* Weissenberg, 1911, is a microsporidian highly pathogenic for several holarctic estuarine smelts, e.g., *Osmerus eperlanus eperlanus* in Northern Europe and *O. e. mordax* in North America. In some regions, its prevalence may reach up to 100%. Elongate oval spores average 4.7 × 2.2 μm. Xenomas, located mainly in the intestine, measure mostly 2–4 mm (Fig. 7.13), rarely more. In severe infections, they may cause intestinal obstruction or may destroy the tissue completely and may occur in all organs. If such serious impairment of viability is combined with a stress situation, mass kills may occur, such as were known after the spawning season in Lake Erie in 1969, where a mass of smelts were washed ashore along a 120 km stretch (Nepszy and Dechtiar, 1972). Thus whole stocks of smelts may be lost.

G. *luciopercae* Dogel and Bykhovski, 1939, is found as a frequent parasite of *Stizostedion lucioperca* in freshwater and estuarine habitats in Europe and Asia. It forms elongate-oval spores averaging 4.5 × 2.1 μm. Minute xenomas, of 0.4 mm in size, with centrally located host cell nucleus are found in intestinal submucosa, rarely elsewhere in the body (Grupcheva and Lom, 1980). Heavy infections impair intestinal function and may inflict a serious situation on pikeperch. Revision of this species will probably result in its transfer into another genus.

G. *plecoglossi* Takahashi and Egusa, 1977, is a common parasite of cultured *Plecoglossus altivelis* in Japan, *Oncorhynchus mykiss* is susceptible to experimental infections. Elongate ellipsoidal spores average 5.8 × 2.1 μm in size. Xenomas up to 5 mm in size are found in all body organs and also appear as conspicuous bulges at the surface. There may be a considerable injury produced by the pressure atrophy in conjunction with the damage done by the host reaction and the parasite is a threat to cultures of the fish. G. *plecoglossi* is being used as an experimental model (e.g., Lee *et al.*, 2004).

G. *stephani* (Hagenmüller, 1899) is a common parasite of flatfish (*Pleuronectes flesus*, *P. platessa*, *Pseudopleuronectes americanus*, *Parophrys vetulus*), also occurs in *Limanda limanda*, *L. ferruginea*, *Liopsetta glacialis*,

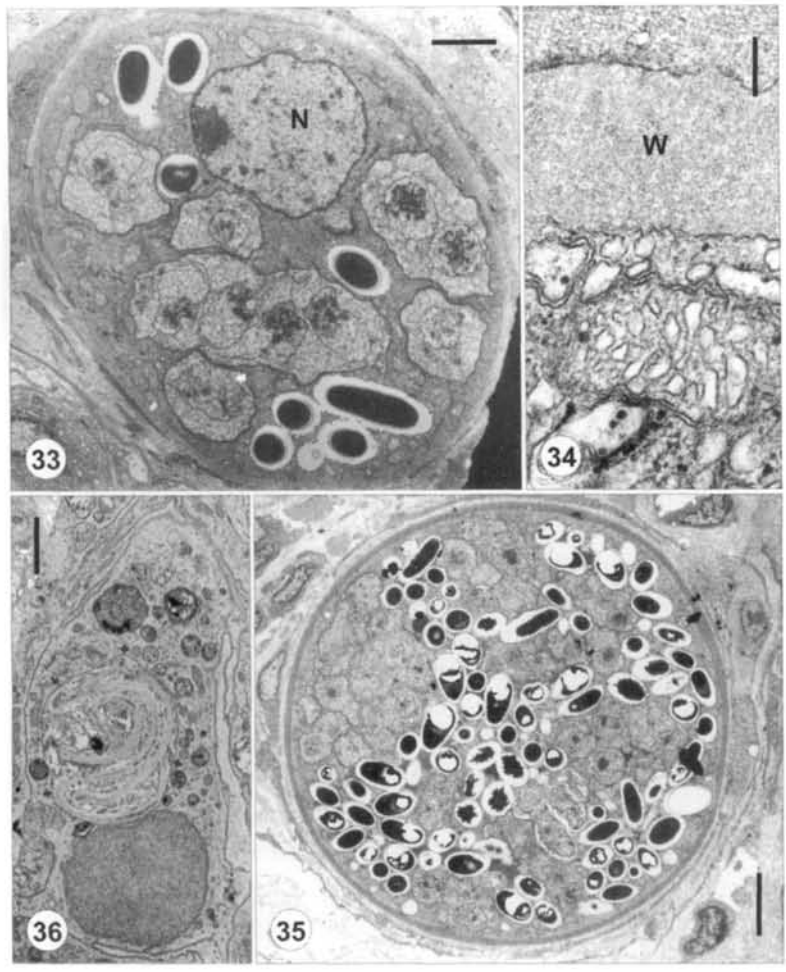
*Rhombus flesus*, and *Platichthys stellatus*. It forms xenomas about 1 mm in diameter mainly in subepithelial connective tissue of the digestive tract, in heavy infections also in other body organs, and is distributed in seas of the Holarctic region. The species may cause considerable morbidity and mortality, especially in yearling fish; losses exceeding 60% were confirmed experimentally (Bekhti and Bouix, 1985; Cali *et al.*, 1986). Elongate oval, sometimes ovoid spores average 4.7 × 2.7 μm. Mass of xenomas may completely replace the usual structure of the intestinal wall, which becomes thick and rigid, assuming a chalk-white pebbled appearance.

The fish may survive a relatively high intensity of infection, as far as the xenomas are located on the serosal side of the intestine, thus not depleting the nutritional needs of the host. Otherwise, the intestine eventually becomes non-functional, being also often quite occluded. Intestinal failure then results in death. As such, G. *stephani* may be one of the limiting factors in the growth of feral and cultured populations of, e.g., plaice and flounders. The presence or absence of infection in a given offshore fish stock can mark nursery grounds in which, due to lower temperatures, the infection cannot take place (Olson and Pratt, 1973). Degree of water contamination may strongly affect the infection. Khan (2004) found that while at pristine sites the infection was limited to the digestive tract, fish taken near sites contaminated with paper and pulp mill effluents had a multifocal infection including liver and kidney. This might have been associated with immunosupression due to the contaminant. Mathieu-Daude *et al.* (1992) suggest that this species is synonymous with G. *anomala*, which has to be confirmed.

## Group 3

Genus *Loma* Morrison and Sprague, 1981 is characterized by a granulofibrillar xenoma wall (Fig. 7.34) up to 0.5 μm thick, covering the cell plasmalemma. Xenomas are up to 1 mm in size, with a central hypertrophic nucleus. Developmental stages are intermingled with mature spores throughout the xenoma. Merogony proceeds from uninucleate cells to cylindrical plasmodial stages, all invested with a cisterna of endoplasmic reticulum. Sporogony produces up to eight spores within a SPV. There are 16 named species and eight described as 'sp'.

Morphological differentiation of species infecting gills of marine gadiform and some other fish is not easy. Recent SSU rDNA analysis of *Loma* spp. completed by fine structural studies will hopefully initiate the




**Figs. 7.33 to 7.35** A *Loma acerinae* xenoma. **7.33**—An early xenoma with meronts and mature spores; N—host cell nucleus. Scale bar = 2 μm. **7.34**—Thick xenoma wall (w). Scale bar = 200 nm. **7.35**—Grown xenoma, with host cell nucleus beyond the plane of section; scale bar = 5 μm.

**Fig. 7.36** A macrophage digesting a spore, chitinous spore walls are still seen in the cytoplasm. Scale bar = 2 μm.

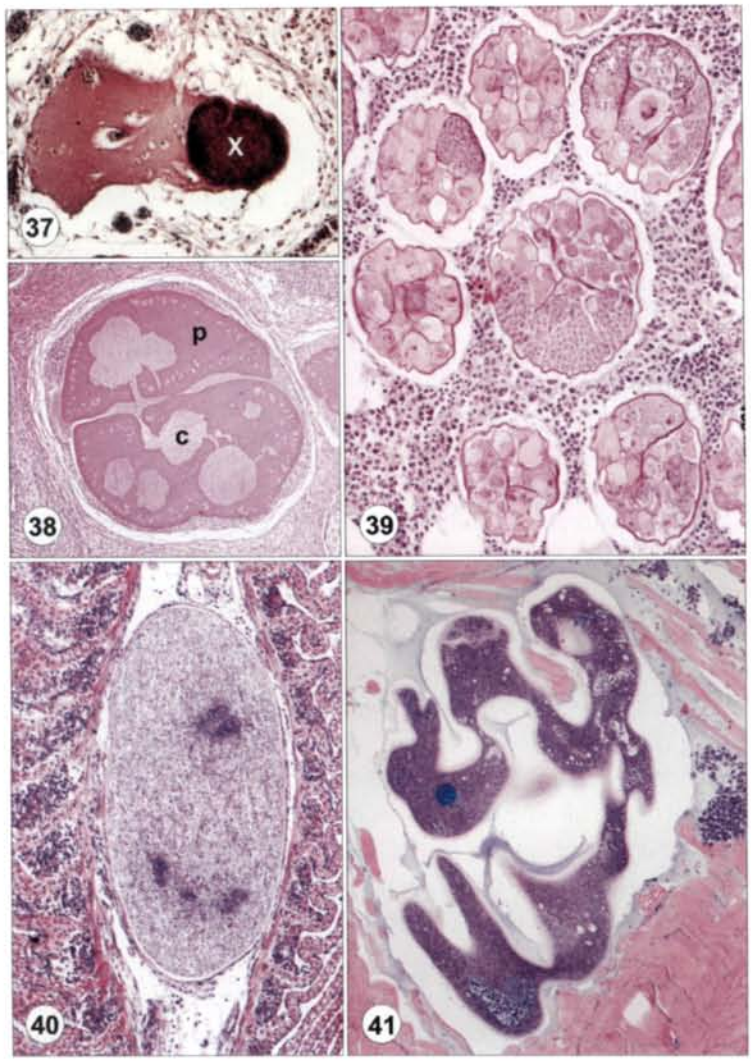
solution of the intricate taxonomy of the genus (Brown, 2005) with many closely related species. In her 2005 thesis, Brown has prepared, using molecular and electron-microscopical techniques, the description of additional three species from gadiform fish and two species from scorpaeniform fish. The overall damage to the gills depends on the intensity of infection.

*L. branchialis* (Nemeczek, 1911) Morrison and Sprague, 1981, (type species of the genus) forms xenomas on gill filaments of *Gadus melanogrammus* in North Atlantic (Fig. 7.40). Ovoid spores average 4.1 × 2.3 μm (Fig. 7.5). The separateness of the closely related species *L. morhua* Morrison and Sprague, 1981, described from the gills of *Gadus morhua* from the American region of North Atlantic and deemed identical with the preceding (e.g., Canning and Lom, 1986) was confirmed by molecular methods by Brown (2005). Ovoid spores average 4.3 × 2.4 μm. In addition to the gills, also heart and liver may be affected in heavy infections of *G. morhua*.

*Loma camerounensis* (Fomena *et al.*, 1992) is a pathogen of tilapia *Oreochromis niloticus*, a fish commonly cultured in West Africa. It forms xenomas in the connective tissue of gut submucosa. In view of large xenomas protruding into the body cavity and up to 94% prevalence, the parasite may play a negative role in fish culture (Fomena *et al.*, 1992).

*L. salmonae* (Putz *et al.*, 1965) Morrison and Sprague, 1981, is one of the most studied fish microsporidians as a pathogen causing large economic losses in cultured salmonids. It is widespread in rainbow trout *Oncorhynchus mykiss*, in *O. masou* and *O. nerka* throughout North America and Japan and has probably been imported to Europe with farmed *O. kisutch*. Laboratory infections of all seven American species of *Oncorhynchus* and of *Salmo trutta* have been successfully achieved, while other salmonids and some non-salmonid fish were resistant (Shaw and Kent, 1999). *L. salmonae* was initially identified as a freshwater pathogen but infections persist after salmon were transferred to seawater. In the curious absence of reliable recent data, one has to refer to the records of unlikely size variation of ellipsoidal spores of 3 to 7.5 × 1.6 × 2.8 μm. This may be linked with the problem of identity of this species. Lovy *et al.* (2004), using an ultrastructural study, suggested that *L. salmonae*, found in net-pen reared *O. tshawytscha*, has two variants, one specific for salmonids of the genus *Oncorhynchus*, the other for *Salvelinus fontinalis*. Brown (2005), applying molecular analysis, confirmed that these are two real, cryptic species.

*L. salmonae* is an important cause of disease in net pen-reared *Oncorhynchus tshawytsha* known as microsporidial gill disease (MGD). There is a variety of gross symptoms of severe infection. In addition to visible xenomas on the gills, it is the gill pallor, petechial hemorrhaging of the gills and skin, ascites and even hemorrhagic pyloric ceca. Growth may




**Figs. 7.37, 7.38** *Spraguea lophii* xenoma. **7.37**—Early stage, the parasite mass (X) occupies only part of the ganglion cell of *Lophius piscatorius*. Bodian stain, × 620. **7.38**—advanced xenoma; the parasite mass at the periphery (p) with *Nosemoides*-type spores is stained differently from *Nosema*-type spores in the centre (c). H&E, × 70.

**Fig. 7.39** *Ichthyosporidium giganteum*: 'cystic' stages supposedly preceding formation of huge xenomas. Compartments contain different stages of merogonial proliferation. H&E, × 225.

**Fig. 7.40** *Loma branchialis* xenoma in the gills of *Melanogrammus aeglefinus*. H&E, × 130.

**Fig. 7.41** Lobose, folded xenoma of *Tetramicra brevifilum* in the muscle of *Scophthalmus maximus*. H&E, × 160. (From Lom and Dyková, 1992, with the permission of *Folia Parasitologica*).

be reduced, swimming efficiency impaired and eventually, mortality rates of young fish increases. Detailed description of pathological changes of systemic infection can be found in Hauck (1984), such as necrosis of cartilage, occlusion of arteries, hyperplasia of gill and heart tissues. In the gills, vasculitis, vascular thrombosis and hyperplasia may be observed (Kent *et al.*, 1989; Speare *et al.*, 1989). *L. salmonae* is highly infective: cohabitation of only five infected *O. mykiss* with 45 naive fish for a period as short as 1 hour resulted in over 95% prevalence of infection in cohabiting fish (Becker *et al.*, 2005).

Several species were described from organs other than gills, especially digestive tract, of some other fish. Correct generic assignment of some of them warrants revision; an example is *Loma acerinae* (Jírovec, 1930) Lom and Pekkarinen, 1999. It forms xenomas up to 0.35 mm in the intestinal wall of the European freshwater fish *Gymnocephalus cernuus* (Figs. 7.32 to 7.35). The elongated ellipsoidal spores average 4.6 × 2.2 μm. It is a rather innocuous parasite; it can be used, however, as a model species for study of morphogenesis of xenoma structures.

Genus *Ichthyosporidium* Vávra and Sprague, 1976, produces xenomas of two kinds. There are globular, compartmentalized cystic structures (up to 200 μm, Fig. 7.39) in which merogony proceeds by binary fission, some cells being diplokaryotic. It is these structures which supposedly produce extremely large, internally undivided, lobose xenomas (up to 4 mm) bearing microvilli at the surface. Sporogony sequence is located at the periphery and mature spores in the centre. Life cycle in the fish seems to be very complex and is insufficiently known. Sporonts and spores are diplokaryotic. There exist two named species.

*Ichthyosporidium giganteum* (Thélohan, 1895) Swarzewsky, 1914, infects coastal fishes along the Atlantic coasts of France (*Crenilabrus melops*), Portugal (*Ctenolabrus rupestris*) and U.S.A. (*Leiostomus xanthurus*) in which the prevalence was 13–25% (Schwartz, 1963) and in the Black Sea (*Crenilabrus ocellatus*) and possibly many others. The cells originally infected are probably connective or fat tissue cells. Diplokaryotic spores are ovoid, averaging 6 × 4 μm in size. Eventually, the parasite produces a large, disfiguring ventral swelling all the way from head to anal fin, made up from hyperplastic host tissue pervaded by xenomas. Severe infections impress as having an unfavourable prognosis, yet exact data have not been recorded.

Genus *Pseudoloma* (Matthews *et al.*, 2001). The features of this xenoma-forming genus are reflected in the definition of its only species.

*Pseudoloma neurophilia* (Matthews *et al.*, 2001). The original and only definition only says that uninucleate spores occur in segregated aggregates of 8 to 16 in a SPV. A molecular definition has been added stating that signature sequences in SSU rDNA at position 168-174 are TTTGTT and at position 1271-1276 are TTATTT. Spores average 5.4 × 2.7 μm. Meronts were not observed in what was described as xenomas. There is only one single species.

*Pseudoloma* is the most widespread infectious agent of zebra fish *Danio rerio* kept as an important laboratory model for studies of basic mechanisms of vertebrate developmental genetics in many laboratories. It affects the spinal cord and ventral nerve root ganglia but may also spread into somatic muscle. Then it is associated with severe multifocal myodegeneration and myositis. In heavy infections, fish are severely emaciated with skeletal deformities. The infection can often be found in kidney, ovaries and sometimes within eggs, which indicates that vertical transmission or contamination of offspring may, too, play a role in transmission of the parasite. Perhaps the parasite is transmitted to progeny with sexual products rather than within eggs (Kent and Bishop-Stewart, 2003). A sensitive PCR test, specific for this microsporidian, has been developed (Kent and Whipps, 2005).

## Group 4

Genus *Spraguea* Morrison and Sprague, 1981 is a dimorphic microsporidian featuring two types of spores in two developmental sequences within the fish host, both without SPV formation. Xenomas develop from an initially restricted peripheral zone of a ganglion cell (Fig. 7.7), which has a single hypertrophic nucleus and is covered by a simple cell membrane. In the type species, *S. lophii* (Doflein, 1898) found in the European anglerfishes, there is one developmental sequence with isolated nuclei producing ellipsoidal spores by cleavage of a multinucleate sporogonial plasmodium. Another sequence with all stages diplokaryotic produces slender, curved spores by disporoblastic sporogony (Loubès *et al.*, 1979). In the second species known, *S. americana* (Takvorian and Cali, 1986), found in American and Japanese anglerfishes, the diplokaryotic sequence is missing (Freeman *et al.*, 2004).

*Spraguea lophii* (Doflein, 1898) Vávra and Sprague, 1976, is commonly found in *Lophius piscatorius* and *L. budegassa* in the Mediterranean, Atlantic and North Sea coasts of Europe. The oval single-nucleated spores average 4.2 × 2.5 μm, the slender and curved binucleate spores average 3.7 × 1.4 μm. The degraded cytoplasm of the ganglion cell forms a thick envelope around the microsporidian mass (Fig. 7.38). Infected cells in a ganglion assume together a bunch-of-grape shape, connective tissue and glial cells proliferate between the xenomas, causing pressure atrophy of the non-infected cells. Afterwards, the entire spore mass turns into a tumour-like granuloma encased with connective tissue envelope. Although most of the ganglia may be infected, the host's condition does not seem to be impaired and the infection is life-long.

Genus *Tetramicra* Matthews and Matthews, 1980. The xenoma has a single, reticulate nucleus and simple, microvillous surface projections (Fig. 7.42) by which several xenomas may interlock. Developmental stages and spores are intermingled in the xenoma. Merogony comprising uni- and multinucleate cylindrical cells ends with production of binucleate stages that enter tetrasporogonic sporogony. All stages develop in vacuoles of host´s origin. All four sporoblasts are for some time interconnected to form a clover-like shape. No SPV formation. A single species is known.

*Tetramicra brevifilum* Matthews and Matthews, 1980, is a potential threat to turbot farming. It infects the connective tissue in skeletal muscles of turbot-of-the-year *Scophthalmus maximus* along the coasts of Western Europe. Xenomas up to 0.2 mm, often of lobose or folded-over shape (Fig. 7.41) may aggregate and then appear as nodules visible through the skin. Ovoid spores up to 4.8 × 2 μm in size have a conspicuous inclusion in the posterior vacuole, unique among all other fish microsporidia. In heavy infections, xenomas may also be found in the intestine, kidney, liver and spleen (Estévez *et al.*, 1992).

Infected muscle fibres appear degenerate, severe infection inactivates large parts of body musculature, which may be in the process of infection even when liquefied. Swimming is impaired, which in turn leads to poorer feeding rate, causing in feral fish starvation and exposure to increased predation. Xenoma formation is associated with a plethora of pathologic phenomena like pressure atrophy, displacement of muscle fibers, necrosis of infected tissues, canalization in connective tissue of the spinal column, focal hemorrhaging, fibrosis and necrosis. Rupture of grown xenomas leads to cell infiltration and deposition of collagen, while released spores are

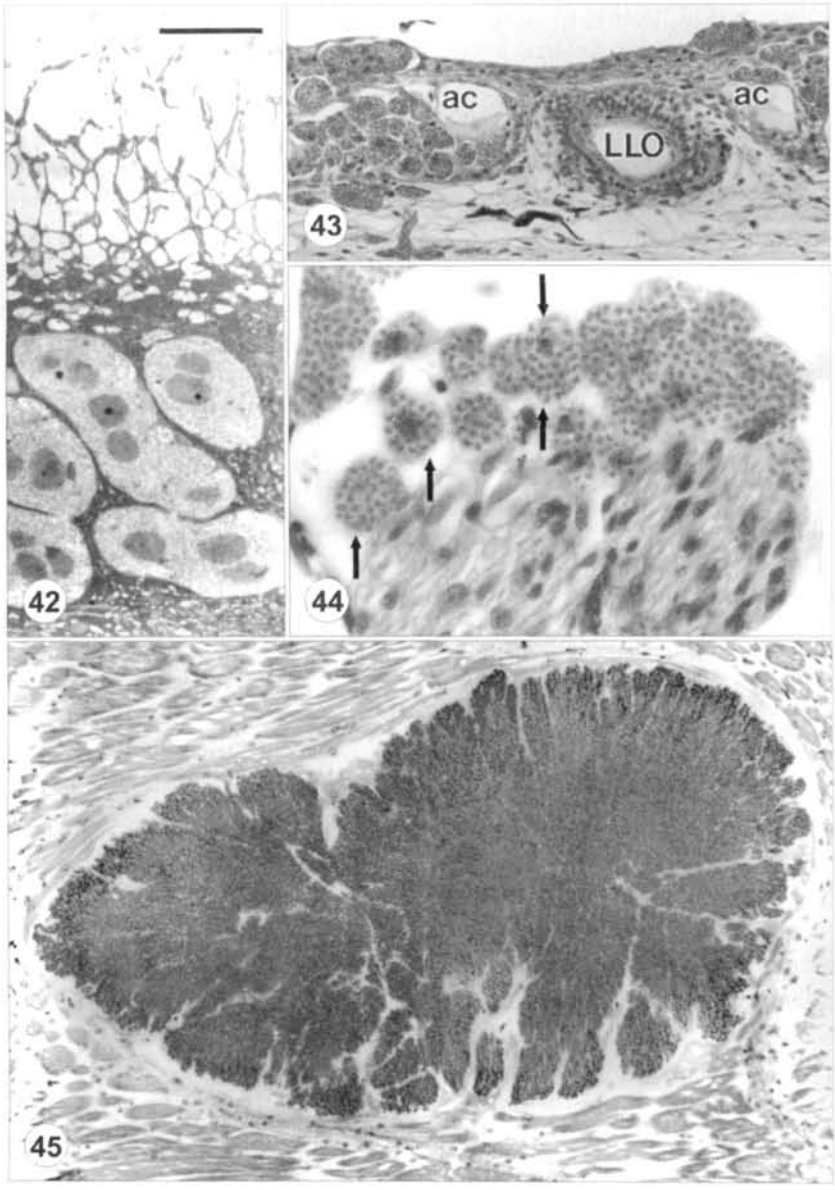



**Fig. 7.42** Microvillous meshwork at the surface of *Tetramicra brevifilum* xenoma. Bar = 1 μm.

**Figs. 7.43 to 7.45** Infection with *Kabatana arthuri*. **7.43.** Section through epidermis with macrophages laden with spores. Alarm cells (ac), lateral line organ (LLO), × 250. **7.44.** Spore-replete macrophages (arrows) close to a peripheral nerve, × 990. **7.45.** Muscle tissue of *Pangasius sutchi* with a large focus of *K. arthuri* infection. × 160.

captured and perhaps digested by macrophages. Even after heavy infection the fish may recover fully. Pathologic phenomena are depicted to a greater detail in Matthews and Matthews (1980), Estévez *et al.* (1992) and Figueras *et al.* (1992).

Matthews and Matthews (1980), although successful in infecting the fish by intramuscular injection, failed to infect them perorally or by cohabitation. Figueras *et al.* (1992) also experienced the failure and thus they resorted to the claim that fish were infected by ingesting crustaceans, which fed on spores released from infected fish. The infection was perhaps facilitated when the recipient fish were under stress.

Genus *Microgemma* Ralphs and Matthews, 1986. Forms xenomas up to only about 0.5 mm in size, covered by a simple plasmalemma raised into microvilli. The reticulated host cell nucleus lies between the peripheral band of mitochondria and the centre of the cell, where developmental stages are intermingled with mature spores. Multinucleate meronts divide by plasmotomy and then enter directly into sporogony by exogenous budding. The nuclei are always single. Seven species are known.

*Microgemma hepaticus* Ralphs and Matthews, 1986, infects the liver of fry of the marine fish *Chelon labrosus* off the Cornwall coast (U.K.). Multinucleate meronts encased with a cisterna of host endoplasmic reticulum divide by plasmotomy. Sporogony proceeds by a single exogenous budding off the sporogonic plasmodium and later by multiple budding and fragmentation of the plasmodium. Spores are pyriform with average size of 4.2 × 2.4 μm. Large lesions may be followed by necrosis (Ralphs and Matthews, 1986).

M. *ovoidea* (Thélohan, 1895) Amigo *et al.* (1996), commonly infects marine fishes *Motella tricirrata, Cepola rubescens* and *Mullus barbatus* along the French coasts. Ovoid spore average 2.5 × 1.5 along the French coasts. Ovoid spore average 2.5 × 1.5 μm. Xenomas appear as cyst-like formations in the liver, sometimes inficing a heavy damage and often leading to the death of the host (Amigó *et al.*, 1996).

Genus *Kabatana* (Lom *et al.*, 2000) is not xenoma-forming. There are no SPVs; all stages are without diplokarya. Cylindrical multinucleate meronts proliferate by serial constriction within disintegrated sarcoplasm, without any boundary or cyst. Eventually, the uninucleate cells transform into uninucleate sporonts, which divide once more, functioning as sporoblast mother cells and giving rise to spores. Four species are known.

*K. seriolae* (Egusa, 1982) Lom *et al.*, 2000, has been described as parasite of skeletal muscle of *Seriola quinqueradiata* in Japanese maricultures. Spores are mostly ovoid, averaging 3.3 × 2.2 μm in size. The lesions are macroscopic and comprise diverse parasite stages within amorphous, cheese-like substance from the disintegrated muscle. The disease is called 'beko' in Japanese (Egusa 1982), gross symptoms being concave surface depressions in places where the muscle tissue has been liquefied by the parasite action.

*K. takedai* (Awakura, 1974) Lom *et al.*, 2001, infects skeletal muscles of salmonids – of six species of the genus *Oncorhynchus* (especially *O. masou* and *O. keta*) and of two species of the genus *Salvelinus* (especially *S. leucomaenis*) in Japan. Ovoid spores average 3.4 × 2 μm in size. It has been paid special attention as the major pathogen of cultured salmonids. In some fish species at warmer temperatures, the prevalence may reach up to 100%, i.e., the infection is temperature dependent and may be restricted to warmer periods of year (Urawa, 1989). The parasite is reported to grow well at 18°C, development was retarded between 11°C and 15°C and it stops growing at 8°C. Therefore rearing smolts below 15°C has been applied to control the infection. In chronic cases or at low temperature, the infection develops only in heart muscle. At warmer ambient temperature, parasitic lesions replete with *Kabatana* stages are cyst-like bodies up to 3 × 6 mm in size, sometimes showing through the skin. In acute cases, where there are as many as 130 'cysts'/gram of muscle tissue, high mortality and heavy losses are incurred both in cultured and feral fish. Fish that survived the infection were immune to re-infection for up to 11 years (Awakura and Kurahashi, 1967).

*K. arthuri* Lom *et al.*, 1999, is a parasite of trunk muscles of a Southeast Asian freshwater fish, *Pangasius sutchi*; infected fish may be commonly found in fish farms. Spores of rather variable shape, mostly oviform, average 3.1 × 1.9 μm (Fig. 7.3). The exospore surface is divided into regular, small fields. Muscle lesions replete with spores (Fig. 7.45) may show through the surface; spores released from this mass are ingested by macrophages (Fig. 7.44) which also concentrate in the epidermis (Fig. 7.43) through which the spores may be released into water (Dyková and Lom, 2000).

## Group 5

The genus *Nucleospora* (Hedrick *et al.*, 1991) differs from other fish-infecting microsporidia by invading nuclei of the host cells, by forming

polar tube precursors already prior to the division of sporogonial plasmodium and prior to thickening of its plasmalemma. Spores have the size of only 2 μm. Two named species and two species described as 'sp.' are known.

*Nucleospora salmonis* (Hedrick *et al.*, 1991) infects hemoblasts of salmonids *Oncorhynchus tshawytscha*, *O. nerka* and *O. mykiss* in the Pacific Northwest of the U.S.A. and in Canada; in the last host it also occurs in France. Similar unassigned intranuclear *Nucleospora* were recorded in *Cyclopterus lumpus* and *Hippoglossus hippoglossus* from the Atlantic (Mullins *et al.*, 1994; Nilsen *et al.*, 1995). The species was at first temporarily assigned to the genus *Enterocytozoon* as *E. salmonis* (Hedrick *et al.* 1991). However, 20.1% genetic divergence in rRNA genes, backed by differences in host class and site in the cell supported the erection of a new genus.

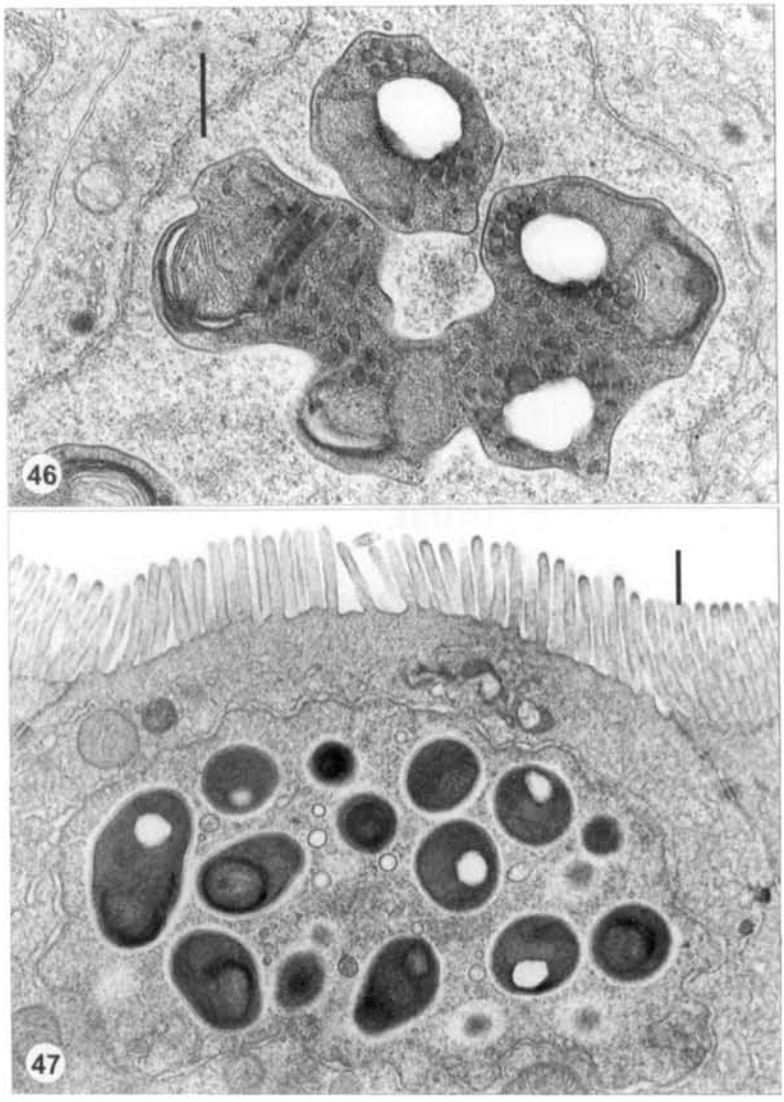
Spores reach only the size of only 2 × 1 μm. The species is primarily found in haematopoietic cells, less frequently in rodlet cells, epithelial cells of the urinary tubules and mesangial cells of the glomerulus. Infected lymphoblasts and plasmablasts show massive proliferation, suggesting neoplasia. In fact, *Nucleospora* infections were usually associated with a simultaneous neoplastic condition with massive lymphoproliferation known as plasmacytoid leukemia (PL) in *O. tshawytscha*. *N. salmonis* is perhaps not the primary cause of this condition. Viral etiology of PL was suggested (Shaw and Kent, 1999) as well as the possibility that the lymphoproliferative disorders are in fact two diseases due to two different agents.

*Nucleospora secunda* Lom and Dyková (2002) infects nuclei of enterocytes of the ornamental fish *Nothobranchius rubripinnis* (Figs. 7.46, 7.47). Spores in tissue embedded for electron microscopy average 1.6 × 0.8 μm in size. Disease symptoms were not observed; in heavy infections they can be expected.

## UNASSIGNED GENERA

Genus *Amazonspora* Azevedo and Matos (2003) has only one species, *A. hassar*. It forms xenomas in the Amazonian fish *Hassar orestis*, without a pronounced pathogenic activity. The same applies to the genus *Microfilum* (Faye *et al.*, 1991) with a single species M. *lutjani* Faye *et al.* (1991), producing xenomas in the gills of the marine fish *Lutjanus fulgens*.

An uncertain position in the above outlined classification also has genus *Myosporidium* Baquero *et al.*, with a single species M. *merluccius*




**Figs. 7.46, 7.47** Ultrastructure of *Nucleospora secunda*. **7.46.** Sporogonial plasmodium segmenting into separate sporoblasts, bar = 500 nm. **7.47.** Mature spores within the nucleus of an enterocyte of *Nothobranchius rubripinnis*, bar = 1 μm.

Baquero *et al.*, 1995, producing xenomas in the trunk muscle of *Merluccius capensis/paradoxus* complex in Namibia.

Collective group *Microsporidium* Balbiani, 1884 comprises 18 named and 33 species described as 'sp'. Future proper description will lead to their ultimate generic assignment.

*M. sulci* (Rašín, 1936) Lom and Dyková, 1992 (syn. *Cocconema sulci* Rašín, 1936) commonly infects oocytes of *Acipenser ruthenus* and *A. guldenstadti* in Volga, Kura and Danube river basins. The incomplete observations of Lom and Dyková (1992) recorded cylindrical meronts and sporogony stages with diplokarya. SPVs up to 25 μm in size contained numerous spherical spores 2.5 μm in diameter. Developmental stages were stratified in the infected oocyte, mature spores appearing first around the nucleus. In view of high prevalence and destruction of the oocyte, this parasite may well prove to be a harmful species.

In addition to true fish parasites, there are about 9 microsporidian species of the genera *Nosema*, *Unicaryon* and of the collective group *Microsporidium* infecting myxosporean and helminth parasites living in marine fish (Lom, 2002).

## OUTLOOK FOR THE FUTURE

In recent years, the increased interest in research of fish microsporidia has provided us better understanding in many directions, e.g., in morphology, life cycles and biology, pathogenesis, immune response and molecular biology. We are still sorely missing the understanding of the relation to the fish host cell and ways that lead to the establishment of such an amazing partnership as the xenoma formation is. Perhaps the development of a successful *in vitro* culture of xenoma-forming microsporidian will throw light on the still little known points in microsporidian biology and host-parasite relations.

The economic importance of microsporidia in fish cultures is an established fact and will continue to represent a pivotal stimulus for all-important aspects of the study of these agents. Studies of host specificity will certainly have an impact on looking for sources of infection from feral fish and for better understanding of microsporidian epizootiology.

Continuing research of molecular biology techniques (using marker genes of SSU rRNA, elongation factor 1α, RNA polymerase largest subunit II and perhaps others) will undoubtedly help to identify microsporidian strains found in the field. It may also reveal the kinship of separate species and elaborate an array of diagnostic methods and as a side benefit, an improved understanding of their phylogeny and its relationship to eukaryote tree of life.

Further development of chemotherapeutic drugs will help to cope with microsporidioses in fish cultures. One can expect trials on the

possible use in aquacultures of drugs being developed against microsporidia of man.

Microsporidia infecting fish will certainly continue to be an excellent material for studies of fundamental questions on the still insufficiently known mode of microsporidian spore hatching and cell invasion, relation to fish host cell and on microsporidian genomics.

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## References

Amigó, J.M., H. Salvadó, M.P. Gracia and C.P. Vivarès. 1996. Ultrastructure and development of *Microsporidium ovoideum* (Thélohan, 1895) Sprague, 1997, a microsporidian parasite of the red band fish (*Cepola macrophthalma* L.). *European Journal of Protistology* 32: 532-538.

Awakura, T. 1974. Studies on the microsporidian infection in salmonid fishes. *Scientific Reports of the Hokkaido Fish Hatchery* 29: 1-96.

Awakura, T. and S. Kurahashi. 1967. Studies on the *Pleistophora* disease of salmonid fish. III. On prevention and control of the disease. *Scientific Reports of the Hokkaido Fish Hatchery* 22: 51-68.

Baquero, E., M. Rubio, I.N.S. Moura, N.J. Pieniazek and R. Jordana. 2005. *Myosporidium merluccius* n.g., n.sp. infecting muscle of commercial hake (Merluccius sp.) from fisheries near Namibia. *Journal of Eukaryotic Microbiology* 52: 476-483.

Becker, J.A. and D.J. Speare. 2004a. Impact of a water temperature shift on xenoma clearance and recovery time during a *Loma salmonae* (Microsporidia) infection in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 58: 185-191.

Becker, J.A. and D.J. Speare. 2004b. Ultraviolet light control of horizontal transmission of *Loma salmonae*. *Journal of Fish Diseases* 27: 177-180.

Becker, J.A., D.J. Speare and I.R. Dohoo. 2005. Effect of the number of infected fish and acute exposure period on the horizontal transmission of *Loma salmonae* (Microsporidia) in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 244: 1-9.

Bekhti, M. and G. Bouix. 1985. Evolution of xenomas and the double role of polynuclear neutrophils in *Glugea stephani* infection in *Platichthys flesus*. *Annales de Parasitologie Humaine et Comparée* 60: 509-522.

Brown, A.M.V. 2005. *Molecular evolution, systematics and ecology of microsporidia from fishes and crustaceans*. Ph.D. Thesis. The University of British Columbia, Vancover, Canada.

Cali, A., P.M. Takvorian, J.J. Ziskowski and T.K. Sawyer. 1986. Experimental infection of American winter flounder (*Pseudopleuronectes americanus*) with *Glugea stephani* (Microsporida). *Journal of Fish Biology* 28: 199-206.

Canning, E.U. and J. Lom. 1986. *The Microsporidia of Vertebrates*. Academic Press, London.

Chatton, E. 1920. Un complexe xéno-parasitaire morphologique at physiologique de *Neresheimeria paradoxa* chez *Fritillaria pellucida*. *Comptes Rendus de l'Académie des Sciences, Paris* 171: 55-57.

Docker, M.F., R.H. Devlin, J. Richard and M.L. Kent. 1997. Sensitive and specific polymerase chain reaction assay for detection of *Loma salmonae* (Microsporea). *Diseases of Aquatic Organisms* 29: 13-20.

Dyková, I. 1995. Phylum Microspora. In: *Fish Diseases and Disorders*, P.T.K. Woo (ed.). CABI International, Wallingford, Oxon, U.K., Vol. 1, pp. 149-179.

Dyková, I. and J. Lom. 1978. Tissue reaction of the three-spined sticklebacks *Gasterosteus aculeatus* L. to infection with *Glugea anomala* (Moniez, 1887). *Journal of Fish Diseases* 1: 83-90.

Dyková, I. and J. Lom. 1980. Tissue reaction to microsporidian infections in fish. *Journal of Fish Diseases* 3: 265-283.

Dyková, I. and J. Lom. 2000. Histopathology of *Kabatana arthuri* (Microspora) infection in sutchi catfish, *Pangasius sutchi*. *Folia Parasitologica* 47: 161-166.

Egidius, E. and O. Soleim. 1986. *Pleistophora ehrenbaumi*, a microsporidian parasite in wolffish, *Anarhichas lupus*. *Bulletin of the European Association of Fish Pathologists* 6: 13-14.

Egusa, S. 1982. A microsporidian species from yellowtail juveniles, *Seriola quinqueradiata*, with 'Beko' disease. *Fish Pathology* 1: 187-192.

Estévez, J.R., R. Iglesias, J. Leiro, F.M. Ubeira and M.L. Sanmartin. 1992. An unusual site of infection by a microsporean in the turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms* 13: 139-142.

Figueras, A., B. Novoa, M. Santarém, E. Martínez, J.M. Alvarez, A.E. Toranzo and I. Dyková. 1992. *Tetramicra brevifilum*, a potential threat to farmed turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms* 14: 127-135.

Findley, A.M., E.H. Weidner, K.R. Carman, Z. Xu and J.S. Godbar. 2005. Role of the posterior vacuole in *Spraguea lophii* (Microsporidia) spore hatching. *Folia Parasitologica* 52: 111-117.

Fischtal, J.H. 1944. Observation on a sporozoan parasite of the eelpout, *Zoarces anguillaris*, with an evaluation of candling methods for its detection. *Journal of Parasitology* 30: 35-36.

Fomena, A., F. Coste and G. Bouix. 1992. *Loma camerounensis* sp. nov. (Protozoa: Microsporida) a parasite of *Oreochromis niloticus* Linnaeus, 1757 (Teleost: Cichlidae) in fish-rearing ponds in Melen, Yaoundé, Cameroon. *Parasitological Research* 78: 201-208.

Freeman, M.A., H. Yokoyama and K. Ogawa. 2004. A microsporidian parasite of the genus *Spraguea* in the nervous tissues of the Japanes anglerfish *Lophius litulon*. *Folia Parasitologica* 51: 167-176.

Frixione, E., L. Ruiz, J. Cerbon and H.H. Undeen. 1997. Germination of *Nosema algerae* (Microspora) spores – Conditional inhibition of $D_2O$, ethanol and $Hg^{2+}$ suggests dependence of water influx upon membrane hydration and specific transmembrane pathways. *Journal of Eukaryotic Microbiology* 44: 109-116.

Fujiyama, I., S. Urawa, H. Yokoyama and K. Ogawa. 2002. Investigation on the transmission stage of the microsporidian *Kabatana takedai* in salmonids. *Bulletin of the National Salmon Resources Center*, No. 5: 1-6.

Garcia, L.S. 2002. Laboratory identification of the microsporidia. *Journal of Clinical Microbiology* 40: 1892-1901.

Grupcheva, G. and J. Lom. 1980. Protozoan parasites of fishes from Bulgaria I. *Glugea luciopercae* and the description of three new *Trichodina* species. *Folia Parasitologica* 27: 289-294.

Guzmán, O., R. Enriquez and J. Schottelius. 2001. Detection of spores of *Nucleospora salmonis* in frozen tissue material from Atlantic salmon. *Bulletin of the European Association of Fish Pathologists* 21: 81-83.

Hauck, A.K. 1984. A mortality and associated tissue reactions of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), caused by the microsporidan *Loma* sp. *Journal of Fish Diseases* 7: 217-229.

Hedrick, R.P., J.M. Groff and D.V. Baxa. 1991. Experimental infections with *Enterocytozoon salmonis* Chilmonczyk, Cox, Hedrick (Microsporea): an intranuclear microsporidium from chinook salmon *Oncorhynchus tshawytscha*. *Diseases of Aquatic Organisms* 10: 103-108.

Kabata, Z. 1959. On two little-known microsporidia of marine fishes. *Parasitology* 49: 309-315.

Kano, T., T. Okauchi and H. Fukui. 1982. Studies on *Pleistophora* infection in eel, *Anguilla japonica*. II. Preliminary tests for application of fumagillin. *Fish Pathology* 17: 107-114.

Kent, M.L. and J.K. Bishop-Stewart. 2003. Transmission and tissue distribution of *Pseudoloma neurophilia* (Microsporidia) of zebrafish, *Danio rerio* (Hamilton). *Journal of Fish Diseases* 2: 423-426.

Kent, M.L. and S.V. Dawe. 1994. Efficacy of fumagillin DCH against experimentally induced *Loma salmonae* (Microsporea) infection in chinook salmon *Oncorhynchus tshawytscha*. *Diseases of Aquatic Organisms* 320: 231-233.

Kent, M.L. and C. Whipps. 2005. A sensitive and specific PCR test for *Pseudoloma neurophilia*, a common microsporidian pathogen of zebrafish (*Danio rerio*) in research facilities. *Abstracts of the 80th Annual Meeting of the American Society of Parasitologists*, Mobile, Alabama, July, 2005, p.62.

Kent, M.L., D.G. Elliot, J.M. Groff and R.P. Hedrick. 1989. *Loma salmonae* (Protozoa, Microspora) infections in seawater reared coho salmon *Oncorhynchus kisutch*. *Aquaculture* 80: 211-222.

Khan, R.A. 2004. Effect, distribution and prevalence of *Glugea stephani* (Microspora) in winter flounder (*Pleuronectes americanus*) living near two pulp and paper mills in Newfoundland. *Journal of Parasitology* 90: 229-233.

Larsson, J.I.R. 1986. Ultrastructure, function and classification of microsporidia. In: *Progress in Protistology*, J.O. Corliss and D.J. Patterson (eds.). Biopress, Bristol, Vol. 1, pp. 325-390.

Larsson, J.I.R. 1999. Identification of Microsporidia. *Acta Protozoologica* 38: 161-197.

Lee, S.J., H. Yokoyama and K. Ogawa. 2003. Rapid *in situ* hybridisation technique for the detection of fish microsporidian parasites. *Fish Pathology* 38: 117-119.

Lee, S.J., H. Yokoyama and K. Ogawa. 2004. Modes of transmission of *Glugea plecoglossi* (Microspora) via the skin and digestive tract in an experimental model using rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 27: 435-444.

Lom, J. 2002. A catalogue of described genera and species of microsporidians parasitic in fish. *Systematic Parasitology* 53: 81-99.

Lom, J. and I. Dyková. 1992. *Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science*, Vol. 26. Elsevier, Amsterdam.

Lom, J. and I. Dyková. 2005. Microsporidian xenomas in fish seen in wider perspective. *Folia Parasitologica* 52: 69-81.

Lom, J. and F. Nilsen. 2003. Fish microsporidia: fine structural diversity and phylogeny. *International Journal of Parasitology* 33: 107-127.

Lom, J. and M. Pekkarinen. 1999. Ultrastructural observations on *Loma acerinae* (Jírovec, 1930) comb. n. (Phylum Microsporidia). *Acta Protozoologica* 38: 61-74.

Lom, J. and J. Vávra. 1963. The mode of sporoplasm extrusion in microsporidian spores. *Acta Protozoologica* 1: 81-89.

Lom, J., I. Dyková, W. Körting and H. Klinger. 1989. *Heterosporis schuberti* n.sp., a new microsporidian parasite of aquarium fish. *European Journal of Protistology* 25: 129-135.

Lom, J., I. Dyková, C.H. Wang, C.F. Lo and G.H. Kou. 2000. Ultrastructural justification of the transfer of *Pleistophora anguillarum* Hoshina, 1959 to the genus *Heterosporis* Schubert, 1969. *Diseases of Aquatic Organisms* 43: 225-231.

Lores, B., M.J. Rosales, C. Mascaró and A. Osuna. 2003. *In vitro* culture of *Glugea* sp. *Veterinary Parasitology* 112: 185-196.

Loubès, C., J. Maurand and R. Ormières. 1979. Etude ultrastructurale de *Spraguea lophii* (Doflein, 1898), Microsporidie parasite de la Baudroie: essai d'interpretation du dimorphisme sporal. *Protistologica* 15: 43-54.

Lovy, J., D.W. Wadowska, G.M. Wright and D.J. Speare. 2004. Morphological characterization and notes on the life cycle of the newly discovered variant of *Loma salmonae* (Putz, Hofman and Dunbar) from a natural infection of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). *Journal of Fish Diseases* 27: 609-616.

Luna, L. 1968. *Manual of Histologic Staining Methods of the Armed Forces Institutes of Pathology*, 3rd Edition, Mc-Graw Hill, New York.

Mathieu-Daude, F. and N. Faye, F. Coste, J.F. Manier and A. Marques. 1992. Occurrence of microsporidiosis in marine culture gilt-head bream from the Languedoc coast; a problem of specificity in the genus *Glugea* (Protozoa, Microspora). *Bulletin of the European Association of Fish Pathologists* 12: 67-70.

Matos, E., L. Coral and C. Azevedo. 2003. Ultrastructural details of the xenoma of *Loma myrophis* (phylum Microsporidia) and extrusion of polar tube during autoinfection. *Diseases of Aquatic Organisms* 54: 203-207.

Matthews, R.A. and B.F. Matthews. 1980. Cell and tissue reactions of turbot *Scophthalmus maximus* (L.) to *Tetramicra brevifilum* gen. n., sp. n. (Microspora). *Journal of Fish Diseases* 3: 495-515.

Meyer, A. 1952. Veränderungen des Fleisches beim Katfisch. *Fischereiwelt* 1952: 57-58.

Michel, C., J. Maurand, C. Loubès, S. Chilmonczyk and P. de Kinkelin. 1989. *Heterosporis finki*, a microsporidian parasite of the angelfish *Pterophyllum scalare*: Pathology and ultrastructure. *Diseases of Aquatic Organisms* 7: 103-109.

Mullins, J.E., M. Powell, D.J. Speare and R. Cawthorn. 1994. An intranuclear protozoon in lumpfish *Cyclopterus lumpus*. *Diseases of Aquatic Organisms* 20: 7-13.

Nagel, M.L and R.C. Summerfelt. 1977. Nitrofurazon for control of the microsporidian parasite *Pleistophora ovariae* in golden shiners. *Progressive Fish Culturist* 39: 18-23.

Nepszy, S.J. and A.O. Dechtiar. 1972. Occurrence of *Glugea hertwigi* in Lake Erie rainbow smelt (*Osmerus mordax*) and associated mortality of adult smelt. *Journal of Wildlife Diseases* 14: 233-239.

Nigrelli, R.F. 1946. Studies on the marine resource of southern New England. V. Parasites and diseases of the ocean pout *Macrozoarces americanus*. *Bulletin of the Bingham Oceanographic College, Yale University* 9: 187-202.

Nilsen, F., A. Ness and A. Nylund. 1995. Observations on an intranuclear microsporidian in lymphoblasts in farmed Atlantic halibut larvae (*Hippoglossus hippoglossus* L.). *Journal of Eukaryotic Microbiology* 42: 131-135.

Olson, R.E. 1981. Effects of low temperature on the development of the microsporidian *Glugea stephani* in English sole (*Parophrys vetulus*). *Journal of Wildlife Diseases* 17: 559-562.

Olson, R.E. and I. Pratt. 1973. Parasites as indicators of English sole (*Parophrys vetulus*) nursery grounds. *Transactions of the American Fisheries Society* 102: 405-411.

Pekkarinnen, M. and J. Lom. 1999. Early xenomas of *Loma acerinae* (Microspora) raised experimentally in intestines of *Gymnocephalus cernuus*. *Proceedings of the 5th International Symposium on Fish Diseases*, 9-13 August 1999, České Budějovice, p.109.

Pekkarinen, M. and J. Lom. 2002. *Ovipleistophora* gen. n., a new genus for *Pleistophora mirandellae*-like microsporidia. *Diseases of Aquatic Organisms* 48: 133-142.

Price, R.L. 1982. Incidence of *Pleistophora cepedianae* (Microsporida) in gizzard shad (*Dorosoma cepedianum*) of Carlyle Lake, Illinois. *Journal of Parasitology* 68: 1117-1168.

Pulsford, A. and R.A. Matthews. 1991. Macrophages and giant cells associated with a microsporidian parasite causing liquefaction of the skeletal muscle of the Norway pout, *Trispoterus esmarkii* (Nilsson). *Journal of Fish Diseases* 14: 67-78.

Ralphs, A.S. and R.A. Matthews. 1986. Hepatic microsporidiosis of juvenile grey mullet, *Chelon labrosus* (Risso), due to *Microgemma hepaticus* gen. nov. sp. nov. *Journal of Fish Diseases* 9: 225-242.

Reimschuessel, R., R.O. Bennet, E.B. May and M.M. Lipsky. 1987. Eosinophilic granular cell response to a microsporidian infection in a sergeant major fish, *Abudefduf saxatilis* (L.). *Journal of Fish Diseases* 10: 319-322.

Rodríguez-Tovar, L.E., D.W. Wadowska, G.M. Wright, D.B. Groman, D.J. Speare and D.S. Whelan. 2003a. Ultrastructural evidence of autoinfection in the gills of Atlantic cod *Gadus morhua* infected with *Loma* sp. (phylum Microsporidia). *Diseases of Aquatic Organisms* 57: 227-230.

Rodríguez-Tovar, L.E., G.M. Wright, G.M. Wadowska, D.J. Speare and R.J.F. Markham. 2003b. Ultrastructural study of the late stages of *Loma salmonae* development in the gills of experimentally infected rainbow trout. *Journal of Parasitology* 89: 464-474.

Sanchez, J.G., D.J. Speare, R.J.F. Markham and S.R.M. Jones. 2001a. Experimental vaccination of rainbow trout against *Loma salmonae* using a live low-virulence variant of *L. salmonae*. *Journal of Fish Biology* 59: 442-448.

Sanchez, J.G., D.J. Speare, R.J.F. Markham, G.M. Wright and F.S.B. Kibenge. 2001b. Localization of the initial developmental stages of *Loma salmonae* in rainbow trout (*Oncorhynchus mykiss*). *Veterinary Pathology* 38: 540-554.

Schuberg, A. 1910. Über Microsporidien aus dem Hoden der Barbe und durch sie verursachte Hypertrophie der Kerne. *Arbeiten des Kaiserlichen Gesundheitsamte (Berlin)* 33: 401-434.

Schwartz, F.J. 1963. A new *Ichthyosporidium* parasite of the spot (*Leiostomus xanthurus*): A possible answer to recent oyster mortalities. *Progressive Fish Culturist* 25: 181-184.

Shaw, R.W. and M.L. Kent. 1999. Fish Microsporidia. In: *The Microsporidia and Microsporidiosis*, M. Wittner and L.M. Weiss (eds.). American Society for Microbiology, Washington, D.C., pp. 418-446.

Sheehy, D.J., M.P. Sissenwine and S.B. Saila. 1974. Ocean pout parasites. *Marine Fisheries Review* 36: 29-33.

Speare, D.J., J. Brackett and H.W. Ferguson. 1989. Sequential pathology of the gills of coho salmon with a combined diatom and microsporidian gill infection. *Canadian Veterinary Journal* 30: 571- 575.

Sprague, V., J.J. Becnel and E.I. Hazard. 1992. Taxonomy of phylum Microspora. *Critical Reviews in Microbiology* 18: 285-395.

Summerfelt, R.C. 1972. Studies on the transmission of *Pleistophora ovariae*, the ovary parasite of the golden shiner (*Notemigonus crysoleucas*). In: *Final Report, Project* 4—*R. National Marine Fisheries Service*, pp. 1-19.

Summerfelt, R.C. and M.C. Warner. 1970. Geographical distribution and host parasite relationships of *Pleistophora ovariae* (Microsporida, Nosematidae) in *Notemigonus crysoleucas*. *Journal of Wildlife Diseases* 6: 457-465.

Sutherland, D., S. Marcquenski, J. Marcino, J. Lom, I. Dyková, F. Nilsen, S.T. Cooper, H.M. Hsu, W. Jahns and J. Hoyle. 2003. *Heterosporis* sp. (Microspora: Pleistophoridae): A new parasite from *Perca flavescens*, *Stizostedion vitreum* and *Esox lucius* in Minnesota, Wisconsin and Lake Ontario. *Abstracts of papers presented at the 78th Meeting of the American Society of Parasitologists*, p. 50.

Takvorian, P.M. and A. Cali. 1986. The ultrastructure of spores (Protozoa: Microsporidia) from *Lophius americanus*, the anglerfish. *Journal of Protozoology* 33: 570-575.

T'sui, W.H., C.H. Wang and C.F. Lo. 1988. On the *Plistophora* (*Pleistophora*) infection in eel II. The development of *Plistophora anguillarum* inexperimentally infected elvers, *Anguilla japonica*. *Bulletin of the Institute of Zoology, Academia Sinica, Taiwan* 27: 249-258.

Undeen, A.H. and E. Frixione. 1990. The role of osmotic pressure in the germination of *Nosema algerae* spores. *Journal of Protozoology* 37: 561-567.

Urawa, S. 1989. Seasonal occurrence of *Microsporidium takedai* (Microsporida) infection in masu salmon, *Oncorhynchus masou*, from the Chitose river. *Physiology and Ecology, Japan* Spec. Vol. 1: 587-598.

Vossbrinck, C.R. and B.A. Debrunner-Vossbrinck. 2005. Molecular phylogeny of the Microsporidia: ecological, ultrastructural and taxonomic considerations. *Folia Parasitologica* 52: 131-142.

Weber, R. and D.A. Schwartz. 1999. Laboratory diagnosis of microsporidiosis. In: *The Microsporidia and Microsporidiosis*, M. Wittner and L.M. Weiss (eds.). American Society for Microbiology, Washington, D.C., pp. 315-362.

Weissenberg, R. 1949. Cell growth and cell transformation induced by intracellular parasites. *Anatomical Record* 103: 517-518.

Weissenberg, R. 1968. Intracellular development of the microsporidian *Glugea anomala* Moniez in hypertrophying migratory cells of the fish *Gasterosteus aculeatus* L., an example of the formation of 'xenoma tumors'. *Journal of Protozoology* 15: 44-57.

Williams, B.A.P. and P.J. Keeling. 2005. Microsporidian mitochondrial proteins: Expression in *Antonospora locustae* spores and identification of genes coding for two further proteins. *Journal of Eukaryotic Microbiology* 52: 271-276.

Wittner, M. and L.M. Weiss (eds.). 1999. *The Microsporidia and Microsporidiosis*. American Society for Microbiology, Washington, D.C.

Wongtavatchai, J., P.A. Conrad and R.P. Hedrick. 1995. *In vitro* characteristics of the microsporidian: *Enterocytozoon salmonis*. *Journal of Eukaryotic Microbiology* 42: 401-405.

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CHAPTER

# 8

# Amoeboid Protists as Parasites of Fish

**Iva Dyková**

Detailed knowledge of causes and combined factors of diseases, as well as research on the emerging and potential pathogens is of paramount importance for expanding fish farming based on technologies interconnected with natural water resources. In comparison with other fish-infecting eukaryotic microorganisms, amoebae have not received adequate attention to date. The purpose of this chapter is to summarize my own research experience and data available in the literature on this group of true and potential fish pathogens.

## GENERAL CHARACTERISTICS

Amoebae infecting or colonizing fish include two main groups of organisms that differ substantially in their way of living. Some amoebae are known to be specific endocommensals and some are free-living, of which those, known as amphizoic, are the most important ones with respect to

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fish health. According to the new classification of protists based on current molecular phylogenies (Adl *et al.*, 2005), amoeboid protists found in fish belong to two taxonomic super-groups: Amoebozoa, grouping together most of lobosean families sensu Page (1991), and Excavata containing Heterolobosea with three families, Acrasidae, Gruberellidae and Vahlkampfiidae.

Specific endocommensals described from the digestive tract of various fish species that are suggestive of microaerophilic entamoebids of mammals include several species belonging to the genera *Entamoeba*. *E. gadi* Bullock, 1966, *E. molae* Noble and Noble, 1966, and *E. salpae* Alexeieff, 1912, were described from marine fish, *E. ctenopharyngodoni* Chen, 1955, and E. *pimelodi* Cunha and Penido, 1926, from freshwater fish. *Schizamoeba salmonis* Davis, 1926, tentatively assigned to the same family Entamoebidae was described from the stomach of *Oncorhynchus mykiss*, *O. tshawytscha*, *O. kisutch* and *Salvelinus fontinalis*. Amoebae of the genus *Entamoeba* lack mitochondria and have a reduced Golgi apparatus. They form cysts with densely staining aggregations of RNA, the chromatoids. The nucleus has small central nucleolus and chromatine bodies on the periphery. The cytoplasm of trophozoites forms pseudopodia as semi-eruptive bulges. *Schizamoeba salmonis* forms small uninucleate as well as multinucleate trofozoites and cysts with three up to many nuclei. Except for the poster by Qi-Zhong Zhang recently presenting a new species, *E. siluri*, the agent of intestinal lesions in *Silurus meridionalis*, no data about this group of amoebae infecting fish were published since the 1960s. The basic data and documentation available on this group of amoebae were summarized in Lom and Dyková (1992).

All other amoebae infecting fish are free-living naked amoebae, which can, under certain conditions, colonize fish organs and tissues. Some of them proved to be serious pathogens and primary agents of diseases; all others can be considered potential pathogens.

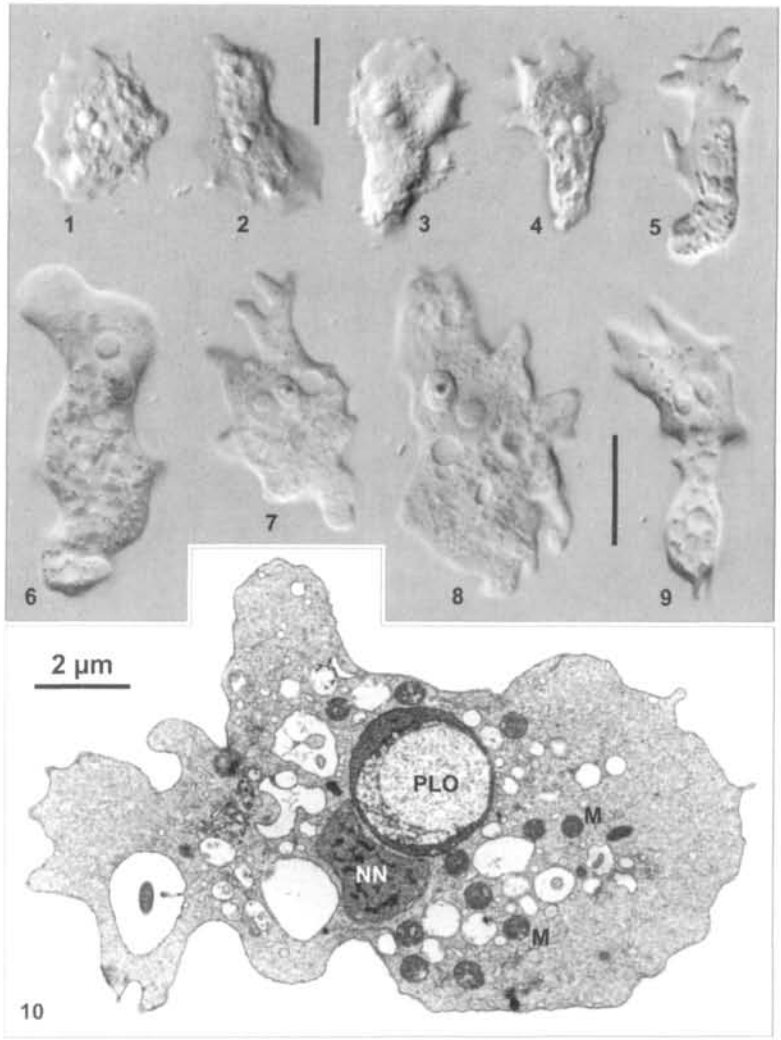
Free-living naked amoebae are highly variable and diverse eukaryotic organisms, ubiquitously distributed in soil and aquatic habitats. They prevail among protozoans in freshwater habitats and constitute regular components in coastal waters and marine sediments. Amoebae previously considered to be entirely free-living, while also capable of invading host tissues, are referred to as amphizoic (Page, 1974). The diversity of free-living amoebae is remarkable. More than 60 genera are known. Contrary to *Entamoeba* and *Schizamoeba* species, free-living amoebae possess

mitochondria and Golgi apparatus. The taxonomic criteria available for generic and species determination of amphizoic amoebae isolated from organs of fish are naturally the same as described for free-living species.

## AMPHIZOIC AMOEBAE RECORDED AS PATHOGENS OF FISH, AGENTS OF LESIONS

Among amoebae known to be able to colonize organs of fish, representatives of only a few genera were described as agents of disease conditions. *Neoparamoeba pemaquidensis* was described as an example of extraordinary pathogenic species of amphizoic amoeba, the agent of amoebic gill disease (AGD) in Atlantic salmon, *Salmo salar*, and turbot, *Psetta maxima*, formerly known as *Scophthalmus maximus* (Kent *et al.*, 1988a; Roubal *et al.*, 1989, Dyková *et al.*, 2000). Although AGD has emerged as one of the most severe health problems in the salmon fish industry (Munday *et al.*, 1990, 1993; Rodger and McArdle, 1996; Clark and Nowak, 1999; Nowak *et al.*, 2002), there was delay in determining AGD agents from various localities. In the first conclusive report on the etiology of AGD, Kent *et al.* (1988a) determined the agent as *Paramoeba pemaquidensis*, most probably because they overlooked the classification of the Rhizopoda published by Page in 1987. In this article, *Paramoeba* was reserved for species covered with microscales and *P. pemaquidensis* and *P. aestuarina* lacking such scales were transferred to the newly established genus *Neoparamoeba*. *Neoparamoeba* strains isolated from gills of different hosts and from localities distant to each other were found to differ only in the size of trophozoites (Figs. 8.1–8.9). Neither light nor electron microscopy allows distinguish species within the genus *Neoparamoeba*. Molecular characterization of morphologically similar gill-isolated *Neoparamoeba* strains revealed that in addition to *N. pemaquidensis*, also *N. branchiphila* have to be taken into consideration as an agent of AGD (Dyková *et al.*, 2000, 2005b). A recently described species, *Neoparamoeba perurans*, has been found a predominating agent of AGD (Young *et al.*, 2007, 2008b).

An undetermined amoeba of the family Cochliopodidae reported as an agent of the so-called nodular gill disease (NGD) (Daoust and Ferguson, 1985; Noble *et al.*, 1994, 1997; Speare, 1999) and *Thecamoeba hoffmani* (Sawyer *et al.*, 1974, 1975) were reported to be pathogenic for freshwater salmonids. Unfortunately, none of the records of amoebic gill disease attributed to amoebae of the family Cochliopodidae included an




**Figs. 8.1–8.10** Trophozoites of *Neoparamoeba* strains of different origin as seen in light microscope equipped with Nomarski DIC (Figs. 8.1–8.9) and transmission electron microscope (Fig. 8.10). **Figs. 8.1–8.3** Trophozoites of *N. pemaquidensis* strains isolated from gills of farmed *Salmo salar*. **Figs. 8.4–8.5.** Trophozoites of *N. branchiphila* strains isolated from *S. salar* and *Psetta maxima*. **Figs. 8.6–8.8.** Trophozoites representatives of Mediterranean strain isolated from gills of *Dicentrarchus labrax*. **Fig. 8.9.** Trophozoite of *N. pemaquidensis* from gills of turbot. **Fig. 8.10.** Trophozoite of *N. pemaquidensis* reference strain. PLO—endosymbiont (*Perkinsiella amoebae*-like organism), NN—nucleus, M—mitochondria of *N. pemaquidensis*. Scale bar for Figs. 8.1–8.5 as well as for Figs. 8.6–8.9 = 10 µm.

exact diagnosis of the agent. The first well-documented *Cochliopodium* sp. found to infect fish was that described by Dyková *et al.* (1998b) from an asymptomatic fish.

The description of *Thecamoeba hoffmani* is based on the morphology of trophozoites, as observed in tissue sections and wet mounts (Sawyer *et al.*, 1974, 1975). Unfortunately, the morphology was documented in histological sections only. Despite incomplete description and documentation of *T. hoffmani*, the relationship between this agent and gill disease associated with mortalities in salmonid fingerlings turned attention to the pathogenic potential of amoebae for freshwater fish.

The taxonomic identity of amoebae associated with etiology of proliferative gill disease (PGD) described by Hoffmann *et al.* (1992) in rainbow trout (*Oncorhynchus mykiss*) and those reported by Roger and Gaines (1975) in *Tilapia aurea* has remained undetermined. Also, the agent of an amoebic gill infestation outbreak in fingerlings of freshwater pallid sturgeon *Scaphirhynchus albus* has not been identified (Smith *et al.*, 2002).

Nash *et al.* (1988) described gill swelling associated with systemic infection caused by amoebae, which they tentatively assigned to the genus *Acanthamoeba*.

The first case of systemic infection caused by amoebae in fish was reported by Taylor (1977). He established systemic infections in experimental fishes using *Acanthamoeba* strain of fish origin. Systemic infection caused by *Naegleria australiensis* was found in a hybrid of *Clarias macrocephalus* × *gariepinus* collected in a fish farm in Thailand (Dyková *et al.*, 2001).

Spontaneous amoebic infections characterized by granulomatous organ lesions were described in goldfishes for the first time by Voelker *et al.* (1977). Lom and Dyková (1992) and Steinhagen *et al.* (1993) briefly described similar infections. Although amoebae were postulated to be the causative agents of lesions in the three publications mentioned, the ultrastructural study (Dyková *et al.*, 1996) did not identify the agent as *Rosculus ithacus*, the only 'true amoeba' that was isolated from these infected goldfishes. Since no mitochondria were observed in cytoplasm of agents of granulomatous lesions, the nature of these organisms remains open to speculations. The trophozoites that can be assigned to Acrasida on the basis of their morphology are frequently isolated together with other amphizoic amoebae.

There are also several reports on so called X-cells. They were described in more than 20 species of fishes from different families, mainly pleuronectids and gadoids (Kent *et al.*, 1988b). Those described from the liver of coho salmon, *Oncorhynchus kisutch*, can possibly be related to multinucleate trophozoites of amoebae known e.g., in *Mastigamoeba* spp. (Dyková *et al.*, 1993; Dyková and Lom, 2007).

## AMOEBIC GILL DISEASE (AGD)

Although it sounds as general term, the name Amoebic Gill Disease is reserved for the disease condition caused by *Neoparamoeba* species. This disease has been diagnosed in a number of fish species cultured in the marine environment worldwide. AGD affects cultures of salmonids in Australia (Munday *et al.*, 1990; Clark and Nowak, 1999), Ireland (Rodger and McArdle, 1996; Palmer *et al.*, 1997), U.S.A. (Kent *et al.*, 1988a; Douglas-Helders *et al.*, 2001b), Spain, France and Chile (Groman, Bustos and Carson, unpubl.). The same disease has been diagnosed in cultured turbot in Spain (Dyková *et al.*, 1995) and South Africa (Dyková, unpublished).

### Disease Symptoms

Non-specific behavioural signs indicative of AGD can be noticed and evaluated best by experienced farm staff. The advanced phase of disease is distinguished by gross abnormalities in the external appearance of the fish gills. The colour and structure abnormalities (white patches) observed in experimental infections of *N. pemaquidensis* in Atlantic salmon do not always coincide with AGD in salmons sampled from farms (Clark and Nowak, 1999). An excessive production of mucus and changes in colour of gill tissue predominates among gross lesions of sub-clinically and clinically diseased turbots.

## DISEASE DIAGNOSTIC

The diagnostic methods developed during the research of AGD in Atlantic salmon were summarized by Zilberg *et al.* (1999), Douglas-Helders *et al.* (2001a), Elliot *et al.* (2001) and Nowak *et al.* (2002). Dyková and Novoa (2001) published comments on diagnostic procedures and their pitfalls experienced during the research of AGD in turbot.

Among the diagnostic methods recommended for AGD affecting cultured Atlantic salmon, inspection of wet mounts of gill tissue and

mucus scraped from gills, air dried mucus smears stained with Quick Dip® or subjected to IFAT, and histological examination were reported (Kent *et al.*, 1988a; Zilberg *et al.*, 1999). Recently, an assay based on PCR amplification of 18S rRNA genes of *Neoparamoeba* spp. and species-specific oligonucleotide probes that hybridise 18S rRNA were designed as important diagnostic tools (Young *et al.*, 2008a).

## Preliminary Diagnosis of AGD

The examination of wet mounts of gill tissue and mucus scraped from gills is of diagnostic value in advanced stages of AGD, when the gills are heavily loaded with amoebae. The recognition of the agent requires a certain experience. Trophozoites that move slowly in dense mucus resemble those observed in histological sections. The cytoplasm of trophozoites is almost always highly vacuolated. In the mucus mixed with seawater, amoebae can also be observed as floating star-like stages with fine radiating pseudopodia. Trophozoites can be concentrated by washing off gill arches in seawater followed by low speed centrifugation, or after their adhesion, from the bottom of Petri dish or any other container. When a drop of centrifugation sediment is placed on coverslip into wet chamber for about 30 minutes, trophozoites attach to coverslip. Then their morphology can be observed in hanging drop preparation using depression slide. Preliminary diagnosis based on the light microscopy requires the recognition of flattened, irregularly fan-shaped trophozoites with short blunt projections of hyaloplasmic zone (Figs. 8.1–8.9). Next to the nucleus, cytoplasm of trophozoite contains remarkable structure originally called parasome. For a safe generic assignment of such amoebae, the attempt to isolate them and culture for purposes of transmission electron microscopy is recommended (Fig. 8.10).

In order to visualize the nucleus and the so-called parasome, which in fact is a symbiotic organism (*Perkinsiella amoebae*) known to occur in the cytoplasm of trophozoites of three genera of the family Paramoebidae (Dyková *et al.*, 2003a), trophozoites attached to slides can be stained with hematoxylin and eosin, using Feulgen reaction or DNA staining (Hoechst 33258). All tested fixatives (Davidson's fixative, ethanol-sublimate and 95% ethanol, respectively) applied immediately after removing the seawater retain the characteristic shape of trophozoites attached to slides. This is considered important in view of the fact that in air-dried preparations, whatever stain used, trophozoites loose their characteristic

shape and size and can hardly be compared with live amoebae. When pre-cleaned slides are used, the adhesion of trophozoites is firm and loss of organisms minimal.

### Final Diagnosis of AGD

Since agents of AGD (together with other amoebae) can be detected in or isolated from asymptomatic fishes, the presence of agent on gills has to be supplemented with other diagnostic methods. Histological examination that visualises symbiotic organism in trophozoites can help to attribute gill lesions to the genus *Neoparamoeba*. (Fig. 8.15). To determine the *Neoparamoeba* species responsible for lesions, *in situ* hybridization with species-specific oligonucleotide probes is available (Young *et al.*, 2008a).

## PATHOGENESIS OF AGD

A comprehensive material available for histological investigation of AGD in turbot, *Psetta maxima*, covered different stages of the infection and allowed inference of the sequence of gill tissue changes, starting with the most delicate one up to the loss of branchial structure in large segments of gill filaments (Dyková *et al.*, 1995). The gill tissue changes initiate and develop mostly in the secondary lamellae. Their thickening (Figs. 8.12, 8.13) due to hypertrophy and hyperplasia of epithelium associated with the attachment of amoeba trophozoites and accumulation of amoebae in the interlamellar spaces are followed by the lamellar fusion (Fig. 8.14). Neighbouring secondary lamellae first adhere to one another and then fuse completely, either partly or focally. Along with the fusion of hypertrophic secondary lamellae, additional alterations occur. The most important ones are regressive changes manifested as desquamation of the epithelium, local disturbances of blood circulation, and progressive changes represented by inflammation. In the initial stage of lamellar thickening, the numerous chloride cells are found also in the distal parts of secondary lamellae. The major phenomenon observed in connection with incomplete fusion of leaf-like secondary lamellae is the formation of cavities or channels between lamellae (Fig. 8.14) that contain proliferating amoebae. In the advanced stage of infection, secondary lamellae of long segments of gill filaments are fused and disfigured by the inflammatory changes. The origin of cavities in the confluent parts is clearly discernible from the presence of the lamellar epithelium or capillaries. The fusion of tips of secondary lamellae, associated with the oedematous separation of

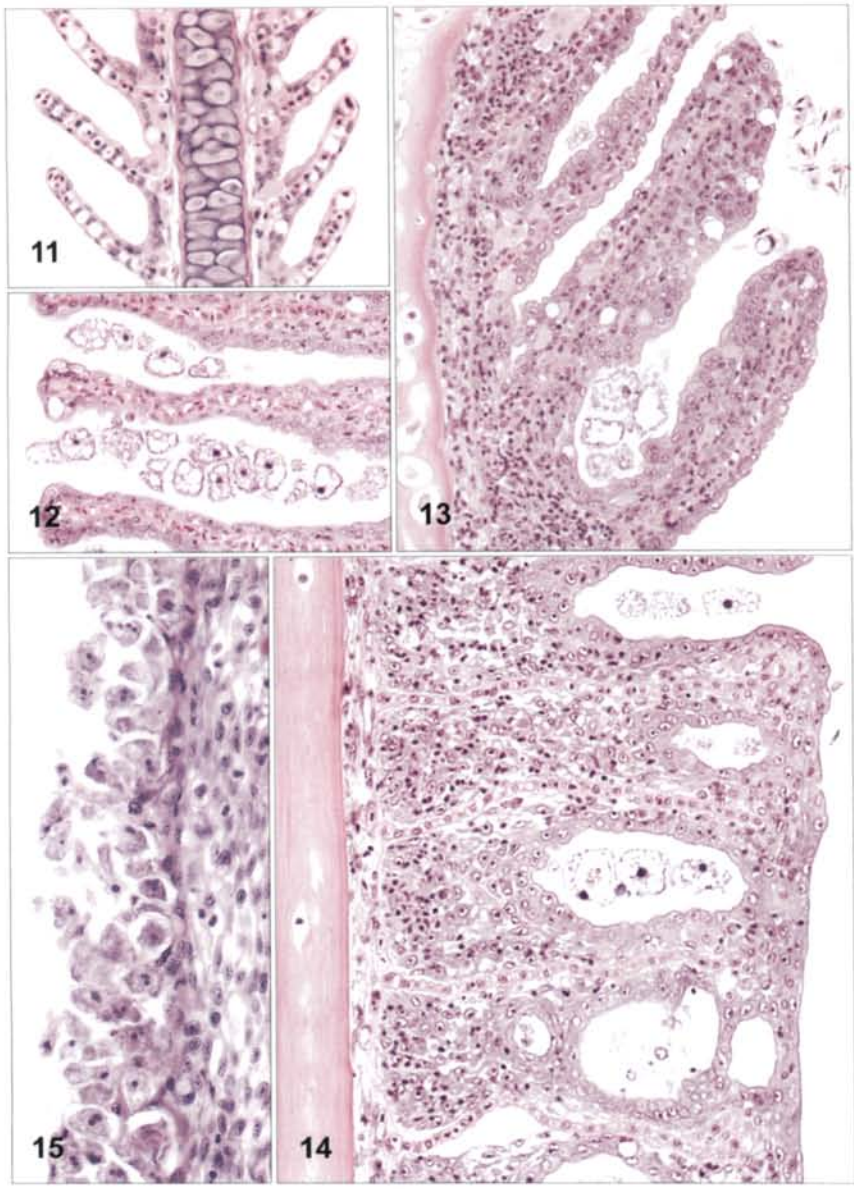



**Fig. 8.11** Histology of a short segment of healthy gill filament of *Salmo salar* to compare it with lesions produced by *Neoparamoeba pemaquidensis* in turbot, *Psetta maxima*, H & E × 320. **Figs. 8.12 and 8.13.** Hypertrophy of gill secondary lamellae caused by *N. pemaquidensis*, H & E × 280. **Fig. 8.14.** Cavities formed between adjacent secondary lamellae laid out with hypertrophic epithelium. H & E × 280. **Fig. 8.15.** Dense layer of trophozoites covering fused tips of secondary lamellae, H & E × 350.

the epithelium in the distal part of the gill lamellar layer, is rather exceptional and difficult to distinguish from post-mortal changes. In such cases, an almost continuous layer of amoebae is observed on the surface of gill filaments (Fig. 8.15). While in the initial stage of the lamellar fusion the multiplying trophozoites localized in newly formed cavities are still surrounded by hypertrophied epithelium, later on in the stage of proliferative inflammation, the thin layer of connective tissue forms a discrete wall around the agent. The number of amoebae localized on the modified gill surface notably diminishes in the late stage of infection with the most pronounced inflammatory changes, while small accumulations of trophozoites and their rounded stages can still be present in the cavities formed originally between two adjacent secondary lamellae (Fig. 8.14). In this stage, the loss of gill respiratory surface area is most pronounced.

Series of studies based on natural and experimental infections confirmed the primary pathogenic role of *Neoparamoeba pemaquidensis* also in AGD of Atlantic salmon (Adams and Nowak, 2001, 2003, 2004). The sequence of gill lesions was found almost identical in both fish hosts, turbot and Atlantic salmon. The analyses of distribution patterns indicated a significant difference in the number of lesions and their severity. The mean number of lesion numbers and pathological severity in the dorsal region of the second gill arch were significantly higher than that found ventrally (Adams and Nowak, 2001).

## DISEASE TREATMENT AND PROPHYLAXIS

At present, the most successful treatment of AGD in sea-caged Atlantic salmon is the use of freshwater baths (of 2–3 h duration) administered when the fish are experiencing outbreaks of AGD during temperatures in the range of 12–20°C (Findlay and Munday, 1998). The freshwater bathing is not fully effective in killing amoebae (Parsons *et al.*, 2001). The efficiency of bathing can be enhanced by reduction of total hardness of freshwater. The use of soft freshwater (19.3–37.4 mg $L^{-1}$ $CaCO_3$) reduces the number of surviving amoebae and can reduce bathing frequency (Roberts and Powell, 2003). In fish experiencing their first exposure to AGD, levamisole added to freshwater bath (1.25, 2.5 and 5.0 mg/L) caused significant decrease in subsequent mortality of the fish. This response could be explained by the enhancement of the non-specific immune system. Although these results are encouraging, their application in the field is not straightforward because farms may not know how many episodes of AGD their fish have been previously exposed to (Zilberg *et al.*,

2000). Moreover, the treatment additive increases total cost of freshwater bath.

### Economic Importance

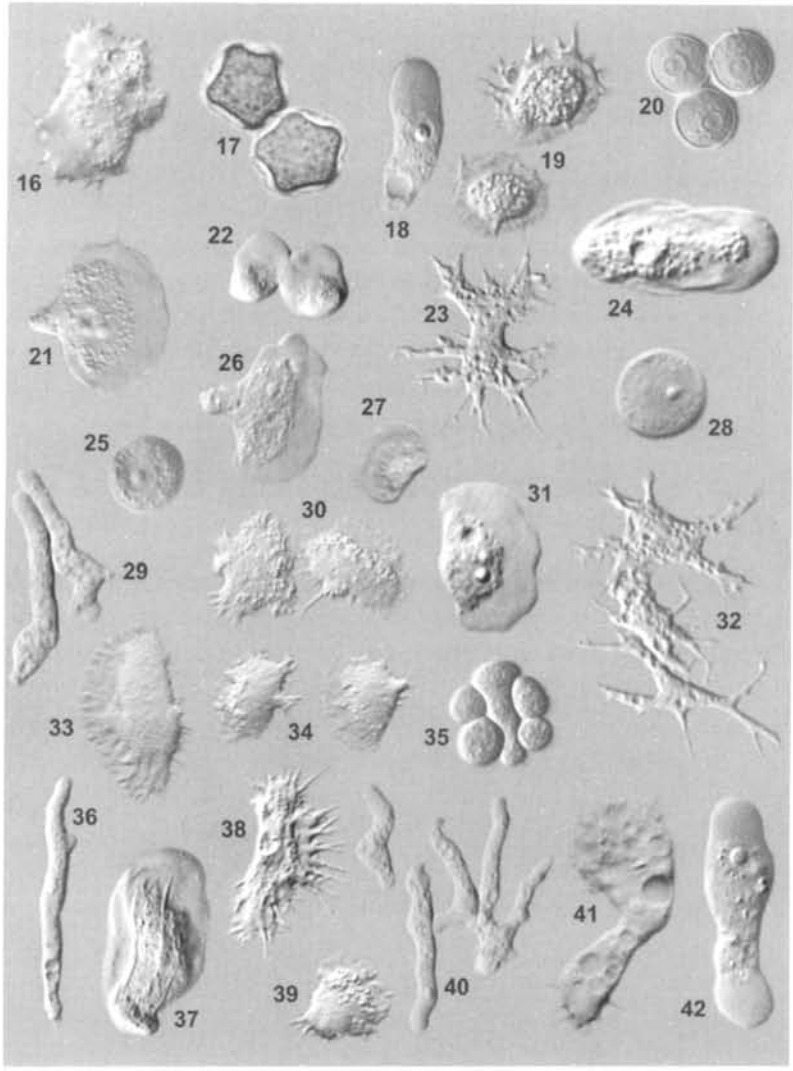
AGD remains the most important disease associated with sea-cage culture of Atlantic salmon in Tasmania, Australia. Current treatment has significant impact on the industry. A total up to 14% of production costs was reported by Adams and Nowak (2001) as an equivalent to over 10% of the industry GVP by Elliot *et al.* (2001). To the best of our knowledge, the data on the impact of AGD on turbot production costs were not published. It is possible that an emergency harvest relatively easy applicable in industrial ongrowing facilities can minimize loses.

## AMPHIZOIC AMOEBAE ISOLATED FROM ORGANS OF ASYMPTOMATIC FISH

Although series of studies revealed that free-living amoebae infect fish as well as some aquatic invertebrates, the reasons why amoebae can convert from free-living to innocuous epi- or endobionts or infectious agents of diseases are enigmatic. The study of amoebae capable of colonizing fish tissues is undoubtedly the most important step towards resolving this enigma. In general, the morphological diversity and polymorphism of amoebae with poorly documented descriptions of species sometimes make even generic diagnosis of newly isolated strains extremely difficult.

Among marine fish species, attention was focused in particular on *Psetta maxima* (Dyková *et al.*, 1999a) and *Salmo salar*. In addition, individual representatives of four marine species belonging to three different families have been tested to date. The assemblage of freshwater fish examined to date comprises more than 30 species belonging to 12 families. In both freshwater and marine fish, the results of isolation attempts have exceeded expectations in the number of isolated strains and their diversity (Dyková *et al.*, 2004).

Flattened amoebae of the family Vannellidae were the most numerous among strains isolated from tissues of marine fish. Representatives of the genera *Vannella*, *Platyamoeba* (Figs. 8.21, 8.22, 8.27) and *Flabellula* (Figs. 8.30, 8.33, 8.34, 8.39) were isolated from the gills of asymptomatic as well as clinically diseased fish with AGD. In addition to the amoebae isolated and successfully cultured, two different species, *Pseudoparamoeba* sp. and *Corythionella* sp. (Euglyphidae) were detected in material embedded for




**Figs. 8.16–8.42** Trophozoites of amphizoic amoebae isolated from organs of fish as observed in hanging drop preparations in Nomarski DIC. **Figs. 8.16, 8.17, 8.41.** Trophozoites and cysts of *Acanthamoeba* sp. **Figs. 8.18, 8.20, 8.42.** Trophozoites and cysts of *Naegleria* sp. **Fig. 8.19.** Trophozoites of *Cochliopodium minus*. **Figs. 8.21, 8.22, 8.27.** Trophozoites of vannelid amoebae isolated from marine fish. **Figs. 8.23, 8.32, 8.35.** Trophozoites of *Filamoeba sinensis*. **Figs. 8.24, 8.37.** Trophozoites of *Thecamoeba hilla*. **Figs. 8.25, 8.29, 8.36, 8.40.** Cyst and trophozoites of *Hartmannella vermiformis*. **Figs. 8.26, 8.31.** Trophozoites of vannelid amoebae isolated from freshwater fish. **Figs. 8.28, 8.38.** Cyst and trophozoite of *Protacanthamoeba bohemica*. **Figs. 8.30, 8.33, 8.34, 8.39.** Trophozoites of *Flabellula* spp.

TEM from primary isolates growing on agar plates. Branched 'buds' of *Corallomyxa* were observed in seawater medium used for primary isolations (Dyková *et al.*, 1999a).

*Thecamoeba* sp. isolated from gill tissue of turbot was tentatively, on the basis of trophozoite morphology, identified with *T. hilla* Schaeffer, 1926, which is one of the five marine species of this genus described thus far (Figs. 8.24, 8.37). This amoeba strain is mentioned below because trophozoites sub-cultured for more than three years contain an eukaryotic symbiont.

In freshwater fish, vannelids also belong to the most common amphizoic amoebae isolated from various organs (Figs. 8.26, 8.31). Ultrastructural features (types of glycocalyx) defined by Page (1980, 1983, 1988, 1991) allow to assign them to the genus *Vannella* Bovee, 1965 or *Platyamoeba* Page, 1969. Except for size differences, host origin and locality, no other features useful for species diagnosis or description of new species are available within this group of amoebae. Sequence-based phylogenetic analyses of SSU rRNA genes from a data set representing a total of 29 strains of flattened amoebae strongly indicated that morphological features differentiating these genera do not reflect phylogenetic relationships of representative strains. Contrary to previous studies, strains of this expanded assemblage formed clusters that did not reflect their environmental origin. Monophyletic groups were of mixed origins and contained freshwater as well as marine strains of both genera isolated in geographically distant localities of various continents. These noteworthy findings were supported by results of phylogenetic analyses of selected strains based on ITS sequences (Dyková *et al.*, 2005a).

Ten *Acanthamoeba* strains (Figs. 8.16, 8.41) isolated from organs of asymptomatic freshwater feral fish were characterized morphologically and their ribosomal RNA gene sequences were compared with 53 other *Acanthamoeba* strains of different origin (Dyková *et al.*, 1999b). The morphology of cyst (Fig. 8.17) analogous with the group II (MGII) varied extensively within and among clones and gave no clear answer as to species identification. Sub-generic classification was based on analyses of diagnostic fragments of 18S rDNA sequences that are widely used in large *Acanthamoeba* data sets (Stothard *et al.*, 1998). Such analyses assigned all fish-isolates to T4 subset of MG2 that contains strains commonly isolated from cases of human infections, especially of *Acanthamoeba* keratitis. Screening focused on gill infecting amphizoic amoebae has revealed the presence of *Acanthamoeba* trophozoites on the gills of ornamental fish in most freshwater aquarium systems sampled to date.

Eighteen out of 40 *Naegleria* strains isolated from organs of five species of freshwater fishes (*Blicca bjoerkna*, *Clarias* hybrid, *Oncorhynchus mykiss*, *Perca fluviatilis*, *Salvelinus fontinalis*) have been studied in detail (Dyková *et al.*, 2001). Six strains, representatives of groups with slightly different size of cysts, were selected and corresponding clones derived from them were subjected to sequence- and riboprinting RFLP-PCR analysis of the small subunit rRNA genes. One strain isolated from the brain of a fish with systemic infection was identified with *N. australiensis*, the species originally described from flood drainage water in South Australia and later found to be pathogenic for homoiotherm host. Another five strains appeared in phylogenetic trees to be closely related to *N. clarki*. Surprisingly, none of the clones assayed by molecular methods grouped with *N. gruberi*, which was considered for a long time to be a widely distributed species. For comparison with a new assemblage of *Naegleria* strains isolated mostly from gills of feral fish (Figs. 8.18, 8.20, 8.42), a set of 37 *Naegleria* spp. determined by De Jonckheere (2002) on the basis of their ITS rDNA sequences will be available.

Scale-bearing amoebae belonging to the genus *Cochliopodium*, another suspected pathogen of fish, were isolated from three different hosts, *Perca fluviatilis*, *Gobio gobio* and *Silurus glanis*. The strain isolated from gills and other organs of perch, *P. fluviatilis* was identified with *C. minus* Page, 1976 (Fig. 8.19). Light microscopical generic assignment was confirmed by ultrastructural study of both, the strain isolated from perch and environmental strain of *C. minus* (CCAP 1537/1A from the U.K. National Culture Collection) serving as a control (Dyková *et al.*, 1998b). The morphology of another two strains isolated from the spleen of *Gobio gobio* and gills of *Silurus glanis* resembled that of *C. minus* and formed monophyletic group in phylogenetic analyses based on sequences of SSU rRNA gene.

Since 1978 when Sawyer *et al.* isolated *Vexillifera bacillipedes* Page, 1960 from kidney of rainbow trout and incorrectly designated it as the agent of proliferative kidney disease (PKD), only one more species of this genus has been recorded. The strain isolated from the liver of perch, *Perca fluviatilis*, could not be identified with any of the freshwater *Vexillifera* species characterized by TEM and was, therefore, described as a new species *V. expectata* (Dyková *et al.*, 1998). Hexagonal glycostyles discovered on the surface coat were similar to those of marine species of the genus.

A thus far unique representative of the genus *Nuclearia* Cienkowski, 1865 was isolated from the gills of the roach, *Rutilus rutilus*, collected from a pond in South Bohemia. The host fish was kept in captivity more than two months prior to isolation attempts. Based on the morphological description and sequence-based phylogenetic analyses, the strain was classified as a new species, *Nuclearia pattersoni* (Dyková *et al.*, 2003b). The analysis of information available to date shows that more data on nucleariid amoebae are needed to determine their relationships within the genus and with other, insufficiently characterized species of similar genera.

Based on morphological and molecular characterization, four amoeba strains isolated from organs of freshwater fish (from the liver of *Tinca tinca*, spleen of *Cyprinus carpio*, kidney of *Oreochromis niloticus* and gills of *Perca fluviatilis*) were identified as *Hartmannella vermiformis* Page, 1967 (Figs. 8.25, 8.29, 8.36, 8.40) (Dyková *et al.*, 2005e).

The gills of *Salmo salar* fry, which has been used for salmon restocking of the river Elbe basin in the Czech Republic, were found infected with amoebae that were similar to *Saccamoeba* spp. when observed in the light microscope. High level of SSU rRNA gene sequence similarity with *S. limax* (strain F-13, ATCC 30942) was, however, at variance with results of comparative ultrastructural study. The strain from gills of *S. salar* differed from the previous and only ultrastructural description of *S. limax* by Page (1985), having regularly arranged, straight tubular, non-branching mitochondria cristae (Dyková *et al.*, 2002).

An amphizoic amoeba isolated from the gills of *Carassius gibelio* (Bloch, 1782) sampled in Hubei province, China was described as a new species of the genus *Filamoeba* Page, 1967. *F. sinensis* (Figs. 8.23, 8.32, 8.35) represents the second species described within the genus (Dyková *et al.*, 2005c).

A new species of amphizoic amoeba denominated *Protacanthamoeba bohemica* (Figs. 8.28, 8.38) was isolated from the liver of *Tinca tinca*. As a first representative of the genus, the strain was subjected to phylogenetic analyses. The results demonstrated close relationship of *P. bohemica* with *Acanthamoeba* spp. and *Balamuthia mandrillaris* (Dyková *et al.*, 2005d).

Strains of minute amoebae recognized on the basis of their morphology as belonging to Acrasida are frequently isolated together with other species of naked amoebae from organs of fish. They can also be considered potential fish pathogens. Many amoeba strains isolated from fish organs, e.g., those belonging to the genera *Echinamoeba* and *Flabellula*, *Vexillifera*, are in the process of species identification. There are also many

strains in which morphological study was not sufficient for generic assignment.

Etiological diagnosis, which identifies the cause of lesions, is of primary importance in any disease problem. In general, eukaryotic microorganisms are not easy to determine in tissues. This applies in particular to trophic stages of amoebae that lack rigid structures and are extremely changeable when subjected to different conditions.

In general, the key steps in the process of determination of amoebae as suspected agents of disease conditions include examination of gross lesions if present, sampling of tissues for isolation attempts, which are crucial for future identification of amoebae, and direct proof of pathogenicity of amoebae in histological sections. There are, however, limitations for each step of the procedure. Gross lesions are mostly non-specific. Nevertheless, squash preparations from macroscopic lesions, in which amoebae are revealed, can be indicative for isolation attempts and histopathological examination. The technique used for isolation of amoebae from organs of fish has been briefly described in Dyková and Lom (2004). If the isolation attempt is successful, the primary isolate contains a homogeneous cell population, from which the strain and clonal cultures can easily be established. Less successful attempts result in isolation of a mixture of different amoebae. The process of separation of different cell populations used to be time consuming. The suspected agent could be lost before adequate culturing conditions are prepared for it and/or because of rapid overgrowth of other simultaneously isolated species and bacteria. The identification of isolated amoebae (i.e., clones derived from established strains) requires as a rule combination of morphological and non-morphological methods (see taxonomic criteria). When fresh material is available and tissues can be sampled simultaneously for isolation attempts and histological examination, conclusive results on etiology of examined lesions can be expected. The etiology of most cases of suspected amoebic infections, observed incidentally in routinely examined histological material, remains obscure. Examination of histological sections, irrespective of staining method, rises in most cases only suspicion of amoebic etiology of lesions. Tentative etiology can be confirmed relatively easily only in the case of *Neoparamoeba* spp., agents of AGD. Histological examination that visualises symbiotic organism in trophozoites can help to attribute gill lesions to the genus *Neoparamoeba*. (Fig. 8.15). To determine the *Neoparamoeba* species responsible for lesions, *in situ* hybridization with species-specific oligonucleotide probes is available (Young *et al.* 2008a).

Since the ultimate goal in studies of pathogenicity of amphizoic amoebae would be to identify these organisms directly in histological sections, the development of specific diagnostic tools is desirable even for genera and species that are considered potential pathogens on the basis of current knowledge.

## AMPHIZOIC AMOEBAE ISOLATED FROM FISH AS HOSTS OF OTHER ORGANISMS

In all their habitats, free-living amoebae are exposed to other organisms. They are able to ingest and feed on many of them. Some bacteria as well as certain prokaryotes are able to infect amoebae, proliferate inside their cells and kill them or establish symbiotic associations (Daniels *et al.*,1966; Lee *et al.*, 1985; Bradley and Marciano-Cabral, 1996). Only a few studies have been devoted to symbiotic associations of amoebae with algae (Lee *et al.*, 1985; Karpov *et al.*, 1991). The other prostistan endobionts of amoebae have been neglected. Whatever type of relationship is established with other microorganisms that share the same habitat, there is always possibility that amphizoic amoebae can introduce these organisms, including agents of diseases, to the fish.

The routine employment of transmission electron microscopy in the research of generic and species composition of amoebae infecting freshwater and marine fish have expanded the knowledge of organisms living in the cytoplasm of amoebae and opened wide field for new discoveries. This can be illustrated with the following examples.

After a long period of axenization, *Acanthamoeba* trophozoites of strains isolated from the liver of *Perca fluviatilis* and brain of *Leuciscus cephalus* continued to host bacteria in their cytoplasm. Virus-like particles were found by electron microscopy in trophozoites of *Acanthamoeba* strain isolated from the spleen of *Silurus glanis*. In addition to the frequent occurrence of facultative endosymbionts, stable symbiotic relationships have also been recognized between amphizoic amoebae of fish origin and bacteria. The cytoplasm of a newly described species of amphizoic amoeba, *Nuclearia pattersoni* isolated from the gills of *Rutilus rutilus*, contained rod-shaped bacteria, which using phylogenetic analyses could be classified as *Rickettsia* sp. related to endosymbionts of leeches (Dyková *et al.*, 2003b). Trophozoites of *N. pattersoni* harboured rickettsial cells over a long period of their subculturing.

In amphizoic amoebae isolated from organs of marine fish, in addition to prokaryotes, also eukaryotes have been recognized as endosymbiotic partners. A species of the genus *Labyrinthula* Cienkowski, 1867 (Myxomycota) was found as a permanent resident in the cytoplasm of *Thecamoeba hilla* Schaeffer, 1926, isolated from gills of *Psetta maxima*. The co-existence of both organisms sustainable for more than three years has to be interpreted as stable type of symbiotic association.

The symbiotic organisms resembling *Perkinsiella amoebae* Hollande, 1980 described previously in *Janickina* spp. from chaetognaths, were found in trophozoites of all *Neoparamoeba* strains isolated from turbot and salmon (Dyková *et al.*, 1998a, 2003a). Similar organisms were recorded from seven species belonging to three genera of the family Paramoebidae. Comparative study of these organisms in trophozoites of six *Neoparamoeba* strains isolated from gills of turbot, *P. maxima*, revealed their mutual similarity. The morphology of *Perkinsiella amoebae*-like organisms (PLOs) supported Hollande's hypothesis (Hollande, 1980) about their kinetoplastid origin. The first conclusive results on this hypothesis were obtained using two marker genes (Dyková *et al.*, 2003a). The recognition of euglenozoan spliced leader RNA (SL RNA) gene sequences in the genomic DNA of endosymbionts from five *Neoparamoeba* strains, together with the acquisition of one SSU RNA gene sequence, allowed to specify the relationship of the endosymbionts with kinetoplastids. Phylogenetic analyses of SSU rRNA gene sequence data currently available revealed close relationship of the first sequenced *Perkinsiella amoebae*-like organism with *Ichthyobodo necator* (Dyková *et al.*, 2003a).

## PROSPECTS OF FUTURE RESEARCH

Research of amphizoic amoebae of fish is still in its infancy in spite of the results briefly mentioned above. This relates to both the benefits their study may offer to fish pathology and husbandry and to the significance these data may have for fundamental knowledge.

Determination of the prevalence of amphizoic amoebae in fish and definition of their potential to inflict disease conditions upon their hosts is of basic interest for fish parasitology. A prerequisite to progress in this direction is further development of convenient methods of diagnostics and generic and species determination of amoebic agents. It is obvious that morphological characteristics alone are not sufficient to characterize and identify all-important taxa and morphological differences may be

misleading in classifying amoebae. The investigation into interspecific and intraspecific diversity of selected taxons may help to uncover alternative biological approaches for disease control of some important pathogens, such as *Neoparamoeba* spp.

The number of free-living and amphizoic amoebae that have been characterized by both morphological and molecular methods makes up only a small fraction of the species described thus far. This also applies to amoeba strains of fish origin. Further progress is necessary on both fields. Detection of signature sequences in newly described species could greatly facilitate future identification and comparisons. Research on marker genes should be aimed at specific diagnostic tools for individual genera and species of amoebae, which should be followed by construction of diagnostic primers suitable for *in situ* hybridization in histological sections. The identification of amphizoic amoebae in histological material can significantly contribute to reveal their pathogenicity.

New data on symbiotic associations established between amoebae and other organisms open an intriguing vista for future researchers. The presence of viable pathogenic bacteria inside free-living amoebae offers a new challenge for studies on interrelations between parasitic organisms. The role of amoebae as vectors of human pathogens has been well studied. An untouched field is, however, the significance of amphizoic amoebae for transmission and dispersion of specific fish pathogens.

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## References

Adams, M.B. and B.F. Nowak. 2001. Distribution and structure of lesions in the gills of Atlantic salmon, *Salmo salar* L. affected with amoebic gill disease. *Journal of Fish Diseases* 24: 535-542.

Adams, M.B. and B.F. Nowak. 2003. Amoebic gill disease (AGD): sequential pathology in cultured Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 26: 601-614.

Adams, M.B. and B.F. Nowak. 2004. Sequential pathology after initial freshwater bath treatment for amoebic gill disease in cultured Atlantic salmon *Salmo salar* L. *Journal of Fish Diseases* 27: 163-173.

Adl, S.M., A.G.B. Simpson, M.A. Farmer, R.A. Andersen, O.R. Anderson, J.R. Barta, S.S. Bowser, G. Brugerolle, R.A. Fensome, S. Fredericq, T.Y. James, S. Karpov, P. Kugrens, J. Krug, C.E. Lane, L.A. Lewis, J. Lodge, D.H. Lynn, D.G. Mann, R.M. Mccourt, L. Mendoza, Ø. Moestrup, S.E. Mozley-Standridge, T.A. Nerad, C.A. Shearer, A.V. Smirnov, F.W. Spiegel and M.F.J.R.Taylor. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology* 52: 399-451.

Bradley, S.G. and F. Marciano-Cabral. 1996. Diversity of free-living naked amoeboid organisms. *Journal of Industrial Microbiology* 17: 314-321.

Clark, A. and B. Nowak. 1999. Field investigations of Amoebic Gill Disease in Atlantic salmon, *Salmo salar* L., in Tasmania. *Journal of Fish Diseases* 22: 433-443.

Daniels, E.W., E.P. Brever and R.R. Kudo. 1966. *Pelomyxa palustris* Greef. II. Its ultrastructure. *Zeitschrift für Zellforschung* 73: 367-383.

Daoust, P.Y and H.W. Ferguson. 1985. Nodular gill disease in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 8: 511-522.

De Jonckheere, J.F. 2002. A century of research on the amoeboflagellate genus *Naegleria*. *Acta Protozoologica* 41: 309-342.

Douglas-Helders, M.G., J. Carson, T. Howard and B. Nowak. 2001a. Development and validation of a new dot blot test for the detection of *Paramoeba pemaquidensis* (Page) in fish. *Journal of Fish Diseases* 24: 273-280.

Douglas-Helders, M.G., S. Saksida, S. Raverty and B. Nowak. 2001b. Temperature as a risk factor for outbreaks of Amoebic Gill Disease in farmed Atlantic salmon (*Salmo salar*). *Bulletin of the European Association of Fish Pathologists* 21: 114-116.

Dyková, I. and J. Lom. 2004. Advances in the knowledge of amphizoic amoebae infecting fish. *Folia Parasitologica* 51: 81-96.

Dyková, I. and J. Lom. 2007. *Histopathology of Protistan and Myxozoan Infections in Fishes*. Academia, praha.

Dyková, I. and B. Novoa. 2001. Comments on diagnosis of amoebic gill disease (AGD) in turbot, *Scophthalmus maximus*. *Bulletin of the European Association of Fish Pathologists* 21: 40-44.

Dyková, I., A. Figueras and B. Novoa. 1993. X-cell lesions in the liver of coho salmon *Oncorhynchus kisutch*. *Diseases of Aquatic Organisms* 15: 171-174.

Dyková, I., A. Figueras and B. Novoa. 1995. Amoebic gill infection of turbot, *Scophthalmus maximus*. *Folia Parasitologica* 42: 91-96.

Dyková, I., J. Lom, B. Macháčková and T.K. Sawyer. 1996. Amoebic infections in goldfishes and granulomatous lesions. *Folia Parasitologica* 43: 81-90.

Dyková, I., A. Figueras, B. Novoa and J. Fernandez Casal. 1998a. *Paramoeba* sp., an agent of amoebic gill disease of turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms* 33: 137-141.

Dyková, I., J. Lom and B. Macháčková. 1998b. *Cochliopodium minus*, a scale-bearing amoeba isolated from organs of perch *Perca fluviatilis*. *Diseases of Aquatic Organisms* 34: 205-210.

Dyková, I., B. Macháčková and H. Pecková. 1998c. *Vexillifera expectata* sp. n. and other non-encysting amoebae isolated from organs of freshwater fish. *Folia Parasitologica* 45: 17-26.

Dyková, I., A. Figueras and B. Novoa. 1999a. Epizoic amoebae from the gills of turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms* 38: 33-38.

Dyková, I., J. Lom, J.M. Schroeder-Diedrich, G.C. Booton and T.J. Byers. 1999b. *Acanthamoeba* strains isolated from organs of freshwater fishes. *Journal of Parasitology* 85: 1106-1113.

Dyková, I., A. Figueras and Z. Perić. 2000. *Neoparamoeba* Page, 1987: light and electron microscopic observations on six strains of different origin. *Diseases of Aquatic Organisms* 43: 217-223.

Dyková, I., I. Kyselová, H. Pecková, M. Oborník and J. Lukeš. 2001. Identity of *Naegleria* strains isolated from organs of freshwater fishes. *Diseases of Aquatic Organisms* 46: 115-121.

Dyková, I., M. Veverková, I. Fiala and B. Macháčková. 2002. A free-living amoeba with unusual pattern of mitochondrial structure isolated from Atlantic salmon, *Salmo salar* L. *Acta Protozoologica* 41: 415-419.

Dyková, I., I. Fiala, J. Lom and J. Lukeš. 2003a. *Perkinsiella amoebae*-like endosymbionts of *Neoparamoeba* spp. relatives of the kinetoplastid *Ichthyobodo*. *European Journal of Protistology* 39: 53-61.

Dyková, I., M. Veverková, I. Fiala, B. Macháčková and H. Pecková. 2003b. *Nuclearia pattersoni* sp. n. (Filosea), a new species of amphizoic amoeba isolated from gills of roach (*Rutilus rutilus*), and its rickettsial endosymbiont. *Folia Parasitologica* 50: 161-170.

Dyková, I., L. Boháčová, I. Fiala, B. Macháčková, H. Pecková and H. Dvořáková. 2005a. Amoebae of the genera *Vannella* Bovee, 1965 and *Platyamoeba* Page, 1969 isolated from fish and their phylogeny inferred from SSU rRNA gene and ITS sequences. *European Journal of Protistology* 41: 219-230.

Dyková, I., B.F. Nowak, P.B.B. Crosbie, I. Fiala, H. Pecková, M.B. Adams, B. Macháčková and H. Dvořáková. 2005b. *Neoparamoeba branchiphila* n. sp., and related species of the genus *Neoparamoeba* Page, 1987: morphological and molecular characterization of selected strains. *Journal of Fish Diseases* 28: 49-64.

Dyková, I., H. Pecková, I. Fiala and H. Dvořáková. 2005c. *Filamoeba sinensis* sp. n., a second species of the genus *Filamoeba* Page, 1967, isolated from gills of *Carassius gibelio* (Bloch, 1782). *Acta Protozoologica* 44: 75-80.

Dyková, I., M. Veverková-Fialová, I. Fiala and H. Dvořáková. 2005d. *Protacanthamoeba bohemica* sp. n., isolated from the liver of tench, *Tinca tinca* (Linnaeus, 1758). *Acta Protozoologica* 44: 369-376.

Dyková, I., Z. Pindová, I. Fiala, H. Dvořáková and B. Macháčková. 2005e. Fish-isolated strains of *Hartmannella vermiformis* Page, 1967: morphology, phylogeny and molecular diagnosis of the species in tissue lesions. *Folia Parasitologica* 52: 295-303.

Elliot, N.G., F. Wong and J. Carson. 2001. Detection and abundance of *Paramoeba* species in the environment. CSIRO Marine Research, Hobart.

Fiala, I. and I. Dyková. 2003. Molecular characterisation of *Neoparamoeba* strains isolated from gills of *Scophthalmus maximus*. *Diseases of Aquatic Organisms* 55: 11-16.

Findlay, V.L. and B.L. Munday. 1998. Further studies on acquired resistance to amoebic gill disease (AGD) in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 21: 121-125.

Hoffmann, R.W., M. El-Matbouli and T. Fischer-Scherl. 1992. A proliferative gill disease (PGD) in rainbow trout (*Oncorhynchus mykiss*). *Bulletin of the European Association of Fish Pathologists* 12: 139-140.

Hollande, A. 1980. Identification du parasome (Nebenkern) de *Janickina pigmentifera* à un symbionte (*Perkinsiella amoebae* nov. gen.-nov. sp.) apparenté aux flagellés Kinetoplastidiés. *Protistologica* 16: 613-625.

Karpov, S.A., A.V. Goodkov and M.A. Marinich. 1991. The variety of algae-bearing invertebrates from inner lakes of the Valamo island. *Zoologicheskii Zhurnal* 70: 5-10. (In Russian).

Kent, M.L., T.K. Sawyer and R.P. Hedrick. 1988a. *Paramoeba pemaquidensis* (Sarcomastigophora, Paramoebidae) infestation of the gills of coho salmon *Oncorhynchus kisutch* reared in sea water. *Diseases of Aquatic Organisms* 5: 163-169.

Kent, M.L., M.S. Myers, S.R. Wellings nd R.A. Elston. 1988b. An internal X-cell pseudotumor in a black croaker (*Cheilotrema saturnum*). *Journal of Wildlife Diseases* 24: 142-145.

Lee, J.J., A.T. Soldo, W. Reisser, M.J. Lee, K.W. Jeon and H.D. Görtz. 1985. The extent of algal and bacterial endosymbioses in Protozoa. *Journal of Protozoology* 32: 391-403.

Lom, J. and I. Dyková. 1992. *Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science*, Vol. 26. Elsevier, Amsterdam.

Munday, B.L., C.K. Foster, F.R. Roubal and R.J.G. Lester. 1990. Paramoebic gill infection and associated pathology of Atlantic salmon, *Salmo salar*, and rainbow trout, *Salmo gairdneri*, in Tasmania. In: *Pathology in Marine Science*, F.O. Perkins and T.C. Cheng (eds.). Academic Press, London, pp. 215-222.

Munday, B.L., K. Lange, C. Foster, R.J.G. Lester and J. Handlinger. 1993. Amoebic gill disease of sea-caged salmonids in Tasmania waters. *Tasmanian Fisheries Research* 28: 14-19.

Nash, G., M. Nash and H.J. Schlotfeld. 1988. Systemic amoebiasis in cultured European catfish, *Silurus glanis* L. *Journal of Fish Diseases* 11: 57-71.

Noble, A., R. Herman, E. Noga and G. Bullock. 1994. Description of a recurrent amoeba gill infestation in rainbow trout (*Oncorhynchus mykiss*) cultured in a semiclosed recirculating system. *International Symposium of Aquatic Animal Health, Seattle, Washington, September* 4-8, 1994. *Abstract* W-10.1.

Noble, A.C., R.L. Herman, E.J. Noga and G.L. Bullock. 1997. Recurrent amoebic gill infestation in rainbow trout cultured in a semiclosed water recirculation system. *Journal of Aquatic Animal Health* 9: 64-69.

Nowak, B., J. Carson, M.D. Powell and I. Dyková. 2002. Amoebic Gill Disease in the marine environment. *Bulletin of the European Association of Fish Pathologists* 22: 144-147.

Page, F.C. 1974. *Rosculus ithacus* Hawes, 1963, Amoebida, Flabellulidea and the amphizoic tendency in amoebae. *Acta Protozoologica* 13: 143-154.

Page, F.C. 1980. A light- and electron-microscopical comparison of limax and flabellate marine amoebae belonging to four genera. *Protistologica* 16: 57-78.

Page, F.C. 1983. *Marine Gymnamoebae*. Institute of Terrestrial Ecology, Culture Centre of Algae and Protozoa, Cambridge, England.

Page, F. 1985. The limax amoebae: Comparative fine structure of the Hartmannellidae (Lobosea) and further comparisons with the Vahlkampfiidae (Heterolobosea). *Protistologica* 21: 361-383.

Page, F.C. 1987. The classification of naked amoebae of phylum Rhizopoda. *Archiv für Protistenkunde* 133: 199-217.

Page, F.C. 1988. A new key to freshwater and soil Gymnamoebae. Freshwater Biological Association, Ambleside, Cumbria.

Page, F.C. 1991. Nackte Rhizopoda. In: *Nackte Rhizopoda un Heliozoea*, F.C. Page and F.J. Siemensma (eds.). Gustav Fischer Verlag, Stuttgart, Vol. 2, pp. 8-170.

Palmer, R., J. Carson, M. Ruttledge, E. Drinan and T. Wagner. 1997. Gill disease associated with *Paramoeba* in sea reared Atlantic salmon in Ireland. *Bulletin of the European Association of Fish Pathologists* 17: 112-114.

Parsons, H., M. Powel, D. Fisk and B. Nowak. 2001. Effectiveness of freshwater bathing as treatment against Amoebic Gill Disease in Atlantic salmon. *Aquaculture* 195: 205-210.

Roberts, S.D. and M.D. Powell. 2003. Reduced total hardness of freshwater enhances the efficacy of bathing as a treatment for amoebic gill disease in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 26: 591-599.

Rodger, H.D. and J.F. McArdle. 1996. An outbreak of amoebic gill disease in Ireland. *Veterinary Record* 139: 348-349.

Rogers, W.A. and J.L. Gaines Jr. 1975. Lesions of protozoan diseases in fish. In: *The Pathology of Fishes*, W.E. Ribelin and G. Migaki (eds.). The University of Wisconsin Press, Madison, Wisconsin, pp. 117-141.

Roubal, F.R., R.J.G. Lester and C.K. Foster. 1989: Studies on cultured and gill-attached *Paramoeba* sp. (Gymnamoebae: Paramoebidae) and the cytopathology of paramoebic gill disease in Atlantic salmon, *Salmo salar* L., from Tasmania. *Journal of Fish Diseases* 12: 481-493.

Sawyer, T.K., J.G. Hnath and J.F. Conrad. 1974. *Thecamoeba hoffmani* sp. n. (Amoebida: Thecamoebidae from gills of fingerling salmonid fish. *Journal of Parasitology* 60: 677-682.

Sawyer, T.K., G.L. Hoffman, J.G. Hnath and J.F. Conrad. 1975. Infection of salmonid fish gills by aquatic amebas (Amoebida: Thecamoebidae). In: *The Pathology of Fishes*, W.E. Ribelin and G. Migaki (eds.). The University of Wisconsin Press, Madison, pp. 143-150.

Sawyer, T.K., P. Ghittino, S. Andruetto, P. Pernin and M. Pussard. 1978. *Vexillifera bacillipedes* Page, 1969, an amphizoic amoeba of hatchery rainbow trout in Italy. *Transactions of the American Microscopical Society* 97: 596-600.

Smith, S.A., K.P. Hughes and J. Luoma. 2002. Amoebic gill infestation in pallid sturgeon, *Scaphirhynchus albus*. *Bulletin of the European Association of Fish Pathologists* 22: 400-402.

Speare, D.J. 1999. Nodular gill disease (Amoebic gill infestation) in char, *Salvelinus alpinus*. *Journal of Comparative Pathology* 121: 277-282.

Steinhagen, D., S. Jendrysek and W. Körting. 1993. Amöbiasis bei Goldfishen. *Kleintierpraxis* 38: 469-474.

Stothard, D.R., J.M. Schroeder-Diedrich, M.H. Awwad, R.J. Gast, D.R. Ledee, S. Rodriguez-Zaragoza, C.L. Dean, P.A. Fuerst and T.J. Byers. 1998. The evolutionary history of the genus *Acanthamoeba* and the identification of eight new 18S rRNA gene sequence types. *Journal of Eukaryotic Microbiology* 45: 45-54.

Taylor, P.W. 1977. Isolation and experimental infection of free-living amoebae in freshwater fishes. *Journal of Parasitology* 63: 232-237.

Voelker, F.A., M.R. Anver, A.V. McKee, H.W. Casey and G.R. Brenniman. 1977. Amebiasis in goldfish. *Veterinary Pathology* 14: 247-255.

Young, N.D., P.B.B. Crosbie, M.B. Adams, B.F. Nowak and R.N. Morrison. 2007. *Neoparamoeba perurans* n. sp., an agent of amoebic gill disease of Atlantic salmon (*Salmo salar*). *International Journal for Parasitology* 37: 1469-1481.

Young, N.D., I. Dykova, B. Nowak and R.N. Morrison. 2008a. Development of a diagnostic PCR to detect *Neoparamoeba perurans*, agent of amoebic gill disease (AGD). *Journal of Fish Diseases*. (In Press).

Young, N.D., I. Dykova, K. Snekvik, B.F. Nowak and R.N. Morrison. 2008b. *Neoparamoeba perurans* is a cosmopolitan aetiological agent of amoebic gill disease. *Diseases of Aquatic Organisms*. (In Press).

Zilberg, D. and B.L. Munday. 2005. Phylum Amoebozoa. In: *Fish Diseases and Disorders*, P.T.K. Woo (ed.). 2nd Edition. CABI Publishing, Wallingford, Oxon, U.K., Vol. 1, pp. 1-15.

Zilberg, D., B. Nowak, J. Carson and T. Wagner. 1999. Simple gill smear staining for diagnosis of amoebic gill disease. *Bulletin of the European Association of Fish Pathologists* 19: 186-189.

CHAPTER

# 9

# Diseases Caused by Flagellates

**Pilar Alvarez-Pellitero**

## INTRODUCTION

In this chapter on Diseases Caused by Flagellates we will study fish diseases produced by organisms traditionally known as flagellates *sensu lato*, mainly dinoflagellates, diplomonads and kinetoplastids.

The taxonomy and systematic of these groups of protists have altered repeatedly from the creation of the corresponding taxa. Successive classifications have been proposed by the Society of Protoozologists in the previous few decades, trying to incorporate the insights of the new scientific findings. Many traditional groups are no longer valid and have been abandoned (Hausmann *et al.*, 2003). Recently, a new higher level classification of eukaryotes has been proposed by Adl *et al.* (2005), using data gathered from modern morphological approaches, biochemical pathways, and molecular phylogenetics, which are generally complementary. Adl *et al.* (2005) adopted a hierarchical system without formal rank designations such as 'class' or 'order' so as to avoid the

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common problem of a single change causing a cascade of changes to the system. In addition, this approach has the advantage of being more flexible and easier to modify.

In this classification of Adl *et al.* (2005), the organisms traditionally included in 'flagellates' have been assigned to different super-groups and groups. Thus, dinoflagellates are included in the super-group Chromalveolata (Adl *et al.*, 2005), whereas both diplomonads and kinetoplastids belong to the super-group Excavata (Simpson, 2003). Kinetoplastids are classified, according to Moreira *et al.* (2004), on the basis of an updated view of the kinetoplastid phylogeny founded on 18S rRNA gene sequence comparison. In this classification, the class Kinetoplastea is divided into two new subclasses: Prokinetoplastina to include the genera *Perkinsiella* and *Ichthyobodo*, and Metakinetoplastina, including the apical kinetoplastid genera. In Tables 9.1-9.3, the

**Table 9.1** Classification of dinoflagellids according to Adl *et al.* (2005).

| *Group and rank* | *Diagnosis* | *Remarks (genera considered)* |
|---|---|---|
| **SUPERGROUP CHROMALVEOLATA** Adl *et al.* 2005 | Plastid from secondary endosymbiosis with an ancestral archaeplastid; plastid secondary lost or reduced in some; with tertiary reacquisition of a plastid in others | The proposed union of Alveolata Cavalier-Smith, 1991 with the Chromista *sensu* Cavalier-Smith, 1998 is tentative |
| . **Alveolata** Cavalier-Smith, 1991 | Cortical alveolae; alveolae sometimes secondarily lost; with ciliary pit or micropore; cristae tubular or ampulliform | |
| .. **Dinozoa** Cavalier-Smith, 1991, emend. Cavalier-Smith and Chao, 2004, emend. Adl *et al.*, 2005 | Usually with extranuclear spindle within cytoplasmic channels through the nucleus; cortical alveoli typically discrete and inflated; often with bipartite trichocyst with a dense square-sectioned basal rod and wised hollow trichocyst filament | |
| ... **Dinoflagellata** Bütschli, 1885, emend. Fensome *et al.*, 1993 | Cells with two flagella in the motile stage, one transverse and one longitudinal; typically, transverse flagellum ribbon like, with multiple waves beating to the cell's left, and longitudinal flagellum beating posteriorly, with only one or few waves; typically with dinokaryotic nucleus that lack histones, and chromosomes that remain condensed during interphase | Phylum Dinoflagellata Bütschli, 1885 in Dodge and Lee (2000) |
| .... **Blastodiniales** Chatton, 1906 | Parasitic with dinokaryon during part of the life cycle only; not highly vacuolated. Fossils unknown | Blastodiniphyceae Fensome *et al.*, 1993 (*Amyloodinium*, *Piscinoodinium*, *Crepidoodinium*) |

Table 9.2 Classification of diplomonads according to Adl *et al.* (2005).

| *Group and rank* | *Diagnosis* | *Remarks (genera considered)* |
|---|---|---|
| **SUPERGROUP EXCAVATA** Cavalier-Smith, 2002, emend. Simpson 2003 | Typically with suspension-feeding groove (cytostome) of the 'excavate' type, presumed to be secondarily lost in many taxa; feeding groove used for capture and ingestion of small particles from feeding current generated by a posteriorly directed flagellum (F1); right margin and floor of groove are supported by parts of the R1 microtubular root, usually also supported by non-microtubular fibres (B fibre, composite fibre), and the left margin by the R2 microtubular root and C fibre | For flagella, microtubular root and fibre terminology, see also Simpson (2003) |
| . **Fornicata** Simpson, 2003 | Lacking typical mitochondria; with single kinetid and nucleus, or one pair each of kinetids and nuclei; two to four kinetosomes per kinetid; usually with a feeding groove or cytopharyngeal tube associated with each kinetid | |
| .. **Eopharyngia** Cavalier-Smith, 1993 | Single kinetid and nucleus, or one pair each of kinetids and nuclei; usually four kinetosomes and flagella per kinetid (occasionally three or two); usually with feeding grooves or cytopharyngeal tubes; mitochondrial homologues and dictyosomes inconspicuous/ transients | |
| ... **Diplomonadida** Wenyon, 1926, emend. Brugerolle *et al.*, 1975 | With a pair of kinetids and two nuclei, each kinetid usually with four kinetosomes and flagella (sometimes three or two), or uncommonly, one kinetid and nucleus; at least one flagellum per kinetid directed posteriorly, associated with a cytopharingeal tube or groove, or lying axially within the cell; various non-microtubular fibres supporting the nucleus and cytopharyngeal apparatus: free-living or endobiotic; often parasitic | Order Diplomonadida in Brugerolle and Lee (2000) |
| .... **Hexamitinae** Kent, 1880, emend. Brugerolle *et al.*, 1975 | With functional feeding apparatuses; with an alternate code (TAR codon for glutamine). *Hexamita, Spironucleus, Trepomonas* | (*Hexamita, Spironucleus*) |

classification of the groups and subgroups of flagellates considered here (dinoflagellates, diplomonads and kinetoplastids) is presented as per the system of Adl *et al.* (2005). Groups, subgroups and their main diagnostic features are according to this system. However, remarks on the correspondence of these groups with traditional taxa are included in some cases for a better comprehension.

Table 9.3 Classification of kinetoplastids according to Moreira *et al.* (2004) and Adl *et al.* (2005).

| *Group and rank* | *Diagnosis* | *Remarks (genera considered)* |
|---|---|---|
| SUPERGROUP EXCAVATA | See Table 9.2 | |
| . **Euglenozoa** Cavalier-Smith, 1981, emend. Simpson | Cells with two (occasionally one, rarely more) flagella, inserted in an apical/subapical flagellar pocket; with rare exceptions, emergent flagella with paraxonemal rods; usually with tubular feeding apparatus associated with flagellar apparatus; basic flagellar apparatus consisting of two functional kinetosomes and three asymmetrically arranged microtubular roots; mostly with discoidal cristae | Phylum Euglenozoa in Cavalier-Smith (1981) |
| .. **Kinetoplastea** Honigberg, 1963 | Euglenozoa with a kinetoplast, which is a large mass (or masses) of fibrillar DNA (kDNA) in the mitochondrion, often in close association with the flagellar bases | Order Kinetoplastida in Honigberg (1963) |
| ... **Prokinetoplastina** Vickerman in Moreira *et al.*, 2004 | An independent early emerging clade in 18S rRNA phylogenies. Most recent common ancestor of *Ichthyobodo* (ectoparasites of fish) and *Perkinsiella* (endosymbiont ("parasome") of certain amoebae). Polykinetoplastic | Range of Subclass in Moreira *et al.* (2004) (*Ichthyobodo*) |
| ... **Metakinetoplastina** Vickerman in Moreira *et al.*, 2004 | An independent late-emerging clade in 18S rRNA phylogenies. With a node-based definition: the clade stemming from the most recent ancestor of *Bodo, Crithidia, Cryptobia, Dimastigiella, Leishmania, Procryptobia, Rhynchobodo, Trypanoplasma, Trypanosoma.* Eu-, pan- or polykinetoplastic. Free-living and parasite species described | Range of Subclass in Moreira *et al.* (2004) |
| .... **Parabodonida** Vickerman in Moreira *et al.*, 2004 | Eu- or pankinetoplastic kDNA not in network; biflagellate, without mastigonemes: posterior flagellum attached or free; phagotrophic or osmotrophic; cytostome, when present, anterolateral; free-living or commensal/parasitic. Node: *Cryptobia, Parabodo, Procryptobia, Trypanoplasma* | Range of Order in Moreira *et al.* (2004) (*Cryptobia, Trypanoplasma*) |
| .... **Trypanosomatida** Kent, 1880, emend. Moreira *et al.* 2004 | Eukinetoplastid with kDNA network; uniflagellate with flagellum lacking mastigonemes and emerging from anterior pocket, or emerging laterally and attached to the body; phagotrophic or osmotrophic; cytostome, when present, simple and close to the flagellar pocket; exclusively parasitic | Range of Order in Moreira *et al.* (2004) (*Trypanosoma*) |

**CHROMALVEOLATA** Adl *et al.*, 2005
**ALVEOLATA** Cavalier-Smith, 1991
**DINOZOA** Cavalier-Smith, 1991, emend. Cavalier-Smith and Chao, 2004, emend. Adl *et al.*, 2005
**DINOFLAGELLATA** Bütschli, 1885, emend. Fensome *et al.*, 1993
**BLASTODINIALES** Chatton, 1906

Dinoflagellates include morphologically diverse organisms found in marine and freshwater habitats. Most of the nearly 2500 described species are free-living flagellates, whereas the number of endosymbionts or parasites is limited. Special attention has been paid to this group of organisms, as some species are responsible for the red or brown 'tides', sometimes involving species producing toxins, filtered by molluscs with subsequent environmental or sanitary significance (Dodge and Lee, 2000). Amongst these harmful algal bloom species, the heterotrophic dinoflagellate *Pfiesteria piscicida* has been repeatedly linked to fish kills (Burkholder, 2001).

Dinoflagellates parasitizing fish belong to Blastodiniales Chatton, 1906, having a dinokaryon only part of the life cycle. They are extracellular parasites quite varied morphologically, with a complex life cycle, including a soporogenic stage which produces infective dinospores, and sometimes one or more cyst stages. Most blastodiniales parasitizing fish are included in the family Oodinaceae Chatton, 1920, and the main representative genera are *Amyloodinium*, *Crepidoodinium* and *Piscinoodinium*.

**AMYLOODINIUM** Brown and Hovasse, 1946
**AMYLOODINIUM OCELLATUM** (Brown, 1931) Brown and Hovasse, 1946

## Morphology and Life Cycle

Trophonts are unpigmented, pear-shaped to ovoid cells, mostly upon 150 μm, with a cellulose wall consisting of a theca with amphiesmal plates, and a central nucleus with a large karyosome. The cytoplasm, lacking chloroplasts, presents a mass of refringent protein granules surrounding the nucleus and is packed with stark grains. In the basal region, a plate bearing numerous rhizoids which penetrate the fish tissues attaches the

parasite to the host cells. From the upper face of the attachment plate extends a long motile tentacle, the stomopode (Fig. 9.7).

The life cycle of *A. ocellatum* (Plate 9.1) includes several parasite stages. Flagellate dinospores, 12-15 μm in diameter, are the free-swimming, migratory, infective stage of the cycle. The ultrastructural features of *Amyllodinium* cf. *ocellatum* dinospores were studied by Landsberg *et al.* (1994), who reported that dinospores were anteroposteriorly compressed, thinly armoured peridinioid type, having an epitheca slightly shorter than the hypotheca. Dinospores measured 6.1 ± 0.8 μm in length, 11.7 ± 0.5 μm in width and 11.1 ± 1.2 μm in depth. The longitudinal flagellum is located below the peduncle and is 20-25 μm long. The transverse flagellum is located in the cingulum (Plate 9.1, E).

Upon reaching the fish host, dinospores transform into trophont, the feeding, growing stage parasitizing fish tissues (Plate 9.1, A-C). When the trophont reaches a certain volume, it detaches from the host, retracts its rhizoids, rounds up and falls to the bottom, becoming a tomont. This tomont stage secretes a wall forming a cyst in which dinospores are formed after several divisions (Plate 9.1, D) (Lom and Dyková, 1992). According to Paperna (1984a), the length of the cycle depends on temperature. Tomont division is limited to 16-30°C and takes the minimum time between 23-27°C. However, tomonts maintained below 15°C can recover their ability to produce dinospores when returned to 25°C (see Noga and Levy, 1999). Tolerance to salinity was dependent on water temperature and was the widest at 24-25°C. A wide variation in tolerance to salinity and temperature was evident in tested populations and also in individual tomonts in each population. Such variability may have important faunistic and epizootic implications. The recent finding of Roberts-Thomson *et al.* (2006) regarding the dispersion of infective dinospores via aerosol droplets is very relevant in relation to parasite transmission and to management of aquaculture facilities.

## Disease Symptoms, Aetiology/Pathogenesis

Gills are usually the primary site of infection, although heavy infections may also involve skin, fins and eyes. Skin infection can lead to a dusty appearance ('velvet disease'). Other clinical signs of amyloodiniosis include anorexia, dyspnoea, scratching and pruritus. Massive infections are frequently associated to mortalities, both in mariculture and sea aquaria, mainly at high temperatures. Death is usually attributed to

anoxia, but osmoregulatory dysfunction and microbial secondary infections could be involved in acute mortalities associated to mild infections (Noga and Levy, 1999). Slight or mild infections cause little pathology, but in heavy infections, histopathological lesions include gill inflammation, haemorrhages, hyperplasia and necrosis (Figs. 9.1-9.6).

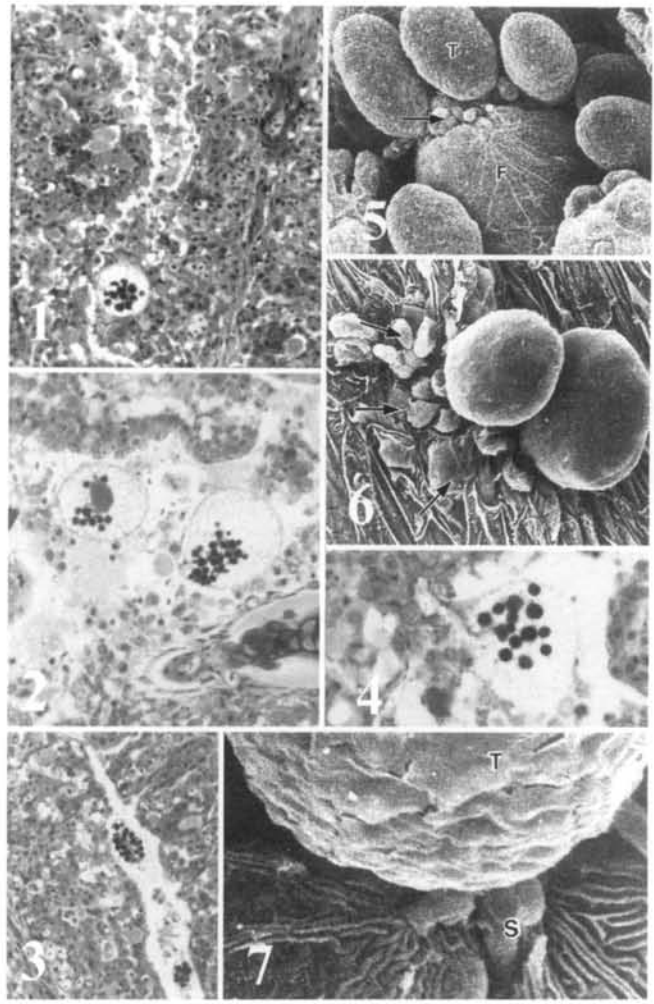
A great part of histopathological damage is due to the trophont attachment disc, whose rhizoids—enlarged at their bases—are embedded into the host cells (Plate 9.1, A, Fig. 9.4), and can sever off parts of these cells by a constant pull (Figs. 9.5, 9.6) (Lom and Lawler, 1973). Vacuolar degeneration of the cells penetrated by the parasite rhizoids was evident at the site of attachment and cloudy swelling was apparent in the surrounding epithelial cells (Figs. 9.2-9.4). In heavy infections gill filament underwent hyperplasia (Figs. 9.1, 9.3) with consequent lamellar fusion (Paperna, 1980).

## Diagnosis

The first approach to diagnose gross skin amyloodiniosis is the direct observation of fish skin, using indirect illumination or a dark background (Noga and Levy, 1999). However, even if the typical 'velvet' appearance is observed, diagnosis must be confirmed by microscopic identification of parasite stages on fresh or histological preparations. Tomonts or trophonts of different sizes can be observed in fresh scrapings of skin and gills of infected fish (even in slight or mild infections, Plate 9.1, B) and in histological sections. Specific antibodies have been found in naturally and experimentally infected fish and can also be detected by ELISA (Smith *et al.*, 1992, 1994; Cobb *et al.*, 1998a, b; Cecchini *et al.*, 2001). The SSU ribosomal gene of *Amyloodinium* has been sequenced (Levy and Noga, 2005) and a highly specific PCR assay for detecting *A. ocellatum* has been developed (Levy *et al.*, 2007) (see also section on Molecular studies).

## Treatment and Prophylaxis

Several treatments assayed against amyloodiniosis proved to be ineffective. The main reason is the existence of a resting cyst phase (the tomont) which is extremely refractory to the chemical action, and usually responsible for re-infections. Freshwater baths (2-4 minutes) or copper sulphate (0.75 mg/l, 12-14 days) have been used against trophonts or dinospores, respectively, with variable results. Higher concentrations of free copper must be avoided considering its toxicity. Other




**Figs. 9.1-9.7** *Amyloodinium ocellatum*. Figs. 1-4. Resin sections stained with toluidine blue showing histopathological damage of the gill epithelium of *Dicentrarchus labrax* (Fig. 9.1) and *Sparus aurata* (Figs. 9.2-9.4). Notice the extensive epithelial hyperplasia and necrosis of the areas surrounding the parasites (Figs. 9.1, 9.3), the necrosis and detachment of epithelium (Figs. 9.1, 9.2) and the swelling of cells near the attachment area (Figs. 9.3, 9.4). Fig. 9.1: × 210; Fig. 9.2: × 290; Fig. 9.3: × 230; Fig. 9.4: × 480. Figs. 9.5-9.7. Scanning electron microscope images of parasitic stages from *Oreochromis mossambicus*. Fig. 9.5. Numerous trophonts (T) on the tip of gill filament (F). Local erosion of epithelium in the site of trophont's attachment (arrow) × 840. Fig. 9.6. Trophonts on the skin. Note the distortion of epithelial tissue in the site of trophont's attachment (arrows) × 880. Fig. 9.7. Penetration of a stomopode (S) into epithelial cell of a tilapia gill; × 4720. Figs. 9.2-9.4 courtesy of Dr. Sitjà-Bobadilla from the Instituto de Acuicultura Torre de la Sal (CSIC) (Castellón, Spain). Figs. 9.5-9.7. From Kuperman and Matey (1999), with permission of Diseases of Aquatic Organisms (Inter-Research) (courtesy of Dr. Matey).

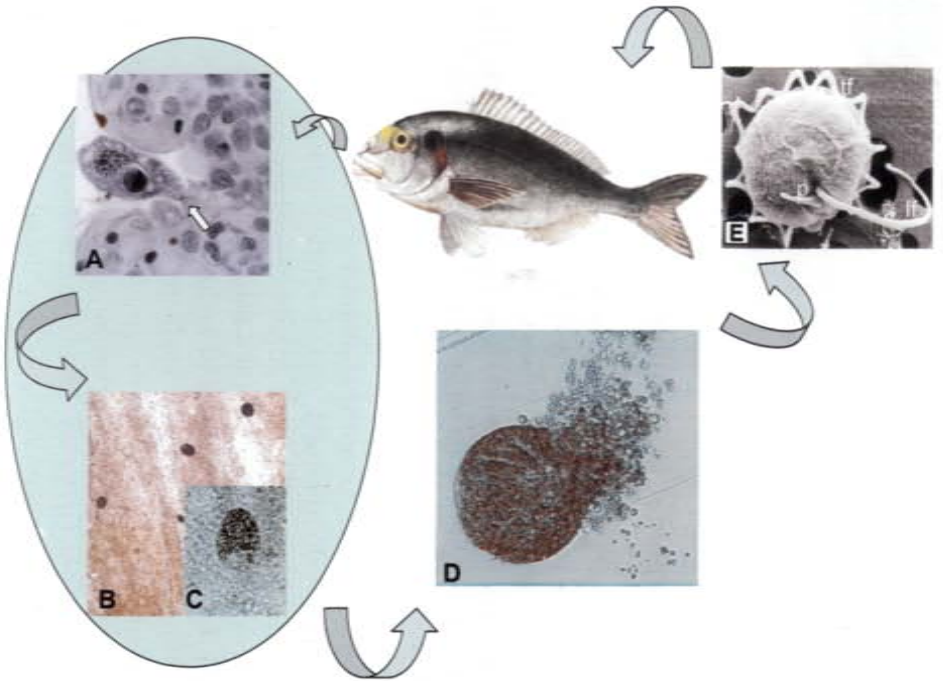



**Plate 9.1** Lyfe cycle of *Amyloodinium ocellatum* in gilt-head seabream *Sparus aurata*. **A-C**: Stages in the fish host. **A**. Trophont attached to the gill epithelium showing the rhizoids (arrow). Toluidine blue staining of a resin section. × 860. **B-C**. Trophonts in fresh scrapings of infected gills. **B:** × 80; **C**: × 260. **D-E**. Stages in the environmental waters. **D**. Fresh preparation of a tomont releasing dinospores. ×300. **E.** Scanning electron microscope image of a dinospore. Hypothecal view showing longitudinal flagellum (lf), transverse flagellum (tf) and peduncle (p). × 3930. Fig. A from Alvarez-Pellitero *et al.* (1995) with permission of Journal of Fish Diseases (Blackwell Publishing). Fig. E from Landsberg *et al.* (1994) with permission of Diseases of Aquatic Organisms (Inter-Research). Courtesy of Dr. Landsberg.

chemotherapeutics have been assayed, including formalin, nitrofurazone, furanace, malachite green and acriflavin (Paperna, 1984b), but some are not approved for use in food fish (reviewed in Noga and Levy, 1999). However, formalin was considered efficacious in the control of *A. ocellatum* in bullseye puffer fish *Sphoeroides annulatus* (Tetraodontidae) in a recent study by Fajer-Ávila *et al.* (2003). Oestmann *et al.* (1995) focussed on the potential value of brine shrimp (*Artemia salina*) as a bioremediation measure for this parasite. Promising results have been reported using lasalocid (Oestmann and Lewis, 1996a) and hydrogen peroxide (Montgomery-Brock *et al.*, 2001). Abreu *et al.* (2005) emphasized the need of cleaning the biofilm of tanks (to eliminate the tomonts) after treatment of infected fish with copper sulphate (1.5 mg Cu/L for 24 h during 7 d) in order to prevent recurrent amyloodiniosis.

## Fish Hosts, Geographical Distribution and Epidemiology

*A. ocellatum* shows almost no host preference, as the number of susceptible fish species is over 100 representing more than 40 families (Lawler, 1977; Lom, 1981). Thus, very few marine species are refractory and many species have been found to be infected in aquaria and in cultures. The most significant reports of *Amyloodinium* and amyloodiniosis, with special emphasis on outbreaks in cultured fish, are summarized in Table 9.4. These selected reports confirm the wide host range, covering several teleostean orders (Perciformes, Gonorhynchiformes, Cyprinodontiformes, Pleuronectiformes, Tetraodontiformes), and also include elasmobranchs. Marine and euryhaline fish are affected. Epizootics have been increasingly recorded in different cultured and aquarium fish. Although most initial reports came from fish cultured in tropical and subtropical waters, the infection is increasingly common in the Mediterranean area, where it is considered to be one of the main threats to mariculture, as it can produce epizootics in several economically important species (*Sparus aurata*, *Diplodus puntazzo*, *Dicentrarchus labrax*, *Seriola dumerili* and mullets). The geographical distribution is very wide, so this dinoflagelllate can be considered cosmopolitan. Besides the Mediterranean Sea, amyloodiniosis has been reported in the Red Sea, the Northern and Southern Atlantic seas and the Pacific Ocean. In addition, numerous fish species can be affected in ornamental and exhibition aquaria. Epizootics are especially severe in fry and juvenile fish in intensive pond cultures, but more so in cage cultures and in broodstocks. The parasite can tolerate a wide range

**Table 9.4** Main records of *Amylooodinium* sp. in marine or brackish waters.

| *Fish hosts*<br>*Class, Order, Family, Species* | *Geographic location* | *Type of habitat or facility* | *Fish age* | *Parasite identification* | *Infection severity* | *References* |
|---|---|---|---|---|---|---|
| **ACTINOPTERYGII**<br>PERCIFORMES | | | | | | |
| **Moronidae** | | | | | | |
| *Dicentrarchus labrax* | Mediterranean Sea (Greece, Italy Spain, Tunisia, Turkey)<br>North Adriatic Sea (Italy)<br>Atlantic (Portugal)<br>Red Sea (Israel) | Wild<br>Cage farms<br>Intensive open and closed earth ponds | Fingerlings, juveniles, occasionally broodstock | *A. ocellatum* or *Amyloodinium* | Outbreaks and subclinical in cultures | Ghittino *et al.* (1980)<br>Paperna (1980)<br>Alvarez-Pellitero *et al.* (1993)<br>Alvarez-Pellitero (1995) (2004)<br>Çagirðan and Tokþen (1996)<br>Duarte *et al.* (2000) |
| *Morone saxatilis* | North America | Wild and cultured | | *A. ocellatum* | outbreaks | Plumb (1991) |
| *Morone saxatilis* × *M. crhysops* | North America (North Carolina, U.S.A.) | Ponds | Juveniles | *A. ocellatum* | | Noga *et al.* (1991) |
| **Sparidae** | | | | | | |
| *Sparus aurata* | Mediterranean Sea (Greece, Italy, France, Morocco, Tunisia)<br>North Adriatic Sea (Italy)<br>Red Sea (Israel) | Wild<br>Cage farms<br>Intensive open and closed earth ponds | Larvae fingerlings, juveniles, occasionally broodstock | *A. ocellatum*<br>*Oodinium* sp.<br>*Amyloodinium* | Outbreaks and subclinical | Paperna and Baudin-Laurencin (1979)<br>Paperna (1980)<br>Barbaro and Francescon (1985)<br>Alvarez-Pellitero (1995, 2004)<br>Alvarez-Pellitero *et al.* (1995) |
| *Diplodus puntazzo* | Mediterranean Sea (Greece, Morocco) | Cage farms<br>Wild | Juveniles, broodstock | *A. ocellatum*<br>*Oodinium* sp. | Outbreaks and subclinical | Rigos *et al.* (1998)<br>Alvarez-Pellitero (2004) |
| **Mugilidae** | | | | | | |
| Mullets<br>*Mugil cephalus* | Mediterranean Sea (Italy, Tunisia)<br>Mississippi<br>Pacific Ocean<br>Taiwan (Yun-Chia-Nan area)<br>Philippines | Intensive and semi-intensive cultures, lagoons<br><br><br><br>Farms | Several ages | *Amyloodinium*<br>Amyloodiniosis<br>*Amyloodinium* sp.<br><br>*Amyloodinium*-like | Outbreaks and subclinical<br><br><br><br>Subclinical | Alvarez-Pellitero (2004)<br>Paperna and Overstreet (1981)<br><br><br><br>JiannHsiung *et al.* (2001)<br>Baticados and Quinitio (1984) |
| **Carangidae** | | | | | | |
| *Seriola dumerili* | Mediterranean Sea (Italy) | Intensive | Juveniles | *A. ocellatum* | Outbreaks | Aiello and D'Alba, (1986) |

*(Table 9.4 Contd.)*

*(Table 9.4 Contd.)*

| | | | | | | |
|---|---|---|---|---|---|---|
| **Cichlidae** | | | | | | |
| *Oreochromis mossambicus* | Salton Sea, California, U.S.A. | Wild (eutrophic, hypersaline) | Juveniles | *A. ocellatum* | Outbreaks | Kuperman and Matey (1999) |
| **Sciaenidae** | | | | | | |
| *Sciaenops ocellatus* | North America<br>Central Taiwan | Wild and cultured<br>Ponds | | *Amyloodinium* sp. | Outbreaks<br>Outbreaks | Plumb (1991)<br>Ching Yu *et al.* (2002) |
| **Latidae** | | | | | | |
| *Lates calcarifer* | Indian Ocean (Thailand) | Cage cultures | | | Subclinical (survey) | Chonchuenchob *et al.* (1986) |
| **Lutjanidae** | | | | | | |
| *Lutjanus argentimaculatus* | Philippines | Hatchery (intensive) | Juveniles | *A. ocellatum* | Outbreaks | Cruz-Lacierda *et al.* (2004) |
| *Lutjanus griseus* | La Restinga lagoon, Margarita Island, Venezuela | Wild (lagoon) | Juveniles | *A. ocellatum* | Subclinical | Fuentes Zambrano *et al.* (2003) |
| **Rachycentridae** | | | | | | |
| *Rachycentron canadum* | Taiwan | Nursery (intensive) | Juveniles | *A. ocellatum* | Outbreaks and subclinical | Liao *et al.* (2004) |
| **Polynaemidae** | | | | | | |
| *Polydactylus sexfilis* | Pacific Ocean (Hawai) | Commercial farm | | | | Ostrowsky and Molnár (1998)<br>Montgomery-Brock *et al.* (2001) |
| GONORHYNCHIFORMES | | | | | | |
| **Chanidae** | | | | | | |
| *Chanos chanos* | Philippines | Hatchery (intensive) | Fry | *A. ocellatum* | outbreaks | Cruz-Lacierda *et al.* (2004) |
| CYPRINODONTIFORMES | | | | | | |
| **Cyprinodontidae** | | | | | | |
| *Aphanius dispar* | Mediterranean Sea, Gulf of Suez | Experimental tanks | Adults | *A. ocellatum* | Outbreaks | Paperna (1980) |

*(Table 9.4 Contd.)*

*(Table 9.4 Contd.)*

| | | | | | | |
|---|---|---|---|---|---|---|
| PLEURONECTIFORMES | | | | | | |
| **Paralichthydae** | | | | | | |
| *Paralichthys orbygnyanus* | Brazil | Experimental tanks | Broodstock | *A. ocellatum* | Outbreaks | Abreu *et al.* (2005) |
| *Paralichthys dentatus* | Atlantic Ocean North America (Virginia, USA) | | | *Amyloodinium* | Health consequences | Hughes and Smith (2003) |
| *Psetta maxima* | Atlantic Sea (Portugal) | Experimental tanks | Juveniles | amyloodiniosis | | Ramos and Oliveira (2001) |
| TETRAODONTIFORMES | | | | | | |
| **Tetraodontidae** | | | | | | |
| *Sphoeroides annulatus* | Mazatlán, Sinaloa, Mexico | Cultures | Juveniles | *A. ocellatum* | outbreaks | Fajer-Ávila *et al.* (2003) |
| *Takifugu rubripes* | Pacific Ocean (Taiwan) | Cage cultures | | *A. ocellatum* | outbreaks | Taiwei *et al.* (2001) |
| **ELASMOBRANCHII** | | | | | | |
| RAJIFORMES | | | | | | |
| **Dasyatidae** | | | | | | |
| *Dasyatis americana* | North America (Tennessee, U.S.A.) | Aquarium | | *Amyloodinium* | Clinical signs | Keller (2006) |

of salinity (Paperna, 1984a), so it can be present in marine and estuarine habitats, lagoons and ponds, from hypersaline to brackish waters. High temperatures favour the infection which can be severe in several tropical ornamental fish also.

### **PISCINOODINIUM** Lom 1981

(syn. *Oodinium* Chatton, 1912 pro parte)

*Piscinoodinium* differs from *Amyloodinium* in the presence of chloroplasts and in lacking a stomopode (Lom, 1981; Lom and Dyková, 1992). *Piscinoodinium* spp. parasitize several freshwater fish, mainly farmed and aquarium tropical fish. On occasions, they have been reported without specific ascription, though the best-known species is *P. pillulare*.

***Piscinoodinium pillulare*** (Schäperclaus, 1954) Lom, 1981

(syn. *Oodinium pillularis* Schäperclaus 1951 and *Oodinium limneticum* Jacobs, 1946)

#### Morphology and Life Cycle

The piriform or sac-like trophont measures up to 160 μm long, and is covered by a theca without thecal plates. At the periphery of the cell lie disc-shaped chloroplasts. From the attachment disc radiate numerous rod-like organelles (rhizocysts), which penetrate and are firmly embedded deep in the epithelial cells of the host. Further morphological and ultrastructural details can be found in Lom (1981), Lom and Schubert (1983) and Lom and Dyková (1992).

The developed trophont drops off the host's surface, sinks to the bottom, rounds off, and becomes a tomont. Without secreting a common envelope, successive divisions occur in the tomont to produce 128 or 256 tomites which differentiate into gymnospores (about 10-19 × 7-12 μm) (summarized from Lom and Dyková, 1992).

#### Disease Symptoms, Aetiology/Pathogenesis

In heavily infected fish, external signs are similar to those of amyloodiniosis, including the velvety appearance of the body surface. A rust-coloured appearance and a dense covering of mucus, as well as mass mortalities of some heavily infected tropical pond fish, were reported by Shaharom-Harrison *et al.* (1990). Some aquarium fish can succumb of the disease in less than a week of exposure (Lom and Dyková, 1992). Histopathological damage consists mainly of gill lamellar fusion and

epithelial hyperplasia, though epithelial degeneration and necrosis can eventually appear. Shaharom-Harrison *et al.* (1990) considered piscinoodiniosis of cultured tropical fish related entirely to parasite-induced gill pathology.

## Diagnosis

Piscinoodiniosis can be diagnosed using similar procedures to those described for *Amyloodinium*.

## Treatment and Prophylaxis

Different chemicals have been used for treatment of *Piscinoodinium* infections, including methylene blue, acriflavine, copper, salt (NaCl) or potassium permanganate. However, some products are not well tolerated by certain fish species and may be toxic to nitrifying bacteria and many aquatic plants (reviewed in Noga and Levy, 1999). Copper sulphate (alone or in combination with NaCl) was used in continuous bath treatment of *Puntius gonionotus* fry infected with *Piscinoodinium* sp. A significant reduction of the level of parasites was obtained with the doses ranging from 1 ppm to 0.4 ppm, but high mortality was noted within 6-7 h to 24 h. NaCl alone and methylene blue did not have any effect in removing the parasite (Siti and Rokiah, 1988a).

## Fish Hosts, Epidemiology and Geographical Distribution

*P. pillulare* was initially reported as *Oodinium pillularis* by Schäperclaus (1951) from wild *Carassius carassius* in Germany and transferred to the newly created genus *Piscinoodinium* by Lom (1981). According to Lom and Dyková (1992), the flagellate has also been observed in trout yearling and eel cultures in warmed water in Germany. However, it affects mainly tropical aquarium fish, and has been spread with their introduction in different countries. Outbreaks by *P. pillulare* or *Piscinoodinium* sp. have been recorded mainly in Asian and South American tropical ornamental fish as also in the fish in recipient temperate countries.

The main records of *Piscinoodinium* spp. listed by both host and geographical distribution are included in Table 9.4. The parasite has been reported at least in cyprinids, cichlids, osphronaemids and callichthyds from Asia, Central and West South Africa and South America. Differences in susceptibility between fish species have been described,

**Table 9.5** Main records of pathogenic Oodinidae in Teleostei from fresh waters.

| *Fish hosts*<br>*Order, Family, Species* | *Geographic location* | *Type of habitat/facility* | *Parasite identification* | *Infection severity* | *References* |
|---|---|---|---|---|---|
| CHARACIFORMES | | | | | |
| **Anostomidae** | Franca, Sao Paulo, Brazil | Fish farms | *Piscinoodinium pillulare* | High intensity | Tavares-Dias *et al.* (2001) |
| *Leporinus macrocephalus* | | | | | |
| **Characidae** | | | | | |
| *Piaractus mesopotamicus* | | | | | |
| *P. mesopotamicus* × *Colossoma macropomum* | | | | | |
| CYPRINIFORMES | | | | | |
| **Cyprinidae** | | | | | |
| *Carassius auratus* | Sri Lanka | Intensive tanks and mud ponds (farms, ornamental fish) | *Piscinoodinium* sp. | Occasional | Thilakaratne *et al.* (2003) |
| *Cyprinus carpio* | | | | | |
| *Aristichthys nobilis* | Malaysia<br>Brazil | Ponds | *Piscinoodinium pillulare* | Subclinical | Shaharom-Harrison *et al.* (1990)<br>Martins *et al.* (2001) |
| *Puntius gonionotus* | Malaysia | Ponds | *Piscinoodinium pillulare* | Mortality | Siti and Rokiah (1988b) |
| *Ctenopharyngodon idella* | | | | Subclinical | Shaharom-Harrison *et al.* (1990) |
| *Leptobarbus hoevenii* | | | | Subclinical | |
| PERCIFORMES | | | | | |
| **Cichlidae** | | | | | |
| *Pelvicachromis teniatus* | Central and West Africa, South America | Aquaria | Dinoflagellate | Outbreaks | Steinhagen *et al.* (1999) |
| **Osphronaemidae** | | | | | |
| *Colisa* sp. | Sri Lanka | Export farms | *Piscinoodinium* sp. | Occasional | Thilakaratne *et al.* (2003) |
| *Betta splendens* | Sri Lanka | Export farms | *Piscinoodinium* sp. | Occasional | Thilakaratne *et al.* (2003) |
| | Uruguay | Ornamental fish farms | *P. pillulare* | Epizootics | Carnevia and Speranza (2003a) |
| SILURIFORMES | | | | | |
| **Callichthydae** | | | | | |
| *Corydoras* spp. | UK (origin Colombia and Brazil) | Intensive export farms<br>Holding facilities | *Piscinoodinium* sp. | Subclinical and with clinical signs | Ferraz and Sommerville (1998) |
| *Brochis splendens* | | | | | |

being *Puntius gonionotus* one of the most affected hosts (Shaharom-Harrison *et al.*, 1990).

***Crepidoodinium*** Lom and Lawler (in Lom, 1981)

The trophont is attached to the host surface by a large, flat adhesive surface bearing projections just touching the host cells. It lacks a stomopode and has well-developed chloroplasts (Lom, 1981).

***Crepidoodinium cyprinodontum*** (Lawler, 1967) Lom, 1981

(syn. *Oodinium cyprinodontum* Lawler, 1967)

It is only known from the gills of three estuarine cyprinodontid fish (*Fundulus* sp., *Cyprinodon variegatus* and *Lucania parva*) from North Carolina (U.S.A.), with no pathogenicity or disease symptoms; hence, it is considered a non-pathogenic symphoriont (Lom and Dyková, 1992). Readers are referred to Lom and Lawler (1973) for ultrastructural morphology.

***Crepidoodinium australe*** Lom, Rhode and Dyková, 1993

It was described from the gills of *Sillago ciliata* from Australia as an ectocomensal, no inflicting obvious injury upon the host cells. Details on morphology and ultrastructure can be found in Lom *et al.* (1993).

## Economical Importance of Dinoflagellosis

The greatest impact of parasitic dinoflagellates is produced under intensive pisciculture conditions. *Amyllodinium ocellatum* is the most relevant species from the economic point of view, as it is one of the most persistent and pathogenic ectoparasites of warm-water marine fish, causing significant losses of mariculture stocks (Paperna, 1984b). *Piscinoodinium pillulare* can also produce losses in aquacultured and aquarium fish.

## *In vitro* Cultivation

One of the main constraints for parasite studies is the lack of reliable and continuous sources in case of many species, allowing the necessary investigation to implement adequate control and immunoprophylactic measures.

Noga and Bower (1987) maintained the DC-1 strain of *A. ocellatum* using gnotobiotic guppies (*Poecilia reticulata*), and Noga (1987, 1992) achieved its propagation in G1B cell culture. According to Levy and Noga (2005), this system has allowed the continuous *in vitro* propagation of the

dinoflagellate for over 15 years, and the parasites have remained pathogenic. Another method for *A. ocellatum* propagation was developed by Oestmann and Lewis (1996b), using aggregates of red drum *Sciaenops ocellatus* dorsal fin cells in suspension. Using this procedure, the parasite was maintained *in vitro* for 10 complete life cycles with no observed decreased viability.

## Immunological Studies

Studies on fish immune responses against parasites have substantially increased in recent years, and some information is also available for dinoflagellates. Differences in susceptibility between fish species have been reported. Some of them are considered specifically resistant to infection, mainly those which can tolerate low oxygen levels, or have certain properties of mucus and epithelia (see Noga and Levy, 1999). In addition, some observations had suggested a resistance of fish to infection following repeated challenges. The existence of an acquired immunity was demonstrated in *Oreochromis aureus* immunised with the parasite, as specific antibodies were detected in an ELISA (Smith *et al.*, 1992). Anti-*Amyloodinium ocellatum* antibodies were also found in sera of cultured stripped bass having recovered from an amyloodiniosis outbreak (Smith *et al.*, 1994). Further observations of Cobb *et al.* (1998a, b) in *Amphiprion frenatus* confirmed the association of acquired immunity with the antibody response. Cecchini *et al.* (2001) found specific antibodies also in Mediterranean sea bass naturally infected by amyloodiniosis.

However, the obtained information has not allowed to develop immunoprophylatic measures and no practical vaccines are presently available. Interestingly, the natural host produced antibiotics, known as histone-like proteins (HLPs), are highly lethal to *Amyloodinium* trophonts (Noga *et al.*, 2001, 2002), which opens a new way of control using the innate immunity of fish.

## Molecular Studies

Recent advances on dinoflagellates taxonomy and evolution are mainly based on molecular studies. The sequencing of the small subunit (SSU) ribosomal gene of *Amyloodinium* has given insights into its phylogenetic relationships, besides to obtain preliminary data for molecular diagnosis (Levy and Noga, 2005). The phylogenetic analysis of this sequence has confirmed the placement of *A. ocellatum* in the family Blastodiniphyceae

together with *Piscinoodinium* and *Crepidoodinium* (Litaker *et al.*, 1999). However, according to Levy and Noga (2005), data on the SSU rDNA gene of an apparent *Piscinoodinium* sp. (infecting the skin of a tropical freshwater fish) places this organism in the family Gymnodiniphycidae. Moreover, ultrastructural features also differ from the known *Piscinoodinium* species. Five nucleotide sequences of *A. ocellatum* (18S and 5.8S, ITS and rRNA) are currently available in GenBank.

## Outlook, Recent Developments and Future Prospects

Recent findings have confirmed the importance of dinoflagellates, mainly *Amyloodinium ocellatum*, in fish culture. Although both *in vivo* and *in vitro* propagation are available for this species, axenic culture has not been obtained yet, and similar methods are necessary for *Piscinoodinium*.

The preliminary information on SS rRNA gene sequence of *A. ocellatum* (Levy and Noga, 2005) could be used for molecular diagnosis, but the test must be developed and validated. Further molecular research on a higher number of dinoflagellates is also needed to clarify their taxonomic and phylogenetic status and to eventually apply the obtained information for diagnosis and control purposes. Specific PCR assays are already available for the toxic species *Pfiesteria psicicida* and other estuarine heterotrophic dinoflagellates, permiting their detection in algal cultures and estuarine water samples (Oldach *et al.*, 2000). A specific PCR assay for detecting *A. ocellatum* in fish tissues on water samples has also been developed (Levy *et al.*, 2007). The existence of a protective immune response against *A. ocellatum* opens a promising future to further investigations especially focused on the development of immunoprophylactic measures.

**FORNICATA** Simpson, 2003
**EOPHARYNGIA** Cavalier-Smith, 1993
**DIPLOMONADIDA** Wenyon, 1926, emend. Brugerolle *et al.*, 1975

Fish diplomonads include 'diplozoic forms', consisting of two karyomastigonts mutually integrated in binary axial symmetry. In each karyomastigont, a nucleus is closely associated with four basal bodies arranged in two pairs, and one flagellum is always directed backward and is generally associated with a ventral cytostome. Three microtubular fibres originating from the basal body periphery make part of the cytoskeleton.

In these 'diplozoic forms' the cell is like a double organism with two nuclei, two sets of four flagella and two cytostomal apparatuses. An additional character of diplozoic forms is the funnel-like construction of the cytostome, which can reach as far as the posterior end of the cell (Hausmann *et al.*, 1993). They reproduce by longitudinal binary fission, and parasitic species form resistant cysts which ensure the transmission (Brugerolle and Lee, 2000). The parasite species are mostly found in the vertebrate intestine and cloaca, but they can also invade other organs.

**HEXAMITINAE** Kent, 1880, emend. Brugerolle *et al.*, 1975

In spite of the common occurrence of diplomonads, the knowledge on their morphological and ultrastructural characteristics is scarce in some cases, and the inadequate recognition of genus and species contributes to the existing confusion (Poynton and Sterud, 2002a; Poynton *et al.*, 2004). Three diplomonad genera, *Hexamita*, *Spironucleus* and *Octomitus* have been reported from fish, and the pathologic problems are mainly produced by the species described in the two former genera. They occur in the digestive tract and systemically in numerous fish species.

## HEXAMITOSIS/SPIRONUCLEOSIS AND INVOLVED DIPLOMONADS

### Taxonomical Update

*Hexamita* and *Spironucleus* are morphologically very similar, which has led to certain taxonomic confusion in the adscription of species to a determined genus (Vickerman, 1990a; Brugerolle and Lee, 2002; Poynton and Sterud, 2002a). The need of ultrastructural studies to an accurate genus and species adscription of fish diplomonads has been stressed. The transmission electron microscopy studies of Bruguerolle (1974, 1975) and Bruguerolle *et al.* (1973, 1980) were essential for a reliable determination of genera. The main diagnostic features are summarized below.

### *Hexamita* Dujardin, 1838

*Hexamita* spp. have an oval or pyriform cell body (6-35 μm), truncated or tapered posteriorly, bearing 6 anterior locomotor flagella and 2 posterior trailing ones (Fig. 9.8). Two rounded nuclei closely apposed each other are located at the anterior end. Each set of flagella is inserted anteriorly on the external side of each nucleus. The recurrent flagella emerge posteriorly as trailing flagella. They are lying in longitudinal canals which open

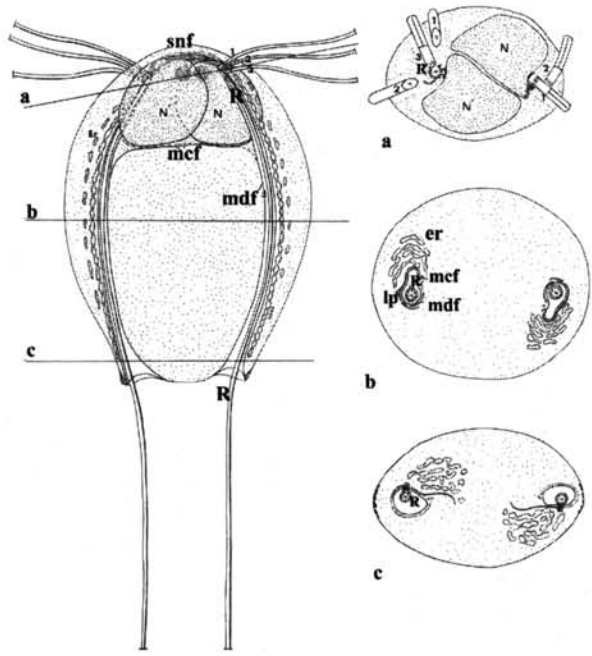



**Fig. 9.8** Schematic representation of *Hexamita*. a, b, c, transversal sections at different levels. General disposition of nuclei N, N', kinetosomes and flagella 1, 2, 3, R (recurrent flagellum kinetosome) and associated fibres: supranuclear fibre (snf), crossed microtubular fibre (mcf), direct microtubular fibre (mdf); lp: lamina with a periodic fine structure; er: ergastoplasm. From Bruguerolle (1974) with permission of Protistologica (now European Journal of Protistology, Elsevier). Courtesy of Dr. Bruguerolle from the Lab. De Biologie des Protistes, Université Blaise Pascal de Clermont-Fd, France.

posteriorly forming 2 apertures which are the cytostome of the cell, and the canals are equivalent to cytopharynges (Brugerolle, 1974). The genus includes several free-living species and other endocommensal or parasitic ones.

Transmission occurs via cysts and/or trophozoites which may survive for some time in water (Lom and Dyková, 1992).

## *Spironucleus* Lavier, 1936

Ovoid or cigar-shaped cell body (5-10 μm) tapered posteriorly bearing 6 anterior flagella and 2 posterior trailing ones (Fig. 9.9). Considering their similar morphology, the differentiation of *Spironucleus* and *Hexamita* at light microscope is very difficult and is mainly based on the more

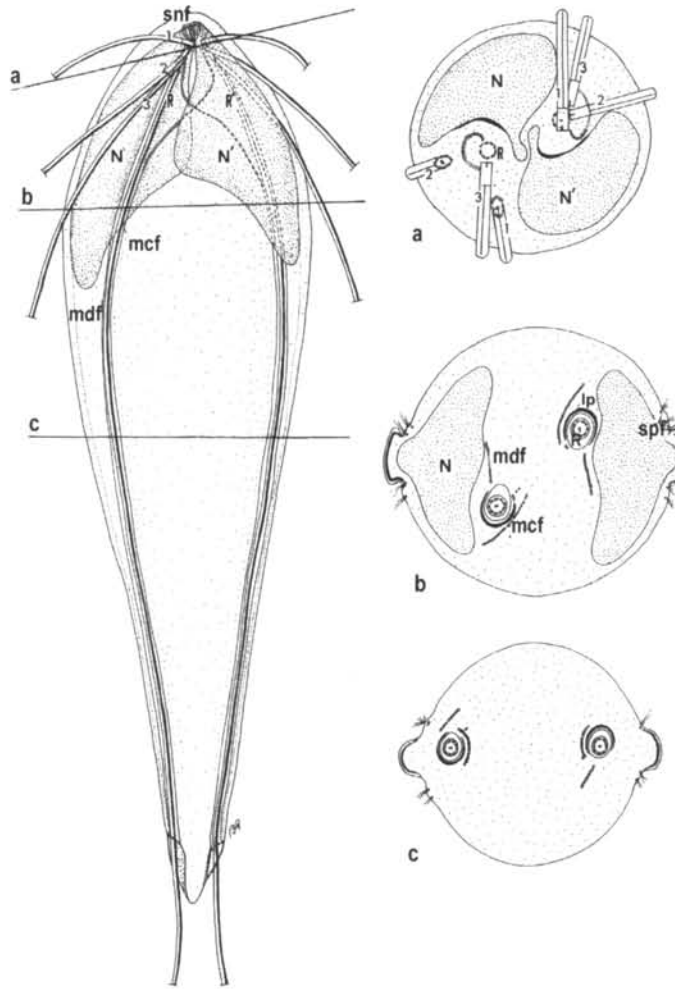



**Fig. 9.9** Schematic representation of *Spironucleus*. a, b, c, transversal sections at different levels. General disposition of nuclei N, N', kinetosomes and flagella 1, 2, 3, R (recurrent flagellum kinetosome) and associated fibres: supranuclear fibre (snf), crossed microtubular fibre (mcf), direct microtubular fibre (mdf) and subpellicular lateral fibre (spf); Lp: lamina with a periodic fine structure. From Bruguerolle *et al.* (1973) with permission of Protistologica (now European Journal of Protistology, Elsevier). Courtesy of Dr. Bruguerolle from the Lab. De Biologie des Protistes, Université Blaise Pascal de Clermont-Fd, France.

elongated shape of the former and its S shaped or spiralled nuclei (Brugerolle and Lee, 2000). Transmission electron microscopy has confirmed the shape of the nuclei and the position of the 2 recurrent

flagella between the two nuclei in contrast with *Hexamita*, where they are located in the external surface of the nuclei (Brugerolle *et al.*, 1973).

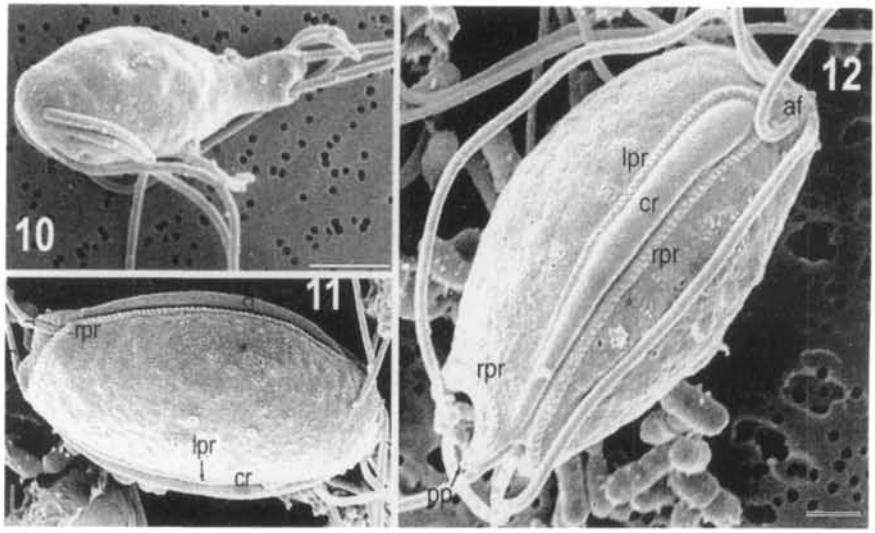
***Hexamita salmonis*** (Moore, 1923) Wenyon, 1926 (syn. *Octomitus salmonis* Moore, 1923) is one of the most reported species. However, some records of this parasite should be reviewed in the light of the new information on the taxonomy of this group. Poynton and Sterud (2002a) suggested new guidelines for species description of diplomonad flagellates of fish, and recommend the use of both, scanning and transmission electron microscopy, for an accurate identification of genus and species. Following this criterion, Poynton *et al.* (2004) synonymized *Hexamita salmonis sensu* Ferguson (1979) with *Octomitus salmonis sensu* Moore (1922), and Davis (1926), and renamed them ***Spironucleus salmonis*** n. comb. Poynton *et al.* (2004) emphasized that the proposed synonymy deals only with above referred organisms, so they cannot determine which other organisms reported as *Octomitus salmonis* and *Hexamita salmonis* actually belong to *Spironucleus salmonis*. As a matter of fact, Kulda and Nohýnková (1979) had already pointed out that some species described in *Hexamita* should be assigned to *Spironucleus*, and Kent *et al.* (1992) suggested that *H. salmonis* could perhaps not be retained in the genus *Hexamita*.

Poynton *et al.* (2004) recognized 4 species from fish in the genus ***Spironucleus*, *S. salmonis*, *S. barkhanus*** (Fig. 9.10), ***S. torosa*** and ***S. vortens*** (Figs. 9.11, 9.12). Although a possible synonymy of *S. barkhanus* and *S. salmonis* was suggested, both genera were retained on the basis of ultrastructural and pathogenic differences until cross-infections studies have been done. In addition, *S. vortens* is very similar to *S. elegans* (typical of amphibians but also reported from fish), and their possible synonymy has been also discussed (Poynton *et al.*, 1995; Poynton and Sterud, 2002b). Readers are referred to Poynton *et al.* (2004) for ultrastuctural details of piscine *Spironucleus* spp. A new species, ***S. salmonicida*** has been recently created to designate the marine genotype of *S. barkhanus* causing systemic spironucleosis in farmed salmonids (Jørgensen and Sterud, 2006).

Considering this taxonomic confusion (see also below, Diagnostic), the fish diplomonadosis are considered together, although the disease described as hexamitiasis/hexamitosis might correspond in some occasions to spironucleosis.

## Disease Symptoms, Aetiology/Pathogenesis

Hexamitosis has been described as typical of weak fish, rarely occurring in natural conditions. In cultured fish, it is generally observed as a secondary




**Figs. 9.10-9.12** Scanning electron microscope images of *Spironucleus* spp. Fig. 9.10. Blood trophozoite of *Spironucleus barkhanus* from *Salvelinus alpinus*. Scale bar = 2 μm. Figs. 9.11, 9.12. *Spironucleus vortens*. Surface morphology of cultivated forms. Fig. 9.11. Dorsal or ventral view of the flagellate showing the two compound lateral ridges (cr). Note the rope-like periodic appearance of the left and right peripheral ridges (lpr, rpr). Fig. 9.12. Lateral view of the flagellate showing one of the compound lateral ridges (cr). Scale bars = 1 μm. Fig. 9.10 from Sterud *et al.* (2003) with permission of Diseases of Aquatic Organisms (Inter-Research). Figs. 9.11, 9.12 from Sterud and Poynton (2002), with permission of Journal of Eukaryotic Microbiology (Blackwell Publishing). Courtesy of Dr. Sterud.

infection, or in fish under stress or other predisposing factors. Direct mortality is mainly recorded in fry and ornamental fish.

Intestine is the primary site of infection, though diplomonads can invade the gall bladder. Trophozoites, the most frequent stages, are usually found free in the lumen. The anterior intestine and the pyloric region are the preferred locations for *Hexamita salmonis* in farmed rainbow trout fry, but in heavily infected fish parasites occur through the entire length of the gut (Uldal and Buchmann, 1996). This intestinal hexamitosis is usually subclinical, but it may become more pathogenic due to fish immunodepression, stress associated to changes in temperature, diet, water physicochemical conditions or handling, and the presence of secondary infections. In those cases, clinical signs include nervous behaviour of fish, anorexia and emaciation. Cysts can appear in the intestine.

In contrast with this syndrome clinically evident only as catarrhal enteritis, severe infections with high mortalities due to systemic

hexamitosis were reported in post-smolt and adult *Salmo salar* from Norway (Mo *et al.*, 1990; Poppe *et al.*, 1992) and *Oncorhynchus tshawytscha* from British Columbia (Canada) (Kent *et al.*, 1992). The diplomonad involved in the Norwegian outbreaks was further identified as *Spironucleus barkhanus* (Sterud *et al*, 1997). The Canadian diplomonad (whose ascription to *Hexamita salmonis* was considered doubtful by Kent *et al.* (1992) is now classified also as *S. barkhanus* (see below, Molecular studies). Clinical signs and gross pathology in naturally infected *Salmo salar* include lesions in the musculature, liver spleen and kidney, and exophthalmia (Poppe *et al.*, 1992; Poppe and Mo, 1993). Similar external signs, besides anaemia, abdominal distension, and skin lesion and ulceration were found in experimental infections of *S. salar* with *S. barkhanus* (Guo and Woo, 2004a). Internal lesions consisted of whitish to yellowish granulomatous nodules in the liver and spleen or haemorrhages of the internal abdominal wall. Hexamitids have been implicated in the hole-in-the-head disease of tropical fish (Ferguson and Moccia, 1980). Paull and Matthews (2001) reported *S. vortens* as a possible cause of this disease in aquarium cichlids (*Symphysodon discus* and *Pterophyllum scalare*). Fish presented lesions confined chiefly to the head region and varying in extension according to the infection severity. Splenomegaly was noted in systemic infections.

Stress or immunodepression might also be involved in the appearance of the systemic infections reported, not only in salmonids but also in cyprinids, eels and tropical freshwater fish. In these infections, trophozoites are also found in the blood, heart, kidney, liver, spleen, eye, brain, muscles, serosa and abdominal cavity. According to Siddall *et al.* (1992), attachment to intestinal epithelium, invasion of mucosa, and dissemination of infection are only seen in *Spironucleus*, and *Hexamita* species exist freely in the intestine. The recent ascription of diplomonads involved in systemic infections to *Spironucleus* (Sterud *et al.*, 1997; Poynton *et al.*, 2004), would confirm this criterion. Whether other reports on systemic invasions by *Hexamita* might be erroneous or not remains to be elucidated.

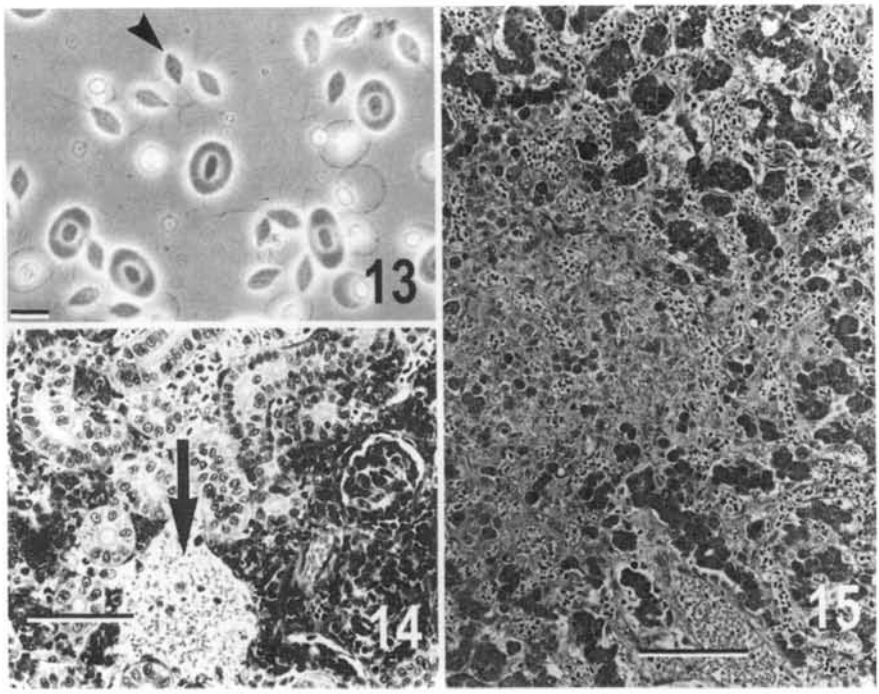
Different histopathological changes associated to hexamitid infections have been described. Again, some lesions reported in *Hexamita* infections could actually be produced by *Spironucleus*, according to the criteria of some authors. We can distinguish between intestinal damage and lesions of other organs and tissues. Catarrhal enteritis is the main pathology of the typical disease initially described as hexamitosis/spironucleosis. In contrast, Atlantic salmon post-smolts with systemic

hexamitosis/spironucleosis have whitish granulomatous nodules in liver and kidney (Poppe *et al.*, 1992). In large fish (4-5 kg), the livers and kidneys show multifocal necrosis, with oedema, congestion, haemorrhages and fibrosis. In advanced infections, fish have severe muscular degeneration, cholangiocephalitis and perihepatitis, encephalitis and meningitis. In Chinook salmon systemic infections, flagellates appear in the blood (Fig. 9.13), the liver can exhibit petechial haemorrhages, oedema, congestion and inflammation (Fig. 9.15), and the renal interstitium can be hyperplasic (Fig. 9.14) (Kent *et al.*, 1992). In *Salvelinus alpinus* infected by *Spironucleus barkhanus*, despite huge numbers of flagellates in the heart (Plate 9.2, A) and in the vasculature (on many occasions, apparently intracellularly), the tissues of the organs were remarkably unaffected, in contrast to systemic spironucleosis in farmed Atlantic salmon (Sterud *et al.*, 2003). Cyprinids with intestinal 'spironucleosis' may have enteritis, liver necrosis and serous exudates in the abdominal cavity, whereas in aquarium fish the parasite can produce mortalities and invade the blood and other parts of the body (Molnár, 1974). Tropical fish can show enteritis, gall bladder inflammation, gastritis and perforation with serosal granuloma in the stomach (Ferguson, 1989).

## Diagnosis

As clinical signs are mostly unspecific, diagnosis is based on the microscopic examination of scrapings from various sites of the gastrointestinal tract, and of the blood. Flagellates appear as oval-shaped spots. It is also useful to observe wet mounts of such material diluted in saline (NaCl 0.9%), using bright field, phase contrast or Nomarski illumination at 400 × (Fig. 9.13). The protargol impregnation of Bouin's fixed suspensions of intestinal contents can aid to observe the flagella and microtubules (see Woo and Poynton, 1999). These methods allow to confirm the involvement of a diplomonad in the morbid process and to apply the advisable measures for control and treatment.

The identification of the genus and species involved in hexamitosis/spironucleosis is more difficult (see above). According to Poynton *et al.* (2004), ultrastructural studies following the guidelines of Poynton and Sterud (2002a) are needed to establish the true identity of parasites. Using these criteria, the four piscine *Spironucleus*, recognized by Poynton *et al.* (2004) can be discriminated.




**Figs. 9.13-9.15** Hexamitid from Chinook salmon, *Oncorhynchus tshawytscha*. Fig. 9.13. Flagellates in the blood (arrowhead). Wet mount preparation, phase contrast. Fig. 9.14. Focal necrosis and heavy infection in liver. Fig. 9.15. Renal interstitial hyperplasia and proliferative glomerulonephritis. Scale bars = 10 μm (Fig. 9.13) and 50 μm (Figs. 9.14, 9.15). From Kent *et al.* (1992), with permission of Diseases of Aquatic Organisms (Inter-Research). Courtesy of Dr. Kent.

Guo and Woo (2004b) reported the detection of *Spironucleus barkhanus* in the blood of experimentally infected *Salmo salar* using the wet mount examination (WME), the hematocrit centrifuge technique (HCT; Woo, 1969) and the hemocytometer (HCM) techniques, being the later used for quantification. WME allowed detecting the parasites also in fish mucus and ascites. In addition, an ELISA was developed to detect specific antibodies. It could be useful in epidemiological studies, although validation in field conditions is required.

Molecular methods could aid to solve these diagnostic problems. Sequencing has been achieved for some genes of *S. vortens* and *S. barkhanus* (Keeling and Doolittle, 1996, 1997; Jørgensen and Sterud, 2004) (see below Molecular studies), but a molecular diagnostic assay is as yet unavailable. The ultrastructure and molecular diagnosis of *S. salmonis* from German rainbow trout has been recently studied by Fard

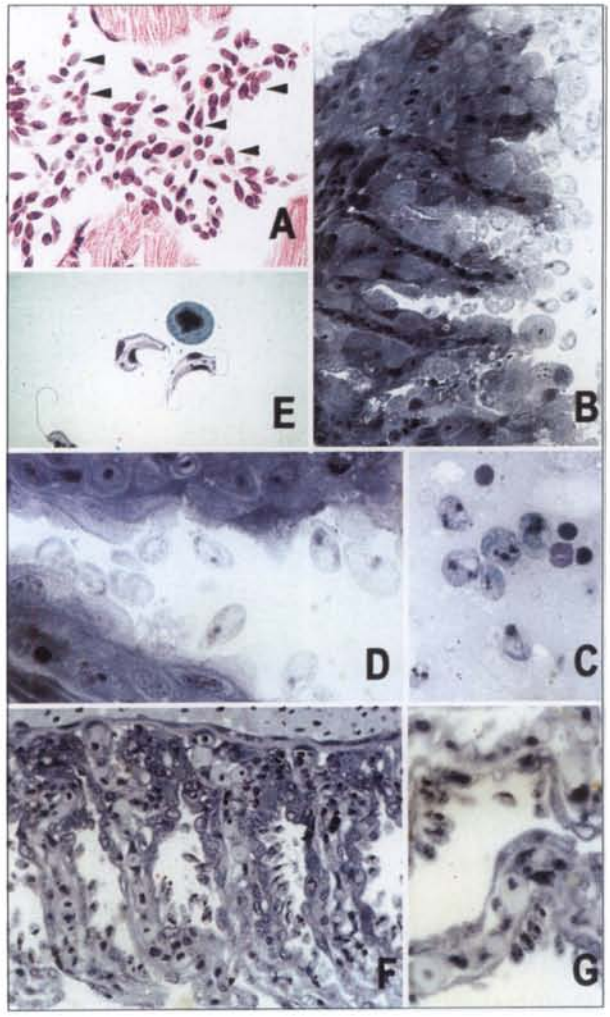



**Plate 9.2** **A.** *Spironucleus barkhanus*. Trophozoites (arrows) whithin the ventricular lumen of the heart in a histological section stained with haematoxylin and eosin × 480. **B-D.** *Ichthyobodo* sp. from the gills of eel *Anguilla anguilla*. **B, D**: Abundant attached forms lining the gill epithelium. A great part of the gill epithelium has been destroyed or slughed and the remaining epithelial cells appear swollen. B: × 330; D: × 740. **C**. Free-swimming forms in a smear of affected gills. × 780. **E**. *Cryptobia salmositica* and a red blood cell from an experimentally infected rainbow trout *Oncorhynchus mykiss*. × 730. **F-G**. *Cryptobia* sp. from the gills of gilt-head seabream *Sparus aurata*. The flagellates appear lining the epithelium but no or scarce damage is observed. F: × 550; G: × 780. B, D, F, G: Resin sections stained with toluidine blue. C, E: Giemsa staining. Fig. A from Sterud *et al.* (2003) with permission of Diseases of Aquatic Organisms (Inter-Research). Courtesy of Dr. Sterud. Fig. E from Woo (2003) with permission of Journal of Fish Diseases (Blackwell Publishing). Courtesy of Dr. Woo.

*et al.* (2007). According to these authors, the SSU rRNA gene sequence clearly distinguished *S. salmonis* from *S. barkhanus*, *S. salmonicida* and *S. vortens*.

## Treatment and Prophylaxis

Good husbandry and nutrition are essential to reduce the presence and severity of outbreaks. Treatments with furazolidone have been used (Ferguson, 1979). Several nitroimidazoles—such as dimetridazole and metronidazole—in the food have been recommended, but they are not licensed for its use in fish for human consumption (Gratzek, 1988). The four nitroimidazoles tested by Tojo and Santamarina (1998a) were proven to be effective in eliminating hexamitiasis in rainbow trout, whereas only four out of 21 non-nitroimidazoles assayed eliminated the infection. Dimetridazole, metronidazole and mebendazole were the most effective of the seven assayed chemotherapeutic agents in inhibiting the *in vitro* growth of *Spironucleus vortens* (Sangmaneedet and Smith, 1999). Albendazole, febendazole and magnesium sulphate produced a lower effect.

## Fish Hosts, Epidemiology and Geographical Distribution

The actual host range and distribution of *Hexamita* spp. and *Spironucleus* spp. is difficult to analyse, considering the existing difficulties in the ascription to genera and species.

*Spironucleus salmonis* (*sensu* Poynton *et al.*, 2004) has the hosts and distribution of the corresponding renamed species, *Hexamita salmonis sensu* Ferguson (1979) (juvenile farmed rainbow trout from Northern Ireland) and *Octomitus salmonis* Moore, 1922 (referred as *Hexamita salmonis* by Wenyon, 1926) (*Salvelinus fontinalis*, *Salmo trutta*, *Oncorhynchus mykiss* and *Salvelinus namaycush* from a hatchery in Bath, New York State, East Coast of North America). Hexamitosis by *Hexamita salmonis* was reported by Uldal and Buchmann (1996) in farmed rainbow trout in Denmark. Whether some initial and further records of *Hexamita salmonis* (*Octomitus salmonis*) and *Hexamita* sp. could actually be *Spironucleus*, remains to be elucidated.

*Spironucleus barkhanus* was described from wild grayling *Thymallus thymallus*, from the river Glomma (southeastern Norway). The flagellates from adult Atlantic salmon *Salmo salar* farmed in six seawater farms in northern Norway (Mo *et al.*, 1990; Poppe *et al.*, 1992) and kept in culture (ATCC no. 50377) were also ascribed to this species (Sterud *et al.*, 1997).

*S. barkhanus* was further recorded from estuarine *Salvelinus alpinus* from northern Norway by Sterud *et al.* (1998) who hypothesised that feral char could be the source of spironucleosis affecting sea-caged Atlantic salmon. The hexamitid producing systemic disease in seawater pen-reared *Oncorhynchus tshawytscha* reported from Kent *et al.* (1992) in British Columbia (Canada), is now classified as *S. barkhanus* (see below, Molecular studies). Thus, the differences between European and North American species, suggested by Moore (1922), cannot be sustained in the light of the new findings.

*Spironucleus vortens* was described from the freshwater angel fish *Pterophyllum scalare* (Cichlidae) from Florida (U.S.A.). Poynton and Sterud (2002b) found it in *Leuciscus idus* in a river of Southeast Norway, thus extending its geographical distribution. Molnár (1974) reported the presence of diplomonad flagellates in several cyprinids from the river Danube (*Chondrostoma nasus* and *Barbus barbus*), pond carps (*Hypophthalmichthys nobilis* and *Cyprinus carpio*) and aquarium fish (mainly the angel fish *Pterophyllum scalare*) in Hungary. Molnár (1974) identified these flagellates as *Spironucleus*, probably *Spironucleus elegans*, the species to which *S. vortens* could be a synonymous (see Poynton and Sterud, 2002b). *Spironucleus* sp. was also reported in grass carp (*Ctenopharyngodon idella*) introduced in Hungary from China and the Soviet Union (Molnár, 1971). Discus, *Symphysodon discus*, and angel fish obtained from local aquarists within the Southwest of the U.K. and suffering from hole-in-the-head disease, were found infected by *S. vortens* (Paull and Matthews, 2001). Diplomonads reported as *Spironucleus* have been found associated to outbreaks of discus and other cichlid fish, though in mixed infections with other parasites (Yanong *et al.*, 2004) (see below, *Cryptobia iubilans*). An unidentified *Hexamita* sp. has been also reported from other aquarium fish, as *Paracheirodon innesi* and *P. axelrodi* (Characidae) imported in Australia from Singapore, Indonesia and Hong Kong (Evans and Lester 2001).

Diplomonads are less frequent in marine fish. *S. torosa* parasitizes Atlantic cod, *Gadus morhua*, haddock, *Melanogrammus aeglefinus* and shaite, *Polachius virens* from Norway (Poynton and Morrison 1990; Sterud, 1998a). The finding in *Lota lota* is considered a probable introduction to this freshwater host (Sterud, 1998b).

## Economical Importance

The systemic disease is the most important cause of loses, mainly in cultured and aquarium fish. Apart from *Spironucleus* affecting mainly

salmonid fish, Bricknell *et al.* (2006) identified *S. torosa* as one of the potential threats to the incresing intensive culture of gadoid fish, as farmed fish could get infected from the wild hosts.

## *In vitro* Culture

Axenic culture of *Hexamita salmonis* in a balanced organic medium (M199 with several supplements) was reported by Uzmann and Hayduk (1963). The hexamitid from *Oncorhynchus tshawytscha* found by Kent *et al.* (1992) was deposited in the American Type Culture Collection (ATCC) (50329 and 50330). *Spironucleus barkhanus* was described by Sterud *et al.* (1997), using flagellates isolated from *Thymallus thymallus* and cultured in bile supplemented TYI-S-33 and those isolated from Atlantic salmon and sent to the ATTC (50377). This modified TYI-S-33 medium has been used in new cultures of *S. barkhanus* (Sterud, 1998c), and to routinely maintain *Spironucleus vortens* (Poynton *et al.*, 1995). Both cultures have been deposited at the ATCC (50386 and 50467, respectively). Buchmann and Uldal (1996) isolated *H. salmonis* from rainbow trout and maintained it in axenic culture in minimum essential medium (MEM) supplemented with a bile solution (Keister's modified TYI-S-33). They found a pH, temperature and bile dependence of the *in vitro* growth. Paull and Matthews (2001) developed a modified method of that described by Buchman an Uldal (1996) for the detection, isolation and long-term maintenance of *S. vortens*. According to Sangmaneedet and Smith (2000), the optimal condition for the growth of *S. vortens* cultivated *in vitro* is 25°C and pH 6.5 to 7.5 without supplementation of bile, differing from *H. salmonis* (Buchmann and Uldal, 1996).

## Immunological Studies

Scarce information is available on the fish immune response to diplomonads, and has been mainly obtained from histopathological studies. In systemic hexamitosis/spironucleosis, an inflammatory response is quite common in kidney, liver and gut (Kent *et al.*, 1992; Poppe *et al.*, 1992; Sterud *et al.*, 2003). In *Salvelinus alpinus* infected by *Spironucleus barkhanus*, a cellular response, comprising lymphocytes and macrophages, as well as abundant intracellular flagellate stages, have been described (Sterud *et al.*, 2003). Although these authors did not identify the host cells, some of them could be macrophages or other phagocytic cells. This phagocytic response would be able to explain the moderate lesions

observed in the tissues in this fish, in contrast with the severity of systemic spironucleosis in Atlantic salmon.

## Molecular Studies

Diplomonads are flagellate protozoa that lack identifiable mitochondria, peroxisomes, and permanent Golgi dyctiosomes. Whether or not these structures really were absent in their ancestors or have since then been lost, remains to be seen (Keeling and Doodlittle, 1997). Most of the attention paid to the molecular biology of diplomonads has been concentrated on *Giardia*, though other diplomonads have been recently studied including some piscine *Hexamita* and *Spironucleus*. To define the distribution of genetic codes within the diplomonads, Keeling and Doolittle (1997) studied several genes of species different from *Giardia*, including the piscine *Hexamita* 50330 (the diplomonad found by Kent *et al.*, 1992 in farmed Chinook salmon), and *Spironucleus vortens*. The sequences of the elongation factor-1α (EF-1α), alpha-tubulin and the gamma subunit of eukaryotic translation initiation factor-2 (eIF-2γ), showed that the variant genetic code in which TAA and TAG encode glutamine rather than termination is utilized by almost all diplomonads, with the single exception of *Giardia*.

Molecular tools can be a good complement of ultrastructural studies to discriminate between genera and species and to accurately describe new species. In addition, they could give insights to develop quick and reliable molecular diagnostic methods. Sequences of the EF-1α, eIF-2γ, alpha-tubulin and SSU rRNA genes of *Spironucleus vortens*, and diplomonad 50330 were obtained by Keeling and Doodlittle (1996, 1997). In addition, two genes of diplomonad 50380 were sequenced, the alpha-tubulin gene (Keeling and Doodlittle, 1997), and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (Rozario *et al.*, 1996). Recently, the SSU rRNA gene sequence was also obtained for *S. barkhanus* from farmed and wild Arctic charr *Salvelinus alpinus* (Jorgensen and Sterud, 2004) and compared with the corresponding sequences of the *Hexamita* 50330 strain (presently considered to be *S. barkhanus*), and *S. barkhanus* from Atlantic salmon, Norway. Jorgensen and Sterud (2004) found a great similarity between *S. barkhanus* from farmed Artic charr, Chinook salmon (50330) and Atlantic salmon, suggesting that systemic spironucleosis may be caused by specific strains/variants of this parasite, and confirming the results of Keeling and Doolittle (1997) for diplomonad strains ATCC 50330 and 50380, using the alpha-tubulin gene. Thus, both strains are referred to in GenBank as synonymous of *S. barkhanus*. However,

Jorgensen and Sterud (2004) found two genotypes of *S. barkhanus* from wild and farmed *Salvelinus alpinus*, respectively, with such genetic differences that their conspecificity should be questioned. Further studies confirmed such differences, and thus the marine pathogenic genotype of *S. barkhanus* was redescribed as *S. salmonicida* (Jørgensen and Sterud, 2006).

## Outlook, Recent Developments and Future Prospects

Gene sequences of some fish diplomonads have been used in recent molecular phylogenetic studies. According to Kolisko *et al.* (2005) the analysis of the SS rRNA gene sequence of several enteromonad and diplomonad genera (including *Spironucleus vortens* and *S. barkhanus*) indicate that the dyplokaryotic cell of diplomonads arose several times independently, or that the monokaryotic cell of enteromonads originated by secondary reduction of dyplokaryotic stage. In another study of Andersson *et al.* (2003), the analysis of 15 genes present in the genome of *S. barkhanus* and *Giardia lamblia* (with high sequence similarity) revealed frequent lateral gene transfer (LTG) in these organisms and demonstarted that LGT is a significant evolutionary mechanism among diplomonads in particular and protists in general.

Diagnosis improvement is one of the challenges in fish diplomonads. Ultrastructural studies are a useful tool to complete the morphological descriptions of some species, and molecular studies have given insights on the phylogeny and relationships between close species. However, currently, a clarification of the genera and species actually existing in this group is still lacking. Keeling and Doolittle (1997) suggested that *Hexamita* 5330 from Chinook salmon and *Hexamita* 50386 from Atlantic salmon (both referred as *Spironucleus barkhanus* by Jorgensen and Sterud, 2004 and in GenBank) are likely to belong to neither the genus *Hexamita* nor the genus *Spironucleus* and most likely should be given the distinction of a unique genus when complete morphological descriptions have been made. Further studies are needed to accurately identify the species involved in hexamitosis/spironucleosis and the existence of species or strains causing severe systemic disease. Several nucleotide and protein sequences for *S. barkhanus* (43 and 38, respectively) and *S. vortens* (22 and 6) are currently available in GenBank, and some of them have been used in comparative phylogenetic studies. A closer genetic comparison of additional genes and other isolates of the parasites could aid this purpose. The involvement of host factors in the course and virulence of the disease should also be clarified.

**EUGLENOZOA** Cavalier-Smith, 1981, emend. Simpson

**KINETOPLASTEA** Honigberg, 1963

It corresponds to the Class Kinetoplastea Honigberg, 1963 emend. Vickerman, 1976 in the classification of Moreira *et al.* (2004). Most fish parasitic kinetoplasteans belong to the Subclass Metakinoplastina Vickerman in Moreira *et al.* (2004), except for *Ichthyobodo*, which is classified in the Subclass Prokinetoplastina Vickerman in Moreira *et al.* (2004) (Table 9.2).

The name of the taxon refers to the typical characteristic, the kinetoplast, a segment of the unique mitochondrion with an unsually high content of DNA (kDNA). During the cell cycle, kinetoplast and nuclear DNA synthesis are synchronous. The structure of kDNA differs between groups. The typical structure in Trypanosomatida consists of a network of circular DNA molecules that is physically connected to the flagellar base via the mitochondrial membrane. There are two types of kDNA circles: a few large maxicircles (25-50 per kinetoplat, each 20-38 kb) and a large number of minicrcles (5,000-27,000, each 0.46-2.5 kb). Parabodonida present an eu- or pan-kDNA not in network (see below). The uninucleated kinetoplastid cell possesses two flagella, one of which is often reduced (Hausmann *et al.*, 2003). These flagellates also have unique features with respect to their energy and carbohydrate metabolism, as they possess peculiar peroxisomes called glycosomes, which play a central role in such metabolism (Hannaert *et al.*, 2003).

Kinetoplastids reproduce usually by longitudinal binary fission, although sexual processes seem to occur occasionally, as synaptonemal complexes (suggesting the occurrence of meiosis) have clearly been demonstrated in a few cases. However, mating is not obligatory to complete the life cycle (Hausmann *et al.*, 2003). These flagellates are abundant in nature either as free-living consumers of bacteria or as parasites of animals, plants, or other protists.

**PROKINETOPLASTINA** Vickerman in Moreira *et al.*, 2004

**PROKINETOPLASTIDA** Vickerman in Moreira *et al.*, 2004

According to Moreira *et al.* (2004), neither the subdivision of the former suborder Bodonina in the two families Bodonidae and Cryptobiidae (Vickerman, 1976) nor the two subfamilies Bodoninae and Cryptobiinae (Zhukov, 1991) are compatible with the 18SS rRNA and Hsp90 phylogenetic trees (see below, Molecular studies).

***Ichthyobodo*** (Pinto, 1928) (= *Costia* Leclerq, 1890)

This genus was previously included in the family Bodonidae Bütschli, 1887 (Suborder Bodonina Hollande, 1952) (Vickerman, 1976, 2000), one of the families not supported by recent molecular studies.

The species of *Ichthyobodo* present two flagella extending posterior-laterally from a deep flagellar pocket. The long tubular mitochondrion contains numerous nucleoids, so multiple kinetoplasts are spread throughout the body (polykinetoplastic). *Ichthyobodo* spp. are ectoparasites on fish, and their life-cycle includes one free-swimming dispersive phase (Plate 9.2, C), alternating with the attached, feeding stage Plate 9.2, B, D; Fig. 9.16).

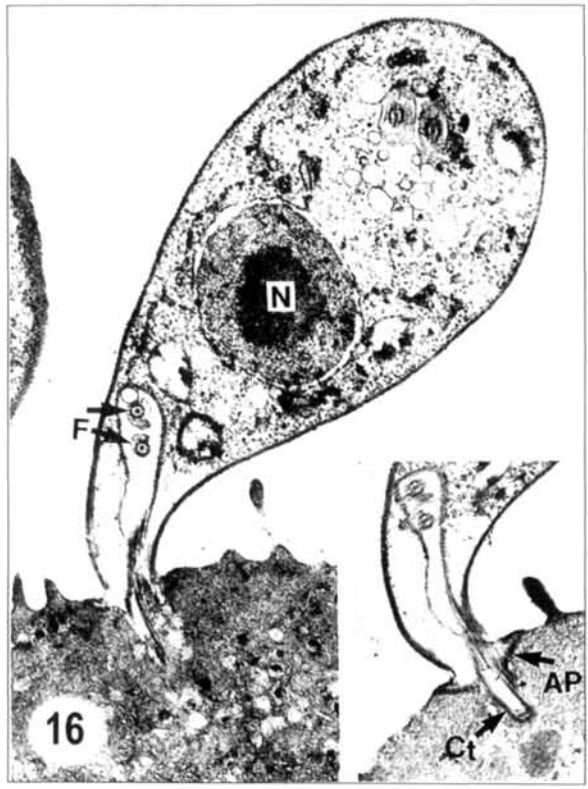



**Fig. 9.16** *Ichthyobodo* sp. from common dab, *Limanda limanda* L. Trophozoite attached to the secondary lamellar epithelial cell. N: parasite nucleus; F: flagellum (×10700). Inset: Attachment plate (AP) with cytostomeal tube (Ct) (× 11250). From Diamant (1987) with permission of Journal of Fish Diseases (Blackwell Publishing). Courtesy of Dr. Diamant.

*Ichthyobodo necator* (Henneguy, 1883) Pinto, 1928 (Syn. *Costia necatrix* Leclerq, 1890)

The causal agent of the fish ichthyobodosis is the best-known species in the genus. Formerly known as *Costia necatrix* (an invalid name from which costiasis is derived), it is an important cosmopolitan fish parasite which can produce heavy mortality, especially in hatcheries.

## Morphology and Life Cycle

The parasitic forms, with a pyriform shape, attach to the epithelial cells by a hypertrophied proboscis, which protrudes into the host cell, whereas flagella are pointed away from the fish surface. The flagellate ingests the host cell cytoplasm through the terminal cytostome transformed in an attachment plate. The free-swimming phase lacks this attachment organ. This free, non-feeding form has a flat, slightly asymmetrical, oval body, averaging $10 \times 5$ μm. It is strongly convex dorsally and slightly concave ventrally. Two unequal flagella extend from the flagellar pocket. The cytostome opens at the right cell margin, at the border of the pocket. The long mitochondrion zig-zags through the cytoplasm and contains numerous tiny kinetoplasts with DNA nucleoids. The transformation from the swimming to the attached form occurs in a few seconds. The parasite divides by binary fission, this process starting by doubling of the flagellar number, which can lead to confusion about the number of flagella when dividing forms are found (Lom and Dyková, 1992; Vickerman, 2000).

## Disease Symptoms, Aetiology/Pathogenesis

Clinical signs are non-specific and depend on the infection severity. In light infections, the fish may twist in the water or rub against objects or tank walls. Fish with heavy infections can become listless or anorexic. Parasites are found on the skin and gills of affected fish. The body surface may present a white-blue cloudy layer, spots, discolouration, and mucus secretion. Fins can be destroyed, and both fins and gills may appear swollen. Pérez and Mainardes-Pinto (1998) reported an outbreak of *I. necator* in fingerlings of the characidae *Piaractus mesopotamicus* cultured in Brazil, and atributed the high mortality to asphyxiation. The action of the flagellate results in destruction of epithelial or epidermal cells producing widespread pathological changes. Skin histopathological lesions include spongiosis, vacuolation and oedema, followed by

degeneration and sloughing of epidermis. Affected gills show hyperplasia and lamellar fusion, sometimes accompanied with swelling and necrosis of epithelial cells (Plate 9.2, B, D) (Lom and Dyková, 1992; Woo and Poynton, 1999).

These clinical signs and histopathological damage have been described in both freshwater and marine fish, and may be enhanced in salmonids transferred to seawater. Urawa (1996) evaluated the effect of *Ichthyobodo* on growth, survival and seawater adaptation in juvenile chum salmon (*Oncorhynchus keta*) in an experimental infection. Increased mortality with respect to control fish was observed, as well as the typical epidermal lesions, leading to a marked effect on osmoregulation which consequently reduced marine survival of anadromous hosts. In the marine fish *Limanda limanda*, Diamant (1987) also described the disruption of lamellar epithelial integrity which might osmotically compromise the fish in a mixed infection of *Ichthyobodo* and X-cells.

Fry and juvenile fish are more prone to suffer severe infections and even mortalities, mainly in stress situations.

## Diagnosis

Upon suspicious clinical signs, diagnosis is confirmed by the examination of mucus smears from skin and gills and the observation of flagellates at light microscope. Diagnosis is easier from alive or recently dead fish, when the moving flagellates can be observed. Anaesthetics can reduce the parasite motility and even produce its detaching. This effect was observed with unbuffered tricaine and was avoided using the buffered anaesthetic (Callahan and Noga, 2002). Smears can be fixed and stained with Giemsa staining.

Light microscope observations allow for diagnosis at the genus level, information necessary for assaying treatments. However, the species discrimination is not easy. Some authors suggest ultrastructural details as an aid to species determination, but recent molecular studies (Todal *et al.*, 2004; Callahan *et al.*, 2005) have revealed the difficulties to establish species in the *Ichthyobodo* complex (see below, section on Molecular studies).

## Treatment and Prophylaxis

Formalin baths (1:4000) of 15-30 min had been usually recommended as rather effective in salmonids, but this treatment must be avoided

according to the present legal dispositions in many countries. The same can be said for malachite green and other effective chemicals. According to Tojo *et al.* (1994), bithionol baths (25 mg/l for 3 h or 2 consecutive days) are very effective in eliminating the parasite from rainbow trout. Cyprinids can be treated with copper sulphate (50 ppm) for 1-2 min or sodium chloride (10,000 ppm) for 15-30 min (up to 5% for larger fish). The rise of water temperature over the optimal range for the flagellates (about 32°C) has also been recommended, but this measure can be unviable depending on the fish species (see Woo and Poynton, 1999). The efficacy of the medicinal plant *Buddleja cordata* (Tepozán) against costiasis was demonstrated in Mexico in bath trials using infected tilapia (*Oreochromis* sp.), and the active fraction was identified as a phenylpropanoid glycoside called verbascoside (Díaz Sánchez *et al.*, 2000). Other alternatives to formalin baths have been tested. Holten *et al.* (2003) reported the potential of Detarox AP (Aco Kjemi TBL AS) (a disinfectant containing 20% hydrogen peroxide and 4-5% peracetic acid) in the treatment of *I. necator*, although two treatments were required for complete removal of the parasites from naturally infected *Salmo trutta*. In addition, bronopol (bromo-2-nitropropane-1,3-diol) has been recommended for the treatment of ichthyobodosis in salmonid fish (J.C. Braidwood, 2003; patent publication number EP1310246, application number EP20030002673 19970729).

Most of the 32 drugs evaluated by Tojo and Santamarina (1998b) in orally treating *I. necator* infected rainbow trout were ineffective. Only metronidazole, secnidazole and triclabendazole (a non-carbamate benzimidazole) proved to be efficacious.

## Fish Hosts, Epidemiology and Geographical Distribution

*Ichthyobodo necator* was originally described from diseased brown trout *Salmo trutta*, and further recorded from other freshwater salmonids and cyprinids. Host specificity is low, as it has been reported from numerous fish species, belonging to different families, mainly cyprinids, salmonids, anguillids, platyfish, catfish, perchs, etc. The flagellate has been frequently associated to outbreaks in cultured and aquarium fish, mainly in fry and in certain specific conditions. Apart from the numerous cyprinids used worldwide with ornamental purpose, the poecilids *Poecilia reticulata*, *P. sphenops* and *Xiphophorus helleri* and the cichlid *Symphysodon aequifasciata* have been reported to suffer epizootics in Sri Lanka

(Thilakaratne *et al.*, 2003) and in Uruguay (Carnevia and Speranza, 2003a), respectively. Brazilian cichlids and the characidae *Piaractus mesopotamicus* were also found to be infected (Eiras *et al.*, 1995; Pérez and Mainardes-Pinto, 1998; Todal *et al.*, 2004). *I. necator* is included among the most important parasites in imported freshwater aquarium fish in Western Europe (Caffara and Tampieri, 2001). It is also very frequent in freshwater cultured salmonids, *Oncorhynchus mykiss*, *O. masou*, *Salmo trutta*, *Salvelinus fontinalis* and *Salmo salar*. Amongst cultured cyprinids, several species can be affected in Europe, Asia, South Africa, North America and South America. In addition, *Ichthyobodo* is one of the parasites most frequently recorded in a recent survey of cultured fish in the Mediterranean area (Alvarez-Pellitero, 2004), so the parasite can be considered cosmopolitan in nature. According to Svobodova and Kolarova (2004), ichthyobodosis is one of most important protozoal disease of tench fry in Central and Eastern Europe. High mortality rates by *I. necator* were reported in perch (*Perca fluviatilis*) intensively cultured in warm water effluents in Belgium (Grignard *et al.*, 1996). *Ichthyobodo* sp. was also found in *Anguilla anguilla* cultured in Spain (Plate 9.2, B-D) (author's unpubl. obs.).

In the marine environment, *I. necator* infections were reported from Atlantic salmon smolts transferred to seawater net pens in Scotland (Ellis and Wootten, 1978; Bruno, 1992). In addition, Urawa and Kusakari (1990) confirmed experimentally that *I. necator* can survive on anadromous salmon when the host fish migrate to sea water. Following the first record of *Ichthyobodo* sp. in the marine fish *Pleuronectes platessa* from Scotland (Bullock and Robertson, 1982), further findings from strictly marine fish led to the consideration that the flagellates found in marine fish might belong to a separate species (Morrison and Cone, 1986; Diamant, 1987; Bruno, 1992). This hypothesis was confirmed by the failure in cross transmiting *I. necator* from chum salmon (*Oncorhynchus keta*) to Japanese flounder (*Paralichthys olivaceus*) and *Ichthyobodo* from flounder to chum salmon (Urawa and Kusakari, 1990). Urawa (1998) reported findings of *Ichthyobodo* in strictly marine fish belonging to several families in 8 orders (Clupeiformes, Dactylopteriformes, Gadiformes, Gasterosteiformes, Perciformes, Pleuronectiformes, Salmoniformes and Tetraodontiformes), and concluded that the *Ichthyobodo* of marine fish may be regarded as a separate species from *I. necator*, though they cannot be differentiated by morphological observations. Recent molecular studies have given new insights in the *Ichthyobodo* taxonomy, confirming the fact that *Ichthyobodo necator* is a complex of sibling species (Todal *et al.*, 2004;

Callahan *et al.*, 2005) and that some marine fish are infected by species different from *I. necator*. Some of the recognized marine species could have a very wide range of fish hosts and geographic distribution (see below, Molecular studies).

In Table 9.6, the main reports of *Ichthyobodo* from cultured marine or anadromous fish are presented. In Mediterranean cultured fish, the flagellate was recorded in marine fish from 6 countries. Several commercially important fish are also affected in the Atlantic Ocean. Apart from salmonids cultured in sea water, the flatfish turbot, halibut and olive flounder, and the gadoid cod have been found to be infected by *Ichthyobodo*. In addition, it has been frequently recorded from wild marine and euryhaline fish, and 16 species belonging to 8 families were listed in the review by Urawa *et al.* (1998). Some fish hosts—as gadoid fish—are commercially important species whose intensive culture is in rapid development. Bricknell *et al.* (2006) identified *Ichthyobodo* as one of the potential threats to these fish under culture conditions. A further record in wild marine fish is a new finding in *Dicentrarchus labrax* caught in the Aveiro's Estuary (Portugal) (Duarte *et al.*, 2000). *Ichthyobodo* was present in juvenile mullets (*Mugil platanus*) caught in Uruguay with farming purposes (Carnevia and Speranza, 2003b). The geographic distribution of *Ichthyobodo* is very wide and covers several countries in Europe, Asia, America, and seldom Oceania, although records in marine fish have been more frequent in the northern hemisphere.

## Molecular Studies

The sequence analysis of Moreira *et al.* (2004) stating the branching of *Ichthyobodo necator* and *Perkinsiella* cf. *amoebae* very deeply at the base of kinetoplastid tree, as suggested by Callahan *et al.* (2002) and Dyková *et al.* (2003), is correct and not the result of an artefactual attraction towards the out-group sequences.

Marine and freshwater isolates of the *Ichthyobodo necator* complex from different hosts and geographic origins (Norway, U.S.A., Japan, Brazil, South Africa and Singapore) have been genetically compared, and the existence of several species has been demonstrated (Todal *et al.*, 2004; Callahan *et al.*, 2005). Todal *et al.* (2004) found certain morphological differences between species belonging to two different *Ichthyobodo* lineages, A and B, and concluded that further detailed morphometrical and ultrastructural studies on the recognized molecular species could reveal previously unrecognized characteristics.

**Table 9.6** Main reports of *Ichthyobodo* spp. from cultured marine fish or salmonids cultured in sea water.

| *Fish hosts*<br>*Order, Family, Species* | *Geographic location* | *Type of habitat/facility* | *Fish age* | *Parasite identification* | *Infection severity* | *References* |
|---|---|---|---|---|---|---|
| PERCIFORMES | | | | | | |
| **Ephippidae** | | | | | | |
| *Chaetodipterus faber* | Western Atlantic Ocean, North Carolina, U.S.A. | Aquarium | | *Ichthyobodo*-like | Clinical signs | Beck *et al.* (1996) |
| **Moronidae** | | | | | | |
| *Dicentrarchus labrax* | Mediterranean Sea (France, Greece, Morocco) | Intensive | Fry and juveniles | Costiasis | Outbreaks (in fry) and subclinical | Alvarez-Pellitero (1995, 2004) |
| **Sparidae** | | | | | | |
| *Sparus aurata* | Mediterranean Sea (Cyprus, Greece, Spain) | Intensive and semi-intensive | Fry and juveniles | Costiasis<br>*Ichthyobodo* sp. | Outbreaks and subclinical | Alvarez-Pellitero (2004)<br>Alvarez-Pellitero *et al.* (1995) |
| | South Atlantic Ocean (Spain) | | | | | |
| | Aegean Sea (Turkey) | | | | Outbreaks | Toksen (2000) |
| *Diplodus puntazzo* | Mediterranean Sea (Cyprus) | Intensive | | *Ichthyobodo* | | Alvarez-Pellitero (2004) |
| PLEURONECTIFORMES | | | | | | |
| **Paralichthydae** | | | | | | |
| *Hippoglossus hippoglossus* | Atlantic Sea | Farming | | *Ichthyobodo* | Clinical signs | Bergh *et al.* (2001) |

*(Table 9.6 Contd.)*

*(Table 9.6 Contd.)*

| | | | | | | |
|---|---|---|---|---|---|---|
| *Paralichthys olivaceus* | Pacific Ocean Japan | Cultures | Juveniles, broodstock | *Ichthyobodo necator*, *Ichthyobodo* sp. | Outbreaks | Reviewed in Urawa *et al.* (1998) |
| *Scophthalmus maximus* | Norway | Cultures | | *I. necator* | | Broderud and Poppe (1986) (cit. Urawa *et al.*, 1998) |
| SALMONIFORMES | | | | | | |
| **Salmonidae** | | | | | | |
| *Salmo salar* | Atlantic Ocean (Scotland) | Net pens in sea water | smolts | *I. necator* | | Ellis and Wootten (1978) |
| | | Cultured in sea water | Post-smolts | *Ichthyobodo* sp. | Subclinical | Bruno (1992) |
| | Atlantic Ocean Norway | Cultured in sea water | Smolts and post-smolts | *Ichthyobodo* | | Todal *et al.* (2004) |
| GADIFORMES | | | | | | |
| **Gadidae** | | | | | | |
| *Gadus morhua* | Atlantic Ocean Norway | Net pens | Juveniles | *Ichthyobodo* | | Todal *et al.* (2004) |

Both the SSU rRNA phylogenetic analysis and the ITS rRNA sequence data (Callahan *et al.*, 2005) support grouping the isolates examined into a complex of nine different species. Most isolates studied by Callahan *et al.* (2005) could be assigned to four of the eight *Ichthyobodo* 'species' groups defined by Todal *et al.* (2004), except for the isolate from rainbow trout from North Carolina (U.S.A.) designated species IX. In spite of the initial results of Todal *et al.* (2004)—suggesting the existence of distinct marine, freshwater and anadromous species of *Ichthyobodo*—some of nine species recognised by Callahan *et al.* (2005) can parasitize different host species and are not necessarily restricted to specific habitats. Thus, the same species was obtained from Japanese flounder, a strictly marine fish, and the freshwater koi, goldfish and channel catfish. The possibility of moving between marine and freshwater hosts has serious implications for aquaculture industries, as the spread of species and strains may be more virulent upon exposure to new hosts.

**METAKINETOPLASTINA** Vickerman in Moreira *et al.*, 2004

**PARABODONIDA** Vickerman in Moreira *et al.*, 2004

Fish parasites described in the genera *Cryptobia* and *Trypanoplasma* belong to the order Parabodonida in the subclass Metakinetoplastina according to Moreira *et al.* (2004)

***Cryptobia*** Leidy, 1846 and

***Trypanoplasma*** Laveran and Mesnil, 1901

## Taxonomic Remarks

The genera *Cryptobia* and *Trypanoplasma* have a controversial taxonomic position. *Tryanoplasma*, created by Laveran and Mesnil (1901) to designate a haemoflagellate parasite of the freshwater fish *Scardinius erythrophthalmus*, from France, was considered a synonym of *Cryptobia* by Crawley (1909). This synonymy was not generally accepted and some authors retained *Trypanoplasma* for haematozoic species (see Lom and Dyková, 1992). Woo (2003) is inclined to assign all the species to *Cryptobia* until non-haematozoic species have been studied. However, Woo (1994) had previously proposed two subgenera within *Cryptobia*, *Trypanoplasma* for the normally haematozoic species with an indirect life cycle (two hosts) and *Cryptobia* for the species non-haematozoic with direct life cycle (one host). According to Wright *et al.* (1999), molecular evidence also calls into question the validity of the genus *Trypanoplasma*. The recent molecular studies of Moreira *et al.* (2004) also support this

point of view. Therefore, until a definitive elucidation of the taxonomic position of both genera and their possible synonymy is available, we will continue to retain the original name for the considered species.

Most flagellates described in *Cryptobia* or *Trypanoplasma* parasitize mainly freshwater fish, although some species are found in marine fish. Some haematozoic species are the most pathogenic.

## HAEMATOZOIC SPECIES FROM FRESHWATER FISH

***Cryptobia salmositica*** (Katz 1951) (syn. *Trypanoplasma lynchi* Katz, 1951) Initially described from Coho salmon (*Oncorhynchus kisutch*), this species has been deeply studied by Woo and co-workers. Readers are referred to the reviews of Woo and Poynton (1999) and Woo (2001, 2003) for an extensive compilation of the different aspects of cryptobiosis. The most relevant information on *C. salmositica* and cryptobiosis is presented below.

### Morphology and Life Cycle

Elongated flagellate measuring 6-25 × 1.3-4.0 μm. Two flagella originate from the flagellar pocket at the anterior end. The anterior flagellum measures 6.5-27 μm and the recurrent flagellum is attached to the body and ends as a posterior free flagellum (4.0-17.0 μm). The kinetoplast is large and anterior to the nucleus, which is located in the anterior part of the organism (Plate 9.2, E) (Katz 1951; Lom and Dyková, 1992; Woo and Poynton, 1999). Ultrastructural details can be found in Paterson and Woo (1983), who referred to the presence of a contractile vacuole in the haematozoic forms.

Direct and indirect transmission can occur in *C. salmositica*. In indirect transmission, leeches (*Piscicola salmositica*) become infected when fed on parasitized fish and can, in turn, infect new fish. The flagellate multiplies in the leech crop, and its morphology is similar to the slender ectoparasitic form of *C. salmositica* from the fish mucus. Direct transmission from fish to fish can also occur, and mucus parasites seem to be mainly involved. Transmission is favoured in stress and crowding situations, and may be very important in hatcheries (reviewed in Woo and Poynton, 1999).

The flagellate multiplies by longitudinally binary fission and newly divided forms are slender. The first indication of division is the production of two new flagella, followed by duplication of the kinetoplast and nucleus. The parasites undergo a series of regular morphological changes during

their life cycle. Bloodstream forms are crescent-shaped and slender, although broader forms may be found in ascites. Metacyclic forms from leeches are much more slender than blood forms (Lom and Dyková, 1992).

## Disease Symptoms, Aetiology/Pathogenesis

The external clinical signs of cryptobiosis in *Oncorhynchus* include exophthalmia, general oedema, abdominal distension and anorexia. Some fish can appear emaciated. At necropsy, ascites, splenomegalia and hepatomegalia can also be seen. The flagellate produces high mortality in cultured fish, which can reach 100% in absence of treatment. Other fish seem to be susceptible, but they do not suffer from this disease. This is the case of sculpins and *Salvelinus fontinalis*, which are considered reservoir hosts of the parasite (Woo and Poynton, 1999).

Parasites are present in the blood, where they multiply, so the parasitemia can fluctuate, mainly in the acute phase of infection. Mycrocytic and hypocromic anaemia is one of the main consequences of cryptobiosis, and lesions are present in the haematopoietic tissues in the acute phase of the disease. The severity of both, the lesions and the anaemia, is related to parasitemia, and also to the extravascular location of parasites. Haemodilution, splenomegalia and haemolysis contribute to the anaemia. Haemolysis is caused by secretions of living parasites or by antigens liberated when parasites are disrupted due to complement fixing antibodies in infected fish. The lytic antigen (haemolysin) lyses red cells directly whereas the immune complex-forming antigen forms immune-complexes on red cells with specific antibodies, which result in vascular/extravascular haemolysis (Thomas and Woo, 1988). Congestion of blood vessels and occlusion of capillaries with parasites can occur (Putz, 1972), which combined with anaemia can lead to reduction of oxygen delivery to tissues and vital organs (Woo and Wehnert, 1986).

Fish show immunodepression and low haemolytic activity of complement. Histopathology (reviewed in Woo and Poynton, 1999) consists of focal haemorrhages, a generalized inflammatory reaction and lesions in connective tissues and reticulo-endothelial systems of juvenile rainbow trout. Endovasculitis and mononuclear infiltration also occur and are followed by tissue necrosis and extravascular location of parasites. Necrosis, haematopoietic depletion and anaemia are probably involved in fish mortality (Bahmanrokh and Woo, 1994).

## Diagnosis

According to Woo and Poynton (1999), clinical signs can be used for preliminary diagnosis. For specific diagnosis, parasites can be detected using the wet mount technique, using fresh smears of blood and ascitic fluid, collected from living fish or within minutes of dying. The observation under bright field or phase contrast microscopy can aid to detect parasites, mainly in slight or mild infections. The identification can be confirmed in Giemsa stained smears (Plate 9.2, E). The haematocrit centrifuge technique (HCT) (Woo, 1969) can be useful in detecting low chronic infections.

Different methods can also be used for indirect diagnosis, based on the detection of specific antibodies to C. *salmositica*. The microscopic immuno-substrate-enzyme technique (MISET), and the immunofluorescent antibody techniques (IFAT) are available (Woo, 1990), as well as an ELISA (Sitjà-Bobadilla and Woo, 1994). A specific DNA probe (1.2 kbp) was developed by Li and Woo (1996) to distinguish C. *salmositica* from other haematozoic *Cryptobia* spp. using blood dried on filter paper (dried blood DNA dot blot, DBDB technique). DBDB is a reliable and rapid method for the identification of C. *salmositica*, though it is less sensitive than HCT and its use as a diagnostic technique may require further research.

## Treatment and Prophylaxis

The trypanocidal drug isometamidium chloride (Samorin) is effective against C. *salmositica* when inoculated intramuscularly into infected rainbow trout and chinook salmon in which it can be used as both therapeutic and prophylactic (Woo, 2001). The effectiveness of the drug is significantly enhanced when conjugated to a monoclonal antibody or to polyclonal antibodies from immune fish. This strategy was designed to control outbreaks of the disease in broodstocks.

The information obtained on *in vitro* culture and immune response studies has allowed developing immunoprophylactic strategies. As reviewed in Woo (2001, 2003) two vaccines have been developed. The first strategy is based on the use of C. *salmositica* attenuated under *in vitro* conditions (see below). The vaccine strain has remained avirulent and protective for about 12 years. This avirulent strain lacks the 200-kDa metalloprotease involved in the haemolysis produced by the virulent strain. The vaccine strain can be used in streams and rivers, where it can

be delivered by the leech vector. The second approach is to use a recombinant cystein protease to vaccinate young fish before their release from hatcheries. Studies on its efficacy, the duration of protection and the best delivery system are needed before its use as a vaccine.

In addition, a monoclonal antibody (mAb-001), produced against the 200-kDa glycoprotein, is therapeutic and prophylactic against the parasite.

## Fish Hosts, Epidemiology and Geographical Distribution

*C. salmositica* was firstly described from the blood of coho salmon, *Oncorhynchus kisutch* in Washington State, U.S.A. (Katz, 1951). It is primarily a parasite of salmonids, as all North American *Oncorhynchus* spp., besides *Salmo trutta* and *Prosopium williamsoni* are affected. It has also been reported in several species of sculpins, cyprinids (e.g., *Rhinichthys cataractae*), sucker (*Catostomus commersoni*) and stickleback (*Gasterosteus aculeatus*) (see Lom and Dyková, 1992). The torrent sculpin, *Cottus rhotheus* is considered an important reservoir host of *C. salmositica* although careful cross-transmission studies are needed for confirmation. Moreover, findings in other teleosts, as cyprinids or suckers are questionable as these fish have their own *Cryptobia* which are not infective to salmonids. In addition, these fish cannot be experimentally infected by *Cryptobia* (reviewed in Woo, 2003).

This flagellate has a discontinous distribution in freshwater streams along the Pacific Coast of North America from northern California to southern British Columbia (Lom and Dyková, 1992). The prevalence in young salmon is variable between years and geographical areas. Experimental studies showed that infected pre-smolt salmon retained their infections when transferred to salt water, and mortality was not reduced (Bower and Margolis, 1985). In the Fraser River drainage (British Columbia, Canada) prevalence in returning salmon can reach 100% by December. The parasite is considered an important pathogen of salmon in semi-natural and intensive culture facilities on the west coast of North America (reviewed in Woo, 1993). Differences in susceptibility between fish species are commented below (see Immunological studies).

## *In vitro* Culture

*Cryptobia salmositica* was initially cultured in Hank's solution with 10% of heat-inactivated foetal calf serum. The parasite multiplies by longitudinal

binary fission as blood forms, and the newly divided forms are slender in shape. Cultures are always infective when inoculated into fish. However, minimum essential medium (MEM) supplemented with serum vitamins and nutrients is the medium of choice, as the parasite multiplies rapidly, and can be repeatedly subcultured (reviewed in Woo and Poynton, 1999). A strain that has been cultured for several years produces low parasitaemias and does not cause disease in fish but actually protects them (Woo and Li, 1990). This avirulent strain has remained infective, non-pathogenic and protective for well over a decade (Woo, 2003) and, thus, it can be used as a live attenuated vaccine (see below, Treatment and prophylaxis).

## Immunological Studies

Several aspects of the immune response to *C. salmositica* have been widely studied. According to the information compiled by Woo (2003), both innate and adaptative factors are involved in the immune responses against this flagellate. The respiratory burst activity and the proportion of peripheral granulocytes increased in fish vaccinated with the live *C. salmositica* vaccine (Chin and Woo, 2005). The natural antiprotease $\alpha_2$ macroglobulin can neutralise the metallopretase involved in *C. salmositica* virulence, as demonstrated in charr resistant to the disease (Zuo and Woo, 1977a, b). Cell-mediated responses were also demonstrated by the presence of macrophages that have engulfed parasites, and using delayed-type hypersensitivity and the macrophage inhibition migration test. Specific antibodies (aglutinating, neutralizing and complement fixing) were detected in sera of the fish that recover from the disease and in experimentally infected fish, and are involved in protective immunity. Specific antibodies fix complement to lyse the parasite by the classical pathway. Titres of complement fixing antibodies in recovered and vaccinated fish rise significantly after *C. salmositica* challenge. This classical anamnesis response also confirms that protective immunity is in part due to a humoral response in infected fish (reviewed in Woo, 2003). The effectiveness of the immune response has allowed developing immunoprophylactic strategies that are commented below.

Surface antigens have also been studied in *Cryptobia salmositica* (reviewed in Woo, 2003). The virulent strain was found to have a more negative surface charge than the avirulent strain obtained by prolonged *in vitro* culture (see above). There are also differences in expression of

surface carbohydrate residues and glycoproteins on cell membranes of the pathogenic and attenuated strain; specifically, the increased surface carbohydrate residues in the latter coincide with its loss of virulence. A monoclonal antibody (mAb-001) was produced against the 200-kDa glycoprotein, whose epitope (Cs-gp200) has a phospahtidylinostitol residue which anchors the conformational polypeptide to the surface membrane. Cs-gp200 has high mannose components and it appears as a doublet in the pathogenic strain and as a single band in the vaccine strain. The monoclonal antibody mAb-001 aglutinates the parasite but does not fix the complement in order to lyse it.

Susceptibility to cryptobiosis varies between individuals of the same species as well as between different species. Woo (2003) distinguished between resistance to infection (pathogen-resistant animal) and resistance to disease (pathogen-tolerant animal), and both are related to innate immunity. Many teleosts (e.g., cyprinids) cannot be infected by *C. salmositica*, and some laboratory raised brook charr, *Salvelinus fontinalis* are also refractive to infection (*Cryptobia*-resistant fish), while others can be infected without developing clinical disease (*Cryptobia*-tolerant fish). In the *Cryptobia*-tolerant charrs, the immune system readly controls the infection, thus allowing a rapid recovering. Genetic variations in resistance to this haemoflagellate were also identified in Coho salmon *Oncorhynchus kisutch* and Sockeye salmon *O. nerka*. Differences in the resistance mechanisms, the selection pressure applied to each species, the genetic control of resistance, or some combination of these factors may be involved (Bower *et al.*, 1995). Chin *et al.* (2004) found differences in susceptibility and antibody production against both pathogenic and vaccine strains of *C. salmositica* between different full-sib families of *Salmo salar*, having increased susceptibility associated with a delayed antibody response.

***Trypanoplasma borreli*** Laveran and Mesnil 1901

(Syn: *Trypanoplasma cyprini* Plehn, 1903, *T. keysselitzi* Minchin, 1909; *T. tincae* Schäperclaus, 1954; *Cryptobia kahibulaewi* Chernova, 1984, *T. carassii* (Kashkovski, 1974)

## Morphology and Life Cycle

It is an elongated parasite with considerable variations in shape and size. Measurements of fixed and stained specimens are 24.4 ± 3.0 × 4.9 ± 1.2 μm, anterior flagellum 14.6 ± 3.6 μm, posterior-free flagellum

7.9 ± 2.7 μm (Kruse *et al.*, 1989a). The forms in acute infection are slender (Fig. 9.17), spindle-shaped, whereas the 'adult forms' of the chronic infection are larger, with wide, lobed loops of the undulating membrane (Lom and Dyková, 1992). Ultrastructural details can be found in Brugerolle *et al.* (1979).

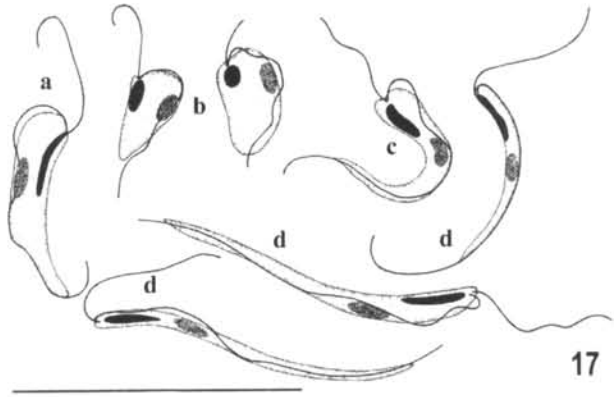



**Fig. 9.17** *Trypanoplasma borreli*. Schematic representation of different life cycle stages. **a**: blood stream stage in the peak parasitaemia in goldfish; **b**: transformation in stubby forms in the leech; **c**: slender form of the proliferative leech phase; **d**: transformation into the elongated metacyclic stages. Scale bar = 20 μm. From Lom and Dyková (1992): Protozoan parasites of fish, Developments in Aquaculture and Fisheries Science, vol. 26 (Elsevier). Courtesy of Drs. Lom and Dyková.

*T. borreli* has an indirect life cycle. The leech *Haemiclepsis marignata* was the firstly described vector, but experimental infections have been achieved using *Piscicola geometra* as intermediate host and common carp as fish host (Kruse *et al.*, 1989b). In the leech crop, trypanoplasms exhibit a regular sequence of forms differing in size and shape (Fig. 9.17), starting with the stubby, rounded shaped stages and ending in the slender forms, representing the metacyclic infective stage, wich move to the proboscis of the leech (Lom and Dyková, 1992).

## Disease Symptoms, Aetiology/Pathogenesis

The flagellate is quite common in the blood and tissues of several freshwater cyprinids in Europe and can produce losses in cultured carp, tench and crucian carp (Lom, 1979). Experimental infections can induce mortality in carp and goldfish. The pre-patent period in carp fingerlings lasts 8 days at 20°C, and the peak is reached 19 to 39 days p.i. Mortality

may appear as soon as 2 days p.i., but the chronic phase in survivors may last until 140 days p.i. (Lom and Dyková, 1992).

The pathology and histopathology have been studied in laboratory infections (Lom and Dylová, 1992; Bunnajirakul *et al.*, 2000). Heavily infected carps showed external signs consisting of abdominal distension, exophthalmia, gill pallor, petechiae and swimming disorders, described as 'sleeping sickness of carp'. In addition, congestion of liver sinusoids, focal necrosis of hepatic tissue, accumulation of erythrocytes in the spleen and anaemia were observed, as well as tubulonephrosis, glomerulitis and marked inerstitial hyperplasia in the kidney. The destruction of excretory renal structures leads to a severe disturbance of osmoregulation, which, together with a decrease in oxygen uptake due to anaemia, is likely to be a major cause of death.

## Diagnosis

The procedures for the detection of *T. borreli* are similar to those described for *C. salmositica*.

## Fish Hosts, Epidemiology and Geographical Distribution

*T. borreli* has been described from different cyprinids. Following the first finding in *Scardinius erythrophthalmus* in France (Laveran and Mesnil, 1901), the parasite has been found in many other cyprinids in Eurasia, and may cause losses in cultures. It also occurs—according to some reports—in North America (Lom and Dyková, 1992). After cross-transmission studies, the host range includes at least *Cyprinus carpio*, *Carassius auratus*, *Tinca tinca*, *Carassius carassius*, *Leuciscus idus*, *Phoxinus phoxinus*, *Rutilus rutilus* and *Gobio gobio*. Prevalence may reach 100% in some carp stocks (Lom, 1979; Lom and Dyková, 1992).

## *In vitro* Culture

*T. borreli* has been successfully cultured in different media. However, in cultures in diphasic blood agar medium (SNB-9), supplemented with vitamins and antibiotics, the parasite loses its infectivity after 10 to 14 d of primary culture (Pecková and Lom, 1990) or after 6 subcultures (Nohýnková, 1984). Steinhagen *et al.* (2000) developed an *in vitro* culture system using a mixture of Hank's balanced salt solution (HBSS, 45%), L15 (22.5%), Earle's minimum essential medium (MEM, 22.5%) and 10% of

distilled water, supplemented with 5 to 10% of heat-inactivated pooled carp serum, at temperatures of 15, 20 and 25°C. Flagellates cultured in such conditions retained their infectivity for carp for at least 90 d (5 passages). In contrast, RPMI 1640 medium appears no to be suitable for cultivating the fish-infecting *T. borreli*.

## Immunological Studies

Some information has been obtained on the immune response of carp against *T. borreli* using *in vitro* and *in vivo* studies. The involvement of innate defence mechanisms—evidenced by the participation of phagocytosis by monocite/macrophages in the elimination of the pathogen (Lom and Dyková, 1992)—has been confirmed in further studies. Leucocytosis and increase in the number of granulocytes and in the lymphocyte size were reported in experimentally infected common carps (Steinhagen *et al.*, 1990; Scharsack *et al.*, 2000). Hamers and Goerlich (1996) demonstrated a stimulation of haematopoiesis using the same experimental model. At the maximum and during the chronic phase of parasitaemia, infected carp showed an increased percentage of S-phase cells in anterior kidney, trunk kidney and thymus compared to non-infected fish. Such leukocyte proliferation was also found in *in vitro* assays (Hamers, 1994). In contrast, an *in vitro* suppression of leukocyte proliferation has been reported (Jones *et al.*, 1995; Scharsack *et al.*, 2000). In addition, head-kidney neutrophils of carp are functionally modulated *in vitro* by *T. borreli*, as demonstrated by the reduction in phagocytosis activity and NO production (Scharsack *et al.*, 2003a, b). These results suggest that the parasite can produce an immunodepression as in the case of *Cryptobia salmositica*. Saeij *et al.* (2002) reported a NO-inducing activity of *T. borreli* (occurring also *in vivo*) that may be an adaptation developed to ensure survival and immune evasion in the fish host, as production of NO was detrimental to the host. Recent findings have demonstrated the expression of different immune factors and cytokines in the carp response to *T. borreli*. Saeij *et al.* (2003a), found enhanced expression of TNFα (higher for TNF2 than for TNF1) in carp head-kidney phagocytes when *T. borreli* was added *in vitro*. Up-regulated expression of TNFα, Il-1β and mRNAs for acute response proteins (complement factor 3, serum amyloid A and alpha-2 macroglobulin) was also observed *in vivo* (Saeij *et al.*, 2003a).

Adaptative immune response was demonstrated by the presence of parasite-specific antibodies in carps inoculated with live *T. borreli*, and

antibody production seems to correlate with clearance of infection (Jones *et al.*, 1993). According to Saeij *et al.* (2003a), specific antibodies against *T. borreli* are able to lyse the parasite in presence of complement, by the classical pathway (as described for *Cryptobia salmositica*).

Differences in susceptibility of fish hosts to infection have been reported. Wiegertjes *et al.* (1995) found a higher production of specific antibodies in outbred carps resistant to infection than in isogenic homozygous carp lines highly susceptible to this flagellate. In relation to the involved mechanisms, two carp lines differing in their resistance to *T. borreli* were typed for the TNF2 polymorphism and association between one isoform and resistance was found (Saeij *et al.*, 2003b). Scharsack *et al.* (2004) found differences in the spectrum of immunomodulatory mediators produced by lymphocyte cells between susceptible and resistant carps, and suggested the involvement of a strong polyclonal activation in susceptible lines.

## HAEMATOZOIC SPECIES FROM MARINE FISH

*Trypanoplasma bullocki* Strout, 1965

This haematozoic parasite can cause disease and mortality in flatfish in North America.

### Morphology and Life Cycle

In air-dried smears, body dimensions are 12.5-23.1 × 1.2-4.5 μm. Anterior and posterior flagellum measurements are 8.3-19.1 μm and 4.4-15.7 μm, respectively. A detailed description can be found in Strout (1965).

*T. bullocki* is transmitted by the bloodsucking leech *Calliobdella vivida*, occurring in estuaries and near shore from Newfoundland to northern Gulf of Mexico. The flagellates concentrate in the leech drop where they divide in slender forms (Burreson, 1982a).

### Disease Symptoms, Aetiology/Pathogenesis

Clinical signs in summer flounder include exophthalmia, abdominal distension with ascites and sluggishness (Burreson, 1982b; Burreson and Zwerner, 1984). Fish also show anaemia, splenomegalia and haemorrhaging in the ventral musculature. These symptoms appear in

experimentally infected fish in 5 weeks and mortality occurs at 11 weeks after infection.

In acute infections, the parasite occurs extravascularly mainly in haematopoietic organs and interstitial adipose tissue. Necrosis can be present in the liver and the spleen, as well as in the intestine together with oedema (Burreson, 1982b). Focal glomerular lesions have been described in the kidney (Newman, 1978).

### Diagnosis

The procedures for the detection of *T. bullocki* are similar to those described for *C. salmositica*.

### Fish Hosts, Epidemiology and Geographical Distribution

This flagellate has been found in winter flounder (*Pseudopleuronectes americanus*), summer flounder (*Paralichthys dentatus*), along the Atlantic coast of North America, and in southern flounder (*Paralichthys dentostigma*), hogchoker (*Trinectes maculatus*) and croacker (*Micropogonias undulatus*) from the southern Atlantic coast to the northern Gulf of Mexico. Smooth flounder (*Liopsetta putnami*) and summer flounder are commonly infected by the parasite in the Chesapeake Bay. Estuarine and inshore fish are included (reviewed in Woo and Poynton, 1999).

According to Burreson (1999), the prevalence of *T. bullocki* is low in migratory fish of Chesapeake Bay, due to their short exposition periods to the vector leech, *Caliobdella vivida*, which has a marked seasonality. However, high prevalence and mortality have been reported in juvenile summer flounders in autumn, whereas the number of infected fish decreased in winter (Burreson, 1982b).

## OTHER HAEMATOZOIC CRYPTOBIA AND TRYPANOPLASMA

*Trypanoplasma beckeri* was described by Burreson (1979) from wild *Scorpaenichthys marmoratus* in Oregon coastal waters, and *Cryptobia neghmei* was found in *Paralichthys* spp. from the Chilean coast in the Southern Pacific Ocean (Khan *et al.*, 2001). A high prevalence of an unidentified haematozoic *Cryptobia* sp. from Chilean *Scarthychthyis viridis* (Blenniidae) was reported by Díaz and George-Nascimento (2002).

## *Cryptobia* spp. from the Digestive Tract

*Cryptobia iubilans* Nohynková, 1984

Several *Cryptobia* spp. are intestinal parasites of fish, but the only pathogenic species is *C. iubilans*, common in cichlid fish (Lom and Dyková, 1992). It can occur not only in the stomach and intestine but also in other organs of infected host. The ovoid or elongated body (19 × 5 μm) has an anterior flagellum extending from the flagellar pocket and a recurrent flagellum which adheres to the flattened ventral side of the body and continues as a long free-trailing flagellum.

### Disease Symptoms, Aetiology/Pathogenesis

The parasite usually causes a granulomatous gastritis, and may be associated with chronic low-level mortality. Poor water quality, high stocking density and nutritional problems may increase the severity of the infection. In fish with active infections, *C. iubilans* is most commonly found in the stomach but may also appear in the intestinal tract and other organs. A systemic form of the disease has been reported in captive East African and Central American cichlids, in association with acute mortalities and loss of 50% of affected animals. The sick fish showed anorexia, and became progressively more listless and moved to the surface with apparent signs of hypoxia. In systemic infections, the parasite can be found in blood and several organs (liver, gall bladder, kidney, ovary, brain and eye). In advanced stages of the disease, fish showed severe anaemia (Floyd and Yanong,1999). The parasite can appear in mixed infection with other digestive flagellates (*Spironucleus*, *Hexamita*), Apicomplexa (*Cryptosporidium*), moderate numbers of the nematode *Capillaria* or bacteria (Yanong *et al.*, 2004).

### Diagnosis

The infection can be diagnosed at light microscope in fresh preparations of scrapings of the digestive mucosa. In mixed infections with hexamitids, flagellates can be distinguished by their morphology. The presence of the typical granulomatous gastritis, typical of *C. iubilans* can help to diagnose this intestinal cryptobiosis.

### Treatment and Prophylaxis

There is no effective treatment for enteric *Cryptobia*. Some Florida farms have used sulfadimethoxine that seems to help control mortalities but did

not eliminate the parasite. Bath treatments with dimetridazol or 2-amino-5-nitrothiazol may help to decrease its prevalence (Yanong *et al.*, 2004). The disease can be introduced in aquaria with infected fish so a strict quarantine protocol must be applied to prevent its introduction (Floyd and Yanong, 1999).

## Fish Hosts, Epidemiology and Geographical Distribution

This gastrointestinal *Cryptobia* has been reported in East African and Central American cichlids, including *Herichthys cyanoguttatus*, *Cichlasoma meeki*, *C. nigrofasciatum* and *C. octofasciatum*. In addition, there are several findings in discus (*Symphysodon discus*) and other cichlids from farms and aquaria of different North American localities. An outbreak of the systemic form of *C. iubilans* occurred in 1995 at the Chicago Shedd Aquarium, resulting in loss of 50% of East African cichlids, including *Cyphotilapia frontosa*, *Dimidiochromis compressiceps* and *Aulonocara stuartgranti*. The infection spread to *Cichlasoma* spp. and was also detected in 98% of apparently healthy fish, including *Cichlasoma* spp., *Haplochromis macula*, *Labeotropheus fuelleborni*, *Pseudotropheus zebra* and *P. elongatus* (Floyd and Yanong, 1999). Between 1998 and 2000, *C. iubilans* was diagnosed in *S. aequifasciatus* and other cichlids from different farms in Florida, New York and Rhode Island (Yanong *et al.*, 2004).

The *Cryptobia* sp. reported in the gut of *Gyrinocheilus aymonieri* (Gyrinocheilidae, Cypriniformes) imported in Australia from Singapore could be a new species (Evans and Lester, 2001).

## ECTOPARASITIC *Cryptobia* SPP.

*Cryptobia branchialis* Nie (Chen, 1956)

According to Kuperman *et al.* (2002), these flagellates are pear-shaped or elongated unicellular organisms, measuring 7.5-11.6 × 2.8-4.6 μm when fixed. The short anterior flagellum is 6.1-10.2 μm in length. The long recurrent flagellum 13.8-28.2 μm, extending posteriorly, attaches the parasite to the gill epithelium. A detailed ultrastructural description can be found in Kuperman *et al.* (2002). Direct fish-to-fish transmission seems to occur (Woo and Poynton, 1999).

It was first described from the gills of several pond fish from China. Further findings have been recorded in a large number of freshwater fish in Europe, Asia, North America and the Philippines, as a parasite of

cultured freshwater fish, sometimes after introduction from other countries (Molnár, 1971; see also Kupermann *et al.*, 2002). *C. branchialis* has also been found in *Oreochromis mossambicus* from the Salton Sea, a salt Californian lake (Kupermann *et al.*, 2002). Eiras *et al.* (1995) reported *Cryptobia* sp., in mixed infections with other ectoparasites, on the gills of farmed Brazilian Characidae (*Piaractus mesopotamicus* and *Colossoma macropomum*) and Prochilodontidae (*Prochilodus lineatus*).

Other ectoparasitic *Cryptobia* spp. also occur in marine fish. In the Atlantic Sea, six species of marine and estuarine fish were found heavily infected by an ectoparasitic *Cryptobia* sp. in Chesapeake Bay (USA) (Burreson and Sypek, 1981). In the Red Sea, **Cryptobia eilatica** Diamant 1990 parasitizes the gills of cultured gilthead seabream, *Sparus aurata* and wild black-spot seabream, *Diplodus noct* in Eilat (Diamant, 1990) (Fig. 9.18). The *Cryptobia* spp. found in the gills of *Sparus aurata* kept in culture in the Mediterranean coasts of Spain (Plate 9.2, F-G) (Alvarez-Pellitero *et al.* 1995) and in France (Blanc *et al.*, 1989) could belong to the same species. An unidentified *Cryptobia* sp. was also recorded from wild and cultured sea bass, *Dicentrarchus labrax*, in the Spanish Mediterranean coast (Alvarez-Pellitero *et al.*, 1993). In a recent survey on fish diseases in the Mediterranean area (Alvarez-Pellitero, 2004), *Cryptobia* sp. was reported from the gills of cultured *D. labrax*, *S. aurata* and *Diplodus puntazzo* from Greece, frequently in mixed infections with monogeneans. Ectoparasitic *Cryptobia* are capable of tolerating a relatively wide range of salinity. *C. eilatica*, thriving at the Red Sea at a salinity of 39-41 ppt, is able to survive at 10 ppt for several days, but not in fresh water (Diamant, 1990). Similarly, *Cryptobia* sp. found in gilt-head seabream in France was eradicated by lowering the water salinity to 0% for 50 min (Blanc *et al.*, 1989). *C. branchialis*, firstly considered a freshwater species, seems to be more halotolerant than *C. eilatica*, as it can survive at a salinity as high as 43 ppt (Kupermann *et al.*, 2002).

## Disease Symptoms, Aetiology/Pathogenesis of Ectoparasitic *Cryptobia* spp.

The actual pathogenicity of ectoparasitic *Cryptobia* has been object of controversia. Different damages to the fish gills had been described, including overproduction of mucus, destruction of gill epithelium, and formation of thrombi, followed by death (Chen, 1956). Moreover, *C. branchialis* had been involed in mortalities in cultured carp, goldfish

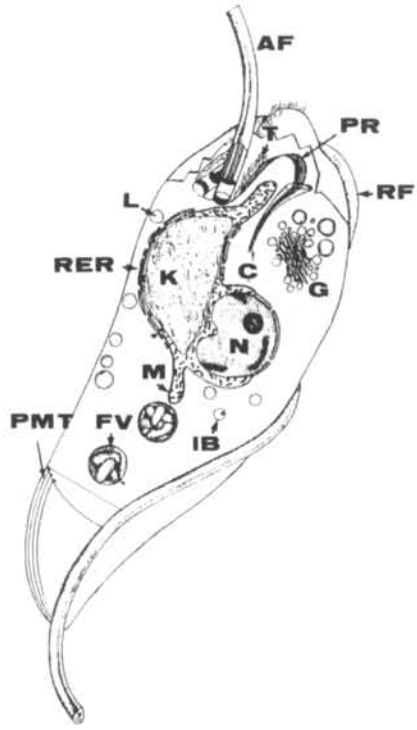



**Fig. 9.18** *Cryptobia eilatica*. Line diagram illustrating the main structural features, as reconstructed from electron micrographs. AF: anterior flagellum; C: cytopharynx; FV: food vacuoles; G: Golgi body; IB: inclusion body; K: kinetoplast; L: lipid vacuole; M: mitochondria; N: nucleus; PMT: pellicular microtubules; PR: paraxial rod; RER: rough enplasmic reticulum; RF: recurrent flagellum. From Diamant (1990) with permission of Journal of Protozoology (now Journal of Eukaryotic Microbiology) (Blackwell Publishing). Courtesy of Dr. Diamant.

and catfish (see Woo and Poynton, 1999), and *Cryptobia* sp. was also implicated in mortalities of cultured gilt-head seabream in France (Blanc *et al.*, 1989). In contrast, subclinical infections were produced by *Cryptobia* spp. in Mediterranean sea bass and gilt-head seabream in Spain (Alvarez-Pellitero *et al.*, 1993, 1995). Moreover, in ultrastructural studies, no alterations were found in the sites of attachment of *C. branchialis* (Lom, 1980) or *C. eilatica* (Diamant 1990). Thus, the reported mortalities in different aquacultured fish associated with ectoparasitic *Cryptobia* spp. infections were attributed to other undetected pathogens. In contrast, Kuperman *et al.* (2002) described important damages in the gills of tilapia fry infected with *C. branchialis*, mainly consisting of swollen filaments and

partial reduction of lamellae, with a thick film of mucus covering both structures. Epithelial cells were hypertrophied and blood vessels appeared expanded and stuffed with erythrocytes. All these changes may depress the oxygen consumption.

## Diagnosis

Ectoparasitic *Cryptobia* can be detected at light microscope in fresh or Giemsa stained smears or in histological sections of fish gills.

## Economic Importance of Cryptobiosis

Haematozoic species cause the most significant losses in affected fish stocks. *Cryptobia salmositica* is the species with the highest repercussion on fish production. Outbreaks of cryptobiosis can occur in hatcheries, and pre-spawning and post-spawning salmonids, with subsequent economic impact on cultures. Apart from mortality, cryptobiosis may affect fish nutrition and reproduction (Woo, 2003). *Trypanoplasma borreli* may also cause losses in cyprinid cultures. Amongst non-haematozoic species, *Cryptobia iubilans* can be involved in losses in aquarium fish, mainly in mixed infections.

## Molecular Studies

Numerous studies on the molecular taxonomy and phylogeny of kinetoplastids have been published in the last two decades. Most analyses are based on the small subunit rRNA gene sequences, though some other genes have been studied. The new order Parabodonida, created by Moreira *et al.* (2004), corresponds to one of the three major bodonid clades retrieved in their sequence analysis. Phylogenetic trees based on 18SS rRNA and Hsp90 genes support robust monophyletic groups for clades 2 and 3, corresponding to the new orders Parabodonida and Eubodonida, respectively.

The synonymy of *Trypanoplasma* with *Cryptobia*, initially proposed by Crawley (1909), has been confirmed by different analysis of rRNA gene sequences (Wright *et al.*, 1999; Dolezel *et al.*, 2000; Maslov *et al.*, 2001; Callahan *et al.*, 2002). The sequence analysis of Moreira *et al.* (2004) demonstrated that the fish *Cryptobia* and *Trypanoplasma* studied (*C. salmositica*, *C. catostomi*, *C. bullocki* and *Trypanoplasma borreli*) form a monophyletic group, corroborating the previous idea that most *Cryptobia*

spp. and *Trypanoplasma* should be united into a single genus. In contrast, *Cryptobia helicis* does not form a monophyletic group with fish *Cryptobia*. and, thus, more sequences of non-molluscan invertebrate hosts are needed to clarify its phylogenetic position. The inclusion of non hematozoic species of fish *Cryptobia* and *Trypanoplasma* should also be advisable as an aid to clarify the suggested synonymy of both genera.

The uridine (U) insertion-deletion RNA editing that occurs in the mitochondrion of trypanosomatids was found to occur in the piscine *Trypanoplasma borreli*. However, the small guide RNA-like transcripts are encoded in tandemly repeated 1-kb sequences instead of minicircles as in trypanosomatids. The presence of pan-editing in this lineage is consistent with an ancient evolutionary origin of this process (Maslov and Simpson, 1994).

**TRYPANOSOMATIDA** Kent, 1880, emend. Moreira *et al.* 2004

This order corresponds exactly to the suborder Trypanosomatina and family Trypanosomatidae of previous classifications (Moreira *et al.*, 2004). These exclusively parasitic kinetoplastids are characterized by the presence of the unique glycosomes and a singular smooth flagellum, which is the homologue of the flimmer-bearing anterior flagellum of the bodonids. It either swings freely or it is connected with the cell surface at several adhesion spots, thus forming an undulating membrane (Hausmann *et al.*, 2003). Kinetoplast relatively small and usually compact, with kDNA in the form of a network of catenated circles invariably associated with the flagellum base and usually in a distinct capsular region of the mitochondrion; kDNA composed of 25-50 homogeneous maxicircles and 5,000 to 27,000 usually heterogeneous minicircles. The trypanosomatids contain genera of outstanding medical, veterinary or agricultural importance (*Trypanosoma*, *Leishmania*, *Phytomonas*). The main trypanosomatids occurring in fish belong to the genus *Trypanosoma* (Vickerman, 2000).

***Trypanosoma*** Gruby, 1843

Haemoflagellates with digenetic life cycle, found as blood parasites in all vertebrate classes including elasmobranchs and bony fish.

Numerous trypanosome species have been described from freshwater and marine fish. However, neither generic nor specific distinctions are easy to draw. A plethora of haematozoan species are listed in the literature, many of which were described on the basis of minor morphologic and morphometric differences (Becker, 1970). Moreover, as fish host

specificity was accepted, some new species were created after their hosts. Therefore, several species could be synonymous and the taxonomy needs revision (Lom and Dyková, 1992).

## Morphology and Life Cycle

The life cycle includes multiplicative and developmental stages in the blood and tissues of vertebrates and in the gut of an invertebrate vector (a leech in fish trypanosomes). Within each species, the different stages occurring in both hosts differ in size and shape and receive different names. The trypomastigote (the usual blood stage) and epimastigote stages are common to nearly all life-cycles, amastigotes more unusual and promastigotes rare (Vickerman, 2000).

In the blood trypomastigote, the definitive stage, the flagellum is situated directly on or at some distance from the posterior end. A functional cytostome exists in the vector forms and is also present in the bloodstream trypomastigotes of some species. The mass of the DNA fibres (the nucleoid) of the kinetoplast is localized at one end of the mitochondrion and faces the kinetosoma of the flagellum (Lom and Dyková, 1992).

The parasites are transmitted to fish through the bite of infected leeches. After a pre-patent period, the flagellates appear in the blood as slender forms which divide repeatedly, increasing parasitaemia. Some species show pleomorphism in the fish host and successive forms differing in shape, size, flagellar length and other characteristics are found. Division takes place by binary fission, starting with the production of a new flagellum, followed by a kinetoplast and the nuclear division by closed pleuromitosis. Thereafter, the cell divides longitudinally, starting anteriorly (Lom and Dyková, 1992).

Leeches become infected when they feed on the blood of infected fish. After ingestion, the flagellates undergo a series of changes leading to the developmental forms amastigote, sphaeromastigote, epimastigote and the metacyclic filiform trypomastigotes in the leech digestive tract. The flagellates migrate to the leeche's proboscis sheath and are infective to the fish host in which they develop into the typical slender trypomastigotes. The developmental stages in the cycle are illustrated in Fig. 9.19, using the example of *Trypanosoma murmanense* (Khan, 1976).

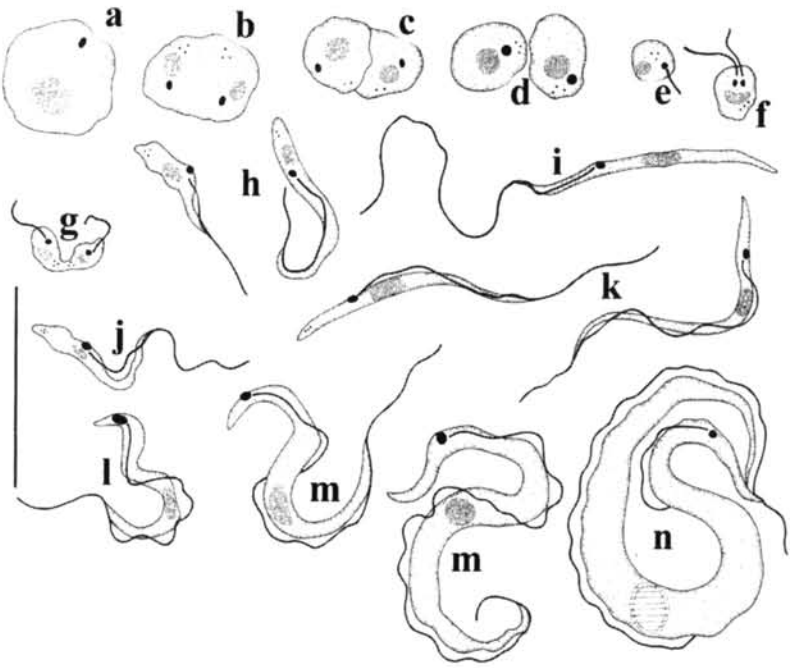



**Fig. 9.19** *Trypanosoma murmanense*. **a-k**: Developmental stages from the leech. Amastigote (**a**) and its division (**b**, **c**, **d**); sphaeromastigote and its division (**e**, **f**, **g**); epimastigotes (**h**, **i**); transitional stage (**j**); metacyclic trypanomastigotes (**k**). **l-n**: Blood stream stages from Atlantic cod after leech feeding. After 10 days (**l**), 18 days (**m**) and 45 days (**n**). Scale bar = 20 μm. After Khan (1976), as reproduced in Lom and Dyková (1992): Protozoan Parasites of Fish, Developments in Aquaculture and Fisheries Science, Vol. 26 (Elsevier). Courtesy of Dr. Khan and Drs. Lom and Dyková.

## TRYPANOSOMOSIS OF FRESHWATER FISH

There are numerous *Trypanosoma* spp. described from freshwater fish, but scarce information on its pathological importance is available in most cases. The best known species is *Trypanosoma carassii*, firstly detected in *Carassius carassius*. According to Lom and Dyková (1992), *T. danilewski* is a synonym of *T. carassii*. However, some authors refer to this parasite as *T. danilewski* (see Woo and Poynton, 1999).

***Trypanosma carassii*** Mitrophanow, 1883

(Syn.; *T. danilewski* Laveran and Mesnil, 1904, *T. wincheniense* Qadri, 1962, *T. tincae* Laveran and Mesnil, 1904)

Initially described from *Carassius carassius*, it has been commonly found in several fish species, mainly cyprinids. According to Lom and

Dyková (1992), the average size is about 21 × 2.3 μm in peak parasitaemia, although young slender forms may be about 15.6 μm, and stout rarely occurring forms of the chronic phase of infection are 26-32 (sometimes even 40) μm × 3-5.3 μm. The leech vectors are *Piscicola geometra* and *Hemiclepsis marginata*. In fish blood, the parasites have a trypomastigote morphology and they transform into proliferating epimastigotes upon transfer to the vector. Details on the life cycle and division can be found in Qadri (1962), Lom (1979), Woo (1981a) and Paterson and Woo (1984).

## Disease Symptoms, Aetiology/Pathogenesis

Most information on the pathogeny of *T. carassi* has been obtained from experimental infections. The parasite multiplies rapidly in goldfish and can produce anaemia and anorexia (Dyková and Lom, 1979; Islam and Woo, 1991). The severity of anaemia is directly related to parasitaemia and is partly produced by haemolysin (Islam and Woo, 1991). The most pronounced histopathological changes were located in the haematopoietic organs, spleen, liver and kidney, though no inflammatory reaction was observed (Dyková and Lom, 1979).

## Diagnosis

During the acute phase of the disease, parasites can be easily detected by examination of a drop of freshly colleted blood under the light microscope (Woo and Poynton, 1999). The morphometric details are better observed in blood smears stained with Giemsa. The haematocrit centrifuge technique (HCT, Woo, 1969) can allow detecting infections that are not found using the wet mount technique.

## Fish Hosts, Epidemiology and Geographic Distribution

As summarized by Woo and Poynton (1999), this flagellate is not host specific, and has been reported in *Cyprinus carpio*, *Carassius auratus*, *Tinca tinca* (Cypriniformes) and *Anguilla* sp. (Anguilliformes) in Europe, and in *Saccobranchus (= Heteropneustes) fossilis* (Siluriformes) in India (Qadri, 1962). It has also been found in other cyprinids, as *Rutilus rutilus* and *Tinca tinca* from breeding ponds in Poland (Wita and Ovcharenko, 1997), and *Abramis brama*, *Carassius auratus gibelio* and *Cyprinus carpio* from the Upper-Reservoir of the Kis-Balaton system (Hungary) (Székely and

Molnár 1996/1997). In addition, the flagellate was infective when inoculated experimentally into *Barbus cohnus, Danio malabaricus, Catostomus commersoni, Notropis cornutus, Etheosoma caeruleum* and *Ictalurus nebulosus* (Woo and Black, 1984), whereas other hosts, as *Oncorhynchus mykiss, Perca fluviatilis* or *Lepomis gibbosus* were refractory in nature (Lom and Dyková, 1992).

### *In vitro* Culture

Although *T. carassii* can be cultivated *in vitro*, some media do not support the growth of certain strains, or infectivity for fish may decrease with subcultures (reviewed in Woo and Poynton, 1999). Wang and Belosevic (1994) reported the rapid multiplication of a strain in serum-free MEM with other supplements. However, growth was improved by addition of fish serum (Bienek and Belosevic, 1997) and in macrophage or fibroblast conditioned medium (Bienek and Belosevic, 1999). Overath *et al.* (1998) succeeded in propagate the parasite in MEM with different supplements (including 5% of foetal bovine serum and 5% of carp serum), an adaptation from formulations used for growth of *T. brucei* bloodstream forms. The cultured forms obtained in such medium were experimentally infective to goldfish and considered indistinguishable from those derived from fish blood based on morphology, ultrastructure and immunostaining with a monoclonal antibody to a surface epitope.

### Immunological Studies

Goldfish that have recovered from previous *T. carassii* infection are protected on subsequent homologous challenge (Islam and Woo, 1991; Woo and Poynton, 1999). The involvement of an adaptative immune response was demonstrated by the presence of neutralizing antibodies in the plasma of immune fish (Woo, 1981b). Passive immunization was also obtained by injecting fish with IgM purified from serum of recovered carp (Overath *et al.*, 1999). In experiments carried out on the course of infection of *T. carassii* in carp, the kinetics of the immune response was studied. Anti-parasite antibodies became detectable 2 weeks after infection and remained high for many months. In addition, fish that have controlled an acute infection with a clone were not only protected against a homologous challenge infection, but also against the infection with parasite lines derived from carp in the chronic phase on infection (Overath *et al.*, 1999). Immunization of goldfish with excretory/secretory

(ES) products of the parasite conferred protection against infection only when ES were administered with Freund's complete adjuvant, suggesting the involvement of cell-mediate response (Bienek *et al.*, 2002). *In vitro* studies demonstrated the involvement of the alternative complement pathway in the lysis of enzyme treated *T. carassii* and that protective surface proteins of the parasite were necessary for the resistance of normal trypanosomes to lysis (Plouffe and Belosevic, 2004). The role of NO in immune modulation during *T. carassii* infection in carp was studied by Saeij *et al.* (2002). The flagellate inhibited LPS-induced NO production *in vitro*, which could be a strategy to deactive specific function of phagocytes and ensure long-term survival of the parasite.

Other studies on surface proteins and antigens have been made. Lischke *et al.* (2000) identified and partially characterized glycophosphatidylinositol (GPI)-anchored, mucin-like surface glycoproteins (containing sialic acid in the carbohydrate moiety) from blood-stream forms of *T. carassii*. This surface is, thus, similar to that of non-Salivarian trypanosomes, as *T. cruzi*, with subsequent limitation in antigenic variation, as observed in the experimental studies of Overath *et al.* (1999).

***Trypanosoma granulosum*** Laveran and Mesnil, 1902

It is a pleomorphic species of the European eel, *Anguilla anguilla*, transmitted by the hematophagous leech *Hemiclepsis marginata*. In experimental infections of farmed eels (Zintl *et al.*, 2000), the pleomorphism of this species was confirmed, as body length ranged between 13.9 and 60.3 μm. Parasitaemia levels were lower than in field observations, in which multiple infections are likely to occur (Zintl *et al.*, 1997).

The available information on the pathogeny of *T. granulosum* is scarce. A decrease in hematocrit values was found in naturally infected eels, but in concomitant infections with *Anguillicola crassus* (Boon *et al.*, 1990). Other authors consider that the flagellate has only minor effects on host health, despite its high prevalence (Aguilar *et al.*, 2005).

Eels can be infected in fresh, brackish and marine waters, though prevalences are usually higher in freshwater, the habitat of the intermediate host (Aguilar *et al.*, 2005). *T. granulosum* has been found in wild eels from different origins, as Portugal (Eiras, 1988; Saraiva and Eiras, 1996), Spain (Aguilar *et al.*, 2005), western Ireland (Zintl *et al.*, 1997), Denmark (Koie, 1988), Netherlands (Boon *et al.*, 1990) and Poland

(Wierzbicka and Orecka-Grabda, 1994). Prevalences were generally higher in lakes than in stream sites.

Davies *et al.* (1995) reported a good growth of *T. granulosum* in one semi-defined medium, a modified version of SDM-79 without MEM F-14, though poor growth was obtained in other media. The growth of the flagellate was inhibited *in vitro* by D, L-alpha-difluoromethylornithine (DFMO) that is known to inhibit the growth of African mammalian trypanosomes and is used to treat human trypanosomosis.

## OTHER *Trypanosoma* SPP. FROM FRESHWATER FISH

There are several records of *Trypanosoma* spp. from wild and cultured fresh water fish, some of them with pathogenic effects on the fish hosts. However, the involved species was not identified in many cases.

***Trypanosoma mukasai*** Hoare, 1932, was found in African catfish, *Clarias lazera*, from Egypt (Alyain *et al.*, 1994). The development of the flagellate was studied in the leech host *Batracobdelloides tricarinata* (Negm-Eldin, 1997). The flagellates can survive for 10 months in the leech, and for 7 months in the blood of experimentally infected catfish. Trypanosomes tentatively identified as *T. mukasai* were reported from several catfish in Botswana (Smit *et al.*, 2000, 2004). Further molecular studies (Davies *et al.*, 2005) have allowed identifying two different genotypes among the Botswana trypanosomes.

A different species, *T. markevitschi*, was recorded from other Egyptian catfish, *Clarias mossambicus*, in the river Nile. In experimental infections, some histopathological changes were detected, in spite of the lack of gross lesions and the low degree of parasitaemia. Histopathological effects consisted of myocardial degeneration, interstitial lymphocytic miocarditis, spleen hemosiderosis, vascular changes in the liver, and alterations of the kidney haematopoietic tissue (Afifi, 1996). Prevalences of 42.3% and 8%, respectively, were reported for *Trypanosoma* sp. in naturally infected Siluriformes, *Chrysichthys auratus* (Bagridae) and *Clarias gariepinus* (Clariidae) from Egypt, and anaemia was also observed in the former species (Ahmed, 2001).

Several Asian fish may harbour *Trypanosoma* spp. *T. trichogasteri* can produce anaemia in the belontid fish *Colisa fasciatus* from an Indian pond. *T. punctati* Hasan and Quasim, 1962 was detected in the blood of *Channa punctatus* (Channidae), a significant species in fisheris and aquaculture, in Kerala (India) (Shanavas, 1991). Experimental infection was obtained by

allowing the infective leeches (*Hemiclepsis marginata*) to feed on parasite-free fish. Attempt to transmit the infection to *Clarias dussunieri*, *Heteropneustes fossilis* and *Mystus malabaricus* were unsuccessful. A different species (*T. mukundi*) caused anaemia in the siluroid catfish *Heteropneustes fossilis* (Saha *et al.*, 1997). Other Indian fish, *Barilius bendelisis*, naturally infected with *Trypanosoma* sp. showed blood changes indicative of anaemia and increase in the proportion of haemoblasts, small lymphocytes, basophiles and monocytes (Rauthan *et al.*, 1995). Trypanosomosis has been also reported in cultured *Cichlasoma managuense* (= *Parachromis managuensis*) (Cichlidae) from southern Taiwan (Hsu *et al.*, 1995). Infected fish showed anorexia, black discoloration of the body surface, sluggish swimming and weakness, and protrusion of the eyes. At necropsy, splenomegalia and pale gills were observed. Anaemia and several histopathological changes were also detected.

*Trypanosoma* is also included in a review of diseases of Neotropical fish in Brazil (Thatcher and Brites Neto, 2004).

## *Trypanosoma* SPP. OF MARINE AND ESTUARINE FISH

Most species have been described from wild fish, and the actual pathogenic concern is unknown. *T. murmanense* is one of the species more frequently recorded.

### *Trypanosoma murmanense* Nikitin, 1927

#### Morphology and Life Cycle

It is a pleomorphic trypanosome with small, intermediate and large forms (Fig. 9.19). Morphometric details of *T. murmanense* from naturally infected fish can be found in Khan (1972). Cod (*Gadus morhua*) is the main fish host and the leech *Johanssonia arctica* the intermediate host (Khan, 1976).

#### Clinical Signs, Aetiology/Pathogenesis

The pathogenesis of *T. murmanense* infection was studied after experimental leech-transmission to four species of marine fish (Khan, 1985). High mortality occurred in juvenile Atlantic cod and winter flounder. At necropsy, anaemia was the most common pathological feature in young infected fish, but the condition factor, and somatic indices of liver, spleen and heart were altered in some fish groups.

## Fish Hosts, Epidemiology and Geographical Distribution

This trypanosome was initially described from cod of the Barents Sea and further reported from the North West Atlantic in coastal Newfoundland. It can infect other perciform and also pleuronectiform fish in natural infections, and has been experimentally transmitted to numerous fish species (Khan, 1977; Khan *et al.*, 1980).

Karlsbakk (2004) recorded another *Trypanosoma* sp. from cod, apparently distinct from *T. murmanense*, considering the smaller size, the nearly central nucleus, and the transmission by a different vector, the leech *Calliobdella nodulifera*. Ranzani-Paiva *et al.* (1997) found no influence of *Trypanosoma* sp. in naturally infected mullets (*Mugil platanus*) from the estuarine region of Cananeia (Brazil).

Other *Trypanosoma* spp. have been found in wild fish, but their real pathological impact is difficult to evaluate. Some of them were compiled by Lom and Dyková (1992) and Woo and Poynton (1999).

## Molecular Studies

The uncertainty of the taxonomy and phylogeny of fish trypanosomes leading to the abundance of probable synonymies has been stressed by different authors (Becker, 1970; Lom, 1979; Lom and Dyková, 1992). As reviewed by Gibson *et al.* (2005), one confusing factor is the variability in length during infection and the existence of pleomorphic species. Cross-transmission experiments do not solve the problems, as many fish trypanosomes are not host-specific, or failure of infection may be due to immunity of recipient fish among other factors. Following the demonstration of the similarity of species considered different by isoenzyme analyses (Letch, 1979; Zajicek, 1991), molecular methods have provided further tools to examine the relationships of fish trypanosomes. The limited host specificity of piscine trypanosomes was confirmed by Figueroa *et al.* (1999) in an analysis of the 12S rRNA from several freshwater fish strains. Overath *et al.* (1999), using partial sequences of the nuclear SSU rRNA genes, found that isolates from 4 species of freshwater fish fall into two closely related subgroups (Types A and B). Interestingly, both types could be isolated from the same individual fish, depending on the culture conditions used. All the trypanosomes studied by Figueroa *et al.* (1999) belonged to Type B.

In a further attempt to elucidate genetic relationships among freshwater fish trypanosomes, Gibson *et al.* (2005) analyzed SSU rRNA

gene sequences and random amplification of polymorphic DNA (RAPD). All trypanosomes from freshwater fish fell in a single clade (subdivided into 3 groups), which sits with a larger, robust clade containing trypanosomes from marine fish and various amphibious vertebrates. The RAPD analysis also showed 3 groups, which are congruent with those identified in the SSU rRNA phylogeny. These groups do not separate purported trypanosome species distinguished by morphology or host origin. Using the same techniques, Davies *et al.* (2005) found two genotypic groups in morphologically similar trypanosomes from Okavango Delta fishes, supposed to belong to *T. mukasai*; so they concluded that the identification of this flagellate remains a problem.

Kinetoplast structure has also been studied in fish trypanosomes and sometimes used with comparative purposes. Zajíček *et al.* (1991) reported maxicircle and minicircle sizes in *T. carassii* that fall into the published value range for kinetoplastids. Freshwater fish trypanosomes represent a compact separated group within the genus as deduced from comparison of kinetoplast conserved regions (Baron *et al.*, 1995; Kolesnikov *et al.*, 1995). In addition, the size of minicircles was conserved among freshwater, but not in marine, fish trypanosomes, and two populations of kDNA minicircles were found in the latter (Jirků *et al.*, 1995).

The phylogenetic position of trypanosomes has also been controversial. The first molecular studies were based on comparisons of genes encoding mitochondrial and nuclear ribosomal RNAs (rRNA) and showed trypanosomes to be paraphyletic. On the contrary, Figueroa *et al.* (1999), after analyzing the 12S rRNA of several *Trypanosoma* strains from freshwater fish, concluded that fish trypanosomes form a monophyletic group, with *T. brucei* as a sister group. Further studies provided support for monophyly. Hamilton *et al.* (2004) tried to reconstruct the trypanosome phylogeny using numerous strains from different vertebrate and invertebrate hosts, being fish trypanosomes represented by *T. boisoni* from the Senegal marine ray *Zanobatus atlanticus*, *T. granulosum* from eel *Anguilla anguilla*, and *Trypanosoma* sp. from catfish *Clarias angolensis*. The phylogenetic analysis of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene supported the monophyly of the genus *Trypanosoma*, whereas the SSU rRNA gene produced equivocal results.

Trans-sialidase-like and mucin-like genes were identified in *T. carassii* by Agüero *et al.* (2002), who also found genes not previously described in trypanosomatids. The presence of sialilated mucins had already been

demonstrated in this flagellate by Lischke *et al.* (2000). Such mucins are considered to be essential for the survival of mammalian trypanosomes in the host (Pereira Chiolcola *et al.*, 2000).

## OUTLOOK ON FISH EUGLENOZOA. RECENT DEVELOPMENTS AND FUTURE PROSPECTS

The availability of *in vitro* cultivation for many fish kinetoplastids have provided the necessary material for genomic and molecular studies and for implementing experimental models allowing the study of different aspects of the host/parasite relationship. The development of axenic *in vitro* culture for other species is an advisable goal.

The taxonomy of some groups has been revised in the light of transmission and scanning electron microscope studies, as in the case of *Ichthyobodo* spp. However, morphological features have proven to be insufficient to an accurate discrimination of some genus and species. Many species of kinetoplastids hitherto described might be invalid, mainly in the case of *Trypanosoma*, for which species have been frequently named after their hosts and with incomplete descriptions. In such circumstances, molecular taxonomic and systematic studies are especially relevant as an aid to clarify this confusing situation. The application of molecular tools has allowed to improve the knowledge of phylogenetic relationships of fish flagellates and to review their higher level classification (Cavalier-Smith, 2002; Simpson, 2003; Simpson *et al.*, 2004; Moreira *et al.*, 2004; von der Heyden *et al.*, 2004; Adl *et al.*, 2005; von der Heyden and Cavalier-Smith, 2005; Simpson *et al.*, 2006a). However, the ascription to the lower level taxa, mainly genus and species, remains controversial in many cases. Molecular taxonomy can aid to the species definition, but it is not exempt of real difficulties, as determining what level of similarity defines a species, the extent of variation within species and the key taxonomic characters which may differ for each group of organisms (Gibson, 2003).

Thus, confusion still exists in the specific ascription of marine *Ichthyobodo*. Recent molecular studies have allowed confirming the existence of several species besides *I. necator* (Todal *et al.*, 2004; Callahan *et al.*, 2005), but the definitive elucidation of the existing species, as well as of their hosts and distribution, are lacking. The molecular study of *Ichthyobodo* from other habitats and hosts not included in previous analysis (as those of Mediterranean marine fish) could aid to such purpose.

The controversial situation of the genera *Cryptobia* and *Trypanoplasma* and their unresolved synonymy is to be mentioned. Recent molecular studies ( Wright *et al.*, 1999; Dolezel *et al.*, 2000; Maslov *et al.*, 2001) have confirmed the synonymy of *Trypanoplasma* with *Cryptobia* initially proposed by Crawley (1909) and further accepted by other authors (Vickerman, 1976, 1990b; Woo, 2003). New studies, including a higher number of species from different invertebrate hosts and non-haematozoic piscine species could allow to get a demonstration of the identity of both genera, and to elucidate the importance of habitat and life cycle in their definition (Moreira *et al.*, 2004).

Trypanosomes are a clear example of the difficulties of applying the morphological criteria of the traditional taxonomy, and even the biological species concept, as their predominant mode of reproduction is asexual. The available molecular studies on the taxonomy of some fish trypanosomes have not allowed clarifying their status definitively, and most of them confirmed the lack of correspondence between host, geographic distribution and morphology, with molecular data. Therefore, new studies using more genes are necessary. The availability of polymorphic DNA markers will facilitate future studies of biological variation (Gibson *et al.*, 2005).

Most of the previous phylogenies of kinetoplastids had been based on analysis of variation in SSU rRNA genes, multi-copy genes that evolve by concerted evolution. Single-copy, protein-coding genes under a different set of evolutionary constraints should be also included in the analysis. This is the case of the GADPH gene, which evolves slowly and under a different set of evolutionary constraints to the SSU rRNA gene. The analysis of the GADPH gene supports the monophyly of the genus *Trypanosoma* and the hypothesis of trypanosomes evolved from an ancestral insect parasite, which adapted to a vertebrate/insect transmission cycle (Hamilton *et al.*, 2004). According to a recent review of Simpson *et al.* (2006b), several unsual features of trypanosomatid genomes are common in kinetoplastids and pre-date the adoption of parasitism, which evolved at least four times in Kinetoplastida. Some fish trypansomes have been included in wide molecular phylogenetic studies of kinetoplastids, and some of their genes have been sequenced. Several GenBank nucleotid and protein sequences are currently available for *Ichthyobodo* sp. (8 nucleotids), *I. necator* (3 nucleotids; 1 protein), *Trypanoplasma borreli* (24; 21), *Cryptobia salmositica* (7; 6), C. *bullocki* (1), C. *catostomi* (1), *Trypanosoma carassii* (1,938; 21) and T. *granulosum* (4; 2).

In relation to taxonomy, the improvement of diagnosis through the obtaining of fast and reliable diagnostic tools requires special attention. A preliminary approach to diagnostic using clinical signs and light microscope observations can be done for most pathogenic flagellates, and in some cases, it allows the immediate application of control measures. However, the generic and specific ascription is very necessary to assess the real importance of the parasitosis, as differences in virulence can exist. Efforts should be directed to the development of PCR diagnostic assays for the most relevant pathogenic flagellates, as no PCR test is presently available for fish euglenozoans. The available sequences could be used as a basis of further development of diagnostic tools.

## Immune Response and Immunoprophylaxis

Fish immune response has been studied for several flagellates, and the involvement of innate and adaptive responses has been demonstrated in most cases. *Cryptobia salmositica* is the best-tudied species and the available knowledge has allowed developing an experimental attenuated vaccine and obtaining the preliminary information to prepare a future recombinant vaccine (reviewed in Woo, 2001, 2003). Infections of *Trypanoplasma borreli* and *Trypanosoma carassii* in carp have been frequently used as models for different studies, including the mechanisms involved in the immune response. Recently, the role of classsically activated macrophages (caMF) and alternatively activated macrophages (aaMF) was investigated in carp infected with *T. borreli* and *T. carassii*. A differential polarization was demonstrated, as *T. borreli*- and *T. carassii*-infected carps are more prone to increase nitrite production by caMF or arginase activity by aaMF, respectively (Joerink *et al.*, 2006). The expression of some genes involved in the immune response has been studied. Saeij *et al.* (2003a) found up-regulated expression of TNF$\alpha$, Il-1$\beta$ and mRNAs in *Trypanoplasma borreli* infected carps. Further studies are needed on other fish/flagellate models in order to elucidate the mechanisms involved in the fish immune response and the further application to flagellosis control.

Therefore, even for the most studied flagellates, new studies are necessary for an effective application of immunoprophylactic measures, and basic studies are required for the other species. In addition, provided scientific challenges could be overcome, the development of commercial piscine vaccines is constrained by economic and practical difficulties.

Once a technology and a product is selected, a development plan is necessary to assure that formulations are safe, stable and efficacious. Disease risk, vaccine efficacy and market price must be evaluated to assess the feasibility of vaccination as a mangement tool (Ellis, 2001; Thorarinsson and Powell, 2006).

Other approaches to control some piscine flagellate infections have been assayed besides the classical treatments based on the use of different chemicals. The stimulation of the innate immune system could be promising. A combination of trypanocidal drugs with monoclonal antibodies has been reported by Woo (2001) for salmonid cryptobiosis. This author (Woo, 2003) considered the production of transgenic salmonids an option to control cryptobiosis. The mechanisms involved in the cytotoxic action of the trypanocidal drug suramin have been studied using *Trypanoplasma borreli*, and a blocking of nucleotide triphosphate binding to ribosomal protein L3 was demonstrated (Avliyakulov *et al.*, 2000).

## Pathogenic and Virulence Mechanisms. Antigenic Composition

The aetiology and pathogeny of fish flagellates are generally well known but scarce information is available on the ultimate virulence mechanisms in most cases. The best studied species regarding pathogenic mechanisms is *Cryptobia salmositica*. Haemolysis is one of the main causes of anaemia and it can occur directly (by a haemolysin) or through the formation of immune-complexes on red cells and lysis by complement. A metalloprotease (200 kDa) has been isolated from this flagellate. The comparison of the virulent *C. salmositica* with the avirulent strain attenuated *in vitro* has allowed elucidating some virulence mechanisms, as well as the study of infection in individual fish or species showing differences in susceptibility to the disease. (See above and the reviews of Woo and Poynton, 1999; Woo, 2003.)

Two different groups are recognized among trypanosomes, Salivarian trypanosomes showing antigenic variation and non-Salivarian trypanosomes lacking such variation. Thus far no trypanosome from non-mammalian vertebrates appeared closely related to Salivaria (Overath *et al.*, 2001). Salivarian trypanosmes have the surface dominated by the coat formed by variant surface glycoproteins (VSGs) that are highly immunogenic and readily eliminated by the humoral immune response.

Therefore, the successive expression of antigenically different variants is a necessary requirement for establishing a chronic infection. In contrast, the piscine *T. carassii* does not seem to show antigenic variation and its surface is covered by mucin-like carbohydrate-rich glycoproteins, attached to the membrane by glycosyl-phosphatidylinositol (GPI) residues, whose corresponding genes have been identified (Agüero *et al.*, 2002). The surface of this fish trypanosome is thus very similar to that of the mammalina *T. cruzi* (Overath *et al.*, 1999, 2001), and all the properties are consistent with the deduction that *T. carassii* belong to the non-Salivarian branch of the pylogenetic tree (Overath *et al.*, 2001).

Surface antigens have also been studied in *Cryptobia salmositica* (reviewed in Woo, 2003) in both virulent and avirulent strains, and an increased surface carbohydrate residues in the attenuated strain (which contributes to avoiding the action of complement) was observed. A phosphatidylinositol residue is also present in *C. salmositica*, and it is located in the epitope of the 200-kDa surface membrane glycoprotein. The nucleotide sequences of this metalloproteinase gene (MSP-1), as well as those of a cysteine proteinase (49 kDa Cysp1 mRNA and complete CYS mRNA9, a methionine adenosyltransferase), have been deposited in GenBank (Jesudhassan and Woo; Hontzeas, Kim and Woo, unpublished).

The mitochondrion of kinetoplastid protozoa is a remarkable organelle with unusual properties, which are unique in this group. According to Lukeš *et al.* (2005), considerable progress has been made in elucidating the complex machinery of DNA editing and in understanding of the structure and replication of kinetoplast DNA. The uridine (U) insertion-deletion RNA editing that occurs in the mitochondrion of trypanosomatids was found to occur in the piscine *Trypanoplasma borreli* (Lukeš *et al.*, 1994; Maslow and Simpson, 1994). The structure and replication of kinetoplast have been mainly studied in mammalian trypanosomes, but some information is available for fish trypanosomes (see above). As reviewed in Lukeš *et al.* (2002), there are variations in kDNA structure amongst different kinetoplastids and the network form found in traypanosomes is not present in other groups. Amongst fish parasites, *Cryptobia branchialis* seems to have a pan-kDNA, similar to that of *Cryptobia helicis*, which fill most mitochondrial matrix and with supercoiled minicircles. The most unusual type, the mega-kDNA is found in *T. borreli*, with a fairly uniform distribution throughout a large region of

the mitochondrial matrix, containing minicircle-like sequences tandemly linked into large molecules (Maslov and Simpson, 1994). The kDNA replication has been mainly studied for the kDNA network of *Trypanosoma brucei* and *T. cruzi* (also reviewed in Lukeš *et al.*, 2002), involving not only exchange of nuclear genes but also exchange of minicircles and maxicircles, in which a fusion of the mitochondria from the two parental cells is implicated. The nucleus-kinetoplastid interactions have also been studied, and a possibility of exchange of molecules between both structures has been suggested (Kallinikova and Kondratieva, 2000).

Therefore, although knowledge on RNA editing and kinetoplast replication have substantially improved, much less is known about the developmental regulation of RNA editing, its integration with other RNA maturation processes, stability of mitochondrial mRNAs, or evolution of editing process itself. The improvement of the knowledge of the complexity of this interesting organelle may enable us to fight these often medically important protozoans (Lukeš *et al.*, 2005), and these advances can be further applied to piscine species.

## References

Abreu, P.C., R.B. Robaldo, L.A. Sampaio, A. Bianchini and C. Odebrecht. 2005. Recurrent amyloodiniosis on broodstock of the Brazilian flounder *Paralichthys orbignynanus*: Dinospore monitoring and prophylactic measures. *Journal of the World Aquaculture Society* 36: 42-50.

Adl, S.M., A.G.B. Simpson, M.A. Farmer, R.A. Andersen, O.R. Anderson, J.R. Barta, S.S. Bowser, G. Brugerolle, R.A. Fensome, S. Fredericq, T.Y. James, S. Karpov, P. Kugrens, J. Krug, C.E. Lane, L.A. Lewis, J. Lodge, D.H. Lynn, D.G. Mann, R.M. McCourt, L. Mendoza, Ø. Moestrup, S.E. Mozley-Standridge, T.A. Nerad, C.A. Shearer, A.V. Smirnov, F.W. Spiegel and M.F.J.R. Taylor. 2005. The new higher level classification of Eukaryotes with emphasis on the taxonomy of Protists. *Journal of Eukaryotic Microbiology* 52: 399-451.

Afifi, S.H. 1996. Histopathology of catfish, mud barbell *Clarias mossambicus* infected with *Trypanosoma markewitschi* under laboratory conditions. *Assiut Veterinary Medical Journal* 35: 72-83.

Agüero, F., V. Campo, L. Cremona, A. Jäger, J.M. Di Noia, P. Overath, D.O. Sánchez and A.C. Frasch. 2002. Gene discovery in the freshwater fish parasite *Trypanosoma carassii*: Identification of *trans*-transialidasa-like and mucin-like genes. *Infection and Immunity* 70: 7140-7144.

Aguilar, A., M.F. Álvarez, J.M. Leiro and M.L. Sanmartín. 2005. Parasite populations of the European eel (*Anguilla anguilla* L.) in the rivers Ulla and Tea (Galicia, Northwest Spain). *Aquaculture* 249: 85-94.

Ahmed, S.M. 2001. A study on trypanosomiasis in some freshwater fishes of Assiut Governorate. *Assiut Veterinary Medical Journal* 45: 117-131.

Aiello, P. and A. D'Alba. 1986. *Amyloodinium ocellatum* infestation in yellowtail, *Seriola dumerili* intensively reared in Sicily, Italy. *Bulletin of the European Association of Fish Pathologists* 6: 110-111.

Alyain, S.A., M.A.E.M. Soheir and N.A.M. Mahnoud. 1994. A revision study on Nile catfish (*Clarias lazera*) in upper Egypt after 20 years. *Veterinary Medical Journal Giza* 42: 21-26.

Alvarez-Pellitero, P. 1995. Study of parasitic and infectious (bacterial and viral) diseases in seabream (*Sparus aurata*) and Mediterranenan sea bass (*Dicentrarchus labrax*) reared in the Mediterranenan environment: Survey, epidemiology and control. In: *FAR 1988-92, Synopsis of R & D Projects,* Aquaculture upgrading of fishery products, European Commission, Directorate-General XIV Fisheries, Luxembourg, Vol. I, pp. 21-26.

Alvarez-Pellitero, P. 2004. Report about fish parasitic diseases. In: *Mediterranean Aquaculture Diagnostic Laboratories*, P. Alvarez-Pellitero, J.L. Barja, B. Basurco, F. Berthe and A.E. Toranzo (eds.). Zaragoza: CIHEAM (Centre International des hautes Études Agronomiques Mediterrannéennes)/FAO (Série B, Études et Recherches) No. 49, Options Mediterranéennes, pp. 103-130.

Alvarez-Pellitero, P., A. Sitjà-Bobadilla and A. Franco-Sierra. 1993. Protozoan parasites of wild and cultured sea bass, *Dicentrarchus labrax* (L.), from the Mediterranenan area. *Aquaculture and Fisheries Management* 24: 101-108.

Alvarez-Pellitero, P., A. Sitjà-Bobadilla, A. Franco-Sierra and O. Palenzuela. 1995. Protozoan parasites of gilt-head seabream, *Sparus aurata* L., from different culture systems in Spain. *Journal of Fish Diseases* 18: 105-115.

Andersson, J.O., Å.M. Sjögren, A.A.M. Davis, T.M. Embley and A.J. Roger. 2003. Phylogenetic analyses of diplomonad genes reveal frequent lateral gene transfers affecting eukaryotes. *Current Biology* 13: 93-104.

Avliyakulov, N.K, J. Lukeš, A.V. Kavaja, B. Liedberg, I. Lundström and S.P.S. Svensson. 2000. Suramin blocks nucleotide triphospahte binding to ribosomal protein L3 from *Trypanoplasma borreli. European Journal of Biochemistry* 267: 1723-1731.

Bahmanrokh, M. and P.T.K. Woo. 2001. Relations between histopathology and parasitaemias in *Oncorhynchus mykiss* infected with *Cryptobia salmositica*, a pathogenic haemoflagellate. *Diseases of Aquatic Organisms* 46: 41-45.

Barbaro, A. and A. Francescon. 1985. Parassitosi da *Amyloodinium ocellatum* (Dinophyceae) su larve di *Sparus aurata* allevate in un impinato di riproduzione artificialer. *Oebalia* 11: 745-752.

Baron, L.I., V.Yu. Yurchenko and A.A. Kolesnikov. 1995. Structure of minicircle kinetoplast DNA from a river perch tryapanosome. *Molecular Biology (New York)* 29: 801-805.

Baticados, M.C. and G.F. Quinitio. 1984. Occurrence and pathology of an *Amyloodinium*-like protozoan parasite on the gills of grey mullet, *Mugil cephalus*. *Helgoländer Meeresuntersuchungen* 37: 595-601.

Beck, K., G. Lewbart and G. Piner. 1996. The occurrence of *Ichthyobodo*-like organism on captive Atlantic spadefish *Chaetodipterus faber* (Broussonet). *Journal of Fish Diseases* 19: 111-112.

Becker, C.D. 1970. Haematozoa of fishes, with emphasis on North American records. In: *A Symposium on Diseases of Fishes and Shellfishes*, S.F. Sniezsko (ed.). Special Publication No. 5. American Fisheries Society, Washington, pp. 82-100.

Bergh, Ø, F. Nilsen and O.B. Samuelsen. 2001. Diseases, prophylaxis and treatment of the Atlantic hablibut *Hippoglossus hippoglossus*: a review. *Diseases of Aquatic Organisms* 48: 57-74.

Bienek, D.R. and M. Belosevic. 1997. Comparative assessment of growth of *Trypanosoma danilewskyi* (Laveran & Mesnil) in medium containing fish or mamalian serum. *Journal of Fish Diseases* 20: 217-221.

Bienek, D.R. and M. Belosevic. 1999. Macrophage or fibroblast-conditioned medium potentiates growth of *Trypanosoma danilewskyi* Laveran & Mesnil, 1904. *Journal of Fish Diseases* 22: 359-367.

Bienek, D.R., D.A. Plouffe, G.F. Wiegertjes and M. Belosevic. 2002. Immunization of goldfish with excretroy/secretory molecules of *Trypanosoma danilewskyi* confers protection against infection. *Developmental and Comparative Immunology* 26: 649-657.

Blanc, E., A. Marques, G. Bouix, G. Brugerolle and G. Breuil. 1989. *Cryptobia* sp. from the gills of the gilt head seabream *Sparus aurata*. *Bulletin of the European Association of Fish Pathologists* 9: 81-82.

Boon, J.H., Y. Zuxu and G.H.R. Booms. 1990. Effects of *Anguillicola crassus* and *Trypanosoma granulosum* infecions in the peripheral blood cells of European eel (*Anguilla anguilla*). *Bulletin of the European Association of Fish Pathologists* 10: 143-145.

Bower, S.M. and L. Margolis. 1985. Effects of temperature and salinity on the course of infection with the haemoflagellate *Cryptobia salmositica* in juvenile Pacific salmon (*Oncorhynchus* spp.). *Journal of Fish Diseases* 8: 25-33.

Bower, S.M., R.E. Withler and B.E. Riddell. 1995. Genetic variation in resistance to the hemoflagellate *Cryptobia salmositica* in coho and sockeye salmon. *Journal of Aquatic Animal Health* 7: 185-194.

Bricknell, I.R., J.E. Bron and T.J. Bowden. 2006. Diseases of gadoid fish in cultivation: a review. *ICES Journal of Marine Science* 63: 253-266.

Brugerolle, G. 1974. Contribution a l'étude cytologique et phylétique des diplozoaires (Zoomastigophora, Diplozoa, Dangeard 1910). III. Étude ultrastructurale du genre *Hexamita* (Dujardin 1838). *Protistologica* 1: 83-90.

Brugerolle, G. 1975. Contribution a l'étude cytologique et pylétique des diplozoaires (Zoomastigophora, Diplozoa, Dangeard 1910). VI. Caractères généraux des diplozoaires. *Protistologica* 11: 111-118.

Brugerolle, G. and J.J. Lee. 2000. Order Diplomonadida. In: *The Illustrated Guide to the Protozoa*, J.J. Lee, G.F. Leedale and P. Bradbury (eds.). Society of Protozoologists, Lawrence, Kansas, pp. 1125-1135.

Brugerolle, G., L. Joyon and N. Oktem. 1973. Contribution a l'étude cytologique et phylétique des diplozoaires (Zomastigophorea, Diplozoa, Dangeard 1910). II. Étude ultrastructurale du genre *Spironucleus* (Lavier 1936). *Protistologica* 9: 495-502.

Brugerolle, C., J. Lom, E. Nohynková and L. Joyon. 1979. Comparison et évolution des structures cellulaires chez plusieurs éspéces de bodonides et cryptobiides

appartenant aux genres *Bodo*, *Cryptobia* et *Trypanoplasma* (Kinetoplastida, Mastigophora). *Protistologica* 15: 197-221.

Brugerolle, C., I. Kunstýr, J. Senaud and K.T. Friedhoff. 1980. Fine structure of trophozoites and cysts of the pathogenic diplomonad *Spironucleus muris*. *Zeitschrift für ParasitenKunde* 62: 47-61.

Bruno, D.W. 1992. *Ichthyobodo* sp. on farmed Atlantic salmon, *Salmo salar* L., reared in the marine environment. *Journal of Fish Diseases* 15: 349-351.

Buchman, K. and A. Uldall. 1996. Temperature, pH and bile dependent in vitro cultivation of *Hexamita salmonis* from rainbow trout *Oncorhynchus mykiss* intestine. *Diseases of Aquatic Organisms* 24: 169-172.

Bullock, A.M. and D.A. Robertson. 1982. A note on the occurrence of *Ichthyobodo necator* (Henneguy 1883) in a wild population of juvenile plaice, *Pleuronectes platessa* L. *Journal of Fish Diseases* 5: 531-533.

Bunnajirakul, S., D. Steinhagen, U. Hetzel, W. Körting and W. Drommer. 2000. A study of sequential histopatology of *Trypanoplasma borreli* (Protozoa: Kinetoplastida) in susceptible common carp *Cyprinus carpio*. *Diseases of Aquatic Organisms* 39: 221-229.

Burkholder, J.M. 2001. Chronic impacts from toxic microalgae on finfish, shellfish and human health. In: *Waters in Peril*, L. Bendell-Young and P. Gallaugher (eds.). Kluwer Academic Publishers, Dordrecht, pp. 103-126.

Burreson, E.M. 1979. Structure and life cycle of *Trypanoplsma beckeri* sp. n. (Kinetoplastida), a parasite of the cabezon, *Scorpaenichthys marmoratus*, in Oregon coastal waters. *Journal of Protozoology* 26: 343-347.

Burreson, E.M. 1982a. The life cycle of *Trypanoplasma bullocki* (Zoomastigophorea, Kinetoplastida). *Journal of Protozoology* 29: 72-77.

Burreson, E.M. 1982b. Trypanosomiasis in flounder along the Atlantic coast of the United States. In: *Les Antigenes des Micro-organismes Parasites des Poissons*, D.P. Anderson, M. Dorson and Ph. Dubourget (eds.). Collection Foundation Merieux, France, pp. 251-260.

Burreson, E.M. 1999. Phylum Annelida: Hirudinea as vectors of disease agents. In: *Fish Diseases and Disorders. Protozoan and Metazoan Infections*, P.T.K. Woo (ed.). CABI Publishing, Wallingford, Oxon, U.K., Vol. 1, pp. 599-629.

Burreson, E.M. and J.P. Sypek. 1981. *Cryptobia* sp. (Mastigophorea, Kinetoplastida) from the gills of marine fishes in Chesapeake Bay. *Journal of Fish Diseases* 4: 519-522.

Burreson, E.M. and D.E. Zwerner. 1984. Juvenile summer flounder, *Paralichthys dentatus*, mortalities in western Atlantic Ocean caused by the haemoflagellate *Trypanoplasma bullocki*, evidence from field and experiemntal studies. *Helgoländer Meeresuntersuchungen* 37: 343-352.

Caffara, M. and M.P. Tampieri. 2001. Parassitosi dei pesci ornamentali dulciaquaicoli. *Obiettivi e Documenti Veterinari* 22: 68-69.

Çağirgan, H. and E. Tokşen. 1996. Yurdumuzda kültürü yapilan levrek (*Dicentrarchus labrax*) juvenillerinde ilk defa görülen *Amyloodinium oellatum* (Dinoflagellata) infestasyonu. *Pendik Veteriner Mikrobioyoloji Dergisi* 27: 197-205.

Callahan, H.A., and E.J. Noga. 2002. Tricaine dramatically reduces the ability to diagnose protozoan ectoparasite (*Ichthyobodo necator*) infections. *Journal of Fish Diseases* 25: 433-437.

Callahan, H.A., R.W. Litaker and E.J. Noga. 2002. Molecular taxonomy of the suborder Bodonina (Order Kinetoplastida), including the important fish parasite, *Ichthyobodo necator*. *Journal of Eukaryotic Microbiology* 49: 119-128.

Callahan, H.A., R.W. Litaker and E.J. Noga. 2005. Genetic relationships among members of the *Ichthyobodo necator* complex: Implications for the management of aquaculture stocks. *Journal of Fish Diseases* 28: 111-118.

Carnevia, D. and G. Speranza. 2003a. Enfermedades diagnosticadas en peces ornamentales tropicales de criaderos de Uruguay: I. Parasitosis. *Veterinaria (Montevideo)* 38: 29-34.

Carnevia, D. and G. Speranza. 2003b. Seasonal variations in parasites found in mullet (*Mugil platanus* Günther, 1880) juveniles captured on the Uruguayan coast of the River Plate. *Bulletin of the European Association of Fish Pathologists* 23: 245-249.

Cavalier-Smith, T. 1981. Eukaryotic kingdoms: Seven or nine? *Biosystems* 14: 461-481.

Cavalier-Smith, T. 1991. Cell diversification in heterotrophic flagellates. In: *The Biology of Free-living Heterotrophic Flagellates*, D.J. Patterson and J. Larsen (eds.). Clarendon Press, Oxford, pp. 113-131.

Cavalier-Smith, T. 1998. A revised six-kingdom system of life. *Biological Reviews* 73: 203-206.

Cavalier-Smith, T. 2002. The phagotrophic origin of eukaryotes and phylogenetic classification of protozoa. *International Journal of Systematic, Ecology and Microbiology* 52: 297-354.

Cecchini, S., M. Saroglia, G. Terova and F. Albanesi. 2001. Detection of antibody response against *Amyloodinium ocellatum* (Brown, 1931) in serum of naturally infected European sea bass by an enzyme-linked immunosorbent assay (ELISA). *Bulletin of the European Association of Fish Pathologists* 21: 104-108.

Chen, C.L. 1956. The protozoan parasites from four species of Chinese pond fishes, *Ctenopharyngodon idellus*, *Mylopharingodon piceus*, *Aristichthys nobilis* and *Hypophthalmichthys molitrix*. I. The protozoan parasites of *Ctenopharyngodon idellus*. *Acta Hydrobiologica Sinica* 1: 123-164.

Chin, A. and P.T.K. Woo. 2005. Innate cell-mediated immune response and peripheral leukocyte populations in Atlantic salmon, *Salmo salar* L., to a live *Cryptobia salmositica* vaccine. *Parasitology Research* 95: 299-304.

Chin, A., B.D. Glebe and P.T.K. Woo. 2004. Humoral response and susceptibility of five full-sib families of Atlantic salmon, *Salmo salar* L., to the haemoflagellate, *Cryptobia salmositica*. *Journal of Fish Diseases* 27: 471-481.

ChingYu, T., S. ChinWen, L. YuHsing and W. WayShyan. 2002. *Amyloodinium* spp. and *Centrotestus* sp. infection in red drum (*Sciaenops ocellatus*). *Taiwan Veterinary Journal* 28: 305-311.

Chonchuenchob, P., S. Sumpawapol and A. Mearoh. 1986. Diseases of cage-cultures sea bass (*Lates calcarifer*) in southwestern Thailand. In: *Management of Wild and Cultured Sea Bass/Barramundi (Lates calcarifer)*, J.W. Copland and D.L. Grey (eds.). *Proceedings of an International Workshop held at Darwin, N. T. Australia, 24-30 Septemeber 1986*, pp. 194-197.

Cobb, C.S., M.G. Levy and E.J. Noga. 1998a. Acquired immunity to amyloodiniosis is associated with an antibody response. *Diseases of Aquatic Organisms* 34: 125-133.

Cobb, C.S., M.G. Levy and E.J. Noga. 1998b. Development of immunity by the tomato clownfish *Amphiprion frenatus* to the dinoflagellate parasite *Amyloodinium ocellatum*. *Journal of Aquatic Animal Health* 10: 259-263.

Crawley, H. 1909. Studies on blood parasites. II. The priority of *Cryptobia* Leidy 1846 over *Trypanoplasma* Laveran and Mesnil, 1901. *Bulletin of the US Bureau of Animal Industry* 119: 16-20.

Cruz-Lacierda, E.R., Y. Maeno, A.J.T. Pineda and V.E. Matey. 2004. Mass mortality of hatchery-reared milkfish (*Chanos chanos*) and mangrove red snapper *(Lutjanus argentimaculatus*) caused by *Amyloodinium ocellatum* (Dinoflagellida). *Aquaculture* 236: 85-94.

Davies, A.J., C. Mastri, D.E. Thorbon and D. Mackintosh. 1995. Experiments with growth of the eel trypanosome, *Trypanosoma granulosum* Laveran & Mesnil, 1902, in semi-defined and defined media. *Journal of Fish Diseases* 18: 599-608.

Davies, A.J., W. Gibson, V. Ferris, L. Basson and N.J. Smith. 2005. Two genotypic groups of morphologically similar fish trypasnosomes from the Okavango Delta, Botswana. *Diseases of Aquatic Organisms* 66: 215-220.

Davis, H.S. 1926. *Octomitus salmonis*, a parasitic flagellate of trout. *Bulletin Bureau of Fisheries (Washington)* 42: 9-26.

Diamant, A. 1987. Ultrastrucutre and pathogenesis of *Ichthyobodo* sp. from wild common dab, *Limanda limanda* L., in the North Sea. *Journal of Fish Diseases* 10: 241-247.

Diamant, A. 1990. Morphology and ultrastructure of *Cryptobia eilatica* n. sp. (Bodonidae: Kinetoplastida), an ectoparasite from the gills of marine fish. *Journal of Protozoology* 37: 482-489.

Díaz, F. and M. George-Nascimento. 2002. Estabilidad temporal de las infracomunidades de parásitos en la borrachilla *Scartichthys viridis* (Valenciennes, 1836) (Pisces: Blenniidae) en la costa central de Chile. *Revista Chilena de Historia Natural* 75: 641-649.

Díaz Sánchez, B.R., M. Jiménez-Estrada and A. Auró de Ocampo. 2000. Evaluación del efecto parasiticida de los extractos acuoso y metabólico de *Buddleja cordata* HBK (Tezopán) sobre *Costia necatrix* en tipalia (*Oreochromis* sp.). *Veterinaria México* 31: 189-194.

Dodge, J.D. and J.L. Lee. 2000. Phylum Dinoflagellata Bütschli, 1885. In: *The Illustrated Guide of the Protozoa*, J.L. Lee, G.F. Leedale and P. Bradbury (eds.). Second Edition. Society of Protozoologists. Allen Press Inc., Lawrence, Kansas, pp. 656-689.

Dolezel, D., M. Jirků, D.A. Maslov and J. Lukeš. 2000. Phylogeny of the bodonid flagellates (Kinetoplastida) based on small-subunit rRNA gene sequences. *International Journal of Systematic and Evolutionary Microbiology* 50: 1943-1951.

Duarte, N., N. Rosa, M.J. Santos and J. Rebelo. 2000. Estudo de ectoparasitas das brânquias do robalo (*Dicentrarchus labrax* L.) da Ria de Aveiro. *Revista de Biologia (Lisboa)* 18: 59-68.

Dyková, I. and J. Lom. 1979. Histopathological changes in *Trypanosoma danilewskyi* Laveran and Mesnil, 1904 and *Trypanoplsma borreli* Laveran and Mesnil, 1902 infections of goldfish, *Carassius auratus* (L.). *Journal of Fish Diseases* 2: 381-390.

Dyková, I., I. Fiala, J. Lom and J. Lukeš. 2003. *Perkinsiella amoebae*-like endosymbionts of *Neoparamoeba* spp., relatives of the kinetoplastid *Ichthyobodo*. *European Journal of Protistology* 39: 37-52.

Eiras, J.C. 1988. *Trypanosoma granulosum* Laveran and Mesnil (1902) (Protozoa: Kinetoplastida) infecting *Anguilla anguilla* L. in Portugal. *Ciencias Biológicas et Ecológicas Systematicas (Portugal)* 8: 29-36.

Eiras, J.C., M.J.T. Ranzani-Paiva, C.M. Ishikawa, A.C. Alexandrino and A.C. Eiras. 1995. Ectopasrasites of semi-intensively farmed tropical freshwater fish *Piaractus mesopotamicus*, *Prochilodus lineatus* and *Colossoma macropomum* in Brazil. *Bulletin of the European Association of Fish Pathologists* 15: 148-151.

Ellis, A.E. and R. Wootten. 1978. Costiasis of Atlantic salmon, *Salmo salar* L. smolts in sea water. *Journal of Fish Diseases* 1: 389-393.

Ellis, R.W. 2001. Product development plan for new vaccine technologies. *Vaccine* 19: 1559-1566.

Evans, B.B. and R.J.G. Lester. 2001. Parasites of ornamental fish imported into Australia. *Bulletin of the European Association of Fish Pathologists* 21: 51-55.

Fajer-Ávila, E.J., I. Abdo-de la Parra, G. Aguilar-Zarate, R. Contreras-Arce, J. Zaldívar-Ramírez and M. Betancourt-Lozano. 2003. Toxicity of formalin to bullseye puffer fish (*Sphoeroides annulatus* Jennyns, 1843) and its effectiveness to control ectoparasites. *Aquaculture* 223: 41-50.

Fard, M.R.S., A. Jørgensen, E. Sterud, W. Bleiss and S.L. Poynton. 2007. Ultrastructure and molecular diagnosis of *Spironucleus salmonis* (Diplomonadida) from rainbow trout *Oncorhynchus mykiss* in Germany. *Diseases of Aquatic Organisms* 75: 37-50.

Ferguson, H.W. 1979. Scanning and transmission electron microscopic observations on *Hexamita salmonis* (Moore, 1922) related to mortalities in rainbow trout fry *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 2: 57-67.

Ferguson, H.W. 1989. *Systemic Pathology of Fish*. Iowa State University Press, Ames, Iowa.

Ferguson, H.W. and R.D. Moccia. 1980. Disseminated hexamitiasis in Siamese fighting fish. *Journal of American Veterinary Medical Association* 177: 854-857.

Ferraz, E. and C. Sommerville. 1998. Pathology of *Piscinoodinium* sp. (Protozoa: Dinoflagellida), parasites of the ornamental freshwater catfishes *Corydoras* spp. and *Brochis splendens* (Pisces: Callichthyidae). *Diseases of Aquatic Organisms* 33: 43-49.

Figueroa, F., W.E. Mayer, J. Lom, I. Dyková, M. Weller, H. Pecková and J. Klein. 1999. Fish trypanosomes: Their position in kinetoplastid phylogeny and variability as determined from 12S rRNA kinetoplast sequences. *Journal of Eukaryotic Microbiology* 46: 473-481.

Floyd, R.F. and R. Yanong. 1999 (revised 2002). *Cryptobia iubilans* in cichlids. *Series of the Veterinary Medicine-Pathobiology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, document VM104. http:// edis.ifas.ufl.edu.*

Fuentes Zambrano, J.L., C. Silva Rojas and Y. Reyes León. 2003. Parásitos en juveniles de *Lutjanus griseus* (Pisces: Lujanidae) de la Laguna de la Restinga, Isla de Margarita, Venezuela. *Interciencia* 28: 463-468.

Ghittino, P., A. Binami, A. Annibali and L. Boni. 1980. Prima segnalazione di grave Oodiniasi in branzini (*Dicentrarchus labrax*) allevati intensivamente in acqua salmastra. *Revista Italiana di Piscicultura e Ittiopatologia* 15: 122-127.

Gibson, W. 2003. Species concepts for trypanosomes: from morphological to molecular definitions? *Kinetoplastid Biology and Disease* 2: 10 (htpp://www.kinetoplastids.com/content/2/1/10).

Gibson, W.C., J. Lom, H. Pecková, V.R. Ferris and P.B. Hamilton. 2005. Phylogenetic analysis of freshwater fish trypanosomes from Europe using SSU rRNA gene sequences and random amplification of polymorphic DNA. *Parasitology* 130: 405-412.

Gratzek, J.B. 1988. Parasites associated with ornamental fish. *Veterinary Clinics of North America, Small Animal Practice (Tropical Fish Medicine)* 18: 375-399.

Grignard, J.C., C. Mélard and P. Kestemont. 1996. A preliminary study of parasites and diseases of perch in intensive culture. *Journal of Applied Ichthyology* 12: 195-199.

Guo, F.C. and P.T.K. Woo. 2004a. Experimental infections of Atlantic salmon *Salmo salar* with *Spironucleus barkhanus*. *Diseases of Aquatic Organisms* 61: 59-66.

Guo, F.C. and P.T.K. Woo. 2004b. Detection and quantification of *Spironucleus barkhanus* in experimentally infected Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 61: 175-178.

Hamers, R. 1994. Studies on carp lymphoid cells from kidney, peripheral blood and spleen stimulated *in vitro* with blood parasites and super-antigen. *Bulletin of the European Association of Fish Pathologists* 14: 191-194.

Hamers, R. and R. Goerlich. 1996. Flow cytometric DNA analysis of the haematopoietic tissue of carp *Cyprinus carpio* during experimental infection with the haemoflagellate *Trypanoplasma borreli*. *Diseases of Aquatic Organisms* 24: 129-134.

Hamilton, P.B., J.R. Stevens, M.W. Gaunt, J. Gidley and W.C. Gibson. 2004. Trypanosomes are monophyletic: Evidence from genes for glyceraldehyde phosphate dehydrogenase and small subunit ribosomal RNA. *International Journal for Parasitology* 34: 1393-1404.

Hannaert, V., F. Bringaud, F.R. Opperdoes and P.A.M. Michels. 2003. Evolution of energy metabolism and its compartimentation in Kinetoplastida. *Kinetoplastid Biology and Disease* 2: II. http://www.kinetoplastids.com/content/2/I/II.

Haussmann, K., N. Hülsmann and R. Radek. 2003. *Protistology*. Third Edition. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, Germany.

Holten, K., B. Skjelstad, A. Aunsmo and H.R. Skjelstad. 2003. Effekt av Detarox ®AP mot ichthyobodose (*Ichthyobodo necator*). *Nosrk Veterinaertidsskrifft* 115: 719-723.

Honigberg, B.M. 1963. A contribution to systematics of the non-pigmented flagellates. In: *Progress in Protozoology*, J. Ludvik, J. Lom and J. Vavra (eds.). Academic Press, New York, p. 68.

Hsu, J.P., S.Y. Huang, T.F. Peng and H.H. Hung. 1995. Case report: Trypanosomiasis in cultured freshwater fish (*Cichlasoma managuense*). *Journal of the Chinese Society of Veterinary Science* 21: 111-116.

Hughes, K.P. and S.A. Smith. 2003. Common and emerging diseases in commercially cultured summer flounder, *Paralichthys dentatus*. *Journal of Applied Aquaculture* 14: 163-178.

Islam, A.K.M.N. and P.T.K. Woo. 1991. Anemia and its mechanism in goldfish *Carassius auratus* infected with *Trypanosoma danylewski*. *Diseases of Aquatic Organisms* 11: 37-43.

JiannHsiung, W., L. JieLin, C. HongYou, W. ChenTain, W. ChenHen and T. KueiHsiung. 2001. A survey of diseases in cultured brood grey mullet (*Mugil cephalus* Linnaeus) in the Yun-Chia-Nan area of Taiwan. *Journal of the Chinese Society of Veterinary Science* 27: 89-93.

Jirků, M., A.A. Kolesnikov, O. Benada and J. Lukeš. 1995. Marine fish and ray trypanosomes have large kinetoplast minicircle DNA. *Molecular and Biochemical Parasitology* 73: 279-283.

Joerink, M., M. Forlenza, C.M.S. Ribeiro, B.J. de Vries, H.F.J. Savelkoul and G.F. Wiegertjes. 2006. Differential macrophage polarisation during parasite infections in common carp (*Cyprinus carpio* L.). *Fish and Shelfish Immunology* 21: 561-571.

Jones, S.R., M. Palmen and W.B. van Muiswinkel. 1993. Effects of inoculum route and dose on the immune response of common carp, *Cyprinus carpio* to the blood parasite, *Trypanoplasma borreli*. *Veterinary Immunology and Immunopathology* 36: 369-378.

Jones, S.R., G.F. Wiegertjes and W.B. van Muiswinkel. 1995. Modulation of carp (*Cyprinus carpio*) cellular immune function *in vitro* caused by the blood parasite, *Trypanoplasma borreli* Laveran and Mesnil. *Fish and Shellfish Immunology* 5: 381-383.

Jørgensen, A. and E. Sterud. 2004. SSU rRNA gene sequence reveals two genotypes of *Spironucleus barkhanus* (Diplomonadida) from farmed and wild Arctic charr *Salvelinus alpinus*. *Diseases of Aquatic Organisms* 62: 93-96.

Jørgensen, A. and E. Sterud. 2006. The marine pathogenic genotype of *Spironucleus barkhanus* from farmed salmonids redescribed as *Spironucleus salmonicide* n. sp. *Journal of Eukaryotic Microbiology* 53: 531-541.

Kallinikova, V.D. and T.Yu. Kondratieva. 2000. Nucleo-kinetoplast interactions and variability in the kinetoplastida. *Protistology* 1: 152-160.

Karlsbakk, E. 2004. A trypanosome of Atlantic cod, *Gadus morhua* L., transmitted by the marine leech *Calliobdella nudulifera* (Malm, 1863) (Piscicolidae). *Parasitology Research* 93: 155-158.

Katz, M. 1951. Two new hemoflagellates (genus *Cryptobia*) from some western Washington teleosts. *Journal of Parasitology* 37: 245-250.

Keeling, P.J. and W.F. Doolittle. 1996. Alpha-tubulin from early-diverging eukaryotic lineages and the evolution of the tubulin family. *Molecular Biology and Evolution* 13: 1297-1305.

Keeling, P.J. and W.F. Doolittle. 1997. Widespread and ancient distribution of noncanonical genetic code in diplomonads. *Molecular Biology and Evolution* 14: 895-901.

Keller, C.J. *Amyloodinium* in Elasmobranchs. *31st Eastern Fish Health Workshop, Mt. Pleasant, South Carolina, 27-31 March 2006*, p. 18.

Kent, M.L., J. Ellis, J.W. Fournie, S.C. Dawe, J.W. Bagshaw and D.J. Whitaker. 1992. Systemic hexamitid (Protozoa: Diplomonadida) infection in seawater pen-reaed chinook salmon *Oncorhynchus tshawytscha*. *Diseases of Aquatic Organisms* 14: 81-89.

Khan, R.A. 1972. On a trypanosome from Atlantic cod, *Gadus morhua*. *Canadian Journal of Zoology* 50: 1051-1054.

Khan, R.A. 1976. The life cycle of *Trypanosoma murmanensis* Nikitin. *Canadian Journal of Zoology* 54: 1840-1849.

Khan, R.A. 1977. Susceptibility of marine fish to trypanosomes. *Canadian Journal of Zoology* 55: 1235-1241.

Khan, R.A. 1985. Pathogenesis of *Trypanosoma murmanensis* in marine fish of the northwestern Atlantic following experimental transmission. *Canadian Journal of Zoology* 63: 2141-2144.

Khan, R.A., M. Barrett and J. Murphy. 1980. Blood parasites of fish from northern Atlantic Ocean. *Canadian Journal of Zoology* 58: 770-781.

Khan, R.A., V. Lobos, F. Garcias, G. Muñoz, V. Valdebenito and M. George-Nascimiento. 2001. *Cryptobia neghmei* sp. n. (Protozoa: Kinetoplastida) in two species of flounder, *Paralichthys* spp. (Pisces: Paralichthydae) off Chile. *Revista Chilena de Historia Natural* 74: 763-767.

Koie, M. 1988. Parasites in European eel *Anguilla anguilla* (L.) from Danish freshwater, brackish and marine localities. *Ophelia* 29: 93-118.

Kolesnikov, A.A., M. Jirků, H. Pecková, A. Polák and D.A. Maslov. 1995. Analysis of kinetoplast DNA of freshwater fish trypanosomes. *Folia Parasitologica* 42: 251-254.

Kolisko, M., I.C. Cepicka, V. Hampl, J. Kulda and J. Flegr. 2005. The phylogenetic position of enteromonads: A challenge for the present models of diplomonad evolution. *International Journal of Systematic and Evolutionary Microbiology* 55: 1729-1733.

Kruse, P., D. Steinhagen, W. Korting and K.T. Friedhoff. 1989a. Morphometrics and redescritpion of *Trypanoplasma borreli* Laveran and Mesnil, 1901 (Mastigophora, Kinetoplastida) from experimentally infected common carp (*Cyprinus carpio* L.). *Journal of Protozoology* 36: 408-411.

Kruse, P., D. Steinhagen, W. Korting and K.T. Friedhoff. 1989b. Development of *Trypanoplasma borreli* (Mastigophora, Kinetoplastida) in the leech vector *Piscicola geometra* and its infectivity for the common carp, *Cyprinus carpio*. *Journal of Parasitology* 75: 527-530.

Kulda, J. and E. Nohynková. 1979. Flagellates of the human intestine and of intestine of other species. In: *Parasitic Protozoa*, J.P. Kreier (ed.). Academic Press, New York, Vol. II, pp. 1-138.

Kuperman, B.I. and V.E. Matey. 1999. Massive infestation by *Amyloodinium ocellatum* (Dinoflagellida) of fish in a highly saline lake, Salton Sea, California, USA. *Diseases of Aquatic Organisms* 39: 65-73.

Kuperman, B.I., V.E. Matey and S.B. Barlow. 2002. Flagellate *Crytptobia branchialis* (Bodonida: Kinetoplastida), ectoparasite of tilapia from the Salton Sea. *Hydrobiologia* 437: 93-102.

Landsberg, J.H., K.A. Steidinger, B.A. Blakesley and R.L. Zondervan. 1994. Scanning electron microscope study of dinospores of *Amyllodinium* cf. *ocellatum*, a pathogenic dinoflagellate parasite of marine fish, and comments on its relationship to the Peridiniales. *Diseases of Aquatic Organisms* 20: 23-32.

Laveran, A. and F. Mesnil. 1901. Sur les flagellés à membrane ondulante des poisons (genres *Trypanosoma* Gruby et *Trypanoplasma* n. gen.). *Comptes Rendus Academie des Sciences (Paris)* 133: 670-675.

Lawler, A. 1977. The parasitic dinoflagellate *Amyoodinium ocellatum* in marine aquaria. *Drum and Croaker* 17: 17-20.

Letch, C.A. 1979. Host restriction, morphology and isoenzymes among trypanosomes of some freshwater fishes. *Parasitology* 79: 107-117.

Levy, M.G. and E.J. Noga. 2005. Controlling parasitic dinoflagellates of fish, with special emphasis on molecular genetics and immunity. *Acta Zoologica Sinica* 51: 550-553.

Levy, M.G., M.F. poore, A. Colorni, E.J. Noga, M.W. Van der Sea and R.W. Zitaker. 2007. A highly specific PCR assay for detecting the fish ectoparasite *Amyloodinium ocellatum*. *Diseases of Aquatic Organisms* 73: 219-226.

Li, S. and P.T.K. Woo. 1996. A *Cryptobia salmositica* (Kinetoplastida: Sarcomastigophora) species-specific DNA probe and its uses in salmonid cryptobiosis. *Diseases of Aquatic Organisms* 25: 9-14.

Liao, I.C., T-S. Huang, W-S. Tsai, C-M. Hsueh, S-L. Chang and E.M. Leaño. 2004. Cobia culture in Taiwan: current status and problems. *Aquaculture* 237: 155-165.

Lischke, A., C. Klein, Y-D. Stierhof, M. Hempel, A. Mehlert, I.C. Almeida, M.A.J. Ferguson and P. Overath. 2000. Isolation and characterization of glycosylphosphatidylinositol-anchored mucin-like surface glycoproteins from bloodstream forms of the freshwater-fish parasite *Trypanosoma carassii*. *Biochemical Journal* 345: 693-700.

Litaker, R.W., P.A. Tester, A. Colorni, M.G. Levy and E.J. Noga. 1999. The phylogenetic relationship of *Pfiesteria piscicida*, Cryptoperidiniopsoid sp. *Amyloodinoum ocellatum* and a *Pfiesteria*-like dinoflagellate to other dinoflagellates and apicomplexans. *Journal of Phycology* 35: 1379-1389.

Lom, J. 1979. Biology of trypanosomes and trypanoplasms in fish. In: *Biology of the Kinetoplastida*, W.H.R. Lumsden and D.A. Evans (eds.). Academic Press, London, Vol. 2, pp. 269-337.

Lom, J. 1980. *Cryptobia branchialis* Nie from fish gills: ultrastrucural evidence of ectocommensal function. *Journal of Fish Diseases* 3: 427-436.

Lom, J. 1981. Fish invading dinoflagellates: a synopsis of existing and newly proposed genera. *Folia Parasitologica* 28: 3-11.

Lom, J. and I. Dyková. 1992. Protozoan parasites of fish. *Developments in Aquaculture and Fisheries Science*, Vol. 26, Elsevier, Amsterdam.

Lom, J. and A.R. Lawler. 1973. An ultrastructural study on the mode of attachment in dinoflagellates invading gills of Cyprinodontidae. *Protistologica* 9: 293-309.

Lom, J. and G. Schubert. 1983. Ultrastructural study of *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom, 1981 with special emphasis on its attachement to the fish host. *Journal of Fish Diseases* 6: 411-428.

Lom, J., K. Rhode and I. Dyková. 1993. *Crepidoodinium australe* n. sp., an ectocommensal dinoflagellate from the gills of *Sillago ciliata*, an estuarine fish from the New South Wales coast of Australia. *Diseases of Aquatic Organisms* 15: 63-72.

Lukeš, J., G.J. Arts, J. Van der Burg, A. deHaan, F. Opperdoes, P. Sloof and R. Benne. 1994. Novel patterns of editing regions in mitochondrial transcripts of the cryptobiid *Trypanoplasma borreli*. *EMBO Journal* 13: 5086-5098.

Lukeš, J., D.L. Guilbride, J. Votýpka, A. Zíková, R. Benne and P.T. Englund. 2002. Kinetoplast DNA network: Evolution of an improbable structure. *Euykaryotic Cell* 1: 495-502.

Lukeš, J., H. Hashimi and A. Zíková. 2005. Unexplained complexity of the mitochondrial genome and transcriptome in kinetoplastid flagellates. *Current Genetics* 48: 277-299.

Martins, M.L., J.R.E. Moraes, P.M. Andrade, S.H.C. Schalch and F.R. Moraes. 2001. *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom, 1981 (Dinoflagellida) infection in cultivated freshwater fish from the northeast region of Sao Paulo State, Brazil. Parasitological and pathological aspects. *Brazil Journal of Biology* 61: 639-644.

Maslov, D.A. and L. Simpson. 1994. RNA editing and mitochocrial genomic organization in the cryptobiid kinetoplastid protozoan *Trypanoplasma borreli*. *Molecular and Cellular Biology* 14: 8174-8182.

Maslov, D.A., S.S. Podlipaev and J. Lukeš. 2001. Phylogeny of the Kinetoplastida: Taxonomic problems and insights into the evolution of parasitism. *Memorias do Instituto Oswaldo Cruz* 96: 397-402.

Mo, T.A., T.T. Poppe and L. Iversen. 1990. Systemic hexamitosis in salt-water reared Atlantic salmon. *Bulletin of the European Association of Fish Pathologists* 10: 69-70.

Molnár, K. 1971. Protoozoan diseases of the fry of herbivorous fishes. *Acta Veterinaria Academiae Scientiarum Hungaricae* 21: 1-14.

Molnár, K. 1974. Data on 'Octomitosis' (Spironucleosis) of cyprinids and aquary fishes. *Acta Veterinaria Academiae Scientiarum Hungaricae* 24: 99-106.

Moore, E. 1922. *Octomitus salmonis*, a new species of intestinal parasite in trout. *Transactions of the American Fisheries Society* 52: 74-97.

Moreira, D., P. López-García and K. Vickerman. 2004. An updated view of kinetoplstid phylogeny using environmental sequences and a closer outgroup: Proposal for a new classification of the class Kinetoplastea. *International Journal of Systematic and Evolutionary Microbiology* 54: 1861-1875.

Montgomery-Brock, D., V.T. Sato, J.A. Brock and C.S. Tamaru. 2001. The application of hydrogen peroxide as a treatment for the ectoparasite *Amyloodinium ocellatum* (Brown, 1931) on the Pacific threadfin *Polydactylus sexfilis*. *Journal of the World Aquaculture Society* 32: 250-254.

Morrison, C.M. and D. Cone. 1986. A possible marine form of *Ichthyobodo* sp. on haddock, *Melanogrammus aeglefinus* (L.) in the north west Atlantic Ocean. *Journal of Fish Diseases* 9: 141-142.

Negm-Eldin, M.M. 1997. *Trypanosoma mukasai* (Hoare, 1932) in its biological vector *Batrachodeloides tricarinata* (Blanchard, 1897) and their life cycles. *DeutscheTierärztliche Wochenschrift* 104: 215-219.

Newman, M.W. 1978. Pathology associated with *Cryptobia* infection in a summer flounder (*Paralichthys dentatus*). *Journal of Wildlife Diseases* 14: 299-304.

Noga, E.J. 1987. Propagation in cell culture of the dinoflagllate *Amyloodinium*, an ectoparasite of marine fish. *Science* 236: 1302-1304.

Noga, E.J. 1992. Immune response to ectoparasitic protozoa: The infectivity assay. In: *Techniques in Fish Immunology*, J.S. Stolen, T.C. Fletcher, D.P. Anderson, S.L. Kaattari and A.F. Rowley (eds.). SOS Publications, Fair Haven, New Jersey, Vol. 2, pp. 167-176.

Noga, E.J. and C.E. Bower. 1987. Propagation of the marine dinoflagllate *Amyloodinium ocellatum* in germ-free conditions. *Journal of Parasitology* 73: 924-928.

Noga, E.J. and M.G. Levy. 1999. Dinoflagellida (Phylum Sarcomastigophora). In: *Fish Diseases and Disorders*, P.T.K Woo (ed.). CABI Publishing, Wallingford, Oxon, U.K., Vol. 1, pp. 1-25.

Noga, E.J., S.A. Smith and J.H. Landsberg. 1991. Amyloodiniosis in cultured hybrid striped bass (*Morone saxatilis* × *M. chrysops*) in North Carolina. *Journal of Aquatic Animal Health* 3: 294-297.

Noga, E.J., Z. Fan and U. Silphaduang. 2001. Histone-like proteins from fish are lethal to the parasitic dinoflagellate *Amyloodinium ocellatum*. *Parasitology* 123: 57-65.

Noga, E.J., Z. Fan and U. Silphaduang. 2002. Host site of activity and cytological effects of histone-like proteins on the parasitic dinoflagellate *Amyloodinium ocellatum*. *Diseases of Aquatic Organisms* 52: 207-215.

Nohynková, E. 1984. A new pathogenic *Cryptobia* from freshwater fishes: a light and electron microscope study. *Protistologica* 20: 181-195.

Oestmann, D.J. and D.H. Lewis. 1996a. Effects of 3,N-methylglucamine lasalocid on *Amyloodinium ocellatum*. *Diseases of Aquatic Organisms* 24: 179-184.

Oestmann, D.J. and D.H. Lewis. 1996b. Improved cell culture propagation of *Amyloodinium ocellatum*. *Diseases of Aquatic Organisms* 24: 173-178.

Oestmann, D.J., D.H. Lewis and B.A. Zettler. 1995. Clearance of *Amyloodinium ocellatum* dinospores by *Artemia salina*. *Journal of Aquatic Animal Health* 7: 257-261.

Oldach, D.W., C.F. Delwiche, K.S. Jacobsen, T. Tengs, E.G. Brown, J.W. Kempton, E.F. Schaefer, H.A. Bowers, H.B. Glasgow, Jr, J.A.M. Burkholder, K.A. Steidinger and P.A. Rublee. 2000. Heteroduplex mobility assay-guided sequence discovery: Elucidation of the small subunit (18S) rDNA sequences of *Pfiesteria piscicida* and related dinoflagellates from complex and algal culture and environmental sample DNA pools. *Proceedings of the National Academy of Sciences of the United States of America* 97: 4303-4308.

Ostrowski, A.C. and A. Molnár. 1998. Pacific threadfin *Polydactylus sexfilis* (moi) hatchery manual. *Publication Number 132, Center for Tropical and Subtropical Aquaculture, Waimanalao, Hawai, USA.*

Overath, P., J. Ruoff and Y.-D. Stierhof. 1998. Cultivation of bloodstream forms of *Trypanosoma carassii*, a common parsite of freshwater fish. *Parasitology Research* 84: 343-347.

Overath, P., J. Haag, M.G. Mameza and A. Lischke. 1999. Freshwater fish trypanosomes: definition of two types, host control by antibodies and lack of antigenic variation. *Parasitology* 119: 591-601.

Overath, P., J. Haag, A. Lischke and C. O'hUigin. 2001. The surface structure of trypanosomes in relation to their molecular phylogeny. *International Journal for Parasitology* 31: 468-471.

Paperna, I. 1980. *Amyloodinium ocellatum* (Brown, 1931) (Dinoflagellida) infestations in cultured marine fish at Eilat, Red Sea: Epizootiology and pathology. *Journal of Fish Diseases* 3: 363-372.

Paperna, I. 1984a. Reproduction cycle and tolerance to temperature and salinity of *Amyloodinium ocellatum* (Brown, 1931) (Dinoflagellida). *Annals de Parasitologie Humaine et Comparée* 59: 7-30.

Paperna, I. 1984b. Chemical control of *Amyloodinium ocellatum* (Brown, 1931) (Dinoflagellida) infections: *In vitro* tests and treatment trials with infected fishes. *Aquaculture* 38: 1-18.

Paperna, I. and F. Baudin-Laurencin. 1979. Parasitic infections of the sea bass *Dicentrarchus labrax* and the gilt-head seabream *Sparus auratus* in mariculture facilities in France. *Aquaculture* 16: 173-175.

Paperna, I. and R.M. Overstreet. 1981. Parasites and diseases of mullets (Mugilidae). In: *Aquaculture of Grey Mullets*, O.H. Oren (ed.). *International Biological Programme* 26. Cambridge University Press, pp. 411-493.

Paterson, W.B. and P.T.K. Woo. 1983. Electron microscopic observations on the bloodstream form of *Cryptobia salmositica* Katz, 1951 (Kinetoplastida: Bodonida). *Journal of Protozoology* 30: 431-437.

Paterson, W.B. and P.T.K. Woo. 1984. Ultrastructural studies on mitosis in *Trypanosoma danilewski* (Mastigophora, Zoomastigophora). *Canadian Journal of Zoology* 62: 1167-1171.

Paull, G.C. and R.A. Matthews. 2001. *Spironucleus vortens*, a possible casuse of hole-in-the-head disease in cichlids. *Diseases of Aquatic Organisms* 45: 197-202.

Pecková, H. and J. Lom. 1990. Growth, division and morphology of flagellates of the genus *Trypanoplasma* (Protozoa, Kinetoplastida) in the *in vitro* culture. *Parasitology Research* 76: 553-558.

Pereira-Chioccola, V.L., A. Acosta-Serrano, I. Correia de Almeida, M.A. Ferguson, T. Souto-Padron, M.M. Rodriguez, L.R. Travassos and S. Shenackman. 2000. Mucin-like molecules from a negatively charged coat that protects *Trypanosoma cruzi* trypomastigotes from killing by human anti-alpha-galactosyl antibodies. *Journal of Cell Science* 113: 1299-1307.

Pérez, A.C.A. de and C.S.R. Mainardes Pinto. 1998. Ocorrência de *Ichthyobodo necator* (Henneguy, 1883) em alevinos de pacu *Piaractus mesopotamicus* (Homberg, 1887) de criatório. *Revista Brasileira de Medicina Veterinária* 20: 58-59.

Plouffe, D.A. and M. Belosevic. 2004. Enzyme treatment of *Trypanosoma danilewskyi* (Laveran & Mesnil) increases its susceptibility to lysis by the alternative complement pathway of goldfish, *Carassius auratus* (L.). *Journal of Fish Diseases* 27: 277-285.

Plumb, J.A. 1991. Major diseases of stripped bass and redfish. *Veterinary and Human Toxicology* 33, Supplement 1: 34-39.

Poppe, T.T. and T.A. Mo. 1993. Systemic, granulomatous hexamitosis of farmed Atlantic salmon: interaction with wild fish. *Fisheries Research* 17: 147-152.

Poppe, T.T., T.A. Mo and L. Iversen. 1992. Disseminated hexamitosis in sea-caged salmon *Salmo salar*. *Diseases of Aquatic Organisms* 14: 91-97.

Poynton, S.L. and C. Morrison. 1990. Morphology of diplomand flagellates: *Spironucleus torosa* n. sp. from Atlantic cod *Gadus morhua* L. and haddock *Melanogrammus aeglefinus* (L.) and *Hexamita salmonis* Moore from brooktrout *Salvelinus fontinalis* (Mitchill). *Journal of Protozoology* 37: 369-383.

Poynton, S.L. and E. Sterud. 2002a. Guidelines for species descriptions of diplomonad flagellates from fish. *Journal of Fish Diseases* 25: 15-31.

Poynton, S.L. and E. Sterud. 2002b. *Spironucleus vortens* (Diplomonadida) in the ide, *Leciscus idus* (L.) (Cyprinidae): A warm water hexamitid flagellate found in Northern Europe. *Journal of Eukaryotic Microbiology* 49: 137-145.

Poynton, S.L., W. Fraser, R. Francis-Floyd, P. Rutledge, P. Red and T.A. Nerad. 1995. *Spironucleus vortens* n. sp. from the freshwater angelfish *Pterophyllum scalare*: morphology and culture. *Journal of Eukaryotic Microbiology* 42: 731-742.

Poynton, S.L., M.R.S. Fard, J. Jenkins and H.W. Ferguson. 2004. Ultrastructure of *Spironucleus salmonis* n. comb. (formerly *Octomitus salmonis* sensu Moore 1922, Davis 1926, and *Hexamita salmonis* sensu Ferguson 1979), with a guide to *Spironucleus* species. *Diseases of Aquatic Organisms* 60: 49-64.

Putz, R.E. 1972. Biological studies on the hemoflagellates *Cryptobia cataractae* and *Cryptobia salmositica*. *Technical Paper Bureau of Sport Fishery and Wildlife* 63: 3-25.

Qadri, S.S. 1962. An experimental study of the life cycle of *Trypanosoma danilewskyi* in the leech, *Hemiclepsis margianata*. *Journal of Protozoology* 9: 254-258.

Ramos, P. and J.M. Oliveira. 2001. Amiloodiniose em pregado, *Psetta maxima* (L.). *Revista Portuguesa de Ciencias Veterinárias* 96: 201-205.

Ranzani-Paiva, M.J.T., C.M. Ishikawa, B.S-S. de Campos and A.C. Eiras. 1997. Haematological characteristics associated with parasitism in mullets, *Mugil platanus* Günther, from the estuarine region of Cananéia, São Paulo, Brazil. *Revista Brasileria de Zoologia* 14: 329-339.

Rauthan, J.V.S., S.P. Grover and P. Jaiwal. 1995. Studies on some haematological changes in a hill stream fish *Barilius bendelisis* (Hamilton) infected with trypanosomes. *Flora and Fauna (Jhansi)* 1: 165-166.

Rigos, G., P. Christophilogiannis, M. Giahnishi, A. Andriopoulou, M. Koutsodimoy, I. Nengas and M. Alexis. 1998. *Amyloodinium ocellatum* infestation of sharpsnout seabream, *Puntazzo puntazzo* Cetti. *Bulletin of the European Association of Fish Pathologists* 18: 198-200.

Roberts-Thomson, A., A. Barnes, D.S. Fielder, R.J.G. Lester and R.D. Adlard. 2006. Aerosol dispersal of the fish pathogen, *Amyloodinium ocellatum*. *Aquaculture* 257: 118-123.

Rozario, C., L. Morin, A.J. Roger, M.W. Smith and M. Müller. 1996. Primary structure and phgylogentic relationships of glyceraldehyde-3-phosphate dehydrogenase genes of free-living and parasitic diplomonad flagellates. *Journal of Eukaryotic Microbiology* 43: 330-340.

Saeij, J.P.J., W.B. Van Muiswinkel, A. Groeneveld and G.F. Wiegertjes. 2002. Immune modulation by fish kinetoplastid parasites: a role for nitric oxid. *Parasitology* 124: 77-86.

Saeij, J.P.J., B.J. de Vries and G.F. Wiegertjes. 2003a. The immune response of carp to *Trypanoplasma borreli*: Kinetics of immune gene expression and polyclonal lymphocyte acivation. *Developmental and Comparative Immunology* 27: 859-874.

Saeij, J.P.J., R.J.M. Stet, B.J. de Vries, W.B. van Muiswinkel and G.F. Wiegertjes. 2003b. Molecular and functional characterization of carp TNF: A link between TNF polymorphism and trypanotolerance? *Developmental and Comparative Immunology* 27: 29-41.

Saha, S., H. Sumit and B. Samir. 1997. Natural prevalence of *Trypanosma mukundi* in *Heteropneustes fossilis* (Bloch) and related haematological analysis. *Journal of Aquaculture in the Tropics* 12: 79-87.

Sangmaneedet, S. and S.A. Smith. 1999. Efficacy of various chemotherapeutic agents on the growth of *Spironucleus vortens*, an intestinal parasite of the freshwater angelfish. *Diseases of Aquatic Organisms* 38: 47-52.

Sangmaneedet, S. and S.A. Smith. 2000. *In vitro* studies on optimal requirements for the growth of *Spironucleus vortens*, an intestinal parasite of the freshwater angelfish. *Diseases of Aquatic Organisms* 39: 135-141.

Saraiva, A. and J.C. Eiras. 1996. Parasite community of european eel *Anguilla anguilla* L. in the river Este, Northern Portugal. *Research and Reviews in Parasitology* 56: 179-183.

Schäperclaus, W. 1951. Der *Colisa*-Parasit, ein neuer Krankheitserreger bei Aquarienfischen. *Aquarien Terrarien* Z. 4: 169-171.

Scharsack, J.P., D. Steinhagen, W. Korting, W. Leibold and J. Schuberth. 2000. Flow cytometric analysis of proliferative responses of carp *Cyprinus carpio* peripheral blood leukocytes to mitogens and to the hemoflagellate *Trypanoplasma borreli*. *Diseases of Aquatic Organisms* 41: 203-210.

Scharsack, J.P., D. Steinhagen, C. Kleczka, J.O. Schmidt, W. Korting, W. Leibold and H.J. Schuberth. 2003a. Head kidney neutrophils of carp (*Cyprinus carpio* L.) are functionally modulated by the haemoflagellate *Trypanoplasma borreli*. *Fish and Shellfish Immunology* 14: 389-403.

Scharsack, J.P., D. Steinhagen, C. Kleczka, J.O. Schmidt, W. Korting, W. Leibold and H.J. Schuberth. 2003b. The haemoflagellate *Trypanoplasma borreli* induces the production of nitric oxide, which is associated with modulation of carp (*Cyprinus carpio* L.) leucocyte functions. *Fish and Shellfish Immunology* 14: 207-222.

Scharsack, J.P., D. Steinhagen, W. Körting, B. Wagner, W. Leibold and H.J. Schuberth. 2004. Some immune parameters in carp *Cyprinus carpio* susceptible and resistant to the haemoflagellate *Trypanoplasma borreli*. *Diseases of Aquatic Organisms* 60: 41-48.

Shaharom-Harrison, F.M., I.G. Anderson, A.Z. Siti, N.A.M. Shazili, K.J. Ang and T.I. Azmi. 1990. Epizootics of Malaysian cultured freshwater pond fishes by *Piscinoodinium pillulare* (Schäperclaus 1954) Lom 1981. *Aquaculture* 86: 127-138.

Shanavas, K.R. 1991. *Tripanosoma punctati* Hasan and Quasim, 1962 from *Channa punctatus* Bloch in Kerala, India, with observations on its vector-phase development and transmision. *Archiv für Protistenkunde* 140: 201-208.

Siddal, M.E., H. Hong and S.S. Desser. 1992. Phylogeneteic analysis of the Diplomonadida (Wenyon, 1926) Brugerolle, 1975: evidence for heterochrony in protozoa and against *Giardia lamblia* as a 'missing link'. *Journal of Protozoology* 39: 361-367.

Simpson, A.G.B. 2003. Cytoskeletal organization, phylogenetic affinities and systematics in the contentious taxon Excavata (Eukaryota). *International Journal of Systematic and Evolutionary Microbiology* 53: 1759-1777.

Simpson, A.G.B., E.E Gill, H.A. Callahan, R.W. Litaker and A.J. Roger. 2004. Early evolution within kinetoplastids (Euglenozoa), and the late emergence of trypanosomatids. *Protist* 155: 407-422.

Simpson, A.G.B., Y. Inagaki and A.J. Roger. 2006a. Comprehensive multigene phylogenies of excavate protists reveal the evolutionary positions of primitive eukaryotes. *Molecular Biology and Evolution* 23: 615-625.

Simpson, A.G.B., J.R. Stevens and J. Lukeš. 2006b. The evolution and diversity of kinetoplastid flagellates. *Trends in Parasitology* 22: 168-174.

Siti, A.Z. and L.A. Rokiah. 1988a. Treatment of *Puntius gonionotus* (Bleeker) fry infested with *Piscinoodinium pillulare* with emphasis on use of $CuSo_4$. In: *Maximising Livestock Productivity*, J. Vadiveloo and C.C. Wong (eds.). *Proceedings of the 11th Annual*

*Conference Malaysian Society of Animal Production, 19-30 March 1988*, No. 11, pp. 65-78.

Siti, A.Z. and L.A. Rokiah. 1988b. Behavioural and morphological characteristics in *Puntius gonionotus* (Bleeker) with *Piscinoodinium pillulare* infestation. In: *Maximising Livestock Productivity*, J. Vadiveloo and C.C. Wong (eds.). *Proceedings of the 11th Annual Conference Malaysian Society of Animal Production, 19-30 March 1988*, No. 11, pp. 69-72.

Sitjà-Bobadilla, A. and P.T.K. Woo. 1994. An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against the pathogenic haemoflagellate, *Cryptobia salmositica* Katz, and protection against cryptobiosis in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum) inoculated with a live vaccine. *Journal of Fish Diseases* 17: 399-408.

Smit, N.J., A.J. Davies and J.G. Van As. 2000. A tryponosome from silver catfish (*Schilbe intermedius*) in the Okavango Delta, Botswana. *Bulletin of the European Association of Fish Pathologists* 20: 116-119.

Smit, N.J., J.G. Van As and A.J. Davies. 2004. Fish trypanosomes from the Okavango Delta, Botswana. *Folia Parasitologica* 51: 299-303.

Smith, S.A., M.G. Levy and E.J. Noga. 1992. Development of an enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to the parasitic dinoflagellate *Amyloodinium ocellatum* in *Oreochromis aureus*. *Veterinary Parasitology* 42: 145-155.

Smith, S.A., M.G. Levy and E.J. Noga. 1994. Detection of anti-*Amyloodinium ocellatum* antibody from cultured hybrid striped bass (*Morone saxatilis* × M. *chrysops*) during an epizootic of Amyloodiniosis. *Journal of Aquatic Animal Health* 6: 79-81.

Steinhagen, D., P. Kruse and W. Körting. 1990. Some hematological observations on carp, *Cyprinus carpio* L., experimentally infected with *Trypanoplasma borreli* Laveran and Mesnil 1901 (Protozoa: Kinetoplastida). *Journal of Fish Diseases* 13: 157-162.

Steinhagen, D., M. Biffar and W. Korting. 1999. A dinoflagellate parasite from tropical fish. *Bulletin of the European Association of Fish Pathologists* 19: 24-27.

Steinhagen, D., W. Hedderich, A. Skouras, J.P. Scharsack, J. Schuberth, W. Leibold and W. Korting. 2000. *In vitro* cultivation of *Trypanoplasma borreli* (Protozoa: Kinetoplastida), a parasite from the blood of common carp *Cyprinus carpio*. *Diseases of Aquatic Organisms* 41: 195-201.

Sterud, E. 1998a. Ultrastructure of *Spironucleus torosa* Poynton & Morrison, 1990 (Diplomonadida: Hexamitidae) in cod *Gadus morhua* and shaite *Pollachius virens* from southeastern Norway. *European Journal of Protistology* 34: 69-77.

Sterud, E. 1998b. Electron microscope identification of the flagellate *Spironucleus torosa* (Hexamitidae) brom burbot *Lota lota* (Gadidae) with comments upon its probable introduction to this freshwater host. *Journal of Parasitology* 84: 947-953.

Sterud, E. 1998c. *In vitro* cultivation and temperature-dependent growth of two strains of *Spironucleus barkhanus* (Diplomonadida: Hexamitidae) from Atlantic salmon *Salmo salar* and grayling *Thymallus thymallus*. *Diseases of Aquatic Organisms* 33: 57-61.

Sterud, E., T.A. Mo and T.T. Poppe. 1997. Ultrastructure of *Spironucleus barkhanus* n. sp. (Diplomonadida: Hexamitida) from grayling *Thymallus thymallus* (L.) (Salmonidae)

and Atlantic salmon *Salmo salar* L. (Salmonidae). *Journal of Eukaryotic Microbiology* 44: 399-407.

Sterud, E., T.A. Mo and T.T. Poppe. 1998. Systemic spironucleosis in sea-farmed Atlantic salmon *Salmo salar*, caused by *Spironucleus barkhanus* transmitted from feral Artic char *Salvelinus alpinus*? *Diseases of Aquatic Organisms* 33: 63-66.

Sterud, E., T.T. Poppe and G. Borno. 2003. Intracellular infection with *Spironucleus barkhanus* (Diplomonadidi: Hexamitidae) in farmed Artic char *Salvelinus alpinus*. *Diseases of Aquatic Organisms* 56: 155-161.

Strout, R.G. 1965. A new hemoflagellate (genus *Cryptobia*) from marine fishes of northern New England. *Journal of Parasitology* 51: 654-659.

Svobodova, Z. and J. Kolarova. 2004. A review of the diseases and contaminant related mortalities of tench (*Tinca tinca* L.). *Veterinary Medicine-Czech* 49: 19-34.

Székely, C. and K. Molnár. 1996/97. Preliminary survey of the parasite fauna of some important fish specis in the Upper-Reservoir of the Kis-Balaton system. *Parasitologia Hungarica* 29/30: 45-54.

TaiWei, C., H. TsaiChuan, C. ChiungHsiang, W. TzuHsiung, H. ChaoTsung, K. ChinHsien and C. PehHeng 2001. Case report: An outbreak of amyloodiniosis in puffer (*Takifugu rubripes*) in Taiwan. *Journal of Chinese Society of Veterinary Science* 27: 143-147.

Tavares-Dias, M., M.L. Martins and F.R. Moraes. 2001. Fauna parasitária de peixes oriundos de pesque-pague do municipio de Franca, São Paulo, Brasil. I. Protozoarios. *Revista Brasileira de Zoologia* 18: 67-79.

Thatcher, V.E. and J. Brites Neto. 1994. Diagnóstico, prevenção e tratamento das enfermidades de peixes neotropicais de água doce. *Revista Brasileira de Medicina Veterinaria* 16: 111-127.

Thilakaratne, I.D.S.I.P., G. Rajapaksha, A. Hewakopara, R.P.V.J. Rajapakse and A.C.M. Faizal. 2003. Parasitic infections in freshwater ornamental fish in Sri Lanka. *Diseases of Aquatic Organisms* 54: 157-162.

Thomas, P.T. and P.T.K. Woo. 1988. *Cryptobia salmositica*, *in vitro* and *in vivo* study on the mechanism of anaemia in infected rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 11: 425-431.

Thorarinsson, R. and D.B. Powell. 2006. Effects of disease risk, vaccine efficacy, and market price on the economics of fish vaccination. *Aquaculture* 256: 42-49.

Todal, J.A., E. Karlsbakk, T.E. Isaksen, H. Plarre, S. Urawa, A. Mounton, E. Hoel, C.W.R. Koren and A. Nylund. 2004. *Ichthyobodo necator* (Kinetoplastida). A complex of sibling species. *Diseases of Aquatic Organisms* 58: 9-16.

Tojo, J.L. and M.T. Santamarina. 1998a. Oral pharmacological treatments for parasitic diseases of rainbow trout *Oncorhynchus mykiss*. I: *Hexamita salmonis*. *Diseases of Aquatic Organisms* 33: 51-56.

Tojo, J.L. and M.T. Santamarina. 1998b. Oral pharmacological treatments for parasitic diseases of rainbow trout *Oncorhynchus mykiss*. III: *Ichthyobodo necator*. *Diseases of Aquatic Organisms* 33:195-199.

Tojo, J.L., M.T. Santamarina, J. Leiro, F.M. Ubeira and M.L. Sanmartín. 1994. Pharmacological treatments against *Ichthyobodo necator* (Henneguy, 1883) in rainbow trout *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 17: 135-143.

Tokşen, E. 2000. First detection of *Ichthyobodo* spp. infection and its treatment in a seabream (*Sparus aurata* L.) farm in Izmir. *Türkiye Parazitoloji Dergisi* 24: 321-325.

Uldal, A. and K. Buchmann. 1996. Parasite host relations. *Heamita salmonis* in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 25: 229-231.

Urawa, S. 1996. Improvement of the marine survival of chum salmon by the control of protozoan infections. *Bulletin Natural Research Institute Aquaculture* Supplement 2: 1-4.

Urawa, S. and M. Kusakari. 1990. The survivability of the ectoparasitic flagellate *Ichthyobodo necator* on chum salmon fry (*Oncorhynchus keta*) in sea water and comparison to *Ichthyobodo* sp. on Japanese flounder (*Paralichthys olivaceus*). *Journal of Parasitology* 76: 33-40.

Urawa, S., N. Ueki and E. Karlsbakk. 1998. A review of *Ichthyobodo* infection in marine fishes. *Fish Pathology* 33: 311-320.

Uzman, J.R. and S.H. Hayduk. 1963. *In vitro* culture of the flagellate protozoan *Hexamita salmonis*. *Science* 140: 290-292.

Vickerman, K. 1976. The diversity of kinetoplastid flagellates. In: *Biology of Kinetopolastida*, W.H.R. Lumdsen and D.E. Evans (eds.). Academic Press, London, pp. 1-34.

Vickerman, K. 1990a. Phylum Zoomastigina: Class Diplomonadida. In: *Handbook of Protoctista*, L. Margulis, J.O. Corliss, M. Melkonian and D.J. Chapman (eds.). Jones & Bratlett, Boston, pp. 167-185.

Vickerman, K. 1990b. Phylum Zoomastigina: Class Kinetoplastida. In: *Handbook of Protoctista*, L. Margulis, J.O. Corliss, M. Melkonian and D.J. Chapman (eds.). Jones & Bratlett, Boston, pp. 200-210.

Vickerman, K. 2000. Order Kinetoplastea Honigberg, 1963. In: *The Illustrated Guide to the Protozoa*, J.J. Lee, G.F. Leedale and P. Bradbury (eds.). Society of Protozoologists, Lawrence, Kansas, pp. 1159-1185.

von der Heyden, S. and T. Cavalier-Smith. 2005. Culturing and environmental DNA sequencing uncover hidden kinetoplastid biodiversity and a major marine clade within ancestrally freshwater *Neobodo designis*. *International Journal of Systematic and Evolutionary Microbiology* 55: 2605-2621.

von der Heyden, S., E.E. Chao, K. Vickerman and T. Cavalier-Smith. 2004. Ribosomal RNA phylogeny of bodonid and diplonemid flagellates and the evolution of Euglenozoa. *Journal of Eukaryotic Microbiology* 51: 402-416.

Wang, R. and M. Belosevic. 1994. Cultivation of *Trypanosma danilewskyi* (Laveran & Mesnil, 1904) in serum-free medium and assessment of the course of infection in goldfish, *Carassius auratus* (L.). *Journal of Fish Diseases* 17: 47-56.

Wenyon, C.M. 1926. *Protozoology. A Manual for Medical Men, Veterinarians and Zoologists*. Vol. I, Bailliere, Tindall & Cox, London.

Wierzbicka, J. and T. Orecka-Garbda. 1994. Protoozoans parasitic on *Anguilla anguilla* (L.) from the Szczecin Lagoon and River Odra mouth. *Acta Ichthyologica et Piscatoria* 24: 3-11.

Wiegertjes, G.F., R.J. Stet and W.B. van Muiswinkel. 1995. Invetigations into the ubiquitous nature of high or low immune responsiveness after divergent selection for

antibody production in common carp (*Cyprinus carpio* L.). *Veterinary Immunology and Immunopathology* 48: 355-366.

Wita, I. and M. Ovcharenko. 1997. Occurrence of trypanosomes, *Trypanosoma carassii*, in blood of roach *Rutilus rutilus* and tench *Tinca tinca*. *Wiadomości Parazytologiczne* 43: 385-392.

Woo, P.T.K. 1969. The haematocrit centrifuge for the detection of trypanosomes in blood. *Canadian Journal of Zoology* 47: 921-923.

Woo, P.T.K. 1981a. *Trypanosoma danilewskyi*, a new multiplication process for *Trypanosoma* (Protozoa, Kinetoplastida). *Journal of Parasitology* 67: 522-526.

Woo, P.T.K. 1981b. Acquired immunity against *Trypanosoma danilewskyi* in goldfish *Carassius auratus*. *Parasitology* 83: 343-346.

Woo, P.T.K. 1990. MISET, an immunological technique for the serodiagnosis of *Cryptobia salmositica* (Sarcomastigophora, Kinetoplastida) infection in *Oncorhynchus mykiss*. *Journal of Parasitology* 76: 389-393.

Woo, P.T.K. 1994. Flagellate parasites of fishes. In: *Parasitic Protozoa*, J.P. Kreier (ed.). Second Edition, Academic Press, London, Vol. 3, pp. 1-80.

Woo, P.T.K. 2001. Cryptobiosis and its control in North American fishes. *International Journal for Parasitology* 31: 566-574.

Woo, P.T.K. 2003. *Cryptobia (Trypanoplasma) salmositica* and salmonid cryptobiosis. *Journal of Fish Diseases* 26: 627-646.

Woo, P.T.K. and G.A. Black. 1984. *Trypanosoma danilewskyi*, host specificity and host's effects on morphometrics. *Journal of Parasitology* 70: 788-793.

Woo, P.T.K. and S. Li. 1990. *In vitro* attenuation of *Cryptobia salmositica* and its use as live vaccine against cyrptobiosis in *Oncorhynchus mykiss*. *Journal of Parasitology* 76: 725-755.

Woo, P.T.K and S.L. Poynton. 1999. Diplomonadida, Kinetoplastida and Amoebida (Phylum Sarcomastigophora). In: *Fish Diseases and Disorders*, P.T.K. Woo (ed.). CABI Publishing, New York, Vol. 1: Protozoan and Metazoan Infections, pp. 27-96.

Woo, P.T.K. and S.D. Wehnert. 1986. *Cryptbia salmositica*, susceptibility of infected trout, *Salmo gairdneri* to environmental hypoxia. *Journal of Parasitology* 72: 392-396.

Wright, A.-D.G., S. Li, S. Feng, D.S. Martin and D.H. Lynn. 1999. Phylogenetic position of kinetoplastids, *Cryptobia bullocki*, *Cryptobia catastomi*, and *Cryptobia salmositica* and monophyly of the genus *Trypanosoma* inferred from small subunit ribosomal RNA sequences. *Molecular and Biochemical Parasitology* 99: 69-76.

Yanong, R.P.E., E. Curtis, R. Russo, R. Francis-Floyd, R.E. Klinger, I. Berzins, K. Kelley and S.L. Poynton. 2004. *Cryptobia iubilans* infection in juvenile discus. *Journal of American Veterinary Medical Association* 24: 1644-1650.

Zajíček, P. 1991. Enzyme polymorphism of freshwater fish trypanosomes and its use for strain identification. *Parasitology* 102: 221-224.

Zajíček, P., O. Benada and A.A. Kolesnikov. 1991. The kinetoplast DNA structure of *Trypanosoma* cf. *carassii*. *Annales de Parasitologie Humaine et Comparée* 6: 256-258.

Zhukov, B.F 1991. The diveristy of bodonids. In: *The Biology of Freeliving Heterotrophic Flagellates*, D.J. Patterson and J. Laesen (eds.). Clarendon Press, New York, pp. 177-184.

Zintl, A., H.P. Voorheis and C.V. Holland. 2000. Experimental infections of farmed eels with different *Trypanosoma granulosum* life-cycle stages and investigation on pleomorphism. *Journal of Parasitology* 86: 56-59.

Zintl, A., W.R. Poole, H.P. Voorheis and C.V. Holland 1997. Naturally occurring *Trypanosoma granulosum* infections in the European eel, *Anguilla anguilla* L. from County Mayo, western Ireland. *Journal of Fish Diseases* 20: 333-341.

Zuo, X. and P.T.K. Woo. 1977a. Natural antiproteases in rainbow trout, *Oncorhynchus mykiss*, and brook charr, *Salvelinus fontinalis*, and the *in vitro* neutralization of fish alpha2-macroglobulin by the metalloprotease from the pathogenic haemoflagellate, *Cryptobia salmositica*. *Parasitology* 114: 375-382.

Zuo, X. and P.T.K. Woo. 1977b. The *in vivo* neutralization of proteases from *Cryptobia salmositica* by α2-macroglobulin in the blood of rainbow trout, *Oncorhynchus mykiss*, and brook charr, *Salvelinus fontinalis*. *Diseases of Aquatic Organisms* 29: 67-72.

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CHAPTER

# 10

# Diseases Caused by Apicomplexans

**Dieter Steinhagen[1,*] and Angela J. Davies[2]**

## INTRODUCTION: GENERAL CHARACTERIZATION OF THE GROUP

Apicomplexans are also known as sporozoans, but both these terms remain controversial (Cavalier-Smith and Chao, 2004). Previously, the Sporozoa (Leuckart, 1879) also included organisms such as microsporidians, myxozoans, and haplosporidians, but this classification has been considered inappropriate for organisms that do not produce spores (Perkins *et al.*, 2000). Protists of the Phylum Apicomplexa (Levine, 1970) or the Subphylum Apicomplexa, Infraphylum Sporozoa (Cavalier Smith and Chao, 2004) are characterized by having an apical complex (see Fig. 10.1A-C, E), an array of structural elements at the anterior end of some developmental stages. The complex includes a conoid comprising

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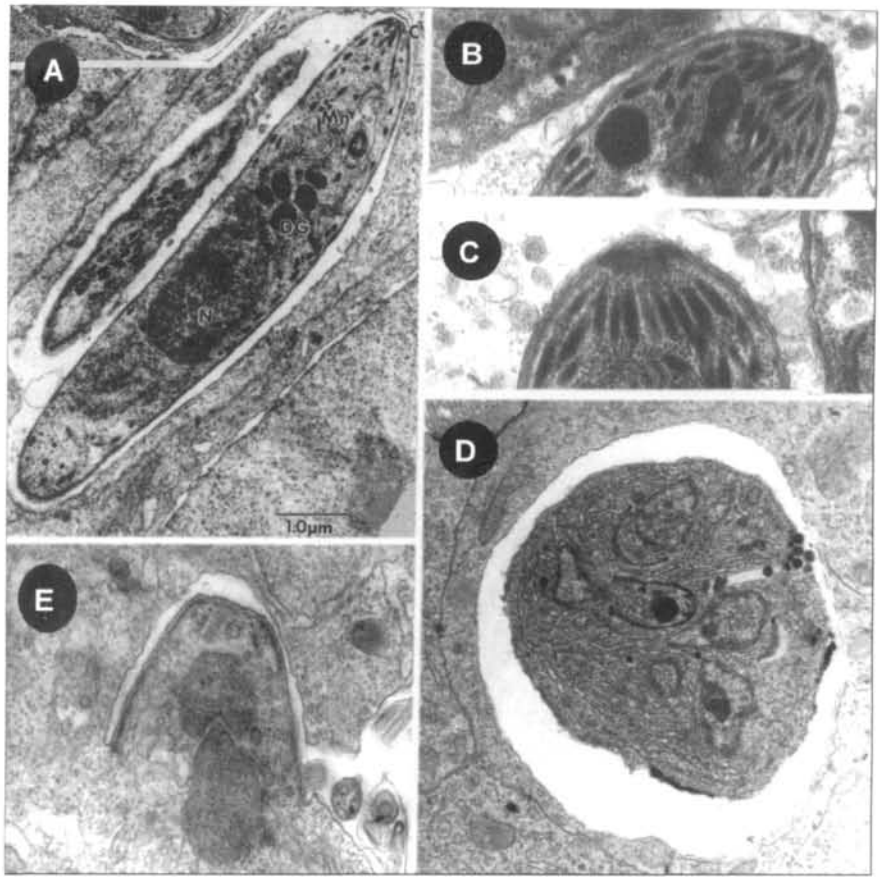


**Fig. 10.1 A-E** Eimeriorine meronts and merozoites. Transmission electron micrographs of developmental stages of *Goussia carpelli* from the intestine of *Cyprinus carpio*. **A**: Longitudinal section of a banana-shaped merozoite showing elements from the apical complex: conoid (C), dense granules (DG), microneme (Mn), N: nucleus. **B**: Merozoite, apical end with conoid (C), dense granules (DG), micronemes (Mn) and cell pellicula (PE) supported by microtubuli. **C**: The conoid is formed by spirally coiled microtubuli. **D**: Meront. Merozoites are formed by endomerogony in close association to nuclei. **E**: High power, newly formed conoid. Mt: microtubulus.

spirally arranged microtubules, one or more polar rings (Fig. 10.1C), electron dense rhoptries, micronemes (Fig. 10.1B, C), and microtubules extending towards the posterior from the anterior pole (Fig. 10.1A-C, E). These structures can be detected by means of transmission electron microscopy, particularly in sporozoite, merozoite, or gamont stages of the Apicomplexa. In the cytoplasm of host-invading stages, dense granules and pellicular invagination known as micropores also occur (Levine, 1985; Perkins *et al.*, 2000). Furthermore, apicomplexans have flattened alveolar

sacs covering the cell surface and a vesicular nucleus, which at division utilizes the centrocones. Their mitochondria have ampulliform or tubular cristae, but apicomplexans generally have no cilia or flagella, except for those associated with the microgametes of some groups. Some taxa may possess all organelles of the apical complex, others only a few elements (Perkins *et al.*, 2000; Cavalier Smith and Chao, 2004).

Apicomplexans are obligate parasites of vertebrate and invertebrate hosts. Humans, livestock and feral vertebrates are hosts to apicomplexans causing diseases such as cryptosporidiosis, malaria, toxoplasmosis, coccidiosis, piroplasmosis and sarcocystosis. Invertebrates, as well as acting as vectors for malaria parasites and other disease-inducing apicomplexans of vertebrates, are themselves hosts to coccidian and gregarine parasites (Perkins *et al.*, 2000). Apicomplexans are particularly ubiquitous in feral fish and often associated with disease under hatchery conditions. Piscine apicomplexans exist in the form of intracellular parasites in cells of the intestine, swimbladder, liver, spleen, testes, kidney or gills, as well as in blood cells such as erythrocytes, lymphocytes, and monocytes (Davies and Ball, 1993).

Organelles of the apical complex allow the invasive stage of apicomplexans, the zoite, the entry of a host cell. The process of cell entry is very rapid and can occur in a few seconds (Sam-Yellowe, 1996). In simple terms, the entry process begins with zoites attaching to the host cell by means of parasite molecules that recognize host surface receptors. Initial loose contact is usually followed by reorientation of the zoite so that apical complex, and especially the protruded conoid, finally lies in direct apposition with the host cell membrane, and firmly anchored by a tight junction. The tight junction between parasite and host cell moves to the posterior end of the zoite as it enters the cell, while the host cell membrane invaginates. Host cell entry is facilitated by discharge of the contents of rhoptries and micronemes, and finally, the zoite lies within the host cell enclosed by a parasitophorus vacuole membrane (Sam-Yellowe, 1996). Within the parasitophorus vacuole, the parasite discharges the contents of its electron dense granules and these—with other secretory products—modify the structure of the parasitophorus vacuole membrane so that it avoids fusion with lysosomes and its contents remain at neutral pH (Cesbron-Delauw, 1994). Despite the wide range of host cells utilized, the events leading to successful invasion of a host cell are essentially similar among the Apicomplexa, although some variations occur (Sam-Yellowe, 1996). None of these events, however, has been studied in any depth in apicomplexan fish parasites.

## Basic Apicomplexan Life Cycle

The basic apicomplexan life cycle includes both sexual and asexual development with three phases of multiplication by multiple fission (schizogony), referred to as merogony, gametogony and sporogony (Perkins *et al.*, 2000). During this development, sporozoites invade a host cell and, in the form of infective trophozoites, de-differentiate (become meronts), grow and divide—often by multiple fission (mergony)—to form merozoites. Merozoites infect new host cells, grow, and give rise to a further generation of merozoites. In most species, there are several merogonic cycles, with the number genetically determined. Eventually, some merozoites become macrogamonts which then develop into macrogametes. Others become microgamonts, and microgametes (formed by gametogony) are usually produced by repeated binary, or multiple, fission (Suborder Eimeriorina Léger, 1911). The zygote is formed by fusion of the macrogamete and microgamete (anisogamous gametes) and may give rise then to oocysts, sporocysts and sporozoites by repeated binary fission or multiple fission (sporogony). Except for the zygote, which is diploid until its first, meiotic, division, all other stages are haploid (Levine, 1985; Perkins *et al.*, 2000).

Both heteroxenous and homoxenous life cycles can be observed. Tissue parasitic Apicomplexa such as eimeriorines usually have no vector or intermediate host. The majority of these organisms are transmitted by ingestion of oocysts. In some groups, however, life cycles have evolved, which include paratenic or obligate vectors (Levine, 1985; Perkins *et al.*, 2000). Haemoparasitic apicomplexans normally develop in two hosts: a vertebrate and a blood-sucking invertebrate. The vertebrate either becomes infected by ingesting the infected invertebrate or during the process of blood sucking. In blood parasitic apicomplexans of fishes, leeches or crustaceans, such as parasitic isopods, act as vectors.

## Systematics

In 2000, the Society of Protozoologists' publication, the *Illustrated Guide to the Protozoa, Volume I*, divided the Phylum Apicomplexa into 3 classes: Perkinsasida Levine, 1978; Conoidasida Levine, 1988; and Aconoidasida Mehlhorn, Peters and Haberkorn, 1980 (Perkins *et al.*, 2000). Whether this classification can be sustained in the light of more recent considerations of protozoan phylogeny is debatable. The division of the Subphylum Apicomplexa, Infraphylum Sporozoa into 3 classes, Coccidea

Leuckart, 1879, Gregarinea Leuckart, 1879, and Haematozoa Vivier, 1982, was recently advocated (Cavalier-Smith and Chao, 2004). The classification adopted in this chapter follows the recommendation of Cavalier-Smith and Chao (2004) at the level of classes, but retains for the most part that of Lom and Dyková (1992) or Perkins *et al.* (2000), for orders, suborders and families (see Table 10.1).

**Table 10.1** Classes, orders (suborders), families, and genera of apicomplexan parasites of fishes. (Adapted from: Lom and Dyková, 1992; Perkins *et al.*, 2000; Cavalier-Smith and Chao, 2004).

Class **Coccidea** Leuckart, 1897
Order **Eucoccidiorida** Léger and Duboscq, 1910
Suborder **Adeleorina** Léger, 1911
Family **Dactylosomatidae** Jakowska and Nigrelli, 1955
Genus ***Dactylosoma*** Labbé, 1894
Genus ***Babesiosoma*** Jakowska and Nigrelli, 1956
Family **Haemogregarinidae** (Neveu-Lemaire) Léger, 1911
Genus ***Haemogregarina*** Danilewsky, 1885
Genus ***Cyrilia*** Lainson, 1981
Genus ***Desseria*** Siddall, 1995
Suborder **Eimeriorina** Léger, 1911
Family **Cryptosporidiidae** Léger, 1911
Genus ***Cryptosporidium*** Tyzzer, 1907
Family **Eimeriidae** Minchin, 1903
Genus ***Eimeria*** Schneider, 1875
Genus ***Epieimeria*** Dykova and Lom, 1981
Genus ***Goussia*** Labbé, 1899
Genus ***Isospora*** Schneider, 1881
Family **Calyptosporiidae** Overstreet *et al.* 1984
Genus ***Calyptospora*** Overstreet *et al.* 1984
Class **Haematozoa** Vivier, 1982
Order **Haemospororida** Danilewsky, 1885
Family **Plasmodiidae** Mesnil, 1903
Genus *Mesnilium* Misra, Haldar and Chakravarty, 1972

## APICOMPLEXAN PARASITES OF FISHES

In fishes, apicomplexans occur in the classes Coccidea and Haematozoa. Members of the Coccidea form the majority of piscine apicomplexans. Apart from the large group of eimeriorine parasites that infects cells of the alimentary tract and other tissues, these organisms include the haemoparasitic dactylosomatids and haemogregarines. In addition, a single organism from the Haematozoa is reported from the blood of fishes.

## Eimeriorine Apicomplexan Parasites of Fishes

Eimeriorine parasites (Coccidea; Eimeriorina) are widespread in fish from fresh, brackish and marine waters. As reported above, a vast number of species has been reported from a large variety of piscine hosts, infecting cells from the alimentary canal and many extra-intestinal tissues (Dyková and Lom, 1983; Davies and Ball, 1993).

Piscine eimeriorine apicomplexans usually have a homoxenous life cycle, but can also be facultatively heteroxenous, or may depend on a true intermediate host for their development (Figs. 10.2–10.4). The gamogonic stages, the macrogamont and microgamont develop independently and the microgamont produces numerous flagellated microgametes. The macrogamont produces a macrogamete, which is fertilized by a microgamete and then develops by sporogony into an oocyst with sporozoites. The oocyst and its contents have a distinct structure, which still forms the basis for identification of species and genera. Within the oocysts, sporozoites are held in an environmentally resistant envelope,

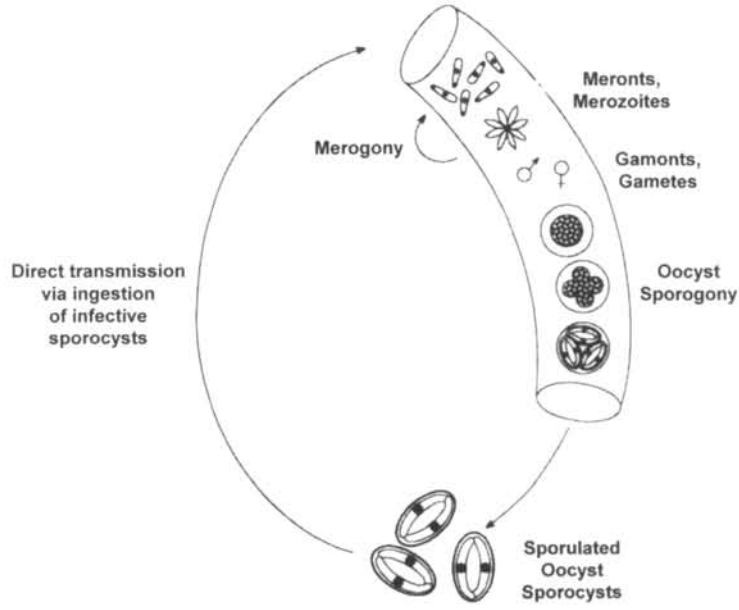


**Fig. 10.2** Monoxenous development of *Goussia iroquoina* from the intestine of *Pimephales promelas*. The infection occurs via ingestion of sporulated oocysts from a contaminated environment. Merogonic, gamogonic and sporogonic development takes place in intestinal epithelial cells of a cyprinid host. Oocysts are liberated in the faeces.

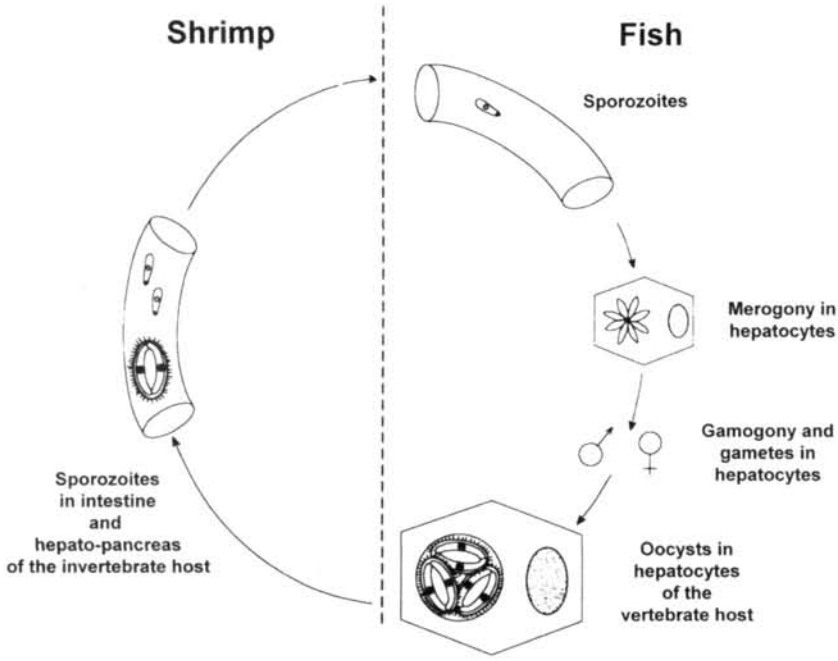


**Fig. 10.3** Heteroxenous development of *Calyptospora funduli* from the liver of *Fundulus grandis*. Palaemonid grass shrimps ingest liver tissue infected with sporulated oocysts of the coccidium. In the intestine of the shrimps, sporozoites excyst from their sporocyst envelope and are found suspended in the intestinal lumen, between intestinal cells and in membrane bound vesicles in the cytoplasm of gut mucosal basal cells. The infection is transmitted to fish after feeding on contaminated shrimps. In the fish, sporozoites most likely reach the liver via the blood stream. Merogonic, gamogonic and sporogonic development take place in hepatocytes.

a series of membranous covers, or commonly a second, often more resistant cyst, referred to as sporocyst. Upon ingestion by a host, sporozoites are released from their cysts, invade the host cells and divide asexually by merogony, binary fission, or endodyogony. Eimeriorine fish parasites normally develop intracellularly within a parasitophorus vacuole.

## Structure of the Developmental Stages

### *Meronts and Merozoites*

In the host cell, the sporozoite de-differentiates into a rounded trophozoite, which develops to a multi-nucleated meront. This then divides, often by multiple fission, to form numerous merozoites, although

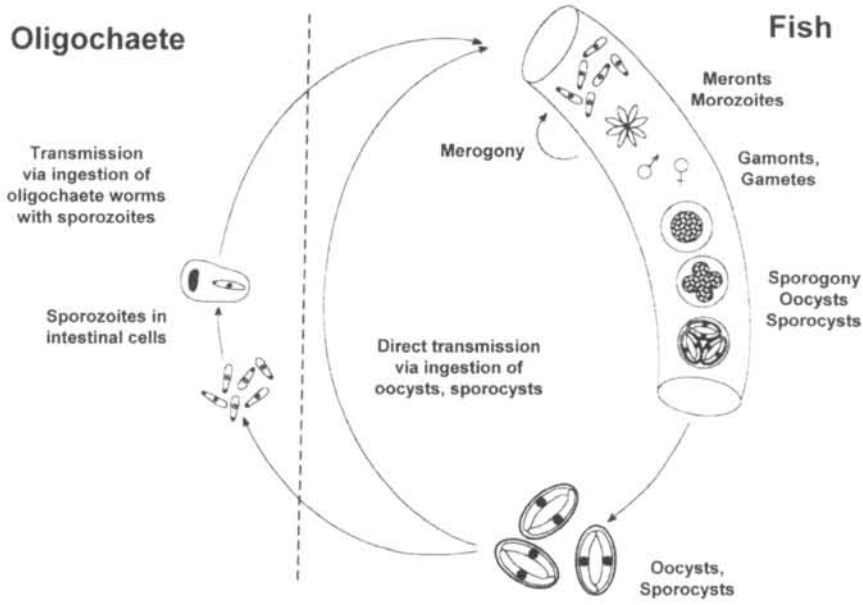


**Fig. 10.4** Facultative heteroxenous development of *Goussia carpelli* from the intestine of *Cyprinus carpio*. Oocysts are ingested from the contaminated environment and initiate merogonic, gamogonic and sporogonic development of the parasite in intestinal epithelium cells. Oocysts also may be ingested by tubificid oligochaetes. In the intestine of the tubificid sporozoites, excyst from their sporocyst shell and are found in cells of the intestinal epithelium of the worm. Transmission of the coccidium also occurs when carp ingest oligochaete worms holding sporozoites.

in *Eimeria* (s.l.) *vanasi*, first generation of merozoites may be formed by endodyogony (Landsberg and Paperna, 1987), producing two merozoites in a parent cell.

In most species, merozoites develop in the meront—either at its surface (ectomerogony) or internally (endomerogony)—and eventually detach from a residual body (Lom and Dyková, 1992). During ectomerogony meront nuclei, each in association with a conoid, develop beneath the cell membrane. The merozoite grows outwards, with the surface membrane of the meront becoming the merozoite's cell membrane. In endomerogony, merozoites originate deep within the meront's cytoplasm, only later growing out from the meront surface (Fig. 10.1D, E). In many species, there are several merogonic cycles, with mature merozoites eventually leaving the infected cells to penetrate new host cells. The number of merozoites produced per meront in a merogonic

cycle varies between 2-32 in *Eimeria* (s.l.) *vanasi* (Landsberg and Paperna, 1987), or 13-18 in *Goussia iroquoina* (Patterson and Desser, 1981c).

Merozoites are banana-shaped cells, possess an apical complex with conoid, polar rings, rhoptries, micronemes, a Golgi complex between the apical region and the nucleus, endoplasmic reticulum and mitochondria (Fig. 10.1A-C; Paterson and Desser, 1981c; Davies, 1990; Alvarez-Pellitero and Sitja-Bobadilla, 2002) and thus are similar to those seen in other Apicomplexa (Scholtyseck, 1979).

### *Gamonts*

Meronts of the final generation, after entering into a new host cell, develop into gamonts. In the initial stage of development, young gamonts cannot be distinguished from trophozoites differentiating into meronts. In laboratory infections, young gamonts can be identified on the basis of a consecutive appearance for merogonic and gamogonic stages (Patterson and Desser, 1981a, b; Landsberg and Paperna, 1987). Young gamonts are often spherical or ellipsoidal in shape, bound by a single cell membrane and lie in a parasitophorus vacuole.

Macrogamonts possess a large nucleus in an almost central position, numerous ribosomes and mitochondria, elaborate endoplasmic reticulum and inclusions with electron lucent and with electron dense material (Fig. 10.5C). Further advanced macrogamonts contain prominent amylopectin granules, lipid inclusions and membrane bound vesicles with electron dense material, which often coalesce into large spherical packets (Fig. 10.5C). These packets were seen, for instance, in maturing macrogamonts and macrogametes of *Goussia iroquoina* (Paterson and Desser, 1981a), *G. laureleus* (Desser and Li, 1986) or *G. carpelli* (Steinhagen, 1991a). These structures have not been considered to be homologous to wall-forming bodies of coccidia from terrestrial vertebrates (Scholtyseck, 1979; Paterson and Desser 1981a), because a contribution of these structures to the formation of oocyst or sporocyst envelopes has not been observed. Organelles that might resemble wall-forming bodies were recorded from macrogamonts and macrogametes of some piscine coccidia, including *Calyptospora funduli* (Hawkins *et al.*, 1983a), *Goussia clupearum* (Morrison and Hawkins, 1984), *Eimeria variabilis* (Davies, 1990), and *Eimeria* (s.l.) *vanasi* (Paperna, 1990). In *E. vanasi*, structures of two kinds of morphology were observed: small, often tear-shaped electron dense vesicles lay in a peripheral position below the

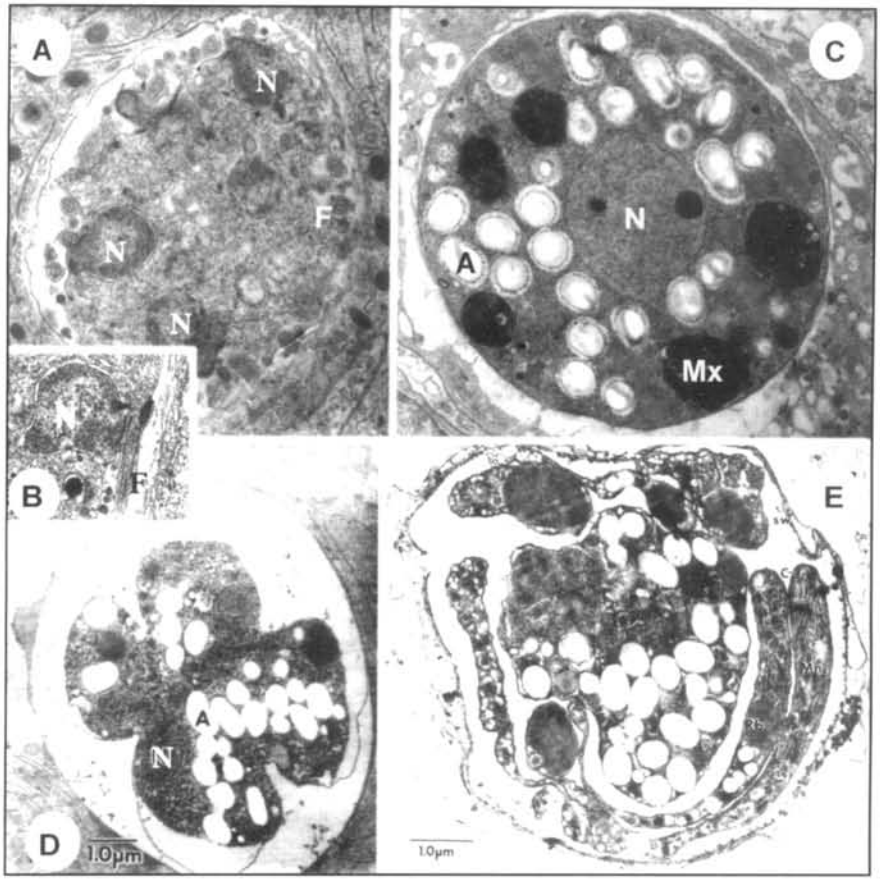


**Fig. 10.5 A-E**. Eimeriorine gamogonic and sporogonic developmental stages. Transmission electron micrographs of *Goussia carpelli* stages from the intestine of *Cyprinus carpio*.

**A**: Microgamont with peripherally arranged nuclei (N), cross sections of flagella (F). **B**: Nucleus in close association to a (F) at the periphery of a microgamont. **C**: Macrogamete with central nucleus (N), amylopection granules (A) and aggregates of electron dense material (Mx). **D**: Young oocyst, simultaneous formation of sporoblasts. N: nucleus, A: amylopectin granule. **E**: Young sporocyst. Two banana-shaped sporozoites split off from a residual body. C: conoid, Mn: micronemes, Rb: refractile body.

macrogamont's cell membrane and larger vesicles with an electron dense core lie in the endoplasmic reticulum within the cytoplasm of the macrogamete (Paperna, 1990). The smaller vesicles resembled morphologically wall-forming bodies type 1 (WF1) of coccidia from other organisms, while the larger vesicles resembled type 2 wall-forming bodies (WF2).

Young microgamonts are multinucleated stages with randomly arranged nuclei. In further advanced stages, the nuclei are peripherally located (Fig. 10.5A). In this position, nuclei may undergo a final division (Steinhagen, 1991a), and the development of microgametes is initiated by the formation of a perforatorium anlage and flagella in close association with the centriole-centrocone complex of the final nuclear division, as seen in G. *carpelli* (Steinhagen, 1991a). The nuclei elongate, protrude along the limiting membrane of the microgamont and are divided in an electron dense portion, which enters the forming microgamete, and an electron lucent portion which is left behind in the microgamont (Fig. 10.5B). In association with the microgamete primordia lies a mitochondrion (Paperna, 1990; Steinhagen, 1991a).

Microgamonts often produce a large number of microgametes. Microgametes are often slender, contain an elongated, condensed nucleus, a mitochondrion and apical perforatorium (Lom and Dyková, 1992). In many species of piscine coccidia (*Calyptospora funduli*, *Goussia iroquoina*, G. *subepithelialis*, G. *carpelli*: see Paterson and Desser, 1981b; Hawkins *et al.*, 1983b; Steinhagen *et al.*, 1990; Steinhagen, 1991a). microgametes have two flagella, although microgametes from *Cryptosporidium* species lack a flagellum (Fayer *et al.*, 1997). Microgametes develop at the surface of a microgamont, detach when mature from the microgamont residuum, and fertilize macrogamonts. Macrogamonts outnumber microgamonts by a ratio of 7 to 1 in *Goussia subepithelialis* or 25 to 1 in G. *cichlidarum* (Lom and Dyková, 1992).

## Oocyst Formation and Sporogony

The process of fertilization of a macrogamont is rarely observed in coccidia (Davies and Ball, 1993). There are several reports of microgametes in the vicinity of a macrogamete in the host cell, in the parasitophorus vacuole of a macrogamete, or even inside of the macrogamete's cytoplasm (Desser and Li, 1984; Paterson and Desser, 1984). Fertilization results in the formation of a zygote that eventually becomes a young oocyst.

Young oocysts of piscine coccidia are usually covered by a thin envelope, closely applied to the host cytoplasm (Lom and Dyková, 1992; Gestal and Azevedo, 2005). This is in contrast to coccidia in homeotherms, where oocyst walls tend to be thick in order to protect the oocyst from environmental challenges (Scholtyseck, 1979). In these species, the oocyst walls are formed from two types of wall-forming bodies

(WF1, WF2), which discharge their contents around the macrogamont. In piscine coccidia, as noted above, homologous structures were only observed in *E.* (s.l.) *vanasi* (see Paperna, 1990), but it is unresolved whether material from these structures contributes to oocyst wall formation.

In oocysts undergoing sporulation, the cytoplasm condenses and, subsequently, four sporoblasts are formed. In most species, exogenous sporulation is noticed (Fig. 10.5D), but in *Goussia cichlidarum*, sporoblasts are formed endogenously and an outer rim of cytoplasm from the sporont remains (Paperna and Landsberg, 1985).

Initially, sporoblasts are covered by a unit membrane, but at later stages of development, the sporoblasts are covered by a bilayered envelope, which transforms into a thick sporocyst wall. The structure of the sporocyst wall varies and is characteristic for different genera of piscine coccidia. Species from the genus *Eimeria* have smooth sporocyst walls, plugged by a Stieda-like body at one pole; in *Goussia* species, the sporocysts have a longitudinal suture, which allows the sporocyst to open like a pea pod (Davies and Ball, 1993). In *Crystallospora*, the suture runs around the equator of the sporocyst, which has the appearance of two hexagonal pyramids fitted base to base, and *Calyptospora* sporocysts have small sporopodia projecting from the sporocyst wall (Duszynski *et al.*, 1979). In *Cryptosporidium* species, a sporocyst wall is not formed, and sporozoites lie without an additional envelope in the oocyst (Fayer *et al.*, 1997; Alvarez-Pellitero and Sitja-Bobadilla, 2002).

The sporoblast also forms sporozoites, which split off from the remaining sporoblast material, leaving behind a sporocyst residuum (Fig. 10.5E). Sporozoites may be ellipsoid, sausage- or club-shaped, and sometimes flexed (Lom and Dyková, 1992). Sporozoites exhibit an apical complex with two polar rings, conoid, micronemes, rhoptires, dense bodies, refractile bodies, nucleus and amylopectin granules (Paterson and Desser, 1984; Morrison and Poynton, 1989; Steinhagen, 1991b). Sporocysts of most piscine coccidia contain two sporozoites, while the cysts from *Cryptosporidium* species contain four.

## Site of Development: Tissue Preference and Intracellular Localization

The majority of eimeriorine apicomplexans from fish hosts invade and develop in the cells of the alimentary canal. Usually the enterocytes are

infected, but not the goblet cells or rodlet cells. Indications for an initial invasion into intraepithelial leukocytes come from laboratory infections of *Oreochromis aureus* hybrids with *Eimeria* (s.l.) *vanasi*. By 5 to 56 h after feeding oocysts to these fish, sporozoites were found free in the gut lumen and in intraepithelial leukocytes, but not in enterocytes. Infected cells were confined to the epithelial layer and were not observed in sub-epithelial locations in the lamina propria. Sporozoites in leukocytes appeared viable because some of them were divided by endodyogeny (Vilenkin and Paperna, 1997).

In enterocytes, developmental stages of gut-dwelling coccidia are localized apical to the nucleus, and large meronts or gamonts deform normal cell architecture. The apical location induces an apical swelling of infected enterocytes and a loss of microvilli (Jendrysek *et al.*, 1994). In some species, the development takes place in a sub-membrane, seemingly epi-cytoplasmatic position. In species from the genus *Epieimeria* as also in some species from the Genus *Goussia*, such as *G. janae* (Lukes and Dyková, 1990), *G. cichlidarum* (Kim and Paperna, 1993a), or *G. trichogasteri* (Kim and Paperna, 1993b), merogonic and gamogonic stages develop in a parasitophorus vacuole formed by apical extensions of the enterocyte which lift the encased parasite above the brush-border layer. *Eimeria* (s.l.) *vanasi* possesses both intra- and epicellular merogonic and gamogonic stages (Landsberg and Paperna 1987; Kim and Paperna, 1992). In species with 'epicytoplasmic' merogonic and gamogonic development, sporogonic stages can be localized in an epicytoplasmic position as well (e.g., *Goussia janae*), deeper in the cytoplasm of the host cell (e.g., *Epieimeria anguillae*, *G. cichlidarum*) or sporogony is exogenous (e.g., *Goussia pigra*). Merogonic and gamogonic development of *Cryptosporidium molnari* also appears in an epicytoplasmic position, while sporogonial stages are located deep in the epithelium (Alvarez-Pellitero and Sitja-Bobadilla, 2002).

Initially, infections with enteric coccidia are focussed in enterocytes at the apical part of some of the mucosal folds. With progressing merogony, the number of coccidial stages in the gut increases, and the infection may spread over the length of the intestine and to cells at the basis of the folds (Jendrysek *et al.*, 1994). In some species, for instance in *Goussia subepithelialis* from common carp *Cyprinus carpio*, the development begins in the epithelium lining and oocysts are concentrated in large aggregates or 'nodules' (Marincek, 1973a, b). This 'nodular coccidiosis' is seen in other species also infecting cyprinds, including *G. balatonica* from the

white bream *Blicca bjoerkna* (Molnar, 1989; Molnar and Baska, 1992) or G. *desseri* from the pike perch *Sander lucioperca* (Molnar, 1996).

A large proportion of piscine coccidia develop in extra-intestinal sites, namely in liver, kidney, spleen, testes, ovary, gall bladder, swimbladder, peritoneum, adipose tissue, and gills (Davies and Ball, 1993). Some species have been studied in detail. These include, for instance, *Calyptospora funduli* (see Hawkins *et al.*, 1983 a, b, 1984), *Goussia clupearum* (Morrison and Hawkins, 1984; Azevedo, 2001) and G. *cruciata* (Gestal and Azevedo, 2005) infecting hepatocytes; *Eimeria sardinae* infecting testes (Morrison and Hawkins, 1984), G. *spraguei* (Morrison and Poynton, 1989) and an undescribed species (Lukes, 1993) infecting kidney tubules; G. *cichlidarum* (Paperna *et al.*, 1986; Kim and Paperna, 1993a) and G. *gadi* (see Morrison *et al.*, 1993a, b) infecting the swimbladder epithelium or the swimbladder's loose connective tissue.

## Life Cycle of Piscine Coccidia

The majority of fish coccidia invade cells of the digestive tract; the oocysts are passed in the faeces and infection occurs via oral contamination. A homoxenous life cycle is common for most gut-dwelling coccidia from homeotherms (Ball *et al.*, 1989) and has been established in the laboratory for *Goussia iroquoina*, G. *subepithelialis*, G. *carpelli*, *Eimeria vanasi* and *Cryptosporidium molnari* (Fig. 10.2). These species were transmitted either by oral inoculation of sporulated oocysts, or by allowing fish to feed on contaminated feed (Marincek, 1973a; Paterson and Desser 1981a; Steinhagen and Körting, 1988; Kim and Paperna, 1992; Sitja-Bobadilla and Alvarez-Pellitero, 2003). In addition, fish were infected with the swimbladder species *Goussia cichlidarum* by feeding on swimbladders containing sporulated oocysts (Kim and Paperna, 1993a). Necrophagia was discussed as an important route for the transmission for fish coccidia with endogenous sporulation and for species with extra intestinal life cycles (Molnar, 1984; Sitja-Bobadilla *et al.*, 2005).

Heteroxenous development of fish coccidia with an invertebrate as an intermediate host was postulated by Landau *et al.* (1975) and Paterson and Desser (1982) and demonstrated for *Calyptospora funduli*, a parasite in the liver of killifishes (Fig. 10.3; Solangi and Overstreet, 1980; Fournie and Overstreet, 1983; Fournie *et al.*, 2000). Liver from gulf killifishes (*Fundulus grandis*) infected with sporulated oocysts of the coccidium were fed to palaemonid grass shrimps. In the intestine of the shrimps, sporozoites

excysted from their sporocyst envelope and were found suspended in the intestinal lumen, between the intestinal cells and in membrane-bound vesicles in the cytoplasm of gut mucosal basal cells (Fournie *et al.*, 2000). Over a period of 4 to 5 days in the gut mucosa of shrimps, sporozoites modified their structure and were then infective for killifish, which acquired a hepatic infection with *Calyptospora funduli* when they were fed on these shrimps (Fournie and Overstreet, 1983).

A role for invertebrates as intermediate hosts was postulated for some species of *Goussia* (Overstreet, 1981; Paterson and Desser, 1982). Subsequently, it could be confirmed that sporozoites of *G. carpelli* and *G. subepithelialis* excysted from sporocysts in the gut of tubificid oligochaetes and invaded cells from the intestinal epithelium of these worms (Steinhagen, 1991b). The oligochaete could serve as a paratenic host at least for *G. carpelli*, but transmission of this species also occurred by faecal-oral contamination from fish to fish (Fig. 10.4; Steinhagen and Körting, 1990).

There is evidence that fish coccidia developing in extra-intestinal sites gain access to their target tissue via the blood stream. In the peripheral blood of killifish, sporozoites of the hepatoparasite *Calyptospora funduli* occurred 4 hr after ingesting grass shrimps infected with this parasite (Fournie *et al.*, 2000). Paterson and Desser (1982) found 'tiny apicomplexan-like meronts' in the vascular endothelium of the liver and swimbladder of common shiners infected with *G. degustii*. This parasite is highly pleomorphic and not only infects the spleen, but also the gall bladder, intestine, kidney, liver and swimbladder of common shiners (Lom *et al.*, 1989). In roach, *E. branchiphila* zygotes and sporogonic stages appear in the gills, where no traces of merogony or gamogony were observed. Dyková *et al.* (1983) assumed a migration of developmental stages of this parasite to the gills via the blood stream.

Sporozoites of some coccidian species from homeotherms penetrate the intestinal intraepithelial lymphocytes and even peripheral blood lymphocytes which can transport sporozoites throughout the body to various organs, including liver and spleen (Ball *et al.*, 1989). In fish, sporozoites from *Eimeria vanasi* were recorded from intestinal lymphocytes, but a dissemination of this species via lymphocytes was not observed (Vilenkin and Paperna, 1997).

The development of fish coccidia in their poikilothermic hosts is strongly influenced by ambient temperature. Decreasing temperature

prolonged morphological development of *Goussia carpelli*, but always resulted in the formation of infective oocysts (Zmerzlaya, 1969; Steinhagen *et al.*, 1989). Low temperature treatment of *Fundulus grandis* infected with *Calyptospora funduli* inhibited the development of all stages, or even eliminated the infection (Solangi *et al.*, 1982).

## Host Specificity

Most eimerian species from warm-blooded vertebrates have a restricted range of hosts and some are extremely host specific, so that chicken parasites, for instance, cannot be transmitted to the closely related turkeys (Levine, 1985). For many fish coccidia, wide host ranges were recorded (Lom and Dyková, 1992; Davies and Ball, 1993), which sometimes included hosts from distant phylogenetic groups. These data were based on oocyst/sporocyst structure and mode of development. Infection experiments with *Calyptospora funduli* (Overstreet *et al.*, 1984), *Eimeria vanasi* (Kim and Paperna, 1992), and *Goussia cichlidarum* (Kim and Paperna, 1993a), supported the view of a less-restricted host range for fish pathogenic coccidia, but this view was challenged recently by cross infection experiments with *Goussia carpelli* (Molnar *et al.*, 2005). The transmission of *G. carpelli* was attempted to fingerlings of an array of eight different cyprinid species via oral contamination and via tubificid oligochaetes in the form of paratenic hosts. Infections were only noted in the intestine of common carp, *Cyprinus carpio* (Molnar *et al.*, 2005). These observations suggest the presence of morphologically similar, but distinct *Goussia* species in cyprinid fishes rather than a single species with a wide host range.

In similar cross infection experiments, *Crytosporidium* species were found to be less host specific. *C. parvum*, for instance, appears to be infectious for more than 100 species of mammals (Fayer *et al.*, 1997), but not for fish (Graczyk *et al.*, 1996). The piscine species *C. molnari* was successfully transmitted to several fish species such as *Dicentrarchus labrax* and *Sparus aurata* (Sitja-Bobadilla and Alvarez-Pellitero, 2003).

## Pathology and Pathophysiology

There exist conflicting reports on the pathogenicity of eimerian fish parasites. Many species have been detected in tissues of feral fish on the occasion of a parasitological survey and induced no or limited pathological effects (Paperna, 1999). Coccidia have also been isolated from anorexic

and lethargic fish, and these coccidia were considered to be related to disease situations (see, for instance, Kent and Hedrick, 1985; Pasnik *et al.*, 2005). In contrast to coccidian species from homeotherms, data on the pathogenicity of fish-infecting species are based on the observation of histopathological alterations, mainly in naturally infected hosts. A few studies only have analyzed laboratory infections, with the focus on histopathological changes in affected tissues, but limited attention has been paid to pathophysiological aspects of fish coccidiosis under laboratory conditions.

Pathogenic effects of coccidia species infecting avian or mammalian hosts were found to be influenced by various factors such as inoculum dose, age of the infected animal, its nutritive or immune status, and by concurrent diseases (Gregory, 1990). These factors have not been sufficiently addressed in the studies conducted on fish coccidiosis. Clinical signs related to infections with gut-dwelling coccidia in fish included depressed behaviour, anorexia, white faeces, abdominal distension and eventually the infected fish died (Kocylowski *et al.*, 1976; Kent and Hedrick, 1985; Steinhagen *et al.*, 1997; Pasnik *et al.*, 2005).

At affected tissue locations, pathological changes become obvious during merogonic development of the parasite with the increase of parasite numbers and dissemination of the infection over the entire intestine. In carp infected with *Goussia carpelli*, enterocytes harbouring growing meronts were deformed, their apical plasma membrane was protruded and apical microvilli were shortened or completely lost (Jendrysek *et al.*, 1994). Cells infected with mature meronts deteriorated and detached from the epithelium lining. In fish with heavy infections during late merogony, and early gamogony, the loss of the covering epithelium progressed from tips to basal regions of mucosal folds and resulted in a destruction of the mucosal architecture. In some individuals, just a narrow fringe of submucosa and a few spots of epithelial tissue remained. In sub-epithelial tissue, an enteritis with infiltration of mononuclear cells and granulocytes (heterophilic as well as 'basophilic' subsets) developed. Mononuclear and granular phagocytes internalized cell debris and parasite stages (Lom and Dyková, 1992; Jendrysek *et al.*, 1994). Subsequently, atrophied folds were covered with squamous or cuboid epithelial cells with few and short microvilli, which were again infected with coccidia (Hemmer *et al.*, 1998).

Developing oocysts are often enveloped by yellow bodies (Kent and Hedrick, 1985; Lom and Dyková, 1992; Jendrysek *et al.*, 1994), which

contain lipofuchsin and often form large aggregates. It was speculated that lipofuchsin might originate from melanomacrophages (Kent and Hedrick, 1985).

Carp infected with G. *carpelli* shed most oocysts immediately after sporulation, over a period of approximately one week, but some spores were present in the faeces of infected carp over a period of up to 48 days (Steinhagen, 1994), most likely released from host cells which were pushed towards the basal membrane or into a sub-epithelial location (Jendrysek *et al.*, 1994).

Epithelial cell dystrophy, necrosis and desquamation, together with atrophy of mucosal folds, were also observed in fish infected with G. *sinensis* (Molnar, 1976) and G. *subepithelialis* (Marincek, 1973b; Dyková and Lom, 1981). Some coccidia, such as G. *janae* (Lukes and Dyková, 1990), *Cryptosporidium molnari* (Alvarez-Pellitero and Sitja-Bobadilla, 2002) or C. *scophthalmi* (Alvarez-Pellitero *et al.*, 2004) infect enterocytes in an apical, sub-membrane (epi-cytoplasmatic) position. The impact of these species on the host is still unclear. In fish with natural infections with C. *molnari and* C. *scophthalmi*, for instance, meronts and gamonts in an epi-cytoplasmic position apparently induced no harm to fish tissue, but zygotes and oocysts, which were located deeper in enterocytes, provoked massive necrosis and sloughing off of infected epithelial cells (Alvarez-Pellitero and Sitja-Bobadilla, 2002; Alvarez-Pellitero *et al.*, 2004).

Similar to processes observed in homeotherms with enteritic coccidiosis (Ruff and Allen, 1990), the architecture of the intestinal mucosa is altered in fish with heavy infections by enteric coccidia: mucosa folds are flattened, infected enterocytes lose their microvillus border, and an increased mitotic activity leads to an increased cell turnover (Hemmer *et al.*, 1998). In consequence, the mucosa is covered by immature, not fully differentiated cells and has a decreased percentage of functionally absorptive cells. Metabolic changes, related to malabsorption of nutrients and retarded growth, however, have not been fully addressed for fish infected with gut-dwelling coccidia.

A decrease in serum sodium and protein level indicated ion loss and the hydration of blood plasma in fish infected with *Goussia carpelli* during the gamogonic and sporogonic phase of development (Steinhagen *et al.*, 1997). A prominent diarrhoea, a loss of water—as seen in enteritic coccidiosis of homeotherms—is not likely to occur in aquatic animals which are surrounded by water. Thorough examinations of coccidia-related effects on the physiology of infected fishes are still lacking.

The pathology of extra-intestinal coccidia is still incompletely known. Renal coccidia such as *Goussia spraguei* (Morrison and Poynton, 1989) induce degeneration of epithelial cells of the kidney tubule, formation of granulomas around infected tubules and occlusion of affected nephrons. The physiological consequences of a portion of non-functioning nephrons due to renal coccidiosis has not been addressed as yet.

Destruction of infected cells and the formation of an inflammatory reaction, which results in the encapsulation of agglomerated oocysts, were commonly observed tissue responses to coccidia infections of liver, spleen, kidney or peri-intestinal adipose tissue in fish (Lom and Dyková, 1992). In these tissues, oocysts were also found in fibrotic capsules of yellowish-brown appearance, known as 'yellow bodies' from infections with enteritic coccidia (Gestal and Azevedo, 2005).

In killifishes, hepatocytes or pancreatic cells are infected by *Calyptospora funduli*. Host cells are damaged by merozoites liberated from meronts, and by gamogonic and sporogonic developmental stages (Solangi and Overstreet, 1980; Hawkins *et al.*, 1981). Inflammatory responses associated with an infiltration of mononuclear and granular phagocytic leukocytes commenced during merogony and progressed during further development of the parasite. In infected fish, liver tissue was gradually eliminated and replaced by encapsulated parasite stages. In some individuals, masses of encapsulated oocysts replaced up to 80% of the host tissue.

In the blue whiting *Micromesistius poutassou*, infections of the liver with an undetermined *Eimeria* species could be related to a clear loss of condition (MacKenzie, 1981).

When summing up these observations, it becomes clear that pathological effects of fish coccidia are related to infection intensity. In host fish with mild infections, little or no damage is usually observed, while in fish with heavy infections, destruction of host cells and replacement by non-functioning cells may interfere with normal physiology of the host tissue. Infections appear to affect mainly juveniles, fish exposed to high numbers of infective stages, as happens under crowded conditions in hatcheries (Sitja-Bobadilla *et al.*, 2005), or under secondary challenge such as adverse husbandry conditions, or even in fish with concurrent diseases. These conclusions may be drawn from coccidian infections in homeotherms (Gregory, 1990), and also from carp under immunosuppression (Steinhagen *et al.*, 1998).

## Immunology

Immune responses of fish to coccidian infections have not been studied in any detail. In the context of host cell deterioration, infiltrations of mononuclear and granular phagocytic leukocytes into infected tissue areas are observed in enteritic coccidiosis and in extra-intestinal coccidia, as already mentioned under 'Pathology'. Infiltrating phagocytes incorporated cellular debris and parasite stages (Solangi and Overstreet, 1980; Jendrysek *et al.*, 1994), and neutrophils isolated from coccidia-infected carp showed an increased phagocytic ability *in vitro* (Studnicka and Siwicki, 1990). Carp infected with *G. subepithelialis* (Studnicka and Siwicki, 1990) or *G. carpelli* (Steinhagen *et al.*, 1997) showed eosinophilia and leukocytosis over a period of time and had elevated serum titres of natural antibodies (Studnicka and Siwicki, 1990). As an immediate response to infections with gut-dwelling coccidia, an increased epithelial cell turnover (Hemmer *et al.*, 1998) and a mucus hypersecretion (Lom and Dyková, 1992) was observed in the intestine of affected individuals.

Fish with sporulated oocysts in the intestine (Molnar, 1976, 1984) or liver (Solangi and Overstreet, 1980) did not acquire an additional infection with the same parasite when re-exposed to infective parasite stages. The basis for protective responses of fish to coccidia infections remains unclear. To a large extent, this has been caused by various difficulties with studies on immune responses of fish to intracellular parasites: a basic understanding of immune processes is limited to a few fish species only and immunological reagents for proper identification of leukocyte subsets, cytokines or chemokines are lacking. With the development of recombinant DNA technology, many of these difficulties can be overcome, and various aspects of the control of coccidia in enteritic and extra intestinal sites could be addressed.

## Taxonomy

The classification of eimeriorine coccidian parasites is traditionally based on the mode of life cycle, the structure of the exogenous stages (=oocyst, sporocyst), and the number of sporocyts and sporozoites (Levine, 1985). An overview of a possible classification has been given in Table 10.1. In many respects, piscine coccidia differ structurally as also in their biology from homeothermic vertebrate coccidia. They have a soft, membranous oocyst envelope, exhibit extreme morphological and biological diversity and, consequently, were divided into several families and genera (see

Table 10.1; Dyková and Lom, 1981; Overstreet *et al.*, 1984, Lom and Dyková, 1992; Davies and Ball, 1993).

The vast majority of coccidia develops tetrasporocystic oocysts with a thin oocyst envelope which morphologically resembles the *Eimeria*-type oocysts known from coccidia of warm-blooded vertebrates. Many piscine species develop in extra intestinal tissues, while a large number of enteritic species develop in an epicytoplasmic position similar to *Cryptosporidium* species. *Eimeria vanasi* even forms both epi- and intra-cytoplasmic generations (Paperna, 1990; Davies and Ball, 1993), and several species of piscine coccidia undergo their entire endogenous development in the nucleus of the host cell (Davies and Ball, 1993). Macrogamonts of most piscine coccidia are lacking true wall-forming bodies, which are known from *Eimeria* species from terrestrial vertebrate hosts (Scholtyseck, 1979; Paperna, 1995). Many coccidia leave the fish in a sporulated stage.

Most species with *Eimeria*-type oocysts are homoxenous and infect their hosts via oral contamination. Some species may be facultatively heteroxenous, for instance *Goussia carpelli*, which can be transmitted via paratenic hosts, namely tubificid oligochaetes (Steinhagen and Körting, 1990).

The taxonomic position of piscine coccidia with *Eimeria*-type oocysts is largely unresolved.

Species which develop a sporocyst shell plugged by a stopper-like structure, named the Stieda body, are traditionally assigned to the genus *Eimeria*, while those tetrasporocystic fish coccidia with a sporocyst wall composed of two joined plates were accommodated in the genus *Goussia* (Dyková and Lom, 1981; Davies and Ball, 1993), which, however, is often synonymized with *Eimeria* (Duszynski *et al.*, 2000b; Upton, 2000).

The classification of eimeriorine species based only on phenotypic characters such as morphology, ultrastructure and life cycles has been seriously questioned (Tenter *et al.*, 2002). A thorough molecular phylogenetic analysis of eimeriorine species from a wide array of different hosts has underlined, for instance, the paraphyletic origin of the current coccidian genus *Eimeria* (Morrison *et al.*, 2004). This analysis, however, did not include piscine species assigned to the genus *Eimeria*. The above-mentioned biological and structural criteria, which are different from homeotherm, but unique to piscine coccidia, were regarded as ancestral traits (Paperna, 1995). This view is further supported by molecular phylogenetic studies performed with the inclusion of a representative from

the Genus *Goussia* (Jirku *et al.*, 2002; Morrison *et al.*, 2004). The molecular data suggest an early branching of the fish-parasitic *Goussia janae* and support a position as a sister taxon to the families Eimeriidae and Sarcocystidae. This hypothesis needs to be confirmed with an inclusion of further fish parasitic coccidia in such analyses.

## Important Genera

As described above, fish coccidia display a wide diversity in structural and biological traits. Traditionally, the species are assigned to the genera *Calyptospora*, *Cryptosporidium*, *Eimeria*, *Epieimeria*, and *Goussia*. Piscine coccidia were not sufficiently included in new concepts for a classification of coccidia (Tenter *et al.*, 2002), which makes a discussions on the taxonomic position of particular species or groups very difficult. Here we present a conservative taxonomy based on the view elaborated by Dyková and Lom (1981, 1983) and Lom and Dyková (1992).

## *Cryptosporidium* Tyzzer, 1907

Members of the eimeriorine family Cryptosporidiidae Léger, 1911 develop on the luminal surface of intestinal or gastric epithelial cells, enclosed in a thin layer of host cell cytoplasm. A unique attachment organelle occurs at the interface between parasite and host cell. The microgametes are non-flagellated, sprorogony of zygotes gives rise to 4 sporozoites that develop in the oocyst envelope, and a sporocyst is not formed (Fayer *et al.*, 1997). Several *Cryptosporidium* species are described from marine and freshwater hosts. They closely resemble species from other vertebrates in respect to morphological development and the structure of developmental stages (Alvarez-Pellitero and Sitja-Bobadilla, 2002). The interest in *Cryptosporidium* findings from fish is beyond taxonomic importance as it is necessary to know if species can be significant for human or domestic animal health. Cross-infection experiments with *C. parvum*, however, confirmed that fish are not susceptible for this agent of human and animal cryptosporidiosis (Graczyk *et al.*, 1996). Data on the biology, host range, structural development, pathology, epidemiology and molecular biology are available on *Cryptosporidium molnari*, a gastric parasite from the marine fishes *Sparus aurata* and *Dicentrarchus labrax* (Alvarez-Pellitero and Sitja-Bobadilla, 2002; Sitja-Bobadilla and Alvarez-Pellitero, 2003; Ryan *et al.*, 2004; Sitja-Bobadilla *et al.*, 2005). Infections were frequent in juvenile fishes with a body weight of 30 to 100 g, with a higher prevalence in *Sparus*

*aurata* than in *Dicentrarchus labrax*. Pathology occurred during gamogonic and sporogonic development with necrosis of gastric epithelium cells.

In a phylogenetic analysis on the basis of the 18S rDNA and on actin loci, a presumably *Cryptosporidium molnari* like isolate from the guppy *Poecilia reticulata* appeared to be genetically distant from all other *Cryptosporidium* species tested and was placed into a separate group basal to all other *Cryptosporidium* species (Ryan *et al.*, 2004).

## *Eimeriidae* Minchin, 1903

The family Eimeriidae contains coccidia that possess a homoxenous life cycle; some members may be facultatively heteroxenous (Upton, 2000). Most fish coccidia traditionally are filed in the *Eimeria*, *Epieimeria* and *Goussia* (Dyková and Lom, 1981; Lom and Dyková, 1992; Davies and Ball, 1993).

## *Eimeria* Schneider, 1875

Species from the genus *Eimeria* develop oocysts with 4 sporocysts, and 1 each with 2 sporozoites. The sporocyst wall is smooth, and has an apical opening, which is plugged by a ring or knob-like structure called Stieda body. In some species, a plug-like structure, the sub-Stieda body, is attached to the inner surface. During excystation, the Stieda body is decomposed and leaves the opening free for the sporozoites to escape. In many species, a sporocyst residuum is left behind.

From piscine tissues, approximately 130 *Eimeria* species were recorded (Dyková and Lom, 1983; Davies and Ball, 1993), which in many respects differ from species found in warm-blooded vertebrates: the oocyst wall is a thin, membranous envelope. The participation of wall-forming bodies in the formation of the oocyst wall could not be confirmed. Most intestinal species are sporulated while still in the host, and many species develop in the extra-intestinal tissues. As mentioned above in the taxonomy paragraph, further phylogenetic work has to prove the assignment of fish parasitic species to the genus *Eimeria*.

From many species, little more than oocyst morphology, the site of the infection and a host species is known (Lom and Dyková, 1992; Davies and Ball, 1993). Ultrastructural data of developmental stages are described for *E. variabilis* (Davies, 1990) and for *E. sardinae* (Morrison and Hawkins, 1984; Morrison, 1991) and *E. sparis* (Alvarez-Pellitero *et al.*, 1997). The

taxonomic position of other species such as the extensively studied *E. vanasi* (Paperna, 1990, 1991; Kim and Paperna, 1992) has been questioned (see below). Biological data on piscine *Eimeria* spp. are scarce. Experimental infections were established for *E. vanasi* (Kim and Paperna, 1992); during the initial phase of the infection, sporozoites were found in intraepithelial leukocytes, and a first multiplication occurred by endodyogeny (Kim and Paperna, 1992).

## *Epieimeria* Dykova and Lom, 1981

Some eimerian species such as *Eimeria anguillae*, *E. sparis*, and *E. isabellae* develop in membranous extension above the brush border of intestinal epithelium cells in a sub-membrane, but seemingly 'epicytoplasmic' position. Merogonic and gamogonic development appears in this epicytoplasmic position, while sporogony is completed intracellularly (Molnar and Baska, 1986; Lom and Dyková, 1992; Alvarez-Pellitero *et al.*, 1997). *Eimeria vanasi* even has intracellular and epicellular generations (Paperna, 1990). To acknowledge this peculiarity in development, Dyková and Lom (1981) erected the genus *Epieimeria*. Phylogenetic studies including molecular biological data are needed to prove the validity of this genus.

## *Goussia* Labbé, 1896

Species from the genus *Goussia* develop tetrasporocystic oocysts with a thin oocyst membrane; the sporocyst envelope is composed of two joined plates which allows it to open the sporocyst like a pea pod. Developmental traits are similar to species assigned to the genus *Eimeria*: most species complete their merogonic, gamogonic and sporogonic development deep in the cytoplasm of the host cell. Some species undergo the merogonic development in an epi-cytoplasmic position similar to the species from the genus *Epieimeria*. Many species develop in extra-intestinal tissues (Davies and Ball, 1993). The genus is often synomymized with the genus *Eimeria* (Duszynski *et al.*, 2000a, b; Upton *et al.*, 2000). In addition, some species, such as *Goussia (Eimeria) vanasi*, are shifted between these two genera (Lom and Dyková, 1992; Paperna, 1995).

Morphological characteristics, ultrastructure of developmental stages, host range, life cycle and pathology have all been studied for an array of *Goussia* species, including *G. gadi* from gadoid fishes, *G. carpelli*,

G. *iroquoina*, G. *sinensis*, and G. *subepithelialis* from cyprinid species, or G. *(Eimeria) vanasi* from cichlid fishes (Marincek, 1973a, b; Molnar, 1976; Paterson and Desser, 1981a, b, c; Landsberg and Paperna, 1987; Kim and Paperna, 1992; Lom and Dyková, 1992; Morrison *et al.*, 1993a, b). Infections of juvenile fishes with the enteritic species such as G. *sinensis*, G. *(E.) vanasi*, and G. *carpelli* were associated with pathology and mortality (Molnar, 1976; Kent and Hedrick, 1985; Landsberg and Paperna, 1987).

The genus *Goussia* traditionally is placed into the family Eimeriidae (Dyková and Lom, 1981; Lom and Dyková, 1992). Some species developed membranous belts at the sporocyst valves near the suture and, therefore, were separated in the subgenus *Plagula* (Overstreet *et al.*, 1984). Because of this character, Overstreet *et al.* (1984) suggested transfer of the Genus *Goussia* to the family Calyptosporidae (see below). The taxonomic position of these coccidian species from piscine hosts has to be confirmed through further phylogenetic analyses.

## *Calyptospora* Overstreet et al., 1984

Eimeriorine coccidia from fish that have an obligate heteroxenous life cycle were placed into the family Calyptosporidae (Overstreet *et al.*, 1984). Species of the Genus *Calyptospora* develop oocysts with 4 sporocysts, which are covered by elongated projections of the sporocyst wall, and which are lacking Stieda- and sub-Stieda bodies, characteristic for *Eimeria* species from warm-blooded hosts (Overstreet *et al.*, 1984; Levine, 1985). *Calyptospora funduli* infects hepatocytes and pancreatic cells of cyprinodont and antheriniform fishes of North America and depends on the transmission by a palaemonid shrimp (Fournie *et al.*, 2000).

A few hours after the ingestion of shrimps with infective sporozoites, some sporozoites were detected in the circulating blood of recipient fishes, and trophozoites and early merogonic developmental stages were found in hepatocytes (Fournie *et al.*, 2000). Merogonic, gamogonic and sporogonic development takes place in hepatocytes or pancreatic cells of infected fishes, and in heavy infections, large aggregations of oocysts can replace large proportions of the liver. Morphological and ultrastructural characteristics, pathology and life cycle traits were studied in detail (Hawkins *et al.*, 1981, 1983a, b, 1984; Fournie and Overstreet, 1983; Fournie *et al.*, 2000).

## Epidemiology

Eimeriorine parasites are widespread in fish from marine and freshwater habitats from almost all biogeographic regions. The majority of species was found in teleosts, and a smaller number of species was reported from elasmobranchs. Thorough lists of described species can be found in reviews published by Lom and Dyková (1992), Davies and Ball (1993), or more recently by Duszynski and co-workers (2000a, b). Most species are predominantly present in juvenile fish (Molnar, 1979; Morrison and Poynton, 1989; Alvarez-Pellitero *et al.*, 2004; Sitja-Bobadilla *et al.*, 2005), but may also be found in ongrowing fish. In cyprinids, some coccidia species develop in nodules in the size of a pinhead in certain segments of the gut (Marincek, 1973a; Molnar, 1989). These species were mainly found in early spring after the hibernation period in ongrowing fish, which suggested a yearly developmental cycle (Marincek, 1973a; Molnar, 1989). In Atlantic herring *Clupea harengus*, the prevalence of *Eimeria sardinae* oocysts correlated with the maturity stage of the testes. It was low in immature fish, increased in ripe and mature individuals and decreased in post-spawning fish (McGladdery, 1987).

Infections with enteritic species are often self-limiting, the oocysts leaving the fish in a sporulated stage when sporulation is completed (Paperna, 1995). In *Eimeria* (s.l.) *vanasi*, however, merozoites are enclosed within macrophages in the lamina propria and might be a source for recurrent infections (Paperna, 1995).

Coccidian infections in extra-intestinal sites appear to be accumulated: in *Micromesistius poutassou*, infections with an undetermined *Eimeria* species rise with increasing age of the fish (MacKenzie, 1981), and *Goussia gadi* in the swimbladder of gadoid fish in the North Atlantic is predominantly found in individuals exceeding 35 cm body length (Morrison *et al.*, 1993a, b).

## Diagnosis and Methods

Coccidia infections in fish can be detected by the presence of spores in fresh or histological preparations of the infected tissues. The fragile oocyst wall makes it difficult to apply concentration methods which are conventionally used for the detection of enteritic coccidia in warm-blooded vertebrates. Merogonic and gamogonic developmental stages can be visualized on histological slides, stained with Giemsa's stain or with haematoxylin and eosin. For the demonstration of macrogamonts, zygotes

or sporogonic developmental stages, the periodic-acid-Schiff (PAS) reaction can be used. With this reaction, carbohydrate stores in coccidia stages are stained purple-red and stand out in sharp contrast to most other cells in the fish tissue. Oocysts and sporocysts from some marine and freshwater fish coccidia (*Eimeria sardinae*, *Goussia harengus*, *G. metchnikovi*) can be detected by intense blue autofluorescence induced by illumination at wavelengths between 410 and 585 nm (Davies and Stewart, 2000).

For species diagnosis, structure and size of spores (oocysts, sporocysts), site of development and the host (range) is conventionally recorded. As pointed out above, cross-infection experiments and molecular data might be needed to distinguish between parasite species with similar morphology from closely related hosts.

Oocysts and sporocysts can be liberated from small tissue pieces or intestinal scrapings by incubation in 0.25-0.5% trypsin solution and decontaminated in 0.03% sodium hypochloride at 4°C. Reliable methods for excystation of sporozoites from piscine coccidia have not been reported.

Sporulation of unsporulated oocysts can be achieved by incubation of separated spores at room temperature in tap water, or seawater supplemented with antibiotics (Davies, 1978). The water should be frequently changed in order to prevent excessive bacterial growth.

Merozoites can be isolated from mucosal scrapings of fish with high numbers of merozoites. The material obtained is then suspended in phosphate buffered saline (PBS) and cleared from tissue particles by gentle centrifugation (200 x g, 1 min). The supernatant is collected, washed several times with PBS by centrifugation (400 x g, 10 min) and then passed through a DE-52 cellulose anion exchange column, pre-treated with PBS + glucose over night (Speer, 1983; Ono *et al.*, 1991; Steinhagen, 1994).

## Cultivation

Apicomplexa depend for their development on a host cell and, therefore, they are difficult to culture. For the induction of laboratory infections with piscine coccidia, fish were reared without previous exposure to the parasite and then kept in tanks holding oocysts shed by hosts with natural infections (e.g., *Goussia iroquoina*: see Paterson and Desser, 1982). In species dependent on an intermediate host, for instance *Calyptospora funduli*, tissue samples from naturally infected fish were fed to their

invertebrate host which then was ingested by parasite free fish (Fournie *et al.*, 2000).

*In vitro* cultivation systems are described for some species from the genera *Eimeria, Isospora*, and *Sarcocystis* from homeotherms (Strout and Schmatz, 1990). These species were either cultivated in tissue culture with cells from avian or mammalian origin, or by inoculation of merozoites or sporozoites into chick embryos (Strout and Schmatz, 1990). Most eimerian species are extremely host specific; chicken parasites, for instance, cannot be transmitted to the closely related turkeys. In tissue culture, many species invade and develop in host cells of various origins. The consensus is that the host cell for most species must be of epithelial origin (Strout and Schmatz, 1990).

Eimerian species from fish hosts have not been cultivated *in vitro* at all. Here much research has to be done in order to examine processes involved in host cell recognition, establishment and development of the parasite in the cell and the effect of factors taking influence on development or survival of the intracellular parasite.

## Treatment

Dispersion of eimerian spores in the aquatic environment most likely takes place through the water supply, spores may also survive in pond sediments, and live food organisms such as oligochaetes in the case of *Goussia carpelli* or grass shrimps in the case of *Calyptospora funduli* can be a source of infection. For prevention of coccidia infections in fish farms, sanitary measures such as water and pond treatment have to be carefully considered. A UV irradiation (20-120 $mJ/cm^2$) of the water supply was not always effective in preventing a *Cryptosporidium molnari* cryptosporidiosis in gilt-head seabream, while a filtration of water over 10 µm pore-size-filter could reduce infection and prevalence levels (Sitja-Bobadilla *et al.*, 2005). For pond treatment, Kocylowski *et al.* (1976) suggested drying out or addition of lime in order to reduce infection levels of carp with *Goussia carpelli*.

Only a few drugs developed for the treatment of coccidiosis in homeotherms were tested on fish coccidia. Solangi and Overstreet (1980) were able to reduce oocyst counts of *Calyptospora funduli* in killifish when fed on a diet containing Monensin. Kocylowski *et al.* (1976) reported that amprolium chloride in the feed at a dose of 5 g $kg^{-1}$ fish reduced the infection intensity of *Goussia carpelli* carp juveniles when fed on 3

occasions at 2 days' intervals. The medication of feed with furazolidone at a dose of 30 mg $kg^{-1}$ fish and supplied over a period of 3 days was recommended as treatment for fish coccidiosis in older reports (Musselius *et al.*, 1965), while Schmahl *et al.* (1989) suggested the use of toltrazurile, without suggesting an effective dose.

## Future Perspectives

Infections of fish with eimeriorine apicomplexans are widespread, showing extreme biological and structural diversity, and exhibiting remarkable differences from parasites of warm-blooded vertebrates. Biological and structural data, as well as first molecular data, indicate that the evolution of piscine coccidia has to be re-considered and most likely occurred independently from species in homeothermic hosts. Phylogenetic and evolutionary analyses with the inclusion of a representative number of fish parasitic species will certainly provide new insights into the evolution of eimeriorine apicomplexan parasites.

Despite the wide distribution of infections, the pathogenicity of fish coccidia is not well understood. There are reports about coccidian-related losses of juvenile fishes in hatcheries, but factors which contribute to—or foster disease situations—are completely unknown. Many coccidia infect cells from the gut mucosa and infections are limited to a short period of time. After a first infection, fish may be refractory for secondary infections with the same parasite. Processes which control host parasite interactions such as parasite invasion, establishment and colonization of host tissues and which lead to a limitation of the infection are also not known. This corresponds to our limited understanding of mucosal immune responses in fish, and here fish coccidia might provide excellent experimental models for studies on pathogen interactions with molecular and cellular components of the piscine mucosal barrier. Most of these studies, however, depend on the establishment of reproducible experimental infection systems, which might prove a difficult task for many species.

## Other Apicomplexan Parasites of Fishes

In addition to the eimeriorine coccidia, other apicomplexans occur in fishes and include dactylosomatids, haemogregarines (Coccidea; Adeleorina) and a single haematozoan organism (Haematozoa; Haemospororida). All these parasites develop primarily within the host blood cells. Despite being less well known than the eimeriorines and often

inadequately described, these apicomplexans are surprisingly widely distributed among fishes of the Gnathostomata, although none has been reported from the Agnatha (Davies and Johnston, 2000). Of the three types of apicomplexans, haemogregarines form the largest group and are the most commonly detected. Dactylosomatids are seen less often, and only one species of haematozoan has been described from fishes. All three types of apicomplexans occur in economically important fishes, as well as in those with no known commercial value.

## General Characteristics, Including Geographical Distribution

Dactylosomatids have been observed in marine fishes (Saunders, 1960), in species able to undergo anadromous and catadromous migration (Fantham *et al.*, 1942; Cruz and Davies, 1998), and in hosts living in freshwater environments (Smit *et al.*, 2003a; Davies *et al.*, 2005). Haemogregarines have been reported from all the continents (Davies, 1995). They occur most frequently in fishes from marine habitats, including those from the coastal margins, reefs, and the open oceans, with many records originating in Europe, North America, Australia and New Zealand. However, they also exist in fishes from brackish water, in catadromous and anadromous hosts, and in fishes from freshwater ditches, rivers and lakes (Davies, 1995). Furthermore, haemogregarines have been detected in the blood of fishes from some extreme aquatic environments, such as the Antarctic and Arctic oceans (Barber *et al.*, 1987; Barber and Mills Westermann, 1988; Siddall and Burreson, 1994), and from great depths in North Atlantic waters (Khan *et al.*, 1992; Davies and Merrett, 2000). In contrast, haematozoan has been found only in a freshwater fish from India (Misra *et al.*, 1972).

In haematological preparations, especially stained blood films from fishes, all three types of these apicomplexans are generally detected in cells of the red cell series. However, some haemogregarines seem confined to leukocytes, or develop in leukocytes and red cells. Other haemogregarines and the haematozoan may occur generally in organs such as liver, kidney and spleen, or specifically in the reticulo-endothelial system (Davies and Johnston, 2000). In blood films, dactylosomatids, haemogregarines and the haematozoan, depending on the stage, generally appear as rather rounded or vermicular intracellular organisms and here—especially in erythrocytes—some may divide by binary fission, or demonstrate one or more characteristic cycles of merogony, yielding merozoites from meronts.

In dactylosomatids and several types of haemogregarines, final stages of merogony (pregamontic merogony) in the fish host lead to the formation of immature sexual stages, the gamonts, whereas pigmented gametocytes are the products of erythrocytic merogony in the haemosporine. Gamonts, or gametocytes, vary considerably in appearance, but are usually vermicular stages, with a distinct nucleus, cytoplasmic granules and vacuoles, and sometimes an anterior cap. Single or multiple gamonts may occur within erythrocytes, and within a single host may all appear identical (monomorphic), or, infrequently, sexual dimorphism may be evident.

The mode of transmission of dactylosomatid, haemogregarine and haematozoan apicomplexans between fishes is largely unknown, but life cycles are generally considered to be heteroxenous, with fishes acting as intermediate hosts. Blood-sucking leeches are likely to be definitive hosts and possible vectors for all three groups of these apicomplexans (Siddall, 1995; Davies and Johnston, 2000), but some haematophagous isopods also support fish haemogregarine development (Davies and Johnston, 2000; Davies and Smit, 2001; Smit and Davies 2004). Within leeches and isopods, gamonts or gametocytes mature, fertilization occurs and sporogony involving formation of sporozoites from oocysts follows. Post-sporogonic merogony may also be evident in these invertebrate hosts.

## Important Genera

The non-eimeriorine apicomplexans of fishes may be classified as in Table 10.1. The dactylosomatids include two genera (*Dactylosoma* Labbé, 1894 and *Babesiosoma* Jakowska and Nigrelli, 1956), the haemogregarines, three genera (*Haemogregarina* Danilewsky, 1885; *Cyrilia* Lainson, 1981 and *Desseria* Siddall, 1995) and the haematozoan, a single genus (*Mesnilium* Misra *et al.*, 1972). These six genera fall into two main groups, the adeleorine apicomplexans (*Dactylosoma, Babesiosoma, Haemogregarina, Cyrilia, Desseria*) and the haematozoan apicomplexan (*Mesnilium*). The phylogeny of the haemogregarines has been discussed by Davies and Johnston (2000).

## Dactylosomes and Babesiosomes

Adeleorine apicomplexans of the genus *Dactylosoma*, the dactylosomes, undergo primary merogony forming 6-16 merozoites in a hand, or fan-like

formation, in erythrocytes (see Table 10.2). Secondary merogony in these same host cells, yielding 6 merozoites which subsequently become intraerythrocytic gamonts, has been demonstrated for an anuran (frog) dactylosome (Barta, 1991), although not in fishes. Oocysts may produce 30 or more sporozoites in leeches, as shown experimentally for the anuran dactylosome (Barta, 1991), but transmission has not been demonstrated from leeches to any ectothermic vertebrate, let alone fishes. Barta (1991) noted 2 species of dactylosomes from fishes that he considered valid. These were *Dactylosoma salvelini* Fantham *et al.* (1942) from the brook trout, *Salvelinus fontinalis*, in Eastern Canada and *Dactylosoma lethrinorum* Saunders, 1960 from emperors, *Lethrinus* spp., in the Red Sea. Only the stages occurring in the red cell series have been described for both these species.

Members of the genus *Babesiosoma*, the babesiosomes, undergo primary and secondary cross-like (cruciform) merogony yielding 4 merozoites on each occasion, within erythrocytes (Table 10.2). Tertiary merogony may also exist for one species from fishes (Negm-Eldin, 1998). Gamonts form within the same host cells from secondary or tertiary merozoites—and probably in leeches—these gamonts associate (syzygy), and gametes fuse following gametogenesis, forming an ookinete (motile zygote). This penetrates the leech intestinal epithelium. Oocysts produce 8 sporozoites (sporogony) and post-sporogonic production of merozoites follows within the leech salivary cells. Transmission to the vertebrate host probably occurs when the leech feeds.

Barta (1991) listed 6 species of babesiosomes from fishes that he considered valid, but Siddall *et al.* (1994) transferred 3 of these to the genus *Haemohormidium* Henry, 1910 (see below). Later, Cruz and Davies (1998) renamed a fish haemogregarine as a babesiosome, and Negm-Eldin (1998) suggested that a variety of babesiosome-like infections reported from African fishes should be united in the single species. Thus, as few as 3 valid species of fish babesiosomes may remain currently: *Babesiosoma bettencourti* (França 1908) from the European eel, *Anguilla anguilla*, in Portugal; *Babesiosoma mariae* (Hoare, 1930) from many species of freshwater fishes and the leech *Batracobdelloides tricarinata* in Africa; and *Babesiosoma tetraogonis* Becker and Katz, 1965 from suckers, *Catostomus* spp., from California, U.S.A. As in dactylosomes, the only stages occurring in the red cell series have been observed for fish babesiosomes and Negm-Eldin (1998) reported that he was able to transmit *B. mariae*, using *B. tricarinata* as vector, from *Tilapia nilotica* to wild-caught African fishes

**Table 10.2** Summary of the development of dactylosomatids (*Dactylosoma*, *Babesiosoma*), haemogregarines (*Haemogregarina* (*s.l.*), *Cyrilia*, *Desseria*) and the haematozoan (*Mesnilium*) in fishes and invertebrates, taken from a variety of sources (see main text).

| *Genus of apicomplexan* | *Development in fish host* | | | *Development in invetebrate host (identity)* |
|---|---|---|---|---|
| | *Tissues* | *White cell series* | *Red cell series* | |
| *Dactylosoma* | Unknown | Unknown | Hand, or fan-like primary (6-16 merozoites) and possibly secondary (6 merozoites) merogony. Gamonts present. | Unknown. Possibly, oocyst with 30+ sporozoites (leech?). |
| *Babesiosoma* | Unknown | Unknown | Cruciform primary (4 merozoites) and secondary (4 merozoites) merogony; tertiary merogony in one species. Gamonts present. | Syzygy, gametogenesis, ookinetes, oocysts with 8 sporozoites. Post-sporogonic merogony (leech). |
| *Haemogregarina* (*s.l.*) | Possible merogony in internal organs | Possible merogony and gamonts in white cell series | Erythrocytic binary fission, or meronts undergoing merogony, and gamonts (single or multiple) present. | Syzygy, gametogenesis, oocysts generally with 8 sporozoites Post-sporogonic merogony (leech, isopod). |
| *Cyrilia* | Unknown | Unknown | Vermicular meronts, and monomorphic or dimorphic gamonts present. | Syzygy, gametogenesis, oocysts with 20+ sporozoites (leech). |
| *Desseria* | Merogony in internal organs | Merogony known in at least one species | Merogony absent, but gamonts, often single within red cells (monomorphic or dimorphic) present. | Syzygy, gametogenesis, oocysts with 16+ sporozoites Post-sporogonic merogony (leech). |
| *Mesnilium* | Merogony (yielding many merozoites) in reticulo-endothelial cells | Unknown | Merogony (6-8 merozoites) and pigmented macro- and microgametocytes present. | Oocysts with sporozoites (leech). |

of the genera *Bagrus, Chrysichthys, Clarias, Cyprinus, Labeo, Synodontis* and *Tilapia.* [Examples of development stages of *B. mariae* from in the erythrocytes of a fish from Botswana are shown in Fig. 10.6.]

## Haemogregarines

Adeleorine apicomplexans falling within the family Haemogregarinidae, the haemogregarines, are thought to be closely related to dactylosomatids. One genus of this family, *Haemogregarina*, has become a 'taxonomic repository' for haemogregarines awaiting proper description (Perkins *et al.*, 2000). Furthermore, the term *Haemogregarina* sp. (*sensu lato*) is often applied to haemogregarines where sporogonic development is unknown (Perkins *et al.*, 2000). Siddall (1995) was responsible for dividing the fish haemogregarines among the three genera discussed below.

Members of the genus *Haemogregarina* have been recorded from a broad range of vertebrates, including fishes, and generally have intraerythrocytic meronts that are characteristically vermicular (Table 10.2). Binary fission or merogony occurs in white and red blood cells, or fixed tissue cells, and single or multiple gamonts exist primarily in erythrocytes. In invertebrate hosts such as leeches and isopods, syzygy and gametogenesis are followed by sporogony. Oocysts generally yield 8 or more naked sporozoites and there is post-sporogonic production of merozoites. Transmission to the vertebrate host may occur through transfer of merozoites by bite from the invertebrate, or possibly by the invertebrate being eaten, although neither of these mechanisms of transfer has been demonstrated for fish haemogregarines.

By synonymizing several species of the genus *Haemogregarina* and transferring another haemogregarine from the genus *Cyrilia*, Siddall (1995) was able to list 13 species of fish haemogregarines in the genus *Haemogregarina* (*sensu lato*). All of these haemogregarines occurred in marine fishes, the majority in Osteichthyes, but some in Chondrichthyes, for example, *Haemogregarina* (*s.l.*) *carchariasi* (Laveran, 1908) from sharks, such as a *Carcharias* sp. and *Haemogregarina* (*s.l.*) *delagei* (Laveran and Mesnil, 1902) from rays, *Raja* spp. Furthermore, *Haemogregarina* (*s.l*) *bigemina* (Laveran and Mesnil, 1901) was remarkable for its apparent broad host range. It may infect as many as 96 species of teleost fishes in 70 genera and 34 families, but this distribution has been questioned (Davies *et al.*, 2004); it also develops in gnathiid isopods, its likely definitive hosts and vectors (Davies *et al.*, 2004; Smit and Davies, 2004).

In contrast, *Haemogregarina* (*s.l.*) *uncinata* (Khan, 1978) from eelpouts, *Lycodes* spp., was the only species in Siddall's list demonstrated to have a leech-definitive host, namely *Johanssonia arctica* (see Khan, 1978).

Siddall's (1995) list was later amended by Smith (1996), who added another 4 fish haemogregarines. New species of haemogregarines from marine teleosts have been described since (Davies and Merrett, 2000; Smit and Davies, 2001). Thus, there are at least 19 species fish haemogregarines currently grouped within the 'repository' genus *Haemogregarina* (*s.l.*), although for two of these, *H.* (*s.l.*) *bigemina* and *H.* (*s.l.*) *uncinata*, sporogonic development in the invertebrate host has been demonstrated. (Examples of a number of *Haemogregarina* (*s.l.*) spp. in their fish hosts are shown in Fig. 10.7).

Haemogregarines of the genus *Cyrilia* exist only in the erythrocytes of freshwater fishes, where they form vermicular meronts and, in one species, dimorphic gamonts. In the invertebrate hosts (leeches), syzygy and sporogony occur within the intestinal epithelium, and oocysts produce 20 or more sporozoites. Transfer of sporozoites to the vertebrate host is presumed to occur when the leeches feed (Table 10.2). Only two fish haemogregarines are currently placed in this genus. These are *Cyrilia lignieresi* (Laveran, 1906) from the marbled swamp eel *Synbranchus marmoratus* and the leech *Haementaria lutzi* in Brazil, and *Cyrilia nili* (Wenyon, 1909) from the snakehead *Ophiocephalus obscurus* and the leech *Batracobdelloides tricarinata* in Egypt (Lainson, 1981; Negm-Eldin, 1999). Lainson (1981) originally placed the marine haemogregarine *H. uncinata* Khan, 1978 in the genus *Cyrilia*, although Siddall (1995) relegated it to the genus *Haemogregarina* (*s.l.*). Furthermore, Negm-Eldin (1999) was responsible for transferring *Desseria nili* (Wenyon, 1909) to the genus *Cyrilia*. This species, unlike *C. lignieresi*, has monomorphic gamonts in the fish host.

Members of the genus *Desseria*, like *Cyrilia*, are reported only from fishes. They undergo possible merogony in the internal organs of fishes, and at least one species, *Desseria nototheniae* (Barber, Mills Westermann and Storoz, 1987), multiplies in mononuclear leukocytes (Barber *et al.*, 1987). However, unlike *Haemogregarina* and *Cyrilia* spp., *Desseria* spp. do not exhibit merogony in erythrocytes (Table 10.2). Single gamonts usually exist in erythrocytes, and at least one species, from gurnards in Europe, *Desseria rovignensis* (Minchin and Woodcock, 1910), appears to be dimorphic (Minchin and Woodcock, 1910). In invertebrates (leeches)

*Desseria* spp. apparently undergo gametogenesis and sporogony in intestinal epithelial cells. Oocysts produce more than 16 sporozoites which invade further leech intestinal cells to produce 4 merozoites. These merozoites then migrate forward to the salivary cells and transmission presumably occurs when merozoites are passed to the fish as the leech feeds.

The genus *Desseria* contains the largest number of species of fish haemogregarines, but it is difficult to judge exactly how many. Currently, around 34 species would be a good estimate. Siddall (1995) has listed 41 species of *Desseria* from both marine and freshwater fishes, and later Smith (1996) added another marine species. However, some species have since been relegated to other genera and others have been synonymized with pre-existing species, or probably should be (Davies and Johnston, 2000; Smit *et al.*, 2003b). Finally, some others may be eimeriorine coccidia, rather than haemogregarines (Lom and Dyková, 1992). (Examples of some *Desseria* spp. in their fish hosts are shown in Fig. 10.7.)

## Haematozoans

The genus *Mesnilium* falls within the Family Plasmodiidae, containing ten genera that can cause malaria and related diseases in a range of vertebrates. *Mesnilium* is found only in fishes, with only one species known. Exoerythrocytic merogony producing many merozoites occurs in reticulo-endothelial cells and further meronts (yielding 6-8 merozoites each) and pigmented micro- (male) and macrogametocytes (female) exist in erythrocytes (Table 10.2). Likely vectors are leeches, where oocysts are produced and sporozoites invade the salivary glands. Transfer of sporozoites to the vertebrate host presumably occurs by leech bite. *Mesnilium malariae* (Misra *et al.*, 1972) was described from the spotted snakeshead, *Ophiocephalus punctatus*, and the leech, *Hirudinaria granulosa*, in India (Misra *et al.*, 1972) (Table 10.2).

## Similar Infections in Fish Blood Cells

Organisms of unknown status found in fish blood cells that may be apicomplexans include *Globidiellum*-like organisms from a variety of marine fishes (Davies, 1995). Representatives of the genera *Haemohormidium* (Henry, 1910) [see Fig. 10.7] and *Haematractidium* (Henry, 1910) also occur in marine fish erythrocytes, but neither genus is likely to be apicomplexan (Siddall *et al.*, 1994) and affinities with

microsporidia have been suggested (Davies *et al.*, 2003). Some viral infections of fish erythrocytes can also resemble apicomplexans (Davies, 1995; Davies and Johnston, 2000). The general pathological status of most of these infections is unknown, although some have an adverse effect on their host cells (Davies, 1995; Davies and Johnston, 2000).

## Disease Symptoms and Pathogenesis

It is difficult to assess the disease potential of dactylosomatids, haemogregarines and the haematozoan on their respective host fishes because most infections have been studied in wild-caught fishes, where the infection course is unknown. Dactylosomatids, found so far only in fish red cells, appear to have little effect on these, even during merogony, except perhaps for inducing slight changes in cell shape and displacement of host cell nuclei. This group of apicomplexans also normally infects low numbers of circulating red cells (typically $<1/10{,}000$ erythrocytes) (Smit *et al.*, 2003a); so effects on the host in this respect are also likely to be slight. Fishes with dactylosomatid infections, thus, exhibit little that can be described as disease symptoms.

Haemogregarines are also generally considered of insignificant disease potential where fishes are concerned, but this can be difficult to judge for the same reasons as those noted above. As for dactylosomes, prevalence of infection and parasitaemias are often low in fish haemogregarine infections (Davies, 1995). However, the remarkable prevalence (100% in some adult populations) and persistence (20-30 years) of *H. bigemina* infections in the blood of shanny, *Lipophrys pholis*, in Wales and Portugal have been reported (Davies *et al.*, 2004; Dias *et al.*, 2005). Furthermore, the prevalence of haemogregarine infections in the tissues of Atlantic mackerel, *Scomber scombrus*, can reach 98-100% (MacLean and Davies, 1990). High parasitaemias are also sometimes reported (for example, up to 85% of leukocytes infected), which may affect young fishes adversely (Laird, 1953).

Davies (1995) reviewed the pathology associated with fish haemogregarine infections and the effects were summarized as changes in circulating blood cells and tissue responses. Changes in immature and mature red cells, and the blood picture generally, can include altered cell counts, such as anaemia in some cases, decreased thrombocyte levels in others, but also increased erythroblast, erythrocyte and leukocyte counts. Changes in individual host cells can involve modifications in size,

alterations in quality or quantity of host cell cytoplasm, nuclear modification and displacement (Fig. 10.7). Overall enlargement of host erythroblasts and erythrocytes in haemogregarine infections is a fairly common occurrence, but other infected red cells may increase in width, but decrease in length, or become smaller or misshapen, suggesting a collapse of cytoskeletal components (Davies, 1995). Cytoplasm of infected erythrocytes and leukocytes may become almost vestigial (Fig. 10.7), exhibit lengthy extensions, or develop metachromatic pigmentation. Red cell nuclei can be displaced, grooved or indented, flattened or stretched, hypertrophied or pyknotic, depending on the type of haemogregarine present (Fig. 10.7). Intraleucocytic stages of haemogregarines can also increase the size of neutrophils, lymphocytes and monocytes, induce cytoplasmic tendrils (Davies, 1995), or destroy host cells (Fig. 10.7).

Perhaps the most intriguing aspects of the disease-status of fish haemogregarines has been their association with a proliferative disease of farmed turbot, *Scophthalmus maximus*, and a similar, though not as extensive, nodular disease of Atlantic mackerel, *S. scombrus* (Ferguson and Roberts 1975, 1976; Kirmse and Ferguson, 1976; MacLean and Davies, 1990; Davies, 1995). In both cases, the relationship between the haemogregarines and the host cellular responses is not clear and a causal effect, therefore, is not proven. Turbot, however, were rendered unsaleable by the presence of nodules. In these turbot, external and internal nodular lesions of various sizes were evident. Microscopically, these comprised accumulations of macrophages, sometimes associated with a liquefied centre, and surrounded by a fibrous capsule. Many of these macrophages, and leukocytes from buffy coat blood preparations from the turbot, contained up to 4 haemogregarines, identified as *Haemogregarina* (*s.l.*) *sachai* (Kirmse, 1978, 1980). In mackerel, lesions found in kidney and spleen also consisted mainly of macrophages, many containing haemogregarines, and surrounded by a fibroblastic response (MacLean and Davies, 1990). In addition, some mackerel with tissue lesions had intraleucocytic haemogregarines in peripheral blood films (MacLean and Davies, 1990). Haemogregarine-like organisms have also been observed in similar nodules in cultured gilthead seabream, *Sparus aurata*, and in wild-caught dusky spinefoot, *Siganus luridus*, from the Red Sea, but blood stages were not observed (Paperna, 1979).

Finally, fish (*Ophiocephalus punctatus*) infected with *Mesnilium malariae* have minute reddish patches on the skin. Fish with larger surface lesions—oozing blood—and with scale and fin ray loss have also been

observed. Increased body temperature and altered respiration rates are other symptoms of the infection, which allegedly kills fish quite rapidly (Misra *et al.*, 1972; Haldar and Mukherjee, 1979).

## Diagnosis

Dactylosomatid, haemogregarine and the haematozoan infections are diagnosed in fishes primarily using conventional thin blood films, but they can also be detected in fresh blood by phase-contrast microscopy (Eiras, 1987), followed by examination of thin films.

Preparation of thin blood films can influence the appearance of these parasites. If fresh blood is left too long before the smears are made, haemogregarines, in particular, may escape from their host cells. Blood films must also be free of contamination and damage from both fresh and seawater. However, even blood films from fishes trawled from great ocean depths can be of value (Davies and Merrett, 2000).

Thin blood preparations are stained by suitable Romanowskyi methods, such as buffered Giemsa, May Grünwald Giemsa and Leishman stains. However, for speedy diagnosis, Diff-Quik (Dade Behring Inc., Newark, USA) and similar rapid haematological stains may be applied (McKiernan *et al.*, 2004). Since these apicomplexans are usually rather small and parasitaemias are often light, a 100X oil immersion objective is essential for their proper visualization. It may also be necessary to screen considerable areas of the blood film before infection is detected. Digital camera and image analysis attachments to modern microscopes make recording images and the accurate measurement of such small organisms much more straightforward than was the case previously. Distinguishing the six genera of apicomplexans relies on identification of development stages in blood films (Table 10.2), although a single blood film rarely contains all the stages necessary to make a diagnosis, and division stages may be particularly difficult to locate.

Although not used routinely in blood-inhabiting apicomplexan studies, tissue squashes and impressions can be useful adjuncts to thin blood films. Prepared with the same types of haematological stains as blood films, they may help detect cryptic tissue stages. Histological sections, treated with a variety of staining methods, may also be helpful for observing associated tissue nodules and other lesions, although it can be difficult to locate individual apicomplexans in such preparations. Like blood films, tissue preparations also need to be examined with a 100X oil objective.

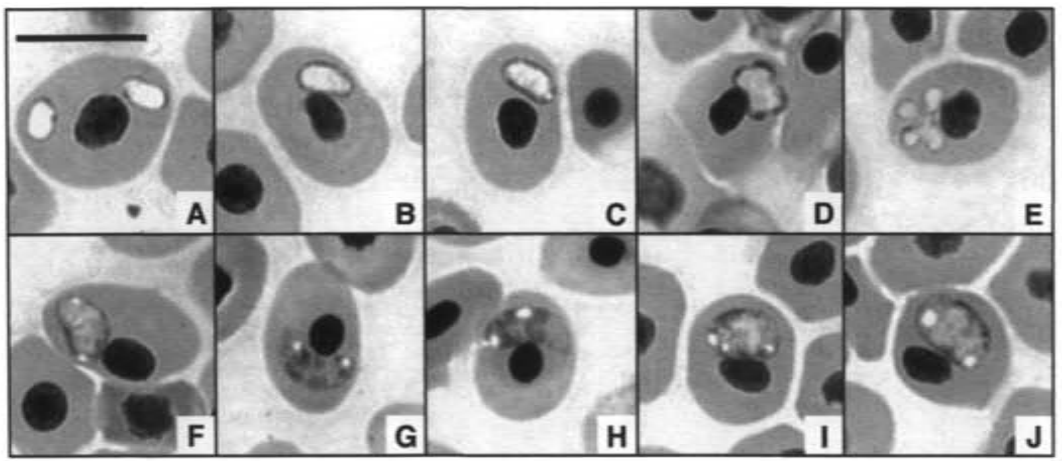


**Fig. 10.6** *Babesiosoma mariae* in the erythrocytes of the freshwater cichlid *Serranochromis angusticeps* from Botswana. **A-C:** paired and single late trophozoites or early meronts; **D, E:** characteristic cruciform merogony; **F:** immature gamont; **G-J:** mature gamonts (curved and broad types). Blood film Giemsa-stained. Bar scale= 10 µm for **A-J**. (From Smit *et al.*, 2003a, and reproduced by kind permission of the Editor-in-Chief of *Folia Parasitologica*.)

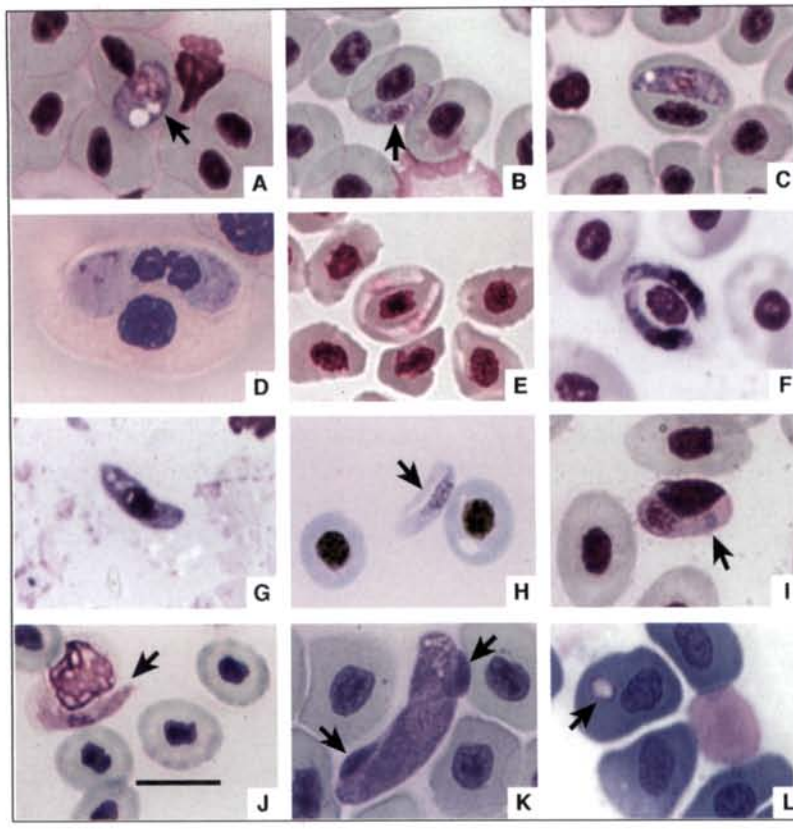


**Fig. 10.7** Haemogregarines (**A-K**) and a *Haemohormidium*-like infection (**L**) in Giemsa-stained blood films from a variety of marine (**A-H, J-L**) and freshwater (**I**) fishes. Intraerythrocytic stages (**A-F, I, J-L**), extracellular forms (**G, H**) and intraleukocytic stage (**J**). **A-C:** A haemogregarine, possibly *Desseria platessae*, in a flounder, *Paralichthys* sp., from Brazil (**A**, meront; **B**, immature gamont; **C**, mature gamont); from material kindly supplied by L. Amado. **D:** Large mature gamont of *Haemogregarina hemiscylli* in the epaulette shark, *Hemiscyllium ocellatum*, from Australia; from material kindly supplied by J. McKiernan. **E:** Paired gamonts of *Haemogregarina bigemina* from the shanny *Lipophrys pholis* from Wales. **F, G:** Paired and single meronts, or immature gamonts, of probable *Haemogregarina callionymi* in the dragonet *Callionymus dracunculus* from Wales; from material kindly supplied by M. Johnston. **H:** Mature gamont of *Haemogregarina koppiensis* showing a characteristic recurved tail, in a pufferfish *Amblyrhynchotes honckenii* from South Africa; from material kindly supplied by N. Smit. **I:** Gamont of *Haemogregarina vltavensis,* filling the erythrocyte cytoplasm and displacing the host cell nucleus, in the perch *Perca fluviatilis* from the Czech Republic; from materal kindly supplied by J. Lom. **J:** Mature gamont of a *Haemogregarina* sp. destroying the large lymphocyte of a triggerfish *Sufflamen* sp. from Australia; from material kindly supplied by A. Grutter. **K:** Large mature gamont of *Desseria nototheniiae* carrying portions of the disrupted host erythrocyte of a cod ice-fish *Notothenia* sp. from the Antarctic; from material kindly supplied by M. Ranzani Paiva. **L:** *Haemohormidium*-like infection from the blenny *Parablennius cornutus* in South Africa; from material kindly supplied by N. Smit. Bar scale= 10 μm for **A-L**.

Finally, transmission electron microscopy is enormously helpful in distinguishing intracellular apicomplexans from other non-apicomplexan infections within the same host cells. Apicomplexans within blood and tissue cells will exhibit some, or all, of their characteristic ultrastructure (the apical complex and its associated organelles), which other infections lack (Figs. 10.6, 10.7).

## Treatment, Prophylaxis and Economic Importance

The disease-inducing status of adeleorine and haematozoan apicomplexans has been little studied or understood, and they are usually considered of little economic importance, even though some occur in fishes of great commercial value. Furthermore, where fishes are farmed, there is no evidence that such infections can transfer to these stocks from endemic, wild, non-commercial fishes. It follows that there are no standard treatments or prophylactic measures in operation to control these infections in fishes.

## Outlook and Recent Developments

Apicomplexan blood parasites of fishes are widespread, but much more information is needed of their distribution in areas such as Africa, Asia and South America. Of the six genera considered above, some *Haemogregarina* spp. and perhaps *Mesnilium* are associated with the most tissue damage in fishes, but there are also indications that haemogregarines cause considerable changes to individual blood cells. Almost all infections have been studied in naturally infected fish and, consequently, little is known of the impact of such apicomplexans on young fishes, or those older fishes that do not survive and do not, therefore, get caught. There is no update on the apicomplexan-associated lesions first reported in farmed turbot during the 1970s and in wild mackerel in 1990. Further reports of *Mesnilium* infections are also lacking. Have such infections persisted? There is good evidence that a few species of adeleorine and haematozoan apicomplexans are likely leech- or isopod-transmitted and there is, thus, the possibility of leeches, but especially isopods, breeding in fish farms and aquaria (Smit and Davies, 2004). However, unless these blood-inhabiting apicomplexans are studied more intensively in future, it is likely that little more will be learnt about these interesting organisms.

## References

Alvarez-Pellitero, P. and A. Sitja-Bobadilla. 2002. *Cryptosporidium molnari* n. sp. (Apicomplexa: Cryptosporidiidae) infection two marine fish species, *Sparus aurata* L. and *Dicentrarchus labrax* L. *International Journal for Parasitology* 32: 1007-1021.

Alvarez-Pellitero, P., O. Palenzuela and A. Sitja-Bobadilla. 1997. Ultrastructure and cytochemistry study of *Eimeria sparis* (Protozoa: Apicomplexa) stages from the intestine of gilthead seabream *Sparus aurata* L. (Pisces: Teleostei). *Parasitology Research* 83: 126-136.

Alvarez-Pellitero, P., M.I. Quiroga, A. Sitja-Bobadilla, M.J. Redondo, O. Palenzuela, F. Padros, S. Vazquez and J.M. Nieto. 2004. *Cryptosporidium scophthalmi* n. sp. (Apicomplexa: Cryptosporidiidae) from cultured turbot *Scophthalmus maximus*. Light and electron microscope description and histopathological study. *Diseases of Aquatic Organisms* 62: 133-145.

Azevedo, C. 2001. Fine structure of sporogonic stages of *Goussia clupearum* (Apicomplexa: Eimeriidae) in the liver of infected fish (*Belone belone* L.), using light and electron microscopy. *Parasitology Research* 87: 326-330.

Ball, S.J., R.M. Pittilo and P.L. Long. 1989. Intestinal and extraintestinal life cycles of eimeriid coccidia. *Advances in Parasitology* 28: 1-54.

Barber, D.L. and J.E. Mills Westermann. 1988. *Haemogregarina georgianae* n.sp. A new haemogregarine parasite from the Antarctic teleost *Parachaenichthys georgianus*. *Systematic Parasitology* 11: 65-71.

Barber, D.L., J.E. Mills Westermann and P. Storoz. 1987. *Haemogregarina nototheniae* n. sp. from the blood of Antarctic nototheniids. *Systematic Parasitology* 10: 135-147.

Barta, J.R. 1991. The Dactylosomatidae. *Advances in Parasitology* 30: 1-37.

Cavalier-Smith, T. and E.E. Chao. 2004. Protalveolate phylogeny and systematics and the origins of Sporozoa and dinoflagellates (Phylum Myzozoa nom. nov.). *European Journal of Protistology* 40: 185-212.

Cesbron-Delauw, M.-F. 1994. Dense granule organelles of *Toxoplasma gondii*: Their host-parasite relationship. *Parasitology Today* 10: 293-296.

Cruz, C. and A.J. Davies. 1998. Some observations on *Babesiosoma bettencourti* (França, 1908) n. comb. (syns *Haemogregarina bettencourti* França 1908; *Desseria bettencourti* Siddall, 1995) from eels, *Anguilla anguilla* L., in Portugal. *Journal of Fish Diseases* 21: 443-448.

Davies, A.J. 1978. Coccidian parasites of intertidal fishes from Wales: Systematics, development, and cytochemistry. *Journal of Protozoology* 25: 15-21.

Davies, A.J. 1990. Ultrastructural studies on the endogenous stages of *Eimeria variabilis* (Thélohan, 1883) Reichenow 1921, from *Cottus (Taurulus) bubalis* Euphrasem (Teleostidae: Cottidae). *Journal of Fish Diseases* 13: 447-461.

Davies, A.J. 1995. The biology of fish haemogregarines. *Advances in Parasitology* 36: 117-203.

Davies, A.J. and S.J. Ball. 1993. The biology of fish coccidia. *Advances in Parasitology* 32: 294-366.

Davies, A.J. and M.R.L. Johnston. 2000. The biology of some intraerythrocytic parasites of fishes, amphibian and reptiles. *Advances in Parasitology* 45: 1-107.

Davies, A.J. and N.R. Merrett. 2000. Haemogregarines and other blood infections from deep demersal fish of the Porcupine Seabight, north-east Atlantic. *Journal of the Marine Biological Association of the United Kingdom, Plymouth* 80: 1095-1102.

Davies, A.J. and N.J. Smit. 2001. The life cycle of *Haemogregarina bigemina* (Adeleina: Haemogregarinidae) in South African hosts. *Folia Parasitologica* 48: 169-177.

Davies, A.J. and N.J. Smit. 2001. The life cycle of *Haemogregarina bigemina* (Adeleina: Haemogregarinidae) in South African hosts. *Folia Parasitologica* 48: 169-177.

Davies, A.J. and B. Stewart. 2000. Autofluorescence in the oocysts of marine and freshwater fish coccidia. *Folia Parasitologica* 47: 157-158.

Davies, A.J., C.C. Reed and N.J. Smit. 2003. An unusual intraerythrocytic parasite of *Parablennius cornutus* from South Africa. *Journal of Parasitology* 89: 913-917.

Davies, A.J., N.J. Smit, P.M. Hayes, A.M. Seddon and D. Wertheim. 2004. *Haemogregarina bigemina* (Protozoa: Apicomplexa:Adeleorina)—Past, present and future. *Folia Parasitologica* 51: 99-108.

Davies, A.J., W. Gibson, V. Ferris, L. Basson and N.J. Smit. 2005. Two genotypic groups of morphologically similar fish trypanosomes from the Okavango Delta, Botswana. *Diseases of Aquatic Organisms* 66: 215-220.

Desser, S.S. and L. Li. 1984. Ultrastructural observations on the sexual stages and oocyst formation in *Eimeria laureleus* (Protozoa: Coccidia) of perch, *Perca flavescens*, from Lake Sasajewun, Ontario. *Zeitschrift für Parasitenkunde* 70: 153-164.

Dias, P.J., J.C. Eiras and A.J. Davies. 2005. A six-month duration, follow-up study of *Haemogregarina bigemina* at Foz do Douro, North Portugal, between ten and twenty years on. *Bulletin of the European Association of Fish Pathologists* 25: 161-165.

Duszynski, D.W., D.W. Solangi and R.M. Overstreet. 1979. A new and unusual eimerian (Protozoa: Eimeriidae) from the liver of the gulf killifish *Fundulus grandis*. *Journal of Wildlife Diseases* 15: 543-552.

Duszynski, D.W., L. Couch and S.J. Upton. 2000a. *The Coccidia of the World* (available at: http://www.k-state.edu/parasitology/worldcoccidia/, cited at: Nov. 27, 2005).

Duszynski, D.W., L. Couch and S.J. Upton. 2000b. Coccidia (Eimeriidae) of Cypriniformes (cyprinids). In: *The Coccidia of the World*, D.W. Duszynski, L. Couch and S.J. Upton (eds.). (available at: http://biology.unm.edu/biology/coccidia/cyprin.html, cited at: Nov. 27, 2005).

Dyková, I. and J. Lom. 1981. Fish coccidia: critical notes on life cycles, classification and pathogenicity. *Journal of Fish Diseases* 4: 487-505.

Dyková, I. and J. Lom. 1983. Fish coccidia: An annotated list of described species. *Folia Parasitologica* 30: 193-208.

Dyková, I., J. Lom and G. Grupcheva. 1983. *Eimeria branchiphilia* sp. nov. sporulating in the gill filaments of roach, *Rutilus rutilus* L. *Journal of Fish Diseases* 6: 13-18.

Eiras, J.C. 1987. Detection of *Haemogregarina bigemina* (Protozoa: Apicomplexa) infection in fish erythrocytes by phase-contrast microscopy. *Journal of Fish Biology* 31: 291-292.

Fantham, H.B., A. Porter and L.R. Richardson. 1942. Some haematozoa observed in vertebrates in eastern Canada. *Parasitology* 32: 199-226.

Fayer, R., C.A. Speer and J.P. Dubey. 1997. The general biology of *Cryptosporidium*. In: *Cryptosporidium and Cryptosporidiosis*, R. Fayer (ed.). CRC Press, Boca Raton, pp. 1-41.

Ferguson, H.W. and R.J. Roberts. 1975. Myeloid leucosis associated with sporozoan infections in cultured turbot (*Scophthalmus maximus* L.). *Journal of Comparative Pathology* 85: 317-326.

Ferguson, H.W. and R.J. Roberts. 1976. A condition simulating lymphoma, associated with sporozoan infection in cultured turbot (*Scophthalmus maximus* L.). *Progress in Experimental Tumour Research* 20: 212-216.

Fournie, J.W. and R.M. Overstreet. 1983. True intermediate hosts for *Eimeria funduli* (Apicomplexa) from estuarine fishes. *Journal of Protozoology* 30: 672-675.

Fournie, J.W., W.K. Vogelbein, R.M. Overstreet and W.E. Hawkins. 2000. Life cycle of *Calyptospora funduli* (Apicomplexa: Calyptosporidae). *Journal of Parasitology* 86: 501-505.

Gestal, C. and C. Azevedo. 2005. Ultrastructure of *Goussia cruciata* (Apicomplexa: Coccidia) infecting the liver of horse mackerel, *Trachurus trachurus* (L.), from Ibero-Atlantic waters. *Journal of Fish Diseases* 28: 125-132.

Graczyk, T.K., R. Faye and M.R. Carnfield. 1996. *Cryptosporidium parvum* is not transmissible to fish, amphibians, or reptiles. *Journal of Parasitology* 82: 748-751.

Gregory, M.W. 1990. Pathology of coccidial infections. In: *Coccidiosis of Man and Domestic Animals*, P.L. Long (ed.). CRC Press, Boca Raton, pp. 235-261.

Haldar, D.P. and M. Mukherjee. 1979. An annotated list of protozoan parasites from *Ophicephalus punctatus* Bloch, a common food-fish. *Archiv für Protistenkunde* 121: 392-400.

Hawkins, W.E., M.A. Solangi and R.M. Overstreet. 1981. Ultrastructural effects of the coccidium *Eimeria funduli* Duszynski, Solangi and Overstreet, 1979 on the liver of killifishes. *Journal of Fish Diseases* 4: 281-295.

Hawkins, W.E., M.A. Solangi and R.M. Overstreet. 1983a. Ultrastructure of the macrogamont of *Eimeria funduli*, a coccidium parasitizing killifishes. *Journal of Fish Diseases* 6: 33-43.

Hawkins, W.E., M.A. Solangi and R.M. Overstreet. 1983b. Ultrastructure of the microgamont and microgamete of *Eimeria funduli*, a coccidium parasitizing killifishes. *Journal of Fish Diseases* 6: 45-57.

Hawkins, W.E., J.W. Fournie and R.M. Overstreet. 1984. Ultrastructure of the interface between stages of *Eimeria funduli* (Apicomplexa) and hepatocytes of the longnose killifish, *Fundulus similis*. *Journal of Parasitology* 70: 232-238.

Hemmer, N., D. Steinhagen, W. Drommer and W. Körting. 1998. Changes of intestinal epithelial structure and cell turnover in carp *Cyprinus carpio* infected with *Goussia carpelli* (Protozoa: Apicomplexa). *Diseases of Aquatic Organisms* 34: 39-44.

Jendrysek, S., D. Steinhagen, W. Drommer and W. Körting. 1994. Carp coccidiosis: Intestinal histopathology and cytopathology under *Goussia carpelli* infection. *Diseases of Aquatic Organisms* 20: 171-182.

Jirku, M., D. Modry, J.R. Slapeta, B. Koudela and J. Lukes. 2002. Thy phylogeny of *Goussia* and *Choleoeimeria* (Apicomplexa, Eimeriorina) and the evolution of excystation structure in coccidia. *Protist* 153: 379-390.

Kent, M.L. and R.P. Hedrick. 1985. The biology and associated pathology of *Goussia carpelli* (Léger and Stankovitch) in goldfish *Carassius auratus* (Linnaeus). *Fish Pathology* 20: 485-494.

Khan, R.A. 1978. A new hemogregarine from marine fishes. *Journal of Parasitology* 64: 35-44.

Khan, R.A., W. Threlfall and W.S. Whitty. 1992. Haematozoa of deep-sea demersal fish of the northwest Atlantic Ocean. *Canadian Journal of Zoology* 70: 1926-1933.

Kim, S.H. and I. Paperna. 1992. Fine structure of epicytoplasmic stages of *Eimeria vanasi* from the gut of cichlid fish. *Diseases of Aquatic Organisms* 12: 191-197.

Kim, S.H. and I. Paperna. 1993a. Development and fine structure of intracytoplasmic and epicytoplasmic meronts, merozoites and young macrogamonts of the cichlid fish swimbladder coccidium *Goussia cichlidarum*. *Diseases of Aquatic Organisms* 15: 51-61.

Kim, S.H. and I. Paperna. 1993b. Endogenous development of *Goussia trichogasteri* (Apicomplexa: Eimeriidae) in the intestine of gourami *Trichogaster trichopterus*. *Diseases of Aquatic Organisms* 17: 175-180.

Kirmse, P.D. 1980. Observations on the pathogenicity of *Haemogregarina sachai* Kirmse, 1978, in farmed turbot *Scophthalmus maximus* (L.). *Journal of Fish Diseases* 3: 101-114.

Kirmse, P.D. and H.W. Ferguson. 1976. *Toxoplasma*-like organisms as the possible causative agents of a proliferative condition in farmed turbot (*Scophthalmus maximus*). In: *Wildlife Diseases*, L.A. Page (ed.). Plenum Press, New York, pp. 561-564.

Kocylowski, B., J. Zelazny, J. Antychowicz and J. Panczyk. 1976. Incidence of carp coccidiosis and its control. *Bulletin of the Veterinary Institute of Pulawy* 20: 12-17.

Lainson, R. 1981. On *Cyrilia gomesi* (Neiva and Pinto, 1926) gen. nov. (Haemogregarinidae) and *Trypanosoma bourouli* Neiva and Pinto in the fish *Synbranchus marmoratus* : Simultaneous transmission by the leech *Haementeria lutzi*. In: *Parasitological Topics: A Presentation Volume to P.C.C. Garnham F.R.S. on the Occasion of his 80th Birthday, 1981*, E.U. Canning (ed.). Society of Protozoologists Lawrence, Kansas, pp. 150-158.

Laird, M. 1953. The Protozoa of New Zealand intertidal zone fishes. *Transactions of the Royal Society of New Zealand* 81: 79-143.

Landau, I., M. Marteau, Y. Golvan, A.G. Chabaud and Y. Boulard. 1975, Heteroxénie chez les coccidies intestinales de poissons. *Compte Rendu Hebdomadaire des Sécances de l'Académie des Sciences, Série D* 281: 1721-1723.

Landsberg, J.H. and I. Paperna. 1987. Intestinal infections by *Eimeria* (s.l.) *vanasi* n.sp. (Eimeriidae: Apicomplexa, Protozoa) in cichlid fish. *Annales de Parasitologie Humaine et Comparée* 62: 283-293.

Levine, N.D. 1985. Phylum Apicomplexa Levine. 1970. In: *An Illustrated Guide to the Protozoa*, J.J. Lee, S.H. Hutner and E.C. Bovee (eds.). Society of Protozoologists, Lawence, KS, pp. 322-374.

Lom, J. and I. Dyková. 1992. Protozoan Parasites of Fishes. *Developments in Aquaculture and Fisheries Science*, Vol. 26. Elsevier, Amsterdam.

Lom, J., S.S. Desser and I. Dyková. 1989. Some little-known and new protozoan parasites of fish from Lake Sasajewun, Algonquin Park, Ontario. *Canadian Journal of Zoology*, 67: 1372-1379.

Lukes, J. 1993. A coccidian (Apicomplexa: Eimeriidae) with extracytoplasmally located stages in the kidney tubule of golden carp (*Carassius auratus gibelio* L.) (Cyprinidae). *Folia Parasitologica* 40: 1-7.

Lukes, J. and I. Dykova. 1990. *Goussia janae* n.sp. (Apicomplexa, Eimeriorina) in dace *Leuciscus leuciscus* and Chubb *L. cephalus*. *Diseases of Aquatic Organisms* 8: 85-90.

Mackenzie, K. 1981. The effect of *Eimeria* sp. infection on the condition of blue whiting, *Micromesistius poutassou*. *Journal of Fish Diseases* 4: 473-486.

MacLean, S.A. and A.J. Davies. 1990. Prevalence and development of intraleucocytic haemogregarines from northwest and northeast Atlantic mackerel, *Scomber scombrus*. *Journal of Fish Diseases* 13: 59-68.

Marincek, M. 1973a. Développement *d'Eimeria subepithelialis* (Sporozoa, Coccidia)-parasite de la carpe. *Acta Protozoologica* 12: 195-216.

Marincek, M. 1973b. Les changements dans le tube digestif dez *Cyprinus carpio* á la suite de l'infection par *Eimeria subepithelialis* (Sporozoa, Coccidia). *Acta Protozoologica* 12: 217-224.

McGladdery, S.E. 1987. Potential of *Eimeria sardinae* (Apicomplexa: Eimeriidae) oocysts for distinguishing between spawning groups and between first- and repeat spawning Atlantic herring (*Clupea harengus harengus*). *Canadian Journal of Fisheries and Aquatic Sciences* 44: 1378-1385.

McKiernan, J.P., A.S. Grutter and A.J. Davies. 2005. Reproductive and feeding ecology of parasitic gnathiid isopods of epaulette sharks (*Hemiscyllium ocellatum*) with consideration of their role in the transmission of a haemogregarine. *International Journal for Parasitology* 35: 19-27.

Minchin, E.A. and H.M. Woodcock. 1910. Observations on certain blood parasites of fishes occurring at Rovigno. *Quarterly Journal of the Microscopical Society* 55: 113-154.

Misra, K.K., D.P. Haldar and M.M. Chakravarty. 1972. Observations on *Mesnilium malariae* gen. nov., spec. nov. (Haemosporidia, Sporozoa) from the freshwater teleost, *Ophiocephalus punctatus* Bloch. *Archiv für Protistenkunde* 114: 444-452.

Molnar, K. 1976. Histological study on coccidiosis in silver carp and the bighead by *Eimeria sinensis* Chen, 1956. *Acta Veterinaria Academiae Scientiarum Hungaricae* 26: 303-312.

Molnar, K. 1979. Studies on coccidia of Hungarian pond fishes. In: *Coccidia and Further Prospects of their Control. International Symposium Prague*, pp. 179-183.

Molnar, K. 1984. Some peculiarities of oocyst rejection of fish coccidia. *Symposia Biologica Hungarica (Akademia Kiado, Budapest)* 23: 87-97.

Molnar, K. 1989. Nodular and epicellular coccidiosis in the intestine of cyprinid fishes. *Diseases of Aquatic Organisms* 7: 1-12.

Molnar, K. 1996. Nodular coccidiosis of the pikeperch *Stizostedion lucioperca* and Volga perch *Stizostedion volgensis*. *Diseases of Aquatic Organisms* 27: 35-41.

Molnar, K. and F. Baska. 1986. Light and electron microscopic studies an *Epieimeria anguillae* (Léger and Hollande, 1922), a coccidium parasitizing the European eel, *Anguilla anguilla*. *Journal of Fish Diseases* 9: 99-110.

Molnar, K. and F. Baska. 1992. Electron microscopic study of developmental stages of *Goussia balatonica* and *G. pannonica* (Apicomplexa Eimeriorina), parasites of white bream *Blicca bjoerkna*. *Diseases of Aquatic Organisms* 13: 95-101.

Molnar, K., G. Ostoros and F. Baska. 2005. Cross-infection experiments confirm the host specificity of *Goussia* spp. (Eimeriidae: Apicomplexa) parasitising cyprinid fish. *Acta Protozoologica* 44: 43-49.

Morrison, C.M. 1991. Further observations on the sporogony of *Eimeria sardinae* in the testis of the herring *Clupea harengus*. *Canadian Journal of Zoology* 69: 1017-1024.

Morrison, C.M. and W.E. Hawkins. 1984. Coccidians in the liver and testes of the herring *Clupea harengus*. *Canadian Journal of Zoology* 62: 480-493.

Morrison, C.M. and S.L. Poynton. 1989. A new species of *Goussia* (Apicomplexa, Coccidia) in the kidney tubules of the cod, *Gadus morhua* L. *Journal of Fish Diseases* 12: 533-560.

Morrison, C.M., J.P. Leger and C.A. Morrison. 1993a. Light and electron microscopic study of the pathology and merogony of *Goussia gadi* (Apicomplexa: Coccidia) in the swimbladder wall of haddock (*Melanogrammus aeglefinus*). *Diseases of Aquatic Organisms* 17: 113-125.

Morrison, C.M., J.P. Leger and C.A. Morrison. 1993b. Gametocytes of *Goussia gadi* (Apicomplexa: Coccidia) in the swimbladder wall of haddock (*Melanogrammus aeglefinus*). *Diseases of Aquatic Organisms* 17: 127-135.

Morrison, D.A., S. Bornstein, P. Thebo, U. Wernery, J. Kinne and J.G. Mattsson. 2004. The current status of the small subunit rRNA phylogony of the coccidia (Sporozoa). *International Journal for Parasitology* 34: 501-514.

Musselius, V.A., V.I. Laptev and N.S. Ivanova. 1965. On the coccidiosis of the common carp. *Trudi VNIIPRH* 13, 69-78. (In Russian).

Negm-Eldin, M.M. 1998. Life cycle, host restriction and longevity of *Babesiosoma mariae* Hoare 1930 (Apicomplexa: Dactylosomatidae). *Deutsche Tierärztliche Wochenschrift* 105: 367-374.

Negm-Eldin, M.M. 1999. Life cycle, host restriction and longevity of *Cyrilia nili* (*Haemogregarina nili* Wenyon, 1909) n.comb. *Deutsche Tierärztliche Wochenschrift* 106: 191-199.

Ono, K., K. Horn and A.O. Heydorn. 1991. Purification of *in vitro* excysted *Sarcocystis* sporozoites by passage through a modified DE 52 anion-exchange column. *Parasitology Research* 77: 717-719.

Overstreet, R.M. 1981. Species of *Eimeria* in nonepithelial sites. *Journal of Protozoology* 28: 258-260.

Overstreet, R.M., W.E. Hawkins and J.W. Fournie. 1984. The coccidian genus *Calyptospora* n.g. and Family Calyptosporidae n.fam. (Apicomplexa), with members infecting primarily fishes. *Journal of Protozoology* 31: 332-339.

Paperna, I. 1979. Sporozoan infection in cultured *Sparus aurata* L. and wild *Siganus luridus*. *Annales de Parasitologie Humaine et Comparée* 54: 385-392.

Paperna, I. 1990. Fine structure of the gamonts of *Eimeria* (s.l.) *vanasi*, a coccidium from the intestine of cichlid fishes. *Diseases of Aquatic Organisms* 9: 163-170.

Paperna, I. 1991. Fine structure of *Eimeria* (s.l.) *vanasi* merogony stages in the intestinal mucosa of cichlid fishes. *Diseases of Aquatic Organisms* 10: 195-201.

Paperna, I. 1995. Ultrastructural and developmental affinities of piscine coccidia. *Diseases of Aquatic Organisms* 22: 67-76.

Paperna, I. 1999. Parasites in warmwater aquaculture. In: *Fish Diseases and Disorders*, P.T.K. Woo (ed.). CABI Publishing, Wallingford, Oxon, UK, pp. 162-165.

Paperna, I. and J.H. Landsberg. 1985. Ultrastructure of oogony and sporogony in *Goussia cichlidarum*, Landsberg and Paperna, 1985, a coccidian parasite in the swimbladder of cichlid fish. *Protistologica* 21: 349-359.

Paperna, I., J.H. Landsberg and N. Feinstein. 1986. Ultrastructure of the macrogamont of *Goussia cichlidarum* Landsberg and Paperna, 1985, a coccidian parasite in the swimbladder of chchlid fish. *Annales de Parasitologie Humaine et Comparée* 61: 511-520.

Pasnik, D.J., S.A. Smith and D.S. Lindsay. 2005. Intestinal coccidiosis in bluegill, *Lepomis macrochirus*. *Journal of Parasitology* 91: 967-970.

Paterson, W.B. and S.S. Desser. 1981a. Ultrastructure of macrogametogenesis, macrogametes and young oocysts of *Eimeria iroquoina* Molnar and Fernando, 1974 in experimentally infected fathead minnow (*Pimephales promelas*, Cyprinidae). *Journal of Parasitology* 67: 496-504.

Paterson, W.B. and S.S. Desser. 1981b. An ultrastructural study of microgametogenesis and the microgamete in *Eimeria iroquoina* Molnar and Fernando, 1974, in experimentally infected fathead minnow (*Pimephales promelas*, Cyprinidae). *Journal of Parasitology* 67: 314-324.

Paterson, W.B. and S.S. Desser. 1981c. An ultrastructural study of *Eimeria iroqouina* Molnar and Fernando 1974, in experimentally infected fathead minnow (*Pimephales promelas*, Cyprinidae). 3. Merogony. *Journal of Protozoology* 28: 764-775.

Paterson, W.B. and S.S. Desser. 1982. The biology of two *Eimeria* species (Protista: Apicomplexa) in their mutual fish hosts in Ontario. *Canadian Journal of Zoology* 60: 764-775.

Paterson, W.B. and S.S. Desser. 1984. Ultrastructural observations on fertilization and sporulation in *Goussia iroquoina* (Molnar and Fernando, 1974) in experimentally infected fathead minnows (*Pimephales promelas*, Cyprinidae). *Journal of Parasitology* 70: 703-711.

Perkins, F.O., J.R. Barta, R.E. Clopton, M.A. Peirce and S.J. Upton. 2000. Phylum Apicomplexa Levine, 1970. In: *An Illustrated Guide to the Protozoa*, J.J. Lee, G.F. Leedale and P. Bradbury (eds.). 2nd Edition. Society of Protozoologists, Lawrence, Kansas, Vol. 1, pp. 190-369.

Ruff, M.D. and P.C. Allen. 1990. Pathophysiology of coccidial infections. In: *Coccidiosis of Man and Domestic Animals*, P.L. Long (ed.). CRC Press, Boca Raton, pp. 263-280.

Ryan, U., A. O'Hara and L. Xiao. 2004. Molecular and biological characterisation of a *Cryptosporidium molnari*-like isolate from a guppy (*Poecilia reticulata*). *Applied and Environmental Microbiology* 70: 3761-3765.

Sam-Yellowe, T.Y. 1996. Rhoptry organelles of the Apicomplexa: their role in host cell invasion and intracellular survival. *Parasitology Today* 12: 308-316.

Saunders, D.C. 1960. A survey of the blood parasites in the fishes of the Red Sea. *Transactions of the American Microscopical Society* 79: 239-259.

Schmahl, G., H. Taraschewski and H. Mehlhorn. 1989. Chemotherapy of fish parasites. *Parasitology Research* 75: 503-511.

Scholtyseck, E. 1979. *Fine Structure of Parasitic Protozoa*. Springer-Verlag, Berlin.

Siddall, M.E. 1995. Phylogeny of adeleid blood parasites with a partial systematic revision of the haemogregarine complex. *Journal of Eukaryotic Microbiology* 42: 116-125.

Siddall, M.E. and E.M. Burreson. 1994. The development of a haemogregarine of *Lycodes raridens* from Alaska in its definitive leech host. *Journal of Parasitology* 80: 567-575.

Siddall, M.E., S.S. Desser and L.N. Measures. 1994. Light and electronmicroscopic examination of the so-called piroplasms of fishes from Atlantic Canada and systematic revision of the Haemohormidiidae (incertae sedis). *Journal of Parasitology* 80: 1018-1025.

Sitja-Bobadilla, A. and P. Alvarez-Pellitero. 2003. Experimental transmission of *Cryptosporidium molnari* (Apicomplexa: Coccidia) to gilthead seabream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.). *Parasitology Research* 91: 209-214.

Sitja-Bobadilla, A., F. Padros, C. Aguilera and P. Alvazez-Pellitero. 2005. Epidemiology of *Cryptosporidium molnari* in Spanish gilthead seabream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.) cultures: from hatchery to market size. *Applied and Environmental Microbiology* 71: 131-139.

Smit, N.J. and A.J. Davies. 2001. An encapsulated haemogregarine from the evileye pufferfish in South Africa. *Journal of the Marine Biological Association of the United Kingdom, Plymouth* 81: 751-754.

Smit, N.J. and A.J. Davies. 2004. The curious life-style of the parasitic stages of gnathid isopods. *Advances in Parasitology* 58: 289-412.

Smit, N.J., J.G. Van As and A.J. Davies. 2003a. Observations on *Babesiosoma mariae* (Apicomplexa: Dactylosomatidae) from the Okavango Delta, Botswana. *Folia Parasitologica* 50: 85-86.

Smit, N.J., J.G. Van As and A.J. Davies. 2003b. Taxonomic re-evaluation of the South African fish haemogregarine, *Desseria fragilis*. *Journal of Parasitology* 89: 151-153.

Smith, T.G. 1996. The genus *Hepatozoon* (Apicomplexa: Adeleina). *Journal of Parasitology* 82: 565-585.

Solangi, M.A. and R.M. Overstreet. 1980. Biology and pathogenesis of the coccidium *Eimeria funduli* infecting killifishes. *Journal of Parasitology* 66: 513-526.

Solangi, M.A., R.M. Overstreet and J.W. Fournie. 1982. Effect of low temperature on development of the coccidium *Eimeria funduli* in the Gulf killifish. *Parasitology* 84: 31-39.

Speer, C.A. 1983. The Coccidia. In: *In vitro Cultivation of Protozoan Parasites*, J.B. Jensen (ed.). CRC Press, Boca Raton, pp. 1-64.

Steinhagen, D. 1991a. Ultrastructural observations on merogonic and gamogonic stages of *Goussia carpelli* (Apicomplexa, Coccidia) in experimentally infected common carp *Cyprinus carpio*. *European Journal of Protistology* 27: 71-78.

Steinhagen, D. 1991b. Ultrastructural observations on sporozoite stages of piscine coccidia: *Goussia carpelli* and *G. subepithelialis* from the intestine of tubificid oligochaetes. *Diseases of Aquatic Organisms* 10: 121-125.

Steinhagen, D. 1994. Methods for isolation and purification of *Goussia carpelli* (Protozoa: Coccidia)—developmental stages from the intestine of infected common carp. *Bulletin of the European Association of Fish Pathologists* 14: 200-203.

Steinhagen, D. and W. Körting. 1988. Experimental transmission of *Goussia carpelli* (Leger and Stankovitch, 1921, Protista: Apicomplexa) to common carp *Cyprinus carpio* L. *Bulletin of the European Association of Fish Pathologists* 8: 112-113.

Steinhagen, D. and W. Körting. 1990. The role of tubificid oligochaetes in the transmission of *Goussia carpelli*. *Journal of Parasitology* 76: 104-107.

Steinhagen, D., W. Körting and W.B. van Muiswinkel. 1989. Morphology and biology of *Goussia carpelli* (Protozoa: Apicomplexa) from the intestine of experimentally infected common carp *Cyprinus carpio*. *Diseases of Aquatic Organisms* 6: 93-98.

Steinhagen, D., J. Lukes and W. Körting. 1990. Ultrastructural observations on gamogonic stages of *Goussia subepithelialis* (Apicomplexa, Coccidia) from common carp *Cyprinus carpio*. *Diseases of Aquatic Organisms* 9: 31-36.

Steinhagen, D., B. Oesterreich and W. Körting. 1997. Carp coccidiosis: Clinical and haematological observations of carp infected with *Goussia carpelli*. *Diseases of Aquatic Organisms* 30: 137-143.

Steinhagen, D., K. Hespe and B. Ellmer. 1998. *Goussia carpelli* (Protozoa: Coccidia) infection in stressed and immunosuppressed common carp *Cyprinus carpio*. *Diseases of Aquatic Organisms* 34: 199-204.

Strout, R.G. and D.M. Schmatz. 1990. *In vitro* culture of coccidia/biochemistry. In: *Coccidiosis of Man and Domestic Animals*, P.L. Long (ed.). CRC Press, Boca Raton, pp. 221-233.

Studnicka, M. and A. Siwicki. 1990. The nonspecific immunological response in carp (*Cyprinus carpio* L.) during natural infections with *Eimeria subepithelialis*. *Israeli Journal of Aquaculture–Bamidgeh* 42: 18-21.

Tenter, A.M., J.R. Barta, I. Beveridge, D.W. Duszynski, H. Mehlhorn, D.A. Morrison, R.C.A. Thompson and P.A. Conrad. 2002. The conceptual basis for a new classification of the coccidia. *International Journal of Parasitology* 32: 595-616.

Upton, S.J. 2000. Suborder Eimeriorina Léger 1911. In: *The Illustrated Guide to the Protozoa*, J.J. Lee, G.F. Leedale and P. Bradbury (eds.). 2nd Edition. Society of Protozoologists, Lawrence, Kansas, pp. 318-339.

Vilenkin, M. and I. Paperna. 1997. Development of sporozoites of the piscine coccidium *Eimeria* (sensu lato) *vanasi* in gut epithelial lymphocyte-like cells. *Folia Parasitologica* 44: 91-98.

Zmerzlaya, E.I. 1969. Eife cycle of *Eimeria carpelli* Léger et Stankovitch. In: *Progress in Protozoology. Third International Congress on Protozoology, Leningrad*, 1969, July 2-10, p. 362.

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CHAPTER

11

# Diseases Caused by Ciliophora

**Angelo Colorni**

The phylum Ciliophora Doflein, 1901, includes highly evolved protozoa that propel their bodies and gather food by means of short hair-like projections called cilia, whose movement is coordinated in a rhythmical, metachronous (wave-like) pattern. Cilia have a characteristic structure—the axoneme—consisting of nine pairs of microtubules surrounding an inner pair and anchored to the cell by a basal body, the kinetosome. The kinetosomes are interconnected by rows of fibrils called kinetodesmata that form extremely elaborate locomotor structures termed kineties. This internal system persists even when the cell is devoid of somatic cilia. A semi-rigid cortical pellicle functions as an exoskeleton, protecting and maintaining body shape. Ciliates are dikaryotic, with nuclear dimorphism: one or more polyploid vegetative macronuclei regulate the cell metabolism and have somatic functions, while one or more diploid micronuclei—repository of the genomic DNA organized into chromosomes—are involved in the generative processes (Steinbrück, 1990; Lynn and Small,

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2000). The two nuclear genomes differ in their organization to varying degrees, depending on the ciliate species (Anderson, 1988; Steinbrück, 1990). Macronuclei eventually disintegrate and are replaced by growth of a micronucleus (in size and ploidy) to become a new macronucleus (Jahn *et al.*, 1949). Macronuclei divide amitotically, and loss or under-replication of some moderately repetitive DNA sequences may accompany their differentiation (Yao and Gorovsky, 1974).

The Ciliophora are today classified in the Alveolates, a lineage that, on the basis of molecular evolution studies, also includes the Apicomplexa and the dinoflagellates (Patterson, 2000). Over 8,000 species of ciliated protozoa are known. Most are phagotrophic, directing food particles by means of specialized ciliary structures, the oral kinetids, through the cytostome and into the cytopharynx (Lynn and Small, 2000). The great majority of ciliates are free-living; many others are associated with invertebrates and vertebrates as harmless symbionts or epiphoronts, while a relatively few species have evolved a true parasitic lifestyle. Overlapping relationships exist, sometimes transitional, and possibly reflecting a shift from one lifestyle to another. In the aquatic environment, short reproduction times, high prolificacy, direct transmission and generally low species-specificity compensate for the difficulties of ciliated parasites in intercepting a suitable host in the vastness of a lake or a sea. In the confined environment of an aquarium or pond, these same traits are ideal for the parasites' rapid proliferation, so that an entire fish stock may be overwhelmed within a few days. Adaptation to obligatory parasitism involves the establishment of a fairly high degree of compatibility with their hosts, as indicated by the invasiveness—limited to the fish's gill and skin epithelial layers—of species like *Ichthyophthirius multifiliis* and *Cryptocaryon irritans*. These species also rely exclusively on piscine hosts as a nutrition source and have developed a complex, polymorphic life cycle. Their destructive feeding activity leads to an accelerated and excessive division of the host epithelial cells (hyperplasia), through which the infested fish attempts to repair the damage. Osmoregulatory disturbance, respiratory distress, and excretory impairment may soon follow. Secondary bacterial or mold infections can further aggravate the condition.

In addition, many species of normally saprozoic ciliates can colonize superficial tissues of severely stressed, debilitated or moribund fish. Reproduction in this group of ciliates is simple; occurring by transverse binary fission, with resting stages occasionally produced by some species.

They are generally amenable to culture *in vitro* on chemically defined media. The outcome of the dynamic interaction between these opportunistic pathogens and the compromised defense mechanisms of the host determines whether or not they succeed in invading deeper tissues and often causing lethal injuries.

Both obligatory and opportunistic pathogenic ciliates share a general lack of discrimination in their host selection, and the ultimate damage they inflict in particular on captive fish can be equally devastating.

This chapter is arranged in two main parts in which the major pathogenic and opportunistic ciliates—first those associated with freshwater fish, then those associated with marine fish—are described. The peritrichs, with sessile and mobile members living in both environments, connect the two sections. A description of the enteric ciliates concludes the chapter. The taxonomic overview (Table 11.1) is based almost entirely on Lynn and Small (2000).

## FRESHWATER

### Ichthyophthiriosis

#### Agent: *Ichthyophthirius multifiliis* Fouquet, 1876

Responsible for freshwater White Spot Disease, *Ichthyophthirius multifiliis* Fouquet, 1876 (Class Oligohymenophorea, Order Hymenostomatida, Family Ichthyophthiriidae) has a worldwide distribution, from the sub-Arctic to the tropics, and virtually no fish specificity, making 'Ich' one of the most common protozoan infections of fish living in fresh waters. Predominant gross signs include small white spots all over the fish body (Fig. 11.1). Diagnosis can be confirmed by microscopic observation of the ciliate in skin smears or gill clips. *Ichthyophthirius multifiliis* is holotrichous; i.e., its cilia are distributed on the entire cell, and it revolves within the fish epithelial layers with a typical slow motion. Variants of *I. multifiliis* have been described, based on differences in nuclear morphology, temperature tolerance, host range (Nigrelli *et al.*, 1976) and serotype (Dickerson *et al.*, 1993). However, none of the variations reported thus far has been substantial enough, or possibly studied in sufficient depth, so as to justify the separation of *Ichthyophthirius* into distinct species. Worldwide distribution of *I. multifiliis* is the apparent result of widespread translocation of cultured and ornamental fish. The presence of *I. multifiliis* in autochthonous fish in remote areas of the world suggests that many

**Table 11.1** Taxonomy of the main ciliate protozoan parasites of fish (to the genus level, according mainly to Lynn and Small (2000). *Helicostoma*, *Porpostoma* and trichodinids according to Corliss (1979) and Lom and Dyková (1992). *Cryptocaryon irritans* re-classified according to Wright and Colorni (2002).

| *Phylum* | *Subphylum* | *Class* | *Subclass* | *Order* | *Suborder* | *Family* | *Genus* | *Species* |
|---|---|---|---|---|---|---|---|---|
| Ciliophora Doflein, 1901 | Postciliodesmatophora Gerassimova and Seravin, 1976 | Heterotrichea Stein, 1859 | | Licnophorida Corliss, 1957 | | Licnophoridae Bütschli, 1887 | *Licnophora* Claparède, 1867 | *hippocampi* |
| | Intramacronucleata Lynn, 1996 | Spirotrichea Bütschli, 1889 | Hypotrichia Stein, 1859 | Euplotida Small and Lynn, 1985 | Euplotina Small and Lynn, 1985 | Euplotidae Ehrenberg, 1838 | *Euplotes* Ehrenberg, 1830 | |
| | | Oligohymenophoreade Puytorac *et al.*, 1974 | Hymenostomatia Delage and Hérouard, 1896 | Hymenostomatida Delage and Hérouard, 1896 | Ophryoglenina Canella, 1964 | Ichthyophthiriidae Kent, 1881 | *Ichthyophthirius* Fouquet, 1876 | *multifiliis* |
| | | | | | Tetrahymenina Fauré-Fremiet in Corliss, 1956 | Tetrahymenidae Corliss, 1952 | *Tetrahymena* | *corlissi*, *faurei*, *pyriformis*, *rostrata* |
| | | | Scuticociliatia Small, 1967 | Philasterida Small, 1967 | | Uronematidae Thompson, 1964 | *Uronema* Dujardin, 1841 | *marinum*, *nigricans* |
| | | | | | | Parauronematidae Small and Lynn, 1985 | *Miamiensis* Thompson and Moewus, 1964 | *avidus* |
| | | | | | | Philasteridae Kahl, 1931 | *Philasterides* Kahl, 1931 | *dicentrarchi* |
| | | | | | | | *Helicostoma* Cohn, 1866 | *buddenbrocki* |
| | | | | | | | *Porpostoma* Möbius, 1888 | *notatum* |

*(Table 11.1 Contd.)*

*(Table 11.1 Contd.)*

| | | | | | |
|---|---|---|---|---|---|
| Peritrichia Stein, 1859 | Sessilida Kahl, 1933 | | Epistylididae Kahl, 1933 | *Epistylis* Ehrenberg, 1830 | *colisarum, lwoffi, niagarae* |
| | | | | *Apiosoma* Blanchard, 1885, syn. *Glossatella* | *amoebea, applanatum, baueri, campanulata, campanulatum, carpelli, caulata, constricta, cotti, curvinucleata, cylindriformis, leuciscus, lotae, micralesti, micropteri, minimicronucleata, minutum, mothlapitsis, obliqua, phiala, piscicola percae, piscicola, robustum, tenera, tretzeri, triangularis, viridis* |
| | | | Vorticellidae Ehrenberg, 1838 | *Vorticella* Linnaeus, 1767 | |
| | | | | *Carchesium* Ehrenberg, 1830 | |
| | | | Zoothamniidae Sommer, 1951 | *Zoothamnium* Bory de St. Vincent, 1826 | |
| | | | Scyphidiidae Kahl, 1933 | *Ambiphrya* Raabe, 1952 | *ameiuri, neobolae* |

*(Table 11.1 Contd.)*

(Table 11.1 Contd.)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | *Riboscyphidia* Jankovski, 1985 (syn. *Scyphidia* Dujardin, 1841) | *arctic, marionidermata, epibranchialis, constricta* |
| | | | | | | Operculariidae Fauré-Fremiet in Corliss, 1979 | *Propyxidium* Corliss, 1979 | |
| | | | | Mobilida Kahl, 1933 | | Trichodinidae Claus, 1874 | *Trichodina* Ehrenberg, 1830 | *acuta, anguilla, bassonae, borealis, borokensis, californica, cancilae, canningensis, canton, carassii, centrostrigata, circinantis, cottidarium, cribbi, ctenogobii, cubanensis, domerguei, donghuensis, equatorialis, fahaka, frequentis, fugu, fultoni, heterodentata, hexagrammi, hippoglossi, indica, intermedia, izyumovae, jadranica, jarmilae, janovice, japonica, jiroveci, jupermani, kamchatika, kazubski, kuleminae,* |

(Table 11.1 Contd.)

| | |
|---|---|
| | *kupermani, labrisomi, lepsii, mandarin, maritinkae, matsu, meridionalis, minima, modesta, murmanica, mutabilis, mystusi, nigra, nobilis, opeongoensis, ophiocephalus, orientalis, ovonucleata, pagoda, pediculus, perforata, porocephalusi, prowazeki, puytoraci, rectispina, rectuncinata, reticulata, rhinobatae, rostrata, saintjohnsi, schizothoracis, scorpenae, siluri, spheroidesi, symmetrica, strelkovi, tenuidens, tenuiformis, trendafilovi, truttae, uretra, urinaria, valasquezae, valkanovi, wulai, xenopodos* |
| *Vauchomia* Mueller, 1938 | *nephritica, renicola* |

*(Table 11.1 Contd.)*

*(Table 11.1 Contd.)*

*(Table 11.1 Contd.)*

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | *Hemitrichodina* Basson and van As, 1989 | *robusta* |
| | | | | | *Trichodinella* Srámek-Husek, 1953 | *epizootica, lawleri, lomi, obtusa, percarum, subtilis* |
| | | | | | *Dipartiella* Shtein, 1961 | *simplex* |
| | | | | | *Paratrichodina* Lom, 1963 | *africana, alburni, corlissi, erectispina, incisa, obliqua, phoxini* |
| | | | | | *Tripartiella* Lom, 1959 | *bulbosa, bursiformis, clavodonta, copiosa, dactylodentata, kashkovskii, lata, obtusa, tilapiae* |
| Phyllopharyngeade Puytorac *et al.*, 1974 | Phyllopharyngiade Puytorac *et al.*, 1974 | Chlamydodontida Deroux, 1976 | | Chilodonellidae Deroux, 1970 | *Chilodonella* Strand, 1928 | *cucullulus, piscicola* (formerly *C. cyprini*), *hexasticha, uncinata* |
| | | Dysteriida Deroux, 1976 | | Hartmannulidae Poche, 1913 | *Brooklynella* Lom and Nigrelli, 1970 | *hostilis* |
| | Suctoria Claparède and Lachmann, 1858 | Endogenida Collin, 1912 | | Trichophryidae Fraipont, 1878 | *Capriniana* (formerly *Trichophrya*) | *piscium, sinensis* |
| Prostomatea Schewiakoff, 1896 | | Prorodontida Corliss, 1974 | | Cryptocaryonidae Wright and Colorni, 2002 | *Cryptocaryon* Brown, 1951 | *irritans* |

*(Table 11.1 Contd.)*

*(Table 11.1 Contd.)*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Litostomatea Small and Lynn, 1981 | Trichostomatia Bütschli, 1889 | Vestibuliferida de Puytorac *et al.*, 1974 | | Balantidiidae Reichenow in Doflein and Reichenow, 1929 | *Balantidium* Claparède and Lachmann, 1858 | *barbi, ctenopharyngodonis, mrigalae, pangasi, polyvacuolum* |
| | | | Haptoria Corliss, 1974 | Haptorida Corliss, 1974 | | Enchelyidae Ehrenberg, 1838 | *Enchelys* O.F. Müller, 1773 | *parasitica* |
| | | | | Pleurostomatida Schewiakoff, 1896 | | Amphileptidae Bütschli, 1889 | *Amphileptus* Ehrenberg, 1830 | *branchiarum* |

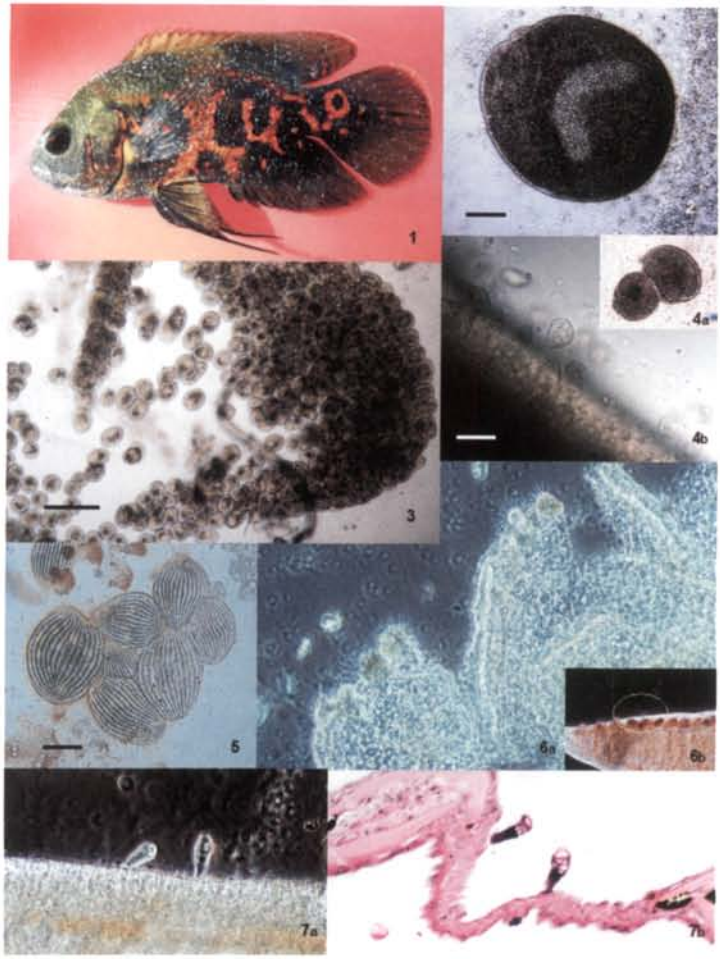


**Fig. 11.1** 'White Spot Disease' by *Ichthyophthirius multifiliis* in oscar, *Astronotus ocellatus* (Photo: A. Colorni, Eilat, Israel). **Fig. 11.2.** *Ichthyophthirius multifiliis* trophont. Note the horseshoe shaped macronucleus (courtesy of M.L. Fioravanti and collaborators, Laboratory of Fish Pathology, Dept. of Public Veterinary Health and Animal Pathology, Univ. of Bologna, Italy). Bar=150 μm. **Fig. 11.3.** *Ichthyophthirius multifiliis* tomites forced out from a tomont (courtesy of M.L. Fioravanti and collaborators, Laboratory of Fish Pathology, Dept. of Public Veterinary Health and Animal Pathology, Univ. of Bologna, Italy). Bar=100 μm. **Fig. 11.4a, b.** *Chilodonella piscicola* (courtesy of M.L. Fioravanti and collaborators, Laboratory of Fish Pathology, Dept. of Public Veterinary Health and Animal Pathology, Univ. of Bologna, Italy). Bar=80 μm. **Fig. 11.5.** *Tetrahymena* sp. (courtesy of M.L. Fioravanti and collaborators, Laboratory of Fish Pathology, Dept. of Public Veterinary Health and Animal Pathology, Univ. of Bologna, Italy). Bar=20 μm. **Fig. 11.6a, b.** *Capriniana* sp. (courtesy of E.J. Noga, NCSU, Coll. Veterinary Medicine, Dept. of Clinical Sciences, Raleigh, North Carolina, U.S.A.). **Fig. 11.7a, b.** *Apiosoma* sp., fresh smear and histological section (courtesy of E.J. Noga, NCSU, Coll. Veterinary Medicine, Dept. of Clinical Sciences, Raleigh, North Carolina, U.S.A.).

populations, in particular those in the tropics, are a mix of indigenous and introduced strains (Paperna, 1996). With the exception of tadpoles that developed Ichthyophthiriosis after being experimentally exposed to high concentrations of theronts (Gleeson, 1999), *I. multifiliis* is not known to infect non-piscine hosts.

During its parasitic stage (trophont), *I. multifiliis* grows in the fish integument up to a size (variable according to the strain, permanence on the host, etc.) of 30-1,000 (rarely 1,500) μm, becoming macroscopically visible as tiny cutaneous vesicles or 'white spots'. The trophonts' constant motion and feeding activities cause severe hyperplasia, which allows the parasite to grow in a sheltered form within the thickened tissue. In heavy infections, individual burrows coalesce to form a maze shared by several trophonts. Osmoregulatory imbalance, respiratory distress, and excretory impairment may soon follow (Hines and Spira, 1974a; Ventura and Paperna, 1985). Epithelial erosion favors secondary bacterial and mycotic infections. Complete maturation of the parasitic trophont requires 3-7 days at 21-22°C (Ewing and Kocan, 1987; Gratzek, 1993), up to 11 days at 15°C and nearly 30 days at 10°C (Gratzek, 1993). The mature trophont leaves the fish, and drops to the substratum and encysts. The gelatinous cyst hardens and—protected by its thin wall—the organism soon starts dividing (stage of tomont). After several rounds of divisions into immature daughter cells (tomites) (Fig. 11.3), hundreds of infective, ciliated cells (theronts) break free from each tomont, actively dispersing into the environment.

Theronts of *I. multifiliis* are elongated and covered by 36 to 48 meridional kineties, converging anteriorly around the pre- and post-oral sutures. Their size varies according to the size of the mother-tomont and number of divisions that occurred within it, ranging 25-70 × 15-22 μm (l × w). The watchglass (Lieberkühn's) organelle—a DNA-containing refractile granule about 3 μm in diameter—is located in the wall of the buccal cavity. A stiff 10-15 μm long caudal cilium may be present. A contractile vacuole discharges through 3 to 5 excretory pores. The macronucleus is ellipsoidal, about 8 × 12 μm, and the micronucleus is subspherical, 3 μm in diameter (Canella and Rocchi-Canella, 1976; Lom and Dyková, 1992; Hoffman 1999). Theronts can survive for a few days, but their infectivity significantly declines after the first 24 hours post-excystment. Once contact with a suitable host is established, adhesion to the host epithelium is aided by a sticky material released by the theront's secretory organelles (pellicular mucocysts). Penetration is facilitated by a

knob-like structure (perforatorium) at the anterior end of the cell, and is completed in as little as 5 min (Canella and Rocchi-Canella, 1976; Ewing *et al.*, 1985). Presence of hyaluronidase (Uspenskaya, 1963), the enzyme that hydrolyzes hyaluronic acid, the intercellular 'glue', may explain the theront's quick burrowing and obliteration of its entry path. Mature trophonts are basically oval to round, but pleiomorphic and quite plastic, uniformly covered with cilia arranged in up to 2,000 meridional kineties. The cytostome is located in the anterior third of the body. The prebuccal space is cylindrical and lined with up to 15 kineties closed to form complete circles. Specialized buccal organelles are either negligible or absent. There is no organelle of Lieberkühn at this stage. The macronucleus is conspicuous and characteristically horseshoe-shaped (Fig. 11.2). Adhering to it is the small, oval to round micronucleus (Canella and Rocchi-Canella, 1976; Lom and Dyková, 1992; Hoffman, 1999).

The time necessary for the parasite to complete its life cycle is temperature dependent and ranges from 3-4 days at 21-24°C, 10-14 days at 15°C, and up to 5 weeks at 10°C (Meyer, 1974). Trophonts evicted from the tissue before their scheduled time of spontaneous departure fail to develop into tomonts and eventually die (Paperna, 1996). Trophonts that exit the fish prematurely when the fish dies cannot infect a new host. Only the larger protomonts encyst (55-70 μm) and produce viable tomites (aciliate daughter cells) (Ewing *et al.*, 1986).

Thanks to their small size, *I. multifiliis* theronts are able to disperse in aerosols generated from infected water (Bishop *et al.*, 2003). Fish culture facilities, whether tanks constantly aerated or ponds exposed to prevailing winds, are particularly susceptible to this pathway of transmission.

### Treatment

A wide array of treatments has been suggested to combat ichthyophthiriosis, an obvious sign that none of them is universally satisfactory. *Ichthyophthirius multifiliis* trophonts are generally unaffected by chemotherapeutants. If the fish can endure it, a temporary (7-10 days) rise in temperature (≥30°C) has been suggested when a chemical treatment may not be well tolerated by cohabiting plants or invertebrates. However, the temperature needed for effective treatment seems to vary among isolates (Noga, pers. comm.). When logistically feasible, removing all fish from the culture system for at least three weeks should eliminate

the parasite, which is allegedly unable to survive without a piscine host. However, at low temperatures, a much longer time may be required. During this time, or at least for the first week, daily transfers of the fish from one treatment tank to another at 25°C (the tanks being dried and cleaned between uses) should rid the fish of all the trophonts and allow the safe re-introduction of the fish into the main aquarium (Gratzek, 1993; Noga, 1996). Low temperature slows the entire life cycle (Noe and Dickerson, 1995) and the parasite may lie dormant for a long time. Removal of the upper layer of gravel from the aquarium is advisable. For ornamental fish, Noga (1996) suggests two consecutive treatments with a synergistic combination of formalin (25 ppm) and malachite green (0.1 mg/l) administered one day apart, during which, up to 50% of the water should be changed. All plants should be removed before treatment. In case of pond fish, copper sulfate is still the treatment of choice. Therapeutic concentrations vary with the water alkalinity. Schlenk *et al.* (1998) suggested a concentration of 0.4 mg/l for at least five days. Copper ions are bound and inactivated by organic matter and precipitate as copper carbonate in hard and/or alkaline water, so that levels need to be monitored frequently (which is normally done using commercial kits) and adjusted in order to maintain the therapeutic concentration. It should be kept in mind, however, that copper is not only very toxic to a large variety of invertebrates and algae, but to fish as well at concentrations near to those parasiticidal. Gill tissue is particularly sensitive, but liver, kidney, and nervous systems can also be seriously harmed (Cardeilhac and Whitaker, 1988). In addition, copper is immunosuppressive, increasing susceptibility to other pathogens (Hetrick *et al.*, 1979; Baker *et al.*, 1983). Residues may be a problem in foodfish, making a withdrawal period of several weeks mandatory before the treated fish can be marketed. Formalin (25 mg/l in static treatment) is effective at eliminating infestations and reducing fish-to-fish transmission when used daily (Tieman and Goodwin, 2001). Potassium permanganate (at least 1.25 mg/l daily for 10 days, Strauss and Griffin, 2002) and sodium chloride (5 g/l for 7-14 days, Selosse and Rowland, 1990; 4 g/l for 45 days, Miron *et al.*, 2003) were effective on various species of catfish. Electrotherapy (Farley and Heckmann, 1980) and oral chemotherapy (Luzardo-Alvarez *et al.*, 2003; Shinn *et al.*, 2003) were also tested with only a modest degree of success.

Fish that recover from infection or have been immunized with experimental vaccines produce humoral and cutaneous antibodies and

can acquire a certain degree of resistance (Hines and Spira, 1974b; Wahli and Meier, 1985; Cross and Matthews, 1993; Dickerson and Clark, 1996). These refractory fish may rapidly expel infective theronts (Wahli and Matthews, 1999) or become carriers by retaining a sub-clinical chronic infection (Paperna, 1996). Immobilization antigens (i-antigens), a class of predominantly surface membrane proteins in *I. multifiliis*, appear to be the targets of the host immune response (Xu *et al.*, 1995). Despite the many attempts to develop an immunoprophylactic treatment and the moderate success at experimental level (Goven *et al.*, 1980, 1981, Clark *et al.*, 1988; Burkart *et al.*, 1990; Ling *et al.*, 1993; He *et al.*, 1997; Lin *et al.*, 2002; Sigh and Buchmann, 2002), no effective, long-lasting protection has ever been achieved warranting production of a commercial vaccine. Loss of viability or senescence hampers long-term propagation of *I. multifiliis* on fish (Houghton and Matthews, 1986; Burkart *et al.*, 1990; Noe and Dickerson, 1995; Xu and Klesius, 2004). Attempts to culture it axenically *in vitro* have failed (Ekless and Matthews, 1993; Nielsen and Buchmann, 2000).

## Chilodonellosis

### Agents: *Chilodonella* spp.

Ciliates belonging to the genus *Chilodonella* (Class Phyllopharyngea, Order Chlamydodontida, Family Chilodonellidae) are cosmopolitan and have been transcontinentally dispersed via translocation of their cultured fish hosts (Paperna, 1996). They have great ecological adaptability to a wide range of temperatures, and have been reported in fresh, estuarine and brackish water, from cold to tropical environments. Chilodonellids are either heart-shaped or kidney-shaped, ventrally flattened or slightly concave, and dorsally convex. They glide with slow circular movements, scraping the epithelia of the fish skin and gills by means of their oral cytoskeletal armament and feed on the cell debris, causing severe irritation and consequent hyperplasia. The cytostome is located ventrally, surrounded by three kineties, and leads into a tubular cytopharynx curved at its inner end (cyrtos). Excretory pores were described by Wiles *et al.* (1985). A distinct unciliated ventral field separates the two ciliary bands. There is a single, oval macronucleus with one adjacent micronucleus.

Most species are free-living. Some (e.g., *Chilodonella cucullulus* and C. *uncinata*) can occasionally harm fish that are weakened by a harsh winter (Migala and Kazubski, 1972; Langdon *et al.*, 1985; Shapiro and Snovski, 1997), but C. *piscicola* (formerly C. *cyprini*) (Fig. 11.4a, b) and

C. *hexasticha*, common pests in fish aquaria and fish cultures, have been recognized as true pathogens. Simultaneous infections by these two species are rare, but when they do occur, then one species dominates or displaces the other (Kazubski and Migala, 1974; Paperna, 1991, 1996), whereas co-infections with other ciliates (trichodinids and sessile peritrichs) during winter are not uncommon (Paperna *et al.*, 1984). Clinical signs of chilodonellosis include corneal opacity, discolored, slimy, abraded or tattered skin, and emaciation. Reactive hyperplasia—sometimes quite severe—fills interlamellar spaces leading to clubbing and fusion of secondary lamellae. Capillary occlusion and necrosis often follow (Paperna and van As, 1983).

*Chilodonella piscicola* has an asymmetrical body, 30-80 × 20-62 μm (l × w) in size, a notch at the posterior margin which gives the parasite a foliate shape. The number of kineties ranges from 7-15 in the right arched ciliary band, and 8-14 in the straighter left band. Two contractile vacuoles, one located anteriorly on the right, the second posterior is on the left, and numerous digestive vacuoles are present. *Chilodonella piscicola* infests fingerlings more often than adults (Urawa and Yamao, 1992; Rintamäki *et al.*, 1994) and, despite its preference for cyprinids, appears to be more virulent in debilitated non-cyprinids (cichlids, catfish, etc.) (Paperna, pers. comm.).

*Chilodonella hexasticha* differs from C. *piscicola* by being on the average smaller (30-67 × 20-51 μm), by having fewer and more widely spaced kineties (5-7 in the right arched ciliary band, 6-9 in the straight left band), and by the absence of a notch at the posterior body margin. C. *hexasticha* is common among cyprinids (Kazubski and Migala, 1974), catfish (Hoffman *et al.*, 1979) and cichlids (Paperna and van As, 1983).

Low winter temperatures and other forms of stress (low oxygen, high nitrite levels, organic stress, etc.) act as predisposing factors. Transmission is direct. *Chilodonella* spp. die within a few hours when removed from the host (Gratzek, 1993; Lom, 1995), indicating a high degree of dependence on their host for survival, but during unfavorable conditions C. *piscicola* can encyst and survive for a long time (Bauer and Nikolskaya, 1957).

### Treatment

A formalin treatment of 25 ppm for 4-8 h (Gratzek, 1993) or of 150-250 ppm for 30-60 min (Lom, 1995) is sufficient to eliminate the parasite. Addition of sodium chloride at 0.1-0.3% for 24-48 h (Gratzek, 1993; Lom,

1995) or 1-3% for 15-30 min (Swarm and Fitzgerald, 1992; Lom, 1995) is also effective. Raising the water temperature to 25°C for several days is another suggested treatment (Lom, 1995), although its effectiveness is probably indirect and—at least for species of fish from temperate or tropical habitats—related to the ameliorated environmental conditions for the host.

## Tetrahymenosis

Agents: *Tetrahymena* spp.

Tetrahymenids (Class Oligohymenophorea, Subclass Hymenostomatia, Order Hymenostomatida, Suborder Tetrahymenina) are typically free-living holotrichous ciliates, pear-shaped, 100 × 30 μm (l × w) in size, with a radially symmetrical body, quilt-like pellicle and 20-35 meridian kineties (Fig. 11.5). The buccal cavity is a deep pouch, with specialized oral ciliature consisting of four membranes: the para-oral membrane on the right side and three serially arranged membranelles on the left side. Tetrahymenids have a worldwide distribution and affect a broad range of hosts. Normally saprozoic, some species thrive when the water is warm, moderately hard, alkaline and rich in organic matter. With the deterioration of the culture conditions, they can become highly invasive and pathogenic for the stressed fish, penetrating into the muscle, entering the bloodstream and ending up in capillaries of the gills, kidney, eye and brain. Clinical signs of tetrahymenosis include mucus hyperproduction, whitish patches of sloughing skin, raised scales and, in severe cases, deep ulcerative dermatitis and myositis. The disease is common among aquarium fish, with guppies, cichlids and tetras being particularly sensitive (Hoffman *et al.*, 1975; Astrofsky *et al.*, 2002; Pimenta Leibowitz *et al.*, 2005), but infections have also been observed in farmed fish (Ferguson *et al.*, 1987). Although many records of fish tetrahymenosis exist, identification to species level has often been presumptive. At least four species, *Tetrahymena corlissi*, *T. pyriformis*, *T. faurei* and *T. rostrata*, have been reported as histophagous on fish, but *T. corlissi* is probably the most common and pathogenic species, disintegrating host tissue and feeding on cell debris (Lom, 1995). *Tetrahymena corlissi* is about 55 × 30 μm (l × w) in size, with 25-31 meridian kineties and a caudal cilium. Its movement was described as resembling that of a spiraling football. The macronucleus is single and ellipsoidal, and a single micronucleus lies close to it. A single contractile vacuole is located posteriorly. *T. corlissi* can produce small

reproductive cysts (2-8 tomites). *Tetrahymena pyriformis* has fewer (17-21) kineties, no caudal cilium, and reproduces via binary fission. Similar to other facultative parasitic ciliates, *Tetrahymena* species are culturable *in vitro* (Elliot, 1973; Gardionio *et al.*, 1973; Lynn, 1975). However, frequent loss of infectivity may indicate selective growth of less virulent strains from a mixed population (Paperna, pers. comm.).

### *Treatment*

Tetrahymenids are sensitive to most parasiticides, including formalin. However, an established deep infection is unlikely to be significantly affected by an external treatment.

## Other Freshwater Facultative Parasites

*Amphileptus branchiarum* (Class Litostomatea, Order Pleurostomatida) has been reported within the gill epithelium of cyprinids and other freshwater species cultured in Asia (Bykhovskaya-Pavlovskaya *et al.*, 1962), Europe (Ergens and Lom, 1970), and the U.S.A. (Mitchell and Smith, 1988). It has a ciliated dorsal surface, a naked ventral surface, two macronuclei surrounding the micronucleus, numerous contractile vacuoles and a row of trichocysts along the posterior margin of the body. This ciliate seldom causes disease epizootics but is responsible for epithelial hyperplasia and cell displacement in branchial tissue.

### *Sessile Suctorians*

*Capriniana* (syn. *Trichophrya*) *piscium* (Class Phyllopharyngea, Subclass Suctoria, Order Endogenida) is a sessile ciliate that commonly infests gills of channel catfish (Noga, 1996), but has also been reported on fish from colder waters, such as rainbow trout (Buchmann and Bresciani, 1997), perch (Halmetoja *et al.*, 2000), and Arctic char (Hofer *et al.*, 2005). Heavy infestations can interfere with their host's respiration. The adult stage has characteristic tentacles emanating from an amorphous body devoid of cilia and cytostome (Fig. 11.6a, b). It settles on the fish by means of a scopuloid, an adhesive area that is analogous—but not homologous—to the scopula of sessile peritrichs (see below). The tentacles are almost completely retractile. Their capitate ends bear numerous haptocysts, which upon contact, impale and immobilize the prey (such as other ciliates) which is then cytolyzed and ingested through the tentacle's transient cytostome. *Capriniana piscium* also feeds on bacteria and suspended organic debris. It

reproduces by budding off discoidal 'larval' stages, motile by means of nine equatorial kineties that colonize a new host (Corliss, 1979; Lom and Dyková, 1992; Lom, 1995). *Capriniana* may be resistant to formalin and require treatment with copper (Noga, 1996).

## Genera with Both Freshwater and Marine Species

### *Sessile Peritrichs*

Sessile peritrichs (Class Oligohymenophorea, Subclass Peritrichia, Order Sessilida) have a cylindrical, conical or bell-shaped naked body and attach themselves to fish integuments by means of a holdfast organelle (scopula) at the aboral pole. An adoral ciliary spiral encircles a vaulted epistomial disc, that is retracted when disturbed (Fauré-Fremiet *et al.*, 1962). Sessile ciliates are fouling organisms on various kinds of animal hosts in fresh, brackish, and marine water. They may be solitary (e.g., most *Apiosoma*, [syn. *Glossatella*] (Fig. 11.7a, b), *Ambiphrya* (Fig. 11.8), *Propyxidium*, *Riboscyphidia* and *Scyphidia* spp.) or stalked and bearing a colony of several zooids (up to 8 in *Epistylis lwoffi*, up to 16 in dichotomous branches in *Epistylis colisarum*, both freshwater species) (Lom and Dyková, 1992). The stalk of *Epistylis* spp. (Fig. 11.9) is non-contractile, which distinguishes them from other peritrichous species such as *Vorticella* (solitary but often gregarious), *Carchesium* (colonial, each zooid with an independently contractile myoneme) and *Zoothamnium* (when colonial, zooids share continuous myonemes, making the entire colony contractile) (Lynn and Small, 2000). In *Vorticella* spp., the stalk recoils and the cell body shrinks rapidly in response to a stimulus. The macronucleus may be conical with its apex pointing downward, or ellipsoidal (as in *Apiosoma* spp.), ribbon-shaped (as in *Ambiphrya* spp.), or horseshoe-shaped (as in *Epistylis* spp.). The 'larval' stages (telotrochs) are free-swimming by means of an equatorial ciliary wreath.

Sessile peritrichs colonize debilitated individuals whose integuments have lost their repellent properties, and use the host as a substratum for attachment and transportation. Although they do not seem to be capable of producing lysis or initiating mechanical lesions, their proliferation on damaged skin has deleterious aggravating effects. Fish infected by the bacterium *Aeromonas hydrophila* produce typical sores that can rapidly be colonized by *Epistylis*, producing a condition named 'red sore' (Esch *et al.*, 1976; Miller and Chapman, 1976). Large colonies of *Epistylis* expanded the lesions, leading to perforation of the opercular bone in tilapia

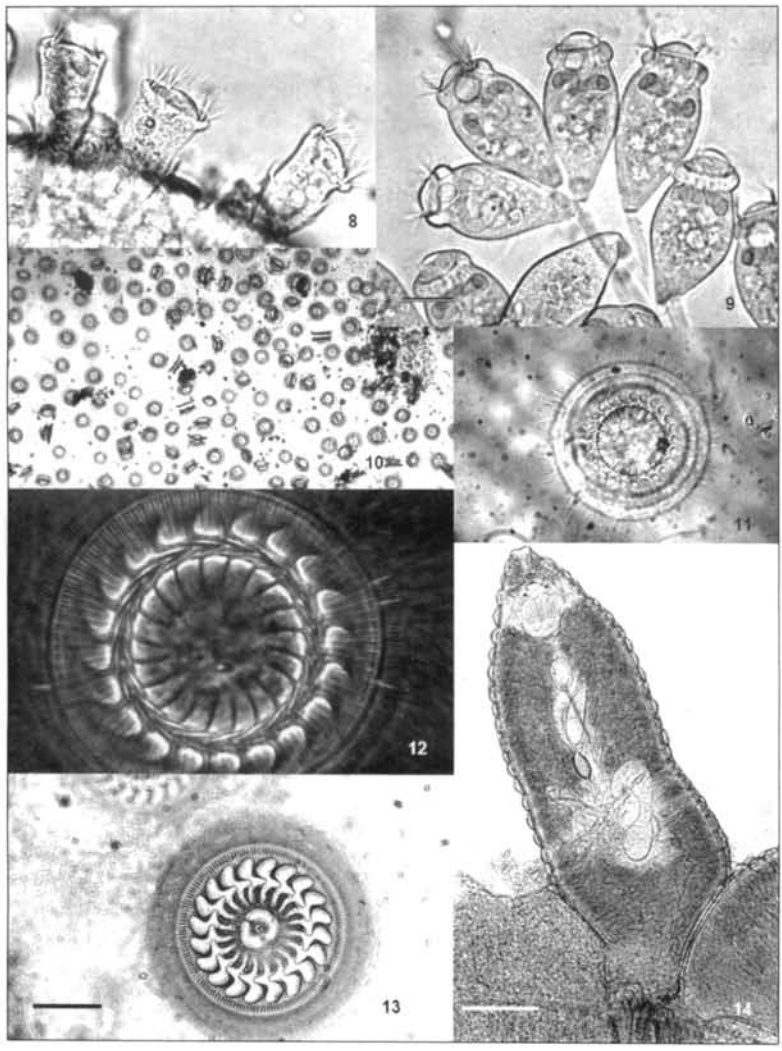



**Fig. 11.8** *Ambiphrya* sp. on gills of sea bass *Dicentrarchus labrax* cultured in brackish water ponds (Photo: A. Colorni, Eilat, Israel). **Fig. 11.9.** *Epistylis* sp. (courtesy of M.L. Fioravanti and collaborators, Laboratory of Fish Pathology, Dept. of Public Veterinary Health and Animal Pathology, Univ. of Bologna, Italy). Bar=25 μm.

**Figs. 11.10–11.12** *Trichodina* spp., fresh smears (courtesy, respectively, of A. Mitchell, USDA in Stuttgart, Arkansas; F.P. Meyer, U.S. Fish and Wildlife Service; and G.L. Hoffman, US Fish and Wildlife Service). **Fig. 11.13.** *Trichodina* sp. from the gills of European seabass *Dicentrarchus labrax* cultured on the Israeli coast of the Red Sea (courtesy of A. Diamant, Israel Oceanographic and Limnological Research, National Center for Mariculture, Eilat, Israel), Klein's dry silver impregnation. Bar=20 μm. **Fig. 11.14.** Hyperparasitism of *Trichodina* sp. on the monogenean gill fluke *Diplectanum aequans*. Fresh mount. (Photo: A. Colorni, Eilat, Israel). Bar=200 μm.

(Paperna, 1991). On the gills, sessile peritrichs interfere with gas exchange. On fish eggs, they reduce the hatching rate. Substratum preference and specificity were studied by Viljoen and van As (1983), who found that several South African peritrichs associated with fish were not only morphologically distinct from those settling on inanimate objects, but also specific to their piscean substrata. However, metabolic independence from the live host is supported by the fact that these ciliates feed on water-borne bacteria and particles, and are often indicators of organically polluted water. The formation of a resting cyst obtained under laboratory conditions in a marine *Vorticella* sp. (Jung and Rho, 1999) indicates their potential ability to survive adverse environmental conditions.

### Treatment

In freshwater, sessile peritrichs can usually be controlled with formalin (25-50 ppm/3 h or longer; in ponds, treatment can be continuous) or sodium chloride (1.5%/1 h or 3%/1-2 min) baths. In seawater, a formalin treatment 150-250 ppm/30-60 min or a freshwater bath have been recommended (Lom, 1995; Noga, 1996).

### Trichodinids

Trichodinids (Class Oligohymenophorea, Subclass Peritrichia, Order Mobilida) (Figs. 11.10–11.13) are bell- or saucer-shaped peritrichous ciliates commonly occurring worldwide on the skin and gills of fish in both freshwater and marine environments. Trichodinids can be easily recognized by their adhesive disc containing a crown of hard denticles, whose characteristics (number, arrangement and shape of their proximal projections—the thorns, and their distal projections—the blades) are the primary features used to differentiate the many described species (van As and Basson, 1989; Lom, 1995; Dove and O'Donoghue, 2005). Their diameter ranges mostly between 50-100 μm (Lom, 1995). The buccal cavity is long, deep and funnel-like (infundibulum), and the adoral spiral of cilia can make from ½ to 3 turns around it (Lom and Dyková, 1992). Structure of the adoral ciliary ring and size of the horseshoe-shaped macronucleus are also of major taxonomic significance. No resting stages have been observed. Reproduction is by perpetual binary fission.

Trichodinids associated with fish form a large group that includes at least seven genera (*Trichodina*, *Vauchomia*, *Hemitrichodina*, *Trichodinella*, *Paratrichodina*, *Dipartiella*, *Tripartiella*) (Lom and Dyková, 1992). Over 190

species have been described, from the northernmost to the southernmost latitudes through the temperate and tropical belts. Most disease agents are *Trichodina* spp. (Lom, 1995). Transmission is direct, from fish to fish, and host-specificity is variable, but generally low. In fact, some freshwater species (e.g., *Trichodina acuta*, *T. nigra*) can also live on tadpoles and copepods (Chen, 1963), while some other species (e.g., *T. jadranica*) occur on both marine and freshwater hosts (Lom and Dyková, 1992). Sparse individuals gliding on or temporarily adhering to the skin and/or gill epithelium and feeding on bacteria and mucus cause little or no noticeable harm to healthy fish. However, on a stressed or otherwise debilitated fish, these ciliates can proliferate massively. Their tactile stimuli cause irritation, leading to mucus hyperproduction and hyperplasia. Their repeated adherence and suction activity eventually damage and severely erode the gills. In such cases, cell debris and red blood cells are often found in the ciliate's food vacuoles (Lom and Dyková, 1992; Lom, 1995). Within several hours of the host's death, some species leave the host and, depending upon temperature, may survive for several days in the water (Davis, 1961). Other species, such as *T. scorpenae*, die quickly upon removal from their host, indicating a stricter physiological bond (Cheng, 1986). Trichodinids commonly occur in association with other ectoparasitoses, especially monogenean helminthes (Noble, 1963; Barker *et al.*, 2002) and even as hyperparasites (Colorni and Diamant, 2005) (Fig. 11.14). Several species are endozoic, infecting the fish urinary bladder, kidney, oviducts, and gastrointestinal tract (da Cunha and Pinto, 1928; Aschurova and Stein, 1972; Khan, 1972; Lom and Haldar, 1976; Li and Desser, 1983; Basson, 1989; Basson *et al.*, 1990; van As and Basson, 1996; Karlsbakk *et al.*, 2002). Although the damage to their host has not always been assessed, endozoic trichodinids are considered to be true pathogens (Eudokimova *et al.*, 1969; Khan, 1972).

Guidelines for the identifications to species level of this large group and abundant illustrative material are found in Basson and van As (1989), van As and Basson (1989), Lom and Dyková (1992) and Lom (1995).

### *Treatment*

Freshwater trichodinids are sensitive to sodium chloride and formalin and the recommended treatments are as for *Chilodonella*. Marine species are sensitive to formalin and the recommended treatment is 100-200 ppm/1 h. A freshwater bath can also be effective.

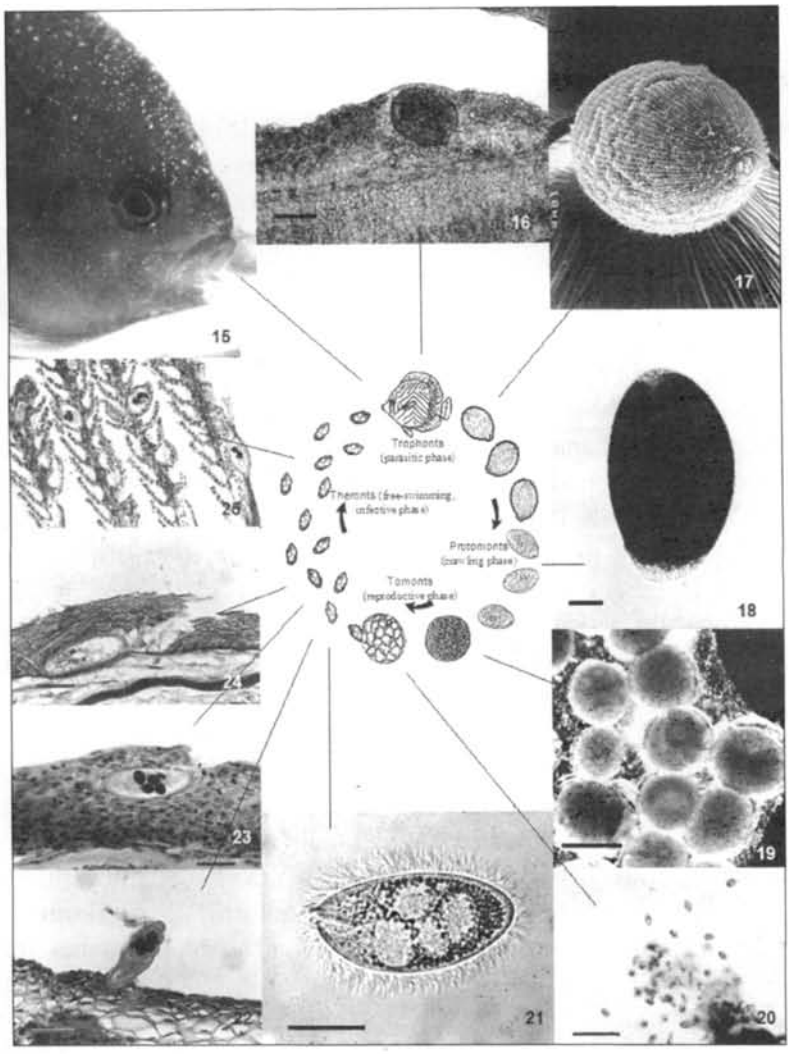
## MARINE

### Cryptocaryonosis

Agent: *Cryptocaryon irritans* Brown, 1951

Responsible for marine White Spot Disease, *Cryptocaryon irritans* Brown, 1951 (Class Prostomatea, Order Prorodontida, Family Cryptocaryonidae) is a holotrichous ciliate that parasitizes marine fishes in tropical, subtropical and temperate seas. With the rapid development of intensive mariculture, this parasite—once known only from marine aquaria—has become an increasingly frequent pest in commercial enterprises. In nature, different fish species seem to vary in their susceptibility to *C. irritans* (Diggles and Lester, 1996c), a phenomenon occasionally observed in cultured fish (Rigos *et al.*, 2001), although captivity generally tends to level host susceptibility (Burgess and Matthews 1995a; Colorni and Burgess, 1997; Tonguthai and Leong, 2000). *Cryptocaryon irritans* invades the epithelium of skin, eyes and gills, compromising the physiological function of these organs. The clinical signs of cryptocaryonosis include pinhead-sized whitish nodules (Fig. 11.15), mucus hyperproduction, epithelial hyperplasia, corneal cloudiness, skin discoloration and, with the disruption of the gills' lamellar structure, severe respiratory distress. In heavy infections, several trophonts share interlocked burrows, osmoregulatory balance becomes increasingly difficult to maintain and gas exchange in severely damaged gills gradually fails.

*Cryptocaryon irritans* has a direct, quadriphasic life cycle, with one parasitic and three off-host stages (Figs. 11.15–11.25). The trophonts (feeding, parasitic stage) are spheroidal to pear-shaped and revolve continuously within the host epithelium (Fig. 11.16). In young trophonts, the macronucleus consists of four linked bead-like segments twisted in a crescent-shaped alignment. Adjacent to it are up to seven micronuclei. A groove-like buccal apparatus extends from the apex downward. A crown of pointed ciliar triplets fused at the tip delimits a small (about 20 μm) cytostome, and is thought to have abrasive function. Radially ridged walls lead to a shallow cytopharynx. Feeding on body fluids and cell debris, the trophonts' growth phase culminates when they spontaneously exit the host after 3-7 days with a peak at 4-5 days (Colorni, 1985) (Fig. 11.17) and sink to the bottom. The exit of trophonts from the host is influenced by circadian rhythms, as the great majority exit in pre-dawn darkness (Burgess and Matthews, 1994b). Several organelles are apparently




**Figs. 11.15–11.25** *Cryptocaryon irritans* life cycle (Photos: A. Colorni, Eilat, Israel). **Fig. 11.15.** Marine 'White Spot Disease' by *Cryptocaryon irritans* in sea goldie *Anthias squamipinnis*. **Fig. 11.16.** *Cryptocaryon irritans* trophont in gill filament. Bar = 100 μm. **Fig. 11.17.** *Cryptocaryon irritans* trophont-protomont (SEM micrograph, × 370: E. Klein, Weizmann Institute, Rehovot, Israel). **Fig. 11.18.** *Cryptocaryon irritans* aciliated protomont. Bar = 50 μm. **Fig. 11.19.** *Cryptocaryon irritans* dividing tomonts. Note the irregular cell division. Bar = 200 μm. **Fig. 11.20.** *Cryptocaryon irritans* excysting theronts (infective stage). Bar=200 μm. **Fig. 11.21.** *Cryptocaryon irritans* theront. Bar = 20 μm. **Fig. 11.22–11.24.** *Cryptocaryon irritans* theront forcing its way into the skin of a juvenile seabream *Sparus aurata*. The entire process, from first contact to settlement on the basal lamina may take less than 5 min. Bars = 25 μm. **Fig. 11.25.** *Cryptocaryon irritans* very young trophonts.

activated in negotiating the passage from the feeding stage in the fish epithelia to the free-swimming stage in seawater (Colorni and Diamant, 1993). Having shed the cilia and flattened its surface ridges (Matthews *et al.*, 1993), what is now a protomont (Fig. 11.18) crawls on the bottom for up to 18 hours with active 'probing' movements of the apical pole. Then its movements gradually slow to immobility, and adhesion to a solid substratum and encystment ensue, with the cyst wall hardening in 8-12 hours (Colorni, 1985). Tomont size varies according to the geographical isolate, and whether the protomont departed from a live fish or upon the death of the fish. An isolate of possibly Hawaiian or Indo-Pacific origin measured 170 × 94.5 to 441 × 252 μm (l × w) (Nigrelli and Ruggieri, 1966), a Red Sea isolate measured 160 × 150 to 370 × 310 μm (Colorni, 1985), while the diameter of Australian isolates, ranging from 155 μm to 720 μm, was correlated with water temperature and species of fish infected (Diggles and Lester, 1996a, b, c). Host death accelerates the transition from trophont to protomont. Trophonts that exit the fish prematurely when the fish dies cannot infect a new host and the protomonts encyst, including those originating from <24-hour-old trophonts. However, tomonts must be at least 50 μm in diameter to produce viable tomites (aciliate daughter cells) (Colorni, 1985). Tomonts undergo a sequence of often-asymmetrical binary fissions, producing numerous tomites (Fig. 11.19). Although mature trophonts on the same fish often spontaneously exit within a narrow time period (16-18 hours), tomont development and subsequent theront (infective stage) release (Fig. 11.20) are very asynchronous, even when the tomonts are incubated under identical conditions (Nigrelli and Ruggieri, 1966; Colorni, 1985; Burgess and Matthews, 1994a). Theronts are oval to claviform, approximately (l × w) 50-70 × 20-30 μm in size (Fig. 11.21) (Colorni, 1985). However, their number per tomont varies with geographic location, host species, and tomont size (Colorni and Burgess, 1997). Theronts spiral actively and are attracted to fish. A long caudal cilium was occasionally observed. The function of this flagellum-like organelle is unclear but, appearing non-motile, is unlikely natatory. Host invasion is very rapid and often completed within five minutes (Figs. 11.22–11.25). The presence of hyaluronidase (Colorni, 1992) may account for the theront's rapid penetration and healing of the piercing path. Theronts' life span is less than 24 hours for most isolates and though it can occasionally extend up to 48 hours, their infectivity decreases considerably after the first 6-8 hours post-excystment. The theronts' emergence from the tomonts is

influenced by circadian rhythms and takes place during the dark phase (Burgess and Matthews, 1994b). Excystment was observed to occur as soon as three and as late as 72 days, peaking at 6 ± 2 days (Colorni, 1985). There is no relationship between tomont size and time of excystment. Asynchronous excystment of the infective theronts prevents simultaneous spending of all tomonts, facilitates theront dispersal over time, and appears to have evolved as a survival strategy.

*Cryptocaryon irritans*' taxonomic position has remained unclear for many years, with this ciliate being considered *incertae sedis* (Canella, 1972) or erroneously included in the Ichthyophthiriidae. Only recently was it assigned to the Cryptocaryonidae (Wright and Colorni, 2002) (see below, Taxonomy of Parasitic Ciliates). Variants of *C. irritans* have been detected in geographically distant sites (Diggles and Adlard, 1995, 1997; Diggles and Lester, 1996b; Yambot *et al.*, 2003), some outside the tropics, in temperate waters (Diamant *et al.*, 1991; Diggles and Adlard, 1997; Jee *et al.* 2000). However, none of the diversities reported thus far have been substantial enough to justify the separation of *Cryptocaryon* into different species. *Cryptocaryon irritans* is not known to infect non-piscine hosts or non-teleost fish.

## Treatment

The difficulty in eradicating *C. irritans* from marine aquaria and mariculture systems stems from its complex life cycle. Virtually invulnerable when protected by the host epithelium as a trophont and by an impervious cyst as a tomont, the parasite is most defenseless after leaving the host as a protomont and exiting the cyst as a theront. Chemical treatments may, thus, be more effective if administered late at night. When logistically feasible, four consecutive transfers of the fish at three-day intervals (the tanks being dried and cleaned between uses) interrupt the life cycle of the parasite and eradicate the disease within 7-10 days. The same method can be used to prevent its introduction into uncontaminated tanks. A hyposalinity treatment administered with the same frequency can be just as effective. However, since its success is based on damaging the tomonts by osmotic shock, dilution of the seawater to ¼ of its original salinity must be carried out very rapidly (within 30 min.) and maintained for at least three hours (Colorni, 1985, 1987). If the freshwater source is tap water, it should be first dechlorinated by sodium thiosulfate treatment. An historical account of *C. irritans* and the

chemical, physical and immunological methods or attempts to control it were reviewed by Colorni and Burgess (1997).

A certain degree of immunity observed in fish that survived previous infections (Burgess and Matthews, 1995b; Dickerson and Clark, 1996; Yoshinaga and Nakazoe, 1997) has raised the prospect of a vaccine as a prophylactic measure. *Cryptocaryon irritans* genomic expression library was screened to identify immunogenic proteins (Lewis *et al.*, 2004). Attempts to culture *C. irritans in vitro* were made, but development of this ciliate never progressed beyond a small, intermediate theront-trophont stage (Colorni, 1992; Yambot and Song, 2004).

## Brooklynellosis

### Agent: *Brooklynella hostilis*

*Brooklynella hostilis* (Class Phyllopharyngea, Subclass Phyllopharyngia, Order Dysteriida, Family Hartmannulidae) (Fig. 11.26) is a cosmopolitan cyrtophorine ciliate and the only member of the genus *Brooklynella* Lom and Nigrelli, 1970 (Lynn and Small, 2000). It is known to occur normally in small numbers on wild fish in tropical ecosystems. Although it may exhibit mild pathogenicity, such natural infections are generally asymptomatic. *B. hostilis* is, nevertheless, able to express high virulence on debilitated hosts, and has been held responsible for acute mortalities in marine fish from several tropical regions (Gallet de Saint Aurin *et al.*, 1990; Landsberg and Blakesley, 1995; Lom, 1995; Noga, 1996; Diamant, 1998). In the Florida Keys, toxic algae may have acted as principal stressors, rendering several species of reef fish susceptible to infections by this and other opportunistic organisms (Landsberg, 1995). *Brooklynella hostilis* glides on its ventral side, feeding on gill and skin debris and ingesting blood cells (Blasiola, 1992; Noga, 1996; Diamant, 1998). In the gills, haemorrhage, aneurysms, fusion of the terminal lamellae, epithelial hyperplasia and desquamation are produced, while sloughing of the skin can be quite extensive.

*Brooklynella hostilis* has a slightly compressed bean-shaped body, with a flat ventral side and a vaulted, cilia-free dorsal side. Its size ranges from 55-86 × 25-50 μm (l × w) (Lom and Dyková, 1992; Landsberg and Blakesley, 1995; Diamant, 1998), and its ventral posterior end is characteristically notched. The macronucleus is oval and surrounded by several micronuclei. The ciliary rows are present only ventrally and (with the exception of a small posterior area where a glandular attachment

organelle is located) cover the entire ventral surface, converging anteriorly along a prebuccal suture. There are 8-10 post-oral kineties; a left band of 12-15 kineties which do not reach the posterior margin of the body stopping short of the glandular organelle, and a right band of long 8-11 kineties arching along the anterior and posterior margins. The cytopharyngeal armature is well developed, with the cytostome reinforced by supporting skeletal rods and equipped with 3 short ciliary rows (Lom and Corliss, 1971; Lom, 1984; Lom and Dyková, 1992). Numerous food vacuoles are present (Blasiola, 1992; Diamant, 1998).

### *Treatment*

A flush treatment of 150-250 ppm of formalin for 30-60 min or 50 ppm of acriflavine for one to several days, or an indefinite immersion in 0.42 ppm cupric acetate in 5.26 ppm formalin in 4.8 ppm Tris buffer are among the recommended treatments (Lom, 1995). An antibacterial treatment to control bacterial infections that may have developed during parasitization of the host should follow (Blasiola, 1992).

## Scuticociliatosis

Scuticociliates (Class Oligohymenophorea, Subclass Scuticociliatia), for a long time regarded as harmless scavengers, are increasingly being recognized as responsible for systemic infections in fish (Figs. 11.27–11.30). Characterized by a great biodiversity regarding their habitats, their identification has been often uncertain or controversial and, in several instances, no further reports have followed the first account.

*Uronema marinum* Dujardin, 1841, is a ubiquitous member of the Uronematidae, a family characterized by a well-defined oral ciliature comprising one small external undulating membrane and three adoral membranelles aligned with the long axis of the body and a non-ciliated anterior pole. *Uronema marinum* is small, 38-32 μm × 20-13 μm (l × w), elongated, ovoid, somewhat laterally flattened, holotrichous, with 10-16 bipolar ciliary meridians, and a 34-40 μm long non-motile caudal cilium (Cheung *et al.*, 1980; Curds *et al.*, 1983). A rather short triangular cytostome is situated anteriorly just above the equator (Curds *et al.*, 1983). A ciliature modified to serve a tactile or adhering function forms a localized thigmotactic field (Lom, 1995). The macronucleus is single, spherical to oval, and located near the mouth. A single micronucleus lies close to it. A contractile vacuole is visible at the posterior end of the cell.

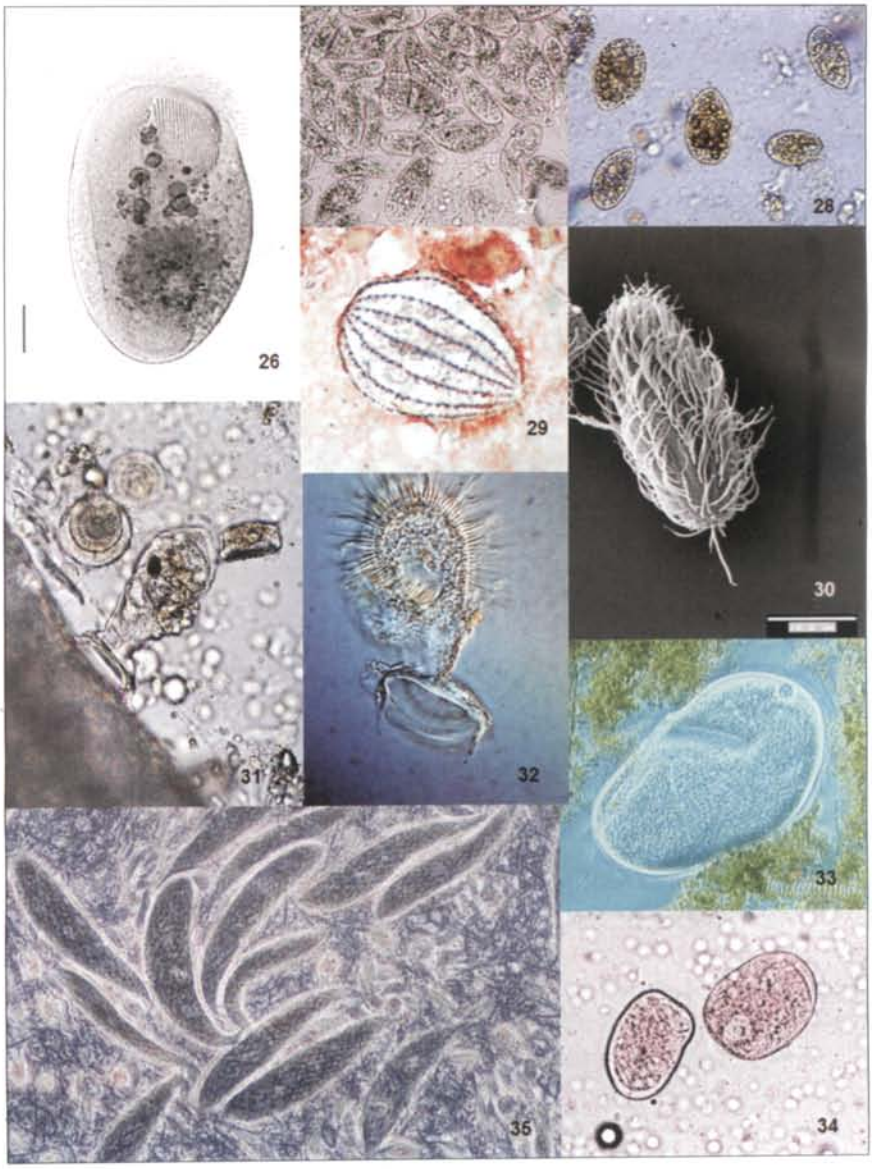



**Fig. 11.26** *Brooklynella hostilis* (courtesy of R. Overstreet and Hongwei Ma, Gulf Coast Research Laboratory, Univ. of Southern Mississippi, Ocean Springs, U.S.A.). Bar = 10 μm. **Fig. 11.27–29.** Scuticociliates from seahorse (Bar = 20 μm), *Dentex dentex* (Bar = 15 μm) and *Pagellus* sp. (Bar = 10 μm), respectively (courtesy of M.L. Fioravanti and collaborators, Laboratory of Fish Pathology, Dept. of Public Veterinary Health and Animal Pathology, Univ. of Bologna, Italy). **Fig. 11.30.** Scuticociliate (dorsal view) from cultured

*Fig. 11.26 Contd.*

A cytoproct is located in the posterior half of the body. *Uronema marinum* is normally bacteriophagic (Plunket and Hidu, 1978) and forms resting cysts in starved cultures (Curds *et al.*, 1983). Conjugation occurs by fusion of the anterior ends of the body (Curds *et al.*, 1983).

*Uronema marinum* is free-living, found in a wide range of temperatures and salinities, and is an opportunistic pathogen with no obvious host specificity, infecting fish predisposed by environmental stress. When enabled to express its highly invasive potential, it penetrates through the skin into the musculature, feeding on tissue debris and blood cells, and forming deep ulcers. Kidney, heart, neural canal (spinal cord) and urinary bladder may be invaded eliciting little or no inflammatory response from the host, but muscle is the tissue in which damage is generally most severe (Cheung *et al.*, 1980). *Uronema marinum* seems to be able to destroy—and protect itself from—the host's phagocytes (Kwon *et al.*, 2002; Lee *et al.*, 2004). Secretion of metalloproteases might be responsible for the tissue degradation and pre-digestion (Lee *et al.*, 2003). While external lesions may develop into haemorrhagic and open necrotic wounds with extensive sloughing of the epidermis, severe lesions may also be inconspicuous (Thompson, 1963; Cheung *et al.*, 1980). *Uronema marinum* can be cultured *in vitro* (Hanna and Lilley, 1974; Lee *et al.*, 2003).

Similar to *U. marinum*, but smaller and with fewer somatic kineties, *Uronema nigricans* was reported to cause a lethal encephalitis in southern bluefin tuna *Thunnus maccoyii* cage cultured in Australia (Munday *et al.*, 1997). The ciliate was pear-shaped with a slightly flattened and oblique anterior margin, its size ranging from 22-32 μm to 12-18 μm (l × w). The somatic ciliature was arranged in 12-14 kineties. Macronucleus and micronucleus, both single and ovoid, lay adjacent to each other. A caudal

*Fig. 11.26 Contd.*

turbot *Scophthalmus maximus* (SEM micrograph, courtesy of A. Sitjà-Bobadilla, P. Alvarez-Pellitero and O. Palenzuela, Instituto de Acuicultura de Torre de la Sal, Ribera de Cabanes, Castellón, Spain). **Fig. 11.31–32.** *Licnophora* sp. on the gills of a wild devil firefish *Pterois miles* and a lizardfish *Synodus variegatus* from the Red Sea coast of Israel (courtesy of A. Diamant, Israel Oceanographic and Limnological Research, National Center for Mariculture, Eilat, Israel). **Fig. 11.33.** *Balantidium* sp. from the intestine of an unidentified ornamental Mediterranean Sea fish (courtesy of M. Caffara, Laboratory of Fish Pathology, Dept. of Public Veterinary Health and Animal Pathology, Univ. of Bologna, Italy). Bar = 15 μm. **Fig. 11.34.** *Balantidium sigani* in Red Sea rabbitfish *Siganus rivulatus* (courtesy of A. Diamant, Israel Oceanographic and Limnological Research, National Center for Mariculture, Eilat, Israel). **Fig. 11.35.** Unidentified enteric ciliate in Red Sea angelfish *Genicanthus caudovittatus* (courtesy of A. Diamant, Israel Oceanographic and Limnological Research, National Center for Mariculture, Eilat, Israel).

cilium was present. An inflammatory response was mounted by the fish, with lesions along the olfactory nerves and meningitis associated with lymphocytic reaction. The ciliate apparently colonized the fish olfactory rosettes, then ascending the olfactory nerves reached the brain. Low water temperature (<18°C) and immune status of the fish were the probable predisposing factors. *Uronema nigricans* has been recorded from both freshwater and marine habitats and grows readily *in vitro* (Pérez-Uz, 1995).

*Miamiensis avidus* (Parauronematidae) was first isolated from sea horses caught in the bay waters of Miami, Florida, U.S.A. (Thompson and Moewus, 1964), and, more recently, from olive flounder *Paralichthys olivaceus* in China (Song and Wilbert, 2000) and Korea (Jung *et al.*, 2005). In England, *Helicostoma buddenbrocki* (Philasteridae), together with *Uronema* sp. and *Euplotes* sp. (Euplotidae), was reported on dead and moribund sole (*Solea solea*), young eels (*Anguilla anguilla*) and blennies (*Zoarces viviparus*), while plaice (*Pleuronectes platessa*) became infested by cohabitation (Purdom and Howard, 1971). *Philasterides dicentrarchi* (30-42 μm × 10-22 μm in size, 14 somatic kineties) massively infested the abdominal cavity of European sea bass *Dicentrarchus labrax* recovering from the winter in southern France (Dragesco *et al.*, 1995). *Philasterides dicentrarchi* was also observed in turbot, *Scophthalmus maximus* (Iglesias *et al.*, 2001; Leiro *et al.*, 2004) cultured in Spain. According to Song and Wilbert (2000) and Palenzuela *et al.* (2005), *P. dicentrarchi* is actually a misidentification or a junior synonym for *M. avidus*.

Another histophagous philasterid, *Porpostoma notatum*, was observed infesting the compromised skin of larvae and post-larvae of cultured seabream *Sparus aurata* in Israel (Paperna, 1984). Unidentified scuticociliates were reported to infect the skin, gills, brain, viscera and muscle of cultured Japanese flounder *Paralichthys olivaceus* (Yoshinaga and Nakazoe, 1993), and the visceral connective tissue associated with the digestive tract, muscle tissue and gill filaments of turbot *Scophthalmus maximus* in Spain (Dyková and Figueras, 1994).

### Treatment

*Uronema marinum* and other marine scuticociliates can be treated by repeated 10-15 minute dips in freshwater, followed by a 10-15 minute dip in 5 ppm methylene blue (Lom, 1995). However, established deep infections will unlikely be significantly affected by external treatments.

## Other Marine Facultative Parasites

*Licnophora* sp. (Class Heterotrichea, Order Licnophorida) is a sessile, heterotrichous (with more than a single type of cilia) ectosymbiont with an hourglass-shaped body that temporarily attaches to a host by a ciliated, aboral, adhesive disc. When settled on fish, both ends appear discoid, with the oral region of the anterior disc surrounded by serial polykinetids, and the conspicuous posterior adhesive disc displaying peripheral rings of cilia (Lynn and Small, 2000). *Licnophora hippocampi* was reported from cultured sea horses *Hippocampus trimaculatus* in China (Meng and Yu, 1985), and a yet unidentified species was observed on the gills of a wild devil firefish *Pterois miles* (Figs. 11.31, 11.32) and a lizardfish *Synodus variegatus* from the Red Sea coast of Israel (Diamant and Colorni, unpublished data).

## Enteric Ciliates

### *Freshwater and Marine Balantidiosis—The Endosymbiont Ciliates in Marine Fish*

The intestinal tracts of some freshwater and marine fish are inhabited by balantidiids. *Balantidium*, the only genus in the family Balantidiidae (Class Litostomatea, Subclass Trichostomatia, Order Vestibuliferida), comprises species living as endosymbionts in a broad variety of hosts, from insects to primates (Lynn and Small, 2000). Balantidiids are holotrichous ciliates with an apico-ventral vestibular groove less than one-half the body length that leads into the oral cavity (Figs. 11.33, 11.34). In fish, turning from harmless symbionts into histophagous parasites by some predisposing factors, they break through the epithelial gut lining and invade deeper layers. Mild enteritis (Feng, 1992) to extensive ulceration, necroses, mucous exudates and granulomatous lesions have been described (Lom and Dyková, 1992; Lom, 1995). Several species, including *Balantidium pangasi* and *B. mrigalae* (Mukherjee and Haldar, 1986; Mukherjee, 1990), *B. ctenopharyngodonis*, *B. polyvacuolum*, *B. barbi* (Astakhova and Stepanova, 1972; Lom and Dyková, 1992) and other species (Ky, 1971) were reported in the midgut and hindgut of Asian cyprinids. In Europe, *B. ctenopharyngodonis* was responsible for enteric ulcerative lesions in grass carp imported from the Far East (Molnár, 1971; Molnár and Reinhardt, 1978). In the marine environment, *Balantidium* spp. have been reported from the intestinal lumen of herbivorous fish associated with coral reefs. The parasitic fauna of the rabbitfishes *Siganus rivulatus* and *S. argenteus*

from the Gulf of Eilat (Red Sea) included *Balantidium sigani* (Fig. 11.34). *Siganus rivulatus* is a Lessepsian immigrant and *B. sigani* was found as well in individuals captured in the Mediterranean Sea. *Balantidium sigani* inhabits the hindgut, is slightly oval, usually slightly dorso-ventrally flattened, 41.6-104.4 × 28.7-73.1 μm (l × w) and possesses 85-90 kineties. The cytostome is at the end of a long apico-ventral slit (vestibulum). A single contractile vacuole and a cytopyge are located posteriorly. The parasite exhibits circular abrasive motion on the intestinal villi (Diamant and Wilbert, 1985).

The alimentary tract of several species of surgeonfishes from widely diverse geographical areas (Sea of Cortez, Mexico; the Indian Ocean off South Africa; Great Barrier Reef, Australia; Guam, Territory of the U.S.A. Tuvalu Islands, Pacific Ocean) were inhabited by various enteric ciliates (Grim *et al.*, 2002 and references therein). Altogether, two orders (Clevelandellida and Vestibuliferida) and three families (Nyctotheridae, Balantiidae, and Pycnotrichidae) were represented. However, no lesions were observed in any of the fish. Enteric ciliates, with no apparent adverse effects on the host, were also found in at least two Pomacanthidae (angelfish) from the Red Sea (Grim *et al.*, 1996; Diamant, unpublished data, Fig. 11.35).

## Taxonomy of Parasitic Ciliates

The taxonomy of parasitic ciliates is in many cases still a controversial issue. Features of considerable potential value for identification of taxa have often been overlooked, and species described in the past without the use of silver impregnation techniques are virtually impossible to confirm. Lom and Dyková (1992) consider identification of trichodinids based on unsilvered specimens unreliable. Advances in molecular biology have provided new insights into phylogenetic evolution and relatedness among protozoa. In particular, the sequence of the 18S small sub-unit ribosomal RNA (SSU rRNA) provides ample genomic information because they are reasonably large (about 1800 nucleotides in length), highly conserved, and because available data for comparison have increased rapidly in recent years (Jung *et al.*, 2005). This approach allows protozoa that share a relatively recent ancestry to be grouped into discrete clusters with generally high genomic homogeny. Unfortunately, the definition of a taxonomic category based on genomic similarity is no less vulnerable to subjective arbitrariness, and the question remains of how much genetic

diversity is permissible in a discrete cluster for its members to be regarded as a single taxon. In particular, the concept of 'species' for these organisms, with the binomial nomenclature of convenience, may need to be thoroughly revised in the near future. The ability to make a newly isolated ciliate fit into its taxon on the basis of its evolutionary development can, nevertheless, be of practical usefulness. If known members of the same family do not have a 'clean bill' concerning their pathogenicity, any related ciliate may be justifiably regarded as a potential offender. 'Commensalism' in particular should always be looked at with suspicion.

In Table 11.1, the taxonomy of the major ciliate parasites of fish is summarized according to the most recent authoritative reference (Lynn and Small, 2000). Although molecular clusters rarely fit into the hierarchical Phylum-Class-Order-Family-Genus structure, this traditional taxonomic system, in its various classification schemes each with different degrees of scholarly disagreement with one another, remains to date the major reference for the classification of eukaryotic microorganisms. Furthermore, the two approaches are not incompatible. The SSU rRNA trees often correlate with taxa based on ultrastructural features (Wright and Lynn, 1995; Patterson, 2000; Itabashi *et al.*, 2002; Lynn, 2003) or have contributed to the correction of several major 'historical' misidentifications. For example, *Cryptocaryon irritans* has been long considered to be the 'marine counterpart' of *Ichthyophthirius multifiliis*, as though the two ciliates were closely related organisms that simply lived in different aquatic habitats. Both are holotrichous, histophagous fish parasites, penetrate the epithelia as far as—but virtually never beyond—the basal lamina, have polymorphic life cycles with a benthic encystment and palintomic reproduction, have a direct life cycle (i.e., do not require an intermediate host), and have the same dispersal mechanism of actively swimming infective forms. Consequently, and despite the fact that several cytological studies did not support a systematic relationship between the two (Canella, 1972; Colorni and Diamant, 1993; Diggles, 1997), *C. irritans* was traditionally included in the Ichthyophthiriidae. Studies at the molecular levels (Diggles and Adlard, 1995, 1997) confirmed the cytological data. Recently, on the basis of the complete SSU rRNA gene sequence of a *C. irritans* isolate from the Red Sea, this ciliate was assigned to a new family, the Cryptocaryonidae, and its taxonomic affiliation relocated up to subphylum level (order Prorodontida, class Prostomatea, subphylum Intramacronucleata) (Wright and Colorni, 2002). The striking similarities between *C. irritans* and *I. multifiliis* were, thus, unequivocally

shown to be the result of an adaptive convergence of life histories rather than phylogenetic proximity.

Genomic studies of *Tetrahymena* and its 'marine counterpart' *Uronema*, of *Chilodonella* and its 'marine counterpart' *Brooklynella*, of some trichodinid (e.g., *Trichodina jadranica*), sessiline peritrich and uronematid (e.g., *Uronema nigricans*) species allegedly occurring in both freshwater and marine environments, may likewise reveal a greater phylogenetic distance than morphological similarities suggest.

## References


Anderson, O.R. 1988. *Comparative Protozoology*. Springer-Verlag, Berlin.

Aschurova, M. and G.A. Stein. 1972. Parasitic ciliates Peritricha, Urceolariidae from fishes of the Alpine Pamirs the Murgab Basin. *Parazitologiya* 6: 476-480. (In Russian).

Astakhova, T.V. and G.A. Stepanova. 1972. Parasitic fauna of the white amur (*Ctenopharyngodon idella*) in pond and spawning-nursery fisheries in the Volga delta. *Parazitologiya* 6: 364-368. (In Russian).

Astrofsky, K.M., J.M. Schech, B.J. Sheppard, C.A. Obenschain, A.M. Chin, M.C. Kacergis, E.R. Laver, J.L. Bartholomew and J.G. Fox. 2002. High mortality due to *Tetrahymena* sp. infection in laboratory maintained zebrafish (*Brachydanio rerio*). *Comparative Medicine* 52: 363-367.

Baker, R.J., M.D. Knittel and J.L. Fryer. 1983. Susceptibility of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), and rainbow trout, *Salmo gairdneri* Richardson, to infection with *Vibrio anguillarum* following sublethal copper exposure. *Journal of Fish Diseases* 6: 267-275.

Barker, D.E., D.K. Cone and M.D.B. Burt. 2002. *Trichodina murmanica* (Ciliophora) and *Gyrodactylus pleuronecti* parasitizing hatchery-reared winter flounder, *Pseudopleuronectes americanus* (Walbaum): Effects on host growth and assessment of parasite interaction. *Journal of Fish Diseases* 25: 81-89.

Basson, L. 1989. An endoparasitic trichodinid (Ciliophora: Peritrichia) from the urinary system of *Barbus trimaculatus* Peters, 1852. *South African Journal of Zoology* 24: 260-269.

Basson, L., and J.G. van As. 1989. Differential diagnosis of the genera in the family Trichodinidae (Ciliophora: Peritrichida) with the description of a new genus ectoparasitic on freshwater fish from southern Africa. *Systemic Parasitology* 13: 153-160.

Basson, L., J.G. van As and L. Fishelson. 1990. A new species of *Trichodina* (Ciliophora: Peritrichia) from the intestine of the surgeonfish *Acanthurus xanthopterus*. *International Journal of Parasitology* 20: 785-787.

Bauer, O.N. and N.P. Nikolskaya. 1957. *Chilodonella cyprini* (Moroff, 1902), biology and epizootological importance. *Izvestiia Vsesoiuznogo Nauchno-Issledovatel'skogo Instituta Ozernogo u Rechnogo Rybnogo Khozjastva* 119: 116-123. (In Russian).

Bishop, T.M., A. Smalls, G.A. Wooster and P.R. Bowser. 2003. Aerobiological (airborne) dissemination of the fish pathogen *Ichthyophthirius multifiliis* and the implications in

fish health management. In: *Biosecurity in Aquaculture Production Systems: Exclusion of Pathogens and other Undesirables*, L. Cheng-Sheng and O.B. Patricia (eds.). The World Aquaculture Society, Baton Rouge, U.S.A.

Blasiola, G.C. 1992. Diseases of ornamental marine fishes. In: *Aquariology: Fish Diseases and Water Chemistry*, J.B. Gratzek and J.R. Matthews (eds.). Tetra Press, Blacksburg, U.S.A., pp. 97-122.

Buchmann, K. and J. Bresciani. 1997. Parasitic infections in pond-reared rainbow trout *Oncorhynchus mykiss* in Denmark. *Diseases of Aquatic Organisms* 28: 125-138.

Burgess, P.J. and R.A. Matthews. 1994a. A standardized method for the *in vivo* maintenance of *Cryptocaryon irritans* (Ciliophora) using the grey mullet *Chelon labrosus* as an experimental host. *Journal of Parasitology* 80: 288-292.

Burgess, P.J. and R.A. Matthews. 1994b. *Cryptocaryon irritans* (Ciliophora): photoperiod and transmission in marine fish. *Journal of the Marine Biological Association of the United Kingdom*, Plymouth 74: 445-453.

Burgess, P.J. and R.A. Matthews. 1995a. Fish host range of seven isolates of *Cryptocaryon irritans* (Ciliophora). *Journal of Fish Biology* 46: 727-729.

Burgess, P.J. and R.A. Matthews. 1995b. *Cryptocaryon irritans* (Ciliophora): Acquired protective immunity in the thick-lipped mullet, *Chelon labrosus*. *Fish and Shellfish Immunology* 5: 459-468.

Burkart, M.A., T.G. Clark and H.W. Dickerson. 1990. Immunization of channel catfish, *Ictalurus punctatus* Rafinesque, against *Ichthyophthirius multifiliis* (Fouquet): Killed versus live vaccines. *Journal of Fish Diseases* 13: 401-410.

Bykhovskaya-Pavlovskaya, I.E., A.V. Gusev, M.N. Dubinina, N.A. Izyumova, T.S. Smirnova, I.L. Sokolovskaya, G.A. Shtein, S.S. Shul'man and V.N. Epshtein. 1962. Key to the parasites of freshwater fish of the U.S.S.R. *Zoologicheskii Institut Akademiya Nauk S.S.S.R., Moscow-Leningrad* (translated from Russian and edited by U.S. Department of Commerce, Office of Technical Services, Washington D.C., No. TT64-11040, 1964, 919 pp.).

Canella, M.F. 1972. Ce qu'on ne connaît pas sur un holotriche ectoparasite des poissons marins, découvert par le Dr. Sikama et appelé *Cryptocaryon irritans* par Miss Brown. Contributions à la connaissance des Ciliés, VII. *Annals of the University of Ferrara (Italy), Nuova Serie, Sezione III*: 107-132.

Canella, M.F. and I. Rocchi-Canella. 1976. Biologie des Ophryoglenina (ciliés hyménostomes histophages). *Annals of the University of Ferrara (Italy), Nuova Serie, Sezione III*: 1-510.

Cardeilhac, P.T. and B.R. Whitaker. 1988. Copper treatments: uses and precautions. In: *The Veterinary Clinics of North America, Tropical Fish Medicine*, M.K. Stoskopf (ed.). W.B. Saunders Co., Philadelphia, 18: 435-448.

Chen, C.L. 1963. Studies on ectoparasitic trichodinids from freshwater fish, tadpole and crustacean in China. *Acta Hydrobiologica Sinica* 3: 99-111.

Cheng, T.C. 1986. Ciliophora: The Ciliates. In: *General Parasitology*, T.C. Cheng (ed.). 2nd Edition. Academic Press, Orlando, pp. 234-251.

Cheung, P.J., R.F. Nigrelli and G.D. Ruggieri. 1980. Studies on the morphology of *Uronema marinum* Dujardin (Ciliata: Uronematidae) with a description of the histopathology of the infection in marine fishes. *Journal of Fish Diseases* 3: 295-303.

Clark, T.G., H.W. Dickerson and R.C. Findly. 1988. Immune response of channel catfish to ciliary antigens of *Ichthyophthirius multifiliis*. *Developmental and Comparative Immunology* 12: 581-594.

Colorni, A. 1985. Aspects of the biology of *Cryptocaryon irritans*, and hyposalinity as a control measure in cultured gilt-head seabream *Sparus aurata*. *Diseases of Aquatic Organisms* 1: 19-22.

Colorni, A. 1987. Biology of *Cryptocaryon irritans* and strategies for its control. *Aquaculture* 67: 236-237.

Colorni, A. 1992. *Biology, pathogenesis and ultrastructure of the holotrich ciliate Cryptocaryon irritans* Brown 1951, *a parasite of marine fish*. Ph.D. Thesis. Hebrew University of Jerusalem, Jerusalem, Israel.

Colorni, A. and A. Diamant. 1993. Ultrastructural features of *Cryptocaryon irritans*, a ciliate parasite of marine fish. *European Journal of Protistology* 29: 425-434.

Colorni, A. and P. Burgess. 1997. *Cryptocaryon irritans* Brown 1951, the cause of 'white spot disease' in marine fish: An update. *Aquarium Sciences and Conservation* 1: 217-238.

Colorni, A. and A. Diamant. 2005. Hyperparasitism of trichodinid ciliates on monogenean gill flukes of two marine fish. *Diseases of Aquatic Organisms* 65: 177-180.

Corliss, J.O. 1979. *The Ciliated Protozoa: Characterization, Classification and Guide to the Literature*. Pergamon Press, Oxford.

Cross, M.L. and R.A. Matthews. 1993. *Ichthyophthirius multifiliis* Fouquet (Ciliophora): The location of sites immunogenic to the host *Cyprinus carpio* L. *Fish and Shellfish Immunology* 3: 13-24.

Curds, C.R., M.A. Gates and D.M. Roberts. 1983. *British and Other Freshwater Ciliated Protozoa*, Cambridge University Press, Cambridge.

da Cunha, M.A. and C. Pinto. 1928. *Trichodina fariai* n. sp. cilié peritriche endoparasite de poisson marin. *Comptes Rendues de la Societé de Biologie (Paris)* 90: 1570-1571.

Davis, H.S. 1961. *Culture and Diseases of Game Fishes*. 3rd Edition. University of California Press, Berkeley.

Diamant, A. 1998. *Brooklynella hostilis* (Hartmannulidae), a pathogenic ciliate from the gills of maricultured seabream. *Bulletin of the European Association of Fish Pathologists* 18: 33-36.

Diamant, A. and N. Wilbert. 1985. *Balantidium sigani* sp. nov., a trichostome ciliate from Red Sea rabbitfish (Pisces, Siganidae). *Archiv für Protistenkunde* 129: 13-17.

Diamant, A., G. Issar, A. Colorni and I. Paperna. 1991. A pathogenic *Cryptocaryon*-like ciliate from the Mediterranean Sea. *Bulletin of the European Association of Fish Pathologists* 11: 122-124.

Dickerson, H.W. and T.G. Clark. 1996. Immune response of fishes to ciliates. *Annual Review of Fish Diseases* 6: 107-120.

Dickerson, H.W., T.G. Clark and A.A. Leff. 1993. Serotypic variation among isolates of *Ichthyophthirius multifiliis* based on immobilization. *Journal of Eukaryotic Microbiology* 40: 816-820.

Diggles, B.K. 1997. Some information on the morphology of *Cryptocaryon irritans* from SouthEast Queensland, Australia. *European Journal of Protistology* 33: 200-210.

Diggles, B.K. and R.D. Adlard. 1995. Taxonomic affinities of *Cryptocaryon irritans* and *Ichthyophthirius multifiliis* inferred from ribosomal RNA sequence data. *Diseases of Aquatic Organisms* 22: 39-43.

Diggles, B.K. and J.G. Lester. 1996a. Influence of temperature and host species on the development of *Cryptocaryon irritans*. *Journal of Parasitology* 82: 45-51.

Diggles, B.K. and J.G. Lester. 1996b. Variation in the development of two isolates of *Cryptocaryon irritans*. *Journal of Parasitology* 82: 384-388.

Diggles, B.K. and J.G. Lester. 1996c. Infections of *Cryptocaryon irritans* on wild fish from southeast Queensland, Australia. *Diseases of Aquatic Organisms* 25: 159-167.

Diggles, B.K. and R.D. Adlard. 1997. Intraspecific variation in *Cryptocaryon irritans*. *Journal of Eukaryotic Microbiology* 44: 25-32.

Dove, A.D.M. and P.J. O'Donoghue. 2005. Trichodinids (Ciliophora: Trichodinidae) from native and exotic Australian freshwater fishes. *Acta Protozoologica* 44: 51-60.

Dragesco, A., J. Dragesco, F. Coste, C. Gasc, B. Romestand, J.-C. Raymond and G. Bouix. 1995. *Philasterides dicentrarchi*, n. sp., (Ciliophora, Scuticociliatida), a histophagous opportunistic parasite of *Dicentrarchus labrax* (Linnaeus, 1758), a reared marine fish. *European Journal of Protistology* 31: 327-340.

Dyková, I. and A. Figueras. 1994. Histopathological changes in turbot *Scophthalmus maximus* due to a histophagous ciliate. *Diseases of Aquatic Organisms* 18: 5-9.

Ekless, L.M. and R.A. Matthews. 1993. *Ichthyophthirius multifiliis*: axenic isolation and short-term maintenance in selected monophasic media. *Journal of Fish Diseases* 16: 437-447.

Elliot, A.M. 1973. *Biology of Tetrahymena*. Dowden, Hutchinson and Ross, Stroudsburg, U.S.A.

Ergens, R. and J. Lom. 1970. *Agents of Parasitic Diseases of Fishes*. Academia Praha, Prague, Czech Republic. (In Czech).

Esch, G.W., T.C. Hazen, R.V. Dimock and J.W. Gibbons. 1976. Thermal effluent and the epizootiology of the ciliate *Epistylis* and the bacterium *Aeromonas* in association with centrarchid fish. *Transactions of the American Microscopical Society* 95: 687-693.

Eudokimova, E.B., I.G. Kuznecova and G.A. Shtein. 1969. Parasitic ciliates of the family Urceolariidae (Peritricha, Mobilia) of some fishes of southwest Atlantic *Zoologicheskii Zhurnal* 48: 1451-1455. (In Russian).

Ewing, M.S. and K.M. Kocan. 1987. *Ichthyophthirius multifiliis* (Ciliophora) exit from gill epithelium. *Journal of Protozoology* 34: 309-312.

Ewing, M.S., K.M. Kocan and S.A. Ewing. 1985. *Ichthyophthirius multifiliis* (Ciliophora) invasion of gill epithelium. *Journal of Protozoology* 32: 305-310.

Ewing, M.S., M.E. Lynn and S.A. Ewing. 1986. Critical periods in development of *Ichthyophthirius multifiliis* (Ciliophora) populations. *Journal of Protozoology* 33: 388-391.

Farley, O.G. and R. Heckmann. 1980. Attempts to control *Ichthyophthirius multifiliis* Fouquet (Ciliophora: Ophryoglenidae) by chemotherapy and electrotherapy. *Journal of Fish Diseases* 3: 203-212.

Fauré-Fremiet, E., P. Favard and N. Carasso. 1962. Etude au microscope électronique des ultrastructures d'*Epistylis anastatica* (Cilié Péritriche). *Journal de Microscopie* 1: 287-312.

Feng, S. 1992. Studies on a new ciliate, *Balantidium fulinensis* sp. nov., from the intestine of fishes. *Acta Hydrobiologica* Sinica/Shuisheng Shengwu Xuebao 16: 66-70.

Ferguson, H.W., B.D. Hicks, D.H. Lynn, V.E. Ostland and J. Bailey. 1987. Cranial ulceration in Atlantic salmon *Salmo salar* associated with *Tetrahymena* sp. *Diseases of Aquatic Organisms* 2: 191-195.

Gallet de Saint Aurin, D., J.C. Raymond and V. Vianas. 1990. Marine finfish pathology: Specific problems and research in the French West Indies. *Advances in Tropical Aquaculture, Tahiti, Feb. 20-March 4, 1989. AQUACOP, IFREMER Actes de Colloque* 9: 143-160.

Gardionio, E., M. Crerar and R.E. Pearlman. 1973. Growth and cloning of *Tetrahymena pyriformis* on solid medium. *Journal of Bacteriology* 116: 1170-1176.

Gleeson, D.J. 1999. Experimental infection of striped marshfrog tadpoles (*Limnodynastes peronii*) by *Ichthyophthirius multifiliis*. *Journal of Parasitology* 85: 568-570.

Goven, D.A., D.L. Dawe and J.B. Gratzek. 1980. Protection of channel catfish against *Ichthyophthirius multifiliis* Fouquet by immunization. *Journal of Fish Biology* 17: 311-316.

Goven, D.A., D.L. Dawe and J.B. Gratzek. 1981. *In vitro* demonstration of serological cross-reactivity between *Ichthyophthirius multifiliis* Fouquet and *Tetrahymena pyriformis* Lwoff. *Developmental and Comparative Immunology* 5: 283-289.

Gratzek, J.B. 1993. Parasites associated with freshwater tropical fishes. In: *Fish Medicine*, M.K. Stoskopf (ed.). W.B. Saunders Co., Philadelphia, pp. 573-590.

Grim, J.N., K.S. Reed and L. Fishelson. 1996. Description of a new species of ciliate, *Metanyctotherus paulini* n. sp., symbiont from the intestines of the angelfish *Apolemichtys xanthotis*. Assessment of special status for extant metanyctotherans. *Archiv für Protistenkunde* 146: 341-347.

Grim, J.N., K.D. Clements and T. Byfield. 2002. New species of *Balantidium* and *Paracichlidotherus* (Ciliophora) inhabiting the intestines of four surgeonfish species from the Tuvalu Islands, Pacific Ocean. *Journal of Eukaryotic Microbiology* 49: 146-153.

Halmetoja, A., E.T. Valtonen and E. Koskenniemi. 2000. Perch (*Perca fluviatilis* L.) parasites reflect ecosystem conditions: a comparison of a natural lake and two acidic reservoirs in Finland. *International Journal for Parasitology* 30: 1437-1444.

Hanna, B.A. and D.M. Lilley. 1974. Growth of *Uronema marinum* in chemically defined medium. *Marine Biology* 26: 153-160.

He, J., Z. Yin, G. Xu, Z. Gong, T. Jin Lam and Y. Min Sin. 1997. Protection of goldfish against *Ichthyophthirius multifiliis* by immunization with a recombinant vaccine. *Aquaculture* 158: 1-10.

Hetrick, F.M., M.D. Knittel and J.L. Fryer. 1979. Increased susceptibility of rainbow trout to infectious hematopoietic necrosis virus after exposure to copper. *Applied and Environmental Microbiology* 37: 198-201.

Hines, R.S. and D.T. Spira. 1974a. Ichthyophthiriasis in the mirror carp *Cyprinus carpio* (L.) III. Pathology. *Journal of Fish Biology* 6: 189-200.

Hines, R.S. and D.T. Spira. 1974b. Ichthyophthiriasis in the mirror carp *Cyprinus carpio* (L.) V. Acquired Immunity. *Journal of Fish Biology* 6: 373-378.

Hofer, R., W. Salvenmoser and J. Fried. 2005. Population dynamics of *Capriniana piscium* (Ciliophora, Suctoria) on the gill surface of Arctic char (*Salvelinus alpinus*) from high mountain lakes. *Archiv für Hydrobiologie* 162: 99-109.

Hoffman, G.L. 1999. *Parasites of North American Freshwater Fishes*. 2nd Edition, Comstock Publishing Associates, Ithaca.

Hoffman, G.L., M. Landolt, J.E. Camper, D.W. Coats, J.L. Stookey and J.D. Burek. 1975. A disease of freshwater fishes caused by *Tetrahymena corlissi* Thompson, 1955, and a key for identification of holotrich ciliates of freshwater fishes. *Journal of Parasitology* 61: 217-223.

Hoffman, G.L., S.L. Kazubski, A.J. Mitchell and C.E. Smith. 1979. *Chilodonella hexasticha* (Kiernik, 1909) (Protozoa, Ciliata) from North American warmwater fish. *Journal of Fish Diseases* 2: 153-157.

Houghton, G. and R.A. Matthews. 1986. Immunosuppression of carp (*Cyprinus carpio* L.) to *Ichthyophthirius* using the corticosteroid triamcinolone acetonide. *Veterinary Immunology and Immunopathology* 12: 413-419.

Iglesias, R., A. Parama, M.F. Alvarez, J. Leiro, J. Fernandez and M.L. Sanmartin. 2001. *Philasterides dicentrarchi* (Ciliophora, Scuticocilia tida) as the causative agent of scuticociliatosis in farmed turbot *Scophthalmus maximus* in Galicia (NW Spain). *Diseases of Aquatic Organisms* 46: 47-55.

Itabashi, T., K. Mikami, J. Fang and H. Asai. 2002. Phylogenetic relationships between *Vorticella convallaria* and other species inferred from Small Subunit rRNA gene sequences. *Zoological Science* 19: 931-937.

Jahn, T.L., E.C. Bovee and F.F. Jahn. 1949. Phylum Ciliophora. In: *How to Know the Protozoa*. 2nd Edition. WCB McGraw-Hill, Boston, pp. 199-266.

Jee, B-Y., K.H. Kim, S-I. Park and Y.-C. Kim. 2000. A new strain of *Cryptocaryon irritans* from the cultured olive flounder *Paralichthys olivaceus*. *Diseases of Aquatic Organisms* 43: 211-215.

Jung, M.-M. and S. Rho. 1999. Cyst formation of the marine ciliate, *Vorticella* sp. using MCCF medium. *Journal of the Korean Fisheries Society* 32: 317-319.

Jung, S.-J., S.-I. Kitamura, J.-Y. Song, I.-Y. Joung and M.-J. Oh. 2005. Complete small subunit rRNA gene sequence of the scuticociliate *Miamiensis avidus* pathogenic to olive flounder *Paralichthys olivaceus*. *Diseases of Aquatic Organisms* 64: 159-162.

Karlsbakk, E., P.E. Aspholm, V. Berg, N.R. Hareide and B. Berland. 2002. Some parasites of the small-eyed rabbitfish, *Hydrolagus affinis* (Capello, 1867) (Holocephali), caught in deep waters off SW Greenland. *Sarsia* 87: 179-184.

Kazubski, S.L. and K. Migala. 1974. Studies on the distinctness of *Chilodonella cyprini* (Moroff) and *Ch. hexasticha* (Kiernik) (Clamydodontidae, Gymnostomatida), ciliate parasites of fishes. *Acta Protozoologica* 13: 9-39.

Khan, R.A. 1972. Taxonomy, prevalence, and experimental transmission of a protozoan parasite, *Trichodina oviducti* Polyanskii (Ciliata: Peritrichida), of the thorny skate, *Raja radiata* Donovan. *Journal of Parasitology* 58: 680-685.

Kwon, S.R., C.S. Kim, J.K. Chung, H.H. Lee and K.H. Kim. 2002. Inhibition of chemiluminescent response of olive flounder *Paralichthys olivaceus* phagocytes by the scuticociliate parasite *Uronema marinum*. *Diseases of Aquatic Organisms* 2: 119-122.

Ky, H. 1971. New Ciliata from the intestine of freshwater fishes of North Vietnam. *Acta Protozoologica* 8: 261-282.

Landsberg, J.H. 1995. Tropical reef-fish disease outbreaks and mass mortalities in Florida, USA: What is the role of dietary biological toxins? *Diseases of Aquatic Organisms* 22: 83-100.

Landsberg, J.H. and B.A. Blakesley. 1995. Scanning electron microscope study of *Brooklynella hostilis* (Protista, Ciliophora) isolated from the gills of gray and French angelfish in Florida. *Journal of Aquatic Animal Health* 7: 58-62.

Langdon, J.S., N. Gudkovs, J.D. Humphrey and E.C. Saxon. 1985. Deaths in Australian freshwater fishes associated with *Chilodonella hexasticha* infection. *Australian Veterinary Journal* 62: 409-413.

Lee, E.H., C.S. Kim, J.B. Cho, K.J. Ahn and K.-H. Kim. 2003. Measurement of protease activity of live *Uronema marinum* (Ciliata: Scuticociliatida) by fluorescence polarization. *Diseases of Aquatic Organisms* 54: 85-88.

Lee, E.H., S.M. Kim, S.R. Kwon, S.K. Kim, Y.K. Nam and K.-H. Kim. 2004. Comparison of toxic effects of nitric oxide and peroxynitrite on *Uronema marinum* (Ciliata: Scuticociliatida). *Diseases of Aquatic Organisms* 58: 255-260.

Leiro, J., J.A. Arranz, R. Iglesias, F.M. Ubeira and M.L. Sanmart. 2004. Effects of the histiophagous ciliate *Philasterides dicentrarchi* on turbot phagocyte responses. *Fish and Shellfish Immunology* 17: 27-39.

Lewis, T.D., I. Misumi, C.D. Kelley, M.L. Kent and J.-A.C. Leong. 2004. Expression library immunization for development of vaccines for use in aquaculture. *Journal of Shellfish Research* 23: 301.

Li, L.-X. and S.S. Desser. 1983. *Trichodina algonquinensis*, a new species of Peritrich ciliate from Ontario freshwater fish, and observations on its transmission. *Canadian Journal of Zoology* 61: 1159-1164.

Lin, Y., T.L. Lin, C.-C. Wang, X. Wang, K. Stieger, R. Klopfleisch and T.G. Clark. 2002. Variation in primary sequence and tandem repeat copy number among i-antigens of *Ichthyophthirius multifiliis*. *Molecular and Biochemical Parasitology* 120: 93-106.

Ling, K.H., Y.M. Sin and T.J. Lam. 1993. Protection of goldfish against some common ectoparasitic protozoans using *Ichthyophthirius multifiliis* and *Tetrahymena pyriformis* for vaccination. *Aquaculture* 116: 303-314.

Lom, J. 1984. Diseases caused by protistans. In: *Diseases of Marine Animals*, O. Kinne (ed.). Biologische Anstalt Helgoland, Hamburg, Vol. 4, pp. 114-168.

Lom, J. 1995. Trichodinidae and other ciliates. In: *Fish Diseases and Disorders*, P.T.K. Woo (ed.). CAB International, Oxon, U.K., Vol. 1, pp. 229-262.

Lom, J. and J.O. Corliss. 1971. Morphogenesis and cortical ultrastructure of *Brooklynella hostilis*, a dysteriid ciliate ectoparasitic on marine fishes. *Journal of Protozoology* 18: 261-281.

Lom, J. and D.P. Haldar. 1976. Observations on trichodinids endocommensal in fishes. *Transactions of the American Microscopical Society* 95: 527-541.

Lom, J. and I. Dyková. 1992. Ciliates (Phylum Ciliophora Doflein, 1901). In: *Protozoan Parasites of Fishes*, Developments in Aquaculture and Fisheries Science Vol. 26. Elsevier, Amsterdam, pp. 237-288.

Luzardo-Alvarez, A., J. Martinez-Mazagatos, M.T. Santamarina-Fernandez, F.J. Otero-Espinar and J. Blanco-Mendez. 2003. Oral pharmacological treatments for ichthyophthiriosis of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 220: 15-25.

Lynn, D.H. 1975. The life cycle of the histophagous ciliate *Tetrahymena corlissi* Thompson, 1955. *Journal of Protozoology* 22: 188-195.

Lynn, D.H. 2003. Morphology or molecules: How do we identify the major lineages of ciliates (Phylum Ciliophora)? *European Journal of Protistology* 39: 356-364.

Lynn, D.H. and E.B. Small. 2000. Phylum Ciliophora Doflein, 1901. In: *The Illustrated Guide to the Protozoa*, J.J. Lee, G.F. Leedale and P. Bradbury (eds.). 2nd Edition. Society of Protozoologists, Lawrence, Kansas, Vol. I, pp. 371-656.

Matthew, B.F., R.A. Matthew and P.J. Burgess. 1993. *Cryptocaryon irritans* Brown 1951 (Ichthyophthiriidae): The ultrastructure of the somatic cortex throughout the life cycle. *Journal of Fish Diseases* 16: 339-349.

Meng, Q. and K. Yu. 1985. A new specie of Ciliata, *Licnophora hippocampi* sp. nov. from seahorse, *Hippocampus trimaculatus* Leach, with consideration of its control in the host. *Acta Zoologica Sinica* 31: 65-69.

Meyer, F.P. 1974. Parasites of freshwater fishes; II, Protozoa 3. *Ichthyophthirius multifiliis*. *Fish Disease Leaflet* 2. United States Department of the Interior, Fish and Wildlife Service, Washington, D.C.

Migala, K. and S.L. Kazubski. 1972. Occurrence of nonspecific ciliates on carps (*Cyprinus carpio* L.) in winter ponds. *Acta Protozoologica* 9: 329-337.

Miller, R.W. and W.R. Chapman. 1976. *Epistylis* and *Aeromonas hydrophila* in fishes from North Carolina reservoirs. *Progressive Fish Culturist* 38: 165-168.

Miron, D.S., L.V.F. Silva, J.I. Golombieski and B. Baldisserotto. 2003. Efficacy of different salt (NaCl) concentrations in the treatment of *Ichthyophthirius multifiliis*-infected silver catfish, *Rhamdia quelen*, fingerlings. *Journal of Applied Aquaculture* 14: 155-161.

Mitchell, A.J. and C.E. Smith. 1988. *Amphileptus branchiarum* (Protozoa: Amphileptidae) in pond reared fish in Arkansas. *Journal of Wildlife Diseases* 24: 642-646.

Molnár, K. 1971. Protozoan diseases of the fry of herbivorous fishes. *Acta Veterinaria Academiae Scientiarum Hungaricae* 21: 1-14.

Molnár, K. and M. Reinhardt. 1978. Intestinal lesions in grasscarp *Ctenopharyngodon idella* (Valenciennes) infected with *Balantidium ctenopharyngodonis* Chen. *Journal of Fish Diseases* 1: 151-156.

Mukherjee, M. 1990. Fish diseases due to protozoan parasites and their relation to the environment. *National Symposium on Environmental Impacts on Animals and Aquaculture, Kalyani (India)*, 14-16 May 1988, G.K. Manna and B.B. Jana (eds.), pp. 261-264.

Mukherjee, M. and D.P. Haldar. 1986. Two new ciliates of the genus *Balantidium* Claparede and Lachmann, 1858 from fish intestine. *Journal of Bengal Natural History Society* 5: 6-17.

Munday, B.L., P.J. O'Donoghue, M. Watts, K. Rough and T. Hawkesford. 1997. Fatal encephalitis due to the scuticociliate *Uronema nigricans* in sea-caged, southern bluefin tuna *Thunnus maccoyii*. *Diseases of Aquatic Organisms* 30: 17-25.

Nielsen, C.V. and K. Buchmann. 2000. Prolonged *in vitro* cultivation of *Ichthyophthirius multifiliis* using an EPC cell line as substrate. *Diseases of Aquatic Organisms* 42: 215-219.

Nigrelli, R.F. and G.D. Ruggieri. 1966. Enzootics in the New York Aquarium caused by *Cryptocaryon irritans* Brown, 1951 (=*Ichthyophthirius marinus* Sikama, 1961), a histophagous ciliate in the skin, eyes and gills of marine fishes. *Zoologica* 51: 97-102.

Nigrelli, R.F., K.S. Pokorni and G.D. Ruggieri. 1976. Notes on *Ichthyophthirius multifiliis*, a ciliate parasitic on freshwater fishes, with some remarks on possible physiological races and species. *Transactions of the American Microscopical Society* 95: 607-613.

Noble, E.R. 1963. The relation between *Trichodina* and metazoan parasites on gills of fish. In: *Progress in Protozoology*, J. Ludvik, J. Lom and J. Vávra (eds.). Czechoslovak Academy of Sciences Publishing House, Prague, pp. 521-523.

Noe, J.G. and H.W. Dickerson. 1995. Sustained growth of *Ichthyophthirius multifiliis* at low temperature in the laboratory. *Journal of Parasitology* 8: 1022-1024.

Noga, E.J. 1996. *Fish Disease Diagnosis and Treatment*. C.V. Mosby-Yearbook Inc., St. Louis.

Palenzuela, O., T. Yoshinaga and P. Alvarez-Pellitero. 2005. Conserved rDNA gene sequences among parasitic Scuticociliates of flatfish. What, if anything, is *Philasterides dicentrarchii? EAFP 12th International Conference, Copenhagen*, 11-16 September 2005, *Book of Abstracts*, O-066, p. 70.

Paperna, I. 1984. Review of diseases affecting cultured *Sparus aurata* and *Dicentrarchus labrax*. In: *L'Aquaculture du Bar et des Sparidés*, G. Barnabé and R. Billard (eds.). Institut National de la Recherche Agronomique Publication, Paris, pp. 465-482.

Paperna, I. 1991. Diseases caused by parasites in the aquaculture of warm water fish. *Annual Review of Fish Diseases* 1: 155-194.

Paperna, I. 1996. Parasites, infections and diseases of fishes in Africa—An update. CIFA Technical Paper No. 31, Rome, FAO, 220 pp.

Paperna, I. and J.G. van As. 1983. The pathology of *Chilodonella hexasticha* (Kiernik). Infections in cichlid fishes. *Journal of Fish Biology* 23: 441-450.

Paperna, I., J.G. van As and L. Basson. 1984. Review of diseases affecting cultured cichlids. In: *International Symposium on Tilapia in Aquaculture*, L. Fishelson and Z. Yaron (eds.). Proceedings of the Tel Aviv University Press, pp. 174-184.

Patterson, D.J. 2000. Changing views of protistan systematics: the taxonomy of Protozoa—An overview. In: *The Illustrated Guide to the Protozoa*, J.J. Lee, G.F. Leedale and P. Bradbury (eds.). 2nd Edition. Society of Protozoologists, Lawrence, Kansas, Vol. I, pp. 2-9.

Pérez-Uz, B. 1995. Growth rate variability in geographically diverse clones of *Uronema* (Ciliophora: Scuticociliatida). *Federation of European Microbiological Societies, Microbiology Ecology* 16: 193-204.

Pimenta Leibowitz, M., R. Ariav and D. Zilberg. 2005. Environmental and physiological conditions affecting *Tetrahymena* sp. infection in guppies, *Poecilia reticulata* Peters. *Journal of Fish Diseases* 28: 539-547.

Plunket, L. and H. Hidu. 1978. The role of *Uronema marinum* (Protozoa) in oyster hatchery production. *Aquaculture* 15: 219-225.

Purdom, C.E. and A.E. Howard. 1971. Ciliate infestations: a problem in marine fish farming. *Journal du Conseil International pour l'Exploration de la Mer* 33: 511-514.

Rigos, G., M. Pavlidis and P. Divanach. 2001. Host susceptibility to *Cryptocaryon* sp. infection of Mediterranean marine broodfish held under intensive culture

conditions: A case report. *Bulletin of the European Association of Fish Pathologists* 21: 33-36.

Rintamäki, P., H. Torpström and A. Bloigu. 1994. *Chilodonella* spp. at four fish farms in Northern Finland. *Journal of Eukaryotic Microbiology* 41: 602-607.

Schlenk, D., J.L. Gollon and B.R. Griffin. 1998. Efficacy of copper sulfate for the treatment of ichthyophthiriasis in channel catfish. *Journal of Aquatic Animal Health* 10: 390-396.

Selosse, P.M. and S.J. Rowland. 1990. Use of common salt to treat ichthyophthiriasis in Australian warmwater fishes. *Progressive Fish-Culturist* 52: 124-127.

Shapiro, J. and Z. Snovski. 1997. The effect of the 1991/1992 winter upon the fishing industry of Lake Kinneret, Israel. *Fisheries Management and Ecology* 4: 249-252.

Shinn, A.P., R. Wootten, I. Cote and C. Sommerville. 2003. Efficacy of selected oral chemotherapeutants against *Ichthyophthirius multifiliis* (Ciliophora: Ophyroglenidae) infecting rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 55: 17-22.

Sigh, J. and K. Buchmann. 2002. Comparative analysis of cross-reactivity between *Ichthyophthirius* and *Tetrahymena*. *Bulletin of the European Association of Fish Pathologists* 22: 37-44.

Song, W. and N. Wilbert. 2000. Redefinition and redescription of some marine Scuticociliates from China, with report of a new species, *Metanophrys sinensis* nov. spec. (Ciliophora, Scuticociliatida). *Zoologischer Anzeiger* 239: 45-74.

Steinbrück, G. 1990. Recent advances in the study of ciliate genes. *European Journal of Protistology* 26: 2-14.

Strauss, D.L and B.R. Griffin. 2002. Efficacy of potassium permanganate in treating ichthyophthiriasis in channel catfish. *Journal of Aquatic Animal Health* 14: 145-148.

Swarm, L. and S. Fitzgerald. 1992. Use and application of salt in aquaculture. *North Central Regional Aquaculture Center in cooperation with USDA*, Publ. No. 105, 2 pp.

Thompson, J.C. 1963. A redescription of *Uronema marinum*, and a proposed new family, Uronematidae. *Virginia Journal of Science* 15: 80-87.

Thompson, J.C. and L. Moewus. 1964. *Miamiensis avidus* n. g., n. sp., a marine facultative parasite in the ciliate order Hymenostomatida. *Journal of Protozoology* 11: 378-381.

Tieman, D.M. and A.E. Goodwin. 2001. Treatments for Ich infestations in channel catfish evaluated under static and flow-through water conditions. *North American Journal of Aquaculture* 63: 293-299.

Tonguthai, K. and T.S. Leong. 2000. Diseases of Cage Cultured Fish in Marine and Brackishwater Cage Aquaculture in Asia. *Proceedings of the First International Symposium on Cage Aquaculture in Asia*, I. Chiu Liao and C. Kwei Lin (eds.). Asian Fisheries Society, Manila and World Aquaculture Society—South Eastern Chapter, Bangkok, pp. 217-224.

Urawa, S. and S. Yamao. 1992. Scanning electron microscopy and pathogenicity of *Chilodonella piscicola* (Ciliophora) on juvenile salmonids. *Journal of Aquatic Animal Health* 4: 188-197.

Uspenskaya, A.V. 1963. Hyaluronidase in the various stages of life history of *Ichthyophthirius multifiliis*. *Dokladi Akademii Nauk SSSR* 151: 1476-1478.

van As, J.G. and L. Basson. 1989. A further contribution to the taxonomy of the Trichodinidae (Ciliophora: Peritrichia) and a review of the taxonomic status of some fish ectoparasitic trichodinids. *Systematic Parasitology* 14: 157-179.

van As, J.G. and L. Basson. 1996. An endosymbiotic trichodinid, *Trichodina rhinobatae* sp. n. (Ciliophora: Peritrichia) found in the lesser guitarfish, *Rhinobatos annulatus* Smith, 1841 (Rajiformes: Rhinobatidae) from the South African coast. *Acta Protozoologica* 35: 61-67.

Ventura, M.T. and I. Paperna. 1985. Histopathology of *Ichthyophthirius multifiliis* infections in fishes. *Journal of Fish Biology* 27: 185-203.

Viljoen, S., and J.G. van As. 1983. A taxonomic study of sessile peritrichians of a small impoundment with notes on their substrate preferences. *Journal of the Limnological Society of Southern Africa* 9: 33-42.

Wahli, T. and R.A. Matthews. 1999. Ichthyophthiriasis in carp *Cyprinus carpio*: Infectivity of trophonts prematurely exiting both the immune and non-immune host. *Diseases of Aquatic Organisms* 36: 201-207.

Wahli, T. and W. Meier. 1985. Ichthyophthiriasis in trout: investigations of natural defence mechanisms. In: *Fish and Shellfish Pathology*, A.E. Ellis (ed.). Academic Press, London, pp. 347-352.

Wiles, M., D.K. Cone and P.H. Odense. 1985. Studies on *Chilodonella cyprini* and *C. hexasticha* (Protozoa, Ciliata) by scanning electron microscopy. *Canadian Journal of Zoology* 63: 2483-2487.

Wright, A.-D.G. and D.H. Lynn. 1995. Phylogeny of the fish parasite *Ichthyophthirius* and its relatives *Ophryoglena* and *Tetrahymena* (Ciliophora, Hymenostomatia) inferred from 18S ribosomal RNA sequences. *Molecular Biology and Evolution* 12: 285-290.

Wright, A.-D.G. and A. Colorni. 2002. Taxonomic re-assignement of *Cryptocaryon irritans*, a marine fish parasite. *European Journal of Protistology* 37: 375-378.

Yambot, A.V. and Y.-L. Song. 2004. Short term *in vitro* culture of *Cryptocaryon irritans*, a protozoan parasite of marine fishes. *Fish Pathology* 39: 175-181.

Yambot, A.V., Y.-L. Song and H.-H. Sung. 2003. Characterization of *Cryptocaryon irritans*, a parasite isolated from marine fishes in Taiwan. *Diseases of Aquatic Organisms* 54: 147-156.

Yao, M.-C. and M.A. Gorovsky. 1974. Comparison of the sequences of macro- and micronuclear DNA of *Tetrahymena pyriformis*. *Chromosoma* 48: 1-18.

Yoshinaga, T. and J. Nakazoe. 1993. Isolation and *in vitro* cultivation of an unidentified ciliate causing scuticociliosis in Japanese flounder (*Paralichthys olivaceus*). *Fish Pathology* 28: 131-134.

Yoshinaga, T. and J. Nakazoe. 1997. Acquired protection and production of immobilization antibody against *Cryptocaryon irritans* (Ciliophora, Hymenostomatida) in mummichog (*Fundulus heteroclitus*). *Fish Pathology* 32: 229-230.

Xu, D.-H. and P.H. Klesius. 2004. Two year study on the infectivity of *Ichthyophthirius multifiliis* in channel catfish *Ictalurus punctatus*. *Diseases of Aquatic Organisms* 59: 131-134.

Xu, C., T.G. Clark, A.A. Leff and H.W. Dickerson. 1995. Analysis of the soluble and membrane-bound immobilization antigens of *Ichthyophthirius multifiliis*. *Journal of Eukaryotic Microbiology* 42: 558-564.

CHAPTER

# 12

# Myxozoan Diseases

**Stephen W. Feist**

## INTRODUCTION

Members of the phylum Myxozoa are common parasites of fish and some invertebrates and are also the causative agents of several economically important diseases in aquaculture systems and wild stocks. They are highly specialized metazoans with often complex life cycles in their hosts, characterized by the formation of multicellular spores with polar capsules and extrudible polar filaments. They have been recognized since the beginning of the nineteenth century (Jurine, 1825; Müller, 1841; Shulman, 1966) and many new species and genera continue to be reported in the scientific literature. Although from several early studies their importance as disease agents was recognized, it was not until the growth of freshwater aquaculture during the latter half of the twentieth century that interest in the group increased significantly. Whirling disease caused by *Myxobolus cerebralis*, proliferative kidney disease (PKD) caused by *Tetracapsuloides bryosalmonae* and ceratomyxosis caused by *Ceratomyxa shasta* are perhaps the best-known examples affecting salmonids.

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Infections with *Enteromyxum leei* and *E. scophthalmi* in mariculture situations can also give rise to significant mortalities. Increasingly, there is evidence that these and other myxozoan pathogens can have a significant effect on wild fish populations.

Since the discovery of oligochaete and, more recently, bryozoan obligatory hosts in the life cycle of several freshwater species (Markiw and Wolf, 1983; Canning *et al.*, 2000), it is apparent that such a host may be required for a number of myxozoan life cycles (Kent *et al.*, 2001). This has been the stimulus for new studies into the biology of the group. Furthermore, with the utilization of molecular phylogenetic techniques, significant changes have been made in the taxonomy of the phylum, particularly in the suppression of the class Actinosporea (Kent *et al.*, 1994a; Fiala, 2006). The discovery that the bryozoan parasite *Buddenbrokia plumatellae* was a myxozoan (Canning *et al.*, 1996; Monteiro *et al*, 2002) and the erection of a new class Malacosporea to accommodate this species both provided the impetus finally resulting in the acceptance that myxozoans were metazoans rather than protozoans (Siddall *et al.*, 1995; Canning *et al.*, 1996). Most of the molecular evidence suggests that Myxozoa are highly specialized bilaterians (Smothers *et al.*, 1994; Schlegel *et al.*, 1996; Anderson *et al.*, 1998; Okamura *et al.*, 2002; Zrzavý and Hypša, 2003). However, the situation is still not entirely resolved since recent studies on actin cDNA sequence of *Myxobolus cerebralis* indicate branching outside the metazoans (Kelley *et al.*, 2004) and phylogenetic analyses of multiple coding genes demonstrated that *Buddenbrokia* is a cnidarian worm (Jiménez-Guri *et al.*, 2007a, b). Recent reviews have been published on the taxonomy of the phylum (Kent *et al.*, 2001; Lom and Dyková, 2006) and an extensive review on the biodiversity and evolution of the Myxozoa has been provided by Canning and Okamura (2004).

This chapter provides a brief summary of the biology of the Myxozoa, their characteristics, taxonomy and life-cycle. Emphasis is given to those parasites of fish which give rise to clinical disease in their hosts. Further sections provide information on diagnostic methods, treatment and control and future trends.

## MORPHOLOGY AND LIFE CYCLES

Within fish hosts, myxozoan stages are either histozoic (within tissues) or coelozoic (inhabiting cavities) and most organs in the fish hosts can be infected. For indirect life cycles, infection to the fish host occurs following

discharge of the actinospore or malacospore polar filament and penetration of the infective sporoplasm cells through the epithelial boundary of the skin, gills or alimentary tract (Kallert *et al.*, 2007). Infection proceeds by proliferation of intracellular or intercellular pre-sporogonic stages, which migrate to the site of sporogony. Several species also possess extrasporogonic proliferative stages, which provide an additional means of parasite proliferation and are able to migrate to target organ(s) and develop into sporogonic stages. They are able to persist in the host tissues after mature spores have been formed. Sporogony proceeds within plasmodia or pseudoplasmodia via the differentiation of sporogonic cells alone or within another cell called the pericyte. Immature spores or sporoblasts comprise capsulogenic, valvogenic and sporoplasmic cells, which form the polar capsules, valves and sporoplasm cells of mature spores respectively. Sporoplasm cells undergo limited morphological change as they mature but nuclear atrophy and reduction in cytosol volume occur in capsulogenic and valvogenic cells, with thickening of the cell wall of the valvogenic cell to form hard shell valves meeting along the sutural line of the mature spore. Pseudoplasmodia may be monosporic or disporic, producing one or two spores. Plasmodia give rise to up to several hundred (possibly thousands) of spores, depending on the species. Mature myxospores are usually between 10-20 μm in length or width. Spores comprise between 2-12 shell valves, 1-13 polar capsules and the infective sporoplasm, which normally consists of one binucleate or two uninucleate cells, further containing characteristic cytoplasmic sporoplasmosomes. However, *Polysporoplasma* can contain up to 12 sporoplasm cells. Sporal shell valves are held together along a sutural line that splits in order to release the infective amoeboid sporoplasm(s) within the oligochaete definitive host. The shell valves can be smooth or ridged and in some genera, for example *Henneguya* and *Myxobilatus*, there may be protrusions from the shell valves. The polar capsules contain a coiled polar filament and the arrangement and number of coils within the capsule are of taxonomic value.

Within the digestive tract of the definitive oligochaete hosts, the myxospore sporoplasm cells are released and a merogonic phase of actinospore development occurs, generally within the intestinal epithelium. The multinucleate cell undergoes plasmotomy to produce numerous uninucleate cells that invade other intercellular spaces. These cells then either undergo further nuclear division, giving rise to more multinucleate stages, which divide, producing more uninucleate cells

which, in turn, fuse so as to produce binucleate stages. Nuclear division then produces a four-cell stage and two of these cells become pericytes enveloping two sporogonic cells, which together form an early pansporocyst. The two enveloping pericytes undergo mitosis twice to produce eight somatic cells. The sporogonic cells undergo meiotic division to produce 16 diploid gametocytes composed of eight α-cells and eight β-cells. Each α-cell fuses with a β-cell to produce a zygote, the sexual phase of development. The pansporocyst consists of eight zygotes surrounded by eight somatic cells. Sporogony proceeds by division and subsequent differentiation of the zygote in order to produce the typical triradiate actinospore form. The spore body contains the sporoplasm with a variable number of infective germ cells, the exact number being species dependent. Valvogenic cells have variable morphology, depending on the species or collective group and spores have either three or six valves although the exception to this is the actinospore collective group Tetractinomyxon that has four spore valves. Each caudal process contains a single nucleus and the length can range from 10 μm for members of the collective group Neoactinomyxum, to several hundred μm for Triactinomyxon and Raabeia types. Polar capsules have a similar structure to those of the myxospore stage but generally with fewer coils of the polar filament and a characteristic plug or stopper of dense material (Lom and Dyková, 1997; Alvarez-Pellitero *et al.*, 2002). Release of actinospores from infected oligochaetes follows a circadian pattern (Yokoyama *et al.* 1993a; Özer and Wootten, 2001) and seem to be viable in the water column for several days (Yokoyama *et al.* 1993a; Xiao and Desser, 2000b). The presence of mucous from the specific fish host—alone or in combination with mechanical cues—stimulates mature actinospores to release the sporoplasm to initiate new infections (Yokoyama *et al.*, 1993a, 1995a; Kallert *et al.*, 2005).

The development of malacosporeans in freshwater bryozoan hosts (Class Phylactolaemata) does not follow the patterns observed in the myxospore-actinospore life-cycle (Canning and Okamura, 2004). Malacospores develop within hollow rounded sacs or larger vermiform forms, in the case of *B. plumatellae*, measuring up to 2 mm in length. The earliest stages so far observed in the bryozoan hosts are the so-called cryptic stages present in the epithelium of the body wall. These stages persist for several weeks and form clusters of cells, eventually forming small spherical or vermiform sacs which becomes detached from the epithelial surface and enters the body cavity of the zooid. Here they grow

in size and divide by budding. In *T. bryosalmonae*, the wall of the sac in which spores develop consists of a single layer of flattened (mural) cells. Proliferation of these cells leads to the formation of a sac wall. Within the sac, sporogonic cells take one of two forms—either as sporoplasmogenic (pale) cells or denser, stellate cells. The sporoplasmogenic cells, containing characteristic cytoplasmic sporoplasmosomes, undergo a series of meiotic divisions. Division of the sporoplasmogenic cells leads to the production of two uninucleate sporoplasms, both of which may contain a secondary cell derived by endogenous budding. Within the sporoblast, the sporoplasms are enveloped by a group of stellate cells, which differentiate to form eight valve cells and four capsulogenic cells. In *B. plumatellae*, sacs the layer of mural cells enclose four longitudinal bundles of muscle and an internal layer of 'B-cells' with additional 'A-cells' which separate and form connections between the muscle blocks. These are thought to have a secretory function. As the sac grows, proliferation of B-cells occurs by binary fission and endogeny and some undergo meiotic division and form discrete groups of cells, which mature and differentiate to form the components of malacospores. Synchronous maturation of the spores occurs within the sac, which contains up to several hundred spores. Unlike myxospores, the valve cells do not degenerate at maturity and remain 'soft'. The formation of an external tube prior to inversion into the capsule appears to be an extremely transitory event (Canning *et al.*, 1996, 1999, 2002; Tops *et al.*, 2005). However, unlike actinospores and myxospores, a cap or stopper-like structure does not cover the exit pore of the polar filament, nor do the valve walls cover it. Mature spores, approximately 19 μm in diameter, comprise eight cells forming the valvular cells, four spherical to sub-spherical polar capsules contained within pyriform capsular cells at the apical end of the spore and two sporoplasms, each of which may contain a secondary cell (McGurk *et al.*, 2005). The 'soft' spore shell valve cells contain haplosporosomes, elongate nuclei, mitochondria and lipid vacuoles and are joined together by septate junctions. Malacospores are short lived, surviving for less than 24 hours in the water column (Gay *et al.*, 2001; De Kinkelin *et al.*, 2002).

Discrimination of malacospore species is currently based on differences in the development of spores and DNA sequence, as there are limited number of features of taxonomic value (Canning and Okamura, 2004). Morphologically identical histozoic malacospore forms occur in salmonid hosts (causing PKD) and in common carp (*Cyprinus carpio*)

(Voronin, 1993; Voronin and Chernysheva, 1993). In carp, sporogonic forms have not been observed whilst in salmonids, sporogony can occur in the renal tubules (Kent *et al.*, 2000; Hedrick *et al.* 2004).

The disease has been experimentally induced in naïve rainbow trout (*Oncorhynchus mykiss*) exposed to the bryozoan stages of the parasite (Feist *et al.*, 2001; Longshaw *et al.*, 2002). The parasite enters the fish via the mucous cells of the skin epithelium and not via the gut epithelium. Morris *et al.* (2000b) showed that the parasite was present in the gill epithelium after three days exposure at an enzootic field site. It appears likely that all external epithelia exposed to the environment are suitable sites for infection. Entry of the parasite into the fish is apparently rapid, with *T. bryosalmonae* cells present in the skin of rainbow trout at 1 minute post-exposure (Longshaw *et al.*, 2002). Parasites are transported around the body via the blood, during which time they may undergo a series of multiplications (Kent and Hedrick, 1985). These extrasporogonic stages undergo further development primarily within the kidney and spleen, but can also be localized within many other tissues and organs of the host where they may elicit a vigorous host response. The earliest identifiable stage of *T. bryosalmonae* (PKX cells) in the fish host tissues is a mononucleate primary cell containing prominent cytoplasmic granules. Following endogenous cleavage, secondary cells are produced within the primary cell. Secondary cells may also themselves divide by binary fission and form tertiary cells by endogeny. In rainbow trout cultured within the United Kingdom, this is most often the final stage observed in fish. *T. bryosalmonae* undergoes sporogony within the lumen of renal tubules and collecting ducts in some hosts, particularly in salmonids from the United States (Kent and Hedrick, 1985). The spores are characterized as being ovoid with indistinct valves and two polar capsules (Kent *et al.*, 2000; Hedrick *et al.*, 2004). Experimental horizontal transmission of spores from the salmonid host to naïve bryozoans have been attempted without success (Tops *et al.*, 2004). However, recent success in studies exposing bryozoans to the effluent of tanks containing experimentally infected brown trout show that this route is possible (Morris and Adam, 2006a). Taticchi *et al.* (2004) described the presence of malacosporean-like vermiform stages, similar to *B. plumatellae*, hatching from *Plumatella fungosa* flotablasts. This finding suggests the possibility of parasite survival and dispersal in the statoblast stages and that other hosts may not be required. In addition, vertical transmission within invertebrate hosts can occur, where bryozoan colony fragmentation and paratomic annelid fission

are intra-clonal propagation mechanisms capable of increasing the prevalence of myxozoan infected bryozoans and oligochaetes (Morris and Adams, 2006).

## CLASSIFICATION OF THE PHYLUM MYXOZOA GRASSÉ, 1970

The phylum Myxozoa Grassé, 1970, currently contains two classes, the Myxosporea Bütschli, 1881, and the Malacosporea Canning, Curry, Feist, Longshaw and Okamura, 2000. The class Myxosporea currently consists of the order Bivalvulida composed of three suborders and twelve families and the order Multivalvulida containing two families. The classification of the Phylum Myxozoa provided here is according to Grassé (1970) and Lom and Noble (1984). Previous classification of the order Multivalvulida included several families. Whipps *et al.* (2003, 2004a) suppressed the multivalvulid families Pentacapsulidae, Hexacapsulidae and Septemcapsulidae based on molecular evidence so that the genera *Pentacapsula*, *Hexacapsula* and *Septemcapsula* become junior synonyms of *Kudoa*. Additional multivalvulid families have been proposed but require confirmation of their current taxonomic status (Lom and Dyková, 2006). Classification schemes are still largely reliant on morphological characters of the spore stages, although molecular data for phylogenetic analyses are becoming more important (Xiao and Desser, 2000a).

Class Myxosporea Bütschli, 1881
- Order Bivalvulida Schulman, 1959
  - Suborder Platysporina Kudo, 1919
    - Family Myxobolidae Thélohan, 1892, with thirteen genera.
  - Suborder Sphaeromyxina Lom and Noble, 1984
    - Family Sphaeromyxiidae Lom and Noble, 1984, with one genus.
  - Suborder Variisporina Lom and Noble, 1984
    - Family Alatosporidae Shulman *et al.*, 1979, with three genera.
    - Family Auerbachiidae Evdokimova, 1973, with two genera.
    - Family Ceratomyxidae Doflein, 1899, with four genera.
    - Family Chloromyxidae Thélohan, 1892, with three genera.
    - Family Fabesporidae Naidenova and Zaika, 1969, with one genus.
    - Family Myxidiidae Thélohan, 1892, with four genera.

Family Ortholineidae Lom and Noble, 1984, with six genera.

Family Parvicapsulidae Shulman, 1953, with two genera.

Family Sinuolineidae Shulman, 1959, with eight genera.

Family Sphaerosporidae Davis, 1917, with six genera.

Order Multivalvulida Shulman, 1959

Family Kudoidae Meglitsch, 1947, with one genus.

Family Trilosporidae Shulman, 1959, with two genera.

Class Malacosporea Canning, Curry, Feist, Longshaw and Okamura, 2000

Order Malacovalvulida Canning *et al.*, 2000

Family Saccosporidae Canning, Okamura and Curry, 1996, with two genera.

Seventeen collective groups of actinospore are recognized as follows: Antonactinomyxon, Aurantiactinomyxon, Echinactinomyxon, Endocapsa, Guyenotia, Hexactinomyxon, Hungactinomyxon, Neoactinomyxum, Ormieractinomyxon, Pseudotriactinomyxon, Raabeia, Siedleckiella, Sphaeractinomyxon, Synactinomyxon, Tetractinomyxon, Tetraspora and Triactinomyxon.

## HOST RANGE AND DISTRIBUTION

Myxozoans are distributed throughout marine, estuarine and freshwater environments worldwide and are common parasites of teleost fish with a few species infecting elasmobranchs (Arthur and Lom, 1985; Stoffregen and Anderson, 1990; Heupel and Bennett, 1996). Amphibians and reptiles are occasional hosts (Mutschmann, 2004; Eiras, 2005a; Garner *et al.*, 2005; Helke and Poynton, 2005). A single case of infection in a digenean has been reported (Overstreet, 1976) and a *Kudoa* sp. causing myoliquefaction in octopus has been reported (Yokoyama and Masuda, 2001). Myxozoan actinospore stages infect a number of oligochaete invertebrate hosts with a few species recorded from marine polychaetes and sipunculids (Køie, 2000; Køie *et al.*, 2004). Freshwater bryozoans are the definitive hosts for malacosporeans (Tops *et al.*, 2005). Recently, homeothermic vertebrates have also been discovered to be hosts. A myxozoan-like parasite in the brain of European mole (*Talpa europaea*) (Friedrich *et al.*, 2000), the presence of myxozoans in inflammatory lesions of the bile ducts and hepatic parenchyma of anatid ducks (Lowenstine *et al.*, 2002) and a new genus and species occuring in the liver of common

shrews have been reported (Dyková *et al.*, 2007; Prunescu *et al.*, 2007). In addition, there have been several reports of myxozoan spores in human stool samples from patients suffering from abdominal pain or diarrhoea associated with enteric pathogens or who were immunocompromised. These are likely to have arisen through the ingestion of infected fish material and evidence of development within human hosts remains lacking (McClelland *et al.*, 1997; Boreham *et al.*, 1998; Lebbad and Wilcox, 1998; Moncada *et al.*, 2001; Hessen and Zamzame, 2004).

Some species appear to be highly specific to their host, whilst others may be specific at the family level (e.g., *M. cerebralis* and *T. bryosalmonae* in salmonid hosts) or wider (e.g., *M. aeglefini* in both gadoid and pleuronectid hosts) (Nielsen *et al.*, 2002). Some myxozoans have either become established or at least demonstrate the potential to become established in other geographical regions via the anthropogenic translocation of the hosts. Examples of this include *M. cerebralis* (Bartholomew and Reno, 2002; Whipps *et al.*, 2004); *M. artus* (Ogawa *et al.*, 1992); *M. koi* (Crawshaw and Sweeting, 1986); *M. pseudodispar* (Kent *et al.*, 1996); *Sphaeromyxa sevastopoli* (Pronin, *et al.*, 1997); *Sphaerospora molnari* (Hedrick *et al.*, 1990) and *S.* cf. *chinensis* (Kaup *et al.*, 1995). Lowers and Bartholomew (2003) highlighted that the importation of oligochaetes into the United States carried a risk of translocating Myxozoa from Europe to the United States. However, the paucity of data on the diversity of myxozoans in many hosts and geographic range for most species makes it difficult to arrive at firm conclusions regarding possible translocation of these parasites.

In marine fish, myxosporeans (and other parasites) have been proposed as useful tags for stock discrimination on the basis that these provide useful indications on ecologically discrete populations rather than purely genetic stocks which may spawn in a known location but later migrate to separate feeding grounds. The fact that myxospore infections in fish hosts can persist for the lifetime of the host is an important factor enabling their use as biological tags. Kabata (1963) discriminated stocks of bib (*Trisopterus luscus*) using *Ceratomyxa arcuata* and *Myxidium sphaericum* and stocks of haddock (*Melanogrammus aeglefinus*) using *Myxidium* sp., *Leptotheca* sp. and *Sphaeromyxa hellandi*. *M. aeglefini* has been used in studies of stock structure of plaice (*Pleuronectes platessa*) (van Banning *et al.*, 1978) and also to examine the migration routes of cod (*Gadus morhua hiemalis*) (Timofeeva and Marasaeva, 1984). *Myxidium*

spp. and *Zschokkella hildae* have been used to discriminate stocks of North Sea (*Gadus morhua*) in northern Norway (Hemmingsen *et al.*, 1991; Larsen *et al.*, 1997). More recent studies highlight the potential use of gall bladder and muscle myxosporeans for stock identification of starspotted dogfish (*Mustelus manazo*) from Japan and Taiwan (Yamaguchi *et al.*, 2003), horse mackerel (*Trachurus trachurus*) (Campbell, 2005) and gadoids (MacKenzie *et al.*, 2005) from the northeast Atlantic and Mediterranean seas. A similar approach has been utilized for studies on the migration and stock structure of Pacific salmonids (Awakura *et al.*, 1995; Urawa and Nagasawa, 1995; Urawa *et al.* 1998). Margolis (1993) reported a court case in Canada where the prevalence of M. *arcticus* in sockeye salmon was used to demonstrate that the fish were caught outside of the commercial fishing season.

Myxozoans can show seasonal and annual variations in prevalence, which are related to a number of factors including availability of hosts and ambient temperature influencing parasite development. Although the definitive hosts (oligochaetes and bryozoans) can release actinospore stages throughout the year, most are released in spring and summer as the ambient temperature rises (Xiao and Desser, 1998; El-Mansy *et al.*, 1998a, b; Gay *et al.* 2001; Özer *et al.*, 2002; Oumouna *et al.*, 2003; Tops and Okamura, 2003). Consequently, following infection and a period of development, the prevalence of infection in fish (based on the presence of mature spore stages) is often highest in the autumn and winter (Andrews, 1979; Gonzalez-Lanza and Alvarez-Pellitero, 1985; Mitchell, 1989; Brummer-Korvenkontio *et al.*, 1991). Significant inter-annual variations in prevalence within fish hosts can occur (Awakura *et al.*, 1995; Molnár, 1998; Molnár and Székely, 1999; Pampoulie *et al.*, 2001). The seasonality of infection in wild fish is less clear and may often be masked when multiple age classes are present within a sample (Roubal, 1994).

## PRINCIPAL DISEASES

### Salmonid Diseases

#### *Whirling Disease*

Whirling disease caused by *Myxobolus cerebralis* is amongst the best-studied myxosporean diseases. First recorded in brown trout (*Salmo trutta*) from Germany at the beginning of the twentieth century, the disease has subsequently been recorded more widely throughout Europe, South

Africa, Russia, New Zealand and in a number of North American states. The parasite is thought to have expanded its range internationally via the movement of live fish and fish products (Bartholomew and Reno, 2002; Bartholomew and Wilson, 2002) and molecular evidence suggests that M. *cerebralis* was spread from a limited geographical source (Andree *et al.*, 1999a; Whipps *et al.*, 2004b).

Until the mid-1980's, the disease was of significant economic importance in salmonid aquaculture, particularly where fish were reared in earthen ponds. The recognition of the requirement for the oligochaete host *T. tubifex* in the life cycle of the parasite (Wolf and Markiw, 1984) offered the effective management solution of removal or replacement of the sediment habitat of the oligochaetes, thereby breaking the life cycle. Alternative control methods such as irradiation of inflow water with ultraviolet light and the use of sand filtration have also been shown to be effective in removing infective stages (Hoffman, 1975; Nehring *et al.*, 2003). The disease is currently not regarded as such a serious threat to the salmonid industry as it once was. However, in some regions of the United States, M. *cerebralis* is responsible for declines of wild rainbow trout and cutthroat trout (Nehring and Walker, 1996; Hedrick *et al.*, 1998; Bartholomew *et al.*, 2005) and the effects on wild populations have continued to be a grave management concern.

Most salmonid species are susceptible, although there are marked differences in the severity of the disease between species. Rainbow trout (and steelhead trout) (*O. mykiss*) are the most susceptible, with brook trout (*Salvelinus fontinalis*) showing reduced susceptibility and Atlantic salmon (*Salar salar*) and grayling (*Thymallus* spp.) being highly resistant (Hedrick *et al.*, 1998). In addition, susceptibility is influenced by fish age, size, parasite dose and environmental factors (Sollid *et al.*, 2003). Juvenile fish with predominantly cartilagenous skeleton are most at risk of developing clinical disease. Adult fish can be infected but remain asymptomatic for the disease (Markiw, 1992). Clinical signs in juveniles include melanization of the tail region and skeletal deformities. The characteristic 'whirling' motion and tail chasing of infected fish is thought to be a consequence of constriction and pressure on the spinal cord (Rose *et al.*, 2000).

Susceptible fish acquire the infection following exposure to waterborne triactinomyxon actinospores released from tubificids. Recent studies have shown that the parasite is extremely specific to *T. tubifex*

(Granath and Gilbert, 2002) with specific geneotypes of *T. tubifex* displaying differential susceptibilities (Beauchamp *et al.*, 2001). More recent studies have shown that resident rainbow trout in habitats with populations of more susceptible genotypes of *T. tubifex*, are more severely impacted by whirling disease than fish from sites harbouring more-resistant *T. tubifex* lineages (Beauchamp *et al.*, 2005). Persistence of infections in oligochaetes for up to a year has been shown under laboratory conditions and under natural conditions, possibly for the lifetime of the oligochaete (El-Matbouli *et al.*, 1999a, b; Granath, Jr. and Gilbert, 2002).

Actinospores attach to the epithelial surfaces of the fish host and the infective sporoplasm enter either interepithelialy or via mucous cells (Markiiw, 1989; El-Matbouli *et al.*, 1995, 1999b). Sporoplasm cells multiply and migrate into the dermal layers of the skin, the peripheral nerves and into the central nervous system. During the neurogenic phase of infection up to approximately 20 days post-infection, the parasites appear to be normally unrecognized by the host immune system. However, such infections have been associated with mass mortality of Atlantic salmon smolts held in seawater (Frasca *et al.*, 1998, 1999). Although gross clinical signs were absent, mild to severe focal encephalitis involving glial cells, macrophages and lymphocytes in the optic tectum of the mesencephalon were reported.

Sporogony within plasmodia is initiated when the parasite stages reach the cartilaginous tissues. This occurs primarily in the cranial region but can occur in other locations where cartilage is present. Proliferative stages unable to reach the cartilage are thought to be eliminated by the host immune system. Infected cartilage becomes eroded by the parasite and phagocytic cells rapidly enter affected areas. Focal to diffuse granulomatous lesions are formed, frequently containing mature spores. *M. cerebralis* spores are highly variable in morphology, but in general are oval to circular in valvular view and measure 7-10 × 7-10 μm in size. Polar capsules are equal in size, measuring 4-6 × 3-3.5 and contain 5-6 turns of the polar filament. Spores infective to *T. tubifex* are released from infected live hosts (Nehring *et al.*, 2002) or upon death.

### *Proliferative Kidney Disease*

Proliferative kidney disease (PKD) caused by the malacosporean *Tetracapsuloides bryosalmonae* has long been recognized as an important disease of mainly salmonid fishes, particularly in aquaculture situations. In

Europe, during the expansion of rainbow trout culture in the 1970's, the disease caused serious losses in hatcheries and several ongrowing sites and has remained a constraint to trout production to the present day. However, the disease was first described in Germany at the beginning of the twentieth century (Hofer, 1906; Plehn, 1924) affecting rainbow trout and brown trout (Clifton-Hadley *et al.*, 1984). The disease is now recorded from many European countries as also in North America, where it was first detected in Idaho, U.S.A. in 1981 (Smith *et al.*, 1984) with subsequent reports from British Columbia and Newfoundland (Brown *et al.*, 1991). Phylogenetic analysis of the internal transcribed spacer 1 (ITS-1) rDNA region from samples collected from several European and North American locations indicates the existence of a North American clade with a subset of Italian and French sequences and a separate clade of isolates from the rest of Europe (Henderson and Okamura, 2004). The authors concluded that there was historic colonization from North America to southern Europe pre-dating fisheries movements. In addition, the lack of southern European lineages in the rest of Europe strongly suggests that *T. bryosalmonae* does not spread via fish by aquaculture or fisheries related activities.

Although most reports of PKD involve rainbow or steelhead trout, outbreaks in brown trout and Atlantic salmon in Europe and in chinook salmon (*O. tschawytscha*) and coho salmon (*O. kisutch*) in North America are common (Hedrick *et al.*, 1993). Cultured California golden trout (*O. mykiss aguabonita*) are susceptible (Morris *et al.*, 2003b) and North American Kokanee and Chum salmon have been shown to be susceptible experimentally. In Europe, Arctic charr (*Salvelinus alpinus*) and grayling (*T. thymallus*) are also known to be hosts for the parasite, and may exhibit signs of the disease (Feist and Bucke, 1993). Pike (*E. lucius*) is the only non-salmonid fish known to be susceptible (Seagrave *et al.*, 1981; Morris *et al.*, 2000a). Brook trout (*S. fontinalis*) can become infected but do not exhibit clinical signs of PKD (Feist and Bucke, 1993). The most common bryozoan hosts for *T. bryosalmonae* are *Plumatella emarginata*, *Fredericella sultana* and *F. indica* (Okamura and Wood, 2002). Juvenile fish are most at risk from the disease but naïve fish of any age are susceptible. Frequently, 100% of susceptible stocks become infected. Clinical signs are seen typically between June and September, when temperatures reach 15°C or greater (Clifton-Hadley *et al.*, 1986) (Fig. 12.1A). The impact of the disease amongst wild fish populations is uncertain (Feist *et al.*, 2002)

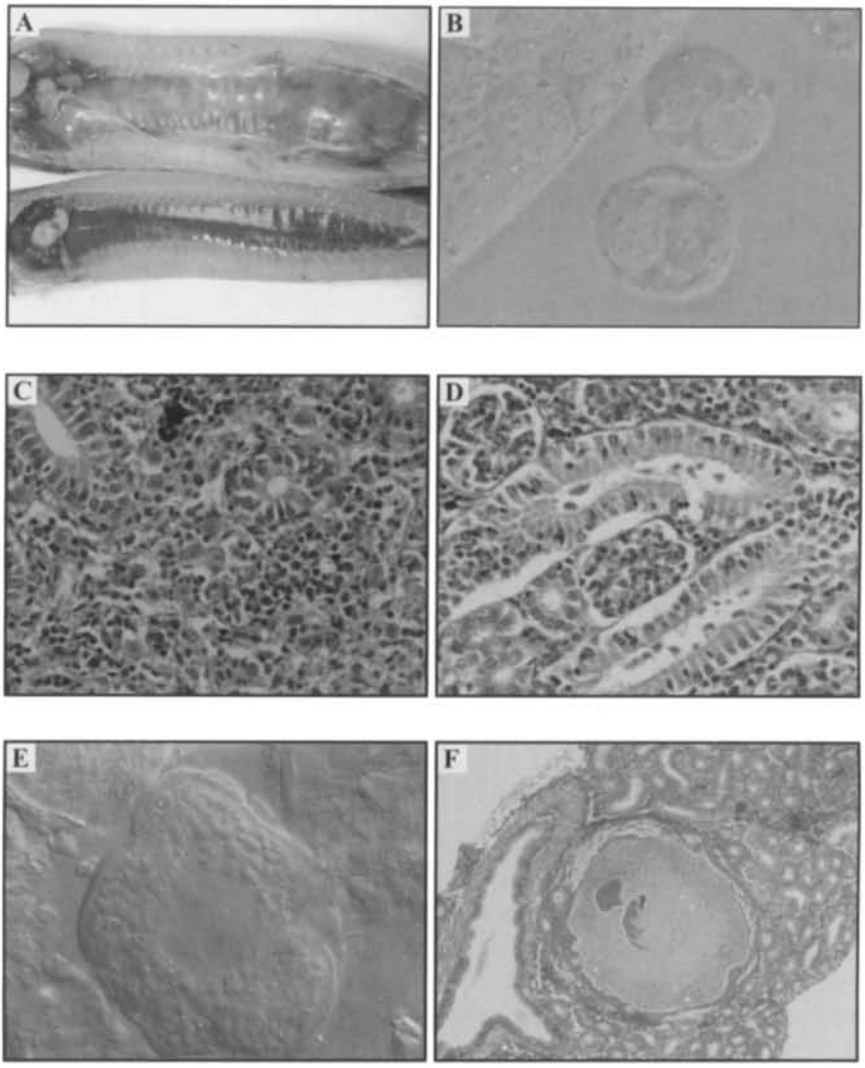


**Fig. 12.1** **A** – Renal hypertrophy in juvenile rainbow trout (*Oncorhynchus mykiss*) caused by proliferative kidney disease. Compare with normal kidney below. **B** – Fresh spore of *Tetracapsuloides bryosalmonae* released from the bryozoan *Fredericella sultana*. Normarski interference contrast. **C** – Extrasporogonic proliferative stages of *T. bryosalmonae* in the renal interstitium of a brown trout (*Salmo trutta*). **D** – Sporogonic stages of *T. bryosalmonae* within renal tubules of brown trout. **E** – Xenoma of extrasporogonic stages of *Myxidium lieberkuehni* within a glomerulus from pike (*Esox lucius*) kidney. Fresh preparation, Normarski interference contrast. **F** – Low power view of *M. lieberkuehni* xenoma in pike kidney. Note displacement of excretory elements and thin fibrous capsule surrounding the xenoma.

but PKD has been implicated as an important factor in the declines of wild salmonids in Switzerland (Wahli *et al.*, 2002; Borsuk *et al.*, 2006) and extensive mortalities of Atlantic salmon fry have been reported from the river Åelva in Norway, associated with elevated summer temperatures and reduced water flow (Sterud *et al.*, 2007).

The disease in the fish hosts is seasonal in nature with infections occurring during late spring and the summer months when water temperatures are on the increase. However, the parasite persists in bryozoan colonies throughout the year (Gay *et al.*, 2001; Tops *et al.*, 2004), proliferating as infected colonies increase growth with the rise in temperatures during the spring and summer (Tops *et al.*, 2006). Infection of fish hosts occurs following contact with spores (Fig. 12.1B) and the entry of the sporoplasms into the fish through the skin and gill epithelium, either interepithelially or intracellularly via mucous cells (Morris *et al.*, 2000b; Longshaw *et al.*, 2002). Parasites are usually observed within renal interstitial tissues about 4 weeks post-exposure with lesions developing shortly thereafter. External clinical signs of the disease are abdominal distension, bilateral exophthalmia and pale gills. Internally, marked renal and splenic hypertrophy is typical and the kidney may have a mottled appearance.

Proliferative histozoic extrasporogonic stages of *T. bryosalmonae* consist of a primary cell measuring approximately 25 to 30 μm in diameter and containing one or more secondary cells and occasional tertiary cells inside. The cytoplasm of the primary cell contains numerous characteristic sporoplasmosomes morphologically identical to those in the sporoplasm cell of the bryozoan stage with a characteristic bar-like invagination (Ferguson and Adair, 1977; Ferguson and Needham, 1978; Feist, 1997; Kent *et al.*, 1998). These are produced by the Golgi apparatus and are frequently observed aligned with the plasma membrane of the primary cell. Pericyte formation has been observed (Feist and Bucke, 1987). Other constituents of the primary cell include mitochondria, endoplasmic reticulum, lipoid inclusions and a variety of vesicles, some of which become enlarged and contain membranous material and are visible at the light microscope level in the form of refractile granules. Secondary and tertiary cells are bound by a cytoplasmic membrane and lack the sporoplasmosomes exhibited in the primary cells. Each can contain microfibrillar arrays, which are associated with active cell growth and division. Proliferation of extrasporogonic *T. bryosalmonae* occurs by plasmotomy or release of secondary cells following rupture of the primary cell.

The parasite proliferates rapidly within the host and, via the blood stream, it is able to reach most organs and tissues where the principal host response is a proliferation of lymphocytes, plasma cells and phagocytic cells resulting in focal or multifocal inflammation. The kidney and spleen are usually the primary site of infection (Fig. 12.1C). Interstitial haematopoietic cell hyperplasia with degenerative changes to the excretory elements and vascular pathology, occasionally with haemaglobin crystallization occur (Clifton-Hadley *et al.*, 1987). In chronic renal infections, granulomatous lesions with phagocytic cells surrounding the parasites are commonly seen (MacConnell *et al.*, 1989) and fibrosis may be present. These changes can persist in the spleen after remission in other organs, even when parasites have been destroyed. In the skeletal musculature, severe granulomatous myositis can result (Fernández-de-Luco *et al.*, 1997). Fish suffering from clinical PKD exhibit suppression of key elements of the innate immune response, thereby showing increased susceptibility to secondary infections (Chilmonczyk *et al.*, 2002). However, fish surviving the infection become resistant to re-infection and generally do not display disease symptoms.

Despite most parasites being eliminated by the host, sufficient numbers are able to traverse the renal tubule epithelium, becoming coelozoic within the tubules and undergo sporogony (Fig. 12.1D). This occurs rarely in European rainbow trout, more frequently in North American steelhead trout and most prominantly in Arctic charr where large numbers of ovoid spores measuring 12 μm in length and 7 μm in width are formed (Kent *et al.*, 2000). The spores are formed within monosporous pseudoplasmodia and contain two sub-spherical polar capsules and are then surrounded by indistinct valvogenic cells. Hedrick *et al.* (2004) described apparently more advanced stages in the urine of fish recovering from PKD. These were sub-spherical with width of 16 μm and height of 14 μm with two spherical polar capsules. As indicated earlier in this chapter, these stages appear infective to susceptible bryozoan hosts.

### *Ceratomyxosis*

Ceratomyxosis, caused by *Ceratomyxa shasta*, is an important disease of hatchery-reared and wild salmonids from the Pacific northwest of North America. The parasite was first described by Noble (1950) and subsequently became recognized as a pathogen amongst hatchery stocks in particular, with juvenile wild salmonids and pre-spawning adults also becoming infected (Ratliff, 1983; Hendrickson *et al.*, 1989; Bartholomew

*et al.*, 1989a). The geographic range of the parasite extends across the major catchments of the Columbia, Sacramento, Klamath and Rogue rivers in the United States (Hoffmaster *et al.*, 1988) and the Fraser river in Canada. Several Alaskan river systems also harbour the infective stage of the parasite. In addition, migrating anadromous salmonids can carry the infection outside the normal range of the parasite (Margolis and Evelyn, 1975). Susceptibility varies between salmonid species and strains of the same species. Those strains originating from waters enzootic for the parasite often exhibit increased resistance as compared to strains from non-enzootic waters (Ching and Munday, 1984; Ibarra *et al.*, 1991, 1992) and the fish surviving the infection are typically undersized and emaciated (Tipping, 1988). Susceptible fish acquire the infection from Tetractinomyxon actinospores released from the freshwater polychaete *M. speciosa* (Bartholomew *et al.*, 1997). The parasite invades the epithelium of the posterior intestine, initially resulting in localized lymphocytic infiltration, leading eventually to widespread enteritis. Infections may become disseminated with large numbers of developmental stages and spores associated with necrosis and haemorrhaging of infected tissues, including visceral organs (Bartholomew *et al.*, 1989b) (Fig. 12.4F). External clinical signs vary according to the salmonid species and include anorexia, lethargy, abdominal distension, darkened colouration and exophthalmia.

### *Parvicapsula Species*

*Parvicapsula minibicornis* is a pathogen of several Pacific salmonid species from Canada with prevalence rates from 47 to 100% (Jones *et al.*, 2003). The infection is acquired in the lower reaches of spawning rivers where fish congregate before migration to the spawning grounds up river. Conversely, smolts returning to the sea become exposed to the parasite, but whether infections result in mortality during the marine phase is unknown. There are no distinctive grossly visible clinical signs but infected fish exhibit renal hypertrophy. Trophozoites reside within Bowman's space and capillaries of the glomeruli and occasionally within the renal tubule epithelium. Sporogonic stages occur within the lumen of renal tubules. Pyriform spores contain two equal-sized pyriform polar capsules and measure 11 μm in length and 7.5 μm in width (Kent *et al.*, 1997). The disease has been associated with prespawn mortality among Fraser River stocks of sockeye salmon (*O. nerka*) in British Columbia (St-Hilaire *et al.*, 2002). Under experimental conditions, heavily infected

fish have a reduced repeat swimming performance and lessened ability to recover from exercise (Wagner *et al.*, 2005).

Mortality of farmed *Salmo salar* associated with *Parvicapsula pseudobranchicola* has been reported (Karlsbakk *et al.*, 2002). The *Parvicapsula* species reported by Sterud *et al.* (2003) is likely to be *P. pseudobranchicola* based on tissue location, pathogenesis and geographic location, despite minor differences in spore dimensions. Clinical signs include increased surface swimming, lethargy, lack of feeding and exophthalmia. Petechial haemorrhaging of infected pseudobranchs is observed and eventual blindness of the host due to impaired pseudobranch function which supplies blood to the eye has been suggested (Karlsbakk *et al.*, 2002; Sterud *et al.*, 2003).

A possibly related *Parvicapsula* species has caused severe outbreaks of disease marine-cultured coho salmon in North America (Hoffman, 1984). Other Pacific salmonids and Atlantic salmon are also susceptible. Infection occurs 8 to 10 weeks post-introduction to an infected site with up to 50% losses being reported (Johnstone, 1984). The kidney is the primary site of infection and appears hypertrophied and haemorrhagic. Renal tubules become occluded with trophozoites and developing spores. Tubule epithelium may also harbour sporulating trophozoites. Histologically, the tubule epithelium becomes atrophied. The release of parasite stages to the renal interstitium following rupture of affected tubules elicits only a mild host response. Glomerular infections have not been reported with this species. However, pseudobranch infections, similar to those occuring in cultured Atlantic salmon infected with *P. pseudobranchiola* have been reported (Yasutake and Elliott, 2003), adding support to the view that they are the same species.

### Salmonid Myxosporeans of Lesser Importance

*Myxobolus arcticus* in Pacific salmonids is generally regarded as non-pathogenic but can be found at prevalences approaching 100% (Kent *et al.*, 1993; Awakura *et al.*, 1995). However, fish infected with M. *arcticus* have reduced mean swimming speed as compared to uninfected fish (Moles and Heifetz, 1998). The infective stage is a triactinomyxon actinospore released from the oligochaete *Styrodrilus heringianus*. Mature myxospores develop in the neural tissue 3-4 months after exposure (Kent *et al.*, 1993). The parasite has proved to be useful as a biological tag to identify the river of origin of sockeye salmon in British Columbia

(Margolis, 1993). Infections with *Myxobolus neurobius* in brown trout (*Salmo trutta*), Atlantic salmon (*S. salar*) and grayling (*Thymallus thymallus*) in Europe and Asia and Pacific salmonids in North America (Gonzales-Lanza and Alvarez-Pellitero, 1984; Maloney *et al.*, 1991) are usually non-pathogenic. However, pressure atrophy of adjacent tissues can occur in heavy infections. A similar *Myxobolus* sp. causes 'sleeping disease' in cultured masu and amago salmon in western Japan [Murakami (1979) cited by Urawa and Awakura (1994)]. Infections with *Myxobolus squamalis* affecting the scale pockets of coho and chum salmon can, in severe cases, reduce the market value of whole fish (Kent *et al.*, 1994b).

*Sphaerospora truttae* has been recorded from brown trout (*S. trutta*), grayling (*T. thymallus*) and Atlantic salmon (*S. salar*). Susceptible fish are infected during the summer months and extrasporogonic stages can be found in the blood and renal interstitial tissues later in the year. These stages are transmissible to naïve fish by intraperitoneal injection. Disporic pseudoplasmodia and spores develop within renal tubules (McGeorge *et al.*, 1996a, b).

Infections with *Henneguya salminicola* in the somatic muscle of Atlantic and Pacific salmon are of economic importance with affected fish having reduced market values (Fig. 12.2E). In heavy infections, where cysts are numerous, filleting may be impossible. *Henneguya zschokkei* from whitefishes (*Coregonus* spp.) induces similar pathological changes. Several authors have considered these two parasites as a single species but recent studies indicate that they are distinct species (Kent *et al.*, 2001; Eiras, 2002).

*Kudoa thyrsites* can be a significant pathogen of pen-reared Atlantic and coho salmon (Harrell and Scott, 1985; Barja and Toranzo, 1993; Kent, 2000) and can occasionally be found in wild-caught sea trout (*Salmo trutta*) (Holliman, 1994). Affected fish exhibit pale streaks running along the muscle fibres and larger areas can be affected (Moran and Kent, 1999; Moran *et al.*, 1999c). There is little host response to the parasite, although chronic multifocal myositis occurs along with rupture of plasmodia (Moran *et al.*, 1999b). The muscle may be soft and myoliquefaction caused by a proteolytic enzyme can occur postmortem, even during refrigeration. *K. thyrsites* is not host specific, infecting approximately 20 different marine fish species worldwide. The infectious stage of this parasite is unknown but experimental transmission has been achieved by injection of blood from infected fish which harbour proliferative stages of *K. thyrsites* (Moran *et al.*, 1999a).

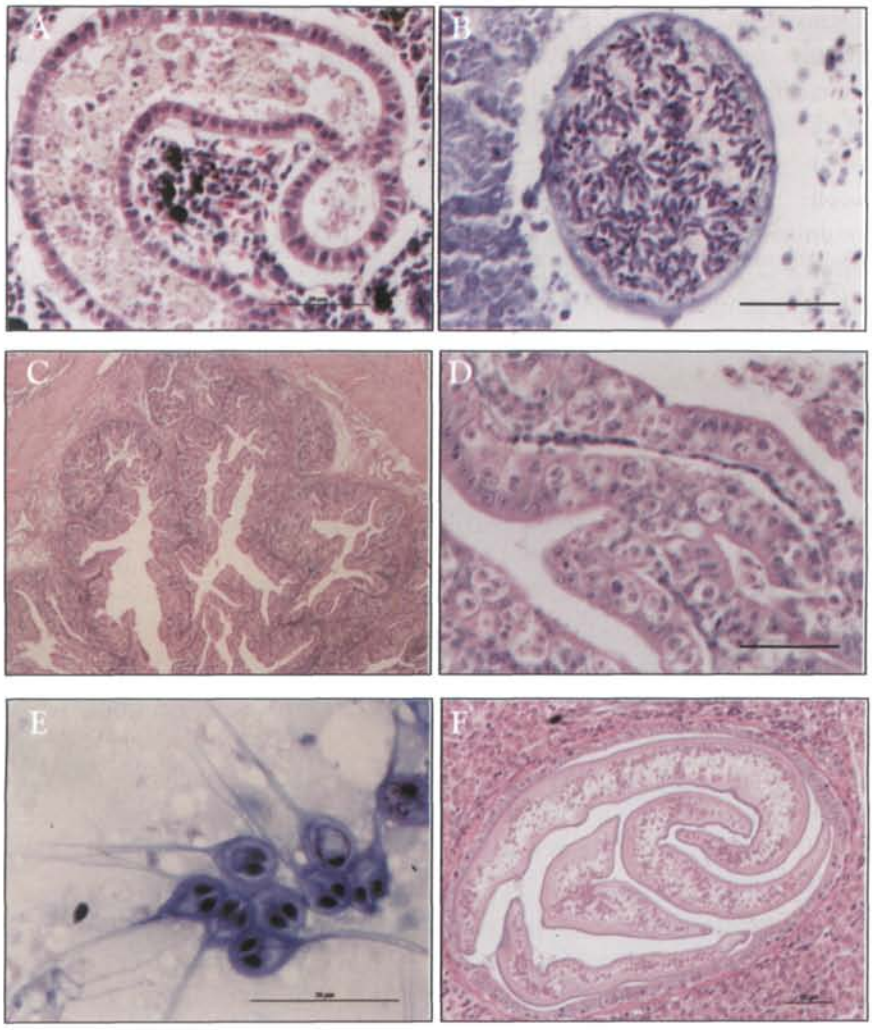


**Fig. 12.2** **A** – Histological section of brown trout (*Salmo trutta*) kidney, showing numerous plasmodia and spores of *Chloromyxum schurovi* within a renal tubule. H&E, Bar = 50 μm. **B** – Cyst of *Myxidium rhodei* surrounded by a thin connective tissue capsule, from the kidney of roach (*Rutilus rutilus*). Giemsa stain, Bar = 50 μm. **C** – Section through the intestine of gilthead seabream (*Sparus aurata*) with intense infection with *Enteromyxum leei*. H&E. **D** – High power view of Figure C showing the presence of numerous stages of *E. leei* within the intestinal epithelium. H&E, Bar = 50 μm. **E** – Spores of *Henneguya salmonicola* from the muscle of *Oncoryhnchus* sp. Giemsa stain, Bar = 30 μm. **F** – Plasmodia of *Myxidium truttae* within the hepatic bile duct of brown trout. The duct is distended with minimal compression of the epithelium and surrounding hepatocytes. Bar = 50 μm.

Gall bladder infections with *Chloromyxum truttae* are frequently reported in brown trout (*S. trutta*) and other salmonids (Alvarez-Pellitero, Pereira-Bueno and Gonzalez-Lanza, 1982; Lom and Dyková, 1992; Feist and Rintamäki, 1994). External clinical signs of disease are generally absent but heavy infections may result in emaciation, and yellowish discolouration of the skin and fins in cultured stocks. Internally, discoloured liver, swollen gall bladder and enteritis have been reported and, in extreme cases, mortalities can occur (Shulman, 1966). Infections with *Chloromyxum schurovi* are common in brown trout *S. trutta* and Atlantic salmon *S. salar* in Europe (Lom and Dyková, 1993; Shulman and Ieshko, 2003). Sporogonic stages reside in the tubule lumens and in heavy infections, attenuation and occasional necrosis of the tubule epithelium occurs (Fig. 12.2A). Presporogonic stages can be found in Bowman's space of the glomerulus and putative extrasporogonic stages lodge in hepatic sinusoids.

An unidentified myxosporean parasite designated coho kidney parasite (CKX), causes mortalities amongst spawning coho salmon in British Columbia and Washington (Jones *et al.*, 2004). The parasite was present in up to 80% of returning fish. Parasite stages infect the renal interstitial tissues and also occur intracellularly in the tubule epithelium as well as within the tubule lumens. Luminal stages appeared to degenerate and sporogony was not observed. Co-infections with a *Myxidium* species were detected but DNA sequence data revealed the parasite to be closely related to *Sphaerospora oncorhynchi*. The authors hypothesized that CKX stages represent an abortive extrasporogonic development of a *Sphaerospora* or *Myxidium* species.

Several other myxosporean parasites that utilize salmonids as hosts have been recognized, but most of them are rarely reported to cause significant disease symptoms in their hosts. Amongst these are *Myxidium truttae*, *M. salvelini* and *M. minteri*. Trophozoites of the gall bladder-infecting *M. truttae* also invade the bile duct and ductules, resulting in attenuation of the epithelial lining, distension and proliferation of the ducts and peribiliary fibrosis (Fig. 12.2F). Parasites reaching the hepatic parenchyma result in hepatocellular necrosis (Walliker, 1968; Kent *et al.*, 1994b). Infections with *M. minteri* are common in Pacific salmonids and brook trout where mild renal tubule degeneration can result. *M. salvelini* appears to be non-pathogenic. Several Pacific northwest *Oncorhynchus* and *Salmo* species are hosts for the muscle parasite *Myxobolus insidiosus*

(Wyatt and Pratt, 1963; Amandi *et al.*, 1985). Fusiform plasmodia invade the skeletal muscle and can be found intracellularly within myofibrils. Heavy infections appear as white streaks in the flesh.

## Diseases of Catfish

### *Proliferative Gill Disease*

Proliferative gill disease (PGD) is a major disease of farmed channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*) in the southern United States and California. The disease is caused by the extrasporogonic stage of *Henneguya ictaluri*. Significant mortalities occur in naïve fish irrespective of size or age, although juveniles are generally more susceptible (Pote *et al.*, 2000). Outbreaks occur primarily during the spring and, to a lesser extent, in the autumn. Clinical signs include listlessness, reduced appetite and gross gill pathology where lamellae are swollen and may be eroded and bleeding. Histologically, the parasite elicits a strong granulomatous bronchitis. Parasite stages can be found in the visceral organs and the brain without discernable host reaction. Styer *et al.* (1991) and Bellem and Pote (2002) experimentally demonstrated that the life cycle alternated between the fish host and the oligochaete *Dero digitata*, within which an Aurantiactinomyxon stage develops. Both stages in the life cycle have been confirmed using molecular techniques (Pote *et al.*, 2000; Hanson *et al.*, 2001; Whitaker *et al.*, 2001, 2005).

A second pathogenic *Henneguya* sp. in the gills of *Ictalurus punctatus* is *Henneguya exilis* Kudo, 1929 (Current and Janovy, 1978). The presence of plasmodia at the base of the secondary lamellae (epithelial filamental type of Molnár, 2002a) elicits a severe tissue proliferation in this atypical site. Known as 'lamellar disease', it has been associated with significant mortalities of fingerlings. The formation of cysts in other sites is not pathogenic. The alternate stage is an Aurantiactinomyxon type in the oligochaete *Dero digitata* (Lin *et al.*, 1999). Infections with *Henneguya suprabranchiae* in catfish (*Clarias gariepinus*) in Egypt produce large intestinal cysts measuring up to 4.5 mm in diameter (El-Mansy, 2002). Cysts form in the outer layer of the intestine and as they increase in size, they induce pressure atrophy of the smooth muscle layer, which eventually breaks down, weakening the intestine.

## Cyprinid Diseases

### *Diseases of Cultured Carp*

Renal sphaerosporosis in carp is caused by *Sphaerospora renicola* with up to 100% prevalence in cultured common carp throughout Europe. The disease has also been reported from Israel and Australia. Pathological changes, induced by coelozoic disporic pseudoplasmodia and spores, include tubule dilation and attenuation of the epithelium. In severe infections, the epithelium becomes atrophied and necrotic with associated nephritis and impairment of renal function. Marked hypertrophy of renal tubule cells and stenosis of affected tubules, which may be present during winter months, are thought to be due to co-infection with extrasoprogonic stages of *H. cyprini*, rather than *S. renicola*.

Spores are infective to the oligochaete *B. sowerbyi*, which release Neoactinomyxum actinospores (Grossheider and Körting, 1993; Molnár *et al.*, 1999). *S. renicola* has two proliferative extrasporogonic cycles, in the blood and swimbladder wall. Blood stages, which appear mobile in fresh preparations, comprise primary cells containing a number of secondary and tertiary cells. These invade the swimbladder where they continue to proliferate, increase in size and induce swimbladder inflammation (SBI) (Molnár, 1988a; Dyková *et al.*, 1990). They also penetrate into the renal tubule lumens and undergo sporogonic development. In the swimbladder, the stages comprise primary cells containing up to approximately 50 secondary cells, each containing one or two tertiary cells. Proliferation is achieved by rupturing the primary cell in order to release the secondary cells, which themselves grow and multiply by internal division to repeat the cycle.

Affected carp may display reduced or abnormal swimming behaviour and poor growth. The parasites induce a marked inflammatory response and hyperplasia of the connective and epithelial tissues, resulting in marked thickening of the swimbladder wall. Oedema and haemorrhaging are frequently present. Outgrowths of infected tissue from the tunica externa of the swimbladder into the visceral cavity can result in peritonitis and renal hypertrophy can occur (Kovács-Gayer, 1983). Prognosis for fish with SBI is poor and mortalities may occur (Körting, 1982; Csaba *et al.*, 1984; Dyková and Lom, 1988a; Molnár, 1988a). Swimbladder stages can be transmitted experimentally (Molnár and Kovács-Gayer, 1986).

Gill sphaerosporosis of carp, caused by *Sphaerospora molnari*, is principally known as a pathogen of fingerling carp but has also been

reported in crucian carp (*Carassius carassius*) in Europe (Dyková and Lom, 1988a). In mild mild cases, local circulatory disorders and dystrophic changes also occur. In heavy infections, marked hyperplasia of the respiratory epithelia occurs with epithelial tissue largely being replaced by sporogonic stages (Fig. 12.4C). Infections of the epithelial lining of the branchial cavity and head have also been reported. The parasites produce monosporic pseudoplasmodia. In contrast to the disseminated gill infections with *S. molnari*, other species such as *Myxobolus basilamellaris* and *M. koi* form cysts in the gills of *C. carpio*. For *M. koi* these may be of variable size and are interlamellar, with minimal host response or as larger cysts inducing hypertrophy of the branchial epithelium and clubbing of the gill filaments (Yokoyama *et al.*, 1997). Cysts of *M. basilamellaris* develop in a basifilamental position, deforming adjacent lamellae and restricting the passage of nerves and blood vessels (Kovács-Gayer and Molnár, 1983). Heavy infections can impair gill function.

*Thelohanellus nikolskii* was originally described from the fins of wild Amur carp and was thought to have been introduced into Europe via introductions of this species (El-Matbouli *et al.*, 1992a). Plasmodia of *T. nikolskii* develop on the outer surfaces of fin rays forming macroscopic cysts and result in deformation of the cartilaginous elements (Molnár, 1982, 2002b; Moshu and Molnár, 1997). In severe infections, clusters of cysts form and affected fin rays may break away, compromising swimming ability. The parasite also infects the scales of common carp (Moshu and Molnár, 1997). Koi carp *C. carpio haematopterus* are also susceptible to infections by *T. nikolskii* but less so than common carp. Goldfish *C. auratus* appear to be resistant (Molnár, 2002c). Myxospores of *T. nikolskii* are infective to *T. tubifex* with an Aurantiactinomyxon actinospore being formed (Székely *et al.*, 1998).

Originally described from the mesentary of koi carp, *T. hovorkai* infects many tissues and organs and causes haemorrhagic thelohanellosis, which can result in chronic mortalities in affected carp stocks (Molnár and Kovács-Gayer, 1986b). The disease is most severe in fry and mature spores may be found by two months post-hatching. Naïve fish of any age appear to be susceptible (Dyková and Lom, 1988a). Disease symptoms include haemorrhaging and oedema in skin lesions. Histologically, destruction of the vascular capillary network associated with the release of mature spores into the surrounding tissues has been described (Yokoyama *et al.*, 1998). The parasite alternates through the oligochaete *Branchiura sowerbyi*,

producing an Aurantiactinomyxon type actinospore (Székely *et al.*, 1998). Actinospores enter the fish host via the gills so as to initiate infection (Yokoyama and Urawa, 1997). *Thelohanellus kitauei* causes serious disease in carp (C. *carpio nudus*) (Egusa and Nakajima, 1981). The parasite forms large cysts on the intestinal submucosa. Development of the cysts is rapid during July with maturation leading to cyst rupture and release of spores occurring in August and September (Rhee *et al.*, 1990, 1993).

Heavy infections with M. *artus* in the skeletal muscle of carp can give rise to an 'irregular and uneven' appearance to the body surface (Ogawa *et al.*, 1992). Cardiac infections can also occur. Within the muscle tissue, plasmodia are of two types. The larger located between myofibrils and the smaller intracellularly within myofibrils (Fig. 12.3C). Rupture of mature plasmodia and spores results in an inflammatory response from the host lysis of adjacent muscle fibres. Phagocytic cells transport released spores to other organs in the body via the blood, with many eventually becoming trapped and degraded in macrophage aggregates in haematopoietic organs. Release of spores is not dependent on the death of the host, being released, possibly via the intestine and skin (Yokoyama *et al.*, 1996). Infections of the musculature in carp by M. *cyprini* are considered to cause only sub-clinical damage to the muscle and local necroses in other organs but can, in some cases, cause hydropic degeneration and dropsy (Molnár and Kovács-Gayer, 1985).

Infections with *Myxobolus encephalicus* in carp fry were first described from Germany. Affected fish exhibit loss of equilibrium and a circling motion. Emaciation and enophthalmia may occur in young fry. Plasmodia of M. *encephalicus* attach to the endothelial lining of the blood vessels of the meninges of the brain and cause dilation of the vessels and focal perivascular oedema without significant inflammation. Sprorgonic stages from ruptured vessels invoke a severe granulomatous response (Dyková *et al.*, 1986; Dyková and Lom, 1988a).

### *Diseases of Other Cyprinid Species*

A large number of *Myxobolus* species have been described from the musculature of cyprinid fishes (Eiras *et al.*, 2005). Discrimination on purely morphological features of the spore stage and location in the host can be problematic and it is likely that there are many species that are junior synonyms of already established species. The use of DNA sequence data for the validation of species with similar morphology is proving a

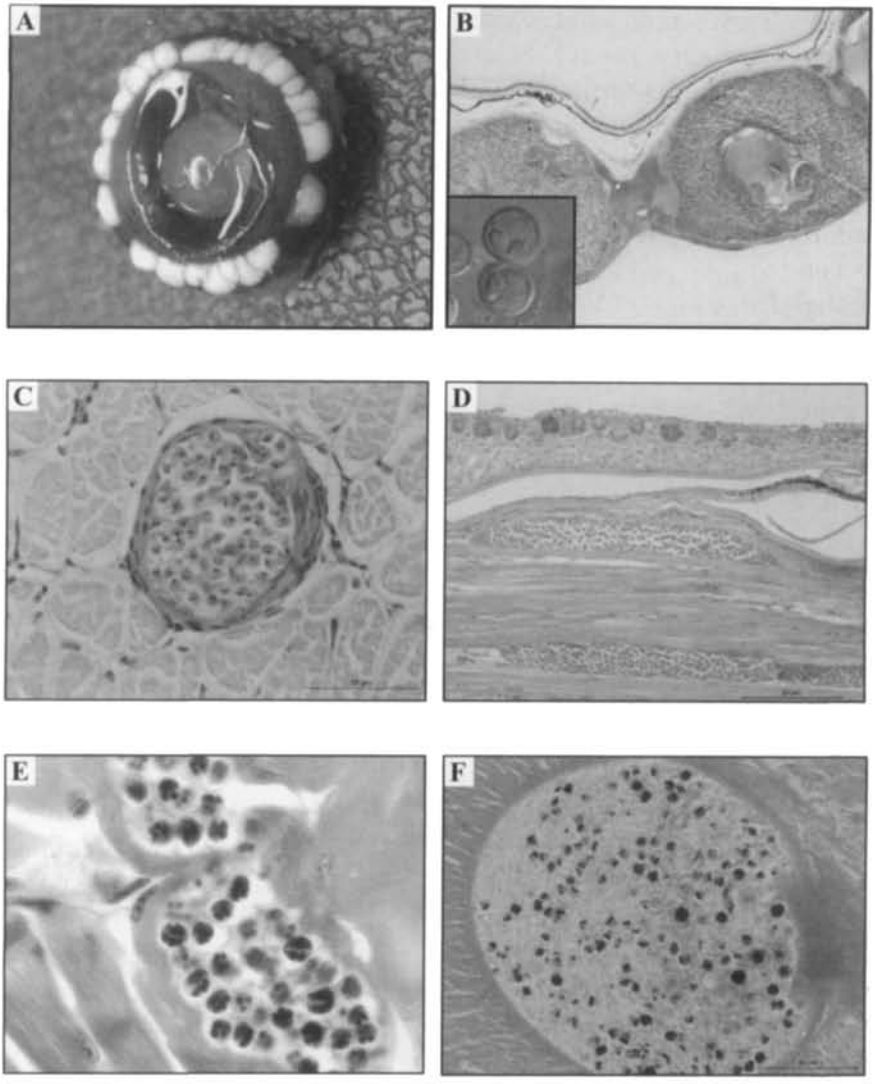


**Fig. 12.3** **A** – Multiple cysts of *Myxobolus aeglifini* in the eye of poor cod (*Trisopterus minutus*) from the North Sea. **B** – Low power view of *M. aeglifini* cysts in the scleral cartilage of whiting (*Merlangius merlangus*). Inset – spores of *M. aeglifini* in valvular view. **C** – Cyst of *Myxobolus artus* in the muscle of carp (*Cyprinus carpio*). **D** – Pseudocysts of a *Kudoa* sp. in the myofibrils of common goby (*Pomatoschistus microps*). **E** – Cownose ray (*Rhinoptera bonasus*) with intra-myofibrillar *Kudoa* sp. infection. **F** – Giemsa stained section through a *Kudoa* sp. pseudocyst in sandbar shark (*Carcharhinus plumbeus*).

valuable approach (Molnár *et al.*, 2002; Easy *et al.*, 2005). For several muscle-invading myxobolids, the pathogenic mechanisms are similar. Growth of cysts enlarges infected muscle fibres and indices pressure atrophy of adjacent myofibrils (Longshaw *et al.*, 2005). Whilst remaining intracellular within myofibrils, the parasites are protected from the host immune response. This situation changes once sporogenesis is completed and rupture of the cysts occurs. Spores and other stages are then recognized by the host and a granulomatous response involving epitheloid-type cells and phagocytic cells frequently follows. Infected myofibrils are destroyed and localized blockage of vessels and necrosis of the surrounding tissue can occur. Spores are transported around the body via the blood and lymphatic system to various sites, including the thymus, liver, kidney, spleen, gills and intestine. It is likely, that as shown for *M. artus*, other muscle-infecting myxobolids are shed from the host during life as well as postmortem. A few species have been reported to cause significant disease outbreaks which can manifest themselves as conspicuous swellings which gave rise to the term 'lump disease' used for a variety of myxosporean infections in freshwater fishes (Shulman, 1966). *Myxobolus pfeifferi* in the muscle has caused epizootics of 'barbel boil disease', but there have been no reports of epidemics in barbel in recent years. New species of muscle-infecting myxobolids in cyprinids continue to be described. For example, *Myxobolus lentisuturalis* produces large cysts in the dorsal musculature of Prussian carp *Carassius gibelio* which give rise to localized swelling and necrosis of affected muscle tissue (Dyková *et al.*, 2002b).

Gill-infecting species are frequently considered to be serious pathogens, as pathological responses to their presence can significantly impair respiratory function. However, in many cases, the tissue response is rather localized, especially for cyst-forming species. In general, juvenile fish appear more susceptible to intense infections. Juvenile bighead carp (*Aristichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) are susceptible to interlamellar and basifilamental infections with *M. pavlovskii* (Molnár, 2002a), which restrict respiratory function (El-Matbouli *et al.*, 1992). The infective stage is a Hexactinomyxon-type actinospore released from the oligochaete *T. tubifex* (El-Matbouli and Hoffman, 1991).

Several myxosporeans infect the skin, scales or subcutaneous tissues. Such infections are unsightly and for ornamental species this results in

reduced market value whilst others can result in mortality. In goldfish, *Myxobolus diversus* in the fins produces obvious cysts on the fins but has not been reported to cause mortalities (Molnár and Székely, 2003) and *M. rotundus* causes large visible cysts on the body surface (Lu *et al.*, 2003). In contrast, *Thelohanellus wuhanensis* is a serious disease of *Carassius auratus gibelio* the skin which causes to mortality of infected fry (Wang *et al.*, 2001). Infections with *M. drjagini* giving rise to 'twist disease': produce small plasmodia in the skin of the operculum, head and buccal cavity of silver carp in China (Wu *et al.*, 1975), although El-Mansy and Molnár (1997a) did not consider that it caused losses in Hungary. The parasite alternates through *T. tubifex* and produces a triactinomyxon type actinospore.

Hoferellosis in goldfish caused by *H. carassii* causes 'kidney enlargement disease' or 'kidney bloater' (KED) (Ahmed, 1974; Molnár *et al.*, 1989). The disease was first reported in Japan and subsequently from North America and Europe. Clinical signs include unilateral or bilateral abdominal distension, protrusion of scales and loss of balance. Infections in gibel carp do not seem to induce any significant disease (El-Matbouli *et al.*, 1992). The disease is seasonal in nature, with fish becoming infected during the late summer and autumn, developing clinical signs the following spring when mortalities may result (Yokoyama *et al.*, 1990). Internally, marked renal hypertrophy is caused by a cystic papillomatous hyperplasia of the tubule epithelium, which becomes metaplastic in response to the parasite. Non-excretory renal elements are unaffected. Extrasporogonic stages of *H. carassii* invade tubule epithelial cells with each cell containing one or two parasites (Fig. 12.4D). These stages consist of primary cells up to 17 μm in diameter, with secondary, tertiary and even quaternary cells contained within them. Affected cells become elongate and protrude into the lumen of the tubule. Eventually, these cells rupture and the released parasites become coelozoic. These plasmodia are frequently attached to the intact epithelium via cytoplasmic processes insinuated between epithelial cells, and initiate sporogony. There remains uncertainty about the life cycle of *H. carassii* since two oligochaete species, *B. sowerbyi* and *Nais* cf *elinguis*, have been identified as hosts for the actinospore stage of *H. carassii* producing Neoactinomyxon and Aurantiactinomyxon actinospores, respectively (Yokoyama *et al.*, 1993b; Trouillier *et al.*, 1996). In addition, transmission experiments have not been successful in inducing typical disease symptoms in susceptible fish (Trouillier *et al.*, 1996).

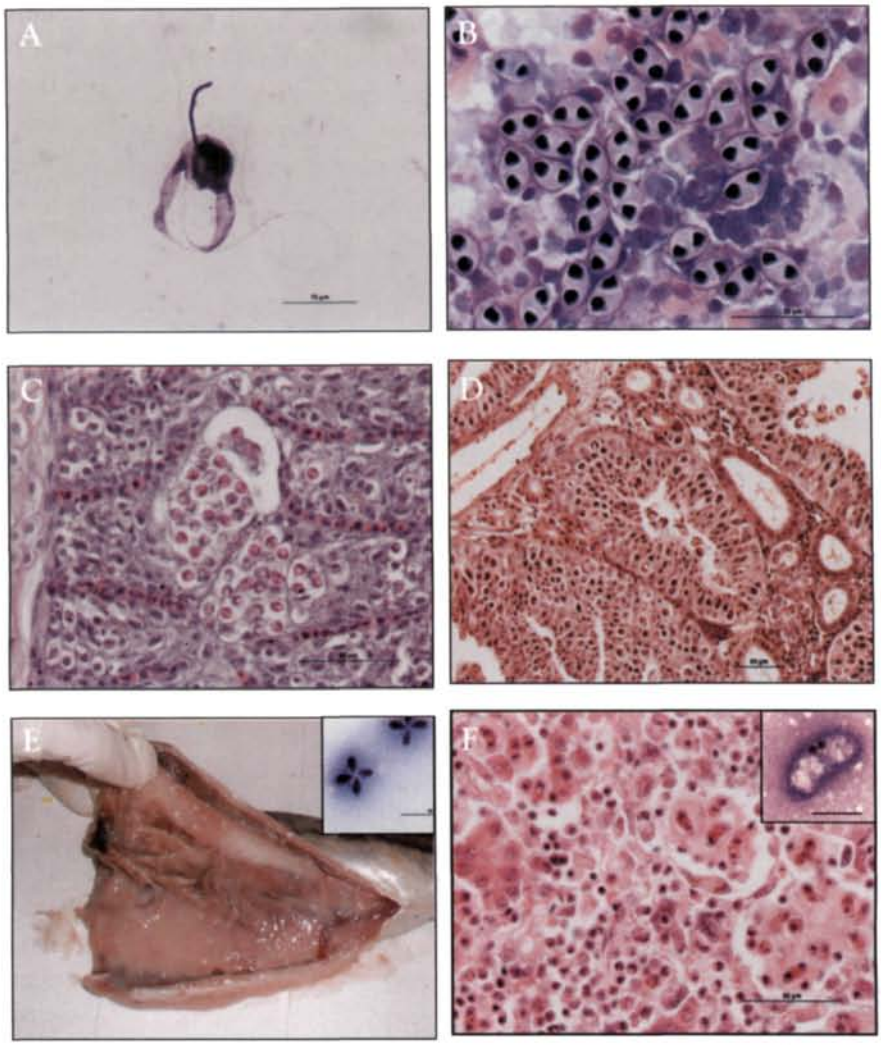


**Fig. 12.4** **A** – Fixed Giemsa stained spore of *Davisia longibrachia* from the urinary bladder of dragonet (*Callionymus lyra*) from the Irish Sea. Bar = 15 μm. **B** – Fixed Giemsa stained spores of *Myxidium giardi* from the kidney of European eel (*Anguilla anguilla*). Bar = 25 μm. **C** – Common carp (*Cyprinus carpio*) containing spores of *Sphaerospora molnari*. Note fusion of secondary gill lamellae. H&E, Bar = 50 μm. **D** – Section through a kidney of goldfish (*Carassius auratus*) with 'kidney enlargement disease'. Numerous stages of *Hoferellus carassii* are present in the hyperplastic renal tubule epithelium. H&E, Bar = 50 μm. **E** – Myoliquefaction of Atlantic mackerel (*Scomber scombrus*) infected with *Kudoa thyrsites*. Inset – Giemsa stained spore of *K. thyrsites*. **F** – Spores and developmental stages of *Ceratomyxa shasta* in the kidney of *Oncorhynchus* sp. associated with necrosis of tubule epithelium. H&E, Bar = 50 μm. Inset – Giemsa stained spore of *C. shasta*. Bar = 15 μm.

*Sphaerospora tincae* has been responsible for outbreaks of histozoic sphaerosporosis amongst cultured juvenile tench (*Tinca tinca*) from Germany. Prevalence is generally low, but can reach 100% and significant losses have been reported. Large numbers of disporic pseudoplasmodia replace the haematopoietic tissues of the pronephros, resulting in hypertrophy of the organ. Inflammation is usually absent. Concurrent infections with the coelozoic *Sphaerospora galinae* exert no discernable pathologic effect (Lom *et al.*, 1985).

Mortalities in wild fish caused by myxosporean infections are rare since severely diseased fish are rapidly removed from the ecosystem by predators. However, there is increasing evidence that chronic myxosporean disease adversely affects populations (see sections on whirling disease and proliferative kidney disease). Amongst juvenile cyprinids in the U.K., infections with *Myxobolus buckei* cause serious disease in resulting in marked spinal compression in infected fish. Chub (*Leuciscus cephalus*) appear to be the most susceptible to this parasite. Normal and burst swimming of infected fish is compromised and few juvenile fish survive to adulthood (Longshaw *et al.*, 2003). Fish become infected prior to ossification of the vertebral column and the parasite develops initially within the remnants of the embryonic notochord. Pathological changes include mild hypertrophy and deformation of the zygapophyseal elements of the vertebrae to complete fusion of affected portions of the vertebral column with infiltration of inflammatory cells and connective tissue. It also results in pressure atrophy of the spinal cord and adjacent blood vessels. Similar vertebral anomalies occur in cyprinid fishes from Oregon, U.S.A. In these cases, pathology is associated with with co-infections of digenean metacercariae *Apophallus* sp. and a *Myxobolus* sp. similar to M. *cyprini* (Kent *et al.*, 2004).

*Myxidium rhodei* is a common parasite of the roach (*Rutilus rutilus*) and other cyprinids in Europe (Dyková *et al.*, 1987; Alvarez-Pellitero, 1989; Athanassapoulou and Sommerville, 1993; Longshaw *et al.*, 2005). Plasmodia are frequently found in Bowman's space within the glomerulus, resulting in atrophy of the glomerular tuft but with minimal host response. Histozoic development within the haemopoietic interstitial tissues results in an intense granulomatous inflammatory reaction, which is often effective in destroying the parasite (Fig. 12.2B). Mortalities have not been associated with M. *rhodei* infections (Dyková *et al.*, 1987).

## Diseases of Non-cyprinid Freshwater Fish

Infections of *Henneguya* species infecting gills often produce large macroscopic cysts. Pikeperch (*Stizostedion lucioperca*) from Lake Balaton in Hungary are hosts of *Henneguya creplini* (Molnár, 1998). Plasmodia are large, reaching 900 × 750 μm in size, with between 30-60 plasmodia per fish. During cyst growth, there is usually little or no host cell response. However, following sporogenesis and rupture of plasmodia, epithelial cell proliferation, overgrowth of the damaged plasmodium and cell necrosis becomes apparent. Infections in pike (*E. lucius*) and perch (*Perca fluviatilis*) caused by *H. psorospermica* are common. These also give rise to multiple large intralamellar plasmodia and subsequent fusion of the lamellae. Severe inflammation and necrosis also occur with *H. psorospermica* infections following maturation and rupture of the cysts. Infections with *H. piaractus* in the gills of captive *Piaractus mesopotamicus* can cause mortalities (Martins and de Souza, 1997; Martins *et al.*, 1997). Affected fish exhibit decreased feeding, lethargy, erratic swimming and loss of equilibrium. Haemorrhaging and severe inflammation occurs in the gill epithelium and larger cysts cause pressure atrophy on adjacent lamellae.

Myxosporean infections in the gonads of fish are relatively rare, but they have the potential to significantly reduce the fecundity of affected fish. Severe oocytic infections with *Myxobolus dahmeyensis* lead to parasitic castration in *Tilapia zilli* and *Sarotherodon melanotheron* (Gbankoto *et al.*, 2001). Other species such as *Sphaerospora ovophila* and *M. algonquinensis* produce large cysts up to 500 μm and 800 μm in diameter, respectively, in the connective tissues of the ovaries of *Notemigonus crysoleucas* (Xiao and Desser, 1997). Infections with *H. amazonica* within the ovarian stroma and follicles of *Hoplosternum littorale* do not appear to elicit significant pathological host response (Torres *et al.*, 1994).

Mitchell *et al.* (1985) described infections with *M. hendricksoni* in fathead minnows (*Pimephales promelas*). Macroscopic cysts up to 1.5 mm in diameter throughout the brain were reported without signs of behavioural anomalies. However, *M. balantiocheirli* in the brain of tricolor sharkminnow (*Balantiocheilos melanopterius*) induces uncoordinated darting and rolling movements in infected fish (Levsen *et al.*, 2004). Plasmodia of *M. cotti* in bullheads (*Cottus gobio*) from the Czech Republic, southern England and Germany infect axons and several regions of the brain and spinal cord (Lom *et al.*, 1989b; El-Matbouli *et al.*, 1990).

Intra-axonal myxosporean plasmodia have also been described from the spinal cord and brainstem of the common shiner (*Notropis cornutus*) (Ferguson *et al.*, 1985). Histopathology of brain-infecting myxosporeans is often unremarkable (Kent and Hoffman, 1984; Langdon, 1990; Dzulinksy *et al.*, 1994), although encephalitis occurs with presporogonic and sporogonic stages with some species (Dyková and Lom, 1988a; Frasca *et al.*, 1998). Skeletal deformities associated with neural myxosporidiosis occur in infections with *Triangula percae* in redfin perch (*P. fluviatilis*) from Australia. Juveniles appear most susceptible to the infection and develop marked spinal lordosis. The parasites are located in the white matter of the mesencephalon, diencephalon and the medulla oblongata (Langdon, 1987). Infections with *Myxobolus sandrae* also cause lordosis and vertebral compression in the same host (Lom *et al.*, 1991).

Renal infections with *Myxidium lieberkuehni* are common in pike throughout its range. Usually there is little host response but renal hypertrophy and greyish mottled appearance reminiscent of proliferative kidney disease can occur in juvenile fish. Two proliferative extrasporogonic stages occur. The first invades the endothelial cells of the glomerulus resulting in grossly enlarged cells containing numerous parasites and a hugely hypertrophied nucleus, the whole structure constituting a parasitic xenoma (Fig. 12.1E and F). Many infected glomeruli become infiltrated by host phagocytes and granulation tissue and are destroyed by the host. The cells within the xenoma frequently contain secondary and tertiary cells. These are released from surviving xenomas and pass down the proximal tubule where they develop into small sporogonic plasmodia. These increase in size as they pass down the tubule, eventually populating the distal regions, collecting ducts and urinary bladder (Lom *et al.*, 1989a; Feist, 1997). Large polysporic plasmodia are found attached to the epithelial lining of the urinary bladder. Intense infections can be seen with the naked eye since plasmodia impart an orange colouration due to the pigment granules contained in the plasmodia. A second extrasporogonic stage occurs intracellularly within the epithelial cells of the collecting ducts. These become hypertrophied and degenerate without xenoma formation. The affected epithelium becomes hyperplastic, forming papillomatous folds into the lumen of the duct which itself becomes extremely dilated. *Henneguya oviperda* infects the ovaries of adult pike in Europe. Plasmodia are intracellular and result in atrophy and necrosis of affected oocytes. A large proportion of the ovary may be affected. This parasite appears to be rare

and a more detailed assessment of its development and pathogenicity are required.

Coelozoic plasmodia of *Myxidium* and *Zschokkella* species frequently become lodged in the biliary collecting ducts in the liver where they can be associated with attenuation of ductule epithelium, proliferation of bile ducts, inflammation of the surrounding tissues (pericholangitis) peribiliary fibrosis and occasionally hepatocellular necrosis (Lom and Dyková, 1981; Bucher *et al.*, 1992). Serious disease and mortalities occur in eels (*Anguilla anguilla*) infected with *Myxidium giardi* (Hine, 1980; Ventura and Paperna, 1985). Parasites can be histozoic, giving rise to granulomatous lesions in several tissues including the kidney where it can result in extensive damage to the excretory elements (Fig. 12.4B). Skin infections reduce the market value of affected fish. Coelozoic infections occur in the gall bladder and urinary bladder, usually without significant pathological changes (Paperna *et al.*, 1987).

## Diseases of Mariculture Species

Descriptions of myxosporeoses of marine netpen cultured and wild anadromous and freshwater salmonids have been described earlier in this chapter. This section provides information on infections in *inter alia* European sea bass (*Dicentrarchus labrax*), gilt-head seabream (*Sparus aurata*) and turbot (*Scophthalmus maximus*).

*Sphaerospora testicularis* infects the seminiferous tubules in the testis of *D. labrax*. Infections lead to damage to the germ and Sertoli cells, occlusion of the tubular lumens, testicular hyalinization and hypertrophy and abdominal ascites. In severe cases, parasitic castration can occur and is of particular concern if broodstock are infected (Sitja-Bobadilla and Alvarez-Pellitero, 1990, 1993a). A second *Sphaerospora* species, *S. dicentrarchi* also infects wild and farmed *D. labrax* in Spain and Italy at up to 100% prevalence (Sitja-Bobadilla and Alvarez-Pellitero, 1993a; Fioravanti *et al.*, 2004). The parasite is histozoic, infecting connective tissues of various organs including the digestive tract and kidney. Heavy infections in juvenile fish can result in mortality.

Several myxosporeans have been reported in gilt-head seabream. Glomerulonephritis in cultured fish from the Mediterranean and Atlantic coast of Spain is caused by *Polysporoplasma sparis* (Sitjà-Bobadilla and Alvarez-Pellitero, 1995). External clinical signs are absent. Parasites also infect the renal tubule epithelium and lumen and in heavy infections,

atrophy of renal interstitial tissue with inflammation and haemorrhage is present (Palenzuela *et al.*, 1999). The presence of between four and twelve sporoplasm cells is a characteristic feature of the spores. Infections with *Leptotheca sparidarum* also infect the renal tubules, glomeruli and ureters. Pathological changes to the tubule epithelial cells and glomeruli include attenuation and vacuolation of the epithelium (Sitjà-Bobadilla and Alvarez-Pellitero, 2001). Gross changes to the kidney are usually absent. Common dentex (*Dentex dentex*) are additional hosts of this pathogen. Ceratomyxosis caused by *Ceratomyxa sparusaurati* is a significant disease of cultured gilt-head seabream in the Mediterranean region, with severe infections giving rise to significant mortalities (Sitjà-Bobadilla *et al.*, 1995; Palenzuela *et al.*, 1997). The parasite is coelozoic within the gall-bladder and causes cellular hypertrophy, degeneration and sloughing, further giving rise to inflammation and ascites. Infections with *Kudoa iwatai* occur in gilt-head seabream, grey mullet (*Mugil cephalus*), sea bass and several other wild fish species (Diamant *et al.*, 2005). In gilt-head seabream, polysporous plasmodia up to 5 mm in diameter primarily infect the trunk musculature, although many other tissues are also be infected.

Systemic infection with *Kudoa lutjanus* in cultured red snapper (*Lutjanus erythropterus*) from Taiwan cause anorexia and low-level mortalities (Wang *et al.*, 2005). Other *Kudoa* species causing disease in cultured species include *Kudoa* (=*Pentacapsula*) *neurophila* causing severe granulomatous meningoencephalomyelitis in juvenile striped trumpeter (*Latris lineata*) in Tasmania (Grossel *et al.*, 2003) and *Kudoa* (=*Septemcapsula*) *yasunagai* which forms large parasitic cysts in the brain of cultured Chinese seabass (*Lateolabrax japonicus*) and *Oplegnathus fasciatus* in China and Japan (Hsieh and Chen, 1984).

*Enteromyxum leei* is a serious pathogen of cultured gilt-head seabream and several other cultured marine species in the Mediterranean area (Diamant, 1992, 1998; Diamant *et al.*, 1994; Le Breton and Marques, 1995; Padros *et al.*, 2001). The parasite infects the digestive tract causing severe enteritis (Fig. 12.2C and D), which can lead to emaciation and mortality. Direct horizontal transmission occurs between susceptible fish. Experimental infections in *S. aurata* resulted in prevalence levels of up to 33% and the onset of mortalities within two weeks of exposure by cohabitation or exposure to effluent from a tank containing infected fish (Diamant, 1997). Emaciation disease of tiger puffer (*Takifugu rubripes*) in the Kyushu district of Japan—also caused by *E. leei*—has been noted since 1996 with mortalities up to 60% (Tin Tun *et al.*, 2000). Yanagida *et al.*

(2004) clarified the taxonomy of enteric myxozoans in tiger puffer recognizing that *Enteromyxum leei* (formerly *Myxidium* sp. TP) was one of three myxozoans to have been detected in infected fish. The second, *Myxidium fugu* has been reassigned as *Enteromyxum fugu* with the third species being *Leptotheca fugu* (Ogawa and Yokoyama, 2001). Clinical signs of *E. leei* infection in *T. rubripes* include emaciation, enophthalmia and the presence of bony ridges on the head. Internally, enteritis with sloughing of the intestinal mucosa is the main feature. More recently, the disease has been confirmed in cultured Japanese flounder *Paralichthys olivaceus*. Externally, affected fish exhibit distended abdomen, enophthalmia and rectal prolapse (Yasuda *et al.*, 2005). Both *E. leei and E. fugu* have been transmitted by feeding infected tissues, co-habitation with infected fish and by exposure of effluent from tanks containing infected fish (Yasuda *et al.*, 2002; Yanagida *et al.*, 2004). *E. fugu* and *L. fugu* do not appear to be pathogenic (Tin Tun *et al.*, 2002).

Similar severe enteric pathology occurs in cultured turbot *Scophthalmus maximus* infected with *E. scophthalmi* (Branson *et al.*, 1999; Palenzuela *et al.*, 2002). Transmission is also direct via cohabitation with infected fish, exposure to water contaminated with spores and developmental stages and by the oral administration of infected intestinal tissue. Mortalities of up to 100% occur within three weeks of exposure to infected material. Early intracellular stages within epithelial cells can be detected in the anterior intestine within eight days following oral intubation. Proliferative stages become abundant intraepithelially and within the lumen of the intestine and can also be found in the blood, muscle, heart, gills and skin (Redondo *et al.*, 2002, 2003). That infections are highly virulent and mature spores rarely found suggests that turbot are accidental hosts for *E. scophthalmi* (Redondo *et al.*, 2002, 2004).

Infections with *Myxobolus buri* cause severe scoliosis in cultured *Seriola quinqueradiata* in Japan (Egusa, 1985). This parasite is synonymous with *M. acanthogobii* infecting the neural tissue of yellowfin goby (*Acanthogobius flavimanus*) (Yokoyama *et al.*, 2004a). Several regions of the brain were affected, including the olfactory and optic lobes, cerebellum and medulla oblongata. Parasite stages become surrounded by inflammatory tissue and a fibrous capsule. *M. acanthogobii* also infects cultured Japanese mackerel (*Scomber japonicus*), producing cysts in several regions of the brain and causing scoliosis in affected fish (Yokoyama *et al.*, 2005b). *Kudoa amamiensis* is also a serious pathogen of cultured

*S. quinqueradiata* in Japan (Egusa and Nakajima, 1980). Heavily infected fish are unmarketable. Scoliosis in grey mullet from Japan is caused by *Myxobolus spinacurvatura*. Cysts approximately 1mm in diameter are found in several regions in the brain including the olfactory and optic lobes as well as mesenteric tissue and spleen (Maeno *et al.*, 1990).

Renal infections with *Sphaerospora epinepheli* occur in wild and cultured groupers (*Epinephelus malabaricus*) from Thailand. Affected fish exhibit a variety of pathological changes in the glomeruli and tubules. Vacuolation of tubule epithelial cells and dilation of glomerular capillaries associated with degeneration of endothelial cells. Severely affected glomeruli become necrotic and fibrosed. Interstitial haematopoietic tissues remain unaffected (Supamattaya *et al.*, 1991, 1993). Chronic mortalities of Chinese sea bass in Japan are caused by *Henneguya lateolabracis*. Affected fish are anaemic with congestion and lamellar hypertrophy of the gills. Large irregular plasmodia of *H. lateolabracis* develop in the bulbus arteriosus of the heart. Release of spores from cardiac cysts travel via the blood stream to the gills where they occlude the capillaries inducing epithelial necrosis (Yokoyama *et al.*, 2003). Similar cardiac infections in net pen cultured red seabream (*Pagrus major*) in Japan are caused by *Henneguya pagri* (Yokoyama *et al.*, 2005c). The principal site of infection is the bulbus arteriosus of the heart but haematogenous transport of spores to the gills gives rise to extensive branchial pathology. Prevalence reaches 75% and chronic infections are lethal.

## Diseases of Wild Marine Fish

Myoliquefaction is a feature of at least eleven *Kudoa* spp. infections that is of major concern for commercial fisheries with the potential for rendering fish unmarketable. Moran *et al.* (1999c) provide a review of these species and others continue to be discovered (Yokoyama *et al.*, 2004b; Yokoyama and Itoh, 2005a). Examples include *K. clupeidae* in Atlantic herring (*Clupea harengus*), *K. musculoliquefaciens* in swordfish (*Xiphias gladius*), *K. paniformis* in Pacific hake (*Merluccius productus*) and *K. thyrsites* in Atlantic mackerel (*Scomber scombrus*) (Figs. 12.3D and 12.4E).

Ulcerative lesions in juvenile Atlantic menhaden (*Brevoortia tyrannus*) have been attributed to a number of aetiological agents including bacteria, fungi and harmful algal blooms. Vogelbein *et al.* (2002)

conclusively demonstrated that the ulceration could be initiated by micropredation of epithelial cells by *Pfiesteria shumwayae*. However, spores and plasmodia of *Kudoa clupeidae* are invariably found in menhaden with ulcerations, associated with inflammatory tissue surrounding the ulcers and within the somatic musculature. This suggests that *K. clupeidae* may be the primary aetiological agent (Reimschuessel *et al.*, 2003; Webb *et al.*, 2005), at least in some epidemic outbreaks of ulceration in menhaden.

Adult bullseye puffer (*Sphoeroides annulatus*) collected off the coast of Mexico are infected with *Kudoa dianae*. Cysts were located in mesenteric tissues of 20% of the fish examined. Intact pseudocysts within muscle fibres do not elicit a host response, but as for other muscle-invading myxosporeans, exposure of spores and developmental stages to the host immune system following rupture of the sarcolemma results in focal inflammation and fibrosis. With *K. dianae* infections parasites are phagocytosed by macrophages and transported to the mucosal epithelium where they can totally replace the epithelial tissue (Dyková *et al.*, 2002a). The parasite *K. ovivora* infects oocytes of *Thalassoma bifasciatum* and other labroid fishes in a similar fashion to *H. oviperda* in pike in freshwater (see above). Sporogonic plasmodia mature along the inner wall of vitellogenic oocytes with spores generally present in mature oocytes. Eggs are non-viable and infected females exhibit reduced growth, fecundity and/or spawning activity (Swearer and Robertson, 1999).

Infections with *M. aeglefini* are widespread in a range of marine flatfish and gadoid hosts in the North Atlantic, North Sea and Baltic Sea (Lom and Dyková, 1992; Nielsen *et al.*, 2002). Plasmodia invade the cranial cartilage and are visible usually in the form of spherical white cysts on the surface of the cartilage or embedded within the matrix. There is no inflammatory response. In gadoids such as whiting (*Merlangius merlangus*) and poor cod (*Trisopterus minutus*), cysts are formed in the scleral cartilage (Fig. 12.3A and B). The function of infected eyes does not appear to be compromised. Japanese porous-head eelpout (*Allolepis hollandi*) are reported to be hosts of *M. aeglefini* infecting cartilage and skeletal muscle. However, this species may represent a morphologically similar but genetically different species of *Myxobolus* (Yokoyama and Wakabayashi, 2000). Red drum (*Sciaenops ocellatus*) from the Atlantic Ocean off Florida is host to *Parvicapsula renalis* in the kidney tubules where infections can result in renal hypertrophy (Landsberg, 1993). Proximal tubules of the posterior kidney harbour extrasporogonic and sporogonic stages that induce vacuolation of the tubule epithelium.

The gall bladder is a common site of infection for myxosporean parasites in freshwater and marine fish species and, in most cases, the parasites do not appear to harm the host. However, as can be seen from the preceding sections, several gall bladder-infecting species are known to be pathogenic. Less attention has been given to studies on the pathogenicity of infections in non-commercial marine fish species and it is likely that several species can induce significant pathological response. Infections are thought to reach the gall bladder via hepatic sinusoids to the bile ductules and finally the gall bladder itself (Dyková and Lom, 1988b). The following are examples where histopathological changes have been described. Five-bearded rockling (*Ciliata mustela*) are hosts to *Zschokkella russeli* with prevalences up to 89% at intertidal localities in Wales (Davies, 1985). Plasmodia are located in the hepatic bile ducts in the liver, floating freely in the bile and loosely attached to the gall bladder epithelium, which appears unaffected. Hepatic pathology includes cholangiofibrosis, pericholangitis and flattened ductal epithelium with reduced microvilli. Degenerative changes to the epithelial lining of the gall bladder can also occur. Infections with *Ceratomyxa drepanopsettae* in the gall bladder of Atlantic halibut (*Hippoglossus hippoglossus*) induce epithelial necrosis (Morrison *et al.*, 1996) and similar pathology occurs with *Zschokkella mugilis* in mullets of the genera *Mugil* and *Liza* from the Mediterranean (Sitjà-Bobadilla and Alvarez-Pellitero, 1993b). Rabbitfish (*Signanus luridus*) from the Red Sea infected with *Zschokkella icterica* exhibit clinical signs of ascites and jaudice in severe infections. Large plasmodia block the hepatic bile ductules, resulting in cholestasis, necrosis of the ductules, cholangitis and hepatocellular necrosis (Diamant and Paperna, 1992). Heavy infections with *Myxidium gadi* in saithe (*Pollachius virens*) and pollack (*Pollachius pollachius*) from the North Sea cause atrophy and hardening of the gall bladder. Replacement of the bile with parasite stages and hyperplastic epithelial lining result in a pale appearance (Feist and Bucke, 1992). Gross pathological changes to the liver have not been reported.

Very few records of myxosporean infections in elasmobranchs exist and these are restricted to *Kudoa* species infecting the musculature (Stoffregen and Anderson, 1990; Heupel and Bennett, 1996) and *Sphaerospora araii* infecting the kidney of longnose skate (*Raja rhina*) (Arthur and Lom, 1985). In these cases, the infections were not thought to be detrimental to the hosts. Additional *Kudoa* infections have been

recorded in captive cownose ray (*Rhinoptera bonasus*) and sandbar shark (*Carcharhinus plumbeus*) (Fig. 12.3E and F) (Feist and Longshaw, unpublished). A study examining the parasito fauna of starspotted dogfish *Mustelus manazo* in Japan and Taiwan identified gall bladder infections with unidentified species of *Ceratomyxa* and *Chloromyxum* that may be of use for stock discrimination (Yamaguchi *et al.*, 2003). It is likely that additional records will be reported from elasmobranchs since these fish have not so far attracted the same degree of attention as teleosts. However, it is interesting to speculate why this ancient group appears to be relatively free from myxosporean infections compared to other marine fish species.

## DIAGNOSTIC METHODS

As for all disease conditions, the initial diagnosis is based on the clinical appearance, history of stock and assessment of risk factors in aquaculture situations. Accurate diagnosis of myxozoan infections does not rely solely on morphological characteristics of mature spores at the light microscope level any more and most descriptions of new species in addition utilize molecular techniques and ultrastructure. However, accurate descriptions of fresh material using the established criteria set out by Lom and Arthur (1989) and Lom *et al.* (1997) are a fundamental requirement. A variety of microscopic techniques can be applied, ranging from bright field to phase and interference contrast. Fixed smears or imprints can be stained with a variety of techniques (Wolf and Markiw, 1979; Clifton-Hadley *et al.*, 1983; Baska and Molnár, 1988; Schachner and Holzer, 2001). Standard histological staining methods such as haematoxylin and eosin (H&E), Giemsa and Gram stains, Periodic acid Schiff (PAS) and trichrome methods can prove useful in detecting parasite stages in tissue sections as well as visualizing host cell and tissue responses. Labelling parasite stages using biotinylated lectins is used for characterization of carbohydrate groups (Castagnaro *et al.*, 1991; Marín de Mateo *et al.*, 1996; Muñoz *et al.*, 1999a, 2000; Morris and Adams, 2004; Knaus *et al.*, 2005) and fluorescent dyes are useful for detecting parasites in fixed material (Yokoyama and Urawa, 1997; Yasuda *et al.*, 2005).

Monoclonal and polyclonal antibodies have been raised against *T. bryosalmonae* (Adams *et al.*, 1992; Marín de Mateo *et al.*, 1996; Saulnier and de Kinkelin, 1996; Morris *et al.*, 1997, 2000a), *C. shasta* (Bartholomew *et al.*, 1989b, 2004), *K. thyrsites* (Chase *et al.*, 2001),

*H. salminicola* (Clouthier *et al.*, 1997), *M. cerebralis* (Griffin and Davis, 1978; Markiw and Wolf, 1978; Hamilton and Canning, 1988; Young and Jones, 2005), *Sphaerospora dicentrarchi* (Muñoz *et al.*, 1998, 1999b) and *M. rotundus* (Lu *et al.*, 2002, 2003). These provide excellent tools for detection, but since parasite stages display differential antigenicity dependent on maturity, limitations with using monoclonal antibodies can occur (Young and Jones, 2005). Antigen capture enzyme-linked immunosorbent assay (ELISA) for the detection of *Kudoa thyrsites* in Atlantic salmon has shown increased sensitivity compared to examination of histological sections and good agreement with PCR analysis (Taylor and Jones, 2005). An ELISA for specific circulating antibodies against *E. scophthalmi* has also been developed (Sitjà-Bobadilla *et al.*, 2004). This technique offers excellent potential for the serological detection of light infections and for screening of field collected samples.

Molecular techniques have provided significant improvements in diagnostic sensitivity in the oligochaete and fish hosts as well as furthering understanding into parasite biology. In particular, the use of PCR to link the alternate stages of myxozoan lifecycles (Andree *et al.*, 1997; Bartholomew *et al.*, 1997; Lin *et al.*, 1999; Pote *et al.*, 2000; Feist *et al.*, 2001; Hanson *et al.*, 2001; Whitaker *et al.*, 2005). Primers have been developed that target both the 18S and ITS regions of the rDNA (Andree *et al.*, 1999a) and include generic myxozoan primers (Kent *et al.*, 2001), genus-specific (Andree *et al.*, 1999b) and species-specific primers (Hervio *et al.*, 1997; Saulnier and de Kinkelin, 1997; Andree *et al.*, 1998; Kent *et al.*, 1998, Morris *et al.*, 2002; Yanagida *et al.*, 2005). The use of real-time quantitative PCR offers the potential for providing information on the severity of *M. cerebralis* infections (Cavender *et al.*, 2004) and to detect parasite stages in river water (Hallett and Bartholomew, 2006). A nested-PCR method for detection of *M. acanthogobii* has proved successful in detecting the parasite in cultured yellowtail and Japanese mackerel as well as in wild fish species (Miyajima *et al.*, 2005). The development of *in situ* hybridization technique to visualize parasite stages in tissue sections and by immunogold labelling, in ultrathin sections under the electron microscope has been extremely useful for studies on pathogenesis and development within the host (Antonio *et al.*, 1998; Frasca Jr. *et al.*, 1999; Morris *et al.*, 1999, 2000b; Longshaw *et al.*, 2002; Holzer *et al.*, 2003).

Recent developments that allow histological 'tissue arrays' to be easily prepared provide opportunities for screening of large numbers of tissue

samples using a variety of non-specific and specific methods, as outlined above. The technique relies on the ability to precisely sub-sample small cylindrical tissue blocks for regions of interest and to arrange these in a multiple block that allows subsequent sections containing a large number of specimens. The method will be of particular use for disseminated infections but of more limited application for focal of multifocal infections where sections at particular levels may miss the parasite of interest. Laser capture microdissection (LCMD) techniques offer an elegant solution to the problem of dealing with rare or localized infections. Precise sampling—with the potential to isolate individual cells from frozen or fixed material from histological sections—offers the ability to obtain material 'uncontaminated' with host genetic material and, therefore, suitable for DNA sequencing and even analysis of gene expression. This approach has not yet been tested for myxozoa and the technical issues related to extraction of material from minute samples will certainly prove challenging. However, extraction procedures have been developed and since the possibilities for investigation of pathogenesis and parasite development are significant, the use of LCMD sampling for analysis of myxozoan material will certainly be reported in the near future.

## TREATMENT AND PROPHYLAXIS

It is not suprising that efforts for treatment and control have focussed on diseases of cultured fish. Management strategies aimed at reducing exposure to the infective stage and reducing stressors on fish remain the most effective means to control disease (see review by Yokoyama, 2003). Reduced water temperature suppresses the effects of PKD (Clifton-Hadley *et al.*, 1986) and fish exposed late in the season, before ambient temperatures fall, survive the infection and appear less susceptible to the disease the following year. Since the infective stage is present throughout the year in at least one bryozoan host (Gay *et al.*, 2001; Tops and Okamura, 2003), measures to reduce the numbers and distribution of bryozoan hosts in and around aquaculture facilities need to be evaluated. In farm control of myxosporeans has been achieved by drying of earthen ponds and fallowing (usually a temporary measure until parasite and oligochaete abundance increase again). Substrate replacement in carp ponds was effective in controlling haemorrhagic thelohanellosis caused by *T. hovorkai* (Liyanage *et al.*, 2003). Replacement of traditional earthen ponds with concrete or plastic tanks and raceways also significantly reduces

possibilities for oligochaete colonization. Other control measures, including treatment of inflow water by filtration or with ultraviolet irradiation, chlorination or ozone have proved efficacious in hatchery situations, but are relatively expensive for large-scale application. Management initiatives for the control of whirling disease and potentially other fish pathogens in the wild—using a risk assessment-based approach—are dependent on local situations where such analysis indicate the most promising options to follow (Bartholomew *et al.*, 2005). These may incude attempts to eradicate localized populations of infected oligochaetes or introduction of resistant strains of fish.

Chemotherapeutants such as Fumagillin, Toltrazuril have been shown to be effective under experimental conditions but currently, none are licensed for use in aquaculture facilities because of unacceptable toxicity at therapeutic levels, bioaccumulation and concerns regarding teratogenic and carcinogenic properties (Alderman, 1985; Wishkovsky *et al.*, 1990; Gerundo *et al.*, 1991). Treatments with Malachite green, Fumagillin and its analog TNP-470 have been shown to control PKD (Alderman and Clifton-Hadley, 1988; Hedrick *et al.*, 1988b; Higgins and Kent, 1998; Le Gouvello *et al.*, 1999). However, Morris *et al.* (2003a) showed that in rainbow trout, TNP-470 had similar toxicity to fumagillin. An experimental oral administration of a combined treatment with the anticoccidial drugs salinomycin and amprolium was found to be effective in controlling natural infections with *Myxobolus* in sharpsnout seabream (Karagouni *et al.*, 2005). Parasites were almost completely eliminated after one month of treatment and no evidence of drug toxicity was detected histologically. The prospect for widespread introduction of effective drug therapy for myxosporeoses seems unlikely, although the need for continued research into potential therapeutants remains.

Several studies have detected circulating antibodies to myxozoan parasites and evidence of acquired immunity following exposure to *C. shasta*, *M. cerebralis* and *T. bryosalmonae* in salmonids, *E. scophthalmi* in turbot and *M. artus* in carp (Griffin and Davis, 1978; Foot and Hedrick, 1987; Furuta *et al.*, 1993; Bartholomew, 1998; Sitjà-Bobadilla *et al.*, 2004, 2007). This suggests that there is potential for vaccine development against the diseases caused by these agents. However, the identification of protective antigens has as yet proved elusive.

## FUTURE DEVELOPMENTS

During the past two decades, there have been enormous advances in the study of Myxozoa, particularly in aspects of phylogeny and taxonomy, life cycles and the understanding of infections in mariculture species. This, together with the equally rapid development of diagnostic tools, has advanced knowledge in diseases caused by these organisms at an unprecedented rate. There is no doubt that aquaculture will continue to grow in importance as a source of protein for the human population, particularly since capture fisheries in most parts of the globe are under intense pressure. Disease is a limiting factor for this production and remains so for existing diseases such as PKD. The lack of approved therapeutants and no immediate prospect of vaccines have led to the introduction of innovative management strategies by the industry. However, the expectation remains high that current research programmes will lead to a cost-effective vaccine for diseases such as PKD. This is likely to be a long-term quest, bearing in mind the paucity of vaccines against metazoan parasites in general.

Molecular systematics using 18s rDNA sequences will continue to be of importance for studies on the phylogeny of myxozoans (Kent *et al.*, 2001) and also for correlation of myxospore and actinospore stages (for example, Holzer *et al.*, 2004). Analysis of other gene sequences, including the ITS region (Andree *et al.*, 1999a) and the recently investigated actin cDNA sequence (Kelley *et al.*, 2004), are likely to provide further insights into myxozoan systematics and phylogeny. However, it is by using a combination of the available techniques that provides the confidence required to establish new species or propose higher-level taxonomic changes. As more sequences become available, it is inevitable that the validity of established taxa will be called into question. Such an example is provided by Diamant *et al.* (2004), where the 18s rDNA sequence analysis of the marine species *Sphaeromyxa zaharoni* indicated close relatedness to *Myxidium* species, which also has a broadly similar morphology. However, the authors have rightly guarded against taxonomic change on the basis of sequence analysis from only one species and a single gene.

Increasing awareness and concern regarding the quality of aquaculture and fisheries products is likely to continue. With regard to fish diseases—including infections with myxozoan parasites—pressure is likely to provide assurance to the consumer on the 'health' of the product. It is

inevitable that unfilleted fish harbouring parasitic infections will reach the market. Although it is highly unlikely that humans can be infected with myxozoan parasites, there is evidence that mammals can act as hosts and mice exposed to *Kudoa* sp. exhibit antigen specific IgG1 and IgE responses (Martínez de Velasco and Cuéllar, 2003). The fact that myxozoans may be allergenic with the potential for inducing hypersensitivity reactions to humans suggests the need for further study.

The investigation of disease caused by myxozoans will continue to be a priority, not least because with the continued expansion of aquaculture across the globe, new or re-emerging pathogens will appear. Techniques for their identification and diagnosis of the diseases thay cause are already in place but there is likely to be increasing emphasis on improving the understanding of the role of these pathogens amongst wild stocks and invertebrate hosts. In fish, the application of serological techniques will be important in the development of epidemiological models for risk assessment and potentially predictive modelling (Taylor and Jones, 2005). Concerning the pathogenesis of disease: techologies genomics, proteomics and metabolomics offer significant potential for unravelling host responses at all stages of infection, including identification of immune-regulatory genes (Holland *et al.*, 2003) and physiological markers of early infection prior to antibody production.

Although the pressing need to provide solutions for disease in aquaculture situations will continue, we are also beginning to better appreciate the impact of infections caused by myxozoans and other pathogens on wild populations. This is an area requiring further research, especially in order to develop capacity to predict the possible effects of global climate change on the prevalence and distribution of myxozoans as well as relatively short-term anthropogenic interventions to freshwater and coastal ecosystems. This will also require increased understanding of the behaviour and physiology of invertebrate and fish hosts. A more holistic ecosystem approach to the study of disease in wild populations is required, such as that being applied to tackle whirling disease in North American salmonids. The assessment of brown trout declines in Swiss rivers—where PKD is regarded as a significant contributing factor—using a Baysian probability network provides a site-specific assessment tool and a predictive capacity to estimate the effect of proposed management actions (Borsuk *et al.*, 2006). This approach, which incorporates data on natural variables and anthropogenic influences, offers potential for improved management of wild fisheries to mitigate the effects of disease.

## References


Adams, A., R.H. Richards and M.M. de Mateo. 1992. Development of monoclonal antibodies to PK'X', the causative agent of proliferative kidney disease. *Journal of Fish Diseases* 15: 515-521.

Ahmed, A.T.A. 1974. Kidney enlargement disease of goldfish in Japan. *Japanese Journal of Zoology* 17: 37-65.

Alderman, D. 1985. Malachite green: A review. *Journal of Fish Diseases* 8: 289-298.

Alderman, D. and R.S. Clifton-Hadley. 1988. Malachite green therapy of proliferative kidney disease in rainbow trout: Field trials. *Veterinary Record* 122: 103-106.

Alvarez-Pellitero, P. 1989. *Myxidium rhodei* (Protozoa: Myxozoa: Myxosporea) in cyprinid fishes from NW Spain. *Diseases of Aquatic Organisms* 7: 13-16.

Alvarez-Pellitero, P., J. Pereira-Bueno and M.C. Gonzalez-Lanza. 1982. On the presence of *Chloromyxum truttae* Léger, 1906 in *Salmo trutta fario* from Leon (Duero basin, NW Spain). *Bulletin of the European Association of Fish Pathologists* 2: 4-7.

Alvarez-Pellitero, P., K. Molnár, A. Sitjà-Bobadilla and C. Székely. 2002. Comparative ultrastructure of the actinosporean stages of *Myxobolus bramae* and M. *pseudodispar* (Myxozoa). *Parasitology Research* 88: 198-207.

Amandi, A., R.A. Holt and J.L. Fryer. 1985. Observations on *Myxobolus insidiosus* (Myxozoa: Myxosporea), a parasite of salmonid fishes. *Fish Pathology* 20: 287-304.

Anderson, C.L., E.U. Canning and B. Okamura. 1998. A triploblast origin for Myxozoa? *Nature (London)* 392: 346-347.

Andree, K.B., S.J. Gresoviac and R.P. Hedrick. 1997. Small subunit ribosomal RNA sequences unite alternate actinosporean and myxosporean stages of *Myxobolus cerebralis* the causative agent of whirling disease in salmonid fish. *Journal of Eukaryotic Microbiology* 44: 208-215.

Andree, K., E. MacConnell and R.P. Hedrick. 1998. A nested chain reaction for the detection of genomic DNA of *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 34: 145-154.

Andree, K.S., M. El-Matbouli, R.W. Hoffman and R.P. Hedrick. 1999a. Comparison of 18S and ITS-1 rDNA sequences of selected geographic isolates of *Myxobolus cerebralis*. *International Journal for Parasitology* 29: 771-775.

Andree, K., C. Székely, K. Molnár, S.J. Gresoviac and R.P. Hedrick. 1999b. Relationships among members of the genus *Myxobolus* (Myxozoa: Bivalvulidae) based on small subunit ribosomal DNA sequences. *Journal of Parasitology* 85: 68-74.

Andrews, C. 1979. The occurrence of *Henneguya psorospermica* Thélohan, 1895 (Myxosporidia) on perch, *Perca fluviatilis* L., from Llyn Tegid, Wales. *Journal of Fish Diseases* 2: 27-33.

Antonio, D.B., K.B. Andree, T.S. McDowell and R.P. Hedrick. 1998. Detection of *Myxobolus cerebralis* in rainbow trout (*Oncorhynchus mykiss*) and oligochaete tissues using a non-radioactive *in situ* hybridisation protocol. *Journal of Aquatic Animal Health* 10: 338-347.

Arthur, J.R. and J. Lom. 1985. *Sphaerospora araii* n. sp. (Myxosporea: Sphaerosporidae) from the kidney of a longnose skate (*Raja rhina* Jordan and Gilbert) from the Pacific Ocean off Canada. *Canadian Journal of Zoology* 63: 2902-2906.

Athanassopoulou, F. and C. Sommerville. 1993. A comparative study of the myxosporeans *Myxidium rhodei* Léger, 1905 and *Myxidium pfeifferi* Auerbach, 1908 in roach, *Rutilus rutilus*. *Journal of Fish Diseases* 16: 27-38.

Awakura, T., K. Nagasawa and S. Urawa. 1995. Occurrence of *Myxobolus arcticus* and *M. neurobius* (Myxozoa: Myxosporea) in masu salmon *Oncorhynchus masou* from northern Japan. *Scientific Reports of the Hokkaido Salmon Hatchery* 49: 35-40.

Barja, J.L. and A.E. Toranzo. 1993. Myoliquefaction post-mortem caused by the myxosporean *Kudoa thyrsites* in reared Atlantic salmon in Spain. *Bulletin of the European Association of Fish Pathologists* 13: 86-88.

Bartholomew, J.L. 1998. Host resistance to infection by the myxosporean parasite *Ceratomyxa shasta*: A review. *Journal of Aquatic Animal Health* 10: 112-120.

Bartholomew, J.L. and P.W. Reno. 2002. The history and dissemination of whirling disease. In: *Whirling Disease: Reviews and Current Topics*, J.L. Bartholomew and J.C. Wilson (eds.). *American Fisheries Society, Symposium* 29, Bethesda, Maryland, pp. 3-24.

Bartholomew, J.L. and J.C. Wilson. 2002. *Whirling Disease: Reviews and Current Topics*. *American Fisheries Society, Symposium* 29, Bethesda, Maryland. 247 pp.

Bartholomew, J.L., J.S. Rohovec and J.L. Fryer. 1989a. *Ceratomyxa shasta*, a myxosporean parasite of salmonids. *Fish Disease Leaflet No. 80. U.S. Fish and Wildlife Service*.

Bartholomew, J.L., C.E. Smith, J.S. Rohovec and J.L. Fryer. 1989b. Characterisation of a host response to the myxosporean parasite, *Ceratomyxa shasta* (Noble), by histology, scanning electron microscopy and immunological techniques. *Journal of Fish Diseases* 12: 509-522.

Bartholomew, J.L., M.J. Whipple, D.J. Stevens and J.L. Fryer. 1997. The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. *Journal of Parasitology* 83: 859-868.

Bartholomew, J.L., E. Ray, B. Torell, M.J. Whipple and J.R. Heidel. 2004. Monitoring *Ceratomyxa shasta* infection during a hatchery rearing cycle: Comparison of molecular, serological and histological methods. *Diseases of Aquatic Organisms* 62: 85-92.

Bartholomew, J.L., B.L. Kerans, R.P. Hedrick, S.C. MacDiarmid and J.R. Winton. 2005. A risk assessment based approach for the management of whirling disease. *Reviews of Fisheries Science* 13: 205-230.

Baska, F. and K. Molnár. 1988. Blood stages of *Sphaerospora* spp. (Myxosporea) in cyprinid fishes. *Diseases of Aquatic Organisms* 5: 23-28.

Beauchamp, K.A., R.D. Kathman, T.S. McDowell and R.P. Hedrick. 2001. Molecular phylogeny of tubificid oligochaetes with special emphasis on *Tubifex tubifex* (Tubificidae). *Molecular Phylogenetics and Evolution* 19: 216-224.

Beauchamp, K.A., G.O. Kelley, R.B. Nehring and R.P. Hedrick. 2005. The severity of whirling disease among wild trout corresponds to the differences in genetic composition of *Tubifex tubifex* populations in central Colorado. *Journal of Parasitology* 91: 53-60.

Bellem, A.M.G. and L.M. Pote. 2002. Portals of entry and systematic localisation of proliferative gill disease organisms in channel catfish *Ictalurus punctatus*. *Diseases of Aquatic Organisms* 48: 37-42.

Boreham, R.E., S. Hendrick, P.J. O'Donoghue and D.J. Stenzel. 1998. Incidental finding of *Myxobolus* spores (Protozoa: Myxozoa) in stool samples from patients with gastrointestinal symptoms. *Journal of Clinical Microbiology* 36: 3728-3730.

Borsuk, M.E., P. Reichert, A. Peter, E. Schager and P. Burkhardt-Holm. 2006. Assessing the decline of brown trout (*Salmo trutta*) in Swiss rivers using a Baysian probability network. *Ecological Modelling* 192: 224-244.

Branson, E., A. Riaza and P. Alvarez-Pellitero. 1999. Myxosporean infection causing intestinal disease in farmed turbot *Scophthalmus maximus* (L.), (Teleostei: Scophthalmidae). *Journal of Fish Diseases* 22: 395-399.

Brown, J.A., J.P. Thonney, D. Holwell and W.R. Wilson. 1991. A comparison of the susceptibility of *Salvelinus alpinus* and *Salmo salar ouananiche* to proliferative kidney disease. *Aquaculture* 96: 1-6.

Brummer-Korvenkontio, H., E.T. Valtonen and O.N. Pugachev. 1991. Myxosporean parasites in roach, *Rutilus rutilus* (Linnaeus), from four lakes in central Finland. *Journal of Fish Biology* 38: 573-586.

Bucher, F., R. Hofer and M. El-Matbouli. 1992. Prevalence and pathology of *Zschokkella nova* (Myxosporea) in the liver of bullhead *Cottus gobio* from a polluted river. *Diseases of Aquatic Organisms* 14: 137-143.

Campbell, N. 2005. The myxosporean parasitofauna of the Atlantic horse mackerel, *Trachurus trachurus* (L.) in the northeast Atlantic Ocean and Mediterrean Sea. *Acta Parasitologica* 50: 97-101.

Canning, E.U. and B. Okamura. 2004. Biodiversity and evolution of the Myxozoa. *Advances in Parasitology* 56: 43-131.

Canning, E.U., B. Okamura and A. Curry. 1996. Development of a myxozoan parasite *Tetracapsula bryozoides* n.g., n.sp. in the body cavity of *Cristatella mucedo* (Bryozoa, Phylactolaemata). *Folia Parasitologica* 43: 249-261.

Canning, E.U., A. Curry, S.W. Feist, M. Longshaw and B. Okamura. 1999. *Tetracapsula bryosalmonae* n. sp. for PKX organism, the cause of PKD in salmonid fish. *Bulletin of European Association of Fish Pathologists* 19: 203-206.

Canning, E.U., A. Curry, S.W. Feist, M. Longshaw and B. Okamura. 2000. A new class and order of myxozoans to accommodate parasites of bryozoans with ultrastructural observation on *Tetracapsula bryosalmonae* (PKX organism). *Journal of Eukaryotic Microbiology* 47: 456-468.

Canning, E.U., S. Tops, A. Curry, T.S. Wood and B. Okamura. 2002. Ecology, development and pathogenicity of *Buddenbrockia plumatellae* Schröder, 1910 (Myxozoa, Malacosporea) (syn. *Tetracapsula bryozoides*) and establishment of *Tetracapsuloides* n. gen. for *Tetracapsula bryosalmonae*. *Journal of Eukaryotic Microbiology* 49: 280-295.

Castagnaro, M., M. Marín de Mateo, C. Ghittino and R.P. Hedrick. 1991. Lectin histochemistry and ultrastructure of rainbow trout *Oncorhynchus mykiss* kidneys affected by proliferative kidney disease. *Diseases of Aquatic Organisms* 10: 173-183.

Cavender, W.P., J.S. Wood, M.S. Powell, K. Overturf and K.D. Cain. 2004. Real-time quantitative polymerase chain reaction (QPCR) to identify *Myxobolus cerebralis* in rainbow trout *Oncorhynchus nykiss*. *Diseases of Aquatic Organisms* 60: 205-213.

Chase, J.C., J.A. Dawson-Coates, J.D. Haddow, M.H. Stewart, L.R. Haines, D.J. Whitaker, M.L. Kent, R.W. Olafson and T.W. Pearson. 2001. Analysis of *Kudoa*

*thyrsites* (Myxozoa: Myxosporea) spore antigens using monoclonal antibodies. *Diseases of Aquatic Organisms* 45: 121-129.

Chilmonczyk, S, D. Monge and P. De Kinkelin. 2002. Proliferative kidney disease: Cellular aspects of the rainbow trout, *Oncorhynchus mykiss* (Walbaum), response to parasitic infection. *Journal of Fish Diseases* 25: 217-226.

Clouthier, S.C., D.J. Gunning, R.W. Olafson and W.W. Kay. 1997. Antigenic characterization of *Henneguya salminicola*. *Molecular and Biochemical Parasitology* 90: 543-548.

Ching, H.L. and D.R. Munday. 1984. Susceptibility of six Fraser chinook salmon stocks to *Ceratomyxa shasta* and the effects of salinity on certaomyxosis. *Canadian Journal of Zoology* 62: 1081-1083.

Clifton-Hadley, R.S., R.H. Richards and D. Bucke. 1983. Method for the rapid diagnosis of proliferative kidney disease in salmonids. *Veterinary Record* 112: 609.

Clifton-Hadley, R.S., D. Bucke and R.H. Richards. 1984. Proliferative kidney disease of salmonid fish: A review. *Journal of Fish Diseases* 7: 363-377.

Clifton-Hadley, R.S., R.H. Richards and D. Bucke. 1986. Proliferative kidney disease (PKD) in rainbow trout, *Salmo gairdneri*: Further observations on the effects of water temperature. *Aquaculture* 55: 165-171.

Clifton-Hadley, R.S., D. Bucke and R.H. Richards. 1987. A study of the sequential clinical and pathological changes during proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 10: 335-352.

Crawshaw, M.T. and R.A. Sweeting. 1986. *Myxobolus koi* Kudo, 1919: A new record for Britain. *Journal of Fish Diseases* 9: 465-467.

Csaba, G., E. Kovács-Gayer, L. Bekesi, M. Bucsek, J. Szakolczai and K. Molnár. 1984. Studies into the possible protozoan aetiology of swimbladder inflammation in carp fry. *Journal of Fish Diseases* 7: 39-56.

Current, W.L. and J. Janovy. 1978. Comparative study of ultrastructure of interlamellar and intralamellar types of *Henneguya exilis* Kudo from channel catfish. *Journal of Protozoology* 25: 56-65.

De Kinkelin, P., M. Gay and S. Forman. 2002. The persistence of infectivity of *Tetracapsula bryosalmonae*-infected water for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 25: 477-482.

Diamant, A. 1992. A new pathogenic histozoic *Myxidium* (Myxosporea) in cultured gilthead seabream *Sparus aurata* L. *Bulletin of the European Association of Fish Pathologists* 12: 64-66.

Diamant, A. 1997. Fish-to-fish transmission of a marine myxosporean. *Diseases of Aquatic Organisms* 30: 99-105.

Diamant, A. 1998. Red drum *Sciaenops ocellatus* (Sciaenidae), a recent introduction to Mediterranean mariculture, is susceptible to *Myxidium leei* (Myxosporea). *Aquaculture* 162: 33-39.

Diamant, A. and I. Paperna. 1992. *Zschokkella icterica* sp. nov. (Myxozoa, Myxosporea), a pathogen of wild rabbitfish *Siganus luridus* (Ruppell, 1829) from the Red Sea. *European Journal of Protistology* 28: 71-78.

Diamant, A., J. Lom and I. Dyková. 1994. *Myxidium leei* n. sp., a pathogenic myxosporean of cultured seabream *Sparus aurata*. *Diseases of Aquatic Organisms* 20: 137-141.

Diamant, A., C.M. Whipps and M.L. Kent. 2004. A new species of *Sphaeromyxa* (Myxosporea: Sphaeromyxina: Sphaeromyxidae) in devil firefish, *Pterois miles* (Scorpaenidae), from the northern Red Sea: Morphology, ultrastructure and phylogeny. *Journal of Parasitology* 90: 1434-1442.

Diamant, A., M. Ucko, I. Paperna, A. Colorini and A. Lipshitz. 2005. *Kudoa iwatai* (Myxosporea: Multivalvulida) in wild and cultured fish in the Red Sea: Redescription and molecular phylogeny. *Journal of Parasitology* 91: 1175-1189.

Davies, J.A. 1985. *Zschokkella russelli* Tripathi (Myxozoa: Myxosporea) from five-bearded rockling, *Ciliata mustela* L. (Teleostei: Gadidae) in Wales. *Journal of Fish Diseases* 8: 229-308.

Dyková, I. and J. Lom. 1988a. Review of pathogenic myxosporeans in intensive culture of carp (*Cyprinus carpio*) in Europe. *Folia Parasitologica* 35: 289-307.

Dyková, I. and J. Lom. 1988b. *Chloromyxum reticulatum* (Myxozoa: Myxosporea) in the liver of burbot (*Lota lota* L.) and its migration to the final site of infection. *European Journal of Protistology* 23: 258-261.

Dyková, I., J. Lom and M. Cirkovic. 1986. Brain myxoboliasis of common carp (*Cyprinus carpio*) due to *Myxobolus encephalicus*. *Bulletin of the European Association of Fish Pathologists* 6: 10-12.

Dyková, I., J. Lom and G. Grupcheva. 1987. Pathogenicity and some structural features of *Myxidium rhodei* (Myxozoa: Myxosporea) from the kidney of the roach *Rutilus rutilus*. *Diseases of Aquatic Organisms* 2: 109-115.

Dyková, I., J. Lom and W. Körting. 1990. Light and electron microscopic observations on the swimbladder stages of *Sphaerospora renicola*, a parasite of carp (*Cyprinus carpio*). *Parasitology Research* 76: 228-237.

Dyková, I., E.J. Fajer Avila and I. Fiala. 2002a. *Kudoa dianae* sp. n. (Myxosporea: Multivalvulida), a new parasite of bullseye puffer, *Sphoeroides annulatus* (Tetraodontiformes: Tetraodontidae). *Folia Parasitologica* 49: 17-23.

Dyková, I., I. Fiala and P. Nie. 2002b. *Myxobolus lentisuturalis* sp. n. (Myxozoa: Myxobolidae), a new muscle-infecting species from the Prussian carp, *Carassius gibelio* from China. *Folia Parasitologica* 49: 252-258.

Dyková, I., T. Tymal, I. Fiala and J. Lom. 2007. New data on *Soricimyxum fegati* (Myxozoa) including analysis of its phytogenetic position inferred from the SSU rRNA gene sequence. *Folia Parasitologica* 54: 272-276.

Dzulinsky, K., D.K. Cone, G.T. Faulkner and R. Cusak. 1994. Development of *Myxobolus neurophilus* (Guilford, 1963) (Myxosporea) in the brain of yellow perch (*Perca flavescens*) in Vinegar Lake, Nova Scotia. *Canadian Journal of Zoology* 1180-1185.

Easy, R.H., S.C. Johnson and D.K. Cone. 2005. Morphological and molecular comparison of *Myxobolus procerus* (Kudo, 1934) and *M. intramusculi* n. sp. (Myxozoa) parasitising muscles of the troutperch *Percopsis omiscomaycus*. *Systematic Parasitology* 61: 115-122.

Egusa, S. and K. Nakajima. 1980. *Kudoa amamiensis* n. sp. (Myxosporea: Multivalvulida) found in cultured yellowtails and wild damsel fishes from Amami-Oshima and Okinawa, Japan. *Bulletin of Japanese Society of Scientific Fisheries* 46: 1193-1198.

Egusa, S. and K. Nakajima. 1981. A new myxozoa *Thelohanellus kitauei*, the cause of intestinal giant cystic disease in carp. *Fish Pathology* 15: 213-218.

Egusa, S. 1985. *Myxobolus buri* sp. n. (Myxosporea: Bivalvulida) parasitic in the brain of *Seriola quinqueradiata* Temminck *et* Schlegel. *Fish Pathology* 19: 239-244.

Eiras, J.C. 2002. Synopsis of the species of the genus *Henneguya* Thélohan, 1892 (Myxozoa: Myxosporea: Myxobolidae). *Systematic Parasitology* 52: 43-54.

Eiras, J.C. 2005. An overview on the myxosporean parasites in amphibians and reptiles. *Acta Parasitologica* 50: 267-275.

Eiras, J.C., K. Molnár and Y.S. Lu. 2005. Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea: Myxobolidae). *Systematic Parasitology* 61: 1-46.

El-Mansy, A. 2002. Immature stages and re-description of *Henneguya suprabranchiae* (Myxosporea: Myxobolidae), an intestinal parasite of the catfish *Clarias gariepinus* in the River Nile, Egypt. *Diseases of Aquatic Organisms* 51: 179-186.

El-Mansy, A. and K. Molnár. 1997. Extrapiscine development of *Myxobolus drjagini* Akhmerov, 1954 (Myxosporea: Myxobolidae) in oligochaete alternate hosts. *Acta Veterinaria Hungarica* 45: 427-438.

El-Mansy, A., Cs. Székely and K. Molnár. 1998a. Studies on the occurrence of actinosporean stages of fish myxosporeans in a fish farm of Hungary, with description of *Triactinomyxon, Raabeia, Aurantiactinomyxon* and *Neoactinomyxon* types. *Acta Veterinaria Hungarica* 46: 259-284.

El-Mansy, A., Cs. Székely and K. Molnár. 1998b. Studies on the occurrence of actinosporean stages of myxosporeans in Lake Balaton, Hungary, with the description of *Triactinomyxon, Raabeia* and *Aurantiactinomyxon* types. *Acta Veterinaria Hungarica* 46: 437-450.

El-Matbouli, M. and R.W. Hoffman. 1991. Experimental transmission of *Myxobolus cerebralis* and *Myxobolus pavlovskii* and their development in tubificids. *Fischerei-Forschung, Rostock* 29: 70-75. (In German).

El-Matbouli, M., T. Fischer-Scherl and R.W. Hoffmann. 1990. Light and electron microscopic studies on *Myxobolus cotti* El-Matbouli and Hoffmann, 1987 infecting the central nervous system of the bullhead (*Cottus gobio*). *Parasitological Research* 76: 219-227.

El-Matbouli, M., T. Fischer-Scherl and R.W. Hoffman. 1992. Present knowledge on the life cycle, taxonomy, pathology, and therapy of some *Myxosporea* spp. important for freshwater fish. *Annual Review of Fish Diseases* 2: 367-402.

El-Matbouli, M., R.W. Hoffmann and C. Mandok. 1995. Light and electron microscopic observations on the route of the triactinomyxon-sporoplasm of *Myxobolus cerebralis* from the epidermis into rainbow trout cartilage. *Journal of Fish Biology* 46: 919-935.

El-Matbouli, M., T.S. McDowell, D.B. Antonio, K.B. Andree and R.P. Hedrick. 1999a. Effect of water temperature on the development, release and survival of the triactinomyxon stage of *Myxobolus cerebralis* in its oligochaete host. *International Journal for Parasitology* 29: 627-641.

El-Matbouli, M., R.W. Hoffman, H. Schoel, T.S. McDowell and R.P. Hedrick. 1999b. Whirling Disease: host specificity and interaction between the actinosporean stage of *Myxobolus cerebralis* and rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 35: 1-12.

Feist, S.W. 1997. Pathogenicity of renal myxosporeans of fish. *Bulletin of the European Association of Fish Pathologists* 17: 209-214.

Feist, S.W. and D. Bucke. 1987. Ultrastructural aspects of PKX, the causative agent of proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 10: 323-327.

Feist, S.W. and D. Bucke. 1992. *Myxidium gadi* Georgévitch, 1916 infections in saithe *Pollachius virens* L. from the North Sea. *Bulletin of the European Association of Fish Pathologists* 12: 211-214.

Feist, S.W. and D. Bucke. 1993. Proliferative kidney disease in wild salmonids. *Fisheries Research* 17: 51-58.

Feist, S.W. and P. Rintamäki. 1994. *Chloromyxum truttae* Legér, 1906 infection from cultured salmonids from Finland. *Bulletin of the European Association of Fish Pathologists* 14: 51-54.

Feist, S.W., M. Longshaw, E.U. Canning and B. Okamura. 2001. Induction of proliferative kidney disease (PKD) in rainbow trout (*Oncorhynchus mykiss*) via the bryozoan *Fredericella sultana*, infected with *Tetracapsula bryosalmonae*. *Diseases of Aquatic Organisms* 45: 61-68.

Feist, S.W., E.J. Peeler, R. Gardiner, E. Smith and M. Longshaw. 2002. Proliferative kidney disease and renal myxosporidiosis in juvenile salmonids from rivers in England and Wales. *Journal of Fish Diseases* 25: 451-458.

Ferguson, H.W. and B. McC. Adair. 1977. Protozoa associated with proliferative kidney disease in rainbow trout (*Salmo gairdneri*). *Veterinary Record* 100: 158-159.

Ferguson, H.W. and E.A. Needham. 1978. Proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 1: 91-108.

Ferguson, H.W., J. Lom and I. Smith. 1985. Intra-axonal parasites in the fish *Notropis cornutus* (Mitchell). *Veterinary Pathology* 22: 194-196.

Fernández-de-Luco, D., M.A. Peribáñez, L. García and J.A. Castillo. 1997. Granulomatous myositis in rainbow trout *Oncorhynchus mykiss* affected by proliferative kidney disease (PKD). *Diseases of Aquatic Organisms* 31: 49-54.

Fiala, I. 2006. The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. *International Journal for Parasitology* 36: 1521-1534.

Fioravanti, M.L., M. Caffara, D. Florio, A. Gustinelli and F. Marcer. 2004. *Sphaerospora dicentrarchi* and *S. testicularis* (Myxozoa: Sphaerosporidae) in farmed European seabass (*Dicentrarchus labrax*) from Italy. *Folia Parasitologica* 51: 208-210.

Foott, J.S. and R.P. Hedrick. 1987. Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. *Journal of Fish Biology* 30: 477-483.

Frasca, S., S.L. Poynton, A.B. West and H.J. Van Kruiningen. 1998. Epizootiology, pathology, and ultrastructure of the myxosporean associated with parasitic encephalitis of farmed Atlantic salmon *Salmo salar* in Ireland. *Diseases of Aquatic Organisms* 32: 211-225.

Frasca Jr., S., D.R. Linfert, G.J. Tsongalis, T.S. Gorton, A.E. Garmendia, R.P. Hedrick, A.B. West and H.J. Van Kruiningen. 1999. Molecular characterization of the myxosporean associated with parasitic encephalitis of farmed Atlantic salmon *Salmo salar* in Ireland. *Diseases of Aquatic Organisms* 35: 221-233.

Friedrich, C., E. Ingolic, B. Freitag, G. Kastberger, V. Hohmann, G. Skofitsch, U. Neumeister and O. Kepka. 2000. A myxozoan-like parasite causing xenomas in the brain of the mole, *Talpa europaea* L., 1758 (Vertebrata, Mammalia). *Parasitology* 121: 483-492.

Furuta, T., K. Ogawa and H. Wakabayashi. 1993. Humoral imune response of carp *Cyprinus carpio* to *Myxobolus artus* (Myxozoa: Myxobolidae) infection. *Journal of Fish Biology* 43: 441-450.

Garner, M.M., J.L. Bartholomew, C.M. Whipps, R.W. Nordhausen and P. Raiti. 2005. Renal myxozoanosis in Crowned River turtles *Hardella thurjii*: Description of the putative agent *Myxidium hardella* n. sp. by histopathology, electron microscopy, and DNA sequencing. *Veterinary Pathology* 42: 589-595.

Gay, M., B. Okamura and P. de Kinkelin. 2001. Evidence that infective stages of *Tetracapsula bryosalmonae* for rainbow trout *Oncorhynchus mykiss* are present throughout the year. *Diseases of Aquatic Organisms* 46: 31-40.

Gbankoto, A., C. Pampoulie, A. Marques and G.N. Sakiti. 2001. *Myxobolus dahomeyensis* infection in ovaries of Tilapia species from Benin (West Africa). *Journal of Fish Biology* 58: 883-886.

Gerundo, N., D.J. Alderman, R.S. Clifton-Hadley and S.W. Feist. 1991. Pathological effects of repeated doses of malachite green: A preliminary study. *Journal of Fish Diseases* 14: 521-532.

Gonzalez-Lanza, C. and P. Alvarez-Pellitero. 1984. *Myxobolus farionis* n. sp. and *M. ibericus* n. sp. of *Salmo trutta* m. *fario* from the Deuro basin (NW Spain). Description and population dynamics. *Angewandte Parasitologie* 25: 181-189.

Granath, W.O. and M.A. Gilbert. 2002. The role of *Tubifex tubifex* (Annelida: Oligochaeta: Tubificidae) in the transmission of *Myxobolus cerebralis* (Myxozoa: Myxosporea: Myxobolidae). In: *Whirling Disease: Reviews and Current Topics*, J.L. Bartholomew and J.C. Wilson (eds.). *American Fisheries Society, Symposium* 29, Bethesda, Maryland, pp. 79-85.

Grassé, P. -P. 1970. Embranchement des Myxozaires. In: *Précis de Zoologie 1, Invértebrés*, P.-P. Grassé, R.A. Poisson and O. Tuzet (eds.). Mason et Cie, Paris, pp. 107-112.

Griffin, B.R. and E.M. Davis. 1978. *Myxosoma cerebralis*: detection of circulating antibodies in infected rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Board of Canada* 35: 1186-1190.

Grossel, G.W., I. Dykova, J. Handlinger and B.L. Munday. 2003. *Pentacapsula neurophila* n. sp. (Multivalvulida) from the cental nervous system of striped trumpeter, *Latris lineata* (Forster). *Journal of Fish Diseases* 26: 315-320.

Grossheider, G. and W. Körting. 1993. Experimental transmission of *Sphaerospora renicola* to common carp *Cyprinus carpio* fry. *Diseases of Aquatic Organisms* 16: 91-95.

Hallett, S.L. and J.L. Bartholomew. 2006. Application of real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in river water samples. *Diseases of Aquatic Organisms* 71: 109-118.

Hamilton, A.J. and E.U. Canning. 1988. The production of mouse anti-*Myxosoma cerebralis* antiserum from Percoll®-purified spores and its use in immunoflourescent labelling of Historesin®-embedded cartilage derived from infected rainbow trout. *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 11: 185-190.

Hanson, L.A., D. Lin, L.M.W. Pote and R. Shivaji. 2001. Small subunit rRNA gene comparisons of four actinosporean species to establish a polymerase chain reaction test for the causative agent of proliferative gill disease in channel catfish. *Journal of Aquatic Animal Health* 13: 117-123.

Harrell, L.W. and T.M. Scott. 1985. *Kudoa thyrsites* (Gilchrist) (Myxosporea: Multivalvulida) in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 8: 329-332.

Hedrick, R.P., M.L. Kent, R.J. Toth and J.K. Morrison. 1988a. Fish infected with *Sphaerospora* spp. Thélohan (Myxosporea) from waters enzootic for proliferative kidney disease of salmonids. *Journal of Protozoology* 35: 13-18.

Hedrick, R.P., P.F. Groff and T. McDowell. 1988b. Oral administration of Fumagillin DCH protects chinook salmon *Oncorhynchus tshawytscha* from experimentally induced proliferative kidney disease. *Diseases of Aquatic Organisms* 4: 165-168.

Hedrick, R.P., J.M. Groff and T.S. McDowell. 1990. Gill sphaerosporosis in goldfish (*Carassius auratus*). *Journal of Wildlife Diseases* 26: 558-560.

Hedrick, R.P., E. MacConnell and P. de Kinkelin. 1993. Proliferative kidney disease of salmonid fish. *Annual Review of Fish Diseases* 3: 277-290.

Hedrick, R.P., M. El-Matbouli, M.A. Adkison and E. MacConnell. 1998. Whirling disease—Re-emergence among wild trout. *Immunological Reviews* 166: 365-376.

Hedrick, R.P., D.V. Baxa, P. De Kinkelin and B. Okamura. 2004. Malacosporean-like spores in urine of rainbow trout react with antibody and DNA probes to *Tetracapsuloides bryosalmonae*. *Parasitology Research* 92: 81-88.

Helke, K.L. and S.L. Poynton. 2005. *Myxidium mackiei* (Myxosporea) in Indo-Gangetic flap-shelled turtles *Lissemys punctata andersonii*: Parasite-host interaction and ultrastructure. *Diseases of Aquatic Organisms* 63: 215-230.

Hemmingsen, W., I. Lombardo and K. MacKenzie. 1991. Parasites as biological tags for cod, *Gadus morhua* L., in northern Norway: A pilot study. *Fisheries Research* 12: 365-373.

Henderson, M. and B. Okamura. 2004. The phylogeography of salmonid proliferative kidney disease in Europe and North America. *Proceedings of the Royal Society of London* B 271: 1729-1736.

Hendrickson, G.L., A. Carleton and D. Manzer. 1989. Geographic and seasonal distribution of the infective stage of *Ceratomyxa shasta* (Myxozoa) in northern California. *Diseases of Aquatic Organisms* 7: 165-169.

Hessen, E.M. and M.L. Zamzame. 2004. *Myxobolus* sp.: A possible new opportunistic parasite in immunocompromised patients in Ismailia. *Journal of the Egyptian Society of Parasitology* 34: 925-930.

Hervio, D.M.L., M.L. Kent, J. Khattra, J. Sakanari, H. Yokoyama and R.H. Devlin. 1997. Taxonomy of *Kudoa* species (Myxosporea), using a small-subunit ribosomal DNA sequence. *Canadian Journal of Zoology* 75: 2112-2119.

Heupel, M.R. and M.B. Bennett. 1996. A myxosporean parasite (Myxosporea: Multivalvulida) in the skeletal muscle of epaulette sharks, *Hemiscyllium ocellatum* (Bonnaterre), from the Great Barrier Reef. *Journal of Fish Diseases* 19: 189-191.

Higgins, M.J. and M.L. Kent. 1998. TNP-470, the analogue of fumagillin-DCH, controls PKX in naturally infected sockeye salmon, *Oncorhynchus nerka* (Walbaum), underyearlings. *Journal of Fish Diseases* 21: 455-457.

Hine, P.M. 1980. A review of some species of *Myxidium* Bütschli, 1882 (Myxosporea) from eels (*Anguilla* spp.). *Journal of Protozoology* 27: 260-267.

Hofer, B. 1906. *Handbuch der Fischkrankheiten*. Schweizerbart'sche Verlagsbuchandlung, Stuttgart.

Hoffman, G.L. 1975. Whirling disease (*Myxosoma cerebralis*) control with ultraviolet irradiation and effect on fish. *Journal of Wildlife Diseases* 11: 505-507.

Hoffman, G.L. 1984. Two fish pathogens, *Parvicapsula* sp. and *Mitraspora cyprini* myxosporea, new to North America. *Symposia Biologica Hungarica* 23: 127-135.

Hoffmaster, J.L., J.E. Sanders, J.S. Rohovec, J.L. Fryer and D.G. Stevens. 1988. Geographic distribution of the myxosporean parasite, *Ceratomyxa shasta* Noble, 1950, in the Columbia River basin. *Journal of Fish Diseases* 11: 97-100.

Holland, J.W., C.R.W. Gould, C.S. Jones, L.R. Noble and C.J. Secombes. 2003. The expression of immune-regulatory genes in rainbow trout, *Oncorhynchus mykiss*, during a natural outbreak of proliferative kidney disease (PKD). *Parasitology* 126: S95-S102.

Holliman, A. 1994. Sea trout, *Salmo trutta*, a new host for the myxosporean *Kudoa thyrsites* (Gilchrist)? *Veterinary Record* 134: 524-525.

Holzer, A.S., C. Sommerville and R. Wootten. 2003. Tracing the route of *Sphaerospora truttae* from the entry locus to the target organ of the host, *Salmo salar* L., using an optimized and specific *in situ* hybridization technique. *Journal of Fish Diseases* 26: 647-655.

Holzer, A.S., C. Sommerville and R. Wootten. 2004. Molecular relationships and phylogeny in a community of myxosporeans and actinosporeans based on their 18s rDNA sequences. *International Journal for Parasitology* 34: 1099-1111.

Hsieh, S.R. and C.L. Chen. 1984. *Septemcapsula yasunagai* gen. et sp. nov., Representative of a new family of the class Myxosporea. *Acta Zootaxonomie Sinica* 9: 225-227.

Ibarra, A.M., G.A.E. Gall and R.P. Hedrick. 1991. Susceptibility of two strains of rainbow trout *Oncorhynchus mykiss* to experimentally induced infections with the myxosporean *Ceratomyxa shasta*. *Diseases of Aquatic Organisms* 10: 191-194.

Ibarra, A.M., R.P. Hedrick and G.A.E. Gall. 1992. Inheritance of susceptibility to *Ceratomya shasta* (Myxozoa) in rainbow trout and the effect of length of exposure on the liability to develop ceratomyxosis. *Aquaculture* 104: 217-229.

Jiménez-Guri, E., B. Okamura and P.W.H. Holland. 2007a. Origin and evolution of a myxozoan worm. *Integrative and Comparative Biology* 47: 752-758.

Jiménez-Guri, E., H. Philippe, B. Okamura and P.W.H. Holland. 2007b. *Buddenbrokia* is a cnidarian worm. *Science* 317: 116-118.

Johnstone, A.K. 1984. *Pathogenesis and life cycle of the myxosporean Parvicapsula sp. infecting marine cultured coho salmon*. Ph.D. Dissertation University of Washington, Seattle, Washington.

Jones, S.R.M., G. Prosperi-Porta, S.C. Dawe and D.P. Barnes. 2003. Distribution, prevalence and severity of *Parvicapsula minibicornis* infections among anadromous salmonids in the Fraser River, British Columbia, Canada. *Diseases of Aquatic Organisms* 54: 49-54.

Jones, S., G. Prosperi-Porta, S. Dawe, J. Blackbourn, K. Taylor, G. Lowe and A. Osborn. 2004. Proliferative renal myxosporidiosis in spawning coho salmon (*Oncorhynchus kisutch*) in British Columbia and Washington. *Folia Parasitologica* 51: 221-227.

Jurine, L.L. 1825. *Histoire des poissons du lec Leman*. Memoires de la Société de Physique et Histoire Naturelle, Volume 3.

Kabata, Z. 1963. Parasites as biological tags. *International Commission on Northwest Atlantic Fisheries*, Special Publications 4: 31-37.

Kallert, D.M., M. El-Matbouli and W. Haas. 2005. Polar filament discharge of *Myxobolus cerebralis* actinospores is triggered by combined non-specific mechanical and chemical cues. *Parasitology* 131: 609-616.

Kallert, D.M., S. Ponader, E. Eszterbauer, M. El-Matbouli and W. Haas. 2007. Myxozoan transmission via actinospores: new insights into mechanisms and adaptations for host invasions. *Parasitology* 134: 1741-1750.

Karagouni, E., F. Athanassopoulou, A. Lytra, C. Komis and E. Dotsika. 2005. Antiparasitic and immunmodulatory effect of innovative treatments against *Myxobolus* sp. infection in *Diplodus puntazzo*. *Veterinary Parasitology* 134: 215-228.

Karlsbakk, E., P.A. Sæther, C. Høstlund, K.R. Fjellsøy and A. Nylund. 2002. *Parvicapsula pseudobranchiola* n. sp. (Myxozoa), a myxosporidian infecting the pseudobranch of cultured Atlantic salmon (*Salmo salar*) in Norway. *Bulletin of the European Association of Fish Pathologists* 22: 381-387.

Kaup, F.-J., E.-M. Kuhm and W. Körting. 1995. Licht- und elektronenmikroskopische untersuchungen zur sporogenese von *Sphaerospora molnari* in den kiemenlamellan des karpfens (*Cyprinus carpio*). *Berliner und Münchener Tierärztliche Wochenschrift* 108: 206-214.

Kelley, G., M.A. Adkinson, C.M. Leutenegger and R.P. Hedrick. 2003. *Myxobolus cerebralis*: identification of a cathepsin Z-like protease gene (MyxCP-1) expressed during parasite development in rainbow trout, *Oncorhynchus mykiss*. *Experimental Parasitology* 105: 201-210.

Kelley, G., K.A. Beauchamp and R.P. Hedrick. 2004. Phylogenetic comparison of the Myxosporea based on an actin cDNA isolated from *Myxobolus cerebralis*. *Journal of Eukaryotic Microbiology* 51: 660-663.

Kent, M.L. 2000. Marine netpen farming leads to infections with some unusual parasites. *International Journal for Parasitology* 30: 321-326.

Kent, M.L. and G.L. Hoffman. 1984. *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. from the brain of South American knife fish, *Eigemannia virescens* (V.). *Journal of Protozoology* 31: 91-94.

Kent, M.L. and R.P. Hedrick. 1985. Transmission of the causative agent of proliferative kidney disease (PKD) with the blood and spleen of infected fish; Further evidence that the PKX parasite belongs to the phylum Myxozoa. *Bulletin of the European Association of Fish Pathologists* 5: 39-42.

Kent, M.L., D.J. Whitaker and L. Margolis. 1993. Transmission of *Myxobolus arcticus* Pugachev and Khokhlov, 1979, a myxosporean parasite of Pacific salmon, via a triactinomyxon from the aquatic oligochaete *Stylodrilus heringianus* (Lumbriculidae). *Canadian Journal of Zoology* 71: 1207-1211.

Kent, M.L., L. Margolis and J.O. Corliss. 1994a. The demise of a class of protists: taxonomic and nomenclatural revisions proposed for the protist phylum Myxozoa Grassé, 1970. *Canadian Journal of Zoology* 72: 932-937.

Kent, M.L., L. Margolis, D.J. Whitaker, G.E. Hoskins and T.E. McDonald. 1994b. Review of myxosporea of importance in salmonid fisheries and aquaculture in British Columbia. *Folia Parasitologica* 41: 27-37.

Kent, M.L., J.W. Bagshaw, J. Nener and B. Raymond. 1996. *Myxobolus cyprini* Doflein, 1898, in peamouth: first report of this myxosporean in the western hemisphere. *Journal of Aquatic Animal Health* 8: 159-162.

Kent, M.L., D.J. Whitaker and S.C. Dawe. 1997. *Parvicapsula minibicornis* n. sp. (Myxozoa: Myxosporea) from the kidney of sockeye salmon (*Oncorhynchus nerka*) from British Columbia, Canada. *Journal of Parasitology* 83: 1153-1156.

Kent, M.L., J. Khattra, D.M.L. Hervio and R. Devlin. 1998. Ribosomal DNA sequence analysis of the PKX myxosporean and their relationship to members of the genus *Sphaerospora*. *Journal of Aquatic Animal Health* 10: 12-21.

Kent, M.L., J. Khattra, R.P. Hedrick and R.H. Devlin. 2000. *Tetracapsula renicola* n. sp. (Myxozoa: Saccosporidae); the PKX myxozoan—The cause of proliferative kidney disease of salmonid fishes. *Journal of Parasitology* 86: 103-111.

Kent, M.L., K.B. Andree, J.L. Bartholomew, M. El-Matbouli, S.S. Desser, R.H. Devlin, S.W. Feist, R.P. Hedrick, R.W. Hoffman, J. Khattra, S.L. Hallett, R.J.G. Lester, M. Longshaw, O. Palenzuela, M.E. Siddall and C.X. Xiao. 2001. Recent advances in our knowledge of the Myxozoa. *Journal of Eukaryotic Microbiology* 48: 395-413.

Kent, M.L., V.G. Watral, C.M. Whipps, M.E. Cunningham, C.D. Criscione, J.R. Heidel, L.R. Curtis, J. Spitsbergen and D.F. Markle. 2004. A digenean metacercaria (*Apophallus* sp.) and a myxozoan (*Myxobolus* sp.) associated with vertebral deformities in cyprinid fishes from the Willamette Rover, Oregon. *Journal of Aquatic Animal Health* 16: 116-129.

Køie, M. 2000. First record of an actinosporean (Myxozoa) in a marine polychaete annelid. *Journal of Parasitology* 86: 871-872.

Køie, M., C.M. Whipps and M.L. Kent. 2004. *Ellipsomyxa gobii* (Myxoza: Ceratomyxidae) in the common goby *Pomatoschistus microps* (Teleostei: Gobiidae) uses *Nereis* spp. (Annelida: Polychaeta) as invertebrate hosts. *Folia Parasitologica* 51: 14-18.

Körting, W. 1982. Protozoan parasites associated with swimbladder inflammation (SBI) in young carp. *Bulletin of the European Association of Fish Pathologists* 2: 25-28.

Kovács-Gayer, E. 1983. Histopathological studies on protozoan swimbladder inflammation of carp fry. *Parasitologica Hungarica* 16: 39-46.

Kovács-Gayer, É. and K. Molnár. 1983. Studies on the biology and pathology of the common carp parasite *Myxobolus basilamellaris* Lom et Molnár, 1983 (Myxozoa: Myxosporea). *Acta Veterinaria Hungarica* 31: 91-102.

Knaus, M. and M. El-Matbouli. 2005. Characterisation of carbohydrate binding sites in developmental stages of *Myxobolus cerebralis*. *Parasitological Research* 97: 505-514.

Landsberg, J.H. 1993. Kidney myxosporean parasites in red drum *Sciaenops ocellatus* (Sciaenidae) from Florida, USA, with a description of *Parvicapsula renalis* n. sp. *Diseases of Aquatic Organisms* 17: 9-16.

Langdon, J.S. 1987. Spinal curvatures and an encephalotrophic myxosporean, *Triangula percae* sp. nov. (Myxozoa: Ortholineidae), enzootic in redfin perch, *Perca fluviatilis* L., in Australia. *Journal of Fish Diseases* 10: 425-434.

Langdon, J.S. 1990. Observations on new *Myxobolus* species and *Kudoa* species infecting the nervous system of Australian fishes. *Journal of Applied Ichthyology* 6: 107-116.

Larsen, G., W. Hemmingsen, K. MacKenzie and D.A. Lysne. 1997. A population study of cod, *Gadus morhua* L., in northern Noorway using otoloith structure and parasite tags. *Fisheries Research* 32: 13-20.

Lebbad, M. and M. Wilcox. 1998. Spores of *Henneguya salminicola* in human stool specimens. *Journal of Clinical Microbiology* 36: 1820.

Le Breton, A. and A. Marques. 1995. Occurrence of a histozoic *Myxidium* infection in two marine cultured species: *Puntazzo puntazzo* and *Pagurus major*. *Bulletin of the European Association of Fish Pathologists* 15: 210-212.

Levsen, A., T. Alvik and S. Grotmol. 2004. Neurological symptoms in tricolour sharkminnow *Balantiocheilos melanopterus* associated with *Myxobolus balantiocheili* n. sp. infecting the central nervous system. *Diseases of Aquatic Organisms* 59: 135-140.

Lin, D., L.A. Hanson and L.M. Pote. 1999. Small subunit ribosomal RNA sequence of *Henneguya exilis* (Class Myxosporea) identifies the actinosporean stage from an oligochaete host. *Journal of Eukaryotic Microbiology* 46: 66-68.

Liyanage, Y.S., H. Yokoyama and H. Wakabayashi. 2003. Evaluation of a vector-control strategy of haemorrhagic thelohanellosis in carp, caused by *Thelohanellus hovorkai* (Myxozoa). *Diseases of Aquatic Organisms* 55: 31-35.

Lom, J. and J.R. Arthur. 1989. A guideline for the preparation of species descriptions in Myxosporea. *Journal of Fish Diseases* 12: 151-156.

Lom, J. and I. Dyková. 1981. Pathogenicity of some protozoan parasites of cyprinid fishes. In: *Fish Pathogens and Environment in European Polyculture*, J. Olah, K. Molnár and Z. Jeney (eds.). *Proceedings of an International Seminar on Fish, Pathogens and Environment in European Polyculture*, June 23-27, 1981. Szarvas, Hungary, Fisheries Research Institute, Szarvas. pp. 146-169.

Lom, J. and I. Dyková. 1992. *Protozoan Parasites of Fish. Developments in Aquaculture and Fisheries Science*, Vol. 26. Elsevier Amsterdam.

Lom, J. and I. Dyková. 1993. Scanning electron microscopic revision of common species of the genus *Chloromyxum* (Myxozoa: Myxosporea) infecting European freshwater fishes. *Folia Parasitologica* 40: 161-174.

Lom, J. and I. Dyková. 1997. Ultrastructural features of the actinosporean phase of Myxosporea (Myxozoa): A comparative study. *Acta Protozoologica* 36: 83-103.

Lom, J. and I. Dyková. 2006. Myxozoan genera: definition and notes on taxonomy, life cycle terminology and pathogenic species. *Folia Parasitologica*. (In Press).

Lom, J. and E.R. Noble. 1984. Revised classification of the Myxosporea Bütschli, 1881. *Folia Parasitologica* 31: 193-205.

Lom, J., W. Körting and I. Dyková. 1985. Light and electron microscope redescription of *Sphaerospora tincae* Plehn, 1925 and *S. galinae* Evlanov, 1981 (Myxosporea) from the tench, *Tinca tinca* L. *Protistologica* 21: 487-497.

Lom, J., I. Dyková and S. Feist. 1989a. Myxosporea-induced xenoma formation in pike (*Esox lucius* L.) renal corpuscles associated with *Myxidium lieberkuehni* infection. *European Journal of Protistology* 24: 271-280.

Lom, J., S.W. Feist, I. Dyková and T. Kepr. 1989b. Brain myxoboliasis of bullhead, *Cottus gobio* L., due to *Myxobolus jiroveci* sp. nov.: Light and electron microscope observations. *Journal of Fish Diseases* 12: 15-27.

Lom, J., A.W. Pike and I. Dyková. 1991. *Myxobolus sandrae* Reuss, 1906, the agent of vertebral column deformities of perch *Perca fluviatilis* in northeast Scotland. *Diseases of Aquatic Organisms* 12: 49-53.

Lom, J., J. McGeorge, S.W. Feist, D. Morris and A. Adams. 1997. Guidelines for the uniform characterisation of the actinosporean stages of parasites of the phylum Myxozoa. *Diseases of Aquatic Organisms* 30: 1-9.

Longshaw, M., R.M. LeDeuff, A.F. Harris and S.W. Feist. 2002. Development of Proliferative Kidney Disease (PKD) in rainbow trout, *Oncorhynchus mykiss*, following short-term exposure to *Tetracapsula bryosalmonae* infected bryozoans. *Journal of Fish Diseases* 25: 443-449.

Longshaw, M., P. Frear and S.W. Feist. 2003. *Myxobolus buckei* sp. n. (Myxozoa), a new pathogenic parasite from the spinal column of three cyprinid fish species from the United Kingdom. *Folia Parasitologica* 50: 251-262.

Longshaw, M., P.A. Frear and S.W. Feist. 2005. Descriptions, development and pathogenicity of myxozoan (Myxozoa: Myxosporea) parasites of juvenile cyprinids (Pisces: Cyprinidae). *Journal of Fish Diseases* 28: 489-508.

Lowenstine, L.J., B.A. Rideout, M. Gardner, M. Busch, M. Mace, J. Bartholomew and C.H. Gardiner. 2002. Myxozoanosis in waterfowl: A new host record? *Proceedings of the American Society of Zoo Veterinarians* 2002: 86-87.

Lowers, J.M. and J.L. Bartholomew. 2003. Detection of myxozoan parasites in oligochaetes imported as food for ornamental fish. *Journal of Parasitology* 89: 84-91.

Lu, Y.S., P. Nie and B.J. Sun. 2002. Detection of *Myxobolus rotundus* (Myxozoa: Myxosporea) in skin mucus of crucian carp *Carassius auratus auratus* using a monoclonal antibody. *Diseases of Aquatic Organisms* 54: 171-173.

Lu, Y.S., M. Li, Y.S. Wu and J.G. Wang. 2003. Antigenic study of *Myxobolus rotundus* (Myxozoa: Myxosporea) using monoclonal antibodies. *Journal of Fish Diseases* 25: 307-310.

MacConnell, E., C.E. Smith, R.P. Hedrick and C.A. Speer. 1989. Cellular inflammatory response of rainbow trout to the protozoan parasite that causes proliferative kidney disease. *Journal of Aquatic Animal Health* 1: 108-118.

MacKenzie, K., C. Kalavati, M. Gaard and W. Hemmingsen. 2005. Myxosporean gall bladder parasites of gadid fishes in the North Atlantic: Their geographical distributions and an assessment of their economic importance in fisheries and mariculture. *Fisheries Research* 76: 454-465.

Maeno, Y., M. Sorimachi, K. Ogawa and S. Egusa. 1990. *Myxobolus spinacurvatura* sp. n. (Myxosporea: Bivalvulida) parasitic in deformed mullet, *Mugil cephalus*. *Fish Pathology* 25: 37-41.

Maloney, R., R.J. Cawthorn, M. Markiw and D. Groman. 1991. The occurrence of *Myxobolus neurobius* (Myxosporea) in wild young Atlantic salmon and Arctic char in Newfoundland. *Journal of Aquatic Animal Health* 3: 146-147.

Margolis, L. 1993. A case of forensic parasitology. *Journal of Parasitology* 79: 461-462.

Margolis, L. and T.P.T. Evelyn. 1975. *Ceratomyxa shasta* (Myxosporida) disease in chum salmon (*Oncorhynchus keta*) in British Columbia. *Journal of the Fisheries Research Board Canada* 32: 1640-1643.

Marín de Mateo, M., J. McGeorge, D. Morris and M.L. Kent. 1996. Comparative studies of PKX and *Sphaerospora* spp. from salmonids using lectin and monoclonal antibody staining techniques. *Journal of Fish Diseases* 19: 55-63.

Markiw, M.E. and K. Wolf. 1978. *Myxosoma cerebralis*: Fluorescent antibody techniques for antigen recognition. *Journal of the Fisheries Research Board of Canada* 35: 828-832.

Markiw, M.E. and K. Wolf. 1983. *Myxobolus cerebralis* (Myxozoa: Myxosporea) etiological agent of salmonid whirling disease requires tubificid worm (Annelida: Oligochaeta) in its life cycle. *Journal of Protozoology* 30: 561-564.

Markiw, M.E. 1989. Portals of entry for salmonid whirling disease in rainbow trout. *Diseases of Aquatic Organisms* 6: 7-10.

Markiw, M.E. 1992. Experimentally induced whirling disease I. Dose response of fry and adults of rainbow trout exposed to the triactinomyxon stage of *Myxobolus cerebralis*. *Journal of Aquatic Animal Health* 4: 40-43.

Martínez de Velasco, G. and C. Cuéllar. 2003. Humoral immune responses induced by *Kudoa* sp. (Myxosporea: Multivalvulida) in BALB/c mice: Oral administration, immunization and cross-reactions with *Myxobolus aeglifini* (Myxosporea: Bivalvulida). *Parasite Immunology* 25: 449-456.

Martins, M.L. and de V.N. Souza. 1997. *Henneguya piaractus* n. sp. (Myxozoa: Myxobolidae), a gill parasite of *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae), in Brazil. *Revista Brasilia Biologica* 57: 239-245.

Martins, M.L., V.N. Souza, F.R. Moraes, J.R.E. Moraes, A.J. Costa and U.F. Rocha. 1997. Pathology and behavioural effects associated with *Henneguya* sp. (Myxozoa: Myxobolidae) infections of captive pacu *Piaractus mesopotamicus* in Brazil. *Journal of the World Aquaculture Society* 28: 297-300.

McClelland, R.S., D.M. Murphy and D.K. Cone. 1997. Report of spores of *Henneguya salminicola* (Myxozoa) in human stool specimens: Possible source of confusion with human spermatozoa. *Journal of Clinical Microbiology* 35: 2815-2818.

McGeorge, J., C. Sommerville and R. Wootten. 1996a. Epizootiology of *Sphaerospora trutta* (Myxozoa: Myxosporea) infections of Atlantic salmon *Salmo salar* at freshwater smolt-producing hatcheries in Scotland. *Diseases of Aquatic Organisms* 26: 33-41.

McGeorge, J., C. Sommerville and R. Wootten. 1996b. Transmission experiments to determine the relationship between *Sphaerospora* sp. from Atlantic salmon, *Salmo salar* L. and *Sphaerospora truttae* Fischer-Scherl, El-Matbouli & Hoffman, 1986, a revised description for *S. truttae*. *Folia Parasitologica* 43: 107-116.

McGurk, C., D.J. Morris, J.E. Bron and A. Adams. 2005. The morphology of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) spores released from *Fredericella sultana* (Bryozoa: Phylactolaemata). *Journal of Fish Diseases* 28: 1-6.

Mitchell, L.G. 1989. Myxobolid parasites (Myxozoa: Myxobolidae) infecting fishes of western Montana, with notes on histopathology, seasonality, and intraspecific variation. *Canadian Journal of Zoology* 67: 1915-1922.

Mitchell, L.G., C.L. Seymour and J.M. Gamble. 1985. Light and electron microscopy of *Myxobolus hendricksoni* sp. nov. (Myxozoa: Myxobolidae) infecting the brain of the fathead minnow, *Pimephales promelas* Rafinesque. *Journal of Fish Diseases* 8: 75-89.

Miyajima, S., H. Yokoyama, Y. Fukuda, K. Okamoto and K. Ogawa. 2005. A PCR method to detect *Myxobolus acanthogobii* (Myxozoa: Myxosporea), the causative agent of skeletal deformities of marine fishes. *Fish Pathology* 40: 197-199.

Moles, A. and J. Heifetz. 1998. Effects of the brain parasite *Myxobolus arcticus* on sockeye salmon. *Journal of Fish Biology* 52: 146-151.

Molnár, K. 1982. Biology and histopathology of *Thelohanellus nikolskii* Akhmerov, 1955 (Myxosporea, Myxozoa), a protozoan parasite of the common carp (*Cyprinus carpio*). *Zeitschrift für Parasitenkunde* 68: 269-277.

Molnár, K. 1988a. Presporogonic development of *Sphaerospora renicola* Dyková and Lom, 1982 in the swimbladder of common carp *Cyprinus carpio* L. *Journal of Fish Diseases* 11: 489-497.

Molnár, K. 1988b. Development of *Myxobilatus legeri* in cyprinid fishes. *Diseases of Aquatic Organisms* 4: 181-187.

Molnár, K. 1998. Taxonomic problems, seasonality and histopathology of *Henneguya creplini* (Myxosporea) infection of the pikeperch *Stizostedion lucioperca* in Lake Balaton. *Folia Parasitologica* 45: 261-269.

Molnár, K. 2002a. Site preference of fish myxosporeans in the gill. *Diseases of Aquatic Organisms* 48: 197-207.

Molnár, K. 2002b. Site preference of myxosporean spp. on the fins of some Hungarian fish species. *Diseases of Aquatic Organisms* 52: 123-128.

Molnár, K. 2002c. Differences between the European carp (*Cyprinus carpio carpio*) and the coloured carp (*Cyprinus carpio haematopterus*) in susceptibility to *Thelohanellus nikolskii* (Myxosporea) infection. *Acta Veterinaria Hungarica* 50: 51-57.

Molnár, K. and E. Kovács-Gayer. 1985. The pathogenicity and development within the fish host of *Myxobolus cyprini* Doflein, 1898. *Parasitology* 90: 549-555.

Molnár, K. and E. Kovács-Gayer. 1986a. Experimental induction of *Sphaerospora renicola* (Myxosporea) infection in common carp (*Cyprinus carpio*) by transmission of SB-protozoans. *Journal of Applied Ichthyology* 2: 86-94.

Molnár, K. and E. Kovács-Gayer. 1986b. Biology and histopathology of *Thelohanellus hovorkai* Achmerov, 1960 (Myxosporea: Myxozoa) a protozoan parasite of the common carp (*Cyprinus carpio*). *Acta Veterinaria Hungarica* 34: 67-72.

Molnár, K. and C. Székely. 1999. *Myxobolus* infection of the gills of common bream (*Abramis brama* L.) in Lake Balaton and in the Kis-Balaton reservoir, Hungary. *Acta Veterinaria Hungarica* 47: 419-432.

Molnár, K. and Cs. Székely. 2003. Infection in the fins of the goldfish *Carassius auratus* caused by *Myxobolus diversus* (Myxosporea). *Folia Parasitologica* 50: 31-36.

Molnár, K., T. Fischer-Scherl, F. Baska and R.W. Hoffmann. 1989. Hoferellosis in goldfish *Carassius auratus* and gibel carp *Carassius auratus gibelio*. *Diseases of Aquatic Organisms* 7: 89-95.

Molnár, K., A. El-Mansy, Cs. Székely and F. Baska. 1999. Experimental identification of the actinosporean stage of *Sphaerospora renicola* Dyková and Lom 1982 (Myxosporea: Sphaerosporidae) in oligochaete alternate hosts. *Journal of Fish Diseases* 22: 143-153.

Molnár, K., E. Eszterbauer, Cs. Székely, Á Dán and B. Harrach. 2002. Morphological and molecular biological studies of intramuscular *Myxobolus* spp. of cyprinid fish. *Journal of Fish Diseases* 25: 643-652.

Moncada, L.I., M.C. López, M.I. Murcia, S. Nicholls, F. León, O.L. Guío and A. Corredor. 2001. *Myxobolus* sp., another opportunistic parasite in immunosuppressed patients? *Journal of Clinical Microbiology* 39: 1938-1940.

Montiero, A.S., B. Okamura and P.W.H. Holland. 2002. Orphan worm finds a home: *Buddenbrockia* is a myxozoan. *Molecular Biology and Evolution* 19: 968-971.

Moran, J.D.W. and M.L. Kent. 1999. *Kudoa thyrsites* (Myxozoa:Myxosporea) infections in pen-reared Atlantic salmon in the Northeast Pacific Ocean with a survey of potential non-salmonid reservoir hosts. *Journal of Aquatic Animal Health* 11: 101-109.

Moran, J.D.W., D.J. Whitaker and M.L. Kent. 1999a. Natural and laboratory transmission of the marine myxozoan parasite *Kudoa thyrsites* to Atlantic salmon. *Journal of Aquatic Animal Health* 11: 110-115.

Moran, J.D.W., L. Margolis, J.M. Webster and M.L. Kent. 1999b. Development of *Kudoa thyrsites* (Myxozoa: Myxosporea) in netpen-reared Atlantic salmon determined by light microscopy and polymerase chain reaction test. *Diseases of Aquatic Organisms* 37: 185-193.

Moran, J.D.W., D.J. Whitaker and M.L. Kent. 1999c. A review of the myxosporean genus *Kudoa* Meglitsch, 1947, and its impact on the international aquaculture industry and commercial fisheries. *Aquaculture* 172: 163-196.

Morris, D.J. and A. Adams. 2006a. Transmission of *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea), the causative agent of salmonid proliferative kidney disease, to the freshwater bryozoan *Fredericella sultana*. *Parasitology* 133: 701-709.

Morris, D.J. and A. Adams. 2006b. Transmission of freshwater myxozoans during the asexual propagation of invertebrate hosts. *International Journal for Parasitology* 36: 371-377.

Morris, D.J., A. Adams and R.H. Richards. 1997. Studies of the PKX parasite in rainbow trout via immunohistochemistry and immunogold electron microscopy. *Journal of Aquatic Animal Health* 9: 265-273.

Morris, D.J., A. Adams and R.H. Richards. 1999. *In situ* hybridization of DNA probes to PKX, the causative organism of proliferative kidney disease (PKD). *Journal of Fish Diseases* 22: 161-163.

Morris, D.J., A. Adams, S.W. Feist, J. McGeorge and R.H. Richards. 2000a. Immunohistochemical and PCR studies of wild fish for *Tetracapsula bryosalmonae* (PKX), the causative organism of proliferative kidney disease. *Journal of Fish Diseases* 23: 129-135.

Morris, D.J., A. Adams and R.H. Richards. 2000b. *In situ* hybridisation identifies the gill as a portal of entry for PKX (Phylum Myxozoa), the causative agent of proliferative kidney disease in salmonids. *Parasitology Research* 86: 950-956.

Morris, D.C., D.M. Morris and A. Adams. 2002. Development of improved PCR to prevent false positives and false negatives in the detection of *Tetracapsula bryosalmonae*, the causative agent of proliferative kidney disease. *Journal of Fish Diseases* 25: 483-490.

Morris, D.J., A. Adams, P. Smith and R.H. Richards. 2003a. Effects of oral treatment with TNP-470 on rainbow trout (*Oncorhynchus mykiss*) infected with *Tetracapsuloides bryosalmonae* (Malacosporea), the causative agent of proliferative kidney disease. *Aquaculture* 221: 51-64.

Morris, D.J., M. Longshaw and A. Adams. 2003b. California golden trout *Oncorhynchus mykiss aguabonita* are susceptible to proliferative kidney disease. *Journal of Aquatic Animal Health* 15: 184-187.

Morrison, C.M., D.J. Martell, C. Leggiardo and D. O'Neil. 1996. *Ceratomyxa drepanopsettae* in the gall bladder of Atlantic halibut, *Hippoglossus hippoglossus*, from the northwest Atlantic Ocean. *Folia Parasitologica* 43: 20-36.

Moshu, A. and K. Molnár. 1997. *Thelohanellus* (Myxozoa: Myxosporea) infection of the scales in the European wild carp *Cyprinus carpio carpio*. *Diseases of Aquatic Organisms* 28: 115-123.

Müller, J. 1841. Über psorospermien. *Archive Anatomie, Physiologie für Wissenschaft* 5: 477-496.

Muñoz, P., A. Sitjà-Bobadilla and P. Álvarez-Pellitero. 1998. Immunohistochemical characterization of a polyclonal antibody against *Sphaerospora dicentrarchi* (Myxosporea: Bivalvulida), a parasite from sea bass (*Dicentrarchus labrax* L.) (Teleostei: Serranidae). *Parasitology Research* 84: 733-740.

Muñoz, P., O. Palenzuela, P. Álvarez-Pellitero and A. Sitjà-Bobadilla. 1999a. Comparative studies on carbohydrates of several myxosporean parasites of fish using lectin histochemical techniques. *Folia Parasitologica* 46: 241-247.

Muñoz, P., O. Palenzuela, A. Sitjà-Bobadilla and Álvarez-Pellitero. 1999b. Immunohistochemical reactivity of polyclonal antibodies against *Sphaerospora testicularis* and *Ceratomyxa labracis* (Myxosporea: Bivalvulida), with other myxosporean parasites. *International Journal for Parasitology* 29: 521-525.

Muñoz, P., A. Sitjà-Bobadilla and P. Álvarez-Pellitero. 2000. Ultrastructural localisation of carbohydrates in four myxosporean parasites. *Parasite* 7: 185-191.

Mutschmann, F. 2004. Pathological changes in African hyperoliid frogs due to a myxosporidian infection with a new species of *Hoferellus* (Myxozoa). *Diseases of Aquatic Organisms* 60: 215-222.

Nehring, R.B. and P.G. Walker. 1996. Whirling disease in the wild: The new reality in the intermountain west. *Fisheries* 21: 28-30.

Nehring, R.B., K.G. Thompson, K.A. Taurman and D.L. Shuler. 2002. Laboratory studies indicating that living brown trout *Salmo trutta* expel viable *Myxobolus cerebralis* myxospores. In: *Whirling Disease: Reviews and Current Topics*, J.L. Bartholomew and J.C. Wilson (eds.). *American Fisheries Society, Symposium* 29, Bethesda, Maryland, pp. 125-134.

Nehring, R.B., K.G. Thompson, K.A. Taurman and W. Atkinson. 2003. Efficacy of passive sand filtration in reducing exposure of salmonids to the actinospore of *Myxobolus cerebralis*. *Diseases of Aquatic Organisms* 57: 77-83.

Nielsen, C.V., M. Køie, C. Székely and K. Buchmann. 2002. Comparative analysis of 18S rRNA genes from *M. aeglefini* Auerbach, 1906 isolated from cod, plaice and dab, using PCR-RFLP. *Bulletin of the European Association of Fish Pathologists* 22: 201-205.

Noble, E.R. 1950. On a myxosporidian (protozoan) parasite of California trout. *Journal of Parasitology* 36: 457-460.

Ogawa, K. and H. Yokoyama. 2001. Emaciation disease of cultured tiger puffer *Takifugu rubripes*. *Bulletin of the National Research Institute of Aquaculture* (Supplement) 5: 65-70.

Ogawa, K., K.P. Delgahapitiya, T. Furuta and H. Wakabayashi. 1992. Histological studies on the host response to *Myxobolus artus* Akmerov, 1960 (Myxozoa: Myxobolidae) infection in the skeletal muscle of carp, *Cyprinus carpio* L. *Journal of Fish Biology* 41: 363-371.

Okamura, B. and T.S. Wood. 2002. Bryozoans as hosts of *Tetracapsula bryosalmonae*, the PKX organism. *Journal of Fish Diseases* 25: 469-475.

Okamura, B., A. Curry, T.S. Wood and E.U. Canning. 2002. Ultrastructure of *Buddenbrockia* sp. identifies it as a myxozoan and verifies the bilaterian origin of the Myxozoa. *Parasitology* 124: 215-223.

Oumouna, M., S.L. Hallett, R.W. Hoffman and M. El-Matbouli. 2003. Seasonal occurrence of actinosporeans (Myxozoa) and oligochaetes (Annelida) at a trout hatchery in Bavaria, Germany. *Parasitology Research* 89: 170-184.

Overstreet, R.M. 1976. *Fabespora vermicola* sp. n., the first Myxosporidian from a platyhelminth. *Journal of Parasitology* 62: 680-684.

Özer, A. and R. Wootten. 2001. Release of actinosporean and myxosporean spores from their hosts, with special reference to both stages of *Sphaerospora truttae* (Myxozoa, Myxosporea). *Acta Parasitologica* 46: 103-112.

Özer, A., R. Wootten and A.P. Shinn. 2002. Survey of actinosporean types (Myxozoa) belonging to seven collective groups found in a freshwater salmon farm in Northern Scotland. *Folia Parasitologica* 49: 189-210.

Padrós, F., O. Palenzuela, C. Hispano, O. Tosas, C. Zaraza, S. Crespo and P. Álvarez-Pellitero. 2001. *Myxidium leei* (Myxozoa) infections in aquarium-reared Mediterranean fish species. *Diseases of Aquatic Organisms* 47: 57-62.

Palenzuela, O., A. Sitjà-Bobadilla and P. Álvarez-Pellitero. 1997. *Ceratomyxa sparusaurati* (Protozoa: Myxosporea) infections in cultured gilt-head seabream *Sparus aurata* (Pisces: Teleostei) from Spain: Aspects of the host-parasite relationship. *Parasitology Research* 83: 539-548.

Palenzuela, O., P. Álvarez-Pellitero and A. Sitjà-Bobadilla. 1999. Glomerular disease associated with *Polysporoplasma sparis* (Myxozoa) infections in cultured gilt-head seabream, *Sparus aurata* L. (Pisces: Teleostei). *Parasitology* 118: 245-256.

Palenzuela, O., M.J. Redondo and P. Álvarez-Pellitero. 2002. Description of *Enteromyxum scophthalmi* gen. nov., sp. nov. (Myxozoa), an intestinal parasite of turbot (*Scophthalmus maximus* L.) using morphological and ribosomal RNA sequence data. *Parasitology* 124: 369-379.

Pampoulie, C., A. Marques, E. Rosecchi, J.L. Bouchereau and A.J. Crivelli. 2001. Long-term monitoring on the occurrence of a myxosporean parasite *Kudoa camarguensis* (Myxosporean) on the common goby (Teleostei, Pisces) *Pomatoschistus microps*. *Diseases of Aquatic Organisms* 45: 69-71.

Paperna, I., A.H. Hartley and R.H.M. Cross. 1987. Ultrastructural studies on the plasmodium of *Myxidium giardi* (Myxosporea) and its attachment to the epithelium of the urinary bladder. *International Journal for Parasitology* 17: 813-819.

Plehn, M. 1924. *Praktikum der Fischkrankheiten* Schweizerbartsche Verlagsbuchhandlung, Stuttgart.

Pote, L.M., L.A. Hanson and R. Shivaji. 2000. Small subunit ribosomal RNA sequences link the cause of proliferative gill disease in channel catfish to *Henneguya* n. sp. (Myxozoa: Myxosporea). *Journal of Aquatic Animal Health* 12: 230-240.

Pronin, N.M., G.W. Fleischer, D.R. Baldanova and S.V. Pronina. 1997. Parasites of the recently established round goby (*Neogobius melanostomus*) and tubenose goby (*Proterorhinus marmoratus*) (Cottidae) from the St. Clair River and Lake St. Clair, Michigan, USA. *Folia Parasitologica* 44: 1-6.

Prunescu, C.-C., P. Prunescu, Z. Pucek and J. Lom. 2007. The first finding of myxosporean development from plasmodia to spores in terrestrial mammals: *Soricimyxum fegati* gen. et sp. n. (Myxozoa) from *Sorex araneus* (Soricomorpha). *Folia Parasitologica* 54: 159-164.

Ratcliff, D.E. 1983. *Ceratomyxa shasta*: longevity, distribution, timing, and abundance of the infective stage in central Oregon. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 1622-1632.

Redondo, M.J., O. Palenzuela, A. Riaza, A. Macías and P. Álvarez-Pellitero. 2002. Experimental transmission of *Enteromyxum scophthalmi* (Myxozoa), an enteric parasite of turbot *Scophthalmus maximus*. *Journal of Parasitology* 88: 482-488.

Redondo, M.J., O. Palenzuela and P. Álvarez-Pellitero. 2003. *In vitro* studies on viability and proliferation of *Enteromyxum scophthalmi* (Myxozoa), an enteric parasite of cultured turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms* 55: 133-144.

Redondo, M.J., O. Palenzuela and P. Álvarez-Pellitero. 2004. Studies on transmission and life cycle of *Enteromyxum scophthalmi* (Myxozoa), an enteric parasite of turbot (*Scophthalmus maximus* L.). *Folia Parasitologica* 51: 188-198.

Reimschuessel, R., C.M. Gieseker, C. Driscoll, A. Baya, A.S. Kane, V.S. Blazer, J.J. Evans, M.L. Kent, J.D.W. Moran and S.L. Poynton. 2003. Myxosporean plasmodial infection associated with ulcerative lesions in young-of-the-year Atlantic menhaden in a tributary of the Chesapeake Bay, and possible links to *Kudoa clupeidae*. *Diseases of Aquatic Organisms* 53: 143-166.

Rhee, J.K., H.C. Kim and B.K. Park. 1993. Efficacy of fumagillin against *Thelohanellus kitauei* infection of Israel carp, *Cyprinus carpio nudus*. *Korean Journal of Parasitology* 31: 57-63.

Rhee, J.K., J.O. Kim, P.G. Kim and B.K. Park. 1990. Prophlyactic and therapeutic studies on intestinal giant-cystic disease of the Israel carp caused by *Thelohanellus kitauei*. I. Course of formation and vanishment of the cysts. *Korean Journal of Parasitology* 28: 1183-1194.

Rose, J.D., G.S. Marrs, C. Lewis and G. Schisler. 2000. Whirling disease behaviour and its relation to pathology of brain stem and spinal cord in rainbow trout. *Journal of Aquatic Animal Health* 12: 107-118.

Roubal, F.R. 1994. Histopathological and ecological aspects of *Henneguya* and *Myxobolus* (Myxosporea) infections in *Acanthopagrus australis* (Günther) (Pisces: Sparidae) from Moreton Bay, Australia. *Journal of Fish Diseases* 17: 495-512.

Saulnier, D. and P. de Kinkelin. 1996. Antigenic and biochemical study of PKX, the myxosporean causative agent of proliferative kidney disease of salmonid fish. *Diseases of Aquatic Organisms* 27: 103-114.

Saulnier, D. and P. de Kinkelin. 1997. Polymerase chain reaction primers for investigations on the causative agent of proliferative kidney disease of salmonids. *Journal of Fish Diseases* 20: 467-470.

Schachner, O. and A. Holzer. 2001. Gram staining and lectin binding properties of Myxosporea and Sporozoea. *Biotechnic and Histochemistry* 76: 15-22.

Schlegel, M., J. Lom, D. Stechmann, D. Bernhard, I. Leipe, I. Dyková and M.L. Sogin. 1996. Phylogenetic analysis of complete small subunit ribosomal RNA coding region of *Myxidium lieberkuehni*: Evidence that Myxozoa are Metazoa related to the Bilateria. *Archiv für Protistenkunde* 147: 1-9.

Schröder, O. 1910. *Buddenbrokia plumatellae*, eine neue Mesozoenart aus *Plumatella repens* L. und *Pl. fungosa* Pall. *Zeitschrift für Wissenschaftliche Zoologie* 96: 525-537.

Seagrave, C.P., D. Bucke, E.B. Hudson and D. McGregor. 1981. A survey of the prevalence and distribution of proliferative kidney disease (PKD) in England and Wales. *Journal of Fish Biology* 4: 437-439.

Shulman, S.S. 1966. *Myxosporidia of the USSR*. Nauka, Moscow-Leningrad. Translated for the US Department of the Interior. 1988. Amerind Publishing Company, New Delhi, India.

Shulman, B.S. and E.P. Ieshko. 2003. *Chloromyxum schurovi* sp. n.—A new myxosporidian species (Myxosporea: Sphaerosporidae) from salmonid fishes (Salmonidae). *Parazitologiia* 37: 246-247.

Siddall, M.E., D.S. Martin, D. Bridge, S.S. Desser and D.K. Cone. 1995. The demise of a phylum of protists: Phylogeny of Myxozoa and other parasitic Cnidaria. *Journal of Parasitology* 8: 961-967.

Sitjà-Bobadilla, A. and P. Álvarez-Pellitero. 1990. *Sphaerospora testicularis* sp. nov. (Myxosporea: Sphaerosporidae) in wild and cultured sea bass, *Dicentrarchus labrax* (L.), from the Spanish Mediterranean area. *Journal of Fish Diseases* 13: 193-203.

Sitjà-Bobadilla, A. and P. Álvarez-Pellitero. 1993a. Ultrastructural and cytochemical observations on the sporogenesis of *Sphaerospora testicularis* (Protozoa: Myxosporea) from Mediterranean sea bass, *Dicentrarchus labrax* L. *European Journal of Protistology* 29: 219-229.

Sitjà-Bobadilla, A. and P. Álvarez-Pellitero. 1993b. *Zschokkella mugilis* n. sp. (Myxosporea: Bivalvulida) from mullets (Teleostei: Mugilidae) of Mediterranean waters: Light and electron microscopic description. *Journal of Eukaryotic Microbiology* 40: 755-764.

Sitjà-Bobadilla, A. and P. Álvarez-Pellitero. 1995. Light and electron microscopic description of *Polysporoplasma* n. g. (Myxosporea: Bivalvulida), *Polysporoplasma sparis* n. sp. from *Sparus aurata* (L.) and *Polysporoplasma mugilis* n. sp. from *Liza aurata* L. *European Journal of Protistology* 31: 77-89.

Sitjà-Bobadilla, A. and P. Álvarez-Pellitero. 2001. *Leptotheca sparidarum* n. sp. (Myxosporea: Bivalvulida), a parasite from cultured common dentex (*Dentex dentex* L.) and gilthead seabream (*Sparus aurata* L.) (Teleostei: Sparidae). *Journal of Eukaryotic Microbiology* 48: 627-639.

Sitjà-Bobadilla, A., O. Palenzuela and P. Álvarez-Pellitero. 1995. *Ceratomyxa sparusaurati* n. sp. (Myxosporea: Bivalvulida), a new parasite from cultured gil-thead seabream (*Sparus aurata* L.) (Teleostei: Sparidae): Light and electron microscopic description. *Journal of Eukaryotic Microbiology* 42: 529-539.

Sitjà-Bobadilla, A., M.J. Redondo, M.A. Macias, I. Ferreiro, A. Riaza and P. Álvarez-Pellitero. 2004. Development of immunohistochemistry and enzyme-linked immonosorbent assays for the detection of circulating antibodies against *Enteromyxum scophthalmi* (Myxozoa) in turbot (*Scophthalmus maximus* L.). *Fish and Shellfish Immunology* 17: 335-345.

Sitjà-Bobadilla, A., O. Palenzuela, A. Riaza, M.A. Macias and P. Alvarez-Pellitero. 2007. Protective acquired immunity to *Enteromyxum scophthalmi* (Myxozoa) is related to specific antibodies in *Psetta Maxima* (L.) (Teleostei). *Scandinavian Journal of Immunology* 66: 26-34.

Smith, C.E., J.K. Morrison, H.W. Ramsay and H.W. Ferguson. 1984. Proliferative kidney disease: First reported outbreak in North America. *Journal of Fish Diseases* 7: 207-216.

Smothers, J.F., C.D. von Dohlen, L.H. Smith and R.D. Spall. 1994. Molecular evidence that the myxozoan protists are metazoans. *Science* 265: 1719-1721.

Sterud, E., P. Simolin and A. Kvellestad. 2003. Infection by *Parvicapsula* sp. (Myxozoa) is associated with mortality in sea-caged Atlantic salmon *Salmo salar* in northern Norway. *Diseases of Aquatic Organisms* 54: 259-263.

Sterud, E., T. Forseth, O. Ugedal, T.T. Poppe, A. Jørgensen, T. Bruheim, H.-P. Fjeldstad and T.A. Mo. 2007. Severe mortality in wild Atlantic salmon *Salmo salar* due to proliferative kidney disease (PKD) caused by *Tetracapsuloides bryosalmonae* (Myxozoa). *Diseases of Aquatic Organisms* 77: 191-198.

St-Hilaire, S., M. Boichuk, D. Barnes, M. Higgins, R. Withler, J. Khattra, S. Jones and D. Kieser. 2002. Epizootiology of *Parvicapsula minibicornis* in Fraser River sockeye salmon, *Oncorhynchus nerka* (Walbaum). *Journal of Fish Diseases* 25: 107-120.

Stoffregen, D.A. and W.I. Anderson. 1990. A myxosporidian parasite in the skeletal muscle of a black-tip reef shark, *Carcharhinus melanopterus* (Quoy and Gaimard, 1824). *Journal of Fish Diseases* 13: 549-552.

Styer, E.L., L.R. Harrison and G.J. Burtle. 1991. Experimental production of proliferative gill disease in channel catfish exposed to a myxozoan-infected oligochaete, *Dero digitata*. *Journal of Aquatic Animal Health* 3: 288-291.

Supmattaya, K., T. Fischer-Scherl, R.W. Hoffmann and S. Boonyaratpalin. 1991. *Sphaeropsora epinepheli* n. sp. (Myxosporea: Sphaerosporidae) observed in grouper (*Epinephelus malabaricus*). *Journal of Protozoology* 38: 448-454.

Supamattaya, K., T. Fischer-Scherl, R.W. Hoffmann and S. Boonyaratpalin. 1993. Light and electron microscopic observations on presporogonic and sporogonic stages of *Sphaeropsora epinepheli* (Myxosporea) in grouper (*Epinephelus malabaricus*). *Journal of Eukaryotic Microbiology* 40: 71-80.

Swearer, S.E. and D.R. Robertson. 1999. Life history, pathology, and description of *Kudoa ovivora* n. sp. (Myxozoa, Myxosporea): An ovarian parasite of Caribbean labroid fishes. *Journal of Parasitology* 85: 337-353.

Székely, C., A. El-Mansy, K. Molnár and F. Baska. 1998. Development of *Thelohanellus hovorkai* and *Thelohanellus nikolskii* (Myxosporea: Myxozoa) in oligochaete alternate hosts. *Fish Pathology* 33: 108-114.

Taticchi, M.I., A. Gustinelli, M.L. Fioravanti, M. Caffara, G. Pieroni and M. Prearo. 2004. Is the worm-like organism found in the statoblasts of *Plumatella fungosa* (Bryozoa, Phylactolaemata) the vermiform phase of *Tetracapsuloides bryosalmonae* (Myxozoa, Malacosporea)? *Italian Journal of Zoology* 71: 143-146.

Taylor, K. and S. Jones. 2005. An enzyme linked immunosorbent assay for the detection of *Kudoa thyrsites* in Atlantic salmon. *Aquaculture* 250: 8-15.

Timofeeva, S.V. and E.F. Marasaeva. 1984. The parasite fauna of two forms of cod in the Kandalakhsh Bay, White Sea. In: *Ekologo-parazitologicheskie Severnykh Morei*. Kol'skii Filial, Academiya Nauk SSSR, Apatity, USSR. pp. 62-76. (In Russian).

Tin Tun, H. Yokoyama, K. Ogawa and H. Wakabayashi. 2000. Myxosporeans and their hyperparasitic microsporeans in the intestine of emaciated tiger puffer. *Fish Pathology* 35: 145-156.

Tin Tun, K. Ogawa and H. Wakabayashi. 2002. Pathological changes induced by three myxosporeans in the intestine of cultured tiger puffer, *Takifugu rubripes* (Temminck and Schlegel). *Journal of Fish Diseases* 25: 63-72.

Tipping, J.M. 1988. Ozone control of ceratomyxosis: survival and growth benefits to steelhead and cutthroat trout. *Progressive Fish-Culturist* 50: 202-210.

Tops, S. and B. Okamura. 2003. Infection of bryozoans by *Tetracapsuloides bryosalmonae* at sites endemic for salmonid proliferative kidney disease. *Diseases of Aquatic Organisms* 57: 221-226.

Tops, S., D.V. Baxa, T.S. McDowell, R.P. Hedrick and B. Okamura. 2004. Evaluation of malacosporean life cycles through transmission studies. *Diseases of Aquatic Organisms* 60: 109-121.

Tops, S., A. Curry and B. Okamura. 2005. Diversity and systematics of the Malacosporea (Myxozoa). *Invertebrate Biology* 124: 285-295.

Tops, S., W. Lockwood and B. Okamura. 2006. Temperature-driven proliferation of *Tetracapsuloides bryosalmonae* in bryozoan hosts portends salmonid declines. *Diseases of Aquatic Organisms* 70: 227-236.

Torres, A., E. Matos and C. Azevedo. 1994. Fine structure of *Henneguya amazonica* (Myxozoa) in ovarian follicles of *Hoplosternum littorale* (Teleostei) from the Amazon River. *Diseases of Aquatic Organisms* 19: 169-172.

Troullier, A., M. El-Matbouli and R.W. Hoffman. 1996. A new look at the life-cycle of *Hoferellus carassii* in the goldfish (*Carassius auratus auratus*) and its relation to 'kidney enlargement disease' (KED). *Folia Parasitologica* 43: 173-187.

Urawa, S. and T. Awakura. 1994. Protozoan diseases of freshwater fishes in Hokkaido. *Scientific Reports of the Hokkaido Fish Hatchery* 48: 47-58.

Urawa, S. and K. Nagasawa. 1995. Prevalence of *Myxobolus arcticus* (Myxozoa: Myxosporea) in five species of Pacific salmon in the North Pacific Ocean and Bering Sea. *Scientific Reports of the Hokkaido Salmon Hatchery* 49: 11-19.

Urawa, S., K. Nagasawa, L. Margolis and A. Moles. 1998. Stock identification of chinnok salmon (*Oncorhynchus tshawytscha*) in the North Pacific Ocean and Bering Sea by parasite tags. *North Pacific Anadromous Fish Commision Bulletin* 1: 199-204.

Van Banning, P., J.F. Veen and P.J. van Leeuwen. 1978. The myxosporidian parasite (*Myxobolus aeglefini* Auerbach, 1906) and its use as parasitological tag for plaice of the eastern North Sea. *International Council for the Exploration of the Sea* CM G:48: 22pp.

Ventura, M.P. and I. Paperna. 1985. Histopathology of *Myxidium giardi* Cepede, 1906 infection in European eels, *Anguilla anguilla* L. in Portugal. *Aquaculture* 43: 357-368.

Vogelbein, W.K., V.J. Lovko, J.D. Shields, K.S. Reece, P.L. Mason, L.W. Haas and C.C. Walker. 2002. *Pfiesteria shumwayae* kills fish by micropredation not exotoxin secretion. *Nature (London)* 418: 967-970.

Voronin, V.N. 1993. PKX like organism in common carp during swimbladder inflammation: Further evidence of an association with the myxosporean *Sphaerospora renicola*. *Bulletin of the European Association of Fish Pathologists* 13: 127-129.

Voronin, V.N. and N.B. Chernysheva. 1993. An intracellular gill parasite as the possible agent of mortality during swimbladder inflammation in common carp, *Cyprinus carpio* L. *Journal of Fish Diseases* 16: 609-611.

Wagner, G.N., S.G. Hinch, L.J. Kuchel, A. Lotto, S.R.M. Jones, D.A. Patterson, J.S. Macdonald, G. Van Der Kraak, M. Shrimpton, K.K. English, S. Larsson, S.J. Cooke, M.C. Healey and A.P. Farrell. 2005. Metabolic rates and swimming performance of adult Frase River sockeye salmon (*Oncorhynchus nerka*) after a controlled infection with *Parvicapsula minibicornis*. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2124-2133.

Wahli, T., R. Knuesel, D. Bernet, H. Segner, D. Pugovkin, P. Burkhardt-Holm, M. Escher, M. and H. Schmidt-Posthaus. 2002. Proliferative kidney disease in Switzerland: Current state of knowledge. *Journal of Fish Diseases* 25: 491-500.

Walliker, D. 1968. Studies on *Myxidium oviforme*, a myxosporidian parasite of Irish salmon, *Salmo salar*. *Parasitology* 58: 839-844.

Wang, G.T., W.J. Yao, J., G. Wang and Y.S. Lu. 2001. Occurrence of thelohanellosis caused by *Thelohanellus wuhanensis* (Myxosporea) in juvenile allogynogenetic silver crucian carp, *Carassius auratus gibelio* (Bloch), with an observation on the efficacy of fumagillin as a therapeutant. *Journal of Fish Diseases* 24: 57-60.

Wang, P.-C., J.-P. Huang, M.-A. Tsai, S.-Y. Cheng, S.-S. Tsai, S.-D. Chen, S.-P. Chen, S.-H. Chiu, L.-L. Liaw, L.-T. Chang and S.-C. Chen. 2005. Systemic infection of *Kudoa lutjanus* n. sp. (Myxozoa: Myxosporea) in red snapper *Lutjanus erythropterus* from Taiwan. *Diseases of Aquatic Organisms* 67: 115-124.

Webb, S.R., G.C. Garman, S.P. McIninch, T.A. Nerad, M.T. Peglar, P.M. Gillevet and B.L. Brown. 2005. Etiology of ulcerative lesions of Atlantic menhaden (*Brevoortia tyrannus*) from James River, Virginia. *Parasitology Research* 97: 358-366.

Whipps, C.M., R.D. Adlard, M.S. Bryant and M.L. Kent. 2003. Two unusual myxozoans, *Kudoa quadricornis* n. sp. (Multivalvulida) from the muscle of goldspotted trevally (*Carangoides fulvoguttatus*) and *Kudoa permulticapsula* n. sp. (Multivalvulida) from the muscle of Spanish mackerel (*Scomberomorus commersoni*) from the Great Barrier Reef, Australia. *Journal of Parasitology* 89: 168-173.

Whipps, C.M., G. Grossel, R.D. Adlard, H. Yokoyama, M.S. Bryant, B.L. Munday and M.L. Kent. 2004a. Phylogeny of the Multivalvulidae (Myxozoa: Myxosporea) based upon comparative rDNA sequence analysis. *Journal of Parasitology* 90: 618-622.

Whipps, C.M., M. El-Matbouli, R.P. Hedrick, V. Blazer and M.L. Kent. 2004b. *Myxobolus cerebralis* internal transcribed spacer (ITS-1) sequences support recent spread of the parasite to North America and within Europe. *Diseases of Aquatic Organisms* 60: 105-108.

Whitaker, J.W., L.M.W. Pote, L. Khoo, R. Shivaji and L.A. Hanson. 2001. The use of polymerase chain reaction assay to diagnose proliferative gill disease in channel catfish (*Ictalurus punctatus*). *Journal of Veterinary Diagnostic Investigation* 13: 394-398.

Whitaker, J.W., L.M.W. Pote and L.A. Hanson. 2005. Assay to detect the actinospore and myxospore stages of proliferative gill disease in oligochaetes and pond water. *North American Journal of Aquaculture* 67: 133-137.

Wishkovsky, A., J.M. Groff, D.J. Lauren, R.J. Toth and R.P. Hedrick. 1990. Efficacy of fumagillin against proliferative kidney disease and its toxic side effects in rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Fish Pathology* 25: 141-146.

Wolf, K. and M.E. Markiw. 1979. *Myxosoma cerebralis*: a method for staining spores and other stages with silver nitrate. *Journal of the Fisheries Research Board of Canada* 36: 88-89.

Wolf, K. and M.E. Markiw. 1984. Biology contravenes taxonomy in the Myxozoa: New discoveries show alternation of invertebrate and vertebrate hosts. *Science* 225: 1449-1452.

Wu, P.-H., Z.-H Chang, J. Chang, Y.-S Chen and L.-F. Huang. 1975. Twist disease of *Hypophthalmichthys molitrix* in Hangchow region of Chekiang Province. *Acta Zoologica Sinica* 21: 190-196.

Wyatt, E.J. and I. Pratt. 1963. *Myxobolus insidiosus* n. sp., a myxosporidian from the musculature of *Oncorhynchus tshawytscha* (Walbaum). *Journal of Parasitology* 49: 951-955.

Xiao, C.X. and S.S. Desser. 1997. *Sphaerospora ovophila* n. sp. and *Myxobolus algonquinensis* n. sp. (Myxozoa, Myxosporea), ovarian parasites of fish from Algonquin Park, Ontario, Canada. *Journal of Eukaryotic Microbiology* 44: 157-161.

Xiao, C.X. and S.S. Desser. 1998. Actinosporean stages of myxozoan parasites of oligochaetes from Lake Sasajewun, Algonquin Park, Ontario—New forms of Triactinomyxon and Raabeia. *Journal of Parasitology* 84: 998-1009.

Xiao, C.X. and S.S. Desser. 2000a. Cladistic analysis of myxozoan species with known alternating life cycles. *Systematic Parasitology* 46: 81-91.

Xiao, C.X. and S.S. Desser. 2000b. The longevity of actinosporean spores from oligochaetes of Lake Sasajewun, Algonquin Park, Ontario, and their reaction to fish mucus. *Journal of Parasitology* 86: 193-195.

Yamaguchi, A., H. Yokoyama, K. Ogawa and T. Taniuchi. 2003. Use of parasites as biological tags for separating stocks of the starspotted dogfish *Mustelus manazo* in Japan and Taiwan. *Fisheries Science* 69: 337-342.

Yanagida, T., Y. Nomura, T. Kimura, Y. Fukuda, H. Yokoyama and K. Ogawa. 2004. Molecular and morphological redescriptions of enteric myxozoans, *Enteromyxum leei* (formerly *Myxidium* sp. TP) and *Enteromyxum fugu* comb. n. (syn. *Myxidium fugu*) from cultured tiger puffer. *Fish Pathology* 39: 137-143.

Yanagida, T., M.A. Freeman, Y. Nomura, I. Takami, Y. Sugihara, H. Yokoyama and K. Ogawa. 2005. Development of a PCR-based method for the detection of enteric myxozoans causing the emaciation disease of cultured tiger puffer. *Fish Pathology* 40: 23-28.

Yasuda, H., T. Ooyama, K. Iwata, Tin Tun, H. Yokoyama and K.Ogawa. 2002. Fish-to-fish transmission of *Myxidium* spp. (Myxozoa) in cultured tiger puffer suffering from emaciation disease. *Fish Pathology* 37: 29-33.

Yasuda, H., T. Ooyama, A. Nakamura, K. Iwata, O. Palenzuela and H. Yokoyama. 2005. Occurrence of the myxosporean emaciation disease caused by *Enteromyxum leei* in cultured Japanese flounder *Paralichthys olivaceus*. *Fish Pathology* 40: 175-180.

Yasutake, W.T. and D.G. Elliott. 2003. Epizootiology and histopathology of *Parvicapsula* sp. in coho salmon *Oncorhynchus kisutch*. *Diseases of Aquatic Organisms* 56: 215-221.

Yokoyama, H. 2003. A review: Gaps in our knowledge on myxozoan parasites of fishes. *Fish Pathology* 38: 125-136.

Yokoyama, H. and K. Masuda. 2001. *Kudoa* sp. (Myxozoa) causing a post-mortem myoliquefaction of North-Pacific giant octopus *Paroctopus dofleini* (Cephalopoda: Octopodidae). *Bulletin of the European Association of Fish Pathologists* 21: 266-268.

Yokoyama, H. and S. Urawa. 1997. Fluorescent labelling of actinospores for determining the portals of entry into fish. *Diseases of Aquatic Organisms* 30: 165-169.

Yokoyama, H. and S. Wakabayashi. 2000. *Myxobolus aeglefini* found in the skeletal muscle of porous-head eelpout *Allolepis hollandi* from the Sea of Japan. *Fisheries Science* 66: 963-966.

Yokoyama, H., K. Ogawa and H. Wakabayashi. 1990. Light and electron microscopic studies on the development of *Hoferellus carassii* (Myxosporea), the causative organism of kidney enlargement disease of goldfish. *Fish Pathology* 25: 149-156.

Yokoyama, H., K. Ogawa and H. Wakabayashi. 1993a. Some biological characteristics of actinosporeans from the oligochaete *Branchiura sowerbyi*. *Diseases of Aquatic Organisms* 17: 223-228.

Yokoyama, H., K. Ogawa and H. Wakabayashi. 1993b. Involvement of *Branchiura sowerbyi* (Oligochaeta: Annelida) in the transmission of *Hoferellus carassii* (Myxosporea: Myxozoa), the causative agent of kidney enlargement disease (KED) of goldfish *Carassius auratus*. *Fish Pathology* 28: 135-139.

Yokoyama, H., K. Ogawa and H. Wakabayashi. 1995. Chemoresponse of actinosporean spores of *Myxobolus cultus* to skin mucus of goldfish *Carassius auratus*. *Diseases of Aquatic Organisms* 21: 7-11.

Yokoyama, H., T. Danjo, K. Ogawa, K., T. Arima and H. Wakabayashi. 1996. Hemorrhagic anemia of carp associated with spore discharge of *Myxobolus artus* (Myxozoa: Myxosporea). *Fish Pathology* 31: 19-23.

Yokoyama, H., D. Inoue, A. Kumamaru and H. Wakabayashi. 1997. *Myxobolus koi* (Myxozoa: Myxosporea) forms large- and small-type 'cysts' in the gills of common carp. *Fish Pathology* 32: 211-217.

Yokoyama, H., Y.S. Liyanage, A. Sugai and H. Wakabayashi. 1998. Hemorrhagic thelohanellosis of color carp caused by *Thelohanellus hovorkai* (Myxozoa: Myxosporea). *Fish Pathology* 33: 85-89.

Yokoyama, H., H. Kawakami, H. Yasuda and S. Tanaka. 2003. *Henneguya lateolabracis* sp. n. (Myxozoa: Myxosporea), the causative agent of cardiac henneguyosis in Chinese sea bass *Lateolabrax* sp. *Fisheries Science* 69: 1116-1120.

Yokoyama, H., M.A. Freeman, T. Yoshinaga and K. Ogawa. 2004a. *Myxobolus buri*, the myxosporean parasite causing scoliosis of yellowtail, is synonymous with *Myxobolus acanthogobii* infecting the brain of yellowfin goby. *Fisheries Science* 70: 1036-1042.

Yokoyama, H., C.M. Whipps, M.L. Kent, K. Mizuno and H. Kawakami. 2004b. *Kudoa thyrsites* from Japanese flounder and *Kudoa lateolabracis* n. sp. from Chinese sea bass: Causative myxozoans of post-mortem myoliquefaction. *Fish Pathology* 39: 79-85.

Yokoyama, H. and N. Itoh. 2005a. Two multivalvulid myxozoans causing post mortem myoliquefaction: *Kudoa megacapsula* n. sp. from red barracuda (*Sphyraena pinguis*) and *Kudoa thyrsites* from splendid alfonso (*Beryx splendus*). *Journal of Parasitology* 91: 1132-1137.

Yokoyama, H., N. Itoh and S. Tanaka. 2005a. *Henneguya pagri* n. sp. (Myxozoa: Myxosporea) causing cardiac henneguyosis in red sead bream, *Pagrus major* (Temminck & Schlegel). *Journal of Fish Diseases* 28: 479-487.

Yokoyama, H., M.A. Freeman, N. Itoh and Y. Fukuda. 2005b. Spinal curvature of cultured Japanese mackerel *Scomber japonicus* associated with a brain myxosporean, *Myxobolus acanthogobii*. *Diseases of Aquatic Organisms* 66: 1-7.

Young, C.A. and S.R.M. Jones. 2005. Epitopes associated with mature spores not recognised on *Kudoa thyrsites* from recently infected Atlantic salmon smolts. *Diseases of Aquatic Organisms* 63: 267-271.

Zrzavý, J. and V. Hypša. 2003. Myxozoa, *Polypodium*, and the origin of the Bilateria: The phylogenetic position of 'Endocnidozoa' in light of the rediscovery of *Buddenbrockia*. *Cladistics* 19: 164-169.

CHAPTER

13

# Diseases Caused by Monogenea

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## INTRODUCTION

Monogenea (Phylum Platyhelminthes) are common ectoparasitic flatworms of marine, brackish water and freshwater fishes. Together with parasitic Copepoda (Chapter 18), Monogenea are the most diverse assortment of ectoparasites of fishes. Monogeneans generally live on the external surfaces including the 'skin' or general body surfaces, fins, the head, gills, eyes and oral and branchial cavities. Some monogenean species parasitize internal sites with openings to the exterior such as the nasal tissue, urogenital system (including the cloaca and rectal gland) and sometimes the body cavity (Table 13.1). Exceptionally, a few species infect the digestive tract, heart musculature or blood vessels (Table 13.1; Whittington, 2005). Despite these exceptions, the Monogenea are widely referred to as ectoparasitic flukes of fish (e.g., Hayward, 2005;

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Table 13.1 Major differences between the 2 groups of Monogenea that parasitize fish.

| *Attribute* | *Monopisthocotylea* | *Polyopisthocotylea* |
|---|---|---|
| **Fish hosts** | Agnatha (jawless fish, e.g., hagfish)<br>Chondrichthyes (cartilaginous fish, e.g., elephant fish, sharks, rays)<br>Teleostei (bony fish, e.g., most freshwater, brackish water and marine species) | Chondrichthyes<br>Dipnoi (lungfish, e.g., the Australian lungfish)<br>Teleostei |
| **Sites (= microhabitats) parasitized (listed from most to least common)** | Gills; 'skin' of external surfaces; fins; branchial chamber; oral cavity; nasal tissue; eye surface; urogenital system (cloaca and rectal gland of Chondrichthyes; ureters; kidneys; oviducts); body cavity; digestive tract; heart muscle; blood vessels | Gills; branchial chamber including the inside of the operculum; buccal cavity |
| **Nutrition source** | Epithelial cells; perhaps mucus<br>Gut contents grey or colourless; rarely strongly pigmented | Blood<br>Gut contents commonly darkly pigmented (intense brown or black) |
| **Mobility** | Mostly highly mobile | Mostly sedentary |
| **Morphology of haptor (= posterior attachment organ; see Figs. 13.1, 13.2, 13.3)** | Single attachment unit; symmetrical<br>Specific and characteristic attachment structures include muscular divisions (septa) into loculi (e.g., Fig. 13.1A) and various hard sclerotized elements known variously as hooklets, hamuli, accessory sclerites, tiny additional sclerites (resembling teeth, studs and/or spines) and squamodisc (Figs. 13.2, 13.3)<br>Within these parameters there are a diversity of morphologies dependent on family (Fig. 13.3A-F) | Comprises multiple attachment units; mostly symmetrical, but some asymmetrical<br>Specific and characteristic attachment structures include muscular suckers and/or multiple clamps bearing a skeleton of hard sclerites<br>Suckers/clamps may number from 3 symmetrically arranged pairs to many hundreds of clamps arranged asymmetrically<br>Within these parameters there are a diversity of morphologies dependent on family (Fig. 13.3G, H) |

Whittington, 2005) which distinguishes them from the Digenea that are principally endoparasitic or internal flukes (e.g., Cribb, 2005; Chapter 14).

The unique and characteristic morphological feature of the Monogenea that easily distinguishes this class from their wholly parasitic flatworm relatives, the tapeworms (Eucestoda), digeneans and aspidogastreans (collectively the Trematoda), is the posterior attachment

organ known as the haptor (Figs. 13.1, 13.2). The haptor is the main organ used by monogeneans to attach to the fish. The huge diversity of haptor structure (Figs. 13.1, 13.2, 13.3) and its adaptability enables attachment to a range of different host substrates and is a major reason for the evolutionary success of Monogenea and their radiation across aquatic vertebrate hosts. The range of fish groups parasitized by monogeneans includes primitive jawless fishes (Agnatha), chimaeras, sharks and rays (collectively the cartilaginous fishes or Chondrichthyes), lungfish (Dipnoi) and the bony fishes (Teleostei) (Table 13.1).

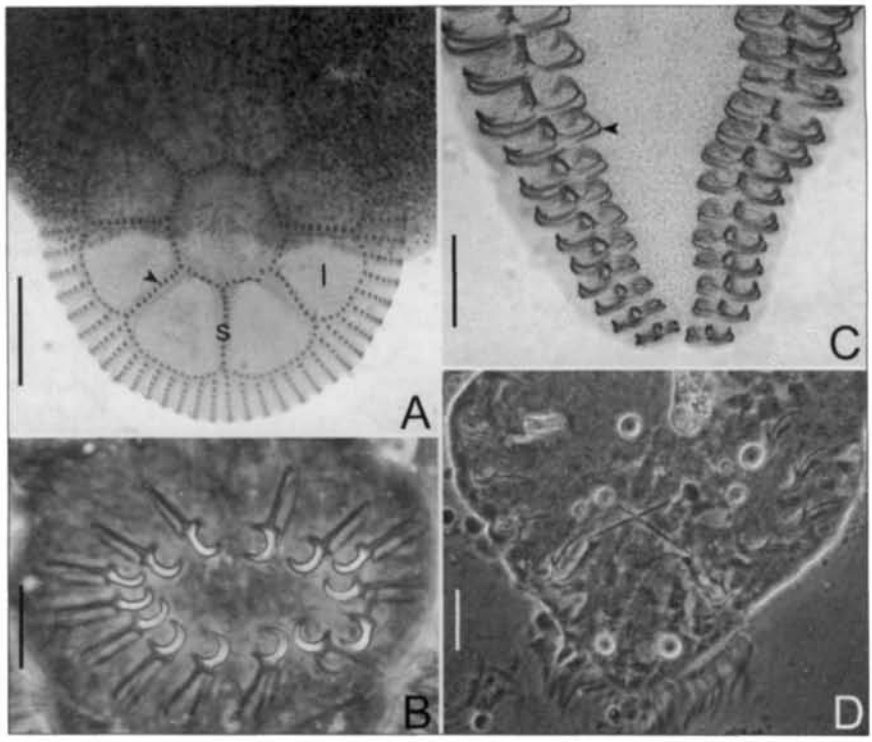


**Fig. 13.1** Adult and larval haptors of a representative Monopisthocotylea (*Dendromonocotyle* species: Monocotylidae) and Polyopisthocotylea (*Zeuxapta seriolae*: Heteraxinidae). A. *Dendromonocotyle torosa*, adult haptor. Single attachment unit divided into loculi (l) by septa (s). Note ventral accessory sclerites (arrowhead) surmounting septa. B. *Dendromonocotyle* sp. larval haptor with 14 hooklets; hamuli absent. C. *Zeuxapta seriolae*, adult haptor with numerous individual clamps (arrowhead) each equipped with a sclerotized skeleton. D. *Zeuxapta seriolae*, larval haptor with 5 small pairs of hooklets and 1 large hooklet pair. Scale bars: A = 500 μm; B, D = 10 μm; C = 100 μm.

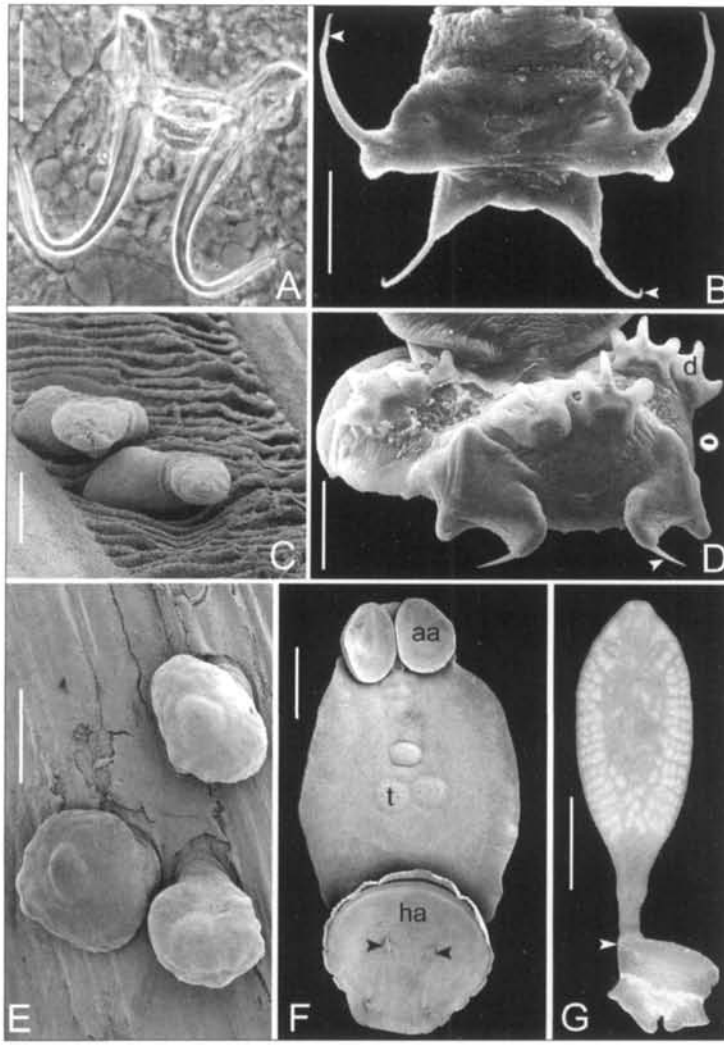


**Fig. 13.2** Haptor morphology of, and attachment by, adult monopisthocotylean monogeneans. A. Hamuli of *Gyrodactylus kobayashii* (Gyrodactylidae). B. Haptor of a Dactylogyridae species with 2 pairs of opposing hamuli (arrowheads). C. *Tetrancistrum* sp. (Dactylogyridae) attached between secondary gill lamellae. D. Haptor of *Neoheterocotyle rhynchobatidis* (Monocotylidae) with 6 dorsal haptoral accessory structures (d) and well-formed ventral hamuli (arrowhead) for attachment. E. *Anoplodiscus australis* (Anoplodiscidae) attached by glue to caudal fin of their sparid host. F. Ventral view of whole body of *Benedenia seriolae* (Capsalidae): note disc-like anterior attachment organs (aa), haptor (ha) and pair of central accessory sclerites (arrowheads); pair of testes (t) can also be seen. G. *Pseudoleptobothrium aptychotremae* (Microbothriidae) firmly attached (by glue) to denticle of fiddler ray; arrowhead marks point of attachment. Scale bars: A = 20 µm; B, D = 50 µm; C = 100 µm; E, G = 500 µm; F = 1 mm.

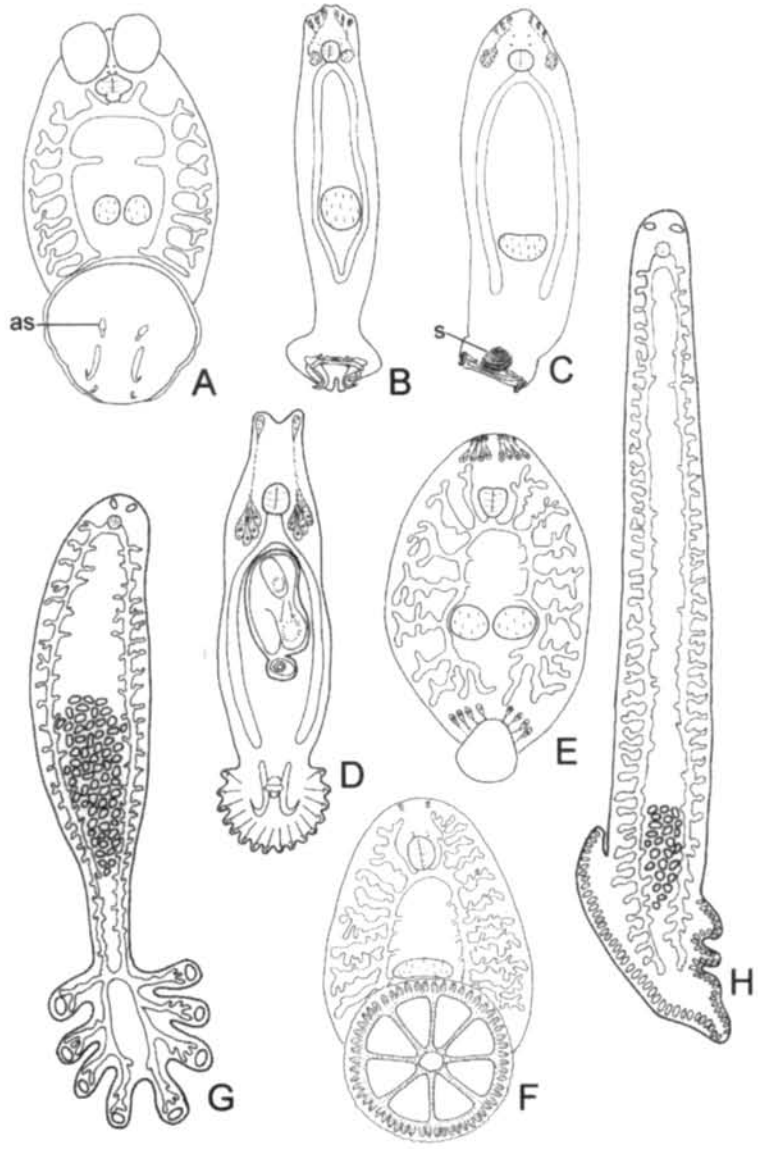


**Fig. 13.3** Outline illustrations of representative Monopisthocotylea (A–F) and Polyopisthocotylea (G and H) Monogenea (note: anterior ends of parasites uppermost). Each taxon represented here is an exemplar dealt with in detail in *Important Monogenean Pathogens.* A. *Benedenia seriolae* (Capsalidae); note central accessory sclerites (as). B. *Haliotrema abaddon* (Dactylogyridae). C. *Diplectanum aequans* (Diplectanidae) with squamodisc (s). D. *Gyrodactylus salaris* (Gyrodactylidae). E. *Dermophthirius* sp. (Microbothriidae). F. *Dendromonocotyle* sp. (Monocotylidae). G. *Neoheterobothrium hirame* (Diclidophoridae). H. *Zeuxapta seriolae* (Heteraxinidae). Not drawn to scale.

In this chapter, we shall follow the most widely used classification which subdivides class Monogenea into 2 subclasses, Monopisthocotylea and Polyopisthocotylea. This division is based largely on the site (= microhabitat) parasitized, the diet and related factors (e.g., gut pigmentation), parasite mobility and haptor morphology (Table 13.1). It should be noted that some refer to Monogenea as Monogenoidea and subdivide the group into the subclasses Polyonchoinea (= Monopisthocotylea) and Heteronchoinea (= Polyopisthocotylea) (see Boeger and Kritsky, 2001, for further information).

Monopisthocotyleans can infect a broad diversity of microhabitats on fish, feed on epithelial cells and perhaps on mucus, they can be highly mobile and often move readily across epithelial surfaces (Table 13.1). Their haptor is usually equipped with a variety of hard, proteinaceous structures forming a single symmetrical attachment unit (Table 13.1; Figs. 13.1A, 13.2B, D, F, G, 13.3A-F). In contrast, polyopisthocotyleans mostly infect fish gills, consume a diet of blood, are principally stationary and generally do not move across large distances, especially when they become adults. Their haptor is either symmetrical or asymmetrical and is divided into multiple units equipped with clamps and/or suckers capable of grasping or sucking onto host gill tissue (Table 13.1; Figs. 13.1C, 13.3G, H). Two specialized families of polyopisthocotyleans, the Polystomatidae and the Sphyranuridae, parasitize aquatic and semi-aquatic tetrapods in the Amphibia (frogs, toads and urodeles) and Reptilia (freshwater turtles), but a single fish species, the Australian lungfish *Neoceratodus forsteri* (Dipnoi), is infected by a polystomatid monogenean, *Concinnocotyla australensis* (see Pichelin *et al.*, 1991). Between 4,000 and 5,000 Monogenea species are currently described (e.g., Whittington, 1998, 2005, for marine Monopisthocotylea; Hayward, 2005, for marine Polyopisthocotylea), but undoubtedly many new species await discovery and description.

Monogenea means 'single generation' which reflects the fact that these parasitic flatworms have a single host life cycle with no intermediate hosts (Fig. 13.4) unlike tapeworms (Chapter 15) and digeneans (Chapter 14). Adult monogeneans usually lay their eggs (oviparous) directly into the water and when they hatch, a free-swimming ciliated or crawling unciliated larva (=oncomiracidium) must find and infect its fish host in order to complete the life cycle (Fig. 13.4). The viviparous *Gyrodactylus* species (Monopisthocotylea: Gyrodactylidae), which give birth to live young, are the exception to oviparity and spread mostly by contagion

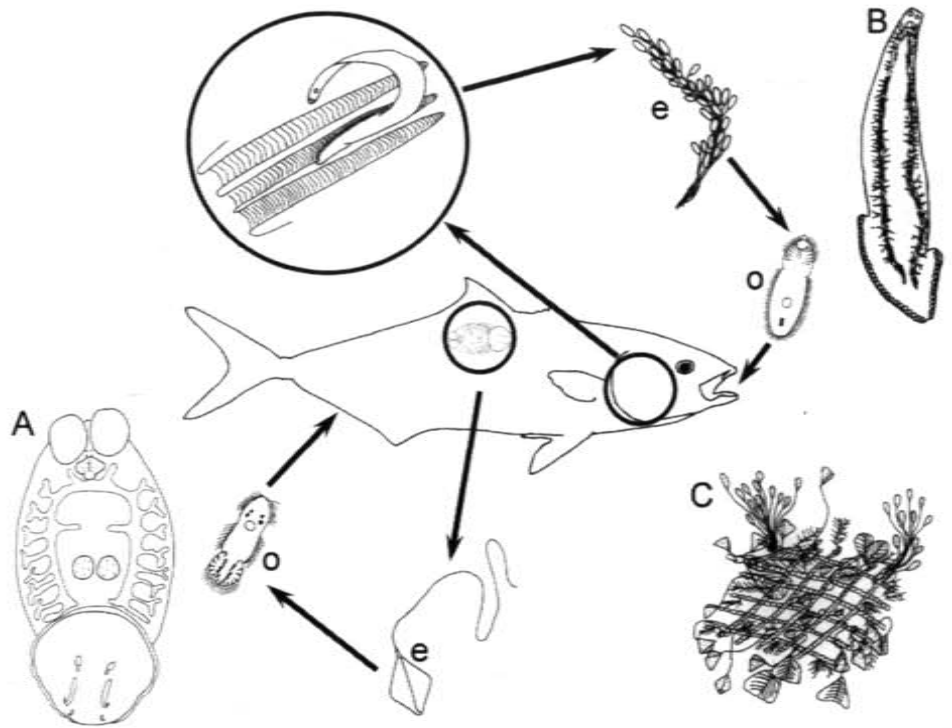


**Fig. 13.4** Life cycles of (A) *Benedenia seriolae* (Monopisthocotylea: Capsalidae) from the 'skin' and (B) *Zeuxapta seriolae* (Polyopisthocotylea: Heteraxinidae) from the gills of several *Seriola* species (Carangidae). Eggs, e; larva or oncomiracidium, o. In intense *Seriola* species aquaculture (e.g., Japan, see Fig. 13.9A), eggs of each monogenean species may tangle on filamentous or leafy algae and other fouling organisms that grow on sea cage material (C).

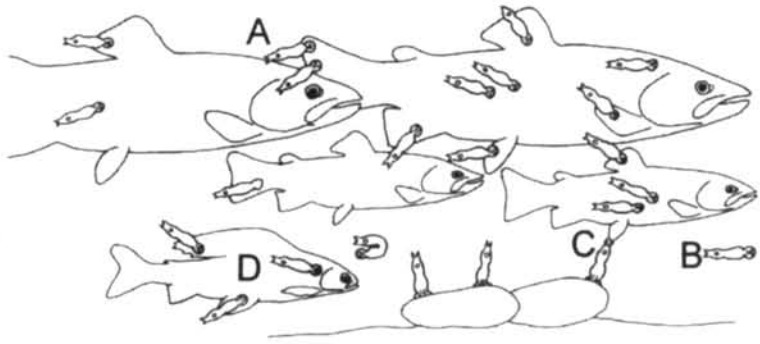


**Fig. 13.5** Life cycle, based on *Gyrodactylus salaris* (Monopisthocotylea: Gyrodactylidae) from the external surfaces of *Salmo salar* (Salmonidae), showing how viviparous Monogenea may spread by: contagion (A) (based on Kearn, 1994) when host fish contact each other; drifting individuals in the water current (B) and temporary settlement on a substrate (C). For *G. salaris*, closely related salmonids such as grayling (*Thymallus thymallus*) can also be hosts (D).

(Fig. 13.5). Monogeneans are generally strictly host specific (i.e., 1 species of Monogenea may infect only a single fish species). There are, however, some notable exceptions such as the notorious pathogen *Neobenedenia melleni* (Monopisthocotylea: Capsalidae) which may infect >100 teleost species (e.g., Whittington and Horton, 1996; Bullard *et al.*, 2000a). Ongoing studies also suggest that a *Microcotyle* species (Polyopisthocotylea: Microcotylidae) may also have broad host specificity (Hayward, 2005). With no intermediate hosts in the life cycle and the restricted host range of most monogenean species due to strict host specificity, some Monogenea species can be common and significant pathogens for confined fish in aquaculture, in large public aquaria and in small home aquaria. Alarmingly, published reports of the pathogenic effects of Monogenea on captive fishes increase every year.

## THE APPROACH TAKEN

This chapter demonstrates the emerging economic importance of Monogenea to captive biomasses of fish. Worldwide, however, there are <100 specialists on monogenean parasites and these research scientists do not necessarily focus principally on disease problems. Instead, there is an extensive blend of investigations into fundamental (e.g., systematics and taxonomy; life cycle adaptations; attachment structures; physiology; specificity; ecology; evolution) and applied (e.g., problematic species; pathology; treatment; immunology) topics of their biology. Syntheses of

relevant information either centering, or with emphasis, on 'diseases' caused by Monogenea have, with varying levels of detail, been published previously (e.g., Rohde, 1984; Thoney and Hargis, 1991; Cone, 1995; Noga, 2000; Buchmann and Bresciani, 2006). Concise general accounts of marine Monogenea with some space devoted to their economic importance were provided by Hayward (2005) and Whittington (2005). Our approach here is to use our combined knowledge of monogenean parasites to communicate a wide-ranging overview of what we consider the most important, interesting and relevant features about the biology and adaptations of Monogenea from fish. From this, we demonstrate how and why these parasites can impact on fish health using examples selected from published accounts as well as our own experiences and observations on Monogenea. We regularly receive requests from staff at aquarium supply businesses, public display aquaria and aquaculture facilities for advice about parasite identification, problematic infections, monitoring procedures and ways to reduce Monogenea populations. The content and format of what we present here attempts to embrace and answer the types of questions we receive by placing the focus on the biological context of the parasites' interactions with their hosts. Some particular issues caused by specific parasites are also highlighted as exemplar species. Our intention is to provide those personnel dealing with fish and their diseases with a framework of actions and resources, including techniques, to broadly identify the Monogenea family or species in question, why it may be causing problems, information on symptoms and pathology and possible treatments. We have drawn on an extensive volume of published literature about the biology of monogenean parasites. We hope this contribution assists those with interests and occupations in parasites, parasitology and diseases of fish to make informed decisions and offers resources, solutions and/or ideas to remedy any problems encountered.

## GENERAL CHARACTERISTICS RELATED TO PARASITE BIOLOGY

Monogeneans range from 300 μm long, e.g., some Dactylogyridae and Gyrodactylidae (Monopisthocotylea; Fig. 13.3B, D) to >3 cm long for some Capsalidae (Monopisthocotylea; Fig. 13.3A), Chimaericolidae, Diclidophoridae (Fig. 13.3G) and Hexabothriidae (Polyopisthocotylea). The body of small species tends to resemble a slightly flattened, translucent cylinder (Dactylogyridae; Diplectanidae; Gyrodactylidae), whereas the body shape of larger monopisthocotyleans (Capsalidae;

Microbothriidae; Monocotylidae) and polyopisthocotyleans (Diclidophoridae; Heteraxinidae) can be leaf-shaped and sometimes strongly flattened dorsoventrally (e.g., Fig. 13.2F). Haptor morphology is diverse among monogeneans (Figs. 13.1-13.3). Its structure and appearance depends on the family of Monogenea encountered. More than 50 families are recognized (Boeger and Kritsky, 2001; Hayward, 2005; Whittington, 2005) and representatives of those most commonly associated with fish diseases are shown in Figure 13.3.

The monogenean haptor is important for 2 major reasons. (1) Knowledge of its specific structure and morphology is critical for the identification of any monogenean parasite; and (2) the manner in which the haptor secures attachment to fish may play a large part in any damage and pathology caused to the host. The single symmetrical unit comprising the monopisthocotylean haptor develops from a simple attachment structure in oncomiracidia equipped with 14 or 16 small sclerotized hooklets (<15 μm long) mostly arranged around the edge (Fig. 13.1B). Some monopisthocotylean oncomiracidia (e.g., Capsalidae) may hatch with additional, larger haptoral sclerites (Fig. 13.4A). The larval haptor attaches the tiny monopisthocotylean oncomiracidium (from 50–600 μm long) to the newly infected host fish (Figs. 13.1B, 13.4A, 13.6E, F). Monopisthocotylean oncomiracidia may infect the external surfaces of their host (Fig. 13.4A) and then migrate to their specific microhabitat, but the larvae of some species are known to locate their specific sites directly (Chisholm and Whittington, 2003). The diet of Monopisthocotylea is epithelial cells and some may eat mucus. As the parasite grows and matures, the haptor of Monopisthocotylea can develop 1 or 2 pairs of large additional hooks called hamuli (Figs. 13.2A, B, D, and F) which usually assist in maintaining secure attachment by piercing the host's epithelium. In some adult monopisthocotyleans, the haptor may also develop extra structures or sclerites to further assist firm attachment. Additional structures include central, prop-like accessory sclerites in Capsalidae (Figs. 13.2F, 13.3A, 13.4A), formation of muscular compartments called loculi (e.g., Monocotylidae; Fig. 13.1A) and development of tiny stud-like sclerites ventrally (Fig. 13.1A) and/or dorsal structures (Monocotylidae; see Fig. 13.2D) or tiny spines (e.g., the squamodisc of Diplectanidae; see Fig. 13.3C). Despite the presence of robust and/or minute sclerites to prevent slippage, the haptor of some monopisthocotyleans may rely more on suction as the primary means of attachment (e.g., many Capsalidae and Monocotylidae). Another innovation in haptoral attachment by some

Monopisthocotylea is the use of adhesive secretions. Species in some families have developed posterior adhesives to supplement attachment by sclerotized hooks and hamuli (e.g., Dactylogyridae; Fig. 13.2B, C) whereas adult Microbothriidae, which infect sharkskin, have a haptor with neither sclerites nor loculi. Instead, microbothriids firmly cement themselves to shark denticles (Fig. 13.2G) by glues secreted from gland cells that open onto the haptor surface. Adult *Anoplodiscus* species (Anoplodiscidae) use adhesives not to cement themselves to host epidermis but to the basement membrane (Roubal and Whittington, 1990; also see Fig. 13.2E).

Monopisthocotyleans also have adhesive glands at their anterior ends, which help attach the parasites to the moist, mucus-covered epithelium that they inhabit. Anterior secretions are deployed from gland duct openings that may open directly at the front of the parasite (Dactylogyridae, Fig. 13.3B; Diplectanidae, Fig. 13.3C; Gyrodactylidae, Fig. 13.3D; Monocotylidae, Fig. 13.3F), through weak muscular grooves (Microbothriidae, Fig. 13.3E) or via specialized disc-like organs that function as suckers and adhesive pads (Capsalidae, Figs. 13.2F, 13.3A). Anterior attachment is usually temporary and highly mobile monopisthocotyleans often move rapidly like a leech across host epithelia by alternating attachment by the haptor and the anterior secretions. Tissue adhesion by anterior glues from a few Monopisthocotylea has been studied to determine how immediate chemical attachment to, and instant detachment from, wet slimy host surfaces occurs (Whittington and Cribb, 2001). Use or involvement of chemical secretions in monogenean attachment, especially by the haptor where there is potential for prolonged interaction between a secretion of parasite origin and host tissue, may contribute to pathogenesis.

The larger, often more extensive symmetrical or sometimes asymmetrical haptor observed in adult polyopisthocotyleans (Figs. 13.1C, 13.3G, H) develops from a simple symmetrical structure in larvae (Fig. 13.1D). Polyopisthocotylean oncomiracidia possess a haptor armed with between 8 and 10 (Hexabothriidae) or up to 16 (Chimaericolidae) sclerotized hooklets (<15 – 25 μm long) arranged mostly around the periphery of the attachment organ (Fig. 13.1D). Additional haptoral sclerites (e.g., 1 or 2 pairs of hamuli and sometimes even a pair of small clamps) may be present in the haptor of some freshly hatched larvae of some families. Polyopisthocotylean oncomiracidia mostly infect fish via the inhalant, respiratory current (e.g., Fig. 13.4B) when newly invaded larvae (from 150 – 350 μm long) settle on and attach to the host's gill epithelium

using hooklets, hamuli and, if present, clamps. Parasites are likely to begin to ingest blood almost immediately (Roubal and Diggles, 1993) and commence growth and development. Haptoral clamps begin to develop soon after larval invasion. Depending on their structure, clamps either attach by suction to flat surfaces in the branchial chamber or mechanically grasp individual, or groups of, gill lamellae (e.g., Llewellyn, 1958; Kearn, 2004; Hayward, 2005) in the manner of a bulldog clip used to secure several sheets of paper. The number of clamps in many families is predetermined and set at 3 or 4 symmetrically arranged pairs (e.g., Fig. 13.3G), but species in some families may develop numerous clamps (sometimes >200 pairs) arranged either symmetrically or asymmetrically (Figs. 13.1C, 13.3H). In some families, some larval haptoral structures such as specific hooklets and hamuli may be lost before parasites reach maturity, but hamuli do persist in adults of some groups. Unlike the majority of Monopisthocotylea where haptoral sclerites, including sometimes large, often robust and pointed hamuli that physically impale host epithelial tissue, the clamps of most polyopisthocotyleans simply grasp the gill lamellae. While this action may compress gill epithelium over an extended area, especially in large multiclamped species, it is generally accepted that clamps of Polyopisthocotylea rarely provoke a host tissue response (but see Montero *et al.*, 2004). Adhesive secretions in the haptor and at the anterior end of adult polyopisthocotyleans are rarely reported. Larvae of several polyopisthocotylean species do possess anterior gland cells (Whittington *et al.*, 2000) and these are probably used in the early stages of infection, for migration to their preferred microhabitat in the gill chamber. When they reach adulthood, the combination of a lack of anterior adhesive secretions and in some species, a plethora of tiny haptoral attachment clamps (Figs. 13.1C, 13.3H), probably contributes to their rather sedentary lifestyle.

Another major and significant difference between Monopisthocotylea and Polyopisthocotylea is their nutrition source. With the possible exception of a bizarre monopisthocotylean family, the Amphibdellatidae, which for some of their life cycle occupy the heart and circulatory system of rays (Euzet and Combes, 1998; Whittington, 2005), all Monopisthocotylea feed on host epithelial cells. Their nutritional source, therefore, is amply available wherever these parasites live (Table 13.1) from the external body surfaces, gills and eyes to the urogenital system and digestive tract. The mechanism of feeding has been studied in remarkably few Monogenea. In Monopisthocotylea, the pharynx can be a large

bipartite structure comprising strong musculature and extensive, intrinsic gland cells (e.g., Capsalidae; Fig. 13.3A) or a small spheroidal, mostly muscular, structure provided with intrinsic and/or extrinsic gland cells (e.g., Dactylogyridae, Gyrodactylidae, Monocotylidae; Fig. 13.3B, D, F, respectively). During feeding, the pharynx is both protruded via the mouth and simultaneously pushed against adjacent host epithelium (e.g., in the Capsalidae) or epithelial cells are drawn into the oral and pharyngeal cavities by suction (e.g., in Dactylogyridae, Gyrodactylidae, and Monocotylidae). Proteolytic products from the extensive array of pharyngeal gland cells separate host epithelial cells and begin to digest them. Contractions of the pharynx force disassociated host epithelial cells into the parasite's gut where intracellular digestion occurs. At relatively low parasite burdens, epithelial feeding combined with mobility may spread any wounds inflicted by Monopisthocotylea over a wide host area. Small monopisthocotylean species such as diplectanids and gyrodactylids tend to have a simple bifurcate gut (Fig. 13.3C, D) but some families possess a single gut tube (Tetraonchoididae). The gut of some species can be confluent posteriorly (e.g., some Dactylogyridae; Fig. 13.3B) and in large Monopisthocotylea, the gut may be highly branched (most Capsalidae; a few Monocotylidae; Figs. 13.3A, F, respectively). With the exception of *Dendromonocotyle* species (Monocotylidae), which may incorporate host 'skin' pigment into their dendritic gut and some microbothriid species, the gut contents of monopisthocotylean monogeneans usually lack pigmentation or may appear grey because fish epithelial cells are rarely pigmented.

In stark contrast, gut contents of polyopisthocotylean monogeneans are commonly intensely pigmented and appear dark brown or black due to haematin-rich residues, a direct result of their blood diet (e.g., Fig. 13.4B). Living on fish gill tissue, most Polyopisthocotylea need only breach the thin epithelial wall of a gill lamella to access their plentiful and renewable nutrition source. There are astonishingly few published accounts of feeding by Polyopisthocotylea. The accepted mechanism is that the anterior end is applied to the epithelial surface of gill tissue, perhaps aided by buccal suckers in species that possess them. The mouth then contacts the gill tissue; the pharynx is protruded and is possibly used to rupture the thin branchial epithelium. Blood may spurt, because of pressure, or be forced into the gut via peristaltic contractions of the small muscular pharynx (Fig. 13.3G, H). In the gut, blood proteins are digested intracellularly. The gut of most polyopisthocotyleans is bifurcate and may sometimes be fused posteriorly, but in many large species it has lateral and

median branches to increase its surface area (Fig. 13.3G, H). There is poor knowledge on the regularity of feeding and the volume of blood ingested at each meal. However Ogawa *et al.* (2005a) quantified that adult *Heterobothrium okamotoi* (Polyopisthocotylea: Diclidophoridae) could consume >84 μL of host tiger puffer blood, *Takifugu rubripes* (Tetraodontidae), during the parasite's estimated 2-month lifespan.

The digestive system of flatworms is closed (i.e., one way) and Monogenea egest undigested food via the mouth. While Polyopisthocotylea have dark gut contents, if an individual has recently regurgitated waste haematin, the gut may be colourless until feeding resumes.

When Monogenea reach adulthood on their specific host, they generally inhabit a precise site. Their site specificity can be relatively broad, e.g., *Entobdella soleae* (Monopisthocotylea: Capsalidae) may live anywhere on the lower 'skin' surface of its sole host, *Solea solea* (Soleidae) (see Kearn, 1998, 2004). Other species have a quite specific site even on 'skin', e.g., *Dendromonocotyle* species (Monopisthocotylea: Monocotylidae) may preferentially inhabit the dorsal surface of the rostrum and between the eyes of rays (Fig. 13.9F). Monogenea that infect gills may occupy a specific microhabitat encompassing only a few gill lamellae in a particular part of a specific gill arch or arches, e.g., *Kuhnia* species (Polyopisthocotylea: Mazocraeidae) on mackerel, *Scomber* species (Scombridae; see Rohde, 1993). Some researchers believe that the 'biological function' of the strong site specificity exhibited by Monogenea is to promote opportunities to mate. All monogeneans are hermaphroditic and while most are probably capable of self-fertilization, it is generally believed that to produce viable eggs, the majority of species require cross-fertilization (e.g., Kearn *et al.*, 1993). The male reproductive system of most Monogenea species matures before the female reproductive system (= protandry). So, during parasite growth and development, a monogenean individual can have a sexually mature male reproductive system capable of producing sperm but an immature female reproductive system incapable of producing eggs. Here, we define a monogenean that has sexually mature male *and* female reproductive systems as *fully* sexually mature (i.e., capable of insemination *and* laying eggs).

Once fertilization is achieved and a monogenean is fully sexually mature, most adult parasites usually shed their eggs directly into the water column (Fig. 13.4). There are a few exceptions to this. Some adults hold onto laid eggs outside the body until they hatch, others attach eggs to host tissue and a few store eggs inside the body until they hatch internally

(ovoviviparity) when oncomiracidia escape into the water (Whittington, 2005). An evolutionary extension of these strategies is the viviparity displayed by most members of the Gyrodactylidae which give birth to live, non-swimming, highly developed individuals that reinfect the host directly and spread among a host population by contagion (Fig. 13.5).

Eggs laid by the diversity of oviparous Monogenea are themselves diverse in shape and size. Eggs can be spherical, oval, ovoid, prolate spheroids, fusiform or tetrahedral and may be laid singly or can be tethered together in bundles, strings or chains (Kearn, 1986) (Figs. 13.4, 13.6A-D). Egg morphology can be a useful diagnostic feature. Monogenean eggs are encapsulated by a proteinaceous, tanned eggshell which confers protection to the developing embryo from digestion (Kearn, 1986) and bacteria and proves to be particularly resistant to attack from various chemical treatments.

Monogeneans have evolved an array of intricate and fascinating strategies that increase their chances of finding and successfully infecting their specific fish at natural host densities experienced in the wild. Egg shape may increase their chances of staying in the vicinity of a host when hatching occurs. For example, monogeneans that infect pelagic hosts such as marlin (Istiophoridae) and tuna (Scombridae) may have eggs with appendages (Fig. 13.6A) which slow their sedimentation rate and increase their likelihood of remaining in surface waters where hosts are located (Kearn, 1986). Long filamentous appendages (Fig. 13.6B) may entangle eggs on substrates or retain egg bundles in areas where fish may congregate (Kearn, 1986). Two polyopisthocotylean species are known to lay eggs rhythmically which may further enhance opportunities for host infection (Macdonald and Jones, 1978; Mooney *et al.*, 2006). We anticipate that egg-laying rhythms may be more widespread among Monogenea.

Egg hatching in monogeneans has been linked to light periodicity, variations in light intensity (i.e., shadows), chemicals in host mucus and tissues and mechanical disturbances (Kearn, 1981; Chisholm and Whittington, 2000; Whittington *et al.*, 2000). This range of stimuli is believed to relate either to the general habits of the host or to the presence of the host in the vicinity of eggs, thereby maximizing the chances for successful infection. Studies demonstrate that some monogenean oncomiracidia may remain viable inside eggs for long periods waiting for specific chemical hatching cues, e.g., up to 83 days for *Acanthocotyle lobianchi* (Monopisthocotylea: Acanthocotylidae; see Macdonald, 1974) and up to 88 days for *Hexabothrium appendiculatum* (Polyopisthocotylea: Hexabothriidae; see Whittington, 1987). Hatched oncomiracidia (mostly

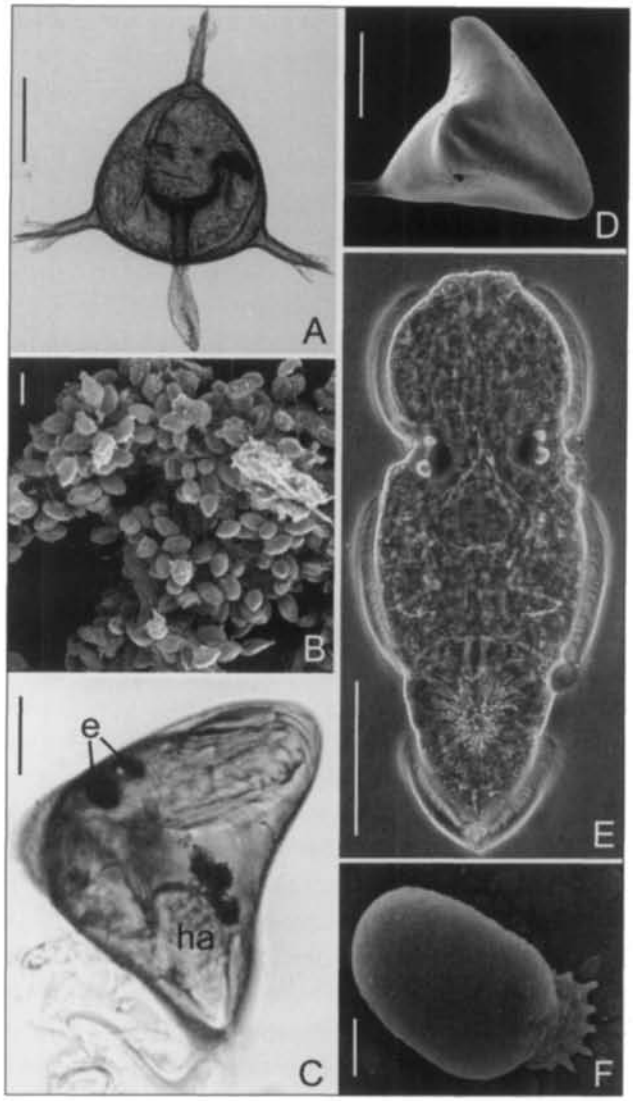


**Fig. 13.6** Monogenean eggs and oncomiracidia. A. Single tetrahedral egg of *Capsala laevis* (Monopisthocotylea: Capsalidae); appendages on each pole slow egg sedimentation rates. B. Oval eggs of *Zeuxapta seriolae* (Polyopisthocotylea: Heteraxinidae) tethered together by filaments; long egg strings may readily entangle on substrates (see also Fig. 13.4C). C. Embryonating oncomiracidium (note eyespots [e] and haptor [ha]) of *Dendromonocotyle* sp. (Monopisthocotylea: Monocotylidae) within a tetrahedral egg. D. Tetrahedral egg of *Neobenedenia* sp. (Monopisthocotylea: Capsalidae). E. Free-swimming *Dendromonocotyle* sp. oncomiracidium (note ciliation). F. Oncomiracidium of *Acanthocotyle lobianchi* (Monopisthocotylea: Acanthocotylidae) newly attached to host ray skin by haptoral hooklets. Scale bars: A, D, E = 50 µm; B = 100 µm; C, F = 20 µm.

ciliated; sometimes unciliated) may only be viable for up to 48 h and perhaps for even less time at higher water temperatures. Most ciliated larvae (e.g., Fig. 13.6E) have a peak swimming speed of approximately 4 mm $s^{-1}$ (Whittington *et al.*, 2000), which is considerably slower than the fish they infect. Many hatched larvae display specific behaviours (e.g., phototaxis, geotaxis, rheotaxis and chemotaxis; see Whittington *et al.*, 2000), which again, increase the probability of successful infection of wild fishes. Evolution of viviparity in the Gyrodactylidae dispenses with freely deposited eggs and these Monogenea transfer from fish to fish when contact occurs (e.g., shoaling, mating, fighting and other social interactions; e.g., Fig. 13.5A). Bakke *et al.* (1992) stressed that other dispersal strategies may also occur (e.g., live, detached parasites drifting in plankton, Fig. 13.5B; parasites resting on inert substrates, Fig. 13.5C; transmission via 'transport' hosts, Fig. 13.5D) and live fish coming into contact with recently dead, infected fish.

For most Monogenea, the following important life cycle parameters are poorly studied or completely unknown: *generation time* (period elapsed between an egg being laid and an oncomiracidium hatching from that egg infecting a host and maturing to become an egg-laying adult); *time to reach full sexual maturity* (period elapsed between host infection by an oncomiracidium and its maturation to an egg-laying parasite); *fecundity* (egg output per unit time per parasite); whether fecundity changes with parasite age; *longevity* (average parasite lifespan). Due to the huge diversity of Monogenea on a variety of fish species, no generalization to cover all combinations of monogenean species and host fish species can be made. Typically, however, the rate of development (whether eggs or parasites) is directly related to water temperature (i.e., at low water temperatures, laid eggs and parasites develop more slowly than at higher water temperatures). Some simplifications for a few important life history details can be inferred, however, at the level of monogenean family.

For example, studies by Cone and Burt (1981, 1985) on *Urocleidus adspectus* (Monopisthocotylea: Dactylogyridae) from the freshwater yellow perch, *Perca flavescens* (Percidae), suggest that the parasite is unable to lay eggs at 4°C and the almost total absence of juveniles in winter fish samples indicates no parasite transmission occurs. As seasonal water temperatures increase, egg production occurs and predictably, parasite activity and egg laying increase while generation time decreases. At 20°C, generation time is about 14 days and at natural lake temperatures over a 4-month summer period in Canada, at least 8 parasite generations can be completed. Juvenile parasites collected throughout this period confirm that perch are

continually infected over summer. Perch acquire infections in the first few months of life and appear to lose and reacquire parasites annually. Ciliated oncomiracidia invade perch anywhere on the body surface and attach beneath scales. From there, they migrate anteriorly to reach the gills by entering via the opercula and buccal cavities. Parasites mature to egg-laying adults on the gills. Length-frequency distributions of parasites on different age classes of hosts indicate that *U. adspectus* grows larger on larger hosts.

Roubal and Diggles (1993) investigated the biology of *Polylabroides multispinosus* (Polyopisthocotylea: Microcotylidae) from gills of *Acanthopagrus australis* (Sparidae) in Australia. A ciliated oncomiracidium hatches bearing 5 hooklet pairs, 1 pair of hamuli and 1 pair of clamps approximately 9, 7 and 6 days after laying at 20, 24 and 28°C, respectively. In experimental infections at 24°C, stages developmentally similar to oncomiracidia were found attached to gills and, after 1 to 2 days, juveniles with 3 clamp pairs already contained haematin in the gut. Parasites reached full sexual maturity after 20 days, when they possessed between 35 and 45 clamp pairs, demonstrating the rapidity of their growth and continued to add clamp pairs after full maturity. *Polylabroides multispinosus* has a generation time of about 30 days at 24°C.

These examples underline the importance and value of monogenean parasite-fish host 'models' (i.e., associations amenable to manipulation either in aquaria and/or in the field) to generate useful core data about the biology and life history of Monogenea species on their specific host. Use of convenient models is especially constructive if determinations of important life cycle parameters can be made for Monogenea *in vivo* because this reflects most accurately the biology of the parasites *in situ* (i.e., when attached to the host) and, therefore, is most likely to replicate natural events and conditions. For example, *in vivo* assessment of parasite fecundity measures the number of eggs laid per parasite per unit time for monogeneans attached to their host, whereas an *in vitro* assessment measures the *unnatural* situation of adult parasites removed from the host and left to starve and lay eggs in an unaerated dish of water.

## CONSEQUENCES OF THE DISSIMILAR BIOLOGIES OF MONOPISTHOCOTYLEA AND POLYOPISTHOCOTYLEA

An overview of differences between these 2 monogenean subclasses was presented above. Their different impacts on fish health relate to differences in attachment, mobility and diet.

## Attachment

The mostly non-invasive use of haptoral clamps or suckers by Polyopisthocotylea results in their predominantly benign attachment. There exist only a few cases of pathology and, where reported, it is due to an unusual attachment method. The Hexabothriidae is a family whose species infect the gills of sharks and rays. Hexabothriids have 6 suckers, each armed with a single, curved sclerite terminating in a pointed, recurved spine which may impale gill epithelium. Hyperplasia of shark gill tissue has been reported in captive hammerhead sharks, *Sphyrna tiburo* (Sphyrnidae) caused by *Erpocotyle tiburonis* (Hexabothriidae; see Bullard *et al.*, 2001). Haptoral suckers of most hexabothriids, however, usually operate using suction and in normal attachment, suction is unlikely to provoke a strong host reaction (Kearn, 2004). Adult *Neoheterobothrium* species (Diclidophoridae) can reach lengths of >30 mm and their clamps embed deeply into the muscle tissue of the buccal cavity of the Japanese flounder, *Paralichthys olivaceus* (Paralichthyidae) causing dermal necrosis and stimulating infiltration by inflammatory cells and fibroblasts (Anshary and Ogawa, 2001; Ogawa, 2002; Hayward, 2005). *Hexastoma* species (Hexastomatidae) attach unusually because the haptor powerfully compresses the secondary gill lamellae of host tuna, *Thunnus* species (Scombridae), causing gill tissue to swell which essentially strengthens attachment by the parasite (Hayward, 2005). There are no significant reports of any posterior glandular secretions in adult Polyopisthocotylea.

In contrast, the vast array of different sclerotized attachment devices in the haptors of Monopisthocotylea (Figs. 13.1A, 13.2, 13.3A–F) and whether or not they penetrate host epithelial tissue can result in a range of pathological responses from none to extreme. Whittington (2005) concluded that at 'natural' intensities on healthy wild hosts, monopisthocotyleans rarely stimulate a strong tissue response. Their small hooklets must certainly grip epithelial cells by penetration (e.g., in gyrodactylids, Fig. 13.7; see also Buchmann and Bresciani, 2006) and the larger ventrally directed and pointed hamuli of some capsalids and monocotylids also definitely impale host epidermis (Kearn, 1964, and Chisholm and Whittington, 1998, respectively). Some small Monopisthocotylea possess 2 pairs of hamuli, 1 pair ventrally directed and the other pair dorsally directed (e.g., Dactylogyridae; Diplectanidae; Figs. 13.2B, 13.3B, C), which adapts them perfectly to attach between adjacent secondary gill lamellae (Fig. 13.2C). Even though these monogeneans

inhabit gill tissue, they still feed on epithelial cells. Their hamuli penetrate gill epithelium and there are several reports of mechanical damage inflicted to carp (Cyprinidae, by dactylogyrids; e.g., Bychowsky, 1957; Prost, 1963), sea bass (Moronidae, by diplectanids; e.g., Oliver, 1977), bream (Sparidae, by dactylogyrids and diplectanids; e.g., Roubal, 1986; 1989) and dhufish (Glaucosomatidae, by dactylogyrids; e.g., Kritsky and Stephens, 2001, Fig. 13.9G). This is by no means an exhaustive list. Adult *Dactylogyrus extensus*, a common dactylogyrid of common carp, *Cyprinus carpio* (Cyprinidae), provokes a tissue response and hyperplasia (excessive tissue proliferation) entirely encloses the haptor of the parasite. Younger parasites, however, elicit no such response (Kearn, 2004). Roubal (1989) reported a similar occurrence for adult *Lamellodiscus major* (Diplectanidae) on *Acanthopagrus australis* (Sparidae). There may be at least 2 reasons for these marked tissue responses by these dactylogyrid and diplectanid species. (1) Perhaps adults of these species are less mobile than most other Monopisthocotylea and therefore they may eventually aggravate a greater host reaction, which ultimately results in them becoming entirely sedentary. (2) Unlike Polyopisthocotylea, many larval and adult Monopisthocotylea possess extensive posterior gland secretions. Perhaps the observed pathology associated with the haptor of *D. extensus* and *L. major* is associated with posterior chemical secretions. It is presumed that posterior secretions in dactylogyrids and diplectanids have an adhesive role or perhaps a histolytic function to soften (Kearn, 2004), or even help hamuli to penetrate into, host gill tissue.

## Mobility

With the possible exceptions of examples just discussed, the Microbothriidae which glue their haptor to shark denticles (Fig. 13.2G and also see Kearn, 1965) and *Anoplodiscus* species (Anoplodiscidae) that cement the haptor to teleost fin rays (e.g., Roubal and Whittington, 1990 and Fig. 13.2E), it is widely considered that the majority of other Monopisthocotylea, whether juvenile or adult, are highly mobile and move, perhaps continually, to minimize the impacts of their attachment armature and feeding activities (see below). Polyopisthocotylea, however, are largely immobile when adult and if large scale movements on hosts are required, these probably occur shortly after invasion and before parasites develop their characteristic adult attachment structures of suckers and/or clamps. Indeed, the evolution of these relatively inert and benevolent attachment mechanisms may be linked directly with their ability to remain sedentary.

**Fig. 13.7** Scanning electron micrograph of *Gyrodactylus salaris* (Monopisthocotylea: Gyrodactylidae) attached by the hooklets to the skin of an Atlantic salmon, *Salmo salar* (Salmonidae). Scale bar = 10 μm.

## Diet

Mobility by most Monopisthocotylea means that their browsing and feeding on epithelial tissue is mainly spread over a broad host area. If feeding were concentrated in a restricted zone, this behaviour would more likely elicit a strong host response. A typical feeding wound inflicted by an epithelial feeding monogenean probably heals rapidly because cells multiply and migrate across the wound to seal it and restore its integrity (Kearn, 1998). In a fish heavily infected by epithelial browsers (e.g., Fig. 13.8A), even the ability to move to new pastures may provide a limited respite because fish are eventually unable to replace their epithelial cells fast enough (Fig. 13.8C). A downward spiral in fish health may occur swiftly. Sick fish may eat less due to reduced appetite. They become weaker and may eventually die. An epizootic of *Benedenia monticellii* (Monopisthocotylea: Capsalidae) reported on *Liza carinata* (Mugilidae) by Paperna *et al.* (1984) adds authenticity to this example. As epithelial cells of infected mullet were consumed by burdens of >50, and in some cases >100, parasites, specimens of *B. monticellii* began to overrun the entire fish surface. Not surprisingly, as epithelium for consumption by parasites

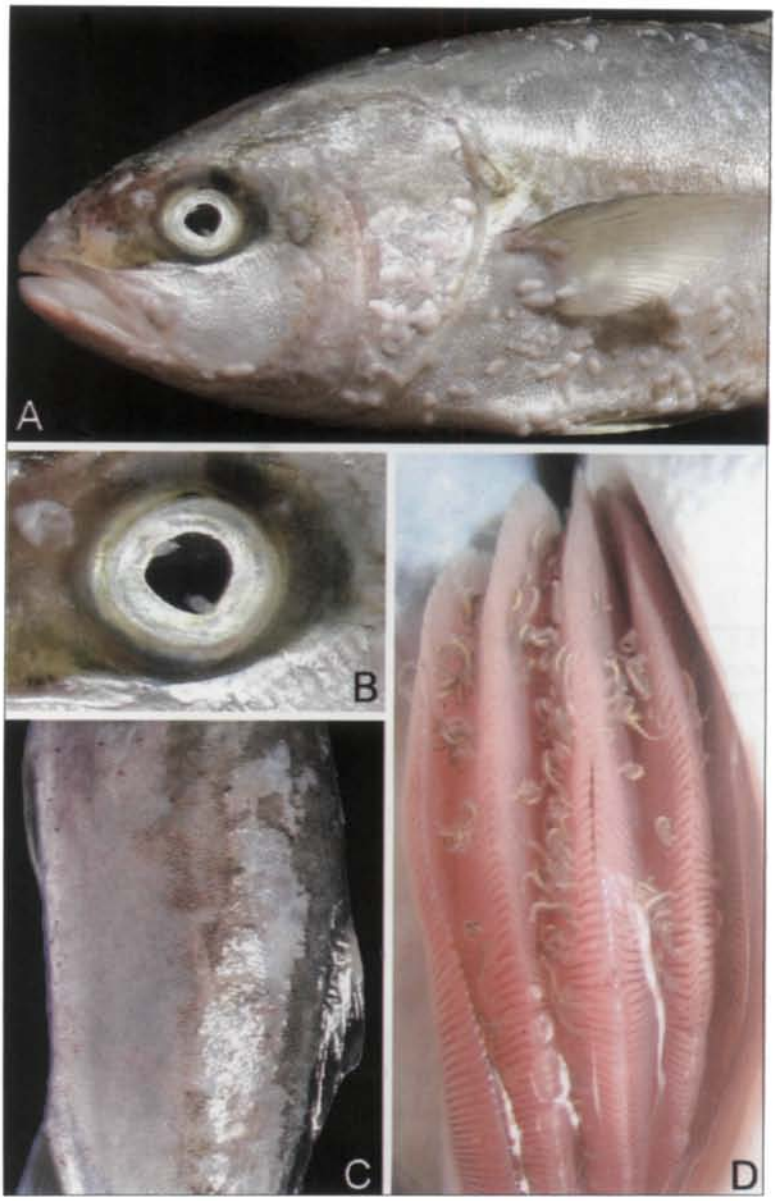


**Fig. 13.8** Heavy monogenean infections promoted experimentally on yellowtail kingfish, *Seriola lalandi* (Carangidae). A. Heavy infection of the 'skin'-dwelling monopisthocotylean, *Benedenia seriolae* (Capsalidae). The parasites appear white and conspicuous after bathing in freshwater. B. *Benedenia seriolae* on the cornea of the eye. C. Skin lesions caused by acute *B. seriolae* infections. D. Gills (note pale colour indicating anaemia) with a moderate to heavy burden of *Zeuxapta seriolae* (Polyopisthocotylea: Heteraxinidae).

became limited, parasite density declined (presumably the parasites began to starve?) and those that remained appeared to avoid areas of necrosis and occurred on 'skin' remaining near the dorsal fin (Paperna *et al.*, 1984).

The immobility of Polyopisthocotylea does not mean that they feed on blood continually or at the same site. As for Monopisthocotylea, their feeding is discontinuous but there exist virtually no data about the regularity and meal size of either parasite group. The remarkable flexibility of Polyopisthocotylea can result in their largely tubular or slightly dorsoventrally flattened body extending to more than 5 or 6 times their resting length (Figs. 13.4B, 13.8D). This considerably extends the range of feeding sites available to an attached, sedentary parasite. Therefore, different gill lamellae, different hemibranchs and even different gill arches can be reached by the wandering mouth of a sedentary monogenean. This significantly reduces the chances of stimulating a host gill tissue response by feeding only at the same site. After feeding, the wounds generated can continue to leak blood. Despite this, the effect and impact of feeding by natural populations of polyopisthocotyleans on fish hosts is unstudied (Kearn, 2004). While elevated polyopisthocotylean burdens on fish in confinement are documented to cause problems such as anaemia (Fig. 13.8D) in aquaria (Longshaw, 1996) and in fish culture (e.g., Yoshinaga *et al.*, 2001a, b; Nakayasu *et al.*, 2002), there is an ongoing debate whether infections are unequivocally associated with, and solely responsible for the disease. Other factors relating to host health may work synergistically to effect the condition of fish (Hayward, 2005). Nevertheless, Ogawa *et al.* (2005a) determined that the volume of blood consumed by *Heterobothrium okamotoi* (Diclidophoridae) increased as the parasites grew and adults ingested 0.69 ± 0.93 μL of host blood in 12 h.

## A BROAD OVERVIEW OF PROBLEMATIC MONOGENEA AND HOW THEY CAN EFFECT HOST FISH

Monogenean parasites and their ancestors have evolved over >400 million years with aquatic vertebrates. The result is the finely tuned, highly adapted and very successful diversity of fish ectoparasites we are familiar with today. The parasite biologies emphasized above evolved to ensure that the monogenean life cycle (Fig. 13.4) can be completed to cope with the biology of their specific fish host(s) at natural population densities (e.g., a tight shoal swarming in shallows; a smaller, more dispersed shoal in deeper water near reef outcrops; relatively solitary cosmopolitan fishes

migrating across expansive oceans). It is commonly accepted that under natural conditions experienced by fish in ponds, lakes, rivers, estuaries, seas and oceans, monopisthocotylean and polyopisthocotylean Monogenea typically cause no deleterious effects to their hosts. Indeed, published reports of mass mortalities in *wild* fish populations attributed directly to Monogenea are exceptional. In fact, the only case we know of is an epizootic outbreak of *Benedenia monticellii* (Monopisthocotylea: Capsalidae) reported by Paperna and Overstreet (1981) and Paperna *et al.* (1984) that killed many wild specimens of *Liza carinata* (Mugilidae) in the Gulf of Suez in the mid-1970s. While this capsalid epizootic on wild mullet is seemingly highly extraordinary, environmental conditions such as heavy oil pollution may have played a role (Paperna and Overstreet, 1981). Paperna *et al.* (1984), however, indicated that epizootics by *B. monticellii* on *L. carinata* in the Gulf of Suez may *not* be exceptional but perhaps recur during winter months when low water temperatures (<18°C) may inhibit the immune system of mullet. Nevertheless, under 'normal' environmental conditions, the long co-evolutionary period of parasite-host associations is thought to have resulted in a harmonious balance between Monogenea and fish. Generally, it is not in the interests of any parasite to harm its host because damage to the host ultimately damages the activities of the parasite. However, we stress that there is exceedingly little information about 'natural' population levels of Monogenea on fish hosts and the effect these parasite burdens may have (Hayward, 2005; Whittington, 2005). Although a technically difficult field to study, this is an important and potentially fruitful research area in the future.

The same innate intricacies of the direct monogenean life cycle outlined previously can have profound consequences when fish are manipulated and held at artificially high densities. This is commonly a direct result of human activities and our desire to keep fish in aquaria at home, to display fish in commercial aquaria for educational, tourist and business purposes or to grow and farm fish for food via aquaculture. When fish are artificially confined, the egg morphologies, laying rhythms, hatching stimuli, extended egg viabilities and different oncomiracidial behaviours discussed earlier, which ensure monogenean transmission between *wild* fish, can promote uncharacteristically high and intense monogenean populations on *captive* fish (Fig. 13.8A, D). Furthermore, the eggs may tangle and accumulate on artificial surfaces such as sea cage nets (Figs. 13.4C, 13.9D), substrates in tanks and aquaria (Fig. 13.9C) or the eggs may even accrue due to ineffective aquarium filtration. The combined result can be swarms of oncomiracidia that hatch from masses

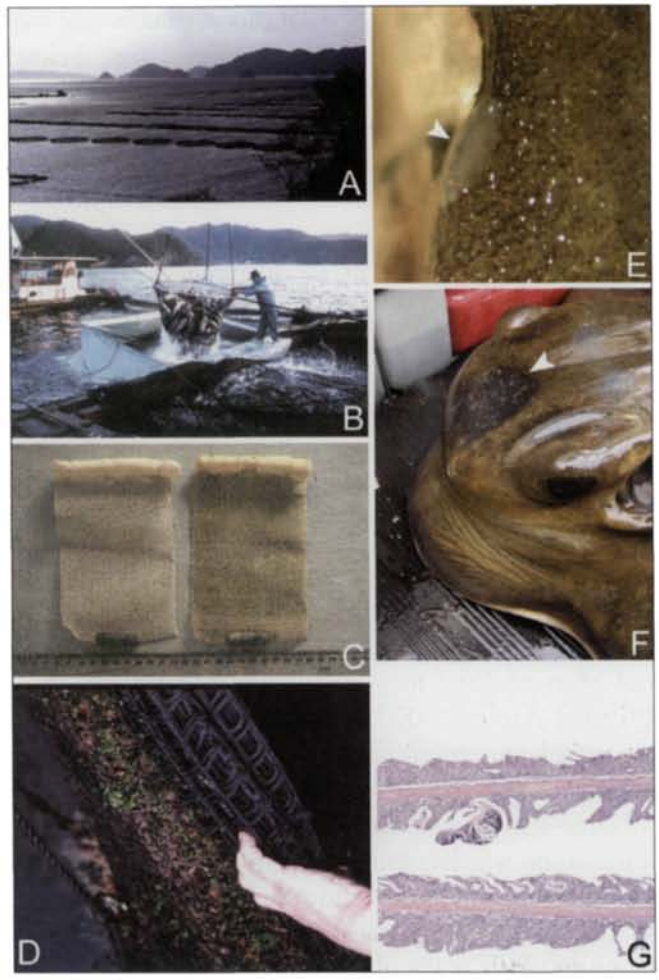


**Fig. 13.9** Aquaculture and Monopisthocotylea Monogenea. A. High density sea cage aquaculture of kingfish (*Seriola* species: Carangidae) in south western Japan. B. Freshwater bathing to kill *Benedenia seriolae* (Capsalidae) infecting kingfish in sea cage culture in south western Japan. A large net scoops parasitized fish from a sea cage into a tarpaulin liner shallowly filled with freshwater. C. Comparison of clean mesh (left) and mesh which has been placed in a tank with fish infected by Monogenea (right). Darker mesh colour is the result of monogenean eggs fouling the mesh. D. Algae-fouled sea cage material which can entangle and trap monogenean eggs (see also Fig. 13.4C). E. *Pseudoleptobothrium aptychotremae* (Microbothriidae) on 'skin' denticles of the southern fiddler ray (*Trygonorrhina fasciata*: Rhinobatidae); the monogenean appears as a raised gelatinous bump (arrowhead). F. *Dendromonocotyle bradsmithi* (Monocotylidae) infection between eyes of a wild caught southern eagle ray (*Myliobatis australis*: Myliobatidae); host skin is darkened in region of infection (arrowhead). G. Gill hyperplasia caused by *Haliotrema abaddon* (Dactylogyridae) infections on west Australian dhufish, *Glaucosoma hebraicum* (Glaucosomatidae).

of eggs in close proximity to a large aggregation of confined fish that may be stressed and therefore immunocompromized (see below). Freshly hatched oncomiracidia may, therefore, have a relatively short distance to travel before they are able to attach to and successfully invade their specific fish host. The consequences of this comparatively effortless and even *more* simplified direct monogenean life cycle can be disastrous for fish health.

The following sections present an overview of why and how some Monogenea can affect the health of fish. We provide general information on: fish in confinement; how other activities can spread and/or promote monogenean infections; some recent advances in immunological knowledge about fish; and how all this relates to the different biologies of their Monogenea. We also present some general data on disease symptoms and associated pathology. Specific cases concerning *Important Monogenean Pathogens* are dealt with later.

## Captive Fish

Confining fish in artificial environments—initially in ponds—began thousands of years ago and may have been practiced in Mesopotamia, Egypt and China. Around 1000 A.D., the Chinese pioneered keeping goldfish indoors in large ceramic pots. Today, it is estimated there are >60 million people whose pastime is to keep fish in home aquaria. The popularity of large-scale public aquaria escalated from the 1850s when the first British aquarium was launched in Regent's Park Zoological Gardens, London. Now, many major cities worldwide boast a large marine aquarium that displays local and exotic marine and freshwater fishes and other aquatic organisms. Fish aquaculture is regarded by many as a recent development, but it may have started in China in the fifth century BC and in Egypt in the second century BC! We do not know whether Monogenea caused disease-related problems to early captive fishes, but it is highly likely that they did. Early published accounts of Monogenea effecting fish in confinement include the outbreak of *Neobenedenia melleni* (as *Epibdella melleni*; Monopisthocotylea: Capsalidae) on several marine fish species in the New York Aquarium (MacCallum, 1927; Jahn and Kuhn, 1932; Nigrelli and Breder, 1934) and various Dactylogyridae (Monopisthocotylea) infections of farmed freshwater fishes in the USSR during the 1930s to the 1950s (Bychowsky, 1957).

Captive fish are prone to stress for several reasons. (1) A higher than normal population density (i.e., more fish individuals stocked per unit volume of water). (2) Less than optimal nutrition from 'artificial' diets. (3)

Poor water quality due to low dissolved oxygen and perhaps increased nitrite and nitrate levels from elevated organic loads due to accumulated uneaten food and/or waste products of fish. (4) Changes to the pH of water can be an additional outcome of accumulated organic products. If captive fish remain stressed, their ability to resist any disease agent diminishes. Ultimately, stressed fish may succumb to sickness because their immune system is compromised, thereby weakening their natural defenses against pathogens. Under these circumstances, captive fish in aquaria and different forms of aquaculture enclosures (ponds; land-based tanks with recirculation systems; flow-through systems; floating sea cages) are clearly susceptible to the direct life cycle of Monogenea. Characteristics of the biology of these parasites mean that monogeneans can proliferate rapidly, leading to an exponential population growth in a short period. Unless corrective measures are introduced, the consequences can be loss of treasured specimens in a home aquarium, death of prize and expensive species in public display aquaria or mortalities of valuable stock in a commercial fish farm. Outbreaks of Monogenea on confined hosts in aquaria and aquaculture are increasingly common worldwide and will, undoubtedly, continue to be documented regularly.

## Other Activities that may Contribute to Problems by Monogenea

Harmful effects caused by so-called 'introduced' species from human activities on species diversity, their ecology and the ensuing economic ramifications receive increasing coverage. While there are probably more cases of 'introduced' parasites than have been documented (Torchin and Kuris, 2005), historically the Monogenea provide some cruel examples of damage that can occur to fish stocks and economies after their accidental exposure to a new system or region. Outbreaks by *Neobenedenia melleni* (Monopisthocotylea: Capsalidae, see above) in the New York Aquarium continue today 80 years since it was introduced. Furthermore, it is likely that this and/or closely related species continually plague public aquaria (Bullard *et al.*, 2003) and their suppliers worldwide. Catastrophic mortalities of ship sturgeon, *Acipenser nudiventris* (Acipenseridae), which is native to the Aral Sea, were caused in 1936–1937 when stellate sturgeon, *A. stellatus* (Acipenseridae), were introduced into the Aral Sea from the Caspian Sea, together with their monogenean parasite, *Nitzschia sturionis* (Monopisthocotylea: Capsalidae), causing an epizootic (Zholdasova, 1997; Whittington and Chisholm, 2003). Today, the Aral

Sea form of *A. nudiventris* is virtually extinct (Zholdasova, 1997)! A similarly devastating event occurred in Norway in the 1970s when *Gyrodactylus salaris* (Monopisthocotylea: Gyrodactylidae; see Figs. 13.3D, 13.5, 13.7) was introduced—perhaps on several independent occasions—into Norwegian rivers on resistant Baltic salmon stocks of Atlantic salmon, *Salmo salar* (see Bakke *et al.*, 2002). An epidemic of *G. salaris* occurred on Norwegian *S. salar* stocks and spread, possibly helped by translocation of infected fish between hatcheries (Johnsen and Jensen, 1986, 1988; Malmberg, 1993), to >40 river systems and >30 hatcheries. Sterud *et al.* (1998) reported that individual salmon parr can be infected by >10,000 specimens of *G. salaris* and this viviparous species is now known in western Sweden, the White Sea coast of Russia, Finland, Denmark, Germany and Norway (Bakke *et al.*, 2002). Differences in the susceptibility of local fish stocks (i.e., immunologically naïve) compared with the introduced fish species or strains carrying the invading alien parasites precipitated these disasters on sturgeon and salmon.

Outbreaks in marine sea cage aquaculture by other Monogenea may be a result of international trade of fish stocks that carry parasites. Import of amberjack fingerlings, *Seriola dumerili* (Carangidae), into Japan from Hainan and Hong Kong is held responsible for the introduction of *Neobenedenia girellae* (Monopisthocotylea: Capsalidae) into Japanese marine aquaculture (Ogawa *et al.*, 1995). Two freshwater gill Monogenea species, *Pseudodactylogyrus anguillae* and *P. bini* (Monopisthocotylea: Pseudodactylogyridae), are common pests in eel (Anguillidae) culture. These parasites were introduced into North America and Europe from Asia via imported Japanese eel, *Anguilla japonica*, and have now parasitized wild North American and European eel, *A. rostrata* and *A. anguilla*, respectively (Hayward *et al.*, 2001a).

The examples given above underline those cases where the introduction of a monogenean species has been either categorically identified or assumed. There are likely to be cases around the world, presently unidentified, where Monogenea species have been unknowingly spread from the endemic range of their host species, perhaps due to the escape or deliberate release of aquarium or cultured fishes into natural water bodies such as streams, rivers or estuaries. It should be remembered that these fish can carry their ectoparasites with them and, if appropriate, related fish species are available in the new environment, it is not unreasonable that the 'alien' parasites may establish and live on the new hosts. Harris (1986) considered that infections of *Gyrodactylus turnbulli* and *G. bullatarudis* (Monopisthocotylea: Gyrodactylidae) on guppies

(Poeciliidae) have been transported widely around the world on fish used ineffectively in waterways for mosquito control programs and via the ornamental fish trade. At least 2 feral populations of guppies have been established in Britain and Harris (1986) warns about the capacity for 'alien' gyrodactylids to transfer to, and then establish on, native British fish stocks. Clearly, there is similar scope for transfers by other viviparous and oviparous Monogenea *anywhere* in the world if environmental conditions, parasite biology and characteristics of potential host species are conducive. Thus caution must be exercised when fish species are transferred out of their natural environments, whether accidentally or deliberately.

In the marine environment, uptake of huge quantities of seawater for ballast by massive ocean-going ships in one port and its discharge in a different port has rendered modern shipping a major vector for global transport of a variety of marine organisms to new regions. However, Torchin and Kuris (2005) consider that the impact of ballast water on the distribution of marine pathogens is undecided. For Monogenea, it is most unlikely that free-swimming oncomiracidia could survive a long sea voyage in ballast water, but viable eggs waiting for a hatching cue could be distributed in this way, as could small fish hosts carrying their monogenean fauna. Another less commonly considered mode of transport for small fish and their parasites is by their 'hitch hiking' among the flora and fauna that foul ship hulls. Large seafaring ships may carry several thousands of tonnes of fouling organisms and are basically unlegislated floating aquaria. The immigration, quarantine and customs departments of most countries rarely consider the ramifications of these introduction methods in their import risk assessments. We think these methods of distributing some monogenean species are possible and anticipate that there will be documented occurrences in the future.

## Immunological Advances

Our understanding of fish immunology has lagged behind our knowledge of mammalian immunology. In the last few years, there has been a surge of information about the immune systems of fish largely driven by the need to understand the ways to control fish diseases. In the field of Monogenea, this has certainly been the case over the previous decade and will continue to be an important and progressive research focus. Fish immunity is likely to have critical implications throughout the monogenean life cycle, including consequences for host specificity (whether larval invasion is successful and whether a parasite can persist on a host), establishment and

maintenance (via attachment and feeding), perhaps for egg manufacture and for population growth and its final size. It is not the intention here to provide a detailed account of the interplay between fish immunity and Monogenea, but we do present a summary of important issues. For more details, refer to the following reviews: Bakke *et al.* (1991, 1992, 2002), Buchmann (1999), Kearn (1999, 2004) and Buchmann and Bresciani (2006).

The importance of environmental water temperature and its relationship to the immune response of fish infected by Monogenea is well known. At higher temperatures, hosts generally have stronger immunity (Paperna *et al.*, 1984; Andersen and Buchmann, 1998; Deveney *et al.*, 2001). There may be occasions, however, when fish species are maintained in culture at a temperature range outside that which they normally experience. This practice may contribute to the stresses imposed on captive fish. The majority of research on monogeneans and host immunity has focused on salmonids in their normal temperature range, motivated by the necessity to understand and try to control the gyrodactylid, *Gyrodactylus salaris* (Monopisthocotylea: Gyrodactylidae) in Europe. These studies show that considerable variation exists in the level of immune response launched by individual salmon of the same strain or species and by different salmon species. An example using Norwegian and Baltic stocks (= strains) of *S. salar* was presented earlier. This individual variation in immunity by salmonids against gyrodactylids clearly exists in many other, and perhaps all, fish species against Monogenea infections. In a given fish population—whether wild or in culture—we have observed that some fish specimens may be heavily parasitized by monogeneans but others may be only lightly infected or perhaps uninfected. In wild populations, infection dynamics will also play a part in this variability or 'overdispersed distribution'. Extremes in individual variations of parasite burdens are usually most obvious in captive fish populations and although this may also be due to a 'self cure' mechanism—perhaps involving acquired immunity—it is plain that some fish individuals have a stronger innate immune system than others. This is no different from the differing susceptibilities among a group of humans to a range of illnesses such as the common cold and influenza. From salmonid research, a spectrum of immune responses against gyrodactylids is apparent. This encompasses outright 'susceptibility' (the parasite burden increases unrestrained until the fish dies) through 'responder fish' (parasite burden may rise but is controlled by the host) to 'innate resistance' (the parasite burden never

threatens and the population is eventually eliminated by the host). In a fish strain or species, this spectrum of immune response appears to be influenced genetically.

Usually among a healthy population of fish, monogenean burdens will not grow exponentially to kill the hosts and this relates to nutritional and other physiological factors including host immunity (Buchmann and Bresciani, 2006). The role of the hosts' immune responses against infections by monogeneans and the impact of a lowered immune system can be demonstrated experimentally. If glucocorticosteroids are experimentally administered to salmonids, their susceptibility to *G. salaris* increases (Harris *et al.*, 2000). This *artificially* demonstrates what may happen *naturally* because stressed fish release cortisol, a corticosteroid hormone, which acts to suppress the immune system.

Parasite diet and feeding likely influences the type of immune response mounted against Monogenea. Fish blood is the stronghold of many circulating immune factors and so polyopisthocotyleans would seem more directly exposed to immunological defenses than monopisthocotyleans. Furthermore, monogenean digestive enzymes may operate as antigens to stimulate a host response. Released either into the circulatory system (by Polyopisthocotylea) or into epithelial cells (by Monopisthocotylea) during feeding activities from intrinsic and/or extrinsic gland cells in the pharynx and/or from the gut, these proteolytic enzymes deserve further study. If their importance in eliciting an immune response in fish is confirmed, it is possible this could be exploited to develop anti-monogenean vaccines as has been the case with the excretory-secretory components such as gut glycoproteins in other ectoparasites (e.g., cattle tick, *Boophilis microplus*; see Richardson *et al.*, 1993).

Studies by Jahn and Kuhn (1932) and Nigrelli and Breder (1934) on the persistent pest *Neobenedenia melleni* (Monopisthocotylea: Capsalidae) on several teleost species in the New York Aquarium were the first to indicate that the 'skin' of some fish contained defense elements against monogeneans. The full spectrum of resistance mentioned previously (susceptible, responder, resistant) was determined between infected members of the same fish family against *N. melleni*. Acquired immunity (= acquired protection) among the infected fish was apparent because naïve fish carried large parasite burdens but after repeated exposure to parasites, infection intensities were less marked. Other monopisthocotylean Monogenea also induce acquired immunity against reinfection, e.g., *Dactylogyrus* species (Dactylogyridae; see Paperna, 1963,

1964; Molnár, 1972); various *Gyrodactylus* species (Gyrodactylidae; see Lester, 1972; Lester and Adams, 1974; Bakke *et al.*, 2002); *Pseudodactylogyrus* species (Pseudodactylogyridae; see Slotved and Buchmann, 1993); another *Neobenedenia* species (Capsalidae; see Bondad-Reantaso *et al.*, 1995a). Buchmann (1999) reviewed innate and acquired responses against monopisthocotyleans. Presently, it appears that no specific antibodies (= immunoglobulins) have a role in killing Monopisthocotylea, but non-specific reactions involving complement factors (from host liver cells and macrophages) and humoral elements (e.g., lectins, lysozyme, C-reactive protein, proteases, peptides, leukotrienes and prostaglandins) are important. Cytokines present in host epithelium may be involved in the coordination of the various interactions (Lindenstrøm *et al.*, 2003, 2004). Cells involved in inflammatory reactions in parasitized epithelium include basophils, eosinophils, neutrophils and macrophages (Buchmann, 1999). While non-specific factors are dominant and are likely to be extremely localized in the response of fish against monogeneans feeding on epithelium, the contribution of specific responses via antibodies and lymphocytes against Monopisthocotylea is not discounted (Buchmann, 1993a, 1999).

Fish blood is more intimately associated with fish epithelial surfaces via the gill lamellae and, therefore, specific antibodies may be more effective against gill parasites (Buchmann, 1999), whether or not they eat blood. Furthermore, the activities of blood feeding by the Polyopisthocotylea may directly result in exposing parasite antigen (e.g., proteolytic secretions; anticoagulants?) to host blood. Moreover, the delicate barrier between parasite and host across the gills and their blood diet indicates that defensive host elements can explicitly contact polyopisthocotyleans. Antiparasitic responses demonstrated by the production of detectable antibodies have been confirmed in hosts infected by the polyopisthocotyleans *Heterobothrium okamotoi* (Diclidophoridae; see Wang *et al.*, 1997; Nakane *et al.*, 2005) on *Takifugu rubripes* (Tetraodontidae) and *Discocotyle sagittata* (Discocotylidae; see Rubio-Godoy *et al.*, 2003a) on *Oncorhynchus mykiss* (Salmonidae), but *Heteraxinoides xanthophilis* (Heteraxinidae) apparently stimulates no antiparasitic response in *Leiostomus xanthurus* (Sciaenidae; see Thoney and Burreson, 1988). Despite these few studies with varied results, adaptive antibody responses by fish infected with polyopisthocotylean Monogenea are not necessarily rare. The diversity of monogenean parasite-fish host combinations may dictate a similar diversity in host

response. Furthermore, although only a few of the Monogenea studied so far may stimulate immunoglobulins, their protective significance is not established and may be outweighed by cellular or humoral elements (Buchmann and Bresciani, 2006).

Infected fish clearly mount several responses against monogeneans, either innate or acquired. So far, there have been few studies to investigate vaccines against monogeneans. An early example used homogenates of *Dactylogyrus vastator* (Monopisthocotylea: Dactylogyridae) from the gills of carp, *Cyprinus carpio* (Cyprinidae) injected intraperitoneally into the host. When fish treated with this crude vaccine were 'challenged' by *D. vastator* infections, some protection against these epithelial parasites was conferred (Buchmann and Bresciani, 2006). The knowledge that elevated antibody levels have been detected in some fish infected by polyopisthocotyleans has led to a few immunization trials. Homogenates of *Microcotyle sebastis* (Polyopisthocotylea: Microcotylidae) from gills of *Sebastes schlegeli* (Sebastidae) conferred some protection against reinfection. In this case, even administration of the adjuvant of the vaccine alone stimulated some protection suggesting some innate and non-specific pathways operated as well as acquired protection (Kim *et al.*, 2000). Homogenate only trials (i.e., no adjuvant) using *Discocotyle sagittata* (Polyopisthocotylea: Discocotylidae) injected intraperitoneally provided protection to rainbow trout, *Oncorhynchus mykiss* (Salmonidae) (see Rubio-Godoy *et al.*, 2003b). Ogawa (2002) reported antibody production in response to intraperitoneal injection of homogenates of *Heterobothrium okamotoi* (Polyopisthocotylea: Diclidophoridae) from gills of tiger puffer, *Takifugu rubripes* (Tetraodontidae). It provided a degree of protection only for adults infecting the branchial wall, but no protection against immature parasites on gill tissue. Subsequent experiments involving naïve fish that can carry >16,000 parasites on gill tissue and 2-year-old specimens of *T. rubripes* continually infected by adult *H. okamotoi* for >1 year allowed the proposal of some hypotheses about protection in this parasite-fish association. The protective mechanism in *T. rubripes* is thought to operate at least at 3 levels based on observations of the relative composition of developmental stages: (1) when oncomiracidia first invade fish, (2) when the first pair of attachment clamps develop and (3) when *H. okamotoi* relocates from gills to the wall of the branchial cavity when adult. From this study, Nakane *et al.* (2005) determined that fish with persistent infections produced antibodies against *H. okamotoi* but no antibody was detected in naïve fish, indicating

that persistent infection does stimulate at least some protection. However, factors responsible for effective and protective 'prevention' against reinfection by *H. okamotoi* require further study.

All studies cited above concerned investigations into the ability of parasite homogenates to induce protection against *gill*-dwelling monogeneans. Similar studies using homogenates of Monopisthocotylea that infect host *skin* have been unable to confer any protection when injected into fishes. For example, although Bondad-Reantaso *et al.* (1995a) recorded acquired immunity in Japanese flounder, *Paralichthys olivaceus* (Paralichthyidae) infected by *Neobenedenia girellae* (Monopisthocotylea: Capsalidae), no protection was achieved after homogenate injection. There has been a similar finding using crude homogenates of *Benedenia seriolae* (Monopisthocotylea: Capsalidae) from the 'skin' of *Seriola lalandi* (Carangidae) in South Australia (pers. comm., Ingo Ernst and Clinton Chambers).

To summarize, Monogenea are an ever-present parasite group on fish in the natural environment. The immune system of fish has evolved to cope with these invaders just as it has done in any other parasite-host system. Future advances in immunological knowledge about fish will undoubtedly help fuel further methods and studies to develop better ways to confer some protection against Monogenea in economically important fish species in commercial culture. Monogenea obviously inflict a range of pathogenic effects on their fish hosts. It is interesting that in many cases, the reactions of hosts infected by Monogenea are only expressed *partially*, which suggests that some sort of immunosuppression or immune evasion strategies are deployed by the parasites. How monogeneans tackle these issues and express appropriately active molecules remains to be discovered. It is likely, however, that the mechanisms employed are similar to those used by helminths of mammals (e.g., Secombes and Chappell, 1996).

## Disease Symptoms

Infections by Monogenea are best identified by physically locating parasites on the fish. This is the routine method for wild hosts because prevalence and intensity can be characteristically low and infections are rarely linked with any 'disease'. The *Disease Diagnostics* section below provides more comprehensive details about detecting, locating and identifying Monogenea on fish. On captive fish, monogenean infections may not present overt symptoms until there are a large number of

parasites. An obvious benefit, therefore, is to be aware of the appearance and behaviour of normal, healthy fish so that any changes can be rapidly identified. Assessing the health of any non-human is difficult because of the inability to ask questions and receive informed answers about symptoms. Therefore, 'suspected' symptoms are largely unconfirmed reports based on qualitative or subjective assessments. For instance, it is commonly 'assumed' that large numbers of capsalid Monogenea grazing on epithelial cells on the surface of a fish cause 'irritation' to the host, which may result in the 'flashing' behaviour (i.e., rubbing the affected areas of the body against available substrates) associated with 'skin' problems. However, there are no unequivocal data to support the notion that Monogenea may 'irritate' or cause 'itchiness' to fish. More quantitative assessments of the effects of Monogenea can be achieved using histological analyses where identification of inflammatory and infiltrating cells clearly indicates that infected fish mount a response against the parasite.

Nevertheless, behavioural responses by fish are associated with infections by Monogenea. Monopisthocotylea on the epithelium of the body, head and fins are associated with flashing but how much this is connected with the activities of the monogeneans and how much is linked with possible secondary infections by fungi, viruses and bacteria is as yet unknown. Worsening infections may become more apparent when discolouration of the 'skin', ragged fins and/or haemorrhagic lesions appear (Fig. 13.8C), probably all coupled with Monogenea and secondary agents. Symptoms of heavy Monogenea infections on gills may be noticed initially by erratic swimming and especially 'piping' (i.e., fish remain near the air-water interface, perhaps trying to access more oxygen) indicating respiratory issues. Hyperventilation or 'gilling' (i.e., fish ventilate heavily and display increased frequency of opercular movements indicative of laboured respiration) can also indicate heavy monogenean infections on gills. Other behavioural symptoms can include lack of appetite, erratic swimming behaviour, lethargy and general malaise.

Physical symptoms relate more specifically to the biology of the parasite. Lesions on the 'skin' of sharks, rays and teleosts (e.g., Figs. 13.8 A–C, 13.9F) can be due to feeding and attachment activities of Monopisthocotylea. Problems from attachment can be attributed to mechanical and/or chemical (from secretions) aggravation. Heavy infections worsened by secondary infections are often accompanied by cellular infiltration, necrosis and damage not only to epithelia but also to underlying dermis and muscle tissue. Monogenea infecting gills can

stimulate hyperplasia (Fig. 13.9G), cellular infiltration and haemorrhagic lesions. Hypersecretion of mucus from the gills by some fish species is assumed to be a host response against gill-dwelling Monogenea. In extreme circumstances, the occlusion of the respiratory surfaces by mucus can lead to asphyxia. Polyopisthocotyleans can cause blood loss, leading to anaemia, which often presents as unusually pale gills (Fig. 13.8D). Continuing, untreated heavy infections by Monogenea at any site can cause osmotic imbalance. As acute infections by Monogenea can progress rapidly, the severe and irreversible damage caused by them and exacerbated by secondary pathogens can kill fish.

## DISEASE DIAGNOSTICS

Here we shall provide a brief account of techniques and resources to diagnose Monogenea infections including screening live fish for monogenean eggs. Specific case studies are dealt with later under *Important Monogenean Pathogens*.

Choosing the most appropriate protocol to treat fish infected by monogeneans is completely dependent on pathogen identification. Therefore, the proper removal, fixation (= preservation) and preparation of the parasites are *critical* to the diagnostic process. In this section, we use the generic term 'water'. Unless specified otherwise, water refers to seawater when dealing with marine monogeneans and freshwater when dealing with freshwater monogeneans. Monogeneans on the 'skin' can be difficult to detect either because they are tiny (e.g., gyrodactylids) or because they are on hidden sites such as lip folds and branchiostegal membranes (some capsalids; see Whittington, 2004) or the parasites are cryptic because they are camouflaged (e.g., *Dendromoncotyle* species) or transparent (e.g., some capsalids; see Whittington, 1996).

If many monogenean specimens are available, it is important to ensure that 2 or 3 live, unflattened worms are preserved *directly* in undiluted high grade ethanol (Analytical Reagent grade; i.e., containing few impurities) and avoiding *all* contact with other chemicals, especially formalin, for future molecular analyses.

### Screening for Eggs

This method is outlined briefly because the same technique can be used to: simply determine whether a fish is infected by monogeneans; assess monogenean fecundity *in vivo*; collect eggs for experiments to determine

embryonation period; determine egg-laying rhythms. Fish under investigation are isolated in a small tank in a volume of aerated water sufficient to maintain the host without causing it undue stress. The type and size of fish will determine the length of the isolation period. Sharks, rhinobatid rays and most teleosts tend to produce less mucus than most batoids. Isolated fish should be monitored carefully to ensure they are comfortable and receive sufficient aeration. After a predetermined period (1 h, 3 h, 6 h, 12 h, 24 h), the isolated host is removed either to a new isolation tank or a holding tank and the water it was held in is filtered through a fine mesh filter with a mesh size no more than 65 μm. A filter of this size should collect eggs of most Monogenea but those of small parasites such as dactylogyrids and diplectanids may require an even finer mesh. The speed of water filtration depends on fish type and isolation period. The contents of the mesh filter can be back flushed into specimen jars for examination using a binocular microscope to search for live monogenean eggs. Eggs collected in this manner can be used to determine the embryonation time at different temperatures; egg-laying rhythms; egg-hatching rhythms; egg-hatching cues; adult fecundity *in vivo*. The methods may need some refinement depending on host species, host size and host type as well as parasite species, parasite fecundity and parasite size.

## Removal of Live Monogenea from Live Hosts

It is preferable to obtain monogeneans from live or very freshly dead hosts for 2 reasons: (1) live, moving monogeneans are far easier to see and (2) the parasites can decompose rapidly after the death of the fish, especially at high water temperatures. Lifting the fish gently out of water and examining the 'skin' carefully, illuminated by a strong beam of light from a torch may reveal 'skin'-dwelling monogeneans as raised shiny bumps (Fig. 13.9E, F). 'Skin' monogeneans can be removed from live fish by carefully sliding a scalpel blade held horizontally beneath the haptor to sever their attachment and then brushing them delicately off the blade into a Petri dish of water using a fine artist's paintbrush. Monogeneans can be removed using fine forceps but *extreme* care should be taken not to damage the parasites—especially the haptor—which is usually critical for identification. Gill monogeneans are difficult to remove from live fish safely and humanely. Heavily infected fish, however, may be anaesthetized (e.g., using AQUI-S, clove oil or MS222) and small clips of the tips of 10–15 gill filaments can be taken and examined using a microscope. Short dechlorinated freshwater dips for marine species (or saltwater dips for

freshwater species) or dilute formalin baths will quickly kill monogeneans on fish 'skin' and render them opaque or white (Fig. 13.8A), making them much easier to detect. Dead monogeneans still attached to the 'skin' can be removed carefully following the procedure outlined above or simply by rubbing them gently with the fingertips. The bath water should also be scanned for dead, detached parasites of all sizes including small, recently invaded recruits. Monogeneans killed and removed in this way should be placed immediately in 10% formalin to fix them and avoid degradation. Fish can also be bathed for a short period in a high concentration of praziquantel (e.g., 20 mg $L^{-1}$ for 15 – 30 min) which will remove, but not kill, many adult monogeneans and some small stages from several sites (e.g., 'skin', gills, nasal tissue, cloaca). Monogeneans left too long in praziquantel are unsuitable for identification (weakened; unattached; 'floppy'; near death) so the bath water should be filtered quickly through fine mesh (mesh size at least 65 μm) and detached parasites put immediately into a Petri dish of water to recover (for at least 2 h). The monogeneans should be examined using a microscope to ensure they have recovered fully (some will revive sufficiently to lay eggs and attach to glass) before they are processed for mounting. The disadvantage of this praziquantel bath technique is the microhabitat of the monogenean cannot be readily determined.

## Removal of Live Monogenea from Freshly Killed Hosts

The entire body surface of a freshly killed small fish can be submerged in a dish of water and examined using a binocular microscope for moving parasites. For larger freshly dead fish, the entire body surface should be scanned by the naked eye for monogeneans and individual fins can be removed and examined using a binocular microscope. Body surfaces can be scraped gently using a scalpel blade and then brushing mucus and any parasites into a dish of water. The gills should be dissected out, cut into the separate arches, shaken in a container of water to remove blood and excess mucus and then placed in a Petri dish of water and examined using a binocular microscope for moving parasites. Monogeneans can be removed from gills by sliding a fine needle or probe under the haptor so as to dislodge the parasite. The monogenean may attach to the probe by the haptor, by anterior secretions or simply in a film of host mucus, but it can be removed by putting the probe beneath the water in a small Petri dish and flicking the probe gently using a second probe. Individual detached parasites can also be picked up using a Pasteur pipette but care should be

taken as many monogeneans have a penchant for attaching firmly within the pipette! Alternatively, individual parasites can be picked up in the surface tension of an Irwin loop (a small loop made from a single strand of thin copper wire) and flicked gently using a probe below the water surface in a Petri dish. Gills of batoids can be particularly difficult to examine because they produce copious amounts of mucus, which often obscures parasites or makes them difficult and/or frustrating to remove. Shaking the gills vigorously in water will remove much of the mucus. Monogeneans attach firmly to gill surfaces and it is most unlikely they will dislodge during shaking.

## Removal of Monogenea from Fixed Hosts

It is generally difficult to obtain beneficial monogenean material from dead, preserved hosts and especially from frozen fish. Many parasites may die in a curled state and be unsuitable for mounting (see below). Parasite specimens removed from frozen hosts, however, may be suitable for molecular analysis and can be dropped directly into undiluted analytical grade ethanol. If this is done, care should be taken to ensure that some reference specimens suitable for mounting are also preserved. If fish must be fixed prior to examination, they should be placed directly into 5 or 10% formalin in a well-sealed container. For small elasmobranchs, especially rays, the gill slits, body cavity and cloacal opening should be extended by incisions in order to allow rapid entry and perfusion of the fixative. After fixation for at least a day (longer for large fish), the specimen can be transferred to 70% ethanol for longer-term storage. For bigger fish, especially large elasmobranchs, individual gill arches and nasal tissue of freshly killed fish should be dissected out, shaken in water to remove blood and mucus and placed in containers of 10% formalin and later (preferably after a week) transferred to 70% ethanol. Tissues should be soaked thoroughly in several changes of freshwater to remove excess fixatives prior to careful examination for parasites using a dissecting microscope. Material should be examined in a fume hood or using an extraction fan to remove any lingering fixative fumes. The bottom of all containers should be scanned carefully for any detached parasites. Removed parasites should be put in labelled vials of 70% ethanol for storage until mounting.

## Monogenea Fixation and Processing

Material in optimum condition for detailed study is best obtained *alive*. Significant amounts of information about structure, morphology, anatomy

and biology can be obtained by examining live specimens using a low power binocular microscope or a high power compound microscope. Once the living monogeneans are removed from the host, they can be killed in a number of ways for subsequent mounting. Care must be taken at this stage that the parasites are relatively flat when killed because it is difficult to mount and identify curled or contracted specimens. Monogeneans that attach to their host using suction will often reattach to the bottom of a glass Petri dish. For marine monogeneans, the water in the dish can be removed and replaced quickly with freshwater which will kill the parasites in a near flattened state. Freshwater monogeneans can be killed in a near flattened state by pipetting them (in a small volume of water) directly into a small (10 ml) Pyrex beaker of freshly boiled water. The dead parasites can then be transferred carefully (using a pipette or a fine artist's paintbrush) into an appropriately labelled vial of 10% formalin for fixation and future processing. Monogeneans killed and fixed in these ways are in a state that mirrors the size, shape and proportions of the parasites when alive and as such are also excellent for scanning electron microscopy and histological studies.

Ideally, if time permits, some live parasites should be placed in a droplet of water on a microscope slide and covered carefully with a coverslip. Using a binocular microscope, one must ensure that the worm is uniformly flattened and not twisted. Twisted specimens can be coaxed to untwist by adding a few extra drops of water next to the coverslip or by manipulating them with fine needles if necessary. Monogeneans should be partially flattened under controlled pressure by applying filter paper or tissue to the edge of the coverslip to wick up the excess fluid. To kill and preserve parasites in a flattened state, place a small drop of 10% buffered formalin to one side of the coverslip and draw it across the parasite by touching filter paper or tissue to the opposite side. Alternatively, the slide with the parasite in a drop of water can be passed over a naked flame from a lighter or alcohol lamp for a few seconds to kill the monogenean. Once dead, excess water/fixative should be added to the slide so the coverslip floats off and the monogenean can be transferred carefully to a vial containing fixative using a fine artist's paintbrush. If the flattened specimen is firmly attached to the coverslip or slide, carefully slide a few bristles of the artists' brush under the parasite to dislodge it. Tiny Monogenea such as Dactylogyridae, Diplectanidae and Gyrodactylidae are best studied alive initially, but to fix and prepare semi-permanent mounts simultaneously, ammonium picrate-glycerin (= Malmberg's

fixative) can be used (Malmberg, 1957; *GyroDb* on the Internet). This method is optimal to prepare slides of characteristic features such as attachment and copulatory sclerites. Edges of the coverslip for specimens prepared using Malmberg's fixative can be sealed with clear nail varnish.

Preserved specimens are then stained, dehydrated, cleared and mounted on a microscope slide using standard parasitological techniques (Meyer and Olsen, 1980; Pritchard and Kruse, 1982; Berland, 2005). For permanent slides of monogeneans, it is preferable that *single* specimens be mounted beneath a coverslip in Canada balsam as this natural resin has been shown to stand the test of time (>150 years). Other commercial resins such as Permount or Depex can become cloudy over time and may obscure the specimen or render it completely useless. Serial sections prepared using standard histological techniques (Presnell and Schreibman, 1997) are most useful for pathological evaluations but they may also assist in tracing the path of hard to see ducts or in locating small structures in parasites not visible in whole mounts. It should be noted, however, that histological sections alone cannot be used easily to identify monogeneans to species and, therefore, it is necessary to prepare whole mounts. All slides and vials should be labelled clearly with the identity of the parasite and host, collection locality of the host, the microhabitat of the parasite, collection date, name of the collector and identifier and any other data deemed important.

## Depositing Specimens

Once specimens are mounted on microscope slides or fixed in high-grade ethanol for subsequent molecular analysis, it is *critical* that they are available for study so species identity can be confirmed and reassessed in the future if required. When a monogenean outbreak occurs, it is extremely frustrating for us to learn that no specimens are available for examination. Even personal collections which may represent years of carefully collected data and curatorial effort can be lost if arrangements are not made for its transfer to a safe haven after a hobbyist, fish health professional or researcher retires or dies. Unfortunately, skillfully prepared material is often left on laboratory shelves or in cupboards and can eventually be misplaced or discarded by others not realizing its significance. Therefore, we cannot stress strongly enough the importance of *depositing* voucher specimens, either as unmounted specimens in 70% ethanol or in undiluted, analytical grade ethanol, or mounted on microscope slides, into *recognized* museums, to ensure that they are

available for study by current and future researchers. Museums selected should be institutions with a mandate and funds to provide enduring curatorial support and to provide loans to bona fide researchers. A local parasitology society (e.g., the American Society of Parasitology, The Australian Society for Parasitology, The British Society for Parasitology, The Parasitology Section of the Canadian Society of Zoologists, The European Association of Fish Pathologists, The Malaysian Society of Parasitology and Tropical Medicine to name a few) can advise the most appropriate museum for specimen deposition.

## DISEASE TREATMENTS

A wide-ranging discussion of important issues in treating fish infected by Monogenea is divided into the following major topics of control: mechanical, biological, chemical and treatments against eggs.

### Mechanical Control

In most cases, wild fish have low-level monogenean infections, which do not appear to effect fish adversely (but see earlier and Buchmann and Bresciani, 2006, for exceptions). We discussed above that stressed fish have lowered resistance to monogenean infections. Therefore, reducing stock densities and ensuring good water quality should be initial steps to manage monogenean proliferation. Filtration of inflow and outflow water in recirculating systems may not eliminate all monogenean eggs and oncomiracidia, but it may help to prevent population explosions. Lowering water temperatures may also help reduce monogenean loads (Cheung *et al.*, 1982; Chisholm *et al.*, 2004) by decreasing monogenean fecundity and increasing generation time, which are both related to temperature. Eggs laid by adults may have appendages that entangle on sea cages. Indeed, introducing a substrate such as netting (Fig. 13.9C) is a procedure used in our laboratory to promote heavy monogenean infections deliberately in experimental fish (Ernst and Whittington, 1996; Glennon *et al.*, 2005). This technique keeps eggs in close proximity to confined fish and can promote heavy reinfections. Regular cleaning or replacement of badly fouled sea cages may significantly reduce the number of monogenean eggs retained in the system. However, this may not be feasible in all circumstances. For example, *Heterobothrium okamotoi* (Polyopisthocotylea: Diclidophoridae), which parasitizes the tiger puffer, *Takifugu rubripes* (Tetraodontidae), can lay eggs in strings 2–3 m long which readily

entangle on culture nets (Ogawa, 2002). At summer temperatures (25°C), egg hatching occurs 4 days after laying and changing culture nets this frequently would be inconceivable! Reducing egg loads by placing additional fouling substrates within the sea cage and removing them before hatching may reduce overall numbers of oncomiracidia in the system but the effectiveness of this approach has not been determined (Ogawa, 2002).

## Biological Control

There is evidence to suggest that some cleaner fish species may be an effective means to control monogeneans. The cleaning goby, *Gobiosoma genie* (now *Elacatinus genie*) and the neon goby, G. *oceanops* (now *E. oceanops*: Gobiidae) significantly reduced infections of *Neobenedenia melleni* (Monopisthocotylea: Capsalidae) on cultured Florida red tilapia but the bluehead cleaner wrasse, *Thalassoma bifasciatum* (Labridae) did not (Cowell *et al.*, 1993). *Labroides dimidiatus* (Labridae) had an effect on the size frequency and abundance of the capsalid *Benedenia lolo* (Monopisthocotylea: Capsalidae) infecting 'skin' of the thick-lipped wrasse, *Hemigymnus melapterus* (Labridae; see Grutter *et al.*, 2002). Incidental cleaning interactions by fish involving microbothriids and gyrodactylids have also been recorded (Grutter, 2002). It is interesting to note that pigmented (i.e., camouflaged) *B. lolo* on the pigmented part of its labrid host suffered less predation than unpigmented specimens. There is evidence to suggest that darkly pigmented *Dendromonocotyle* species that parasitize the dark dorsal surface of their ray hosts may also avoid predation by cleaner fish (Chisholm *et al.*, 2004).

## Chemical Control

Over the years, numerous treatments have been developed to manage monogenean infections on fish with variable results. It is beyond the scope of this chapter to list every treatment and concentration trialed. Detailed reviews of therapies used and their efficacy can be found in Schmahl *et al.* (1989), Thoney and Hargis (1991), and Schmahl (1991, 1993). A recent synopsis of chemicals to rid monogenean infections on elasmobranchs, which tend to be more sensitive to chemicals than teleosts, was made by Benz and Bullard (2004). Here, we present a brief overview of common treatments. More specific treatment protocols for 8 species and 2 genus groups in 8 families that we identify as *Important Monogenean Pathogens* are

given in case studies (see later). Ultimately, however, the treatment used is dependent on the species of monogenean, the sensitivity of the host, the fate of the host (i.e., whether a display fish or food fish), the system (closed versus open) and the legislation governing the use(s) of the chemical. Sensitivity of fish to chemicals tested is an important issue. Some of the case studies highlighted later under *Important Monogenean Pathogens* demonstrate that some chemical trials were ineffective at killing Monogenea but were effective at killing the host! Thus, extreme caution must be taken to select dosages carefully and monitor fish in trials attentively. Sodium chloride, formaldehyde, copper sulphate, hydrogen peroxide, mebendazole, trichlorphon and praziquantel are common treatments for monogenean infections and we review these below. We also provide a synopsis of recent work on in-feed treatments. Preliminary studies on vaccinations were covered earlier under *Immunological Advances*.

### *Freshwater and Saltwater*

Baths using saltwater (to treat freshwater fish) and dechlorinated freshwater (to treat marine fish) are probably the most common methods to rid fish of monogeneans. These treatments are not restricted by drug legislation and there are no withholding periods. Generally, a 10–15 min freshwater bath is used to rid marine fish of parasites (Thoney and Hargis, 1991) but fish need to be monitored carefully. This method can be used to treat a single fish or many fish. In Japan, stocks from entire sea cages of *Seriola* species (Carangidae) are scooped into large tarpaulins of freshwater (Fig. 13.9B) for 5–7 min to remove the 'skin' parasite *Benedenia seriolae* (Monopisthocotylea: Capsalidae; see Figs. 13.2F, 13.3A, 13.4A, 13.8A). Typically, a 40–50 ppt saltwater bath for approximately 5 min is used to remove monogeneans from freshwater fish. Using either treatment, 'skin' parasites are killed rapidly but may not fall off the fish. Gill parasites appear to be more resistant to saltwater or freshwater baths, perhaps because they can shelter between gill lamellae. Often the host also shuts its opercula during treatment and, therefore, the entry and flow of water over the gills may be reduced. Saltwater and freshwater baths are stressful to the host and bath durations above are merely a guide. Marine elasmobranchs are particularly sensitive to freshwater treatments and fish must be monitored closely.

### Formalin

Formalin baths, another common treatment for monogeneans, can be extremely stressful to fish and should probably be used as a last resort. Nevertheless, formalin is approved for use in aquaculture in several countries. Formalin concentration and treatment duration varies considerably depending on parasite species, fish species and system type. Typically, fish are dipped in a separate formalin bath (100–500 ppm for 15 min to 1 h; see Thoney and Hargis, 1991) but entire aquaria or ponds can be treated for extended periods at low concentrations (25–60 ppm). A formalin bath of 200 ppm for 1 h was the most effective treatment to remove all *Microcotyle* sp. (Polyopisthocotylea: Microcotylidae) from gills of cultured red porgy, *Pagrus pagrus* (Sparidae) in sea cages off Crete (Katharios *et al.*, 2006).

### Copper Sulphate

This chemical has been used extensively in the aquarium trade and in aquaculture. It is most effective if used at low dosages for a prolonged period and has generally been successful in treating sharks infected with microbothriids. These monogeneans are especially difficult to eradicate because they attach firmly using a cement-like glue to host denticles (Figs. 13.2G, 13.9E, 13.13B). Poynton *et al.* (1997) found that a treatment of 0.25 ppm for 85 days eradicated all *Neodermophthirius harkemai* (Monopisthocotylea: Microbothriidae) from the 'skin' of the lemon shark, *Negaprion brevirostris* (Carcharhinidae). Elasmobranchs and juvenile teleosts are extremely sensitive to copper sulphate and must be monitored carefully throughout treatment. Administration of copper sulphate is difficult because it binds readily to organic matter and levels must be checked regularly. Its use is generally confined to marine systems because it is highly toxic in the low pH conditions of freshwater (Thoney and Hargis, 1991).

### Hydrogen Peroxide

This strong oxidizing agent has been used effectively to eliminate monogeneans. It has the added benefit that it has no food safety issues and as such, it has been approved for use as a bath treatment in Japanese and Australian aquaculture industries. Rach *et al.* (2000) found that a total of 3 treatments of hydrogen peroxide (up to 560 $mgL^{-1}$ for 30 min every 2 days) removed all *Gyrodactylus* (Monopisthocotylea: Gyrodactylidae)

specimens from the 'skin' of the rainbow trout, *Oncorhynchus mykiss* (Salmonidae). Similarly, Buchmann and Kristensson (2003) found that all *G. derjavini* were eradicated when exposed to hydrogen peroxide in the form of sodium percarbonate (80 $mgL^{-1}$ for 18 h). Mansell *et al.* (2005) found that a hydrogen peroxide treatment of 300 ppm for 10 min removed all *Zeuxapta seriolae* (Polyopisthocotylea: Heteraxinidae) from gills of the yellowtail kingfish, *Seriola lalandi* (Carangidae). Changes in some blood parameters (lactate and osmolality) of *S. lalandi* were detected after treatment. However, these changes were less than those caused by the parasite and it was concluded that treatment was less harmful to fish than chronic parasite infections. Higher concentrations of hydrogen peroxide can be toxic to fish and, therefore, treatment duration and concentration need to be selected carefully (Kiemer and Black, 1997). Furthermore, hydrogen peroxide toxicity is directly proportional to water temperature and therefore treatments safe for fish at lower temperatures may prove highly toxic at higher temperatures (Rach *et al.*, 1997). Fish farms in South Australia provide additional oxygenation when treating at high summer water temperatures to prevent fish mortality.

### *Mebendazole*

Benzimidazole based compounds bind to the microtubule subunit protein, tubulin, and disrupt microtubule structure and cell transport functions. Several chemicals in this group have been trialed for their efficacy against monogeneans (e.g., albendazole, fenbendazole, flubendazole, luxabendazole, mebendazole, oxfendazole, parbendazole, tricladbendazole and thiabendazole; see Buchmann, 1993b; Buchmann and Bresciani, 2006 and references therein). Mebendazole (Vermox®) is one of the more widely used compounds in this group, but its efficacy can vary. Goven and Amend (1982) found that a 24-h treatment of 0.01 $mgL^{-1}$ removed all *Gyrodactylus elegans* (Monopisthocotylea: Gyrodactylidae) from the goldfish *Carassius auratus* (Cyprinidae) but had no effect on *Dactylogyrus vastator* (Monopisthocotylea: Dactylogyridae) from the same host species. Continuous bath treatments of 1 ppm eradicated infections of *Pseudodactylogyrus anguillae* and *P. bini* (Monopisthocotylea: Pseudodactylogyridae) from the cultured European eel, *Anguilla anguilla* (Anguillidae) within 72 h (Buchmann and Bjerregaard, 1990). Conversely, a treatment of 400 ppm for 1 h was recently shown to have no effect on *Microcotyle* sp. (Polyopisthocotylea: Microcotylidae) infections on *Pagrus pagrus* (Sparidae) kept in 70 L experimental tanks

(Katharios *et al.*, 2006). There are also indications that monogeneans become resistant to mebendazole after prolonged exposure (Buchmann *et al.*, 1992; Waller and Buchmann, 2001).

### *Trichlorphon*

The widely used organophosphate insecticide trichlorphon (also known as Dipterex®, Dylox®, Lepidex®, Neguvon®) has been in use since the early 1960s and is usually added directly to an aquaculture or aquarium system to eradicate monogeneans. It acts by inhibiting cholinesterase activity in the nervous system and paralyzes the monogenean (Buchmann and Mellergaard, 1988). Repeated dosages between 0.3 and 1 ppm trichlorphon eliminated *Pseudodactylogyrus* species from Japanese eels (Imada and Muroga, 1979; Chan and Wu, 1984). However, Buchmann *et al.* (1987) suggested that resistance may develop because trichlorphon treatments up to 5 ppm had no effect on *Pseudodactylogyrus* species on European eels. Other studies have also found monogeneans to be resistant to trichlorphon (Goven *et al.*, 1980). There is increasing evidence to suggest that prolonged exposure to trichlorphon causes more problems for the host than the monogenean infection and in some cases, the chemical may be responsible for host death. Thoney (1990) found that cownose rays, *Rhinoptera bonasus* (Rhinopteridae), treated with trichlorphon to eliminate *Benedeniella posterocolpa* (Monopisthocotylea: Capsalidae) fed poorly, had poor colour and produced excess 'skin' mucus. Juvenile blotched fantail rays, *Taeniura meyeni* (Dasyatidae), eventually died after exposure to repeated trichlorphon treatments to control *Dendromonocotyle* (Monopisthocotylea: Monocotylidae) infections. Autopsies revealed extensive damage to the kidneys and liver and death was attributed to the treatments and not to the parasite infection (Chisholm *et al.*, 2004). Thus, extreme care should be exercised when using trichlorphon given the potential long-term toxic effects and resistance issues.

### *Praziquantel*

The anthelmintic praziquantel has proven to be one of the more outstanding chemotherapeutants that specifically targets platyhelminths. It was introduced in 1975 and is considered the drug of choice in the control of schistosome and cestode infections in humans and animals of veterinary importance. It has also been shown to be effective in eradicating Monogenea on fish. Remarkably, the exact mode of action of praziquantel is still unknown. Exposure to the drug induces almost

immediate muscle contractions and vacuolization of the flatworm's tegument which is thought to be the result of calcium flux across the tegumental membrane (see Köhler, 2001 and references therein). However, much work is still required to elucidate the exact mode of action and determine why praziquantel is selective to platyhelminth parasites. Generally, praziquantel baths of 10 – 20 $mgL^{-1}$ for 1 – 3 h effectively remove 'skin' monogeneans from teleosts and elasmobranchs (Schmahl and Mehlhorn, 1985; Thoney, 1990; Thoney and Hargis, 1991; Whittington and Ernst, 2002). A single 4-min bath of 100 ppm praziquantel removed all specimens of the gill monogenean *Microcotyle sebastis* (Polyopisthocotylea: Microcotylidae) from the rockfish, *Sebastes schlegeli* (Sebastidae: see Kim and Cho, 2000). However, these short duration-high concentration protocols may not be effective in removing juvenile stages of gill/nose-dwelling monogeneans or 'skin'-dwelling microbothriids. Chisholm and Whittington (2002) demonstrated that while adult stages of monocotylids infecting the gills and nasal tissue of the shovelnose ray *Rhinobatos typus* (Rhinobatidae) were flushed out soon after a 2 h treatment of 20 $mgL^{-1}$ praziquantel began, smaller stages retracted between the secondary lamellae and survived. This work suggests that so-called reinfections after treatment may be attributed to monogeneans that remain on the gills after treatment but have been overlooked due to their minute size, cryptic microhabitat and behaviour. Furthermore, if adult monogeneans are not flushed from the host during this treatment, they can recover fully within 2 h of the host being returned to seawater and lay viable eggs which embryonate and hatch normally (Chisholm and Whittington, 2002). As noted above, it is unlikely that microbothriids, which glue themselves to their shark host, would be killed by a short duration-high concentration treatment. Two 40-h baths (48 h apart) in 5 $mgL^{-1}$ praziquantel in seawater successfully eradicated all gill and nasal monogeneans on the shovelnose ray (Chisholm and Whittington, 2002). This protocol also appears effective in killing *Pseudoleptobothrium aptychotremae* (Microbothriidae) infections from the southern fiddler ray, *Trygonorrhina fasciata* (Rhinobatidae; Glennon, Chisholm and Whittington, unpublished data). Thoney and Hargis (1991) suggested that exposure duration is more critical than praziquantel concentration and recommended longer treatments at lower concentrations, especially since high concentrations of praziquantel can be toxic. Buchmann (1987) demonstrated that eels were paralyzed within 18 min of exposure to 120 ppm praziquantel and juvenile fish showed signs of distress at higher praziquantel concentrations (20 $mgL^{-1}$ for 90 min; Thoney and Hargis, 1991).

### Oral Treatments

In-feed treatments are effective ways to deliver anthelminthics to fish directly. They have the added advantage that they negate the need to handle fish which is often the most stressful (to fish) and labour intensive part of any treatment regime. Orally administered praziquantel is an approved treatment in Japan for control of *Benedenia seriolae* (Monopisthocotylea: Capsalidae) on the 'skin' of *Seriola* species (Carangidae). Kim and Choi (1998) demonstrated that a single dose of mebendazole at a rate of 50 $mgkg^{-1}$ body weight (BW) significantly reduced the numbers of *M. sebastis* (Polyopisthocotylea: Microcotylidae) on gills of cultured *Sebastes schlegeli* (Sebastidae). They also found that diets supplemented with the surfactant microbial agent, bithionol, (100 $mgkg^{-1}$ BW and 200 $mgkg^{-1}$ BW) reduced *M. sebastis* infections. Oral treatments of praziquantel have also proven effective and intubation at 200 $mgkg^{-1}$ BW lowered *M. sebastis* infections on *S. schlegeli* (see Kim *et al.*, 1998). Furthermore, the efficacy of an oral praziquantel treatment can be increased almost 2-fold by combining it with cimetidine, a chemical that inhibits the host's metabolism of praziquantel (Kim *et al.*, 2001). By raising the bioavailability of the administered praziquantel, cimetidine ultimately reduces the total treatment costs. This is an important issue since the cost of praziquantel can be prohibitive. So far the efficacy of anthelminthics administered orally has been tested primarily using a single blood-feeding gill polyopisthocotylean species, *M. sebastis*, as the target pathogen. It would be expected that praziquantel should be incorporated quickly into the host's bloodstream and be available for uptake by the blood-feeding parasite. Less clear is the potential efficacy of in-feed treatments in the removal of epithelial grazing monopisthocotyleans. Undoubtedly, the drug will take longer to be incorporated into epithelial cells and dosages administered for longer durations may be required for effective control. Given this fact, it is not surprising that Janse and Borgsteede (2003) reported that a single oral dose of praziquantel ranging between 10 $mgkg^{-1}$ BW and 40 $mgkg^{-1}$ BW had no effect on *Clemacotyle australis* infections (but likely to be *Dendromonocotyle torosa*, Monocotylidae; see below) on *Aetobatus narinari* (Myliobatidae). Hirazawa *et al.* (2004) showed that an oral treatment of 40 $mgkg^{-1}$ BW for 11 days was more effective in treating the spotted halibut, *Verasper variegatus* (Pleuronectidae), for *Neobenedenia girellae* (Monopisthocotylea: Capsalidae) infections than 150 $mgkg^{-1}$ BW for 3 days. Fish fed on the

higher dosage for a short period experienced lack of appetite and vomited food while fish fed the lower dosage rate for a longer period did not display adverse symptoms.

To comprehend possible oral praziquantel dose strategies, Tubbs and Tingle (2006a, b) investigated the chemical's bioavailability and pharmokinetics in kingfish, *Seriola lalandi*. These studies indicated that at oral doses of 40, 50 and 150 $mgkg^{-1}$ BW $day^{-1}$, praziquantel concentrations in plasma and skin were highly variable and may be sub-optimal to treat the gill and skin monogeneans infecting *S. lalandi*. Williams *et al.* (2007) observed that *S. lalandi* rejected feed pellets that had been surface-coated with praziquantel, especially in trials using 100 and 150 $mgkg^{-1}$ BW $day^{-1}$ indicating palatability issues. However if praziquantel was administered by intubation, fewer *B. seriolae* and *Zeuxapta seriolae* (Polyopisthocotylea: Heteraxinidae) occurred on medicated fish compared with control fish (Williams *et al.*, 2007).

The efficacy of natural agents including 3 essential oils (orange oil, peppermint oil and cinnamon oil) and caprylic acid (the fatty acid in palm oil) against *Heterobothrium okamotoi* (Polyopisthocotylea: Dichidophoridae) which infects the tiger puffer, *Takifugu rubripes* (Tetraodontidae), have also been investigated. Caprylic acid showed the most promising anthelmintic properties (Hirazawa *et al.*, 2000, 2001a, b).

### Treatments against Eggs

Treatments presented above are targeted against parasites after hatching and are mostly focused against the adults. The tanned eggshell of flatworm parasites has proven particularly resistant in terrestrial and aquatic environments. Eggs laid by Monogenea are no exception and few chemical treatments have proven effective to reduce their hatching success. Desiccation for 3 min to 3 h (e.g., Cecchini and Cognetti-Varriale, 2003; Sharp *et al.*, 2004; Ernst *et al.*, 2005) has been demonstrated to be an effective method to prevent egg hatching but can only be used efficiently in closed facilities to clean equipment in hatcheries and in ponds that can be dried out over extended periods.

## IMPORTANT MONOGENEAN PATHOGENS

Below, we present by way of selected case studies, some examples of Monogenea that have been responsible for epizootics and disease on their fish hosts. This is not intended as an extensive list but the examples are chosen to reflect species, genera and/or families of monogeneans reported

to wreak havoc to the health of mostly captive fish in aquaculture and aquaria. Exemplars, presented alphabetically beginning with Monopisthocotylea, are: Capsalidae (*Benedenia seriolae*, Figs. 13.2F, 13.3A, 13.4A, 13.8A-C, 13.10A; *Neobenedenia* species, Figs. 13.6D, 13.10B); Dactylogyridae (*Dactylogyrus vastator*, Fig. 13.11; *Haliotrema abaddon*, Figs. 13.3B, 13.9G, 13.11B); Diplectanidae (*Diplectanum aequans*, Figs. 13.3C, 13.11); Gyrodactylidae (*Gyrodactylus salaris*, Figs. 13.3D, 13.5, 13.7, 13.12); Microbothriidae (*Dermophthirius* species, e.g., Figs. 13.2G, 13.3E, 13.9E, 13.13); and Monocotylidae (*Dendromonocotyle* species, Figs. 13.1A, B, 13.3F, 13.9F, 13.14). For Polyopisthocotylea, exemplars are: Diclidophoridae (*Neoheterobothrium hirame*, Fig. 13.3G) and Heteraxinidae (*Zeuxapta seriolae*, Figs. 13.1C, D, 13.3H, 13.4B, 13.6B, 13.8D).

## MONOPISTHOCOTYLEA

### Capsalidae

#### *General Information*

The Capsalidae is a family comprising approximately 45 genera and about 200 described species parasitizing 'skin', fins and gills of a wide diversity of marine elasmobranch and teleost fishes including sturgeon (Whittington, 2004). Capsalids range in size, depending on the species, from 2 mm long to nearly 3 cm long. They are all oviparous. There is limited morphological diversity within the family, which makes distinguishing between genera and species particularly difficult, even for a specialist parasitologist. Pathogens of captive fish are known from the genera *Benedenia* (see below), *Benedeniella* (see Thoney, 1990), *Entobdella* (e.g., Anderson and Conroy, 1968; Svendsen and Haug, 1991) and *Neobenedenia* (see below). A report of an epizootic by the capsalid *Benedenia monticellii* on wild mullet, *Liza carinata* (Mugilidae) in the Red Sea was mentioned earlier. Here, we have selected 2 capsalids that can be pathogenic to captive fish. *Benedenia seriolae* is a species that is representative of the family. The other example, *Neobenedenia* is enigmatic for several reasons: *Neobenedenia* is a genus comprising several species but there are questions about how many species there are and how to distinguish between them. The most widely known 'species', *N. melleni*, may have exceptionally broad host specificity, unlike almost all other Monogenea. *Neobenedenia* species can cause significant health issues to fish in aquaria and in culture.

## *Benedenia seriolae* (Yamaguti, 1934) Meserve, 1938

*Benedenia seriolae* has been a notorious pathogen of some *Seriola* species (Carangidae) in Japanese sea cage culture since the 1960s, causing considerable health problems on commercial farms throughout southwestern Japan (see Whittington *et al.*, 2001a, b for reviews). This capsalid species is reported from the 'skin' of *S. quinqueradiata*, *S. dumerili*, *S. lalandi* and *S. rivoliana* in Japanese culture (Whittington *et al.*, 2001b; Ernst *et al.*, 2002) and from *S. lalandi* in New Zealand and South Australia (Sharp *et al.*, 2001). Growth of carangid culture in other parts of the world may mean that *B. seriolae* could also become a problem in Chile, Ecuador, the USA (Hawaii), México and the western Mediterranean Sea.

*Benedenia seriolae* roams across the external 'skin' surfaces of *Seriola* species, in the wild and in culture, resulting in generic references to it as 'skin fluke'. On hosts in captivity, specimens of *B. seriolae* may be observed on the eye in heavy infections (Fig. 13.8B). This is a large monogenean species with a resting (i.e., unextended) length from 4 to 12 mm and resting breadth from 1 to 6 mm for adults (Figs. 13.2F, 13.10A). The haptor is a large, almost circular sucker-like organ (Figs. 13.2F, 13.3A) bearing a pair of blunt accessory sclerites that protrude from the centre, 2 pairs of hamuli and 14 hooklets around the edge. Despite the presence of large haptoral sclerites, the haptor can generate strong suction and attaches readily to glass and plastic surfaces. No detailed studies of haptoral attachment or feeding by *B. seriolae* have been published and any pathology caused has not been assessed critically. Instead, there are numerous reports that refer to the damage caused by 'skin flukes' to various *Seriola* species, but injuries are not quantified. Forty-five wild specimens of *S. lalandi* angled by recreational fishers off the eastern coast of North Island, New Zealand were parasitized by an average of 28 *B. seriolae* specimens per fish (Sharp *et al.*, 2001).

Despite persistent problems from *B. seriolae* in Japan, it is only relatively recently that studies have been published about its biology. Early research in the mid-1960s was published by Japanese scientists (see Egusa, 1983 for a review), reflecting the regional nature of the problem at that time. Now that *B. seriolae* is also a recognized and serious pathogen in commercial ventures in Australia and New Zealand, there is broader interest in its biology. Egg embryonation period is inversely related to water temperature. At 35 ppt salinity, eggs of *B. seriolae* from *S. quinqueradiata* in Japan hatched after 16 days at 14°C and after 5 days at 28°C (Ernst *et al.*, 2005). Oncomiracidia continued to hatch mostly over

a 2-day period at 20° – 28°C, over 3 days at 16° and 18°C and over 4 days at 14°C. At all temperatures, >90% of oncomiracidia emerged during the first 2 days of hatching. Mean hatching success was >70% for eggs incubated between 14° and 28°C, but no eggs embryonated and hatched at 30°C, a temperature that appears to exceed the physiological tolerance of *B. seriolae* eggs. Embryonation period remained similar at salinities of 25, 30 and 35 ppt, but eggs took longer to embryonate at salinities below and above these levels (Ernst *et al.*, 2005). For *B. seriolae* on *S. lalandi* in New Zealand, at 13°, 17.5° and 21±1°C, peak egg hatching took place 22, 11 and 9 days, respectively, after laying, oncomiracidia continued to hatch for 4 days and water temperature did not influence mean hatching success (Tubbs *et al.*, 2005).

On *S. quinqueradiata* in Japan, *B. seriolae* reaches full sexual maturity at 22°C after 14 days (Kearn *et al.*, 1992a). At 13°, 18° and 21°C, specimens of *B. seriolae* attained full sexual maturity at 48, 25 and 20 days, respectively on *S. lalandi* in New Zealand (Tubbs *et al.*, 2005). In experimental infections of *S. lalandi* in South Australia, *B. seriolae* reached full sexual maturity at 41, 24, 16 and 14 days at 14, 18, 22 and 26 ± 0.5°C, respectively (Lackenby *et al.*, 2007). Assessments of fecundity *in vitro* vary widely: 27 eggs/parasite/h at 20°C for a single parasite from *S. quinqueradiata* in Japan (Kearn *et al.*, 1992a); approximately 20 eggs/parasite/24 h at 13°C, 61 eggs/parasite/24 h at 17.5°C and 46 eggs/parasite/24 h at 21°C for parasites from *S. lalandi* in New Zealand (Tubbs *et al.*, 2005); approximately 8 eggs/parasite/h at 20 ± 0.5°C for parasites from *S. lalandi* in South Australia (Lackenby *et al.*, 2007). There is no published fecundity value for *B. seriolae* determined experimentally *in vivo*, but we expect it to be considerably higher than values *in vitro*. Ernst *et al.* (2002) estimated *in vivo* fecundity at 100 eggs/parasite/h and extrapolated that if this were accurate and each of 15,000 fish in a single sea cage was infected by only 5 adult *B. seriolae*, potentially 180 million parasite eggs could be laid every day! In aquaculture, however, parasite burdens can be appreciably greater than 5 adult parasites per fish. Eggs of *B. seriolae* are laid singly and the uterus is incapable of storing eggs, so they are released individually. The eggs are tetrahedral (Fig. 13.4A) and possess a fine filamentous appendage at least 2 – 4 mm long (Kearn *et al.*, 1992a). In sea cage culture, drifting eggs may tangle on sea cage netting and associated fouling organisms (Figs. 13.4C, 13.9D). A preliminary study indicated a relationship between egg numbers on cage material and the biomass and type of fouling flora and fauna present. As many as 64,000 *B. seriolae* eggs

per square metre of nylon cage material were counted from a fish farm in south western Japan (Ernst *et al.*, 2002).

When exposed experimentally to alternating 12 h periods of illumination and darkness at 23°C, eggs of *B. seriolae* hatch rhythmically, mostly during hours of daylight with a suggestion of early and late peaks in larval emergence (Kearn *et al.*, 1992b). Hatched larvae are ciliated (Fig. 13.4A) but their longevity is currently unknown.

### Disease Symptoms

Sharp *et al.* (2001) noted that heavily infected specimens of *S. lalandi* in New Zealand showed reduced appetite, tended towards emaciation and displayed flashing behaviour. Continued flashing led to severe 'skin' abrasions which became secondarily infected by fungi, bacteria (e.g., *Vibrio*) and viruses. Ernst *et al.* (2005) noted that feeding by *B. seriolae* on *S. quinqueradiata* 'skin' can cause lesions on heavily infected specimens which, with secondary infections, can lead to mortalities. Egusa (1983) presented similar symptoms for *Seriola* species in Japanese culture and by Sharp *et al.* (2004) for *S. lalandi* in New Zealand. Sharp *et al.* (2004) stated that hooklets and hamuli of *B. seriolae* pierce the host epidermis and even penetrate the dermis but if this occurs, it may only take place in especially severe infections. There is a strong need for a careful study of how *B. seriolae* attaches to its hosts and how its feeding activities effect host epithelia. 'Skin' lesions from an acute infection by *B. seriolae* promoted experimentally on *S. lalandi* are shown in Figure 13.8C.

In *S. lalandi* culture in Spencer Gulf, South Australia, heavy epizootic infections by *B. seriolae* are characterized by the following chronology of symptoms (pers. comm., Ingo Ernst): 'flashing' behaviour; appearance of dark patches of epithelium, perhaps from the feeding of the parasites; a reduction in fish condition as judged by poor growth and reduced food conversion ratio; severe epithelial damage presenting as lesions, perhaps due to an inability of heavily infected hosts to repair damaged 'skin' effectively; appearance of secondary infections; loss of appetite; potential for mass mortalities if there is no intervention to treat monogenean and secondary infections. It should be noted that epizootics of *B. seriolae* may occur most commonly in culture during summer months when the increase in ambient seawater temperature accelerates the rate at which the life cycle proceeds (i.e., more rapid egg embryonation, parasite development and attainment of full sexual maturity; Lackenby *et al.*, 2007).

### *Disease Diagnostics*

Although adult *B. seriolae* can be large, their body is often transparent making them inconspicuous on fish 'skin' (Whittington, 1996). Depending on their specific location on the host, parasites may be pigmented and therefore concealed (Whittington, unpublished observation). However, once an investigator is familiar with what to look for, skills in finding them, especially if adults are present, will develop rapidly. Signs that fish are flashing, dark 'skin' patches and reduced fish condition may all indicate that a closer examination of captive *Seriola* species is necessary. Careful restraint of fish will permit scrutiny of the flanks, dorsal 'shoulder' areas and belly for *B. seriolae*. Parasites often appear as a slightly raised, gelatinous 'blister' which to the uninitiated could look like excess mucus. Live parasites can be removed for identification by gently sliding a sharp scalpel blade held parallel against the fish 'skin' surface beneath the haptor to break the suction (see earlier). Care must be taken not to damage valuable fish specimens should the fish become agitated and flail around. A fine artist's paintbrush assists the removal of parasites to a dish of seawater. Bathing an infected individual in dechlorinated freshwater for up to 5 min will kill any *B. seriolae* (see Chambers and Ernst, 2005) and render them opaque and easy to see on the fish surface (Fig. 13.8A). Freshwater, however, does not necessarily *remove* these parasites from host 'skin' due to their strong, suctorial attachment. Treated monogenean specimens can be removed by rubbing body surfaces gently after the host is returned to seawater. Furthermore, freshwater treatment serves to distinguish *B. seriolae*, which has a tapering body shape anteriorly and a relatively large haptor, in comparison to body size, from specimens of *Neobenedenia* species (see next taxon below) which are characteristically smaller, broader bodied anteriorly and have a relatively small haptor in comparison to body size (Fig. 13.10; also consult Kinami *et al.*, 2005). Some sample specimens should be flattened and mounted permanently on glass slides as described earlier for specific identification.

There are >20 described species of *Benedenia* and *B. seriolae* (Figs. 13.2F, 13.3A, 13.10A) is best distinguished from other species by: its large size; large, centrally located accessory sclerites; long, conspicuous but relatively slender anterior hamuli which are curved towards their tips; the large, cone-shaped chamber immediately behind the dorsal vaginal pore (Whittington *et al.*, 2001c). Tetrahedral eggs are not a useful diagnostic feature because many monopisthocotyleans lay them, including all known

*Benedenia* species. Samples of parasites should be preserved in high-grade ethanol because nucleotide sequence data can be compared with that of other species stored in GenBank®.

## Disease Treatment and Prophylaxis

The threat by *B. seriolae* to *Seriola* sea cage farms in Japan (Fig. 13.9A) was thought to have been resolved from the late 1960s to the early 1980s because its presence and impact seemingly declined. However, it is possible that this respite in infection pressure was due to use of an effective, but highly toxic, antifoulant paint, tributyl tin oxide (TBTO) on cage materials (Egusa, 1983). TBTO was thought by some to be toxic to eggs and oncomiracidia of *B. seriolae* and when the use of TBTO was discontinued in mid-1990, epizootics of this monopisthocotylean reappeared (Whittington *et al.*, 2001b). The freshwater bathing treatment cited by Egusa (1983) is still widely used in Japan to treat epizootics (Fig. 13.9B), but certainly confers no protection to treated fish which become reinfected immediately by oncomiracidia in the surrounding water or when they hatch from embryonating eggs nearby (Fig. 13.4C). Bathing in hydrogen peroxide administered at 300 ppm for 10 min is used in South Australia for *S. lalandi* infected by *B. seriolae* and *Zeuxapta seriolae* (Polyopisthocotylea: Heteraxinidae) because it is effective and has no implications regarding food safety (Mansell *et al.*, 2005). Freshwater is too expensive and rare in the dry state of South Australia to be a viable treatment. Furthermore, sea cages in the Spencer Gulf of South Australia are further offshore than in the intense Japanese industry (Fig. 13.9A), further contributing to the difficulties of using freshwater. Hydrogen peroxide should be used with caution, however, because it presents toxicity issues to some fish species (e.g., Arndt and Wagner, 1997; Kiemer and Black, 1997; Rach *et al.*, 1997) and its toxicity can be strongly correlated with an increase in water temperature (Rach *et al.*, 1997).

Freshwater baths for 5 min are used regularly in laboratory experiments to obtain individuals of *Seriola* species that are free of *B. seriolae* (e.g., Chambers and Ernst, 2005), but while all specimens are killed, not all individuals are detached immediately. Sharp *et al.* (2004) determined that baths for infected *S. lalandi* in seawater containing a low praziquantel dose (2.5 ppm) for 24 or 48 h were >99% effective at removing the parasites. Specimens of *B. seriolae* removed by 24-h exposure to 2.5 ppm praziquantel continued to lay viable eggs. Sharp *et al.* (2004) also reported that a formalin bath at 250 and 400 ppm for 1 h followed by

a 5-min freshwater bath removed only approximately 80% of *B. seriolae* but these treatments did inhibit egg production by treated parasites. Chambers and Ernst (2005) reported that a 5-min freshwater bath *alone* (i.e., no formalin) killed *B. seriolae* outright. It is likely, therefore, that all *B. seriolae* were killed in the treatment trials of Sharp *et al.* (2004) using dilute formalin baths followed by a 5-min freshwater dip. Parasite death was probably assured by freshwater treatment, but some monogeneans did not fall off the fish, thereby confounding their results. We have noticed this phenomenon among several species of Monogenea that attach largely by suction (e.g., Capsalidae; Monocotylidae) and dead parasites may commonly remain firmly attached by the sucker-like haptor to glass and plastic surfaces for several hours after death.

Praziquantel is the active compound in Hadaclean® (Bayer), an oral treatment approved for *B. seriolae* infecting *S. quinqueradiata* in Japan (Williams *et al.*, 2007). No oral anthelmintic is approved for use in the Australian kingfish industry. Experiments in South Australia on *S. lalandi* fed daily doses of praziquantel surface-coated onto feed pellets for 6 days at 50 and 75 $mgkg^{-1}$ BW $day^{-1}$ had fewer *B. seriolae* than fish fed higher daily doses for 3 days at 100 and 150 $mgkg^{-1}$ BW $day^{-1}$ but pellet palatability was a problem (Williams *et al.*, 2007).

The kinds of treatment strategies investigated by Sharp *et al.* (2004) and Williams *et al.* (2007) and those currently in use in South Australia via hydrogen peroxide bathing (Mansell *et al.*, 2005) require dual treatments timed strategically to first kill all adult parasites on fish (treatment 1) followed by a second treatment to kill immature recruits invading previously bathed fish from larvae in the water and hatching eggs left in the system (treatment 2). The strategic timing of treatment 2 is critical to kill all newly recruited stages but before any attain full sexual maturity to lay more eggs. The timing, therefore, must accommodate environmental variables (e.g., water temperature and salinity) and *all* cages on a single farm must be treated over a short time duration for maximum benefit. Lackenby *et al.* (2007) generated data on the effect of water temperature on the development, growth rates and age at full sexual maturity for *B. seriolae* from farmed *S. lalandi* in South Australia. At seawater temperatures between 14 and 26°C representing the approximate annual seawater temperature range for farmed *S. lalandi* in Spencer Gulf, South Australia, quadratic equations derived from growth of the anterior hamuli of *B. seriolae* provide a simple tool to help implement strategic treatment plans for commercial kingfish farms (Lackenby *et al.*, 2007).

Eggs of *B. seriolae* from *S. lalandi* in New Zealand could not be prevented from hatching unless exposed to air for >3 h (Sharp *et al.*, 2004). Ernst *et al.* (2005) prevented eggs of *B. seriolae* (laid by parasites removed from *S. quinqueradiata* from southwestern Japan) from hatching by desiccation for 3 min, by immersion in water at 50°C for 30 s or by treatment with 25% ethanol for 3 min. These methods involving the prevention of egg hatching are appropriate for the management and quarantine of fish in closed systems rather than open systems such as sea cages.

Chambers and Ernst (2005) devised an innovative series of field experiments to generate information about the infection dynamics of *B. seriolae*, recognizing that the greatest source of reinfection to farmed *Seriola* stocks that have been treated was from parasite populations on adjacent sea cages. The effect of tidal currents on dispersal of *B. seriolae* eggs and on the rate of parasite infection using 'sentinel' fish to measure infection pressure was assessed within and between adjacent sea cage leases in South Australia. It was determined that the density of *B. seriolae* eggs in the plankton and infection pressure on sentinel fish was strongly influenced by the direction of tidal currents. Within farm leases, eggs were recovered in plankton samples only at sites in line with tidal current and fish sentinels had greater infection rates in line with and not perpendicular to, or across, current flow. Between leases, infection pressure on sentinel fish decreased with increasing distance from the parasite source. These findings suggest that *B. seriolae* eggs can disperse extensive distances and to achieve independent management units for effective parasite management, separate leases may need to be >8 km apart. There are some sobering messages for farm layout in the study by Chambers and Ernst (2005). Currently, sea cages are usually organized in rows in line with current flow for purposes of operational efficiency, cage mooring and to maintain sea cage shape, but results from Chambers and Ernst (2005) suggest that this same layout may *enhance* parasite dispersal between cages. Strategic positioning of sea cages perpendicular to water current direction may provide prospects to manage infections more efficiently although this arrangement may also impact negatively on other operational efficiencies. This suggestion has credibility in an environment where sea cages are currently relatively dispersed (e.g., in Spencer Gulf, South Australia), but in intense farming environments where sea cages are closely spaced (e.g., in Japan; Fig. 13.9A), opportunities to realize these advantages are impossible.

## Geographical Distribution

*Benedenia seriolae* is reported on wild *Seriola* species throughout most of their circumglobal distribution across tropical and sub-tropical regions (Indo-West Pacific, Micronesia, eastern Pacific, Western Atlantic). Epizootics are known from Japan, New Zealand and South Australia but are not reported in culture from the Mediterranean Sea. *Benedenia seriolae* on captive *Seriola* species is also reported in public display aquaria (Whittington, 1996).

## Economic Importance

In Japan, *Benedenia seriolae* has threatened commercial sea cage farming of *Seriola* species (current annual production can reach 150,000 tonnes) since the 1960s. *Benedenia seriolae* infects *S. dumerili*, *S. lalandi*, *S. quinqueradiata* and *S. rivoliana* in Japanese culture (Ernst *et al.*, 2002). In some regions of Japan, *S. dumerili* appears especially susceptible to infection by *B. seriolae* and can exert constraints on what fish species is farmed, stocking densities and the size and type of cage used (Whittington *et al.*, 2001b; Ernst *et al.*, 2002). *Benedenia seriolae* can kill captive stocks but its most insidious and grave effect is the cost to manage and treat infections (Ernst *et al.*, 2005) which can be as high as 20% of the total fish production costs (including expenses to cover treatments, personnel costs, lost growth, decreased food conversion as well as mortalities). Bathing treatments using freshwater or hydrogen peroxide are especially expensive, for they require a great deal of labour and infrastructure, interrupt feeding regimes and stress the fish.

Problems experienced in the culture of *Seriola* species in Japan by *B. seriolae* are surfacing in South Australia and New Zealand. Expansion of *Seriola* species culture elsewhere (e.g., South and Central America, Hawaii and the Mediterranean) may result in future epizootics of *B. seriolae* in these regions.

## *Neobenedenia* species

*Neobenedenia* Yamaguti, 1963 is a genus of capsalids reported to contain 6 described species (Whittington and Horton, 1996), but there is some disagreement about likely synonymous species (e.g., is *N. girellae* the same as *N. melleni*? see Whittington and Horton, 1996; Kinami *et al.*, 2005). In general, *Neobenedenia* species have proven to be confusing, especially *N. melleni*, and a current hypothesis is that *N. 'melleni'* may comprise a

cryptic species complex of perhaps several species that are morphologically indistinguishable (Whittington, 2004). Research in progress in our laboratory is investigating this suggestion using morphological and molecular genetic techniques. In this section, we have covered many reports under the single heading of *Neobenedenia* species because of the difficulties in discriminating between species using traditional morphological methods.

*Neobenedenia melleni* (MacCallum, 1927) Yamaguti, 1963 was first reported (as *Epibdella melleni*; see MacCallum, 1927) from the 'skin' and eyes of various chaetodontid, ephippid, pomacanthid and tetraodontid fishes held in public display tanks at the New York Aquarium. Since its discovery, this parasite has been a persistent pathogen on tropical, subtropical and a few temperate water teleost species in the New York Aquarium, in other public display aquaria and in tanks of aquarium suppliers around the world. Infections by monogeneans identified as *N. melleni* have also been reported from wild and/or cultivated fishes globally (see *Geographical Distribution* below). Outbreaks attributed to *N. melleni* on cultivated fish in floating sea cages or in land-based recirculation systems can also be pathogenic and have contributed to significant losses of stocks.

While most Monogenea are generally host specific, *N. melleni* appears to be highly aberrant because it is reported from >100 teleost species in >30 families from 5 orders, but it is not known from elasmobranchs (Whittington and Horton, 1996; Bullard *et al.*, 2003). The infamy and apparent ubiquity of *N. melleni*, however, may be a product of it being a cryptic species complex (see above). Further research will indicate whether this is the case. Complications that have contributed to the identity issue concerning *N. melleni* include the fact that uncertainty shrouds its type-host species because MacCallum (1927) mounted pooled parasite specimens from *different* host species at the New York Aquarium on the *same* slides. It is frustrating that voucher specimens of *N. melleni* from other host species from different localities are rarely lodged in properly curated museum collections, which means that there may not be 'reference specimens' to confirm parasite identity (see section on *Depositing Specimens* earlier).

In addition to its low host specificity, *Neobenedenia* species appear to have low site specificity and may infect the 'skin', fins, eyes, gill cavity and nasal cavity of hosts (Jahn and Kuhn, 1932; Whittington and Horton, 1996; Bullard *et al.*, 2000a). This broad site specificity may, however, be

related to severe epizootics. The cornea of the eye lacks a supply of blood and Llewellyn (1957) suggested that such a site away from blood-borne antibodies may be immunologically inert. However, the cornea also lacks mucous cells (Kearn, 1999) and since fish mucus is known to be immunologically active (Buchmann, 1999), then the apparent lack of anti-parasitic activity against *Neobenedenia* on fish eyes may be related to this.

Like their close relative *Benedenia seriolae* (see above), *Neobenedenia* species feed on epithelial cells. The haptor is structurally similar to that of *B. seriolae* and although there has been no study of its attachment, it probably relies heavily on suction but is likely assisted by some penetration into epithelia by the large and conspicuous anterior hamuli. *Neobenedenia* species are 2 to 7 mm long. To our knowledge, the only assessment on mean *Neobenedenia* infection intensity from a wild host is 11.8 *N. girellae* per specimen of the halfmoon, *Medialuna californiensis* (Kyphosidae) and specimens that were heavily infected (up to 42 parasites per fish) showed no pathology (Gaida and Frost, 1991), but many of these parasites were small. It remains to be determined whether the association of *Neobenedenia* species at natural population levels with a wild, unstressed host is always benevolent.

The following 2 accounts pertain to studies on *Neobenedenia* species in outbreaks. Jahn and Kuhn (1932) reported differences in the morphology of the tetrahedral egg laid *in vitro* by the same adult specimen of *N. melleni* from the New York Aquarium. Usually, the egg had 1 long filamentous appendage and 2 shorter, hooked appendages, but some eggs had no hooks (Fig. 13.6D) and others allegedly had 3 hooked appendages but no long filamentous appendage. It is possible that these differences were due to abnormalities induced by starvation of parasites after removal from a fish host. Jahn and Kuhn (1932) measured the filamentous appendage at 0.8 to 1.2 mm long. Eggs hatched after 5 to 8 days (no temperature was specified), the oncomiracidia were ciliated, 225 μm long and 60 μm wide, and swam actively for 6 h before settling at the bottom of the dish. The most complete picture of *Neobenedenia* biology is from a laboratory infection of *N. girellae* established on Japanese flounder, *Paralichthys olivaceus* (Paralichthyidae) studied by Bondad-Reantaso *et al.* (1995b). When exposed to a natural illumination cycle, eggs hatched after 8 days at 18°C, 7 days at 20°C, 5–6 days at 25°C and 4 days at 27–30°C, but failed to hatch at 15°C even after 3 weeks. Oncomiracidia ranged from 180–210 μm long and at 25°C, full sexual maturity on a host was

reached after 10–11 days at a minimum body length of 2.1 mm. An assessment of mean fecundity *in vitro* at 27–30°C for 1 h after removal from the host was 35.4 eggs/parasite/h for large specimens (3.9–5.2 mm long) and 12.2 eggs/parasite/h for small specimens (2.1–2.9 mm long). Unfortunately, there is no assessment of fecundity for a *Neobenedenia* species *in vivo*.

## Disease Symptoms

The outbreak of *N. melleni* in the New York Aquarium in 1926 destroyed the cornea of many fish (MacCallum, 1927). Jahn and Kuhn (1932) confirmed this symptom even in mild infections and noted that destruction of the whole eye—aided by secondary bacterial infections—could occur if infections were not treated. Heavy infections (>2,000 adults on a single fish) also severely injured the epithelial tissue of the general body surface causing scale loss and exposure of extensive regions of connective and muscle tissue, symptoms that inevitably killed the fish (Jahn and Kuhn, 1932). Kaneko *et al.* (1988) noted parasites attached to the anterodorsal region of the head and corneal surface of tilapia, *Oreochromis mossambicus* (Cichlidae), and in heavy infestations (>400 specimens per fish), the parasites covered the head and dorsal surface along the length of the dorsal fin. These fish displayed hyperirritability, excessive mucus production and discoloured 'skin' (dull grey to black), showed loss of epithelium and scales and had haemorrhagic lesions. The eyes were again a focus of infection and pathology: in early stages, the cornea was opaque but in later stages, corneal ulceration, rupture of the eye and degeneration of internal structures was followed by scarring and blindness. Untreated fish became more heavily infected (Kaneko *et al.*, 1988). Koesharyani *et al.* (1999) described opaque eyes, excessive mucus secretion from the body and anorexia in *Cromileptes altivelis* (Serranidae). Other common symptoms include flashing, which may exacerbate epithelial damage and secondary infections, and loss of appetite which heightens loss of condition in *Verasper variegatus* (Pleuronectidae) (see Hirazawa *et al.*, 2004). It is unclear how much pathology is a direct result of the attachment and feeding of *Neobenedenia* and how much is caused by secondary pathogens such as bacteria.

## Disease Diagnostics

*Neobenedenia* species are commonly between 2 and 4 mm long, but can be up to 7 mm long and are among the smallest parasites in the Capsalidae.

Live specimens of *Neobenedenia* species can be transparent, occasionally pigmented or opaque and at low population densities, these parasites can go unnoticed. This may account for the relative scarcity of records from potential fish hosts in the wild (Bullard *et al.*, 2003). Gaida and Frost (1991) presented a technique to detect *Neobenedenia* infections on wild fish by applying alcohol-formalin-acetic acid (AFA) by pipette to the external surfaces of a fish. The method using the fixative was more effective for recovering *Neobenedenia* infections than simple visual inspection of untreated fish because it turned parasites opaque.

Methods outlined above for *B. seriolae*, including freshwater bathing and removal of parasites using scalpel blade and fine paintbrush, also apply for the removal of *Neobenedenia* species in heavy infections. Kinami *et al.* (2005) showed that simple measurement of *N. girellae* and *B. seriolae* specimens killed by freshwater baths could help discriminate between them. The 2 species were readily distinguished when the length of the anterior adhesive organs of *B. seriolae* (Fig. 13.2F) and *N. girellae* were plotted against the total parasite length (Kinami *et al.*, 2005). We emphasize the importance of preparing some specimens as *permanent* mounts on glass slides for specific identification and deposition in an appropriate museum collection after consultation with parasite specialists. In view of the apparent ubiquity of *Neobenedenia* species and the countless queries that shroud them, the need to follow this protocol is *especially* critical.

The major morphological distinction between the genera is that a vagina is present in *Benedenia* species and absent in *Neobenedenia* species (Whittington and Horton, 1996; Whittington *et al.*, 2001c). In practice, however, it can be very difficult to find the vagina in many *Benedenia* species unless the observer has access to live material and is confident about what to look for. For this reason, failure to observe a narrow vaginal duct in a capsalid does not necessarily mean the specimen is a species of *Neobenedenia*. The freshwater bath treatment highlighted above may provide a broad method to discriminate most *Benedenia* species from most *Neobenedenia* species. Other characteristic differences are that the body of some, but not all, *Neobenedenia* species tends to be broader anteriorly (Fig. 13.10B) rather than tapering anteriorly in *B. seriolae* (Fig. 13.10A). Also, the anterior adhesive organs of *Neobenedenia* species are relatively small, may appear 'hooded' and the haptor is also small relative to parasite size (Fig. 13.10). Eggs of all *Neobenedenia* species described are tetrahedral (Fig. 13.6D), but some may have hooked appendages on 2 of the 4 poles

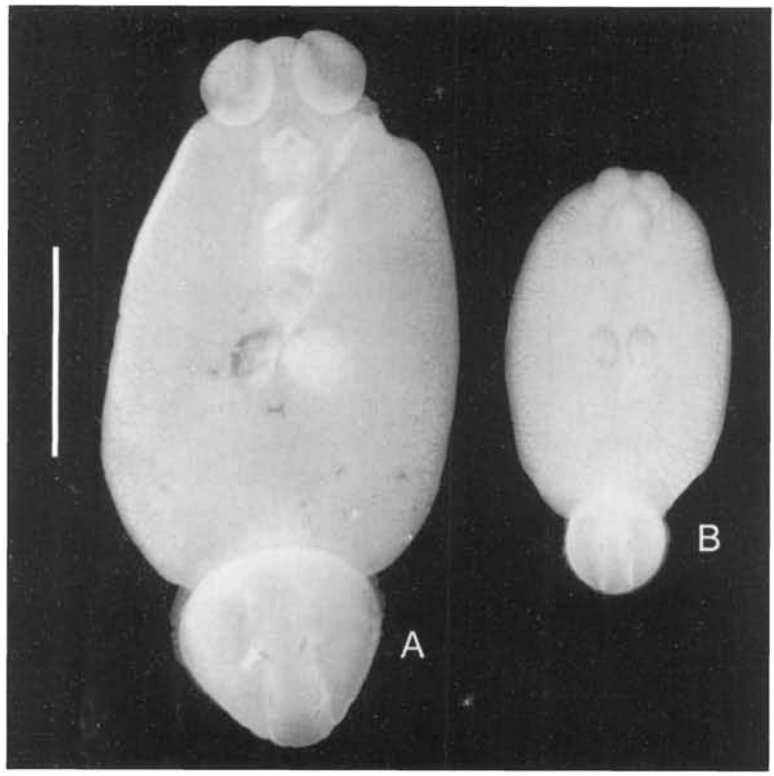


**Fig. 13.10.** Comparison of *Benedenia seriolae* and a *Neobenedenia* species (Monopisthocotylea: Capsalidae) in ventral view from *Seriola* species (Carangidae) following freshwater treatment. A. An adult specimen of *Benedenia seriolae* from the 'skin' of a wild specimen of *Seriola lalandi* off Kangaroo Island, South Australia. B. An adult specimen of a *Neobenedenia* species from the 'skin' of *S. rivoliana* in culture in Ecuador. Note the relative differences in body shape, haptor shape and size and the shape and size of the anterior attachment organs. Scale bar = 2 mm.

in addition to a filamentous appendage (e.g., MacCallum, 1927; Jahn and Kuhn, 1932). This account of how to diagnose a *Neobenedenia* infection may appear vague but it reflects the difficulties we face in understanding this mysterious monogenean genus. In view of the possibility that *Neobenedenia* may contain at least 1 species complex (Whittington, 2004), it is strongly advised that some parasite samples from any outbreaks experienced are preserved in high-grade ethanol for molecular analysis. We are happy to receive samples of suspected *Neobenedenia* species prepared for morphology and genetic analysis to help our on-going project that seeks to better understand the genus.

## Disease Treatment and Prophylaxis

Infected tilapia from the epizootic attributed to *N. melleni* in sea cages in Kaneohe Bay, Hawaii were treated successfully using a 2-min dip in freshwater. Kaneko *et al.* (1988) reported that freshwater dips between 15 s and 105 s were not 100% effective whereas treatment for 120 s and 150 s killed all parasite specimens and there was 100% survival of the treated tilapia. When a regime of 2-min freshwater baths was applied across infected cages every 2–4 weeks, the *N. melleni* population declined markedly after several repetitions. Ellis and Watanabe (1993) examined the effects of hyposalinity on survival of eggs *in vitro* and on juvenile and adult *N. melleni in situ* on Florida red tilapia in Caribbean culture to determine a therapy during growout in seawater. A 5-day treatment with hyposaline water at 15 $gL^{-1}$ salinity was recommended because it prevented egg hatching *in vitro* and eliminated juveniles and adults *in situ*. It was pointed out, however, that this therapy has not been verified on a commercial scale. In the same parasite-host system, Cowell *et al.* (1993) evaluated 3 species of tropical cleaner fish (the bluehead wrasse, *Thalassoma bifasciatum* [Labridae], the neon goby, *Gobiosoma oceanops* [now *Elacatinus oceanops*; Gobiidae] and the cleaning goby, *G. genie* [now *E. genie*; Gobiidae]) for their abilities to remove *N. melleni*. Final infection levels on tilapia kept without cleaners were significantly higher in 2 of 3 trials. Monogeneans were identified in the gut of each cleaner species but Cowell *et al.* (1993) reported that the gobies were superior cleaners to the labrid and may provide a viable biological control method for *Neobenedenia* in seawater tilapia culture.

Nigrelli and Breder (1934) recognized that the 'skin' of some fish in the New York Aquarium could mount a defence against *Neobenedenia* infections and noted that some developed resistance for a significant period, some developed partial resistance and some were always susceptible. These early observations were extended by Bondad-Reantaso *et al.* (1995a) who studied the immune response of Japanese flounder, *Paralichthys olivaceus* (Paralichthyidae), against *N. girellae*. They confirmed that fish acquired protection against secondary or challenge infections by *N. girellae* assessed by a reduction in number and size of monogeneans on the primed fish but detected no significant difference in serum antibody levels between primed and control flounder. In another experiment, while antibodies were detected in sera of fish injected intraperitoneally using sonicated parasite antigen in Freund's complete adjuvant, no significant

difference was observed in parasite counts between fish immunized by antigen and control fish receiving an injection of phosphate-buffered saline. Limited acquired protection, therefore, against *N. girellae* develops in *P. olivaceus* but serum antibody plays no role in this immunity. Recently, Hatanaka *et al.* (2005) discovered that *N. girellae* expresses an antigen that elicits antibodies in rabbits, which have an agglutination/immobilization activity *in vitro* against *N. girellae* oncomiracidia. The antigen is expressed on the surface of larval cilia and an intraperitoneal injection, of either sonicated or intact ciliary proteins with adjuvant, stimulated agglutination/immobilization antibodies in sera from *P. olivaceus*. The antigen identified may be useful for vaccination but it is not yet known whether fish antibodies against this antigen prevent infection by *N. girellae* (see Hatanaka *et al.*, 2005). Similar work on oncomiracidia of *N. girellae* by Ohashi *et al.* (2007a) purified and identified glycoproteins with attachment-inducing properties which may play a role in host specificity. Behavioural responses by *N. girellae* oncomiracidia to brightness and black and white contrast could lead to the development of a simple, practical and cheap method to control outbreaks (Ishida *et al.*, 2007).

A chemical therapeutant administered orally in feed against *N. girellae* infecting spotted halibut, *Verasper variegatus* (Pleuronectidae), was investigated by Hirazawa *et al.* (2004). A low-dose treatment of the anthelmintic praziquantel administered at 40 $mgkg^{-1}$ BW $day^{-1}$ for 11 days had a strong antiparasitic effect against *N. girellae*. Appetence of fish fed at a higher dose (150 $mgkg^{-1}$ BW $day^{-1}$ for 3 days) was a problem because the fish vomited highly medicated pellets, whereas this was not the case at the lower dose over 11 days. Ohashi *et al.* (2007b) determined antiparasitic effects of calcium and magnesium ion-free buffers and claimed the lack of these metallic ions *in vitro* and *in vivo* disrupted intercellular junctions in *N. girellae*, decreased their survival and could provide a control method.

## Geographical Distribution

*Neobenedenia* species are apparently widespread on a miscellany of tropical, sub-tropical and some temperate water fishes globally. It is conceivable that these parasites are spread unwittingly worldwide within the aquarium trade. Published records of *Neobenedenia* outbreaks (normally attributed to *N. melleni*) in aquaria include: New York Aquarium (MacCallum, 1927; Jahn and Kuhn, 1932; Nigrelli and Breder,

1934); Chicago Aquarium and Philadelphia Aquarium (Nigrelli and Breder, 1934); Bermuda Aquarium (Whittington and Horton, 1996); Shark Reef Aquarium, Las Vegas and Alaska SeaLife Center, Seward, Alaska (Bullard *et al.*, 2003). *Neobenedenia melleni* is also known from a tropical fish clearing house in California, USA (Bullard *et al.*, 2003). We know of unpublished records of *Neobenedenia* species from many teleost species in aquaria from various localities in Australia, México and Taiwan and on teleosts in tanks of aquarium suppliers from Australia and New Zealand. Monogeneans, usually identified as *N. melleni*, have been reported from wild and/or cultivated fishes in the east Pacific (California, México, Chile), mid-Pacific (Hawaii) and west Pacific (eastern Australia, China) Ocean, Caribbean Sea, Red Sea and Gulf of México. In Japan, *N. girellae* is known from several commercially important species in aquaculture (Ogawa and Yokoyama, 1998).

One of the most disturbing aspects of *Neobenedenia* infections in aquaculture is the fact that the source of infection is not always clear. While Ogawa *et al.* (1995) were of the opinion that the origin of the *N. girellae* infection in Japan was via unregulated importation of amberjack fry, the sources of *Neobenedenia* epizootics on tilapia in Hawaii and on barramundi in Queensland, Australia, are undetermined (Kaneko *et al.*, 1988 and Deveney *et al.*, 2001, respectively). In each case, it seems likely that a *Neobenedenia* species may live naturally on a wild fish species in each locality, but these parasite-host associations are currently unreported.

## Economic Importance

The persistence of outbreaks by *Neobenedenia* species in public aquaria and in the tanks of businesses that supply large aquaria has been noted above. We are unaware of a monetary value attributed to attempts to monitor and control *Neobenedenia* in aquaria or to the losses of valuable fish specimens, but the cost must be considerable. Occurrence of *Neobenedenia* epizootics has constrained the culture of some fish species in tropical and sub-tropical regions due to their virulence and apparent broad host specificity (e.g. in Hawaii by Kaneko *et al.*, 1988; in the Caribbean by Cowell *et al.*, 1993 and Mueller *et al.*, 1994; in south east Asia by Leong, 1997; in Bali by Koesharyani *et al.*, 1999; in México by Flores-Crespo and Flores-Crespo, 2003). Susceptibility to *Neobenedenia* infections has also been viewed as a potential problem to further

development of sea cage culture of cobia (*Rachycentron canadum*; Rachycentridae) in Taiwan (Liao *et al.*, 2004) and *Paralichthys olivaceus* (Paralichthyidae) and *Verasper variegatus* (Pleuronectidae) in Japan (Hirazawa *et al.*, 2004). Epizootics of *Neobenedenia* species have killed sea caged stocks of tilapia (*O. mossambicus*) in Hawaii (Kaneko *et al.*, 1988), Japanese flounder (*P. olivaceus*) and longfin amberjack (*Seriola rivoliana*; Carangidae) in Japan (Ogawa *et al.*, 1995), other carangids (*S. quinqueradiata*, *S. dumerili*), red seabream (*Pagrus major*; Sparidae) and tiger puffer (*Takifugu rubripes*; Tetraodontidae) in Japan (Ogawa and Yokoyama, 1998). In 3 weeks during the winter of 2000, a *Neobenedenia* outbreak killed 50 tonnes of barramundi (*Lates calcarifer*; Latidae) valued at 500,000 Australian dollars in sea cages off Queensland, Australia (Deveney *et al.*, 2001) highlighting how potent and swift these epizootics can be.

## Dactylogyridae

### *General Information*

The Dactylogyridae is a diverse and speciose family, species of which parasitize the gills of freshwater and marine teleost fish. *Dactylogyrus* has >900 described species that predominantly infect freshwater Cyprinidae (see Gibson *et al.*, 1996), although they are occasionally reported on Percidae (see Valtonen *et al.*, 1990; Cone *et al.*, 1994; Hayward, 1997) and other families (Gibson *et al.*, 1996). Because of this diversity, Dactylogyridae species are excellent models for studies on parasite-host co-evolutionary processes (Šimková *et al.*, 2004).

Dactylogyrids are small, oviparous parasites. The gut is bifurcate and confluent posteriorly (Fig. 13.3B). The haptor is generally armed with 2 pairs of hamuli (each pair connected by a supporting bar) and 14 hooklets, but some of these sclerites may be secondarily reduced or lost in some species. For example, the ventral pair of hamuli are reduced to spicules in *Dactylogyrus* species. Dactylogyrids, like diplectanids (see below), attach between adjacent secondary gill lamellae using their opposing pairs of hamuli (Figs. 13.2B, C, 13.3B, 13.9G, 13.11A). The pathological effects of dactylogyrid infections are dependent on the species and intensity of infection. Here we give brief accounts of 2 dactylogyrid species: *D. vastator* in freshwater and *Haliotrema abaddon* in the marine environment, both of which are pathogens in aquaculture.

## *Dactylogyrus vastator* Nybelin, 1924

*Dactylogyrus vastator* is a gill parasite of common carp, *Cyprinus carpio* (Fig. 13.11), and goldfish, *Carassius auratus* (Cyprinidae). Endemic to Asia, *D. vastator* has been introduced into Russia, Europe and North America on fish hosts. It is an especially prevalent pathogen of carp fingerlings cultured in fish ponds worldwide. *In vitro* and *in vivo* estimates of fecundity differ and are directly proportional to water temperature (Bychowsky, 1957). *Dactylogyrus vastator in vivo* lays about 24 eggs day$^{-1}$ at 28°C (Paperna, 1963). This rate decreases as water temperature drops. Eggs are oval, approximately 90 μm long and have a small appendage at the abopercular end (Fig. 13.11). At higher temperatures, the life cycle is particularly rapid. Between 24 and 28°C, eggs can hatch within 2–3 days of being laid and in one instance, hatching occurred just 18 h after laying (Paperna, 1963)! The oncomiracidia can survive for approximately 24 h but they only appear active during the first 3–5 h suggesting that they may

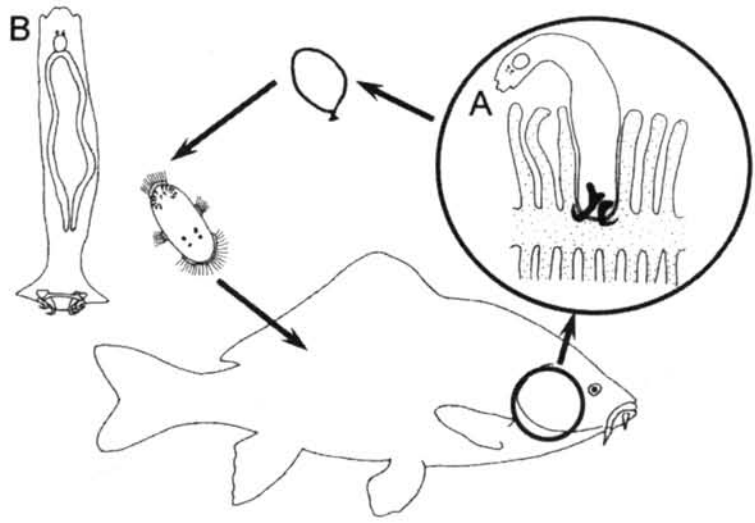


**Fig. 13.11** Generalized life cycle of small monopisthocotylean Monogenea that typically occupy a microhabitat between adjacent secondary gill lamellae (A) using opposing pairs of hamuli (only 1 of each pair drawn; see also Fig. 13.2B) that penetrate gill epithelium. Based on *Dactylogyrus vastator* (Dactylogyridae) from gills of common carp, *Cyprinus carpio* (Cyprinidae). There is unresolved controversy about whether eggs of *D. vastator* can 'overwinter' in a diapause-like state (Paperna, 1963). Note that oncomiracidia of *D. vastator* may also be inhaled passively into the branchial chamber. Other exemplar Monopisthocotylea species with a similar life cycle and microhabitat include *Haliotrema abaddon* (Dactylogyridae) (B) and *Diplectanum aequans* (Diplectanidae).

only be infective for the first few hours after hatching. The oncomiracidia are approximately 125 μm long, ciliated and their haptor is armed with 14 hooklets (Fig. 13.11). It has been suggested that the larvae first attach to the 'skin' of the host and then migrate to their final site, the gills (Fig. 13.11). However, Paperna (1963) determined that carp fingerlings became infected by larvae inhaled passively into the gill chamber and that attachment of larvae first to host 'skin' was extremely rare. Once on the gills, development is rapid and at 28°C, they can reach full sexual maturity within 5 days of invasion. Incidental observations suggest that at 28°C the generation time of *D. vastator* is between 12 and 13 days (i.e., 2 to 3 days for egg embryonation + 10 days from invasion to death; Paperna, 1963). Bychowsky (1957) suggested that the lifespan is probably 20–25 days at summer temperatures and even longer, 6–7 months, during winter months. Paperna (1963) noted that adult *D. vastator in vitro* is able to survive for about 24 h and continue to lay eggs. The egg-laying rate of detached *D. vastator* may actually increase initially but later the eggs become malformed.

The presence of 'overwintering' eggs in *D. vastator* is controversial. Several studies have suggested that when water temperatures fall in autumn, *D. vastator* lays large 'winter' eggs that hibernate at the bottom of the water body and hatch in spring when water temperatures increase and fingerlings are abundant. Paperna (1963) believes that some eggs undergo diapause until spring but that they do not differ significantly in size or shape from non-diapausing eggs. Others argue that egg size is extremely variable and that there is no evidence that any larger overwintering eggs are produced (Bychowsky, 1957). In this case, the prevalence of newly hatched larvae in the spring is attributed to the production of eggs from the few adult *D. vastator* that survived the winter on their host (Bychowsky, 1957). Clearly, this issue needs to be resolved.

### Disease Symptoms

Outbreaks generally occur in late spring and early summer when temperatures are optimum (20–25°C). Fish infected with *D. vastator* are usually lethargic, the opercula may open (gilling) and the gills, which may appear pale, secrete significant amounts of mucus. Severe hyperplasia of gill filament epithelium occurs and interferes with respiratory function resulting in death of the fish (Paperna, 1963). *Dactylogyrus vastator* prefers to attach to the tip of the gill filaments and hyperplasia may therefore only

occur in regions where parasites attach, leaving the rest of the filament functional (Paperna, 1963).

### Disease Diagnostics

The gill filaments of live fish should be inspected by noting the colour of the gill lamellae, the quantity of mucus and whether filament tips are hyperplastic. If infection is suspected, dissection of fish is usually required for parasite detection. Individual gill arches of a freshly killed fish should be placed in a Petri dish of water and scanned for monogeneans using a binocular dissecting microscope set at the highest magnification. Gill filaments and mucus can also be placed onto a microscope slide and examined for monogeneans under a dissecting microscope using transmitted light or a compound microscope. If the fish are heavily infected, detection may be made from gill filament snips without killing the fish. In this instance, the fish is sedated, the tips of 10 to 15 primary gill lamellae are removed using sharp scissors and examined under a compound microscope for monogeneans. Specimens of dactylogyrids for permanent mounts are best prepared using the ammonium picrate-glycerine method of Malmberg (1957; see also '*GyroDb*' on the Internet). Specific identification of the monogenean is based on morphological characteristics of the adult (Nybelin, 1924).

### Disease Treatment and Prophylaxis

Baths in sodium chloride (25 $gL^{-1}$ for 3–10 min) have been used with mixed results because adult parasites are often resistant. Applications directly into the pond or as baths of the organophosphate Dipterex® (0.25–0.50 ppm) (Sarig *et al.*, 1965; Schmahl, 1991) and praziquantel may also be effective (Schmahl and Mehlhorn, 1985; Lim *et al.*, 2005). Prost (1963) recommends prophylaxis by stopping the life cycle of *D. vastator* in carp farm ponds. Infected ponds should be drained annually to destroy any potentially overwintering eggs by desiccation. Water from infected ponds should not be transferred to ponds where uninfected fry reside. There are indications that host immune mechanisms are involved in the regulation of parasite loads (Buchmann and Bresciani, 2006).

### Geographic Distribution

*Dactylogyrus vastator* is endemic to Asia but has been introduced into Europe, Russia, Israel and North America on fish.

### *Economic Importance*

Farmed common carp production was nearly 14% of the total global freshwater aquaculture production in 2002 and in Europe that year, common carp production was 144,602 tonnes. We could find no estimates of costs associated with *D. vastator* infections. However, because culture of common carp is widespread, *D. vastator* would certainly contribute significantly to production costs and mortalities. Similarly, *D. vastator* infections must have significant economic consequences in the goldfish aquarium trade. Furthermore, incalculable funds will undoubtedly be spent on disease monitoring and regulatory processes.

### *Haliotrema abaddon* Kritsky and Stephens, 2001

*Haliotrema* is a highly diversified genus containing more than 150 described species (Cribb *et al.*, 2002). A number of *Haliotrema* species are pathogens of marine fish including *H. abaddon* which infects the gills of maricultured west Australian dhufish, *Glaucosoma hebraicum* (Glaucosomatidae) (see Pironet and Jones, 2000; Stephens *et al.*, 2003). This is a prized recreational and commercial saltwater fish whose potential as a species for aquaculture was assessed in Western Australia (Pironet and Neira, 1998; Stephens *et al.*, 2003). *Haliotrema abaddon* (Figs. 13.3B, 13.11B) was described only recently and features that characterize it are given in Kritsky and Stephens (2001). Oval eggs approximately 80 μm long are laid singly and have a long appendage at one end. The eggs take between 7 and 10 days to embryonate at 25°C after which time a free-swimming ciliated oncomiracidium emerges apparently spontaneously (i.e., no cue is required but this requires verification). Oncomiracidia attach to the bottom of Petri dishes and cilia stop beating within a few hours of hatching. No information is currently available on *H. abaddon* fecundity, generation time or longevity. In some heavily infected dhufish, strands containing multiple eggs of *H. abaddon* were entangled on the gill lamellae; this would increase larval infection success via autoinfection if they embryonate and hatch *in situ*. This is the first record of *Haliotrema* eggs attached to host gills and it is unclear whether this is a 'real' infection strategy or simply an anomalous consequence of eggs being trapped on inflamed and mucus covered gills in exceptionally high monogenean infections. The presence of white necrotic gill tissue in the region of the egg strands suggests that they may restrict blood supply to lamellae (Kritsky and Stephens, 2001).

### Disease Symptoms

The prevalence of *H. abaddon* on 32 wild caught dhufish was 100% but the intensity of infection was low and there was no evidence of gill pathology (Stephens, 2001). In heavily infected captive fish, however, the gill lamellae displayed significant epithelial hyperplasia (Fig. 13.9G) and fish died presumably due to respiratory failure (Pironet and Jones, 2000; Kritsky and Stephens, 2001).

### Disease Diagnostics

Diagnostic techniques for *H. abaddon* are as described for *D. vastator* above. Kritsky and Stephens (2001) should be consulted for specific identification based on parasite morphology.

### Disease Treatment and Prophylaxis

Control of *H. abaddon* on *G. hebraicum* has been the subject of several studies (Pironet and Jones, 2000; Stephens *et al.*, 2003). Stephens *et al.* (2003) trialed a number of treatments based on previously published protocols but no treatment was 100% effective. Fish bathed in freshwater for 1.5 h were highly stressed and it was not clear if all monogeneans were removed. Fish withstood formalin (25 ppm for 10.5 h) and toltrazuril (12.5 $mgL^{-1}$ for 6 h) baths better than freshwater baths, but some monogeneans remained on fish post-treatment. Monogeneans also remained on fish bathed in trichlorphon (0.5 $mgL^{-1}$ for 30 h) and higher doses of this chemical were toxic to dhufish (Pironet and Jones, 2000). The most promising treatment was praziquantel (2 $mgL^{-1}$ for 30 h). However, to avoid anthelmintic resistance, Stephens *et al.* (2003) recommended rotating treatments of praziquantel, trichlorphon and freshwater baths. Knowledge of specific parasite biology is needed to time the treatments strategically (i.e., before egg laying commences) to minimize their use and maximize their efficacy. Stephens (2001) notes that maintaining water quality and providing a stress-free environment would help to prevent heavy *H. abaddon* infections but acknowledges that this may not be feasible. For example, increased stocking densities and temperatures to maximize fish output are preferred by growers to maximize returns but these same conditions also favour monogenean proliferation.

### Geographic Distribution

*Glaucosoma hebraicum* is endemic to Western Australia and is distributed between Beagle Island in the Dampier Archipelago in the north and Recherche Archipelago in the south (Pironet and Jones, 2000). *Haliotrema abaddon* has thus far only been recorded from hosts in this region.

### Economic Importance

On completion of feasibility studies, it was deemed that the west Australian dhufish was an unsuitable candidate for culture. The relatively slow growth of the fish and spawning difficulties were significant problems that could not be overcome. In addition, these ambush predators required excellent water quality with minimal variation in temperature, chemical or physical properties. They suffered significant stress in less than pristine conditions and succumbed to a number of problems including high burdens of *Haliotrema*. Significant funds were allocated to evaluating the potential of this fish species for culture, but far more would have been spent on fish health issues, including parasite control, had the culture program proceeded untested.

## Diplectanidae

### General Information

The Diplectanidae is a family comprising approximately 20 genera and >250 described species parasitizing the gills of a wide diversity of marine and some freshwater teleost fishes. At first sight, these small oviparous monogeneans can easily be confused with members of the Dactylogyridae (see above) because of their similar size, location between adjacent secondary gill lamellae, the presence of 2 opposing pairs of hamuli with supporting bars (Figs. 13.3C, 13.11A) and 14 hooklets. Most diplectanids can be distinguished readily from dactylogyrids by the presence of circular friction pads known as squamodiscs on the haptor, just anterior to the hamuli and supporting bars (Fig. 13.3C). Squamodiscs are unique to the Diplectanidae. There are usually 2 squamodiscs—one on the ventral surface and an identical structure on the dorsal surface of the haptor. Some, however, have only a single squamodisc (e.g., *Monoplectanum* species; *Pseudorhabdosynochus monosquamodiscusi*) and species in *Lobotrema*, *Murraytrema*, *Murraytrematoides* and *Rhabdosynochus* have no

squamodisc (Desdevises, 2001). Squamodiscs comprise numerous, radially arranged rows of small sclerotized spines and at first glance may appear as tiny raised fingerprint-like structures. The arrangement and number of individual sclerites in the squamodisc is important to differentiate between genera. The squamodiscs supplement attachment by the 2 pairs of hamuli which—although they are orientated dorsally and ventrally—are displaced somewhat laterally on the haptor. The 2 pairs of hamuli tend to be smaller than those of many Dactylogyridae and maintain a connection with each other via 3 elongate bars (1 ventral; 2 dorsal). The posterior part of the bodies of some diplectanids is covered in anteriorly directed scales or tiny spines which may further assist attachment. Other features such as 2 pairs of small eyespots, a small spherical pharynx, a mostly bifurcate gut and the presence of anterior and posterior gland cells are reminiscent of many Dactylogyridae (compare Fig. 13.3B, C). The structure and shape of the sclerotized squamodisc and male copulatory organ is also important for distinguishing between many diplectanid species.

The diplectanid species we have selected to cover in detail is *Diplectanum aequans* but other important species known to cause epizootics include *Furnestinia echeneis* on gilt-head seabream, *Sparus aurata* (Sparidae) in the Mediterranean Sea and Red Sea (Vagianou *et al.*, 2004), *Lamellodiscus major* on yellowfin bream, *Acanthopagrus australis* (Sparidae) in Australia (Roubal, 1986, 1989) and several species on barramundi, *Lates calcarifer* (Latidae) and orange-spotted grouper, *Epinephelus coioides* (Serranidae) in fish culture in south east Asia (Leong, 1997) and northern Australia.

## *Diplectanum aequans* (Wagener, 1857) Diesing, 1858

Adults of this species are approximately 1 mm long. Off the Mediterranean coast of France, *D. aequans* infects the gills of *Dicentrarchus labrax* and *D. punctatus* (Moronidae). Oliver (1977) reported that *D. labrax* is the principal host with a prevalence of 92.7% compared with 13.2% on *D. punctatus*. Prevalences of 99 to 100% are recorded for *Diplectanum aequans* from wild *Dicentrarchus labrax* off Portugal (Santos, 1996; Duarte *et al.*, 2000). Natural infection levels on wild *D. labrax* (as *Morone labrax*) in the English Channel varied widely between 30 and 250 parasites per fish (Paling, 1966) but González-Lanza *et al.* (1991) reported the prevalence and mean intensity as 81% and 112, respectively, for *Diplectanum aequans*

on cultivated sea bass in the Mediterranean. A second diplectanid species, *D. laubieri*, occurs on gills of *Dicentrarchus labrax* in the Mediterranean Sea, but the distributions of these monogenean species differ: adult *Diplectanum aequans* occur at the distal tips of gill lamellae, whereas adult *D. laubieri* occur halfway along gill lamellae (Lambert and Maillard, 1975).

Paling (1966) determined that the dorsal and ventral pairs of hamuli in *D. aequans* penetrate gill tissue by gaffing into the bases of the adjacent secondary gill lamellae between which the parasite sits (Fig. 13.11A). Also, the squamodiscs can be moved by extrinsic muscles and the combination of hamuli and spined, paired squamodiscs which also embed into the gill tissue firmly fastens the monogenean in place. *Diplectanum aequans*, however, is capable of relocating its position on the gills by the alternate use of anterior adhesive glands and the haptor. Secretions from posterior glands in the haptor may contribute to the pathology observed in heavily infected sea bass. *Diplectanum aequans* feeds on epithelial tissue from the gills.

The tiny eggs (side length about 60 μm) of *D. aequans* are tetrahedral and there is a single short appendage approximately 40 μm long. Cecchini *et al.* (1998) studied the influence of water temperature on embryonation. At 10°C, embryonation took 11 days and hatching continued for 8 days; at 15°C, embryonation took 7 days and hatching continued for 5 days; at 20°C, embryonation took 4 days and hatching continued for 2 days; at 25°C, embryonation took 3 days and eggs continued to hatch for 3 more days; at 30°C, eggs embryonated in only 2 days and hatching continued for 3 more days. No hatching occurred when *D. aequans* eggs were maintained for 45 days at 5°C. The percentage of hatched eggs ranged from 83 to 89% between 15 and 30°C but decreased to 75% at 10°C. The hatched oncomiracidium of *D. aequans* is ciliated, swims actively and may remain active for up to 30 h at 13–14°C (Whittington, unpublished observation). The oncomiracidia initially contact either the external epithelial surfaces of the host's body (Fig. 13.11) or the lining of the buccal cavity and attach using their 14 hooklets. Migration occurs to the gills when the hamuli and then the squamodiscs develop (Kearn, 1968). Water temperature has a marked effect on time taken to reach full sexual maturity. *Diplectanum aequans* develops to adulthood in 35 days at 15.5°C, 25 days at 20°C, 15 days at 26°C and in only 9 days at 30°C (Cecchini *et al.*, 1998). Assessment of fecundity *in vitro* for *D. aequans* was 6 – 10 eggs/parasite/h (Cecchini *et al.*, 1998). We are unaware of a fecundity measurement *in vivo*, but expect it to be considerably higher.

## Disease Symptoms

Significant mortalities from *D. aequans* can occur among farmed *Dicentrarchus labrax* (see Silan *et al.*, 1996). The widespread prevalence and high intensity of the parasite, even in wild fish, indicates that it is a strong candidate to cause disease in captive fish. FAQ (2008) list the following gross symptoms for infected sea bass: cloudiness of the 'skin'; epithelial hyperplasia; reddening of areas on the gills associated with excess mucus secretion; gill haemorrhages. Pathology caused by *Diplectanum aequans* appears to be mostly the product of its attachment and wounds attributed to its feeding activities are not mentioned. The most detailed study is that by Oliver (1977) who used scanning electron microscopy and histology to examine the detrimental effects of *D. aequans*. At the site where the hamuli impale the host gill tissue (Fig. 13.11A), Oliver (1977) described hyperplastic tissue and haemorrhaging exacerbated by abrasion from the squamodiscs. Removal of attached parasites revealed oval, scar-like depressions in the gill epithelium bearing deep circular wounds caused by the scuffing squamodiscs and penetrating hamuli, respectively. It is unclear from the work of Oliver (1977) whether the pathology he reported was from infections on wild hosts or those in culture. However, using direct observations and histology, González-Lanza *et al.* (1991) demonstrated that under culture conditions, haemorrhagic gill tissue, hypersecretion of mucus, hyperplasia of gill epithelia and a focus of inflammation and bloody lesions occurred where *D. aequans* attached. Leukocytic infiltration associated with eosinophils was detected and some fusion of gill lamellae was reported. Importantly, González-Lanza *et al.* (1991) pointed out that these symptoms were common to *D. aequans* and *D. laubieri* and considered that both species presented pathological problems to intensive sea bass culture in the Mediterranean. Cruz-e-Silva *et al.* (2000) reported the following histopathological symptoms on parasitized gills from freshwater culture (*Dicentrarchus labrax* is a euryhaline species) in Portugal: hyperplasia; hypertrophy of mucous cells; necrosis of epithelia; and secondary bacterial infection (epitheliocystis). Clearly *Diplectanum aequans* is also euryhaline and capable of surviving in freshwater.

Similar symptoms were reported for another diplectanid, *Lamellodiscus major*, from the gills of the sparid, *Acanthopagrus australis*, but its attachment was associated with extensive cellular infiltration, perhaps more indicative of the sedentary habit of this monogenean species

(Roubal, 1989). *Lamellodiscus acanthopagri*, a mobile species from gills of the same host species, elicited no host response.

## Disease Diagnostics

*Diplectanum aequans* is a small monogenean, as indeed are all diplectanids, and their detection is similar to that described above for the similarly tiny Dactylogyridae. For such small parasites, their movements often betray their presence and it is advisable to study gill filaments or entire gill arches newly removed from a freshly killed fish. Scrapings from the gills of a sick host can also be taken gently. Excess mucus, hyperplastic lamellar tissue and presence of haemorrhagic respiratory tissue may indicate infection by *D. aequans* even before the gills of *Dicentrarchus labrax* are searched thoroughly for parasites. The simultaneous presence of a congener, *Diplectanum laubieri*, is possible but the difference in distribution between adults noted above may help to distinguish between them. The symptoms and pathogenicity caused by *D. aequans* and *D. laubieri* are likely so similar that coinfections by both species should be treated as a single disease ('diplectanosis'). *Diplectanum laubieri* was described incompletely by Lambert and Maillard (1974) and for a more detailed description, consult González-Lanza *et al.* (1991). The 2 species differ in size, in the morphology of the squamodiscs and most significantly in the size and shape of the male copulatory organ (Lambert and Maillard, 1974).

## Disease Treatment and Prophylaxis

Stress is considered to be an important issue in disease outbreaks in cultured sea bass and improved husbandry is recommended as an important factor to reduce or limit it (FAO, 2008). As with the treatment of dactylogyrid infections (see above), many different chemical therapies have been tested against *D. aequans*. However, in many European countries, there is a lack of authorized effective therapeutants for most parasite infections (FAO, 2008) and so, effective husbandry techniques are recommended. Husbandry improvements can include good oxygenation of water, control of waste food and faeces to reduce build up of ammonia and low stocking densities (Silan and Maillard, 1986; Papoutsoglou *et al.*, 1996). Here we present a few treatments that have been tested against *D. aequans*, but extreme caution must be exercised due to the possible toxic effects of some of these agents to the host.

A low dose of trichlorphon (= Neguvon®) administered as a bath at 0.2 $mgL^{-1}$ for at least 48 h killed immature and adult stages on a land-based farm (Cognetti-Varriale *et al.*, 1992). Shorter durations (e.g., 24 h) of trichlorphon—even at doses that were toxic to fish—were not as effective at killing the parasites. Silan *et al.* (1996) tested nitroxinil *in vitro* on parasites attached to gill filaments for 4 h at concentrations ranging from 50 to 2000 $mgL^{-1}$. Depending on the dose, this anthelmintic caused loss of mobility and detachment from gill lamellae. Cruz-e-Silva *et al.* (2000) demonstrated that a dilute formalin treatment (concentration: 1: 4000) for 1 h killed swimming oncomiracidia and pre-adult stages of *D. aequans*. They also determined that treatment using 5 $mgL^{-1}$ of trichlorphon for 30 min killed adult specimens of *D. aequans* but this trichlorphon concentration also killed the fish! Monni and Cognetti-Varriale (2001) analysed the influence of oxygenation on the immune system of sea bass against infections by *D. aequans* on a fish farm off Italy. They detected protein fractions from *D. aequans* that stimulated an immune response and demonstrated enhanced antigenic activity and fewer parasites in fish exposed to hyperoxygenation compared with fish reared in normal oxygenation.

In a study to investigate effective treatments against eggs of *D. aequans*, *in vitro* trials examined formalin at 300 and 100 $\mu LL^{-1}$ $h^{-1}$, trichlorphon at 0.2 $mgL^{-1}$ per 48 h and dehydration of eggs for 4 and 8 h (Cecchini and Cognetti-Varriale, 2003). Their results demonstrated that neither formalin nor trichlorphon—the chemicals most commonly used in land-based culture—could completely control embryonic development or egg hatching in *D. aequans*. Dehydration for 8 h, however, completely inhibited egg hatching. Therefore, use of these chemicals would require a second strategic treatment to completely eradicate infections in land-based culture. Cecchini and Cognetti-Varriale (2003) recommended that a more effective and inexpensive method was to dry tanks for at least 8 h after a production cycle. Of course, this method does not apply to sea cage culture.

## Geographical Distribution

*Diplectanum aequans* principally infects *Dicentrarchus labrax* but is also known from the spotted sea bass, *D. punctatus*. It is likely that *Diplectanum aequans* occurs throughout the ranges of both of these species. Most reports of *D. aequans* centre in the Mediterranean Sea but it is also known from the English Channel, eastern Atlantic Ocean and Red Sea.

### *Economic Importance*

Diplectanosis caused by *D. aequans* (and *D. laubieri*) is one of the most significant ectoparasitic diseases in the culture of sea bass, *Dicentrarchus labrax*. The parasite species occurs on wild sea bass throughout its range but only appears to cause pathogenicity when *D. labrax* is cultured, e.g., off the Mediterranean coast (France, Greece, Spain), Atlantic coast (Portugal) and Red Sea (Israel). *Dicentrarchus labrax* was the first non-salmonid species to be cultivated commercially in Europe and even now remains the most important commercially cultured fish species grown in sea cages in the Mediterranean. The largest producers of *D. labrax* are Greece, Turkey, Italy, Spain, Croatia and Egypt (FAO, 2008). We could find no figures to demonstrate the economic importance of *Diplectanum aequans* to sea bass culture, but the number of studies on this monogenean species, and its congener *D. laubieri*, throughout the Mediterranean region in the last 30 years demonstrates their significance (e.g., Lambert and Maillard, 1974, 1975; Oliver, 1977; Silan and Maillard, 1986, 1990; González-Lanza *et al.*, 1991; Cognetti-Varriale *et al.*, 1992; Santos, 1996; Silan *et al.*, 1996; Bouloux *et al.*, 1998; Cecchini *et al.*, 1998; Cruz-e-Silva *et al.*, 2000; Duarte *et al.*, 2000; Monni and Cognetti-Varriale, 2001; Cecchini and Cognetti-Varriale, 2003; Vagianou *et al.*, 2004).

## Gyrodactylidae

### *Gyrodactylus salaris* Malmberg, 1957

### *General Information*

Currently, 409 valid species of *Gyrodactylus* have been described (Harris *et al.*, 2004) but there is an estimated >20,000 species of *Gyrodactylus* worldwide (Bakke *et al.*, 2002)! It is beyond the scope of this text to provide information on all pathogenic *Gyrodactylus* species. Here we present information on perhaps the most infamous species, *G. salaris* (Figs. 13.3D, 13.5, 13.7). This exemplar differs from all other species selected because *G. salaris* affects *wild* freshwater fish in rivers and lakes. 'Gyrodactylosis', the disease caused by *G. salaris*, is the only monogenean disease listed as 'notifiable' by the OIE (World Organization for Animal Health, formerly known as the Office International des Epizooties). As mentioned earlier, *G. salaris* has been singularly responsible for the catastrophic losses of Atlantic salmon, *Salmo salar*, in Norway after the parasite was inadvertently introduced into the country in the 1970s (Mo,

1994). Further details on most *Gyrodactylus* species can be found on the *Gyrodactylus* Database '*GyroDb*' at http://www.gyrodb.net). This is an excellent web-based resource with current information on *Gyrodactylus* that is maintained and updated regularly by specialists in the field. Additional information on other pathogenic *Gyrodactylus* species and treatments can also be found in Buchmann and Bresciani (2006) and references therein.

*Gyrodactylus salaris* predominantly parasitizes the fins of salmonids in freshwater but it can also infect the 'skin', eyes, nose and more rarely the gills. It is pathogenic on Atlantic salmon (Fig. 13.5), but it has also been reported from the following salmonids: rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), North American brook trout (*Salvelinus fontinalis*) and lake trout (*S. namaycush*), arctic char (*S. alpinus*) and grayling (*Thymallus thymallus*; Fig. 13.5D). *Gyrodactylus salaris* is a freshwater parasite and although it can survive for 1 to 2 days at 15–20 ppt, it cannot survive normal seawater (Soleng and Bakke, 1997). Therefore, transfer of *G. salaris* between rivers through brackish fjord systems is possible (Soleng *et al.*, 1998) but introduction of *G. salaris* on fish populations migrating from full strength seawater is unlikely. However predictive models suggest the strong influence of freshwater inflow supports the hypothesis of inter-river dispersal through fjords (Jansen *et al.*, 2007). *Gyrodactylus salaris* specimens are approximately 500 μm long and often appear as tiny white 'whiskers' on heavily infected fish. They can be differentiated from other species in the genus by the morphology of the haptoral sclerites, including the hooklets, ventral bar and hamuli (Malmberg, 1964) but given that there are >400 other *Gyrodactylus* species described, this can be a frustrating and difficult task for specialists and non-specialists alike. More recently, studies have shown that statistical analyses (such as the nearest neighbour and feed analyses) of specific measurements taken from the hooklets, bars and hamuli can accurately discriminate between closely related *Gyrodactylus* species (McHugh *et al.*, 2000; Shinn *et al.*, 2000, 2001, 2004). Molecular markers, including the mitochondrial cytochrome oxidase 1 (CO1) gene (Hansen *et al.*, 2003), the intergenic spacer (IGS) region of the ribosomal RNA gene (Collins and Cunningham, 2000; Cunningham *et al.*, 2003) and the variable region V4 of the 18S small subunit ribosomal RNA and ribosomal RNA internal transcribed spacers (ITS) 1 and 2 (Matejusová *et al.*, 2001), also accurately discriminate between closely related species.

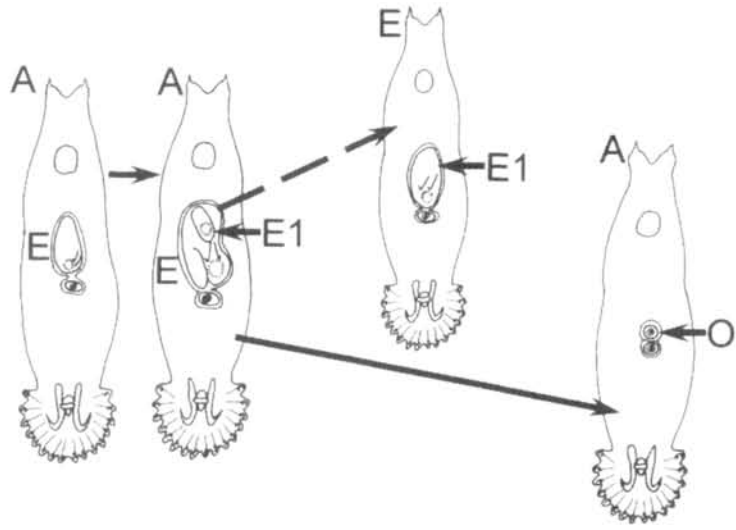


**Fig. 13.12** Diagrammatic representation of viviparity in the Gyrodactylidae (Monopisthocotylea). The embryo (E) inside individual A grows and develops an embryo inside it (E1). Embryo E is born (dashed line) carrying embryo E1 within it, which will undergo further development and may develop its own embryo. After the birth of embryo E, a new oocyte (O) moves into the empty 'uterus' of individual A and will develop into a new embryo. Based loosely on Kearn (1994).

Like all members of the genus, *G. salaris* is differentiated from other monogeneans by its viviparous method of reproduction (Fig. 13.12). Three generations may be represented in 1 worm like 'Russian dolls'; the adult may contain an offspring *in utero*, which is already carrying an embryo itself (Fig. 13.12). Reproduction occurs rapidly and, in some species of *Gyrodactylus*, the first-born daughter can sometimes be born within 24 h of the birth of the parent (Scott, 1982). Thus, it may only take a single individual to start a devastating outbreak and 1 *G. salaris* can become 6 million in just 4 weeks! The formation of embryos may be the result of either sexual, asexual or parthenogenic reproduction (Cable and Harris, 2002). After birth, the offspring attaches to the same fish that hosted the parent. Species of *Gyrodactylus* attach to their host via the 16 hooklets on the haptor which penetrate the host 'skin' (Fig. 13.7). Surprisingly, the sharp pointed hamuli (e.g., Figs. 13.2A, 13.3D, 13.12) do not appear to actively penetrate the 'skin' tissue but instead, they aid attachment by raising the haptor which increases the tension on the hooklets (see Shinn *et al.*, 2003 for further details on the mechanics of attachment). Consult Bakke *et al.* (2007) for a comprehensive review of gyrodactylid biology.

*Gyrodactylus salaris* cannot swim, so infection is usually via direct contact between fish (Fig. 13.5). However, Soleng *et al.* (1999a) have demonstrated that detached drifting individuals of G. *salaris* can infect salmon parr at a relatively high rate (Fig. 13.5B). Detached G. *salaris* (Fig. 13.5C) can survive up to 6 days (Mo, 1987).

### Disease Symptoms

Fish infected with G. *salaris* display increased mucus production and behavioural changes such as flashing and rubbing. White or greyish lesions may appear on the 'skin'. Other symptoms—when fish are closer to death—include fin erosion and secondary fungal infections. Heavy infestations may adversely affect the osmoregulatory ability of the host due to epithelial damage caused by hooklet penetration and result in significant mortality in affected populations.

### Disease Diagnostics

*Gyrodactylus salaris* is very small and, therefore, living parasites are more easily detected by their movements. The whole surface of a freshly killed fish, including the gills and mouth cavity, should be examined thoroughly using a binocular dissecting microscope at high magnification with good illumination from above and preferably a black background below in order to provide a contrast. Monogeneans on the fins of unfixed fish are most easily seen by detaching the fins, placing them in a glass Petri dish of freshwater and examining them using transmitted light. If fish are fixed (preferably in 10% formalin or 70% ethanol), they should be studied in a similar manner. *Gyrodactylus* specimens, which are translucent when alive, turn white when fixed in formalin, while ethanol-preserved specimens are usually only slightly opaque. Care should be taken to examine the sediment in the bottom of the container where fish were fixed for parasites that may have fallen off the fish. Monogeneans should be flattened for morphological analysis as outlined previously, being careful to ensure that the haptor is clearly visible. Gyrodactylid specimens should be fixed and mounted using the ammonium picrate-glycerine method outlined in Malmberg (1957; see also *GyroDb* on the Internet). *Gyrodactylus salaris* is morphologically very similar to G. *teuchis* from brown trout, Atlantic salmon, rainbow trout and North American brook trout, and to G. *thymalli* from grayling. The species can be differentiated

on the basis of the shape of the hooklet sickle. Specimens should also be fixed in undiluted analytical grade ethanol for identification using molecular methods since identification using morphology can be difficult.

### Disease Treatment and Prophylaxis

The introduction of G. *salaris* into Norway led rapidly to the near total extinction of Atlantic salmon in 45 infected rivers. Therefore, drastic control measures have been taken to eliminate outbreaks of G. *salaris* in Norwegian fish farms and in natural waterways. If the pathogen is detected in farms, the facility is closed down, all fish are slaughtered and equipment/tanks are disinfected thoroughly. The farm is reopened only when it is deemed that there are no chances of reinfection. Tank water may also be treated with formaldehyde (Buchmann and Bresciani, 2006). In 2004, none of 34 fish farms examined had G. *salaris* infections.

Rotenone, a non-selective pesticide, has been used to eradicate G. *salaris* in Norwegian rivers. Of the 45 rivers that have been invaded by the monogenean, 15 are now free of G. *salaris* after treatment, 11 have been treated with rotenone and are being monitored and the remainder await treatment. The treated rivers have been restocked from fish eggs and juveniles removed before chemical treatment. This eradication method is extreme because rotenone, which inhibits oxidative phosphorylation, not only kills fish, but also has a large effect on aquatic invertebrates. Furthermore, rotenone treatment is expensive and has failed in some of the larger, more complicated river systems (Poleo *et al.*, 2004). Recently, aluminium and zinc have been shown to have a negative impact on G. *salaris* populations without affecting the fish host or other invertebrates (Soleng *et al.* 1999b, 2005; Poleo *et al.*, 2004). Acidified aluminium treatments are currently being trialed in a number of streams in Norway (Mo, 2004).

Prevention of gyrodactylosis is the most important control measure because G. *salaris* infections are devastating. Most countries have strict guidelines regarding the import of salmonid products. An extensive awareness campaign is active in Scotland, where introduction of G. *salaris* would be catastrophic to their salmon industry, and recreational fishers are urged to decontaminate their fishing equipment after fishing in areas not designated as G. *salaris*-free.

### *Geographical Distribution*

*Gyrodactylus salaris* has been recorded in Norway, Sweden, Finland, Denmark and Germany.

### *Economic Importance*

There is no question that *G. salaris* is the most economically important monogenean species and has earned its notoriety as the single 'notifiable' monogenean by the OIE. It is estimated that *G. salaris* infections have cost the Norwegian Atlantic salmon industry alone more than 480 million € since the pathogen was first introduced 30 years ago. This industry has an estimated annual income of 150 million € and attracts 40,000 tourists each year (Hansen *et al.*, 2004). There is no way to calculate the economic impact of this pathogen in *G. salaris*-free countries where considerable funds are spent annually on monitoring and research and on initiating and sustaining preventative measures.

## Microbothriidae

### *Dermophthirius* species

### *General Information*

There are currently 5 known species of *Dermophthirius* (see Bullard *et al.*, 2004). They infect the 'skin' of carcharinid sharks (Fig. 13.13) and can cause significant husbandry problems in captive animals. *Dermophthirius carcharhinus* and *D. penneri* are not strictly host specific (Bullard *et al.*, 2004) and, therefore, there is potential for host switching if closely related shark species are kept in the same display tank. Species of *Dermophthirius* are distinguished from each other primarily by the morphology of the male copulatory organ (Benz, 1987; Cheung *et al.*, 1988). Adult microbothriids can be distinguished from other monogeneans by the morphology of the haptor which lacks any sclerites (i.e., hooklets and hamuli are absent; Figs. 13.2G, 13.3E). They attach by glueing themselves securely to the placoid scales of host sharks (Figs. 13.2G, 13.9E, 13.13B) using cement secreted by glands opening onto the haptor. Microbothriid attachment causes 'skin' lesions which are likely the result of damage to, or loss of, placoid scales (Bullard *et al.*, 2000b). Unlike almost all other monopisthocotyleans, microbothriids are relatively non-mobile parasites and when they move, they do so slowly, taking perhaps several seconds to detach the haptor when the anterior end is attached firmly. Because they are cemented to

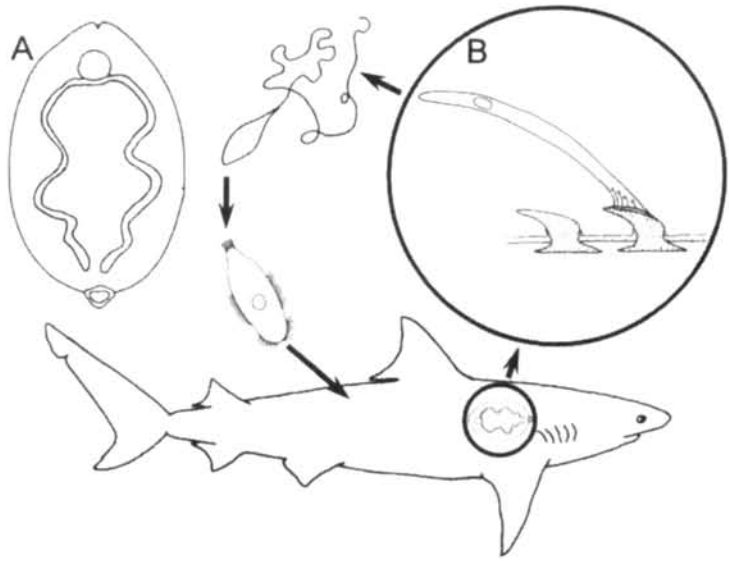


**Fig. 13.13** Life cycle for species of Microbothriidae (Monopisthocotylea), adults (A) of which cement themselves to the surface denticles (B) of their elasmobranch hosts (also see Figs. 13.2G and 13.9E) such as carcharhinid sharks. Based on *Leptocotyle minor* (see Kearn, 1965; Whittington, 1987). The exemplar microbothriids used in the text are *Dermophthirius* species.

their host, they may not be removed readily when exposed to standard treatments such as freshwater dips, formalin or praziquantel baths. Therefore, eliminating microbothriid infections can be very difficult (see below).

No work has been done on egg hatching or larval development and behaviour for species of *Dermophthirius* but these features have been investigated for 2 other microbothriid species. The eggs of *Leptocotyle minor* (Fig. 13.13) from the common dogfish, *Scyliorhinus canicula* (Scyliorhinidae), contained fully embryonated oncomiracidia after 16–18 days when incubated between 13 and 14°C in an LD 12:12 light regime (Whittington, 1987). Eggs remained viable but unhatched even after 88 days and only hatched when eggs were exposed to host 'skin' washings when ciliated oncomiracidia emerged (Whittington, 1987). This strategy ensures that larvae are in close proximity to the host and significantly increases the chances of successful invasion. The strategy also has considerable ramifications for treatment protocols. If a shark is removed from a display tank for treatment, the unhatched eggs remaining in the tank may survive for long periods and hatch later when the shark is

reintroduced into the display tank. Eggs of *Pseudoleptobothrium aptychotremae*, from the southern fiddler ray, *Trygonorrhina fasciata* (Rhinobatidae), were fully embryonated after 8 days at 22°C when exposed to a LD 12:12 light regime and some spontaneous hatching does occur in the last 3 h of the illumination period. Other eggs in the same batches do not hatch spontaneously, indicating perhaps that another hatching cue is required. Mechanical disturbance does not stimulate hatching in *P. aptychotremae* and host 'skin' secretions do not appear to induce hatching but may have been administered at an inappropriate 'concentration'. The ciliated oncomiracidia survived for up to 4 h after hatching (Glennon *et al.*, 2006). Some eggs of *P. aptychotremae* may remain viable for up to 120 days after being laid (Glennon, Chisholm and Whittington, unpublished data). Low hatching success during these experiments suggests that a currently unidentified cue (or cues) may trigger hatching in some eggs. Most importantly, however, eggs of the 2 microbothriid species studied thus far appear to remain viable for prolonged periods suggesting that hatching by some embryonated eggs awaits a host induced cue.

### Disease Symptoms

Sharks infected with microbothriids usually first display erratic swimming behaviour and flashing (Cheung *et al.*, 1982). Subsequently, they develop white or grey patches on the 'skin' and around the mouth (accompanied by excessive mucus production) which can further develop into ulcerated 'skin' lesions (Cheung *et al.*, 1982; Cheung and Ruggieri, 1983; Poynton *et al.*, 1997). These lesions are susceptible to secondary bacterial or viral infections and in heavy infections, can result in fish death (Cheung *et al.*, 1982; Cheung and Ruggieri, 1983). Grimes *et al.* (1985) suggested that *D. nigrellii* may act as a vector for *Vibrio carchariae*. Bullard *et al.* (2000b) found no evidence of secondary bacterial infections in lesions caused by *D. penneri* on wild caught black-tip reef sharks, *Carcharhinus limbatus*, and they suggested that microbothriids may only be pathogenic when large parasite populations proliferate on confined fish.

### Disease Diagnostics

Microbothriids can be very difficult to detect and often appear only as small white flecks on the body surface. Therefore, the first indication of infection may be behavioural changes or discoloured patches on the 'skin' or around the mouth of the shark. Microbothriids can also be found by

lifting the fish slightly out of the water and examining the 'skin' with the aid of a torch while pouring water over the surface. The parasites will appear as small raised shiny bumps on the 'skin' (Fig. 13.9E) and can be carefully removed by scalpel blade and fine paintbrush (see *Removal of Live Monogenea from Live Hosts* above). A scalpel is far preferable because it is easy to damage firmly attached parasites using forceps. Specimens should be fixed and mounted following protocols outlined earlier (see *Monogenea Fixation and Processing* above).

### Disease Treatment and Prophylaxis

Clearly, the presence of resident, unhatched microbothriid eggs in a display tank poses the most significant problem for control. Therefore, the importance of appropriate quarantine measures cannot be stressed enough. Due to their cement (Figs. 13.2G, 13.13B), attached microbothriids are difficult to treat because they are rarely removed from the host easily. Short-term, high-concentration treatments of praziquantel (i.e., a 2 h treatment of 20 $mgL^{-1}$) are generally ineffective because if the parasites do not fall off the host, they will likely recover when the shark is returned to seawater (Chisholm and Whittington, 2002). Freshwater dips are extremely stressful to elasmobranchs and a 15-min treatment had no effect on *Dermophthirius* infections on *Negaprion brevirostris* (see Cheung *et al.*, 1982). Bullard *et al.* (2004) noted that *D. maccallumi* infects the bull shark, *C. leucas*, which is a coastal marine species that can penetrate into freshwater rivers and lakes. While Bullard *et al.* (2004) hypothesized that *D. maccullumi* could not withstand long periods in freshwater, they suggest that this microbothriid species would likely survive a standard freshwater dip treatment. Two formalin dips (15 min at 200 $mgL^{-1}$) within 1 week effectively removed a *Dermophthirius* sp. (since identified as *D. brevirostris*) from *N. brevirostris* but this treatment was extremely stressful to sharks which exhibited excessive mucus production and needed additional aeration during the treatments (Cheung *et al.*, 1982). Reduction of tank water temperature to 19°C lowered the incidence of flashing in lemon sharks but did not eliminate the parasites (Cheung *et al.*, 1982). Cheung *et al.* (1982) found that continuous (1 week to 10 days) treatments of 0.5 ppm Dylox® (trichlorphon) removed all *D. brevirostris* from lemon sharks and symptoms did not redevelop up to 1 year after treatment.

Poynton *et al.* (1997) tried various treatments to remove *Neodermophthirius harkemai* from *Negaprion brevirostris* with different results to those found by Cheung *et al.* (1982). Poynton *et al.* (1997) effectively cleaned the sharks using a single 30-min freshwater dip but the fish were quickly reinfected when returned to the display tank. An *in situ* weekly dose of 0.5 ppm trichlorphon for 3 weeks removed the microbothriids but lesions did not heal (Poynton *et al.*, 1997). This suggests that the trichlorphon did not kill unhatched eggs in the display tank and that perhaps eggs were not resident in the tank treated in the experiments of Cheung *et al.* (1982). Poynton *et al.* (1997) successfully treated isolated sharks with copper sulphate (0.25 ppm for 85 days) and the 'skin' lesions healed but 2 months after the sharks were re-introduced into the display tank, the infections returned. Oral (ranging between 3 and 19 $mgkg^{-1}$ BW daily for 3–8 days) and intramuscular (dosages not given) praziquantel therapies were administered with no success (Poynton *et al.*, 1997).

Consecutive treatments timed to break the life cycle (i.e., after sharks are infected but before new infections mature to reach egg-laying adults) may be the only way to eliminate infections completely once *Dermophthirius* infections are established in a display tank. This requires knowledge of egg embryonation and parasite development times, which are currently unavailable for members of this genus. Egg embryonation times listed above for other microbothriid species and parasite development times recorded for monocotylids (see *Dendromonocotyle* section below) which may be closely related to microbothriids (Olson and Littlewood, 2002; Perkins, Chisholm, Donnellan and Whittington, unpublished data) should provide a useful starting point for design of treatment protocols.

## Geographical Distribution

Species of *Dermophthirius* have been recorded from numerous localities worldwide including the northwestern Atlantic and eastern Atlantic (off Senegal), Costa Rica, Nicaragua, the Gulf of México and the Indian Ocean (off Christmas Island and off South Africa) (Bullard *et al.*, 2004). There are many reports of microbothriids in various public aquaria in the United States (Bullard *et al.*, 2004) and undoubtedly outbreaks of *Dermophthirius* have occurred, but reports have not been published, in other public aquaria worldwide because their carcharhinid hosts are impressive and popular display animals.

The following genera of Microbothriidae are known on sharks from around the world (e.g., *Asthenocotyle*; *Dermophthirius*; *Dermophthiroides*; *Leptobothrium*; *Leptocotyle*; *Microbothrium*; *Neodermophthirius*; *Pseudoleptobothrium*) and this family is poorly studied and understood. It seems likely to us that more disease reports involving species in some of these genera may arise in the future in the aquarium industry.

### *Economic Importance*

The cost of a single elasmobranch purchased from an aquarium supply company can range between 5,000 and 20,000 US dollars, depending on the size and species of animal. These amounts include the cost of the fish, packing and freight. Therefore, the expense to replace an animal that dies from monogenean infections or due to complications from secondary infections or chemical treatments can be significant. Disease treatment can also be costly because chemicals such as praziquantel can be expensive. Hidden costs may also be experienced if the animal must be removed from the display tank and placed into quarantine for treatment. Elasmobranchs, especially large sharks, are generally considerable draw cards for visitors to an aquarium. Lack of imposing animals on display for a long period may result in a reduction in visitor numbers.

## Monocotylidae

### *Dendromonocotyle* species

### *General Information*

*Dendromonocotyle* currently comprises 13 described species and, presently, they are the only monocotylids known to parasitize the 'skin' of stingrays (see Chisholm *et al.*, 2004, 2005; Chisholm and Whittington, 2005 and references therein). They infect the dorsal surfaces of dasyatid (Fig. 13.14) and myliobatid rays and there have been an increasing number of reports (mostly unpublished) of *Dendromonocotyle* outbreaks on rays kept in public aquaria (Janse and Borgsteede, 2003; Chisholm and Whittington, 2004; Chisholm *et al.* 2004, 2005). Characters used to identify *Dendromonocotyle* and a key to species are given in Chisholm *et al.* (2004). The haptor of *Dendromonocotyle* is divided into 1 central and 8 peripheral loculi by septa which are armed with small sclerites (Fig. 13.1A). Muscular papillae, also armed with small sclerites, extend into the marginal valve of

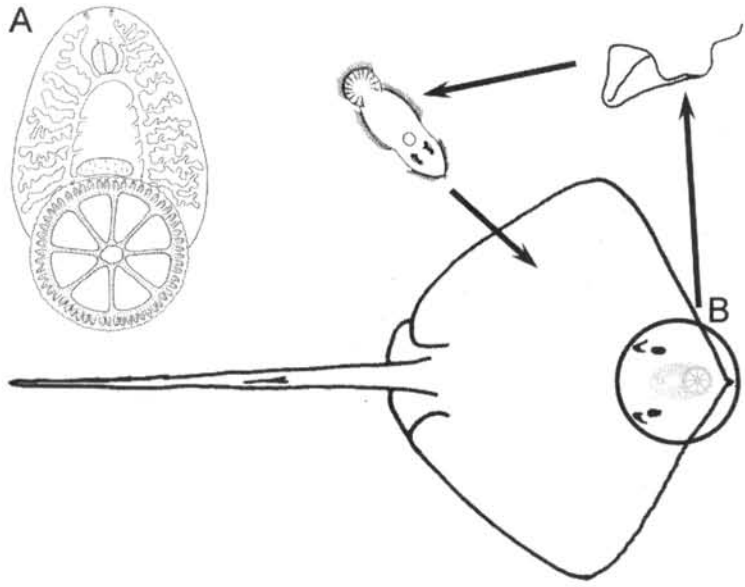


**Fig. 13.14** Life cycle for species of *Dendromonocotyle* (Monopisthocotylea: Monocotylidae), adults (A) of which live on the dorsal 'skin' surfaces of batoids, especially around the rostrum (B) (see also Fig. 13.9F).

the haptor (Fig. 13.1A). The male copulatory organ of *Dendromonocotyle* species is an important diagnostic character. It is generally long and, in juveniles, it is coiled so it can be accommodated in the small body. As the parasite grows the male copulatory organ straightens and, therefore, it is important to use *adult* specimens for species identification. The dorsal attachment site, morphology of the haptor and the highly branched intestine distinguishes *Dendromonocotyle* from other monogeneans infecting rays. *Dendromonocotyle* species are almost exclusively found on the dorsal 'skin' surfaces of their ray hosts but a few small juvenile specimens of *D. lasti* were recorded from the gills of *Himantura* sp. in Moreton Bay, Queensland, Australia (Chisholm and Whittington, 2005). Monogenea on the ventral 'skin' surfaces of rays are most likely species of *Neoentobdella* or *Benedeniella* (Capsalidae) which can be clearly distinguished (see Kearn and Whittington, 2005 and Hendrix, 1994, respectively) from *Dendromonocotyle* by their large haptoral sclerites (accessory sclerites and 2 pairs of ventral hamuli) and lack of septa.

*Dendromonocotyle* is most easily confused with another closely related monocotylid, the monotypic sister taxon *Clemacotyle*. Previously, specimens of *Dendromonocotyle* have been misidentified as *C. australis* (see

Janse and Borgsteede, 2003). *Clemacotyle australis* lives in the gill cavity (not the 'skin') of the white-spotted eagle ray, *Aetobatus narinari* (Myliobatidae), and can be readily recognized by the presence of additional dorsomarginal projections on the haptor, an unsclerotized male copulatory organ and by the unpigmented gut (Young, 1967; Beverley-Burton and Whittington, 1995). *Dendromonocotyle* species incorporate host pigment into the dendritic gut when feeding on the pigmented dorsal 'skin' surface of its host ray (Kearn, 1979). This pigment camouflages the parasite (Fig. 13.9F) and low-intensity infections can be inadvertently overlooked. *Dendromonocotyle* species are generally strictly host-specific but *D. octodiscus* is recorded from 4 closely related dasyatid species (Chisholm *et al.*, 2004).

Egg embryonation, hatching, oncomiracidial behaviour and morphology and adult biology and development have been investigated for several *Dendromonocotyle* species (e.g., Kearn, 1979, 1986; Chisholm and Whittington, 1995, 2004, 2005; Chisholm *et al.*, 2001, 2004). *Dendromonocotyle* species lay tetrahedral eggs (Figs. 13.6C, 13.14) and based on *in vitro* experiments, free-swimming ciliated oncomiracidia (Figs. 13.1B, 13.6E, 13.14) begin to hatch spontaneously after approximately 5 days at 25°C (Young, 1967; Chisholm and Whittington, 1995, 2005). A single batch of eggs laid over 24 h may continue to hatch over a period of 5–7 days. Kearn (1986) noted that *D. kuhlii* eggs may require mechanical disturbance for hatching to occur but recent experiments showed that *D. kuhlii* eggs also hatch spontaneously without mechanical disturbance (Chisholm and Whittington, unpublished data). Longevity of *Dendromonocotyle* oncomiracidia is not known but other monocotylid larvae can survive up to 55 h at 25°C (Chisholm and Whittington, 2003). Most *Dendromonocotyle* larvae are likely to attach directly to the dorsal 'skin' surface of the host (Fig. 13.14), but there is indirect evidence that some larvae of *D. lasti* may first attach to the gills and live there for a short period before migrating to the dorsal 'skin' surface. Alternatively, migration may take place from the ventral to the dorsal surface via the gill chamber (Chisholm and Whittington, 2005). There is currently no information on the time it takes newly attached *Dendromonocotyle* oncomiracidia to develop into fully sexually mature adults but monocotylids from the shovelnose ray, *Rhinobatos typus* (Rhinobatidae), take a minimum of 20 days (*Neoheterocotyle rhinobatidis* from the gills) or 22 days (*Merizocotyle icopae* from the nasal tissue) at 25°C to reach full sexual maturity after host invasion (Chisholm and Whittington, 2003).

## Disease Symptoms

Rays infected with *Dendromonocotyle* species often display changes in behaviour such as erratic swimming, flashing, holding pectoral fins, tail or pelvic fins at unusual angles while swimming and may have an increased respiratory rate. Excess mucus production and discoloured (Fig. 13.9F) or haemorrhagic lesions on the dorsal surface, especially on or around the rostrum or between the eyes, are characteristic in high-intensity infections.

### *Disease Diagnostics*

As mentioned above, gut pigment can camouflage *Dendromonocotyle* specimens and make low-level infections difficult to detect. However, *Dendromonocotyle* specimens often congregate on the rostrum and between the eyes of the ray (Fig. 13.9F) and, therefore, 'skin' scrapings should be taken specifically from these areas and examined microscopically in case infection is suspected. It is advisable to inspect 'skin' scrapings immediately because it is easier to locate live, active parasites among samples of dark dorsal mucus. Alternatively, monogeneans can also be found by lifting the ray slightly out of the water and examining the dorsal surface for short periods with the aid of light from a torch beam while pouring water over the surface of the fish to keep it moist. Parasites will appear as raised shiny 'blisters' on the 'skin' (Fig. 13.9F) and they can then be removed *carefully* with scalpel blade or forceps for identification. Specimens should be mounted on microscope slides following protocols outlined earlier (see *Monogenea Fixation and Processing*).

### *Disease Treatment and Prophylaxis*

Low-level *Dendromoncotyle* species infections can be difficult to detect and therefore rays should be quarantined for at least 1 month, screened for eggs and treated, if necessary, for parasite infections before they are introduced into a display tank. Freshwater dips and formalin baths have been used successfully to remove 'skin' monogeneans from rays, but elasmobranchs are exceptionally sensitive to these treatments and extreme care should be taken if used. Two 5-min dips in 24°C freshwater (14 days apart) removed all *Dendromonocotyle centrourae* from the 'skin' of *Dasyatis centroura* (see Cheung and Whitaker, 1993). The parasites turned opaque within 30 s

and were removed manually during the dips. During treatment, the rays were kept in a quarantine pool with temperature (22°) and salinity (23 ppt) reduced. At low concentrations, praziquantel poses few side effects to elasmobranchs and a single treatment of 20 $mgL^{-1}$ praziquantel for 90 min to 2 h has been shown to be effective in removing monogeneans from the 'skin' of rays (Thoney, 1990; Janse and Borgsteede, 2003). Janse and Borgsteede (2003) noted that a praziquantel bath concentration of 25 $mgL^{-1}$ caused significant stress to *Aetobatus narinari* (Myliobatidae) and they could only be kept in the bath for 45 min. High concentration-short duration praziquantel treatments have been shown to be less effective in removing smaller developmental stages of monogeneans infecting the gills of elasmobranchs (Chisholm and Whittington, 2003). Since it is documented that a few juvenile *Dendromonocotyle* may reside temporarily on ray gills (Chisholm and Whittington, 2005), long duration-low concentration treatments (i.e., 2, 40-h treatments, 48 h apart, using 5 $mgL^{-1}$ praziquantel dissolved in seawater; following Chisholm and Whittington, 2003) should be administered to ensure all gill and 'skin' Monogenea are removed. Janse and Borgsteede (2003) investigated the efficacy of oral praziquantel treatments on *Dendromonocotyle* (likely *D. torosa* but misidentified as *Clemacotyle australis*) from the dorsal surface of *A. narinari* in Burgers' Zoo, Arnhem, Netherlands. A single oral praziquantel dose ranging between 10 $mgkg^{-1}$ BW and 40 $mgkg^{-1}$ BW was delivered to the rays in tablet form in food items ensuring that the food was not regurgitated. The treatment was unsuccessful but this is not surprising because it is unlikely that a single oral dose of praziquantel would provide the necessary levels of the drug to the host's epidermal tissues to effect these epithelial-feeding parasites. Chisholm *et al.* (2004) studied the removal of *D. pipinna* on captive juvenile blotched fantail rays, *Taeniura meyeni* (Dasyatidae), kept in an 18,760 L shallow interactive display pool at the public aquarium, Underwater World, in Mooloolaba, Queensland, Australia. Initially rays were treated successfully in formalin baths (20 ml of 37% formalin in 100 L seawater for 30–60 min; repeated for 14 to 20 days if necessary) and/or by dosing the entire display pool with Lepidex® (=500 $gL^{-1}$ trichlorphon; 1 ml Lepidex® to 2,000 L seawater = 9.38 ml to 18,760 L). However, treated rays rapidly became reinfected by larvae hatching from *D. pipinna* eggs remaining in the display tank and eventually, after repeated treatments, a number of heavily infected rays died. Autopsies revealed significant damage to the liver and kidneys and the

deaths were attributed to the chemical treatments and not to *D. pipinna* infections. Aquarium staff noted that infections were present on the juvenile rays year round, but only became problematic in summer months when water temperatures exceeded 25°C. When action was taken to minimize stress to hosts (i.e., all chemical treatments terminated; water temperature reduced; restriction of staff/public interactions with rays in display pools on hot days), the population explosions of *D. pipinna* on the 'skin' of rays no longer occurred. All rays maintained low-level *D. pipinna* infections but they were healthy and the need to eliminate all monogeneans was deemed unnecessary.

## Geographical Distribution

The distribution of *Dendromoncotyle* species is widespread and the hosts they infect often have circumglobal distributions. Species of *Dendromonocotyle* have been described from wild dasyatid and myliobatid rays caught off the east and west coasts of North America, in the Bahamas, off the east, west and south coasts of Australia, in the Sea of Japan, off Senegal, Africa and off the coast of Israel (Chisholm and Whittington, 2004). *Dendromonocotyle* has also been recorded in public aquaria worldwide and with the continuing expansion of the public aquarium trade, the potential for disease spread is high in this industry. For example, a *Dendromonocotyle* species was found on a specimen of *Himantura uarnak* (Dasyatidae) in a public aquarium in Sweden. When asked to identify the parasite, we discovered that the ray was collected in Australia, sent to an aquarium in Holland and subsequently transported to Sweden. Therefore, it is difficult to ascertain whether the parasite originated in Australia or was acquired in Holland or Sweden. Although species of *Dendomonocotyle* appear to be strictly host specific, *D. octodiscus* can infect 4 host species (Chisholm and Whittington, 2004) and aquarists should be mindful that there may be potential for host switching by this species onto closely related ray species held in the same display tanks.

## Economic Importance

The expenses associated with treating or replacing elasmobranchs infected by monogeneans in public display aquaria were discussed in the previous section dealing with microbothriids.

# POLYOPISTHOCOTYLEA

## Diclidophoridae

### *General Information*

There are approximately 40 genera and 120 described species in the Diclidophoridae, a family of polyopisthocotyleans, most of which have a symmetrical haptor bearing no more than 4 clamp pairs. *Polycliphora* species are exceptional because there are many clamp pairs. In some genera, the clamps are on the end of flexible peduncles (e.g., *Diclidophora*; *Neoheterobothrium*, Fig. 13.3G) but in others the clamps are termed sessile and are located directly in the haptor tissues (e.g., *Heterobothrium*). Like most Polyopisthocotylea, the majority of Diclidophoridae species use their clamps to grab secondary gill lamellae and lie with the longitudinal axis of their body parallel with a primary gill lamella. Some Diclidophoridae species, however, attach to the flat surfaces of the gill arches (e.g., *Choricotyle* species), or migrate from the respiratory surfaces and embed their haptor in gill arch tissue (e.g., *Heterobothrium elongatum*) or in musculature inside the branchial or buccal cavities (e.g., *H. okamatoi* and *Neoheterobothrium hirame*, respectively). Attachment by species of Diclidophoridae that grasp secondary gill lamellae is believed to be benign to their hosts, but species that embed their haptor into tissues can elicit a significant response, even in wild hosts (e.g., *H. elongatum* on *Torquigener pleurogramma* [Tetraodontidae]; see Williams and Lethbridge, 1990). *Heterobothrium ecuadori* causes pathology to gill tissue of *Sphoeroides annulatus* (Tetraodontidae) in experimental culture in México (Flores-Crespo and Flores-Crespo, 2003). At least 2 species of Diclidophoridae are pathogenic when their hosts are farmed in Japanese aquaculture: *H. okamatoi* from the tiger puffer, *Takifugu rupripes* (Tetraodontidae) and *N. hirame* (considered to be *N. affine* by Hayward, 2005) from the olive flounder, *Paralichthys olivaceus* (Paralichthyidae). Ogawa (2002) provides an overview of the effects of these 2 Diclidophoridae species on cultured fish in Japan. There has been an explosion of publications about many aspects of their biology, pathology and the host responses they stimulate. We provide detailed information about *N. hirame* because its description, importance, role in the decline of *P. olivaceus* populations and possible spread from Japan has taken place relatively recently (Hayward *et al.*, 2001b; Ogawa, 2002).

### *Neoheterobothrium hirame* Ogawa, 1999 [possibly synonymous with *N. affine* (Linton, 1898) Price, 1943]

A *Neoheterobothrium* species was first reported at low prevalence on *P. olivaceus* caught in the Sea of Japan in 1993 and was named *N. hirame* (Fig. 13.3G) by Ogawa (1999). It is only reported from *P. olivaceus*. Adult parasites are large with a mean length of 21 mm but can reach >30 mm long. They possess 4 pairs of pedunculate clamps and the haptor is separated from the body by a slim isthmus-like peduncle up to 7 mm long (Fig. 13.3G). The uterus of *N. hirame* is narrow and contains a few eggs which are not connected together and are laid singly (Ogawa, 1999), unlike those of *H. okamotoi* which form long egg chains >2 m long and can entangle on the mesh of sea cages holding infected tiger puffers (Ogawa and Yokoyama, 1998). Immature specimens of *N. hirame* live on the gill filaments, gill arches and gill rakers and it seems that oncomiracidia first settle on the gill lamellae and later migrate via the arches and rakers to the wall of the buccal cavity where they mature to adults. Since the first report of *N. hirame* in 1995, this diclidophorid has been confirmed from wild stocks, broodstock kept in tanks and fish maintained in floating sea cages around the Japanese coast.

At 33 ppt salinity and water temperatures of 10, 15, 20 and 25°C, eggs of *N. hirame* hatched after approximately 24, 13, 7 and 5 days, respectively. At these temperatures, >85% of eggs embryonated successfully whereas at 30°C, hatching success was <5%. Similarly, high hatching success was achieved when eggs were exposed to hyposaline water (one-third and two-thirds of full strength seawater). In hypersaline water (1.5 times full strength seawater), embryonation time increased and hatching success declined. No hatching occurred when eggs were incubated in distilled water and double-strength seawater (Yoshinaga *et al.*, 2000a). The oncomiracidia that emerge from the eggs are ciliated, possess no eyespots and other than the observation that larvae sink in still water but swim freely in agitated water, there have been no studies on their swimming biology or longevity. Ogawa (2002) reported results from experimental infections of *P. olivaceus* by oncomiracidia to determine the progress of infection at different water temperatures. At 15°C, parasites remained on gill filaments until 38 days post-infection, on gill arches and rakers until 59 days post-infection and parasites first appeared on the buccal wall 52 days post-infection. At 20 and 25°C, parasites were first recorded on the buccal wall 31 and 24 days post-infection, respectively. Specimens of *N. hirame*

reached full sexual maturity at 15, 20 and 25°C at 59, 38 and 31 days, respectively. Parasite fecundity *in vivo* was 203, 578, 781 and 651 eggs/parasite/24 h at 10, 15, 20 and 25°C, respectively and studies on egg morphology indicated that 25°C may be near the upper limit for normal egg laying (Tsutsumi *et al.*, 2002). Ogawa (2002) reported that the longevity of *N. hirame* was 122, 66 and 52 days, respectively, at 15, 20 and 25°C.

In the western Sea of Japan, young flounder become infected around June each year and within 2 months, the prevalence and intensity of infection increases dramatically and is associated with a sharp decrease in the juvenile flounder population (Anshary *et al.*, 2001; Ogawa, 2002). Predictably, low water temperature limits the population growth of *N. hirame* on its host (Shirakashi *et al.*, 2005).

### Disease Symptoms

Effects of *N. hirame* on infected flounders can be observed at 2 levels and may relate directly to the attachment and feeding of the parasites. Infection by parasites on respiratory gill surfaces and gill rakers is not associated with any pathology. Attachment by *N. hirame* to the buccal cavity wall, however, is associated with necrosis of the dermis and underlying musculature when the haptor becomes deeply embedded as host tissue surrounds it and much of the haptoral peduncle. Histological studies demonstrate that only mild inflammation occurs on gill lamellae, gill arches and gill rakers, whereas there is a strong infiltration of inflammatory cells and fibroblasts around the embedded clamps. In advanced stages of attachment after prolonged parasitism, the clamps are no longer able to grasp connective or muscle tissue and necrotic and detached cells occur around the haptor (Anshary and Ogawa, 2001). *Paralichthys olivaceus* mounts an immune response against *N. hirame* because different types of leukocytes (granulocytes and monocytes) and cells with large electron-dense granules have been identified in the peripheral blood of infected fish using electron microscopy (Nakayasu *et al.*, 2003). Vacuolation of the surface of parasites that were embedded in the buccal cavity wall may have been associated with these leukocytes. Lymphocyte infiltration, however, was not conspicuous, suggesting to Nakayasu *et al.* (2003) that a non-specific immune response mediated by the granulocytes, monocytes and cells with electron-dense granules is dominant, but they did not exclude involvement of specific antibodies and lymphocytes.

The effect on the host from the blood-feeding activities of *N. hirame* has been contentious. Hayward *et al.* (2001b) considered that the lack of a perfect correlation between the presence of the parasite and host anaemia indicated co-occurrence of a second pathogen, perhaps a viral infection (see also Ogawa, 2002). Yoshinaga *et al.* (2001a, b) and Nakayasu *et al.* (2002), however, have demonstrated that high prevalences of *N. hirame* and the haematological characteristics of *P. olivaceus* are related. Indeed, when *P. olivaceus* was deliberately infected by *N. hirame*, anaemia similar to that experienced in infected wild and cultivated flounder populations around Japan was induced and when the monogenean infections were experimentally removed, the haematological characteristics of fish returned to normal levels (Yoshinaga *et al.*, 2001b). Nakayasu *et al.* (2002) induced anaemia experimentally in *P. olivaceus* by withdrawing blood repeatedly to mimic blood feeding by *N. hirame*. They also determined that 8 weeks were needed for anaemic fish to return to a healthy condition and show normal haematological values.

Infected *P. olivaceus* show reduced appetite, pale gills, low haematocrit levels and low haemoglobin concentrations. Pathology associated with attachment by adult parasites should be obvious but is unlikely to result in overt external symptoms until the buccal cavity wall is examined. Hosts suspected of infection can also be screened for *N. hirame* eggs (see *Screening for Eggs* above).

## Disease Diagnostics

The length of the adults and the blood diet of *N. hirame* means that infections of Japanese flounder by this monogenean can be determined by inspection of the buccal cavity wall and gills. Large clusters of up to 40 long dark parasites embedded in the buccal cavity wall form conspicuous tangled masses (Anshary and Ogawa, 2001) but may be overlooked if only the gills are inspected. The respiratory surfaces of the gills may be pale due to anaemia. Adult *N. hirame* on the buccal cavity wall are best removed using a scalpel blade to dig into the host tissue where the haptor of the parasites is embedded. The size and robustness of these large polyopisthocotyleans means that they can also be removed gently using fine forceps (see below) but these same attributes will challenge even the most meticulous workers to prepare quality, whole mounted specimens. Eggs of *N. hirame* are laid singly and are fusiform, bearing a long straight

appendage at either end. The *Disease Treatment and Prophylaxis* section below indicates the difficulty in removing adult *N. hirame* using chemicals but Anshary *et al.* (2001) described a novel and rapid method to collect immature specimens from gill filaments of preserved and separated gill arches using a magnetic stirrer spinning in a beaker at approximately 1200 rpm for 30 min.

There is some question about whether *N. hirame* is actually distinct from another *Neoheterobothrium* species, *N. affine*, which infects summer flounder, *Paralichthys dentatus* (Paralichthyidae), in the U.S.A. (Hayward *et al.*, 2001b). According to Ogawa (1999), *N. hirame* is similar to *N. affine*, but can be distinguished from it by its body shape. Most known *Neoheterobothrium* species parasitize *Paralichthys* flatfish. Ogawa (1999) should be consulted for a detailed description of *N. hirame* but the comments of Hayward *et al.* (2001b) regarding its possible synonymy with *N. affine* should be noted.

## Disease Treatment and Prophylaxis

The severity of the host tissue response stimulated by the haptor of adults presents significant difficulties for the removal of *N. hirame*. Yoshinaga *et al.* (2001b) commented that manual removal using forceps was required for adults. Immature stages attached to gill lamellae, arches and rakers can be removed by bathing infected fish for 60 min in seawater supplemented with sodium chloride (i.e., 1 L of seawater with 30 g of NaCl dissolved in it; Yoshinaga *et al.*, 2000b) in which the fish do not suffer adversely.

Eggs of *N. hirame* fail to hatch when incubated only in distilled water and double-strength seawater (see above). However, exposure of eggs for 15 min to seawater supplemented with various concentrations of sodium hypochlorite solution (available chlorine concentration <50 ppm), had minimal impact on hatching success. Even a 15-min exposure to seawater supplemented with an available chlorine concentration of 100 ppm killed few *N. hirame* eggs. These findings are disturbing because a routine method to treat wastewater from fish culture facilities in Japan is chlorine treatment at 5 to 50 ppm for 10 min before neutralization for return to the sea (Yoshinaga *et al.*, 2000a). This study demonstrates how resistant the eggs of some Monogenea species can be to chemical treatments.

## Geographical Distribution

*Neoheterobothrium hirame* infections on *P. olivaceus* are confirmed from all areas off Japan where the host occurs except from northern and eastern Hokkaido (Ogawa, 2002). The swift spread widely around Japan has been attributed to the transport of broodstock and release of 'offspring' (= fertilized eggs or 'seeds') nationally. Between 1989 and 1999, regular surveys of 0+ *P. olivaceus* specimens in the Sea of Japan first detected *N. hirame* in 1993 at a prevalence of 3.1% but, by 1998, the prevalence was 89%. While seasonal prevalence of any monogenean species may be expected to fluctuate annually, this striking increase may indicate that the natural population of *P. olivaceus* had little or no innate resistance to infection by *N. hirame* (i.e., they may have been naïve). Furthermore, the appearance of *N. hirame* on *P. olivaceus* off South Korea could be attributed to a recent and continuing extension westwards across the Tsushima and Korea Straits from Japan on wild flounder, although shipments of live fish for aquaculture cannot be discounted (Hayward *et al.*, 2001b). These events prompted Hayward *et al.* (2001b) to propose 2 alternate hypotheses for the sudden appearance of *N. hirame* in Japan on *P. olivaceus*. (1) The monogenean species switched from a different, less well-known flatfish teleost species in Japanese waters to *P. olivaceus*. (2) *N. hirame* may be synonymous with *N. affine*, the latter species being introduced into Japan via shipments of *P. dentatus* from the USA which occur regularly for the Japanese sashimi market. If *N. hirame* and *N. affine* are the same, this diclidophorid is, so far, only known to be pathogenic around Japan on *P. olivaceus*.

## Economic Importance

In 1999, the annual wild catch of *P. olivaceus* in Japan was nearly 7,200 tons. National and local associations throughout coastal Japan have released up to 26 million 'seeds' to enhance wild stocks. The culture of *P. olivaceus* is mainly in land-based systems which produced approximately 7,200 tons in 1999 (Ogawa, 2002). In Korea, *P. olivaceus* is a valuable species with >25,000 tonnes cultured in 1997, a volume double that of the black rockfish, *Sebastes schlegeli* (Sebastidae). In Korea, flounder and rockfish are the top 2 most farmed fish species (Hayward *et al.*, 2001b). *Paralichthys olivaceus* is revered in NE Asia as sashimi. The sudden appearance and apparent spread of *N. hirame* around Japan has impacted seriously on commercial fisheries and on the culture of flounders as

evidenced by the number of recent studies on this monogenean species. *Neoheterobothrium hirame* may threaten industries in Korean waters.

For concise information about the impact of another diclidophorid monogenean, *Heterobothrium okamatoi* on tiger puffer, *Takifugu rubripes* (Tetraodontidae), including images of the histopathology caused by its attachment, consult Ogawa (2002) and references within. Ogawa *et al.* (2005a, b) evaluated blood feeding and egg laying *in vivo* (mean daily output range, 51.2-362 eggs/parasite), respectively. Ohhashi *et al.* (2007) investigated host recognition by larvae and post-larval survivorship in *H. okamotoi* on congeneric and 'alien' host species.

## Heteraxinidae

### *Zeuxapta seriolae* (Meserve, 1938) Price, 1962

#### *General Information*

The Heteraxinidae is a family of marine polyopisthocotyleans containing approximately 20 genera and about 45 described species that mostly parasitize the gills of Carangidae (jacks, pompanos and trevallies). Therefore, its hosts include amberjack, kingfish, yellowtail and other *Seriola* species. Two species of Heteraxinidae have proved pathogenic to farmed stocks of carangids: *Heteraxine heterocerca* from *S. quinqueradiata* and *S. dumerili* and *Zeuxapta seriolae* from *S. dumerili* and *S. lalandi*. Severe infections by *H. heterocerca* on cultivated stocks of *S. quinqueradiata* in Japanese aquaculture have been reported since the 1960s (Egusa, 1983). Here, however, we present information on *Z. seriolae* because there have been increasing reports about its effects on *Seriola* species from pilot culture projects or from farms in the Spanish Mediterranean, New Zealand and Australia. The parasitosis caused by *Z. seriolae* has been termed 'zeuxaptosis' (Grau *et al.*, 2003). Aquaculture of *Seriola* species is expanding in Australia, Chile, Ecuador, USA (Hawaii), México and New Zealand and it is likely that problems caused by *Z. seriolae* may continue in the foreseeable future. The close relationship between *Heteraxine* and *Zeuxapta* is further revealed by their similar effects on captive carangids.

*Zeuxapta seriolae* parasitizes the respiratory gill surfaces (i.e., gill lamellae) of amberjack (*S. dumerili*), yellowtail kingfish (*S. lalandi*) and Samson fish (*S. hippos*) in the wild and *S. dumerili* and *S. lalandi* in aquaculture. It is possible that *Z. seriolae* may infect other *Seriola* species in the wild such as *S. rivoliana* (almaco jack). Adult parasites have a total resting (i.e., unextended) length from 4 to 10 mm. The haptor is

asymmetrical (Figs. 13.3H, 13.4B) bearing 2 clamp rows (Fig. 13.1C) of unequal length: 1 side has approximately 38–44 clamps (the major row); the other side has approximately 27–32 clamps (the minor row). Forty-five wild *S. lalandi* caught by recreational anglers off the east coast of North Island, New Zealand had a mean of 50 *Z. seriolae* per fish (Sharp *et al.*, 2001). Off southern and eastern Australia, prevalence and intensity of *Z. seriolae* on wild *S. lalandi* was 38% and 83% and 6 and 32, respectively (Hutson *et al.*, 2007a).

Escalating problems in *Seriola* species aquaculture have promoted several recent studies into basic aspects of the biology of *Z. seriolae* (Fig. 13.4B). The most detailed published account so far has studied the egg embryonation time and parasite age at full sexual maturity across a range of temperatures (13–21°C), reflecting water temperatures experienced by wild *S. lalandi* in New Zealand (Tubbs *et al.*, 2005). Embryonation period and age at full sexual maturity were inversely related to water temperature. At 13, 17.5 and 21±1°C, peak egg hatching took place 15, 9 and 7 days, respectively, after laying and oncomiracidia continued to hatch for 2 days. Mean hatching success was significantly lower at 13°C than at 17.5 or 21°C. At 13, 18 and 21°C, *Z. seriolae* reached full sexual maturity on *S. lalandi* at >52, 37 and 25 days, respectively. Mean fecundity *in vitro* was 218 eggs/parasite/24 h at 13°C, 309 eggs/parasite/24 h at 17.5°C and 207 eggs/parasite/24 h at 21°C (Tubbs *et al.*, 2005). But, when assessed *in vivo*, fecundity was found to be approximately 571 eggs/parasite/24 h at 18.5°C and 40 ppt salinity (Mooney *et al.*, 2006). Eggs of *Z. seriolae* are laid singly but accumulate inside the extensive uterus of the parasite, probably over a 24-h period, after which they are expelled, sometimes in groups of >1000 eggs, as a continuous string, the separate eggs being bound together by their filamentous strands (Grau *et al.*, 2003; Mooney *et al.*, 2006) (Figs. 13.4B, 13.6B). After laying, egg strings can reach a length of 1,200 to 1,800 mm (Grau *et al.*, 2003). An egg-laying rhythm in *Z. seriolae* *in vivo* is well defined (under LD12:12, 18±0.5°C, 40 ppt salinity) when between 57.4% and 81.8% of all eggs are laid during the first 3 h period after 'dusk' (Mooney *et al.*, 2006). Grau *et al.* (2003) stated that severe zeuxaptosis in culture is likely to be promoted by huge numbers of eggs tangling on netting enclosures (Fig.13.4C).

Adult *Z. seriolae* attach to gill lamellae by their haptoral clamps (Figs. 13.1C, 13.3H, 13.4B). Histological studies of attached parasites indicate that individual clamps grasp 1 or 2 secondary gill lamellae (Montero *et al.*, 2004; Mansell *et al.*, 2005). Differences in pathology as a result of

attachment by adults have been noted between different host *Seriola* species (Grau *et al.*, 2003; Montero *et al.*, 2004; Mansell *et al.*, 2005), which is emphasized below. Some evidence of gill pathology possibly attributed to feeding was noted by Mansell *et al.* (2005). Epizootics of *Z. seriolae* are more likely over summer months when higher ambient seawater temperatures cause the rate of the life cycle to accelerate (less time needed for egg embryonation, parasite development and achievement of full sexual maturity).

### Disease Symptoms

Sharp *et al.* (2001) noted that levels of *Z. seriolae* on captive *S. lalandi* in New Zealand rapidly attained epizootic levels. Signs that characterize infection include pronounced gilling, appetite loss, poor growth, darkening of the 'skin' and colour loss to gills and internal organs (kidney, liver and muscles). Heavy parasite burdens (Fig. 13.8D) caused excess mucus production from gills, hyperplasia of gill epithelium and mechanical gill damage which contributed to osmoregulatory and respiratory dysfunction (Sharp *et al.*, 2001). Similar symptoms were reported by Grau *et al.* (2003) in an epizootic by *Z. seriolae* on *S. dumerili* in culture in the western Mediterranean Sea. Gross internal examination indicated colour loss to all tissues, coelomic cavity lining and mesenteries; yellow discolouration to perivisceral fat; enlargement of the gall bladder perhaps symptomatic of anaemia, jaundice and starvation. Gills were pale (Fig. 13.8D). Grau *et al.* (2003) noted that the 0+ age class of infected *S. dumerili* (BW: 400–500 g) presented no gross external clinical symptoms even though there was a 0.8–1.5% daily mortality rate, which resulted in a final mortality rate of 50% after 8 weeks. Destruction of gill tissue, occasional fusion of gill lamellae and mechanical blockage of interlamellar spaces by egg strings and parasite stages were proposed by Grau *et al.* (2003) to reduce or prevent water flow across the gills and, therefore, effect host respiration. Coupled with anaemia, these issues may have precipitated the mass mortalities of *S. dumerili* observed. These symptoms were apparently independent of infection by epitheliocystis because this bacterium was not reported. Similar symptoms of pale spleen and liver indicative of anaemia and white zones on gill filaments of *S. dumerili* where *Z. seriolae* were attached were reported by Montero *et al.* (2004) in a pilot culture project in Spain. Although parasite intensities up to 731 individuals per fish were recorded, there was no relationship between parasite abundance and fish condition factor. Haematocrit values were significantly lower in the

infected fish and there was a strong, significant negative relationship between Z. *seriolae* abundance and haematocrit values. Clamp attachment led to lamellal fusion and clubbing with apparent epithelial and vascular disruption, but no proliferative cell response occurred. It should be noted that these symptoms presented by Montero *et al.* (2004) were recorded during a dual infection by epitheliocystis and Z. *seriolae*. Loss of equilibrium in infected fish was noted by Sharp *et al.* (2004).

Mansell *et al.* (2005) followed the progress of infection over 8 weeks by Z. *seriolae* on gills of S. *lalandi* in South Australia. Little pathology associated with parasite attachment was noticed and hyperplastic lamellae noted at 6 weeks may have been due to feeding. It seems, therefore, that there is a strong contrast in host response to parasite attachment between S. *dumerili* and S. *lalandi* (compare Grau *et al.*, 2003 and Montero *et al.*, 2004 with Mansell *et al.*, 2005). This may indicate that Z. *seriolae* is better adapted to S. *lalandi* and perhaps only acquired S. *dumerili* as a host relatively recently. Infection intensity on S. *lalandi* peaked 4 weeks post-infection (mean intensity approximately 560 parasites/fish), after which absolute numbers decreased indicative of an immune response. Mean haemoglobin concentration was inversely related to Z. *seriolae* burdens suggesting that these monogeneans cause host anaemia but a delay noticed in this relationship was attributed to larger parasites exerting increased feeding pressure (i.e., consuming more blood) on the fish (Mansell *et al.*, 2005). Comparison of weight and length between uninfected and infected S. *lalandi* implied that parasite infection exerts a significant metabolic cost on the host. All results from the study by Mansell *et al.* (2005) must, however, be viewed within the context of a concurrent infection by epitheliocystis.

## Disease Diagnostics

Infections by Monogenea on gill tissue are more difficult to monitor and often less obvious than those by parasites on the external surfaces of fish. Adult Z. *seriolae* are large, highly noticeable parasites. Gentle lifting of the opercula to inspect the respiratory surfaces of the gills is likely to reveal large numbers of worms attached to gill filaments (Fig. 13.8D). Gills excised from a freshly killed fish should be examined using a binocular dissecting microscope with strong incident illumination. Fine needles or very careful manipulation using forceps can dislodge attached monogeneans, which should then be flattened and prepared for morphological study. The arrangement and number of attachment clamps,

egg morphology and details of the reproductive system are important characters. Currently, *Z. seriolae* is the only species in *Zeuxapta* but can be distinguished from other family members by a combination of its double vaginal ducts, mid-dorsal vaginal pores, unarmed genital atrium and eggs tethered together in bunches by long filament-like appendages that entwine together like braid (Figs. 13.4B, 13.6B). In heavy infections, egg strings of *Z. seriolae* can tangle on host gills (Grau *et al.*, 2003; Montero *et al.*, 2004), but it is unknown whether eggs may remain entangled until they hatch. Parasite specimens preserved in high-grade ethanol will also permit comparison of nucleotide sequence data with information lodged in GenBank®.

## Disease Treatment and Prophylaxis

Only three experimental studies are published on treating *Z. seriolae* infections. Sharp *et al.* (2004) determined that bathing infected fish in seawater containing a low praziquantel dose (2.5 ppm) for 24 or 48 h or in formalin at 400 ppm for 1 h followed by a 5-min freshwater bath were >99% effective at removing *Z. seriolae* from *S. lalandi*, but the parasites were not killed and continued to lay viable eggs. Hatching of *Z. seriolae* eggs could only be prevented if exposed to air for >5 h before egg embryonation reached the development of eyespots. It was concluded that dual treatments would be required should either of these bathing methods be adopted: the initial treatment removes adult parasites and a second strategic treatment is needed to remove immature parasites hatching from eggs left in the system. The timing of the second treatment must accommodate an appropriate period after all resident eggs have hatched but before maturation of the next cohort of parasites.

Experiments to investigate aspects of the biology of *Z. seriolae* have used a bath of 5 $mgL^{-1}$ praziquantel for 10 min to remove all parasites (Mansell *et al.*, 2005), although subsequent on-going studies have suggested that longer exposure to a higher concentration may be required in order to remove all stages of the parasite. The South Australian *S. lalandi* industry uses hydrogen peroxide baths as the treatment of choice for *Z. seriolae* and *B. seriolae* infections because it is effective and has no food safety issues. Mansell *et al.* (2005) used a bath of 300 $mgL^{-1}$ (= 300 ppm) hydrogen peroxide for 10 min to assess the effect of this treatment on the pathophysiology of *S. lalandi*. This treatment effectively removed all *Z. seriolae* but had acute effects on fish health. The size (increase in lactate and osmolality) and extent (no effect on haemoglobin

concentration) of the pathophysiological effects led Mansell *et al.* (2005) to conclude that hydrogen peroxide treatment is clearly beneficial because in its absence, severe Z. *seriolae* infections could promote anaemia and death. Acute effects from hydrogen peroxide bathing, therefore, are less harmful to S. *lalandi* that the effects of persistent infection by Z. *seriolae*.

A study on efficacy of orally administered praziquantel surface-coated onto feed pellets determined that S. *lalandi* fed lower daily doses for 6 days (50 and 75 $mgkg^{-1}$ BW $day^{-1}$) had fewer Z. *seriolae* than fish fed higher daily doses for 3 days (100 and 150 $mgkg^{-1}$ BW $day^{-1}$; Williams *et al.*, 2007). This study noted rejection of medicated pellets by fish indicating palatability problems. When similar praziquantel doses were administered by intubation, there were fewer Z. *seriolae* on gills of medicated compared with control fish. Intubated praziquantel was also effective against new Z. *seriolae* recruits. Therefore oral treatment of praziquantel against Z. *seriolae* could be developed further if suspected palatability problems can be resolved (Williams *et al.*, 2007), but the drug's variable concentration in host plasma from bioavailability and pharmokinetic studies by Tubbs and Tingle (2006a, b) are a cause for concern.

Because of the growth of *Seriola* species aquaculture globally, it is expected there will be further research to develop more effective methods to control Z. *seriolae*. Other avenues to follow include ways to reduce parasite fecundity and strategies to kill eggs or affect their viability.

## Geographical Distribution

*Zeuxapta seriolae* is known widely throughout the eastern and western Pacific Ocean (Galapagos Islands, eastern Australia, New Zealand) and in the Mediterranean Sea. It is very likely to occur throughout the range of *Seriola* species known to be hosts such as S. *dumerili* and S. *lalandi*. It remains to be determined whether Z. *seriolae* infects S. *rivoliana* but it is reported from wild S. *hippos* off the east coast of Australia (Rohde, 1981; Hutson *et al.*, 2007b).

## Economic Importance

In Japan, Z. *seriolae* together with its relative *Heteraxine heterocerca*, have proved to be persistent gill flukes problematic to various carangid species in sea cage culture. Specifically, Z. *seriolae* has caused losses of S. *dumerili* and S. *lalandi* in Japan where 150,000 tonnes of *Seriola* species are produced annually (Mansell *et al.*, 2005). In Japan, there is one report that

>US$230 million is spent annually on medications against all diseases effecting *Seriola* species culture (Sharp *et al.*, 2001). Losses are also known in the expanding sea cage culture of *S. lalandi* in South Australia (Ernst *et al.* 2002) where in 2003–03, the annual harvest was 1,500 tonnes and it is estimated that >5,000 tonnes will be produced by 2005–06. Grau *et al.* (2003) reported mass mortalities of *S. dumerili* in a sea cage farm off the Balearic Islands (western Mediterranean Sea) and Montero *et al.* (2004) reported similar problems to the same host species in a pilot culture project in Murcia, Spain. No clear indication has been provided of the economic burden caused by *Z. seriolae* infections in Australian, New Zealand and Spanish fish culture, but individual farm operations and cooperatives spend significant amounts for allocation to a variety of resources such as personnel (for regular monitoring, reactive bathing and chemical treatments) and to fund research to try to solve the problem. It should be noted that in the Spanish pilot project and in South Australia, a concurrent infection by epitheliocystis (bacteria) might have contributed synergistically to the effects of *Z. seriolae*.

F or the impact of a close relative of *Z. seriolae*, namely *Heteraxine heterocerca*, on the Japanese *Seriola* species industry, see Egusa (1983) and Ogawa and Yokoyama (1998).

## OUTLOOK

We began this chapter stating that Monogenea are *common* ectoparasitic flatworms of fish. The enormous diversity of Monogenea assures that wherever there are fish, there will be monogenean parasites. It is also certain that countless more Monogenea species will be encountered. Monogeneans occur naturally on healthy, wild fish populations. We assume that because most *wild*-caught fish have relatively low prevalences and intensities of Monogenea that the parasites are relatively harmless to their *wild* hosts. Whittington (2005) pointed out that a 'natural' population of 300 to 500 specimens of *Neoheterocotyle rhinobatidis* (Monopisthocotylea: Monocotylidae) commonly encountered on gills of *Rhinobatos typus* (Rhinobatidiae) does not appear to effect host health. We have a significant amount to learn about the natural population dynamics of Monogenea and their interactions with their host fish in the wild. This aspect deserves special attention in the future.

What *is* obvious, however, are the effects Monogenea can have on *captive* fishes which, due to confinement, may be more susceptible to these mostly host-specific parasites with a direct life cycle and incredible

structural, strategic and behavioural adaptations to parasitism (Figs. 13.4, 13.5, 13.11–13.14). Fish populations can succumb to severe infections by Monogenea in a small fish bowl or aquarium tank at home, in large internationally acclaimed public display aquaria and in aquaculture industries around the world. For fish grown in sea cages in coastal waters globally, the problems from monogeneans can be especially relentless because the initial infection reservoir is usually *wild* fish of the same or similar species that are themselves parasitized by Monogenea (Paperna, 1987). Prevention of infections by Monogenea in an open system, therefore, is virtually impossible with our current level of knowledge. There are valuable lessons to learn from other aquatic parasites such as sea lice and their associated disease problems in salmonid aquaculture in the Northern Hemisphere. Initial epizootics of *Lepeophtheirus salmonis* (Copepoda: Caligidae) occurred during the 1960s, shortly after salmonid sea cage culture began in Norway and again in the 1970s in Scotland. *Caligus elongatus* (Copepoda: Caligidae) epizootics effected salmon farming in North America in the 1980s. These epizootics stimulated expensive reactive research into chemicals to control the copepods and studies to investigate parasite biology waned. A range of issues including environmental pressures, parasite resistance and public concerns motivated restrictions on chemical use in salmon farming, therefore compelling industry to develop a *balanced* approach to parasite control via integrated management strategies (Pike and Wadsworth, 1999; Ernst *et al.*, 2002). Integrated management strategies demand intimate knowledge of the pests. For sea lice, extensive data about their biology, ecology and epidemiology resulted from numerous studies, the results of which are now applied to sea lice control. In any battle, there is no substitute for a thorough understanding of the weaponry, tactics and strategies of the enemy. In this chapter, we have emphasized throughout the need to understand *fundamental* features of the biology of Monogenea in meticulous detail so that we are appropriately armed to wage war with those species that promote problems to captive fish.

Can the battle be won? In reality, the kinds of disease problems that face the husbandry of fish confined in aquaria and aquaculture are similar to the kinds of disease problems that faced us when we began farming terrestrial livestock (e.g., sheep, cattle, chickens) hundreds of years ago. Pathogens, including flatworm parasites, continue to present problems to sheep, cattle and chicken farming, but we are far better informed about how to reduce disease, alleviate suffering and we can monitor and react to

epizootics because of a solid foundation of knowledge. Chemical intervention is available, but because of the development of resistance, environmental and health concerns and regulatory control, more responsible and sensitive integrated solutions are necessary. For aquaculture, whether the pathogens to be tackled are viruses, bacteria, protists, copepods or flatworms such as Monogenea, the integrated pest management approach offers much and is the ultimate and responsible goal for long-term industry growth, sustainability and environmental sensitivity. It is naïve to imagine that a single chemical, whether natural or man-made, can be identified and developed to the point where it can be administered to fish in culture to reduce infections by Monogenea. It *is* possible, however, to imagine that a chemical treatment or treatments may be available for use as a *last* resort to control an epizootic of Monogenea if all other integrated management practices fail (e.g., standard husbandry procedures; stocking density; net fouling; feeding; regular parasite monitoring; fallowing; separation of age classes; arrangement of cages on leases). Because of the scale and value of salmonid farming in Europe, *Gyrodactylus salaris* (Monopisthocotylea: Gyrodactylidae) is *the* most significant monogenean species worldwide and is the *only* notifiable Monogenea species listed by the OIE. The devastation and impact *G. salaris* has had on salmon has stimulated significant research and has resulted in a large volume of information about this pathogen which is unsurpassed for any other species of monogenean. Furthermore, the exceptional and atypical method of its reproduction (viviparity; Figs. 13.5, 13.12) means that features of the biology of *G. salaris* and its association with its hosts may not be justifiably applied to, or assumed for, most other Monogenea.

For oviparous Monogenea, what are the key and core pieces of information that we need to understand how to control them? Some areas we have drawn attention to throughout this chapter include: enhanced knowledge about specific and important life cycle parameters (e.g., egg embryonation, larval longevity, time to full sexual maturity, fecundity, longevity of adults, generation time); attachment and associated pathology; adhesives; regularity of feeding and 'volume' of what they eat; the effects of Monogenea on their hosts at 'natural' population levels; hatching cues; resistance of eggs to treatments; the interplay between host stress and infection. Harris *et al.* (2000) have demonstrated experimentally that immunosuppressed salmon are susceptible to infection by *G. salaris* and we expect this to be broadly applicable across other fish-Monogenea

associations. Chisholm *et al.* (2004) noted that dasyatid rays that were less stressed coped better with small infections of monocotylid monogeneans that those that were stressed. Nowhere in this chapter have we focused in detail on 'host switching', i.e., the apparent ability of some parasites to switch from their normal host species to a different host species. Apparent host-switching events, however, have been noted, e.g., devastations caused to sturgeons and salmonids by Monogenea in the Aral Sea and Norway, respectively; *Pseudodactylogyrus* (Monopisthocotylea: Pseudodactylogyridae) infections on European and North American eel; possible switching of hosts in aquaria by *Dermophthirius* species (Monopisthocotylea: Microbothriidae) and *Dendromonocotyle* species (Monopisthocotylea: Monocotylidae); perhaps *Neoheterobothrium affine* (Polyopisthocotylea: Diclidophoridae) switched from *Paralichthys dentatus* to *P. olivaceus* (Paralichthyidae) in Japan. The ability of Monogenea to switch hosts may be strongly related to fish immunity. Clearly, growth in the field of fish immunology will shed considerable light on the association between Monogenea and their fish hosts as well as contributing significantly to potential vaccine research.

Much basic information about Monogenea can be derived from models. Fish species that are easy to keep in captivity offer considerable scope to study important interactions between their parasites and the host. Models are amenable to experimentation and can provide valuable data about parasite parameters *in vivo* such as generation time, time to reach full sexual maturity, fecundity, longevity as well as specific information about egg embryonation, hatching biology, larval behaviour and invasion dynamics. Chigasaki *et al.* (2000) developed a method using a fluorescent stain to follow experimental infections of fish by oncomiracidia (also see Ohhashi *et al.*, 2007). Their method promises to be valuable when applied to other parasite-host associations (e.g., in elasmobranchs by Glennon *et al.*, 2007) to learn more about invasion and any subsequent migrations to the definitive site of the adults. Factors used by oncomiracidia to identify their specific host species have been analysed recently using advanced techniques to extract chemicals from fish tissues (Yoshinaga *et al.*, 2000c, 2002; Ohashi *et al.*, 2007a) and the pH of fish mucus is also considered important (Hirazawa *et al.*, 2003). The identification of a small ciliary surface glycoprotein on oncomiracidia of *Neobenedenia girellae* (Monopisthocotylea: Capsalidae) that can induce an immune response in the host (Hatanaka *et al.*, 2005) is further evidence of how fundamentally critical the early invasive biology of monogenean larvae is. Oncomiracidia

hatch from eggs and the resistance of monogenean eggs, like those of many parasites, is renowned. Considerable research funds have been spent to develop drugs and chemicals to target and effect egg laying and fecundity of parasites in livestock but with varying degrees of success. Another potential line of attack against Monogenea is to better understand egg production and egg structure. Kearn *et al.* (1999) studied the structure of the eggshell of a monogenean, *Entobdella soleae* (Monopisthocotylea: Capsalidae) and specifically the way the detachable lid (= operculum) bonds to the rest of the eggshell. The way this monogenean species manufactures eggs has also been studied in detail (Kearn, 1985; Tappenden and Kearn, 1999a, b). It is possible that more studies of this kind will help pinpoint methods to attack egg production, egg laying or the eggs themselves as an extra method in the arsenal against Monogenea. Ohashi *et al.* (2007c) have identified genes involved in gametogenesis in *N. girellae*. Other studies have focused on understanding the role and physiology of enzymes such as serine proteases in *N. girellae* (see Hirazawa *et al.*, 2006) and coding regions for, and expression of, cysteine proteases in *N. melleni* (see Rao and Yang, 2007). This type of research seeks to identify candidate target molecules for anti-monogenean drugs.

Under several of the *Disease Treatment and Prophylaxis* sections for specific problematic Monogenea species (see above), the fact that some current bathing and chemical treatments have proved uneconomic and stressful to fish has been emphasized. An effective oral treatment by incorporation of appropriate dosages of an active chemical into medicated feed pellets is a preferable method to bathing because it needs no additional labour or infrastructure and requires no handling or crowding of fish which are known stressors. Difficulties in developing efficient and successful oral treatments, however, may pertain to the dietary differences of the 2 monogenean subclasses. Polyopisthocotylea feed on blood and several compounds (mebendazole, bithionol, praziquantel, and caprylic acid) fed orally have proven effective against *Microcotyle sebastis* (Polyopisthocotylea: Microcotylidae). It seems likely that any active chemical is likely to find its way into host blood far faster after ingestion than it will into peripheral tissues such as the epithelium of the 'skin'. Therefore, while some orally administered compounds may well prove to be effective against Polyopisthocotylea, it remains to be determined how effective these compounds will be against the epithelial feeding Monopisthocotylea. In addition to continual efforts to improve nutrition of captive fish via dietary advances of feed pellets, other compounds worth

exploring include various vitamin supplements and immunostimulants to strengthen fish immunity. All these compounds, however, will require appropriate approvals, monitoring and legislation for use. Improved knowledge of fish genetics and future potential to select fish stocks that may be resistant to some parasites is an emerging possibility.

Mindful of the fact that helminth parasites still remain a problem in terrestrial livestock farming, an instant and enduring solution to Monogenea infections on captive fish is highly unlikely. There are, however, several avenues to follow, some of which have been emphasized above. The search for chemical intervention will undoubtedly continue, whether as baths or as in feed therapeutants. An important issue to consider and practice, however, is the *rotation* of effective treatments to reduce the chances of monogeneans developing resistance to chemicals. We have stressed throughout this chapter the importance of assessing the biology of Monogenea *in vivo*. In future, however, with an enhanced comprehension of the biology and nutritional needs of different Monogenea species, the ability to maintain monogenean parasites under *in vitro* culture may considerably extend the range of studies to develop potent chemicals against important pathogenic species. Another fruitful line of attack could be to determine the mechanism of adhesion by the anterior adhesive secretions of Monopisthocotylea. Once we know how adhesion and detachment in these flatworms operates, we are better placed to try to interfere with their attachment to hosts, the benefits of which are obvious. Finally, an advanced knowledge and understanding of something as fundamental as *how* the oncomiracidia of Monogenea recognize their specific host and what their core metabolic requirements are, may help to develop larval traps to lure infective oncomiracidia away from biomasses of captive fish. Whatever the future holds, integration of the most successful outcomes of many multidisciplinary research projects is the *key* to improved and efficient management of Monogenea on captive fishes.

## RECENT DEVELOPMENTS

There is sufficient information available to realize the impact that anthropogenic effects have had on the distributions of some Monogenea. We must learn lessons from our past actions so that similarly devastating events do not recur. Anthropogenic effects, however, can be used in our favour to try to control Monogenea and here, a heightened understanding

of fish immunology, vaccines, drug development and drug targets should all help. However, beneficial recent developments do not all hinge on chemical intervention. The growth in the number of simple and advanced techniques to learn more about monogenean biology escalates every year. This is a strong signal that many innovative research scientists are keen to understand the *fundamental* biology of these fascinating parasites. Continued work on monogenean biology using living specimens on live hosts will reveal much of value. Contemporary molecular methods using advanced genetics and nucleotide sequence data from nuclear and mitochondrial genomes of Monogenea are widely applied for phylogenetic studies and to distinguish between species that are difficult to differentiate using more traditional methods. Classical methods to identify species remain invaluable, but advanced statistical techniques and image recognition software can supplement taxonomy to help separate and identify parasite species.

Electronic communication, the ease with which data can be sent and the shared resources available over the Internet means that there is a wealth of relevant, up-to-date information readily available. Nucleotide sequence data is available in GenBank®. There is an extensive resource on Gyrodactylidae on the Internet at *GyroDb* ('a home for gyrodactylids on the web'; http://www.gyrodb.net/), including information about their biology, host records, and images of haptoral sclerites and even video footage. We anticipate that this innovation could be the first of other similar web sites about different, important groups of Monogenea. *FishBase* (Froese and Pauly, 2007) is another valuable resource on the internet ('a global information system on fishes'; http://www.fishbase.org/) that integrates a wealth of information about all known fish species. Eventually, *FishBase* aims to incorporate information about diseases and aquaculture for relevant fish species, further enhancing its appeal.

We have accentuated the need to take a holistic approach to manage infections by Monogenea on captive fish. This has been well exemplified by the salmon industry in their control of sea lice in the Northern Hemisphere and serves as a useful model to follow. We have discussed much about the individual treatments and control strategies that have been trialled against specific monogenean pathogens. Unless the 'big picture' approach is taken to assimilate all aspects of parasite biology, ecology and epidemiology as well as host stress, nutrition, improved husbandry in all its guises, solutions to the problems will only be partly

effective. Rae (2002) highlights this fact in a review of past and present methods to control sea lice. Integration of methods to minimize the impacts of Monogenea on fish in confinement should heed the messages in Rae (2002). For sea cage farming in particular, the study by Chambers and Ernst (2005) to investigate egg dispersal of *Benedenia seriolae* (Monopisthocotylea: Capsalidae) in South Australian culture of *Seriola lalandi* (Carangidae) by tidal currents has taken the first step to identify 'independent management units'. Failure to view monogenean infections in sea cages at whole farm and inter-farm levels, rather than within a single sea cage, means there can be few prospects to maximize opportunities at minimizing the impacts of monogeneans.

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## References

Andersen, P.S. and K. Buchmann. 1998. Temperature dependent population growth of *Gyrodactylus derjavini* on rainbow trout, *Oncorhynchus mykiss*. *Journal of Helminthology* 72: 9-14.

Anderson, J.I.W. and D.A. Conroy. 1968. The significance of disease in preliminary attempts to raise flatfish and salmonids in sea water. *Bulletin Office Internationales Epizootie* 69: 1129-1137.

Anshary, H. and K. Ogawa. 2001. Microhabitats and mode of attachment of *Neoheterobothrium hirame*, a monogenean parasite of Japanese flounder. *Fish Pathology* 36: 21-26.

Anshary, H., K. Ogawa, M. Higuchi and T. Fujii. 2001. A study of long-term changes in summer infection levels of Japanese flounder *Paralichthys olivaceus* with the monogenean *Neoheterobothrium hirame* in the central Sea of Japan, with an application of a new technique for collecting small parasites from the gill filaments. *Fish Pathology* 36: 27-32.

Arndt, R.E. and E.J. Wagner. 1997. The toxicity of hydrogen peroxide to rainbow trout *Oncorhynchus mykiss* and cutthroat trout *Oncorhynchus clarki* fry and fingerlings. *Journal of the World Aquaculture Society* 28: 150-157.

Bakke, T.A., P.A. Jansen and C.R. Kennedy. 1991. The host specificity of *Gyrodactylus salaris* Malmberg (Platyhelminthes, Monogenea): susceptibility of *Oncorhynchus mykiss* (Walbaum) under experimental conditions. *Journal of Fish Biology* 39: 45-57.

Bakke, T.A., P.D. Harris, P.A. Jansen and L.P. Hansen. 1992. Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms* 13: 63-74.

Bakke, T.A., P.D. Harris and J. Cable. 2002. Host specificity dynamics: observations on gyrodactylid monogeneans. *International Journal for Parasitology* 32: 281-308.

Bakke, T.A., J. Cable and P.D. Harris. 2007. The biology of gyrodactylid monogeneans: the "Russian-Doll killers". *Advances in Parasitology* 64: 161-377.

Beverley-Burton, M. and I.D. Whittington. 1995. *Clemacotyle australis* (Monogenea: Monocotylidae) from the white-spotted eagle ray *Aetobatus narinari* (Rajiformes: Myliobatidae) on the Great Barrier Reef: redescription, emended generic diagnosis, and oncomiracidium. *Journal of Parasitology* 81: 616-625.

Benz, G.W. 1987. *Dermophthirius penneri* sp. n. (Monogenea: Microbothriidae) and ectoparasite of carcharhinid sharks, *Carcharhinus brevipinna* and *Carcharhinus limbatus*. *Proceedings of the Helminthological Society of Washington* 54: 185-190.

Benz, G.W and S.A. Bullard. 2004. Metazoan parasites and associates of chondrichthyans with emphasis on taxa harmful to captive hosts. In: *Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and Their Relatives*, M. Smith, D. Warmolts, D. Thoney and R. Hueter (eds.). Ohio Biological Survey, Ohio, U.S.A., pp. 325-415.

Berland, B. 2005. *Whole Mounts*. Kolej Universiti Sains dan Teknologi Malaysia, Malaysia.

Boeger, W.A. and D.C. Kritsky. 2001. Phylogenetic relationships of the Monogenoidea. In: *Interrelationships of the Plathyhelminthes*, D.T.J. Littlewood and R.A. Bray (eds.). Taylor and Francis, London, pp. 92-102.

Bondad-Reantaso, M.G., K. Ogawa, T. Yoshinaga and H. Wakabayashi. 1995a. Acquired protection against *Neobenedenia girellae* in Japanese flounder. *Fish Pathology* 30: 233-238.

Bondad-Reantaso, M.G., K. Ogawa, M. Fukudome and H. Wakabayashi. 1995b. Reproduction and growth of *Neobenedenia girellae* (Monogenea: Capsalidae), a skin parasite of cultured marine fishes of Japan. *Fish Pathology* 30: 227-231.

Bouloux, C., M. Langlais and P. Silan. 1998. A marine host-parasite model with direct biological cycle and age structure. *Ecological Modelling* 107: 73-86.

Buchmann, K. 1987. The effects of praziquantel on the monogenean gill parasite *Pseudodactylogyrus bini*. *Acta Veterinaria Scandinavica* 28: 447-450.

Buchmann, K. 1993a. A note on the humoral immune response of infected *Anguilla anguilla* against the gill monogenean *Pseudodactylogyrus bini*. *Fish and Shellfish Immunology* 3: 397-399.

Buchmann, K. 1993b. Screening of 22 anthelmintics for their efficacy against the gill-parasitizing monogeneans *Pseudodactylogyrus* spp. from the European eel (*Anguilla anguilla*). *Bulletin of the Scandinavian Society for Parasitology* 3: 11-19.

Buchmann, K. 1999. Immune mechanisms in fish against monogeneans – a model. *Folia Parasitologica* 46: 1-9.

Buchmann, K. and S. Mellergaard. 1988. Histochemical demonstration of the inhibitory effect of Nuvan® and Neguvon® on cholinesterase activity in *Pseudodactylogyrus anguillae* (Monogenea). *Acta Veterinaria Scandinavica* 29: 51-55.

Buchmann, K. and J. Bjerregaard. 1990. Mebendazole treatment of pseudodactylogyrosis in an intensive eel-culture system. *Aquaculture* 86: 139-153.

Buchmann, K. and J. Bresciani. 2006. Monogenea (Phylum Platyhelminthes). In: *Fish Diseases and Disorders: Protozoan and Metazoan Infections*, P.T.K. Woo (ed.). 2nd Edition. CABI Publishing, Wallingford, Oxon, U.K., Vol. 1, pp. 294-341.

Buchmann, K. and R.T. Kristensson. 2003. Efficacy of sodium percarbonate and formaldehyde bath treatments against *Gyrodactylus derjavini* infestations of rainbow trout. *North American Journal of Aquaculture* 65: 25-27.

Buchmann, K., S. Mellergaard and M. Koie. 1987. *Pseudodactylogyrus* infections in eel: a review. *Diseases of Aquatic Organisms* 3: 51-57.

Buchmann, K., A. Roepstorff and P.J. Waller. 1992. Experimental selection of mebendazole-resistant gill monogeneans from the European eel, *Anguilla anguilla* L. *Journal of Fish Diseases* 15: 393-400.

Bullard, S.A., G.W. Benz, R.M. Overstreet, E.H. Williams Jr. and J. Hemdal. 2000a. Six new host records and an updated list of wild hosts for *Neobenedenia melleni* (MacCallum) (Monogenea: Capsalidae). *Comparative Parasitology* 67: 190-196.

Bullard, S.A., S. Frasca Jr. and G.W. Benz. 2000b. Skin lesions caused by *Dermophthirius penneri* (Monogenea: Microbothriidae) on wild-caught blacktip sharks (*Carcharhinus limbatus*). *Journal of Parasitology* 86: 618-622.

Bullard, S.A., S. Frasca Jr. and G.W. Benz. 2001. Gill lesions associated with *Erpocotyle tiburonis* (Monogenea: Hexabothriidae) on wild and aquarium-held bonnethead sharks (*Sphyrna tiburo*). *Journal of Parasitology* 87: 972-977.

Bullard, S.A., R.J. Goldstein, R. Hocking and J. Jewell. 2003. A new geographic locality and three new host records for *Neobenedenia melleni* (MacCallum) (Monogenea: Capsalidae). *Gulf and Caribbean Research* 15: 1-4.

Bullard, S.A., S.M. Dippenaar, E.R. Hoffmayer and G.W. Benz. 2004. New locality records for *Dermophthirius carcharhini* (Monogenea: Microbothriidae) and *Dermophthirius maccallumi* and a list of hosts and localities for species of *Dermophthirius*. *Comparative Parasitology* 71: 78-80.

Bychowsky, B.E. 1957. *Monogenetic Trematodes, their Systematics and Phylogeny*. Izdatel'suo Akademiya Nauk SSSR, Moscow. [In Russian: English translation edited by W.J. Hargis Jr., 1961].

Cable, J. and P.D. Harris. 2002. Gyrodactylid developmental biology: historical review, current status and future trends. *International Journal for Parasitology* 32: 255-280.

Cecchini, S. and A.M. Cognetti-Varriale. 2003. Dehydration is more effective for the control of embryonic development and larval hatching of *Diplectanum aequans* (Monogenea, Diplectanidae) than formalin and trichlorphon. *Aquaculture International* 11: 261-265.

Cecchini, S., M. Saroglia, P. Berni and A.M. Cognetti-Varriale. 1998. Influence of temperature on the life cycle of *Diplectanum aequans* (Monogenea, Diplectanidae), parasitic on sea bass, *Dicentrarchus labrax* (L.). *Journal of Fish Diseases* 21: 73-75.

Chambers, C.B. and I. Ernst. 2005. Dispersal of the skin fluke *Benedenia seriolae* (Monogenea: Capsalidae) by tidal currents and implications for sea-cage farming of *Seriola* spp. *Aquaculture* 250: 60-69.

Chan, B.Z. and B.W. Wu. 1984. Studies on the pathogenicity, biology and treatment of *Pseudodactylogyrus* for eels in fishfarms. *Acta Zoologica Sinica* 30: 173-180.

Cheung, P.J. and G.D. Ruggieri. 1983. *Dermophthirius nigrellii* n. sp. (Monogenea: Microbothriidae), an ectoparasite from the skin of the lemon shark, *Negaprion brevirostris*. *Transactions of the American Microscopical Society* 102: 129-134.

Cheung, P.J. and B. Whitaker. 1993. A new dendromonocotylinid (monogenean) from the skin of the roughtail stingray, *Dasyatis centroura* Mitchill. *Journal of Aquariculture and Aquatic Sciences* 6: 63-68.

Cheung, P.J., R.F. Nigrelli, G.D. Ruggieri and A. Cilia. 1982. Treatment of skin lesions in captive lemon sharks, *Negaprion brevirostris* (Poey) caused by monogeneans (*Dermophthirius* sp.). *Journal of Fish Diseases* 2: 167-170.

Cheung, P.J., R.F. Nigrelli, G.D. Ruggieri and G.L. Crow. 1988. A new microbothriid (monogenean) causing skin lesion on the Pacific blacktip reef shark, *Carcharhinus melanopterus* (Quoy and Gaimard). *Journal of Aquariculture and Aquatic Sciences* 5: 21-25.

Chigasaki, M., M. Nakane, K. Ogawa and H. Wakabayashi. 2000. Standardized method for experimental infection of tiger puffer *Takifugu rubripes* with oncomiracidia of *Heterobothrium okamotoi* (Monogenea: Diclidophoridae) with some data on the oncomiracidial biology. *Fish Pathology* 35: 215-221.

Chisholm, L.A. and I.D. Whittington. 1995. A revision of *Dendromonocotyle* Hargis, 1955 (Monogenea: Monocotylidae) with a description of a new species from *Pastinachus sephen* (Forsskål) (Myliobatiformes: Dasyatididae) from the Great Barrier Reef, Australia. *Journal of Natural History* 29: 1093-1120.

Chisholm, L.A. and I.D. Whittington. 1998. Morphology and development of the haptors among the Monocotylidae (Monogenea). *Hydrobiologia* 383: 251-261.

Chisholm, L.A. and I.D. Whittington. 2000. Egg hatching in three species of monocotylid monogenean parasites from the shovelnose ray *Rhinobatos typus* at Heron Island, Australia. *Parasitology* 121: 303-313.

Chisholm, L.A. and I.D. Whittington. 2002. Efficacy of praziquantel bath treatments for monogenean infections of the *Rhinobatos typus*. *Journal of Aquatic Animal Health* 14: 230-234.

Chisholm, L.A. and I.D. Whittington. 2003. Invasion of the shovelnose ray (*Rhinobatos typus*) by *Neoheterocotyle rhinobatidis* and *Merizocotyle icopae* (Monogenea: Monocotylidae). *Parasitology* 127: 561-570.

Chisholm, L.A. and I.D. Whittington. 2004. Two new species of *Dendromonocotyle* Hargis, 1955 (Monogenea: Monocotylidae) from the skin of *Taeniura meyeni* (Dasyatidae) and *Aetobatus narinari* (Myliobatidae) from aquaria in Queensland, Australia. *Systematic Parasitology* 57: 221-228.

Chisholm, L.A. and I.D. Whittington. 2005. *Dendromonocotyle lasti* n. sp. from the skin and *Monocotyle caseyae* n. sp. (Monogenea: Monocotylidae) from the gills of *Himantura* sp. (Dasyatidae) from Moreton Bay, Queensland, Australia. *Systematic Parasitology* 60: 81-89.

Chisholm, L.A., I.D. Whittington and G.C. Kearn. 2001. *Dendromonocotyle colorni* sp. n. (Monogenea: Monocotylidae) from the skin of *Himantura uarnak* (Dasyatididae) from Israel and a new host record for *D. octodiscus* Hargis, 1955 from the Bahamas. *Folia Parasitologica* 48: 15-20.

Chisholm, L.A., I.D. Whittington and A.D.P. Fischer. 2004. A review of *Dendromonocotyle* (Monogenea: Monocotylidae) from the skin of stingrays and their control in public aquaria. *Folia Parasitologica* 51: 123-130.

Chisholm, L.A., V. Glennon and I.D. Whittington. 2005. *Dendromonocotyle bradsmithi* n. sp. (Monogenea: Monocotylidae) from the skin of *Myliobatis australis* (Elasmobranchii: Myliobatidae) off Adelaide and Perth, Australia: description of adult and larva. *Zootaxa* 951: 1-15.

Cognetti-Varriale, A.M., S. Cecchini and M. Saroglia. 1992. Therapeutic trials against *Diplectanum aequans* (Monogenea), parasite of seabass (*Dicentrarchus labrax*, L.) in intensive farming. *Bulletin of the European Association of Fish Pathologists* 12: 204-206.

Collins, C.M. and C.O. Cunningham. 2000. Characterization of the *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes: Monogenea) ribosomal intergenic spacer (IGS) DNA. *Parasitology* 121: 555-563.

Cone, D.K. 1995. Monogenea (Phylum Platyhelminthes). In: *Fish Diseases and Disorders: Protozoan and Metazoan Infections*, P.T.K. Woo (ed.). CABI Publishing, Wallingford, Oxon, U.K., Volume 1, pp. 289-327.

Cone, D.K. and M.D.B. Burt. 1981. The invasion route of the gill parasite *Urocleidus adspectus* Mueller, 1936 (Monogenea: Ancyrocephalinae). *Canadian Journal of Zoology* 59: 2166-2171.

Cone, D.K. and M.D.B. Burt. 1985. Population biology of *Urocleidus adspectus* Mueller, 1936 (Monogenea) on *Perca flavescens* in New Brunswick. *Canadian Journal of Zoology* 63: 272-277.

Cone, D.K., T. Eurell and V. Beasley. 1994. A report of *Dactylogyrus amphibothrium* (Monogenea) on the gills of European ruffe in western Lake Superior. *Journal of Parasitology* 80: 476-478.

Cowell, L.E., W.O. Watanabe, W.D. Head, J.J. Grover and J.M. Shenker. 1993. Use of tropical cleaner fish to control the ectoparasite *Neobenedenia melleni* (Monogenea: Capsalidae) on seawater-cultured Florida red tilapia. *Aquaculture* 113: 189-200.

Cribb, T.H. 2005. Digenea (endoparasitic flukes). In: *Marine Parasitology*, K. Rohde (ed.). CSIRO Publishing, Melbourne, Australia, pp. 76-87.

Cribb, T.H., L.A. Chisholm and R.A. Bray. 2002. Diversity in the Monogenea and Digenea: does lifestyle matter? *International Journal for Parasitology* 32: 321-328.

Cruz-e-Silva, M.P., M.L. Orge, M.M. Afonso-Roque, M.S. Grazina-Freitas and M. Carvalho-Varela. 2000. *Diplectanum aequans* (Wagener, 1857) Diesing, 1858 (Monogenea, Diplectanidae) in sea bass (*Dicentrarchus labrax* (L.), 1758) from freshwater culture. *Acta Parasitologica Portuguesa* 7: 53-56.

Cunningham, C.O., C.M. Collins, G. Malmberg and T.A. Mo. 2003. Analysis of ribosomal RNA intergenic spacer (IGS) sequences in species and populations of *Gyrodactylus* (Platyhelminthes: Monogenea) from salmonid fish in northern Europe. *Diseases of Aquatic Organisms* 57: 237-246.

Desdevises, Y. 2001. The phylogenetic position of *Furnestinia echeneis* (Monogenea, Diplectanidae) based on molecular data: a case of morphological adaptation? *International Journal for Parasitology* 31: 205-208.

Deveney, M.R., L.A. Chisholm and I.D. Whittington. 2001. First published record of the pathogenic monogenean parasite *Neobenedenia melleni* (Capsalidae) from Australia. *Diseases of Aquatic Organisms* 46: 79-82.

Duarte, N., N. Rosa, M.J. Santos and J. Rebelo. 2000. Study of ectoparasites on the gills of sea bass (*Dicentrarchus labrax* L.) from the Rio de Aveiro. *Revista de Biologia Lisboa* 18: 59-68.

Egusa, S. 1983. Disease problems in Japanese yellowtail, *Seriola quinqueradiata*, culture: a review. *Rapports et Procès-verbaux des Réunion Conseil International pour Exploration de la Mer* 182: 10-18.

Ellis, E.P. and W.O. Watanabe. 1993. The effects of hyposalinity on eggs, juveniles and adults of the marine monogenean, *Neobenedenia melleni*. Treatment of ectoparasitosis in seawater-cultured tilapia. *Aquaculture* 117: 15-27.

Ernst, I. and I.D. Whittington. 1996. Hatching rhythms in the capsalid monogeneans *Benedenia lutjani* from the skin and *B. rohdei* from the gills of *Lutjanus carponotatus* at Heron Island, Queensland, Australia. *International Journal for Parasitology* 26: 1191-1204.

Ernst, I., I. Whittington, S. Corneillie and C. Talbot. 2002. Monogenean parasites in sea-cage aquaculture. *Austasia Aquaculture* February/March 2002: 46-48.

Ernst, I., I.D. Whittington, S. Corneillie and C. Talbot. 2005. Effects of temperature, salinity, desiccation and chemical treatments on egg embryonation and hatching

success of *Benedenia seriolae* (Monogenea: Capsalidae), a parasite of farmed *Seriola* spp. *Journal of Fish Diseases* 28: 157-164.

Euzet, L. and C. Combes. 1998. The selection of habitats among the Monogenea. *International Journal for Parasitology* 28: 1645-1652.

FAO. 2008. Cultured aquatic species information programme - *Dicentrarchus labrax* (Linnaeus, 1758). A Fishery Resource under Fisheries and Aquaculture on the Food and Agriculture Organization (FAO) of the United Nation website. *World Wide Web electronic publication*. http://www.fao.org/fishery/culturedspecies/Dicentrarchus_labrax accessed on 8 January 2008.

Flores-Crespo, J.F. and R.F. Flores-Crespo. 2003. Monogeneo, parásitos de peces en México: estudio recaptilativo. *Técnica Pecuaria en México* 41: 175-192.

Froese, R. and D. Pauly. 2007. *FishBase. World Wide Web electronic publication*. http://www.fishbase.org, version (11/2007), accessed on 27 November 2007.

Gaida, I.H. and P. Frost. 1991. Intensity of *Neobenedenia girellae* (Monogenea: Capsalidae) on the halfmoon, *Medialuna californiensis* (Perciformes: Kyphosidae), examined using a new method for detection. *Journal of the Helminthological Society of Washington* 58: 129-130.

Gibson, D.I., T.A. Timofeeva and P.I. Gerasev. 1996. A catalogue of the nominal species of the monogenean genus *Dactylogyrus* Diesing, 1850 and their host genera. *Systematic Parasitology* 35: 3-48.

Glennon, V., L.A. Chisholm and I.D. Whittington. 2005. *Branchotenthes octohamatus* sp. n. (Monogenea: Hexabothriidae) from the gills of the southern fiddler ray, *Trygonorrhina fasciata* (Rhinobatidae) in South Australia: description of adult and larva. *Folia Parasitologica* 52: 223-230.

Glennon, V., L.A. Chisholm and I.D. Whittington. 2006. Three unrelated species, 3 sites, same host—monogeneans of the southern fiddler ray, *Trygonorrhina fasciata*, in South Australia: egg hatching strategies and larval behaviour. *Parasitology* 133: 55-66.

Glennon, V., L.A. Chisholm and I.D. Whittington. 2007. Experimental infections, using a fluorescent marker, of two elasmobranch species by unciliated larvae of *Branchotenthes octohamatus* (Monogenea: Hexabothriidae): invasion route, host specificity and post-larval development. *Parasitology* 134: 1243-1252.

González-Lanza, C., P. Alvarez-Pellitero and A. Sitjá-Bobadilla. 1991. Diplectanidae (Monogenea) infestations of sea bass, *Dicentrarchus labrax* (L.), from the Spanish Mediterranean area. Histopathology and population dynamics under culture conditions. *Parasitology Research* 77: 307-314.

Goven, B.A. and D.F. Amend. 1982. Mebendazole/trichlorfon combination: a new anthelmintic for removing monogenetic trematodes from fish. *Journal of Fish Biology* 20: 373-378.

Goven, B.A., J.P. Gilbert and J.B. Gratzek. 1980. Apparent drug resistance to the organophosphate dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate by monogenetic trematodes. *Journal of Wildlife Diseases* 16: 343-346.

Grau, A., S. Crespo, E. Pastor, P. Gonzalez and E. Carbonell. 2003. High infection by *Zeuxapta seriolae* (Monogenea: Heteraxinidae) associated with mass mortalities of amberjack *Seriola dumerili* Risso reared in sea cages in the Balearic Islands (western Mediterranean). *Bulletin of the European Association of Fish Pathologists* 23: 139-142.

Grimes, D.J., S.A. Gruber and E.B. May. 1985. Experimental infection of lemon sharks, *Negaprion brevirostris* (Poey), with *Vibrio* species. *Journal of Fish Diseases* 8: 173-180.

Grutter, A.S. 2002. Cleaning symbioses from the parasites' perspective. *Parasitology* 124: 65-81.

Grutter, A.S., M.R. Deveney, I.D. Whittington and R.J.G. Lester. 2002. The effect of the cleaner fish *Labroides dimidiatus* on the capsalid monogenean *Benedenia lolo* parasite of the labrid fish *Hemigymnus melapterus*. *Journal of Fish Biology* 61: 1098-1108.

*GyroDb*. A home for gyrodactylids on the web. *World Wide Web electronic publication*. http://www.gyrodb.net/, accessed on 13 December 2007.

Hansen, H., L. Bachmann and T.A. Bakke. 2003. Mitochondrial DNA variation of *Gyrodactylus* spp. (Monogenea, Gyrodactylidae) populations infecting Atlantic salmon, grayling, and rainbow trout in Norway and Sweden. *International Journal for Parasitology* 33: 1471-1478.

Hansen, H., K. Olstad, T.A. Bakke and L. Bachmann. 2004. Species, subspecies or strains: the taxonomy of *Gyrodactylus salaris* and *G. thymalli* (Monogenea) revisited. *IX European Multicolloquium of Parasitology, Valencia, Spain*, pp. 229-234.

Harris, P.D. 1986. Species of *Gyrodactylus* von Nordmann, 1832 (Monogenea: Gyrodactylidae) from poeciliid fishes, with a description of *G. turnbulli* sp. nov. from the guppy, *Poecilia reticulata* Peters. *Journal of Natural History* 20: 183-191.

Harris, P.D., A. Soleng and T.A. Bakke. 2000. Increased susceptibility of salmonids to the monogenean *Gyrodactylus salaris* following administration of hydrocortisone acetate. *Parasitology* 120: 57-64.

Harris, P.D., A.P. Shinn, J. Cable and T.A. Bakke. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Systematic Parasitology* 59: 1-27.

Hatanaka, A., N. Umeda, S. Yamashita and N. Hirazawa. 2005. A small ciliary surface glycoprotein of the monogenean parasite *Neobenedenia girellae* acts as an agglutination/immobilization antigen and induces an immune response in the Japanese flounder *Paralichthys olivaceus*. *Parasitology* 131: 591-600.

Hayward, C.J. 1997. Helminth ectoparasites of sillaginid fishes (Perciformes: Percoidei) have low species richness. *Folia Parasitologia* 44: 173-187.

Hayward, C.J. 2005. Monogenea Polyopisthocotylea (ectoparasitic flukes). In: *Marine Parasitology*, K. Rohde (ed.). CSIRO Publishing, Melbourne, Australia, pp. 55-63.

Hayward, C.J., M. Iwashita, J.S. Crane and K. Ogawa. 2001a. First report of the invasive eel pest *Pseudodactylogyrus bini* in North America and in wild American eels. *Diseases of Aquatic Organisms* 44: 53-60.

Hayward, C.J., J.H. Kim and G.J. Heo. 2001b. Spread of *Neoheterobothrium hirame* (Monogenea), a serious pest of olive flounder *Paralichthys olivaceus*, to Korea. *Diseases of Aquatic Organisms* 45: 209-213.

Hendrix, S.S. 1994. *Marine Flora and Fauna of the Eastern United States. Platyhelminthes: Monogenea. NOAA Technical Report NMFS 121 of the Fisheries Bulletin.*

Hirazawa, N., T. Ohtaka and K. Hata. 2000. Challenge trials on the anthelmintic effect of drugs and natural agents against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*. *Aquaculture* 188: 1-13.

Hirazawa, N., S.I. Oshima, T. Mitsuboshi and K. Hata. 2001a. The anthelmintic effect of medium-chain fatty acids against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*: evaluation of doses of caprylic acid at different water temperatures. *Aquaculture* 195: 211-223.

Hirazawa, N., S.I. Oshima and K. Hata. 2001b. In vitro assessment of the antiparasitic effect of caprylic acid against several fish parasites. *Aquaculture* 200: 251-258.

Hirazawa, N., S. Oshima, T. Mitsuboshi and S. Yamashita. 2003. Mucus pH of the tiger puffer *Takifugu rubripes* is an important factor for host identification by the monogenean *Heterobothrium okamotoi*. *Parasitology* 127: 225-230.

Hirazawa, N., T Mitsuboshi, T. Hirata and K. Shirasu. 2004. Susceptibility of spotted halibut *Verasper variegatus* (Pleuronectidae) to infection by the monogenean *Neobenedenia girellae* (Capsalidae) and oral therapy trials using praziquantel. *Aquaculture* 238: 83-95.

Hirazawa, N., N. Umeda, A. Hatanaka and A. Kuroda. 2006. Characterization of serine proteases in the monogenean *Neobenedenia girellae*. *Aquaculture* 255: 188-195.

Hutson, K.S., I. Ernst, A.J. Mooney and I.D. Whittington. 2007a. Metazoan parasite assemblages of wild *Seriola lalandi* (Carangidae) from eastern and southern Australia. *Parasitology International* 56: 95-105.

Hutson, K.S., I. Ernst and I.D. Whittington. 2007b. Risk assessment for metazoan parasites of yellowtail kingfish *Seriola lalandi* (Perciformes: Carangidae) in South Australian sea-cage aquaculture. *Aquaculture* 271: 85-99.

Imada, R. and K. Muroga. 1979. *Pseudodactylogyrus microrchis* (Monogenea) on the gills of cultured eels. III. Experimental control by trichlorfon. *Bulletin of the Japanese Society of Scientific Fisheries* 45: 25-29.

Ishida, M., F. Kawano, N. Umeda and N. Hirazawa. 2007. Response of *Neobenedenia girellae* (Monogenea) oncomiracidia to brightness and black-and-white contrast. *Parasitology* 134: 1823-1830.

Jahn, T.L. and L.R. Kuhn. 1932. The life history of *Epibdella melleni* MacCallum, 1927, a monogenetic trematode parasitic on marine fishes. *Biological Bulletin (Woods Hole)* 62: 89-111.

Janse, M. and F.H.M. Borgsteede. 2003. Praziquantel treatment of captive white-spotted eagle rays (*Aetobatus narinari*) infested with monogenean trematodes. *Bulletin of the European Association of Fish Pathologists* 23: 152-156.

Jansen, P.A., L. Matthews and N. Toft. 2007. Geographic risk factors for inter-river dispersal of *Gyrodactylus salaris* in fjord systems in Norway. *Diseases of Aquatic Organisms* 74: 139-149.

Johnsen, B.O. and A.J. Jensen. 1986. Infestation of Atlantic salmon, *Salmo salar*, by *Gyrodactylus salaris* in Norway. *Journal of Fish Biology* 29: 233-241.

Johnsen, B.O. and A.J. Jensen. 1988. Introduction and establishment of *Gyrodactylus salaris* Malmberg, 1957, on Atlantic salmon, *Salmo salar* L., fry and parr in the River Vefsna, northern Norway. *Journal of Fish Diseases* 11: 35-45.

Kaneko, J.J., R. Yamada, J.A. Brock and R.M. Nakamura. 1988. Infection of tilapia, *Oreochromis mossambicus* (Trewavas), by a marine monogenean, *Neobenedenia melleni* (MacCallum, 1927) Yamaguti, 1963 in Kaneohe Bay, Hawaii, USA, and its treatment. *Journal of Fish Diseases* 11: 295-300.

Katharios, P., N. Papandroulakis and P. Divanach. 2006. Treatment of *Microcotyle* sp. (Monogenea) on the gills of cage-cultured red porgy, *Pagrus pagrus* following baths with formalin and mebendazole. *Aquaculture* 251: 167-171.

Kearn, G.C. 1964. The attachment of the monogenean *Entobdella soleae* to the skin of the common sole. *Parasitology* 54: 327-335.

Kearn, G.C. 1965. The biology of *Leptocotyle minor*, a skin parasite of the dogfish, *Scyliorhinus canicula*. *Parasitology* 55: 473-480.

Kearn, G.C. 1968. The development of the adhesive organs of some diplectanid, tetraonchid and dactylogyrid gill parasites (Monogenea). *Parasitology* 58: 149-163.

Kearn, G.C. 1979. Studies on gut pigment in skin-parasitic monogeneans, with special reference to the monocotylid *Dendromonocotyle kuhlii*. *International Journal for Parasitology* 9: 545-552.

Kearn, G.C. 1981. Behaviour of oncomiracidia. *Parasitology* 82: 57-59.

Kearn, G.C. 1985. Observations on egg production in the monogenean *Entobdella soleae*. *International Journal for Parasitology* 15: 187-194.

Kearn, G.C. 1986. The eggs of monogeneans. *Advances in Parasitology* 25: 175-273.

Kearn, G.C. 1994. Evolutionary expansion of the Monogenea. *International Journal for Parasitology* 24: 1227-1271.

Kearn, G.C. 1998. *Parasitism and the Platyhelminths*. Chapman & Hall, London.

Kearn, G.C. 1999. The survival of monogenean (platyhelminth) parasites on fish skin. *Parasitology* 119: S57-S88.

Kearn, G.C. 2004. *Leeches, Lice and Lampreys. A Natural History of Skin and Gill Parasites of Fishes*. Springer, Dordrecht.

Kearn, G.C. and I.D. Whittington. 2005. *Neoentobdella* gen. nov. for species of *Entobdella* Blainville in Lamarck, 1818 (Monogenea, Capsalidae, Entobdellinae) from stingray hosts, with descriptions of two new species. *Acta Parasitologica* 50: 32-48.

Kearn, G.C., K. Ogawa and Y. Maeno. 1992a. Egg production, the oncomiracidium and larval development of *Benedenia seriolae* a skin parasite of the yellowtail *Seriola quinqueradiata*, in Japan. *Publications of the Seto Marine Biological Laboratory* 35: 351-362.

Kearn, G.C., K. Ogawa and Y. Maeno. 1992b. Hatching patterns of the monogenean parasites *Benedenia seriolae* and *Heteraxine heterocerca* from the skin and gills, respectively, of the same host fish, *Seriola quinqueradiata*. *Zoological Science* 9: 451-455.

Kearn, G.C., R. James and R. Evans-Gowing. 1993. Insemination and population density in *Entobdella soleae*, a monogenean skin parasite of the common sole, *Solea solea*. *International Journal for Parasitology* 23: 891-899.

Kearn, G.C., R. Evans-Gowing and T. Tappenden. 1999. The opercular bond in the egg-shell of the monogenean *Entobdella soleae*, a platyhelminth skin parasite of the common sole (*Solea solea*). *Parasitology* 118: 433-438.

Kiemer, M.C.B. and K.D. Black. 1997. The effects of hydrogen peroxide on the gill tissues of Atlantic salmon, *Salmo salar* L. *Aquaculture* 153: 181-189.

Kim, K.H. and E.S. Choi. 1998. Treatment of *Microcotyle sebastis* (Monogenea) on the gills of cultured rockfish (*Sebastes schelegeli*) with oral administration of mebendazole and bithionol. *Aquaculture* 167: 115-121.

Kim, K.H. and J.B. Cho. 2000. Treatment of *Microcotyle sebastis* (Monogenea: Polyopisthocotylea) infestation with praziquantel in an experimental cage simulating commercial rockfish *Sebastes schlegeli* culture conditions. *Diseases of Aquatic Organisms* 40: 229-231.

Kim, K.H., S.I. Park and B.Y. Jee. 1998. Efficacy of oral administration of praziquantel and mebendazole against *Microcotyle sebastis* (Monogenea) infestation of cultured rockfish (*Sebastes schlegeli*). *Fish Pathology* 33: 467-471.

Kim, K.H., Y.J. Hwang, J.B. Cho and S.I. Park. 2000. Immunization of cultured rockfish *Sebastes schlegeli* against *Microcotyle sebastis* (Monogenea). *Diseases of Aquatic Organisms* 40: 29-32.

Kim, K.H., E.H. Lee, S.R. Kwon and J.B. Cho. 2001. Treatment of *Microcotyle sebastis* infestation in cultured rockfish *Sebastes schlegeli* by oral administration of praziquantel in combination with cimetidine. *Diseases of Aquatic Organisms* 44: 133-136.

Kinami, R., J. Miyamoto, T. Yoshinaga, K. Ogawa and Y. Nagakura. 2005. A practical method to distinguish between *Neobenedenia girellae* and *Benedenia seriolae*. *Fish Pathology* 40: 63-66.

Koesharyani, I., Zafran, K. Yuasa and K. Hatai. 1999. Two species of capsalid monogeneans infecting cultured humpback grouper *Cromileptes altivelis* in Indonesia. *Fish Pathology* 34: 165-166.

Köhler, P. 2001. The biochemical basis of anthelmintic action and resistance. *International Journal for Parasitology* 31: 336-345.

Kritsky, D.C. and F.J. Stephens. 2001. *Haliotrema abaddon* n. sp. (Monogenoidea: Dactylogyridae) from the gills of wild and maricultured West Australian dhufish *Glaucosoma hebraicum* (Teleostei: Glaucosomatidae), in Australia. *Journal of Parasitology* 87: 749-754.

Lackenby, J.A., C.B. Chambers, I. Ernst and I.D. Whittington. 2007. Effect of water temperature on reproductive development of *Benedenia seriolae* (Monogenea: Capsalidae) from *Seriola lalandi* in Australia. *Diseases of Aquatic Organisms* 74: 235-242.

Lambert, A. and C. Maillard. 1974. Parasitisme branchial simultane par deux especes de *Diplectanum* Diesing, 1858 (Monogenea, Monopisthocotylea) chez *Dicentrarchus labrax* (L., 1758). *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences, Paris*, 279: 1345-1347.

Lambert, A. and C. Maillard. 1975. Repartition branchiale de deux monogenes: *Diplectanum aequans* (Wagener, 1857) Diesing, 1858 et *Diplectanum laubieri* Lambert et Maillard, 1974 (Monogenea, Monopisthocotylea) parasites simultanes de *Dicentrarchus labrax* (Teleosteen). *Annales de Parasitologie Humaine et Comparée* 50: 691-699.

Leong, T.S. 1997. Control of parasites in cultured marine finfishes in Southeast Asia—an overview. *International Journal for Parasitology* 27: 1177-1184.

Lester, R.J.G. 1972. Attachment of *Gyrodactylus* to *Gasterosteus* and host response. *Journal of Parasitology* 58: 717-722.

Lester, R.J.G. and J.R. Adams. 1974. A simple model of a *Gyrodactylus* population. *International Journal for Parasitology* 4: 497-506.

Liao, I.C., T.S. Huang, W.S. Tsai, C.M. Hsueh, S.L. Chang and E.M. Leaño. 2004. Cobia culture in Taiwan: current status and problems. *Aquaculture* 237: 155-165.

Lim, K.T., J.H. Kim, J.H. Park, N.S. Shin, G.J. Heo and S.C. Park. 2005. Treatment of dactylogyrosis in oscar (*Astronotus ocellatus*). *Journal of Veterinary Clinics* 22: 160-162.

Lindenstrøm, T., K. Buchmann and C.J. Secombes. 2003. *Gyrodactylus derjavini* infection elicits IL-1β expression in rainbow trout skin. *Fish and Shellfish Immunology* 15: 107-115.

Lindenstrøm, T., C.J. Secombes and K. Buchmann. 2004. Expression of immune response genes in rainbow trout skin induced by *Gyrodactylus derjavini* infections. *Veterinary Immunology and Immunopathology* 97: 137-148.

Llewellyn, J. 1957. Host-specificity in monogenetic trematodes. In: *First Symposium on Host-Specificity Among Parasites of Invertebrates*, J.G. Baer (ed.). Impr. P. Attinger, Neuchâtel, Switzerland, pp. 199-212.

Llewellyn, J. 1958. The adhesive mechanisms of monogenetic trematodes: the attachment of species of the Diclidophoridae to the gills of gadoid fishes. *Journal of the Marine Biological Association of the United Kingdom, Plymouth* 37: 67-79.

Longshaw, M. 1996. Mortality of captive herring, *Clupea harengus* L. (Teleostei: Clupeidae) due to *Pseudanthocotyloides heterocotyle* (Van Beneden, 1871) (Monogenea: Polyopisthocotylea: Mazocraeidae). *Bulletin of the European Association of Fish Pathologists* 16: 143-144.

MacCallum, G.A. 1927. A new ectoparasitic trematode, *Epibdella melleni*, sp. nov. *Zoopathologica* 1: 291-300.

Macdonald, S. 1974. Host skin mucus as a hatching stimulant in *Acanthocotyle lobianchi*, a monogenean from the skin of *Raja* sp. *Parasitology* 68: 331-338.

Macdonald, S. and A. Jones. 1978. Egg-laying and hatching rhythms in the monogenean *Diplozoon homoion gracile* from the southern barbel (*Barbus meridionalis*). *Journal of Helminthology* 52: 23-28.

Malmberg, G. 1957. Om förekomsten av *Gyrodactylus* på svenska fiskar (On the occurrence of *Gyrodactylus* on Swedish fishes). *Skrifter Utgivna av Södra Sveriges Fiskeriförening Årsskrift* 1956: 19 – 76. (In Swedish, species descriptions and summary in English).

Malmberg, G. 1964. Taxonomical and ecological problems in *Gyrodactylus*. In: *Parasitic Worms and Aquatic Conditions Proceedings of Symposium*, Prague, Oct 29 – Nov 2, 1962, R. Ergens and B. Rysavy (eds.). pp. 203-230.

Malmberg, G. 1993. Gyrodactylidae and gyrodactylosis of Salmonidae. *Bulletin Français de la Pêche et de la Pisciculture* 328: 5-46.

Mansell, B., M.D. Powell, I. Ernst and B.F. Nowak. 2005. Effects of the gill monogenean *Zeuxapta seriolae* (Meserve, 1938) and treatment with hydrogen peroxide on pathophysiology of kingfish, *Seriola lalandi* Valenciennes, 1833. *Journal of Fish Diseases* 28: 253-262.

Matejusová, I., M. Gelnar, A.J.A McBeath, C.M. Collins and C.O. Cunningham. 2001. Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). *International Journal for Parasitology* 31: 738-745.

McHugh, E.S., A.P. Shinn and J.W. Kay. 2000. Discrimination of the notifiable pathogen *Gyrodactylus salaris* from *G. thymalli* (Monogenea) using statistical classifiers applied to morphometric data. *Parasitology* 121: 315-323.

Meyer, M.C. and O.W. Olsen. 1980. *The Essentials of Parasitology.* 3rd Edition. Wm. C. Brown Company Publishers, Iowa, U.S.A.

Mo, T.A. 1987. Taksonomiske og biologiske undersøkelser, virksomheten i 1986 og forslag til virksomheten i 1987. *Gyrodactylus undersøkelsene ved Zoologisk Museum, Universitetet i Olso, Rapport nr 2*, pp. 1-70. (In Norweigan).

Mo, T.A. 1994. Status of *Gyrodactylus salaris* problems and research in Norway. In: *Parasitic Diseases of Fish*, A.A. Pike and J.W. Lewis (eds.). Samara Publishing Ltd., Dyfed, U.K., pp. 43-56.

Mo, T.A. 2004. The *Gyrodactylus salaris* situation in Norway: an update on current distribution and eradication programs implemented. In: *Diagnosis and Control of Fish Diseases; Workshop on the Sustainable Control of Fish Diseases*, K. Buchmann (ed.). Royal Veterinary and Agricultural University, Copenhagen, Denmark, 18 pp.

Molnár, K. 1972. Studies on gill parasitosis of grass-carp (*Ctenopharyngodon idella*) caused by *Dactylogyrus lamellatus* Achmerow, 1952. IV. Histopathological changes. *Acta Veterinaria Academiae Scientiarum Hungariae* 22: 9-24.

Monni, G. and A.M. Cognetti-Varriale. 2001. Antigenic activity of *Diplectanum aequans* (Monogenea) in sea bass (*Dicentrarchus labrax* L.) held under different oxygenation conditions. *Bulletin of the European Association of Fish Pathologists* 21: 241-245.

Mooney, A.J., I. Ernst and I.D. Whittington. 2006. An egg-laying rhythm in *Zeuxapta seriolae* (Monogenea: Heteraxinidae), a gill parasite of yellowtail kingfish (*Seriola lalandi*). *Aquaculture* 253: 10-16.

Montero, F.E, S. Crespo, F. Padros, F. de la Gandara, A. Garcia and J.A. Raga. 2004. Effects of the gill parasite *Zeuxapta seriolae* (Monogenea: Heteraxinidae) on the amberjack *Seriola dumerili* Risso (Teleostei: Carangidae). *Aquaculture* 232: 153-163.

Mueller, K.W., W.O Watanabe and W.D. Head. 1994. Occurrence and control of *Neobenedenia melleni* (Monogenea: Capsalidae) in cultured tropical marine fish, including three new host records. *Progressive Fish Culturist* 56: 140-142.

Nakane, M., K. Ogawa, T. Fujita, M. Sameshima and H. Wakabayashi. 2005. Acquired protection of tiger puffer *Takifugu rubripes* against infection with *Heterobothrium okamotoi* (Monogenea: Diclidophoridae). *Fish Pathology* 40: 95-101.

Nakayasu, C., T. Yoshinaga and A. Kumagai. 2002. Hematology of anemia experimentally induced by repeated bleedings in Japanese flounder with comments on the cause of flounder anemia recently prevailing in Japan. *Fish Pathology* 37: 125-130.

Nakayasu, C., N. Tsutsumi, T. Yoshitomi, T. Yoshinaga and A. Kumagai. 2003. Identification of Japanese flounder leucocytes involved in the host response to *Neoheterobothrium hirame*. *Fish Pathology* 38: 9-14.

Nigrelli, R.F. and C.M. Breder. 1934. The susceptibility and immunity of certain fishes to *Epibdella melleni*, a monogenetic trematode. *Journal of Parasitology* 20: 259-269.

Noga, E.J. 2000. *Fish Disease. Diagnosis and Treatment.* Iowa State University Press, Iowa, U.S.A.

Nybelin, O. 1924. *Dactylogyrus vastator* n. sp. *Arkiv Für Zoologi* 16: 1-2.

Ogawa, K. 1999. *Neoheterobothrium hirame* sp. nov. (Monogenea: Diclidophoridae) from the buccal cavity wall of Japanese flounder *Paralichthys olivaceus*. *Fish Pathology* 34: 195-201.

Ogawa, K. 2002. Impacts of diclidophorid monogenean infections on fisheries in Japan. *International Journal for Parasitology* 32: 373-380.

Ogawa, K. and H. Yokoyama. 1998. Parasitic diseases of cultured marine fish in Japan. *Fish Pathology* 33: 303-309.

Ogawa, K., M.G. Bondad-Reantaso, M. Fukudome and H. Wakabayashi. 1995. *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963 (Monogenea: Capsalidae) from cultured marine fishes of Japan. *Journal of Parasitology* 81: 223-227.

Ogawa, K., Yasuzaki, M. and Yoshinaga, T. 2005a. Experiments on the evaluation of the blood feeding of *Heterobothrium okamotoi* (Monogenea: Diclidophoridae). *Fish Pathology* 40: 169-174.

Ogawa, K., Yamabata, N. and Yoshinaga, T. 2005b. Egg-laying of the monogenean *Heterobothrium okamotoi* on experimentally infected tiger puffer *Takifugu rubripes*. *Fish Pathology* 40: 111-118.

Ohashi, H., N. Umeda, N. Hirazawa, Y. Ozaki, C. Miura and T. Miura. 2007a. Purification and identification of a glycoprotein that induces the attachment of oncomiracidia of *Neobenedenia girellae* (Monogenea, Capsalidae). *International Journal for Parasitology* 37: 1483-1490.

Ohashi, H., N. Umeda, N. Hirazawa, Y. Ozaki, C. Miura and T. Miura. 2007b. Antiparasitic effect of calcium and magnesium ion-free buffer treatments against a common monogenean *Neobenedenia girellae*. *Parasitology* 134: 229-236.

Ohashi, H., N. Umeda, N. Hirazawa, Y. Ozaki, C. Miura and T. Miura. 2007c. Expression of *vasa* (*vas*)-related genes in germ cells and specific interference with gene functions by double-stranded RNA in the monogenean, *Neobenedenia girellae*. *International Journal for Parasitology* 37: 515-523.

Ohhashi, Y., T. Yoshinaga and K. Ogawa. 2007. Involvement of host recognition by oncomiracidia and post-larval survivability in the host specificity of *Heterobothrium okamotoi* (Monogenea: Diclidophoridae). *International Journal for Parasitology* 37: 53-60.

Oliver, G. 1977. Effet pathogène de la fixation de *Diplectanum aequans* (Wagener, 1857) Diesing, 1858 (Monogenea, Monopisthocotylea, Diplectanidae) sur les branchies de *Dicentrarchus labrax* (Linnaeus, 1758), (Pisces, Serranidae). *Zeitschrift für Parasitenkunde* 53: 7-11.

Olson, P.D. and D.T.J. Littlewood. 2002. Phylogenetics of the Monogenea – evidence from a medley of molecules. *International Journal for Parasitology* 32: 233-244.

Paling, J.K. 1966. The attachment of the monogenean *Diplectanum aequans* (Wagener) Diesing to the gills of *Morone labrax* L. *Parasitology* 56: 493-503.

Paperna, I. 1963. Some observations on the biology and ecology of *Dactylogyrus vastator* in Israel. *Bamidgeh Israel* 15: 8-28.

Paperna, I. 1964. Competitive exclusion of *Dactylogyrus extensus* by *Dactylogyrus vaştator* (Trematoda, Monogenea) on the gills of reared carp. *Journal of Parasitology* 50: 94-98.

Paperna, I. 1987. Solving parasite-related problems in cultured marine fish. *International Journal of Parasitology* 17: 327-336.

Paperna, I. and R.M. Overstreet. 1981. Parasites and diseases of mullets (Mugilidae). In: *Aquaculture of Grey Mullets*, O.H. Oren (ed.). *International Programme* 26. Cambridge University Press, Cambridge, pp. 411-493.

Paperna, I., A. Diamant and R.M. Overstreet. 1984. Monogenean infestations and mortality in wild and cultured Red Sea fishes. *Helgoländer Meeresuntersuchungen* 37: 445-462.

Papoutsoglou, S., M.J. Costello, E. Stamou and G. Tziha. 1996. Environmental conditions at sea-cages, and ectoparasites on farmed European sea-bass, *Dicentrarchus labrax* (L.), and gilt-head sea-bream, *Sparus aurata* L., at two farms in Greece. *Aquaculture Research* 27: 25-34.

Pichelin, S., I. Whittington and J. Pearson. 1991. *Concinnocotyla* (Monogenea: Polystomatidae), a new genus for the polystome from the Australian lungfish, *Neoceratodus forsteri*. *Systematic Parasitology* 18: 81-93.

Pike, A.W. and S.L. Wadsworth. 1999. Sealice on salmonids: their biology and control. *Advances in Parasitology* 44: 234-337.

Pironet, F.N. and J.B. Jones. 2000. Treatments for ectoparasites and diseases in captive Western Australian dhufish. *Aquaculture International* 8: 349-361.

Pironet, F.N. and F.J. Neira. 1998. Hormone-induced spawning and development of artificially reared larvae of the West Australian dhufish, *Glaucosoma hebraicum* (Glaucosomatidae). *Marine and Freshwater Research* 49: 133-142.

Poleo, A., J. Schjolden, H. Hansen, T.A. Bakke, T.A. Mo, B.O. Rosseland and E. Lydersen. 2004. The effect of various metals on *Gyrodactylus salaris* (Platyhelminthes, Monogenea) infections in Atlantic salmon (*Salmo salar*). *Parasitology* 128: 169-177.

Poynton, S.L., T.W. Campbell and H.W. Palm. 1997. Skin lesions in captive lemon sharks *Negaprion brevirostris* (Carcharhinidae) associated with the monogenean *Neodermophthirius harkemai* Price, 1963 (Microbothriidae). *Diseases of Aquatic Organisms* 31: 29-33.

Presnell, J.K. and M.P. Schreibman. 1997. *Humason's Animal Tissue Techniques*, 5th Edition. The Johns Hopkins University Press, Baltimore, U.S.A.

Pritchard, M.H. and G.O.W. Kruse. 1982. *The Collection and Preservation of Animal Parasites*. University of Nebraska Press, Lincoln, U.S.A.

Prost, M. 1963. Investigations on the development and pathogenicity of *Dactylogyrus anchoratus* (Duj. 1845) and *D. extensus* Mueller et var. Cleave, 1932 for breeding carps. *Acta Parasitologica Polonica* 11: 17-48.

Rach, J.J., T.M. Schreier, G.E. Howe and S.D. Redman. 1997. Effect of species, life stage, and water temperature on the toxicity of hydrogen peroxide to fish. *Progressive Fish-Culturist* 59: 41-46.

Rach, J.J., M.P. Gaikowski and R.T. Ramsay. 2000. Efficacy of hydrogen peroxide to control parasitic infestations on hatchery-reared fish. *Journal of Aquatic Animal Health* 12: 267-273.

Rae, G.H. 2002. Sea louse control in Scotland, past and present. *Pest Management Science* 58: 515-520.

Rao, Y.Z. and T.B. Yang. 2007. cDNA cloning, mRNA expression and recombinant expression of a cathepsin L-like cysteine protease from *Neobenedenia melleni* (Monogenea: Capsalidae). *Aquaculture* 269: 41-53.

Richardson, M.A., D.R.J. Smith, D.H. Kemp and R.L. Tellam. 1993. Native and baculovirus-expressed forms of the immunoprotective protein BM86 from *Boophilus microplus* are anchored to the cell membrane by a glycosylphosphatidyl inositol linkage. *Insect Molecular Biology* 1: 139-147.

Rohde, K. 1981. Ultrastructure of the buccal organs and associated structures of *Zeuxapta seriolae* (Meserve, 1938) Price, 1962, and *Paramicrocotyloides reticularis* Rohde, 1978 (Monogenea: Polyopisthocotylea). *Zoologischer Anzeiger* 206: 279-291.

Rohde, K. 1984. Diseases caused by metazoans: helminthes. In: *Disease of Marine Animals. Introduction, Pisces*, O. Kinne (ed.). Biologische Anstalt Helogoland, Hamburg, Vol. 4, pp. 193-320.

Rohde, K. 1993. *Ecology of Marine Parasites. An Introduction to Marine Parasitology*, 2nd Edition. CAB International, Wallingford, Oxon, U.K.

Roubal, F.R. 1986. Studies of monogeneans and copepods parasitizing the gills of the sparid, *Acanthopagrus australis* (Gunther) in northern New South Wales. *Canadian Journal of Zoology* 64: 841-849.

Roubal, F.R. 1989. Comparative pathology of some monogenean and copepod ectoparasites on the gills of *Acanthopagrus australis* (Family Sparidie). *Journal of Fish Biology* 34: 503-514.

Roubal, F.R. and B.K. Diggles. 1993. The rate of development of *Polylabroides multispinosus* (Monogenea: Microcotylidae) parasitic on the gills of *Acanthopagrus australis* (Pisces: Sparidae). *International Journal for Parasitology* 23: 871-875.

Roubal, F.R. and I.D. Whittington. 1990. Observations on the attachment by the monogenean, *Anoplodiscus australis*, to the caudal fin of *Acanthopagrus australis*. *International Journal for Parasitology* 20: 307-314.

Rubio-Godoy, M., J. Sigh, K. Buchmann and R.C. Tinsley. 2003a. Antibodies against *Discocotyle sagittata* (Monogenea) in farmed trout. *Diseases of Aquatic Organisms* 56: 181-184.

Rubio-Godoy, M., J. Sigh, K. Buchmann and R. Tinsley. 2003b. Immunization of rainbow trout *Oncorhynchus mykiss* against *Discocotyle sagittata* (Monogenea). *Diseases of Aquatic Organisms* 55: 23-30.

Sarig, S., M. Lahav and M. Shilo. 1965. Control of *Dactylogyrus vastator* on carp fingerlings with dipterex. *Bamidgeh Israel* 17: 47-52.

Santos, M.J. 1996. Observations on the parasitofauna of wild sea bass (*Dicentrarchus labrax* L.) from Portugal. *Bulletin of the European Association of Fish Pathologists* 16: 77-79.

Schmahl, G. 1991. The chemotherapy of monogeneans which parasitize fish: a review. *Folia Parasitologica* 38: 97-106.

Schmahl, G. 1993. Up to date chemotherapy against Monogenea: a review. *Bulletin Français de la Pêche et de la Pisciculture* 328: 74-81.

Schmahl, G. and H. Mehlhorn. 1985. Treatment of fish parasites. I. Praziquantel effective against Monogenea (*Dactylogyrus vastator, Dactylogyrus extensus, Diplozoon paradoxum*). *Zeitschrift für Parasitenkunde* 71: 727-737.

Schmahl, G., H. Taraschewski and H. Mehlhorn. 1989. Chemotherapy of fish parasites. *Parasitology Research* 75: 503-511.

Scott, M.E. 1982. Reproductive potential of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology* 85: 217-236.

Secombes, C.J. and L.H. Chappell. 1996. Fish immune responses to experimental and natural infection with helminth parasites. *Annual Review of Fish Diseases* 6: 167-177.

Sharp, N.J., C.W. Poortenaar and B.K. Diggles. 2001. Monogenean parasites on NZ kingfish. *Fish Farming International* 28: 2.

Sharp, N.J., B.K. Diggles, C.W. Poortenaar and T.J. Willis. 2004. Efficacy of Aqui-S, formalin and praziquantel against the monogeneans, *Benedenia seriolae* and *Zeuxapta seriolae*, infecting yellowtail kingfish *Seriola lalandi lalandi* in New Zealand. *Aquaculture* 236: 67-83.

Shinn, A.P., J.W. Kay and C. Sommerville. 2000. The use of statistical classifiers for the discrimination of species of the genus *Gyrodactylus* (Monogenea) parasitizing salmonids. *Parasitology* 120: 261-269.

Shinn, A.P., D.I. Gibson and C. Sommerville. 2001. Morphometric discrimination of *Gyrodactylus salaris* Malmberg (Monogenea) from species of *Gyrodactylus* parasitising British salmonids using novel parameters. *Journal of Fish Diseases* 24: 83-97.

Shinn, A.P., J.E. Bron, C. Sommerville and D.I. Gibson. 2003. Comments on the mechanism of attachment in species of the monogenean genus *Gyrodactylus*. *Invertebrate Biology* 122: 1-11.

Shinn, A.P., H. Hansen, K. Olstad, L. Bachmann and T.A. Bakke. 2004. The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salaris* and *G. thymalli* (Monogenea). *Folia Parasitologica* 51: 239-252.

Shirakashi, S., T. Yoshinaga, M. Oka and K. Ogawa. 2005. Larval attachment and development of the monogenean *Neoheterobothrium hirame* under low water temperature. *Fish Pathology* 40: 33-35.

Silan, P. and C. Maillard. 1986. Modalites de l'infestation par *Diplectanum aequens*, monogene ectoparasite de *Dicentrarchus labrax* en aquaculture. Elements d'epidemiologie et de prophylaxie. *Pathologie en Aquaculture Marine* 1986: 139-152.

Silan, P. and C. Maillard. 1990. Comparative structures and dynamics of some populations of helminths, parasites of fishes: the sea bass-*Diplectanum* model. *Acta Oecologica* 11: 857-874.

Silan, P., E. Birgi, C. Louis, F. Clota, A. Mathieu and L. Giral. 1996. Aquaculture et ichtyoparasitologie: action in vitro du nitroxinil (anthelminthique) sur *Diplectanum aequans*, monogene ectoparasite branchial du bar *Dicentrarchus labrax*. *Recueil de Medecine Veterinaire* 172: 401-407.

Šimková, A., S. Morand, E. Jobet, M. Gelnar and O. Verneau. 2004. Molecular phylogeny of congeneric monogenean parasites (*Dactylogyrus*): a case of intrahost speciation. *Evolution* 58: 1001-1018.

Slotved, H.C. and K. Buchmann. 1993. Acquired resistance of the eel *Anguilla anguilla* L. to challenge infections with gill monogeneans. *Journal of Fish Diseases* 16: 585-591.

Soleng, A. and T.A. Bakke. 1997. Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): laboratory studies. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 1837-1845.

Soleng, A., T.A. Bakke and L.P. Hansen. 1998. Potential for dispersal of *Gyrodactylus salaris* (Platyhelminthes, Monogenea) by sea-running stages of the Atlantic salmon

(*Salmo salar*): field and laboratory studies. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 507-514.

Soleng, A., P.A. Jansen and T.A. Bakke. 1999a. Transmission of the monogenean *Gyrodactylus salaris*. *Folia Parasitologica* 46: 179-184.

Soleng, A., A.B.S. Poléo, N.E.W. Alstad and T.A. Bakke. 1999b. Aqueous aluminium eliminates *Gyrodactylus salaris* (Platyhelminthes, Monogenea) infections in Atlantic salmon. *Parasitology* 119: 19-25.

Soleng, A., A.B.S. Poléo and T.A. Bakke. 2005. Toxicity of aqueous aluminium to the ectoparasitic monogenean *Gyrodactylus salaris*. *Aquaculture* 250: 616-620.

Stephens, F.J. 2001. *Health Problems of the West Australian Dhufish, Glaucosoma hebraicum*. Ph.D. Thesis, Murdoch University, Western Australia, Australia.

Stephens, F.J., J.J. Cleary, G. Jenkins, J.B. Jones, S.R. Raidal and J.B. Thomas. 2003. Treatments to control *Haliotrema abaddon* in the West Australian dhufish, *Glaucosoma hebraicum*. *Aquaculture* 215: 1-10.

Sterud, E., P.H. Harris and T.A. Bakke. 1998. The influence of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea) on the epidermis of Atlantic salmon, *Salmo salar* L., and brook trout, *Salvelinus fontinalis* (Mitchill), experimental studies. *Journal of Fish Diseases* 21: 257-263.

Svendsen, Y.S. and T. Haug. 1991. Effectiveness of formalin, benzocaine, and hypo- and hypersaline exposures against adults and eggs of *Entobdella hippoglossi* (Müller), an ectoparasite on Atlantic halibut (*Hippoglossus hippoglossus* L.). Laboratory studies. *Aquaculture* 94: 279-289.

Tappenden, T. and G.C. Kearn. 1999a. An ultrastructural study of the egg assembly region of the female reproductive tract of the monogenean (platyhelminth) skin parasites *Entobdella soleae* and *E. hippoglossi*. *Acta Parasitologica* 44: 39-50.

Tappenden, T. and G.C. Kearn. 1999b. An experimental study of egg assembly in the monogenean (platyhelminth) skin parasite *Entobdella soleae*, using a fast preservation technique and transmission electron microscopy. *Acta Parasitologica* 44: 51-62.

Thoney, D. 1990. The effects of trichlorfon, praziquantel, and copper sulfate on various stages of the monogenean *Benedeniella posterocolpa*, a skin parasite of the cownose ray, *Rhinoptera bonasus* (Mitchill). *Journal of Fish Diseases* 13: 385-389.

Thoney, D.A. and E.M. Burreson. 1988. Lack of specific humoral antibody reponse in *Leiostomus xanthurus* (Pisces: Serranidae) to parasitic copepods and monogeneans. *Journal of Parasitology* 74: 191-194.

Thoney, D.A. and W.J. Hargis Jr. 1991. Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annual Review of Fish Diseases* 1991: 133-153.

Torchin, M.E. and A.M. Kuris. 2005. Introduced Marine Parasites. In: *Marine Parasitology*, K. Rohde (ed.). CSIRO Publishing, Melbourne, Australia, pp. 358-369.

Tsutsumi, N., K. Mushiake, K. Mori, M. Arimoto, T. Yoshinaga and K. Ogawa. 2002. Effects of water temperature on the egg-laying of the monogenean *Neoheterobothrium hirame*. *Fish Pathology* 37: 41-43.

Tubbs, L.A. and M.D. Tingle. 2006a. Bioavailability and pharmacokinetics of a praziquantel bolus in kingfish *Seriola lalandi*. *Diseases of Aquatic Organisms* 69: 233-238.

Tubbs, L.A. and M.D. Tingle. 2006b. Effect of dose escalation on multiple dose pharmacokinetics of orally administered praziquantel in kingfish *Seriola lalandi*. *Aquaculture* 261: 1168-1174.

Tubbs, L.A., C.W. Poortenaar, M.A. Sewell and B.K. Diggles. 2005. Effects of temperature on fecundity in vitro, egg hatching and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriola lalandi*. *International Journal for Parasitology* 35: 315-327.

Vagianou, S.F., F. Athanassopoulou, V. Ragias, D. di Cave, L. Leontides and E. Golomazou. 2004. Prevalence and pathology of ectoparasites of Mediterranean fish, reared under three different environmental and aquaculture conditions in Greece. *Journal of the Hellenic Veterinary Medical Society* 55: 203-216.

Valtonen, E.T., M. Prost and R. Rahkonen. 1990. Seasonality of two gill monogeneans from two freshwater fish from an oligotrophic lake in Northeast Finland. *International Journal for Parasitology* 20: 101-107.

Waller, P.J. and K. Buchmann. 2001. Anthelmintic resistance and parasite control in commercial eel farms: consequences for producers. *Veterinary Record* 148: 783-784.

Wang, G., J.-H. Kim, M. Sameshima and K. Ogawa. 1997. Detection of antibodies against the monogenean *Heterobothrium okamotoi* in tiger puffer. *Fish Pathology* 32: 179-180.

Whittington, I.D. 1987. Hatching in two monogenean parasites from the common dogfish (*Scyliorhinus canicula*): the polyopisthocotylean gill parasite, *Hexabothrium appendiculatum* and the microbothriid skin parasite, *Leptocotyle minor*. *Journal of the Marine Biological Association of the United Kingdom, Plymouth* 67: 729-756.

Whittington, I.D. 1996. Benedeniine (capsalid) monogeneans from Australian fishes: pathogenic species, site-specificity and camouflage. *Journal of Helminthology* 70: 177-184.

Whittington, I.D. 1998. Diversity "down under": monogeneans in the Antipodes (Australia) with a prediction of monogenean biodiversity worldwide. *International Journal for Parasitology* 28: 1481-1493.

Whittington, I.D. 2004. The Capsalidae (Monogenea: Monopisthocotylea): a review of diversity, classification and phylogeny with a note about species complexes. *Folia Parasitologica* 51: 109-122.

Whittington, I.D. 2005. Monogenea Monopisthocotylea (ectoparasitic flukes). In: *Marine Parasitology*, K. Rohde (ed.). CSIRO Publishing, Melbourne, Australia, pp. 63-72.

Whittington, I.D. and L.A. Chisholm. 2003. Biodiversity of marine parasites in Australia: more than just a list of largely invisible creatures. *Records of the South Australian Museum Monograph Series* 7: 51-60.

Whittington, I.D. and B.W. Cribb. 2001. Adhesive secretions in the Platyhelminthes. *Advances in Parasitology* 48: 101-224.

Whittington, I.D. and I. Ernst. 2002. Migration, site-specificity and development of *Benedenia lutjani* (Monogenea: Capsalidae) on the surface of its host, *Lutjanus carponotatus* (Pisces: Lutjanidae). *Parasitology* 124: 423-434.

Whittington, I.D. and M.A. Horton. 1996. A revision of *Neobenedenia* Yamaguti, 1963 (Monogenea: Capsalidae) including a redescription of *N. melleni* (MacCallum, 1927) Yamaguti, 1963. *Journal of Natural History* 30: 1113-1156.

Whittington, I.D., L.A. Chisholm and K. Rohde. 2000. The larvae of Monogenea (Platyhelminthes). *Advances in Parasitology* 44: 138-232.

Whittington, I.D., I. Ernst, S. Corneillie and C. Talbot. 2001a. Sushi, fish and parasites. *Australasian Science* 22: 33-36.

Whittington, I.D., S. Corneillie, C. Talbot, J.A.T. Morgan and R.D. Adlard. 2001b. Infections of *Seriola quinqueradiata* Temminck & Schlegel and *S. dumerili* (Risso) in Japan by *Benedenia seriolae* (Monogenea) confirmed by morphology and 28S ribosomal DNA analysis. *Journal of Fish Diseases* 24: 421-425.

Whittington, I.D., M.R. Deveney and S.J. Wyborn. 2001c. A revision of *Benedenia* Diesing, 1858 including a redescription of *B. sciaenae* (van Beneden, 1856) Odhner, 1905 and recognition of *Menziesia* Gibson, 1976 (Monogenea: Capsalidae). *Journal of Natural History* 35: 663-777.

Williams, A. and R.C. Lethbridge. 1990. The subcutaneous attachment of the monogenean *Heterobothrium elongatum* (Diclidophoridae) in the gills of *Torquigener pleurogramma* (Pisces: Tetraodontidae). *International Journal for Parasitology* 20: 769-777.

Williams, R.E., I. Ernst, C.B. Chambers and I.D. Whittington. 2007. Efficacy of orally administered praziquantel against *Zeuxapta seriolae* and *Benedenia seriolae* (Monogenea) in yellowtail kingfish *Seriola lalandi*. *Diseases of Aquatic Organisms* 77: 199-205.

Yoshinaga, T., I. Segawa, T. Kamaishi and M. Sorimachi. 2000a. Effects of temperature, salinity and chlorine treatment on egg hatching of the monogenean *Neoheterobothrium hirame* infecting Japanese flounder. *Fish Pathology* 35: 85-88.

Yoshinaga, T., T. Kamaishi, I. Segawa and E. Yamamoto. 2000b. Effects of NaCl-supplemented seawater on the monogenean, *Neoheterobothrium hirame*, infecting the Japanese flounder. *Fish Pathology* 35: 97-98.

Yoshinaga, T., T. Nagakura, K. Ogawa and H. Wakabayashi. 2000c. Attachment-inducing capacities of fish tissue extracts on oncomiracidia of *Neobenedenia girellae* (Monogenea, Capsalidae). *Journal of Parasitology* 86: 214-219.

Yoshinaga, T., T. Kamaishi, I. Segawa, K. Yamano, H. Ikeda and M. Sorimachi. 2001a. Anemia caused by challenges with the monogenean *Neoheterobothrium hirame* in the Japanese flounder. *Fish Pathology* 36: 13-20.

Yoshinaga, T., T. Kamaishi, H. Ikeda and M. Sorimachi. 2001b. Experimental recovery from anemia in Japanese flounder challenged with the monogenean *Neoheterobothrium hirame*. *Fish Pathology* 36: 179-182.

Yoshinaga, T., T. Nagakura, K. Ogawa, Y. Fukuda and H. Wakabayashi. 2002. Attachment-inducing capacities of fish skin epithelial extracts on oncomiracidia of *Benedenia seriolae* (Monogenea: Capsalidae). *International Journal for Parasitology* 32: 381-384.

Young, P.C. 1967. A taxonomic revision of the subfamilies Monocotylinae Gamble, 1896 and Dendromonocotylinae Hargis, 1955 (Monogenoidea: Monocotylidae). *Journal of Zoology, London* 153: 381-422.

Zholdasova, I. 1997. Sturgeons and the Aral Sea ecological catastrophe. *Environmental Biology of Fishes* 48: 373-380.

CHAPTER

# 14

# Digeneans as Enemies of Fishes

**Stephen A. Bullard[1,2] and Robin M. Overstreet[3]**

## INTRODUCTION

### What is a Digenean?

Digeneans (Platyhelminthes: Trematoda: Digenea) (Plates 14.1–14.25)—sometimes referred to as flukes, trematodes, digenes, or digenetic trematodes—comprise the vast majority of Trematoda, which is one of the four entirely parasitic classes within the phylum Platyhelminthes. The other classes are the monogeneans (Monogenea or Monogenoidea, with the latter name being the earliest available name proposed for the class [see Boeger and Kritsky, 2001]), tapeworms (Cestoda), and 'turbellarians'. Collectively, the members of each class can be distinguished by their ecological attributes and appearance. Ecologically, most digeneans differ from all other platyhelminths by the combination of having (1) an indirect life cycle, which means they require more than one host to produce viable eggs, (2) a mollusc only as the first intermediate host (with exception to

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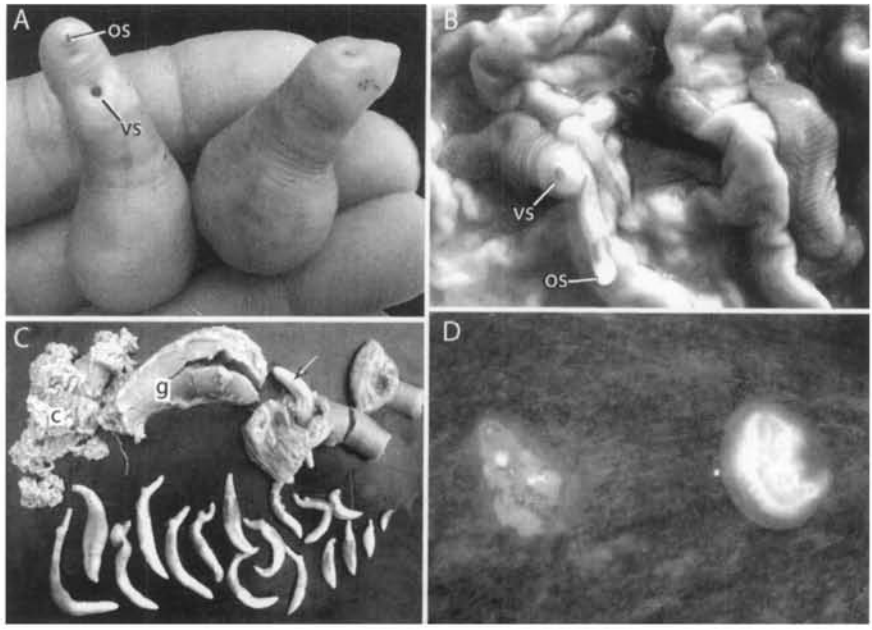


**Plate 14.1** Especially large, exemplary digeneans in fishes. **A.** Two cold-fixed adult specimens of *Hirudinella ventricosa* (Hirudinellidae), each ~60 mm in total length, collected from the stomach of a wahoo (*Acanthocybium solandri*) in the Northern Gulf of Mexico, showing the obvious oral- (os) and ventral suckers (vs). This species is one of the relatively few digeneans having a common name, 'wahoo worm', used by fishermen of pelagic gamefishes. **B.** Stomach of yellowfin tuna (*Thunnus albacares*) infected with several specimens of *H. ventricosa* sensu lato occupying the spaces between rugae; ventral sucker (vs) and oral sucker (os). **C.** Gut (g), primarily the stomach and anterior portion of the intestine, of a longnose lancetfish (*Alepisaurus ferox*) from the western Pacific Ocean. The alimentary canal of this fish was heavily infected with many specimens of the tetraphyllidean cestode *Pelichnibothrium speciosum* (see Scholz *et al.*, 1998) that partially occluded the intestine as well as a group of at least 18 specimens of *Botulus microporus* (Hirudinellidae): largest, which is ~60 mm in total length, to smallest specimens arranged left to right, with one specimen remaining in the gut (arrow). Infection intensities such as this are reportedly common in this host. **D.** Specimens of *Nagmia floridensis* (Gorgoderidae), the largest specimen is ~20 mm in total length, collected from the body cavity of a southern stingray (*Dasyatis americana*) from Horn Island, Mississippi. In life, the ceca of these flukes appeared bright yellow.

some fish blood flukes [Aporocotylidae] that use a polychaete first intermediate host), (3) asexual reproduction in the first intermediate host, and (4) always a vertebrate as the definitive host (although some species have a progenetic metacercaria in arthropod and molluscan second intermediate hosts that may enable the fluke to perpetuate its life cycle when definitive hosts are absent [Buttner (1951)] (Plates 14.8, 14.10–14.14, 14.16, 14.20, 14.22). Morphologically, digeneans differ from other

platyhelminths by the combination of having a non-segmented body, a mouth, and a gut as well as an anterior muscular sucker (oral sucker) surrounding the mouth and a medioventral sucker (termed a ventral sucker or acetabulum) (Fig. 14.1, 14.6, 14.7). Most adult digeneans are about one to several mm in total length. However, several of them are grossly apparent and a few are very large (Fig. 14.1A). As an extreme example, the thread-like body of the didymozoid species *Nematobibothrioides histoidii* (cf. Fig. 14.25F), which infects ocean sunfish (*Mola mola*), can be longer than 12 m. Additional obvious exceptions to this generalized description are the aporocotylids (Plates 14.16–14.21) and unrelated 'monostomes' that lack a medioventral sucker, bucephalids that have a mouth located well-posterior to the anterior end (Fig. 14.6W; Plate 14.14), and 'amphistomes' that have a posteriorly located ventral sucker. For those species not lodged in vessels or within tissues, attachment is usually accomplished with a strong to weak ventral sucker, often accompanied by the anterior oral sucker (Figs. 14.7M-P). The bucephalids attach by the anterior rhynchus, sometimes aided with an incorporated hood, sucker, glandular material, or tentacles. Some diplostomoids attach by an adhesive pad (Erasmus, 1972) or the anterior portion forms a cup, as do some lepocreadiids. Members of the Aspidogastrea, a small group of about 80 freshwater and marine species that is the sister group to the Digenea, have an attachment organ consisting of numerous suckerlets, rugae, or alveoli (Fig. 14.7M) (Rohde, 2005). The syncytial tegument of at least one digenean, the homalometrid *Crassicutis archosargi*, can transform into a thickened adhesive surface—dorsally or ventrally—that is histochemically different from its counterpart on the unattached (free) surface (Overstreet, 1976). Monogeneans, sometimes referred to as monogenetic trematodes, monogenes, or monogenoideans, typically infect external surfaces of fishes, e.g., skin, gill, olfactory lamellae, and fins, and they differ from digeneans by having the combination of a posteriorly located attachment organ (haptor) as well as exhibiting a direct life cycle, which means they require only a single host to produce viable eggs. Tapeworms are usually segmented, and the vast majority of aquatic species have indirect life cycles that typically include a crustacean first intermediate host (Caira and Reyda, 2005). The 'turbellarians' (see Rieger *et al.*, 1991) comprise a large, primarily free-living paraphyletic group of platyhelminths that has several parasitic species (Awerinzew, 1925; Ball and Khan, 1976; Blasiola, 1976; Cannon and Lester, 1988; Benz and Bullard, 2004), at least one of those, i.e., *Paravortex* sp., can cause diseases characterized by host death,

acute focal dermatitis, and secondary infections of '*Vibrio* sp.' (Kent and Olson, 1986). Most turbellarians differ from all other platyhelminths by the combination of lacking an oral sucker, moving in a gliding fashion by using multiciliated epithelial cells, and having a direct life cycle (Cannon, 1986).

## Fishes as Hosts for Digeneans

Herein, we follow Nelson (2006) in using the term 'fish' not as a taxonomic rank but simply as a term that includes all primary craniate lineages (i.e., vertebrates plus hagfishes [Myxiniformes]) except Tetrapoda. Fishes form a paraphyletic, heterogeneous assemblage of vertebrates that, for the most part, live in water, have gills throughout life, have fin-like limbs, and typically comprise the focus taxa in ichthyology courses (Nelson, 2006). Digeneans mature in vertebrates and probably outnumber them. As larvae and juveniles they infect many invertebrates and vertebrates, including fishes, as intermediate hosts (Plates 14.2–14.5), and as adults they infect members of nearly every vertebrate phylum (although primarily restricted to bony fishes [Plates 14.6, 14.7], amphibians, reptiles, birds, and mammals). Collectively, digeneans constitute perhaps the most morphologically and ecologically diverse group of platyhelminths (Yamaguti, 1971). They amass the largest group of monozoic platyhelminths and possibly the largest group of internal metazoan parasites, including ~18,000 nominal species in ~2,500 genera in ~150 families. Fishes host an astonishing number of digeneans. The ~27,977 extant fish species account for over half (51%) of all living vertebrates, and based on the many new digenean species that are named and described each year from fishes, the number of digeneans infecting fishes will soon probably exceed the number of fish species.

## Fish Tissues as Habitats for Digeneans

Adult and larval digeneans in fishes have been reported from nearly every imaginable tissue and organ, including eye (Figs. 14.4A-B, 14.7A, D, 14.9F), brain (Figs. 14.5L, 14.9A-C), gill epithelium (Figs. 14.4G-H, 14.17A-C, E, F, I), skin (Figs. 14.9E, 14.25G), fins (Figs. 14.4J-K, 14.15), immediately beneath the scales (Fig. 14.7I), throat, gill arches (Figs. 14.7L, 14.24A, 14.25A-E), gill filaments (Figs. 14.4G-H, 14.9G; Plate 14.24), gill rakers (Fig. 14.7P), branchial blood vessels (Figs. 14.17D, G-H), cardiac muscle (Figs. 14.4E-F, 14.9H, J-K, 14.18A-C, E), bulbus arteriosus (Figs. 14.4C-D, 14.7H, J, 14.9D, I), ventricle (Fig. 14.4I),

pericardial chamber, musculature of the head and body (Figs. 14.5G-H, 14.7C, F-G, K), body cavity (Fig. 14.1D), mesentery, beneath the serosal lining (Fig. 14.4L; Plate 14.19), mesenteric blood vessels (Figs. 14.19B), ovary, liver (Figs. 14.4L, 14.9L), pancreatic tissue (Figs. 14.18D, F), kidney, spleen, and gall bladder as well as stomach (Fig. 14.1B), intestine (Figs. 14.1C, 14.7E, M-O), and rectum where most of them occur.

Most major groups of digeneans seem concentrated in a particular site in their definitive host, but many groups have aberrant species that infect atypical sites. For example, bucephalids, hemiurids, and cryptogonimids typically mature in the gut of fishes but the bucephalid *Paurorhynchus hiodontis* infects the body cavity of mooneye (*Hiodon tergisus*) (e.g., Manter, 1957); the hemiurid *Gonocerca macroformis* infects the ovary of cod (*Gadus morhua*); the cryptogonimid *Acetodextra ameirui* infects the swimbladder, body cavity, and ovary of ictalurid catfishes (e.g., Paperna and Dzikowski, 2006); and several other published and unpublished examples exist. Adult digeneans that infect extra-intestinal sites in fishes are probably frequently overlooked because few workers carefully examine those sites during routine necropsies. Plus, when specimens are discovered in those unusual sites, the significance of the find may be discounted by default as 'accidental' or as contamination from the gut. Further, the flukes themselves may be misidentified as representing a species of another platyhelminth group renown for infecting that site. Several families of Digenea include species that primarily infect extra-intestinal sites, e.g., vascular system (Aporocotylidae) (Plates 14.16–14.21), somatic musculature (Didymozoidae) (Plates 14.22–14.25), and body cavity and visceral organs (Gorgoderidae) (Fig. 14.1D). On the other hand, others are bona fide ectoparasites, e.g., throat and gill arches of pelagic elasmobranchs (Syncoeliidae) and gill filaments of marine fishes (Hemiuroidea). In the gut or in any of these sites, the intensity of infection can be extreme (e.g., Fig. 14.1C), sometimes with flukes collectively outweighing the host fish (Miyanaga, 1939).

## Host and Site (Tissue/Organ) Specificity

Few, if any, digeneans can infect any site within any single host species or any single site in all or most fish species. Hence, all digeneans exhibit some degree of host and site specificity. 'Host specificity' is a relative term that attempts to pigeonhole a parasite species into a category based on how many groups of hosts that parasite infects. These 'groups' could be defined

in a phylogenetic context (host lineages, e.g., susceptible species, genera, families, or orders) or an ecological context (e.g., herbivorous vs. carnivorous, benthic vs. pelagic, schooling vs. solitary, oceanic vs. estuarine) or some combination of both (Holmes and Price, 1980; Benz and Bullard, 2004). A parasite regarded as highly host specific infects few hosts, e.g., a parasite that matures in only one fish species is highly host specific; whereas, a parasite that matures in numerous fish species has a lower host specificity (see Caira *et al.*, 2003, for additional terminology). Digeneans certainly are not accurately depicted as opportunistic pathogens capable of infecting a wide ecological and phylogenetic spectrum of fishes. In fact, several species seem specific to an ecological type of host(s) or a host lineage such that, for example, related digeneans infect fishes with similar feeding ecologies or similar life histories or that share a common ancestor.

Still, the matter of host and site specificity in digeneans is especially confusing because for an individual species different grades of specificity exist for definitive hosts, second intermediate hosts (in some cases, third intermediate hosts), and the molluscan first intermediate hosts. Some digeneans exhibit a low degree of host specificity, infecting a variety of fishes and other definitive hosts. For example, some heterophyids can mature in several piscivorous birds, rodents, and carnivorous mammals (including dogs and humans), and their metacercariae can infect a broad spectrum of intermediate fish hosts (Paperna, 1991). Other heterophyids have but a single vertebrate definitive, fish intermediate, and molluscan host. Our understanding as well as that of many researchers of host specificity is based on a combination of personal experiences with and literature records of parasites collected from hosts, hence the count of how many kinds of hosts a parasite can infect depends heavily on collection effort. Digeneans as a group are regarded as the least host-specific platyhelminths, but many species, nonetheless, have been reported from a single intermediate or definitive host fish only. It is possible that some species seem to be highly host specific because they have been collected only once or none of the other sympatric hosts that are phylogenetically or ecologically related to that host had been concurrently examined for the presence of that digenean. However, accounting for collection effort, many digeneans indeed seem specific to a single definitive host species. For example, Bullard *et al.* (2006) examined 250 individual sharks comprising 17 species captured from the same geographic region (Northern Gulf of Mexico) and detected an infection of *Selachohemecus*

*benzi* (Aporocotylidae) in the blacktip shark (*Carcharhinus limbatus*) only. Yet, interestingly, other aporocotylids, e.g., *Paradeontacylix* spp. and *Psettarium* spp., seem less host specific to their definitive teleost hosts (Smith, 1997b; SAB, unpublished data). As another example, cercariae of the bucephalid *Bucephaloides gracilescens* encyst in gadid fishes, but not sympatric bothids, pleuronectids, and gobiids, perhaps because of the nature of the gadid-type acoustico-lateralis system which may be required for successful cercarial migration (Crofton and Fraser, 1954; Matthews, 1974a). Of the numerous heterophyids in cyprinidontiform fishes in Mississippi, the great majority infect one or two fishes, and all or nearly all metacercariae of a single species infect a very specific site, e.g., base of gill lamellae, distal portion of gill lamellae, liver, mesentery, intestinal serosa, meninges, conus arteriosus, or ventricle of heart. A distinction can also be drawn between hosts identified from experimental studies and those identified from collections of wild animals. Although some hosts are susceptible to infection under experimental conditions, these same hosts are seldom if ever found to be infected in the wild, indicating that host immunophysiological factors plus environmental factors determine host susceptibility and, by extension, digenean host specificity (Skirnisson *et al.*, 2004). We have also determined that experimental infections with large numbers of cercariae of species having a restricted site will result in a few metacercariae in ectopic sites.

Regarding fish disease, the least host-specific parasites have the potential to infect and cause problems in the most hosts; whereas, the most host-specific parasites are more easily screened for because they infect the fewest hosts. Where relevant to the discussion of fish disease, host specificity is treated for individual taxa included in the Pathogens section. Records of hosts for digeneans can also be derived from the synoptical works of Yamaguti (1971, 1975).

## Scope

We discuss digeneans in the context of fish disease with special reference to those groups (usually families) holding species that as eggs (Plates 14.17–14.19), larvae (Plates 14.2–14.5, 14.9), or adults (Plates 14.6, 14.21, 14.23) comprise pathogens of fishes in the wild or aquaculture. Doubtless, this list will grow as more information is published regarding digeneans in wild fish populations and as the aquaculture industry expands to include more fish species grown in more types of culture

systems, each of which will introduce new parasite problems. Herein, we provide general information about the bauplan and life cycle of digeneans, overview diagnostic methods including taxonomy, histology, and molecular markers, provide references for the biological and chemical control of digenean infections, and discuss dynamics of digenean infections in terms of wild and captive Host-Parasite Relationships (HPRs) (see also Williams, 1967; Paperna, 1995; Paperna and Dzikowsky, 2006). For select families that include fish pathogens, we provide a few specific examples dealing with their morphological features, hosts, sites of infection, life cycles, and pathological alterations to host tissues. Although we have tried to avoid being myopic or redundant in our treatment of digeneans as enemies of fishes, some pertinent references have been omitted for economy of space; however, these references can be obtained by perusing the bibliographies of the papers cited herein. Also, we emphasize that the 'typical digenean' is practically non-existent because of the extensive evolutionary diversification of body plans and life history strategies within this group of platyhelminths. For these reasons, there are exceptions to many of our statements, but we spotlight some of these interesting exceptions and provide references to the primary literature where appropriate.

## First-stop References

We find several references particularly useful for an initial attempt in identification: the synopses by Satyu Yamaguti (Yamaguti, 1971, 1975), the keys to the digeneans published in three volumes by CAB International and the Natural History Museum, London, England, (Gibson *et al.*, 2002; Jones *et al.*, 2005; Bray *et al.*, in preparation), Glenn Hoffman's book on the parasites of North American freshwater fishes (Hoffman, 1999), Stewart C. Schell's keys to the trematodes (Schell, 1970, 1985), and Malcolm McDonald's key to digeneans in waterfowl (McDonald, 1981). K.I. Skrjabin had a long series of Russian books that treated a translation of keys and descriptions of digenean species from various languages into Russian, some of which were then translated from Russian to English (*Trematodes of Animals and Man: Essentials of Trematodolgy*). There exist good treatments of fish digeneans in general reference books dealing specifically with fish parasites (e.g., Rohde, 1984; Williams and Jones, 1994; Paperna, 1995; Benz and Bullard, 2004; Paperna and Dzikowski, 2006), and there are good treatments on diversity,

ecology, and evolution in a variety of books (e.g., Bush *et al.*, 2001; Combs, 2001; Rohde, 2005). Several general textbooks on parasites treat some fish digeneans (e.g., Hyman, 1951; Beaver *et al.*, 1984; Kearn, 1998; Roberts *et al.*, 2004). Miscellaneous reference books like Edward Noga's book covering diagnosis and treatment of fish diseases (Noga, 2000) and the book *Interrelationships of the Platyhelminthes* (Littlewood and Gibson, 2001) treat fish digeneans and provide important references. Numerous other helpful books exist, as do numerous websites (e.g., http://www.diplectanum.dsl.pipex.com/purls; http://asp.unl.edu).

## LIFE CYCLE

We concur with Ginetesinskaya (1961) who stated that the life cycles for digeneans that use fishes as intermediate or definitive hosts vary widely (e.g., Plates 14.8, 14.10–14.14, 14.16, 14.20, 14.22), making it especially difficult to arrange these digeneans into a relatedness scheme that is concordant regarding host phylogeny plus the combination of the life history and morphology of the digenean.

A general pattern for the digeneans is that they have indirect life cycles (Plate 14.8). The life cycle includes a cyclic alternation of homologous generations, of which the miracidium/mother-sporocyst and the daughter germinal-sac generations usually reproduce by apomictic parthenogenesis, i.e., 'true clonality' or a form of reproduction wherein the reduction division of meiosis is suppressed thereby producing offspring that are genetically identical to their mothers and heterozygosity is maintained (Suomalainen, 1950). Polyembryony—the development of more than one embryo from a single egg or ovule—may also occur (James and Bowers, 1967). All digeneans except some aporocotylids (Plates 14.16, 14.20) have a molluscan first intermediate host wherein asexual reproduction occurs at least twice and a vertebrate final (= definitive) host wherein adults undergo sexual reproduction (Yamaguti, 1975). Many species use vertebrates as second intermediate hosts also. The general sequence of digenean life history stages is (1) egg, (2) miracidium, (3) sporocyst (may or may not include daughter sporocyst) then (4) redia (if present, may or may not also include daughter redia), (5) cercaria, (6) metacercaria (if present, encysted, encapsulated, or free), and (7) adult. There is extensive morphological variability among species regarding the appearance and ecology of these life history stages (Plates 14.2, 14.3, 14.5, 14.6), and each life history stage requires some action from each of its

hosts. This concept is best demonstrated by the fact that *in vitro* cultivation of digeneans is extremely difficult, requiring a virtual battery of culture media and conditions (e.g., Kannangara and Smyth, 1974; Smyth, 1990; Stewart *et al.*, 2003b; Fredensborg and Poulin, 2005). Although the various species of digeneans differ regarding the appearance, behavior, and infection strategy of their larvae, phylogenetically related digeneans seemingly share at least some similar life history characteristics (although in many cases, related worms exhibit strikingly different life history characteristics that have occasionally misled digenean systematists). That being said, the life cycles of digeneans can be broken down into six broad categories that relate to the 'directness' of cercarial transmission (Cribb, 2005). The first four include only two hosts, an intermediate host (mollusc) and a definitive host (fish); whereas, the remaining two include three hosts (an additional intermediate host following the molluscan first intermediate host) or an additional host as a carrier host (= paratenic host; a host in which no larval development occurs): (1) the cercaria penetrates the muscle and connective tissue of the definitive host directly and may then migrate to and within the lymphatic system or blood system of that particular host before finding the appropriate site and maturing (in all schistosomatoideans), (2) a large cercaria is ingested by the definitive host before developing in the definitive host's gut (in some species of Haploporidae, Bivesiculidae, Azygiidae, Fellodistomatidae, and Tandanicolidae- a life history strategy that may be the most primitive among digeneans), (3) the cercaria attaches to and matures on the body surface of the definitive host, typically beneath the scales of marine fishes in the Indo-Pacific Region (in all species of Transversotrematidae), (4) a cercaria with cystogenous glands emerges from the first intermediate host (mollusc) and encysts on the surface of a food item of the definitive host (in most or all species of Paramphistomoidea, Pronocephaloidea, Haplosplanchnoidea, and Haploporidae), (5) the cercaria is eaten by a crustacean and then penetrates the crustacean's body cavity or remains in the crustacean's gut before the crustacean is eaten by the definitive fish host, and (6) a cercaria with well-developed penetration glands (sometimes associated with a stylet for penetrating the host, e.g., piercing the joints and appendages of arthropods) penetrates the second intermediate host and resides there as an encysted or unencysted metacercaria before that host is eaten by the definitive host.

## Egg

Eggs are ectolecithal, usually ovoid, and the shell is light yellow to dark brown, depending on the tanning; some measure about 0.1 mm in total length, but 0.025 mm is not unusual for digeneans in fishes. Egg size is widely variable among species and in some cases within an individual; measurements for diagnostic purposes should be restricted to those eggs being deposited or those at the distal end of the uterus. An operculum may or may not be present at one end of the egg. Most digeneans lack visible spines and filaments, unlike those of monogeneans, but some (e.g., some members of Opecoelidae, Monorchiidae, and Hemiuroidea) may have a long or short filament or a knob. Filaments may be unipolar or less often bipolar (Fig. 14.17A) or multipolar. After the egg is formed, it is released through a genital pore that may be shared with the male genital pore, may be separate or, most typically, empties into a common genital atrium. Typically, the egg is passed in the feces of the definitive fish host, although adult flukes that infect extra-intestinal sites may exhibit a different strategy (e.g., most aporocotylids and didymozoids). The egg may hatch after being consumed by the mollusc first intermediate host, it may hatch in the aquatic environment, or occasionally it may hatch in utero. At least some aporocotylids have eggs that hatch while embedded in the host's gill epithelium (Plate 14.17). Within each egg is a single miracidium (Fig. 14.17B), the first infective stage of the parasite. Fertilization is accomplished by cross-fertilization or self-insemination, if only a single hermaphroditic individual is present. For most species, details of the process of egg formation and oogenesis is poorly understood, if even studied at all. However, generally speaking, ova are fertilized within the ootype or uterus before yolk material (vitelline and shell-forming material produced by a vitellarium) floods into the lumen, surrounding the fertilized ova, and soon thereafter the eggshell material coalesces and the egg capsule hardens. Smyth and Clegg (1959), among others, used histochemistry to study the eggshell formation in digeneans and cestodes. Their study showed that the capsule of many digenean eggs comprises a sclerotizing protein that is stabilized and hardened by a quinone-tanning process, which produces the dark brown appearance of some eggs. Sclerotin precursors are secreted by a group of vitelline cells. Dyes and stains reveal up to several different products, depending on the species, being produced by the membrane-bound or free Mehlis gland.

## Miracidium (Plural = Miracidia)

As the only intensely ciliated life-history stage for digeneans, the miracidium (Fig. 14.8.2) is a small, nearly transparent, elongate larva that may or may not have one or two eyespots. Those without pigmented eyespots may have a pair of lamellated ciliary organs with presumptive photoreceptive function (Dunn *et al.*, 1987). The body of most species has several ciliated epidermal plates (e.g., Fig. 14.8A) that facilitate locomotion either within the first intermediate host's gut or in the water column. Many miracidia have a terebratorium, a cone-shaped apical papilla, at the anterior tip. This portion lacks cilia, but is equipped with sensory receptors and gland openings (Brooker, 1972). The miracidium also has an apical gland that produces lytic enzymes/secretions that presumably aid in the penetration. As a general rule, fully embryonated eggs of species with small eggs are eaten by the molluscan host and the miracidium of those with large eggs hatch and seek the tegument of the mollusc. The manner in which the miracidium finds its mollusc host is complex and unknown for the vast majority of species, but secretory products from the mollusc may provide the chemical attractant; after 50 years of intensive research on the subject, still little is confirmed (MacInnis *et al.*, 1974; MacInnis, 1976; Saladin, 1979; Kalbe *et al.*, 1997). Within the mollusc develops a mother sporocyst (Fig. 14.8.3) from the germ cells of the miracidium, and, following that, asexually produced daughter sporocysts or redia (Fig. 14.8.4), in turn, can produce cercariae (Figs. 14.8.5, 14.8.6), the subsequent asexually produced larval form. The miracidium contains within it an astonishing potential for producing large numbers of individual flukes, and cercarial shedding from the first intermediate host can be prolific regarding numbers of cercariae and duration of shedding. Rothchild (1942) recorded an original infection of the heterophyid *Cryptocotyle lingua* in the winkle *Littorina littorea* that produced ~1,000 cercariae per day at seven years post-infection (Beaudette, 1945).

## Sporocyst

Perhaps one of the strangest forms of an animal, sporocysts are germinal sac-like bodies formed after the miracidial transformation within the foot, tentacles, viscera, or mantle of the first intermediate host (always a mollusc except for aporocotylids).

Whether a daughter sporocyst or a redia is produced, usually, but not always, is determined by the fluke's ancestry, i.e., its phylogenetic placement. Mother and daughter sporocysts usually seem indistinguishable and time-sequence experiments may be required to identify which is which. The shape of the sporocyst varies widely from spherical, ovoid, elongate, or branched, depending on the species, the number present in a mollusc, and the tissue infected. The body wall of the sporocyst (brood chamber) is covered by a syncytial tegument that has microvilli; beneath this tegument is a thin layer of connective tissue termed a 'basal lamella' covering subtegumental musculature, cytons, parenchymal cells, and a lumen containing germinal cells (Fried and Haseeb, 1991). The sporocyst lacks a pharynx or mouth (Fig. 14.8.3), and the produced cercariae either escape from a birth pore near the anterior end of the sporocyst or from a rupture in the syncytium. Nutrients are probably absorbed through the permeable tegument (James *et al.*, 1966).

## Redia (Plural = Rediae)

Rediae are diagnosed by their mouth, pharynx, and a sac-like gut (Fig. 14.8.4). Because the mouth may be located at the blunt end of the body, the redia superficially resembles some adult flukes and is considered the primitive stage by some researchers. Rediae can be released through the birth pore or a rupture similar to the release of cercariae. Like sporocysts, a second or third generation of redia can be produced in the life cycle of some species, e.g., some echinostomes (Fried and Haseeb, 1991). Rediae are more active than sporocysts and some can migrate within the mollusc's tissues. Rediae feed on molluscan tissue, and some are predators of sporocysts and other rediae, conspecific or not, within the mollusc. The muscular pharynx of the redia serves to pump food into the gut. Rediae have a well-developed excretory system as well as sub-tegumental muscles and cytons. Fried and Hasseeb (1991) point out that the reason why rediae stop producing other rediae and begin to produce cercariae is indeterminate, but population density may control the switch because echinostomatid rediae transplanted to new snails continue to produce only rediae and not cercariae (Dönges, 1970).

## Cercaria (Plural = Cercariae)

The cercaria is a juvenile digenean that usually has a tail and that, upon losing its tail, migrates within the host (Plates 14.2, 14.8). In addition to

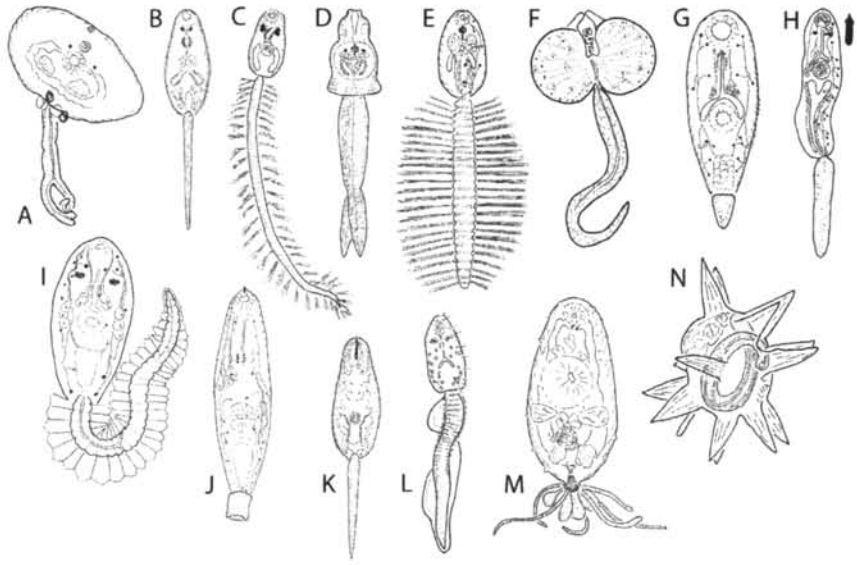


**Plate 14.2** Representative cercariae of digeneans involving a fish in their life cycle. All drawn from Yamaguti (1975). **A.** *Transversotrema laruei* (Transversotrematidae). **B.** *Saccocoelioides sogandaresi* (Haploporidae). **C.** *Lepocreadium album* (Lepocreadiidae). **D.** *Cercaria caribbea* 57 (Bivesiculidae). **E.** *Cercaria caribbea* 53 (Fellodistomidae). **F.** *Phyllodistomum staffordi* (Gorgoderidae). **G.** *Telolicithus pugetensis* (Monorchiidae). **H.** *Stephanostomum tenue* (Acanthocolpidae), note cercarial stylet at right. **I.** *Stephanostomum dentatum* (Acanthocolpidae). **J.** *Podocotyle lepomis* (Opecoelidae). **K.** *Microphallus opacus* (Microphallidae). **L.** *Caecincola parvulus* (Cryptogonimidae). **M.** *Genarchella genarchella* (Hemiuridae). **N.** *Cercaria caribbea* 74 (Hemiuridae).

the tail, most have a mouth, pharynx, intestine, pigmented eyespots, and glands of various types (e.g., cystogenous, penetration, mucus lubricant, or adhesive). The anterior end may have a stylet (xiphidiocercariae) or rasping minute spines with or without glandular secretions. Most have a ventral sucker and some have tegumentary spines and primordia of genital organs. The tail can be forked (Figs. 14.10C, 14.11B, 14.12B, 14.16D, 14.20D), be swollen, have a finfold (Figs. 14.2L, 14.20D), or have setae, finlets, bristles, or other ornamentation (Figs. 14.2C, 14.2E); it can be long or short, with or without a sucker (microcercous), or be absent (cercariaeum); and it can retract its body into a large chamber at the basal part of the tail (cystocercous) (Plate 14.2). Some cercariae infecting fishes as second intermediate and definitive hosts (1) are preyed upon by the second intermediate or definitive host, (2) are encysted on the intermediate host, (3) are encysted on aquatic vegetation, debris, or the water's

surface, (4) actively attached to and penetrated the gills or body surface, or (5) passively entered the buccal cavity and penetrate the gills. Mohandas (1971) and others described the tegumentary papillae of cercariae and their taxonomic significance. Matricon-Gondran (1971) and others provided observations of the nervous system in larval digeneans. Because of the great variety of life history strategies, there is great morphological diversity of cercariae. Cercariae emerge from the first intermediate host (a mollusc or a polychaete in a few blood flukes) and, depending on the species, infect either a second intermediate host (an invertebrate, vertebrate, or substratum vector) or a definitive host fish (Plate 14.8). Some cercariae crawl and a few either do not leave their mollusc host or are shed and then infect it, using the host as both a first and second intermediate host. Most cercariae swim and these may exhibit positive or negative phototaxis (related to specific light wavelengths) or geotaxis (up or down relative to the benthos). Cercariae must find a host quickly because they do not feed in the water column and are short-lived (hours to days, e.g., Kniskern, 1952; Matthews, 1973a).

The diversity of morphological features, with exceptions, usually relates to the phylogenetic group as well as life history strategy. Taxonomically, the origin and shape of the tails, excretory system (number and pattern of flame cells and ducts), and type of penetration organ (if any) are among several important features. For example, Diplostomoidea and Aporocotylidae have furcocercous cercariae with forked tails and rasping penetration organs with associated glands producing proteolytic enzymes; most known members of Azygiidae, Bivesiculidae, and Hemiuridae are also furcocercous, but they are also cystophorous, retracting their body into a large chamber at the basal part of the tail. Heterophyidae has members with a pleurolophocercous cercaria (with a non-divided tail having a finfold) containing a rasping oral organ similar to those above; most members of Haploporidae and Haplosplanchnidae have a long tail and lack penetration glands but have cystogenous glands (gymnocephalus cercariae), allowing them to encyst on a substratum such as submerged aquatic vegetation (Overstreet and Curran, 2005); most members of Opecoelidae (cotylomicrocercous cercariae) have a short sucker-like tail allowing the cercaria to crawl in a leech-like fashion onto the joints of a crustacean intermediate host where it can enter using a stylet. However, not all members of a family have similar cercarial types, and some features, such as the lack of ventral sucker, features of a tail, and

loss of eyespots, have evolved multiple times in multiple digenean lineages.

La Rue (1957) separated digeneans into Epitheliocystida (e.g., Opisthorchioidea, pleurolophocercous cercariae, and Plagiorchioidea, xiphidiocercariae) and Anepitheliocystida (e.g., Brachylaimoidea, forked tailed cercariae, and Echinostomoidea, gymnocephalus cercariae) on the basis of whether or not an epithelial layer lined the excretory bladder of the cercaria. In anepithelial forms, the bladder forms by the union of the two primitive protonephridial ducts in the cercarial embryos. Initial formation of epithelial bladders was similar except that a cellular mass of mesodermal origin supposedly enveloped the region of the bladder to form a layer of cuboidal cells. Cable (1974, 1982) pointed out that research was beginning to show that the epithelial bladders include or constitute syncytial features but that the relationship between the excretory system and the cercarial tail—rather than the form of the bladder—provided a better phylogenetic basis for classification. The tail of primitive taxa forms by molding of the embryo, resulting in ducts extending all along the tail, and, at the other extreme, the tail forms by proliferation between the primary excretory pores, leaving the pores at the junction of the cercarial body and tail. Both processes occur in some intermediate taxa. There is apparently a loss of some structures, such as the paranephridial system (Pearson, 1986) in the more derived taxa, and some tail features such as ornamentation and rudimentation can be misleading in phylogenetic studies (Cable, 1974). More recently, molecular data has provided strength to the majority of families considered in Cable's traditional, but not universally accepted, scheme (Cribb *et al.*, 2001a, b; Olson *et al.*, 2003).

## Metacercaria (Plural = Metacercariae)

The metacercarial life history stage is often encountered by fisherman, ichthyologists, and naturalists who are interested in fishes, with records existing for at least 221 years (Munro, 1785) (Plates 14.3–14.5, 14.7, 14.9). Although often considering the metacercaria a quiescent stage in the second intermediate host, current researchers demonstrate that intricate physiological interactions exist at least between some encysted or encapsulated flukes and their host. In a few cases, the metacercaria of some species produce eggs while in the intermediate host's tissues. Further, in some groups, this juvenile stage neither encysts nor becomes

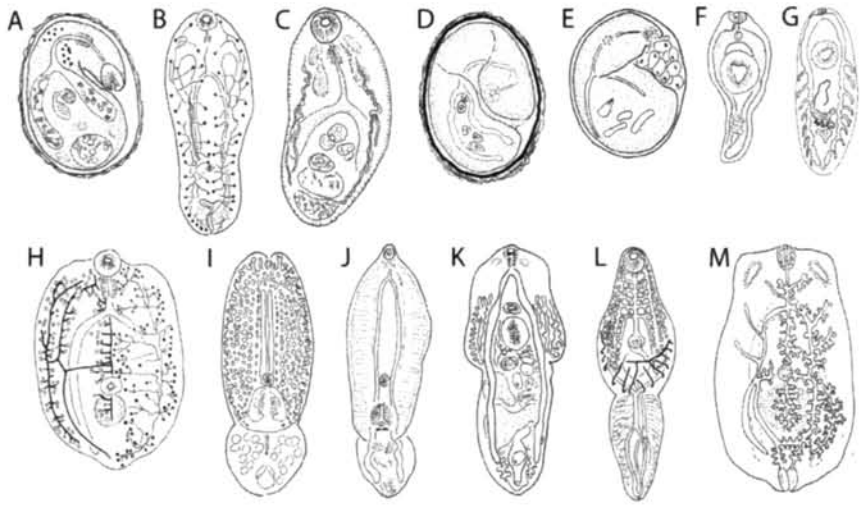


**Plate 14.3** Representative metacercariae of digeneans that infect fishes as second intermediate hosts and mature in piscivorous birds. Mostly derived from Yamaguti (1975). **A.** *Stellantchasmus falcatus* (Heterophyidae), encysted. **B.** *Cryptocotyle lingua* (Heterophyidae), excysted. **C.** *Haplorchis pumilio* (Heterophyidae), excysted. **D.** *Euhaplorchis californiensis* (Heterophyidae), encysted. **E.** *Stictodora tridactyla* (Heterophyidae), encysted. **F.** *Clinostomum marginatum* (Clinostomidae) excysted. **G.** *Euclinostomum indicum* (Clinostomidae), excysted. **H.** *Diplostomum spathaceum* (Diplostomidae). **I.** *Posthodiplostomum minimum* (Diplostomidae). **J.** *Posthodiplostomum cuticola* (Diplostomidae). **K.** *Diplostomum phoxini* (Diplostomidae). **L.** *Uvulifer ambloplitis* (Diplostomidae). **M.** *Apatemon gracilis* (Strigeidae).

encapsulated; it actively feeds and migrates within the fish host and is termed 'free'. Unencysted metacercariae are present in the life histories of several ecologically different types of digeneans. For example, at least *Bacciger* sp. (Fellodistomatidae) infects pelagic cnidarians (ctenophores and jellyfishes) as a second intermediate host. Its cercaria, when placed in a dish with the ctenophore, is carried into the digestive tract by host ciliary action before the cercarial tail is shed. There, it remains unencysted, transforming to a metacercaria and growing to be several times larger than the cercaria without harming its host. Another example of a non-encysted metacercaria is the diplostomulum of *Austrodiplostomum compactum* that infects the eye vitreous humor, optic nerve, brain, and spinal cord of the fish second intermediate host without harming the fish unless present in very large numbers (Overstreet and Curran, 2004).

Every metacercaria develops from a cercaria, whether free or encysted; some remain poorly developed and take 1-3 weeks to mature

when acquired by the definitive host compared with 1-3 days for others. Some metacercariae cause disease in invertebrate (Miller, 1963) and vertebrate hosts and others mature. Aporocotylids lack a metacercaria in the true sense since the unencysted, migrating stage, termed a 'schistosomulum', continues to develop (e.g., Fried and Haseeb, 1991; Bullard and Overstreet, 2002), and some unencysted hemiuroideans (e.g., Syncoeliidae and Hemiuridae) rupture from their intermediate euphasid (syncoelid) host and externally attach to other crustaceans or even remain at the surface of the water. Overstreet (1970) reported larval specimens of *Syncoelium* sp. on a copepod (*Candacia pachydactyla*). Others, such as some hemiurids, may have a paratenic host, one of which feeds on the second intermediate host and maintains the unencysted metacercaria without developing into the following adult stage. For example, chaetognaths, or 'arrow worms', feed on hemiurids and maintain them, making them available for fish definitive hosts to feed on them. On the other hand, *Ferosagitta hispida* (as *Sagitta hispida*) from Biscayne Bay, Florida, presumably feeds on copepods infected with *Parahemiurus merus*. In this case, the free metacercariae fed on the gonads of the arrow worm, produced eggs in one month, and then ruptured out of the arrow worm's coelomic cavity and survived several days in full strength sea water (Overstreet, 1969; Overstreet, unpublished data). Lebour (1923) found a young specimen of *Hemiurus communis* emerging from each of two individual copepods (*Acartia clausi*) that she likened to those reported by Pratt (1898). We have noted that copepod-feeding anchovies often have several free paratenic specimens of *Lecithochirium* sp. in the swimbladder. Despite these hemiuroid and aporocotylid examples, the vast majority of digeneans that mature in fishes possess a metacercaria that encysts in tissue of the intermediate host.

## The Cyst and Encapsulation

We discuss the fish-pathogenic metacercariae below (see Pathogens) and provide a general introduction to the form of the cyst and the host encapsulation here (Plates 14.3–14.5, 14.9). Semantically and in the strictest sense, we define 'cyst' as the layer or layers (Lee and Cheng, 1970) secreted by the parasite (e.g., Figs. 14.5F, J-L) and the 'encapsulation' as the layer produced by the host (e.g., Fig. 14.5F). The term 'cyst' can also include the combination of both host- and parasite-derived components (Leong and Howell, 1971). On the other hand, the term 'capsule' can be

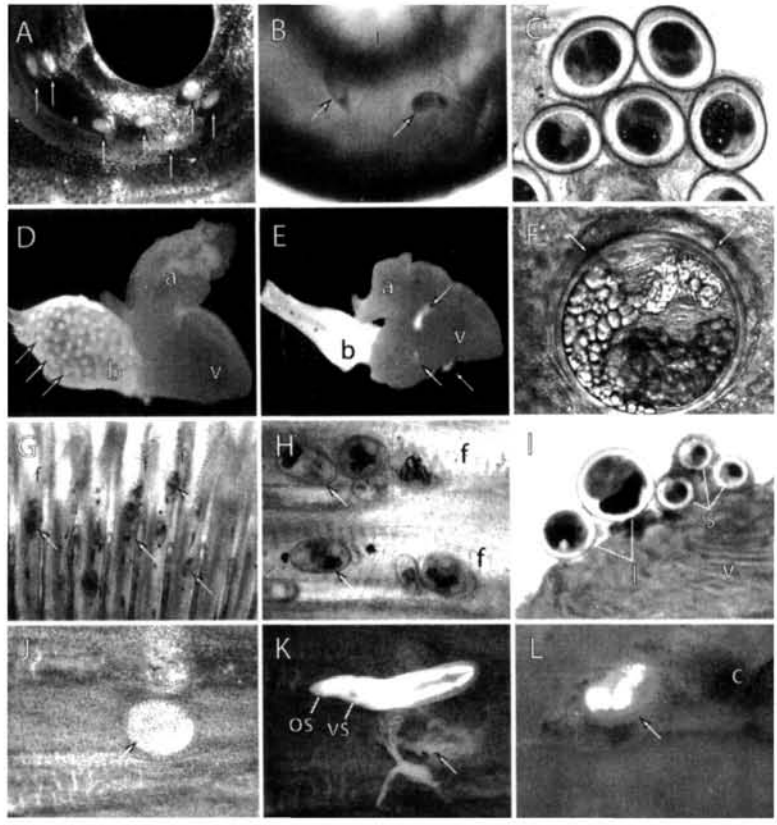


**Plate 14.4** Metacercariae, all encysted (except Figures 14.4B and 14.4D), in wild-caught fishes. **A.** Several bucephalid (*Rhipidocotyle* sp.) metacercariae (arrows) encysted in the eye of a spotted weakfish (*Cynoscion nebulosus*). **B.** Two non-encysting diplostomulum metacercariae (arrows) in the eye about the lens (l) of a centrarchid fish. **C.** Cysts of *Ascocotyle pachycystis* removed from heart of *C. variegatus* showing a thick, refractive cyst wall surrounding the fully developed metacercaria. **D.** Excised heart of sheepshead minnow (*Cyprinodon variegatus*) showing atrium (a), ventricle (v), and bulbus arteriosus (b): only the bulbus arteriosus is infected with many cysts of *Ascocotyle pachycystis* (Heterophyidae) (arrows). **E.** Excised heart of Gulf killifish (*Fundulus grandis*) showing atrium (a), ventricle (v), and bulbus arteriosus (b) infected with encysted bucephalid metacercariae (arrows). **F.** Metacercaria encysted in the heart of a fish. A moderately thick cyst wall surrounds the metacercaria (arrows). **G.** Encysted metacercariae of *Ascocotyle angrense* (Heterophyidae) (arrows) in the gill filaments (f) of *F. grandis*. **H.** Higher magnification of Fig. 14.4G showing several cysts (arrows) in gill filaments (f). **I.** Concurrent infection of two species in the heart ventricle (v) of a fish: note two larger cysts (l) and three smaller cysts (s). **J.** Cyst (arrow) of *Clinostomum* sp. in the fin of a bass (*Morone* sp.). **K.** The forcibly excysted individual metacercaria of Fig. 14.4J showing the oral sucker (os) and ventral sucker (vs) of the metacercaria nearby the pocket of tissue (arrow) that contained the cyst. **L.** Encysted metacercaria (arrow) beneath serosal lining of liver of *F. grandis* associated with nearby ceroid pigment (c).

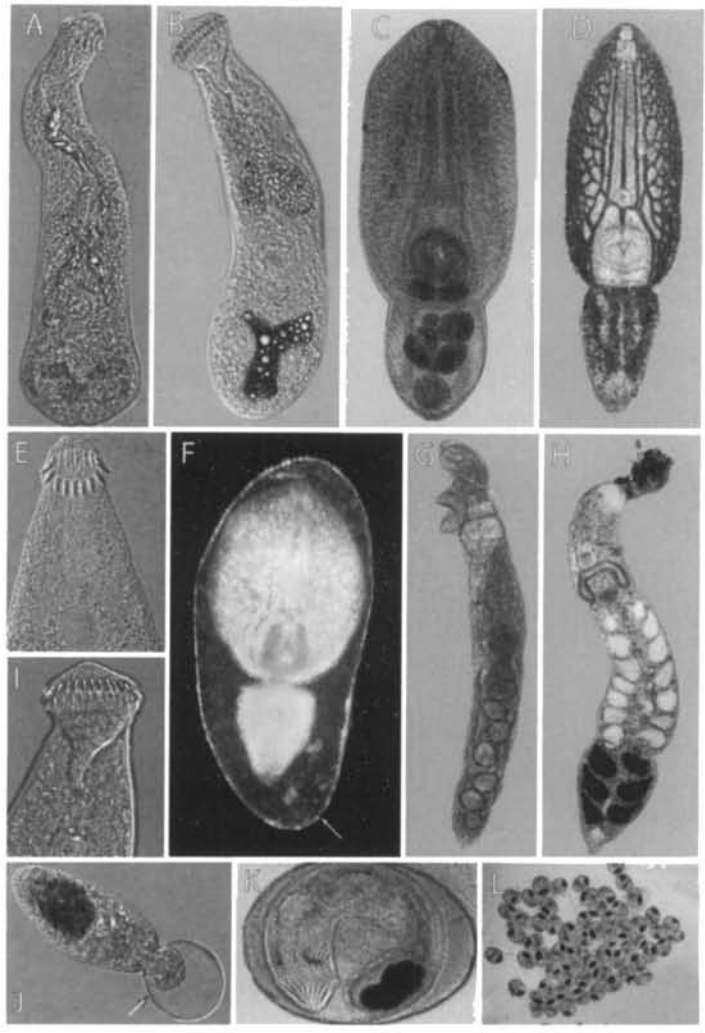


**Plate 14.5** Metacercariae from wild-caught fishes. **A.** *Phagicola diminuta* forcibly excysted from gill of Gulf killifish (*Fundulus grandis*). **B.** *Ascocotyle gemina* (Heterophyidae) forcibly excysted from gill of sheepshead minnow (*Cyprinodon variegatus*). **C.** Neascus metacercaria. **D.** Diplostomoid from mosquitofish (*Gambusia affinis*). **E.** Anterior end of specimen in Fig. 14.5A. **F.** Prodiplostomulum metacercaria excised from *G. affinis*. Note the thin cyst wall (arrow). **G.** Didymozoid metacercaria from bay anchovy (*Anchoa mitchilli*). **H.** A second species of didymozoid metacercaria from the same host individual as in Fig. 14.5G. **I.** Anterior end of specimen in Fig. 14.5B. **J.** Metacercaria emerging from cyst excised from coho salmon (*Oncorhynchus kisutch*). Note the thin, membrane-like cyst wall (arrow). **K.** Cyst from the fin of a flatfish. **L.** A mass of cysts of *Euhaplorchis* sp. excised from the cranium of *F. grandis*.

used for either the combination of the cyst and the fibrotic or epithelioid host response or just the host response. That structure can also be termed a 'connective tissue capsule' (Sommerville, 1981). In fishes, an encapsulation can be demonstrated without the aid of histology in some species, especially some didymozoids and hemiuroids, if the encapsulation surrounding the cyst or parasite is well developed; a fibrotic, semi-elastic coating can become obvious upon removing the cyst or digenean from the host's tissue because it leaves an indentation (Figs. 14.4J, K, 14.7L, 14.24A, 14.25E). In some infections, the skin color of the infected intermediate host may become browned or blackened, a condition referred to as 'melanosis' by Hsiao (1941), who studied heterophyid cysts in cod. This encapsulation comprises flattened epithelioid cells overlying an acellular layer secreted by the encysting cercaria. For at least some flukes, the cyst and encapsulation are permeable (Larson *et al.*, 1988). Many gymnocephalous cercariae produce a cyst wall, sometimes more than a single layer thick, that attaches to aquatic vegetation, the shells of molluscs, or other surfaces as well as remains free in the water column or near the water's surface by means of long filaments or tendrils.

The most commonly reported reaction to encysted metacercarial infections in fish comprises an outer fibroblastic connective tissue capsule surrounding the cyst, but other cells such as leukocytes (Higgins *et al.*, 1977; Sommerville, 1981), hepatocytes (Higgins, 1980), melanocytes, and macrophages may be involved, depending on the species of fluke and the site of infection. Font *et al.* (1984a) showed that the thickness and composition of the cyst of *Phagicola nana* in centrarchids depended on the site in the host. Early histological studies revealed the inner parasite-derived layer and an outer host-derived layer (Hughes, 1928; Hunter and Hunter, 1942). Hunter and Hamilton (1941) studied cysts of the diplostomoid *Uvulifer ambloplitis* in fish muscle and observed that the inner hyaline cyst formed in 2-4 days post-infection, and pigment ('melanin') bearing cells appeared in the outer layers of the fibroblast portion of the cyst during the third week post-infection. Although the connective tissue capsule surrounding encysted metacercariae is frequently superficially described, time-dependant experimental studies documenting the sequential involvement of host inflammatory cells and how they contribute immunologically to the outer layer of the encapsulation are few. Lynch and Bogitsh (1962) used both histology and histochemistry to elucidate the constituents of the cyst and capsule of another diplostomoid, *Posthodiplostomum minimum*, and showed that the

layer adjacent to the parasite cyst demonstrated a faint PAS-positive staining reaction while some cells contained intensely staining PAS-positive granules. They detailed the cyst of *P. minimum* from the liver and pericardium of various freshwater fishes and showed that the cyst wall included 14 parasite-derived amino acids and 16 host-derived ones. Lee and Cheng (1970) described a 3-layered cyst in *Stellantchasmus falcatus* (Heterophyidae) from striated muscles of flathead mullet (*Mugil cephalus*); the acellular inner layer was comprised of complex carbohydrate polyanions rich in acidic groups, while the medial acellular layer was comprised of a neutral mucopolysaccharide including a moiety of carbohydrate polyanions. Stein and Lumsden (1971) described a four-layered capsule in *Ascocotyle pachycystis*. The inner two layers were thought to be of parasite origin and the outer layer was cellular and host-derived. Pike and Burt (1983) showed a complex host interaction with the cyst of *Apophallus brevis* (Heterophyidae) in yellow perch (*Perca flavescens*): the cyst becomes enclosed in an elaborate capsule consisting of material similar to or identical with host bone, but at opposite poles there are canals through which the parasite can escape when eaten by the definitive host. The presence of cells containing granular PAS-positive material lying free in the area of inflammation was a constant feature of the capsule of several fishes, suggesting the possible release of the PAS-positive material by granulocytes contributed to the degeneration of the encapsulating cells. Faliex (1990) infected thinlip mullet (*Liza ramado*) with metacercariae of the bucephalid *Bucephalus minimus* (as *Labratrema minimus*) and documented ultrastructural changes in the host comprising (1) laying down of three layers forming the cyst wall, (2) the evolution of the three layers constituting the fully developed cyst wall, and (3) the passing of lipid droplets through the inner layer from the nearest hepatocytes to the cyst cavity. This study showed that the cyst wall protects the parasite against possible reactions from the host: it is solidified by compaction with the appearance of numerous desmosomes, and the wall permits passing of nutritive reserves that can be used by the parasite.

## Adult

Adults exhibit a wide variety of body forms (Plates 14.1, 14.6, 14.7, 14.21, 14.23–14.25). These forms include a variety of tegumental features associated with attaching to the host, moving about within the host, having sex with themselves or another nearby fluke, and sensing their

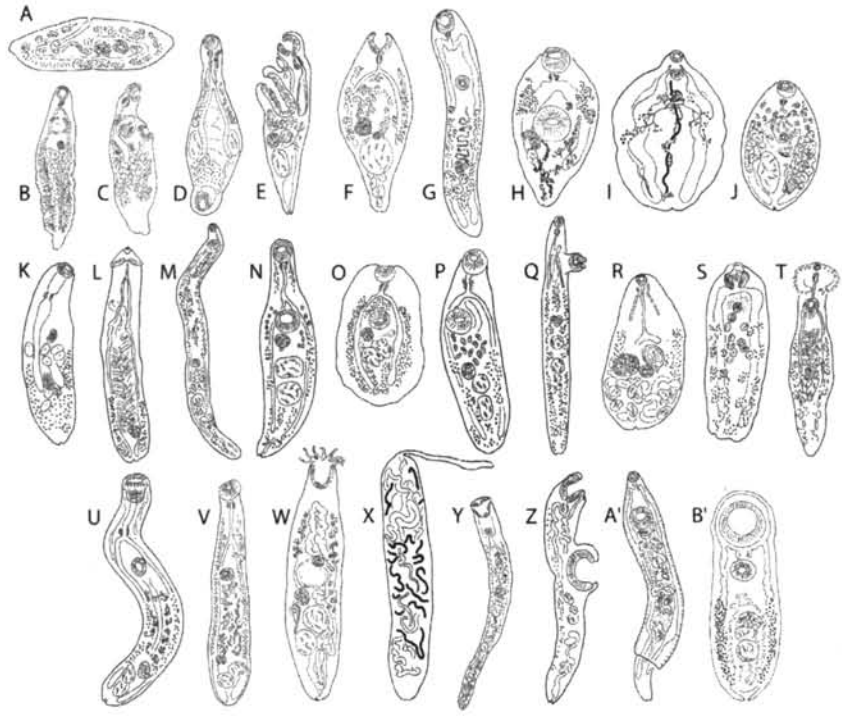


**Plate 14.6** Adult digeneans representing many of the families that mature in fishes. Mostly derived from Yamaguti (1975). **A.** *Transversotrema haasi* (Transversotrematidae). **B.** *Apocreadium mexicanum* (Apocreadiidae). **C.** *Haploporus lateralis* (Haploporidae). **D.** *Cephaloporus monacanthi* (Cephaloporidae). **E.** *Haplosplanchnus pachysomus* (Haplosplanchnidae). **F.** *Bivesicula claviformis* (Bivesiculidae). **G.** *Azygia micropteri* (Azygiidae). **H.** *Fellodistomum agnotum* (Fellodistomidae). **I.** *Anaporrhutum albidium* (Gorgoderidae). **J.** *Monorchis monorchis* (Monorchiidae). **K.** *Zoogonus rubellus* (as *Z. mirus*) (Zoogonidae). **L.** *Barisomum candidulum* (Pronocephalidae). **M.** *Tormopsolus orientalis* (Acanthocolpidae). **N.** *Allocreadium isoporum* (Allocreadidiidae). **O.** *Crassicutis cichlasomae* (Homalometridae). **P.** *Lepocreadium bimarinum* (Lepocreadiidae). **Q.** *Opecoelus sphaericus* (Opecoelidae). **R.** *Microphallus opacus* (Microphallidae). **S.** *Megacreadium tetrodontis* (Plagiorchiidae). **T.** *Caballerotrema brasiliense* (Echinostomatidae). **U.** *Acanthostomum spiniceps* (Acanthostomidae). **V.** *Opisthorchis parasiluri* (Opisthorchiidae). **W.** *Bucephalus polymorphus* (Bucephalidae). **X.** *Didymozoon branchiale* (Didymozoidae). **Y.** *Diplopharyngotrema laterolabracis* (Cryptogonimidae). **Z.** *Syncoelium ragazzii* (Syncoelidae). **A'.** *Hemiurus appendiculatus* (Hemiuridae). **B'.** *Ptychogonimus megastomus* (Ptychogonimidae).

location within the host. Such features include spines (Figs. 14.5B, E, I) and scales (Figs. 14.7Q-S) in many varieties, sensory cilia, sensory papillae, and tegumental ultrastructure. Details of these features remain undocumented for many digeneans because to see them requires light microscopy (LM) using oil immersion, scanning electron microscopy

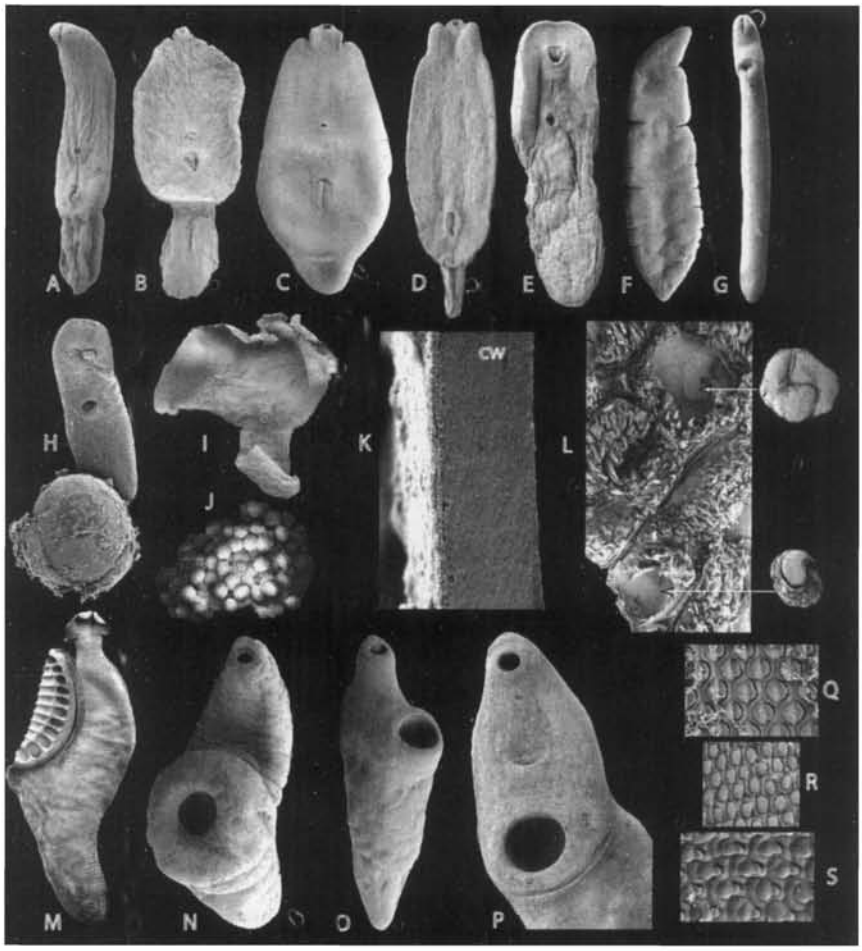


**Plate 14.7** Scanning electron micrographs of larval and adult digeneans collected from fishes. **A.** Non-encysted diplostomid metacercaria from vitreous humor of mosquitofish (*Gambusia affinis*). **B.** Diplostomid adult from intestine of a heron. **C.** Forcibly excysted metacercaria of *Bursacetabulus pelecanus* from musculature of head of channel catfish (*Ictalurus punctatus*). **D.** Non-encysted metacercaria of *Austrodiplostomum compactum* from vitreous humor of *I. punctatus*. **E.** Adult lepocreadiid (*Bianium* sp.) from bluntnose pufferfish (*Sphoeroides pachygaster*). **F.** Non-encysted didymozoid metacercaria from body cavity or musculature of bay anchovy (*Anchoa mitchilli*). **G.** Non-encysted didymozoid from body cavity or musculature of same host individual as Fig. 14.7F. **H.** Metacercaria of *Ascocotyle pachycystis* (Heterophyidae) partially extruding from a cyst collected from the bulbous arteriosus of sheepshead minnow (*Cyprinodon variegatus*). **I.** Forcibly excysted metacercaria of *Scaphanocephalus* sp. (Heterophyidae) from skin of ballyhoo (*Hemiramphus brasiliensis*) from the Florida Keys. **J.** Grape-like mass of cysts (*A. pachycystis*) from the bulbus arteriosus of *C. variegatus*. **K.** Cyst wall (cw) of

*Plate 14.7 contd.*

(SEM) (Plate 14.7), transmission electron microscopy (TEM), histology, or histochemical evaluations. More histochemical studies dealing with taxonomic aspects of papillae have dealt with cercariae than adults. Herein, we provide a bauplan for adult digeneans knowing full well that there are many exceptions. In general, the mouth is anteroventral and usually surrounded by a conspicuous muscular oral sucker. Another sucker, the ventral sucker, or acetabulum, usually occurs on the ventral surface (Figs. 14.1A-B, 14.7N-P; Plate 14.6). Like most monogeneans and turbellarians, but unlike all cestodes, digeneans have a gut. The typical gut plan of a digenean is a mouth that leads to an esophagus that may or may not be surrounded for part of its length by a muscular, bulb-like pharynx. The esophagus leads directly to an intestinal bifurcation that yields at least two blind-ended ceca. The excretory system of digeneans comprises an excretory vesicle, a bladder in the posterior end of the fluke, that is fed by an intricate and oftentimes difficult-to-characterize network of fine ducts that are species-specific and associated with flame cells. The excretory vesicle empties through the terminal portion as a sphincter-controlled excretory pore. When the vesicle communicates with the cecal lumen, the structure is termed a 'uroproct'. This structure, voiding both excretory and digestive products, occurs in a variety of hemiuroids as well as in a few members of the unrelated opecoleoid, lepocreadid, acanthocolpid, and other families. Like other platyhelminths, nearly all digeneans possess both male and female reproductive tracts, and much of the taxonomy of digeneans is based on the relative shape, positions, and sizes of specific features associated with the reproductive systems (e.g., Plate 14.6). The male system can generally be characterized by having one, two, or multiple testes, a fine network of sperm-collecting ducts (vasa efferentia) that in

*Plate 14.7 contd.*

*Bolbophorus damnificus* prodiplostomulum cyst from somatic muscle of *I. punctatus*: the left of the image is the outside of the cyst and the right side of the image, slightly curved, is the inner surface of the cyst. **L.** Adult didymozoids (arrows) 'popped out' of their respective crater-like host encapsulations (see also Fig. 14.24F). **M.** Adult *Lobatostoma ringens* (Aspidogastrea) from gut of Florida pompano (*Trachinotus carolinus*), lateral view. **N.** Hemiurid from gut of red snapper (*Lutjanus campechanus*). **O.** Adult hemiurid, *Lecithocaster confusus*, from gut of skipjack herring (*Alosa chrysochloris*). **P.** Hemiurid from gill raker of Atlantic cutlassfish (*Trichiurus lepturus*), anterior end showing oral- and ventral suckers Fig. 14.7Q through 14.7S show the tegumental 'scales' of digeneans. **Q.** Anteroventral region near mouth of a metacercaria excysted from cardiac muscle of Gulf killifish (*Fundulus grandis*). **R.** Ventral body surface posterior to ventral sucker of adult fluke shown in Fig. 14.7P. **S.** Dextral posteroventral body surface of same metacercaria as Fig. 14.7Q.

some groups entwine throughout the testicular or parenchymal tissue but in most species extend directly from a testis to a larger common collecting duct (vas deferens) that is usually replete with fully mature sperm, a sac that can be thin- or thick-walled and weakly or strongly muscular that includes the seminal vesicle, ejaculatory duct, pars prostatica associated with one or more types of prostate gland cells, and the intromittent organ, which can be either eversible (cirrus) or protrusile. Members of some families do not have the muscular cirrus sac or pouch, and others have an additional seminal vesicle external to the sac. If the male and female ducts join within the sac, the common duct is termed the 'hermaphroditic duct' (a copulatory duct often with spines, papillae, or pads in the Haploporidae and Atractotrematidae and what may be a non-homologous duct termed a sinus organ in some hemiuroids when the internal duct joins with the thick, well-developed genital atrium) and the sac is called a hermaphroditic pouch. The female system can generally be characterized by having an ovary, a field of glandular cells (vitellarium) producing yolk and shell material, associated collecting ducts ending as a reservoir of 'vitelline material', an oviduct that leads from the ovary and combines with the vitelline duct before meeting with the ootype. It is within the ootype that ova from the oviduct are mixed with vitelline material to form an egg. The ootype is surrounded by the Mehlis gland, a loose or compact field of various gland cells that contribute to egg formation. In some flukes, there is a duct that leads dorsally from the oviduct to the external environment (Laurer's canal). Its purpose appears to rid the worm of aged or unviable sperm. In some hemiuroids, this duct does not exit dorsally but ends as a duct or swollen vesicle in the parenchyma surrounded by a membrane enclosing an amorphous granular material, with the complex termed a Juel's organ (Gibson and Bray, 1979). This organ occurs free in the parenchyma and is typically surrounded by a complex of glands. The organ functions to degenerate excess sperm and provide the worm with the ability to recycle materials from the aged sperm. From the ootype extends a uterus wherein developing eggs are accommodated or stored. The uterus can be long or short, convoluted or straight, fill much of the space within the worm, or comprise a short duct; its distal portion is called a metraterm if it is conspicuously muscular or separated by a muscular sphincter. The external opening to the female reproductive tract (female genital pore) may share a common pore with the male reproductive tract or it may be separate. In some flukes, the opening to the male and female reproductive tracts comprises a common pore, in others the pores are

separate, and in most the pores may both open into a common genital atrium which, in turn, opens to the external environment. In some the atrium everts and in most it consists of a muscular rimmed pore that accepts the intromittent organ of a mating individual or, less commonly, of itself when a mate is unavailable.

## DIAGNOSIS

Herein, we define 'diagnosis' as 'identification' of a disease or pathogen. There are direct and indirect methods for identifying digenean infections in fishes. The most direct method comprises taxonomic morphological identification of the specimen and may require LM and SEM. The two indirect methods we discuss comprise the histology of infected tissues and molecular biology, neither of which always ensures that one can identify the pathogen to the level of species.

### Taxonomy

The most precise diagnosis of an infection or disease comprises species- or strain-level identification of the agent (parasite or pathogen). This usually consists of collecting the specimen, using a relevant taxonomic key if available, and comparing that specimen with features in an up-to-date description. In some cases, comparison is necessary with a previously deposited museum specimen or with conspecific or similar species described and illustrated in the primary literature. A few fish diseases are now recognizable using immunological or molecular tools. In general, parasitologists regard morphologically indistinguishable individuals as conspecific, and now such assumptions can be tested using various molecular techniques. Debate exists within biology about what comprises a species, and there are over a dozen concepts of what constitutes a species (Stanford, 1995; Hull, 1999; Wheeler and Meier, 2000). In the context of fish disease and whichever concept is employed, the species identification is important because it allows disease diagnosticians and aquaculture managers to communicate effectively and precisely about a potential disease and its treatment, control, or management. Moreover, it empowers those persons to be proactive, to respond to a problem or crisis, and to identify whether the situation will or could comprise a crisis. This is because the species name, the identification, carries with it a lot of useful biological information and thereby helps answer some important questions. The most relevant in terms of aquaculture and fish disease

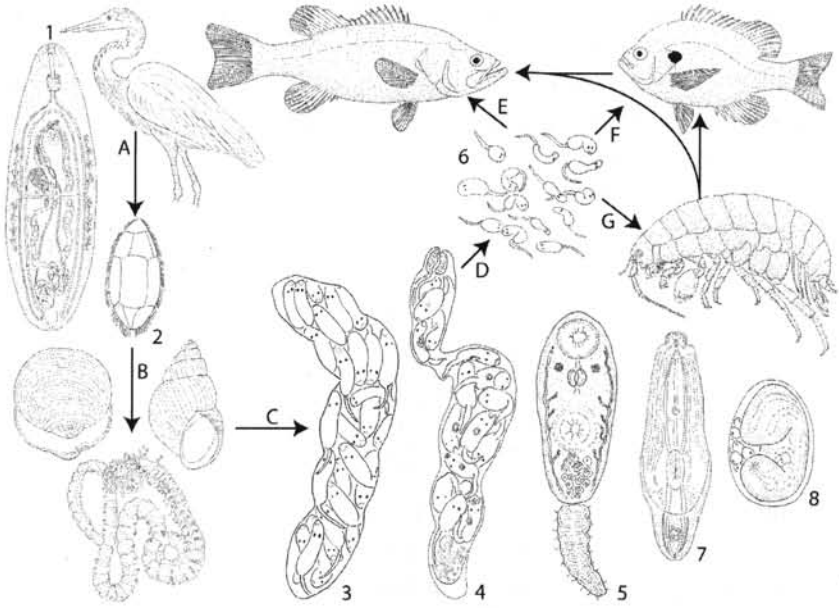


**Plate 14.8** Course of digenean life histories that involve fishes as second intermediate hosts, paratenic hosts, and definitive hosts as well as birds, reptiles, and mammals as definitive hosts. The hypothetical adult fluke (1) in its definitive host releases eggs that exit the definitive host (A) through the gastrointestinal tract or gill epithelium or directly into the water. A ciliated miracidium (2) emerges from the egg or remains within it before infecting the first intermediate host (B), a bivalve, snail, or polychaete, wherein asexual reproduction ultimately yields (C) sporocysts (3) or rediae (4) that each contain few to numerous cercariae (5-6). Depending on the species of digenean, the cercaria can penetrate and infect the definitive host immediately (E) (= two host life cycle) or the cercariae can infect a fish second intermediate host (F) or an invertebrate second intermediate host (G) wherein they develop to a metacercaria that can be free/non-encysted (7) or encysted and/or encapsulated (8) in various tissues of the second intermediate host. This second intermediate host is then preyed upon by the definitive host (= three host life cycle). In some species, a paratenic host can be included wherein the invertebrate second intermediate host is eaten by a fish other than the 'proper' definitive host fish, bird, mammal, or reptile.

being, 'Is this parasite harmful to my fish'? The presence of particular parasites in a farmed fish population should rightfully elicit distress to the farmer; whereas, other parasites should not. For example, a multitude of aporocotylid eggs in the branchial arterioles of a recently suffocated cage-cultured fish should cause concern; whereas, conversely, a few adult tapeworms in the gut of a few of those same fish should not.

Proper taxonomic identification is possible only after the proper collection, fixation, and preparation of specimens. The collection of

taxonomically useful digeneans is challenging for several reasons: (1) flukes die soon after their host dies, (2) flukes are soft-bodied and degrade soon after death, and (3) because of #2 flukes must be preserved immediately after being killed but (4) if live flukes are placed directly into cool or cold fixative they will curl and contract, making them nearly useless for morphological study. A few workers advocate fixing platyhelminths in 95% EtOH after immersion in freshwater to straighten the flukes because they allege that specimens handled in such a way can be useful for both molecular biology and morphological studies. Our collective experience and that of Ronnie Palmer (GCRL)—who has been collecting and fixing platyhelminths for over 35 years—shows that specimens fixed in alcohols consistently yield preparations that are poor (regarding histology, LM, SEM) relative to those fixed in formalin and treated as we discuss below. Further, leaving digeneans in freshwater, although a good technique for forcibly everting the tentacles of trypanorhynchs or the proboscis of acanthocephalans, typically results in pronounced fixation artifacts due to osmotic imbalance, swelling, and the specimens may even rupture. The following is a good standard protocol for collecting taxonomically useful fluke specimens: (1) remove the flukes from the host and isolate them in a clean dish of physiologic saline (about 6-8 grams of salt per L of water), (2) clean the flukes of any debris before pipetting off as much saline as possible from the storage dish, (3) flood the dish with very hot water, and (4) immediately transfer, using a pipette or fine artist's brush, the heat-killed specimens to a vial of 5-10% neutral buffered formalin. If the specimens are thick-bodied, a few can be heat-killed under slight or strong coverslip pressure with heat from an ethanol burner flame. Observing a cover-slipped live specimen with the aid of a compound microscope, especially one that is equipped with differential interference contrast (DIC) optical components, can elucidate delicate membranes and connections between fine ducts that are not always obvious in fixed and stained conspecific specimens. Ultimately, the specimen vial should be labeled with geographic locality, capture date, host species, infected site in host, and collector's name. If possible, a unique, sequential collection number associated with the vial should be logged in an electronic, searchable database. A citrated saline solution is especially useful for the collection of blood flukes because it also functions as an anticoagulant that can be used to keep blood from obscuring flukes. If necessary, seawater (typically ranging from ~20-35 ppt) can be diluted with freshwater in a 1: ~3-5 parts ratio to yield a useable saline solution.

Clean dissection tools are an absolute requirement, since if the dissection tools are tainted with fixative or certain cleaning agents they will contaminate the dish and kill the flukes or cause them to contract. Specimens should be left in fixative for at least 24 hours, stained, and permanently mounted on glass slides using Canada balsam. Good all-around stains for fixed digeneans intended as permanent mounts are hematoxylin stains like a combination of Van Cleave's and Ehrlich's (Bullard and Overstreet, 2003, 2004). Whenever possible, voucher specimens should be deposited in a curated helminthological collection such as the United States National Museum (Beltsville, Maryland, U.S.A.), The British Museum of Natural History (London, England), The Institute of Parasitology, Academy of Sciences of the Czech Republic (Ceské Budeiovice, Czech Republic), or The Manter Laboratory of Parasitology (Lincoln, Nebraska, U.S.A.).

## Histology

The study of histological sections allows one to: (1) critically assess specific structures in a worm for taxonomic or systematic purposes, (2) detect parasites in tissues that normally could not or would not be examined during routine necropsy, and (3) understand the host-parasite interface at a cellular level (Plates 14.9, 14.15, 14.18–14.19, 14.24–14.25). Chitwood and Lichtenfels (1972), Cosgrove (1975), and Orihel and Ash (1995) provided many examples of how particular parasite species appear in histological sections and emphasize the importance of consulting a taxonomic expert for identification of questionable parasites. Histological examination also allows the researcher or fish health diagnostician to assess the efficacy of treatment protocols if the test population is sub-sampled at regular intervals throughout the course of the treatment. We advocate sampling a portion of gill, somatic muscle, heart, kidney, liver, spleen, intestine, and mesentery during routine necropsies of fishes to screen for digenean infections as well as infections of other parasites (e.g., Esch and Huffines, 1973). These tissues can be archived and used later for other purposes but, in any event, the samples serve as a permanent record of the health status of the fish stock. In addition, should an epizootic occur, previously sampled tissues can be re-checked and scrutinized for the presence of the pathogen so that more can be learned about the disease history and process. Histological samples should be fixed immediately after the host is killed because tissue autolysis will yield artifacts and obscure the actual pathological alterations to host

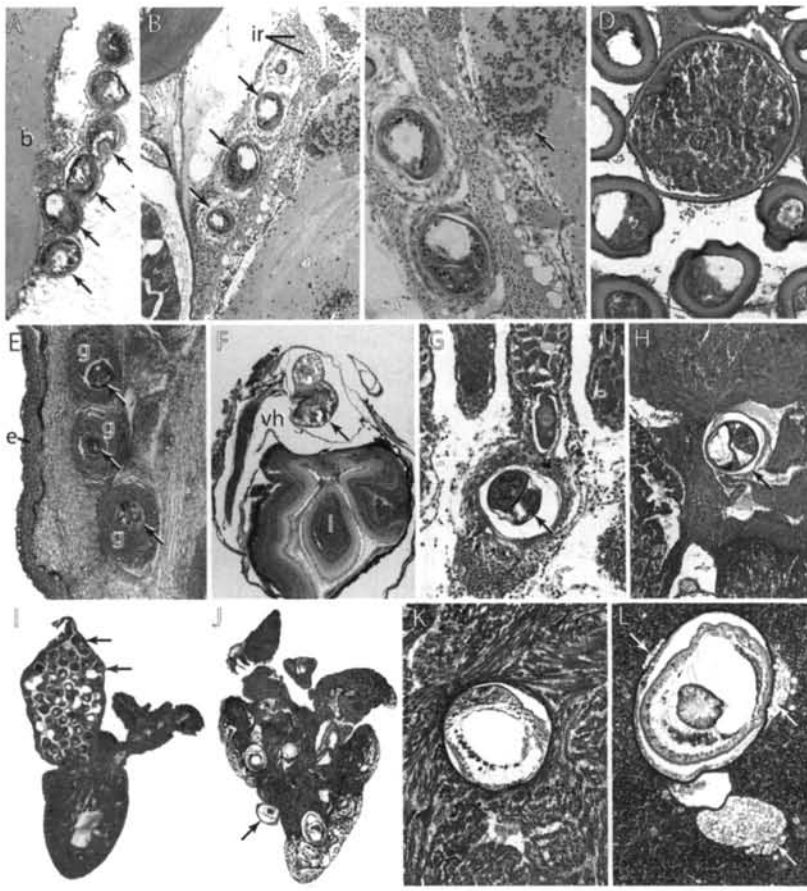


**Plate 14.9** Histological sections of fish tissue infected with larval digeneans. Figs. 14.9A-C. Cysts of *Euhaplorchis* sp. adjacent to the brain of Gulf killifish (*Fundulus grandis*). **A.** Several cysts (arrows) opposing the brain (b). **B.** Cysts (arrows) encapsulated and associated with inflammatory response (ir). **C.** Cysts surrounded by a mild granuloma and basophilic cells (arrow) in adjacent nervous tissue. **D.** The thick-walled cysts of *Ascocotyle pachycystis* (Heterophyidae) in the bulbus arteriosus of sheepshead minnow (*Cyprinodon variegatus*). **E.** Encysted metacercariae (arrows) associated with granulomas (g) beneath the epithelium (e) of a flatfish. **F.** Vitreous humor (vh) of mosquitofish (*Gambusia affinis*) infected by diplostomoid metacercaria (arrow) near lens (l). **G.** Distal tip of gill filament of *F. grandis* showing encysted metacercaria (arrow). **H.** Bucephalid metacercaria (arrow) encysted in cardiac muscle of *F. grandis*. **I.** Heart of sheepshead minnow (*C. variegatus*) showing bulbus arteriosus infected with many cysts (arrows) of *A. pachycystis* (Heterophyidae). **J.** Heart of *F. grandis* infected with several bucephalid metacercariae (one indicated by arrow) encysted beneath the epicardium and within the compact myocardium. **K.** Higher magnification view of bucephalid metacercaria showing either a lack of significant host response or epithelioid encapsulation. **L.** Encysted metacercaria in liver of *F. grandis* associated with concurrent infection of a coccidian (*Calyptospora funduli*) occurring in large groups (arrows); some of which are closely applied to the capsule wall.

tissue induced by the pathogen. For soft tissues, 10% neutral buffered formalin works well and for hard tissue (bone, cartilage) Bouin's fixative or non-buffered formalin yields good preparations. Zinc-formalin is recommended if immunochemical tests are anticipated. Both Bouin's and Lillie's fixatives contain picric acid that demineralizes tissues. If Bouin's fixative is not the initial fixative used, tissues resting in other fixatives subsequently can be 'post-fixed', with seemingly less efficacy, in Bouin's for 24-48 hours before routine histological processing. Relatively small pieces of tissue ($\sim$1 $cm^3$) with their relatively large surface areas yield the best preparations because the fixative can more easily penetrate the sample. If grossly visible parasites are left attached to the infected tissue and processed for histology, a more complete picture of the parasite-host interface can be elucidated in the histological sections. Sometimes, this may not be feasible if only one or a few parasites are present; those specimens should be removed and handled in a way more conducive to taxonomic studies. Doubtless, not finding a parasite in a series of histological sections does not mean that a host is not infected because a typical histological section may only be 0.004 mm in thickness, which captures only a very small portion of a sub-sample of the fish's tissue. If time or equipment are lacking, the tissues can be kept indefinitely before sectioning; however, tissue in Bouin's should be washed and transferred to 70% ethanol after 2-4 days depending on its size.

## Molecular Biology

Comparisons of DNA nucleotide sequences of digeneans are useful for (1) reconstructing digenean phylogeny (Anderson and Barker, 1998; Cribb *et al.*, 2001b; Olson *et al.*, 2003), (2) distinguishing species (Anderson and Barker, 1993), (3) elucidating cryptic species (Sorensen *et al.*, 1998; Overstreet *et al.*, 2002; Dzikowski *et al.*, 2003b, 2004b), (4) studying parasite-host biogeography (Galazzo *et al.*, 2002), (5) facilitating human disease diagnostics (Dzikowski *et al.*, 2004b), and (6) elucidating life histories (Cribb *et al.*, 1998; Levy *et al.*, 2002; Overstreet *et al.*, 2002). The last use is most relevant for this chapter because it is the most applicable to management of and disease diagnostics among afflicted aquaculture fishes. As eluded to previously, understanding the life cycle of a pathogen allows one to take measures that facilitate its management. The use of molecular markers in disease diagnostics is pragmatic in terms of cost and time because (1) it does not require extensive taxonomic expertise, (2) specimens can be collected relatively easily by simply placing them directly

into molecular-grade 95% EtOH, (3) informative loci for several taxa have already been established, and (4) nucleotide sequencing can be performed off-site by any number of inexpensive mail-in/mail-out laboratories. That molecular diagnostics does not require extensive taxonomic expertise is the primary benefit because training one to become a taxonomist is extremely time consuming. Clearly, the most robust studies include molecular comparisons that complement experimental studies with solid taxonomic work. For example, Overstreet *et al.* (2002) worked with *Bolbophorus damnificus* (Bolbophoridae), a fluke that includes a commonly pathogenic prodiplostomulum metacercaria that infects the flesh of pond-reared channel catfish (*Ictalurus punctatus*). They applied morphology to specimens obtained from experimental infections as well as employed a suite of molecular markers to match the prodiplostomulum with an adult of one of two similar-appearing species that concurrently infect the gut of the American white pelican (*Pelecanus erythrorhynchos*). The second species can kill fish hosts, but not the channel catfish. The results gave catfish farmers the ability to understand, and thus manage, the pathogen's life cycle and problems concerning multiple digeneans in the channel catfish, in accidental fishes, and in a variety of birds in and associated with the channel catfish ponds.

## CONTROL

### Pragmatic Considerations

Long-term success in aquaculture means preventing infectious diseases. Presently, the body of literature on controlling digenean infections among cultured fishes is small because (1) relatively few digenean species harm cultured fishes, (2) chemical therapies and biological controls for the few known pathogenic digeneans are often impractical or ineffective in large-scale aquaculture operations (e.g., Bullard and Overstreet, 2002; Paperna and Dzikowski, 2006), (3) known effective treatments have not been given governmental (e.g., United States Food and Drug Administration) approval, and (4) the process of obtaining governmental approval for compounds that target specific agents in specific hosts is very expensive. For those reasons, seldom should one consider expending significant institutional resources to treat a digenean infection in a fish population unless there is an obvious justification to do so, such as if all sick fish harbor large numbers of worms, if the fish are valuable brood stock, if the fish are valuable display specimens, or if the worms reduce the market value of filets, and unless there is a reasonable chance that the treatment

will be effective. Generally speaking, a given disease can be prevented by (1) eliminating the agent, (2) avoiding the agent, or (3) reducing agent virulence or increasing host defense (Kinne, 1984). Since the number of worms in a host (mean infection intensity) is a primary determinate of infection pathogenicity, a control strategy for digenean infections need not aim to completely eradicate the parasite (number 1) which, in any case, is probably not tenable, but it should aim to reduce infection intensity to some acceptable level (numbers 2 and 3), i.e., reduce the parasite population such that fishes are not being killed or marketed filets are not rejected by consumers. Hence, expending institutional resources to achieve and maintain a 'pathogen-free' or 'parasite-free' stock may not always be cost-effective or even necessary. An acceptable low-level of infection that neither kills fishes nor reduces marketability of filets could be acceptable. For example, Hayward *et al.* (2007) showed that three metazoan gill parasites, i.e., the copepods *Pseudocycnus appendiculatus* and *Euryphorus brachypterus* as well as the monogenean *Hexastoma thynni*, all of which exhibit a direct life cycle, although common on southern bluefin tuna (*Thunnus maccoyii*), did not proliferate on the gill of sea-caged tuna over a 5-month farming period. As a result, those parasites were not associated with extra production costs. We have observed a similar phenomenon between red snapper (*Lutjanus campechanus*) and a gill-dwelling monogenean (Ancyrocephalinae). In addition, having knowledge of the host range (host specificity) of the digenean could increase the efficacy of a control strategy. That is, a plan that includes treating or screening/monitoring fishes that are not susceptible to infection is wasteful and nonproductive. Alternatively, fishes that are known to be carrier hosts or highly susceptible to infection should be monitored closely. In addition, pathogenic viruses and bacteria can be vectored by parasites (Cusack and Cone, 1986). Due to these aspects, imported fishes and fish products should be quarantined or examined fresh for infections prior to potential contamination of culture systems (see also Bullard and Overstreet, 2002).

## Management of Snails Shedding Cercariae in Ponds

An effective way to manage or control most digenean infections in ponds or confined areas is to interrupt the fluke's life cycle by killing molluscs, the first intermediate host for nearly all of the fish-pathogenic digeneans (Plates 14.10–14.13). Management by influencing the fluke's life cycle often means chemical control of snails. As indicated by Paperna and

Dzikowski (2006), preventing digenean eggs from entering a freshwater pond or outdoor culture facility is usually futile; culture ponds attract a diverse cadre of migratory birds that each host digeneans (Overstreet and Curran, 2004). Even if the birds do not enter a pond, they often defecate over them. Birds can access an abundance of fishes in those ponds and eat them, and in doing so these infected birds expose snails to digenean miracidia when they defecate in the pond water. Because these birds both eat fish and are associated with the parasite's life cycle, some pond managers prefer to shoot birds. The strategy of eliminating the snail hosts and not the bird hosts can be accomplished through (1) molluscicide application, (2) environmental manipulation, or (3) the introduction of molluscivorous fishes. Pieri and Thomas (1987) proposed that snail populations could hypothetically be reduced by (1) introducing a predator, (2) altering flow characteristics, (3) eliminating submerged aquatic vegetation, or (4) applying a chemical molluscicide (e.g., Francis-Floyd, 1991). Various combinations of such methods commonly have been applied to curb the spread of schistosomiasis and reduce the prevalence of species of *Fasciola* throughout the world as well as managing digeneans involving fishes (McCullough and Mott, 1983; Madsen, 1990; Paperna and Dzikowski, 2006). Any one of these methods requires substantial knowledge of the life history of the target snail and access to the system. For example, an isolated freshwater earthen pond is closed in terms of both water flow through the system and movements of particular invertebrates and fishes. That same system, however, may be open regarding the deposition of parasite eggs that are delivered by birds or other terrestrial vertebrates that visit the pond. On the other hand, a net pen, or floating sea cage, may be open regarding water flow through the system and transit invertebrates that cling to the cage or swim through it, but closed regarding larger vertebrates that visit the cage. So, control of parasites in these various systems may be less or more difficult depending on the system. For freshwater systems, reducing or eliminating the snail population in ponds seems practical; whereas, in a marine fish culture arena, such as floating cages or net pens, such a strategy is not only environmentally reprehensible (e.g., harmful to the endemic invertebrate fauna), but it is often illegal. In any event, given the high turnover rate of water in open marine aquaculture systems, molluscicides probably would have only a short-term effect, requiring a continual input of copper sulfate or some other expensive compound into the surrounding water, an option that also may be economically and environmentally unacceptable.

Reportedly, the only molluscicide that is acceptable or practical for use in ponds and circulation systems is copper sulfate. Other effective molluscicides such as niclosamide and *N*-tritylmorpholine are more toxic to fishes (Cowper, 1971). Ferguson and Hayford (1941) stated that copper sulfate, potassium permanganate, calcium hypochloride, chlorine gas, and derris root powder effectively kill snails. Copper sulfate is also useful as an algicide, and it is cost-effective for large-scale applications because it is inexpensive. Available copper from copper sulfate, however, depends on water chemistry, precipitating out of solution or dissolving into solution, depending on pH and water hardness. For example, the concentration of copper sulfate added to a body of water will test differently relative to the overall quantity added over a period of time and at different pH levels. The use of sodium citrate to keep the copper in solution in some cases effectively reduces the total amount that has to be added. At a relatively low concentration, copper sulfate is toxic to both parasites or snails and fishes but more so to snails and parasites. Copper salt may be applied continuously at a low concentration (1 ppm) or as a low-soluble formulation as copper carbonate or copper oxide to produce long-term effects (Hoffman, 1970). Depending on the fish species, it can be killed by 0.03-0.7 ml/L in soft water and 0.60-0.64 ml/L in hard water (Noga, 2000); however, in ponds adult fish and young of some species will avoid areas given high concentrations of copper. Ponds are typically treated around their margins only because that is where the vegetation and most of the snails reside. Stables and Chappell (1986) effectively delayed repopulation of snails in culture facilities by treating drained ponds or raceways with copper sulphate prior to stocking. Paperna (1991) stated that routine control measures for removing submerged aquatic vegetation could decimate snail populations.

A significant diplostomoid digenean infection in the channel catfish in the southeastern United States provides a good example to appreciate the complexities of chemical treatments, the USDA-recommended method of control. When the pond water has a high total alkalinity like in the Mississippi Delta, Mississippi, copper sulfate that is not citrated will continually precipitate after each treatment, concentrating in the benthos. An acidic episode to the ponds or sediments will release the soluble copper, and the resulting high concentration will kill the fish. All chemicals are toxic to both fish and their parasites (or snail hosts), but control depends on using a dose lethal to the parasite that is not harmful to the fish or those who eat them. According to Drew Mitchell and others

(pers. comm.), 2.5-5.0 ppm copper is best used when applied by tractor in a one-meter-wide swath along the shoreline. Citration of the copper would be preferable to avoid a build up of copper in the sediments. Ponds three hectares or larger only should be treated to properly accommodate the copper. The water temperature should be cooler than 21°C in the spring or fall so as to avoid algal mortality and resulting oxygen depletion in the summer months. In contrast, when the water is 'soft', expressing a low alkalinity like in West Mississippi, a slurry of 1.2-1.5 kg of hydrated lime per meter (0.8-1.0 lb per foot) of shoreline in a one-meter-wide swath is superior. More care is necessary because large amounts of the caustic compound require special storage and handling (David Wise and others, pers. comm.). Regardless of the compound used, treatment should be confined to ponds with high mortalities on calm days to avoid rapid dilution; the fish farmer should realize that recently stocked ponds should not be treated, that all snails will not be killed, and that snails will eventually repopulate the ponds. Infected snails and vulnerable fingerlings are both most common along the shallow shoreline, so all five species of digeneans in southeastern US ponds can be controlled simultaneously. Salt and other compounds would work but are not economical.

As further insight into the US channel catfish industry, millions of dollars worth of fish losses result from infections with the diplostomoid *Bolbophorus damnificus* (Plate 14.10) and other species, all of which will be treated in more detail later. Most commercial channel catfish are cultured in Mississippi, but significant numbers of fish and infections also occur in Louisiana, Arkansas, and Alabama. A small number, apparently 50 or more metacercariae of *B. damnificus* per fingerling, relative to the number of metacercariae of other diplostomoid species in fingerlings can kill these young channel catfish. This number was surmised by maximal numbers in most surviving fingerlings as well as undocumented experimental exposures (Overstreet and Curran, 2004). The bacterium *Edwardsiella ictaluri* which, in experimental infections, can produce about 40% cumulative mortality starting at day seven and leveling off in the second two weeks, can double that mortality over the same period with the same bacterial dose when fish have been infected with a recent digenean infection of approximately four metacercariae per fish. Concurrent groups of fish with either just the low level of worms or no worms or bacteria did not die during the same period (Labrie *et al.*, 2004). Various methods have been attempted and tested to eliminate or control the digenean infections, but using the chemical treatment of snail hosts for this and other

pathogenic digeneans pathogenic to the channel catfish in the same ponds appears to be the best available method (Overstreet and Curran, 2004).

Regarding a snail predator, the black carp (*Mylopharyngodon piceus*), also known as "snail carp", for freshwater systems and seabreams (*Sparus* spp.) for marine and brackish systems are considered by some as the only functional and economical fishes available for use (De Bont and De Bont Hers, 1952; Carothers and Allison, 1968; Taraschewski and Paperna, 1981; Paperna, 1996; Paperna and Dzikowski, 2006). The black carp is strongly discouraged for use in the US primarily because of the high risk of escape into natural waters where it could threaten local molluscs as it has done in Asia (Nico *et al.*, 2005); specimens of it have already been caught in two US states, and three other carps, i.e., grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys nobilis*), and silver carp (*Hypophthalmichthys molitrix*), imported into the US all have escaped, spread rapidly, and become well-established in numerous states (http://nas.er.usgs.gov/) in spite of regulations such as requiring triploids in aquaculture ponds (Nico *et al.*, 2005). Some black carp as well as grass carp were introduced to eliminate parasitic infections of 'yellow grub', i.e., the metacercaria of *Clinostomum* sp. (Plate 14.12), and other parasites. The grass carp is also a fast-growing fish that also grows to a large size, escaping into natural waters in 48 states. It eats the vegetation, reducing the habitat for the snail and thereby reduces the number of infected snails. Manipulating the distance between the hosts can also prove effective. For example, McGladdery *et al.* (1990) simply moved their fish to deeper water so as to avoid cercarial infections by *Stephanostomum tenue* (Acanthocolpidae); trout cages held over water deeper than 4 m were not seriously affected, presumably because infective cercariae could not reach the trout.

## Chemical Treatments in Tanks or Baths

Therapies in the form of treatment with drug compounds have not yet come into their own in the arena of controlling digenean infections in culture conditions, and the most common methods used to control fluke infections were discussed above for ponds. In tanks and closed, small containers, attendants may typically or routinely use various more costly or more toxic chemicals either because the fish are not going to be consumed by people or because individual animals, such as those for use as brood stock or display, have a high economic or research value.

We will discuss the effective compound praziquantel because it is commonly used. It creates vacuoles in the syncytial tegument of platyhelminths (Andrews *et al.*, 1983; Schmahl and Mehlhorn, 1985), and it is regarded as an industry standard for killing a variety of platyhelminths, including digeneans, as well as monogeneans that harm exhibit fishes in public aquaria (Benz and Bullard, 2004). In that arena, the drug is usually administered in water, not in fish feed; however, others have used the chemical in medicated feeds for eliminating metacercariae of *Diplostomum spathaceum* and other metacercariae. Székely and Molnár (1991) showed that at a single dose of 300 mg per kg body mass killed all metacercaria and that three sequential lower doses ranging from 35-100 mg per kg yielded 88-100% efficacy. Chemical baths of 1 mg per L for nine hours or 10 mg per L for one hour showed 100% and 93-94% efficacy, respectively. Zhatkanbayeva and Heckmann (1990) demonstrated that short baths (0.5-4 hours in duration) and long baths (1-8 days in duration) killed metacercariae of *Diplostomum pusillum* and *Apatemon gracilis*. Paperna and Dzikowski (2006) reported that fish farmers in Israel effectively used veterinary formulas (Droncit) of praziquantel to eliminate metacercarial infections of *Centrocestus* spp., *Haplorchis* spp., and *Bolbophorus levantinus* in juveniles of unspecified tilapia that were >70 mm in total length. However, Paperna and Dzikowski (2006) also noted that such treatments are costly and impractical for some farming systems. Lorio (1989) showed that praziquantel baths and injections were more effective than Ivermectin injections (less effective) and Masoten baths (ineffective) in experimental control of metacercariae of yellow grub in channel catfish. Mitchell (1995) tested the efficacy of praziquantel against yellow grub and unidentified metacercariae in sunshine bass (white bass [*Morone chrysops*] × striped bass [*Morone saxatilis*]). Treatments of 0.25 mg per L for 24 hours and 8 mg per L for eight hours were equally effective in reducing the number of live metacercariae. He also provided some evidence that killed metacercariae remaining in the fish tissue released harmful toxins. Doses of praziquantel of 10, 20, or 100 mg per kg of body weight did not kill metacercariae of *Nanophyetus salmincola* in chinook salmon (*Oncorhynchus tshawytscha*) (see Foreyt and Gorham, 1988). Mahmoud *et al.* (2002) used plant extracts to treat digenean infections. Ata *et al.* (1988) tested the efficacy of praziquantel against *Heterophyes heterophyes* and *Fasciola* sp. Other studies have tested its effectiveness on platyhelminths in other hosts (Nollen and Murray, 1978; Perera *et al.*, 1990).

### Host Defense

Howell (1973) provided a mini-review of how parasites avoid the defense mechanisms of their hosts: (1) antigenic variation (Brown, 1971) where, within a parasite population, a wide range of antigenic variants is present and host responses specific for each variant develop sequentially, (2) induction (Capron *et al.*, 1968) where a parasite is considered to possess a range of codes within its genome so that antigens similar to those of a particular host may be synthesized on infection—in this way the parasite is able to mimic the host in which it finds itself, (3) molecular mimicry (Damian, 1964) where natural selection has resulted in the parallel evolution of host and parasite antigens, and (4) selective contamination (Smithers and Terry, 1969), whereby substances derived from the host become associated with the exposed surface of the parasite and disguise it as host tissue. Additionally, the intracellular habitat of some parasites offers protection from host responses (Desowitz, 1970), and parasites may produce cytotoxins (Heath, pers. comm. in Howell, 1973) that destroy reactive host cells. Roberts (1975), Vogelbein *et al.* (1987), and others have provided detailed discussions of melanin and melanin-macrophage aggregates as well as their interactions with the vascular and lymphatic systems to which they seem closely related. Recent immunological studies will be able to better diagnose, evaluate, treat, and manage digenean infections and stress in general (e.g., Robinette and Noga, 2001).

## DISEASE

### The Host-Parasite Relationship (HPR)

Although definitions of 'parasite' include a consideration of trophic interactions between the host and the parasite as well as the harm inflicted upon the host by the parasite (Bush *et al.*, 2001), the degree of harm, or pathogenicity, is especially difficult to quantify in an infected wild fish. Even more elusive is teasing apart the effects of a parasite on a fish population in nature. An organism is pathogenic when it causes disease, and we define disease in the context of a relationship between symbionts. Herein, a parasite and its host (see other definitions in Kinne, 1984). Disease compromises the HPR when the parasite significantly debilitates (causes morbidity) or kills (causes mortality) its host. One must realize, however, that the term 'disease' is loosely defined in most literature, often used as a synonym of 'infection'. For example, Steedman (1991) referred

to the occurrence of 'black spot disease' in stream fish collections from Ontario. The study documented the incidence, actually 'prevalence' (Bush *et al.*, 1997), of infection by a suite of diplostome metacercariae that elicited the typical melanin-associated host encapsulation that gives the fish a black-spotted or speckled appearance. However, no observation of the health status of any infected fish was described. Hence, in this context, which is a typical occurrence in the literature, the author is probably referring to an *infection* (in the strict sense) rather than a *disease*. In contrast and as an aside, others use the term 'infection' to be restricted to microbial agents. In any event, the status of the HPR, or the magnitude of parasite pathogenicity, is a sliding-scale value influenced by the interplay of parasite factors (e.g., toxin production, prevalence, intensity, site in the host, and life cycle), host factors (e.g., immunological status, stage of development, age, sex, nutrition, genetic history, migratory behavior, and presence of secondary bacterial or viral infections), and environmental factors (e.g., water temperature, pH, turbidity, salinity, organic load, pollution, and oxygen concentration) (Krull, 1934b; Overstreet, 1993; Taskinen and Valtonen, 1995). For example, cold water temperatures may increase susceptibility to particular infectious diseases by altering the wild HPR (Couch and Fournie, 1993) or, in specific, by inducing immunosuppression (Avtalion *et al.*, 1976; Ellis, 1982). Expanding on each of these factors is beyond the scope of this chapter, but the status of the HPR seems primarily dependent on the 'geographic setting', such as an ocean basin, estuary, river, pond, or raceway. So, herein we discuss diseases caused by digeneans according to the setting or context of the HPR: 'wild' (in nature) and 'captive' (in aquaculture). We do this because the pathogen status of a parasite, i.e., the nature of the HPR, can shift from benign to pathogenic or visa versa depending on the setting. HPRs are complex and should be considered on a case by case basis because the environment of the host is inextricably linked to the life cycle of the parasite and, therefore, indirectly related to prevalence and intensity of infection; both of which may determine the severity of disease. Rabideau and Self (1953) highlighted the fact that little is known about how most parasites affect their hosts. Van Oosten (1944) and Elliot and Russert (1949) indicated that parasites are of minor importance in a population, but others surmised that parasites do negatively affect fish populations (e.g., Essex and Hunter, 1926; Hubbs, 1927; Reighard, 1929; Cross, 1938; Vernard, 1940; Fantham and Porter, 1947) or impair reproductive capacity (e.g., Reighard, 1929; Hunter, 1942). Some authors

have likened the HPR to a dynamic homeostasis in which the host and parasite each compensate and adapt to the other in a tit-for-tat fashion, whether envisioned as an 'evolutionary arms race' comprising an escalation of adaptive inventions or weaponry on each side (Damian, 1964; Desowitz, 1970; Hayunga, 1989; Combes, 2001) or as a sort of 'Red Queen hypothesis' (see Van Valen, 1973) wherein the parasite or the host must constantly 'race' against each other to keep the HPR in benign stasis. Ultimately, it is these long-term tit-for-tat interactions and jostlings between hosts and parasites that may yield a phenomenon referred to as 'co-evolution', wherein genetic changes in one of the organisms entices or mandates a reciprocal genetic change in the other (Anderson and May, 1982; Hayunga, 1989; Overstreet, 1998).

## Host-Parasite Relationships (HPRs) in Wild Fish

This section treats the HPR between digeneans (as larvae and adults) and fishes (as both intermediate and definitive hosts) primarily. However, we note that many larvae are pathogenic to the molluscan first intermediate host because they castrate it and essentially use it as a vessel for asexual reproduction. So, in that regard, the digenean essentially kills this host, making it incapable of producing offspring. Regarding fishes, however, the picture is generally much different, although we emphasize that the literature on digeneans causing disease among wild fishes is nevertheless limited. No detail is available about the vast majority of HPRs between digeneans and fishes in the marine environment, scant detail exists about most of those HPRs regardless of the environment (marine or freshwater), and only a tiny fraction of HPRs (mostly of medical or veterinary importance and limited to freshwater systems) have been studied extensively. Further, multiple year studies that describe the change in a digenean population in a wild freshwater host population are few (e.g., Kennedy, 1981; Esch *et al.*, 1997) and, to our knowledge, fragmentary in a wild marine fish population.

Given appropriate environmental conditions, benign parasites can be pathogenic. However, we know of no digenean species that is an obligate pathogen of its normal definitive fish host. With possible exception to *Crepidostomum farionis*, a gut fluke that reportedly caused some intestinal inflammation in salmonids (Davis, 1937; Mitchum, 1965; Hoffman, 1975), and freshwater aporocotylids (*Sanguinicola* spp.) that cause gill hyperplasia and may occlude blood vessels (Bullard and Overstreet,

2002), no detailed report describes a debilitating or significant lesion caused by an adult fluke in a freshwater fish (Hoffman, 1975). Marine records of disease are sparse. Based on our experience, most of the digeneans we have collected from wild hosts resemble commensals in their definitive hosts instead of pathogens because infected and uninfected conspecific hosts captured simultaneously from the same locality seem grossly and histologically indistinguishable. Ecologically, it seems counterintuitive that killing the definitive host would increase parasite fitness (as measured by larval transmission) since digenean larvae are discharged within eggs shed in the host's feces, or rarely from eggs lodged in the gill epithelium, of their definitive hosts. Hence, killing the definitive host would not only halt oogenesis and larval dispersion, but the adult flukes would also die. Unless harming the host somehow affords the parasite a means of producing more eggs in a shorter period of time prior to killing the host, it is difficult to envision how killing the normal definitive host would increase parasite fitness. All of that being said, accidental or stressed hosts still are occasionally severely harmed by digeneans.

## Modifying the Behavior of the Intermediate Host

Some flukes induce in their second intermediate host (either fishes or crustaceans) a modification that makes them more likely to be eaten by the definitive host than an uninfected counterpart. This can be accomplished by weakening the host or by otherwise altering the host's behavior. Scant or occasional circumstantial evidence exists that some digeneans decrease fitness of their intermediate fish hosts in this way. Based on the general appearance of infection only, several authors have speculated that digenean infections facilitate the demise of their intermediate hosts by decreasing their ability to avoid predation by the piscine or avian definitive host (Sommerville, 1982; McGladdery *et al.*, 1990; Bergh *et al.*, 2001) and behavior modifications induced by encysted metacercariae have been observed in experimental or field studies (Radabaugh, 1980; Coleman, 1993; Krause and Godin, 1994). Because the oceans are vast and fishes comprise vagile and oftentimes elusive members of that environment, estimating how parasites affect these hosts is challenging, but there is some information derived from freshwater systems. Brassard *et al.* (1982b) found that guppies (*Poecilia reticulata*) exposed to as few as 25 cercariae of *Diplostomum spathaceum* were consistently and significantly more susceptible to predation by brook trout

(*Salvelinus fontinalis*) than were the uninfected controls, and they concluded that the migrating diplostomula of *D. spathaceum* predisposed the fish host to predation by altering their behavior (circular movements and erratic swimming) and appearance (exophthalmia and dark body coloration). Pennycuick (1971) and Lester (1977) attributed mortalities of fishes to infection by species of *Diplostomum* inferred from fish population and infection frequency data (Chappell, 1995). Crowden and Broom (1980) described loss of efficiency and increase in time devoted to feeding in common dace (*Leuciscus leuciscus*) infected by diplostomula of *D. spathaceum*, and, as infection intensity increased, common daces spent more time in the surface layer of the water. These authors speculated that this behavior increased the risk of predation by gulls (Laridae). By observing the predator efficiency of three-spined sticklebacks (*Gasterosteus aculeatus aculeatus*) infected with low levels of *Diplostomum* spp. (as low as four metacercariae per fish), Owen *et al.* (1993) concluded that these infections could significantly reduce vision, decreasing the ability (measured as reactive distance to prey) of the three-spined stickleback to capture amphipod prey. Radabaugh (1980) showed that schools of infected fathead minnows (*Pimephales promelas*) infected with *Ornithodiplostomum ptychocheilus* were less compact and occupied positions closer to the water surface than uninfected individuals in control schools, and he proposed that *O. ptychocheilus* may manipulate the fathead minnow's behavior to increase its vulnerability to predation by the avian definitive host. For trophically transmitted cercariae, some digeneans can manipulate the behavior of their molluscan first intermediate host but occasionally at the cost of the mollusc being preyed upon by a host in which the parasite cannot develop, such as a molluscivorous fish instead of a bird (Mourtisen and Poulin, 2003).

## Digenean-induced Mortalities of Wild Fishes

We know of only a few detailed reports that describe a mortality of wild fishes attributed to a digenean larva: (1) in 1966 and 1984, cercariae and metacercariae of *Bucephalus polymorphus* killed cyprinids in the Seine (France), Wda (Poland) (Hoffman, 1975), and Main (Germany) rivers (Hoffmann *et al.*, 1990), (2) from 1979 through 1982, metacercaria of *Uvulifer ambloplitis* were implicated in mortalities of juvenile bluegill sunfish (*Lepomis macrochirus*) in a pond in North Carolina, USA (Lemly and Esch, 1984a), (3) in 1997 through 1998, metacercariae of *Centrocestus formosanus* endangered fountain darters (*Etheostoma*

*fonticola*) in the Comal River (USA) (Mitchell *et al.*, 2000), and (4) in 2000, two unidentified bucephalid metacercariae caused mortality in populations of two cyprinid species from the Uji River (Japan). Each of these events share the combined element of a water temperature change (warming or cooling), a presumably short-lived but massive pulse-like mingling of the first intermediate host (mollusc), second intermediate host(s) (small cyprinids or centrarchids), and definitive host(s) (predatory fishes or birds), or an introduced mollusc and parasite. In most of these cases, the researchers comment on the seemingly anomalous nature of the mortality. In other instances, some evidence exists that fry or juvenile fishes stranded in or passing through a particular area during a particular season and under specific environmental conditions may encounter intense infections that may debilitate or even kill them (Faliex and Morand, 1994). Adding to the list and regarding diplostomoids, large numbers of brain-encysting metacercariae of *Austrodiplostomum compactum* (as *Diplostomulum mordax*) killed fishes in an Argentinian lake and two adjacent rivers (Szidat and Nani, 1951, 1952). Reported in less detail, various fishes in the former Soviet Union periodically have likewise been killed or debilitated by intense metacercarial infections of *Diplostomum spathaceum* (Nordmann, 1832; Szidat, 1927; Gvozdev, 1950; Petrushevski and Shulman, 1961), *Posthodiplostomum cuticola* (see Dubinin, 1949), and the strigeid *Cotylurus pileatus* (Dubinin, 1952; Petrushevski and Shulman, 1961).

Other studies have assumed the presence of parasite-induced mortality of a wild fish population (Meyer, 1958; Kakonge, 1972; Harrison and Hadley, 1982; Fischer and Kelso, 1988) with seemingly insufficient quantitative data to support the claim. Rather than collecting animals and making direct observations of the HPR, previous authors have proposed methods for detecting parasite-induced mortalities of wild fishes using statistical models (Crofton, 1971; Pennycuick, 1971; Lester, 1977). However, few of these well-cited models include a single novel collection of an animal. Lester (1984) theorized several useful methods for estimating mortality due to parasites in wild fish populations: (1) by necropsy, (2) by determining the frequency of infections known to be eventually lethal, (3) by observing a decrease in the prevalence of a long-lived parasite (or permanent indication of a prior parasitic infection, such as a scar) with host age, (4) by observing a decrease in the variance/mean ratio for the parasites with host age, (5) by comparing the observed frequency of a combination of two independent events with the calculated probability of their occurrence, and (6) by comparing the observed

frequency distribution of the parasite with a projected frequency based on data from lightly infected fish. Kennedy (1984) suggested that a peaked host age-parasite abundance curve, concomitant with a decline in the degree of dispersion of parasites in the older age classes of hosts, can provide evidence of parasite-induced mortality of a fish stock. He used metacercariae of *Diplostomum spathaceum*, *D. gasterostei*, *Tylodelphys calvata*, and *T. podicipina* in relation to fish age in a field collection and found that the mean parasite burden of most of those parasites increased with host age and the maximum mean burden was found in the oldest hosts. Whereas, the variance to mean ratio also increased in one species, it decreased in the oldest hosts of two species. From that he concluded that the decrease could have been due to parasite-induced host mortality but could also be equally due to death of parasites within the host or to changes in the infection rate or a result of the small sample size. He noted that none of the data provided clear and unambiguous evidence of host mortality induced by intense infections of any of the four species, and also that it may be just as difficult to detect and demonstrate parasite-induced mortality in metacercarial-fish host systems as in any other parasite-host system. For certain types of HPRs, convex curves of mean parasite abundance in relation to age (age-density curves) concomitant with a decline in the degree of dispersion in the older age classes of hosts may be evidence of the start of a parasite-induced host mortality (Anderson and Gordon, 1982). This is intuitive and can be stated more simply: if most of the infected individuals are young and few or no parasite is detected in old fish, then young heavily infected fish may have died.

Statistical methods are helpful and provide a framework within which to plug-in empirical data based on field observations (e.g., Falex and Morand, 1994). However, there is a philosophical disconnect between the sub-disciplines of Parasite Ecology and Descriptive Parasitology that is based on the personal experiences and interests of the workers operating in each sub-discipline. Ecologists may model systems sometimes without particularly relevant empirical data or first hand observations of the HPR. For example, they may apply agriculture disease or human disease models to marine HPRs. Anderson and May (1982) claimed that parasites are as important as predators and insect parasitoids in the 'regulation' of host populations, but the problem with such a statement is that there is very little quantitative data to support this claim (Lemly and Esch, 1984a) and a single example does not necessarily express a generalization. On the other hand, field parasitologists rely primarily on field observations of the

HPR based on few individuals and attempt to justify those observations as typifying the condition in most individuals in that system. Both methods are biased, but neither field has produced compelling evidence that any digenean commonly kills its definitive fish host. As recorded in the literature, field parasitologists, including us, tend to regard many adult digeneans as benign symbionts of their fish hosts because it is unusual or practically unheard of to encounter a wild HPR in which the digenean causes a significant pathological alteration. That is, it is extremely rare to collect a fish that is obviously debilitated by one or a multitude of digeneans. Seemingly the most comprehensive method for assessing the wild HPRs comprises a combination of field observations and collections, statistical data analysis, and controlled experimental studies. Few studies have achieved this, but some good examples have been published in freshwater systems. For example, Lemly and Esch (1984a) studied juvenile bluegill sunfish infected by encysted metacercariae of *Uvulifer ambloplitis* at both the individual host level and at the host population level. They used a battery of field observations, statistical analyses, laboratory experiments, and field collections to address how *U. ambloplitis* affected its hosts. Krull (1934a) thought that *Cercaria bessiae* (probably a species of *Crassiphiala*) caused mortalities of wild fish fry based on experimental observations of exposed pumpkinseed (*Eupomotis gibbosus*). Field collections and laboratory experiments should complement each other because, standing alone, they each have inherent weaknesses. In any event, the setting seems to matter, and mortality demonstrated in laboratory experiments may not naturally occur in the wild (Bedinger and Meade, 1967; Hoffman and Hutcheson, 1970; Mitchell *et al.*, 1983). For example, exposing fry or fingerlings to a large number of cercariae may kill the fish, but in nature the fish may seldom or never encounter that level of infection (e.g., Baldwin *et al.*, 1967; Matthews, 1973b; Overstreet and Curran, 2004). Hence, reported occurrences of cercariae or metacercariae killing fishes in aquaculture or under experimental conditions should be regarded as artificial (essentially an overdose, e.g., Kniskern, 1952) because more cercariae are available than in the natural environment or the fish are physically confined to the area of those cercariae and unable leave the area. Again, ecologically, pathogenicity to the second intermediate host or the definitive fish host is of no apparent benefit to the parasite's fitness because the cercariae must develop further in that host to continue the life cycle. Nonetheless, some digeneans are pathogenic in this way under particular aquaculture settings and, in a few reports, in the wild. Hence, this larval stage of flukes is relevant to fish disease.

Why are there not more records of diseased wild fishes infected with digeneans? We concur with Sindermann and Rosenfield (1954) and others who have stated that it is difficult to assess parasite-induced mortalities of wild fishes because 'predators quickly remove weakened and disabled fish, and ... scavengers rapidly remove evidence of death'. Stated differently, moribund or chronically diseased fishes are consumed by predators and are lost from the population before they can be caught and examined (e.g., Van Dobben, 1952). The most obvious explanation, alternatively, is that we do not see diseased wild fishes infected with digeneans because digeneans do not cause disease in wild fishes. However, this is overly simplistic. Sampling effort has not been adequate enough for most taxa to have any clue about what is happening between digeneans and wild fish hosts. This is because working in the ocean is risky and fishing or parasite collections can be hectic onboard boats, especially those operating far from shore. Additionally, fish sometimes are not caught, weather and sea conditions can be dangerous or prohibitive and unpredictable, navigation in the ocean can be challenging and in any case requires a knowledgeable captain, ice and cooler space for maintaining fish carcasses prior to necropsy is limiting, mechanical problems with boats or gear can quash collections, fish distributions may vary seasonally, parasite life cycles are seasonal (MacKenzie, 1968; Bowers, 1969; Lemly and Esch, 1984c; Taskinen *et al.*, 1994; Taskinen, 1998a, b; Flores and Semanas, 2002; Steinauer and Font, 2003), captured fishes may not be infected, and parasites may not be extracted from infected fishes soon enough to yield quality specimens. Perhaps freshwater HPRs are better studied because ponds, streams, lakes, and rivers are relatively confined and more readily accessible over a wider range of weather conditions and with minimal gear requirements (Lemly and Esch, 1983, 1984a, b, c). The way fish are sampled may bias which fish are caught. For example, hook and line fishing may more likely capture healthy fishes; whereas, trawling and gill netting may more likely capture less healthy fish (Couch and Fournie, 1993). More than half a century ago, Fischthal (1948) recognized that the perception of parasite prevalence could be an artifact of the behavior of the collector, or how, when, and where the fisherman samples the host population. Further, the effect on a host population may be a result of infection in fry, and fry and fingerlings in the wild are more difficult to sample than larger fish. In addition, baseline data on 'normal' histological features is completely lacking for most fish populations. As a result of these factors, assessing the status of a wild HPR is a daunting but alluring task;

especially among marine fishes at liberty in the vast ocean. In captivity, however, the effects of digeneans are more readily apparent and more easily studied, and much detail about the life histories and pathological effects of digeneans has been derived from captive HPRs in which a disease condition is manifested.

## Host-Parasite Relationships in Captive Fishes

Digenean epizootics in aquaculture occur in ponds and hatcheries in freshwater as well as in sea cages or net pens in the ocean where they result in significant economic losses by killing the fish outright, devaluing filets, or by mandating costly manipulations of the fish stock. Most of the digeneans that are known pathogens of cultured fishes infect freshwater aquaculture fishes as the second intermediate host that are then preyed upon by a piscivorous bird definitive host (Plates 14.10–14.13). Penetrating cercariae can kill the cultured fish provided infection intensities are high enough, and the number varies considerably, depending on the species. Alternatively and although the fish may not be killed outright, a multitude of encysting metacercaria can devalue the filets. For example, channel catfish are of little value in the processed fish market and of limited value in fee-fishing ponds if they are infected with yellow grub (Lorio, 1989). Paperna (1991) stated that such large and conspicuous metacercariae can escape from their cysts and migrate within the fish. Metacercariae associated with 'black spot' affect market value of both freshwater and marine fish products as will be discussed later. In a few cases restricted entirely to aporocotylids, the adult fluke or its eggs as well as penetrating cercariae can kill fishes in freshwater hatcheries as well as in oceanic net pens (Plates 14.16–14.21). Although not a documented pathogen of an aquaculture fish, some didymozoids, digeneans that as adults are encapsulated in extra-intestinal sites (e.g., gill epithelium), show potential for causing pathological changes to the host's gill epithelium that may be harmful (Plates 14.22–14.25). Many of these specific pathogens will be discussed by family below.

## PATHOGENS

Larval digeneans harm fish hosts more than adult digeneans, and only select species of Aporocotylidae are regarded herein as pathogens of their definitive host fish. Appreciating the differences in potential pathogenicity among different life history stages of digeneans allows one

to critically assess disease diagnosis and prevention, and because of this we spend considerable time discussing specific life histories of select digeneans (Plates 14.10–14.14, 14.16, 14.20, 14.22). No digenean is equally pathogenic throughout all of its life history stages, but cercariae and metacercariae of many species as well as the adults and eggs of a few species are problematic under appropriate conditions.

The digeneans that cause disease in aquaculture and those that are alleged potential pathogens of wild fishes can be separated into three broad functional categories, with some species occupying multiple categories depending on their life history stage. (1) Second intermediate host killed by a multitude of penetrating cercariae. For example, penetrating cercariae may induce erratic swimming behavior and equilibrium loss (Johnson, 1971; Erasmus, 1972) or hemorrhages of blood vessels (Davis, 1936; Ferguson, 1943; Erasmus, 1959; Johnson, 1971). (2) Second intermediate host debilitated, killed, or otherwise devalued by encysted, encapsulated, or free metacercariae in somatic muscle, eye, or other tissues or organs. Many of these larvae are pathogenic because they elicit a strong host response during migrations within the second intermediate host fish (e.g., Hoffman, 1975). (3) Definitive host debilitated or killed by multitude of adult flukes or eggs plus miracidia (e.g., Paperna, 1991; Overstreet, 1993; Bullard and Overstreet, 2002). In each case, the extent of the host's cellular response or reaction depends on the duration and location or path of larval migration in the fish host as well as species differences in how the parasites and hosts interact (e.g., Hoffman, 1975). Extra-intestinal adult flukes or progenetic metacercariae that do not produce or permanently maintain a cyst in the second intermediate or definitive host tend to cause the most significant problems in wild fishes; whereas, flukes that actively penetrate their hosts and encyst in the flesh tend to be especially problematic in aquaculture settings. Below, we present biological information about digeneans that harm fishes to demonstrate the diversity of effects that have been documented for particular groups of digeneans in fishes. We organize these groups according to their hosts, their life history, and which hosts they harm the most. We also provide some cursory information about some other flukes that are occasionally identified as fish pathogens. Herein and simply as a matter of convenience we define a 'bird fluke' as a digenean that matures in a bird (e.g., Plates 14.10–14.13) and a "fish fluke" as a digenean that matures in a fish (e.g., Plates 14.14, 14.16, 14.20, 14.22).

## Bird Flukes whose Cercariae and Metacercariae Harm the Second Intermediate Host Fish

Most of the digeneans presently known to cause disease among fishes in aquaculture or in the wild or to cause economic losses in aquaculture operations fall within this category (e.g., Ferguson and Hayford, 1941; Overstreet *et al.*, 2002). Of those, we discuss examples in the Diplostomoidea (Figs. 14.5C-D, F; Plates 14.10–14.11), Clinostomidae (Plate 14.12), and Heterophyidae (Plate 14.13). Most readily-available literature on fish diseases involves the diplostomoids, although heterophyids may rival them in their economic relevance in fish aquaculture. The general life history pattern for members of these groups may have exceptions that involve some second intermediate hosts other than fishes and definitive hosts that may involve a mammal or reptile, but the ones we focus on here mature in birds, use fishes as second intermediate hosts, and use snails as first intermediate hosts (Plates 14.10–14.13).

## DIPLOSTOMOIDEA

### *Diagnosis*

For the purposes of this chapter, we will emphasize the Bolbophoridae, Diplostomidae, Neodiplostomidae, and Strigeidae. Members of these diplostomoid families, commonly referred to as 'strigeids' or members of 'Strigeidae' in the older literature, can be recognized by their metacercariae in fish (Figs. 14.3H-M, 14.5C-D, F, 14.7A-D, 14.10E). In fact, the metacercariae have historically been provided 'group' names corresponding to the metacercarial forms. The strigeids have a *Tetracotyle*, which contains 'pseudosuckers', also called 'lappets' and other terms, and a composite of several integrated characters: para-oral depressions, microvilliform processes, gland cells with associated ducts, and secretions associated with extra-corporeal digestion. In addition, they have a paranephridial plexus, also called a 'reserve bladder', that networks throughout the entire body, and they encapsulate within a well-defined cyst wall of parasite origin and referred to here as a cyst rather than a capsule, even though the cyst may be encapsulated by host tissue or host-derived material. Another thick-walled metacercaria encysting in fish but lacking the pseudosuckers and having a plexus consisting of four main canals in a circular or oval-shaped body is called a *Prohemistomulum* and

characterizes another diplostomoid family, Cyathocotylidae. Little is known about the *Proterodiplostomulum* that has pseudosuckers and encysts in fish and matures in reptiles. The three most commonly encountered metacercariae in most regions are the *Neascus*, *Diplostomulum*, and *Prodiplostomulum*. The neascus metacercaria encysts, often with a thin cyst wall, and does not have pseudosuckers; the diplostomulum has pseudosuckers and does not encyst; and the prodiplostomulum is in between the two, with pseudosuckers and a true but thin cyst wall, often encircled by a thick host-derived encapsulation. This scheme follows the metacercarial forms and associated families recognized by Shoop (1989) rather than the more recent characterization of Niewiadomska (2002a) for which the metacercarial forms do not fit within family groups; however, she provides helpful diagnoses and keys to the genera (Niewiadomska, 2002b, c), which updates the keys in the synopses by Dubois (1968, 1970).

## Life Cycle

Although disease in pond culture systems is sometimes blamed on one digenean species, diplostomoid life cycles and diseases can be complex and involve multiple parasite species and multiple sets of hosts for those parasites because (1) susceptible bird definitive hosts are transient participants in pond culture systems that feed on infected fishes and defecate in the water, (2) susceptible snail hosts can be abundant in pond systems that include submerged aquatic vegetation, and (3) many ponds include a polyculture of several fishes. This complex of interactions underscores the need for consultation with a taxonomic expert who can reliably identify these animals (both parasitic and free-living members of the fauna). For example, Overstreet *et al.* (2002) studied channel catfish aquaculture facilities in the southeastern region of the United States (US) that were taking heavy losses originally attributed to infections by a single diplostomoid species (*Bolbophorus "confusus"*) from the American white pelican primarily in Mississippi, Louisiana, and Arkansas (Plate 14.10). The infection dynamics of this system were much more complex than originally assumed (Overstreet and Curran, 2004). Two sympatric species of *Bolbophorus* matured in the American white pelican: *B. damnificus* infected the musculature of channel catfish and a species to be named, *Bolbophorus* sp., did not, but *B. confusus* did not occur in the area. Additionally, at least four other pathogenic diplostomoids and a clinostomid infected the channel catfish, and several other species also

infected snails but not channel catfish in the ponds. The cercaria of each species of these channel catfish flukes was restricted to a single snail host species, and at least four different snails, including planorbids (*Planorbella trivolvis* and *Gyraulus parvus*), a physid (*Physella gyrina*), and a lymnaeid, harbored cercariae infective to channel catfish. Two metacercariae (the prodiplostomulum of *B. damnificus* in the skeletal musculature and the diplostomulum of *Bursacetabulus pelecanus* in the brain, spinal cord, optic nerve, and eye) matured in the American pelican while the diplostomulum of two others, *Austrodiplostomum compactum* free in the brain, optic nerve, and eye and *Hysteromorpha triloba* free or encapsulated in the skeletal musculature, usually deep near the vertebral column, matured in cormorants (*Phalacrocorax* spp.) and that of another, *Diplostomum* sp. in the eye lens, matured in gulls. The yellow grub of *Clinostomum marginatum*, a related digenean, occurred free or encapsulated, usually under the dermis, matured in the mouth and esophagus of herons and egrets (Ardeidae). In the same ponds often occurred the prodiplostomulum *Bolbophorus* sp. in mosquitofish (*Gambusia affinis*) and bluegill sunfish, the neascus of *Posthodiplostomum centrarchi* in bluegill sunfish, and the yellow grub of *Clinostomum* sp. in mosquitofish; none of these was seen in the sympatric channel catfish, but all could produce mortalities in their fish hosts.

Host specificity of the various life history stages of diplostomoids is different for different species, but of those that have been well studied, an optimal host in which larval production or larval success is high is usually identified (Chappell, 1995). Some larvae will simply not develop in some hosts (Olson, 1966) and, in some cases, miracidia require particular snail hosts to reproduce asexually. For example, Hoffman (1958) documented the life cycle of *Ornithodiplostomum ptychocheilus* in which adults were obtained from juvenile domestic chickens (*Gallus gallus domesticus*) that were force-fed neascus metacercariae collected from the mesentery and brain of common shiners (*Luxilus cornutus*) (as *Notropis cornutus frontalis*) and fathead minnows as well as those from the mesentery of creek chubs (*Semotilus atromaculatus*) (as *Semotilus a. atromaculatus*). Three snail species, *Physa anatina*, *Stagnicola palustris*, and *Helisoma anceps* were exposed to miracidia but only *P. anatina* yielded a longifurcate monostome-type cercariae after 45 days post-exposure. Waddu and Chappell (1991) demonstrated that *Diplostomum spathaceum* established and survived less well in *Lymnaea stagnalis* than in *Lymnaea peregra*. Although many diplostomid cercariae infect and develop to diplostomula within a range of

small fishes, all require a particular host fish or fishes within which to develop. Cercariae of *Diplostomum baeri eucaliae* infected the snails *Stagnicola palustris* and *Stagnicola palustris elodes*, but out of several small fishes exposed to those cercariae, only the brook stickleback (*Culaea inconstans*) (as *Eucalia inconstans*) was susceptible to infection (Hoffman and Hundley, 1957). Lester and Huizinga (1977) observed that *Diplostomum adamsi* developed in yellow perch only and not lake trout (*Salvelinus namaycush*), white sucker (*Catostomus commersonii*), creek chub, or goldfish (*Carassius auratus*) that were also exposed to the cercariae. Sweeting (1974) infected several fishes with cercariae of *Diplostomum spathaceum* and observed differences in the resulting mean number of diplostomula per fish species. He concluded that physiological differences between host species accounted for considerable differences in infection intensity. Betterton (1974) demonstrated that brown trout (*Salmo trutta trutta*) and rainbow trout (*Oncorhynchus mykiss*) under identical culture conditions and exposed to cercariae of *Diplostomum spathaceum* were differentially susceptible to infection; brown trout had many fewer diplostomula in the eye, with those diplostomula taking three hours longer to reach that site in experimental infections. Mitchell *et al.* (2006) examined the metacercariae of *Bolbophorus* in fathead minnows from a baitfish pond in the Mississippi Delta and discovered one specimen of *B. damnificus* and nine of *Bolbophorus* sp., making the fathead minnow the only known naturally infected non-ictalurid host. Similarly, diplostomoids will mature in particular bird hosts only (Lester and Lee, 1976; Hoffman, 1999; Overstreet *et al.*, 2002).

As already indicated, site specificity of diplostomoids has been studied in second intermediate host fishes. Most larvae migrate to a specific site in their intermediate host. For example, diplostomula of *Cercaria X* had a restricted tissue distribution during migration plus a unimodal distribution along the body length, indicating tissue specificity to the eye lens of three-spined sticklebacks (Erasmus, 1959). So, although penetration by this cercaria could occur all over the body, the resulting distribution of larvae in the fish was not haphazard. In a few exceptional cases, the migrating larva may infect the same organ in several unrelated hosts under experimental conditions, e.g., cercariae of *Diplostomum flexicaudum* penetrated the eye and grew in the lens of 'frog tadpoles, frogs, turtles, chicks, ducklings, and laboratory white mice, rats, guinea pigs, and rabbits' (Ferguson, 1942).

Immune responses to diplostomoid infections are relatively well studied in fishes (Chappell, 1995). Metacercarial antigens enhance survival of wild infected fish (Speed and Pauley, 1985), peritoneal macrophages can be activated *in vitro* and *in vivo* (Whyte *et al.*, 1990), and antibodies for *Diplostomum spathaceum* (in the lens) and *D. gasterostei* (in the retina) are known (Chappell, 1995), but more information is needed before a feasible vaccination strategy can be implemented. So-called 'immunologically privileged' sites, such as nerve tissue and the eye, are the target site for at least several species (e.g., Ferguson, 1943; Ashton *et al.*, 1969; Overstreet and Curran, 2004).

Environmental factors such as water temperature and water flow characteristics as well as habitat characteristics, host density, and fish community structure significantly affect the distribution and larval development of digeneans (Olson, 1966; Stables and Chappell, 1986; Ondračova *et al.*, 2004a, b). Life cycles are seasonal. Temperature affects the (1) ability of miracidia to infect snails, (2) development of asexual reproduction in the snail host, and (3) larval development in the second intermediate host fish. For example, Waddu and Chappell (1991) documented that lower temperatures reduced larval success of miracidia of *Diplostomum spathaceum* in *Lymnaea peregra* and *L. stagnalis*. Fox and Olson (1965) studied egg viability, cercarial shedding, metacercarial development, and adult maturation of *Bolbophorus confusus* relative to temperature. Eggs of that species collected from fecal samples hatched at twelve days at 26.7°C, 14 days at 21.1°C, and 65 days at 15.6°C, with high mortality of eggs and malformation of miracidia at temperatures between 15-20°C. Laboratory infected snails shed cercariae in 30-34 days at 21°C, and maximum numbers of cercariae were shed between 21-24°C. Shedding ceased below 15°C, suggesting that the larvae cannot overwinter in the snail (unless perhaps the snail buries in the sediment, which they can do at least in Mississippi and adjacent states). Results for temperatures were consistent with those of Lemly and Esch (1983), who studied the neodiplostomid *Uvulifer ambloplitis* in pond-dwelling juvenile centrarchids. Lyholt and Buchmann (1996) studied the influence of temperature and light on shedding of the cercaria of *Diplostomum spathaceum* from infected *Lymnaea stagnalis*. Their results showed that cercarial shedding was strongly temperature dependent: 58,000 cercariae per day at 20°C and only 10,000 cercariae per day at 10°C. At constant temperature, cercarial shedding was light/dark dependant. *Planorbella trivolvis* shedding cercariae of *Bolbophorus damnificus* reduced from 345

down to only 2 per ml when cooled from 25 to 15°C (Terhune *et al.*, 2002), but at higher temperatures the shed cercariae could thrive in 2.5 ppt salinity, a level harmful to the host snail (Venable *et al.*, 2000). The snails, however, can bury in the substratum during the winter months, once again producing infections in the spring. In another situation, diplostomula of *D. spathaceum* migrated from the gills or skin to the eye faster at the warmer temperature: 50% of the larvae reached the lens of rainbow trout in <26 hours at 7°C and in <15 hours at 15°C. Karvonen *et al.* (2003) studied transmission, infectivity, and survival of cercariae of *D. spathaceum* in a relatively small container and concluded that while some cercariae could survive for 36 hours, most started dying after 20 hours, and that infectivity of the fish host dropped off when cercariae penetrated the host after twelve hours, suggesting their resources necessary for penetration and migration was significantly depleted at twelve hours. Infection was also found to be frequency dependent, suggesting a higher larval success in culture ponds as opposed to large lakes or free-flowing streams.

Migratory pathways of diplostomid larvae within the host and the optimal site of cercarial penetration apparently differ for different fluke species, with individuals of some species using multiple pathways, e.g., somatic musculature and blood stream. Cercariae that penetrate and migrate in fish tissues can cause obvious muscle necrosis and hemorrhaging soon after penetrating the host (Davis, 1936; Lewert and Lee, 1954a, b; Erasmus, 1959). For example, the cyathocotylid *Neogogatea kentuckiensis* has a small and innocuous metacercarial cyst in the muscle of rainbow trout, but migrating cercariae of *N. kentuckiensis* can kill two-week-old rainbow trout in 2.5 hours when exposed to 30 cercariae each (Hoffman and Dunbar, 1963; Hoffman, 1975). Although the damage to the host may not be lethal, and typically is not, if only small numbers of larvae are acting; greater numbers of penetrating cercariae, however, may kill fishes (Hoffman, 1999). Several authors propose that cercariae are carried passively in the bloodstream (Betterton, 1974; Chappell, 1991); whereas, Hoffman (1958), Erasmus (1959), and Ratanarat-Brockelman (1974) provided evidence that diplostomula traveled through the muscle and connective tissue. Ferguson (1943) exposed the tails of fathead minnows to a single cercaria of *Diplostomum flexicaudum* and in 14 of 18 attempts the cercariae successfully reached one of the eye lenses. In fish that were 5-6.5 cm in total length, parasites that had invaded the tail reached the lens in 1.75-2.0 hours at 0.5 mm per minute, suggesting that they were carried in the blood. Similarly, Hoffman and Hoyme (1958)

exposed 28 brook sticklebacks to cercariae of *Diplostomum baeri eucaliae* for various lengths of time before being fixed and serially sectioned. The distribution of cercariae in the fish indicated that cercariae penetrate the skin of the head and gill primarily before migrating through the subcutaneous tissue and muscle to make their way to the brain. At least some were found in the blood. Hendrickson (1979) studied penetration and migration of *Ornithodiplostomum ptychocheilus* to the brain of fathead minnows and stated that migration of the diplostomulum occurs almost exclusively in the general body musculature and connective tissue, although a few cercariae gain direct access to the nervous system by way of the eyes. Kearn (1998) pointed out that it would be remarkable if the diplostomulum had sufficient secretory material within the penetration glands of the cercaria to sustain an 18-hour migration by tunneling through relatively dense tissues and still retain enough to enter the eye lens. But, regardless of the method of migration or the route taken by the diplostomulum, the backwardly directed spines seemingly function to grip tissue and allow the worm to move forward by extending the anterior end of the body and grappling the host tissue. Some work demonstrates that the site of penetration partly determines larval success. Whyte *et al.* (1991) studied penetration and migration of the diplostomulum of *Diplostomum spathaceum* in the rainbow trout. They exposed different regions of the fish to known numbers of infective cercariae and noted that 40% of penetrating cercariae reached the lens of fish following exposure of the head or entire body; 20% of those injected intraperitoneally, intramuscularly, or intracardially reached the lens; and only 5% established as metacercariae in the lens following exposure of the tail region. However, once the diplostomulum has reached the site of infection, e.g., *D. spathaceum* in the eye lens of fish, they may survive for several years. Hendrickson (1978) showed that metacercariae identified as *D. spathaceum* in Arctic grayling (*Thymallus arcticus*) lived for three years and those of *D. baeri eucaliae* in brook sticklebacks lived for over one year.

### *Specific Pathogens*

We discuss pathogens belonging to Bolbophoridae (*Bolbophorus damnificus* only), Diplostomidae (~eight species), and Neodiplostomidae (~four species) as well as the strigeids *Apatemon gracilis* and *Cotylurus pileatus*. The cyathocotylid *Neogogatea kentuckiensis* was already discussed above.

We present each of these four families and their members that comprise specific pathogens of fishes in alphabetical order, not necessarily in order of their pathogenicity or relevance to fish disease.

## Bolbophoridae (Prodiplostomulum)

### *Bolbophorus damnificus*

Overstreet and Curran (2004) determined that cercariae of four species of diplostomoids can produce mortality in the channel catfish from Louisiana catfish ponds. Time of death depended on the species of worm, virulence of the individual cercaria or metacercaria, number of cercariae entering the host, size of fish, tissues and organs infected, presence of associated bacteria, environmental conditions, and presumably a few other biotic and abiotic factors. *Bolbophorus damnificus* seemed especially pathogenic to the channel catfish compared with other species; fingerlings died within five minutes if exposed to several hundred cercariae; fewer cercariae produced mortalities after longer periods; hydropic degeneration of kidney occurred in some infected fish; chronic infections elicited a thick fibrotic encapsulation around the cyst, depending on the site and other features of infection; hemorrhaging was often associated with the infection. When prodiplostomula occurred just below the dermis, hemorrhaging often was grossly visible, and when channel catfish with a low number of about four prodiplostomula per fish were administered the bacterium *Edwardsiella ictaluri* they started dying at about day seven, which was the same time as channel catfish without the fluke but given the bacterium. However, mortalities of the two groups started to level off about day eleven or twelve, reaching over 80% mortality with the fluke and less than 50% with the bacterium only. Those fluke-infected fish not given the bacterium did not die nor did fluke-infected fish die when given the bacterium at day 28 when the metacercarial cyst wall was well-formed (Labrie *et al.*, 2004). As indicated above, when we exposed a few hundred cercariae to the fingerlings, they died within about five minutes, and some of those fish exposed to fewer cercariae also died. We determined that many exposed fish with 50-100 established metacercariae survived at least one to two weeks. Of the numerous examined 10-cm long fingerlings from a Mississippi pond with chronic infections, all had 48 or fewer metacercariae, suggesting that heavier infections were lethal. We have seen some adult fish with a few hundred established metacercariae, but most have few.

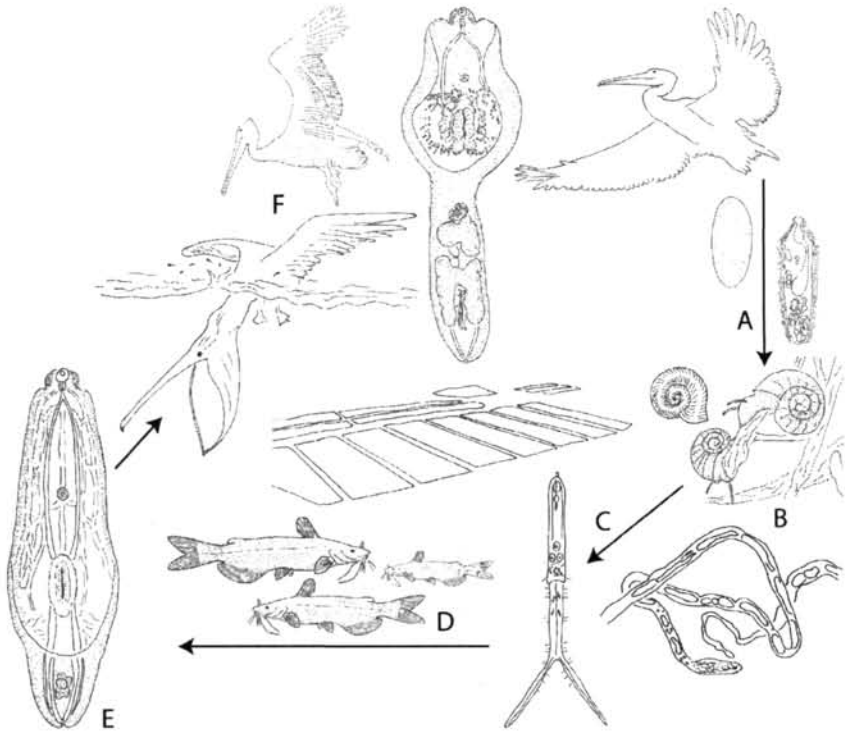


**Plate 14.10** Life cycle of *Bolbophorus damnificus* (Bolbophoridae) involving the marsh rams-horn snail (*Planorbella trivolvis*), the channel catfish (*Ictalurus punctatus*), and the American white pelican (*Pelecanus erythrorhynchos*). Based on Overstreet *et al.* (2002). **A.** The adult diplostomid infects the white pelican's gut and its eggs are deposited in the pond by means of the pelican's feces. **B.** Each egg contains a ciliated miracidium that infects the marsh rams-horn snail only, despite other snails being present in the system. **C.** The miracidium produces mother and daughter sporocysts and a furcocercous cercariae. **D.** Cercariae emerge from the marsh rams-horn snail and penetrate the channel catfish only, lose their cercarial tail, and encyst in and develop as a metacercaria in the dermis or a nearby site. **E.** White pelicans prey on infected channel catfish and acquire the metacercariae.

## Diplostomidae (Diplostomulum)

### *Diplostomum adamsi* and *Diplostomum scudderi*

When 1.6 cercariae of *D. adamsi* per ml were exposed to yellow perch for 60 minutes, the fish died in less than 24 hours, probably as a result of extensive hemorrhaging in the brain during cercarial migration (Lester and Huizinga, 1977). When fewer cercariae (e.g., about 400) were exposed to the yellow perch, most of them migrated to the retina, but a

few occurred in the brain. The cercariae formed a cavity between the photoreceptor layer and the pigment epithelium of the fish eye. In that site, the photoreceptor cells were intact but spaced farther apart than in the normal tissue, suggesting that the retina had been stretched. Free pigment-containing cells were present on their surface. There were fewer nuclei in the inner nuclear layer, and the inner synaptic layer was thinner than normal. The cells of the pigment epithelium were more diffuse than normal, and numerous mononuclear and polymorphonuclear cells, most containing black pigment, were free in the cavity. *Diplostomum scudderi* becomes established in a similar manner in the retina of three-spined sticklebacks. Both fish hosts exhibit cytologic damage, and alterations occur in both layers of the retina, but overall visual loss due to the peripheral location of the parasites was minimal (Lester and Huizinga, 1977).

### *Diplostomum baeri eucaliae*

Cercariae of *Diplostomum baeri eucaliae* penetrate the body surface of brook sticklebacks and migrate to the brain where they tend to localize in the choroid plexus. The organ may become hyperplastic as the metacercariae grow in size and number, resulting in a tumor-like structure that extends posterolaterally from the optic lobe (Hoffman and Hoyme, 1957, 1958; Hoffman, 1975). There are columnar epithelial cells and cells appearing as macrophages surrounding the worms (Hoffman, 1975). Hoffman and Hoyme (1957) showed that the 'tumor' was really an out-pocketing of the dura caused by *Diplostomum baeri eucaliae*; its epithelium was an outgrowth of the choroid plexus which surrounded the worms at the posterolateral aspect of the optic lobes. Many worms could become localized in the inner part of the optic lobes, resulting in considerable brain tissue being re-absorbed, the ependyma becoming hyperplastic, and macrophages accumulating.

### *Diplostomum flexicaudum*

Diplostomula of several species target the vitreous humor or other areas of the eye rather than the lens; one is *Diplostomum flexicaudum*. Van Haitsma (1930) determined that large numbers of experimental cercariae of *D. flexicaudum* caused lesions and death in fishes. In numbers of several hundred, this cercariae also kills tadpoles of American toad (*Bufo americanus*), green frog (*Rana clamitans*), and northern leopard frog (*Rana*

*pipiens*) by causing paralysis and vascular system injury that results in fatal hemorrhage (Davis, 1936). Interestingly, large numbers of these metacercariae occurred in the eye of exposed tadpoles, similar to that in fishes and suggesting that the pathways of cercarial migration may be common among fishes and frogs. Dawes (1952) found that penetration by '*Cercaria* C' killed 'minnows', goldfish, common carp (*Cyprinus carpio*), 'bitterling carp', tench (*Tinca tinca*), stone loach (*Barbatula barbatula*), as well as 'perch and small sticklebacks' overnight.

Larson (1965) studied an atypical pathological effect caused by the metacercaria of a fluke that resembled *D. flexicaudum* in black bullhead (*Ameiurus melas*) (as *Ictalurus melas*) from Minnesota and North Dakota. The cercariae can occasionally be found in the vitreous humor and retina, but they typically localize in the lens. As they grow the lens herniates near the point of attachment to the dorsal ligament to form a dorsally located cyst containing the fully developed metacercariae with associated hemorrhaging. In some cases, the iris can be greatly distorted, and the metacercaria can spill into the protrusion, leaving the remainder of the lens clear for visual function and allowing the fish to avoid predation. In blind fish, the color and feeding behavior becomes altered.

### *Diplostomum phoxini*

Rees (1955) examined hundreds of Eurasian minnows (*Phoxinus phoxinus*) from a pool in Wales and found that each was infected with *Diplostomum phoxini*. This is significant simply because it shows the high prevalence of particular larvae in particular habitats, but this is not necessarily typical. Later (Rees, 1957), she infected Eurasian minnows with cercariae of *D. phoxini* and described the pink color of the brain and the presence of blood in the brain ventricles. She attributed these pathological changes to minor hemorrhages produced by arriving cercariae, but these features were not seen in long-standing infections even though naturally infected Eurasian minnows had brains that were noticeably swollen.

### *Diplostomum spathaceum*

The taxonomy of *D. spathaceum* is confused. Not all material identified as this species is conspecific, and some species considered by some as synonyms of it, such as *D. flexicaudum*, are not. In any event, this species and a few others primarily infect the lens rather than the vitreous humor or retina of the eye. Nordmann (1832) first described a disease caused by

*D. spathaceum* upon discovering 370 diplostomula in the lens of rudd (*Scardinius erythrophthalmus*). Diplostomula cause 'white eye', or an obvious pathological alteration recognizable as a cataract in the lens (Chappell, 1995), and in severe cases the infection can cause blindness, emaciation, and death (Rushton, 1938; Palmer, 1939; Pennycuick, 1971). Usually, cercariae of this species penetrate the eye lens, grow, and occupy the hole created in the lens (Hoffman, 1975), and the infected fish becomes sluggish, cannot feed properly, becomes emaciated, and in some cases dies (Ferguson and Hayford, 1941). Typically, there is very little tissue reaction to the parasite; however, in some infections there is marked proliferation of the lens epithelium adjacent to the parasites, and the lens becomes opaque, resulting in blindness (Ashton *et al.*, 1969). Larsen and Buchmann (in Overstreet and Curran, 2004) exposed five-month-old rainbow trout to 10, 100, 600, or 1,000 cercariae, of which 60-80% were recovered as diplostomula in the lens. The two high doses killed the fish within two days and some fish in the other groups developed abnormal behavior. Brassard *et al.* (1982a) exposed guppies (as *Lebistes reticulatis*) and brook trouts to cercariae and documented an essentially linear relationship between exposure density and cercarial penetration of the host coupled with a host death rate that increased exponentially with parasite intensity in both hosts. They noted other studies (e.g., Pennycuick, 1971) that showed a high degree of overdispersion of *D. spathaceum* in wild fish populations, perhaps ensuring that relatively few fishes succumb to the infection by migrating diplostomula of *D. spathaceum*.

### *Austrodiplostomum compactum*

This fluke occurs as a diplostomulum free in the vitreous humor, brain, and optic nerve of a few fish hosts, including the channel catfish, from North to South America; it matures in cormorants. When in the eye of the channel catfish, it can occur in large numbers and produce a hypertrophied, distorted, disrupted, or otherwise damaged retina (Overstreet and Curran, 2004). A large number of cercariae can invade the nervous system without associated mortality, but in some cases when two different diplostomoid species occur in the same host, the host dies. Regardless of whether or not *Austrodiplostomum mordax* in Argentina is a junior synonym of *A. compactum* as considered by Dubois (1970) (Kohn *et al.*, 1995), it is also considered to be more pathogenic in the brain of

pejerrey (*Odontesthes bonariensis*) when *Tylodelphys destructor* also occurs (Szidat and Nani, 1951, 1952). These authors also showed that many of the fishes from Lake Pellegrini and in both the Limay and Neuquen rivers in Argentina were unfit for sale because of intense infections of the brain-encysting metacercariae of *A. mordax* (as *Diplostomulum mordax*) as well as *T. destructor* that caused poor growth. In Lake Pellegrini, most of the fish were small and emaciated, the digestive tract was practically empty, and the brain was destroyed to different degrees. In young fish, cercariae invaded ventricles of the brain as well as the cerebral ganglia without inflicting much damage, but in the older fish they penetrated the optic lobe, cerebellum, and optic nerve and caused blindness and other symptoms which presumably exposed these fish to predatory piscivorous water birds.

## Other Species having a *Diplostomulum*

The combined effects of cataracts and retinal detachment cause blindness in some species (Shariff *et al.*, 1980). Intense infections of *Diplostomum gigas* in the eye lens of white sucker caused the eyes to be opaque and the vision of the hosts presumably impaired, since the lenses were often flaccid and misshapen (Hughes and Berkhout, 1929). The effects of a few unencysted diplostomula of *Diplostomum huronense* on trout-perch (*Percopsis omiscomaycus*) were considered negligible because the metacercariae were small, transparent, and free in the vitreous humour. Mueller (1972) reported severe diplostomiasis in *Orestias* spp. (Cyprinodontidae) from Lake Titicaca in the Peruvian Andes. Cranial deformations occurred in small infected fishes, but not the larger ones. Mueller (1972) noted that the infections probably did not debilitate the fish because, except for the bulging cranium, infected and uninfected fishes were indistinguishable in condition and flesh quality. Paperna (1995) stated that only in the late stage of infection was a cellular response evident for *Diplostomum* spp. in the eyes of fishes: macrophages aggregated around the debris from ruptured lenses in the damaged periphery of the iris, in the choroid, and around necrotic foci of the optic nerve. Shariff *et al.* (1980) reported severe damage to the eye caused by different diplostomula in rainbow trout from a farm in Perthshire, Scotland, that included exophthalmia, lens discoloration, retinal detachment, and capsule rupture; the latter was involved with a marked inflammatory response and uveitis or phacogenic uveitis, an auto-immune reaction to

the lens protein (Rahi and Gardner, 1976). Epizootics of numerous diplostomula in cultured fishes have been reported nearly worldwide from a variety of hosts.

## Neodiplostomidae (neascus)

### *Posthodiplostomum centrarchi* and *P. minimum*

Neascus metacercariae of these species usually considered as *P. minimum* or one of its subspecies appear as white bladder-like cysts in the liver and other visceral organs of many freshwater fishes. These are referred to as 'white grubs'; *P. centrarchi* is commonly observed in the liver of largemouth bass (*Micropterus salmoides*), bluegill sunfish, and other centrarchids in North America, and *P. minimum* occurs in cyprinids. An initial simultaneous infection of either by many cercariae will kill the fish, but a slow accumulation of cercariae apparently is less pathogenic (Hoffman, 1975). The metacercaria secretes a cyst wall that becomes surrounded by a thin host-derived wall and a connective tissue sheath (Mitchell, 1974; Font *et al.*, 1984a). Migrating cercariae may be problematic, but once encapsulated they elicit little or no additional host response (Spall and Summerfelt, 1970; Mitchell *et al.*, 1982). Grizzle and Goldsby (1996) studied *P. centrarchi* in the liver of largemouth bass from lakes in Florida, and found no correlation between intensity of infection and hematocrit, relative weight, or fish growth, and density of the grub was also unrelated to age, gender, total length, or fish weight. Smitherman (1964) studied the health of bluegill sunfish infected with the white grub and noted that at 103, 353, and 546 metacercariae per fish, growth at the two higher levels was stunted over 32 weeks and increased mortality occurred over a 40-week period. Kidneys of the fish contained nearly 100 neascus and excreted phenol red dye faster than controls, possibly related to kidney dysfunction. Infected livers consumed more oxygen in warmer water. Plasma samples taken nine months post-infection showed higher total globulin and albumin levels than controls. Sections from hearts, livers, spleens, and gonads showed only a slight host reaction to the cysts. In some cases, the cysts caused infarcts in liver tissue. Spall and Summerfelt (1969) noted similar pathological effects by the white grub. Metacercariae may have killed an unusual host, fingerlings of striped bass (as *Roccus saxatilis*), by encysting in atypical sites such as the muscle and orbit of the eye rather than the viscera (Hoffman and Hutcheson, 1970). Mitchell *et al.* (1982) documented a massive infection of a related white grub

(*P. minimum*) in fathead minnows from a commercial baitfish farm in Missouri. More than 2,000 cysts infected one 6-cm TL fish, with 'low-grade mortality' occurring in counterparts. Histological sections showed that an intensive inflammatory response resulted from the infection. The body cavity also had a large volume of ascitic fluid containing many blood cells, 93% of which were leukocytes. Moderate numbers of metacercariae caused a less intense inflammatory response. Visceral infections of 500-2,300 metacercariae in 5.0-6.5 cm total length fathead minnows caused abdominal dropsy with milky ascitic fluid containing 92% leukocytes and 8% erythrocytes. Granulomas developed around degenerating parasites and parasitic debris. Mitchell *et al.* (1983) also documented an unusual case in bluegill sunfish in which neascus in conjunction with cestode plerocercoids of *Proteocephalus* sp. induced signs consistent with chronic liver degeneration and visceral fibrosis. Meyer (1958) examined 1,841 fish from Trumbull Lake, Iowa, for internal helminths and noted an exceedingly high prevalence of black spot (92%) (neascus of *Uvulifer* sp.?) in the flesh and numerous cysts (*P. minimum*) in the liver. Heavily infected minnows were unsightly due to the black cysts, and Meyer (1958) alleged that young fish experienced mass mortality since their numbers had precipitously dropped in routine weekly samples.

### *Posthodiplostomum cuticola*

Encysted neascus of *P. cuticola* produced black spot in the skin and fins of fishes in the former Soviet Union (Petrushevski and Shulman, 1961). This species was recorded in ~50 fish species, with cyprinids comprising most of them. Dubinin (1952) recorded 3,832 metacercariae in a single specimen of carp bream (*Abramis brama*), and Petrushevski and Shulman (1961) found a small specimen of rudd from the Danube Delta Region with twelve metacercariae per square cm. Even though pathogenic in fishes, this or a related species apparently enhanced growth of juvenile roach (*Rutilus rutilus*), rudd, and white bream (*Blicca bjoerkna*) (as *Abramis bjoerkna*) (see Ondračova *et al.*, 2004a). According to Petrushevski and Shulman (1961), *P. cuticola* has been recorded from 45 localities in the former Soviet Union, but most commonly from the Kuban Delta, lower reaches of the Danube, Dnepr, Don, and Volga rivers as well as the coast of the Aral Sea and the Barabinskye Lakes. Dubinin (1949) noted a 1937 Caspian Sea epizootic involving cyprinids that were heavily infected; the population was estimated to have been reduced by 30%, and a high

intensity of infection was associated with the presence of nesting herons nearby. The infected fishes had severe body deformations, muscular injuries, spinal deformities, and severe growth retardation. Ganapati and Rao (1955) reported high mortality rates among infected catla (*Catla catla*), orange-fin labeo (*Labeo calbasu*), and flying barb (*Esomus danricus*) (as *Nuria danrica*) in an Indian fisheries pond attributed to "black grub disease" by an unidentified species; black ovoid patches occurred all over the body of infected fishes. Some cases of 'black spot' involve the eye. Impairment of vision can be aggravated when metacercarial cysts are accompanied by melanophores. Severe infection leads to exophthalmos, cataracts, and complete collapse of the eye. Blindness can be uni- or bi-lateral (Sinderman and Rosenfield, 1954; Mawdesley-Thomas and Young, 1967).

### *Uvulifer ambloplitis*

The life history sequence for this fluke incorporates the snail *Planorbella trivolvis*, a centrarchid fish only (Hoffman and Putz, 1965; Yamaguti, 1975), and kingfishers (*Megaceryle* spp.). Lemly and Esch (1983; 1984a, c) documented the prevalence and intensity of neascus infections in juvenile bluegill sunfish and largemouth bass from Reed's Pond, North Carolina. Bluegill sunfish had 1-269 cysts per fish, with significant differences occurring in intensity among size classes of bluegill sunfish, and largemouth bass had up to twelve per fish. The frequency distribution of *U. ambloplitis* in bluegill sunfish closely fit the negative binomial model, with recruitment beginning in May and ending in September and maximum recruitment occurring in July. The seasonal changes in the degree of overdispersion of *U. ambloplitis* suggested that parasite-related mortality of bluegill sunfish occurred in winter. High prevalence and intensity of cysts in bluegill sunfish at Reed's Pond was attributed to (1) a seasonal aggregation of kingfishers coinciding with maximum snail density, ultimately resulting in relatively high cercarial production in July and (2) the seasonal recruitment of juvenile bluegill sunfish to the littoral zone in July: these two factors produced infective stages of the parasite in all the hosts within a defined area during a short time period. Laboratory experiments confirmed field observations that heavily infected bluegill sunfish died when exposed to cooling, but those held continuously at 20-25°C did not die. Infection significantly reduced total body lipid and body condition of the bluegill sunfish in both field and laboratory experiments. A spike in host oxygen consumption in recently infected fishes coincided

with the host encapsulation process and dropped progressively with time post-infection. Cooling of water in the fall resulted in mortality among fish infected with >50 metacercariae during the winter when the fish were still in a lipid-depleted state. Natural parasite-induced mortality accounted for an estimated 10-20% elimination of young-of-the-year bluegill sunfish each winter. Lively *et al.* (1990) and Weeks (1996) both studied black spot from *Uvulifer* sp. in Mexican pools inhabited by 'strains' (populations) of the headwater livebearer (*Poeciliopsis monacha*) and tested the Red Queen Hypothesis, which states that parasites target the common host genotype, thereby disproportionately reducing that genotype's fitness relative to rarer genotypes in the population. The model assumed that parasites decrease fish fitness, but Weeks (1996) found that *Uvulifer* sp. had no such effect.

## Strigeidae (tetracotyle)

### *Apatemon gracilis*

The life history sequence for this fluke includes a bird (experimentally in juveniles of both the mallard [*Anas platyrhynchos*] and the domestic chicken), the snail host *Lymnaea peregra*, and one of the fish hosts rainbow trout, three-spined stickleback, or stone loach (Blair, 1976) (Plate 14.11). Tetracotyle metacercariae of *A. gracilis* sampled at regular intervals from cohort brook sticklebacks from a swamp on Ile Perrot, Quebec, from mid-summer to fall of 1978 had a prevalence of 100%, with the mean intensity increasing from zero to a plateau of about 44 parasites per fish. Overdispersion of the frequency distribution of parasites in the fish host, as measured by variance to mean ratios, increased to a peak and then decreased significantly while the mean intensity remained constant (Gordon and Rau, 1982). Those authors suggested that these data could be explained by parasite-induced mortality of wild brook sticklebacks or increased predation upon them. Tort *et al.* (1987) showed that *in vitro* heart pumping performance in rainbow trout was reduced by up to 50%, and Watson *et al.* (1992) detailed the pathology associated with an infection in rainbow trout. Development of the lesion initiated only after cercariae encysted and were encapsulated by the host. The lesion progressed from a vascularized layer of fibroblasts and macrophages at eight weeks post-infection to a fibro-granulomatous tissue surrounding the cysts that were attached to the epicardium at eleven weeks post-infection, and then to a thickened, fibrotic pericardial layer and fibro-

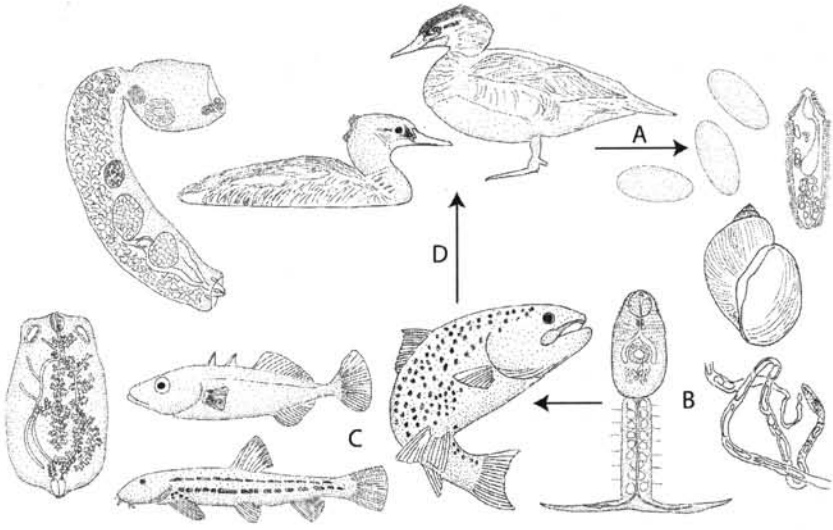


**Plate 14.11** Life cycle of *Apatemon gracilis* (Strigeidae) involving piscivorous birds, in this example a common merganser (*Mergus merganser*), i.e., the type host for *A. gracilis*, a freshwater snail (e.g., the lymnaeid *Lymnaea peregra*), and several freshwater fishes: three-spined stickleback (*Gasterosteus aculeatus*), rainbow trout (*Oncorhynchus mykiss*), and stone loach (*Nemacheilus barbatula*). Based primarily on experimental work by Hoffman (1959) and Blair (1976), but see also the synopsis of Yamaguti (1975) for other snail, fish, and bird hosts. **A.** The adult strigeid infects the piscivorous bird's gut and its eggs are deposited in the water with its feces. **B.** Each egg contains a ciliated miracidium that infects the snail wherein asexual reproduction occurs and yields a pharyngeate, longifurcate, distomatous cercaria that penetrates the second intermediate host fish. **C.** Cercariae penetrate and encyst in the musculature of the second intermediate host fish; some metacercariae develop in the epicardium. **D.** Piscivorous birds are infected after preying upon infected fishes.

granulomatous tissue that accumulated in the sulcus between the ventricle and bulbus arteriosus at twelve weeks' post-infection. In this latter stage, fine blood vessels had proliferated across the surface of the host reaction tissue and a larger, more distinct lesion formed perpendicular to the surface of the compact myocardium.

### *Cotylurus pileatus*

The tetracotyle of this or a similar strigeid that matures in herons affects wild freshwater fish hosts in the former Soviet Union (Pokrovskii, 1961; Petrushevski and Shulman, 1961); 36 species and varieties of fishes serve as intermediate hosts. Up to 2,400 metacercariae have been collected from one specimen of rudd (Dubinin, 1952), and a prevalence of 100%

was recorded in ruffe (*Gymnocephalus cernuus*) (as *Acerina cernua*) from several east Asian lakes, with intensities in the hundreds (e.g., Dogiel, 1932). Ruffe was introduced into lakes Superior and Michigan, US, in the 1980's (Devine *et al.*, 2000), and we know of no record of C. *pileatus* in North America; however, given the broad host specificity of C. *pileatus* to its second intermediate fish host as well as its documented ability to cause epizootics in wild freshwater fish populations, we suspect that this strigeid may at some point cause a disease problem in North America. Dubinin (1952) reported a mass mortality induced by the tetracotyle in juvenile cyprinids of *Rutilus* spp. and *Abramis* spp. in the Volga Delta. Petrushevski and Shulman (1961) stated that an epizootic in Lake Chudskoye in 1954 was marked by perches (*Gymnocephalus* spp.) (as *Acerina* spp.) having several hundred metacercariae each and appearing seriously emaciated and in overall poor condition. They estimated 10-15 dead fish per m of coastline. These infected fish exhibited kidney and liver necrosis, and, in some extremely severe infections, cystomas developed in the ovary. A mass mortality associated with an intense infection by the same or a similar species (*Tetracotyle intermedia*) developed among *Gymnocephalus* spp. (as *Acerina*) in Müggelsee (Schäperclaus, 1954), and many salmonids infected in the pericardial cavity and cardiac muscle succumbed to the infection (Petrushevski and Shulman, 1961).

## CLINOSTOMIDAE (Yellow Grub)

### *Diagnosis*

Clinostomids differ from the other digeneans discussed herein by maturing in the mouth and esophagus of birds. The cercariae of clinostomids are occulate (have eyespot pigment), pharyngeate (have a pharynx), furcocercous (have a forked tail), and lophocercous (have a finfold) with an elongate head organ (Plate 14.12). Metacercariae are stout, linguiform, convex dorsally and concave ventrally; the oral sucker is surrounded by a collar-like fold when retracted (Figs. 14.3F, G). The esophagus is swollen bulbously at the posterior end without forming a typical pharynx, and anlagen of gonads are obvious. The most obvious feature of clinostomid metacercariae is their size and color; they comprise some of the largest metacercariae infecting fishes, and their gut is typically filled with a bright yellow content (Figs. 14.4J, K) that is cause for the name 'yellow grub'. Clinostomid metacercariae range on all continents (Paperna, 1991). Two commonly encountered clinostomids are *Clinostomum complanatum*, a

fluke that ranges in Asia, Europe, and Africa, and *Clinostomum marginatum* that ranges throughout North America (Dzikowski *et al.*, 2004a) to at least the Yucatan Peninsula, Mexico (Scholz *et al.*, 1995). Each of these shows a high degree of morphological variability. However, Dzikowski *et al.* (2004a) demonstrated that they comprise distinct species based on ribosomal DNA. The taxonomy of species in North America is still confused. Gibson (1996) and Hoffman (1999) consider all known North American clinostomids to be *C. complanatum*, but some data indicates that there are two or three species, excluding *C. complanatum* (e.g., Overstreet and Curran, 2004; Tkach, Curran, Overstreet, unpublished data).

### *Life Cycle*

Adult clinostomids infect the laryngo-pharyngeal zone of herons, cormorants, anhingas (*Anhinga* spp.), and pelicans (*Pelecanus* spp.) (e.g., Paperna, 1991; Hoffman, 1999) and in 'accidental' cases, which are rare, humans (e.g., Kamo *et al.*, 1962; Beaver *et al.*, 1984; Overstreet, 2003). Members of one of the four recognized subfamilies infect the esophagus of reptiles (Kanev *et al.*, 2002). Fishes are the primary second intermediate host for the yellow grub. Despite being rather beautiful to parasitologists, the yellow grub presents an unsightly surprise for fishermen and fishmongers. Some species can cause considerable damage to their second intermediate fish hosts in nature and in aquaculture (see below). The generalized life cycle for clinostomids can be exemplified by *Clinostomum 'complanatum'* (e.g., Yamaguti, 1975) (Plate 14.12). The miracidium hatches roughly 19 days after eggs are shed in the buccal cavity of a heron. The miracidium is an active swimmer and penetrates a planorbid snail. The mother sporocyst develops primarily in the digestive gland of these snails at 11-21 days post-infection, with the mother redia developing after 26-69 days post-infection, and the daughter rediae at 27-69 days post-infection. The daughter redia has cuticular folds, a pharynx, ceca, a birth pore posterior to pharynx, and an anterior end with transverse rows of spines. The brevifurcate cercaria penetrates the fish host, and the metacercaria develops after six weeks. The cercaria is typically short-lived, remaining infective for only few-several hours after being shed from the snail. It remains suspended in the water for a short time and is stimulated to activity when the water surface is altered, resulting in tail lashing. The metacercaria encysts in fish muscle and rarely in the gill chamber. Hoffman (1999) thought that clinostomid metacercariae, all of which in

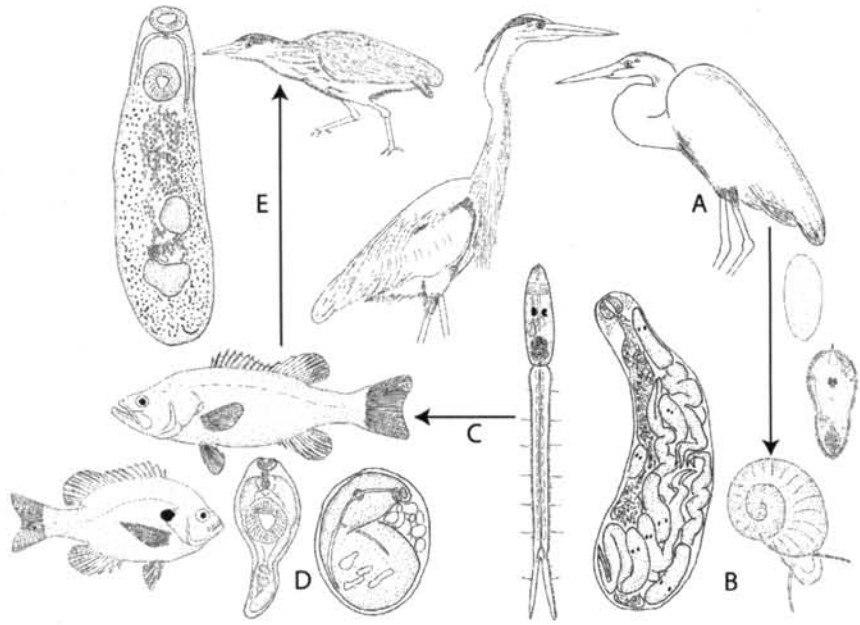


**Plate 14.12** Life cycle of a yellow grub, *Clinostomum* sp. (Clinostomidae), involving a heron (e.g., great blue heron [*Ardea herodias*], snowy egret [*Egretta thula*], and black-crowned night heron [*Nycticorax nycticorax*]), a planorbid snail (e.g., *Planorbella trivolvis*), and a variety of freshwater teleosts including basses (*Micropterus* spp.) and sunfishes (*Lepomis* spp.). Based on Hoffman (1999). **A.** The adult clinostome infecting the mouth of the definitive host heron deposits eggs that are shed in the heron's feces. **B.** Each egg contains a ciliated miracidium that infects the snail wherein asexual reproduction occurs. **C.** A sporocyst produces rediae that yield numerous pharyngeate furcocercous cercariae. **D.** The cercaria penetrates the second intermediate host fish, encysts in the muscles or fins, and develops into a metacercaria, the 'yellow grub'. **E.** The definitive host heron is infected after preying on the infected fish. Multiple herons are illustrated here because this clinostome infects several herons, including those illustrated, as definitive hosts.

North America he regarded as C. *complanatum*, were so broadly infective to such a wide spectrum of hosts that nearly any freshwater fish could be experimentally infected if exposed to cercariae. Such is not true. For example, when the channel catfish as well as other fishes, hosts for what we consider to be *Clinostomum attenuatum* were exposed to thousands of cercariae from *Planorbella trivolvis*, these fishes subsequently were not infected; however, similarly-exposed mosquitofish were infected, primarily in the ovary and other visceral organs (Overstreet and Curran, 2004). Infection intensities ranging from 106-657 metacercariae of C. *marginatum* have been reported from channel catfish captured from a small pond in Tennessee (Edney, 1940). The cyst wall of parasite origin is

thin and permeable (Larson *et al.*, 1988). Infected centrarchids were fed to a young heron, and numerous eggs were collected from the bird's feces three days post-infection. Adult worms, *C. complanatum* in this case, were collected from the mouth of the heron five hours post-infection but not from a duck similarly fed. Several life cycles have been described throughout the world; Ortlepp (1935) fed metacercariae of *Clinostomum vanderhorsti* derived from an infected fish in South Africa to an uninfected young heron, and Dönges (1974) documented the life cycle of *Euclinostomum heterostomum* in Africa.

The large clinostomid metacercariae typically encyst in particular sites in particular fishes (Paperna, 1995). For example, metacercariae of *Clinostomum cutaneum* infect the dermis of cichlids; those of *Clinostomum tilapiae* infect connective tissues primarily around the gills of tilapias (*Oreochromis* spp.); and those of *Euclinostomum heterostomum* infect the peritoneum adjacent to the kidney of several fishes as well as beneath the dermis (Britz *et al.*, 1985).

## Specific Pathogens

### *Clinostomum* spp. and *Euclinostomum* spp.

We regard some of the reports of disease attributed to *Clinostomum* spp. as dubious because they are based solely on gross observations of the infected fish. For example, Ortlepp (1935) photographed an extremely intense infection of metacercariae of *Clinostomum vanderhorsti* that appeared 'like a bunch of grapes' in the body cavity of the mormyrid bulldog (*Marcusenius macrolepidotus*) in Africa. Mosquitofish are on occasion seen as having large numbers of *C. attenuatum* in wild fish in ponds and in experimental infections in Mississippi (Overstreet and Curran, 2004; Overstreet, unpublished data). Schwartz (1956) found metacercariae of *Clinostomum marginatum* in the brindled madtom (*Noturus miurus*) (as *Schilbeodes miurus*), and presumed that, since the internal organs were grossly normal, the fluke must have killed the fish. Thatcher (1981) referenced some pathological changes from the yellow grub from South America. Rabideau and Self (1953), however, showed that there was no correlation between the K-factor (condition) of yellow perch and intense infections of grubs (as *Clinostomum marginatum*). Paperna (1991) stated that intense infections of clinostomids in wild and cultured fishes were rare, but a few recorded cases demonstrated pathogenicity in fish. Natural infections of *C. attenuatum* in the visceral organs and mesentery of mosquitofish in

Mississippi typically number several small to a few large metacercariae, but fish can be experimentally infected with over a hundred worms without killing them within 39 days, however, higher numbers could kill them within a few days (Overstreet and Curran, 2004; pers. obs. Overstreet). Intense infections of metacercariae of *Clinostomum cutaneum* in the dermis caused skin erosion, and *Clinostomum tilapiae* interfered with the function of the operculum in fishes, but did not significantly affect the health of the fish (Paperna, 1991). In some infections there is inflammation and fibrosis surrounding visceral cysts. Paperna (1991) stated that the records of clinostomids causing disease in aquaculture involved (1) few (three to five) cysts of *Euclinstomum heterostomum* in pond-reared Mozambique tilapia (*Oreochromis mossambicus*) fingerlings that ranged from 30-50 mm in total length in Malawi (Paperna, 1980; Kabata, 1985), (2) mass mortality from intense infection by C. *tilapiae* in northern Transvaal, South Africa (Britz *et al.*, 1985), and (3) mortality from intense infections of C. *complanatum* in Oriental weatherfish (*Misgurnus anguillicaudatus*) and ayu (*Plecoglossus altivelis altivelis*) in Taiwan (Lo *et al.*, 1981). Regarding the Malawi infections, those cysts were located in the mesentery but exerted pressure on the viscera. The infected fish had a distended abdominal cavity as if suffering from dropsy, and heavily infected fishes died. The large size of the metacercaria may have influenced pathogenicity. Another clinostomid infection in Indonesia retarded growth and was responsible for some loss of farmed fish in Java during the early 1950s; some weight loss and pronounced exophthalmus were observed in those infected fishes (Kabata, 1985). Dönges (1974) experimentally infected redbelly tilapia (*Tilapia zilli*) with cercariae of *Euclinostomum heterostomum* and noted deaths of hyperinfected fishes at 30 days post-infection. In this species, the metacercariae migrated to and began considerable growth either in the space between the vertebral column and the dorsal peritoneum such as the dorsal region of the swimbladder or in the kidneys. He did not find a single metacercaria in the muscle or cutis. He noted that since the metacercariae continue to grow for months, mortalities of heavily infected fish should be, and were, delayed. For example, a heavily infected, small redbelly tilapia (4.3 cm total length) died 35 days post infection and exhibited 76 growing metacercariae. A longer specimen (9.5 cm total length) died 62 days post infection. This latter fish was exposed a second time, 28 days later, and contained 31 large and about 50 smaller growing metacercariae.

## HETEROPHYIDAE

### *Diagnosis*

Heterophyidae, with its adult species occurring in birds and mammals, contains pathogenic larvae in intermediate fish hosts, some of which constitute zoonotic agents for humans that eat raw or incompletely cooked fishes. Heterophyid metacercariae in fishes are usually distomous, relatively small, and covered with scale-like spines. The Cryptogonimidae, a closely related family also considered a subfamily of Heterophyidae by some researchers including us, differs by maturing in fishes rather than in homeotherms and by having features to accommodate that difference.

The oral sucker and pharynx is obvious, and most have long ceca. The ventral sucker, however, may or may not be well developed and associated with a ventrogenital sac. Heterophyids are known for having a 'gonotyl', but there is much confusion in the literature about what constitutes a gonotyl. Initially, all heterophyid copulatory organs were considered as such, but Pearson (2002) differentiated them and restricted the term to one kind of modification. In *Heterophyes* spp. the organ consists of a modified genital sinus; in *Haplorchis* spp. it is a modified ventral sucker; in *Pygidiopsis* spp. and in *Apophallus* spp. it is an outgrowth of the ventrogenital sac; and in *Acetodextra* spp. it appears to have an outgrowth of the ventrogenital sac but as an eversible tubular invagination of the wall of the sac. The true gonotyl, according to Pearson, is restricted to the outgrowth of the ventrogenital sac, an unbounded, anucleated, typically muscular outgrowth that usually bears the genital pore and is unarmed. Some members, such as those of the '*Ascocotyle-Phagicola*' complex of heterophyines and many cryptogonimines, have a distinctive crown of spines at the anterior end (e.g., Figs. 14.5A-B, E, I). Metacercariae typically occur in specific sites in freshwater, estuarine, and marine fishes, which may or may not be highly specific to a specific heterophyid species. A key to the genera by John Pearson will be included in volume III of the Keys to the Trematoda (Bray *et al.*, in preparation). Several heterophyid species such as *Cryptocotyle lingua* cause 'black spot disease' in addition to the many diplostomoids and a few other digenean groups.

At least one species may be represented in the fossil record; Sinclair (1972) described the metacercarial cyst of *Apophallus brevis*, the so-called 'sand grain grub' of yellow perch. The cyst comprises bone that becomes clustered around the settled cercaria within the peripheral blood vessels. The bony structure has two opposite escape canals for the parasite and has

lines interpreted as the growth rings of the bone. These cysts have also been called 'sub-fossils' (Sinclair, pers. comm. Overstreet).

### *Life Cycle*

The most common life cycle probably involves a single snail species, primarily a restricted site in the fish host, and a few different definitive hosts, often unrelated (Plate 14.13). For example, the life cycle of *Ascocotyle gemina* includes the hydrobiid snail *Littoridinops monroensis*; the

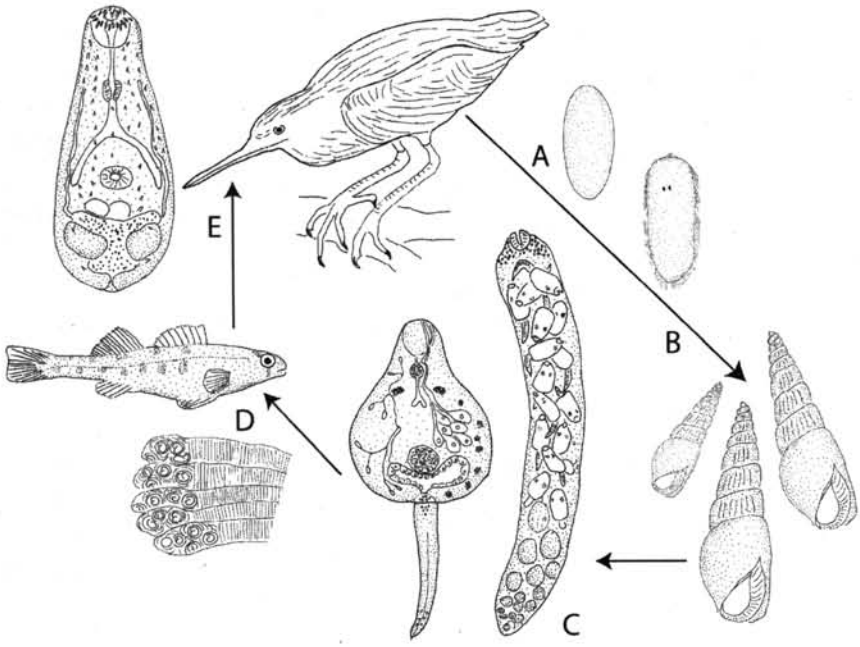


**Plate 14.13** Life cycle of *Centrocestus formosanus* (Heterophyidae) involving the green heron (*Butorides virescens*), the red-rim melania (*Melanoides tuberculatus*), and the fountain darter (*Etheostoma fonticola*). Based on Mitchell *et al.* (2005). **A.** The adult of *C. formosanus* infecting the gut of the definitive host heron deposits eggs, which are operculated (although in some examples indistinct), that are shed in the heron's feces. **B.** The red-rim melania eats the fluke's egg before the miracidium hatches and infects the snail. **C.** Germinal cells of the miracidium ultimately yield a redia that sheds a large number of a pharyngeate cercaria with eyespots and a nearly indistinct dorsal and ventral fin fold. **D.** The cercaria is passively consumed by the second intermediate host fish or actively penetrates that host, drops its tail, and encysts near a cartilage support of the gill filament before developing into a metacercaria. This encysted metacercaria induces a moderate to extensive cartilaginous response. **E.** The heron is infected upon preying on the infected fountain darter.

serosal intestinal tissue as well as mesentery, nervous tissue, gills, musculature, and elsewhere in the sheepshead minnow (*Cyprinodon variegatus*), but not in related sympatric cyprinidontiform fishes, i.e., Gulf killifish (*F. grandis*), longnose killifish (*F. similis*), bayou killifish (*F. pulvereus*), California killifish (*F. parvipinnis*), sailfin molly (*Poecilia latipinna*), or mosquitofish; and the clapper rail (*Rallus longirostris*) as the primary definitive host (Font *et al.*, 1984b), but it also has been reported from the reddish egret (*Egretta rufescens*) (Conti *et al.*, 1986) and the great egret (*Ardea alba*) (Sepulveda *et al.*, 1999) as well as experimentally in ducklings and chicks (Font *et al.*, 1984b). Most other related species have a more restricted site in the fish intermediate host such as the bulbus arteriosus of *C. variegatus* for *A. pachycystis*.

In contrast to having a single fish host, *Centrocestus* cf. *formosanus*, a heterophyid introduced into fresh waters of the contiguous US, infects a wide variety of fish hosts and elicits a different response in some of them, some of which are harmed and even killed. These harmed fishes are a matter of concern because several of them are endangered or are part of the aquarium trade (Mitchell *et al.*, 2005). The only known first intermediate and final host in the Southeast US of this introduced fluke is the exotic snail *Melanoides tuberculatus* and the green heron (*Butorides virescens*), at least after many necropsies of birds that presumably feed on infected fishes (Mitchell *et al.*, 2005) (Plate 14.13), even though we determined that chicks, ducklings, mice, and hamsters can serve as experimental hosts. What is considered by the same name from other countries is probably different but part of a complex that has different snail and bird hosts; all the populations seem to be locally restricted to one or two definitive host species.

*Phagicola nana* represents an example of a species that infects several related fish hosts and numerous definitive hosts. Font *et al.* (1984a) found metacercariae occurring in largemouth bass, redear sunfish (*Lepomis microlophus*), bluegill sunfish, and orange spotted sunfish (*L. humilis*) from estuarine bayous and rivers of Mississippi, Florida, and Georgia. Metacercariae also infect cichlids and characids in Mexico (e.g., Scholz *et al.*, 1997). This heterophyid has a broad host specificity in nature for piscivorous estuarine birds and mammals, including possibly humans that consume improperly prepared centrarchid fishes (Font *et al.*, 1984a). It has also been recovered experimentally as adults in Virginia opossum (*Didelphis virginiana*), northern raccoon (*Procyon lotor*), laboratory white mouse (*Mus musculus*), Syrian hamster (*Mesocricetus auratus*) and chicks.

## Specific Pathogens

### *Apophallus* spp.

Metacercariae of *Apophallus* sp. of cyprinid fishes from the Willamette River, Oregon, have been implicated in vertebral deformities (Kent *et al.*, 2004). All deformed specimens of the chiselmouth (*Acrocheilus alutaceus*) had many metacercariae encysted near the vertebrae; half the specimens of the deformed northern pikeminnow (*Ptychocheilus oregonensis*) had metacercariae associated with the lesion, and metacercariae were near vertebral lesions in three of four affected specimens of the peamouth (*Mylocheilus caurinus*). Bony dysplasia and proliferation occurred in all hosts. When the heterophyid in Canadian Shield lakes was present in conjunction with concurrent infections of xenomas of *Glugea* sp., growth of the fish was reduced, associated with mortality of the young, small individuals. This effect was alleged to be strongest during periods of low energy input or reproductive activity (Johnson and Dick, 2001).

Miller (1942) reported that 'black spot disease', which he observed widely distributed throughout the lakes of Quebec, Canada, in the brook trout was due to encysted metacercariae of *Apophallus imperator* (as A. *brevis*). The snail intermediate host is *Amnicola limosa* and the definitive host is the common loon (*Gavia immer*). Metacercariae were experimentally recovered from brown trout, but rainbow trout and lake trout were refractory to infective cercariae. Black spot is a common condition resulting from metacercariae of a few different species of *Apophallus* (including *Rossicotrema donicum*, which is considered in *Apophallus* by some researchers) throughout North America and Europe (e.g., Lyster, 1940; Warren, 1953; Ivanov and Semenova, 2004). As indicated earlier, *Apophallus brevis* in yellow perch in North America produces a 'boney cyst' and is referred to as the 'sand-grain grub' (Sinclair, 1972). Uncertainty exists about the taxonomic status of several of the species in the *Apophallus*-complex.

### *Ascocotyle* spp.

Several species of *Ascocotyle* occur throughout the Americas, and most of these are site-specific in a single or a few hosts, even though some workers consider closely related species conspecific. A few species modify the behavior of the fish host so that the definitive host more readily feeds on infected rather than non- or lightly infected fish prey. In contrast, some fish can tolerate high numbers of multiple species without suffering any

harmful effects, at least when not under environmental stress. Metacercariae of *Ascocotyle pachycystis*, up to 6,800 per fish, encyst in the bulbus arteriosus of the sheepshead minnow (Figs. 14.4C, D, 14.7J, 14.9D, I). The presence of more than a few cysts, which is common in the Southeast US, causes hypertrophy of the ventricle due to mechanical blockage of normal blood flow by metacercariae in the bulbus (Coleman, 1993). Coleman (1993) determined that swimming performance in fish infected with >100 cysts was generally reduced but not intensity related; performance was most affected at low temperatures and low dissolved oxygen levels. Coleman and Travis (1998) found that recruitment was seasonal and that older fish had more parasites, with almost all older fish in the samples infected. The digenean seemed to reduce winter survivorship based on data involving fish size and decrease in mean intensity during winter.

Hoffman (1975) showed that metacercariae of *Ascocotyle tenuicollis* were found beneath the endothelium in the bulbous arteriosus of moribund mosquitofish collected from a warm spring pond near Salt Lake City, Utah. In some cases they were numerous enough to occlude the lumen of the bulbus arteriosus. In one case, one cyst in the distal end of the bulbus arteriosus was in a position such that it occluded blood flow. Diseased fish exhibited loss of appetite and vigor, immobility at the surface, erratic swimming, and apparent paralysis. We have not noted such behavior in heavily infected mosquitofish in Mississippi.

### *Centrocestus formosanus*

The species tentatively called *C. formosanus* in the US infects gill cartilage of fishes, debilitating or killing wild and cultured fishes, often those cultured by tropical fish producers (Mitchell *et al.*, 2005). This fluke is unusual because there is strong evidence that intense infection by metacercariae of this species kill small fishes in small streams and ponds in nature when not under environmental stress. Also, it infects a wide range of fishes, even though the response to the parasite differs among species. The fountain darter, an endangered species which can serve as the second intermediate host (Plate 14.13), represents one of the more responsive species. The metacercariae induce an unusual inflammatory response comprising a proliferation of host fibroblasts that differentiate into chondroblasts and then chondrocytes that form a cartilaginous encapsulation around the digenean that also is seen in a few other hosts (Blazer and Gratzek, 1985; Vogelbein and Overstreet, 1988). Intense

infections in the fountain darter greatly reduced respiratory surface. Infected fish had flared opercula, shortened or thickened gill filaments, hyperplastic epithelial tissue, and engorged lamellae. Distortion and displaced cartilage support for the filaments was suspected of leading to severe deformities of filaments. These pathological alterations were life threatening for hosts with >800 cysts (9% of the examined fish) and probably killed those fountain darters. The gills of 194 fountain darters in the Comal River, Texas, from May 1997 through May 1998 had 8–1,524 metacercarial cysts (Mitchell *et al.*, 2005). Mitchell *et al.* (2002) experimentally infected several fishes and documented gross pathological alterations to the gill tissue. Cartilage displacement and hyperplasia of lamellar epithelium occurred in sunshine bass and fathead minnow and the response was less dramatic in the golden shiner (*Notemigonus crysoleucas*) and channel catfish. Mitchell *et al.* (2005) and others proposed that the high level of pathogenicity produced by metacercariae of C. cf. *formosanus* in its fish hosts resulted because the parasite was an exotic species producing a newly established HPR in a naive host. Olsen and Pierce (1997) reported metacercariae in steelhead trout, i.e., anadromous rainbow trout, in Oregon. Because of the nature of the infection and its presence in the same geographic localities as the first intermediate host (the exotic snail *Melanoides tuberculatus*), the infection may have been C. cf. *formosanus*. The cysts were located adjacent to the cartilaginous rods of gill filaments and elicited a host response of cartilage proliferation from the perichondrium along with some gill hyperplasia and fusion of lamellae; however, these alterations were insufficient to cause any mortality in the infected trout.

Other species in the complex also have an effect on the fish but usually not so severe as in North America. However, Yanohara and Kagei (1983) reported an epizootic and mass mortality of elvers of farmed Japanese eels (*Anguilla japonica*) infected with large numbers of C. *formosanus*. These infections resulted after the fish were stocked into outdoor earthen ponds. Epizootics of intense infections in Israeli fish ponds involved juveniles of unspecified cichlids and carps (Paperna, 1991).

### *Cryptocotyle lingua*

Metacercariae of *Cryptocotyle lingua* cause 'pigment spot disease' in Atlantic herring (*Clupea harengus*) in the western north Atlantic Ocean, and penetration by many cercariae can rapidly kill Atlantic herring under

experimental conditions (Sinderman and Rosenfield, 1954). Those metacercariae in the skin of European plaice (*Pleuronectes platessa*) are associated with epidermal erosion, myofibrillar necrosis, and a reactive swelling of the intermuscular septa. However, some of these reactions, particularly myofibrillar necrosis, was thought to be caused by a bacterium that may have been vectored by C. *lingua*. The inflammatory reaction to the metacercaria was slight, and McQueen *et al.* (1973) regarded this lack of irritation as evidence of a long standing HPR that evolved with an advanced state of adaptation by the parasite and tolerance by the host. This heterophyid is well known, partially because of the work by Stunkard (1930). Ryder (1884) provided a very early record of *Cryptocotyle lingua*, also known at the time as 'pigment spot disease'.

### *Euhaplorchis californiensis*

Metacercariae of *Euhaplorchis californiensis* (Fig. 14.3D) have a predilection for encysting near or on the meninges or rarely within the outer region of the brain (meningeal lining) of the California killifish (Martin, 1950). The HPR between the California killifish and concurrent infections of the metacercariae of *Euhaplorchis californiensis* and the renicolid *Renicola buchanani* in California may have modified the host behavior enough to make it more susceptible to predation (Lafferty and Morris, 1996). The same or a sister species of *Euhaplorchis californiensis* in Mississippi produces lesions—some hemorrhaging—that possibly produces a similar susceptibility to predation (Figs. 14.9A-C).

### *Haplorchis pumilio*

As with most heterophyids, large numbers of this species can infect the fish host without harm (Fig. 14.3B). Metacercariae infected the dermis, particularly about the connective tissue at the base of fins, without causing a debilitating effect. This included infections in cultured hybrid tilapias (*Oreochromis aurea* × *Oreochromis nilotica*) in Israel (Paperna, 1964a, b; Farstey, 1986) and cultured tilapias (*Oreochromis* spp.) in Kenya (Sommerville, 1982), with a total of 50-60 cysts in the caudal fin of a heavily infected fish. However, and as with most digeneans, large numbers of cercariae produced death to fry within hours. Histology of those infected tissues in fry showed focal hemorrhages in skeletal muscles, indicating that migration of cercariae was through connective tissue. In extremely intense infections, the cercariae occurred in many organs; they

penetrated skin, muscle, kidney, and eyes but were most often found in connective tissue associated with these organs of the fry. Metacercariae established in lightly infected fish did not elicit a significant pathological change except some thickening of the perichondrium near the cysts.

### *Heterophyes* spp.

As with *Haplorchis pumilio* and other heterophyids, metacercariae of various members of *Heterophyes* occur in large numbers in the flesh of their hosts, some in specific mullets. Specimens of the thinlip mullet (as *Mugil capito*) weighing 250 grams had 2,300-6,000 metacercariae per gram of flesh, with an estimated 582,000 in the most heavily infected individual, while those of flathead mullet of comparable size had only 100-150 metacercariae per gram (Paperna, 1975; Paperna and Overstreet, 1981). Usually, intense infections do not appear in both hosts, but those from Bardawil Lagoon in January 1974 with 1,730-6,000 metacercariae per gram of tissue were emaciated, and Paperna (1975) and Paperna and Overstreet (1981) suggested that the young fish with such intense infections were more vulnerable to harm than the older fish.

### *Phagicola nana*

Font *et al.* (1984a) showed that metacercariae can induce more extensive cellular changes, especially during late stages of infection when damaged tissues and dead parasites accumulate. The high level of site specificity indicated for some other heterophyids does not occur for *P. nana*. Cyst characteristics, however, varied according to site. When along the serosa of the pyloric ceca, many cysts were encapsulated by a thick fibrotic capsule; when in fat bodies surrounding the ceca, clusters of cysts evoked a moderate response; when in submucosa, the host response occasionally included fibroblasts, collagen, and a proliferation of recently differentiated smooth muscle cells; when in the spleen, cysts occurred singly or in clusters that were associated with considerable amounts of collagen; when in liver parenchyma, cysts typically occurred singly within a thin fibrous capsule; when in the heart, cysts typically occurred in the muscular tissue; and when in skeletal muscle, cysts occurred singly and had only a thin capsule surrounding them. In general, the metacercariae caused only minor histopathological alterations in fish hosts by inducing formation of the fibrotic host capsule. The infection is of special importance because of the public health risk of eating improperly prepared centrarchids.

### *Stictodora lari*

Cysts of *S. lari* seemingly do not elicit a host reaction until after weeks of infection in fish. The cyst and host encapsulation associated with metacercariae in freshwater fishes has been investigated by Leong and Howell (1971) and Howell (1973). Glass beads and metacercarial cysts of *S. lari* were implanted in the abdominal cavity of mosquitofish to study the sequence of the encapsulation by the host (Howell, 1973). Three days post-implantation the glass beads were encapsulated by host cells; whereas, cysts of *S. lari* remained unencapsulated until 21-23 days later. Howell (1973) proposed both an immunological explanation and a physical one: the cysts remained unencapsulated because they were either immunologically disguised as host tissue or they remained as such because the presence of spikes on the external surface of the cyst inhibit the attachment of host cells. He further asked why it takes so long for the host to encapsulate a parasite cyst? Basically, the cyst includes fish elements and he insinuates that "selective contamination" (e.g., Smithers and Terry, 1969) of the cyst masks the parasite from the host's defenses. Sommerville (1982) likened her results to those of Howell (1973) in that there was very little inflammation and cited an 'avoidance of host response'.

This heterophyid infected Koreans who consumed raw flesh of brackish water fishes, especially that of flathead mullet and yellowfin goby (*Acanthogobius flavimanus*) captured from an estuary near their village (Chai *et al.*, 2002). In addition to *S. lari*, the sampled human population also had concurrent infections of the heterophyids *Heterophyes nocens* (7-328 specimens per human), *Metagonimus yokogawai* (1-36), *Pygidiopsis summa* (1-11), or *Stellantchasmus falcatus* (1-7) as well as the gymnophallid *Gymnophalloides seoi* (1,008-69,125 specimens per human!). The source of this diversity was probably the ayu for *M. yokogawai*, flathead mullet and yellowfin goby for *H. nocens*, *P. summa*, and *S. falcatus*, and an oyster (*Crassostrea gigas*) for *G. seoi*.

## Miscellaneous Heterophyids

Hsiao (1941) described 'a very melanotic cod', from near Race Point, Provincetown, Massachusetts, that was infected with a *Cryptocotyle*-like heterophyid metacercaria throughout the tegumental system, i.e., fins, skin, gill, and cornea, but nowhere else. Multi-layered masses of epidermal cysts caused the cod's skin to appear rugose and warty. The melanotic cod

had extremely high numbers of melanophores relative to cod infected by average numbers of cysts, e.g., 63-121 vs. 9-25 in head region; 40-51 vs. 5 in anal fin, and those in the trunk were too numerous to count accurately. The cornea was so intensely infiltrated by melanophores that it was scarcely distinguishable from the rest of the head. The uninfected regions exhibited no melanosis.

A mass infection by metacercariae of *Procerovum cheni*, a human pathogen, occurred in cultured Japanese eels in Taiwan (Ooi *et al.*, 1999). No individual Japanese eel died from the infection, but ~25,000 of 28,000 (90%) Japanese eels showed swollen, cloudy, white eyes. Cysts were located in the muscle around the eye, subcutaneous tissue, and cartilage. Histology of infected tissues showed edema and hemorrhage of the eye only. When the Japanese eels were transferred to a pond that lacked snails, their eye lesions did not persist, suggesting that the lesions were related to irritation caused by penetrating cercariae. Japanese eels with healthy eyes in a nearby farm were also infected with metacercariae in the muscle. However, the number of the cysts averaged 4.2 per fish, much less than in the first pond. The infection is transmitted by at least herons.

## Fish Flukes whose Cercariae and Metacercariae Harm the Second Intermediate Host Fish

## BUCEPHALIDAE

### *Diagnosis*

Bucephalids differ from all other digeneans by the structure and configuration of the digestive system, configuration of the terminal genitalia, and by the presence of an anterior rhynchus that is used for attachment and disassociated from the sac-like or elongate, non-bifurcated gut (Overstreet and Curran, 2002) (Fig. 14.6W). Bucephalids lack both an oral and ventral sucker, but they have a sucker-like pharynx associated with the mouth. Most species have an intromittent organ ('genital lobe', or 'genital tongue') which partly surrounds the ejaculatory duct and is considered a cirrus by some workers. Some common genera encountered in the literature are *Bucephalus*, *Rhipidocotyle*, and *Prosorhynhcus*, but of these only *Bucephalus* has "tentacles" about the rhynchus, the inspiration for the name.

## *Life Cycle*

Bucephalidae comprises a cosmopolitan family parasitic in marine, brackish-water and freshwater fishes. Lamellibranch bivalves serve as molluscan intermediate hosts for the sporocysts and cercariae. Fishes serve as intermediate hosts for metacercariae, and fishes as well as, at least in one case, an amphibian (*Bucephalopsis kweiyangensis* in the giant salamander *Megalobatrachus japonicus*) serve as definitive hosts (Plate 14.14). The general life history sequence for bucephalids involves eggs from the mature worm being voided with the predatory fish's feces that develop and hatch. The miracidium of at least some bucephalids has jointed appendages (Woodhead, 1929) (Fig. 14.14A) and others lack appendages and have long cilia only (Dickerman, 1954). The miracidium enters a bivalve by the inhalant siphon, penetrates the mollusc, and produces a mother-sporocyst; which for some species can be extensively branched (Kniskern, 1952) (Fig. 14.14B). Most bucephalids show a high degree of host specificity to particular bivalves (Lauckner, 1983). Bucephalid cercariae, like those for many digenean families, exhibit a great deal of morphological variability that hints at their diverse life history strategies (Woodhead, 1936). Cercariae are shed through the exhalant siphon, and many have an 'ox-bow' resemblance. The forked tails of some bucephalid cercariae are filament-like and sticky, and they seem adapted for becoming entangled on the fins or gill rakers of fishes (Wardle, 1988) (Figs. 14.14C, D). Matthews (1973a) observed a sticky secretion originating from the disc-like tail stem of *Prosorhynchus crucibulum*. These cercariae are drifters that move with 'slow and graceful' contractions of the furcae (Woodhead, 1930). After ensnaring a small fish, the cercariae penetrate, encyst, and develop into metacercariae before this second intermediate host fish is consumed by a predatory fish. In the definitive host, they mature, copulate, and produce eggs. Bucephalids seem particularly host- and site-specific in some but not all second intermediate fish hosts (Krull, 1934b). In several species, the metacercariae are well developed and are identifiable by comparison with the adult form (e.g., Matthews, 1973a, b).

*Bucephaloides gracilescens* matures in the intestine and pyloric ceca of angler (*Lophius piscatorius*). Matthews (1974a) studied the life cycle of *B. gracilescens* and found sporocysts comprising a mass of dichotomously branched tubules occupying the gonad hemocoel and kidney of the bivalve *Abra abra*. The sporocyst breaks through the body wall at points

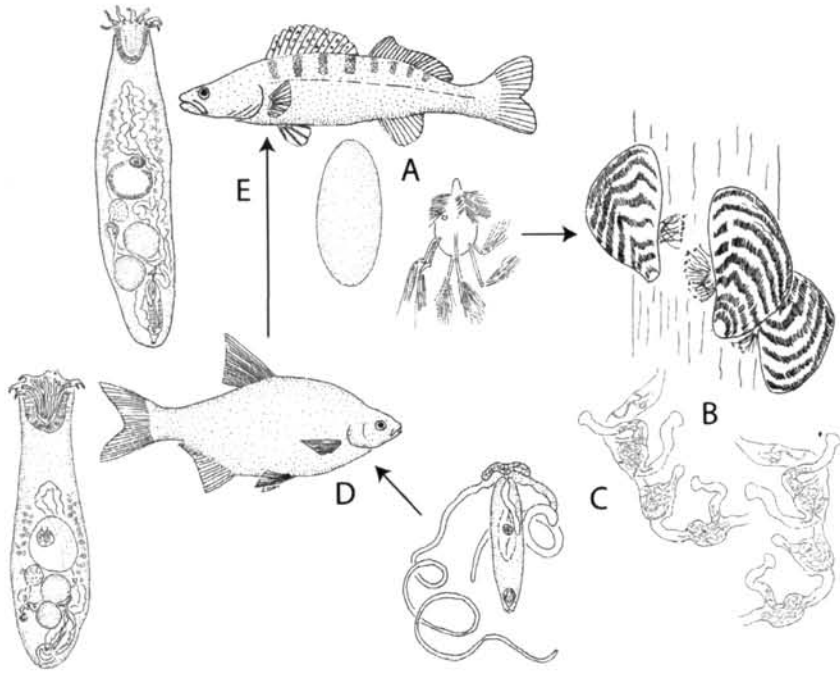


**Plate 14.14** Life cycle of *Bucephalus polymorphus* (Bucephalidae) involving piscivorous freshwater fishes including zander (*Sander lucioperca*) and a variety of cyprinid fishes including white bream (*Blicca bjoerkna*) as well as the first intermediate molluscan host the zebra mussel (*Dreissena polymorpha*). Based on Baturo (1978). **A.** The adult of *B. polymorphus* infecting the gut of the definitive host zander deposits eggs that are dispersed in the zander's feces. **B.** The miracidium infects the zebra mussel wherein a highly branched sporocyst develops. **C.** The sporocyst yields numerous cercariae having extremely long, filamentous furcae with terminal sucker-like depressions and coated with a sticky adhesive substance secreted from a gland at the base of the tail. **D.** The cercaria can float freely in the water whenever furcae extend horizontally with the body hanging downwards and can sink when the furcae are repositioned vertically. Agitation stimulates the cercaria to swim off a substratum. The sticky tail of the cercaria adheres to the scales of a fish and subsequently penetrates the fish skin and encysts in the appropriate site. Mature metacercariae resemble the adult. **E.** The zander is infected by preying on the infected cyprinid.

of weakness and protrudes into the mantle cavity of the bivalve, wherein the sporocyst ruptures and releases cercariae into the exhalent chamber before they are flushed through the exhalent canal. The cercarial tail is twelve times the length of the cercarial body and comprises two contractile and sticky furcae. This cercaria floats, partially contracting its furcae, only to jerk its body slightly upwards and away from the substratum; fully extending the furcae allows drift in the water current as

if 'fishing' for the next host. After attaching to a host, some individual cercariae migrate to nervous tissue possibly by entering the canals of the lateral line system (Crofton and Fraser, 1954). The metacercaria infects the nervous system of at least nine gadoid fishes (Matthews, 1974b), especially the brain or cranial and spinal nerves (Johnston and Halton, 1981). In at least whiting (*Merlangius merlangus*) most cysts infect the intracranial fluid around the brain, auditory capsules, eye muscle, orbit, and spinal nerves. The cyst wall thickness differed in these sites: cysts in the orbit/nasal cavity were thicker than those in the brain (Halton and Johnston, 1982). Metacercariae in the brain cavity were regarded as 'progenetic' because they developed fully differentiated testes, an intromittent organ, an ovary, and a uterus; the most advanced specimens had spermatozoa in the testes and seminal vesicle. However, although the vitellarium had shell precursors such as phenols, it did not react for phenolase, a product essential for egg production (Smyth and Clegg, 1959). After being ingested by its definitive host, the angler, the metacercariae excyst, feed, and mature in the intestine and pyloric ceca, presumably because of the increased levels of nutrients in those sites (Halton and Johnston, 1983). Stewart *et al.* (2003a) studied the cholinergic, serotoninergic, and neuropeptidergic components of the nervous system and compared them between progenetic metacercariae and adults to provide baseline information on the muscles involved in egg assembly. *In vitro* metacercarial development was most rapid at 18°C in a medium containing NCTC 135 supplemented with 25% chicken serum, 25% hen egg yolk, and 25% hen egg albumen, with a gas phase of air. Shell-protein synthesis was triggered by day three and the ovary by day ten. Teratological eggs were evident by day 14 but failed to embryonate (Halton and Johnston, 1983).

*Prosorhynchus crucibulum* has a metacercaria that infects turbot (*Psetta maxima*) (as *Scophthalmus maximus*) as well as three other experimental fishes, i.e., dab (*Limanda limanda*), a probable rockling species (as '*Onus mustelus*'), and sand goby (*Pomatoschistus minutus*) (as *Gobius minutus*), but not brill (*Scophthalmus rhombus*), European plaice, longspined bullhead (*Taurulus bubalis*) (as *Cottus bubalis*), or shanny (*Lipophrys pholis*) (as *Blennius pholis*). Matthews (1973a) followed the development of metacercariae in the connective tissues and muscle of turbot over a 10-month period. Within three to four hours of penetration, the cercaria exuded a hyaline cyst, but at one month post-infection this cyst was eliminated. Matthews (1973a) speculated that this enabled the

metacercaria to feed on host tissue, accounting for an advanced stage of metacercarial development. Despite the metacercariae being clearly identifiable with the adult form, egg production did not commence until excystment in the gut of the definitive host, European conger (*Conger conger*).

### Specific Pathogens

Few bucephalids harm their second intermediate host fish, but we detail this group because some are implicated in parasite-induced host mortalities of wild fishes that range in shallow, small bodies of water such as streams or reservoirs or naturally enclosed, ephemeral habitats such as lagoons, tide pools, and rocky shore habitats. That some metacercariae encyst in or near the brain or eyes of their second intermediate host fish has prompted some researchers to assume that some alteration of biological function might make them more vulnerable to predation by their definitive host (Laffargue *et al.*, 2004), but we emphasize that at present there is scant experimental or histopathological evidence to support that claim for most species (e.g., Pulsford and Matthews, 1984; Wang *et al.*, 2001). Regarding the molluscan first intermediate host, many bucephalids castrate it (e.g., Kniskern, 1952; Tripp, 1973; Stunkard, 1976; Lauckner, 1983; Coustau *et al.*, 1990; Wardle, 1990; da Silva *et al.*, 2002), and because of this much attention has been focused on the infection dynamics of larval forms in commercially valued bivalve populations. Some bucephalids may significantly limit the population size of particular intertidal molluscs: Taskinen and Valtonen (1995) estimated a 31% reduction in the number of glouchidia-bearing clams in the littoral zone of Lake Saravesi, central Finland, and attributed this reduction to the castrating bucephalid *Rhipidocotyle fennica*.

### Bucephalus minimus

This bucephalid is also referred to as *Bucephalus haimeanus* and *Labratrema lamirandi* in the literature. Actually, there are probably at least two species in a complex, the status of which is unsettled (Overstreet and Curran, 2002). This is an estuarine bucephalid that infects a variety of bivalves, the liver of several small estuarine fishes (Matthews, 1973b), including the big-scale sand smelt (*Atherina boyeri*), and matures in European seabass (*Dicentrarchus labrax*) (as *Morone labrax*). Faliex and Morand (1994) investigated the ecological interaction between cercariae of *B. minimus* (as

*Labratrema minimus*) and big-scale sand smelt. Cercarial shedding was seasonal and greatest during May until October. They observed variation in the mean abundance of infection with respect to time and host size, and explained this variation by (1) susceptibility of big-scale sand smelt to multiple successive exposures to and subsequent infections with cercariae (increase in abundance of infection with host length and raising of parasite burden) and to (2) the loss, probably by more rapid death, of heavily infected big-scale sand smelt from each host size group. They deduced that the infection pattern was a dynamic process influenced by: (1) availability of infective cercariae, (2) influx of newborn, naive big-scale sand smelt only, (3) high susceptibility of naive and adult big-scale sand smelt to infection, (4) metacercarial loss, and (5) probably parasite-induced host mortality. Higgins (1980) studied the cyst wall around *B. haimeanus* and related changes in the parasite tegument during its metamorphosis from the cercarial to the metacercarial stage in the two-spotted goby (*Gobiusculus flavescens*). A fibrous cyst wall was derived from secretions from anterior gland cells and the tegument of the encysting metacercaria. These secretions become compacted against the surrounding hepatic cells, ultimately forming a three-zoned inner layer. Compacted hepatocytes form the middle vacuolated layer, and as the cyst grows the adjacent hepatocytes become flattened and incorporated into the cyst, thereby forming the outer nucleated layer.

### *Bucephalus polymorphus*

The life cycle of *B. polymorphus* comprises sporocysts and cercariae in *Dreissena polymorpha*, metacercariae in a variety of cyprinids, and adults in zander (*Sander lucioperca*) (as *Lucioperca lucioperca*) as well as species of *Esox*, *Perca*, *Lota*, and *Gymnocephalus* (as *Acerina*) (Baturo, 1978) (Plate 14.14). Metacercarial infections of *B. polymorphus* among cyprinid fishes in the Main River between Würzburg and Frankfurt, Germany, were studied by Hoffmann *et al.* (1990) during the summer of 1984. High losses of roach, carp bream, white bream, tench, common carp, European chub (*Leuciscus cephalus*) (as *Squalius cephalus*), and European perch (*Perca fluviatilis*) were documented after a rapid change in water temperature from 12-14°C to 20°C. Roach (24-31 cm TL, 220-430 grams) and carp bream (27-36 cm TL, 230-590 grams) were especially emaciated and had a low condition factor (k = 1.0-1.4). Heavily infected fish had large hemorrhages covering the head, mouth, eyes, and about the base of the pectoral or dorsal fins. In other cases, the fins were altered with worn

edges, and fin rays were only residuary. European perch and the breams had opaque eyes, and the eye-lens had metacercariae in the connective tissue of the eyes and in the lens itself; these were identified as diplostomula of *Diplostomum spathaceum*. Metacercariae of *B. polymorphus* were also found beneath the gill epithelium, in lymph spaces, and in the blood vessels along the gill rods or in the connective tissue of the gill arch. The pseudobranchs were affected by migrating parasites at the base as well as in the vessels of the parenchyma. Histological sections revealed large numbers of adults in the mucosal folds of the intestine as well as metacercariae in the subcutis of zander, but no significant pathological alteration to these tissues was detected. The subcutis and gills of these fishes had secondary bacterial infections. In the Main River, cercarial shedding was greatest between 10-20°C and the rapid rise to 20°C probably induced a massive pulse of cercariae. The pathology associated with the penetration of cercariae of *B. polymorphus* included ulcerations of the skin and the proximal parts of the fins as well as changes in the gills due to cyst formation. Cercarial migration and encystment can cause pathological changes in the parietal peritoneum, kidney, liver, brain, muscles, and urinary bladder (Baturo, 1977; Wierzbicka, 1977), including vertebral deformities caused by muscle infections (Baturo, 1980). In addition to temperature, a high concentration of nitrogenous compounds in the water and an alleged inflated population size of the first and second intermediate hosts in this river may have had a synergistic effect in inducing this epizootic: both zander and the *Dressenia* spp. were introduced in the Main River in the late nineteenth century; fishing pressure on cyprinids had decreased due to lessened market demand; and upstream agricultural runoff originating from vineyards along the river caused periodic episodes of eutrophication. Hoffman (1975), although not cited by Hoffmann *et al.* (1990), mentioned a 1966 episode in which *B. polymorphus* caused simultaneous widespread mortalities of cyst-laden cyprinid fishes captured from two other European rivers, the Seine (France) and the Wda (Poland). The pathological signs of the disease were strikingly similar to those reported by Hoffmann *et al.* (1990) and others; the afflicted fishes rubbed their bellies and sides on the river bottom, and the mouth and bases of the caudal and anal fins had marked congestion and hemorrhage. Some fishes were exophthalmic with many opaque areas in the cornea, perhaps attributed to a diplostome. Septic necrosis of fins contributed to the disease, and migrating cercariae caused muscle necrosis and retinal hemorrhages.

### *Dollfustrema vaneyi*

Wang *et al.* (2001) studied the seasonality of *D. vaneyi* in yellow catfish (*Pelteobagrus fulvidraco*) (as 'bullhead catfish', *Pseudobagrus fulvidraco*) in an enclosed reservoir in China. They thought that the higher mean intensity level of metacercariae in fishes that were 14-16 cm TL and the lower levels in smaller and larger fishes included in their study indicated 'removal' (mortality) of the heavily infected fish. Heavily infected fish may have a high probability of being preyed upon. Death of metacercariae could explain this result. However, no evidence of dead or deteriorating metacercariae was found in any fish. Further, the infection level of this bucephalid in yellow catfish was high (overall prevalence = 90%, mean intensity = 136 ± 308 [standard deviation]), and hemorrhaging was a commonly observed occurrence in the infected yellow catfish. Wang *et al.* (2001) stated that further study is needed to assess the pathological effects of this metacercariae in this host.

### *Rhipidocotyle* spp.

Matthews (1968, 1974b) detailed intense metacercarial infections of *Rhipidocotyle johnstonei*. This metacercaria is somewhat unique because it does not exude a cyst, despite becoming encapsulated by the host and eliciting a strong host response. Within this encapsulation, the parasite can apparently feed and produce eggs. In European plaice, this type of infection may cause high mortality rates in young fish as a result of seasonal, intense pulses of cercariae in restricted habitats. The metacercariae elicited a massive localized host response comprising granuloma formation and a fibrotic encapsulation. Pulsford and Matthews (1984) studied the ultrastructural cellular response of the European plaice to this metacercaria. The chronic inflammatory lesion contained cells primarily of the mononuclear phagocyte series, and the surrounding granuloma included monocytes, macrophages, epithelioid cells, and multinucleated cells with peripheral fibrocytes. A few lymphocytes and plasma cells were present in the mature granuloma, suggesting a cell-mediated response. The parasite matured in two months and lived for ten months, with the host encapsulation becoming increasingly fibrotic during that time. Intense infections in young fish caused fin erosion and hemorrhage as well as probably impaired muscle function. These authors suspected that these alterations might increase the risk of predation. In this host, the metacercariae of *R. johnstonei* are progenetic, producing eggs and releasing them into the surrounding granuloma. In the late stage of

infection there was no trace of a parasite cyst and the metacercariae apparently were free to feed on the surrounding granulomatous tissue (Pulsford and Matthews, 1984). Such an intense reaction to a larval digenean is exceptional (Paperna, 1995), and perhaps such a reaction is more common than has been reported in the literature considering progenetic species that either feed on their second intermediate host fishes or deposit eggs in the muscle of those hosts. The humoral response of wild, lake-dwelling roach to homogenized and live cercariae of *Rhipidocotyle fennica* was investigated by Aaltonen *et al.* (1997). These fish had antibodies to this cercaria, and the levels of those antibodies in roach positively correlated with infection levels in roach during late summer. An especially strong host response was observed when roach were exposed to living cercariae shed from the clam *Anodonta piscinalis*.

### Unidentified Bucephalid Metacercariae (Probably *Parabucephalopsis* spp.)

In the first report of its kind from Japan, Ogawa *et al.* (2004) reported intense metacercarial infections of unidentified trematodes of Bucephalidae from freshwater minnows (*Zacco platypus*) and Khanka gudgeons (*Squalidus chankaensis chankaensis*) in the Uji River (Yodo River System) from January to April of 2000 (Fig. 14.15). Heavily infected fishes had numerous cysts in the musculature of the caudal peduncle region (most commonly), about the base of other fins, and in the gill (rarely). Along the banks of the river, some of these fish appeared dead and were easily caught by hand. Most observations of listless fishes were during January and February, and in March and April there was a marked decrease in the number of freshwater minnows in the river, presumably from death of heavily infected individuals. Pathological alterations associated with the cysts included hemorrhagic lesions of the eyes, head, and fin bases, erosions in the peduncle and at the base of the caudal and dorsal fins, and occasional exophthalmia and pale gills. Most cysts were thin-walled (Metacercaria A) and only a few were thick-walled (Metacercaria B). Heavily infected Khanka gudgeons had hemorrhages and dissolution of muscle fibers around the cysts. Because no bacterium, fungus, or virus was detected in an infected fish, it seemed probable that the etiological agents were the two encysted bucephalids. As with the epizootic in the European rivers, this epizootic was thought to be the result of an exotic species and the physical environment of the river. Urabe *et al.* (2001) surveyed molluscs from this same river and found that the

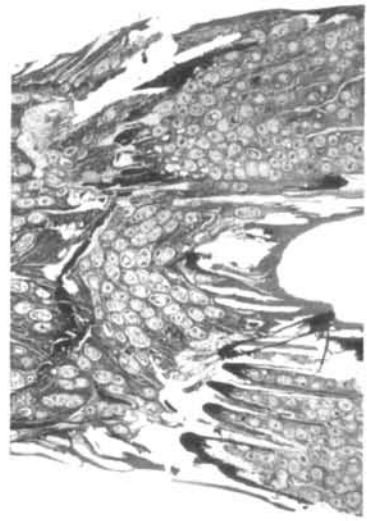

**Plate 14.15** Histological section of bucephalid metacercariae in the caudal region of a Khanka gudgeon (*Squalidus chankaensis chankaensis*) collected from the Uji River in Japan during April 2000. Azan stain. From Ogawa *et al.* (2004).

introduced Asian freshwater mussel (*Limnoperna fortunei*) was infected with a bucephalid sporocyst. Based on that, Ogawa *et al.* (2004) thought that this mussel was the first intermediate host for one or both of the pathogenic bucephalids. Cercarial shedding was seasonal but most active during November through January, the months preceding the period of the epizootic. Low rainfall during this period may have isolated freshwater minnows and Khanka gudgeons in slow-moving, shallow waters of the river where infected mussels were shedding multitudes of bucephalid cercariae. Freshwater minnows examined the following winter during a period of 'normal' water flow were infected, but with many fewer cysts (Urabe *et al.*, 2001), which seemingly added some support for a drought component to the epizootic.

## Fish Flukes whose Adults, Eggs, or Miracidia Harm the Definitive Host Fish

### APOROCOTYLIDAE

Aporocotylidae, the earliest available family-group name and the name presently applied to all 'fish blood flukes' (although many authors in the recent literature refer to this group as 'Sanguinicolidae'), is one of the few

groups containing members that have adults, eggs, and miracidia all capable of harming the definitive host fish. Several aporocotylids fit this pattern.

## Diagnosis

Aporocotylids comprise the only digeneans that as adults are known to reside in the blood of fish. They are a morphologically and ecologically diverse family with adults that infect the heart (Plate 14.18) as well as blood vessels such as branchial arteries and arterioles of marine and freshwater fishes (Plate 14.21). Some adults infect more unusual sites such as body cavity (Overstreet, 1969), pectoral girdles (Montero *et al.*, 2003), and mesenteric vessels (Bullard and Overstreet, 2002) (Plate 14.19). All of these sites are atypical for digeneans, and many infections are probably missed because the fish vascular system is seldom examined critically during routine necropsies. Many adult blood flukes are 2-11 mm in total length (although some are much smaller), thin-bodied, dorsoventrally flat or tube-like, elongate or oval in shape, and opaque or nearly transparent in life. Those that crawl are concave and capable of using the ventral surface of the body as a suction cup that adheres them to smooth surfaces such as the walls of arteries and veins (Bullard and Overstreet, 2003, 2004). Adults of elongate and narrow-bodied species can be entwined within the myocardium, and those of other species are extremely small and apply their thin, delicate bodies to the trabeculae of the heart (Bullard, unpublished data). The vast majority of nominal species have lateral tegumental spines, but the arrangement and function of these spines differs widely among species. Some of those that infect luminal spaces have tread-like spine rows that facilitate attachment and locomotion along luminal walls (Bullard and Overstreet, 2003); some are sedentary as adults, partially embedding themselves in the cardiac muscle, and have sparsely distributed, minute spines that are functionally degenerate (Bullard and Overstreet, 2006); others have larger, ice tong-like spines that probably function to grip host tissue (Bullard *et al.*, 2006). Aporocotylids differ from mammalian blood flukes (Schistosomatidae) because they are usually wider and are hermaphroditic rather than having separate sexes. Aporocotylids are further differentiated from other digeneans by lacking a large, obvious oral sucker in most species and by lacking a ventral sucker in all named species. Although it is a misconception that aporocotylids lack an 'oral sucker', in most species the anterior sucker is diminutive and difficult to understand without studying

living specimens with the aid of a compound microscope or using SEM of well-fixed, i.e., heat-killed specimens. Smith (2002) listed the morphological features that previously have been used to distinguish genera, but several genera need revision. In general, the family remains in a state of taxonomic disarray, and a phylogenetic hypothesis for the group as a whole is lacking.

### *Life Cycle*

Aporocotylids are unique because (1) they lack a distinct second intermediate host, (2) they lack an encysted or encapsulated metacercaria, (3) they mature in an extra-intestinal site (blood or body cavity) in their definitive host fish, (4) their larvae may exit the definitive host through the gill rather than the gut, with some miracidia probably hatching while the egg is lodged in the definitive host's gill epithelium, (5) some marine species have a polychaete rather than a molluscan first intermediate host (e.g., Martin, 1952; Oglesby, 1961; Smith, 1972, 1997a; Køie, 1982; Køie and Petersen, 1988), and (6) they can kill their definitive hosts in aquaculture (Bullard and Overstreet, 2002; Ogawa *et al.*, 2007). The following serves as a generalized life cycle for an aporocotylid (Plate 14.16). For most species, adults release a few pliable, thin-shelled eggs (Plate 14.17), with some doing so on a seasonal basis (Avendaño de MacIntosh and Ostrowski de Núñez, 1998). Most of the eggs become entrapped in gill tissue wherein the egg undergoes a morphological transformation and the miracidium develops. It seems that this transformation varies, depending on the species: for at least *Elaphrobates euzeti*, whose adults infect the heart of red snapper (*Lutjanus campechanus*) and grey snapper (*L. griseus*), the recently deposited egg (Figs. 14.17G-H) is spheroid and lodged in the afferent branchial arteriole. Later, the egg apparently traverses the arteriole's wall and becomes embedded, but not encapsulated, in the gill filament epithelium. In this site, the eggs are much changed: each being more elongated, having polar filaments, and enclosing a ciliated, well-developed miracidium (Fig. 14.17I). For *Sanguinicola inermis*, the miracidium develops and is released, leaving the fish within seven days (Kirk and Lewis, 1993). In gill, the host response to the egg is typically mild, if present at all. Depending on their size, eggs are lodged in the brachial arterioles or the branchial epithelium between arterioles and the capping tissue of the gill filament. After hatching, the miracidium bores through the gill epithelium, leaves the host, and searches for an appropriate snail, bivalve, or polychaete intermediate host.

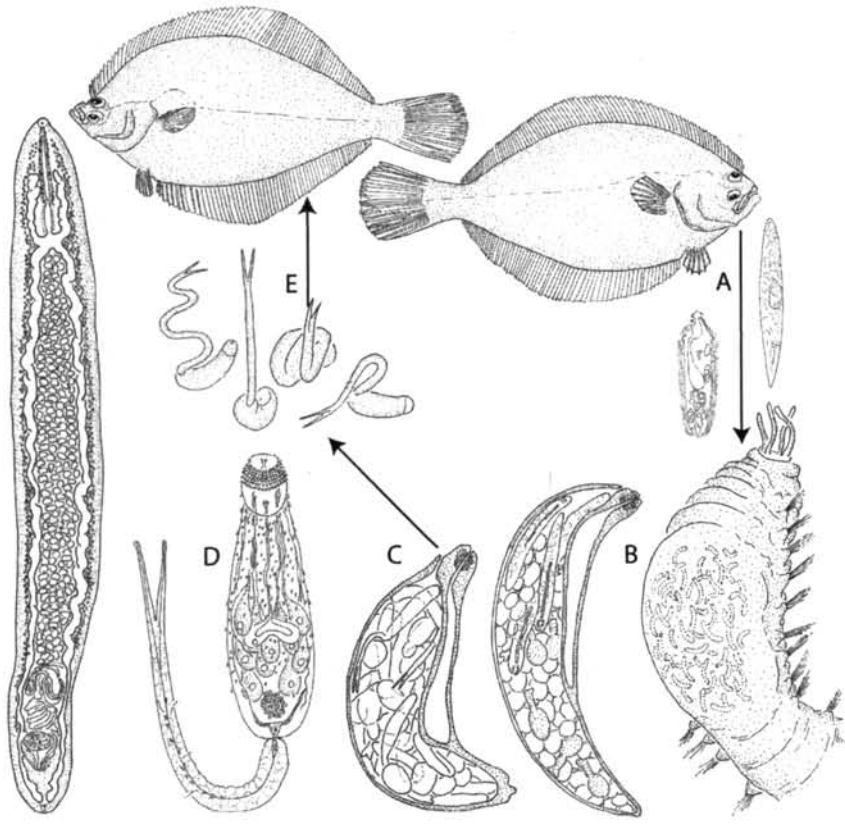


**Plate 14.16** Life cycle of the marine aporocotylid *Aporocotyle simplex* involving flatfishes (*Hippoglossoides platessoides*, *Limanda limanda*, or *Pleuronectes platessa*) and terebellid polychaetes (*Artacama proboscidea* or *Lanassa nordenskioeldi*). Based on Køie (1982) and Køie and Petersen (1988). **A.** The adult of *A. simplex* (depicted at left of figure) infects the vascular system (primarily the heart, ventral aorta, and the gill arteries) of flatfishes, depositing eggs in the blood. Some eggs are carried to the gill wherein the miracidium is released from the egg, emigrates through the gill epithelium, and seeks and penetrates a terebellid polychaete. **B-C.** Mother sporocysts (not illustrated), mother redia, and daughter redia occur in the polychaete's coelomic cavity. **D.** A furcocercous, apharyngeate cercaria with a characteristic cephalic organ develops in the daughter redia. **E.** This cercaria swims actively, penetrates the fish, loses its tail, develops first to a schistosomule and then to an adult before finally migrating to the specific site in the circulatory system of the host where it copulates and produces eggs.

After penetration of that host, sporocysts and rediae develop that yield cercariae that, in turn, penetrate the fish host through its gill, skin, eye, fin, or alimentary tract and develop into a juvenile stage known as a schistosomule before ending up in a specific site in the circulatory system

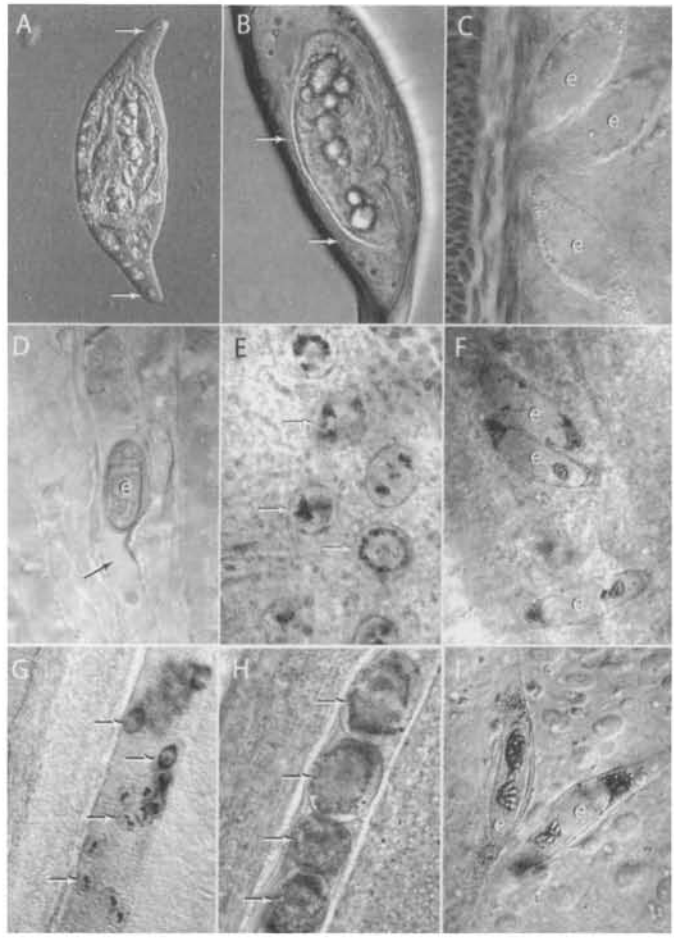


**Plate 14.17** Eggs of extra-intestinal digeneans, primarily aporocotylids, from or in the gill of several marine fishes from the Gulf of Mexico: butterflyfishes (Chaetodontidae) (Figs. 14A-C), grouper (Serranidae) (Figs. 14D-F), and snapper (Lutjanidae) (Figs. 14G-I). All are fresh-preparations in seawater and photographed with differential interference contrast (DIC) optical components. Note that no egg is encapsulated or encysted in the gill of any host. **A.** Egg excised from gill epithelium with the elongate filaments (arrows) typical of many fully developed aporocotylid eggs. **B.** Higher magnification view of miracidium within another egg showing ciliated regions in plane of focus (arrows) and lipid-like droplets. **C.** Three eggs (e) lodged in the gill epithelium. **D.** The lumen of a branchial arteriole (arrow) infected with a didymozoid egg, which is more reniform or ovoid and thick-shelled than aporocotylid eggs. **E.** Spheroid, recently deposited eggs (arrows) in branchial epithelium. **F.** Mature, elongate eggs (e) each having a single well-developed miracidium. **G.** Recently deposited eggs (arrows) amassed in the afferent branchial artery. **H.** Spheroid eggs (arrows) partially occluding the afferent branchial arteriole of a filament, note that these eggs lack fully formed, ciliated miracidia. **I.** A pair of fully developed eggs (e) in the branchial epithelium, each containing a ciliated miracidium and coalesced lipid droplets.

of the fish. All but a few adult aporocotylids reportedly infect the heart or branchial vessels, wherein they ultimately mature, copulate, and release eggs, but we suspect that many additional, unnamed species infect other sites. Life cycles for most blood flukes are cryptic; of the hundred or so known marine species, the life cycle of only one has been elucidated by manipulating experimental hosts. *Aporocotyle simplex* (see Køie, 1982) uses a terebellid polychaete intermediate host (*Artacama proboscidea*) and several pleuronectids, i.e., dab, European plaice, flounder (*Platichthys flesus*), or turbot (as *Scophthalmus 'maximum'*) (see Køie and Petersen, 1988) (Plate 14.16). Undoubtedly, some marine aporocotylids use bivalves, snails, and other polychaetes as the intermediate host. Life cycles of freshwater aporocotylids (*Sanguinicola* spp.) are more numerous in the existing literature and follow the general pattern of *A. simplex*, but all known cycles incorporate a snail first intermediate host (Meade, 1967; Schell, 1974; Hoffman *et al.*, 1985; Kirk and Lewis, 1993; Nolan and Cribb, 2004) (Plate 14.20). Smith (1972, 1997a) provided a useful table with references to various life history information and larval details for aporocotylids, and Smith (1997b) provided a listing of nominal species and known hosts for aporocotylids.

Aporocotylid life cycles can be seasonal. For example, *Paradeontacylix grandispinus* (Fig. 14.21B) and *Paradeontacylix kampachi* (Fig. 14.21A) infect greater amberjack (*Seriola dumerili*) (as yellowtail, *Seriola purpurescens*) and migrate to the branchial vessels to deposit eggs during a specific season, creating peak periods of intense infections and subsequent fish kills (Ogawa *et al.*, 1989, 1993). Some species apparently can overwinter in fishes: Ewens *et al.* (1994) showed that the adult of *Sanguinicola inermis* overwintered in wild common carp and released eggs in the spring. Hine (1978) readily observed adult specimens of *Paracardicoloides yamagutii* in the gills of eels, i.e., shortfin eel (*Anguilla australis*) and New Zealand longfin eel (*A. dieffenbachii*), only when the flukes migrated there to lay eggs in spring and autumn. Maillard and Ktari (1978) found that the uterus of adult specimens of *Hyperandrotrema cetorhini* collected from a basking shark (*Cetorhinus maximus*) either lacked eggs or possessed only a few deformed eggs, suggesting that viable eggs were produced during a specific period other than when collected. An interacting set of abiotic factors probably drives this apparent seasonality, but knowledge on how each of those factors affects the miracidium, schistosomule, and adult for any species is scant, if known at all. Water temperature probably significantly influences the behavior and physiology

of at least the miracidium and cercaria (Overstreet, 1982). The life cycle of *S. inermis* depends on temperature (Ewens *et al.*, 1994), and discrepancies in penetration and migration duration of *S. inermis* may result from different host maintenance temperatures (Smith, 1997a). Temperature may also dictate the developmental time of schistosomules; the schistosomule of *S. inermis* remains in the dermis much longer at low temperature than does the adult infecting large blood vessels (Sommerville and Iqbal, 1991; Kirk and Lewis, 1993). The host response to aporocotylids varies according to host species, site in the host, intensity of infection, season, and environmental conditions. Little information exists regarding the effect of these flukes on wild host populations, but some flukes may weaken their hosts, making them vulnerable prey and reducing their populations, especially during certain seasons. However, probably few wild HPRs significantly influence population-structure.

All fishes probably have a significant immune response to challenging cercarial penetration, schistosomule development and migration, or egg development in non-gill associated tissues, but the response apparently differs according to temperature and host physiology. The elegant series of papers by Richards and colleagues (Richards *et al.*, 1994a, b; 1996a, b, c) have provided considerable information on the response of common carp to *S. inermis*. The fish clearly elicits a cellular response against the adult, egg, and cercaria involving eosinophils, neutrophils, and macrophages. Common carp exposed to extracts of cercariae and adult flukes at 20°C increased lymphocyte production, and at 10°C the adult extract elicited an even greater response. Perhaps the reduced ability of the lymphocytes to proliferate in common carp at lower temperatures in response to the fluke extracts suggests an impairment of the immune system. This would perhaps allow the adult worm to overwinter, since at the higher temperature the adult may survive for only a couple of months (Kirk and Lewis, 1993). Periods of cool weather also correspond to periods of cercarial shedding, enhancing cercarial penetration into the host and subsequent survival of the young worm. The proliferation of lymphocytes in common carp is even more complicated because at 10°C it occurs in the pronephric (kidney) and splenic lymphocytes; whereas, at 20°C most proliferation occurs in the pronephric cells (Richards *et al.*, 1996a). Perhaps during warmer months, a specific humoral immune response mediated by kidney lymphocytes influences infections, but during cool months, a more nonspecific response, probably mediated by T-cells in the spleen, predominates (Richards *et al.*, 1996a). The ability of leukocytes to

adhere to cercariae or encapsulate/degrade eggs appears to be inhibited by live adult worms (Richards *et al.*, 1996c). Giving a marine example, Ogawa *et al.* (1989) presented data on prevalence and intensity of aporocotylids infecting young and old greater amberjack in a net pen. Individuals less than 1-year-old had a relatively high number of eggs in cardiac muscle and gill, suggesting that the older fish acquired some level of immunity to infections after receiving an initial exposure to the flukes a year earlier. We have observed a similar pattern with infections of *Cardicola currani* in red drum (*Sciaenops ocellatus*) and with that of *Cardicola palmeri* in black drum (*Pogonias cromis*) in Mississippi Sound; with infections occurring only in younger age classes of each of the drums (Bullard, 2002). However, whether or not this pattern is related to host-acquired immunity, the geographic location of the intermediate host, the life histories of the definitive hosts, or some combination of these is indeterminate. McLaren and Hockley (1977) demonstrated that blood flukes have a double outer membrane that may protect them from the host immune response by serving as a binding site for host antigens. However, in schistosomes at least, this second outer layer develops only after the schistosomula transforms into the adult form (Brink *et al.*, 1977).

Eggs can cause pathological alterations if they lodge in viscera or connective tissue, wherein the host's inflammatory granulomatous response can result in encapsulation and degradation of the eggs (Plates 14.18–14.19). Overstreet and Thulin (1989) investigated the heart and tissues of several marine teleosts and found that most serranids contained an abundance of macrophage aggregates (MAs) in the ventriculus. These serranids and a few other fishes contained free macrophages among ventricular trabeculae. This unusual abundance of macrophages in these locations may have developed as a means of contending with blood fluke infections. One aporocotylid, *Pearsonellum corventum*, in its serranid host, the leopard coralgrouper (*Plectropomus leopardus*), causes minimal harm to the wild host (Overstreet and Thulin, 1989). In the heart, the macrophages along with neutrophils and lymphocytes surrounded the eggs as well as the adult worm, but no fibrotic encapsulation was evident. Eggs lodged in the ventricular epicardium, pericardium, aortic serosa, mesentery, liver, spleen, and kidney were mostly encapsulated and being degraded in small granulomas, some of which were associated with ceroid or MAs. Moreover, the substantial cellular response of most serranids and a few other fishes also serves to sequester a variety of parasites, prey spines, entire eels, and other foreign bodies within heavily pigmented fibro-

encapsulations (Overstreet and Thulin, 1989). Leknes (1986) found subendothelial macrophages in two teleosts that were not present in other investigated species but did not relate the finding to the presence of parasites. Bullard and Overstreet (2002, 2003) observed aporocotylid eggs in cardiac muscle, gill epithelium, and liver of several wild-caught fish, including red snapper from the Northern Gulf of Mexico (Plates 14.18–14.19). These infections were like most cases in the literature. Eggs in cardiac muscle (which lacked MAs) (Figs. 14.18A, C, E) and liver (Figs. 14.18D, F) were not abundant but were typically present in various stages of degeneration. Recently deposited eggs in early stages of degeneration were encapsulated by a thin epitheliod or fibroid layer. Somewhat degenerated eggs exhibited some yellowish ceroid pigment. Eggs in a later phase of degeneration often displayed more conspicuous light to dark brown ceroid pigment (Fig. 14.18C). Cardiac muscle associated with the degenerating eggs appeared normal except for an infiltration of leukocytes. Based on these observations, we doubt that these eggs caused any significant decrease in the mechanical efficiency of the heart. Eggs in the liver primarily occurred in pancreatic nodules (Fig. 14.18F) and were often surrounded by a granulomatous response. Sequestered eggs in the gill (Fig. 14.17I) mostly contained living miracidia that were readily apparent by their refractive excretory products when observed in fresh wet-mounted preparations. We estimated that fewer than ten of these thin-shelled, elongated eggs in the afferent vessels infected each filament of the wild-caught red snapper. In comparison, mature eggs of *Cardicola currani* in wild-caught red drum from Mississippi Sound differed from those in the red snapper by being more spherical, but neither species elicited a noteworthy host response as is common for eggs of some species in other sites. For example, the eggs plus adults of *Plethorchis acanthus* from the intestine and mesenteric pancreas of wild-caught flathead mullet from Queensland, Australia, provide a good example of an ecologically atypical aporocotylid (Plate 14.19). These adults expand the vessels and accommodate large numbers of surrounding leukocytes. Large clusters of eggs occluded both large and small vessels and elicited an extensive inflammatory response. A given fish typically demonstrated both recently deposited eggs containing miracidia associated with a slight granulomatous response and more advanced cases with eggs in various phases of degeneration. The more advanced cases, especially in heavily infected fish, typically exhibited an extensive inflammatory response involving ceroid pigmentation.

### *Specific Pathogens*

Blood flukes further distinguish themselves from all other digeneans by having a track record of killing their definitive hosts in aquaculture (Bullard and Overstreet, 2002; Ogawa *et al.*, 2007). Although the larval forms of several other digeneans are renowned pathogens of cultured fishes, to our knowledge there is no record of a mass mortality caused by a non-aporocotylid adult digenean killing its definitive host in aquaculture or in the wild. Because aporocotylids infect groups of commercially valued marine and freshwater fishes, e.g., salmons (Salmonidae), tunas (Scombridae), jack fishes (Carangidae), cyprinids (Cyprinidae), sparids (Sparidae), eels (Anguillidae), catfishes (Siluriformes), seabasses (Centropomidae and Latidae), mullets (Mugilidae), cobia (*Rachycentron canadum*) (Rachycentridae), and drums (Sciaenidae), information on aporocotylid-fish HPRs in the wild and in aquaculture is useful to the aquaculture industry regarding disease prophylaxis, diagnosis, and treatment. Confined fishes are especially prone to sanguinicoliasis because in cage-culture and pond systems the definitive host (the cultured fish) can be held nearby or cultured along with infected intermediate hosts. Like so many other parasitic diseases, sanguinicoliasis is intensity dependent, and intensity depends on the proximity of the hosts. Acute and chronic stages of the disease can be lethal, and lethal acute cases comprise fishes killed by penetrating cercariae or exiting miracidia (e.g., Crespo *et al.*, 1992). For example, 2,500 cercariae of *S. inermis* killed common carp within a few hours and produced severe edema and epidermal hemorrhaging (Kirk and Lewis, 1992). However, as with other digenean infections, few cercariae do not typically kill the host. When miracidia exit a host en mass simultaneously, they cause severe hemorrhaging of the secondary lamella capable of killing the fish. Lethal chronic infections comprise a multitude of eggs or adult flukes that involve a vital organ or elicit a severe host response leading to vascular infarcts that embolize and kill fish during periods of intense activity, e.g., during feeding. The lodging of fluke eggs in confined fish may be more numerous, accumulate faster, trigger a stronger immune response, form a more effective blockage, and result in greater infarcts and more reduced blood flow and hypoxia than in a non-confined host. Moreover, in some cases, the host can have severe reactions to the adults or juveniles that also could cause infarcts. Rapidly warming water may amplify pathogenicity by causing the host's immune response to become hyperactive, producing

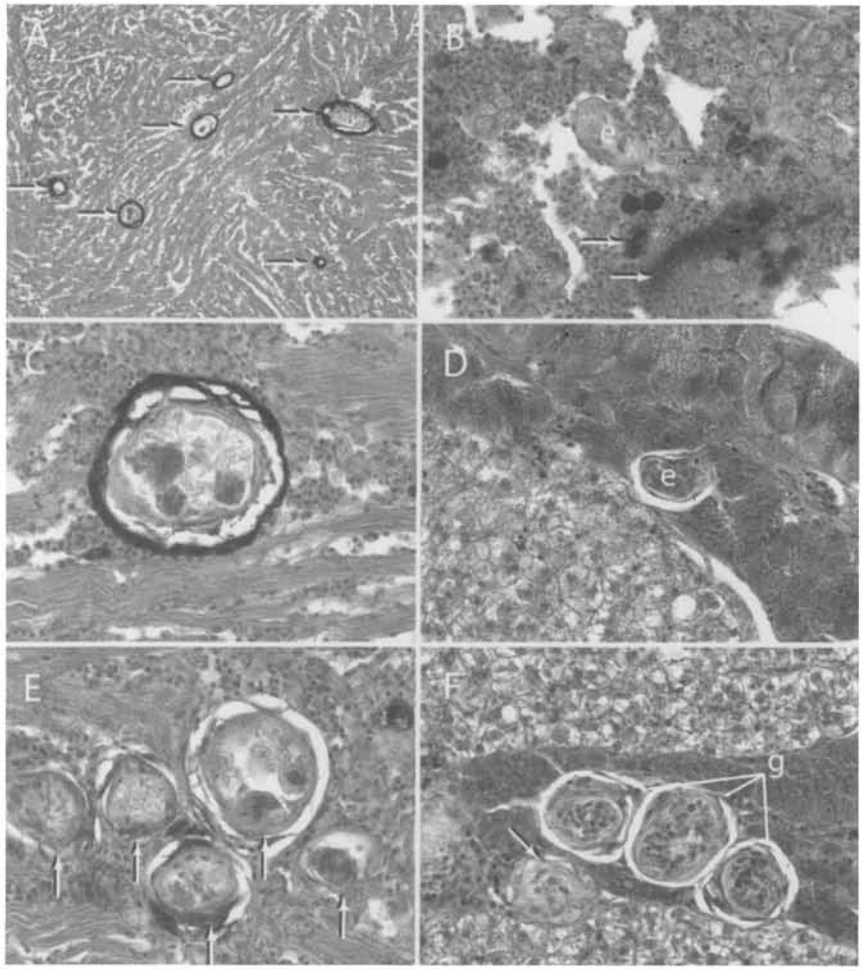


**Plate 14.18** Eggs of *Elaphrobates euzeti* (Aporocotylidae) in cardiac muscle (Figs. 14 A-C, E) and pancreatic nodules (Figs. 14D, F) of red snapper, *Lutjanus campechanus* (Lutjanidae) in the Northern Gulf of Mexico. **A.** Low magnification view of cardiac muscle showing, uniform and continuous ceroid rings (arrows) surrounding degenerating eggs. **B.** High magnification view of an unencapsulated egg (e) amidst a field of rodlet cells accompanied by some ceroid pigment (arrow). **C.** Late-stage, well-formed granuloma with dense, continuous ceroid ring surrounding remnants of egg. **D.** A probable recently lodged egg (e) not yet encapsulated. **E.** A cluster of early stage granulomas (arrows) involving degraded eggs, note that some ceroid pigment is incorporated among the epithelioid cells of the granuloma. **F.** Encapsulated eggs comprising three well-formed granulomas (g) and one nearly eliminated egg (arrowhead).

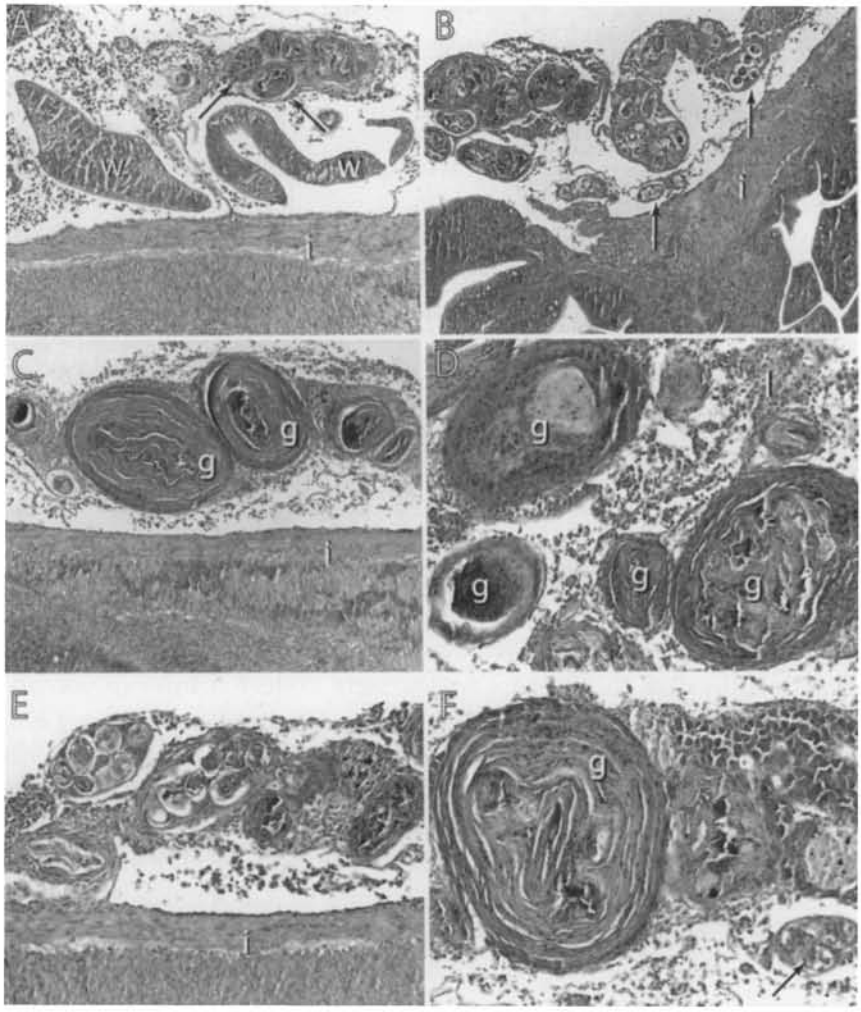


**Plate 14.19** Adults and eggs of *Plethorchis acanthus* (Aporocotylidae) in mesentery of wild flathead mullet (*Mugil cephalus*) from Queensland, Australia. **A.** Adult worm (w) and clusters of eggs (arrows) surrounded by inflammatory cells in dilated mesenteric vein in serosa of intestine (i). **B.** Eggs occluding wide and narrow mesenteric veins (arrows) near intestine (i). **C.** Well-developed epithelioid granulomas (g) near intestinal wall (i). **D.** Several late-stage granulomas (g) that probably once encapsulated eggs. **E.** Large clusters of eggs associated with mesenteric inflammation near intestinal wall (i). **F.** Late stage granuloma (g) near a small cluster of eggs that appear to have been recently encapsulated (arrow).

numerous leukocytes, fibroblasts, and other components that quickly block vessels, killing the fish (Bullard and Overstreet, 2002).

### *Aporocotyle simplex*

Thulin (1980) stated that the grayish gill filaments of infected flatfishes indicated that dead and disintegrating specimens of *A. simplex* (Plate 14.16) had partially reduced blood flow to infected filaments. He speculated that a large number of flukes would probably completely occlude the vessel and obstruct the flow of blood, presumably reducing the physiological efficiency of the gill.

### *Cardicola forsteri*

Based on morphological evidence, adults of this geographically wide-ranging aporocotylid infect the heart of southern bluefin tuna (*Thunnus maccoyii*) in the southwestern Pacific Ocean off Australia (Cribb *et al.*, 2000; Colquitt *et al.*, 2001; Munday *et al.*, 2003; Deveney *et al.*, 2005) as well as northern bluefin tuna (*Thunnus thynnus*) in the northwestern Atlantic Ocean off North Carolina (Bullard *et al.*, 2004) (Fig. 14.21D). Eggs identified as those of *C. forsteri* were collected from cage-reared northern bluefin tuna in the Adriatic Sea (Mladineo and Tudor, 2004); however, because no adult specimen was collected, in our view, the identity of that particular Adriatic tuna blood fluke remains indeterminate. Molecular sequence data indicate that specimens of *C. forsteri* from *T. maccoyii* off Australia and those from *T. thynnus* in the Mediterranean Sea are conspecific (Aiken *et al.*, 2007). The ITS rDNA sequences for these isolates were identical and sequences for the 28S D1-D2 rDNA differed by one in 629 nucleotides. Both tunas are presently 'fattened' or grown in sea cages because they are among the most valued commercial fishes in existence, with some individual bluefin tuna previously selling for US$80,000 in the Japanese sushi and sashimi market (Kantor, 1996). The apparent present-day geographic focus for commercial-scale rearing of southern bluefin tuna is in Port Lincoln, South Australia, and adjacent waters, where production has been estimated at 10,000 tonnes per annum (Cheshire and Volkman, 2004). A risk assessment study conducted by Nowak (2004), stated that "further research is required into the biology and epidemiology of this parasite" (= *C. forsten*). Tunas and other pelagic teleosts harbor at least a few named and unnamed blood flukes (Smith, 1997b; Nolan and Cribb, 2006;

Aiken *et al.*, 2007; Bullard, unpublished data), some of which seem to hold promise as serious potential disease threats. For example, C. *forsteri* has yet to be associated with a captive epizootic, but we consider it here because, given suitable environmental conditions and in the presence of a susceptible intermediate host, we suspect that C. *forsteri* could seriously threaten the culture of southern bluefin tunas in sea cages. Further, we think that the sustainability of offshore cage culture will necessitate control of at least particular aporocotylids and monogeneans.

When documenting the pathological alterations to wild and cultured southern bluefin tuna, Colquitt *et al.* (2001) determined that only cultured tunas were infected with adults and associated eggs of C. *forsteri*. Fluke eggs impacted the afferent filamentary blood vessels where they provoked a marked, but variable, inflammatory response. This inflammation resulted in nodular gill lesions. Because of these minor alterations to the gill, they stated that this species probably was not a pathogen of cultured tunas. Munday *et al.* (2003) reported that cultured southern bluefin tunas infected with C. *forsteri* had increased mucus on the gills. They also stated that infections by C. *forsteri* in this host can be associated with signs of respiratory distress and lethargy as well as slightly increased mortality. Cardiac lesions for this HPR are characterized by myocardial granulomas surrounding eggs of C. *forsteri* and hypertrophy of the cardiac spongiosa; the latter pathological alteration perhaps resulting from increased resistance to blood passing through the partly occluded branchial vessels (Munday *et al.*, 2003). Aiken *et al.* (2006) studied the epidemiology of C. *forsteri* in a total of 210 southern bluefin tuna over a 6-month grow-out season in Port Lincoln. After transfer from tow cages to sea cages in March, the prevalence and intensity of infection increased rapidly and peaked at 2 months, with 100% prevalence and 27 mean intensity (one southern bluefin tuna had 99 specimens of C. *forsteri* in its heart). In the following months the prevalence and mean intensity decreased to 35% and about 3, respectively, and southern bluefin tuna with heavy and light infections had similar condition indices. The drop in infection prevalence and intensity seemingly indicated that the intermediate host for C. *forsteri* did not range near the sea cage holding these tuna. Colquitt *et al.* (2001), on the other hand, observed that infection by C. *forsteri* increased with the time that *T. maccoyii* were held in cages, thereby not ruling out that the intermediate host was in fact present in the vicinity of those sea cages. In any event, locating cages near

the intermediate host(s) provides a serious threat to the health of caged fishes. Watts *et al.* (2001) developed and successfully used a seriological assay that detected infection by C. *forsteri* in southern bluefin tuna.

### *Cruoricola lates*

Adults of C. *lates* infect many sites within barramundi (*Lates calcarifer*), but most commonly mesenteric blood vessels, kidney, pericardial vessels, and the eyes (Fig. 14.21E). Herbert *et al.* (1995) found that the eggs of the fluke infected primarily gill, ventricle, hepatopancreas, and kidney, but only those embedded in the gill and in the heart had developing miracidia. Large groups of miracidia exiting the fish host could cause an inflammatory response and hemorrhaging. In the heart, macrophages encapsulated eggs that were trapped between trabeculae, and the reaction to the eggs lodged in kidney, hepatopancreas, and spleen consisted of fibrocytic encapsulation only. Herbert *et al.* (1995) speculated that probable sub-lethal effects of infection by adults and eggs of C. *lates* could include reduced food conversion ratios and increased susceptibility to secondary infections and disease. Although infection levels in the study were not intense and did not induce a significant host response or disease condition, we agree with some in the industry that suspect this HPR has serious potential to result in disease in the barramundi aquaculture industry of Australia and Southeast Asia. Although some barramundi are produced in environmentally controlled recirculating systems in Australia, the majority of production occurs in ponds or in cages sited within estuaries or rivers in water temperatures exceeding 20°C. The largest producer in Australia uses cages in earthen ponds. In southeast Asia, most fish are caged in rivers, estuaries, and protected marine areas (Allan and Stickney, 2000). Because of the nature of barramundi production, these shallow, warm, enclosed systems can nurture an epizootic if the infected intermediate host infiltrates that type of system. This potential is demonstrated by cases of sanguinicoliasis in freshwater ponds from North America, Europe, and Asia in which confined fish are continually exposed to aporocotylid cercariae. We find it noteworthy that another aporocotylid, *Parasanguinicola vastispina* (Fig. 14.21C) infects barramundi, and we think it too holds equal potential for causing disease problems in barramundi under appropriate environmental conditions, and perhaps especially in freshwater culture systems. The synergistic effect of these flukes in concurrent infections in barramundi has not been addressed.

### *Paradeontacylix grandispinus* and *P. kampachi*

The life cycles for these often concurrent flukes are indeterminate, but a few details regarding their habitat preference and pathological effects are known (Figs. 14.21A-B). These flukes infect the girdles, cephalic kidney, sinus venosus, kidney, and branchial arteries of amberjacks (*Seriola* spp.) only. In a survey of 50 greater amberjack captured from the wild, *P. grandispinus* was the dominant species. It infected the afferent branchial arteries; whereas, nearly half of the adult aporocotylids infected the sinus venosus and heart (Montero *et al.*, 2003). That two congeneric species of blood flukes infect the same individual host is noteworthy and probably related to differences between the microhabitats in the host, i.e., heart versus branchial vessels. Ogawa *et al.* (1993) thought that these flukes matured in approximately two months after cercariae had penetrated the greater amberjack. Sanguinicoliasis involving concurrent infections of these flukes has killed cage-cultured greater amberjack (Ogawa and Egusa, 1986; Ogawa *et al.*, 1989). Mass mortalities of greater amberjack that were cultured in shallow-water floating net cages off the coast of Shikoku Island, Japan, occurred from December to March. Gill filaments of heavily infected dead fish had >1,000 eggs of *P. grandispinus* or *P. kampachi* per filament, causing hyperplasia and extensive papillary formation from endothelial proliferation of the afferent branchial arteries (Ogawa *et al.*, 1989). Nodules of encapsulated eggs in the gill obstructed blood flow through afferent arteries, and those in the ventricular wall were surrounded by atrophied muscle. Heavily infected greater amberjack gasped at the water surface and died soon after being fed, suggesting that the increased oxygen demand during feeding could not be met; these fishes essentially suffocated themselves after rigorous feeding activity. Ogawa and Fukudome (1994) reported a subsequent mass mortality among imported greater amberjack off Kyushu, Japan; these fish exhibited similar signs. Ogawa and Fukudome (1994) suggested that infected greater amberjack imported from China contaminated the culture system and caused mass mortalities of that fish in Japanese systems. In the Mediterranean Sea, the culture of greater amberjack is based on younger, small fishes caught from the wild, reared in sea cages, and fed on natural food of low commercial value, e.g., round sardinella (*Sardinella aurita*), Mediterranean horse mackerel (*Trachurus mediterraneus*), blotched picarel (*Spicara maena*), and bogue (*Boops boops*) (see Gonzalez *et al.*, 1995). These wild-caught greater amberjack bring their parasites with them, and some of those parasites may comprise adult blood flukes. Crespo *et al.*

(1992) and Grau (1994) reported a mass mortality of 0- and 1-year-old cultured greater amberjack from the Spanish Mediterranean Sea off Majorca, Catalonia, and Murcia. The 0-year-class fish probably were killed by epitheliocystis infection, but the 1+-year-old fishes that lacked epitheliocystis and exhibited infections of one or both species of *Paradeontacylix* did die. The heart of infected fishes contained eggs in different stages of development, and the gill had a chronic inflammatory response around egg-thrombi as well as infarcts in afferent arteries caused by eggs and well-developed miracidia (Gonzalez *et al.*, 1995).

Recent collections of *Paradeontacylix* spp. from several amberjacks (*Seriola* spp.) (Hutson and Whittington, 2006; Hutson *et al.*, 2007; Bullard, unpublished data) indicate that (1) species of this genus can infect multiple host species, (2) a host individual may harbor concurrent infections (see also Ogawa and Egusa, 1986; Ogawa *et al.*, 1989; Grau *et al.*, 1999; Montero *et al.*, 1999, 2003), and (3) infections in amberjacks, like those in *Cardicola forsteri* in southern and northern bluefin tunas (Bullard *et al.*, 2004; Cribb *et al.*, 2000), are seemingly as geographically widespread as that of their nearly cosmopolitan hosts. All of which intuitively makes members of this group particularly menacing regarding the health management of cultured amberjacks.

### *Sanguinicola* spp.

Sanguinicola eggs (1954) reported 'great loss among 6 cm carp brood infected with *Sanguinicola* eggs' in large ponds of the Peitz fishery in Germany. Large masses of aporocotylid eggs were in the kidney of these hosts, and eggs inundated the gill (15-30 per square cm of gill area), occluding branchial vessels and causing thrombi. Erickson and Wallace (1959) reported occlusion of branchial vessels by eggs of *Sanguinicola* sp. in a specimen of blacknose shiner (*Notropis heterolepis*) experimentally infected with *Sanguinicola lophophora*. These gills had been destroyed to a large degree, appearing pale with light spots occurring in numerous sites. An epizootic of *Sanguinicola* spp. killed 300,000 rainbow trout in 1955 at the California Department of Fish and Game's Darrah Springs Hatchery, Tehama County, California (Wales, 1958). A similar epizootic of *Sanguinicola klamathensis* killed many of 5,000 cutthroat trout fingerlings (*Oncorhynchus clarkii clarkii*) at the Klamath Hatchery, Klamath County, Oregon. Chappell (1967) collected several specimens belonging to *Sanguinicola* from the heart, ventral aorta, or afferent branchial vessels of several freshwater fishes from Hobhole Drain and River Bain, England,

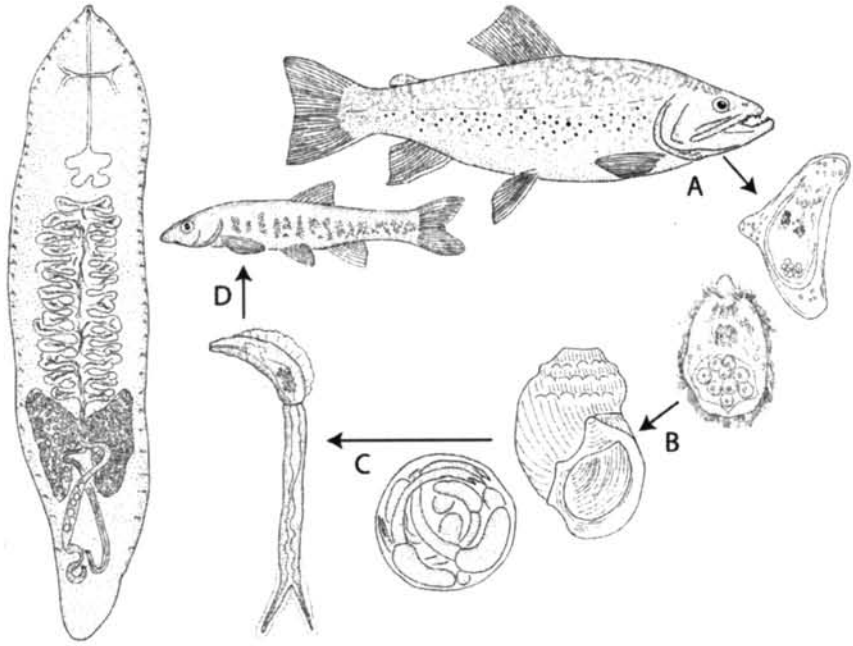


**Plate 14.20** Life cycle of the freshwater aporocotylid *Sanguinicola fontinalis* involving the definitive hosts brook trout (*Salvelinus fontinalis*) or longnose dace (*Rhinichthys cataractae*) and the intermediate host crested mudalia (*Leptoxis carinata*). Based on Hoffman *et al.* (1985). **A.** The adult of *S. fontinalis* (depicted at left of figure) infects the branchial, renal, and cardiac blood vessels of the definitive host fish wherein it deposits eggs. As with *A. simplex*, some eggs are carried to the gill. **B.** The miracidium hatches from the egg, emigrates through the gill epithelium, and seeks and penetrates the snail host. **C.** A sporocyst produces many furcocercous, apharyngeate cercariae (Hoffman *et al.* [1985] did not study the cercaria in detail and reported that a fin fold was absent, but we have illustrated one here modified from that of *Sanguinicola inermis* in Yamaguti [1975]). **D.** The cercaria swims actively, penetrates the fish, loses the tail, develops to a schistosomule and then to an adult, and then migrates to the circulatory system of the host where it copulates and produces eggs.

and predicted that additional *Sanguinicola* spp. would be discovered in other fishes and that these parasites could comprise a cause of fish mortality that had previously gone undetected. In September 1982, an infection by *S. fontinalis* (Plate 14.20) killed ~4,000 brook trout per day from a cultured stock of ~400,000 individuals maintained in raceways at the Pennsylvania Huntsdale State Fish Hatchery (Hoffman *et al.*, 1985). Most of the brook trout from the most severely affected stock were not smaller than those from other ponds, indicating a lethal acute infection. Heavily infected fishes exhibited pale gills and depressed hematocrit

values (Holliday and Fried, 1986). Individual gill filaments had damaged lamellae with obvious hemorrhaging, and, in intense infections, the gills were greatly clubbed. The kidney was swollen and had a generalized nephrosis and moderate edema as well as accumulated basophilic material in both Bowman's capsule and tubule lumina. For lack of an effective treatment, all heavily infected fish had to be destroyed. This aporocotylid uses the snail host *Leptoxis carinata*, from which some cercariae identified as *S. fontinalis* were isolated by Hoffman *et al.* (1985), and it seems specific to the one definitive host, the brook trout, because brown trout and rainbow trout were refractive to infections in the hatchery (Plate 14.20). That blood fluke is just one of the five known species historically assigned to *Sanguinicola* and that infects North American salmonids; the others being *S. davisi*, *S. klamathensis*, *S. alseae*, and *S. idahoensis*. Davis *et al.* (1961) reported intense mortalities of fingerlings of cutthroat trout and rainbow trout infected with *S. davisi*. These fishes were anemic and had gills damaged by fluke eggs and exiting miracidia. Evans and Heckmann (1973) and Evans (1974a, b) reared trout in the presence of infected snails (*Fluminicola fusca*, with a 6% prevalence of infection by *S. klamathensis*) for several months. The exposed trout had 80% mortality after a three month exposure to cercariae of *S. klamathensis*, and hematocrit and oxyhemoglobin levels were significantly reduced in the exposed fish group when compared to uninfected controls. In these fish, the eggs of *S. klamathensis*, when deposited over a period of time, produced progressive pathological alterations that included necrosis and calcification of heart and kidney plus hyperplasia of the brachial epithelium, resulting in poor weight gain and the mass mortality. Schell (1974) reported that the eye of rainbow trout heavily infected with *S. idahoensis* exhibited a bulging retina, thickened iris, and disrupted vascular stroma in addition to affected gills. There is evidence that some potentially pathogenic species of *Sanguinicola* have been introduced by transporting infected carps. Anderson and Shaharom-Harrison (1986) believed that *Sanguinicola armata* was brought into Malaysia by importation of fingerling bighead carp (as *Aristichthys nobilis*) and grass carp, and Kirk and Lewis (1994) reported that *S. inermis* was introduced to Britain when infected fishes were imported from continental Europe in the 1950s and 1960s. They noted that the fluke has since spread to various British rivers by movements of fishes that were caught for display or recreation and subsequently released. Interestingly, infections of *S. inermis* have been sustained in British rivers and hatcheries because the

intermediate hosts occur abundantly there. Inadequate pre-transfer protocols were blamed for subsequent mass mortalities in hatcheries.

### *Psettarium* spp.

Ogawa *et al.* (2007) documented mortalities of cage-cultured tiger puffers, *Takifugu rubripes*, in Japan that were attributed to two species of *Psettarium:* one thought to have originated from China ('*Psettarium* sp. TPC') and the other endemic to Japan ('*Psettarium* sp. TPJ'). First, Tanaka and colleagues reported a 1993 mass mortality of tiger puffers in Wakasa Bay (Japan) that were heavily infected with *Psettarium* sp. TPJ in the visceral vascular system. Later, in 2005, some of the 18,300 tiger puffers that were shipped from China to Japan and maintained in net cages (Seto Inland Sea off Kagawa Prefecture, Japan ) began dying within three days; with more than half of the stock being lost in three months and the mortality being attributed to *Psettarium* sp. TPC (Ogawa *et al.*, 2007). In all but one of the examined, infected tiger puffers imported from China, most of the hosts' organs (liver, spleen, kidney, intestine, gonads, heart, and gill) contained a multitude of eggs belonging to *Psettarium* sp. TPC that occupied a large area of the visceral tissue being examined. Moreover, white spots on the spleen were noted as comprising masses of aporocotylid eggs. Ogawa *et al.* (2007) warned that further introductions of tiger puffers from China should be avoided, even though their study seemed to indicate that the life cycle of the Chinese tiger puffer's blood fluke (*Psettarium* sp. TPC) could not be completed in Japan for lack of a susceptible intermediate host in that region. We find it noteworthy that the same specific definitive host (*T. rubripes*) harbors two seemingly allopatric blood flukes, perhaps indicating the intermediate hosts for these flukes are themselves allopatric, i.e., not ranging in both Japan and China.

### Unidentified Aporocotylid

Padrós *et al.* (2001) investigated the effects of an unidentified aporocotylid infecting gilthead seabream (*Sparus aurata*) cultured in net cages in Northeastern Spain. The infection caused 'trickling' mortalities during the cold seasons of 1999 and 2000, with a prevalence of 83% and 100% in those years, respectively. The adults reportedly infected the kidney; whereas, eggs and miracidia occurred in the branchial vessels only. Some eggs trapped in branchial vessels, mainly at the base of gill filaments, caused some lamellar disruption, hemorrhaging, and a mild to moderate

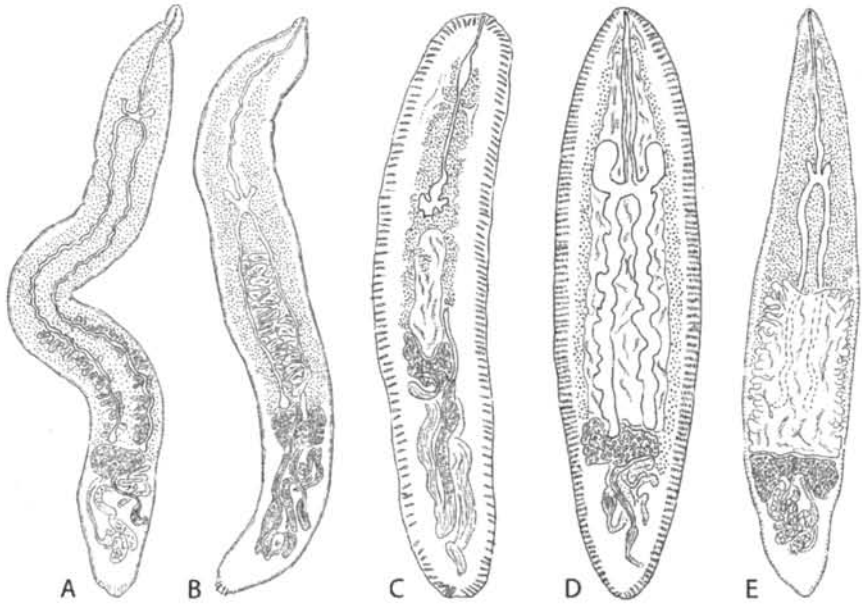


**Plate 14.21** Adults of some aporocotylid species that have been implicated in disease or hold potential for causing disease in aquaculture (see also Plates 14.15 and 14.19 as well as text below for discussion of *Psettarium* spp.). Drawn from original descriptions of those species. **A.** *Paradeontacylix kampachi* from the afferent branchial arteries, ventral aorta, heart, and sinus venosus of yellowtail (*Seriola purpurascens*). **B.** *Paradeontacylix grandispinus* from the afferent branchial arteries and heart of *S. purpurascens*. **C.** *Parasanguinicola vastispina* from branchial arteries, dorsal aorta, and mesenteric venules of barramundi (*Lates calcarifer*). **D.** *Cardicola forsteri* from the heart of southern bluefin tuna (*Thunnus maccoyii*). **E.** *Cruoricola lates* from the blood vessels of the viscera, kidney, pericardium, the filamental arteries, dorsal aorta, and rete mirabile of *L. calcarifer*.

diffuse inflammatory response involving lymphocytes and granulocytes. Aggregates of eosinophilic granular cells occasionally surrounded the eggs but no remarkable epithelial hyperplasia was observed in the gill of any fish. Adult aporocotylids infected the renal parenchyma and were surrounded by a thin fibrotic capsule and melanomacrophages. Some granulomatous substance, probably representing degraded adult flukes, was observed in the renal parenchyma. In the endocardium and ventricular cavity, a mild inflammatory response and a few thrombi but no associated egg or adult was observed. The intensity of infection in these seabream was low, and the gills of these fish were also infected by two monogeneans (*Furnestinia echeneis* and *Microcotyle chrysophrii*) as well as a *Flexibacter*-like bacterium. For this reason, the influence of the blood fluke was difficult to separate.

## DIDYMOZOIDAE

### *Diagnosis*

The systematics of Didymozoidae is described as 'chaotic' by Nikolaeva (1985), including >200 species allocated to >80 genera and >20 subfamilies, many of which are monotypic and mostly described by Yamaguti. Didymozoids comprise a bizarre group of sub-tropical and tropical digeneans whose adults (Plates 14.22–14.25) are in teleosts and (1) encapsulated or encysted in extra-intestinal tissues usually beneath a serosal or epithelial layer, or (2) residing free, usually in the body cavity. Didymozoids seem especially diverse in marine fishes (Nikolaeva, 1985), but a few infect freshwater fishes (Self *et al.*, 1963). Adults lack spination on the body, and many are bipartite, having a long, neck-like forebody and a bulbous, globular hindbody; whereas, others are long and thread-like. These digeneans are oftentimes misidentified by non-specialists who mistake them for aberrant larvae of nematodes or cestodes because (1) they infect extra-intestinal sites, (2) they can be extremely long, thin, delicate, and thread-like (Nobel, 1975) (Figs. 14.24A, 14.25F) or globular (Kamegai and Araki, 1995) (Figs. 14.23A, C, 14.24C-D, 14.25D-E), (3) their suckers, if either is present, are not always obvious, and (4) long, thin specimens can be especially difficult to extract (Fig. 14.24A), resulting in many collections consisting of incomplete specimens. For example, Noble (1975) described perhaps the world's longest digenean, *Nematobibothrioides histoidii*, measuring 1-2 mm wide and at least 12 m long; it infects the somatic muscle of ocean sunfish, wherein it usually forms a tangled mass in the subcutaneous tissue and between muscles and takes many hours to extract. Some fishes host several species. Perera (1992a) studied five species of didymozoids that infect the gills (four species), fins, finlets, operculum, skull bones, and around the eyes (one species) of the blue mackerel (*Scomber australasicus*) off Eden, New South Wales, Australia. Each of these gill worms occupied the space between the basement membrane of the primary epithelium and the efferent artery of the primary gill lamellae. In addition to concurrent didymozoid infections in the definitive host, some intermediate or paratenic fish hosts can also harbor multiple didymozoid species; we have seen juvenile specimens (Figs. 14.5G-H, 14.7F-G) comprising two different species of didymozoids in a single specimen of the bay anchovie (*Anchoa mitchilli*) from Mississippi Sound.

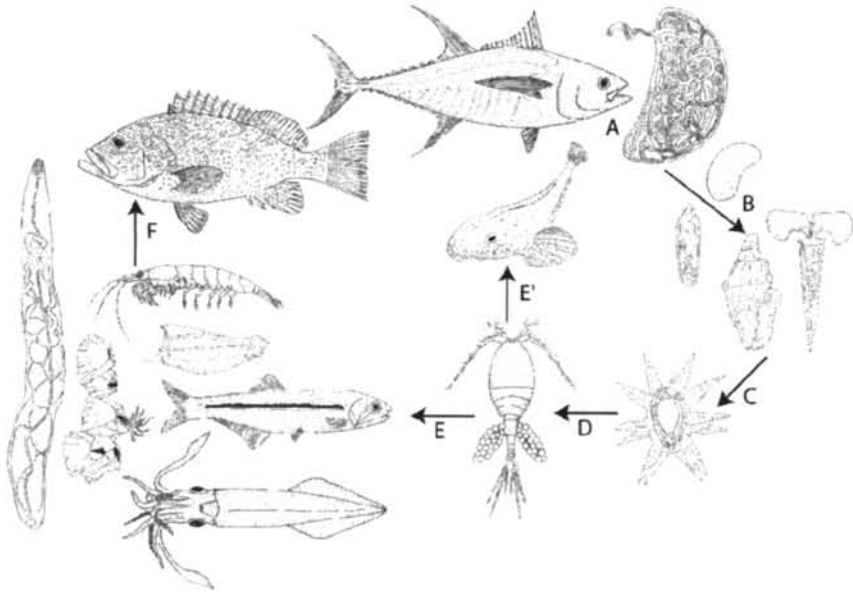


**Plate 14.22** Synoptical and hypothetical life history information for species of Didymozoidae infecting marine fishes. Based primarily on the hypotheses of Nikolaeva (1985) and the present authors. **A.** Adult didymozoid encapsulated in the musculature of a piscivorous fish releases eggs. **B.** Each egg has a miracidium that infects a mollusc, perhaps benthic snails or pelagic gastropods (e.g., 'pteropods'). **C.** Asexual reproduction in this first intermediate host yields a drifting, encased cercaria. **D.** Such cercariae are engulfed by planktonic arthropods (e.g., a cyclopoid copepod). **E'.** Infected plankton are consumed (passively or otherwise) by predatory fish, and the didymozoid matures. **E.** Alternatively, if a paratenic host is employed, the infected planktor is consumed by any variety of small planktivorous organisms, including pelagic fishes (e.g., anchovies [*Anchoa* spp.]), squids, barnacles, siphonophores (Thecosomata), or pelagic crustaceans (e.g., the krills, *Meganyctiphanes* spp.). In these hosts, a large non-encysted, unencapsulated metacercaria develops (depicted at left of figure). These paratenic hosts probably play a significant role in didymozoid life cycles. **F.** The infected paratenic host is consumed by a piscivorous fish, and the larva matures in that host.

Several didymozoids are nearly unique digeneans in two respects. As with schistosomatids, some are considered to be gonochoristic (having separate sexes) rather than hermaphroditic. As with *Paragonomus* spp. and some other species, individuals, either hermaphroditic or not, typically occur paired in host capsules within tissue. When considering gonochoristic members, Pozdnyakov (1993) followed the subfamily divisions of some early workers but placed them in a separate family. However, some gonochoristic members exhibit different degrees of hermaphroditism, whereas 'females' and 'males' are delineated by gonadal development and body shape (Gibson, 2002). Williams (1959) discussed

the complications of using separate sexes for classification, and Anderson and Barker (1998) showed, using molecular sequencing of the second internal transcribed spacer (ITS2), that the gonochoristic *Indodidymozoon pearsoni* and *I. suttiei* were genetically similar to the hermaphroditic species also clearly belonging in *Indodidymozoon*. Most species are hermaphroditic and paired in tissue. For example, *Didymocystis scomberomori* occurs as two similar hemispherical individuals abutted to form a sphere in the tissues of the stomach, intestine, and pyloric ceca of Spanish mackerel (*Scomberomorus maculatus*) and cero (*S. regalis*) in Florida and Mississippi. Different species occur free, encysted, or encapsulated in or adjacent to tissues. When encysted, the parasite component is very thin compared to the thick host portion (e.g., *Kollikeria filicollis* by Williams, 1959) (Figs. 14.24E-F); the parasite component is not always present (Lester, 1980), hence the term 'encapsulation', even though often called a 'cyst' in the literature.

The shape of the adult fluke in some infections seems to be influenced by the site of infection. For example, the body of a didymozoid we collected from the gill of snowy grouper (*Epinephelus niveatus*) mirrored the structure of the gill in which it was encapsulated (Fig. 14.24A), and the specimens we extracted from the gill arch of yellowfin tuna (*Thunnus albacares*) were similarly 'molded' to the pocket of tissue that surrounded them (Figs. 14.7L, 14.25E). Further, adults of *Neometadidymozoon polymorphis* and *Neometadidymozoon tuberosum* infect various sites in their host and exhibit extensive intraspecific morphological variability in body shape because they seem to grow into and produce the space they occupy (Anderson, 1998). However, fin-dwelling species seem relatively consistent regarding the overall body shape. For example, *Rhopalotrema pterygionastes* and *Rhopalotrema elusiva* infect beneath the skin of the pectoral fins of Australian platycephalid fishes and possessed a consistent body shape (Anderson, 1998), and we have collected a didymozoid from Atlantic bigeye (*Priacanthus arenatus*) in the Northern Gulf of Mexico that also is consistent in body shape. The former ecologically driven intraspecific morphological variation in other didymozoids has probably caused taxonomic confusion, particularly when multiple species are described from the same host species primarily based on body shape (e.g., Ching and Madhavi, 1999). Further complicating the use of body shape as a distinguishing taxonomic feature in this group, the body; typical for other soft-bodied digeneans, can be influenced drastically by fixation technique (Kohn *et al.*, 2001). The metacercariae of didymozoids are

easily distinguished from other digeneans by the structure of their gut, which comprises a cecum with a series of connected chambers that can be misinterpreted as two columns of multiple testes. The configuration of the anterior excretory tubules further distinguishes didymozoid metacercariae (Cable, 1956). The diagnostic features of the metacercariae do not necessarily have any bearing on generic affiliation and may change considerably with growth. However, the actual size of both suckers, when present, and chamber, if present at the posterior extremity of the esophagus, may be useful in matching larvae with adults (Anderson, 1999).

## *Life Cycle*

Didymozoids comprise one of the least understood digenean groups regarding their life histories because no complete life cycle for a didymozoid has been published, although many researchers have provided hypothetical infection strategies. A primary reason for this lack of information could stem from the fact that many didymozoids infect hosts such as tunas that are difficult or practically impossible to maintain in experimental systems. The general life cycle pattern that seems most acceptable to most workers is the one synthesized by Nikolaeva (1965, 1985) and depicted by us in Plate 14.22 in which the didymozoid uses four hosts, but we speculate that a three-host cycle probably exists for some species. If the cycle corresponds to that in related hemiuroids, a cystophorous cercaria may develop in a gastropod first intermediate host; the cercaria infects a copepod second intermediate host. In addition to copepods, metacercariae have been reported from one or more species of siphonophore, ctenophore, planktonic polychaete, chaetognath, cirriped, squid, and fish (e.g., Karlsbakk, 2001). At least some of these serve as paratenic hosts. Presumably, those with larvae in the alimentary tract are paratenic hosts, but some or all of those with larvae free in the gill, liver, body cavity, body musculature, and muscles of swimbladder wall (e.g., Karlsbakk, 2001; pers. obs.) would be more likely to be third intermediate hosts. In any event, when eaten by the appropriate fish definitive host, the larva migrates to the appropriate site and develops to the mature worm. Eggs are typically small, about 0.02 mm; they presumably are eaten by the mollusc, and the miracidium, with its crown of anterior spines, penetrates through the alimentary tract and undergoes asexual reproduction.

How these didymozoid exit the fish host is not clear, but they probably are discharged in a variety of ways. Most seem to be disseminated in the

**Plate 14.23** Adult didymozoids in marine and freshwater fishes, demonstrating the diversity of bauplans in Didymozoidae (from Yamaguti, 1971). **A.** *Kamegaia kawakawa* from small intestine of kawakawa, *Euthynnus affinis* (as *E. yaito*). **B.** *Nematobothrium labeonis* from orbit of *Labeo* spp. **C.** *Neodidymozoon macrostoma* from connective tissue or adipose tissue of striped marlin, *Tetrapturus audax* (as *Makaira audax*). **D.** *Osteodidymocodium kawakawa* from posterodorsal part of cranial bone of *E. affinis* (as *E. yaito*). **E.** *Neodiplotrema pelamydis* on the fifth gill arch of skipjack tuna, *Katsuwonus pelamis* (as *Euthynnus pelamys*).

feces of predators having fed on the infected fishes, but others are discharged through an ulcer or orifice when the parasite dies, degenerates, or escapes its capsule. Some worms may even drop out of the ulcer while still alive. Seasonality in the morbidity and mortality of at least *Neometadidymozoon helicus* in dusky flathead (*Platycephalus fuscus*) in Queensland, Australia, appears to be related to host sexual maturation and presumably hormone release by the fish host (Lester, 1980). We have seen a variety of moribund and necrotic worms in groupers, mackerels, and tunas, and the worm eggs in many of these probably pass into the environment.

### *Specific Didymozoid-fish HPRs*

Few detailed pathological studies describe the HPR between didymozoids and their hosts. We are aware of no didymozoid that has caused a mortality of a wild or aquacultured fish. Although Paperna (1995) thought that natural infections of adult didymozoids are inconsequential to the health of wild fishes, we think that definitive hosts that are fed (in aquaculture) or naturally feed on intermediate hosts could develop intense, debilitating infections of these extra-intestinal flukes. And, pathological alterations do in fact occur in some infected fishes. Several species infect the gill, inner operculum, palatal teeth, stomach wall, skin (Fig. 14.25G), or mouth cavity of tunas and groupers, and in the gill of these hosts they can be quite

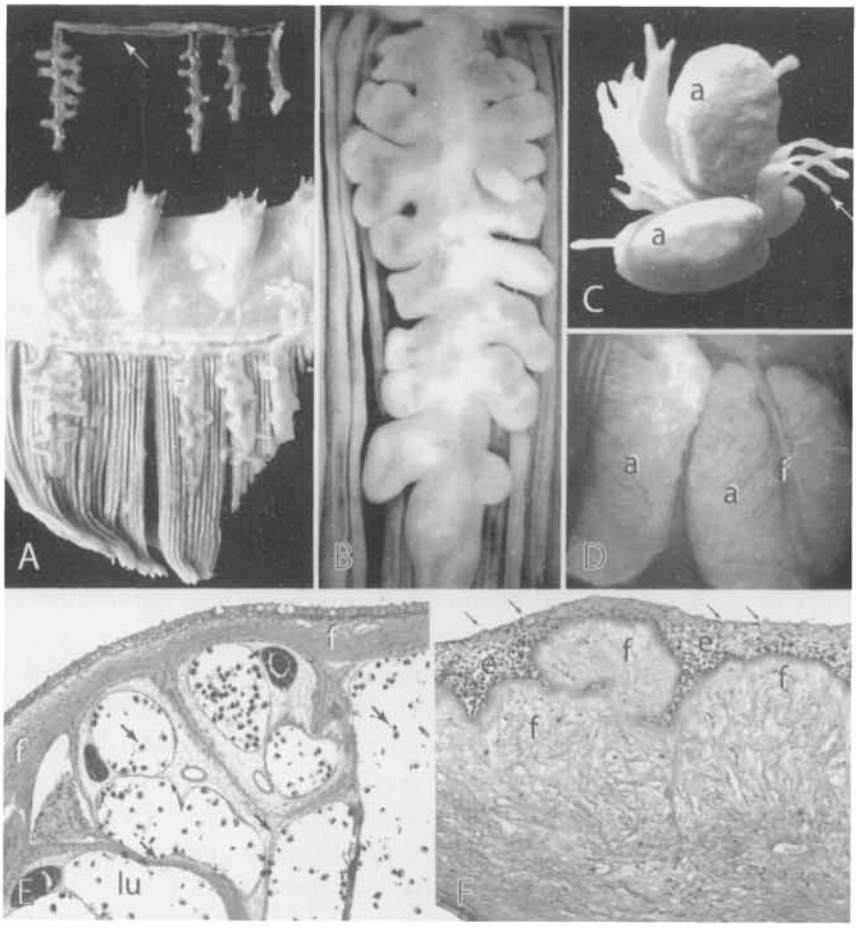


**Plate 14.24** Didymozoids from groupers (Serranidae). **A.** A portion of a probable adult didymozoid specimen (arrow) carefully excised from the branchial epithelium of a misty grouper (*Epinephelus mystacinus*). **B.** Higher magnification view of the same infected tissue as in Fig. 14.24A before the partial specimen was excised from the gill. **C.** Two probable adult specimens (a) of another didymozoid species encapsulated in the gill filaments (arrow) of snowy grouper (*Epinephelus niveatus*). **D.** Didymozoid encapsulated in gill of red grouper (*Epinephalus morio*). There are two probable adult specimens (a), each of which is associated with a gill filament (f). In life, these specimens appeared bright yellow. **E.** Histological sections of encapsulated worms (in Figs. 14.24A, B). The luminal spaces of the uterus (lu) contain spheroid eggs (arrows) and comprise most of the field of view. The adult is encapsulated by a fibrotic response (f), but, as with all adult didymozoids, there is no cyst wall. **F.** Higher magnification view showing the fibrotic host encapsulation (f) underlying a relatively thickened epithelium (e) with numerous goblet cells (arrows) and lymphocytes.

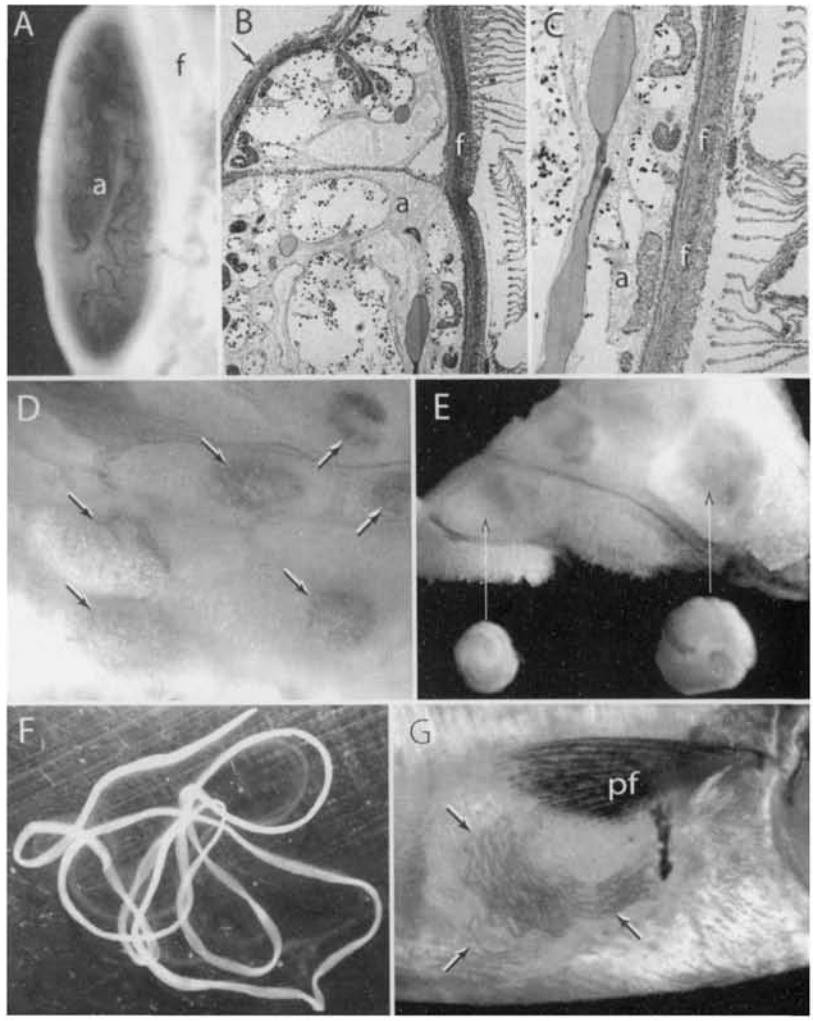


**Plate 14.25** Figures 14A-F. Yellowfin tuna (*Thunnus albacares*) infected with didymozoids. **A.** Capping tissue of a gill filament infected with an adult pair of *Didymocystis* sp. (a). **B.** Histological section of comparable tissue in Fig. 14.25A showing lamellae of gill filament (f), adult didymozoid (a), and gill filament epithelium (arrow). **C.** Higher magnification of Fig. 14.25B showing the intact gill filament (f) opposing the adult didymozoid (a). **D.** Surface of gill arch showing raised mounds (arrows) caused by encapsulated adult didymozoids embedded beneath the epithelium of the gill arch. **E.** Two spheroid specimens removed from the gill arch in Fig. 14.25D. These specimens created smooth-surfaced crater-like depressions in the tissue, and they were used for SEM (see Fig. 14.7L). **F.** Thread-like immature specimen of another species found intact from coelomic and branchial wash. **G.** Flank of a Spanish mackerel (*Scomberomorus maculatus*) infected with an adult didymozoid (demarcated by arrows) located under the epithelium beneath the right pectoral fin (pf).

obvious to an observer and harmful to the host because they modify functional tissues, reduce respiration, occlude alimentary passages, or compress vital organs. At least several adult didymozoids probably feed on host erythrocytes because they (1) reside in a host capsule that is perfused by host blood vessels, (2) have a well-developed oral sucker and pharynx, and (3) have deposits of partly digested erythrocytes in their ceca (Lester, 1980). Yamaguti (1970) thought that didymozoids derived blood nutrients from the blood capillaries of the host and that most apparently fed on blood serum; although Perera (1992a) and others found no evidence for that. *Nematobothrium spinneri* in the muscles of wahoo (*Acanthocybium solandri*) provides an example of a species enclosed in a thick-walled capsule in which connective tissue and blood capillaries are interdigitated with worm coils, perhaps facilitating didymozoid blood feeding (Lester, 1980). As indicated above, many species degenerate in their living host and result in hemorrhaging encapsulations or ulcers, encouraging secondary infections. Perera (1992a), in the study on five species, concluded that the didymozoids did not elicit a host reaction in the gill but noted that chronic stage infections may have a different pathological outcome. Perera (1992b) studied this HPR using TEM, demonstrated that the host encapsulation comprised a layer of columnar epithelial cells and electron-dense filamentous bands around the nucleus of the epithelial cells of the capsule, and speculated that the relatively thickened columnar epithelial layer was a host adaptation to inhibiting the entry of potentially pathogenic bacterial agents through the gill. In addition, chloride cells were more numerous in the capsule.

Overstreet and Edwards (1976) reported a didymozoid that resembled a species of *Unitubulotestis* that entwined within the dermis of southern flounder (*Paralichthys lethostigma*) from the Northern Gulf of Mexico. This didymozoid was directly adjacent to a tumor, a benign subcutaneous mesenchymal fibroma, and, although a philometrid nematode was also closely associated with the tumor, these authors thought it possible that the didymozoid had some involvement in development of the tumor.

## OTHER FLUKES

### Acanthocolpidae

Metacercariae of *Stephanōstomum tenue* were associated with mass mortalities of caged rainbow trout at a fish farm off northern Prince Edward Island, Canada (McGladdery *et al.*, 1990). A fibrogranulomatous

sheath surrounded the cysts and included melanomacrophages, neutrophils, and lymphocytes whereas live metacercariae elicited only a minor inflammatory response. Most cysts occurred around the bulbus arteriosus or loose in the pericardium, and the fibrogranulomatous sheath covering the surface of the bulbous arteriosus may have decreased respiration efficiency (McGladdery *et al.*, 1990). Metacercarial cysts of *S. tenue* also caused severe distention of the pericardium and thoracic region in elvers of the American eel (*Anguilla rostrata*) collected from the Annaquatucket River, Rhode Island, from April through June 1997 (Oliveira and Campbell, 1998). European plaice, turbot, common sole (*Solea solea*), and dab exposed to cercariae of *Stephanochasmus baccatus* each responded differently. European plaice and turbot showed the most intensive response, and in turbot the reaction was considerably accelerated. Mortality of encysted metacercariae occurred in turbot only, and younger European plaice were more severely affected by large cysts that presumably interfered with organ function (Sommerville, 1981).

## Hemiuridae

Ivanchenko and Grozdilova (1971) reported that *Brachyphallus crenatus* had a pathologic effect on cultured herring and led to mortalities among larvae and fry. Chambers *et al.* (2001) reported the adult of *Lecithocladium invasor* from the throat of the whitemargin unicornfish (*Naso annulatus*), humpnose unicornfish (*N. tuberosus*), and bignose unicornfish (*N. vlamingii*); causing marked pathological alterations in the latter fish but no clinical signs. This hemiurid penetrated the esophageal mucosa and caused granulomas that expanded the full thickness of the esophageal wall and protruded both into the esophageal lumen and outward from the serosal surface. Two types of lesions were characterized: (1) large ulcerated, active granulomas with a central cavity containing a single or multiple live feeding worms and large numbers of different bacteria and (2) many smaller chronic fibrinous submucosal nodules that included small or degenerated flukes. Concurrent infection of the posterior esophagus of these fishes with *Lecithocladium chingi* was common but disassociated with the lesions. In the Northern Gulf of Mexico, the hemiurid-like giant stomach worm or 'wahoo worm' *Hirudinella ventricosa* (Figs. 14.1A-B) raises eyebrows and sparks curiosity in those who see it (Overstreet, 1978) in blue water gamefishes such as wahoo, dolphinfishes (*Coryphaena* spp.), and tunas (*Thunnus* spp.). Although it is quite large, few worms typically infect the stomach of their pelagic fish hosts, and they

are probably benign. Infections by some hemiurids can be impressive in their biomass (Fig. 14.1C), but they also seem to have no detrimental affect on their hosts.

### Transversotrematidae

Adults of *Transversotrema patialense* leave pressure and feeding indentations in the body surface of zebra danio (*Danio rerio*) (as *Brachydanio rerio*) (see Mills, 1979), but the lesions associated with infection are minor. Given the extraordinary site of infection under scales, we suspect that intense infections could cause sufficient skin irritation that could result in a disease condition, especially in culture conditions.

## IMPACT AND OPPORTUNITY

All fish probably have parasites, and many of those have digeneans that affect the lives of basic and applied researchers. Digeneans are useful and relevant to other biologists studying non-parasitological problems in other systems. Digenean research is like a tapestry of other biological disciplines because digeneans infect a broad phylogenetic array of hosts (both intermediate and definitive) and have life cycles that are among the most complex of all organisms. Digeneans comprise (1) powerful indicators of trophic interactions among hosts in complex food webs (Huxham *et al.*, 1995; Overstreet, 1997; Marcogliese, 2002), (2) cost-effective sentinels that provide insight on ecosystem health and biodiversity (Overstreet, 1993, 1997; MacKenzie *et al.*, 1995; MacKenzie, 1999; Halmetoja *et al.*, 2000; Ruus *et al.*, 2001; Morley *et al.*, 2002, 2003a, b, c, 2004, 2005a, b; Dzikowski *et al.*, 2003a), (3) biological tags that distinguish otherwise cryptic host populations (Kabata, 1963; Lester, 1990; Arthur, 1997; Lile, 1998; Granovitch and Johannesson, 2000), (4) indicators of host life history information (Scott, 1975; Williams *et al.*, 1992) as well as habitat characteristics and host population structure (Ondračková *et al.*, 2004b), and (5) useful models for community ecology (Bush *et al.*, 1993) and epidemiology (Calvo-Ugarteburu and McQuaid, 1998). Further, some digeneans manipulate the behavior of their intermediate hosts (Lafferty and Morris, 1996; Poulin *et al.*, 1998; Thomas *et al.*, 1998; Combes, 2001; Kuris *et al.*, 2004; Seppala *et al.*, 2004) or cause behavioral (Guthrie and Kroger, 1974) or ontogenetic defects (Johnson *et al.*, 2001), and this complex interaction between parasite and host serves as a fascinating subject for parasitologists, physiologists, developmental biologists,

behavioral ecologists, environmental biologists, and students alike. Some digeneans may even regulate host populations and affect ecosystem structure (Anderson, 1978; Anderson and May, 1979). Further still, some act as alien invaders that infiltrate and cause problems in aquaculture (Overstreet *et al.*, 2002), and others may affect the success of introduced marine species (Torchin *et al.*, 2002). Relating to education, digeneans also comprise ideal models within which to stimulate interest in biology among young students (Rea and Irwin, 1999; Bullard, unpublished data). These examples, when considered together, demonstrate that biologists, clinicians, fishermen, and educators can discuss fish digeneans as beguiling subjects for Evolutionary Biology, Epidemiology, Community Ecology, Fisheries Biology, Conservation Biology, Physiology, Animal Behavior, and Environmental Health.

## Bioindicators

A few examples are included here to supplement a couple of those topic areas. Since the digeneans have a complex life cycle, often highly specific to one or more of the intermediate or final hosts, infections of specific species serve as indicators of diet, migration, and environmental changes. As examples, we know that many 'herbivorous' fishes actually consume significant amounts of protein as intermediate host prey; for instance, monorchiids and bucephalids in mullets indicate that they have been feeding on molluscs and fishes rather than solely on algae, detritus, and other organic matter.

We can determine the importance of some birds that feed on fish in specific areas such as aquaculture ponds, their migratory pattern and their feeding behavior. A paper on the American white pelican discussed how *Phagicola longus*, *Mesostephanus appendiculoides*, and the nematode *Contracaecum multipapillatum* indicated that the bird fed on flathead mullet along coastal areas in contrast with the presence of *Bolbophorus damnificus* and *Bursacetabulus pelecanus*, which indicated it had fed on channel catfish in freshwater aquaculture ponds inland far from the coast. By comparing the relative numbers of these common parasites plus a few other less common parasites as well as the relative age of the parasites as determined by the phase of development, including the number of eggs present, one can determine from where the bird came, how long ago it arrived in the locality of capture, how long it had been there, and on what it had been feeding (Overstreet and Curran, 2004).

We can determine the influence of catastrophic events on biota of a region. For example, Hurricane Katrina, during a short period on 29 August 2005, altered the barrier islands, nearby sediments, and estuarine fauna and flora in Mississippi. This storm, its aftermath, and a following drought either locally eliminated entire mollusc and other invertebrate populations or reduced each or both of them considerably. Consequently, the digeneans depending on those molluscan first intermediate hosts as well as on some second intermediate hosts were absent or sparse in fish or other hosts for various periods after the storm. We examined juveniles of the Atlantic croaker (*Micropogonias undulatus*), a host previously examined extensively and determined to be host for at least 13 local adult and four metacercarial digeneans, with four of those adults found commonly in individuals in Ocean Springs, Mississippi. By nine months after the storm, we encountered single specimens of *Lecithaster confusus*, *Lecithochirium* sp. (immature), *Opecoeloides fimbriatus*, and *Diphtherostomum* sp., but no individual of any of the relatively common species. The single worms were probably acquired by feeding on copepods for the first two, a penaeid shrimp for the third, and a non-crustacean invertebrate for the last one. The common species are acquired by feeding on a bivalve, fish, amphipod, and polychaete. Several digeneans may live longer than a year in the croaker, so we stress the fact that our findings reflect new infections which, based on our historical data of over 35 years, should have occurred in those juveniles during the same season. Additional common cestodes, nematodes, and an acanthocephalan were also lacking from the juveniles.

## Fish Flukes Posing Public Health Risks

Collectively, food-borne digeneans of 70 species, 36 genera, and 13 families infect the gut or liver of approximately 50 million humans worldwide. Digeneans pose considerable public health risks, especially in Asia and the middle East where societies do not rely on pre-packaged foods (e.g., Deardorff and Overstreet, 1991; Fried *et al.*, 2004; Keiser and Utzinger, 2005). The problems are worldwide and relate to changes in human diets, migration of people, international trade of infected stocks and seafood products, an increase in immuno-compromised consumers, environmental changes, and other factors.

Many digenean infections in people result from eating metacercaria from fishes, and several freshwater, estuarine, and marine fishes are responsible hosts. Many of the responsible digeneans have been

misidentified or not identified at all, and no attempt had been made to investigate many cases. Those that have been identified include members of Opisthorchiidae (liver flukes, e.g., *Clonorchis sinensis*, *Opisthorchis viverrini*, *Metorchis* spp.), Heterophyidae (intestinal and ectopic flukes, e.g., *Heterophyes* spp., *Metagonimus* spp., *Centrocestus* spp., *Haplorchis* spp., *Stellantchasmus falcatus*, *Procerovum varium*, *Heterophysopsis continua*, *Stictodora* spp., *Pygidiopsis summa*, and *Phagicola* spp.), Echinostomatidae (intestinal flukes, e.g., *Echinochasmus japonicus* and *Echinostoma* spp.), Troglotrematidae (intestinal fluke, i.e., *Nanophyetus salmincola*), and Clinostomidae (pharyngeal cavity, *Clinostomum* spp.).

Humans are common hosts for some opisthorchiids and heterophyids but 'accidental' hosts for the others. A combination of fish cultured in cages or ponds and those from the wild contribute to this high number of infections. Perhaps the liver flukes are most serious, since humans develop enlargement and chronic irritation of the liver and bile duct, with jaundice, anorexia, cholangitis, numerous complications of the biliary system, pancreatitis, and occasionally cholangiocarcinoma, the latter especially in Northeast Thailand. There is little host-specificity for the fish or snail hosts for either *Clonorchis sinensis* or *Opisthorchis viverrini*. Over 100 fishes host *C. sinensis* and 35 host *Opisthorchis* (e.g., Keiser and Utzinger, 2005). Consequently, *C. sinensis* may infect ~15 million people in China and ~20 million others in Japan, Korea, Taiwan, Vietnam, and adjacent areas (Keiser and Utzinger, 2005; Lun *et al.*, 2005), and *O. viverrini* may infect ~six million people in Thailand (see Sripa *et al.*, 2003a, b), with many of those individuals having concurrent infections that were acquired by eating wild fishes. These digeneans create a major socioeconomic situation because Asians are eating more rather than less raw fish. In fact, human cases of *C. sinensis* have more than tripled in China within a recent ten-year period (Keiser and Utzinger, 2005), a period with a sharp increase in freshwater fish production and an increase in consumption of raw fish dishes. Data on *C. sinensis* infections from Vietnam (De *et al.*, 2003) suggest a lack of acquired immunity; prevalence of infection progressively increases from about 3% in people <ten years old to >50% in those 40-49 years old. The species can apparently survive in a human for 20-25 years (Attwood and Chou, 1978). Heterophyids also are much smaller worms, have a relatively short longevity, and generally occur in the intestine but are still serious. In human hosts, light infections are typically asymptomatic, but infections are usually relatively intense; the intestinal villi become inflamed and atrophy, with the more heavily

infected people having diarrhea, vomiting, nausea, anemia, edema, anorexia, weight loss, eosinophilia, and malaise. Occasionally, the eggs or adults in a human will leave the intestine and produce inflammation or an emboli of the heart, brain, and spinal cord and some patients will die (Kean and Breslau, 1964). Many, often over half the population of villages dependent on fishing, will be infected. These are best documented in Korea, Japan, Vietnam, Laos, Thailand, the Philippines, and Egypt, but diagnostic procedures are not well established and many cases are missed. Infected individuals often harbor more than one species, both because fish often are infected with multiple species and because fish consumers often eat a variety of fishes. Because of increased affluence by some, geographical spreading of cuisines, and increased aquaculture, more fish products are consumed raw, resulting in an increase in human infections. In addition to some of the cuisines indicated by Deardorff and Overstreet (1991), there are many more such as 'koi pla' in Thailand that consists of raw fish with chopped garlic, lemon juice, chili, rice, and vegetables as well as a fermented fish dish called 'plasom'. Raw fish is considered healthier than cooked products by many in Southeast Asia.

Infections with troglotrematids, echinostomes, clinostomes, and diplostomoids are rare. The troglotrematid *Nanophyetus salmincola* perhaps has been common in people who eat raw salmonids in the Arctic and temperate areas, most notably eastern Siberia, but the adult is very small, does not produce symptoms unless 500 or more specimens are present, and is probably overlooked in most cases. In one case, a seafood handler who handled many heavily infected fish did not eat raw fish but smoked cigarettes while working, and he probably acquired the infection from personally contaminating cigarettes with metacercariae and then putting them in his mouth to smoke them (Deardorff and Overstreet, 1991). Most echinostomes are transmitted by molluscs, but a few human pathogens infect fishes (e.g., *Echinochasmus japonicus*, *E. liliputanus*, *Echinostoma hortense*, and *E. cinetorchis* [De *et al.*, 2003]). Usually infections consist of few specimens and often occur concurrently with other digenean infections also acquired from eating raw fish. Clinostomids lodge in the pharynx and produce laryngitis (Beaver *et al.*, 1984; Overstreet, 2003). Metacercariae of diplostomoids typically occur in fishes, but human infections primarily come from mesocercariae in amphibians rather than from fishes. Fish cases are known (e.g., *Alaria* sp.) and human lesions typically involve the eye. Human death is usually attributed to eating raw amphibians (Beaver *et al.*, 1984) or contact with amphibians or their organs such as the gall bladder (anecdotal reports).

Human infections can be controlled by cooking infected products. The US Food and Drug Administration on 21 August 1987 released the following interpretation: 'Fishery products which are not cooked throughout to 60°C or above, must have been or must, before service or sale in ready-to-eat form, be blast frozen to –35°C or below for 15 hours or regularly frozen to –23°C or below for 168 hours' (Deardorff and Overstreet, 1991). Freezing fish at –18°C for 24 hours or heating infected meat to 50°C for a few minutes killed heterophyid metacercariae (Paperna and Overstreet, 1981). Since some worms can live in people for many years, and many people are traveling among countries and immigrating from countries endemic for infective digeneans, no country can remain safe from infections. Fish products should be more carefully inspected for digeneans and other parasites of public health interest as well as for disease agents harmful to fishes.

Human infections are typically detected by eggs in fecal microscopic examinations. Other methods, sometimes less specific but some much more sensitive for C. *sinensis* include ultrasound, computer tomography, magnetic resonance imaging, tissue harmonic imaging, and immunological approaches (Lun *et al.*, 2005). Since the eggs of most harmful digeneans that infect humans have a similar appearance, many agents have been misdiagnosed. On the other hand, eggs of some digeneans not infective to humans have been similarly misdiagnosed, and some seafood products have been incorrectly considered a public health threat. Kamegai (1971) and others before him have reported didymozoid eggs, measuring 30.2-45 × 20.9-30 micrometers, in feces of humans that had eaten raw muscle of flying fishes that harbored infections of ovigerous adult didymozoids (e.g., Figs. 14.17D, 14.24E). Although benign, these eggs can cause worry in humans.

### *Marketability and Esthetics*

Seafood distributors, inspection agents, seafood dealers, commercial fishermen, and recreational fishermen have sent us tissues, including filets, from epinephalids, scombrids, lutjanids, and other fishes with melanistic host responses to parasites that affected the ability to market or consume the products. Some devaluing infections comprised didymozoids and acanthocolpids of somatic muscle, i.e., filets, but other digeneans and parasites also produced a similar appearance. At least one didymozoid previously mentioned herein, *Nematobothrium spinneri*, is of commercial importance in the southwestern Pacific Ocean off Australia because it reduces the market value of wahoo (Lester, 1980).

Regarding aquaculture and infections, remarkably, some digenean infections might even increase market value or yield! Heasman *et al.* (1996) studied castrating metacercarial infections of *Bucephalus* sp. in the commercial scallops *Pecten fumatus* and *Chlamys* [*Mimachlamys*] *asperrima* in Jervis Bay, New South Wales, and reported that the large, turgid, orangish-red gonad of infected *P. fumatus* was attractive to consumers and may have increased marketability of the scallop. Similarly, Bowers (1969) reported that although infections of '*Cercaria bucephalopsis haimeana*' decreased the shell growth rate of common cockles (*Cerastoderma edule*) (as *Cardium edule*), it simultaneously increased the flesh yield of the harvested product. Overstreet (2003) discussed how consumers thought a bucephalid-infected oyster tasted better than a non-infected one and other aspects of purposeful consumption of raw and cooked digeneans as well as other parasites.

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## References

Aaltonen, T.M., E.T. Valtonen and E.I. Jokinen. 1997. Humoral response of roach (*Rutilus rutilus*) to digenean *Rhipidocotyle fennica* infection. *Parasitology* 114: 285-291.

Aiken, H.M., C.J. Hayward and B.F. Nowak. 2006. An epizootic and its decline of a blood fluke, *Cardicola forsteri*, in farmed southern bluefin tuna, *Thunnus maccoyii*. *Aquaculture* 254: 40-45.

Aiken, H.M., N.J. Bott, I. Mladineo, F.E. Montero, B.F. Nowak, and C.J. Hayward. 2007. Molecular evidence for cosmopolitan distribution of platyhelminth parasites of tunas (*Thunnus* spp.). *Fish and Fisheries* 8: 167-180.

Allan, G. and R.R. Stickney. 2000. Barramundi culture. In: *Encyclopedia of Aquaculture*, R.R. Stickney (ed.). John Wiley & Sons, New York. pp. 104-105.

Anderson, G.R. 1998. *Rhopalotrema* (Digenea: Didymozoidae), a new genus from Australian platycephalid fishes. *Systematic Parasitology* 41: 81-86.

Anderson, G.R. 1999. Identification and maturation of the metacercaria of *Indodidymozoon pearsoni*. *Journal of Helminthology* 73: 21-26.

Anderson, G.R. and S.C. Barker. 1993. Species differentiation in the Didymozoidae (Digenea): Restriction fragment length differences in internal transcribed spacer and 5.8S ribosomal DNA. *International Journal for Parasitology* 23: 13-136.

Anderson, G.R. and S.C. Barker. 1998. Inference of phylogeny and taxonomy within the Didymozoidae (Digenea) from the second internal transcribed spacer (ITS2) of ribosomal DNA. *Systematic Parasitology* 41: 87-94.

Anderson, I.G. and F.M. Shaharom-Harrison. 1986. *Sanguinicola armata* infection in bighead carp (*Aristichthys nobilis*) and grass carp (*Ctenopharyngodon idella*) imported in Malaysia. In: *The First Asian Fisheries Forum*, Asian Fisheries Society, Manila, Philippines. J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.). pp. 247-250.

Anderson, R.M. 1978. The regulation of host population growth by parasitic species. *Parasitology* 76: 119-157.

Anderson, R.M. and D.M. Gordon. 1982. Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* 85: 373-398.

Anderson, R.M. and R.M. May. 1979. Population biology of infectious diseases: Part I. *Nature* (London) 280: 361.

Anderson, R.M. and R.M. May. 1982. Co-evolution of hosts and parasites. *Parasitology* 85: 411-426.

Andrews, P.H., R. Thomas, R. Pohlke and J. Seubert. 1983. Praziquantel. *Medical Research Review* 3: 147-200.

Arthur, J.R. 1997. Recent advances in the use of parasites as biological tags for marine fish. In: *Diseases in Asian Aquaculture* III. T.W. Flegel and I.H. MacRae (eds.). Fish Health section, Asian Fisheries Society, Manila, pp. 141-154.

Ashton, N., N. Brown and D. Easty. 1969. Trematode cataract in freshwater fish. *Journal of Small Animal Practice* 10: 471.

Ata, A.A., M.N. el-Khashab, A.A. Mourad and H.M. Soliman. 1988. The effect of praziquantel on *Heterophyes heterophyes*, *Hymenolepis nana* and *Fasciola* sp. infections. *Journal of the Egyptian Society of Parasitology* 18: 243-246.

Attwood, H.D. and S.T. Chou. 1978. The longevity of *Clonorchis sinesis*. *Pathology* 10: 153-156.

Avendaño de MacIntosh, M.F. and M. Ostowski de Núñez. 1998. Seasonal dynamics of *Plehniella coelomica* (Trematoda: Sanguinicolidae) in two catfish, *Pimelodus albicans* and *P. maculatus* (Pisces: Siluriformes), from La Plata River, Argentina. *Folia Parasitologica* 45: 253-255.

Avtalion, R.R., E. Weiss and T. Moalem. 1976. Regulatory effects of temperature upon immunity in ectothermic vertebrates. In: *Comparative Immunology*, J.J. Marchalonis (ed.). Blackwell Scientific Publications, Oxford, pp. 227-238.

Awerinzew, S.A. 1925. Über eine neue Art von parasitären Tricladen. *Zoologischer Anzeiger* 64: 81-84.

Baldwin, N.L., R.E. Millemann and S.E. Knapp. 1967. "Salmon poisoning" disease. III. Effect on experimental *Nanophyetus salmincola* infection on the fish host. *Journal of Parasitology* 53: 556-564.

Ball, I.R. and R.A. Khan. 1976. On *Micropharynx parasitica* Jägerskiöld, a marine planarian ectoparasitic on thorny skate, *Raja radiata* Donovan, from the north Atlantic Ocean. *Journal of Fish Biology* 8: 419-426.

Baturo, B. 1977. *Bucephalus polymorphus* Baer, 1987 and *Rhipidocotyle illense* (Ziegler, 1883) (Trematoda: Bucephalidae): morphology and biology of developmental stages. *Acta Parastologica Polonica* 24: 203-220.

Baturo, B. 1978. Larval bucephalosis in artificially heated lakes of the Konin region, Poland. *Acta Parasitologica Polonica* 25: 307-321.

Baturo, B. 1980. Pathological changes in cyprinid fry infected by *Bucephalus polymorphus* Baer, 1827 and *Rhipidcotyle illensis* (Ziegler, 1883) metacercariae (Trematoda, Bucephalidae). *Acta Parasitologica Polonica* 27: 241-246.

Beaudette, F.R. 1945. Aspergillosis and parasitism in a gull. *Journal of Field Ornithology* 16: 99-101.

Beaver, P.C., R.C. Jung and E.W. Cupp. 1984. *Clinical Parasitology*. 9th Edition. Lea and Febiger, Philadelphia.

Bedinger, C.A. Jr. and T.G. Meade. 1967. Biology of a new cercaria for *Posthodiplostomum minimum* (Trematoda: Diplostomidae). *Journal of Parasitology* 53: 985-988.

Benz, G.W. and S.A. Bullard. 2004. Metazoan parasites and associates of chondrichthyans with emphasis on taxa harmful to captive hosts. In: *The Husbandry of Elasmobranch Fishes*, M.L.F. Smith, D.I. Warmolts, D.A. Thoney and R.E. Hueter (eds.). *Special Publication No. 16, Ohio Biological Survey, Columbus, OH*. pp. 325-415.

Bergh, O., F. Nilsen and O.B. Samuelsen. 2001. Diseases, prophylaxis and treatment of the Atlantic halibut *Hippoglossus hippoglossus*: a review. *Diseases of Aquatic Organisms* 48: 57-74.

Betterton, C. 1974. Studies on the host-specificity of the eyefluke, *Diplostomum spathaceum* in brown and rainbow trout. *Parasitology* 69: 11-29.

Blair, D. 1976. Observations on the life-cycle of the strigeoid trematode, *Apatemon (Apatemon) gracilis* (Rudolphi, 1819) Szidat, 1928. *Journal of Helminthology* 50: 125-131.

Blasiola, G.C. 1976. Ectoparasitic turbellaria. *Marine Aquarist* 7: 53-58.

Blazer, V.S. and J.B. Gratzek. 1985. Cartilage proliferation in response to metacercarial infections of fish gills. *Journal of Comparative Pathology* 95: 273-280.

Boeger, W.A. and D.C. Kritsky. 2001. Phylogenetic relationships of the Monogenoidea. In: *Interrelationships of the Platyhelminthes*. Taylor and Francis, London, pp. 92-102.

Bogitsh, B.J. 1962. The chemical nature of metacercarial cysts. I. Histological and histochemical observations on the cyst of *Posthodiplostomum minimum*. *Journal of Parasitology* 48: 55-60.

Bowers, E.A. 1969. *Cercaria bucephalopsis haimeana* (Lacaze-Duthiers, 1854) (Digenea: Bucephalidae) in the cockle, *Cardium edule* L. in South Wales. *Journal of Natural History* 3: 409-422.

Brassard, P., M.E. Rau and M.A. Curtis. 1982a. Infection dynamics of *Diplostomum spathaceum* cercariae and parasite-induced mortality of fish hosts. *Parasitology* 85: 489-493.

Brassard, P., M.E. Rau and M.A. Curtis. 1982b. Parasite-induced susceptibility to predation in diplostomiasis. *Parasitology* 85: 495-501.

Bray, R.A., D.I. Gibson and A. Jones. *Keys to the Trematoda*, Volume 3. CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K. (In Preparation).

Brink, L.H., D.J. McLaren and S.R. Smithers. 1977. *Schistosoma mansoni*: A comparative study of artificially transformed schistosomula and schistosomula recovered after cercarial penetration of isolated skin. *Parasitology* 74: 73-86.

Britz, J., J.G. Van As and J.E. Saayman. 1985. Occurrences and distribution of *Clinostomum tilapiae* Ukoli, 1966 and *Euclinostomum heterostomum* (Rudolphi, 1809) metacercarial infections of freshwater fish in Venda and Lebowa, southern Africa. *Journal of Fish Biology* 26: 21-28.

Brooker, B.E. 1972. The sense organs of trematode miracidia. In: *Behavioral Aspects of Parasite Transmission*, E.U. Canning and C.A. Wright (eds.). Academic Press, London, pp. 171-180.

Brown, K.N. 1971. Protective immunity to malaria provides a model for the survival of cells in an immunologically hostile environment. *Nature* (London) 230: 163-167.

Bullard, S.A. 2002. *Blood flukes (Digenea: Sanguinicolidae) from commercially and recreationally important fishes in the Gulf of Mexico*. Unpublished M.Sc. Thesis. The University of Southern Mississippi, Ocean Springs, U.S.A.

Bullard, S.A., R. Goodwin, R. Goldstein and R.M. Overstreet. 2004. *Cardicola forsteri* (Digenea: Sanguinicolidae) from the heart of a northern bluefin tuna, *Thunnus thynnus* (Scombridae), in the Northwest Atlantic Ocean. *Comparative Parasitology* 71: 245-246.

Bullard, S.A. and R.M. Overstreet. 2002. Potential pathological effects of blood flukes (Digenea: Sanguinicolidae) on pen-reared marine fishes. *Proceedings of the 53rd Gulf and Caribbean Fisheries Institute*, pp. 10-25.

Bullard, S.A. and R.M. Overstreet. 2003. *Elaphrobates euzeti* gen. and sp. n. (Digenea: Sanguinicolidae) from snappers (Lutjanidae) in the Gulf of Mexico. In:. *Taxonomy,*

*Ecology and Evolution of Metazoan Parasites.* (Livre hommage à Louis Euzet), C. Combes and J. Jourdane (eds.). PUP Perpignan, Vol. 1, pp. 97-113.

Bullard, S.A. and R.M. Overstreet. 2004. Two new species of *Cardicola* Short, 1952 (Digenea: Sanguinicolidae) from the heart of drums (Sciaenidae) in Mississippi and Louisiana. *Journal of Parasitology* 90: 128-136.

Bullard, S.A. and R.M. Overstreet. 2006. *Psettarium anthicum* sp. n. (Digenea: Sanguinicolidae) from the heart of cobia *Rachycentron canadum* (Rachycentridae) in the northern Gulf of Mexico. *Folia Parasitologica* 53: 117-124.

Bullard, S.A., R.M. Overstreet and J.K. Carlson. 2006. *Selachohemecus benzi* n. sp. (Digenea: Sanguinicolidae) from the blacktip shark *Carcharhinus limbatus* in the Northern Gulf of Mexico. *Systematic Parasitology* 63: 143-154.

Bush, A.O., R.W. Heard, Jr. and R.M. Overstreet. 1993. Intermediate hosts as source communities. *Canadian Journal of Zoology* 71: 1358-1363.

Bush, A.O., J.C. Fernández, G.W. Esch and J.R. Seed. 2001. *Parasitism, The Diversity and Ecology of Animal Parasites.* Cambridge University Press. Cambridge.

Bush, A.O., K.D. Lafferty, J.M. Lotz and A.W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* 83: 575-583.

Buttner, A. 1951. La progénèse chez les trématodes digénétiques. Technique et recherches personnelles. *Annals of Parasitologie Humaine et Comparee* 26: 19-66.

Cable, R.M. 1956. *Marine Cercaria of Puerto Rico.* Scientific Survey of Porto Rico and the Virgin Islands. R.W. Miner (ed.). 16: 489-577.

Cable, R.M. 1974. Phylogeny and taxonomy of trematodes with reference to marine species. In: *Symbiosis in the Sea*, W.B. Vernberg (ed.). University of South Carolina Press, Columbia, pp. 173-193.

Cable, R.M. 1982. Phylogeny and Taxonomy of the Malacobothrean Flukes. In: *Parasites: Their World and Ours*, D.F. Mettrick and S.S. Desser (eds.). Elsevier Biomedical Press, Amsterdam, pp. 194-197.

Caira, J.N., K. Jensen and K.E. Holsinger. 2003. On a new index of host specificity. In: *Taxonomy, Ecology and Evolution of Metazoan Parasites.* (Livre hommage à Louis Euzet). C. Combes and J. Jourdane (eds.). PUP Perpignan. Vol. 1, pp. 161-201.

Caira, J.N. and F.B. Reyda. 2005. Eucestoda (true tapeworms). In: *Marine Parasitology*, K. Rohde (ed). CABI Publishing, Wallingford, Oxon, U.K., pp. 92-115.

Calvo-Ugarteburu, G. and C.D. McQuaid. 1998. Parasitism and introduced species: epidemiology of trematodes in the intertidal mussels *Perna perna* and *Mytilus galloprovincialis*. *Journal of Experimental Marine Biology and Ecology* 220: 47-65.

Cannon, L.R.G. 1986. *Turbellaria of the World. A Guide to Families and Genera.* Queensland Museum. Queensland, Australia.

Cannon, L.R.G. and R.J.G. Lester. 1988. Two turbellarians parasitic in fish. *Diseases of Aquatic Organisms* 5: 15-22.

Capron, A., J. Biguet, A. Vernes and D. Afchain. 1968. Structure of antigénique des helminthes. Aspects immunologiques des relations hote-parasite. *Pathologie et Biologie (Paris)* 16: 121-138.

Carothers, J.L. and R. Allison. 1968. Control of snails by the redear (snail cracker) sunfish. *FAO Fisheries Report* 5: 399-406.

Chai, J.-Y., E.-T. Han, Y.-K. Park, S.-M. Guk, J.-H. Park and S.-H. Lee. 2002. *Stictodora lari* (Digenea: Heterophyidae): The discovery of the first human infections. *Journal of Parasitology* 88: 627-629.

Chambers, C.B., M.S. Carlisle, A.D.M. Dove and T.H. Cribb. 2001. A description of *Lecithocladium invasor* n. sp. (Digenea: Hemiuridae) and the pathology associated with two species of Hemiuridae in acanthurid fish. *Parasitology Research* 87: 666-673.

Chappell, L.H. 1967. On the occurrence of blood flukes (Sanguinicolidae: Trematoda) in British freshwater fishes. *Journal of Natural History* 1: 163-167.

Chappell, L.H. 1991. The biology of diplostomid eyeflukes of fishes. *Journal of Helminthology* 69: 97-101.

Cheshire, A.C. and J. Volkman. 2004. Australians net benefits of sustainable fish farming. *Nature* 432: 671.

Ching, H.L. and R. Madhavi. 1999. *Lobatocystis euthynni* sp. n. (Digenea: Didymozoidae) from mackerel tuna (*Euthynnus affinis*) from Sulawesi Island, Indonesia. *Journal of the Helminthological Society of Washington* 66: 25-27.

Chitwood, M.B. and J.R. Lichtenfels. 1972. Identification of parasitic Metazoa in tissue sections. *Experimental Parasitology* 32: 407-519.

Coleman, F.C. 1993. Morphological and physiological consequences of parasites encysted in the bulbous arteriosus of an estuarine fish, the sheepshead minnow, *Cyprinodon variegatus*. *Journal of Parasitology* 79: 247-254.

Coleman, F.C. and J. Travis. 1998. Phenology of recruitment and infection patterns of *Ascocotyle pachycystis*, a digenean parasite in the sheepshead minnow, *Cyprinodon variegatus*. *Environmental Biology of Fishes* 51: 87-96.

Colquitt, S.E., B.L. Munday and M. Daintith. 2001. Pathological findings in southern bluefin tuna, *Thunnus maccoyii* (Castelnau), infected with *Cardicola forsteri* (Cribb, Daintith and Munday, 2000) (Digenea: Sanguinicolidae), a blood fluke. *Journal of Fish Diseases* 24: 225-229.

Combes, C. 2001. *Parasitism: The Ecology and Evolution of Intimate Interactions*. The University of Chicago Press, Chicago.

Conti, J.A., D.J. Forrester and R.T. Paul. 1986. Parasites and diseases of reddish egret (*Egretta rufescens*) from Texas and Florida. *Transactions of the American Microscopical Society* 105: 79-82.

Cosgrove, G.E. 1975. Parasites in tissue sections: recognition and reaction. In: *Pathology of Fishes*, W.E. Ribelin and G. Migaki (eds.). The University of Wisconsin Press, Madison, pp. 205-211.

Couch, J.A. and J.W. Fournie. 1993. Importance of marine fish diseases—an overview. In: *Pathobiology of Marine and Estuarine Organisms*, J.A. Couch and J.W. Fournie (eds.). Advances in Fisheries Science, CRC Press, Boca Raton, pp. 1-24.

Coustau, C., C. Combes, C. Maillard, F. Renaud and B. Delay. 1990. *Prosorhynchus squamatus* (Trematoda) parasitosis in the *Mytilus edulis-Mytilus galloprovincialis* complex: specificity and host-parasite relationships. In: *Pathology in Marine Science*, T.C. Cheng and F.O. Perkins (eds.). Academic Press, London, pp. 291-298.

Cowper, S.G. 1971. *A Synopsis of African Bilharziasis*. H.K. Lewis, London.

Crespo, S., A. Grau and F. Padrós. 1992. Sanguinicoliasis in the cultured amberjack *Seriola dumerili* Risso, from the Spanish Mediterranean area. *Bulletin of the European Association of Fish Pathologists* 12: 157.

Cribb, J.C., G.R. Anderson, R.D. Adlard and R.A. Bray. 1998. A DNA-based demonstration of a three host life-cycle for the Bivesiculidae (Platyhelminthes: Digenea). *International Journal for Parasitology* 28: 1791-1795.

Cribb, T.H. 2005. Digenea (endoparasitic flukes). In: *Marine Parasitology*, K. Rohde (ed). CABI Publishing, Wallingford, Oxon, U.K., pp. 76-87.

Cribb, T.H., R.A. Bray and D.T.J. Littlewood. 2001a. The nature and evolution of the association among digeneans, molluscs and fishes. *International Journal for Parasitology* 31: 997-1011.

Cribb, T.H., R.A. Bray, D.T.J. Littlewood, S.P. Pichelin and E.A. Herniou. 2001b. The Digenea. In: *Interrelationships of the Platyhelminthes*, D.T.J. Littlewood and R. Bray (eds.). Taylor and Francis, London, pp. 168-185.

Cribb, T.H., M. Daintith and B. Munday. 2000. A new blood-fluke, *Cardicola forsteri*, (Digenea: Sanguinicolidae) of southern blue-fin tuna (*Thunnus maccoyii*) in aquaculture. *Transactions of the Royal Society of South Australia* 124: 117-120.

Crofton, H.D. 1971. A quantitative approach to parasitism. *Parasitology* 62: 179-193.

Crofton, H.D. and P.G. Fraser. 1954. The mode of infection of the hake, *Merluccius merluccius* by the trematode *Bucephalopsis gracilescens* (Rud.). *Proceedings of the Zoological Society of London* 124: 105-109.

Cross, S.X. 1938. A study of fish parasite relationships in the Trout Lake region of Wisconsin. *Transactions of the Wisconsin Academy of Science and Arts and Letters* 31: 439-456.

Crowden, A.E. and D.M. Broom. 1980. Effect of the eye fluke, *Diplostomum spathaceum*, on the behavior of dace (*Leuciscus leuciscus*). *Animal Behavior* 28: 287-294.

Cusack, R. and D.K. Cone. 1986. A review of parasites as vectors of viral and bacterial diseases of fish. *Journal of Fish Diseases* 9: 169-171.

da Silva, P.M., A.R.M. Magalhães and M.A. Barracco. 2002. Effects of *Bucephalus* sp. (Trematoda: Bucephalidae) on *Perna perna* mussels from a culture station in Ratones Grande Island, Brazil. *Journal of Invertebrate Pathology* 79: 154-162.

Damian, R.T. 1964. Molecular mimicry: antigen sharing by parasite and host and its consequences. *American Naturalist* 98: 129-149.

Davis, D.J. 1936. Pathological studies on the penetration of the cercaria of the strigeid trematode, *Diplostomum flexicaudum*. *Journal of Parasitology* 22: 329-339.

Davis, D.J. 1937. Care and diseases of trout. *US Department of Commerce, Bureau of Fisheries Investigations. Report.* 35 pages.

Davis, H.S., G.L. Hoffman and E.W. Surber. 1961. Notes on *Sanguinicola davisi* (Trematoda: Sanguinicolidae) in the gills of trout. *Journal of Parasitology* 47: 512-514.

Dawes, B. 1952. Trematode life-cycle enacted in a London pond. Correspondence. *Nature* (London) 170: 72-73.

De, N.V., K.D. Murrell, L.D. Cong, P.D. Cam, L.V. Chau, N.D. Toan and A. Dalsgaard. 2003. The food-borne trematode zoonoses of Vietnam. *Southeast Asian Journal of Tropical Medicine and Public Health* 34 (Supplement 1): 12-34.

De Bont, A.F. and M.J. De Bont Hers. 1952. Mollusc control and fish farming in central Africa. *Nature (London)* 170: 323-324.

Deardorff, T. and R.M. Overstreet. 1991. Seafood-transmitted zoonoses in the US: the fishes and the worms. In: *Microbiology of Marine Food Products*, D.R. Ward and C. Hackney (eds.). AVI, Van Nostrand Reinold, New York. pp. 211-265.

Desowitz, R.S. 1970. Antiparasitic mechanisms in parasitic infections. *Journal of Parasitology* 56 (Section 2): 521-525.

Deveney, M.R., T.J. Bayly, C.J. Johnston and B.F. Nowak. 2005. A parasite survey of farmed southern bluefin tuna, *Thunnus maccoyii* (Castelnau). *Journal of Fish Diseases* 28: 279-284.

Devine, J.A., C.E. Adams and P.S. Maitland. 2000. Changes in reproductive strategy in the ruffe during a period of establishment in a new habitat. *Journal of Fish Biology* 56: 1488-1496.

Dickerman, E.E. 1954. *Paurorhynchus hiodontis*, a new genus and species of trematode (Bucephalidae: Paurorhynchinae n. subfam.) from the mooneye fish, *Hiodon tergisus*. *Journal of Parasitology* 40: 311-315.

Dogiel, V.A. 1932 *Parasitic Diseases of Fishes*, Selkhozgiz, Moscow-Leningrad.

Dönges, J. 1970. The potential number of redial generations in Echinostomatids (Trematoda). *International Journal for Parasitology* 1: 51-59.

Dönges, J. 1974. The life cycle of *Euclinostomum heterostomum* (Rudolphi, 1809) (Trematoda: Clinostomatidae). *International Journal for Parasitology* 4: 79-90.

Dubinin, V.B. 1949. Relation between the distribution of the larvae of parasitic helminths in the fishes of the delta of the Volga and changes in the concentration of birds. *Zoologicheskii Zhurnal* 27: 2.

Dubinin, V.B. 1952. Fauna of helminth larvae of vertebrates of the River Volga Delta. *Parazitologiya sb., Lenningrad* 14: 213-226. (In Russian).

Dubois, G. 1968. Synopsis des Strigeidae et des Diplostomatidae (Trematoda). *Mémoires de la Société Neuchâteloise des Sciences Naturelles* 10: 1-258.

Dubois, G. 1970. Synopsis des Strigeidae et des Diplostomatidae (Trematoda). *Mémoires de la Société Neuchâteloise des Sciences Naturelles* 10: 259-727.

Dunn, T.S., R.E.B. Hanna and W.A. Nizami. 1987. Sensory receptors of the miracidium of *Gigantocotyle explanatum* (Trematoda: Paramphistomidae). *International Journal for Parasitology* 17: 1131-1140.

Dzikowski, R., A. Diamant and I. Paperna. 2003a. Trematode metacercariae of fishes as sentinels for a changing limnological environment. *Diseases of Aquatic Organisms* 55: 145-150.

Dzikowski, R.A., M.G. Levy, M.F. Poore, J.R. Flowers and I. Paperna. 2003b. Genetic and morphologic differentiation of *Bolbophorus confusus* and *B. levantinus* (Digenea: Diplostomatidae), based on rDNA SSU polymorphism and SEM. *Diseases of Aquatic Organisms* 57: 231-235.

Dzikowski, R.A., M.G. Levy, M.F. Poore, J.R. Flowers and I. Paperna. 2004a. *Clinostomum complanatum* and *Clinostomum marginatum* (Rudolphi, 1819) (Digenea: Clinostomidae) are separate species based on differences in ribosomal DNA. *Journal of Parasitology* 90: 413-414.

Dzikowski, R.A., M.G. Levy, M.F. Poore, J.R. Flowers and I. Paperna. 2004b. Use of rDNA polymorphism for identification of Heterophyidae infecting freshwater fishes. *Diseases of Aquatic Organisms* 59: 35-41.

Edney, J.M. 1940. A heavy infestation of *Clinostomum marginatum* metacercariae in channel catfish. *Journal of the Tennessee Academy of Sciences* 15: 371.

Elliot, A.M. and L.R. Russert. 1949. Some conditions characteristic of yellow perch population heavily parasitized by *Clinostomum marginatum*. *Journal of Parasitology* 35: 183-190.

Ellis, A.E. 1982. Differences between the immune mechanisms of fish and higher vertebrates. In: *Microbial Diseases of Fish*, R.J. Roberts (ed.). Academic Press, New York, pp. 1-29.

Erasmus, D.A. 1959. The migration of *Cercaria* X Baylis (Strigeida) within the fish intermediate host. *Parasitology* 48: 173-190.

Erasmus, D.A. 1972. *The Biology of the Trematodes*. Edward Arnold, Ltd., London.

Erickson, D.G. and F.G. Wallace. 1959. Studies on blood flukes of the genus *Sanguinicola*. *Journal of Parasitology* 45: 310-322.

Esch, G.W. and W.J. Huffines. 1973. Histopathology associated with endoparasitic helminths in bass. *Journal of Parasitology* 59: 306-313.

Esch, G.W., E.J. Wetzel, D.A. Zelmer and A.M. Schotthoefer. 1997. Long-term changes in parasite population and community structure: A case history. *American Midlands Naturalist* 137: 369-387.

Essex, H.E. and G.W. Hunter III. 1926. A biological study of fish parasites from the central states. *Transactions of the Illinois State Academy of Science* 19: 151-181.

Evans, W.A. 1974a. The histopathology of cutthroat trout experimentally infected with the blood fluke *Sanguinicola klamathensis*. *Journal of Wildlife Diseases* 10: 243-248.

Evans, W.A. 1974b. Growth, mortality, and hematology of cutthroat trout experimentally infected with the blood fluke *Sanguinicola klamathensis*. *Journal of Wildlife Diseases* 10: 341-346.

Evans, W.A. and R.A. Heckmann. 1973. The life history of *Sanguinicola klamathensis*. *Life Sciences* 13: 1285-1291.

Ewens, E., J.W. Lewis, D.T. Richards, D. Hoole and C. Arme. 1994. Epidemiological studies on the blood fluke: *Sanguinicola inermis* Plehn, 1905, in the common carp (*Cyprinus carpio* L.). *International Symposium on Aquatic Animal Health*, Program and Abstracts, Davis, California, p. 50.

Faliex, E. 1990. Experimental infection of juvenile grey mullet, *Liza ramada*, by metacercaria of *Babratrema minimus* (Digenea: Bucephalidae): ultrastructural analysis of the host-parasite relationship. In: *Pathology in Marine Science*, T. C. Cheng and F.O. Perkins (eds.). Academic Press, London, pp. 299-303.

Faliex, E. and S. Morand. 1994. Population dynamics of the metacercarial stage of the bucephalid trematode, *Labratrema minimus* (Stossich, 1887) from Salses-Deucate lagoon (France) during the cercarial shedding period. *Journal of Helminthology* 68: 35-40.

Fantham, H.B. and A. Porter. 1947. The parasitic fauna of vertebrates in certain Canadian fresh waters, with some remarks on their ecology, structure and importance. *Proceedings of the Zoological Society of London* 117: 609-649.

Farstey, V. 1986. *Centrocestus sp. (Heterophyidae) and other trematode infections of the snail Melanoides tuberculata (Muller, 1774) and cichlid fish in Lake Kinneret.* Unpublished M.Sc. Thesis. Hebrew University of Jerusalem, (Hebrew text, English summary).

Ferguson, M.S. 1942. Development of the metacercariae of *Diplostomum flexicaudum* in the lenses of frogs, turtles, birds, and mammals. *Journal of Parasitology* 28 (Supplement): 9.

Ferguson, M.S. 1943. Migration and localization of an animal parasite within the host. *Journal of Experimental Zoology* 93: 375-400.

Ferguson, M.S. and R.A. Hayford. 1941. The life history and control of an eye fluke. *Progressive Fish Culturist* 54: 1-13.

Fischer, S.A. and W.E. Kelso. 1988. Potential parasite-induced mortality in age 0 bluegills in a floodplain pond of the lower Mississippi River. *Transactions of the American Fisheries Society* 117: 565-573.

Fischthal, J.H. 1948. The over-wintering of black grubs and yellow grubs in fish. *Journal of Parasitology* 35: 191-192.

Flores, V. and L. Semanas. 2002. Infection patterns of *Tylodelphys barilochensis* and *T. crubensis* (Trematoda: Diplostomatidae) metacercariae in *Glaxias maculatus* (Osmeriformes: Galaxiidae) from two Patagonian Lakes and observations on their geographical distribution in the southern Andean region, Argentina. *Journal of Parasitology* 88: 1135-1139.

Font, W.F., R.W. Heard and R.M. Overstreet. 1984a. Life cycle of *Ascocotyle gemina* n. sp. a sibling of *A. sexidigita* (Digenea: Heterophyidae). *Transactions of the American Microscopical Society* 103: 392-407.

Font, W.F., R.M. Overstreet and R.W. Heard. 1984b. Taxonomy and biology of *Phagicola nana* (Digenea: Heterophyidae). *Transactions of the American Microscopical Society* 103: 408-422.

Foreyt, W.J. and J.R. Gorham. 1988. Preliminary evaluation of praziquantel against metacercariae of *Nanophyetus salmincola* in chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Wildlife Diseases* 24: 551-554.

Fox, A.C. and R.E. Olson. 1965. The life cycle of the digenetic trematode *Bolbophorus confusus*, and effects of water temperatures on the developmental stages. *Transactions of the American Microscopical Society* 84: 153-154.

Francis-Floyd, R. 1991. Use of Bayer 73 to control snails in ornamental fish ponds. In: *Aquaculture Report Series*. Florida Department of Agriculture and Consumer Services, Tallahassee, pp. 467-490.

Fredensborg, B.L. and R. Poulin. 2005. *In vitro* cultivation of *Maritrema novaezealandensis* (Microphallidae): the effect of culture medium on excystation, survival and egg production. *Parasitology Research* 95: 310-313.

Fried, B., T.K. Graczyk and L. Tamang. 2004. Food-borne intestinal trematodiasis in humans. *Parasitology Research* 93: 159-170.

Fried, B. and M.A. Haseeb. 1991. Platyhelminthes: Aspidogastrea, Monogenea, and Digenea. In: *Microscopic Anatomy of Invertebrates*, F.W. Harrison and B.J. Bogitsh (eds.). Wiley-Liss, Inc., New York, pp. 141-209.

Galazzo, D.E., S. Dayanandan, D.J. Marcogliese and J.D. McLaughlin. 2002. Molecular systematics of some North American species of *Diplostomum* (Digenea) based on

rDNA-sequence data and comparisons with European congeners. *Canadian Journal of Zoology* 80: 2207-2217.

Ganapati, P.N. and K.H. Rao. 1955. On the occurrence of metacercariae cysts of a diplostome (Trematoda) in freshwater fishes. [Abstract.] *Proceedings of the Indian Science Congress 42nd* (1955), pp. 285-286.

Gibson, D.I. 1996. Trematoda. In: *Guide to the Parasites of Fishes of Canada*, L. Margolis and Z. Kabata (eds.). *Canadian Special Publication of Fisheries and Aquatic Sciences No.* 124, NRC Press, Ottawa.

Gibson, D.I. 2002. Class Trematoda Rudolphi, 1808. In: *Keys to the Trematoda*, Volume 1. D.I. Gibson, A. Jones and R.A. Bray (eds.). CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K.

Gibson, D.I. and R.A. Bray. 1979. The Hemiuroidea: Terminology, systematics and evolution. *Bulletin of the British Museum (Natural History) (Zoology)* 32: 167-245.

Gibson, D.I., A. Jones and R.A. Bray. 2002. *Keys to the Trematoda*, Volume 1. CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K.

Ginetesinskaya, T.A. 1961. The life cycles of fish helminths and the biology of their larval stages. In: *Parasitology of Fishes*, V.A. Dogiel, G.K. Petrushevski and Yu. I. Polyanski (eds.). Translation. Oliver and Boyd, Edinburgh; TFH Publications, reprint Jersey City.

Gonzalez, G., A. Garcia, M. Jover and S. Crespo. 1995. Influence of artificial food on 1+amberjack (*Seriola dumerili*, Risso) sanguinicoliasis and epitheliocystis. *Bulletin of the European Association of Fish Pathologists* 15: 14-16.

Gordon, D.M. and M.E. Rau. 1982. Possible evidence for mortality induced by the parasite *Apatemon gracilis* in a population of brood sticklebacks (*Culea inconstans*). *Parasitology* 84: 41-47.

Granovitch, A. and K. Johannesson. 2000. Digenetic trematodes in four species of *Littorina* from the west coast of Sweden. *Ophelia* 53: 55-65.

Grau, A. 1994. *Aspectos histológicos, ciclo reprodcutor y principales procesos patológicos de Seriola dumerili, Risso 1810 (Carangidae)*. Ph.D. Thesis, Universitat Autönoma de Barcelona, Spain.

Grau, A., F. Riera and E. Carbonell. 1999. Some protozoan and metazoan parasites of the amberjack from the Balearic sea (western Mediterranean). *Aquaculture International* 7(5): 307-317.

Grizzle, J.M. and M.T. Goldsby Jr. 1996. White grub *Posthodiplostomum minimum centrarchi* metacercariae in the liver of largemouth bass: quantification and effects on health. *Journal of Aquatic Animal Health* 8: 70-74.

Guthrie, J.R. and R.L. Kroger. 1974. Schooling habits of injured and parasitized menhaden. *Ecology* 55: 208-210.

Gvozdev, E.V. 1950. Observations on the parasite fauna of fishes of Lake Markakul. *Izvestiya Akademii Nauk Respubliki Kazakhstan, Seriya Khimicheskaya, Nauk S.S.S.R.* B:8.

Halmetoja, A., E.T. Valtonen and E. Koskenniemi. 2000. Perch (*Perca flavescens* L.) parasites reflect ecosystem conditions: A comparison of a natural lake and two acidic reservoirs in Finland. *International Journal for Parasitology* 30: 1437-1444.

Halton, D.W. and B.R. Johnston. 1982. Functional morphology of the metacercarial eyst of *Bucephaloides gracilescens* (Trematoda: Bucephalidae). *Parasitology* 85: 44-52.

Halton, D.W. and B.R. Johnston. 1983. Development *in vitro* of the metacercaria of *Bucephaloides gracilescens* (Trematoda: Bucephalidae). *International Journal for Parasitology* 13: 157-164.

Harrison, E.J. and W.F. Hadley. 1982. Possible effects of black-spot disease on northern pike. *Transactions of the American Fisheries Society* 111: 106-109.

Hayunga, E.G. 1989. Parasites and immunity: tactical considerations in the war against disease—or, how did the worms learn about Clausewitz? *Perspective in Biology and Medicine* 32: 349-370.

Hayward, C.J., H. Aiken and B.F. Nowak. 2007. Metazoan parasites on the gills of southern bluefin tuna (*Thunnus maccoyii*) do not proliferate rapidly after transfer to culture cages. *Aquaculture* 262: 10-16.

Heasman, M.P., W.A.O. Connor and A.W.J. Frazer. 1996. Digenean (Bucephalidae) infections in commercial scallops, *Pecten fumatus* Reeve, and doughboy scallops, *Chlamys* (*Mimachlamys*) *asperrima* (Lamarch), in Jervis Bay, New South Wales. *Journal of Fish Diseases* 19: 333-339.

Hendrickson, G.L. 1978. Observations on strigeoid trematodes from the eyes of southeastern Wyoming fish. I. *Diplostomulum spathaceum* (Rudolphi, 1819). *Proceedings of the Helminthological Society of Washington* 45: 60-64.

Hendrickson, G.L. 1979. *Ornithodiplostomum ptychocheilus*: migration to the brain of the fish intermediate host, *Pimephales promelas*. *Experimental Parasitology* 48: 245-258.

Herbert, B.W., F.M. Shaharom and I.G. Anderson. 1995. Histopathology of cultured sea bass (*Lates calcarifer*) (Centropomidae) infected with *Cruoricola lates* (Trematoda: Sanguinicolidae) from Pulau Ketam, Malaysia. *International Journal for Parasitology* 25: 3-13.

Higgins, J.C. 1980. Formation of the cyst wall and related changes in the structure of the tegument of *Bucephalus haimeanus* (Lacaze-Duthiers, 1854) during its metamorphosis from the cercarial to the metacercarial stage. *Parasitology* 81: 47-59.

Higgins, J.C., D.E. Wright and R.A. Matthews. 1977. The ultrastructure and histochemistry of the cyst wall of *Bucephalus haimeanus* (Lacaze-Duthiers, 1854). *Parasitology* 75: 207-214.

Hine, P.M. 1978. Distribution of some parasites of freshwater eels in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 12: 179-187.

Hoffman, G.L. 1958. Studies on the life cycle of *Ornithodiplostomum ptychocheilus* (Faust) (Trematoda: Strigeoidea) and the 'self cure' of infected fish. *Journal of Parasitology* 44: 416-421.

Hoffman, G.L. 1959. Synopsis of Strigeoidea (Trematoda) of fishes and their life cycles. *Fishery Bulletin of the Fish and Wildlife Service* 60: 39-469.

Hoffman, G.L. 1970. Control methods for snail-borne zoonoses. *Journal of Wildlife Diseases* 6: 262-265.

Hoffman, G.L. 1975. Lesions due to internal helminths of freshwater fishes. In: *Pathology of Fishes*, W.E. Ribelin and G. Migaki (eds.). The University of Wisconsin Press, Madison. pp. 151-187.

Hoffman, G.L. 1999. *Parasites of North American Freshwater Fishes*, 2nd Edition, Comstock Publishing Associates, Cornell University Press, Ithaca, New York.

Hoffman, G.L. and C.E. Dunbar. 1963. Studies on *Neogogatea kentuckiensis* (Cable, 1935) n. comb. (Trematoda: Strigeoidea: Cyathocotylidae). *Journal of Parasitology* 49: 737-744.

Hoffman, G.L., B. Fried and J.E. Harvey. 1985. *Sanguinicola fontinalis* sp. nov. (Digenea: Sanguinicolidae): a blood parasite of brook trout, *Salvelinus fontinalis* (Mitchill), and longnose dace, *Rhinichthys cataractae* (Valenciennes). *Journal of Fish Diseases* 8: 529-538.

Hoffman, G.L. and J.B. Hoyme. 1957. The experimental histopathology of the 'tumor' on the brain of the stickleback caused by *Diplostomum baeri eucaliae* Hoffman and Hundley, 1957 (Trematoda: Strigeoidea). *Journal of Parasitology* 43: 33.

Hoffman, G.L. and J.B. Hoyme. 1958. The experimental histopathology of the "tumour" on the brain of the stickleback caused by *Diplostomum baeri eucaliae* Hoffman and Hundley, 1957 (Trematoda: Strigeoidea). *Journal of Parasitology* 44: 374-378.

Hoffman, G.L. and J.B. Hundley. 1957. The life-cycle of *Diplostomum baeri eucaliae* n. subsp. (Trematoda: Strigeida). *Journal of Parasitology* 43: 613-627.

Hoffman, G.L. and J.A. Hutcheson. 1970. Unusual pathogenicity of a common metacercaria of fish. *Journal of Wildlife Diseases* 6: 109.

Hoffman, G.L. and R.E. Putz. 1965. The black spot *Uvulifer ambloplites* of centrarchid fish. *Transactions of the American Microscopical Society* 94: 143-151.

Hoffmann, R.W., W. Körting, T. Fischer-Scherl and W. Schaufer. 1990. An outbreak of *Bucephalus polymorphus* in a fish of the Main River. *Angewandte Parasitologie* 31: 95-99.

Holliday, C.W. and B. Fried. 1986. Some physiological effects of *Sanguinicola fontinalis* (Trematoda: Sanguinicolidae) infection in the brook trout, *Salvelinus fontinalis*. *Journal of Parasitology* 72: 189-190.

Holmes, J.C. and P.W. Price. 1980. Parasite communities: the roles of phylogeny and ecology. *Systematic Zoology* 29(2): 203-213.

Howell, M.J. 1973. The resistance of cysts of *Stictodora lari* (Trematoda: Heterophyidae) to encapsulation by cells of the fish host. *International Journal for Parasitology* 3: 653-659.

Hsiao, S.C.T. 1941. Melanosis in common cod, *Gadus callaris* L., associated with trematode infection. *Biological Bulletin (Woodshole)* 80: 37-44.

Hubbs, C.L. 1927. The related effects of a parasite on a fish. *Journal of Parasitology* 14: 75-84.

Hutson, K.S. and I.D. Whittington. 2006. *Paradeontacylix godfreyi* n. sp. (Digenea: Sanguinicolidae) from the heart of wild *Seriola lalandi* (Perciformes: Carangidae) in southern Australia. *Zootaxa* 151: 55-68.

Hutson, K.S., I. Ernst and I.D. Whittington. 2007. Risk assessment for metazoan parasites of yellowtail kingfish Seriola lalandi (Perciformes: Carangidae) in South Australia sea-cage aquaculture. *Aquaculture* 271: 85-99.

Hughes, R.C. 1928. Studies on the trematode family Strigeidae (Holostomatidae). No. IX. *Neascus van-cleavei* (Agersborg). *Transactions of the American Microscopical Society* 47: 320-341.

Hughes, R.C. and P.G. Berkhout. 1929. Studies on the trematode family Stigeidae (Holostomidae) No. XV. *Diplostomulum gigas*, sp. nov. *Papers of the Michigan Academy of Science* 10: 483-488.

Hull, D.L. 1999. On the plurality of species: Questioning the party line. In: *Species, New Interdisciplinary Essays*, R.A. Wilson (ed.). The MIT Press, London, pp. 23-48.

Hunter, G.W. III. 1942. Studies on the parasites of fresh-water fishes of Connecticut. *Connecticut Geological and Natural History Survey Bulletin* 63: 228-288.

Hunter, G.W. III and J.M. Hamilton. 1941. Studies on host-parasite reactions to larval parasites. IV. The cyst of *Uvulifer ambloplitis* (Hughes). *Transactions of the American Microscopical Society* 60: 498-507.

Hunter, G.W. III and W.S. Hunter. 1942. Studies on host parasite reactions. V. The integumentary type of strigeid cyst. *Transactions of the American Microscopical Society* 61: 134-140.

Huxham, M., D. Raffaelli and A. Pike. 1995. Parasites and food web patterns. *Journal of Animal Ecology* 64: 168-176.

Hyman, L.H. 1951. *The Invertebrates: Platyhelminthes and Rhynchocoela*. McGraw-Hill Book Co Inc., New York.

Ivanchenko, O.F. and T.A. Grozdilova. 1971. Parasites of young of the year white-sea herring (*Clupea harengus pallasi* natio *maris-albi* Berg) reared under artificial conditions. *Parazitologiya* 5: 233-236. (In Russian).

Ivanov, V.M. and Semenova, N.N. 2004. Life cycle of the trematode *Rossicotrema donicum* (Opisthorchiida, Heterophyidae) in the Volga river delta. *Zoologicheskii Zhurnal* 83: 1206-1215. (In Russian).

James, B.L. and E.A. Bowers. 1967. Reproduction in the daughter sporocyst of *Cercaria bucephalopsis hai meana* (Lacaze-Duthiers, 1854) (Bucephalidae) and the *Cercaria dichotoma* Lebour, 1911 (Von Muller) (Gymnophalidae). *Parasitology* 57: 607-625.

James, B.L., E.A. Bowers and J.G. Richards. 1966. The ultrastructure of the daughter sporocyst of *Cercaria bucephalopsis haimaena* Lacaze-Dutheirs, 1854 (Digenea: Bucephalidae) from the edible cockle, *Cardium edule* L. *Parasitology* 56: 753-762.

Johnson, K.A. 1971. The migration of *Cotylurus erraticus* cercariae (Trematoda: Strigeidae) in rainbow trout (*Salmo gairdneri*) and their effects on the host. *Journal of Parasitology* 57: 244-251.

Johnson, M.W. and T.A. Dick. 2001. Parasite effects on the survival, growth, and reproductive potential of yellow perch (*Perca flavescens* Mitchill) in Canadian Shield Lakes. *Canadian Journal of Zoology* 79: 1980-1992.

Johnson, P.T.J., K.B. Lunde, R.W. Haight, J. Bowerman and A.R. Blaustein. 2001. *Ribeiroia ondatrae* (Trematoda: Digenea) infection induces severe limb malformations in western toads (*Bufo boreas*). *Canadian Journal of Zoology* 79: 370-379.

Johnston, B.R. and D.W. Halton. 1981. Occurrence of *Bucephaloides gracilescens* metacercariae in three species of gadoid fish. *Journal of Fish Biology* 18: 685-691.

Jones, A., R.A. Bray and D.I. Gibson. 2005. *Keys to the Trematoda*, Volume 2. CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K.

Kabata, Z. 1963. Parasites as biological tags. *International Commission on Northwest Atlantic Fisheries Special Publication* No. 4, pp. 31-37.

Kabata, Z. 1985. *Parasites and Diseases of Fish Cultured in the Tropics*. Taylor and Francis, London.

Kakonge, S.A. 1972. Methods for the study of freshwater fish parasites. *University of Waterloo Biology Series* 12: 1-76.

Kalbe, M., B. Haberl and W. Haas. 1997. Miracidial host-finding in *Fasciola hepatica* and *Trichobilharzia ocellata* is stimulated by species-specific glycoconjugates released from the host snails. *Parasitology Research* 83: 806-812.

Kamegai, S. 1971. A new digenetic trematode *Sclerodistomoides pacificus* n. g., n. sp. (Trematoda: Cephaloporidae, Plectognathotrematinae n. subfam.), from intestine of marine fish, *Navodon modestus*. *Research Bulletin of the Meguro Parasitological Museum* 3: 5-11.

Kamegai, S. and J. Araki. 1995. A new digenean (*Didymocystis margolisi* n. sp. (Didymozoidae: Didymozoinae), from the skipjack tuna, *Katsuwonus pelamis*, of Japan. *Canadian Journal of Fisheries and Aquatic Science* 52(Supplement 1): 95-97.

Kamo, H., K. Ogino and R. Hatsushika. 1962. A unique infection of man with *Clinostomum* sp., a small trematode causing acute laryngitis. *Yonago Acta Medica* 6: 37-40.

Kanev, I., V. Radev and B. Fried. 2002. Family Clinostomidae Lühe, 1901. In: *Keys to the Trematoda*, D.I. Gibson, A. Jones and R.A. Bray (eds.). CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K., Vol. 1, pp. 113-120.

Kannangara, D.W.W. and J.D. Smyth. 1974. *In vitro* cultivation of *Diplostomum phoxini* metacercariae. *International Journal for Parasitology* 4: 667-673.

Kantor, M. 1996. Historic rationale, effectiveness, and biological efficiency of existing regulations for the U.S. Atlantic bluefin tuna fisheries: a report to the U.S. Congress. *Final report for Section 310 of Public Law 104-43, The Fisheries Act of 1995. National Marine Fisheries Service, Silver Spring, Maryland, U.S.A.*

Karlsbakk, E. 2001. Didymozoid larvae (Trematoda) in the body musculature of cultured Atlantic herring larvae (*Clupea harengus*). *Acta Parasitologica* 46: 164-170.

Karvonen, A., S. Paukku, E.T. Valtonen and P.J. Hudson. 2003. Transmission, infectivity and survival of *Diplostomum spathaceum* cercariae. *Parasitology* 127: 217-224.

Kean, B.H. and R.C. Breslau. 1964. Cardiac heterophyidiasis. In: *Parasites of the Human Heart*. Grune and Stratton, New York, pp. 95-103.

Kearn, G.C. 1998. *Parasitism and the Platyhelminths*. Chapman & Hall, London.

Keiser, J. and J. Utzinger. 2005. Emerging foodborne trematodiasis. *Emerging Infectious Diseases* 11: 1507-1514.

Kennedy, C.R. 1981. Long-term studies on the population biology of two species of eyefluke, *Diplostomum gasterostei* and *Tylodelphys clavata* (Digenea: Diplostomatidae), concurrently infecting the eyes of perch, *Perca fluviatilis*. *Journal of Fish Biology* 19: 221-236.

Kennedy, C.R. 1984. The use of frequency distribution in an attempt to detect host mortality induced by infections of diplostomatid metacercariae. *Parasitology* 89: 209-220.

Kent, M.L. and A.C. Olson Jr. 1986. Interrelationships of a parasitic turbellarian, (*Paravortex* sp.) (Graffillidae, Rhabdocoela) and its marine fish hosts. *Fish Pathology* 21: 65-72.

Kent, M.L., V.G. Watral, C.M. Whipps, M.E. Cunningham, C.D. Criscione, J.R. Heidel, L.R. Curtis, J. Spitsbergen and D.F. Markle. 2004. A digenean metacercaria (*Apophallus* sp.) and a myxozoan (*Myxobolus* sp.) associated with vertebral deformities in cyprinid fishes from the Willamette River, Oregon. *Journal of Aquatic Animal Health* 16: 116-129.

Kinne, O. 1984. Diseases of marine animals: general aspects. In: *Diseases of Marine Animals*, O. Kinne (ed.). John Wiley & Sons, New York, pp. 13-73.

Kirk, R.S. and J.W. Lewis. 1992. The laboratory maintenance of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae). *Parasitology* 104: 121-127.

Kirk, R.S. and J.W. Lewis. 1993. The life cycle and morphology of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae). *Systematic Parasitology* 25: 125-133.

Kirk, R.S. and J.W. Lewis. 1994. The distribution and host range of species of the blood fluke Sanguinicola in British freshwater fish. *Journal of Helminthology* 68: 315-318.

Kniskern, V.B. 1952. Studies on the trematode family Bucephalidae Poche, 1907. Part 2. The life history of *Rhipidocotyle septpapillata* Krull, 1934. *Transactions of the American Microscopical Society* 71: 317-340.

Kohn, A., B.M.M. Fernandes and M.F.D. de Baptista-Farias. 1995. Metacercariae of *Diplostomum* (*Austrodiplostomum*) *compactum* (Trematoda, Diplostomidae) in the eyes of *Plagioscion squamosissimus* (Teleostei, Sciaenidae) from the reservoir of the hydroelectric power station of Itaipu, Brazil. *Memórias do Instituto Oswaldo Cruz* 90: 341-344.

Kohn, A., A.L. Santos and M.F.D. Baptista-Farias. 2001. Report of *Didymocystis wedli* Ariola, 1902 (Digenea: Didymozoidae) from *Thunnus albacares* in Brazil. *Memórias del Instituto Del Oswaldo Cruz*, Rio de Janeiro 96: 951-954.

Køie, M. 1982. The redia, cercaria and early stages of *Aporocotyle simplex* Odhner, 1900 (Sanguinicolidae) a digenetic trematode which has a polychaete annelid as the only intermediate host. *Ophelia* 21: 115-145.

Køie, M. and M.E. Petersen. 1988. A new annelid intermediate host (*Lanassa nordenskioeldi* Malmgren, 1866) (Polychaeta: Terebellidae) for *Aporocotyle* sp. and a new final host family (Pisces: Bothidae) for *Aporocotyle simplex* Odhner, 1900 (Digenea: Sanguinicolidae). *Journal of Parasitology* 74: 499-502.

Krause, J. and J.J. Godin. 1994. Influence of parasitism on the shoaling behavior of banded killifish, *Fundulus diaphanus*. *Canadian Journal of Zoology* 72: 1775-1779.

Krull, W.H. 1934a. *Cercaria bessiae* Cort and Brooks, 1928, an injurious parasite of fish. *Copeia* 1934: 69-73.

Krull, W.H. 1934b. Studies on the life history of a trematode *Rhipidocotyle septpapillata* n. sp. *Transactions of the American Microscopical Society* 53: 408-415.

Kuris, A.M., M.E. Torchin and K.D. Lafferty. 2004. Parasites in the thoracic ganglion of *Pachygrapsus marmoratus* (Brachyura: Grapsidae) from the coast of Portugal. *Parasite-Journal de la Societe Francaise de Parasitologie* 11: 425-427.

La Rue, G.R. 1957. The classification of digenetic Trematoda: A review and a new system. *Experimental Parasitology* 6: 306-344.

Labrie, L., C. Komar, J. Terhune, A. Camus and D. Wise. 2004. Effect of sublethal exposure to the trematode *Bolbophorus* spp. on the severity of enteric septicemia of catfish in channel catfish fingerlings. *Journal of Aquatic Animal Health* 16: 231-237.

Laffargue, P., G. Baudouin, P. Sasal, C. Arnaud, M.B. Anras and G. Lagardére. 2004. Parasitic infection of sole *Solea solea* by *Prosorhynchus* spp. metacercariae (Digenea, Bucephalidae) in Atlantic nurseries under mussel cultivation influence. *Diseases of Aquatic Organisms* 58: 179-184.

Lafferty, K.D. and A.K. Morris. 1996. Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology* 77: 1390-1397.

Larson, O.R. 1965. *Diplostomulum* (Tematoda: Strigeoidea) associated with herniations of bullhead lenses. *Journal of Parasitology* 51: 224-229.

Larson, O.R., G.L. Uglem and J.L. Kook. 1988. Fine structure and permeability of the metacercarial wall of *Clinostomum marginatum* (Digenea). *Parasitology Research* 74: 352-355.

Lauckner, G. 1983. Diseases in Mollusca: Bivalvia. In: *Diseases of Marine Animals*, O. Kinne (ed.). Biologische Anstalt Helgoland, Hamburg, Germany, pp. 477-879.

Lebour, M.V. 1923. Note on the life history of *Hemiurus communis* Odhner. *Parasitology* 15: 233-235.

Lee, F.O. and T.C. Cheng. 1970. The histochemistry of *Stellantchasmus falcatus* Onji and Nishio, 1915 (Trematoda: Heterophyidae) metacercarial cyst in the mullet *Mugil cephalus* L. and histopathological alterations in the host. *Journal of Fish Biology* 2: 235-243.

Leknes, I.L. 1986. Fine structure and endocytic properties of subendothelial macrophages in the bulbus arteriosus of two bony fish species. *Acta Histochemistry* 79: 155-160.

Lemly, A.D. and G.W. Esch. 1983. Differential survival of metacercariae of *Uvulifer ambloplites* (Hughes, 1927) in juvenile centrarchids. *Journal of Parasitology* 69: 746-749.

Lemly, A.D. and G.W. Esch. 1984a. Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: ecological implications. *Journal of Parasitology* 70: 475-492.

Lemly, A.D. and G.W. Esch. 1984b. Population biology and largemouth bass *Micropterus salmoides*. *Journal of Parasitology* 70: 466-474.

Lemly, A.D. and G.W. Esch. 1984c. Population biology of the trematode *Uvulifer ambloplitis* (Hughes, 1927) in the snail intermediate host, *Helisoma trivolvis*. *Journal of Parasitology* 70: 461-465.

Leong, C.H.D. and M.J. Howell. 1971. Formation and structure of the cyst wall of *Stictodora lari* (Trematoda: Heterophyidae). *Zeitschrift für Parasitenkunde* 35: 340-350.

Lester, R.J.G. 1977. An estimate of the mortality in a population of *Perca flavescens* owing to the trematode *Diplstomum adamsi*. *Canadian Journal of Zoology* 55: 288-292.

Lester, R.J.G. 1980. Host-parasite relations in some didymozoid trematodes. *Journal of Parasitology* 66: 527-531.

Lester, R.J.G. 1984. A review of methods for estimating mortality due to parasites in wild fish populations. *Helgolander Meeresuntersuchungen* 37: 53-64.

Lester, R.J.G. 1990. Reappraisal of the use of parasites for fish stock identification. *Australian Journal of Marine and Freshwater Research* 41: 855-864.

Lester, R.J.G. and H.W. Huizinga. 1977. *Diplostomum adamsi* sp. n.: description, life cycle, and pathogenesis in the retina of *Perca flavescens*. *Canadian Journal of Zoology* 55: 64-73.

Lester, R.J.G. and T.D.G. Lee. 1976. Infectivity of the progenetic metacercariae of *Diplostomum spathaceum*. *Journal of Parasitology* 62: 832-833.

Levy, M.G., J.R. Flowers, M.F. Poore, F.E. Mullen, L.H. Khoo, L.M. Pote, I. Paperna, R. Dzikowski and R.W. Litaker. 2002. Morphologic, pathologic, and genetic investigations of *Bolbophorus* species affecting cultured channel catfish in the Mississippi Delta. *Journal of Aquatic Animal Health* 14: 235-246.

Lewert, R.M. and C.L. Lee. 1954a. Studies on the passage of helminth larvae through host tissues. I. Histochemical studies on the extracellular changes caused by penetrating larvae. *Journal of Infectious Disease* 95: 13-35.

Lewert, R.M. and C.L. Lee. 1954b. Studies on the passage of helminth larvae through host tissues. II. Enzymatic activity *in vivo* and *in vitro*. *Journal of Infectious Disease* 95: 36-51.

Lile, N.K. 1998. Alimentary tract helminths of four pleuronectid flatfish in relation to host phylogeny and ecology. *Journal of Fish Biology* 53: 945-953.

Littlewood, D.T.J. and D.I. Gibson. 2001. *Interrelationships of the Platyhelminthes*. Taylor and Francis, New York.

Lively, C.M., C. Craddock and R.C. Vrijenhoek. 1990. Red queen hypothesis supported by parasitism in sexual and clonal fish. *Nature* (London) 344: 864-866.

Lo, C.F., F. Huber, G.H. Kou and C.J. Lo. 1981. Studies on *Clinostomum complanatum* (Rudolphi, 1819). *Fish Pathology* 15: 219-227.

Lorio, W.J. 1989. Experimental control of metacercariaė of the yellow grub *Clinostomum marginatum* in channel catfish. *Journal of Aquatic Animal Health* 1: 269-271.

Lun, Z.R., R.B. Gasser, D.H. Lai, A.X. Li, X.Q. Zhu, X.B. Yu and Y.Y. Fang. 2005. Clonorchiasis: A key foodbourne zoonosis in China. *Lancet Infectious Diseases* 5: 31-41.

Lyholt, H.C.K. and K. Buchmann. 1996. *Diplostomum spathaceum*: Effects of temperature and light on cercarial shedding and infection of rainbow trout. *Diseases of Aquatic Organisms* 25: 169-173.

Lynch, D.L. and B.J. Bogtish. 1962. The chemical nature of metacercarial cysts. II. Biochemical investigations on the cyst of *Posthodiplostomum minimum*. *Journal of Parasitology* 48: 241-243.

Lyster, L.L. 1940. *Apophallus imperator* sp. nov., a heterophyid encysted in trout, with a contribution to its life history. *Canadian Journal of Research* 18: 106-121.

MacInnis, A.J. 1976. How parasites find hosts: some thoughts on the inception of host-parasite integration. In: *Ecological Aspects of Parasitology*, C.R. Kennedy (ed.). North-Holland Publishing Company, Amsterdam, pp. 3-20.

MacInnis, A.J., W.M. Bethel and E.M. Cornford. 1974. Identification of chemicals of snail origin that attract *Schistosoma mansoni* miracidia. *Nature* (London) 248: 361-363.

MacKenzie, K. 1968. Some parasites of 0-group plaice, *Pleuronectes platessa* L. under different environmental conditions. *Journal of Marine Research* 3: 1-23.

MacKenzie, K. 1999. Parasites as pollution indicators in marine ecosystems: a proposed early warning system. *Marine Pollution Bulletin* 38: 955-959.

MacKenzie, K., H.H. Williams, B. Williams, A.H. McVicar and R. Siddall. 1995. Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. *Advances in Parasitology* 35: 85-144.

Madsen, H. 1990. Biological methods for the control of freshwater snails. *Parasitology Today* 6: 227-241.

Mahmoud, L.H., S.O. Basiouny and H.A. Dawoud. 2002. Treatment of experimental heterophyiasis with two plant extracts, areca nut and pumpkin seed. *Journal of the Egyptian Society for Parasitology* 32: 501-506.

Maillard, C. and M.H. Ktari. 1978. *Hyperandrotrema cetorhini* n. g., n. sp. (Trematoda, Sanguinicolidae) parasite du système circulatoire de *Cetorhinus maximus* (Selacii). *Annales de Parasitologie Humane et Compare* 53: 359-365.

Manter, H.W. 1957. Host specificity and other host relationships among the digenetic trematodes of marine fishes. In: *Premier Symposium sur la Spécifité Parasitaire des Parasites des Vertèbres*, J.G. Baer (ed.). Series B, No. 32, International Union of Biological Sciences, pp. 185-197.

Marcogliese, D.J. 2002. Food webs and the transmission of parasites to marine fish. *Parasitology* 124: S83-S99.

Martin, W.E. 1950. *Euhaplorchis californiensis* n. g., n. sp., Heterophyidae, Trematoda, with notes on its life cycle. *Transactions of the American Microscopical Society* 64: 194-209.

Martin, W.E. 1952. Another annelid first intermediate host of a digenetic trematode. *Journal of Parasitology* 38: 1-4.

Matricon-Godran, M. 1971. Etude ultrastructurale des récepteurs sensoriels tégumentaires de quelques trématodes digénétiques larvaires. *Zeitschrift für Parasitenkunde* 35: 318-333.

Matthews, R.A. 1968. *Studies on the helminth parasites of marine teleosts*. Ph.D. Thesis. University College of Wales, Aberystwyth.

Matthews, R.A. 1973a. The life cycle of *Bucephalus haimeanus* Lacaze-Duthiers, 1854 from *Cardium edule* L. *Parasitology* 67: 341-350.

Matthews, R.A. 1973b. The life cycle of *Prosorhynchus crucibulum* (Rudolphi, 1819) Odhner, 1905, and a comparison of its cercaria with that of *Prosorhynchus squamatus* Odhner, 1905. *Parasitology* 66: 133-164.

Matthews, R.A. 1974a. The life cycle of *Bucephaloides gracilescens* (Rudolphi, 1819) Hopkins, 1954 (Digenea: Gasterostomata). *Parasitology* 3: 1-12.

Matthews, R.A. 1974b. Metacercariae and diseases in marine teleosts. *Proceedings of the Third International Congress of Parasitology*, Munich, Germany, 26-31 August, 1974, 3: 1723.

Mawdesley-Thomas, L. and P.C. Young. 1967. Cutaneous melanosis in a flounder (*Platichthys flesus* L.). *Veterinary Record October* 7th: 2068.

McCullough, F.S. and K.E. Mott. 1983. *The Role of Mulluscicides in Schistosomiasis Control. Document WHO/VBC83.879*, World Health Organization. Geneva, Switzerland.

McDonald, M.E. 1981. *Key to Trematodes Reported in Waterfowl.* Resource Pub. 142 (U.S. Fish and Wildlife Service).

McGladdery, S.E., L. Murphy, B.D. Hicks and S.K. Wagner. 1990. The effects of *Stephanostomum tenue* (Digenea: Acanthocolpidae) on marine aquaculture of the rainbow trout, *Salmo gairdneri*. In: *Pathology in Marine Science*, T.C. Cheng and F.O. Perkins (eds.). Academic Press, London, pp. 305-315.

McLaren, D.J. and D.J. Hockley 1977. Blood flukes have a double outer membrane. *Nature* (London) 269: 147-149.

McQueen, A., K. MacKenzie, R.J. Roberts and H. Young. 1973. Studies on the skin of plaice (*Pleuronectes platessa* L.). III. The effect of temperature on the inflammatory response to the metacercariae of *Cryptocotyle lingua* (Creplin, 1825) (Digenea: Heterophyidae). *Journal of Fish Biology* 5: 241-247.

Meade, T.G. 1967. Life history studies on *Cardicola klamathensis* (Wales, 1958) Meade and Pratt, 1965 (Trematoda: Sanguinicolidae). *Proceedings of the Helminthological Society of Washington* 34: 210-212.

Meyer, F.P. 1958. Helminths of fishes from Trumbull Lake, Clay County, Iowa. *Proceedings of the Iowa Academy of Sciences* 65: 477-516.

Miller, M.J. 1942. Black spot disease of speckled trout. *Revue Canadienne de Biologie* 1: 464-471.

Miller, R.H. 1963. Oysters killed by trematode parasites. *Nature* (London) 197: 616.

Mills, C.A. 1979. Attachment and feeding of the adult ectoparasitic digenean *Transversotrema patialense* (Sorparkar, 1924) on the zebra fish *Brachydanio rerio* (Hamilton-Buchanan). *Journal of Fish Diseases* 2: 443-447.

Mitchell, A.J. 1995. Importance of treatment duration for praziquantel used against larval digenetic trematodes in sunshine bass. *Journal of Aquatic Animal Health* 7: 327-330.

Mitchell, A.J., A.E. Goodwin and M.G. Levy. 2006. *Bolbophorus* infections in cultured fathead minnow. *Journal of Aquatic Animal Health* 18: 55-57.

Mitchell, A.J., A.E. Goodwin M.J. Salmon and T.M. Brandt. 2002. Experimental infection of an exotic heterophyid trematode, *Centrocestus formosanus*, in four aquaculture fishes. *North American Journal of Aquaculture* 64: 55-59.

Mitchell, A.J., R.M. Overstreet, A.E. Goodwin and T.M. Brandt. 2005. Spread of an exotic fish-gill trematode: A far-reaching and complex problem. *Fisheries* 30(8): 11-16.

Mitchell, A.J., M.J. Salmon, D.G. Huffman, A.F. Goodwin and T.M. Brandt. 2000. Prevalence and pathogenicity of a heterophyid trematode infecting the gills of an endangered fish, the fountain darter, in the two central Texas spring-fed rivers. *Journal of Aquatic Animal Health* 12: 283-289.

Mitchell, A.J., C.E. Smith and G.L. Hoffman. 1982. Pathogenicity and histopathology of an unusually intense infection of white grubs (*Posthodiplostomum m. minimum*) in the fathead minnow (*Pimephales promelas*). *Journal of Wildlife Diseases* 18: 51-57.

Mitchell, C.W. 1974. Ultrastructure of the metacercarial cyst of *Posthodiplostomum minimum* (MacCallum, 1921). *Journal of Parasitology* 60: 67-74.

Mitchell, L.G., J. Ginal and W.C. Bailey. 1983. Melanotic visceral fibrosis associated with larval infections of *Posthodiplostomum minimum* and *Proteocephalus* sp. in bluegill, *Lepomis macrochirus* Rafinesque, in central Iowa, U.S.A. *Journal of Fish Diseases* 6: 135-144.

Mitchum, D. 1965. Diseases and parasites of fish. *Wyoming Game and Fish Commission* FW-3-R-12.

Miyanaga, S. 1939. Studies on second intermediate host of *Clonorchis sinesis* in Mukden area. *Journal of Oriental Medicine* 31: 565-568. [In Japanese: English summary page 43].

Mladineo, I. and M. Tudor. 2004. Digenea of Adriatic cage-reared Northern bluefin tuna (*Thunnus thynnus thynnus*). *Bulletin of the European Association of Fish Pathologists* 24: 145-152.

Mohandas, A. 1971. Integumentary papillae and their taxonomic importance in cercariae. *Zeitschrift für Parasitenkunde* 35: 334-339.

Montero, F.E., A. Garcia and J.A. Raga. 1999. First record of *Paradeontacylix* Mcintosh, 1934 species (Digenea: Sanguinicolidae) in Mediterranean amberjack, *Seriola dumerili* (Risso, 1810), culture. *Bulletin of European Association of Fish Pathologists* 19(3):107-109.

Montero, F.E., F.J. Aznar, M. Fernandéz and J.A. Raga. 2003. Girdles as the main infection site for *Paradeontacylix kampachi* (Sanguinicolidae) in the greater amberjack, *Seriola dumerili*. *Diseases of Aquatic Organisms* 53: 271-272.

Morley, N.J., M. Crane and J.W. Lewis. 2002. Toxic effects of cadmium and zinc on the transmission of *Echinoparyphium recurvatum* cercariae. *Journal of Helminthology* 76: 157-163.

Morley, N.J., M. Crane and J.W. Lewis. 2003a. Toxicity of cadmium and zinc to the cercarial activity of *Diplostomum spathaceum* (Trematoda: Diplostomidae). *Folia Parasitologica* 50: 57-60.

Morley, N.J., M. Crane and J.W. Lewis. 2003b. Effects of cadmium and zinc toxicity on orientation behaviour of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae) cercariae. *Diseases of Aquatic Organisms* 56: 89-92.

Morley, N.J., M. Crane and J.W. Lewis. 2003c. Pollution toxicity to the transmission of larval digeneans through their molluscan hosts. *Parasitology* 126: S5-S26.

Morley, N.J., M. Crane and J.W. Lewis. 2004. Influence of cadmium exposure on the incidence of first intermediate host encystment by *Echinoparyphium recurvatum* cercariae in *Lymnaea peregra*. *Journal of Helminthology* 78: 329-332.

Morley, N.J., M. Crane and J.W. Lewis. 2005a. Changes in survival characteristics of *Diplostomum spathaceum* cercariae emerged from cadmium-exposed *Lymnaea stagnalis*. *Journal of Helminthology* 79: 55-59.

Morley, N.J., M. Crane and J.W. Lewis. 2005b. Toxicity of cadmium and zinc mixtures to cercarial tail loss in *Diplostomum spathaceum* (Trematoda: Diplostomidae). *Ecotoxicology and Environmental Safety* 60: 53-60.

Mourtisen, K.N. and R. Poulin. 2003. Parasite-induced trophic facilitation exploited by a non-host predator: A manipulator's nightmare. *International Journal for Parasitology* 33: 1043-1050.

Mueller, J.F. 1972. Cranial deformation in a fish resulting from intracranial parasitism by strigeid metacercariae. *Journal of Parasitology* 58: 183-184.

Munday, B.L., Y. Sawada, T. Cribb and C.J. Hayward. 2003. Diseases of tunas, *Thunnus* spp. *Journal of Fish Diseases* 26: 187-206.

Munro, A. 1785. *Structure and Physiology of Fishes*. C. Elliott., Edinburgh.

Nelson, J.S. 2006. *Fishes of the World*. 3rd Edition. John Wiley & Sons, New York.

Nico, L.G., J.D. Williams and H.L. Jelks. 2005. Black carp: Biological synopsis and risk assessment of an introduced fish. *American Fisheries Society, Special Publication 32, Bethesda, Maryland*, pp. 337.

Niewiadomska, K. 2002a. Superfamily Diplostomoidea Poirier, 1886. In: *Keys to the Trematoda*, D.I. Gibson, A. Jones and R.A. Bray (eds.). CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K., Vol. 1, pp. 159-166.

Niewiadomska, K. 2002b. Family Diplostomidae Poirier, 1886. In: *Keys to the Trematoda*, D.I. Gibson, A. Jones and R.A. Bray (eds.). CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K., Vol. 1, pp. 167-196.

Niewiadomska, K. 2002c. Family Strigeidae Railliet, 1919. In: *Keys to the Trematoda*, D.I. Gibson, A. Jones and R.A. Bray (eds.). CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K., Vol. 1, pp. 231-241.

Nikolaeva, V.M. 1965. Life cycle of trematode family Didymozoidae (Monticelli, 1888) Poche, 1907. *Zoologichesky Zhurnal* 44: 1317-1327.

Nikolaeva, V.M. 1985. Trematodes—Didymozoidae Fuana, Distribution and Biology, W.J. Hargis (ed.). *NOAA Technical Report NMFS 25*, pp. 67-72.

Noble, G.A. 1975. Description of *Nematobibothrioides histoidii* (Noble, 1974) (Trematoda: Didymozoidae) and comparison with other genera. *Journal of Parasitology* 61: 224-227.

Noga, E.J. 2000. *Fish Disease, Diagnosis and Treatment*. Blackwell Publishing Professional, Ames, Iowa.

Nolan, M.J. and T.H. Cribb. 2004. The life cycle of *Paracardicoloides yamagutii* Martin, 1974 (Digenea: Sanguinicolidae). *Folia Parasitologica* 51: 320-326.

Nolan, M.J. and T.H. Cribb. 2006. *Cardicola* Short, 1953 and *Braya* n. gen. (Digenea: Sanguinicolidae) from five families of tropical Indo-Pacific fishes. *Zootaxa* 1265: 1-80.

Nollen, P.M. and H.D. Murray. 1978. *Philophthalmus gralli:* identification, growth characteristics, and treatment of an oriental eyefluke of birds introduced into the continental United States. *Journal of Parasitology* 64: 178-180.

Nordmann, A. von. 1832. Entozoa in the eyes of animals. *Dublin Journal of Medical and Chemical Science* 1: 238.

Nowak, B.F. 2004. Assessment of health risks to southern bluefin tuna under current culture conditions. *Bulletin of the European Association of Fish Pathologists* 24: 45-51.

Ogawa, K., H. Andoh and M. Yamaguchi. 1993. Some biological aspects of *Paradeontacylix* (Trematoda: Sanguinicolidae) infection in cultured marine fish *Seriola dumerili*. *Fish Pathology* 28: 177-180.

Ogawa, K. and S. Egusa. 1986. Two new species of *Paradeontacylix* McIntosh, 1934 (Trematoda: Sanguinicolidae) from the vascular system of a cultured marine fish, *Seriola purpurascens*. *Fish Pathology* 21: 15-19.

Ogawa, K. and M. Fukudome. 1994. Mass mortality caused by blood fluke (*Paradeontacylix*) among amberjack (*Seriola dumerili*) imported to Japan. *Fish Pathology* 29: 265-269.

Ogawa, K., K. Hattori, K. Hatai and S. Kubota. 1989. Histopathology of cultured marine fish, *Seriola purpurascens* (Carangidae) infected with *Paradeontacylix* spp. (Trematoda: Sanguinicolidae) in its vascular system. *Fish Pathology* 24: 75-81.

Ogawa, K., T. Nagano, N. Akai, A. Sugita and K.A. Hall. 2007. Blood fluke infection of cultured tiger puffer *Takifugu rubripes* imported from China to Japan. *Fish Pathology* 42(2): 91-99.

Ogawa, K., T. Nakatsugawa and M. Yasuzaki. 2004. Heavy metacercarial infections of cyprinid fishes in Uji River. *Fisheries Science* 70: 132-140.

Oglesby, L.C. 1961. A new cercaria from an annelid. *Journal of Parasitology* 47: 233-236.

Oliveira, K. and R. Campbell. 1998. The occurrence and pathological effects of *Stephanostomum tenue* (Digenea: Acanthocolpidae) metacercariae in elvers of the American eel. *Journal of Fish Biology* 53: 690-692.

Olsen, R.E. and J.R. Pierce. 1997. A trematode metacercaria causing fill cartilage proliferation in steelhead trout from Oregon. *Journal of Wildlife Diseases* 33: 886-890.

Olson, P.D., T.H. Cribb, V.V. Tkach, R.A. Bray and D.T.J. Littlewood. 2003. Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology* 33: 733-755.

Olson, R.E. 1966. Some experimental fish hosts of the strigeid tematode *Bolbophorus confusus* and effects of temperature on the cercaria and metacercaria. *Journal of Parasitology* 52: 327-334.

Ondračková, M., M. Reichard, P. Jurajda and M. Gelnar. 2004a. Seasonal dynamics of *Posthodiplostomum cuticola* (Digenea, Diplostomatidae) metacercariae and parasite-enhanced growth of juvenile host fish. *Parasitology Research* 93: 131-136.

Ondračková, M., A. Simkova, M. Gelnar and P. Jurajda. 2004b. *Posthodiplostomum cuticola* (Digenea: Diplostomatidae) in intermediate fish hosts: factors contributing to the parasite infection and prey selection by the definitive bird host. *Parasitology* 129: 761-770.

Ooi, H.K., W.S. Wang, C.Y. Tu, H.Y. Chang and C.I. Chenn. 1999. Natural mass infection by heterophyid metacercariae in aquacultured Japanese eel in Taiwan. *Diseases of Aquatic Organisms* 35: 31-36.

Orihel, T.C. and L.R. Ash. 1995. *Parasites in Human Tissues*. ASCP Press, Chicago.

Ortlepp, R.J. 1935. On the metacercariae and adult of *Clinostomum van der horsti* sp. n., a trematode parasite of fishes and herons. *Onderstepoort Journal of Veterinary Research* 5: 51-58.

Overstreet, R.M. 1969. Digenetic trematodes of marine teleost fishes from Biscayne Bay, Florida. *Tulane Studies in Zoology and Botany* 15: 119-176.

Overstreet, R.M. 1970. A syncoeliid (Hemiuridea Faust, 1929) metacercaria on a copepod from the Atlantic equatorial current. *Journal of Parasitology* 56: 834-836.

Overstreet, R.M. 1976. A redescription of *Crassicutis archosargi*, a digenean exhibiting an unusual tegumental attachment. *Journal of Parasitology* 62: 702-708.

Overstreet, R.M. 1978. *Marine Maladies? Worms, Germs, and Other Symbionts from the Northern Gulf of Mexico*. Mississippi-Alabama Sea Grant Consortium. Blossman Printing, Ocean Springs, Mississippi.

Overstreet, R.M. 1982. Abiotic factors affecting marine parasitism. In: *Parasites: Their World and Ours*, D.F. Mettrick and S.S. Desser (eds.). *Fifth International Congress of*

*Parasitology Proceedings and Abstracts*, 7-14 August 1982, Toronto, Canada, Vol. 2, pp. 36-39.

Overstreet, R.M. 1993. Parasitic diseases of fishes and their relationship with toxicants and other environmental factors. In: *Pathobiology of Marine and Estuarine Organisms*, J.A. Couch and J.W. Fournie (eds.). CRC Press, Boca Raton, pp. 111-156.

Overstreet, R.M. 1997. Parasitological data as monitors of environmental health. *Parassitologia* 39: 169-175.

Overstreet, R.M. 1998. Introduction to co-evolution of fish and their parasites and diseases. In: *ICOPA IX (9th International Congress of Parasitology)*, I. Tada, S. Kojima and M. Tsuji Monduzzi (eds.). Bologna, Italy, pp. 21-28.

Overstreet, R.M. 2003. Flavor buds and other delights. *Journal of Parasitology* 89: 1093-1107.

Overstreet, R.M. and S.S. Curran. 2002. Superfamily Bucephaloidea Poche, 1907. In: *Keys to the Trematoda*, D.I. Gibson, A. Jones and R.A. Bray (eds.). CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K., Vol. 1, Chapter 15, pp. 67-110.

Overstreet, R.M. and S.S. Curran. 2004. Defeating diplostomoid dangers in USA catfish aquaculture. *Folia Parasitologica* 51: 153-165.

Overstreet, R.M. and S.S. Curran. 2005. Family Haploporidae Nicoll, 1914. In: *Keys to the Trematoda*, A. Jones, R.A. Bray and D.I. Gibson (eds.). CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K., Vol. 2, pp. 129-165.

Overstreet, R.M., S.S. Curran, L.M. Pote, D.T. King, C.K. Blend and W.D. Grater. 2002. *Bolbophorus damnificus* n. sp. (Digenea: Bolbophoridae) from the channel catfish *Ictalurus punctatus* and American white pelican *Pelecanus erythrorhynchos* in the USA based on life-cycle and molecular data. *Systematic Parasitology* 52: 81-96.

Overstreet, R.M. and R.H. Edwards. 1976. Mesenchymal tumors of some estuarine fishes of the northern Gulf of Mexico. II. Subcutaneous fibromas in the southern flounder, *Paralichthys lethostigma*, and the sea catfish, *Arius felis*. *Bulletin of Marine Science* 26: 41-48.

Overstreet, R.M. and J. Thulin. 1989. Response by *Plectropomus leopardus* and other serranid fishes to *Pearsonellum corventum* (Digenea: Sanguinicolidae), including melanomacrophage centers in the heart. *Australian Journal of Zoology* 37: 129-142.

Owen, S.F., I. Barber and P.J.B. Hart. 1993. Low level infection by eye fluke, *Diplostomum* spp., affects the vision of three-spined sticklebacks, *Gasterosteus aculeatus*. *Journal of Fish Biology* 42: 803-806.

Padrós, F., C. Zarza and S. Crespo. 2001. Histopathology of cultured sea bream *Sparus aurata* infected with sanguinicolid trematodes. *Diseases of Aquatic Organisms* 44: 47-52.

Palmer, E.D. 1939. Diplostomiasis, a hatchery disease of freshwater fishes new to North America. *Progressive Fish Culturist*, Memo 1-31(45): 41-47.

Paperna, I. 1964a. The metazoan parasite fauna of Israel inland water fishes. *Bamidgeh* 16: 3-66.

Paperna, I. 1964b. Parasitic helminths from inland water fishes in Israel. *Israel Journal of Zoology* 13: 1-20.

Paperna, I. 1975. Parasites and disease of the grey mullet (Mugilidae) with special reference to the seas of the Near East. *Aquaculture* 5: 65-80.

Paperna, I. 1980. Parasites, infections and diseases of fish in Africa. *Food and Agriculture Organization, United Nations, CIFA Technical Paper No.* 7.

Paperna, I. 1991. Diseases caused by parasites in the aquaculture of warm water fish. *Annual Review of Fish Disease* 1: 155-194.

Paperna, I. 1995. Digenea (Phylum Platyhelminthes). In: *Fish Diseases and Disorders*, volume 1, P.T.K. Woo (ed.). CABI Publishing, Wallingford, Oxon, U.K., pp. 329-389.

Paperna, I. 1996. Parasites, Infections and Diseases of Fishes in Africa: *An Update. Technical Paper 31, Central Institute of Freshwater Aquaculture, Food and Agriculture Organization, United Nations, Rome.*

Paperna, I. and R. Dzikowski. 2006. Digenea (*Phylum Platyhelminthes*). In: *Fish Diseases and Disorders*, P.T.K. Woo (ed.). 2nd Edition. CABI Publishing, Wallingford, Oxon, U.K., Vol. 1, pp. 345-390.

Paperna, I. and R.M. Overstreet. 1981. Parasites and diseases of mullets (Mugilidae). In: *Aquaculture of Grey Mullets*, O.H. Oren (ed.). *International Biological Programme 26.* Cambridge University Press, Cambridge, pp. 411-493.

Pearson, J.C. 1986. The paranephridial system in the Digenea: Occurrence and possible phylogenetic significance. In: *Parasite Lives*, M. Cremin, C. Dobson and D.E. Moorhouse (eds.). University of Queensland Press, Brisbane, pp. 56-68.

Pearson, J.C. 2002. *Protoheterophyes spuriocirrus* gen. et sp. Nov. (Trematoda, Heterophyidae) described and the nature of the gonotyl in heterophyids explicated. *Acta Parasitologica* 47: 205-207.

Pennycuick, L. 1971. Quantitative effects of three species of parasites on a population of three-spined sticklebacks, *Gasterosteus aculeatus*. *Journal of Zoology* 165: 143-162.

Perera, G., M. Yong, J.R. Ferrer, C. Arrinda and O. Amador. 1990. Effectiveness of three biological control agents against intermediate hosts of snail-mediated parasites in Cuba. *Malacological Review* 23: 47-52.

Perera, K.L.M. 1992a. Light microscopic study of the pathology of a species of didymozoan, Nematobothriinae gen. sp., from the gills of slimy mackerel *Scomber australasicus*. *Diseases of Aquatic Organisms* 13: 103-109.

Perera, K.L.M. 1992b. Ultrastructure of the primary gill lamellae of *Scomber australasicus* infected by a didymozoid parasite. *Diseases of Aquatic Organisms* 13: 111-121.

Petrushevski, G.K. and S.S. Shulman. 1961. The parasitic diseases of fishes in the natural waters of the USSR. In: *Parasitology of Fishes*, V.A. Dogiel, G.K. Petrushevski and Yu.I. Polyanski (eds.). (Translation Edinburgh; Oliver and Boyd; Reprint, TFH Publications, Jersey City), pp. 299-319.

Pieri, O.S. and J.D. Thomas. 1987. Snail host control in the eastern coastal areas of North-East (NE) Brazil. *Memorias de Instituto Oswaldo Cruz, Rio de Janeiro*, 82 (Supplement 4): 197-201.

Pike, A.W. and M.D.B. Burt. 1983. The tissue response of yellow perch, *Perca flavescens* Mitchill to infections with metacercarial cyst of *Apophallus brevis* Ransom, 1920. *Parasitology* 87: 393-404.

Pokrovskii, V.V. 1961. Basic environmental factors determining the abundance of whitefish. *Trudy Soveshchanii* 13: 228-234.

Poulin, R., K. Hecker and F. Thomas. 1998. Hosts manipulated by one parasite incur additional costs from infection by another parasite. *Journal of Parasitology* 84: 1050-1052.

Pozdnyakov, S.E. 1993. The systematics of trematodes of the suborder Didymozoata. *Parazitologiya* 27: 415-425. (In Russian).

Pratt, H.S. 1898. A contribution to the life history and anatomy of the appendiculate distomes. *Zoologisches Jahrbuch für Anatomie* 11: 1-40.

Pulsford, A. and R.A. Matthews. 1984. An ultrastructural study of the cellular response of the plaice, *Pleuronectes platessa* L., to *Rhipidocotyle johnstonei* nom. nov. (pro-*Gasterostomum* sp. Johnstone, 1905) Matthews, 1968 (Digenea: Bucephalidae). *Journal of Fish Diseases* 7: 3-14.

Rabideau, T.R. and J.T. Self. 1953. A study of the effects of black and yellow grubs on *Lepomis cyanellus* and *L. humilis* in a natural environment. *American Midland Naturalist* 50: 402-406.

Radabaugh, D.C. 1980. Changes in minnow, *Pimephales promelas* Rafinesque, schooling behaviour associated with infections of brain-encysted larvae of the fluke, *Ornithodiplostomum ptychocheilus*. *Journal of Fish Biology* 16: 621-628.

Rahi, A.H.S. and A. Gardner. 1976. *Immunopathology of the Eye*. Blackwell Scientific Publications, Oxford.

Ratanarat-Brockelman, C. 1974. Migration of *Diplstomum spathaceum* (Trematoda) in the fish intermediate host. *Zeitschrift für Parasitenkunde* 43: 123-134.

Rea, J.G. and S.W.B. Irwin. 1999. Alien invaders: Larval flukes as an aid to teaching. *Journal of Biological Education* 34: 41-45.

Rees, G. 1955. The adult of diplostomulum stage (*Diplostomum phoxini* (Faust)) of *Diplostomum pelatoides* Dubois and an experimental demonstration of part of the life cycle. *Parasitology* 45: 295-312.

Rees, G. 1957. *Cercaria diplostomi phoxini* (Faust), a furcocercaria which develops into *Diplostomulum phoxini* in the brain of the minnow. *Parasitology* 47: 126-137.

Reighard, J. 1929. A biological examination of Loon Lake, Gogebic County, Michigan, with suggestions for increasing its yield of small mouth bass (*Microterus dolomieu*). *Papers Michigan Academy of Science and Arts Letters* 10: 589-612.

Richards, D.T., D. Hoole, J.W. Lewis, E. Ewens and C. Arme. 1994a. Changes in the cellular composition of the spleen and pronephros of carp *Cyprinus carpio* infected with the blood fluke *Sanguinicola inermis* (Trematoda: Sanguinicolidae). *Diseases of Aquatic Organisms* 19: 173-179.

Richards, D.T., D. Hoole, J.W. Lewis, E. Ewens and C. Arme. 1994b. Ultrastructural observations on the cellular response of carp, *Cyprinus carpio* L., to eggs of the blood fluke *Sanguinicola inermis* Plehn, 1905 (Trematoda: Sanguinicolidae). *Journal of Fish Diseases* 17: 439-446.

Richards, D.T., D. Hoole, J.W. Lewis, E. Ewens and C. Arme. 1996a. Stimulation of carp *Cyprinus carpio* lymphocytes *in vitro* by the blood fluke *Sanguinicola inermis* (Trematoda: Sanguinicolidae). *Diseases of Aquatic Organisms* 25: 87-93.

Richards, D.T., D. Hoole, J.W. Lewis, E. Ewens and C. Arme. 1996b. In vitro polarization of carp leucocytes in response to the blood fluke *Sanguinicola inermis* Plehn, 1905 (Trematoda: Sanguinicolidae). *Parasitology* 112: 509-513.

Richards, D.T., D. Hoole, J.W. Lewis, E. Ewens and C. Arme. 1996c. Adherence of carp leucocytes to adults and cercariae of the blood fluke *Sanguinicola inermis*. *Journal of Helminthology* 70: 63-67.

Rieger, R.M., S. Tyler, J.P.S. Smith III and G.E. Rieger. 1991. Platyhelminathes. Turbellaria. In: *Microscopic Anatomy of Invertebrates*, Wiley-Liss, Inc. New York, Vol. 3, pp. 7-140.

Roberts, L.S., J. Janovy, Jr. and P. Schmidt. 2004. *Foundations of Parasitology*. 7th Edition. McGraw-Hill Co Inc., New York.

Roberts, R.J. 1975. Melanin-containing cells of teleost fish and their relation to disease. In: *The Pathology of Fishes*, W.E. Ribelin and G. Migaki (eds.). The University of Wisconsin Press, Madison, Wisconsin, pp. 399-428.

Robinette, D.R. and E.J. Noga. 2001. Histone-Like Protein (HLP): A novel method for assessing chronic stress in fish. *Diseases of Aquatic Organisms* 44: 97-107.

Rohde, K. 1984. Diseases caused by metazoans: helminths. In: *Diseases of Marine Animals*, O. Kinne (ed.). Biologische Anstalt Helgoland, Hamburg, Vol. 6, pp. 193-320.

Rohde, K. 2005. *Marine Parasitology*. CABI Publishing, Wallingford, Oxon, U.K.

Rothchild, M.A. 1942. Seven-year-old infection of *Cryptocotyle lingua* Creplin in the winkle *Littorina littorea* L. *Journal of Parasitology* 28: 350.

Rushton, W. 1938. Blindness in freshwater fishes. *Nature* (London) 141(3563): 289.

Ruus, A., J.U. Skaare and K. Ingebrigtsen. 2001. Accumulation of the lipophilic environmental contaminant lindane in metacercariae of *Bucephaloides gracilescens* (Trematoda: Bucephalidae) in the central nervous system of bulltrout *Myoxocephalus scorpius*. *Diseases of Aquatic Organisms* 48: 75-77.

Ryder, J.A. 1884. On a skin parasite of the cunner (*Ctenolabrus adspersus*). *Bulletin of the US Fisheries Commission for 1884* 4: 37-42.

Saladin, K. 1979. Behavioral parasitology and perspectives on miracidial host-finding. *Zeitschrift für Parasitenkunde* 60: 197-210.

Schäperclaus, W. 1954. *Fischkrankheiten*. Akademie-Verlag, Berlin.

Schell, S.C. 1970. *How to Know the Trematodes?* W.C. Brown, Dubuque, Iowa.

Schell, S.C. 1974. The life history of *Sanguinicola idahoensis* sp. n. (Trematoda: Sanguinicolidae), a blood parasite of steelhead trout, *Salmo gairdneri* Richardson. *Journal of Parasitology* 60: 561-566.

Schell, S.C. 1985. *Trematodes of North America North of Mexico*. University Press of Idaho, Moscow, Idaho.

Schmahl, G. and H. Mehlhorn. 1985. Treatment of fish parasites. 1. Praziquantel effective against Monogenea (*Dactylogyrus vastator, Dactylogyrus extensus, Diplozoon paradoxum*). *Zeitschrift für Parasitenkunde* 71: 727-737.

Scholz, T., L. Euzet and F. Moravec. 1998. Taxonomic status of *Pelichnibothrium speciosum* Monticelli, 1889 (Cestoda: Tetraphyllidea), a mysterious parasite of *Alepisaurus ferox* Lowe (Teleostei: Alepisauridae) and *Prionace glauca* (L.) (Selachii: Carcharinidae). *Systematic Parasitology* 41: 1-8.

Scholz, T., J. Vargas-Vazquez, L. Aguirre-Macedo and V.M. Vidal-Martinez. 1997. Species of *Ascocotyle* Loos, 1899 (Digenea: Heterophyidae) of the Yucatan Peninsula, Mexico, and notes on their life-cycles. *Systematic Parasitology* 36: 161-181.

Scholz, T., J. Vargas-Vazquez, F. Moravec, C. Vivas-Rodriguez and E. Mendoza-Franco. 1995. Metacercariae of trematodes of fishes from cenotes (=sinkholes) of the Yucatan Peninsula, Mexico. *Folia Parasitologica* 42: 173-192.

Schwartz, F.J. 1956. First record of infestation and death in the ictalurid catfish, *Schilbeodes miurus*, by the parasite *Clinostomum marginatum*. *Copeia* 1954: 250-251.

Scott, J.S. 1975. Incidence of trematode parasites of American plaice (*Hippoglossoides platessoides*) of the Scotian Shelf and Gulf of St. Lawrence in relation to fish length and food. *Journal of the Fisheries Research Board of Canada* 32: 479-483.

Self, J.T., L.E. Peters and C.E. Davis. 1963. The egg, miracidium, and adult of *Nematobothrium texomensis* (Trematoda: Digenea). *Journal of Parasitology* 49: 731-736.

Seppala, O., A. Karvonen and E.T. Valtonen. 2004. Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke: fish interaction. *Animal Behavior* 68: 257-263.

Sepulveda, M.S., M.G. Spaulding, J.M. Kinsella and D.J. Forrester. 1999. Parasites of the great egret (*Ardea albus*) in Florida and a review of the helminths reported for the species. *Journal of the Helminthological Society of Washington* 66: 7-13.

Shariff, M., R.H. Richards and C. Sommerville. 1980. The histopathology of acture and chronic infections of rainbow trout *Salmo gairdneri* Richardson with eye flukes, *Diplostomum* spp. *Journal of Fish Diseases* 3: 455-465.

Shoop, W.L. 1989. Systematic analysis of the Diplostomidae and Strigeidae (Trematoda). *Journal of Parasitology* 75: 21-32.

Sinclair, N.R. 1972. Studies on the heterophyid trematode *Apophallus brevis*, the 'sand-grain grub' of yellow perch (*Perca flavescens*). II. The metacercaria: position, structure, and composition of the cyst; hosts; geographical distribution and variation. *Canadian Journal of Zoology* 50: 577-584.

Sindermann, C.J. and A. Rosenfield. 1954. Diseases of fishes of western North Atlantic III. Mortalities of sea herring (*Clupe harengus*). *Maine Department of Sea Shore Fisheries Bulletin* 21: 1-16.

Skirnisson, K., K.V. Galaktionov and E.V. Kozminsky. 2004. Factors influencing the distribution of digenetic trematode infections in a mudsnail (*Hydrobia ventrosa*) population inhabiting salt marsh ponds in Iceland. *Journal of Parasitology* 90: 50-59.

Smith, J.W. 1972. The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded vertebrates and some comparison with the schistosomes. [Review article] *Helminthological Abstracts Series* A 41: 161-204.

Smith, J.W. 1997a. The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded vertebrates: Part 1. A review of the published literature since 1971, and bibliography. *Helminthological Abstracts* 66: 255-294.

Smith, J.W. 1997b. The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded vertebrates: Part 2. Appendix I: Comprehensive parasite-host list; Appendix II: Comprehensive host-parasite list. *Helminthological Abstracts* 66: 329-344.

Smith, J.W. 2002. Family Sanguinicolidae von Graff, 1907. In: *Keys to the Trematoda*, D.I. Gibson, A. Jones and R.A. Bray (eds.). CABI Publishing and The Natural History Museum, Wallingford, Oxon, U.K., London, Vol. 1, pp. 433-452.

Smitherman, Jr. R.O. 1964. Effects of infections with strigeid trematode, *Posthodiplostomum minimum* (MacCallum), upon the bluegill, *Lepomis macrochirus* Rafinesque. *Dissertation Abstracts* 25: 2115.

Smithers, S.R. and R.J. Terry. 1969. The immunology of schistosomiasis. In: *Advances in Parasitology*, B. Dawes (ed.). Academic Press, London, Vol. 7, pp. 41-93.

Smyth, J.D. 1990. Trematoda: basic problems and approaches. In: *In vitro Cultivation of Parasitic Helminths*, J.D. Smyth (ed.). CRC Press, Boca Raton, pp. 17-43.

Smyth, J.D. and J.A. Clegg. 1959. Egg-shell formation in trematodes and cestodes. *Experimental Parasitology* 8: 286-323.

Sommerville, C. 1981. A comparative study of the tissue response to invasion and encystment by *Stephanochasmus baccatus* (Nicoll, 1907) (Digenea: Acanthocolpidae) in four species of flatfish. *Journal of Fish Diseases* 4: 53-68.

Sommerville, C. 1982. The pathology of *Haplorchis pumilio* (Looss, 1896) infections in cultured tilapias. *Journal of Fish Diseases* 5: 243-250.

Sommerville, C. and N.A.M. Iqbal. 1991. The process of infection, migration, growth and development of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae) in carp, *Cyprinus carpio* L. *Journal of Fish Diseases* 14: 211-219.

Sorensen, R.E., J. Curtis and D.J. Minchella. 1998. Intraspecific variation in the rDNA ITS loci of 37-collar-spined echinostomes from North America: Implications for sequence-based diagnoses and phylogenetics. *Journal of Parasitology* 84: 992-997.

Spall, R.D. and R.C. Summerfelt. 1969. Host-parasite relations of certain endoparasitic helminths of the channel catfish and white crappie in an Oklahoma reservoir. *Bulletin of the Wildlife Disease Association* 5: 48-67.

Spall, R.D. and R.C. Summerfelt. 1970. Life cycle of the white grub, *Posthodiplostomum minimum* (Maccallum, 1921: Trematoda, Diplostomatidae), and observations on host-parasite realtionships of the metacercaria in fish. In: *A Symposium on Diseases of Fish and Shellfishes*, S.F. Snieswzko (ed.). *Special Publication No. 5 of the American Fisheries Society, Washington, DC.*, pp. 218-230.

Speed, P. and G.B. Pauley. 1985. Feasibility of protecting rainbow trout, *Salmo gairdneri* Richardson, by immunizing against the eyefluke, *Diplostomum spathaceum*. *Journal of Fish Biology* 26: 739-744.

Sripa, B., M.R. Haswell-Elkins and P. Sinawat. 2003a. Histological analysis of gallbladder diseases in relation to opisthorchiasis in endemic areas of Thailand. *Acta Tropica* 88: 239-246.

Sripa, B., P. Sithithaworn and S. Sirisinha. 2003b. *Opisthorchis viverrini* and opisthorchiasis: the 21st century review. *Acta Tropica* 88: 169-170.

Stables, J.N. and L.H. Chappell. 1986. *Diplostomum spathaceum* (Rud. 1819): effects of physical factors on the infection of rainbow trout (*Salmo gairdneri*) by cercariae. *Parasitology* 93: 71-79.

Stanford, P.K. 1995. For pluralism and against monism about species. *Philosophy of Science* 62: 70-91.

Steedman, R.J. 1991. Occurrence and environmental correlates of black spot disease in stream fishes near Toronto, Ontario. *Transactions of the American Microscopical Society* 120: 494-499.

Stein, P.C. and R.D. Lumsden. 1971. An ultrastructural and cytochemical study of metacercarial cyst development in *Ascocotyle pachycystis* Schoeder and Leigh, 1965. *Journal of Parasitology* 57: 1231-1246.

Steinauer, M.L. and W.F. Font. 2003. Seasonal dynamics of the helminths of bluegill (*Lepomis macrochirus*) in a subtropical region. *Journal of Parasitology* 89: 324-328.

Stewart, M.T., N.J. Marks and D.W. Halton. 2003a. Neuroactive substances and associated major muscle systems in *Bucephaloides gracilescens* (Trematoda: Digenea) metacercaria and adult. *Parasitology Research* 91: 12-21.

Stewart, M.T., A. Mousley, B. Koubkova, S. Sebelova, N.J. Marks and D.W. Halton. 2003b. Development *in vitro* of the neuromusculature of two strigeid trematodes, *Apatemon cobitidis* and *Cotylurus erraticus*. *International Journal for Parasitology* 33: 413-424.

Stunkard, H.W. 1930. The life history of *Cryptocotyle lingua* (Creplin), with notes on the physiology of the metacercariae. *Journal of Morphology and Physiology* 50: 143-184.

Stunkard, H.W. 1976. The life cycles, intermediate hosts, and larval stages of *Rhipidocotyle transversale* Chandler, 1935 and *Rhipidocotyle intoni* Hopkins, 1954: Life-cycles and systematics of bucephalid trematodes. *Biological Bulletin (Woodshole)* 150: 294-317.

Suomalainen, E. 1950. Parthenogenesis in animals. *Advances in Genetics* 3: 193-253.

Sweeting, R.A. 1974. Investigations into natural and experimental infections of freshwater fish by the common eye-fluke *Diplostomum spathaceum* Rud. *Parasitology* 69: 291-300.

Székely, C. and K. Molnár. 1991. Praziquantel (Droncit) is effective against diplostomosis of grasscarp (*Crenopharyngodon idella*) and silver carp (*Hypophthalmichtys molitrix*) *Diseases of Aquatic Organisms* 11: 147-150.

Szidat, L. 1927. Bieträge zur Entwicklungsgeschichte der Holostomiden. *Zoologischer Anzeiger* 61: 11-12.

Szidat, L. and A. Nani. 1951. Diplostomiasis cerebralis del pejerrey. Una grave epizootia que afecta a la economia nacional producida por larvas de trematodes que destruyen el cerebro de los prejerreyes. *Revista del Instituto Nacional de Investigaciones de las Ciencias Naturales* (Argentina) 1: 323-394.

Szidat, L. and A. Nani. 1952. Nota preliminar sobre una parasitosis grave de los pejerreyes producida por larvas de trematodes de la familia Diplostomidae Poirier 1886 que destruyen el cerebro de los peces. *Revista Medicina Veterinaria* 34: 9-14.

Taraschewski, H. and I. Paperna. 1981. Distribution of the snail *Pirenella conica* in Sinai and Israel and its infection by Heterophyidae and other trematodes. *Marine Ecology Progress Series* 5: 193-205.

Taskinen, J. 1998a. Influence of trematode parasitism on the growth of a bivalve host in the field. *International Journal for Parasitology* 28: 599-602.

Taskinen, J. 1998b. Cercarial production of the trematode *Rhipidocotyle fennica* in clams kept in the field. *Journal of Parasitology* 84: 345-349.

Taskinen, J. and E.T. Valtonen. 1995. Age-, size-, and sex-specific infection of *Anodonta piscinalis* (Bivalvia: Unionidae) with *Rhipidocotyle fennica* (Digenea: Bucephalidae) and its influence on host reproduction. *Canadian Journal of Zoology* 73: 887-897.

Taskinen, J., E.T. Valtonen and T. Mäkelä. 1994. Quantity of sporocysts and seasonality of two *Rhipidocotyle* species (Digenea: Bucephalidae) in *Anodonta piscinalis* (Mollusca: Bivalvia). *International Journal for Parasitology* 24: 877-886.

Terhune, J.S., D.J. Wise and L.H. Khoo. 2002. *Bolbophorus confusus* infections in channel catfish in northwestern Mississippi and effects of water temperature on emergence of cercariae from infected snails. *North American Journal of Aquaculture* 64: 70-74.

Thatcher, V.E. 1981. Patologia de peixes da Amazônia Brasileira, 1. Aspectos gerais. *Acta Amozonica* 11: 125-140.

Thomas, F., F. Renaud, T. De Meeüs and R. Poulin. 1998. Manipulation of host behavior by parasites: Ecosystem engineering in the intertidal zone? *Proceedings of the Royal Society of London* B 265: 1091-1096.

Thulin, J. 1980. A redescription of the fish blood-fluke *Aporocotyle simplex* Odhner, 1900 (Digenea, Sanguinicolidae) with comments on its biology. *Sarsia* 65: 35-48.

Torchin, M.E., K.D. Lafferty and A.M. Kuris. 2002. Parasites and marine invasions. *Parasitology* 124: S137-S151.

Tort, L., J.J. Watson and I.G. Priede. 1987. Changes *in vitro* heart performance in rainbow trout *Salmo gairdneri* Richardson, infected with *Apatemon gracilis* (Digenea). *Journal of Fish Biology* 30: 341-347.

Tripp, M.R. 1973. Hermaphroditism in *Bucephalus*-infected oysters. *Journal of Invertebrate Pathology* 21: 321-322.

Urabe, M., K. Ogawa, T. Nakatsugawa, Y. Imanishi, T. Kondo, T. Okunishi, Y. Kaji and H. Tanaka. 2001. Newly recorded gasterostome trematode (Digenea: Bucephalidae) in the Uji River: the life history, distribution and damage to fishes. *Bulletin of the Kansai Organization for Nature Conservation* 23: 13-21.

Van Dobben, W.H. 1952. The food of the cormorants in the Netherlands. *Ardea* 40: 1-63.

Van Haitsma, J.P. 1930. Studies on the trematode family Strigeidae (Holostomidae). XXI. Life cycle and description of the cercariae of *Cotylurus michiganensis* (La Rue). *Journal of Parasitology* 16: 224-230.

Van Oosten, J. 1944. Factors influencing the growth of fish. *Transactions of the Ninth North American Wild Life Conference*. 24-26 April, 1944, Chicago, Illinois, pp. 177-183.

Van Valen, L. 1973. A new evolutionary law. *Evolution Theory* 1: 1-30.

Venable, D.L., A.P. Gaudé III and P.L. Klerks. 2000. Control of the trematode *Bolbophorus confusus* in channel catfish *Ictalurus punctatus* ponds using salinity manipulation and polyculture with black carp *Mylopharyngodon piceus*. *Journal of the World Aquaculture Society* 31: 158-166.

Vernard, C.E. 1940. Studies on the parasites of Reelfoot Lake fish I. Parasites of the large-mouthed black bass, *Huro salmoides* (Laćepèe). *Report Reelfoot Lake Biological Station of the Tennessee Academy of Science* 4: 43-63.

Vogelbein, W.K., J.W. Fournie and R.M. Overstreet. 1987. Sequential development and morphology of experimentally induced hepatic melano-macrophage centres in *Rivulus marmoratus*. *Journal of Fish Biology* 31 (Supplement A): 145-153.

Vogelbein, W.K. and R.M. Overstreet. 1988. Life-history and pathology of a heterophyid trematode infecting Florida-reared ornamental fishes. *International Association for Aquatic Animal Medicine Proceedings* 19: 138.

Waadu, G.D.B. and L.H. Chappell. 1991. Effect of water temperature on the ability of *Diplostomum spathaceum* miracidia to establish in lymnaeid snails. *Journal of Helminthology* 65: 179-185.

Wales, J.H. 1958. Two new blood flukes of trout. *California Fish and Game* 44: 125-136.

Wang, G.T., W.J. Yao and P. Nie. 2001. Seasonal occurrence of *Dollfustrema vaneyi* (Digenea: Bucephalidae) metacercariae in the bullhead catfish *Pseudobagrus fulvidraco* in a reservoir in China. *Diseases of Aquatic Organisms* 44: 127-131.

Wardle, W.J. 1988. A bucephalid larva, *Cercaria pleuromerae* n. sp. (Trematoda: Digenea), parasitizing a deepwater bivalve from the Gulf of Mexico. *Journal of Parasitology* 23: 285-289.

Wardle, W.J. 1990. Experimental verification of the metacercarial stage of *Rhipidocotyle leisostei* (Trematoda: Bucephalidae) with notes on the natural occurrence of its adult stage in gars in Texas and Virginia. *Journal of Parasitology* 76: 293-295.

Warren, B.H. 1953. A new type of metacercarial cyst of the genus *Apophallus*, from the perch, *Perca flavescens*, in Minnesota. *The American Midland Naturalist* 50: 397-401.

Watson, J.J., A.W. Pike and I.G. Priede. 1992. Cardiac pathology associated with the infection of *Oncorhynchus mykiss* Walbaum with *Apatemon gracilis* Rud. 1819. *Journal of Fish Biology* 41: 163-167.

Watts, M., B.L. Munday and C.M. Burke. 2001. Production, characterisation and diagnostic use of antisera to southern bluefin tuna (*Thunnus maccoyii* Castelnau) immunoglobin. *Aquaculture* 199: 245-257.

Weeks, S.C. 1996. A re-evaluation of the Red Queen model for the maintenance of sex in a clonal-sexual fish complex (Poeciliopsis: Poeciliopsis). *Canadian Journal of Fisheries and Aquatic Sciences* 53: 1157-1164.

Wheeler, Q.D. and R. Meier. 2000. *Species Concepts and Phylogenetic Theory, A Debate.* Columbia University Press, New York.

Whyte, S.K., L.H. Chappell and C.J. Secombes. 1990. Protection of rainbow trout, *Oncorhynchus mykiss* (Richardson), against *Diplostomum spathaceum* (Digenea): the role of specific antibody and activated macrophages. *Journal of Fish Diseases* 13: 281-291.

Whyte, S.K., C.J. Secombes and L.H. Chappell. 1991. Studies on the infectivity of *Diplstomum spathaceum* in rainbow trout (*Oncorhynchus mykiss*). *Journal of Helminthology* 65: 169-178.

Wierzbicka, J. 1977. Trematodes of *Abramis brama*, *A. ballerus* and *Blicca bjoercna* from the Dabie lake, Poland. *Acta Parasitologica Polonica* 25: 1-16.

Williams, H. and A. Jones. 1994. *Parasitic Worms of Fish.* Taylor and Francis Inc, Bristol.

Williams, H.H. 1959. The anatomy of *Köllikeria filicollis* (Rudolphi, 1819), Cobbold, 1860 (Trematoda: Digenea) showing that the sexes are not entirely separate as hitherto believed. *Parasitology* 49: 39-53.

Williams, H.H. 1967. Helminth Disease of Fish. *Helminthological Abstracts* 36: 261-295.

Williams, H.H., K. MacKenzie and A.M. McCarthy. 1992. Parasites as biological indicators of the population biology, migrations, diet, and phylogenetics of fish. *Reviews in Fish Biology and Fisheries* 2: 144-176.

Woodhead, A.E. 1929. Life history studies on the trematode family Bucephalidae. *Transactions of the American Microscopical Society* 48: 256-274.

Woodhead, A.E. 1930. Life history studies on the trematode family Bucephalidae. No. II. *Transactions of the American Microscopical Society* 49: 1-17.

Woodhead, A.E. 1936. A study of the gasterstome cercaria of the Huron River. *Transactions of the American Microscopical Society* 55: 465-476.

Yamaguti, S. 1970. *Trematodes of Hawaiian Fishes*. Keigaku Publishing Company, Ltd. Tokyo, Japan.

Yamaguti, S. 1971. *Synopsis of Digenetic Trematodes of Vertebrates*, Vol. 1 and Vol. 2. Keigaku Publishing Company, Tokyo.

Yamaguti, S. 1975. *A Synoptical Review of Life Histories of Digenetic Trematodes of Vertebrates With Special Reference to the Morphology of Larval Forms*. Keigaku Publishing Company, Tokyo.

Yanohara, Y. and N. Kagei. 1983. Studies on metacercaria of *Centrocestus formosanus* (Nishigori, 1924)-I. Parasitism of metacercariae in gills of young rearing eels, and abnormal deaths of hosts. *Fish Pathology* 17: 237-241.

Zhatkanbayeva, D. and R.A. Heckmann. 1990. Effectiveness of praziquantel against trematodes of fish. In: *Book of Abstracts, VIIth International Congress of Parasitology*, 20-24 August 1990, Paris, pp. 869 (abstract No. 7046).

CHAPTER

# 15

# Diseases Caused by Cestoda

**Joanna Danuta Borucinska**

## INTRODUCTION

Fish are very frequently parasitized by adult and/or larval cestodes. Most wild and many cultured fish will harbor an infection with at least one species of these worms over the course of their life time. Many cestode-fish relationships are believed to have a long evolutionary history, and although that implies minimal pathogenicity, several of the known tapeworm infections do have serious effects on their fish hosts. Accordingly, cestodiasis can act as a population-limiting factor either directly by causing mortality, or indirectly through lowered fecundity or outright sterility. In addition, many cestode infections manifest as behavioral and/or phenotypic changes that increase susceptibility to predation, thus indirectly contributing to mortality. The morbidity associated with cestodiasis may result in decreased production yields in aquaculture, and rejection of the affected fish flesh for human consumption. It is thus agreed that, at least from the anthropocentric

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perspective, cestodes have both an environmental and economical impact by worsening the health of fish from wild and cultured habitats.

Additionally, fish tapeworms are responsible for classic zoonotic diseases such as diplogonoporiasis (Kino *et al.*, 2002) and diphyllobothriasis, also known as 'tapeworm pernicious anemia'. The latter is due to vitamin $B_{12}$ deficiency, a condition that encompasses megaloblastic, macrocytic anemia with thrombocytopenia and leukopenia. This deficiency is a result of a high demand for vitamin $B_{12}$ in the ATP-generating pathways in *Diphyllobothrium latum* and *D. dendriticum*, a feature common to many tapeworm species (Arme *et al.*, 1983; Schantz, 1996; Dick *et al.*, 2001). It has also been shown that some larval tapeworms have the potential to induce anaphylactic reactions in animals that are fed fish meat infected with these larvae, and a possibility for the development of similar hypersensitivity in humans has been suggested (Vazquez-Lopez *et al.*, 2001, 2002).

## General Characteristics of Cestoda

Cestodes are members of the Phylum Platyhelminthes. There are over 5000 recorded species of cestodes, which are subdivided into two major subgroups, the Cestodaria and Eucestoda (tapeworms) (Caira and Reyda, 2005). The Cestodaria consist of two orders: the Gyrocotylidea and Amphilinidea. Eucestoda encompass the remaining 11 orders, including the most pathogenic tapeworms from bony fish, the Caryophyllidea, Pseudophyllidea and Proteocephalidea (Dick and Choudhury, 1995). The orders Tetraphyllidea and Trypanorhyncha are very common in elasmobranchs, but their pathogenicity has not been clearly established (Cheung, 1993; Borucinska and Dunham, 2000; Borucinska and Caira, 2006).

The form and classification of cestodes were provided in great detail by Mackiewicz (1972) and Roberts and Janovy (1996), and morphological characteristics of different genera of cestodes as encountered in tissue sections can be found in Chitwood and Lichtenfels (1973) and Gardiner and Poynton (1999). What follows is a brief description of the particular features that are important in the consideration of identification, pathogenicity and transmission within this group of parasites.

Most adults and many larval cestodes are recognizable macroscopically during necropsy of fish. The body or strobila of most adult cestodes is flattened and it can obtain a length surpassing that of its host

by orders of magnitude. The cephalic portion of the strobila differentiates into an attachment organ, the scolex, which is usually quite minute in comparison to the rest of the body (Fig. 15.1A). The scolex bears morphological features that are frequently characteristic for a particular order of cestodes, which include specialized attachment structures such as hooks, sucker-like organs (bothria, bothridia and acetabula), spined tentacles, a myzorhynchus, and apical organs (Fig. 15.1A-D). In Cestodaria, there is no scolex, and in some tapeworms it degenerates into a *scolex amorphous* (Williams, 1960). For example, in *Khawia iowensis* attached within the gut of the common carp *Cyprinus carpio*, immature worms exhibit a great variation in the shape of the scolex (Calentine and Ulmer, 1961). This variation is due to formations of anterior 'festons' that conform to host tissue patterns generated during attachment (Jara and Szerow, 1981). Many cestodes—both larval and adults—possess a morphologically defined apical organ which can be composed of muscular and/or glandular elements. In others, an apical gland is only detected microscopically as a dispersed population of characteristic granular cells within the matrix of the scolex and/or neck (Whittington and Cribb, 2001). The apical gland cells are large polygonal cells with eosinophilic intracytoplasmic granules seen in H&E stained sections (Figs. 15.2, 15.3). Transmission electron microscopy has revealed that the granules are membrane bound in some worms, and that their content can be secreted to the tegumental surface via apocrine or eccrine mechanisms (Whittington and Cribb, 2001). In some worms, a duct lined with microtubules connects the apical gland cells with the tegument (Fig. 15.3) (Moreno *et al.*, 2001; Zd'árská *et al.*, 2004). Proteins, lipids and glycoproteins were detected within the granules in apical gland cells and most investigators agree that the secretions from these cells aid in penetration of host tissues by plerocercoids (McCormick and Stokes, 1982), facilitate attachment by adult worms (Mackiewicz and McCrae, 1967), possibly contribute to immune mimicry, and also provide protection against host proteolytic enzymes (Whittington and Cribb, 2001).

The scolex is followed by the neck, which is the undifferentiated part of the strobila (Fig. 15.1A). It contains germ cells that generate new segments (proglottids). Along the strobila, the maturation of segments takes place and the most caudad segments detach and are shed in the feces in most tapeworm species. Apolytic cestodes drop gravid segments, enapolytic drop mature but non-gravid segments, and in hyperapolytic

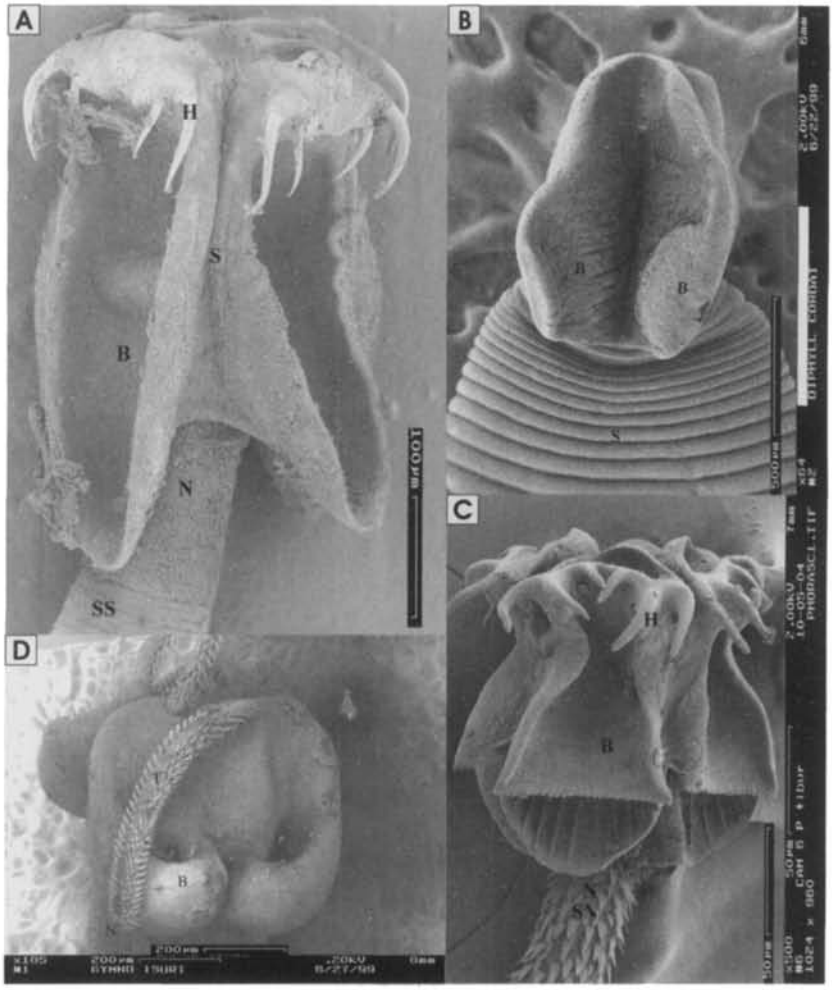


**Fig. 15.1A-D** Courtesy of Dr Janine N. Caira. **A.** *Pedibothrium longispine* from a common nurse shark *Ginglymostoma cirratum*; B – bothridia, H – hooks, N – cephalic peduncle, S – scolex, SS – segmented strobila. **B.** *Diphyllobothrium cordatum* from a Harbor seal *Phoca vitulina*; BB – bothria, S – segmented strobila. **C.** *Phoreiobothrium* sp. from a bonnethead shark *Sphyrna tiburo*; B – bothridia, H – hooks, SN – spines on cephalic peduncle. **D.** *Gymnorhynchus isuri* from a shortfin mako shark *Isurus oxyrinchus*; B – bothria, N – neck, T – tentacles.

tapeworms immature segments are shed (Roberts and Janovy, 1996). In some species yet, there is no shedding of segments and the eggs are released into the external environment via one or more uterine pores. Each segment contains one or several complete sets of male and female

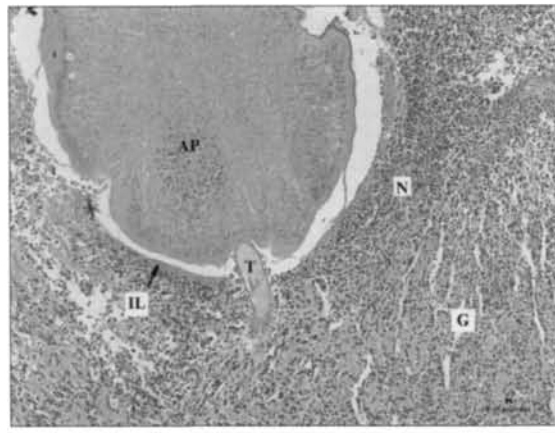


**Fig. 15.2** Adult trypanorhynch tapeworm attached to the pyloric gut of a sicklefin weasel shark, *Hemigaleus microstoma*; the scolex is surrounded by a narrow intermediate layer (IL), the tentacle (T) penetrates into necrotic tissue (N), which blends with granulation tissue (G) effacing the lamina propria; apical gland cells (AP) form a distinct cluster within the scolex.

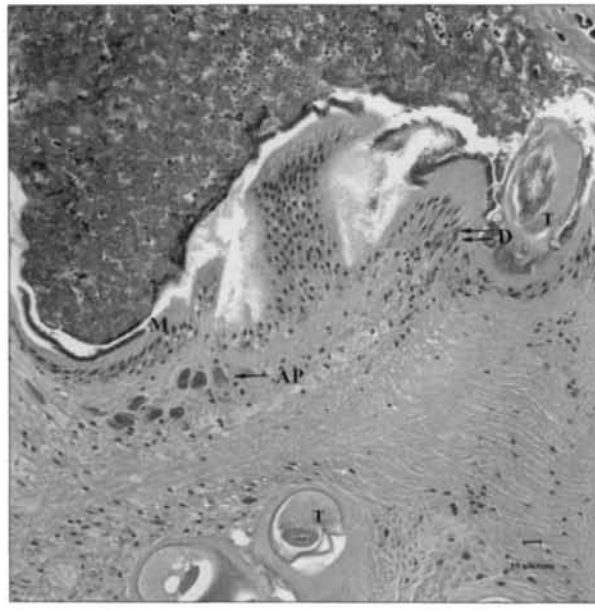


**Fig. 15.3** Adult trypanorhynch tapeworm attached to the duodenal portion of the spiral intestine in a shortfin mako shark *Isurus oxyrinchus*. Partially everted tentacle (T) inserts into necrotic tissue composed of eosinophilic granular material; also apical gland cells (AP) and ducts (D), tentacles within tentacular bulbs and microtriches (M) engaging host tissue can be seen.

reproductive organs, a simplified nervous and excretory system and prominent somatic musculature. There is no digestive tract. Because there is also no body cavity or coelom, the internal organs of cestodes are surrounded by a sparsely cellular matrix, which in many species contains dispersed ovoid to polygonal mineralized bodies (usually 12-32 μm), the calcareous corpuscles (Fig. 15.4) (Roberts and Janovy, 1996). Albeit their function is unknown, their presence is diagnostic for adult and/or larval tapeworms in tissue sections (one has to note though that calcareous corpuscles can be also found in trematodes, but the latter posses a digestive tract which should help in identification).

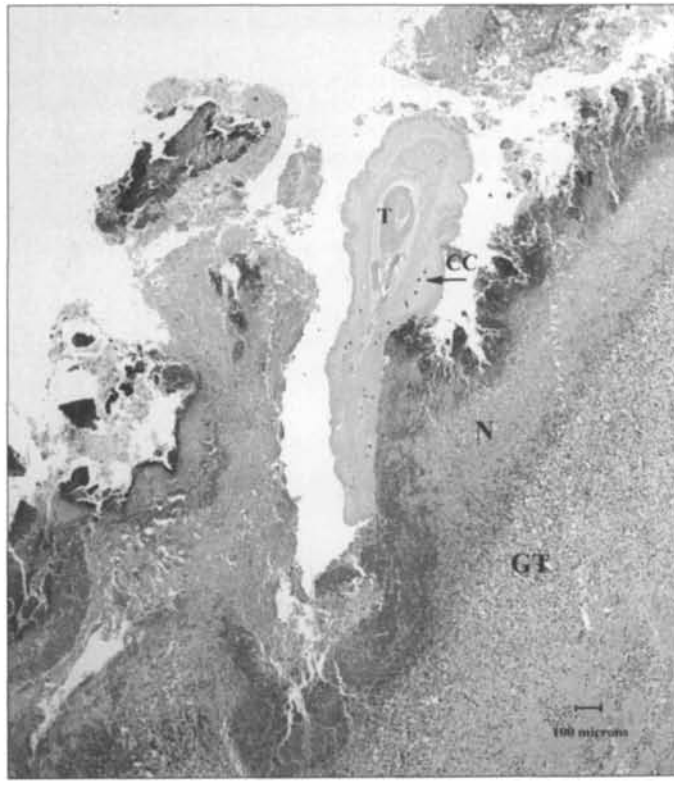


**Fig. 15.4** A necrotic trypanorhynch tapeworm within the distal glandular stomach of a shortfin mako shark *Isurus oxyrinchus*. Calcareous corpuscles (CC) and tentacles (T) within tentacular bulbs can be seen. The gastric mucosa has been effaced by mineralized (M) and necrotic tissues (N) that blend with granulation tissue (GT) in the submucosa and muscularis of the gastric wall.

The body covering of cestodes is termed the tegument. It serves a multitude of functions such as mechanical protection, immune barrier, secretion, digestion and absorption of nutrients. It is a syncytial structure with surface modifications in the form of a glycocalyx and microscopic surface projections, the microtriches (Fig. 15.5A, B). The glycocalyx is a layer of glycolipids secreted by tegumental cells onto the surface of the microtriches. The tegument is rich in phospholipids, phosphotidylcholine being prominent among them. In additional several phosphohydrolases were detected within the tegument. The glycocalyx and the microtriches are functionally and morphologically similar to the brush border within the gut of vertebrates, and they serve similar functions, i.e., transport of macromolecules across the tegument, inactivation and trapping of enzymes (some, like pancreatic amylase derived from host cells), adsorption of bile acids, and contact digestion (Befus and Podesta, 1986). The distribution and form of microtriches varies among the regions of the body of the worm (i.e., scolex and its attachment structures, neck and strobila) and among different species (Izvekova *et al.*, 1997; Caira *et al.*, 2001; Zd'árská *et al.*, 2004). Because of the morphological variability exhibited by microtriches, in addition to absorptive functions mentioned above, they are believed to also aid in attachment, migration of plerocercoids, and immune protection through absorption of masking antibodies (McVicar, 1972; Befus and Podesta, 1986).

## Life Cycle

Fish can serve as intermediate, paratenic and definitive hosts for tapeworms (Schäperclaus, 1992). The life cycle begins when the embryonated eggs containing the larval form, the hexacanth, are shed into water in feces of definitive hosts (a predatory fish or piscivorous bird or mammal). Pseudophyllidea and some Trypanorhyncha have a free-swimming, ciliated larval form, the coracidium. The larvae are ingested by the first intermediate host, an invertebrate (a copepod, mollusc or an oligochete), in which they develop into a first stage larva, usually classified as the procercoid [for a review of invertebrate intermediate hosts and proper nomenclature of larval stages for marine fish see Caira and Reyda (2005)]. If the first intermediate host is consumed by a second intermediate host (a fish) the procercoids migrate into an extra-intestinal site and develop into a second stage larva, which in fish is commonly classified as a plerocercoid or plerocercus. Plerocercoids need to be ingested by a definitive host (fish, bird or mammal) to develop into a mature tapeworm in the gut. The patent period is reached usually within

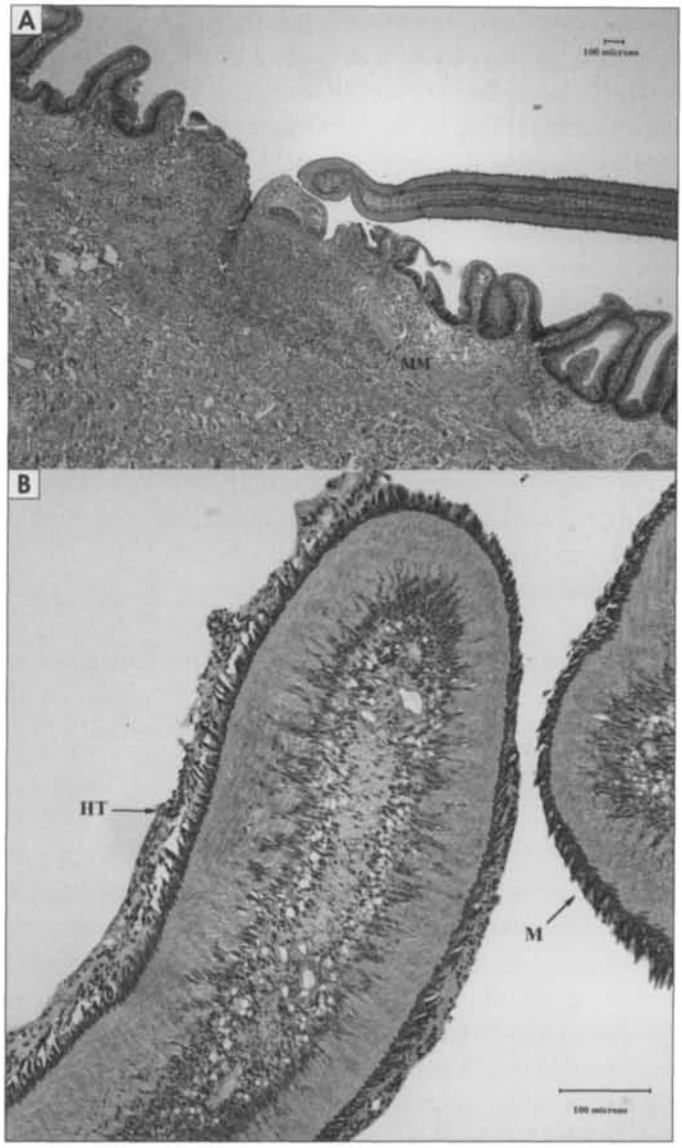


**Fig. 15.5A-B** **A.** A tetraphyllidean tapeworm attached to the spiral intestine of a blue shark *Prionacae glauca*. The strobila has prominent microtriches. The scolex is attached within a mucosal ulcer; glandular bothria closely adhere to a narrow intermediate layer continuous with the muscularis mucosae (MM). The lamina propria is infiltrated diffusely with mononuclear cells. **B.** Strobila of a tetraphyllidean tapeworm within the spiral intestine of a blue shark *Prionacae glauca*. Host tissue (HT) consists of necrotic material and leukocytes which adhere to prominent microtriches (M) on the tegumental surface.

a few weeks. Most tapeworms are hermaphroditic but rarely fertilize their own eggs; in most cases, cross-fertilization occurs among proglottids from different worms. Released eggs reach the environment via feces, thus, completing the life cycle. If infected intermediate hosts are ingested by an aberrant animal, i.e., one that lacks the proper environment for the larvae to mature, the plerocercoids again migrate into an extra-intestinal site and re-encyst. Many of the life cycles of fish cestodes are unknown, i.e., we do not know the intermediate or definitive host. The life cycles of some common fish tapeworms have been reviewed by several authors (Mackiewicz, 1972; Schäperclaus, 1992; Roberts and Janovy, 2005).

## Important Pathogens

Several reports on the most pathogenic adult (Sindermann, 1970; Hoffman, 1975; Dick and Choudhury, 1995) and larval (Williams, 1967; Sapozhnikov *et al.*, 1972; Nie and Pan, 1985; Ferguson, 1989; Mitchell, 1994) cestodes affecting fish both in the aquaculture industry and in the wild have been published. The authors generally agree that pathogenic changes in host tissues are more pronounced in infections with plerocercoids than with adult intestinal worms, and that the degree of pathology is related to the level of adaptation, i.e., no or minimal host injury is associated with cestodes that share a long evolutionary history with their host (Williams and Jones, 1994). Examples of such minimal pathogenicity include *Triaenophorus nodulosus* in perch *Perca* and numerous *Tetraphyllidea* and *Trypanorhyncha* in their shark definitive hosts (Borucinska and Caira, 1993). One has to stress, though, that exceptions to this rule are numerous, and the co-evolutionary relationships of parasites and their host fish cannot be assumed based on the degree of pathology and vice versa. The most significant pathogens in aquaculture and in wild fish include adult intestinal forms of *Bothriocephalus* spp., *Proteocephalus ambloplitis*, *Caryophylleus* spp. (cloweworms), *Khawia* spp., *Hunterella nodulosa*, *Triaenophorus nodulosus*, *T. crassus*, and *Eubothrium* spp. (Bauer, 1962; Williams, 1967; Paperna, 1991; Schäperclaus, 1992; Hoole, 1994; Dick and Choudhury, 1995). Tapeworm larvae causing considerable losses in cultured and wild fish include the plerocercoids of *Ligula* (strapworm disease), *Schistocephalus*, *Triaenophorus*, *Proteocephalus* and *Diphyllobothrium* spp. (see Hoffman and Dunbar, 1961; Esch and Huffines, 1973; Joy and Madan, 1989; Schäperclaus, 1992; Schabuss *et al.*, 2005).

## Disease Symptoms

The degree of pathology in cestodiasis depends on the interplay of several host- and parasite-related factors. With regard to the host, the species, body size/age, gut morphology, organs affected, and the fine balance between the protection and self-inflicted damage caused by the immune response to the parasite, all play a vital role. Also, the presence of concomitant stress caused by husbandry conditions like crowding and malnutrition, infections with other pathogens, exposure to environmental toxins, and immunosuppression (from any of the above) can significantly influence the degree of pathology and outcome of infection with the cestode. With regard to the parasite, one has to consider the intensity of infection, the behavior of the parasite within the host (stationary vs. migrating or 'grazing'), the size and metabolic demands of the worms, the morphology of the holdfast organ, and the level of molecular mimicry, which would generally down-regulate the immune response generated by the host.

Due to the complexity of parasite-host interactions mentioned above, one can observe a plethora of clinical manifestations in fish infected with plerocercoids. Disease symptoms vary from minimal to severe and can include phenotypic and behavioral alternations, growth retardation, weight loss due to disturbances in metabolism and function of internal organs, and mortality (Williams, 1967; Hoffman, 1975; Sweeting, 1977; McCormick and Stokes, 1982; Hoole and Arme, 1983; Rzeczkowska *et al.*, 1988; Joy and Madan, 1989; Weiland and Meyers, 1989; Rodger, 1991; Stoskopf, 1993; Noga, 2000). Acute infections with migrating plerocercoids tend to produce the most tissue damage and clinical signs of disease in the majority of infected fish (Esch and Huffines, 1973; Rosen and Dick, 1983). Contrary to the previous statement, muscle-invading plerocercoids like these of *Diphyllobothrium latum* and *Triaenophorus crassus* can cause no or little clinical symptoms, but the damage to the tissues, especially in high-intensity infections, can be considerable and result in economical loss due to market rejection of the infected fish (Rosen and Dick, 1983; Halvorsen and Andersen, 1984). Severe morbidity and high mortality can be associated with few plerocercoids damaging vital organs such as the myocardium or eyes, or with the high-intensity infections in young fish most commonly encountered in cultured systems (Halvorsen and Andersen, 1984; Weiland and Meyers, 1989).

*Diphyllobothrium* sp. plerocercoids in liver, peritoneum and heart can cause mortality in salmonids (Hoffman and Dunbar, 1961; Weiland and Meyers, 1989; Rodger, 1991; Rahkonen *et al.*, 1996). Larvae of *Gilguinia squali* can invade the eye and cause blindness in pen-reared chinook salmon, *Oncorhynchus tshawytscha* (Kent *et al.*, 1991). The plerocercoids of *Ligula* and *Schistocephalus* can exceed the body mass of the host, and the infection is marked by growth retardation, abdominal distension and diverse metabolic and endocrine alterations (Otto, 1972; Sweeting, 1977; Arme *et al.*, 1983; Szalai *et al.*, 1989; Hoole, 1994; Dick and Choudhury, 1995; Overli *et al.*, 2001; Hecker and Krabbe, 2005). In *Proteocephalus ambloplitis* in smallmouth bass *Micropterus dolomieui* (Esch and Huffines, 1973; McCormick and Stokes, 1982), in *Schistocephalus solidus* in female three-spined sticklebacks *Gasterosteus aculeatus* (Arme and Owen, 1967; Barber and Svensson, 2003), and in ligulosis in the European chub *Coregonus zenithicus* and in roach *Rutilus rutilus* (Sweeting, 1977), damage of the gonads is associated with decreased fecundity or infertility. In addition, the heavy metabolic burden of *Schistocephalus* plerocercoids might delete oocyte maturation, spermatogenesis, and inhibit shedding of oocytes resulting in larger ovaries in the post-spawning period in parasitized fish (Arme *et al.*, 1983). In ligulosis, in addition to reduced gonad mass and decreased plasma levels of the egg-precursor protein, vitellogenin, (Schabuss *et al.*, 2005), reduction in the number of pituitary basophilic cells (presumably the source of gonadotropins) has also been implicated in infertility (Arme *et al.*, 1983; Hoole, 1994).

Hematological findings in fish infected with plerocercoids of *D. dendriticum* included decreased hematocrit, and increased lymphocyte to erythrocyte ratios (Blanar *et al.*, 2005), and neutrophilia, lymphocytosis, and thrombocytopenia in experimentally infected brown trout *Salmo trutta* (Rahkonen and Pasternack, 1998). Packed cell volume was also decreased in three-spined sticklebacks infected with plerocercoids of *S. solidus* (Arme and Wynne, 1967) and in roach with plerocercoids of *Ligula* (Sweeting, 1977). Monocytosis, neutrophilia, low hemoglobin, and decreased total plasma protein coupled with hypergammaglobulinemia were observed in ligulosis (Sweeting, 1977; as reviewed by Arme *et al.*, 1983).

Lastly, phenotypic alterations in fish infected with plerocercoids, including severe abdominal distension and changes in body symmetry and tinctorial alterations, might contribute to increased predation of

parasitized fish as has been shown in fish infected with *Ligula intestinalis* plerocercoids, (Loot *et al.*, 2001). Similarly, a change in behavior observed in fish infected with plerocercoids of *Ligula* and *Schistocephalus* seems to enhance predation and thus transmission to the final host (Mackiewicz, 1988; Dick and Choudhury, 1995; Overli *et al.*, 2001; Brown *et al.*, 2002; Simková *et al.*, 2005).

Some studies on plerocercoid infections in wild fish have noted little or no pathology associated with the larvae which, coupled with increased numbers of plerocercoids in older fish and with a lack of tissue reactions (encystations), prompted a conclusion that there are no immune reactions to the parasites (Meyer and Vik, 1968).

The disease associated with adult intestinal tapeworms in fish can also manifest with a variety of signs (Williams, 1967; Hoffman, 1975; Paperna, 1991). In cultured freshwater fishes, generally no symptoms can be seen in light infections of adult fish, with the exception of khawiosis in carp (Schäperclaus, 1992). Heavy infections with intestinal tapeworms can manifest as behavioral changes, growth retardation, emaciation, anemia, abdominal swelling and bloating, secondary bacterial infections, gut distension, intestinal obstruction and perforation, and severe hemorrhagic enteritis (Williams, 1960; Scott and Grizzle, 1979; Andrews *et al.*, 1981; Pronina and Pronin 1982; Kurovskaya, 1991; Schäperclaus, 1992; Hoole, 1994; Dick and Choudhury, 1995). Larval fish are usually more severely affected by intestinal tapeworms than adult fish, for example bothriocephalosis in young fish can result in mortality approaching 100% (Sapozhnikov *et al.*, 1972; Da-Shu Nie and Jin-Pei Pan, 1985; Ferguson, 1989; Paperna, 1991; Mitchell, 1994).

In wild bony fish as also elasmobranchs, relatively few studies have investigated the pathology of intestinal or systemic cestodiasis (Mackiewicz and McCrae, 1962; McVicar, 1972; Borucinska and Caira, 1993; Borucinska and Dunham, 2000; Borucinska and Caira, 2006) and clinical data are obviously sparse. Because cestodiasis is quite common in marine fish with prevalence as high as 100% reported (Curran and Caira, 1995), and individual fish can harbor thousands of plerocercoids or adults (Sindermann, 1970), one is left to speculate about the potential of morbidity and mortaility in natural cestode infections in elasmobranchs and bony fish in their natural habitats.

## Diagnostics

For identification of adult and larval cestodes, one can refer to published identification keys (Schmidt, 1986; Khalil *et al.*, 1994) or atlases (Chitwood and Lichtenfels, 1973; Gardiner and Poynton, 1999).

## Pathogenesis

### *Interference with Fish Nutrition and Metabolism*

Tapeworms, both larval and adult, can generally have a negative impact on the growth and body mass of fish and can alter their body composition and metabolism (Boyce, 1979; Arme *et al.*, 1983; Deudero *et al.*, 2002; Barber and Svensson, 2003; Blanar *et al.*, 2005). These effects are caused by competition for nutrients, disturbances in digestion and absorption due to gastroenteritis, and by the induction of stress with resulting metabolic alterations (Sinderman, 1972; Pappas, 1983; Kurovskaya, 1991, 2001; Williams and Jones, 1994; Bosi *et al.*, 2005). In cestodes, external digestion takes place primarily by enzymes secreted from tegumental and apical gland cells. Nutrients are absorbed through the tegument by means of diffusion, receptor-mediated transport, and endocytosis (Arme *et al.*, 1983; Pappas, 1983; Befus and Podesta, 1984; Threadgold, 1984; Deudero *et al.*, 2002). This direct nutrient uptake involves the syncytial tegumental cells as well as their specialized microtriches that have been occasionally observed in close contact with the brush-border region of the host gut (McVicar, 1972; Pronina and Pronin, 1982; Izvekova *et al.*, 1997). This latter led to speculations that tapeworms actually use some enzymes secreted by the brush border, and possibly pancreatic enzymes, for their purpose. To facilitate digestion and absorption, the glycocalyx layer on the tegumental surface prevents the diffusion of enzymes and digested molecules away from the worm (Pappas, 1983). Parasite-derived lipases and phosphatases are associated with the apical plasma membrane of the tegument, and host-derived pancreatic amylase and proteases are found between the microtriches; interestingly tapeworms appear to inhibit pancreatic proteolytic enzymes like trypsin and chymotrypsin, and pancreatic lipases (Threadgold, 1984). An additional mechanism of nutrient uptake by some adult cestodes, as in *Echeneibothrium*, involves the myzorhynchus apical pad, which penetrates into the submucosa of the gut where macromolecules from damaged host cells are absorbed (McVicar, 1972; Dick and Choudhury, 1995).

Plerocercoids are believed to assimilate nutrients directly from surrounding tissues by means of absorption through microtriches (Read, 1966) and micropinocytosis by tegumental cells (Hopkins *et al.*, 1978). This absorption occurs after external digestion of host tissues by proteolytic secretions from the tegumental and/or apical glands (Coggins, 1980; Whittington and Cribb, 2001). In schistocephalosis, increased demand for food and oxygen and a decrease in feeding efficiency in fish has been shown, with plerocercoids growing faster than their fish hosts because they depleted their nutrients and contributed to acidosis via their metabolites (Arme *et al.*, 1983). Interestingly, in sticklebacks, *Gasterosteus aculeatus*, experimentally infected with plerocercoids of *Schistocephalus solidus* and fed ad libitum, the infected fish grew faster (excluding parasite weight) and maintained body condition similar to uninfected controls (Arnott *et al.*, 2000). A different experimental study of *S. solidus* in the same fish recorded decreased body weight, decreased perivisceral fat and larger ovaries in the infected individuals (Barber and Svensson, 2003; Barber, 2005). Within the same host/parasite system, Sweeting (1977) also noted that the plerocercoids obtained as much as 50% of the body mass of the infected fish.

### Tissue Pathology Associated with Plerocercoids

As a rule, migrating plerocercoids cause the most damage in fish (Sinderman, 1970; Dick and Choudhury, 1995) and more damage is caused in aberrant hosts and in fish kept in cultured systems (Williams and Jones, 1994). Coggins (1980) described proteolytic secretions from the apical organ in plerocercoids, which are most likely to be used for tissue migration. Similarly, rostellar hooks and suckers of *Neogryporhynchus cheilancristrotus* larvae were postulated to both aid in tissue penetration and to contribute to damage of host tissues (Molnar, 2005). Tissue migration of larval cestodes usually lasts several weeks and is associated with necrosis and an acute inflammatory response with influx of neutrophils, eosinophilic granulocytic leukocytes and macrophages. This acute inflammation, depending on the organ affected, can produce transmural gastritis, peritonitis and necrosis of multiple organs like the liver, kidneys, gonads, and heart (Arme and Wynne, 1967; Arme *et al.*, 1983). In chronic infections, the old tracts of migration, as well as the plerocercoids can be associated with neovascularization and mononuclear infiltrates composed of fibroblasts, macrophages, epithelioid cells,

multinucleated giant cells, lymphocytes, and plasma cells (Molnar, 2005). The final outcome of the migration, with plerocercoids 'established' at their destination site, might include fibrosis and encapsulation (Sharp *et al.*, 1992; Molnar, 2005), and mineralization (Arme *et al.*, 1983). Fibrosis resulting from repair of necrotic tissues, including serosal surfaces of abdominal organs, can result in adhesions of viscera. This, in turn, can lead to infertility, as suggested in *Proteocephalus* sp. infection in bluegill *Lepomis macrochirus* (see Mitchell *et al.*, 1983). The encapsulation of plerocercoids with host-derived connective tissue can be total (as in some *Diphyllobothrium* spp.) or partial (as in *Ligula* spp.) (Dick and Choudhury, 1995). In ligulosis, a layer of leukocytes (macrophages and neutrophils) adheres to the microtriches of the larva, and a layer of fibroblasts, necrotic cells and fibrous tissues forms around them. In spite of this cellular response, no damage to the plerocercoids was seen in cyprinid fishes with ligulosis (Arme *et al.*, 1983). If migrating plerocercoids invade the liver, necrosis, hemorrage and finally hepatic fibrosis occur (Arme *et al.*, 1983; Joy and Madan, 1989; Weiland and Meyers, 1989). In blue sharks *Prionacae glauca* infected with the post-larvae of *Hepatoxylon trichuri* (which attaches to serosal surfaces of abdominal organs, most commonly the liver), changes in hepatic capsule in the form of epithelial hypertrophy and subcapsular fibrosis were described (Campbell and Callahan, 1998). Although in the majority of *H. trichuri* infections, there is no significant reaction to the tentacles penetrating into the hepatic parenchyma (Fig. 15.6) the capsular epithelial changes progress to serosal metaplasia and dysplasia (Borucinska, unpublished) (Fig. 15.7). Pathology in wild salmonids infected with the plerocercoids of *Diphyllobothrium sebago* included fibrous peritonitis and transmural granulomatous gastritis (Van Kruiningen *et al.*, 1987).

### *Tissue Pathology Associated with Adult Intestinal Cestodes*

The intestinal cestodes are considered by many authors to be non- or minimally pathogenic (Williams, 1967; Mackiewicz, 1972; Dick and Choudhury, 1995; Ferguson, 1995). This notion, however, can be easily abolished through the examples of pathology provided below. Just as clinical manifestations of intestinal cestodiasis vary in severity, the histopathological lesions in the gut can range from minimal to severe. Also, the pathology of the gut depends on the stage of infection, i.e., in

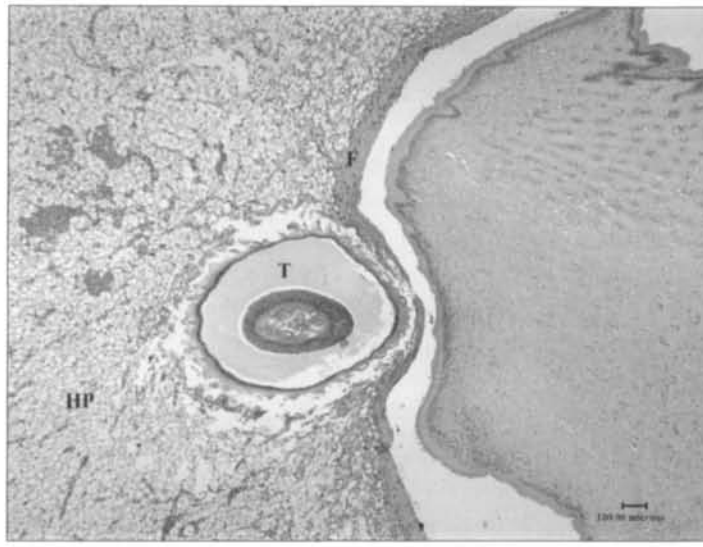


**Fig. 15.6** Larva of *Hepatoxylon trichuri* attached to the liver of a blue shark *Prionace glauca*. The tentacle (T) is inserted into the hepatic parenchyma (HP), and except subcapsular fibrosis (F) there is no host reaction to the parasite.

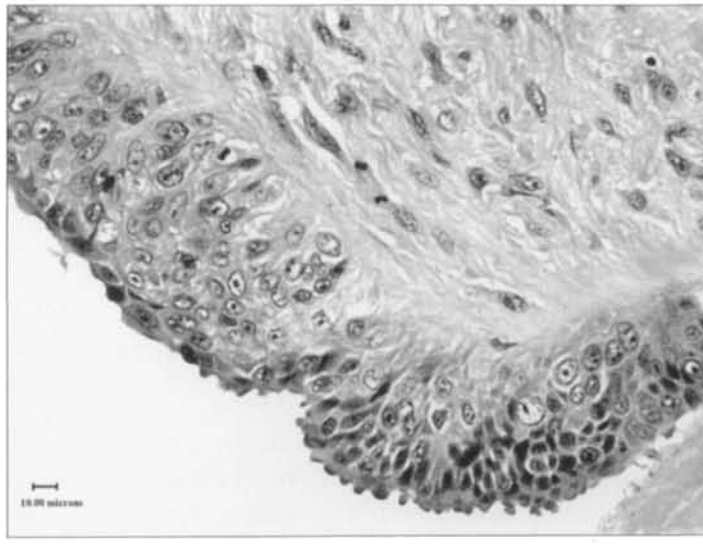

**Fig. 15.7** Liver capsule of a blue shark *Prionace glauca* adjacent to attachment site of *Hepatoxylon trichuri;* there is subcapsular fibroplasia and epithelial metaplasia and dysplasia.

many cestode infections the severity of pathology progresses over a period of time as the parasite burrows deeper into the tissues and host inflammatory responses develop. The mildest form of pathology is manifested as a non-conspicuous lymphocytosis and/or eosinophilic granulocytosis within the lamina propria and/or submucosa, shape conformation of mucosa and scolex, hypertrophy of enterocytes, pressure atrophy of enterocytes, and finally epithelial denudation/erosion with intact basement membrane (Figs. 15.8, 15.9). The most severe lesions can include necrohemorrhagic enteritis and effacement of the gut wall by fibrosis (Bauer *et al.*, 1973; Scott and Grizzle, 1979; Mitchell, 1994; Borucinska and Caira, 2006).

In evaluating the cestode-host interaction, one has to appreciate the obvious that the most opportunity to elicit pathology is created by the scolex morphologically differentiated into a holdfast organ, which actively grasps and/or penetrates host tissues. Some tapeworms apparently never engage in such close attachment, and in those cases, there is no easily appreciable morphological damage to host tissues (Borucinska and Caira, 1993; Williams and Jones, 1994). In cases where the scolex does engage the intestinal wall, a spectrum from no reaction (Fig. 15.9) to severe

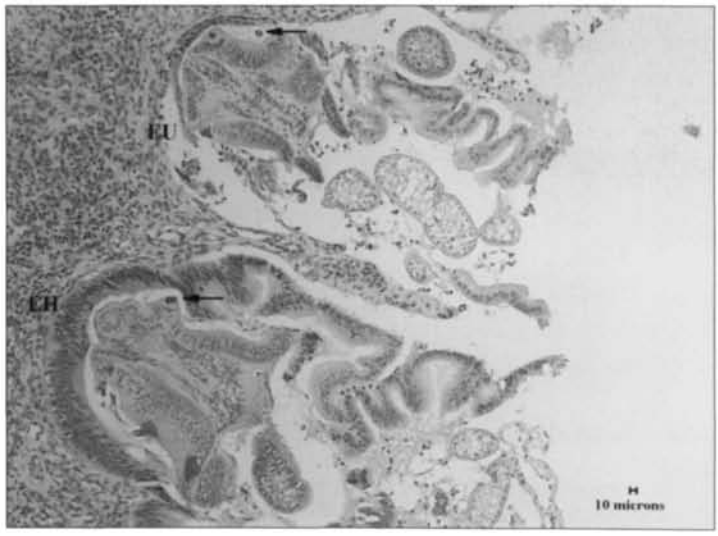


**Fig. 15.8** Two tetraphyllidean tapeworms attached within mucosal pits in the spiral gut of a shortfin mako shark *Isurus oxyrinchus*. There is shape conformation of the mucosa and scolex, enterocyte hypertrophy (EH) and epithelial ulceration (EU), as well as increased cellularity of the lamina propria. Arrows indicate hooks.

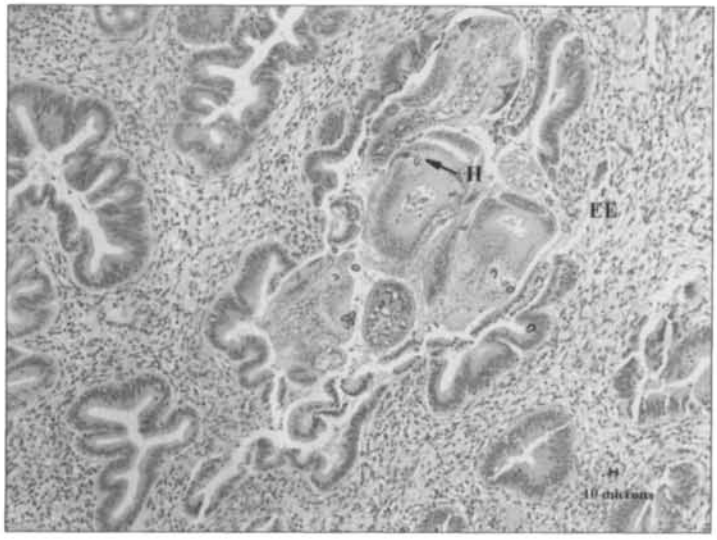


**Fig. 15.9** Four tetraphyllidean tapeworms attached within a mucosal fold in the spiral gut of a shortfin mako shark *Isurus oxyrinchus*. There are focal epithelial erosions (EE) of the mucosa. Arrow indicates a hook.

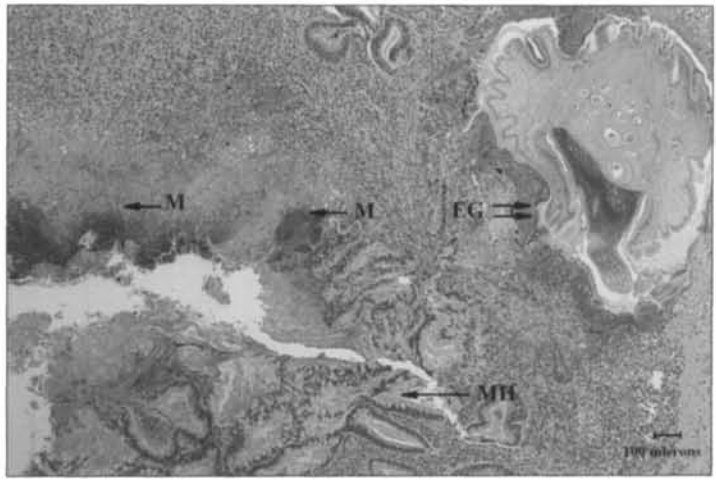


**Fig. 15.10** Adult trypanorhynch tapeworm attached to the duodenal portion of the spiral intestine in a shortfin mako shark *Isurus oxyrinchus*. Mineralized mucosa (M) borders an area of mucous hyperplasia (MH); the scolex is surrounded by degranulated and necrotic eosinophilic granulocytes (EG) and there is total effacement of normal intestinal components by necrosis and granulation tissue.

necrosis can be seen (Fig. 15.10), even in the same parasite-host model. As has been mentioned above, the latter is the result of the stage of the attachment that is minimal damage present early in the attachment and severe damage accompanies either long-term attachment sites, or when the cestode is moving about the mucosa selecting new and better sites for anchorage (see 'grazing' below). The pathology of cestode attachment as related to the presence of specific holdfast organs has been described in bony fishes (Mackiewicz *et al.*, 1972; Scott and Grizzle, 1979; Shostak and Dick, 1986; Karanis and Taraschewski, 1993) and elasmobranchs (McVicar, 1972; Borucinska and Caira, 1993; Williams and Jones, 1994). It is generally agreed upon that the severity of pathology as related to the holdfast organ is correlated with the size of the scolex, the length of the myzorhynchus, depth of penetration, and the presence of hooks and/or spined tentacles on the scolex (Williams and Jones, 1994). From studies of sharks and their intestinal tapeworms, it is evident that when the surface epithelium of the mucosa is not damaged during attachment, no appreciable inflammation is present, and the overall damage to the mucosa is minimal (Figs. 15.8, 15.9). On the other hand, when the basement membrane of the mucosal epithelium is disrupted and mucosal ulceration occurrs, an influx of degranulating eosinophilic granulocytes is always present (Figs. 15.10, 15.11) (Borucinska and Dunham, 2000). It is known that the lymphokines released by granulocytic cells and macrophages include pro-inflammatory amines, growth factors and chemoattractants for other leukocytes. Thus, degranulation of these cells is followed by a cascade of classic pathological events composed, in various proportions, of necrosis, edema, hemorrhage, neovascularization, granulation tissue, granulomatous inflammation and fibrosis. Tentacles and hooks appear to cause more severe mechanical damage to the mucosa than bothria or bothridia. Mechanical destruction created by the holdfast organ is enhanced by external digestion of host tissues by tapeworms. Such digestion is believed to be executed by proteolytic enzymes from the apical and neck glands (Mackiewicz and McCrae, 1967; Karanis and Taraschewski, 1993; Whittington and Cribb, 2001; Zd'árská *et al.*, 2004). The term glandular bothria reflects the fact that secretions from the above glands reach the tegumental surface on the bothria and, thus, facilitate their attachment (Fig. 15.5A). The softening of tissues created by the above process permits tentacle insertion (Fig. 15.12) and bothrial or bothridial adhesion (Fig. 15.13). The latter is achieved by the creation of a cementing eosinophilic intermediate layer that is closely opposed to host

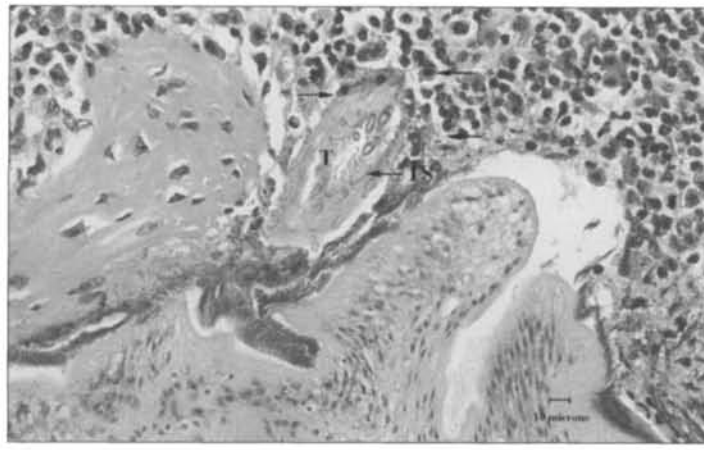


**Fig. 15.11** Adult trypanorhynch tapeworm attached to the duodenal portion of the spiral intestine in a shortfin mako shark *Isurus oxyrinchus*. Arrows point to degranulating eosinophilic granulocytes adjacent to a tentacle (T) which bears prominent tentacular spines (TS).

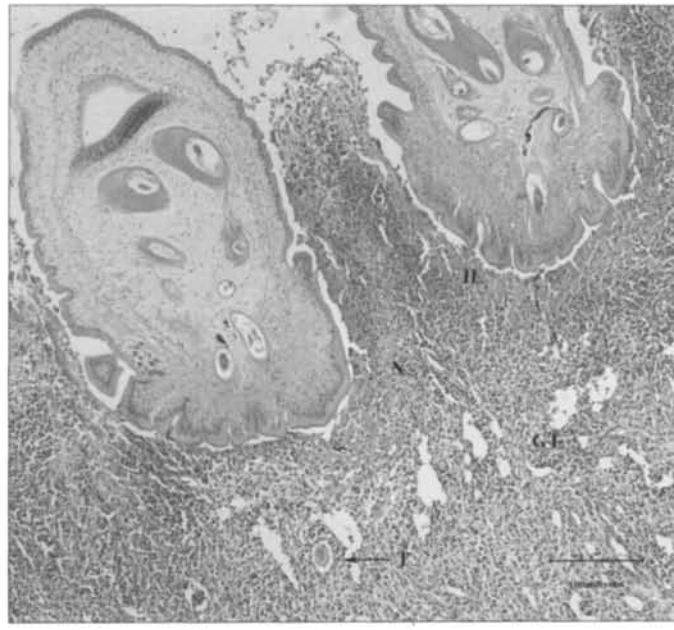


**Fig. 15.12** Adult trypanorhynch species *Heteronybelinia estigmena* attached to the pyloric gut of a bull shark *Carcharhinus leucas*. A narrow rim of intermediate layer (IL), followed by necrosis (N) and granulation tissue (GT) surrounds the scolexes. A cross-section of an everted tentacle (T) is visible deep within the granulation tissue.

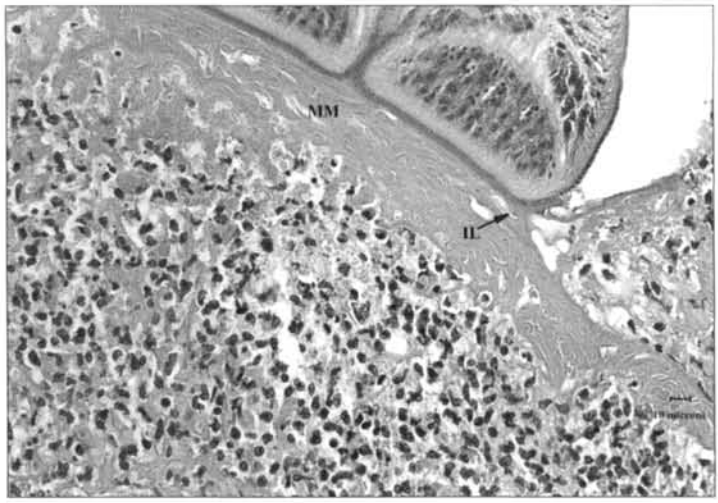


**Fig. 15.13** Adult tetraphyllidean tapeworm attached to the spiral intestine of a blue shark *Prionacae glauca*. Glandular bothria closely adhere to a narrow intermediate layer (IL) continuous with the muscularis mucosae (MM). The lamina propria is infiltrated diffusely with mononuclear cells and eosinophilic granulocytes.

tissues on one side, and microtriches of the worm on the other (Figs. 15.2, 15.12, 15.13). Although most investigators agree that the intermediate layer is composed of materials secreted from the apical and/or neck glands, ultrastructural studies have shown that the goblet cells of the host also discharge mucus into this layer (Karanis and Taraschewski, 1993). The role of scolex microtriches in attachment of adult and larval cestodes has been suggested by many authors (Hoole and Arme, 1983; Karanis and Taraschewski, 1993; Zdarska *et al.*, 2004). Also, a similar function, i.e., attachment along the intestinal mucosa, for microtriches found on the strobila has been shown with *Phyllobothrium thridax* in the European angel shark *Squatina squatina* (Williams and Jones, 1994).

The degree of pathology increases when tapeworms attach in large groups in one location. For example, Mackiewicz (1968) found 119 caryophyllaeid cestodes attached in one cluster in a natural infection of the spotted sucker *Minytrema melanops*. A similar tendency, albeit with lesser number of worms, has been observed by other authors (Borucinska and Caira, 2006) (Figs. 15.12, 15.14).

It has been postulated that intestinal cestodes detach periodically and move in a 'grazing fashion' about the mucosa, and diurnal migration

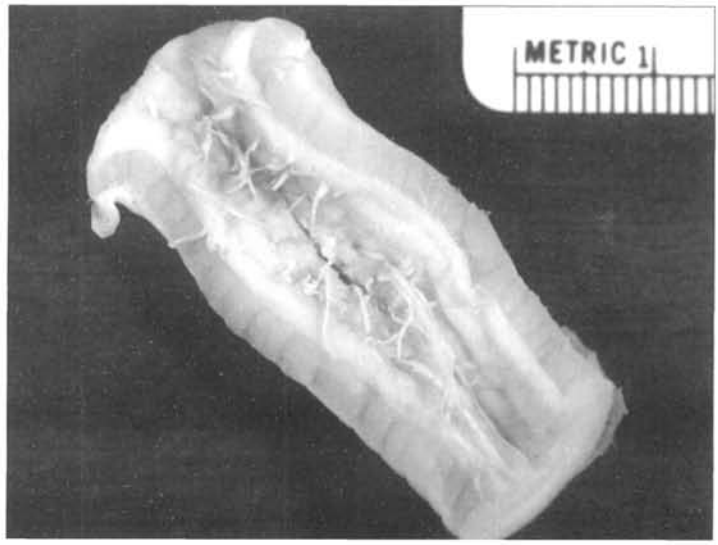


**Fig. 15.14** Numerous adult trypanorhynch tapeworms *Heteronybelinia estigmena* attached to the pyloric gut of a bull shark *Carcharhinus leucas*.

through the small intestine has been documented in some tapeworm infections (Pappas, 1983). This mobility would enable the cestode to escape the humoral- and cell-mediated specific and the non-specific immunity elicited by the prolonged presence in one location (Kern, 1998, Whittington and Cribb, 2001). This mobility might also account for the extensive mucosal lesions seen expanding beyond the point of attachment, a phenomenon frequently observed with intestinal tapeworms (Fig. 15.15A, B), (Pronina and Pronin, 1982; Hoole and Nisan, 1994; Borucinska and Caira, 2006). It is also possible, however, that such parasite-free lesions are the result of the combined cellular and humoral immune response resulting in destrobilation or death and removal of tapeworms from the gut of fish (Fig. 15.4).

Taking into account the phenomena described above, gastrointestinal lesions associated with attachment of intestinal tapeworms can be quite variable in quality and quantity. Provided below is a description of the pathological phenomena associated with intestinal tapeworms in fish that have been reported in the existing literature.

Mucosal necrosis, ranging from minimal to severe, has been described by many authors (Mackiewicz *et al.*, 1972; Scott and Grizzle, 1979; Pronina and Pronin, 1982; Shostak and Dick, 1986). Such necrosis can

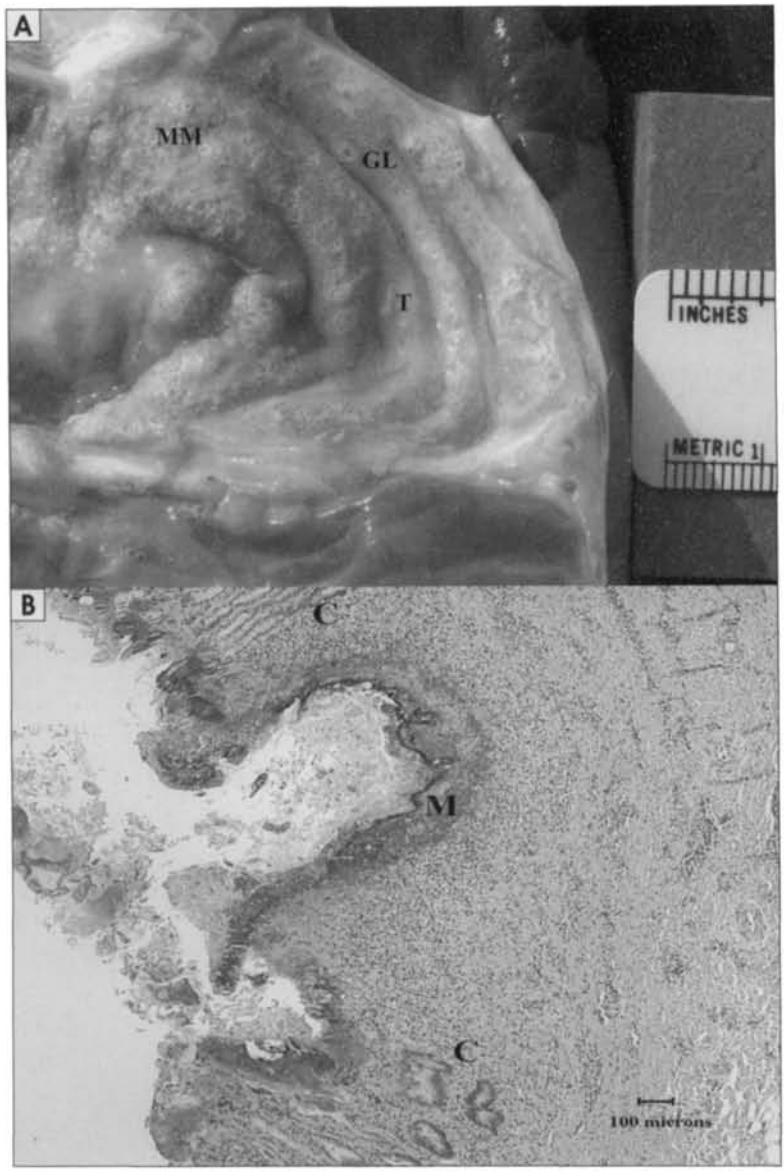


**Fig. 15.15A, B** **A.** Numerous adult trypanorhynch tapeworms (T) attached to the duodenal portion of the spiral intestine in a shortfin mako shark *Isurus oxyrinchus*; there is extensive mucosal mineralization (MM) with numerous 'grazing lesions' seen as ulcers with elevated margins (GL). **B.** Microphotograph of a grazing lesion from Fig. 15.15A; the lesion consists of a deep ulcer lined by mineralized necrotic tissues (MM) and bordered by hyperplastic intestinal crypts (CC).

take the milder form of epithelial erosions, with intact basement membrane (Figs. 15.8, 15.9), or the more severe form of mucosal ulceration with damage extending to the lamina propria or even becoming transmural which occasionally results in intestinal perforation (Mackiewicz *et al.*, 1972; Jara and Szerow, 1981; Hoole and Nisan, 1994; Mitchell, 1994). Acute ulceration is always accompanied by vascular changes in the intestinal wall. In the acute phases of disease these include congestion, edema and hyperemia (Fig. 15.16) (Pronina and Pronin, 1982), and if the necrosis involves the vasculature, hemorrhage is present (Sapozhnikov *et al.*, 1972; Scott and Grizzle, 1979). The necrosis of the epithelium and subepithelial structures as a rule provokes a host response in the form of inflammation. In the acute phase of inflammation eosinophilic granulocytes predominate (Fig. 15.11), and the massive degranulation of these cells creates a layer of eosinophilic granular necrotic material surrounding the scolex (Figs. 15.3, 15.10). In more

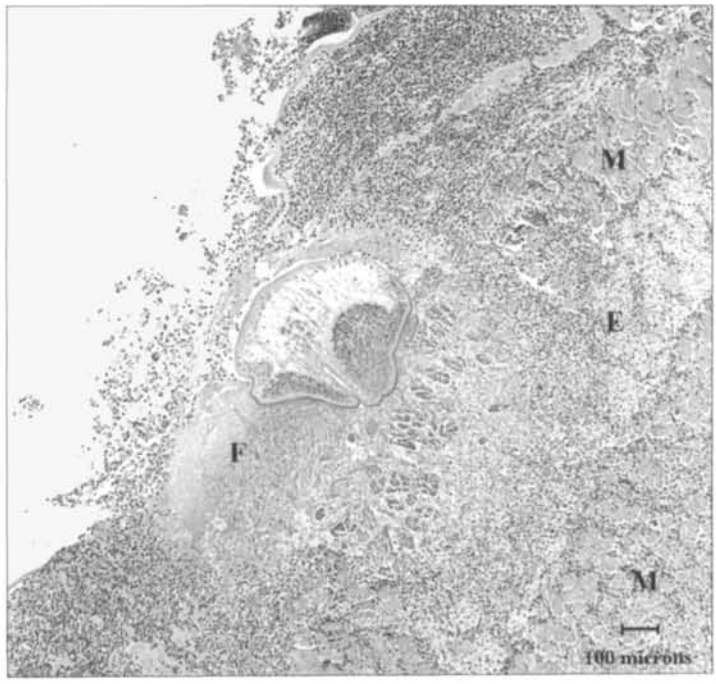


**Fig. 15.16** Scolex of an adult tetraphyllidean tapeworm penetrating into the submucosa of the spiral intestine of a blue shark *Prionace glauca*. There is acute transmural necrosis and hemorrhage with fibrin (F) and edema fluid (E) expanding the muscularis externa and interna (M).

chronic lesions mononuclear cells like macrophages, lymphocytes and fibroblasts predominate within the lesion (Mackiewicz *et al.*, 1972; Scott and Grizzle, 1979; Pronina and Pronin, 1982; Hoole and Nisan, 1994). The chronic inflammation usually develops within a few weeks after injury, and in some fish it takes the form of a granulomatous reaction typified by multinucleated giant cells, epithelioid cells and lymphocytes (Sharp *et al.*, 1991). The signs of tissue repair and/or regeneration appear in chronic lesions. These can take the form of granulation tissue (Figs. 15.4, 15.12), fibrosis (Fig. 15.17) and epithelial hyperplasia/hypertrophy (Figs. 15.8, 15.17). Interestingly, although neovascularization has been documented by several authors (Pronina and Pronin, 1982; Sharp *et al.*, 1991), only a few of the histopathological studies of cestodiasis in fish have mentioned the healing processes by granulation tissue (Shostack and Dick, 1986; Hermanns and Körting, 1987; Borucinska and Dunham, 2000; Borucinska and Caira, 2006).

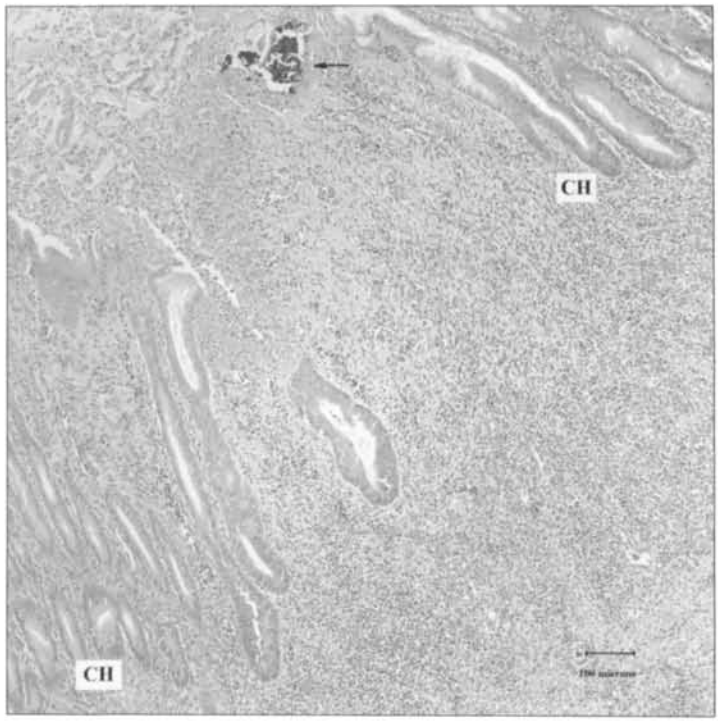


**Fig. 15.17** Chronic lesion in the pyloric gut of a bull shark *Carcharhinus leucas* infected with *Heteronybelinia estigmena*; the mucosa is effaced by necrotic and mineralized tissues (arrow) bordered by hyperplastic crypts (CH); there is transmural enteritis and fibrosis.

**Fig. 15.18** Pyloric gut (PG) at junction with the spiral intestine (SI) and glandular stomach (GS) in a shortfin mako shark *Isurus oxyrinchus*; there is extensive enteritis and mucosal mineralization with numerous 'grazing lesions', arrows point to tapeworms.

Connective tissue proliferation described as fibrosis, associated with scolex attachment, has been reported by several authors in bony fish (Williams, 1960; as reviewed in Sinderman, 1970; Mackiewicz, 1972; Mackiewicz *et al.*, 1972; Bauer *et al.*, 1973; Scott and Grizzle, 1979; Jara and Szerow, 1981; Pronina and Pronin, 1982; Shostak and Dick, 1986). In elasmobranchs, Borucinska and Dunham (2000) reported a submucosal fibroblastic response in the pyloric stomach of a blue shark (*Prionace glauca*), infected with a species of *Tentacularia*.

Although mineralization of necrotic histozoic parasites is frequent, mineralization in association with intestinal cestodes has been rarely reported (Williams and Jones, 1994; Borucinska and Caira, 2006). Mineralized mucosal lesions are frequently associated with parasites attached within the junction between glandular stomach/pyloric gut and pyloric gut/spiral intestine in sharks (Figs. 15.4, 15.10 and 15.18) (Borucinska, unpublished data). The pathogenesis of this mineralization might involve the combination of any of the following: necrosis, low gastric pH, bile salts, pancreatic secretions, and specific parasite secretions, but further studies are needed to elucidate the pathogenesis of such lesions.

Another lesion observed frequently in association with intestinal cestodes is a spectrum of proliferative responses involving the mucosal epithelium and/or underlying connective tissues. A tendency of fish tissues to proliferate in response to injury has been noted previously in bony fishes (Jara, 1959) and elasmobranchs (Borucinska *et al.*, 2002). The most common of these hyperplastic reactions involves goblet cell hyperplasia/mucous metaplasia with a resulting increase in mucus secretion (Fig. 15.10) (Scott and Grizzle, 1979; Jara and Szerow, 1981; Pronina and Pronin, 1982; Shostak and Dick, 1986; Borucinska and Caira, 2006). The presence of such lesions might be due to regional anatomo-physiological features of the mucosa and they are most likely dependent on neuroendocrine factors that enhance mucous differentiation as described below.

Diffuse crypt hypertrophy, hyperplasia, metaplasia and dysplasia in response to extensive mucosal damage causally linked to cestodes have been described from elasmobranchs (Figs. 15.17, 15.19) (Borucinska and Dunham, 2000; Borucinska and Caira, 2006), and regenerative crypt hypertrophy and hyperplasia have been reported from bony fish (Williams and Jones, 1994). The mucosal dysplasia in the gut of the bull shark *Carcharhinus leucas* and the intestinal metaplasia in the glandular stomach of the sicklefin weasel shark *Hemigaleus microstoma* (Borucinska and Caira, 2006) are interesting for a couple of reasons. First, dysplasia and metaplasia are common responses of the mucosa to injury in higher species of animals—including humans—and the compatibility of tissue responses in distantly related species points to similar, evolutionary conserved, mechanisms of cellular healing. Secondly, intestinal metaplasia of gastric glands is a condition predisposing to gastric cancer in humans, and interestingly enough, intestinal metaplasia was reported in a shark with a benign gastric tumor (Borucinska and Bogicevic, 2004). Although no association between this tumor and tapeworm attachment was seen in this case, the paper suggested chronic gastritis as one of the pathogenic mechanisms involved in tumor formation. Thus, the causal relationship between chronic parasitic gastroenteritis and gut tumors in sharks would be an interesting topic to explore. A causal link of neoplastic lesions to parasite-induced chronic inflammation has been made previously in cases of esophageal tumors with *Spirocerca lupi* infections in dogs and *Schistosoma haematobium* and urinary bladder cancer in humans (Roberts and Janovy, 2005).

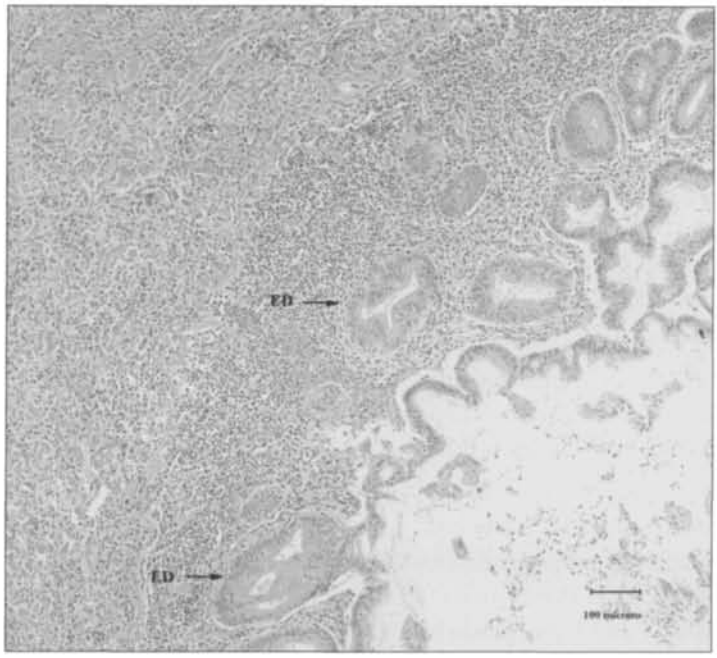


**Fig. 15.19** Pyloric gut of a bull shark *Carcharhinus leucas* infected with *Heteronybelinia estigmena*; the healing of parasite-induced mucosal ulcers is characterized by adenomatous hyperplasia of intestinal crypts with features of mild dysplasia (ED); there is chronic transmural granulomatous enteritis with fragmentation and disorganization of muscle cells within the muscularis.

The final result of healing of parasite-induced lesions in the gut often results in fibrosis which can be associated with mucosal thinning and attenuation (Scott and Grizzle, 1979) and in its most severe form, with atrophic gastritis, as has been described in pike *Esox lucius* infected with *Triaenophorus nodulosus* (Pronina and Pronin, 1982).

## The Role of Host Immunity in the Pathogenesis of Lesions Associated with Cestodiasis in Fish

Immune reactions stimulated by the presence of cestodes can lead to death and removal of the parasites, but they can simultaneously contribute to the development of lesions via pro-inflammatory and/or cytotoxic serum components and cytokines secreted by activated inflammatory cells. The varying degrees of inflammation seen in tissues of different species of fish infected with the same species of cestodes suggests,

that different mechanisms of immunity are activated in particular fish-host systems.

Fish possess an immune system that is similar to higher vertebrates, with both innate and specific (cellular and humoral) immunity (Nakanishi *et al.*, 1999). Within the gut, the first line of defense is created by a protective layer of mucus that contains IgM, and also by immunoglobulins in the bile. The second level of protection consists of immune surveillance executed by intraepithelial lymphocytes of the T, B, and natural killer cell (NK) lineages, as well as antigen presenting cells, the macrophages (Kaattari and Piganelli, 1996). Within the lamina propria, many fish species possess lymphofollicular aggregates which are the sites of B cell generation, and in all fish, granulocytes, macrophages, lymphocytes, and plasma cells can be found scattered in small numbers in the submucosal tissues even in apparently lesion-free guts. The leukocytes are a source of antibodies, pro-inflammatory cytokines, growth factors, and reactive oxygen intermediates. In addition, serum factors—most of which are secreted by liver cells—such as C-reactive proteins and complement have been documented in fish (as reviewed by Taylor and Hoole, 1995). The above components of the innate and specific immunity are activated in fish in response to cestode infections (both adult and larval forms). According to many authors, the immune response to intestinal cestodes in fish is less pronounced than the response elicited by the invasive plerocercoids (as reviewed by Nie and Hoole, 1999), a finding in agreement with data from mammalian host-cestode models (Fraize *et al.*, 2004).

The innate immunity involved in protection against parasites in fish encompasses several mechanisms. These include alterations in the secretion and chemical properties of intestinal mucus, activation of serum complement, increased secretion of C-reactive proteins CRC from hepatocytes, increased secretion of lysozyme from leukocytes, leukocytosis, and granulocytosis with increased generation of reactive oxygen intermediates ROI by neutrophils. In addition, the major histocompatibility complex molecules (MHC) might contribute to different innate responses and thus susceptibility of fish to infections with particular cestodes.

In intestinal cestodiasis, an increase in mucus secretion by intestinal goblet cells, as well as a change from neutral to acidic sulfated and mixed glycoconjugates was observed in brown trout *Salmo trutta* infected with

*Cyathocephalus truncatus* (Bosi *et al.*, 2005). Also, an increase in numbers and altered distribution of neuroendocrine cells within the gut, particularly serotonin and bombesin secreting cells, was detected in the same fish-parasite system (Dezfuli *et al.*, 2003). It is known from studies in mammals that serotonin increases mucous secretion, and that this, coupled with increase in mucus acidity, may aid in the exclusion of tapeworms from the gut. Apart from mucus being a mechanical and chemical barrier preventing attachment and penetration of parasites, mucus in fish contains IgM antibodies; thus, the goblet cell hyperplasia and/or mucous hypersecretion in response to cestodiasis increases the concentration of lumenal immunoglobulins in the gut (Kennedy-Stoskopf, 1993).

Leukocyte adherence to larval stages of tapeworms was documented in cyprinids with ligulosis (Joy and Madan, 1989) and macrophages, eosinophils and lymphocytes were observed on the tegument surface of *Bothriocephalus acheilognathi* in carp *Cyprinus carpio*, where it was shown that this adherence is serum mediated (Hoole and Arme, 1986; Hoole and Nisan, 1994). Since incubation of plerocercoids with heat inactivated serum eliminated the leukocyte adherence, it was believed to be mediated by complement and possibly antibody. As has been shown by ultrastructural studies in rats infected with intestinal *Hymenolepis diminuta*, complement-mediated killing and/or destrobilation of parasites occurs via necrosis of the surface layers of the tegument, with complete lysis of the microtriches (Robinson *et al.*, 1987), and a similar mechanism might operate in fish. C-reactive proteins have been demonstrated in serum of fish infected with bothriocephalosis (Fletcher *et al.*, 1980) and ligulosis (Hoole and Arme, 1983). An increase in lysozyme activity coupled with increased IgM levels in plasma has been reported in carp with intestinal *Ptychobothrium* sp. infection (Dautremepuits *et al.*, 2004). In many infections, granulocytosis (predominantly neutrophilia) followed by lymphocytosis have been reported (Rahkonen and Pasternack, 1999). The neutrophilia has been associated with elevated levels of ROIs, including nitrous oxide (Dautremepuits *et al.*, 2002), which apparently plays a crucial role in protective immune responses in primary infections with larvae of *Schistocephalus* (Scharsack *et al.*, 2004) and *Ligula intestinalis* in gudgeon *Gobio gobio* L. (Taylor and Hoole, 1995). It has also been shown that extracts from the plerocercoids of *Diphyllobothrium dendriticum* induce the production of two potent ROIs, hydrogen peroxide and

superoxide anion from macrophages in the rainbow trout *Onchorhynchus mykiss* (Sharp *et al.*, 1991). Macrophages, in addition to phagocytosis, play a significant role in the scavenging of free radicals generated during leukocyte activation. Melanin, and possibly other pigments, from melanosomes in macrophages quenches the free radicals and it also might protect against lytic enzyme systems and, thus, interfere with parasite invasion (Williams and Jones, 1994). The reported increase of melanomacrophage centers in lymphoid organs of fish in cestode infections must be viewed with caution, as this is a common reaction to environmental stress including pollution and poor water quality.

The diversity of proteins within the major histocompatibility complex (MHC) seems to influence the innate resistance of sticklebacks to infection with *S. solidus* (Kurtz *et al.*, 2004). A high level of MHC diversity resulted in less pathology, decreased worm mass, increased numbers of granulocytes and concomitant increase in their respiratory burst and oxidative killing of parasites. The role of MHC in host specificity or resistance to infection in the cestode/fish system needs to be elucidated further.

Evidence for an activated specific cellular immunity in plerocercoid infections consists of leukocytosis (Rahkonen and Pasternack, 1999; Scharsack *et al.*, 2004), and the fact that the cyst wall surrounding plerocercoids is frequently infiltrated by lymphocytes, macrophages and epithelioid cells (Sharp *et al.*, 1989; Rodger, 1991). Cellular responses to *Ligula* plerocercoids have been described by Hoole and Arme (1983) who observed macrophages and neutrophils damaging the tegument and who, in further studies, demonstrated complement mediated leukocyte adherence and possibly killing of plerocercoids (Hoole and Arme, 1986, 1988). Ultrastructurally phagocytosis of microtriches by macrophages and neutrophils and focal denudation of the tegument to the level of basal lamina was observed in *Ligula* sp. plerocercoids coated with leukocytes (Hoole and Arme, 1986). Although, as described earlier, the neutrophils are a part of the innate immunity, the involvement of macrophages and lymphocytes strongly supports the notion that specific cell-mediated immunity is participating in the larval killing. It has been postulated that cellular adherence to cestodes is enhanced by several opsonins from serum including CRP, complement and IgM (Taylor and Hoole, 1995). The role of cellular immunity in the killing and rejection of adult intestinal cestodes is somewhat less clear (Fig. 15.5B). Adhesion of leukocytes to tegumental

surface of adult *B. acheilognathi* within the intestine of carp was shown by ultrastructural studies to result in desmosome damage and disruption of the tegument (Hoole and Nissan, 1994), but the role of cell-mediated immunity in destrobilation or damage and detachment of the strobila and/or scolex needs to be elucidated in further research.

Although fish synthesize only one class of immunoglobulins—which is equivalent to the mammalian IgM—the importance of humoral specific immunity in fish is well established. IgM is tetrameric in bony fishes, and monomeric or pentameric in elasmobranchs. The piscine antibody has been shown to be active in complement activation as well as neutralization, opsonization and precipitation, and it is found in intestinal and epidermal mucus (Kennedy-Stoskopf, 1993). Although initial attempts to isolate parasite epitope-specific antibodies from sera of fish with cestodiasis have been unsuccessful, the rejection of plerocercoids in fish kept at higher temperatures suggests that this rejection was antibody based (Kennedy and Walker, 1969). In addition, there has been ample morphological evidence of stimulated humoral response in the gut of the majority of fish infected with cestodes. This evidence consists of intralesional plasma cells (Pronina and Pronin, 1982; Hermanns and Körting, 1987), diffuse lymphocytosis within the intestinal wall (Bauer *et al.*, 1973; Pronina and Pronin, 1982; Shostack and Dick, 1986; Karanis and Taraschewski, 1993; Hoole and Nisan, 1994), and the formation of lymphofollicular structures in the submucosa (Fig. 15.20) (Borucinska and Dunham, 2000; Borucinska and Caira, 2006). The explanation for such lymphofollicular hyperplasia might include the presence of B cell-stimulating (Th2 inducing) antigens of worm origin, but this requires confirmation in experimental studies. Such studies are also needed because intralesional bacteria are commonly encountered in cestode-associated lesions in the gut (Borucinska and Caira, 2006), and the role of bacteria as secondary pathogens contributing to the pathogenesis of parasitic lesions and to the immune stimulation needs to be clarified.

Direct evidence of humoral immune responses has been provided by studies in which precipitating antibodies to phosphorylcholine, a haptenic group on the surface of many infectious organisms including the tegument of cestodes (Fletcher *et al.*, 1980; Lal and Ottesen, 1989; Choudhury and Dick, 1994; Dick and Choudhury, 1995) have been detected. Hole and Arme (1988) suggested that the binding of CRP from fish serum to phosphorylcholine within the tegument of plerocercoids creates a

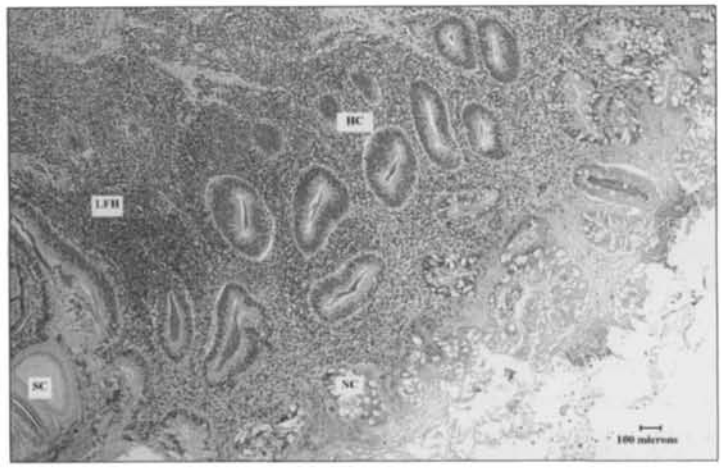


**Fig. 15.20** Adult trypanorhynch tapeworm within the pyloric gut of a sicklefin weasel shark, *Hemigaleus microstoma*; the scolex (SC) attaches within hyperplastic crypts (HC) separated by a lamina propria with prominent lymphofollicular structures (LFH); there is mucous hyperplasia of the edematous and necrotic superficial mucosa (NC).

complete antigen (Ag), which induces the secretion of anti-phosphorylcholine antibodies (Ab). Both the antibody/antigen and CRP-phosphorylcholine complexes are believed to activate the classical complement pathway which most likely results in complement-mediated parasite killing (Hole and Arme, 1983, 1986). It is noteworthy that a combination of complement and antibody-mediated immunity has been suggested to be responsible for the resistance of the Thorny skate *Raja radiata* to the tetraphyllidean cestode *Acanthobothrium quadripartitum* (McVicar and Fletcher, 1970). In addition to the anti-phosphorylcholine antibodies, antibody response to various antigens from plerocercoid homogenates of *Diphyllobothrium* spp. (Sharp *et al.*, 1989, 1992) and *Ligula intestinalis* (Sweeting, 1977) were detected in trout and roach, respectively. Also, plaque-forming cells were found in the pronephros and spleen of roach exposed to extracts of *L. intestinalis* larvae (Williams and Hoole, 1992). In carp, a proliferation of pronephric lymphocytes was induced by an *in vitro* exposure to extracts of *B. acheilognathi* (Nie *et al.*, 1996), and later studies detected antibody response to protein extracts of adult *B. acheilognathi* in sera of carp (Nie and Hoole, 1999). The latter study used experimental carp that received a series of intraperitoneal injections of worm protein extracts; the sera from these fish tested positively for over 200 days post-injection in an ELISA.

## Immune Evasion

The lack of immune reaction to some cestode infections in fish is supported by several observations, including the lack of acquired immunity to most cestodes seen as increased numbers of plerocercoids in older fish and annual re-infections of fish with the same intestinal cestode species. The lack of tissue reactions to attached intestinal cestodes, and a lack of cellular reactions to tissue plerocercoids further support this theory. The latter was described in *D. ditremum* in arctic charr *Salvelinus alpinus* (Halvorsen and Andersen, 1984) and in some cyprinids infected with *Ligula* spp. (Hole and Arme, 1983). Such anergy in fish could be a result of molecular mimicry on part of the cestode, which would be enhanced by a long evolutionary association between the parasite and host. Such molecular mimicry can also be achieved by absorption of host-derived proteins onto the glycocalyx as was observed in plerocercoids of *Ligula* and *Schistocephalus* (Hoole and Arme, 1986). That host-parasite adaptation plays a role in anergy is supported by observations that many tapeworms elicit severe inflammation and thus pathology in incidental fish hosts (Scharsack *et al.*, 2004), while they are not pathogenic to their normal hosts (Hoole and Arme, 1983). As exceptions to every rule exist, only a few specimen of *Khawia* spp. can inflict severe inflammation-mediated damage in its natural host, the carp (Schäperclaus, 1992).

A different mechanism of immune evasion has been suggested for plerocercoids of *Ligula intestinalis* (Hoole, 1994), and *Schistocephalus solidus* (Scharsack *et al.*, 2004). These parasites evade cell-mediated damage by altering granulocyte and lymphocyte activity. Yet another strategy of immune evasion was suggested by Hoole and Arme (1988) who have shown that leukocyte adherence to plerocercoids of *L. intestinalis* is inhibited by phosphorylcholine, a common hapten of the tegumental brush border. The tegument has also been shown to recycle its outer layers including the glycocalyx; fragments of old apical tegumental cell membrane are either endocytosed (in Pseudophyllidea) or shed (in Cyclophyllidea). Such shedding or turnover of antigens leads to immune evasion (Threadgold, 1984). In addition to the mechanisms described above, the use of blocking antibodies that coat the surface of plerocercoids has been postulated as an immunoprotective strategy used by larval cestodes (Sharp *et al.*, 1989).

Evasion of non-specific immune mechanisms is achieved in carp infected with adult cestodes of *Ptychobothrium* sp. through stimulation of

antioxidant enzymes that prevents tegument damage by oxidative burst from activated leukocytes (Dautremepuits *et al.*, 2003). Lastly, immunosuppressive effects based on alterations in neuroendocrine functions compatible with chronic stress have been proposed to play a role in the lack of immune reactions to some parasitic cestodes (Overli *et al.*, 2001).

## Treatment and Prophylaxis

The treatment and prophylaxis of specific cestode infections in fish are provided by several authors (Schäperclaus, 1992; Stoskopf, 1993; Noga, 2000). The drug of choice for intestinal adult tapeworms is Praziquantel. It can be applied as a bath (1-10 mg/liter for 3 hrs) or as medicated food or individual per os dosage of 35-125 mg/kg for up to 3 days. Di-n-butyl tin oxide has also been used in a per os dosage at 250mg/kg for 3 days (Merck Veterinary Manual, 1998). It must be emphasized that the dynamics of drug interactions and drug metabolism and efficacy in fish are largely unknown (Hoole, 1994).

Prevention strategies that could lower morbidity and eliminate mortality in fish with cestode infections should include various husbandry techniques and vaccinations. Husbandry techniques aimed at the removal of intermediate and/or final host are difficult and have been only partially successful (Hoole, 1994; Dick and Choudhury, 1995). A very important element of husbandry aimed at disease prevention on the global scale is the monitoring of fish trade and the translocation of only parasite-free fish.

Vaccine development for tapeworms in fish is hampered by inadequate knowledge of the immune processes active in response to these parasites and currently, there are no vaccines available for the prevention of tapeworm infection in fish.

## Epidemiology

Most species of fish studied harbor larval or adult cestodes (Cheung 1993; Dick and Choudhury, 1995; Caira and Healy, 2004; Palm, 2004), and the number of worms recovered from one fish can vary between a few and several thousand (Calentine and Ulmer, 1961; Curran and Caira, 1995). Schäperclaus, (1992) reported 300 worms per 30 cm of gut in caryophyllosis in cyprinids, and 1,700 worms per fish in eubothriosis in

pyloric caeca of trout. Several hundred plerocercoids per fish can be present, and in cases of *Ligula* and *Schistocephalus*, the plerocercoids can aquire a biomass in excess of 50% of the body weight of the host (Otto, 1972; Dick and Choudhury, 1995). Over-aggregation is a common phenomenon reported from many cestode infections (Dick and Choudhury, 1995). It consists of a small group of fish within a population carrying the majority of the cestode burden. It has been suggested that over-aggregation is caused by feeding patterns of predatory fish, genetic susceptibility to infection, efficiency in immune rejection of parasites, and heterogeneity among hosts in the exposure to infective parasite stages, but the exact cause of it remains unknown (Knudsen *et al.*, 2004).

Because most of the well-studied tapeworm infections of the gut in bony fish have seasonal peaks, conclusions about prevalence and intensity of intestinal tapeworms should be based on more than one observation. Prevalence can be as high as 97% in some populations of overwintering fish, or when the intermediate hosts are abundant (Schäperclaus, 1992; Choudhury, 1995), and then the prevalence declines sharply due to changes in feeding habits of fish or the intrinsic patterns of seasonality and shedding of particular parasite-host systems. Prevalence as high as 100% has been reported from wild fish infected with plerocercoids, and prevalence tends to increase in older fish (Meyer and Vik, 1968; Halvorsen and Andersen, 1984).

The fact that some intestinal tapeworm infections in bony fishes are of limited duration is supported by several field observations from farmed fish (Jara and Szerow, 1981; Nie and Pan, 1985; Karanis and Taraschewski, 1993; Luo *et al.*, 2003). In non-cultured fish, circumstantial data on shedding of intestinal cestodes were provided by Kennedy and Walker (1969) for dace *Leuciscus leuciscus* infected with the cestode *Caryophyllaeus laticeps*, but no other data on lifespan, shedding or seasonality of tapeworms exist for wild bony fishes and elasmobranchs. In bony fish raised in aquaculture systems, most infections have a one-year life cycle with infections occur during summer and fall and adult tapeworms reaching maturity in spring and early summer (Paperna, 1991). Adult tapeworms might be shed during the summer. In pike *Esox lucius* the shedding of *T. crassus* is synchronized with spawning (Shostack and Dick, 1986; Dick and Choudhury, 1995; Simková *et al.*, 2005). Some authors suggest that summer shedding is related to increases in temperature which enhances immunity in fish (Mackiewicz, 1972) with the resultant immune

destrobilation and/or killing of the intestinal cestodes (Bauer *et al.*, 1962; Mackiewicz, 1972; Roberts and Janovy, 1996). Other authors suggest that shedding might be associated with short life spans of worms (Knudsen *et al.*, 2004). Seasonality might also be related to abundance or presence of intermediate hosts (Mackiewicz, 1972; Dick and Choudhury, 1995; Simková *et al.*, 2005).

Many tapeworm species are cosmopolitan in distribution as they follow the distribution of their final host (Dick and Choudhury, 1995; Luo *et al.*, 2003). The latter is exemplified by plerocercoids of *Pyramicocephalus phocorum*, which infect fish in geographical ranges inhabited by its final host, the arctic seal (Sinderman, 1970). In several cases, including *Bothriocephalus* and *Khawia* spp.—which are one of the economically most important pathogens in aquaculture—the translocation of infected definitive host has been known to spread the disease worldwide and often into native, wild fish populations (Hickey and Harris, 1947; Andrews *et al.*, 1981; Hoole, 1994; Dick and Choudhury, 1995; Vincent and Font, 2003). The ability of tapeworms to infect new intermediate or final hosts relates to their specificity. Although host specificity of tapeworms has not been firmly established for most host-parasite systems, adult tapeworms are considered to be more host-specific than plerocercoids (Dick and Choudhury, 1995; Luo *et al.*, 2003; Vincent and Font, 2003), while intestinal tapeworms of sharks are considered to be the most host specific (Williams and Jones, 1994). For example, *Triaenophorus nodulosus* has 78 intermediate host species and only pike *Esox* spp. as its final host. Plerocercoids of *T. crassus* have been described from 39 fish species, but only whitefish and cisco *Coregonus* spp. serve as definitive hosts (Dick and Choudhury, 1995). Specificity might be caused by immune rejection of parasites or feeding habits of fish that exclude appropriate intermediate hosts.

## Economical Importance

The importance of cestode induced disease in fish is significant both in popular game fish (Rahkonnen *et al.*, 1996) and in aquaculture (Weiland and Meyers, 1989). In cultured conditions, cestode infections can manifest in the form of decreased productivity from direct losses through mortality, and indirect losses due to lowered resistance to other stressors, metabolic alterations affecting growth, and decreased marketability of the infected flesh (Sinderman, 1970; Dautremepuits *et al.*, 2003). As noted by

Dick and Choudhury (1995), warm water aquaculture is mostly affected by the now cosmopolitan, but originally Asian, pseudophyllidean tapeworm *Bothriocephalus acheilognathi*. In Europe, Asia and North America, *Caryophylleus* spp., *Khawia* spp., and *Triaenophorus* spp. are impacting fish in both aquaculture and natural habitats.

## Outlook/Recent Developments

There are many factors influencing parasite-host relationships, some of which have been discussed in this chapter. One of the factors that have been poorly studied is the sum of environmental conditions that can alter the parasite-host interaction patterns. Of particular interest are the anthropogenic environmental pollutants because they are widespread in aquatic habitats, and many of them bioaccumulate and biomagnify in the aquatic food chain. The study of parasites as bioindicators of environmental pollution has a long history, which has been reviewed by MacKenzie *et al.* (1995). Most of our knowledge in this area comes from studies on the effects of thermal pollution, metals, and hydrocarbon molecules from oil spills. The data point to a complicated web of interrelationships that include both biotic and abiotic factors that can affect the dynamics of fish-parasite relationships. Some of the parasite-related factors that lead to enhanced toxicity of pollutants in parasitized fish include altered mucosal barriers, immunosuppression due to damage to the lymphopoietic organs, increased stress responses negatively affecting cellular immunity, and lastly, metabolic alterations (Boyce and Yamada, 1977; Dautremepuits *et al.*, 2004; Hecker and Krabbe, 2005). Boyce (1979) documented lowered ability of sockeye salmon *Oncorhynchus nerka* infected with *Eubothrium salvelini* to adapt to salt water. A decreased tolerance of temperature changes was shown in the mosquitofish *Gambusia affinis* infected with *B. acheilognathi* (Granath and Esch, 1983), and increased susceptibility to cadium toxicity was documented in three-spined stickleback *Gasterosteus aculeatus* infected with larve of *S. solidus* (Pascoe and Woodworth, 1980). On the other hand, some field observations suggest that fish from polluted environments might be more susceptible to parasites. This pollution-enhanced susceptibility has not been confirmed in one experimental study of toxaphene and *D. dendriticum* in arctic charr (Blanar *et al.*, 2005), but a different study documented that copper can modulate antioxidant response in carp parasitized by *Ptychobothrium* sp. (Dautremepuits *et al.*, 2002).

On the side of the parasites, the effects of pollution might be manifested as mortality of free-living coracidia or eggs, bio-accumulation of toxins in intestinal cestodes (Sures, 2003) and changes in populations of invertebrate hosts leading to altered transmission patterns.

In addition, some parasitic infections can bias the findings in studies using morphological or physiological parameters in fish as bioindicators of environmental pollution. This has been shown with ligulosis, when fish were used as bioindicators of endocrine disruption (Schabuss *et al.*, 2005).

It is obvious that the above studies provide only a fraction of the knowledge needed to understand the complexity of environmental influences on the parasite-fish system. For example, the influence of sewage and industrial and agricultural pollution on the pathogenesis of cestode infections in fish is largely unknown (MacKenzie *et al.*, 1995).

Another challenge lying ahead in the field of fish parasitology is further elucidation of the mechanisms involved in innate and acquired immunity to cestodes. Immunity in fish is considerably less studied and understood when compared to mammals and some of the findings from studies of immunity to parasites in terrestrial vertebrates could be extrapolated to fish (Rickard, 1983; Meeusen and Balic, 2000). The combination of histopathology and modern molecular techniques should provide better answers about the role played by different host and parasite factors involved in tissue damage, parasite persistence or rejection, and resistance to primary and or secondary infections in fish. Of particular importance in respect to the cestode is the identification of masking epitopes and other factors involved in molecular mimicry. Also important is the identification and purification or cloning of stage-specific antigens and of the secretory products that are crucial for attachment and tissue penetration. With respect to the fish, the cell populations involved in innate and specific immunity, the cytokines that modulate these cell populations, and the protective mechanisms (humoral and/or cellular immunity) active in particular host-parasite systems in natural infections, must be identified. The knowledge gained from these studies will hopefully lead to the development of second or third generation vaccines. We also need to elucidate the role of MHC in resistance to parasite infections with the aim to develop disease-resistant strains of fish for aquaculture production.

In addition to the research that would develop vaccines for piscine cestodes, there is a need to study alternative approaches to disease

management, including the development of infection-resistant invertebrate intermediate hosts, better husbandry techniques to eliminate intermediate hosts, and the generation of fish strains with higher innate resistance to infection (like enhanced mucosal immunity). Lastly, we need to improve our understanding of the relationships between environmental stresses including immunosuppressive and endocrine disrupting toxins and global/local changes in water quality and temperature, and parasitic infections in fish.

Having said all this, it might be wise to understand that the associations between many parasites and their hosts have evolved over thousands of years, and in their perfect manifestation they are part of the equilibrium of life on Earth. It might be thus wiser to abandon man-made 'inventions' like intensive aquaculture, thus eliminating the unnaturally harmful parasite-host relationships created by such artificial habitats.

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## References

Ajello, S.A. 1998. *Merck Veterinary Manual.* 8th Edition. Merck & Co., Inc., Whitehouse Station, New Jersey.

Andrews, C., J.C. Chubb, T. Coles and A. Dearsley. 1981. The occurrence of *Bothriocephalus acheilognathi* Yamaguti, 1934 (*B. gowkongensis*) (Cestoda: Pseudophyllidea) in the British Isles. *Journal of Fish Diseases* 4: 89-93.

Arme, C. and R.W. Owen. 1967. Infection of the three-spined stickleback *Gasterosteus aculeatus L.*, with the plerocercoid larvae of *Schistocephalus solidus* (Müller, 1776), with special reference to pathological effects. *Parasitology* 57: 301-314.

Arme, C., J.F. Bridges and D. Hoole. 1983. Pathology of cestode infections in the vertebrate host. In: *Biology of the Eucestoda*, C. Arme and P.W. Pappas (eds.). Academic Press, London, Vol. 2, pp. 499-538.

Arnott, S.A., I. Barber and F.A. Huntingford. 2000. Parasite-associated growth enhancement in a fish-cestode system. *Proceedings of the Royal Society of London* B267: 657-663.

Barber, I. 2005. Parasites grow larger in faster growing fish hosts. *International Journal of Parasitology* 35: 137-143.

Barber, I. and P.A. Svensson. 2003. Effects of experimental *Schistocephalus solidus* infection on growth, morphology and sexual development of female three-spined sticklebacks, *Gasterosteus aculeatus*. *Parasitology* 126: 359-367.

Bauer, O.N., V.A. Musselius and Y.U. Strelkov. 1973. *Diseases of Pond Fishes.* Israel Program for Scientific Translations, Jerusalem, pp. 115-122.

Befus, A.D. and R.B. Podesta. 1984. Platyhelminth host-prasite interface. In: *Biology of the Integument*, J. Bereiter-Hahn, A.G. Matolsky and K.S. Richards (eds.). Springer-Verlag, Berlin, Vol. I, Invertebrates, pp. 192-201.

Blanar, C.A., M.A. Curtis and H.M. Chan. 2003. Growth, nutritional composition, and hematology of Arctic charr (*Salvelinus alpinus*) exposed to toxaphene and tapeworm (*Diphyllobothrium dendriticum*) larvae. *Archives of Environmental Contamination and Toxicology* 48: 397-404.

Borucinska, J.D. and J.N. Caira. 1993. A comparison of mode of attachment and histopathogenicity of four tapeworm species representing two orders infecting the spiral intestine of the nurse shark, *Ginglymostoma cirratum*. *Journal of Parasitology* 79: 238-246.

Borucinska, J.D. and J.N. Caira. 2006. Mode of attachment and lesions associated with trypanorhynch cestodes in the gastrointestinal tracts of two species of sharks collected from coastal waters of Borneo. *Journal of Fish Diseases* 29: 395-407.

Borucinska, J.D. and A. Dunham. 2000. Lesions associated with attachment of the parasitic cestode *Tentacularia* sp. to the duodeno-spiral junction in blue sharks (*Prionace glauca*) (Linnaeus, 1758). *Journal of Fish Diseases* 23: 353-359.

Bosi, G., S. Arrighi, A. Di Giancamillo and C. Domeneghini. 2005. Histochemistry of glycoconjugates in mucous cells of *Salmo trutta* uninfected and naturally parasitized with intestinal helminths. *Diseases of Aquatic Organisms* 64: 45-51.

Boyce, N.P. 1979. Effects of *Eubothrium salvelini* (Cestoda: Pseudophyllidea) on the growth and vitality of sockeye salmon, *Oncorhynchus nerka*. *Canadian Journal of Zoology* 57: 597-602.

Boyce, N.P. and S.B. Yamada. 1977. Effects of a parasite, *Eubothrium salvelini* (Cestoda: Pseudophyllidea), on the resistance of juvenile sockeye salmon, *Oncorhynchus nerka* to zinc. *Journal of the Fisheries Research Board of Canada* 34: 706-709.

Brown, S.P., G. Loot, A. Teriokhin, C. Brunel and J.F. Guegan. 2002. Host manipulation by *Ligula intestinalis*: a cause or consequence of parasite aggregation? *International Journal of Parasitology* 32: 817-824.

Calentine, R.L. and M.J. Ulmer. 1961. *Khawia iowensis* n. sp. (Cestoda: Caryophyllaeidae) from *Cyprinus carpio* L. in Iowa. *Journal of Parasitology* 47: 795-805.

Campbell, R.A. and C. Callahan. 1998. Histopathological reactions of the blue shark, *Prionace glauca*, to postlarvae of *Hepatoxylon trichiuri* (Cestoda: Trypanorhyncha: Hepatoxylidae) in relationship to scolex morphology. *Journal of Parasitology* 84: 409-421.

Caira, J.N. and F.B. Reyda. 2005. Eucestoda (true tapeworms). In: *Marine Parasitology*, K. Rhode (ed.). CABI Publishing, New York, pp. 92-104.

Caira, J.N., K. Jensen and C.J. Healy. 2001. Interrelationships among tetraphyllidean and lecanicephalidean cestodes. In: *Interrelationships of the Platyhelminthes*, T.D.J. Littlewood and R.A. Bray (eds.). Academic Press, London, pp. 135-158.

Cheung, P. 1993. Parasitic diseases of elasmobranchs. In: *Fish Medicine*, M.K. Stoskopf (ed.). W.B. Saunders Company, Philadelphia, pp. 782-808.

Chitwood, M. and J.R. Lichtenfels. 1973. *Identification of Parasitic Metazoa in Tissue Sections*. Animal Parasitology Institute, U.S. Department of Agriculture, Beltsville, Maryland.

Choudhury, A. and T.A. Dick. 1994. Natural anti-phosphorylcholine (PC) antibodies in lake sturgeon, *Acipenser fulvescens* Rafinesque, 1817 (Chondrostei: Acipenseridae). *Fish and Shellfish Immunology* 4: 399-401.

Coggins, J.R. 1980. Apical end organ structure and histochemistry in plerocercoids of *Proteocephalus ambloplitis*. *International Journal of Parasitology* 10: 97-102.

Curran, S. and J.N. Caira. 1995. Attachment site specificity and the tapeworm assemblage in the spiral intestine of the blue shark (*Prionacae glauca*). *Journal of Parasitology* 81: 149-157.

Dautremepuits, C., S. Betoulle and G. Vernet. 2002. Antioxidant response modulated by copper in healthy or parasitized carp (*Cyprinus carpio* L.) by *Ptychobothrium* sp. (Cestoda). *Biochimica et Biophysica Acta* 1573: 4-8.

Dautremepuits, C., S. Betoulle and G. Vernet. 2003. Stimulation of antioxidant enzymes levels in carp (*Cyprinus carpio* L.) infected by *Ptychobothrium* sp. (Cestoda). *Fish and Shellfish Immunology* 15: 467-471.

Dautremepuits, C., S. Betoulle, S. Paris-Palacios and G. Vernet. 2004. Humoral immune factors modulated by copper and chitosan in healthy or parasitized carp (*Cyprinus carpio* L.) by *Ptychobothrium* sp. (Cestoda). *Aquatic Toxicology* 68: 325-338.

Deudero, S., J.K. Pinnegar and N.V. Polunin. 2002. Insights into fish host-parasite trophic relationships revealed by stable isotope analysis. *Diseases of Aquatic Organisms* 52: 77-86.

Dezfuli, B.S., L. Giari, S. Arrighi, C. Domeneghini and G. Bosi. 2003. Influence of enteric helminthes on the distribution of intestinal endocrine cells belonging to the diffuse endocrine sytem in brown trout, *Salmo trutta*. *Journal of Fish Diseases* 26: 155-166.

Dick, T.A. and A. Choudhury. 1995. Cestoidea (Phylum Platyhelminthes). In: *Fish Diseases and Disorders*, P.T.K. Woo (ed.). CABI Publishing, New York, Vol. I, pp. 391-414.

Dick, T.A., P.A. Nelson and A. Choudhury. 2001. Diphyllobothriasis: Update on human cases, foci, patterns and sources of human infections and future considerations. *Southeast Asian Journal of Tropical Medicine and Public Health* (32 Supplement) 2: 59-76.

Esch, G.W. and W.J. Huffines. 1973. Histopathology associated with endoparasitic helminthes in bass. *Journal of Parasitology* 59: 306-313.

Ferguson, H.W. 1995. *Systemic Pathology of Fish*. Iowa State University Press, Ames, Iowa.

Fletcher, T.C., A. White and B.A. Baldo. 1980. Isolation of phosphorylcholine-containing component from the turbot tapeworm, *Bothriocephalus scorpii* (Mueller), and its reaction with C-reactive protein. *Parasite Immunology* 2: 237-248.

Fraize, M., M.E. Sarciron, D. Saboulard, S. Azzouz, A.L. Debard, G. Bosquet and A.F. Petavy. 2004. An in vitro model to evaluate the cytokine response in *Ecchinococcus* infections. *Parasitological Research* 92: 506-512.

Gardiner, C.H. and S.L. Poynton. 1999. *An Atlas of Metazoan Parasites in Animal Tissues*. Armed Forces Institute of Pahtology, American Registry of Pathology, Washington D.C.

Granath, W.O. Jr. and G.W. Esch. 1983. Temperature and other factors that regulate the composition and infrapopulation densities of *Bothriocephalus acheilognathi* (Cestoda) in *Gambusia affinis* (Pisces). *Journal of Parasitology* 69: 1116-1124.

Halvorsen, O. and K. Andersen. 1984. The ecological interaction between arctic charr, *Salvelinus alpinus* (L.), and the plerocercoid stage of *Diphyllobothrium ditremum*. *Journal of Fish Biology* 25: 305-316.

Hecker, M. and L. Krabbe. 2005. Parasitism in fish: An endocrine modulator of ecological relevance? *Aquatic Toxicology* 72: 195-207.

Hermanns, W. and W. Körting. 1987. Histopathology of *Cyathocephalus truncatus* infections in the rainbow trout. *Deutsche Tierärztliche Wochenschrift* 94: 71-72.

Hickey, M.D. and J.R. Harris. 1947. Progress of the *Diphyllobothrium* epizootic at Pouaphouca Reservoir, Co. Wicklow, Ireland. *Journal of Helminthology* 22: 13-28.

Hoffman, G.L. 1975. Lesions due to internal helminthes in freshwater fishes. In: *The Pathology of Fishes*, W.E. Ribelin and G. Migaki (eds.). The University of Wisconsin Press, Madison, pp. 151-188.

Hoffman, G.L. and C.E. Dunbar. 1961. Mortality of eastern brook trout caused by plerocercoids (Cestoda: Pseudophyllidea: Diphyllobothridiae) in the heart and viscera. *Journal of Parasitology* 47: 399-400.

Hopkins, C.C., L.M. Law and L.T. Threadgold. 1978. *Schistocephalus solidus*: Pinocytosis by the plerocercoid tegument. *Experimental Parasitology* 44: 161-172.

Hoole, D. 1994. Tapeworm infections in fish: past and future problems. In: *Parasitic Diseases of Fish*, A.W. Pike and J.W. Lewis (eds.). Samara and the British Society for Parasitology, London, pp. 119-141.

Hoole, D. and C. Arme. 1983. Ultrastructural studies of the cellular response of fish hosts following experimental infection of the plerocercoid of *Ligula intestinalis* (Cestoda: Pseudophyllidea). *Parasitology* 87: 139-149.

Hoole, D. and C. Arme. 1986. The role of serum in leukocyte adherence to the plerocercoid *Ligula intestinalis* (Cestoda: Pseudophyllidea). *Parasitology* 92: 412-424.

Hoole, D. and C. Arme. 1988. *Ligula intestinalis* (Cestoda: Pseudophyllidea) phosphorylcholine inhibition of leucocyte adherence. *Diseases of Aquatic Organisms* 5: 29-33.

Hoole, D. and H. Nisan. 1994. Ultrastructural studies on intestinal response of carp, *Cyprinus carpio* L., to the pseudophyllidean tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934. *Journal of Fish Diseases* 17: 623-629.

Hopkins, C.A., L.M. Law and L.T. Threadgold. 1978. *Schistocephalus solidus*: Pinocytosis by the plerocercoid tegument. *Experimental Parasitology* 44: 161-172.

Izvekova, G.I., B.I. Kuperman and V.V. Kuzmina. 1997. Digestion and digestive-transport surfaces in cestodes and their fish hosts. *Comparative Biochemical Physiology* 118: 1165-1171.

Jara, Z. and D. Szerow. 1981. Histopathological changes and localization of tapeworm *Khawia sinensis* in the intestine of carps (*Cyprinus carpio* L.). *Wiadomosci Parazytologiczne* 27: 695-703.

Joy, J.E. and E. Madan. 1989. Pathology of black bass hepatic tissue infected with larvae of the tapeworm *Proteocephalus ambloplitis*. *Journal of Fish Biology* 35: 111-118.

Karanis, P. and H. Taraschewski. 1993. Host-parasite interface of *Caryophyllaeus laticeps* (Eucestoda: Caryophyllidae) in three species of fish. *Journal of Fish Diseases* 16: 371-379.

Kaattari, S.T. and J.D. Piganelli. 1996. The specific immune system: Humoral defense. In: *The Fish Immune System: Organism, Pathogen, and the Environment*, G.K. Iwama and T. Nakanishi (eds.). Academic Press, London, pp. 207-254.

Kennedy-Stoskopf, S. 1993. Immunology. In: *Fish Medicine*, M. Stoskopf (ed.). W.B. Saunders Company, Philadelphia, pp. 149-159.

Kennedy, C.R. and P.J. Walker. 1969. Evidence of an immune response by dace, *Leuciscus leuciscus*, to infection by the cestode *Caryophyllaeus laticeps*. *Journal of Parasitology* 55: 579-582.

Kent, M.L., L. Margolis and J.W. Fournie. 1991. A new eye disease in pen-reared chinook caused by metacestodes of *Gilguinia squali* (Trypanorhyncha). *Journal of Aquatic Animal Health* 3: 134-140.

Kern, G.C. 1998. *Parasitism and the Platyhelminths*. Chapman & Hall, London.

Khalil, L.F., A. Jones and R.A. Bray (eds.). 1994. *Keys to Cestode Parasites of Vertebrates*. CAB International Publishing, Wallingford, Oxon, U.K.

Kino, H., W. Hori, H. Kobayashi, N. Nakamura and K. Nagasawa. 2002. A mass occurrence of human infection with *Diplogonoporus grandis* (Cestoda: Diphyllobothriidae) in Shizuoka Prefecture, central Japan. *Parasitology International* 51: 73-79.

Knudsen, R., M.A. Curtis and R. Kristoffersen. 2004. Aggregation of helminths: the role of feeding behavior of fish hosts. *Journal of Parasitology* 90: 1-7.

Kurovskaya, L.Y. 1991. Interrrelations between digestive processes in the system *Bothriocephalus acheilognathi*-carp. *Parazitologiia* 25: 441-449.

Kurovskaya, L.Y. 2001. Effect of the cestoda *Bothriocephalus acheilognathi* on morphology and physiology of carps reared in warm water. *Parazitologiia* 35: 249-256.

Kurtz, J., M. Kalbe, P.B. Aeschlimann, M.A. Häberli, K.M. Wegner, T.H.B. Reusch and M. Milinski. 2004. Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. *Proceedings of the Royal Society of London* B 271: 197-204.

Kuz'mina, V.V. and K.A. Pervushina. 2003. The role of proteinase of the enteral microbiota in temperature adaptation of fish and helminthes. *Doklady Biological Sciences* 391: 135-137.

Lal, R.B. and E.A. Ottesen. 1989. Phosphocholine epitopes on helminth and protozoal parasites and their presence in the circulation of infected human patients. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 83: 652-655.

Loot, G., S. Lek, S.P. Brown and J.F. Guegan. 2001. Phenotypic modification of roach (*Rutilus rutilus* L.) infected with *Ligula intestinalis* L. (Cestoda: Pseudophyllidea). *Journal of Parasitology* 87: 1002-1010.

Luo, H.Y., P. Nie, Y.A. Zhang, W.J. Yao and G.T. Wang. 2003. Genetic differentiation in the population of the cestode *Bothriocephalus acheilognathi* (Cestoda: Pseudophyllidea) as revealed by eight microsatellite markers. *Parasitology* 126: 493-501.

MacKenzie, K., H.H. Williams, B. Williams, A.H. McVicar and R. Siddall. 1995. Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. *Advances in Parasitology* 35: 85-144.

Mackiewicz, J.S. 1968. Two new Caryophyllaeid cestodes from the spotted sucker, *Minytrema melanops* (Raf.) (Catostomidae). *Journal of Parasitology* 54: 808-813.

Mackiewicz, J.S. 1972. Caryophyllidea (Cestoidea): A review. *Experimental Parasitology* 31: 417-512.

Mackiewicz, J.S. 1988. Cestode transmission patterns. *Journal of Parasitology* 74: 60-71.

Mackiewicz, J.S. and R. McCrae. 1967. *Hunterella nodulosa* gen. n, sp. n (Cestoidea: Caryophyllaeidae) from *Catostomus commersoni* (Lacepede) (Pisces: Catostomidae) in North America. *Journal of Parasitology* 48: 798-806.

Mackiewicz, J.S., G.E. Cosgrove and W.D. Gude. 1972. Relationship of pathology to scolex morphology among caryophyllid cestodes. *Zeitschrift für Parasitenkunde* 39: 233-246.

McCormick, H.J. and G.N. Stokes. 1982. Intraovarian invasion of smallmouth bass oocytes by *Proteocephalus ambloplitis* (Cestoda). *Journal of Parasitology* 68: 975-976.

McVicar, A.H. 1972. The ultrastructure of the parasite-host interface of three tetraphyllidean tapeworms of the elasmobranch *Raja naevus*. *Parasitology* 65: 77-88.

McVicar, A.H. and T.C. Fletcher. 1970. Serum factors in *Raja radiata* toxic to *Acanthobothrium quadripartitum* (Cestoda : Tetraphyllidea) a parasite specific to *R. nevus*. *Parasitology* 61: 55-63.

Meeusen, E.N.T. and A. Balic. 2000. Do eosinophils have a role in the killing of helminth parasites. *Parasitology Today* 16: 95-102.

Meyer, M.C. and R. Vik. 1968. Observations on *Diphyllobothrium sebago* plerocercoids in their fish hosts. *Proceedings of the Helminthological Society* 35: 92-96.

Mitchell, A. 1994. XII. Bothriocephalosis. In: *Suggested Procedures for the Detection and Identification of Certain Fish and Shellfish Pathogens*, L.C. Thoesen (ed.). 4th Edition. Fish Health Section, American Fisheries Society, Bethesda, Maryland.

Mitchell, L.G., J. Ginal and W.C. Bailey. 1983. Melanotic visceral fibrosis associated with larval infection of *Posthodiplostomum minimum* and *Proteocephalus* sp. in bluegill, *Lepomis macrochirus* Rafinesque, in central Iowa, USA. *Journal of Fish Diseases* 6: 135-144.

Molnár, K. 2005. Histopathological changes caused by the metacestodes of *Neogryporhynchus cheilancristrotus* (Wedl, 1855) in the gut of the gibel carp, *Carassius gibelio*. *Acta Veterinaria Hungarica* 53: 45-52.

Molnár, K. and I. Berczi. 1965. Demonstration of parasite-specific antibodies in fish blood by agar-gel diffusion precipitation test. *Zeitschrift für Immunologie and Allergie-Forschung* 129: 263-267.

Moreno, M.J., N. Casado, M.A. Urrea-París and F. Rodrigues-Caabeiro. 2001. Evidence of tubulin in the scolex gland ducts of *Gymnorhynchus gigas* plerocercoid (Cestoda: Trypanorhyncha). *Folia Parasitologica* 48: 163-164.

Nakanishi, K., K. Aoyagi, C. Xia, J.M. Dijkstra and M. Ototake. 1999. Specific cell-mediated immunity in fish. *Veterinary Immunology and Immunopathology* 72: 101-109.

Nie, D. and J.P. Pan. 1985. Diseases of grass carp (*Ctenopharyngodon idellus* Valenciennes, 1844) in China, a review from 1953 to 1983. *Fish Pathology* 20: 323-330.

Nie, P. and D. Hoole. 1999. Antibody response of carp, *Cyprinus carpio* to the cestode, *Bothriocephalus acheilognathi*. *Parasitology* 118: 635-639.

Nie, P., D. Hoole and C. Arme. 1996. Proliferation of pronephric lymphocytes of carp, *Cyprinus carpio* induced by extracts of *Bothriocephalus acheilognathi*. *Journal of Helminthology* 70: 127-131.

Noga, E.J. 2000. *Fish Diseases. Diagnosis and Treatment*. Iowa State University Press, Ames, Iowa.

Overli, O., M. Pall, B. Borg, M. Jobling and S. Winberg. 2001. Effects of *Schistocephalus solidus* infection on brain monoaminergic activity in female three-spined sticklebacks *Gasterosteus aculeatus*. *Proceedings in Biological Sciences* 268: 1411-1415.

Otto, F. 1972. Ligulosis in cyprinids. *Berliner und Münchener Tierärztliche Wochenschrift* 85: 235-236.

Paperna, I. 1991. Diseases caused by parasites in the aquaculture of warm water fish. *Annual Review of Fish Diseases* 199: 155-194.

Pappas, P.W. 1983. Host-parasite interface. In: *Biology of Eucestoda*, C. Arme and P.W. Pappas (eds.). Academic Press, New York, pp. 297-334.

Pascoe, D. and J. Woodworth. 1980. The effects of joint stress on sticklebacks. *Zeitschrift der Parasitenkunde* 69: 159-163.

Pronina, S.V. and N.M. Pronin. 1982. The effect of cestode (*Triaenophorus nodulosus*) infestation on the digestive tract of pike (*Esox lucius*). *Journal of Ichthyology* 22: 105-113.

Rahkonen, R. and M. Pasternack. 1998. Effect of experimental *Diphyllobothrium dendriticum* infection on the blood leucocyte pattern of brown trout at two temperature levels. *Boreal Environment Research* 3: 381-386.

Rahkonen, R., J. Aalto, P. Koski, J. Särkkä and K. Jontunen. 1996. Cestode larvae *Diphyllobothrium dendriticum* as a cause of heart disease leading to mortality in hatchery-reared sea trout and brown trout. *Diseases of Aquatic Organisms* 25: 15-22.

Read, C.P. 1966. Nutrition of intestinal helminthes. In: *Biology of Parasites*, E.J.L Soulsby (ed.). Academic Press, New York, pp. 101-126.

Rickard, M.D. 1983. Immunity. In: *Biology of Eucestoda*, C. Arme and P.W. Pappas (eds.). Academic Press, New York, pp. 539-579.

Roberts, L.S. and J. Janovy Jr. 2005. *Gerald D. Schmidt and Larry S. Roberts' Foundations of Parasitology*. 7th Edition. McGraw-Hill Higher Education, New York.

Robinson, R.D., J. Andreassen and C. Arme. 1987. *Hymenolepis diminuta*: ultrastructural observations on complement-mediated tegumental lysis and destrobilation of 4-day-old worms in vitro. *International Journal of Parasitology* 17: 1225-1232.

Rodger, H.D. 1991. *Diphyllobothrium* sp. Infections in freshwater reared salmon (*Salmo salar* L.). *Aquaculture* 95: 7-14.

Rosen, R. and T.A. Dick. 1983. Growth and migration of plerocercoids of *Triaenophorus crassus* Forel and pathology in experimentally infected whitefish. *Canadian Journal of Zoology* 62: 203-211.

Rzeczkowska, A. and M. Honowska. 1988. Biochemical effect of the plerocercoid of *Ligula intestinalis* (L.) on the bream *Abramis brama* (L). *Wiadomosci Parazytologiczne* 34: 19-27.

Sapozhnikov, G.I., G.D. Likhovidov and A.P. Klenov. 1972. Bothriocephaliasis in white amurs. *Veterinariia* 7: 80-81.

Schabuss, M., M. Gemeiner, A. Gleiß, J.W. Lewis, I. Miller, E. Möstl, U. Schober, W. Tschulenk, I. Walter and B. Grillitsch. 2005. *Ligula intestinalis* infection as a potential source in bias in the bioindication of endocrine disruption in the European chub *Leuciscus cephalus*. *Journal of Helminthology* 79: 91-94.

Scharsack, J.P., M. Kalbe, R. Derner, J. Kurtz and M. Milinski. 2004. Modulation of granulocyte responses in three-spined sticklebacks *Gasterosteus aculeatus* infected with the tapeworm *Schistocephalus solidus*. *Diseases of Aquatic Organisms* 59: 141-150.

Schäperclaus, W. 1992. Helminthiases. In: *Fish Diseases*, W. Schäperclaus, H. Kulow and K. Schreckenbach (eds.). A.A. Balkema, Rotterdam, Vol. 2, pp. 729-876.

Schantz, P.M. 1996. Tapeworms (cestodiasis). *Gastroenterology Clinics of North America* 25: 637-653.

Schmidt, G.D. 1986. CRC *Handbook of Tapeworm Identification*. CRC Press, Boca Raton.

Scott, A.L. and J.M. Grtizzle. 1979. Pathology of cyprinid fishes caused by *Bothriocephalus gowkongenesis* Yea, 1955 (Cestoda: Pseudophyllidea). *Journal of Fish Diseases* 2: 69-73.

Sharp, G.J.E., A.W. Pike and C.J. Secombes. 1989. The immune response of wild rainbow trout, *Salmo gairdneri* Richardson, to naturally acquired plerocercoid infections of *Diphyllobothrium dendriticum* (Nitzsh, 1824) and *D. ditremum* (Creplin, 1825). *Journal of Fish Biology* 35: 781-794.

Sharp, G.J.E., A.W. Pike and C.J. Secombes. 1991. Rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) leucocyte interaction with metacestode stages of *Diphyllobothrium dendriticum* (Nitzch, 1824), (Cestoda: Pseudophyllidea). *Fish and Shellfish Immunology* 1: 195-211.

Sharp, G.J.E., A.W. Pike and C.J. Secombes. 1992. Sequential development of the immune response in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) to experimental plerocercoid infection of *Diphyllobothrium dendriticum* (Nitzsch 1824). *Parasitology* 104: 169-178.

Shostak, A.W. and T.A. Dick. 1986. Intestinal pathology in northern pike, *Esox lucius* L., infected with *Triaenophorus crassus* Forel, 1868 (Cestoda: Pseudophyllidea). *Journal of Fish Diseases* 9: 35-45.

Simková, A., J. Jarkovsky, B. Koubková, V. Barus and M. Prokes. 2005. Associations between fish reproductive cycle and the dynamics of metazoan parasite infection. *Parasitological Resarch* 95: 65-72.

Sinderman, C.J. 1970. *Principal Diseases of Marine Fish and Shellfish*. Academic Press, New York.

Stoskopf, M.K. (ed.) 1993. *Fish Medicine*. W.B. Saunders Company, Philadelphia.

Sures, B. 2003. Accumulation of heavy metals in intestinal helminthes in fish: An overview and perspective. *Parasitology* 126: S53-S60.

Sweeting, R.A. 1977. Studies on *Ligula intestinalis*: Some aspects of pathology in the second intermediate host. *Journal of Fish Biology* 10: 43-50.

Szalai, A.J., X. Yang and T.A. Dick. 1989. Changes in numbers and growth of *Ligula intestinalis* in the spottail shiner (*Notropis hudsonius*), and their roles in transmission. *Journal of Parasitology* 75: 571-576.

Taylor, M.J. and D. Hoole. 1995. The chemiluminescence of cyprinid leukocytes in response to zymosan and extracts of *Ligula intestinalis* (Cestoda). *Fish and Shellfish Immunology* 5: 191-198.

Threadgold, L.T. 1984. Parasitic platyhelminths. In: *Biology of the Integument*, J. Bereiter-Hahn, A.G. Matolsky and K.S. Richards (eds.). Springer-Verlag, Berlin, Vol. I, pp. 163-191.

Van Kruiningen, H.J., M.E. Placke and L.D. Wojan. 1987. Diphyllobothrium plerocercoid infestation in landlocked salmon. *Veterinary Pathology* 24: 285-286.

Vazquez-Lopez, C., C. de Armas-Serra, W. Bernardina and F. Rodriguez-Caabeiro. 2002. A 24-kDa collagenase from *Gymnorhynchus gigas* elicits rat ileum hyperactivity and is a target of humoral responses in mice previously given a single oral dose of parasite extract. *Digestive Diseases Science* 47: 935-942.

Vazquez-Lopez, C., C. de Armas-Serra, W. Bernardina and F. Rodriguez-Caabeiro. 2001. Oral inoculation with *Gymnorhynchus gigas* induces anti-parasite anaphylactic antibody production in both mice and rats and adverse reactions in challenge mice. *International Journal of Food Microbiology* 64: 307-315.

Vincent, A.G. and W.F. Font. 2003. Seasonal and yearly population dynamics of two exotic helminths, *Camallanus cotti* (Nematoda) and *Bothriocephalus acheilognathi* (Cestoda), parasitizing exotic fishes in Waianu stream, O'ahu, Hawaii. *Journal of Parasitology* 89: 756-760.

Weiland, K.A. and T.R. Meyers. 1989. Histopathology of *Diphyllobothrium ditremum* plerocercoids in coho salmon *Oncorhynchus kisutch*. *Diseases of Aquatic Organisms* 6: 175-178.

Whittington, I.D and B.W. Cribb. 2001. Adhesive secretions in the Platyhelminthes. *Advances in Parasitology* 48: 102-207.

Williams, H.H. 1960. Some observations on *Parabothrium gadi-pollachii* (Rudolphi, 1810) and *Abothrium gadi* van Beneden, 1870, (Cestoda: Pseudophyllidea) including an account of their mode of attachment and variations in the two species. *Parasitology* 50: 303-322.

Williams, H.H. 1967. Helminth Diseases of Fish. *Helminthological Abstracts* 36: 261-295.

Williams, H.H. and A. Jones. 1994. *Parasitic Worms of Fish*. Taylor and Francis, London.

Williams, M.A. and D. Hoole. 1992. Antibody response of the fish *Rutilus rutilus* to the metacestode of *Ligula intestinalis*. *Diseases of Aquatic Organisms* 12: 83-89.

Zd'árská, Z., T. Scholz and J. Nebesárová. 2004. Ultrastructure of the apical glandular region of the scolex of *Proteocephalus torulosus* (Cestoda: Proteocephalidae). *Folia Parasitologica Praha* 51: 333-338.

CHAPTER

16

# Acanthocephala

**Horst Taraschewski**

## INTRODUCTION

Acanthocephalans, also known as thorny or spiny-headed worms, comprise at least 1000 species. More than half of these tubiform, gutless worms, which reach a length of up to a few centimetres, pass through fish during their life cycle; the others use birds, mammals or other vertebrates as final hosts and do not occur in fish functioning as paratenic hosts. Various fish species serve as final hosts, harbouring the adult parasites in their gut or as paratenic hosts with immature stages of the worms in their body cavity. In addition, intra-intestinal acanthocephalans, immature or reproducing, which parasitize small fishes, may end up in the intestines of larger, predatory fishes via post-cyclic transmission. Thus far, ancient marine fish such as elasmobranches (sharks, rays) have not been found to harbour gravid acanthocephalans in their gut, suggesting that the Acanthocephala did not evolve in the sea but in freshwater where the majority of species occur. Interestingly, they are highly abundant in fish of

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the deep sea and of Antarctic waters (Crompton and Nickol, 1985; Taraschewski, 2000, 2005; Zditowiecki *et al.*, 2004).

Due to their attachment to or penetration through the host's intestinal wall by means of their protrusive proboscis which is armed with hooks (Figs. 16.1-16.3), many acanthocephalans create deep lesions. This conspicuous histopathology has been documented in many sections over the last five decades, and has led to the idea that thorny headed worms belong to the most pathogenic helminths of fishes. Concomitantly, early researchers were also impressed by the huge worm infrapopulations recovered from many hosts and by the shiny colour of the trunk of certain species (Figs. 16.4, 16.5).

In recent years, a few authors have investigated the host-parasite interface of acanthocephalans in fish using different electron microscopical techniques (Taraschewski, 2000). They have also begun to

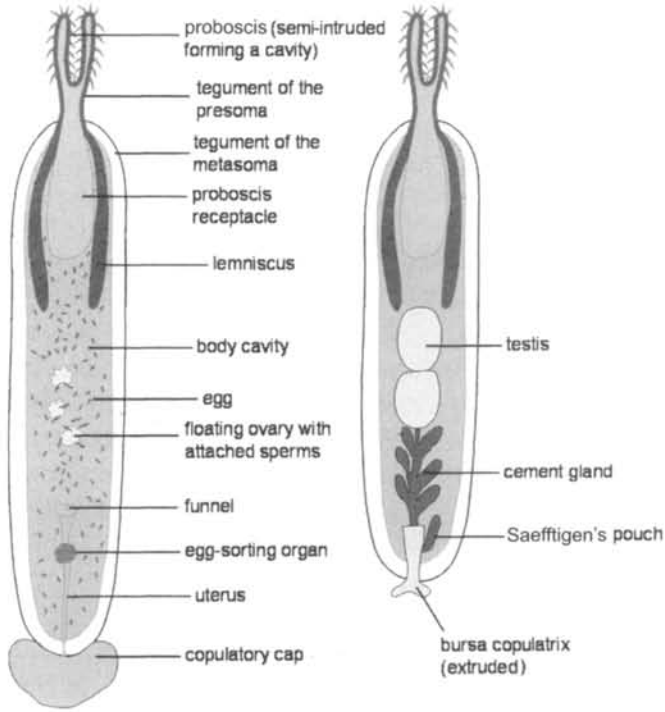


**Fig. 16.1** Schematic drawings of a typical female and male acanthocephalan (short-necked echinorhynchid palaeacanthocephalan parasitising fish). According to Taraschewski (2000, 2005).

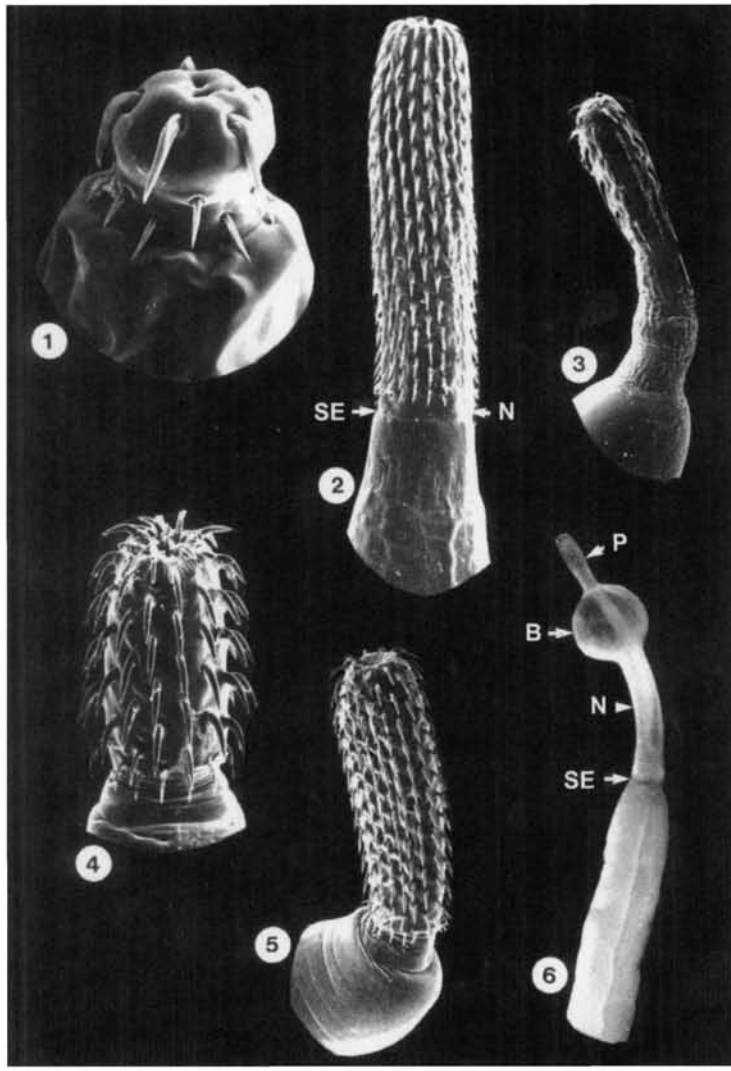


**Fig. 16.2** SEM-micrographs of the anterior part of the body of six European fish parasitizing acanthocephalans shown at different magnifications. Presoma comprising the proboscis (P), the bulbus (B in the case of *P. laevis*) and the neck (N). The fully evaginated proboscis is a post-mortem condition. The tegument of the presoma and of the metasoma is separated by a fibrous septum (SE).

1: *Neoechinorhynchus rutili*
2: *Paratenuisentis ambiguus*
3: *Acanthocephalus anguillae*
4: *A. lucii*
5: *Echinorhynchus truttae*
6: *Pomphorhynchus laevis*

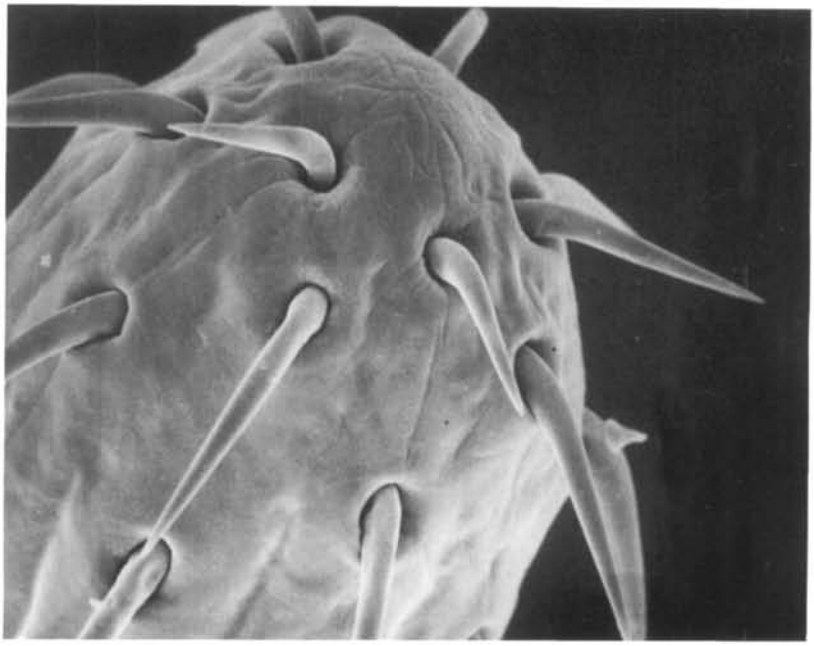

**Fig. 16.3** SEM-micrograph showing the anterior part of the proboscis of *Acanthocephalus anguillae* with its long and tipped hooks.

investigate fitness parameters, as well as the reproductive success of infected and uninfected hosts that may be altered by infection with these parasites (Sasal *et al*,. 2001; Neff and Cargnelli, 2004). Thus, in the near future, the regulation of wild fish populations by acanthocephalans via the modulation of the host's reproductive success will be better understood. There has been very little investigation on parasite-induced mortality, the second decisive factor regulating host populations (Tompkins and Begon, 1999) with respect to associations between acanthocephalans and fish.

Over the last few decades, fishes and their parasites have been increasingly subjected to new trends related to 'global change'. Various marine and freshwater species are now being cultivated in aquacultures in ponds, as well as in submerged cages. However, there is limited literature available showing acanthocephalan as disease agents creating health problems or mortality among cultured fishes (Ogawa, 2005). Another aspect of global change is that fishes have been transcontinentally displaced, mostly for commercial reasons. In parallel, many parasites of fish have become invasive, colonizing new areas and new host species. But

**Fig. 16.4** Light microscopical micrograph of an opened intestine of a barbel (*Barbus barbus*) from the Danube River. In this gut *Acanthocephalus anguillae* forms a very dense infrapopulation, but in between also a few specimens of *Pomphorhynchus laevis* can be figured out due to their orange colour.

again, only a few acanthocephalans have been described as or suspected of having attained a range expansion by benefiting from human activities (Taraschewski, 2006).

Another trend of global change is the globally increasing environmental pollution and its growing public awareness in many industrialized countries. In this context, interest has been raised in fishes and their parasites as indicators of environmental conditions, such as levels of pollution. Acanthocephalans were shown to be the most useful group of all parasites infecting fish investigated thus far, and a vast number of papers dealing with acanthocephalans as accumulation indicators of hazardous metals in the environment have appeared (Sures, 2003, 2004, 2006).

All of the items mentioned above are treated below, but prior to this, introductory remarks on the morphology, development, life cycles and taxonomy of thorny-headed worms are required. Consideration of the interactions across the host-parasite interface, for instance, depends on an understanding of the morphology and fine structure of the parasite's

**Fig. 16.5** Light microscopical micrograph of a young specimen of *Pomphorhynchus laevis*. In older worms, due to allometric growth the metasoma is more voluminous compared to the presoma. Note the orange colour.

proboscis (Figs. 16.1, 16.2). In contrast, the defence cells of the hosts which accumulate at the worms' surface are not described here in detail since they are the subject of other chapters in this book.

Many parasitologists regard the Acanthocephala as belonging to a phylum of its own (Amin, 1985, 1987), but recent molecular studies suggest that they should form the taxon 'Syndermata' together with the Rotatoria (Herlyn *et al.*, 2003).

## TAXONOMY, HOST SPECIFICITY

Acanthocephalans infecting fish belong to the taxa Palaeacanthocephala, Eoacanthocephala and Polyacanthocephala (Amin, 1985, 1987). The latter taxa, which has been little investigated and thus far, comprises only a few species (Amin, 1987; Garcia-Varela *et al.*, 2002), is not considered here. The same is true for members of the Archiacanthocephala which do not parasitize fish.

Many species occurring in the marine environment show more extended distributional ranges than those being transmitted in freshwater

(Taraschewski, 2005). Certain families and genera are rich in species, such as the genera *Acanthocephalus* and *Neoechinorhynchus* (Amin, 1985, 2002). The species *Acanthocephalus lucii* and *A. anguillae* use the same intermediate host, the isopod *Asellus aquaticus*, but show a different preference for definitive hosts and also differ in the mode of attachment to the gut wall of their hosts (Figs. 16.6, 16.7). In addition, the different depth of penetration coincides with a morphological feature: the perforating species (*A. anguillae*) has a long neck while the neck is short in the non-perforating congener *A. lucii* (Fig. 16.2).

According to Holmes (1979), the final hosts of parasites are classified as: (1) **required:** a species of fish in which parasite recruitment, maturation and egg production are sufficient to maintain the parasite's suprapopulation in the absence of other host species, (2) **suitable:** a species in which parasite recruitment, maturation and egg production occur but at a level insufficient to maintain the parasite's suprapopulation in the absence of a required host species or (3) **unsuitable:** a species in which the parasite occurs but does not mature and either contributes to the maintenance of the parasite suprapopulation by acting as a paratenic host or, conversely, results in loss from the system by acting as an ecological sink. The usefulness of this distinction becomes obvious when investigating the life cycles and abundance of acanthocephalans in the field.

Hine and Kennedy (1974) studied a river in England in terms of the host suitability of 12 species of fishes for *Pomphorhynchus laevis*. Gravid worms frequently occurred in only two of these (*Leuciscus cephalus*, *Barbus barbus*), and in another four hosts gravid females could occasionally be found. Nevertheless, a prevalence of 100%, a maximum intensity of 429 and a moderate length of about 10 mm were also encountered in grayling (*Thymallus thymallus*) acting as an unsuitable host. Usually, however, high abundance, high reproductive rate as well as large worm size (resulting from extended longevity) coincide in the 'required' hosts. For most European populations of *P. laevis* as well as *Acanthocephalus anguillae*, chub and barbel function as such hosts, and under certain circumstances, both parasites may form mixed infrapopulations in individuals of one or the other host species (Fig. 16.4).

In general, host-specificity seems to be determined by both intrinsic (evolutionary) and extrinsic (ecological) factors and can vary under

different conditions. In the U.S.A., field studies on *Leptorhynchoides thecatus* have shown that green sunfish (*Lepomis cyanellus*) is a required host and largemouth bass (*Micropterus salmoides*) a suitable host where both fish species occur sympatrically in natural communities. When both species, however, are exposed to *L. thecatus* cystacanths via laboratory infected *Hyalella azteca* amphipods in 'mesocosm aquaria', no significant difference in the prevalence, abundance, percentage of gravid worms, or time to parasite maturation was found between the two host populations. Thus, in the latter case, the apparent host preference in the field has been shown to be largely influenced by host autecology and community structure and does not exclusively reflect evolutionary factors (Olson and Nickol, 1996).

The histopathology of several acanthocephalans occurring in Germany was studied in laboratory trials with hosts of different suitability in parallel and under the same conditions. In addition, various sections from different naturally infected hosts of the same acanthocephalans were used for comparison. The results revealed a high host-specific divergence with respect to each species' growth, size, longevity, and microhabitat preference and to the depth of penetration into the intestinal wall. The hosts' reactions also differed, for instance in the degree of inflammation or encapsulation (see Taraschewski, 2000). No experimental studies have so far been carried out on polymorphid palaeacanthocephalans maturing in seals or whales, commonly found as immature stages inside the body cavity of fishes in marine and brackish waters. Various host records show that the host specificity of this group in fishes seems to be very low, even including sharks and rays (see Taraschewski, 2005), but we do not know anything about the longevity of these encapsulated stages for any acanthocephalan in any fish species.

## MORPHOLOGY, NUTRITION, DEFENCE

The body of a typical acanthocephalan consists of the metasoma (trunk) lying inside the intestinal lumen of the final host, and the presoma (proboscis and neck) inserted into the intestinal wall (Figs. 16.4, 16.7 and 16.16). *In situ*, the proboscis, the major holdfast organ, is usually kept semi-invaginated (Figs. 16.1, 16.6). Only those species that deeply penetrate into or through the intestinal wall occupying a permanent point of attachment may keep the proboscis in a fully everted condition (Fig. 16.11). In most cases, however, a fully evaginated proboscis, as

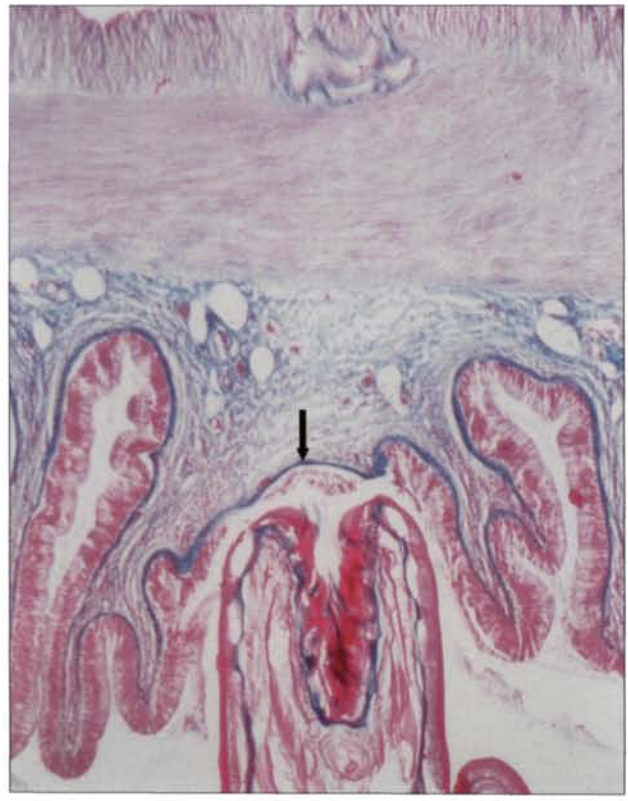

**Fig. 16.6** Paraffin section (Azan stained) of the small intestinal wall of a river perch (*Perea fluviatilis*) and an attached specimen of *Acanthocephalus lucii*. Note the superficial attachment of this short-necked species. The thin (blue) layer of collagen (arrow) between the mucosal epithelium and the tunica propria seems to function as a penetration obstacle. Also note the cellular debris rising from the lesion into the lumen of the gut. The proboscis is semi-intruded.

shown in textbooks and taxonomic studies, is a post-mortem condition (Fig. 16.2). For the taxonomic investigation of the proboscis, hooks are counted and measured in worms which have been relaxed in chilled tap water, stimulating the total evagination of the proboscis.

In both sexes, the acanthocephalan body mainly consists of the body wall, the thick tegument which is a syncytium. The nuclei of the presoma tegument lie within the lemnisci, which are sack-shaped outgrowths of the presoma-tegument projecting into the body. The subtegumental musculature consists of longitudinal and circular muscles. In mature females, which are larger than the males, ovaries, eggs (and sperm from a

**Fig. 16.7** Longitudinal paraffin section (azan stained) through a female specimen of *Acanthocephalus anguillae* inside the intestine of an experimentally infected juvenile goldfish, 7 dpi. Note that the worm's proboscis (invaginated) has perforated the liver, causing necrotic tissue (NT). Inflammatory tissue (IT) has already formed, but connective tissue is not seen yet. PA: pancreas, SP: spleen, MM: melano-macrophage.

copulation) float within the voluminous body cavity. Embryonated eggs are sorted out by an 'egg-sorting organ', and are released into the host's intestinal lumen via the vagina. Females, after sex, carry a copulatory cap which is imposed on them by the males after the injection of sperm. They continue to carry it until they start discharging eggs (Fig. 16.1).

In males, the sexual organs mainly consist of two testicles and one or several cement glands that produce the substance used for 'locking up' the female body at the end of copulation (Fig. 16.1). Among the Eoacanthocephala, a cement reservoir is present (see Taraschewski, 2000, 2005). Interestingly, male worms of *Acanthocephaloides propinquus* in a

gobiid fish were found to have a larger testicular volume when the percentage of male individuals in a host increased, presumably reflecting increasing male-male competition for access to females (Sasal *et al.*, 2000). For morphological distinctions between Eoacanthocephala and Palaeacanthocephala, see Crompton and Nickol (1985) and Taraschewski (2000).

Like tapeworms, acanthocephalans do not have an intestine. They absorb their nutrients through densely packed deep crypts of the outer membrane (Fig. 16.8). Due to this huge resorptive surface, the parasite can efficiently compete with the host in rapid nutrient uptake. The trunk (metasoma) surface is lined by a fuzzy glycocalyx and is the part of their body through which acanthocephalans seem to absorb carbohydrates, amino acids (and fatty acids ?) from the lumen of the hosts' gut, as shown

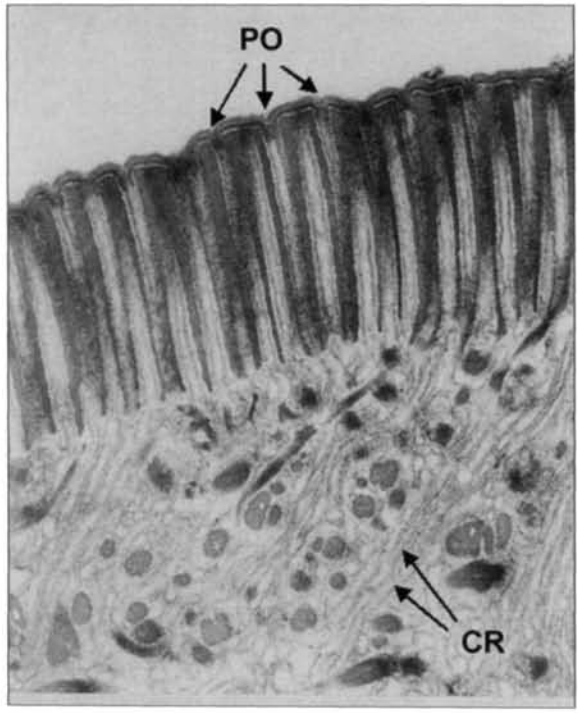


**Fig. 16.8** Transmission electron micrograph of the outer layers of a metasoma-tegument of *Acanthocephalus lucii*. Note the regularly set pores at the surface (PO) communicating with crypts of the outer membrane (CR) forming a dense mesh of canals named vesicular layer.

by in-vitro autoradiographic studies (see Taraschewski, 2000). The tegument of the presoma is separated from that of the metasoma by a fibrous septum (Figs. 16.2, 16.9), which seems to function as a barrier preventing the flow of liquid between the two compartments of the tegument. This is necessary for the in- or evagination of the proboscis or of the entire presoma when the lemnisci function in storing or releasing

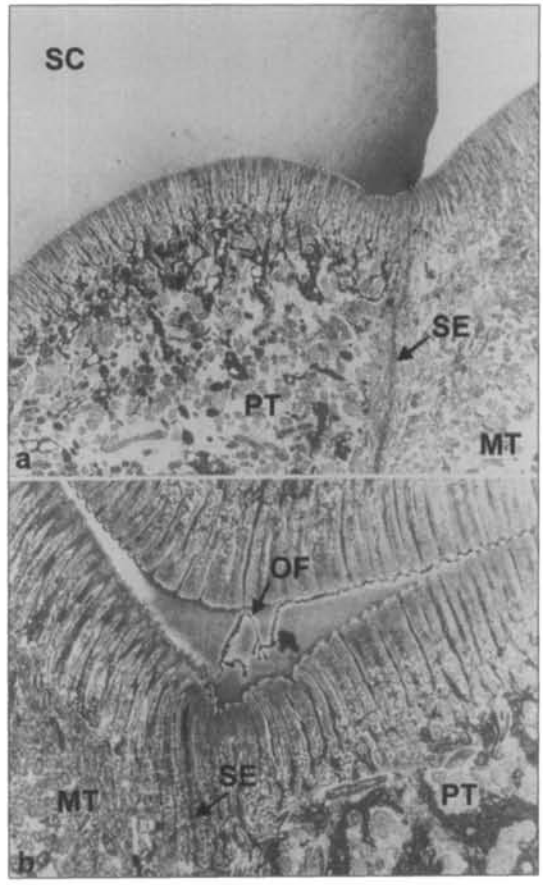


**Fig. 16.9** Ultra-thin sections through the tegument of the long-necked palaeacanthocephalan *Acanthocephalus anguillae* at the septum (SE) between the metasoma (MT) and the presoma tegument (PT). A: Note the conspicuous surface coat (SC) of lipoid matter on the presoma and the abundance of lipoid, osmiophilic substances in the (fused) crypts of the presoma tegument. B: The worm has been sectioned in a fold at the posterior end of the neck, so that the presoma tegument is seen in the right as well as in the upper part of the micrograph. Note that the presoma-surface apparently has shed an osmiophilic film (OF) into the surface coat.

liquid of the presoma tegument. Along the surface of the presoma, the crypts of the outer membrane are less densely set and shorter than in the metasoma, and the tegument as such is thinner (Fig. 16.1). As this part of the parasite's body is surrounded by host tissue, competition with the host for a quick uptake of nutrients as in the lumen of the gut does not seem to play a major role. At the surface of the presoma, the parasites take up lipids deriving from necrotic tissue at the site of attachment (see Taraschewski, 2000). For this task, the semi-invaginated proboscis (Figs. 16.1, 16.6, 16.7) seems to function as a collecting funnel for lipids. Furthermore, it can be concluded from semi-thin and ultra-thin sections that acanthocephalans have the ability to affect the release of lipids (Fig. 16.10), the degree of tissue necrosis (Fig. 16.11) and even the severity of inflammation along their presomal surface (Fig. 16.12). But unfortunately, histochemical studies dedicated to such questions are customarily conducted with laboratory mice or rats and helminths such as *Fasciola hepatica* or *Nippostrongylus brasiliensis* and not with acanthocephalans of fish. However, it has been demonstrated that the perforating acanthocephalan *Pomphorhynchus laevis* discharges a trypsin-like collagenolytic serine proteinase (see Taraschewski, 2000).

The presoma of acanthocephalans parasitizing fish has a lipid-like surface coat which may have a diameter of several microns. Inside this surface coat, films with different osmiophilic properties can be differentiated (Fig. 16.9), suggesting that the defence strategy of shedding or capping the glycocalyx loaded with host antibodies and antiparasitic enzymes is also practiced by palae- and eoacanthocephalans parasitizing fish. It has been shown from the archiacanthocephalan *Moniliformis moniliformis* parasitizing rats which has a fuzzy presomal surface coat well discernable under the electron microscope (see Taraschewski, 2000).

Interestingly, the hooks of the of fish-parasitizing acanthocephalans, which may be long and tipped (Fig. 16.3), are covered by tegument (Figs. 16.13, 16.14), enabling the parasite to inject enzymes, mucins or lipid matter into the host's tissue (Fig. 16.14) and, conversely, to extract lipophilic nutrients from it. Different acanthocephalans of fish have been demonstrated to absorb $^{3}$H-trioleoylgycerol as well as the lipophilic substance $^{3}$H-vitamin A through the pores of their hook tegument (see Taraschewski, 2000). Considering all of these findings and hypotheses, it appears that the acanthocephalan host-parasite interface deserves more attention from workers researching in the fields of histochemistry and immunology.

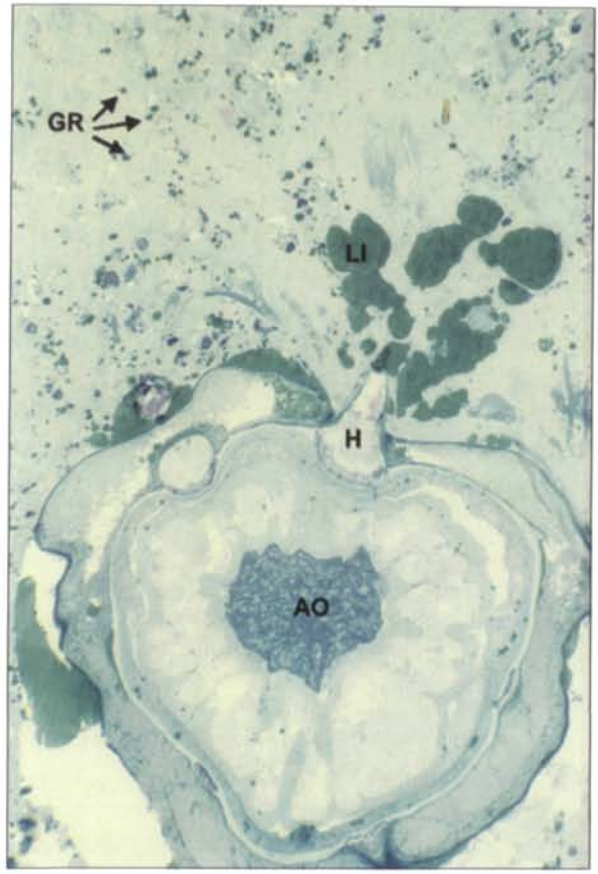


**Fig. 16.10** Oblique semi-thin section through the proboscis of *Neoechinorhynchus rutili* and the surrounding intestinal tissue of a naturally infected juvenile rainbow trout. Note the accumulation of large (greenish) lipid drops (LI) forming from smaller contributory drops deriving from further inside the tissue. Also (blue) granula from degranulated eosinophilic granulocytes (GR) seem to fuse with the lipid drops and with the lipoid surface coat of the presoma. H: hook, AO: apical organ.

## DEVELOPMENT, LIFE CYCLE AND DISTRIBUTION

**Eoacanthocephala** have ovoid to elongated eggs in which the acanthor (the first larva) is surrounded by 4 or 5 envelopes with different characteristics and functions, and by electron-lucent interstices. Thus far, eoacanthocephalans have been found to utilize ostracods, copepods, and in one case (*Paratenuisentis ambiguus*) an amphipod, as intermediate hosts. In the hemocoel of these crustaceans the acanthor develops to

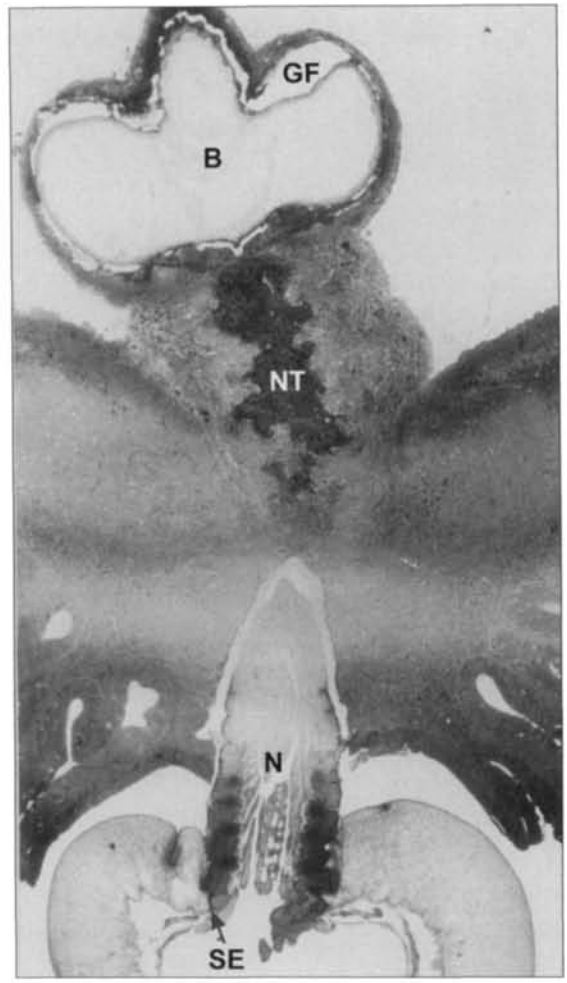


**Fig. 16.11** Semi-thin longitudinal section through the presoma of the long-necked perforating species *Pomphorhynchus laevis* and the surrounding intestinal tissue of its host chub (*Leuciscus cephalus*). The anterior half of the neck is not seen in this section. Note the abundance of necrotic tissue (NT) rich in lipids along the surface of this part of the neck and around the bulbus (B). Around the posterior part of the neck (i.e., in the intestinal wall close to the gut's lumen) the neoplasic host tissue forms a solid belt of cells that do not deteriorate in the vicinity of the worm's surface. GF: gap due to fixation.

the acanthella and finally reaches the stage of cystacanth (Fig. 16.15) which is infective to the final (or to the paratenic) host via food chain transmission. Small fishes as well as arthropods may occur as paratenic hosts, and post-cyclic transmission is well described from

**Fig. 16.12** Longitudinal paraffin section of the anterior third of a young female of *Neoechinorhynchus rutili* attached to the intestinal wall of a naturally infected grayling (*Thymallus thymallus*). During the acute phase of infection the presoma provokes a very heavy inflammation response. ST: stratum compactum, MU: mucosa.

*Neoechinorhynchus rutili* (see Crompton and Nickol, 1985; Taraschewski, 2000). The species richness of the Eoacanthocephala (of neoechinorhynchids) is conspicuous in the New World, which is in contrast to the poverty of species in Europe (Amin, 1985, 1998, 2002).

**Palaeacanthocephala** have elongated eggs comprising four eggshells and interstices. The acanthellae and cystacanths of echinorhynchid acanthocephalans reproducing in fish are found in isopods, amphipods or mysiids. Post-cyclic transmission seems to occur commonly, for instance during the life cycle of *Echinorhynchus gadi* (see Nickol, 2003), but for encapsulated individuals of perforating species it is doubtful whether the infected fish are paratenic hosts or whether they just function as an ecological sink in the life cycle of the respective acanthocephalan (see Taraschewski, 2000). Polymorphida maturing in seals or whales utilize amphipods as their intermediate hosts and fishes as paratenic hosts. Post-cyclic transmission is not known, and fishes serve as true paratenic hosts. In contrast, in the life cycle of polymorphids which reach sexual maturity in water birds or sea otters, for instance, fishes seem to act as an ecological

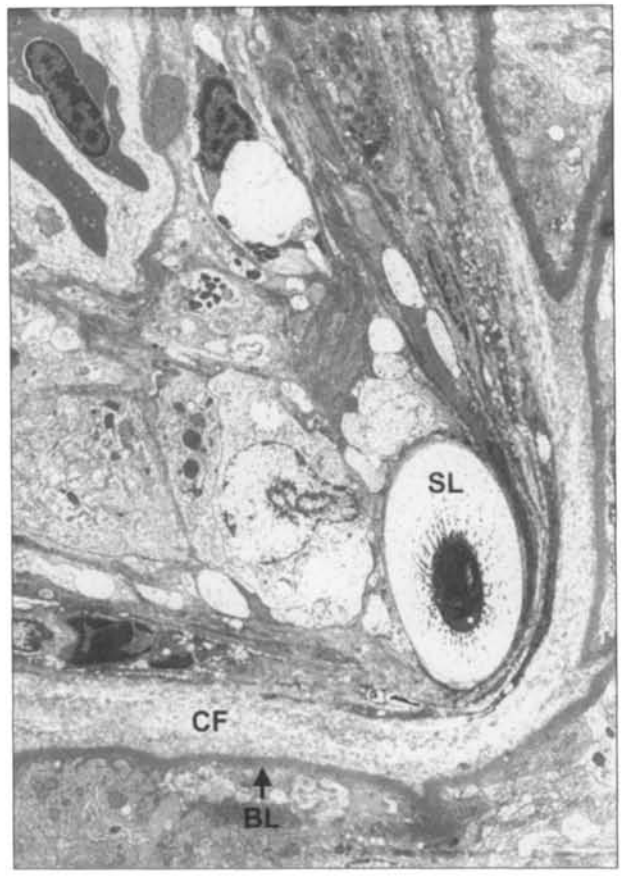


**Fig 16.13** Ultra-thin cross-section through a proboscis hook of *Acanthocephalus lucii* in a naturally infected river perch. Note the outer layer (striped layer SL) of the hook being perforated by crypts of the outer membrane. The hook has perforated the basal lamina (BL) with its layer of collagen fibers (CF) tearing these two layers towards the worm's proboscis (not seen in the micrograph).

sink. In Europe, the isopod *Asellus aquaticus*, an indicator of alpha-mesosaprobic waters, serves as the sole intermediate host of the echinorhynchids *Acanthocephalus anguillae* and *A. lucii* and of the polymorphid perforating species *Filicollis anatis* which can cause mortality among water fowl. Fish were found to serve as an ecological sink for *F. anatis* and, conversely, water birds for the two *Acanthocephalus* species. Palaeacanthocephalans occur in fresh and brackish waters and in the sea without showing preferences for certain continents or oceans.

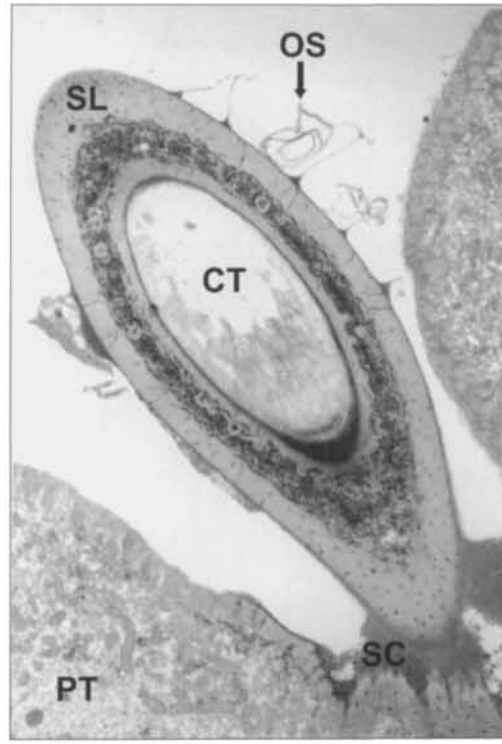


**Fig. 16.14** Ultra-thin oblique section of a hook and surrounding proboscis tegument (PT) of *Paratenuisentis ambiguus*. Note the lipoid surface coat (SC) accumulating around the curved hook and obviously also being discharged from the hook through pores in the hooks striped layer (SL). (CT: connective tissue forming the core of the hook, OS: osmiophilic substance).

## PARASITE ACTION, HOST RESPONSE, IMPACT OF INFECTIONS ON HOST INDIVIDUALS AND POPULATIONS

The following generalizations can be drawn from studies conducted on fishes naturally and/or experimentally infected by acanthocephalans (see Taraschewski, 2000):

- The species with a short neck such as *Neoechinorhynchus rutili*, *Paratenuisentis ambiguus* or *Acanthocephalus lucii* (see Fig. 16.2), always occupy a shallow attachment at the intestinal wall of their hosts not entering the muscular layers. Often the anchorage is so superficial that just the gut epithelium is ruptured (Fig. 16.6) while the posterior half of the proboscis remains unattached (Fig. 16.16). These non-perforating species often seem to change their point of

**Fig. 16.15** Light microscopical micrograph of *Gammarus trigrinus* naturally infected with a cystacanth of *Paratenuisentis ambiguus*. Note the huge, white sausage-shaped larva inside the amphipod's hemocoel.

attachment (exception *N. rutili* and other neoechinorhynchids, see below) each time creating a new lesion. They do not occur in extra intestinal sites within their hosts.

- Species which have a more or less long neck, for instance *Pomphorhynchus* spp. or *Acanthocephalus anguillae* (Fig. 16.2) deeply penetrate into the intestinal wall of their major hosts with their presoma provoking a fibrous nodule visible at the outer surface of the gut (Figs. 16.7, 16.11). However, if the host is little suitable or unsuitable, the size and longevity of the worm may be insufficient for such a connective tissue formation around the presoma. Perforating acanthocephalans seem to occupy permanent sites of attachment and can be found in extra-intestinal positions (Fig. 16.17), especially if they happen to parasitize in a small fish which has a thin intestinal wall. But single specimens may be also found in the viscera of larger hosts.

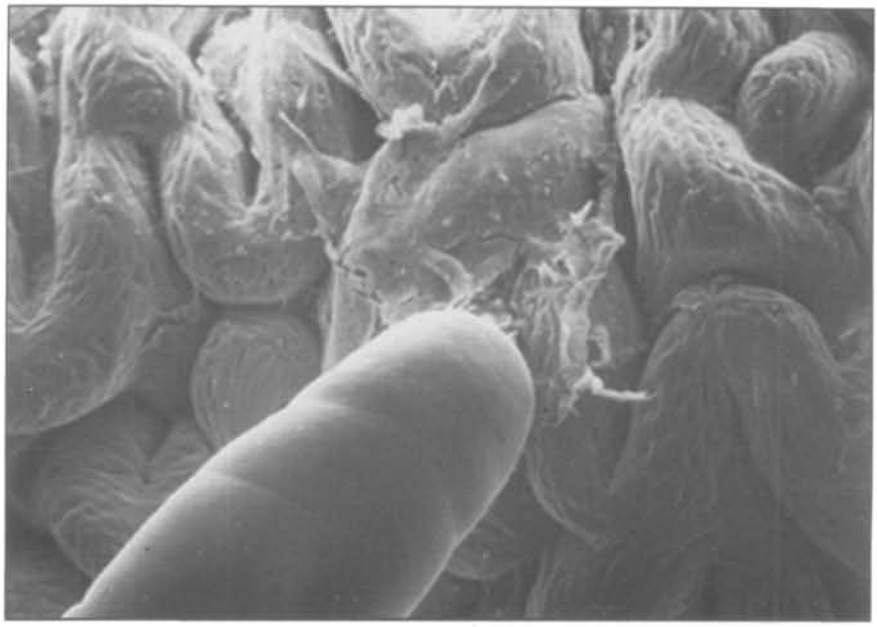

**Fig. 16.16** SEM micrograph showing a specimen of the superficially attaching species *Echinorhynchus truttae* viewed from the lumen of the intestine. A few proboscis hooks are seen. Note the abundance of mucus being discharged by hypertrophic goblet cells around the site of attachment.

- The type of cells accumulating at the worm's presoma follow a certain sequence which also has to do with the acanthocephalans' mode of attachment. During the first few days of an infection, the proboscides of all worms are mainly surrounded by necrotic tissue (Fig. 16.13). Three to five days post-infection, an exteriorly forming belt of inflammatory tissue with hemorrhagic involvement starts forming. From about 10 days onwards after infection, the inflammatory tissue around the presoma of perforating species usually becomes dominated by monocytes/macrophages maturing into epitheloid cells, and an outer belt of connective tissue begins to appear (Fig. 16.11) Later on, this tissue may consist of a solid layer of collagen fibers with a few interspersed fibroblasts. However, this chronic stage of infection is only reached by the perforating species (and partly by *N. rutili*, see below) while the species with shallow attachment seem to change their point of attachment prior to the formation of connective tissue at one site. Accordingly, they

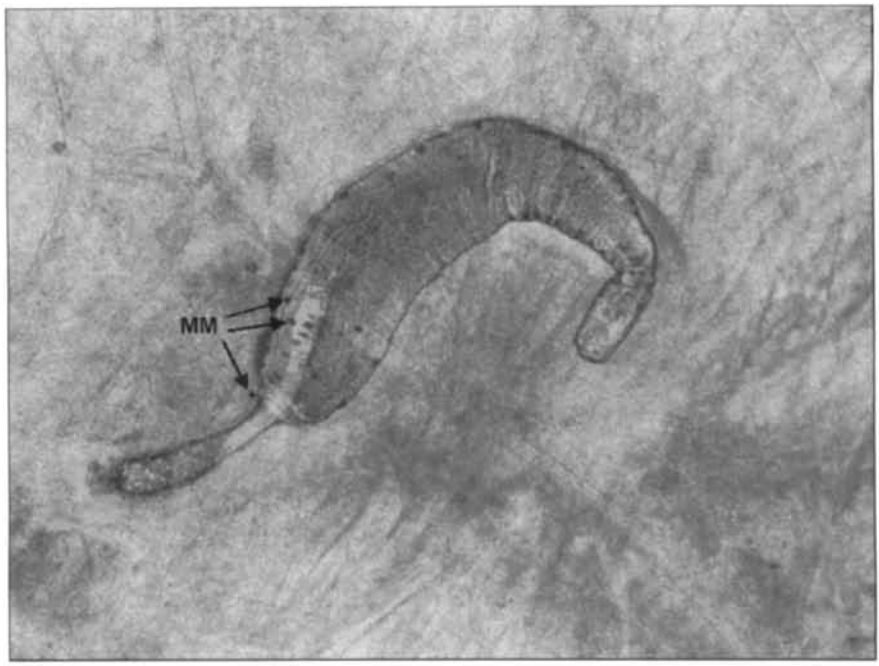


**Fig. 16.17** Squash preparation of an intestinal wall of a chub naturally infected with an intra-peritoneal worm of *Acanthocephalus anguillae*. Note that the worm is shrunk and that melano-macrophages (MM) attach to its surface.

only exhibit a necrotic belt and a belt of inflammatory tissue around their proboscides (Fig. 16.6).

*Neoechinorhynchus cylindratus* is a potentially perforating species depending on the host species. In green sunfish (*Leponis cyanellus*) given 10 cystacanths, the parasites deeply penetrated into the intestinal wall provoking the formation of a collagenous capsule around the presoma which made them remain permanently at one site. If 50 cystacanths were inoculated, the parasites migrated around throughout the intestine obviously due to a crowding effect. Accordingly, their mode of attachment was shallow and little if any connective tissue accumulated around the presoma (Abd El Meguid *et al.*, 1995). These findings clearly indicate a causative link between the time of residence at one site of attachment, the depth of penetration and the formation of connective tissue around the worm's presoma.

- A longitudinal zoning of host's tissue is associated with the presoma of perforating acanthocephalans. Near or inside the peritoneal cavity an inner belt of necrotic tissue is very conspicuous while in proximity to the intestinal mucosa, the defence cells form a closely fitting solid compensatory tissue with a very narrow necrotic belt around the parasite's neck (Fig. 16.11). One may assume that this differential tissue necrosis is modulated by the parasite by secretions at its surface.
- Acanthocephalan proboscis hooks are most attractive towards defence cells of the host (Figs. 16.3, 16.13) which may have to do with the secretion of antigens through the pores in the hooks (Fig. 16.14).
- In fish hosts, the inflammatory tissue around the acanthocephalan presoma is dominated by granulocytes and macrophage/epitheloid cells depending on the duration of attachment at one spot (Fig. 16.7). Conspicuous melanomacrophages also occur in this tissue (Figs. 16.7, 16.17). Furthermore, lymphocytes and plasma cells were documented to be involved in the cellular response against acanthocephalans but seem to be less abundant than in the inflammatory tissue of mammals infected with archiacanthocephalans. Nothing is known about the formation of antibodies by fish infected with acanthocephalans.
- A leakage of host's liquids and necrotic and defence cells from the lesions created by the worms into the gut lumen has been frequently documented from histological sections of acanthocephalans in fish (Fig. 16.6). The loss of plasma proteins through the intestinal wall of the fish *Carpiodes cyprinus* naturally infected with *Neoechinorhynchus carpiodi* has been experimentally quantified and found to be considerable. However other authors studying other host-parasite associations could not find any significant differences in leakage of plasma proteins through the gut wall of experimentally infected fish compared to the uninfected controls. This aspect of acanthocephalan pathogenicity seems to vary with the host-parasite association and with the density of the acanthocephalan infrapopulation.
- Digesta and faecal samples analyzed from young largemouth bass (*Micropterus salmoides*) infected with 10-20 *Neoechinorhynchus cylindratus* revealed that the presence of the worms lowered both

protein digestibility and amino acid availability compared to uninfected conspecifics (Ayala *et al.*, 1993). Unfortunately, no other parameters reflecting the ability of fish-parasitizing acanthocephalans to modify the physiochemical characteristics of their intestinal environment have been studied.

- Goblet cell hyperplasia and an increase in the number of goblet cells leading to a sticky covering of the mucosa around the attached worms have been described. The severity of this response is parasite-density dependant (Fig. 16.16).
- Irrespective of an acanthocephalan's depth of penetration, abrasion, erosion and compression of the gut's mucosal surface adjacent to the upper metasoma ('shoulder') of the worms were noted (Fig. 16.20).
- A local increase in the thickness of the intestinal wall at the parasite's point of attachment and also an increased diameter of other parts of infected host's gut wall which does not harbour acanthocephalans has been observed. This may be combined with a distension of those parts of the intestine which are infected. It has been described from pyloric caeca, the diameter of which can become almost doubled.
- Pyloric caeca, blind sacks of the anterior small intestine of certain fishes may become occluded by a surplus of acanthocephalans.
- Trout species heavily infected with different acanthocephalans were repeatedly recorded to have deformed backbones (Fig. 16.18) and/or shortened gill operculae or fins. If this should be caused by the acanthocephalans it may be related to the high absorption capacity of these worms for Ca and other essential elements (compare Fig. 16.22), which may then not be sufficiently available to the fish.
- Fingerlings of cultivated rainbow trout naturally infected with several specimens of *Neoechinorhynchus rutili* were found to be retarded in their growth compared to conspecifics of the same age. Obviously, infection is combined with a drain of energy and a trade-off between defence and growth.
- Referring to the pattern of attachment, host's defence and tissue formation designed above, *Neoechinorhynchus rutili* (and obviously other members of its family) does not well fit into either of the two categories of acanthocephalan species. Its small round proboscis

**Fig. 16.18** Light microscopical micrograph of a brown trout showing a skeletal deformation leading to a humpy appearance. The fish comes from a population highly infected with *Acanthocephalus anguillae*.

(Fig. 16.2) is not kept semi-invaginated as shown in Figure 16.1 but is maintained in a state of semi-retraction into the metasoma creating a ring-shaped cavity around it and the short neck (Fig. 16.12). It seems to function in collecting nutrients from the necrotic intestinal wall at the worm's point of anchorage. The acute phase of infection is associated with a very heavy inflammation response mainly consisting of granulocytosis and hemorrhage directed against the presoma (Fig. 16.12) especially the hooks. Fused granula from degranulated eosinophilic granulocytes as well as lipid drops of conspicuous size from necrotic host cells fuse, gather at the proboscis and become incorporated into the lipoid surface coat. Despite this pronounced inflammatory reaction, the worms do not seem to be adversely affected and survived at least six months in laboratory maintained naturally infected rainbow trout. So, it is not unlikely that the worms are able to utilize the granules of the eosinophils, which are rich in proteins. The eosinophilia could be induced by the parasite in order to lyse the tissue at the point of attachment as known from other parasites. The chronic stage of infection of this non-perforating

acanthocephalan is characterized by a collagenous encapsulation of the presoma (Fig. 16.19). The dense mesh of collagen fibers exhibit a "negative print" of the proboscis (compare Fig. 16.2) after the worm has been removed from its pit with a forceps (Fig. 16.20). Again, one may hypothesize that this reaction ensuring the permanent attachment at one site is encouraged by the parasite via excretions.

As reported by several authors, perforating archiacanthocephalans such as *Prosthenorchis elegans* and *Oncicola spirula* and palaeacanthocephalans comprising for instance *Filicollis anatis* and *Corynosoma enhydri* parasitizing in warm-blooded vertebrates have repeatedly created mortality among their hosts (monkeys, water fowl, sea otters, respectively). In contrast, non-perforating acanthocephalans parasitizing mammals or birds usually do not seem to be associated with host mortality (see Taraschewski, 2000, 2005). As to acanthocephalans of fish such a distinction related to the mode of attachment is not justifiable in view to morbidity and mortality. Sorting the literature on

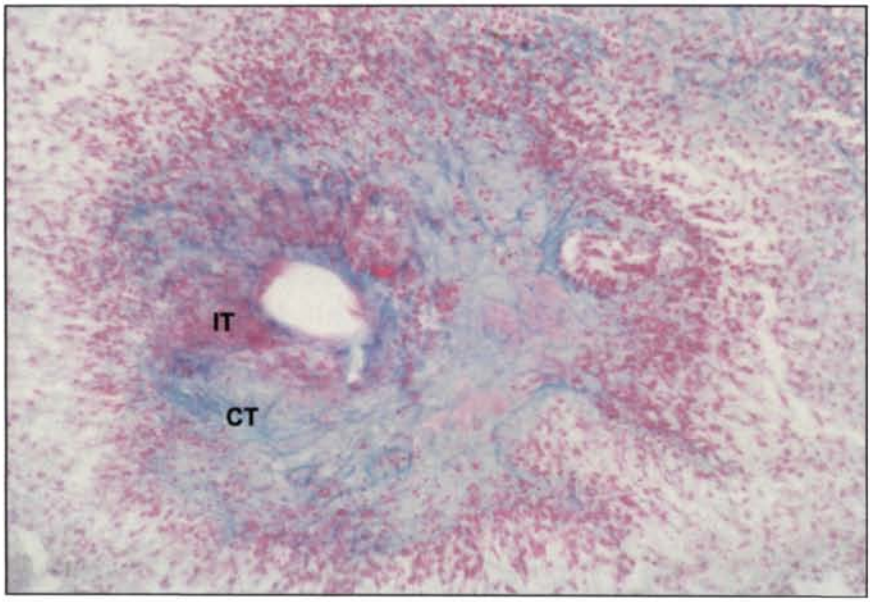


**Fig. 16.19** Paraffin section (Azan stained) of the intestinal wall of a juvenile rainbow trout naturally infected with *Neoechinorhynchus rutili*. The worm has been torn off from its point of attachment creating a pit surrounded by inflammatory tissue (IT) and connective tissue (CT).

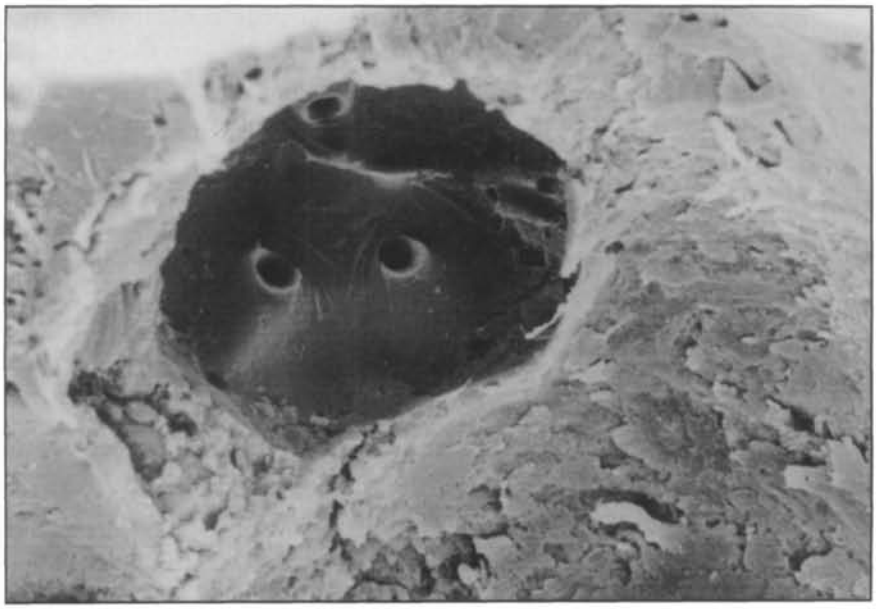

**Fig. 16.20** SEM-micrograph of the lesion in the intestinal wall of a naturally infected juvenile rainbow trout created by an adult worm of *Neoechinorhynchus rutili*. Note that the pit, the worm has been pulled away from by a forceps, is a negative print of the proboscis stabilized by a mesh of collagen fibres as can be seen from parallel histological sections (compare Fig. 16.19). Also note the abrasion and compression of the mucosal surface around the pit created by the anterior metasoma of the worm.

*Pomphorhynchus laevis* and *Acanthocephalus anguillae*, it appears that despite extensive changes in the normal structure of the gut (see Fig. 16.11) the gross pathological effects of infection appear moderate even under high worm intensities (compare Fig. 16.4) and do not seem to exceed the degree of pathogenicity revealed by non-perforating acanthocephalans.

Among warm-blooded hosts perforations of the intestinal wall may be accompanied by subsequent bacterial infections of the peritoneal cavity bringing about the death of the host. Conversely, in fish, secondary bacterial infections either do not seem to be associated with the penetration, or if they do so, they do not seem to be harmful to the cold blooded hosts. In fishes not the tremendous histological alterations created by many acanthocephalans appear to be the major aspects of the parasites' pathogenicity, more so changes of the chemical environment prevailing in the lumen of the gut as well as an altered conversion of energy and its shift from growth and reproduction to defence.

Sasal *et al.* (2001) investigated a population of the gobiid fish *Gobius bucchichii* naturally infected with the acanthocephalan *Acanthocephaloides propinquus* in respect to the fecundity of the host individuals. The percentage of infected fish was high for all size classes and for both males and females. For mature females there was a negative relationship between the gonadosomatic index (gonad weight/body weight) and the parasite intensity. The number of eggs in the gonads was significantly reduced when the fish carried a huge parasite infrapopulation. In contrast, the male hosts' reproductive success did not seem to be affected by the acanthocephalans. Apparently, for females, it is more difficult to compensate the energetic costs of parasitism because of the very high cost of egg production. Interestingly, in hosts with male parental care, different observations were made. In parental males of bluegill sunfish *Lepomis macrochirus* infected with *Leptorhynchoides thecatus*, the (Fulton's) condition factor, energy reserves and the mass of the infrapopulation of the acanthocephalans were negatively correlated with paternity (proportion of young fertilized by an individual) of nesting males (Neff and Cargnelli, 2004). Also sticklebacks (*Gasterosteus aculeatus*), another fish species with paternal care showed an impairment of energy reserves and subsequent reproductive success of males being infected with *Pomphorhynchus laevis* (Bakker and Mundwiler, 1999). All these findings demonstrate a relation between energy consuming activities, fitness and reproductive success on the side of the host and parasite mass on the other.

Mortality created by acanthocephalans does not seem to be a decisive factor regulating the populations of fish species as far as can be ascertained from the literature. However, it is likely that fishes harbouring encysted polymorphid acanthocephalans in their viscera are affected by favouritism, i.e., that they show behavioural changes making them more vulnerable to predation by seals or cetaceans. Thus far, however, this question has not been investigated yet (see Taraschewski, 2000, 2005).

## ACANTHOCEPHALANS AS ALIENS

The displacement of biota is considered a major aspect of global change. As reviewed by Taraschewski (2006), the colonization of target areas by hosts, such as fish, and by parasites are often interwoven. The arrival and

dispersal of a certain host species can be the factor leading to a parasite's establishment (or its disappearance). On the other hand, the loss of its native parasites might encourage an invasive host's colonization success in its new area of distribution. Four patterns of host-parasite co- or independent invasion can be determined.

1. While colonizing recipient areas, hosts may lose the parasites from their source area. This may be linked with the small size of the host's founder population not permitting parasite establishment or with safety measures when species such as rainbow trout became naturalized by intention. According to the parasite escape hypothesis, a host may benefit from this favourable situation by attaining higher population densities and greater individual sizes in the colonized areas when compared to the conspecifics in their native range. However, this hypothesis is mainly applicable for invertebrate hosts such as the highly invasive European shore crab, *Carcinus maenas*, thought to be regulated by castrating parasites only in its European home range. For fish which are globally introduced, like rainbow trout, data are also available documenting a decreasing number of salmonid parasites with increasing distance to the source area of the host (western North America and Kamchatka) (see Poulin, 1998), but no attempt has yet been made to correlate this loss of parasites with the fitness status of fish hosts in their colonized range.

Often, parasites from the donor area of the introduced hosts follow their hosts, although usually with a delay of one or several decades.

2. A host species may establish in a colonized area together with a parasite, with or without a time lag for the latter. The parasite remains associated with its host and does not include hosts from the new territory in its host range.

3. Host and parasite co-invade but, due to the low host specificity of the parasite, it successfully colonizes populations of novel hosts, native or introduced, in the recipient area. The East Asian tapeworm *Bothriocephalus acheilognathi* attained its global distribution through intercontinental introductions of carp and grass carp, but in the target areas many other species of fish turned out to be susceptible hosts.

4. Parasites colonize a new region without their hosts. Several helminths from Japanese eels have colonized populations of other eel species on several continents without Japanese eels being naturalized or cultivated there.

The American eoacanthocephalan *Paratenuisentis ambiguus* (see Fig. 16.2), which performed a dispersal jump across the Atlantic Ocean to Germany, followed model 1 with a time lag with respect to its intermediate host, and model 4 for the final host. As far as can be ascertained, it is the only acanthocephalan which has succeeded in colonizing a new continent within the last 50 years. The American euryhaline amphipod *Gammarus tigrinus*, serving as the sole intermediate host of *P. ambiguus* (Fig. 16.15) parasitizing the American eel *Anguilla rostrata* in North America, was naturalized into the German river Weser in 1957 to replace the indigenous amphipod fauna. The native gammarids had become extinct following pollution from potassium mines. About 25 years later, invasion by the American acanthocephalan occurred. A susceptible target final host, the European eel *A. anguilla*, was naturally present in the river. During the 1990s, both exotics had spread into the river Rhine, dominating the amphipod fauna (*G. tigrinus*) and the intestinal helminth community of the local eels (*P. ambiguus*), respectively. However, after the inauguration of the (Rhine)-Main-Danube Canal, the large, omnivorous Ponto-Caspian amphipod *Dikerogammarus villosus* colonized the river Rhine and eradicated the inferior competitor *G. tigrinus*. As a consequence, *P. ambiguus* lost its status as the dominant intestinal helminth of its novel host, and from 2001 on it has no longer been found in eels investigated from that river in the southwest of Germany. However, single records from other riverine and brackish water biotopes in Germany and The Netherlands show that it still exists in Europe.

For macroparasites of fish, East Asia is the source of several strikingly successful invaders such as the cestodes *Bothriocephalus acheilognathi* and *Khawia sinensis*, the monogeneans *Pseudodactylogyrus anguillae* and *P. bini* and the copepods *Lernaea cyprinacea* and *Ergasilus japonicus*, each of which has colonized several continents. This may have to do with certain evolutionary and ecological conditions prevailing in East Asia, but it also becomes obvious that all of these species are associated with cyprinids or anguillids, several of which have a very high commercial value (*Cyprinus carpio*, *Ctenopharyngodon idella*, *Anguilla* spp.) and are subject to globally organized trade. Furthermore, all six parasites either have monoxenic life cycles or accept a broad range of copepod intermediate hosts, suitable species of which are present in almost every freshwater biotope. The eel acanthocephalan *P. ambiguus* has also successfully colonized a novel final host on a new continent, but in contrast to the latter highly invasive species, this happened following the intentional naturalization of its sole

amphipod intermediate host. Finally, *P. ambiguus* has failed to achieve a permanent establishment due to the eradication of this host by an alien competitor invading its environment after the anthropogenic removal of a geographical barrier (see Taraschewski, 2006). Taking all of what has been discussed above into consideration, it appears that acanthocephalans of fish interacting with specific crustacean intermediate hosts in specific parts of their natural range (see Table 16.1; Amin, 1998) are less likely to become transcontinentally displaced than monogeneans, cestodes or crustaceans parasitizing fish.

## ACANTHOCEPHALANS AS BIO-INDICATORS

In recent years, an increasing number of investigations have been dedicated to parasites in the search for indicators of human impact on the environment, in particular pollution, contributing to a new field of interdisciplinary research called Environmental Parasitology (see Sures, 2003, 2004, 2006). At the same time, parasites have also raised interest because they have the potential to interfere with bio-indication applications by modifying the biomarker responses of their hosts. In both lines of research, fish as hosts have played the major role and acanthocephalans of fish have turned out to be highly efficacious sentinels of metals and other elements in their environment.

(1) In conventional bio-indication procedures, information on the quality of the environment is gained from the physiological reactions of test organisms, such as altered stress hormone or heat shock protein levels or altered survival times. In the meantime, however, many parasites have been demonstrated to affect host responses used in the context of biomarker tests, potentially making the outcome of such studies doubtful. As for acanthocephalans, the larvae of *Pomphorhynchus laevis* lead to a decrease in the median lethal concentrations of the heavy metal cadmium in infected amphipods (*Gammarus pulex*), and in chub, *Leuciscus cephalus*, experimentally infected with adult *P. laevis*, concentrations of the heavy metal Pb (in different organs) ranged below the respective levels of uninfected host individuals. In the liver of perch (*Perca fluviatilis*) the concentrations of several essential elements such as Ca and Zn were negatively correlated with the number of specimens of *Acanthocephalus lucii* harboured by the fishes. Both examples show that acanthocephalans are able to affect the physiological homeostasis of fishes. Still, when different parasites of fishes are compared for their potential to interfere

with physiological bio-indication procedures, acanthocephalans do not seem to play an outstanding role.

(2) Parasites may not just function as disruptive factors in bio-indication trials with free-living organisms, they may also provide valuable information on the chemical state of their environment by changes in occurrence or abundance, or in their physiology or behaviour, thus functioning as **effect indicators**. With respect to behavioural changes, opportunities to observe the behaviour of parasites are limited. Predominantly miracidia and cercariae of digeneans have been tested in behavioural trials. In general, however, mussels (*Dreissena polymorpha* or *Mytilus edulis*) are used for such purposes, as changes in their shell opening frequency, indicating the presence of toxins in the water, can be more easily recorded. The same is true for the presence or absence of proteins (or their respective DNA or RNA sequences) as effect indicators or biomarkers. The induction of metallothionein production, for instance, which is commonly measured, reflects the exposure of cells or organisms to metals such as Cd or Zn.

Effect indication may also aim at monitoring changes in animal populations and/or communities. However, despite the considerable efforts that have been put into linking levels of environmental pollution with parasite abundance, only a few parasite-pollution combinations are known that show predictable changes. As for acanthocephalans, the establishment of a modern sewage plant on the German river Ruhr resulted in a switch of the predominant acanthocephalan species in the gut of the downstream population of chub. *Acanthocephalus anguillae*, being transmitted by *Asellus aquaticus*, almost disappeared while *Pomphorhynchus laevis*, benefiting from the re-immigration of its local intermediate host *Gammarus pulex* into the biotope, attained dense infrapopulations. However, the upgraded water quality indicated in this case could have been classified more accurately and reproducibly by applying a saprobic bio-indicator system.

(3) **Accumulation indicators** (sentinels) are expected to perform a bioconcentration of (environmentally hazardous) substances within their tissues to levels surpassing those prevailing in their environment. Toxic effects on the indicator organism should not accompany this uptake. A well-established free-living sentinel is the zebra mussel, *Dreissena polymorpha*, which accumulates heavy metals as well as organic pollutants.

Thus far, studies involving parasites have mainly focused on the indication of accumulation of heavy metals, and much less frequently researchers have reported elevated levels of organic pollutants in parasites. This is obviously related to different accumulation patterns of hydrophilic and lipophilic substances. Lipophilic chemicals mainly accumulate in fat and therefore become biomagnified along food webs, whereas hydrophilic substances, such as heavy metals, are distributed more evenly among tissues. Parasites, having a low percentage of fat, are not able to bioaccumulate lipophilic substances above the levels of the host tissues.

However, acanthocephalans were found to show metal levels that were often more than a 1,000-fold higher than in the tissues of their fish hosts. The concentrations of Pb and Cd in *Pomphorhynchus leavis* from chub from the river Ruhr were 2,700 (Pb) and 400 (Cd) fold higher than in the musculature of the fish, and exceeded the values known from the ambient water by factors of 11,000 (Pb) and 27,000 (Cd) (Fig. 16.21). This difference in metal content between fishes and their acanthocephalan parasites was slightly less when other fish were analyzed, but in all investigated cases it was impressive. Thus far, among fish parasites, only metazoic endoparasites have been studied with respect to their metal accumulation capacity. Protozoan parasites appear to be too small to provide enough material for a reliable chemical analysis. Beside acanthocephalans in fish, only tapeworms have been shown to perform a certain degree of bioaccumulation of metals, however, to a much lower extent.

The enormous accumulation capacity of acanthocephalans also becomes obvious when species are compared with the zebra mussel, which is considered to be the major established accumulation indicator in freshwaters throughout Europe and also partly in the U.S.A. *Acanthocephalus lucii* from the intestines of perch contained metals and other elements at some 100-fold higher levels than the soft body of the mussel sampled from the same biotope. Furthermore, in this multi-element analysis, the concentrations of Ag and Ti were below detection levels in the zebra mussel but could be quantified from *A. lucii.*

Due to the high levels of metals accumulating in acanthocephalans, these helminths may serve as sentinels in environments with a very low pollution impact, such as the ocean around the Antarctic. Data from the acanthocephalan *Aspersentis megarhynchus*, commonly parasitizing Antarctic fish, revealed high concentrations of Pb, Cd, Ag, Ni, Cu and

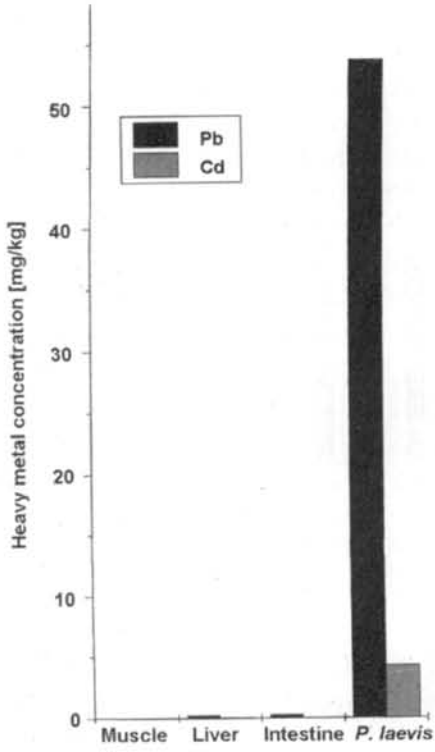


**Fig. 16.21** Relative concentration of lead and cadmium in different tissues of chub of the river Ruhr (Germany) compared to *Pomphorhynchus laevis* inside the intestine of these fishes (see Taraschewski, 2000).

other metals. A few of these elements could only be detected in the worms (Ag, Ni, Co), but not in the fish host *Notothenia corriiceps* (Fig. 16.22).

The north Atlantic species *Echinorhynchus gadi* was also shown to be a useful marine accumulation indicator. Furthermore, experiments with eels infected with *Paratenuisentis ambiguus* and kept under different salinities were conducted. The fishes were exposed to lead either orally or via the maintenance water. As expected, the parasites incorporated higher Pb quantities than the tissues of all exposed eels. Interestingly, there were no differences in the lead levels in the worm bodies either with divergent water salinity or with the mode of metal application. In contrast, the uptake of lead by the eels varied according to the method of exposure to the toxin. These findings underline the universal usefulness of acanthocephalans as sentinel organisms for monitoring bioavailable metals in the aquatic environment.

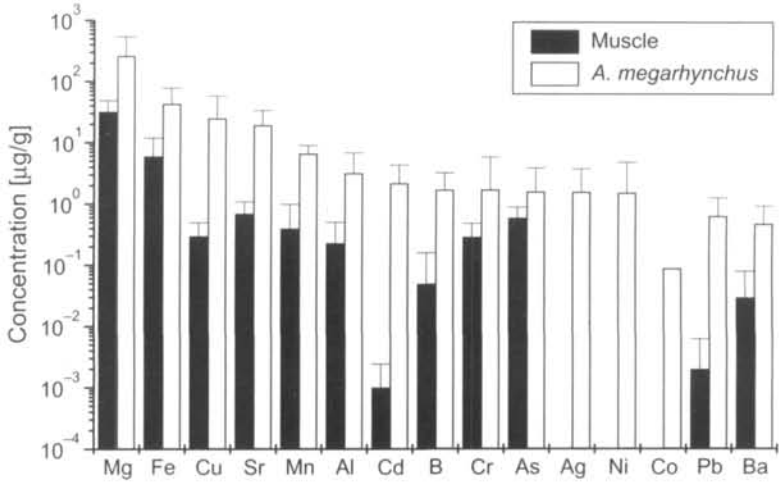


**Fig. 16.22** Element levels in the musculature of the Antarctic fish *Notothenia coriiceps* compared to its intra-intestinal acanthocephalan *Aspersentis megarhynchus* (see Sures *et al.*, 2004).

In recent years, in industrialized countries, increasing environmental pollution with platinum group metals (platinum, palladium and rhodium) has been described. These novel pollutants, deriving from automobile catalytic converters, could be detected, for instance, in the sediments of urban rivers. Due to the assumed low bioavailability of precious metals, highly sensitive accumulation indicators are required if monitoring such metals in the aquatic environment is to be possible. Again, acanthocephalans might prove to be useful sentinels.

The main uptake and accumulation of metals by acanthocephalans takes place when worms are located in the gut of their final hosts. Cystacanths inside the hemocoel of crustaceans harbour about the same amounts of Pb and Cd, or even less, than their intermediate hosts, although metal levels are usually higher in these invertebrates than in the final host. In addition, experimental infections of *Pomphorhynchus laevis* in juvenile goldfish and chub have revealed that Pb accumulation in acanthocephalans is associated with an intra-intestinal habitat of the worms. After 3 weeks of exposure, the intestinal parasites from chub contained 30, 100 and 730 times more Pb than the intestine, liver and muscle of this host, respectively. In contrast, in goldfish most of the acanthocephalan individuals of this perforating species (Fig. 16.2) chose

extra-intestinal positions. These worms from the body cavity were found to have only 0.4, 3.3 and 7.6 times higher Pb levels than the intestine, liver and muscle, respectively, of this host.

In the chub, the Pb concentration in the parasite reaches a steady state after approximately 4 weeks of exposure. Interestingly, in the latter experiment infected chub specimens contained only about half of the Pb in their intestinal wall and in other organs compared to the uninfected control fish. But this apparent sanitary function with respect to heavy metals of intra-intestinal acanthocephalans might be negatively compensated by the absorption of other elements required by the fish. Statistical analyses have revealed that the worms also affect mineral concentrations in the inner organs of fish. Thus, the levels of Ca, Zn and other essential elements in the liver of perch (*Perca fluviatilis*) were negatively correlated with the size of the infrapopulation of *Acanthocephalus lucii*.

Why do these gutless worms absorb such huge quantities of metals and other elements? It is known that acanthocephalans are extremely efficient in taking up bile salts from their hosts, as they seem to be unable to synthesize their own steroids and fatty acids. Accordingly, they rely on the large-scale uptake of substances which have a basic sterol structure from the host's intestinal lumen. For Pb uptake by intra-intestinal acanthocephalans, it has been hypothesized that the bile coming from the liver, where the majority of Pb is removed from the blood, contains steroids with which the heavy metal ions form organometallic complexes passing down the bile duct into the small intestine. These complexes can either become reabsorbed by the intestinal wall of the host or they can be excreted by the faeces of the fish. In infected fish, huge quantities of the organometallic complexes are apparently efficiently taken up by the acanthocephalans concurrently with bile salts. Thus, depending on the size of the respective acanthocephalan infrapopulation, the reabsorbance of bile-bound Pb by the host's intestinal wall is markedly reduced.

The perforating palaeacanthocephalan *Pomphorhynchus laevis* (Fig. 16.5), occurring throughout Europe, was found to have the highest accumulation capacity for metals compared to the other species studied so far. Since the family Pomphorhynchidae and the genus *Pomphorhynchus* include many other species parasitizing fish from fresh and salt waters on several continents, sufficient candidates for a highly efficient accumulation indication of metals should be available. But first

ecologists and toxicologists will have to give up their prejudice against 'ugly' parasites.

## References

Abdel-Meguid, M., G.W. Esch and H.E. Eure. 1995. The distribution and pathology of *Neoechinorhynchus cylindratus* in the intestine of green sunfish, *Leponis cyanellus*. *Parasitology* 111: 221-231.

Adams, A.M., K.D. Murrell and J.H. Cross. 1997. Fish and risk to public health. *Revue Scientifique et Technique, Office International ds Epizooties* 16: 652-660.

Amin, O.M. 1985. Classification. In: *Biology of the Acanthocephala*, D.W.T Crompton and B. Nickol (eds.). Cambridge University Press Cambridge, pp. 27-72.

Amin, O.M. 1987. Key to the families and subfamilies of Acanthocephala with erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). *Journal of Parasitology* 73: 1216-1219.

Amin, O.M. 1998. Marine flora and fauna of the eastern United States: Acanthocephala, *NOAA Technical Reports NMFS*, 135, pp. 1-27.

Amin, O.M. 2002. Revision of *Neoechinorhynchus* Stiles and Hassall, 1905 (Acanthocephala: Neoechinorhynchidae) with keys to 88 species in two subgenera. *Systematic Parasitology* 53: 1-18.

Ayala, C.E., C.C. Kohler and R.R. Stickney. 1993. Protein digestibility and amino acid availability of fish meal fed to largemonth bass infected with intestinal acanthocephalans. *Progressive Fish-Culturist* 55: 275-279.

Bakker, T.C.M. and B. Mundwiler. 1999. Pectoral fin size in a fish species with parenteral care: A condition-dependent sexual trait revealing infection status. *Freshwater Biology* 41: 543-551.

Crompton, D.W.T. and B.B. Nickol. 1985. *Biology of the Acanthocephala*. Cambridge University Press, Cambridge.

Garcia-Varela, M., M.P. Cummings, G. Péarez-ce de León, S.L. Gardner and J.P. Laclette. 2002. Phylogenetic analysis based on 18 S ribosomal RNA gene sequences supports the existence of class Polyacanthocephala (Acanthocephala). *Molecular Phylogenetics and Evolution* 23: 288-292.

Herlyn, H., O. Piskurek, J. Schmidtz, U. Ehlers and H. Zischler. 2003. The phylogeny of the Syndermata (Rotifera: Monogononta, Bdelloidea, Seisonidea; Acanthocephala: Palaeacanthocephala, Eoacanthocephala, Archiacanthocephala). *Molecular and Phylogenetic Evolution* 26: 155-164.

Hine, P.M. and C.R. Kennedy. 1974. Observations on the distribution, specificity and pathogenicity of the acanthocephalan *Pomphorhynchus laevis* (Müller). *Journal of Fish Biology* 6: 521-535.

Holmes, J.C. 1979. Parasite populations and host community structure. In: *Host-Parasite Interfaces*, B.B. Nickol (ed.). Academic Press, New York, pp. 27-46.

Khalil, L.F. 1971. The helminth parasites of African freshwater fishes. Part I. Zoogeographical affinities. *Revue de Zoologie et de Botanique Africaines* 84: 236-263.

Marcogliese, D. and G. Mc Clelland. 1992. *Corynosoma wegeneri* (Acanthocephala: Polymorphida) and *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) larvae in Scotian shelf groundfish. *Canadian Journal of Fisheries and Aquatic Science* 49: 2062-2069.

McCormick, A.L. and B.B. Nickol. 2004. Postcyclic transmission and its effect on the distribution of *Paulisentis missouriensis* (Acanthocephala) in the definitive host *Semotilus atromaculatus*. *Journal of Parasitology* 90: 103-107.

Neff, B.D. and L.M. Cargnelli. 2004. Relationships between condition factors, parasite load and paternity in bluegill sunfish, *Lepomis macrochirus*. *Environmental Biology of Fishes* 71: 297-304.

Nickol, B.B. 2003. Is postcyclic transmission underestimated as an epizootiological factor for acanthocephalams? *Helminthologia* 40: 93-95.

O'Mahony, E.M., D.G. Bradley, C.R. Kennedy and C.V. Holland. 2004. Evidence for the hypothesis of strain formation in *Pomphorhynchus laevis* (Acanthocephala): an investigation using mitochondrial DNA sequences. *Parasitology* 129: 341-347.

Ogawa, K. 2005. Effects in finfish culture. In: *Marine Parasitology*, K. Rohde (ed.). CABI Publishing, Wallingford, Oxon, U.K., pp. 378-391.

Olson, P.D. and B.B. Nickol. 1996. Comparison of *Leptorhynchoides thecatus* (Acanthocephala) recruitment into green sunfish and largemonth bass populations. *Journal of Parasitology* 82: 702-706.

Santos, R.S., M.L. Martins, N.G. Marengoni, C.J. Francisco, R.S. Piazza, H.K. Takahashi and E.M. Onaka. 2005. *Neoechinorhynchus uremai* (Acanthocephala: Neoechinorhynchidae) in *Prochilodus lineatus* (Ostheichthyes: Prochilodontidae) from the Paraná River, Brazil. *Veterinary Parasitology* 134: 111-115.

Sasal, P., E. Jobet, E. Faliex and S. Morand. 2000. Sexual competition in an acanthocephalan parasite of fish. *Parasitology* 120: 65-69.

Sasal, P., E. Faliex, I. De Buron and S. Morand. 2001. Sex discriminatory effect of the acanthocephalan *Acanthocephaloides propinquus* on a gobiid fish *Gobius bucchichii*. *Parasite* 8: 231-236.

Schmidt, D. 1971. Acanthocephalan infections in man, with two new records. *Journal of Parasitology* 57: 582-584.

Sinisalo, T. and E.T. Valtonen. 2003. *Corynosoma* acanthocephalans in their paratenic fish hosts in the northern Baltic Sea. *Parasite* 10: 227-233.

Skirnisson, K., E. Eydal, E. Gunnarson and P. Hersteinson. 1993. Parasites of the arctic fox (*Alopex lagopus*) in Iceland. *Journal of Wildlife Diseases* 29: 440-446.

Sures, B. 2003. Accumulation of heavy metals by intestinal helminths in fish: An overview and perspective. *Parasitology* 126: 53-60.

Sures, B. 2004. Fish acanthocephalans of the genus *Pomphorhynchus* as globally applicable bioindicators for heavy metal pollution in the aquatic environment? *Wiener Klinische Wochenschrift* 116: 19-23.

Sures, B. 2006. How parasitism and pollution affect the physiological homeostasis of aquatic hosts. *Journal of Helminthology* 80: 151-157.

Taraschewski, H. 2000. Host-parasite interactions in Acanthocephala: A morphological approach. *Advances in Parasitology* 46: 1-179.

Taraschewski, H. 2005. Acanthocephala (thorny or spiny headed) worms. In: *Marine Parasitology,* K. Rohde (ed.). CABI Publishing, Wallingford, Oxon, U.K., pp. 116-121.

Taraschewski, H. 2006. Hosts and parasites as aliens. *Journal of Helminthology* 80: 99-128.

Tompkins, D.M. and M. Begon. 1999. Parasites can regulate wildlife populations. *Parasitology Today* 15: 311-313.

Zdzitiwiecki, K. and K. Laskowski. 2004. Helminths of an Antarctic fish *Notothenia coriiceps*, from the Vernadsky Station (Western Antarctica) in comparison with Admiralty Bay (South Shetland Islands). *Helminthologica* 41: 201-207.

CHAPTER

# 17

# Phylum Nematoda

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## INTRODUCTION

Nematodes are common parasites of fishes in both marine and freshwater environments, and several species are also significant as disease-causing agents. At least 17 families of nematodes include species that parasitize fish, five of which appear to be restricted to this group of vertebrates (Anderson, 1984; Dick and Choudhury, 1995). They are most often encountered as adults in the gut of their fish hosts but are also commonly found in/on the viscera as larvae. Some fish nematodes are also found as adults in the skin (e.g., *Cystoopsis* spp., philometrids such as *Philometra* spp. and *Philometroides* spp.), abdominal cavity and viscera (philometrids such as *Philonema* spp.), swimbladder (*Cystidicola* spp., *Anguillicola* spp.), and liver (some capillariids). Larval ascaridoids (anisakids) and *Eustrongylides* spp. may be particularly common in the viscera and flesh of fishes in environments that support populations of fish-eating birds

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(cormorants, pelicans, gulls, herons, grebes, etc.). These nematodes are also commonly encountered in outdoor pond aquaculture operations. Nematodes are less commonly found in the heart and blood vessels and in cysts in the skin.

The decline in commercial fish catches worldwide, accompanied by an increasing awareness of the benefits of fish in our diet has fueled a steady rise of aquaculture operations worldwide, both marine and freshwater. The special conditions (high density of spatially restricted fish hosts) of aquaculture operations have occasionally resulted in fast accumulation rates of parasites leading to heavy infections as well as the rapid spread of parasites among fish being reared. Luckily, and exceptions notwithstanding, most nematode infections are acquired through ingesting intermediate hosts (Anderson, 2000) that may be eliminated from certain aquaculture operations, but this is not always feasible or successful. The socio-economic demands for commercial fish operations, sport fishing, and the aquarium (ornamental) fish trade have resulted in an altogether different kind of problem: the translocation and establishment of exotic parasites into new areas and new hosts (Bauer, 1991; Sures and Knopf, 2004a). Certain environmental conditions (abiotic and biotic) may also exacerbate infection rates and burdens.

Nematode infections impact commercial fisheries, aquaculture operations and sport fishing in two major ways. First, nematodes may cause mortality, affect growth, and compromise the health of the fish resource. Secondly, certain nematode infections, e.g., anisakids in the flesh, may render the fish commercially non-viable due to regulatory laws governing contamination and aesthetics. Furthermore, several nematodes, e.g., the marine anisakids are important zoonotic disease causing agents in humans and a long-standing cause for concern in some marine fisheries (Smith and Wootten, 1978; Berland and Fagerholm, 1994; Ogawa, 1996; Scholz, 1999; Chai *et al.*, 2005). Other nematodes with zoonotic potential, e.g., gnathostomes, are also found in freshwater fishes, mostly in subtropical and tropical regions (Rojas-Molina *et al.*, 1999; Dash *et al.*, 2004) and can be a problem in aquaculture (Stoskopf, 1998). Not surprisingly, Hafsteinnsson and Rizvi (1987) considered nematodes the most economically important group of helminths in fishes, and economic losses can be substantial (Bonnell, 1994).

This chapter focuses on the biology and pathology of disease-causing nematodes of fishes with particular emphasis on advances made since Dick and Choudhury (1995) reviewed the literature on this subject.

## GENERAL CHARACTERISTICS

### Distribution and Specificity

Some families of nematodes with fish as definitive hosts (hosts in which a parasite attains sexual maturity) are very widespread. Families of the order Spirurida and Ascarida are particularly well represented in fishes. Spiruridan families such as Cystidicolidae, Rhabdochonidae and Camallanidae as well as the ascaridan families Anisakidae and Cucullanidae are represented in the Americas, the Eurasian landmass and Africa. The superfamily Dracunculoidea (of which *Anguillicola crassus* and the philometrids are members) is also widely distributed. Some families and species apparently face greater geographical constraints than others; camallanids are much more common and speciose in the Neotropical region than in the nearctic or palaearctic regions (Sood, 1988; Moravec, 1998; Hoffmann, 1999). The reverse is true for rhabdochonids (Vismanis *et al.*, 1987; Moravec, 1998; Hoffmann, 1999). Others are considerably restricted in their occurrence, such as the pinworm family Pharyngodonidae, which is particularly diverse in Neotropical fishes but absent from nearctic (North American) and palaearctic freshwater fishes (in general, pinworms (Oxyurida) are rather rare in fishes). Cosmocercoideans are found in Neotropical freshwater fishes but not in the nearctic or palaearctic regions. Several nematode families have representatives in freshwater and marine environments; this includes all the spiruridan and ascaridan (except for the cosmocercoidean) families mentioned above. Several families, such as cucullanids, anisakids and camallanids seem to more speciose in marine fishes, particularly in teleosts (Ivashkin and Khromova, 1976; Rigby, 1999). At least ten families of nematodes have representatives in chondrichthyans (sharks, skates and rays), of which the family Acanthocheilidae is restricted to this group of fishes (Benz and Bullard, 2004).

Larval nematodes tend to be more widely distributed and common than adults, particularly species of *Contracaecum*, *Anisakis* and *Eustrongylides*. This is partly because larval nematodes of these genera tend to be less specific for their fish hosts than adult nematodes of fishes and also because they are parasites (as adults) of fish-eating birds and mammals that are often wide ranging (Table 17.1). Adult nematodes of fishes follow the same general rules of host-parasite association typical of

**Table 17.1** The major disease causing larval nematodes, host-associations and distribution.

| *Nematode* | *Fish intermediate hosts* | *Definitive hosts* | *Distribution* |
|---|---|---|---|
| *Contracaecum* spp. | Marine/freshwater | Marine: Seals, gulls<br>Freshwater: Gulls, pelicans, cormorants, grebes, herons | Worldwide, common in Northern Atlantic, and in freshwater lakes and ponds |
| *Anisakis simplex* (herring worm, whaleworm) | Herring, salmon, smelt and other pelagic feeders | Mainly toothed whales | Marine: Common problem in North Atlantic waters |
| *Pseudoterranova decipiens* (codworm, sealworm) | Cod, flounder, and other demersal fishes | Mainly seals | Marine: Common problem in North Atlantic waters |
| *Raphidascaris acus* | Perch, roach, etc. | Mainly the northern pike, *Esox lucius* | Nearctic and Palaearctic. |
| *Truttaedacnitis* spp. | Lampreys for *T. truttae*<br>Dead end in trout? | Lampreys (for *T. pybusae*)<br>Trout (for *T. truttae*) | Freshwater: Nearctic, Palaearctic |
| *Hysterothylacium* spp. | Variety of forage and prey species | Cod (for *H. aduncum*)<br>Sunfishes (*H. analarum*) | Marine: Atlantic freshwater: North America |
| *Anacanthocheilus rotundatus* | Cod | | North Atlantic |
| *Eustrongylides* spp. | Various freshwater fishes | Herons, pelicans | Genus worldwide. *E. tubifex*: The Americas<br>Other species on other continents. |

other helminths; most are specific for a monophyletic group of hosts, i.e., hosts of a family or genus, or more rarely for a single species of fish. Generally speaking, a nematode that parasitizes a particular fish species as an adult is often more likely to infect a host species of the same genus or family, even if the hosts are found elsewhere (Bauer, 1991). This has practical implications, as in the case of the eel swimbladder nematode, *Anguillicola crassus*, a parasite of Asian eels that transferred into the European eel and has spread rapidly in Europe (Haenen *et al.*, 1994; Moravec, 1994; Manfredi *et al.*, 1998; Evans and Matthews, 1999; Scholz, 1999; Nimeth *et al.*, 2000; Kirk, 2003; Knopf and Sures, 2004a; Lefebvre and Crivelli, 2004) and to eels in the U.S. (Barse and Secor, 1999; Moser *et al.*, 2001). A similar situation involves *Philometroides cyprini*, a pathogenic carp parasite native to East Asia that was introduced into Europe in the 1960s and now infects cyprinids there (Moravec and Cervinka, 2005). This sort of specificity of nematodes for closely related fishes is also seen in the trout cecal nematode, *Truttaedacnitis truttae*, which infects, and affects, a variety of salmonine hosts (trout and salmon species). There are few species that are able to exploit resources in their hosts across taxonomic lines, e.g., *Camallanus oxycephalus*, which causes rectal and anal pathology and infects a variety of North American freshwater fishes from different families, although in this case too the parasite appears to prefer percomorph fishes. The distribution of at least two other nematode species, such as *Skrjabillanus schigini* and *Sinoichthyonema amuri*, both native to East Asia, have also been altered and extended by introductions into other areas such as Europe (Moravec, 2004). Changes in environmental conditions can also result in changes of nematode abundances in fish as has been seen in *Eustrongylides* sp. (Hirshfield *et al.*, 1983) and *Truttaedacnitis truttae* in Grand Canyon (Cole *et al.*, unpubl.). Such changes usually involve invertebrate intermediate host populations and affect transmission dynamics. An earlier bibliography not mentioned in Dick and Choudhury (1995), that of Natarajan and James (1977) provides a list of primary literature on the diseases and parasites of marine and freshwater fishes of a region, India, that has not been widely tracked.

The information on host-parasite associations, while allowing for certain broad generalizations, makes it difficult to predict the behavior and fate of a nematode species in a new host or a new area into which it is introduced. What is certain, however, is that a nematode's endemicity to

a particular area is no guarantee that it will not successfully establish in another, often very distant, area.

## General Biology

Nematodes, as a group, are readily identifiable by their morphology (Fig. 17.1). They are bilaterally symmetrical, tubular (cylindrical), pseudocoelomate, sexually dimoprhic protostomes with a complete gut (beginning with a mouth and ending in an anus). When alive or even after being thawed, the nematode body is usually translucent or, in the case of the more delicate species, even transparent. In such nematodes, the internal organs are often visible. Most fish nematodes are a whitish to tan color, but some are characteristically reddish due to the color of their pseudocoelomic fluid (camallanids, *Eustrongylides* spp.). This reddish color may be due to their own hemoglobin (as in *Pseudoterranova* larvae in cod muscle) or obtained from feeding on host blood (e.g., *Camallanus* spp.). The external surface of most fish nematodes is usually smooth, athough some nematodes (*Goezia*, *Spinitectus*) have rows of spines on their cuticle. The mouth often possesses prominent lips such as in ascaridans (which have three) that may or may not bear teeth. These lips usually become increasingly prominent in the later larval stages and reach their full expression in the adults. The mouth of the earlier larval stages of ascaridans is usually surrounded by raised lip primordia in the form of bumps, and there is often an anterolateral 'boring tooth', as in anisakids, which they presumably use to help penetrate the dense tissues of their hosts. Spiruridans on the other hand, do not possess the distinct three lips of asacridans. Their lips are typically reduced, often with sublabia and other surrounding structures (*Cystidicola*, *Spinitectus*). Although the mouth opening of spiruridans is often a narrow dorsoventral slit (*Cucullanus*, *Truttaedacnitis*, *Camallanus*), it may be oval (*Spinitectus*, some *Rhabdochona* spp.) or even rounded (*Anguillicola*, *Cystidicola*). Capillarids generally have very simple mouth openings. Some nematodes have a row of teeth guarding the rim of the mouth (*Anguillicola*, *Cystidicola* spp., *Rhabdochona* spp.). The oral cavity of fish nematodes is often reinforced by plates and collars (cucullanids) and longitudinal rib-like structures (camallanids). Most nematodes possess sensory structures such as papillae that surround the mouth and anterior end.

Nematodes have a body wall that lacks circular muscles, which also results in their characteristic side-to-side serpentine or thrashing motion.

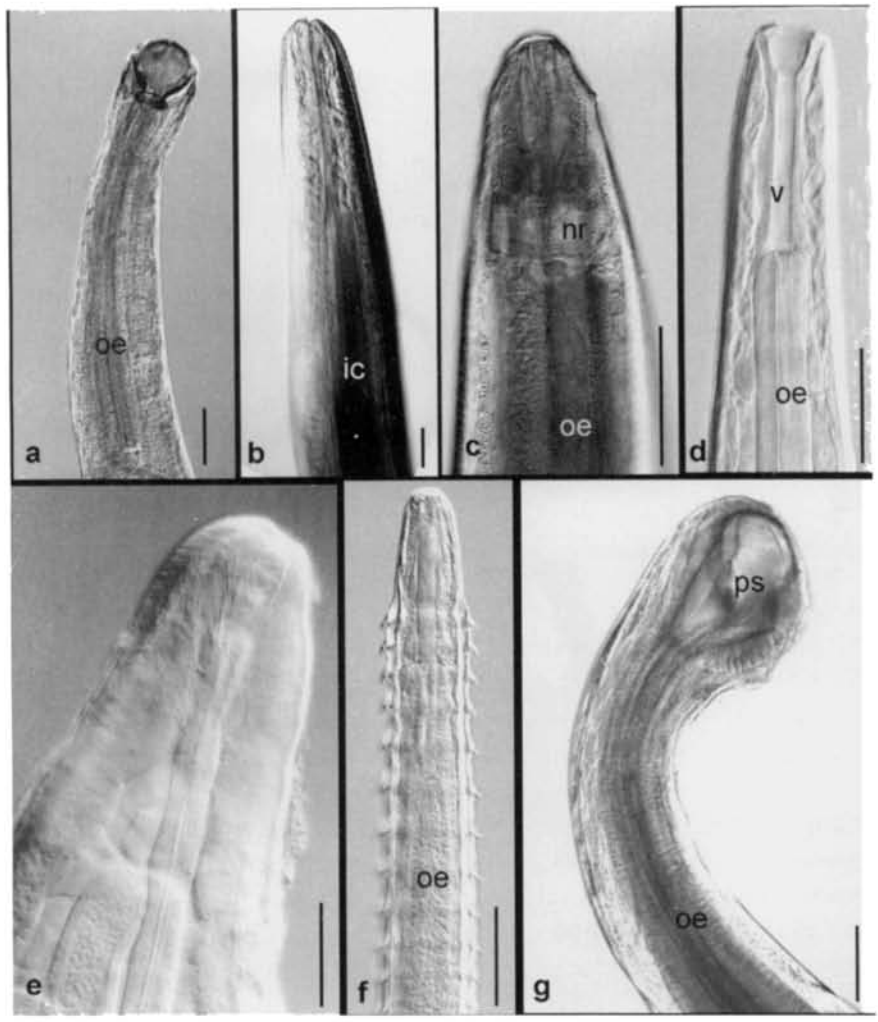


**Fig. 17.1** Gallery of some representative fish nematodes. **a**. *Camallanus ancylodirus*, **b**. *Contracaecum* sp. (L3 larva), **c**. *Eustrongylides* sp. (larva), **d**. *Rhabdochona decaturensis*, **e**. *Philometra* sp. **f**. *Spinitectus* sp., **g**. *Truttaedacnitis truttae*. Scale bars: a, b, c, f and g 100 μm, d 50 μm, e estimated as 100 μm based on known values. Photomicrograph **e** courtesy of Hans-Peter Fagerholm. oe: oesophagus, ic: intestinal caecum, nr: nerve ring, ps: pseudobuccal capsule, v: vestibule.

The longitudinal muscles use the fluid filled pseudocoelomic cavity as a hydrostatic skeleton. The body is covered by a collagenous cuticle, which is shed periodically in the life of a nematode by the process of molting (ecdysis). All nematodes have four larval stages before they reach adulthood (sexual maturity). The five stages in their lives, L1, L2, L3, L4

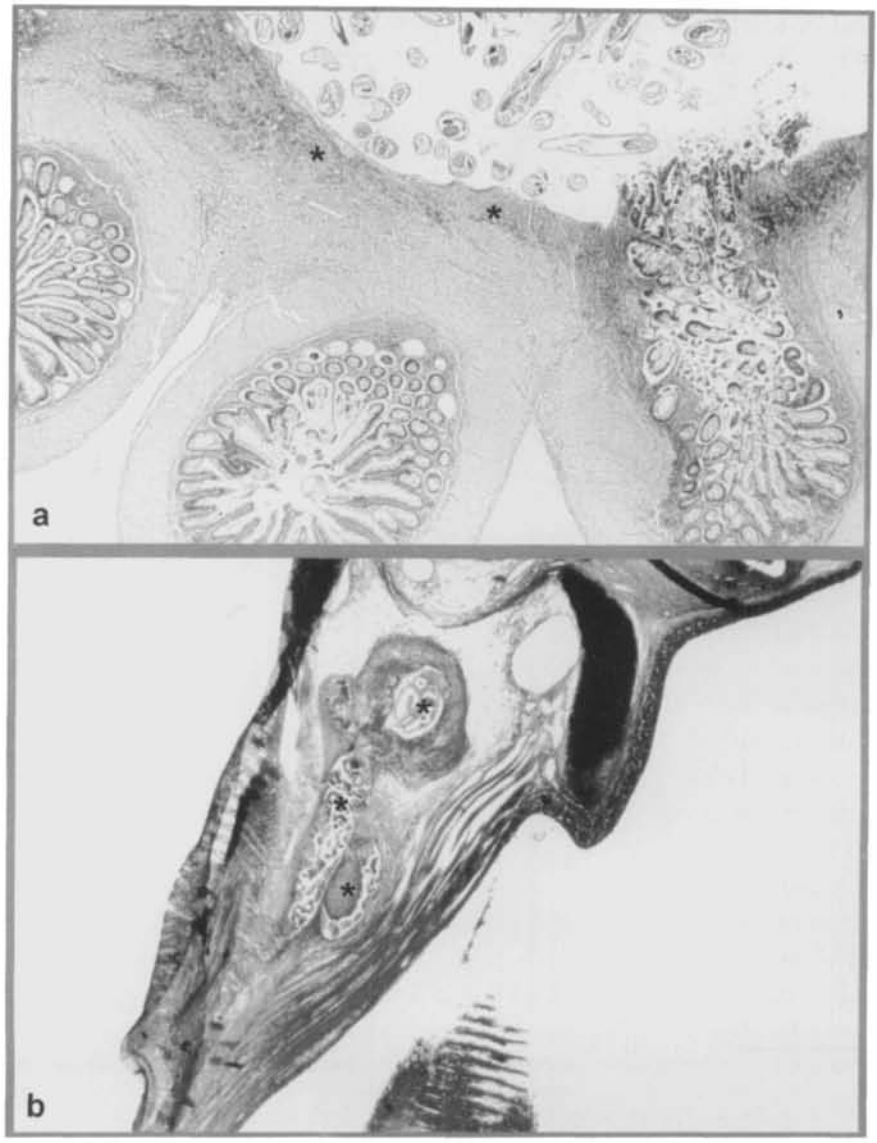


**Fig. 17.2** Examples of histopathology. **a.** Histological section through the cecal duodenal region of rainbow trout, *Oncorhynchus mykiss* naturally infected with *Truttaedacnitis truttae*. Note the loss of epithelium in the duodenum and associated pathology (asterisks) compared to the ceca. **b.** Histological section through a branchial arch of the pike, *Esox lucius*, infected with *Philometra obturans*. Note the distension of the efferent artery and the potential blockage of the blood vessels with worms (asterisks).

and A (Adult), are punctuated by four molts, one between each stage. When fish act as intermediate hosts of nematodes, harboring larval stages that will mature in some other host, they are usually initially infected by the first or second larval stage of the nematode, and sometimes by ingesting eggs containing the very early larval stages (L1, may be L2). Typically, in secernentean nematodes (the vast majority of fish nematodes), the L3 stage is the stage that is infective to the final host and the nematode larval stages that are commonly encountered encysted or encapsulated in fishes are usually the L3 larval stages. Even when an earlier stage establishes itself in a final host, it is still usually the L3 stage that establishes at the final infection site of the adult. A molt often accompanies the process of establishing in a particular host in the life cycle. The differences between male and female of the species become apparent usually in the late L3 stage when the genital primordial begin to differentiate, but often not until later. By the L4 stage, the sex is usually apparent and the copulatory structures become fully functional after the final molt to the adult stage.

The successful infection of a definitive host depends on two major factors: the ecological opportunity (encountering and ingesting the larval stage) and the physiological compatibility (including the immunological interactions) of the nematode and the host. This combination of factors explains why not all parasites are found everywhere and in everything, and also determines the 'specificity' of a particular nematode for its host as well as the nematode fauna of any host.

Fish serve as defintive, intermediate and paratenic (carrier/transport) hosts of nematode parasites. As a result, life cycles can be diverse in this phylum. Most adult nematodes either produce eggs that contain the early larval stages (typically the L1), but some shed L1 larvae (e.g., camallanids) directly. The unembryonated or partially embryonated eggs develop further in the aquatic environment. In capillarids, the eggs containing the larvae are ingested directly by its final definitive fish hosts but this sort of direct life cycle without intermediate hosts is rare among fish nematodes. More typically, larvae that are shed into the water or hatch from eggs are usually ingested by invertebrates of some sort. Williams and Jones (1994) provide a useful summary of the egg hatching times, survival times of eggs or larvae in water and the time taken by various fish nematodes to penetrate and develop in their intermediate hosts. Generally, the development times of the various stages are temperature dependent and

development is often regulated by an optimal range for each species. The invertebrate first intermediate host depends on the type of nematode and its ecological and physiological requirements. Thus, camallanid and philometrid larvae that are shed by gravid females into the water are usually eaten by pelagic and planktonic invertebrate hosts such as copepods, whereas larvae of the codworm, *Pseudoterranova decipiens*, whose eggs sink and whose eggs and early larvae are sticky and adhere to the substrate usually have more benthic and epibenthic intermediate hosts such as isopods and amphipods. The life cycle of *Eustrongylides* spp. is commonly completed in stagnant waters and their first intermediate invertebrate hosts are tubificid oligochaete annelids, which are quite abundant in the sediments of such stagnant, often hypoxic and eutrophic, waters. There is therefore a strong correlation between the biology of the parasite and the ecology of its invertebrate hosts. Generally, arthropods are the most common invertebrate intermediate host of fish nematodes. In freshwater systems, such hosts are often insect larvae, particularly ephemeropteran nymphs, and crustaceans (amphipods, copepods in some). In marine systems, crustaceans are *de facto* the most common fisrt intermediate hosts (copepods, amphipods, isopods). Annelids are occasionally used, e.g., in the life cycle of *Eustrongylides* and possibly some *Truttaedacnitis* spp. In simple life cycles involving an invertebrate intermediate host and a definitive fish host (many spiuridans such as species of *Spinitectus*, *Cystidicoloides*, *Rhabdochona*, *Cystidicola*, *Anguillicola*, philometrids, etc.), the nematode develops to the L3 stage in the hemocoel (body cavity) of its invertebrate host and is then ingested by the fish definitive (final) host, whereupon the nematode molts further and reaches maturity. Many life cycles are more complex.

In several nematode life cycles, the first intermediate host may be a fish instead of, or in addition to, invertebrates. Hatched larvae of *Raphidascaris acus* and *Contracaecum* spp. can infect fish intermediate hosts (Moravec, 1994; Køie and Fagerholm, 1995; Anderson, 2000), although transmission to the fish host is probably greatly facilitated by invertebrate hosts. Direct infection of fish intermediate hosts (*Gambusia holbrooki*), bypassing the tubificid host, has also been shown experimentally in the life cycle of *Eustrongylides ignotus* in Florida (Coyner *et al.*, 2003).

Once the nematode larva has reached the required stage of development in the first intermediate host, it may be ready to infect a definitive (final) fish host by ingestion of the first intermediate host (see

above for examples of 'simple' indirect life cycles). Such two-host life cycles are very common in nematodes that infect the gastrointestinal tract of non-piscivorous fish. In some life cycles, however, there is an additional, second intermediate host (where larval development takes place) or alternatively a paratenic (transport) host where no significant larval development takes place but that helps in transmission. The second intermediate host or transport host is usually a fish that preys on the first intermediate (generally invertebrate) host. This is the case in the life cycle of many anisakids (*Contracaecum* spp., *Anisakis* spp., *P. decipiens*, *Raphidascaris acus*). However, *A. simplex* may reach a stage infective to its final cetacean (whale) host in planktonic shrimp and krill but is usually transmitted to its typical toothed cetacean hosts (Odontoceti) by fish (Smith and Wootten, 1978). In some of these anisakid life cycles, other larger piscivorous fish may accumulate the L3 larvae by feeding on the smaller intermediate fish hosts, and consequently act as paratenic or transport hosts. Transport hosts are also used in the life cycle of the eel swimbladder nematode, *A. crassus*, where adult eels, due to their piscivorous habit, are more likely to be infected by eating smaller prey fish carrying the larvae rather than by ingesting infected copepods. Similarly, lateral transmission and accumulation of larval *Eustrongylides ignotus* (and possibly other *Eustrongylides* spp.) may occur in larger predatory fish (Coyner *et al.*, 2003). Ultimately, these second intermediate and paratenic fish hosts transmit the parasite to the final host, which for anisakids is often the top predator in the food chain (fish-eating bird, mammal or a top piscivorous fish).

The life cycles of some nematodes can be quite complex. In *Truttaedacnitis pybusae*, a parasite of North American freshwater brook lampreys, hatched larvae are ingested by ammocetes of the lampreys, develop in the intestine to the L3 stage and then migrate to the liver. It is unclear how they reach the gut again but presumably they migrate back to the intestine to discharge their eggs. In the case of the trout cecal nematode, *T. truttae*, it is becoming more evident that the biology of the European worm is different from that of the North American form. In Europe, lampreys are considered important intermediate hosts (Moravec, 1994) and trout become infected by ingesting lampreys infected with larvae. Information from surveys (Choudhury *et al.*, 2004) and pathology studies (Cole *et al.*, unpubl. results) show that rainbow trout in the Colorado River in Grand Canyon acquire their infections in other ways, since lampreys are absent from that system. Also, we have found very

small third stage larvae in the gut of rainbow trout that were heavily infected with these nematodes (authors, unpubl. data). The presence of *T. truttae* in trout farms also indicates that lampreys may not be necessary in the biology of North American *T. truttae*. These biological differences between European and North American *T. truttae* are consistent with the morphological differences that have been previously reported between them (Choudhury and Dick, 1996).

Dracunculoids have a two-host life cycle that is characterized by the extensive migrations of the larvae and adults within their fish hosts (Moravec, 1994, 2004). Adult dracunculoids typically occupy extra-intestinal sites in the body, such as the body cavity, gonads, swimbladder, other visceral organs, and under the skin. Dracunculoids such as *A. crassus*, *Phlionema* spp., *Philometra* spp., and *Philometroides* spp are transmitted to their fish hosts typically by copepods that contain the infective L3 stage. More recently, Moravec *et al.* (2005) demonstrated that ostracods could also act as natural first intermediate hosts of *A. crassus*. The L3 burrow through the fish gut and migrate in the body cavity to their final destination where they mature. Often, this journey is interrupted as the larvae spend some time in sites such as the peritoneum of the swimbladder. In the case of *A. crassus*, paratenic (transport) hosts, usually smaller prey fishes, become infected by consuming infected copepods and remain infected with the L3 larvae, that may occasionally develop to the L4 (Moravec, 1994, 1996; Szekely, 2005). The life cycle is completed when such infected paratenic hosts, such as the European perch (*Perca fluviatilis*), are ingested by piscivorous eels. Snails have also been shown to act as paratenic hosts in the life cycle of *A. crassus* (Moravec, 1996). In some philometrid species, females become fertilized in various visceral sites but migrate to reach a site closer to the external aquatic environment. Depending on the species of philometrid, gravid females release larvae into the body cavity or into the aquatic environment by emerging from the host (Molnar *et al.*, 1982; Moravec, 1994; Moravec, 2004).

Aquatic ascaridoids (in this case anisakids) have life cycles that make extensive use of the food web where multiple trophic levels are bridged, often with a myriad of transport hosts. The definitive (final) host of these ascaridoids (anisakids) is often a top predator. Adults live in the stomach and intestines of their final hosts, females release eggs that are carried to the external aquatic medium with the host's feces. The partially

embryonated eggs develop in a temperature dependent manner in the water and the first larval stage usually molts once within the egg and hatches out as an L2 larva, often retaining the L1 cuticle around it as a sheath. Køie (1993) and Køie and Fagerholm (1993), however, interpret their findings to mean that L3, not L2, emerge from the eggs of two anisakids, *Hysterothylacium* and *Contracaecum*. These hatched larvae are ingested by certain invertebrate intermediate hosts (depending on the life cycle) where further development occurs, following which these larvae are further transmitted through the food chain by fish that eat these infected invertebrates (Køie and Fagerholm, 1995; Anderson, 2000). Larvae of *Anisakis simplex* and *Pseudoterranova decipens* develop and grow in invertebrate and fish hosts (Smith, 1983; McClelland *et al.*, 1985; Bowen, 1990; McClelland, 1990). Larvae of *Contracaecum* spp. and *Raphidascaris acus* typically use copepods as paratenic hosts and growth and development takes place in the subsequent fish host (Anderson, 2000). Lateral transmission of L3 stages from smaller prey fish to larger predator fish can cause the L3 of certain anisakids such as *A. simplex* and *P. decipiens* to accumulate in certain larger predator species. The L3 is the stage infective to the definitive host, where the third molt to L4 and the final molt to adult occurs in the stomach or intestine.

Accounts of the life cycles of nematodes are scattered in the literature but Anderson (2000) provides an excellent compilation of known life cycles of nematodes of vertebrates up until that time, including fish nematodes, and Moravec (1994) provides a comprehensive compendium on European fish nematodes, much of it based on his own expertise and contributions to the subject. Specific groups such as the dracunculoids have also been reviewed more recently (Moravec, 2004).

## Diagnostics (Taxonomy and Identification)

Resources used for identifying nematodes often also contain information on the distribution and biology of fish nematodes. For broader level taxonomy, the works of Anderson *et al.* (1974-1975) are still useful. Most compendia that provide keys, lists, and species accounts are regional in nature and vary in their format. They include Hoffman (1999) for the U.S.A. and Canada, Moravec (1994) for Europe, Moravec (1998) for the neotropical region, Sood (1988) for the Indian subcontinent, Bykhovskaya-Pavlovskaya (1962) and Vismanis *et al.* (1987) for the former Soviet Union, and Garrido-Olvera *et al.* (2006) for Mexico. The

works by Moravec (1994, 1998) are highly recommended because they also provide an in-depth account of the morphology and biology of fish nematodes, as well as methods for handling and processing these parasites. Other accounts (Grabda, 1989; Sinderman, 1990; Anderson, 2000) provide morphological and biological information as well as illustrations. Berland's (1989) work, although dated, remains is a well-illustrated and detailed account of the morphology of marine anisakids. Given the importance of eel aquaculture and the potential for translocations of *Anguillicola* spp., accurate species level identification these swimbladder nematodes will be crucial. In this regard the works by Moravec and Taraschewski (1988) and Moravec (2004) are also valuable resources.

The proper recovery of nematodes from the infected fish is important for subsequent identification. Poorly handled and preserved specimens often result in inconclusive identifications. Where possible, live nematodes should be recovered intact and both sexes should be collected if available, because it is often easier to determine the species from both sexes. The nematodes should be cleaned in 0.6% saline or PBS (phosphate-buffered saline) by gentle pipetting or agitation in a petridish, to wash off adhering debris. After this, the nematodes should be fixed by dropping some in hot 10% neutral buffered formalin and some in hot 70% ethanol. Using hot fixatives ensures that the nematodes are fixed in a relaxed position, which makes them easier to study later. Fixation in 10% neutral buffered formalin has the advantage that specimens can be processed for scanning electron microscopy but renders the specimen less transparent during clearing (see below). For future histopathological studies, the infected tissue, with some parasites still attached to it or in it should be fixed in an appropriate fixative. Again, 10% neutral buffered formalin is a routine fixative for these purposes, but other fixatives such as Bouins (aqueous or alcoholic) is also often used (Humason, 1974).

Given the widespread use of molecular techniques these days, some live nematodes should also be fixed separately in 95% or 100% ethanol, making sure that the ethanol does not contain denaturing agents such as ketones, aldehydes, methanol or kerosene, which are harmful to DNA. Isopropanol can be allowed as a denaturing agent. The sample in ethanol should be kept chilled in a regular freezer at –20°C if possible or in a regular refrigerator (~ 4°C) until use. DNA can be extracted from these worms using standard techniques, such as Qiagen's Qiaquick DNEasy DNA extraction kit (Qiagen Inc.).

Most parasitologists and pathologists use light microscopy to identify nematodes but the nematodes have to be 'cleared' (made transparent or translucent) so that their internal anatomy can be studied (nematodes, while generally translucent in life, become opaque when fixed). This clearing is accomplished by placing the nematodes in a small dish filled with 5% glycerine in 70% ethanol and allowing the ethanol and water to evaporate slowly at room temperature or on a mildly warmed slide warmer (if available). This eventually leaves the nematodes in glycerine. The nematodes are then transferred into a drop of that same glycerine (fresh glycerine from the bottle is often thicker and causes the nematode to collapse) on a glass slide and a cover slip (cover glass) placed over the preparation before examining with a compound light microscope. Alternatively, warm lactophenol can also be used to clear the nematodes, in which case nematodes are transferred from the 70% ethanol to lactophenol. The lactophenol method is faster but can occasionally cause specimens to collapse. Differential interference contrast (DIC) optics provides better resolution than regular transmitted light micropscopy. Scanning electron microscopy has also been used to address taxonomic problems (Fredericksen and Specian, 1980; Valter *et al.*, 1982; Weerasooriya *et al.*, 1986; Moravec *et al.*, 1998, 2006; Moravec and Nagasawa, 2000, 2006) but because of the time taken and expense incurred, this is not a routine procedure for identification.

Adult nematodes can be identified using a combination of the keys and reference works cited above. However, it is the larval stages that are the most difficult to identify, particularly to species. The anisakid L3 larvae have been especially problematic but they can usually be identified to genus based on a combination of the following characteristics: presence or absence of a mucron, position of the excretory pore, the boring tooth, alae, striations, the relative proportions of the esophagus and ventriculus, and of the cecum and appendix (when present) (see Choudhury and Dick, 1995). For example, L3 larvae of the three common genera of anisakids in the marine coastal environments of the north Atlantic, *Anisakis*, *Contracaecum* and *Pseudoterranova* can be differentiated by the fact that *Contracaecum* spp. have both an anteriorly directed intestinal caecum and a posteriorly directed ventricular appendix, *Pseudoterranova* spp. have only the caecum, whereas *Anisakis* spp. have neither. All three genera can be differentiated from another common marine anisakid genus, *Hysterothylacium*, by the presence of the excretory pore: anterior near the lips in *Contracaecum*, *Pseudoterranova* and *Anisakis*, posterior near the

nerve ring in *Hysterothylacium* spp. In some cases, experimental infections in laboratory hosts (Dick *et al.*, 1987; Fagerholm, 1988) and *in vitro* culture of nematode larvae (Likely and Burt, 1989, 1992).

Sometimes, pathologists may encounter nematodes in histological sections of fish tissues (Bruno *et al.*, 2006). They can be identified in such sections by their collagenous cuticle (which is clearly discernible), pseudocoelomic cavity, gut, and body wall with a hypodermis. In cross-sections, nematodes typically present a round or oval profile, and their lateral chords are often visible. A nematode in cross-section is difficult to confuse with other common fish parsites such as flatworms (cestodes, digeneans). Acanthocephalans also have a pseudocoelom and may resemble nematodes in histological sections but they lack a gut altogether and do not possess the distinctive collagenous cuticle of nematodes (Bruno *et al.*, 2006). Sometimes, nematodes may be identified in cross-section, at least to family or genus by a combination of distinctive characteristics, e.g., prominent lateral alae, buccal capsule, pseudobuccal capsule, ventricular appendix that are visible in the histological sections. Examining many different histological sections and forming a mental composite may help in making a reasonable diagnosis.

Biochemical and molecular methods have been extensively used in attempts to resolve the taxonomy of anisakids, particularly *Anisakis* spp. Based on allozyme analysis (Mattiucci *et al.*, 1997, 2005) and genetic markers in the rRNA genome (D'Amelio *et al.*, 2000), it appears that the common herring-worm, *Anisakis simplex*, is a complex of at least three sibling species: *A. simplex sensu stricto*, *A. pegreffi* and *A. simplex* C. The three species show differences in host preferences and distribution but pairs of them are also sympatric in certain areas (Mattiucci *et al.*, 1997; Abollo *et al.*, 2003). Molecular studies have also indicated gene flow between pairs of these sibling species where they are sympatric (Abollo *et al.*, 2003; Martín-Sánchez *et al.*, 2005). Biochemical and molecular data have indicated sibling species complexes in other anisakids such as *Hysterothylacium fabri* (Martín-Sánchez *et al.*, 2003), *H. corrugatum* (Mattiucci *et al.*, 1994) and the codworm (sealworm) *Pseudoterranova decipiens* (Appleton and Burt, 1991; Paggi *et al.*, 1991; Brattey and Dividson, 1996; Paggi *et al.*, 2000; Zhu *et al.*, 2002) or to validate single species status of some nematodes such as *Hysterothylacium auctum* (Szostakowska *et al.*, 2001).

Biochemical and molecular methods have also been used for diagnostic work, including diagnosing worms from seafood (reviewed by

La Rosa *et al.*, 2005). More recently, specific 18S rRNA gene primers have been used to detect anisakids in sardines (Santos *et al.*, 2006). Such tools would be very useful for the fish processing industry, especially if this diagnostic tool can be combined with quick estimates of worm burden. Mattiucci *et al.* (1998) used allozyme data to identify species of the three common anisakid genera, *Anisakis*, *Contracaecum* and *Pseudoterranova*, in Japanese waters, and while allozymes continue to be used as genetic markers (Mattiucci *et al.*, 2004, 2005) DNA probes and sequences obtained through PCR-based molecular methods have gained more widespread application. PCR-RFLPs (restriction fragment length polymorphisms) have been successfully used to identify and differentiate between species and genera of nematodes (Zhu *et al.*, 1998; Kijewska *et al.*, 2002; Szostakowska *et al.*, 2002) and PCR-based techniques have also been used to identify anisakids in Japanese Pacific cod (Abe *et al.*, 2005), in fishes from Portugese waters (Pontes *et al.*, 2005; Marques *et al.*, 2006), in the cutlass fish (Shih, 2004), and in several species of fish from the Baltic, Norwegian, Bering and Okhotsk Seas (Kijewska *et al.*, 2000). Despres *et al.* (1995) developed a PCR-based method to differentiate between *Philonema oncorhynchi* and *P. agubernaculum* in Pacific salmonids.

Given the importance of *A. crassus*, it is not surprising that efforts have been made to develop non-invasive diagnostic tools to detect infections by these parasites in eels. Ushikoshi *et al.* (1999) and Knopf *et al.* (2000a, b) have successfully used the enzyme linked immunosorbent assay (ELISA) to detect the presence of specific antibodies in infected eels. Beregi *et al.* (2003) developed radio-diagnostic tools to study swimbladder inflammation as well as its contents. This technique was further developed by Szekely *et al.* (2004, 2005) who applied X-ray computerized tomography and traditional X-ray methods to examine the infected eels. Shin and Chen (2000) reported that the merthiolate-iodine-formalin (MIF) technique, which is relatively inexpensive and widely used for screening egg and larval stages of parasites in fecal samples, was very effective in diagnosing infections in eels. Crean *et al.* (2003) reported anal redness as a diagnostic symptom of *A. crassus* infection in a sample of infected European eels from Northern Ireland and the intensity of the redness was correlated with worm burden. Corroboration of this phenomenon from a wider geographical range would allow this to become a simple and useful non-invasive diagnostic tool.

## PATHOLOGY AND AETIOLOGY

In order to facilitate use by aquaculturists and fisheries biologists, and others interested in diseases caused in fishes by nematodes, we include a list of the disease causing nematodes and their associated pathology by organ system (Table 17.2).

### General Pathology

Nematodes that infect extra-intestinal sites and tissues are generally more pathogenic than those that infect the gastro-intestinal tract although several gut nematodes cause mucosal erosion or burrow into the mucosa and submucosa and cause pathology (Fig. 17.2). Like other parasites, nematodes elicit an immune response that is often non-specific but some nematode infections may generate immunological memory (Ramakrishna and Burt, 1991, 1993; Silva *et al.*, 1999). The presence of antibodies specific for antigens of nematodes has also been documented in fishes (Buchmann *et al.*, 1991; Priebe *et al.*, 1991; Coscia *et al.*, 1997a, b; Coscia and Oreste, 1998, 2000), particularly in experimental infections of eels (*Anguilla* spp.) with *Anguillicola crassus* (Nielsen, 1999; Knopf *et al.*, 2000; Sures and Knopf, 2004). The most common initial immune response to a tissue-invasive nematode, whether it is in the gut or some other organ is similar to localized inflammation or a hypersensitivity response (Fig. 17.2). This is often accompanied by haemorrhaging, oedema and, in chronic infections, peristent necrosis, encapsulation and granuloma formation (Hauck and May, 1977; Elarifi, 1982; Jilek and Crites, 1982; Dunn *et al.*, 1983; Dick, 1987; Eiras and Rego, 1988; Measures, 1988; Ramakrishna and Burt, 1991; Eng and Youson, 1992a, b; Credille *et al.*, 1993; Molnar, 1994; Dick and Choudhury, 1995; Molnar *et al.*, 1995; Meguid and Eure, 1996; Szekely *et al.* 1996; Ghitino, 1998; Saraiva *et al.*, 1998; Borucinska and Heger, 1999; Dezfuli *et al.*, 2000; Borucinska and Frasca, 2002; Conboy and Speare, 2002; Kent *et al.*, 2002; Benz and Bullard, 2004; see Fig. 17.2). Visceral adhesions may also be common in certain infections such as *Philonema agubernaculum* in Pacific salmonids (*Oncorhynchus* spp.) (Meyer, 1960; Nagasawa, 1985, 1989). These clinical symptoms are found in various combinations depending on the type of parasite and the severity of the disease. For example, once a larval anisakid such as an L3 of *Contracaecum* spp. has been encapsulated and attains a dormant state in the fish host, it causes little continued pathology compared to its earlier migratory phase. There may also be other effects due to the growth of the

**Table 17.2** Nematodes causing pathology and disease in fishes.

| *Organ and Organ system* | *Parasite* | *Host* | *Pathology* |
|---|---|---|---|
| Eye (orbit) | *Philometra* sp. | *Lepomis macrochirus* (bluegill) | Exophthalmia (Hoffman, 1975) |
| Eye | *Philometroides okeni* | Sea perch | Exophthalmia (Bauer *et al.*, 1977) |
| Eye (socket) | *P. fulvidraconi* | *Pseudobagrus fulvidraco* | Inflammation, lens turbidity, extrusion of eyeballs, eyes lost in severe cases (Wang, 2002) |
| Brain | Dracunculoidea gen. sp. | *Ginglymostoma cirratum* (nurse shark) | Lesions, meningoencephalitis (Credille *et al.*, 1993) |
| Skin | *Cystoopsis acipenseris* | Sturgeon (Caspian), sterlet | Abdominal nodules (Grabda, 1989), capsules under bony plates, oedema (Bauer *et al.*, 1977) |
| Skin (subcutaneous) | *Huffmanella* sp. (eggs) | *Sebastes* spp. | 'Black-mould syndrome', linear black disfigurations and erosion, inflammation (Conboy and Speare, 2002) |
| Skin (subcutaneous) | *Philonema americana* | *Platichthys stellatus, Lepidopsetta bilineata* | Nodules (Grabda, 1989), Sindermann (1990) (fins) |
| Skin (subcutaneous) | *Philometroides huronensis* | *Catostomus commersoni* | Mechanical disruption and compaction of sub-epidermal tissue; localized inflammation; fibrotic encapsulation of adults (Uhazy, 1978) |
| Skin (subcutaneous) | *Phlyctainophora lamnae* | *Mustelus mustelus* | Tumors in ventral and pectoral fins (Williams, 1967) |
| Skin | *Eustrongylides* sp. | *Bagrus docmac* | Unspecified, encapsulation? (Paperna, 1991) |
| Muscle | *Eustrongylides* sp. | *B. docmac* | Encapsulation, inflammation, necrosis (Paperna, 1991) |
| Muscle | *Eustrongylides* sp. | *Pseudoplatysoma corruscans* | Leucocyte infiltration, necrosis (Eiras and Rego, 1988) |
| Muscle | *Eustrongylides* sp. | *Clarias gariepinus, Haplochromis* sp., *Oncorhynchus mykiss* | Damage, necrosis, cellular infiltration (Paperna, 1974) |
| Muscle | *Eustrongylides* sp. | *Paratrygon* sp. | Encapsulation, leucocyte infiltration (Berland, 1987) |
| Muscle | *Pseudoterranova decipiens* | *Gadus morhua* | Loose, spongy (Grabda, 1989), encapsulation (Kahl, 1938a), chronic granulomatous inflammation (Ramakrishna and Burt, 1991, 1993) |

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|---|---|---|---|
| Muscle | *P. decipiens* | *Osmerus eperlanus* | Affects swimming performance (Sprengel and Leuchtenberg, 1991) |
| Muscle | *Anisakis* sp. | Various marine fishes | Necrosis, encapsulation, inflammation (Sindermann, 1990) |
| Muscle | *Contracaecum microcephalum* | *Oncorhynchus mykiss* | Haemorrhaging (Dick, 1987) |
| Liver | *Capillaria eupomitis* | *Lepomis gibbosus* | Enlarged, anaemic, jaundiced (Hoffman, 1975) |
| Liver (and pancreas) | *C. cichlasomae* | *Cichlasoma urophthalmus* | Cholangiopathy, degeneration of acinar cells (pancreas) (Sima *et al.*, 1996). |
| Liver | *Capillaria* sp. | *Scomber japonicus* | Atrophy, necrosis (Margolis, 1970) |
| Liver | *Truttaedacnitis pybusae* | *Lampetra lamottenii* (brook lamprey) | Intracellular alteration of hepatocytes (mainly Golgi and smooth endoplasmic reticulum); dilation of bile ducts and periductal fibrosis (Eng and Youson, 1992a, b) |
| Liver | *Anisakis simplex* | Marine fishes (cod, redfish) | Tissue destruction, small livers (Margolis, 1970; Grabda, 1989) |
| Liver | *Anisakis* sp. | *Micromesistius potassou* | Impaired function, damage (Smith and Wootten, 1978) |
| Liver | | Marine teleosts | Unspecified pathology (Smith and Wootten, 1978) |
| Liver | | *Merlangius merlangus* | Encapsulation, melanin deposition, leucocyte and fibroblast infiltration (Elarifi, 1982) |
| Liver | *Contracaecum* sp. (possibly *Hysterothylacium*) | Gadidae (cods) | Decrease in liver weight, fat content, condition factor (Margolis, 1970) |
| Liver | *Contracaecum* sp. (possibly *Hysterothylacium*) | *Merlangius merlangus* | Encapsulation, melanin deposition, leucocyte and fibroblast infiltration (Elarifi, 1982) |
| Liver | *Contracaecum* sp. (possibly *Hysterothylacium*) | *Labrus festivus* | Unspecified pathology, role in mortality (Margolis, 1970) |
| Liver | *Hysterothylacium aduncum* | Baltic cod, herring larvae | Weight loss, decrease in mass and fat content (Margolis, 1970) |

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| Liver | *Raphidascaris acus* | *Abramis brama* (bream) | Lesions, rusty and pink or yellowish with dark green hue, congested (Grabda, 1989)<br>Dirty brown color, gall bladder damaged (Bauer *et al.*, 1977) |
| Liver | *R. acus* | *Lota lota*, *Stizostedion vitreum* | Lesions, hemorrhaging, granulomas, necrosis (Dick, unpublished in Dick and Choudhury, 1995) |
| Liver | *R. acus* | *Oncorhynchus mykiss* (experimental) | Lesions, hemorrhaging, granuloma (Dick, unpublished in Dick and Choudhury, 1995) |
| Liver | *R. acus* | *Coregonus clupeaformis* | Lesions, nodules (Dick, unpublished in Dick and Choudhury, 1995) |
| Liver | *R. acus* | *C. nasus* | Pathology, lesions (Choudhury and Dick, unpublished) |
| Liver | *R. acus* | *Perca flavescens* | Lesions, granuloma, necrosis (Poole and Dick, 1984; Szalai and Dick, 1991) |
| Liver | *R. acus* | *Barbatula barbatula* (stone loach) | Cysts, abscess-like formations (Koubkova *et al.*, 2004) |
| Liver | *Philonema opsalichthydis* | *Hypomesus olidus* | Unspecified pathology (Williams, 1967) |
| Liver | *Acanthocheilus rotundatus* | *Gadus morhua* | Fibrosis Kahl (1938b) |
| Pancreas | *Pacreatonema americanum* | *Squalus acanthias* | Pancreatitis, chronic lesions, focal fibrosis, ductal ectasia, necrosis and rupture, hyperplasia of ductules, attenuation and erosion of ductal epithelium (Borucinska and Frasca, 2002) |
| Pancreas (& liver) | *Raphidascaris acus* | *Rutilus rutilus* (roach) | Chronic granulomatous inflammation (Valtonen *et al.*, 1994) |
| Viscera | *Philonema oncorhynchi* | *Oncorhynchus nerka*, *O. keta* | Adhesions, involving mesentery (Nagasawa, 1989; Garnick and Margolis, 1990) |
| Viscera | *P. rubra* | *Morone saxatilis*, *M. americana* | Adhesions, oedema, granulomas (Sindermann, 1990) |
| Viscera: Multiple organ | *P. oncorhynchi* | *Oncorhynchus kisutch* | Long standing infection: diffuse fibrogranulomatous perienteritis, perisplenitis, peripancreatitis, steatitis (Brocklebank *et al.*, 1996) |

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|---|---|---|---|
| Body cavity | *Philometra* sp. | *Canthigaster jactator* | Abdominal protrusion (Deardorff and Stanton, 1983) |
| Body cavity | *Philometra rubra* | *Morone saxatilis* | Peritoneal inflammation, serosal adhesions (Paperna and Zwerner, 1976) |
| Gonads | *Eustrongylides* sp. | *Bagrus docmac* | Necrosis, sterility (Paperna, 1991) |
| Gonads | *Eustrongylides* sp | *Clarius gariepinus*, *Haplochromis* spp. | Deformed and degenerate (Paperna, 1974) |
| Gonads | *Philonema agubernaculum* | *Salmo salar* | Loss of function, sexual impotency 1, ovaries encased, genital pore blocked (Margolis, 1970; Sindermann, 1990) |
| Gonads | *P. globiceps* | *Uranoscopus surinamensis* | Destruction of gonads (Grabda, 1989) |
| Gonads | *P. saltatrix* | *Pomatomus saltatrix* | Hemorrhage, inflammation, oedema, necrosis, follicular atresia (Clarke *et al.*, 2004) |
| Gonads | *Philometra* sp. | *Otolithus argentatus*, *Chrysophrys auratus* | Ovaries destroyed, testes atrophied, replaced by nematode (Williams, 1967; Margolis, 1970; Sindermann, 1990) |
| Gonads | *P. lusiana* | Carp | Unspecified (Margolis, 1970) |
| Gonads | *Philometra* sp. | *Chrysophrys auratus* | Inflammation, fibrosis, granuloma, necrotic foci, swelling (Hine and Anderson, 1982) |
| Gonads | *P. cephalus* | *Mugil cephalus* | Ovaries hypertrophied or atrophied, fibrosis (Ramachandran, 1975; Sindermann, 1990) |
| Gonads | *Philometra* spp. | *Epinephelus septemfasciatus* | Ovary: Lesions, fibrosis, hemorrhaging, atrophy of ova, (Moravec *et al.*, 2002) |
| Gonads | Philometridae gen. sp. | *Carcharhinus limbatus* | Ovary: granuloma (Rosa-Molinar and Williams, 1983) |
| Swimbladder | *Cystidicola stigmatura* | *Salvelinus namaycush* | Lesions, ulcers, hyperemia (Lankester and Smith, 1980; Black, 1984) |
| Swimbladder | *C. farionis* | *Coregonus artedii* | Lesions, inflammation, enlargement, epithelial atrophy, histiocyte and granular cell infiltration (Willers *et al.*, 1991) |

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|---|---|---|---|
| Swimbladder | *C. farionis* | *Oncorhynchus mykiss* | Inflammation (Otto and Korting, 1973) |
| Swimbladder | *C. farionis* | *O. mykiss* | Ulcers (with craters), mucosal hyperemia (Lankester and Smith, 1980) |
| Swimbladder | *Anguillcola crassus* | *Anguilla anguilla* | Thickening of wall, folded internal surface, inflammation, fibrosis, epithelial hyperplasis, cytological changes, infiltration of lamina propria by leucocytes (Würtz and Taraschewski, 2000) |
| Swimbladder | *A. crassus* | *A. anguilla* | Acute: lesions, wall hyperaemia, epithelial hyperplasia<br>Chronic: Inflammation; oedema; tunica propria, submucosa and serosa thickened; monocyte, lymphocyte, histiocyte and plasma cell infiltration (Molnar *et al.*, 1993) |
| Swimbladder | *A. crassus* | *A. anguilla* | Dilation of blood vessels, inflammation, rupture, fibrosis and visceral adhesions (Haenen *et al.*, 1989; Van Banning and Haenen, 1990) |
| Swimbladder | *A. globiceps* | Eels | Haemorrhagic inflammation (Paperna, 1991) |
| Swimbladder | *Philometroides lusiana* | Carp | Swimbladder function impaired (Bauer *et al.*, 1977) |
| GI Tract | *Capillaria* sp. | *Symphysodon* sp. | (Intestine) Unspecified, loss of function, light and dark fecal segments (Lo Wing Yat, 1988) |
| GI Tract | *Camallanus oxycephalus* | *Sander vitreus* | (Rectum) hyperplasia, inflammation, vascularization, loss of epithelium; infiltration of eosinophils, lymphocytes, fibroblasts (Choudhury and Dick, unpublished) Same as above, granulomatous material (Dick and Choudhury, 1995) |
| GI Tract | *Procamallanus spiculogubernaculus* | *Heteropneustes fossilis* | (Oesophagus, stomach) blood loss (results in anaemia) (Sinha and Sinha, 1988) |
| GI Tract | *Cucullanus* (*Cucullanellus*) sp. | Flounders | (Intestine) Extensive pathology (Sindermann, 1990) |

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|---|---|---|---|
| GI Tract | *Cucullanus minutus* | *Platichthys flessus, Plueronectes platessa* | (Gut wall, mesenteric blood vessels) inflammation, necrosis, vessel dilation, capillary destruction, hemorrhaging, encapsulation on mesenteric blood vessels; lymphocyte and histiocyte infiltration (Janiszewska, 1938; Margolis, 1970) |
| GI Tract | *Cystidicoloides ephimeridarum* | *Salmo trutta (fario)* | Localized disruptions of mucosal epithelium without inflammation (Frantová and Moravec, 2003) |
| GI Tract | *Truttaedacnitis truttae* | *Oncorhynchus mykiss* | Tissue destruction in duodenum: lesions with loss of mucosal epithelium, mucosal hyperplasia, hemorrhage and fibrosis in lamina propria (R.A. Cole, unpublished). |
| GI Tract | *T. truttae* | *O. mykiss* | (Pyloric ceca) epithelium loss, mucosal hyperplasia, haemorrhaging, fibrosis. Infiltration of granulocytes and fibroblasts (Dunn *et al.*, 1983) |
| GI Tract | *Spinitectus* sp. | *Lepomis macrochirus* | (Intestine) Inflammation (Hoffman, 1975) |
| GI Tract | *Spinitectus carolini* | *L. macrochirus* | Small intestine (submucosa): Penetration, lesions, aseptic traumatic enteritis, infectious enteritis, desquamative catarrhal inflammation, necrosis, haemorrhage, encapsulation; infiltration with eosinophils, other leucocytes, macrophages, fibroblasts (Jilek and Crites, 1982) |
| GI Tract | *S. jamundensis* | *Prochilodus lineatus* | Stomach (mucosa, muscularis layer): Penetration, lesions, lymphocytic infiltration (Ramallo *et al.*, 2000). |
| GI Tract | *Acanthocheilus nidifex* | *Galeocerdo cuvieri (=tigrinus)* | (Stomach) Inflammation, vascularization, connective tissue deposition (Margolis, 1970) |
| GI Tract | *A. rotundatus* | *Gadus morhua* | (Gut wall) Encapsulation in gut wall (Kahl, 1938b) |
| GI Tract | *Anisakis* sp. | *G. morhua* | (Stomach wall) Encapsulation, ulceration (Sindermann, 1990) |

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|---|---|---|---|
| GI Tract | | *Clupea harengus pallasi* | (Pyloric caeca, intestine) mechanical compression, inflammation, trauma of muscularis externa of pyloric ceca (Hauck and May, 1977) |
| GI Tract | "Contracaecum" (*Hysterothylacium*) *bidentatum* | Sturgeon (Black and Caspian seas) | (Esophagus, stomach, intestine) Inflammation (Bauer *et al.*, 1977) |
| GI Tract | *Goezia leporini* | *Leporinus macrocephalus* | Fluid accumulation, perforation of stomach wall (Martins *et al.*, 2004) |
| GI Tract | *G. sinamora* | *Tilapia aurea, Morone saxatilis* | (Stomach) nodules, lesions (Deardorff and Overstreet, 1980b) |
| GI Tract | *G. pelagia* | *Rachicentron canadum* | (Stomach) nodules, lesions, hemorrhage, mild autolysis of gastric glands (Deardorff and Overstreet, 1980b) |
| GI Tract | *Hysterothylacium aduncum* | *Oncorhynchus mykiss* (farmed) | (Intestine) Unspecified pathology, mortality (Berland, 1987) |
| GI Tract | *H. dollfusi* | *Polyodon spathula* | (Stomach, caeca, intestine) Stomach ulcers, nodules, hemorrhage; infiltration of eosinophils, macrophages and fibroblasts (Miyazaki *et al.*, 1988) |
| GI Tract | *Hysterothylacium* sp. | *Ariopsis guatemalensis, Eleotris picta* | Lesions in the submucosa and muscular layers, occasional enteritis, fibrosis and necrosis (Vazquez Nunez *et al.*, 2004) |
| GI Tract | *Pseudanisakis rotundata* | *Raja radiata* | (Intestine) Inflammation, infiltration of eosinophils (Margolis, 1970) |
| GI Tract | *Raphidascaris acus* | *Esox lucius* | (Intestine) Inflammation (Grabda, 1989) |
| GI Tract | *Proleptus obtusus* | *Scyllium canicola* | (Intestine, pyloric region) hemorrhage, destruction of epithelium, ulceration, penetration into tunica propria and muscularis mucosa, leucocyte infiltration (Schurmans-Stekhoven and Botman, 1932; Margolis, 1970) |

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|---|---|---|---|
| GI Tract | *Proleptus obtusus* | *Hemiscyllium ocellatum* | Minimal or no inflammatory or cellular immune reaction; large amount of connective tissue proliferation at attachment sites (Heupel and Bennett, 1998) |
| Heart & Blood vessels | *Philometra obturans* | *Esox lucius* | Obstruction, parietal thrombi in bulbous arteriosus, endothelium hypertrophied in ventral aorta, obstruction of gill arteries (Moravec and Dykova, 1978; Kall *et al.*, 2004) |
| Heart | *Contracaecum microcephalum* | *Culaea inconstans*, *Pimephales promelas* | Enlarged atrium, thrombi (Dick, 1987) |
| Heart and pericardial cavity | *Philometra saltatrix* | *Pomatomus saltatrix* | Inflammation, blood pooling, necrosis (Cheung, Nigrelli and Rugieri, 1984) |
| Pericardial cavity | *Contracaecum* sp. | Tilapia hybrids, cichlids | Enlargement and stretching of pericardial sac (Paperna, 1991) |

larvae in these ultimate sites of infection, such as in larvae of *Contracaecum* occupying the pericardial cavity of tilapia (Paperna, 1991). On the other hand, the eel swimbladder nematode causes well-documented and extensive pathology as an adult in its final resting place, the swimbladder (Table 17.2).

The severity of the disease caused by nematode infections often depends on the species of host and parasite involved. For example, the nematode *Philometra lateolabracis* did not cause conspicuous atrophy of ovaries in the Australian dhufish, *Glaucosoma hebraicum* (Hesp *et al.*, 2002), unlike several other philometrids in other hosts (Table 17.2, also see below). The eel swimbladder parasite, *A. crassus*, which causes serious disease in European eels is not an important pathogen in its native Asia. Another congener, *A. novazaelandae*, which infects the swimbladder of its native eel host, *Anguilla australis*, is likewise not pathogenic in that fish host (Lefebvre *et al.*, 2004b). As a general principle, introduced parasites that successfully establish in new hosts in new areas can be expected to be more pathogenic in these new hosts, at least initially. After a period of time, pathogenicity and virulence may decrease (Molnar *et al.*, 1994).

Pathology also generally increases as worm burden increases and is also correlated with reinfections, as has been demonstrated experimentally in eels infected with *A. crassus* (Haenen at al, 1996). This conforms to observations in wild eels (Lefebvre *et al.*, 2002) where severely damaged swimbladders are found in larger (older) eels, but such damaged bladders apparently become uninhabitable and harbor few nematodes. Thus, pathology may be cumulative rather than transient, as it may be for many intestinal nematode infections that are seasonal. Most of the typical vertebrate immune cells are involved in the response to nematodes; lymphocytes, granulocytes (mainly neutrophils and eosinophils when present), macrophages and fibroblasts (in encapsulation and granuloma formation) (Ramachandra and Burt, 1991; Reite, 1998, 2005; Reite and Evenson, 2006).

## Mortality

Reports of nematodes causing outright mortality are rare. Several species of anisakid nematodes have been associated with fish mortality: the larvae of the marine *Hysterothylacium aduncum* has been associated with mortalities of larval marine fish (cod or herring) and farmed rainbow trout ( Margolis, 1970; Berland, 1987). The larvae of *Contracaecum* sp. has been

associated with mortalities of African freshwater fishes including tilapia and *Labrus festivus* (Margolis, 1970; Grabda, 1991), and the larvae of the freshwater *Raphidascaris acus* was reported to cause mortality in rainbow trout (Williams, 1967; Dick, unpubl., in Dick and Choudhury, 1995). *Anguillicola crassus* has been reported to cause mass mortality of European eels in Lake Balaton, Hungary (Molnar *et al.*, 1991) and of American eels in an aquaculture facility in Taiwan (Ooi *et al.*, 1996). Infection with *Pseudoterranova decipiens* was reported to decrease survival rates of European smelt *Osmerus eperlanus* interacting with the screening system of a power plant (Rohlwing *et al.*, 1998). Heavy infections of *Philometroides fulvidraconi*, which infects tissues around the eyes, causes mortality of catfish *Pseudobagrus fulvidraconi* in China (Yu *et al.*, 2002). There is some circumstantial evidence that *P. oncorhynchi* may cause mortality in pen-reared coho salmon (Brocklebank *et al.*, 1996).

## Physiological and Behavioral Effects

Nematodes have been reported to cause changes in the body condition, swimming performance, fecundity, hematological profile and other health parameters of fishes. Based on a modified health assessment index (mHAI), McKinney *et al.* (2001) concluded that the health of a population of rainbow trout declined as burdens of the cecal nematode, *Truttaedacnitis truttae*, increased. This corroborates an earlier study (Russell, 1979), which found that growth rates of rainbow trout decreased as worm burden increased even though no effect on swimming performance was reported. In contrast, infection with larvae of the anisakid *Pseudoterranova decipiens* reportedly reduces the swimming speed in smelt (Rohlwing *et al.*, 1998) while in European eels, infections with *P. decipiens* and the swimbladder nematode, *A. crassus*, together have reportedly the same effect (Leuchtenberg, 1991). While there is evidence to suggest that anguillicolosis does not affect swimming performance in the short term (Muenderle *et al.*, 2004), Van den Thillart *et al.* (2005) observed that heavy infections of eels *by A. crassus* did reduce speed and endurance, which may have serious implications for their spawning migration. In laboratory experiments, Dolly Varden charr, *Salvelinus malma*, infected with a single individual *Philonema agubernaculum* (Philometridae) showed reduced ability to capture prey (Moles, 2003) while another related philometrid, *Philonema oncorhynchi* may impact orientation of salmon during the migratory phase (Garnick and Margolis, 1990). In coastal pen-reared coho salmon that had intitially been reared

in a freshwater lake, slow surface swimming salmon as well as moribund fish were found to have pale gills, distended abdomens and their visceral organs showed pathology associated with *P. oncorhynchi* infections (Brocklebank *et al.*, 1996).

Liver infections of *Raphidascaris acus* retard the growth and sexual maturation of yellow perch, *Perca flavescens*, along with a decrease in condition factor (Szalai and Dick, 1991) while liver nematode (unspecified anisakid) infections in cod result in a decrease in the weight and condition factor of the fish (reviewed by Margolis, 1970). Mating and feeding behavior in guppies is altered in fish infected with *Camallanus cotti* (McMinn, 1990). Some philometrid nematodes such as *Philonema tranlucida* and *P. saltatrix* reduce fecundity by damaging the gonads (Ramachandran, 1975; Anyanwu, 1983). The importance of *P. saltatrix* in the culture of Mediterranean perciforms has been stressed by Moravec *et al.* (2003). Infection with larval *Eustrongylides* sp. results in lower body and ovarian mass of mummuchog, *Fundulus heteroclitus*, resulting in lower fecundity (Hirshfield *et al.*, 1983) and *E. ignotus* infections in mosquitofish (*Gambusia affinis*) made them more susceptible to predation (Coyner *et al.*, 2001).

Nematode infections may make natural fish populations susceptible to other, environmental, stressors such as water contamination (Marcogliese *et al.*, 2005), and conversely, environmental stressors such as pollution may make fish more susceptible to infection through immunosuppression (Sures and Knopf, 2004b). Marcogliese *et al.* (2005) reported higher lipid peroxidation levels (a measure of oxidative stress) in yellow perch (*Perca flavescens*) infected with *Raphidascaris acus* larvae at a contaminated site on the St. Lawrence River compared to infected fish from an uncontaminated reference site. Eels infected with *A. crassus* show significantly higher levels of blood (plasma) cortisol when exposed to severe hypoxia compared to uninfected eels (Gollock *et al.*, 2005a). Sometimes the metabolic costs of nematode infections may be expressed in subtle ways; eels infected with *A. crassus* showed a 'slight lag' in glucose metabolism compared to uninfected controls (Gollock *et al.*, 2005b). Sures *et al.* (2001) were able to induce stress in eels with repeated experimental infection of *A. crassus*. At least one study (Kelly *et al.*, 2000) did not find any change in hormonal, metabolic or osmoregulatory status in wild European eels infected with *A. crassus*, suggesting that wild eels 'appear to adapt to chronic parasitism' (Kelly *et al.*, 2000) with *A. crassus*. It is

difficult to extrapolate from experimental studies to observations in natural populations because of the complex dynamics of natural infections and their effects on wild eel populations (Kirk, 2003), e.g., trickle infections, seasonality, ontogenetic changes in eel predator-prey interactions, among other variables.

Changes in hematological (blood) parameters have also been reported in at least two nematode infections. The gut nematode *Goezia leporini* was reported to reduce hematocrit, mean corpuscular volume, haemoglobin concentration and lymphocyte counts with a concomitant increase in neutrophil and eosinophil counts (Martins *et al.*, 2004). A similar decrease in lymphocytes and concomitant increase in granulocyte counts was reported in the European eel over the period of infection with the swimbladder nematode, *A. crassus* (Boon *et al.*, 1990). These findings are not surprising considering the role of these granulocytes, especially eosinophils, in helminth infections (Reite, 1998, 2005; Reite and Evensen, 2006). Boon *et al.* (1990) also reported a decrease of total plamsa protein with higher *A. crassus* burdens and Lefebvre *et al.* (2004) reported increases of spleen size in heavily infected eels which may be related to increased lumphocyte production and activation. However, at least one study (Kelly *et al.*, 2000) has suggested that wild European eels 'appear to adapt to chronic parasitism' with *A. crassus*.

## Other Potential Pathogens

Several species of dracunculoids have been reported from the heart and circulatory system of fishes, e.g., *Pseudodelphis* spp., *Lockenloia sanguinis, Syngnathinema californiense* (Adamson and Roth, 1990; Adamson and Caira, 1991; Moravec *et al.*, 2001; Bennet and Adamson, 2004). *Philometra saltatrix* has been reported from the heart cavity of young-of-the-year bluefish (*Pomatomus saltatrix*) (Cheung *et al.*, 1984). While the precise pathogenic effects have not been clarified, one can reasonably assume that these nematodes would have some effect on the circulation of their hosts. Adult females of the dracunculoid, *Philometra obturans*, occupy gill arteries, and according to Moravec and Dykova (1978) burrow through their walls and cause haemorrhaging. While Kåll *et al.* (2004) did find evidence for arterial distension by these worms, they found no evidence of active penetration and associated haemorrhaging. One species, *Pseudodelphis limnicola* from the heart of the valued food fish, *Percichthys trucha*, in Argentina (Brugni and Viozzi, 2006), should be of

concern, particularly if the host species is cultured and maintained in aquaculture facilties along with wild broodstock. The presence of eggs of several *Huffmanella* species (Family Trichosomoididae) in bone tissue is also noteworthy (Justine, 1987).

## CONTROL AND TREATMENT

### Control

Controlling disease-causing nematodes in naturally infected populations of fishes is difficult and presents numerous logistic problems. In the case of some nematodes, such as larval anisakids, the culling of bird and seal definitive hosts has been suggested as a means of controlling the parasite abundance in the fish hosts, but the reaction to such suggestions has been mixed. Definitve hosts are relatively easy targets because of their size and hence visibility and certainly easier to control than intermediate hosts, especially in the wild. We are not aware of any cases of fish nematodes having been eradicated from a wild fish population. Treating wild fish populations with anthelmintics is not considered to be feasible.

The first step to controlling the establishment of parasites in aquaculture or captive situations (e.g., ornamental fishes) is the proper quarantine of fishes being introduced into the facility from other localities (other hatcheries, natural populations, etc.). This is of particular concern in Asia (Tonguthai, 1997; Kim *et al.*, 2002) but remains an issue worldwide. Quarantine practices are usually used in conjunction with formal certification to ensure that no pathogens enter or leave a population of natural or captive fishes. A survey of export farms in Sri Lanka (Thilakaratne *et al.*, 2003) found *Capillaria* spp. in several ornamental fish species and the treatment and control of these parasites at the source would be a more effective strategy. In view of the efforts to manage and farm eels in Africa and Madagascar, Taraschewski *et al.* (2005) have cautioned against the inadvertent introduction of eel swimbladder nematodes due to farming and stocking, the consequences of which could be severe. Ultimately, the success of such practices depends on continuous monitoring and enforcement.

In aquaculture or captive situations, parasites with direct transmission (e.g., copepods, monogeneans, protistans) are generally of greater concern and more likely to cause problems than parasites with life cycles involving one or more intermediate hosts (e.g., nematodes, cestodes, trematodes). Exceptions such as capillariids—many of which may have direct life cycles

without intermediate hosts—are of particular concern. Nevertheless, parasites with indirect life cycles have been known to establish in aquaculture situations, most often as larval stages in the fish (*Contracaecum* spp., *Eustrongylides* spp.), but also as adults (the trout cecal nematode *Truttaedacnitis truttae* is occasionally present in salmonid aquaculture operations). Knowledge of the life cycles of the nematodes is always an advantage and often critical to developing strategies for control; the recent report of ostracods as natural intermediate hosts of *A. crassus* (Moravec *et al.*, 2005) and its implications for eel aquaculture operations is a case in point.

The most obvious strategy of control/prevention is excluding hosts, both intermediate and definitive. This includes excluding exotic definitive hosts from which parasites may be acquired and natural predators such as piscivorous birds and mammals that are often definitive hosts for the larval stages occurring in the fish. Excluding potential intermediate invertebrate hosts is often difficult because of their size and the ease with which some (such as copepods, ostracods, tubificids, etc.) colonize aquatic environments. Nevertheless, in aquaculture situations, precautions must be taken to isolate any exotic fish transfers into the facility and separate them from fish already present. In recirculation systems, the water supply to these exotic transfers should be kept separate and dip nets, boots and other equipment used to handle any exotic transfers should not be used for other fish. If a natural water supply is being used, ground water is preferable to water from a stream or pond. In the latter case, the water should be passed through a commercial filtering system before it is used for aquaculture operations. This will limit parasite establishment. Exotic fish can also kept out by barricades, curtains or other similar devices. In outdoor aquaculture operations, the facility should be routinely monitored for the build up of invertebrate populations and arrangements should be made to keep fish eating birds away. Defecation by fish-eating birds often introduces nematodes such as *Contracaecum* and *Eustrongylides*, both of which have relatively large and pathogenic larval stages. These parasites only require one intermediate host in order to be transmitted to the fish in the facility, through copepods in the former and through tubificid annelids in the latter. Both these invertebrates are often common in outdoor aquaculture ponds and tanks, and so preventing birds from gaining access to these facilities may be a more efficient option than trying to eliminate these invertebrates. Many of the parasite problems can be prevented with proper husbandry techniques and a good dose of common

sense. Tonguthai (1997) considered the following aspects to be critical to successful prevention of a disease outbreak in captive situtations: desirable water qualities, adequate nutrition, appropriate stocking density, and adherence to other measures such as reducing stress, applying chemotherapy and developing disease-resistant stocks.

## Treatment and Post-infection Measures

The following drugs have been either suggested, recommended or used against fish parasitic nematodes: Mebendazole, Fenbendazole, Levamisole and Piperazine. (Yanong, 2002, www.merckvetmanual.com, Benz and Bullard, 2004). Yanong (2002) lists febendazole and levamisole as two "effective and commonly used dewormers" for nematode infections. Febendazole is used only as a feed additive (1.14 g / lb of food, fed for 3 days, repeated after 2-3 weeks) whereas levamisole can be added to the feed or to the water as a bath treatment (Yanong, 2002). A dose of 1.8 g/ lb of food fed once a week for 3 weeks is considered an effective oral dose, and 2ppm (mg/l) for 24 hours, repeated after 2-3 weeks, is recommended for the bath treatment (Yanong, 2002). Websites of several commercial pet, aquarium fish, and fish medication dealers list 'Paracide D' and as a chemotherapeutic agent for fish nematodes along with dosages but studies supporting the efficacy were unavailable. The online Merck Veterinary Manual (www.merckvetmanual.com) lists treatment with fenbendazole (25 mg/kg for 3 days) but also cautions that 'efficacy has not been firmly established'. It also states that Levamisole (10 mg/L) 'administered as a bath treatment for 3 days has also been recommended'. It cautions against the use of Ivermectin which 'is highly toxic to aquarium fish, particularly cichlids', and states that 'its use is not recommended'. Levamisole has been successfully used against adult and pre-adult *Anguillicola crassus* in eels (Taraschewski *et al.*, 1988; Hartmann, 1989; Geets *et al.*, 1992), causing paralysis, death and expulsion from the swimbladder, but eggs and early larval stages were refractory to this drug. The antibacterial drugs flumequine (FQ) and oxytetracycline (OTC) seem depress circulating granulocytes in experimentally infected eels compared to untreated infected controls but have no effect on the lymphocyte poulations (van Der Heijden *et al.*, 1996). This may have interesting implications in possibly reducing the inflammatory response while allowing for a more effective humoral immune response. Worm burdens were only lowered by FQ treatment. Benz and Bullard (2004) discuss the complications surrounding the use of drugs against nematode infections in captive

chondrichthyans (sharks, rays and skates). Their concerns, such as the difficulty with diagnosing histozoic infections or the actual initial worm burdens of fish being brought in, loss of condition after a period of captivity due to the syngergistic effects of captive stress, etc., can be extended to other fishes as well.

While chemotherapy using commercially manufactured veterinary drugs is the first choice of treatment in many countries, plant extracts have been, continue to be, used to treat helminths in certain parts of the world. Minced garlic (200 mg/l) provided 100% prophylaxis against carp (*Cyrpinus carpio*) infected with *Capillaria* sp. Hexane extracts of garlic were less effective (75%) but aqueous extracts were ineffective. The effectiveness of garlic extract treatment was augmented by the addition of ammonium-potassium tartrate to the water (1.5 mg/l) (Pena *et al.*, 1988). Other studies on fish such as the cichlid *Symphysodon* (discus) indicate that the anthelmintics such as piperazine, trichlorphon and levamisole in the diet were inconsistent in their effects (Lo Wing Yat, 1988).

## Outlook and Conclusions

A review of the existing literature on disease causing nematodes in fishes, particularly since the last major review by Dick and Choudhury (1995), suggests that considerable progress has been made in species descriptions, delineating species boundaries, and understanding the life cycles of many of the pathogenic nematodes. This is especially true for anisakids and the eel swimbladder nematode, *A. crassus*, where molecular data have also been brought to bear on a variety of taxonomic and biogeograhical issues. However, most of our information comes from teleosts and the disease aspects of nematodes in elasmobranchs remain less well known. Furthermore, our understanding of the phylogenetic relationships of the various fish nematodes also remains largely untested by molecular data. These are potential areas of future research.

More importantly (for our purposes here), the interactions of pathogenic nematodes with the fish immune system and their treatment remain less well known. If vaccines are to be developed against pathogenic nematodes, a more sophisticated understanding of the fish immune system will be essential. Progress has been made in understanding the role of melanomacrophage centers in fish pathology (Aguis and Roberts, 2003), the complement system (Boshra *et al.*, 2006), rodlet cells (Koponen and Myers, 2000; Reite, 2005), eosinophilic cells (Reite, 1998), cells involved

in the inflammatory response (Reite and Evenson, 2006), innate immunity (Magnadóttir, 2006), interferons (Robertsen, 2006), and the ontogeny of the immune system (Zapata *et al.*, 2006). Research in comparative immunology is also providing insights into the function of B-cells in basal vertebrates (Li *et al.*, 2006) and has led to the discovery of previosuly unknown Ig isotypes (Danilova *et al.*, 2005). The importance of eel aquaculture has also focused attention on the eel immune system (Nielsen and Esteve-Gassent, 2006) and its relevance to *A. crassus* infections is obvious. More detailed information on the anamnestic response in fishes is going to be essential if vaccination is an eventual strategic goal. The use of probiotics to enhance the innate immune system (Kim and Austin, 2006) also seems a worthy area of research. At the same time, our understanding of antigenic epitopes of the various pathogenic nematodes is still in its infancy despite some progress (Höglund and Pilström, 1994, 1995) but needs to keep pace with the progress made in fish immunology. The integration of all these diverse areas of knowldege will prove critical in the coming years if real progress is to be made in dealing with nematodes that are actually or potentially pathogenic to fishes.

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## References

Abe, N., N. Ohya and R. Yanagiguchi. 2005. Molecular characterization of *Anisakis peregreffi* larvae in Pacific cod in Japan. *Journal of Helminthology* 79: 303-306.

Abollo, E., L. Paggi, S. Pascual and S. D'Amelio. 2003. Occurrence of recombinant genotypes of *Anisakis simplex* s.s. and *Anisakis pegreffii* (Nematoda: Anisakidae) in an area of sympatry. *Infection, Genetics and Evolution* 3: 175-181.

Adamson, M.L. and M. Roth. 1990. Prevalence and intensity of *Pseudodelphis oligocotti* n. gen., n. sp., (Dracunculoidea: Guyanemidae), in the tidepool sculpin, *Oligocottus maculosus* (Scorpaeniformes: Cottidae). *Journal of Parasitology* 76: 509-514.

Adamson, M.L. and J.N. Caira. 1991. *Lockenloia sanguiris* n. gen., n. sp. (Nematoda: Dracunculoidea) from the heart of a nurse shark, *Ginglystoma cirratum*, in Florida. *Journal of Parasitology* 77: 663-665.

Agius, C. and R.J. Roberts. 2003. Melano-macrophage centers and their role in fish pathology. *Journal of Fish Diseases* 26: 499-509.

Anderson, R.C. 1984. The origins of zooparasitic nematodes. *Canadian Journal of Zoology* 62: 317-328.

Anderson, R.C. 2000. *Nematode Parasites of Vertebrates, Their Biology and Transmission.* 2nd Edition. CABI Publishing, Wallingford, Oxon, U.K.

Anderson, R.C., A.G. Chabaud and S. Willmott. (eds.) 1974-1975. *CIH Keys to the Nematode Parasites of Vertebrates*, Nos. 1, 2, & 3. Commonwealth Agricultural Bureaux, Farnham Royal, U.K.

Anyanwu, A.O. 1983. Parasitic infestations of *Pseudotolithus* ssp. off the coast of Lagos, Nigeria. *Journal of Fish Biology* 22: 29-33.

Appleton, T.E. and M.D.B. Burt. 1991. Biochemical characterization of third stage larval sealworm, *Pseudoterranova decipiens* (Nematoda: Anisakidae), in Canadian Atlantic waters using isoelectric focusing of soluble proteins. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 1800-1803.

Barse, A. and D.H. Secor. 1999. An exotic nematode parasite of the American eel. *Fisheries* 24: 6-10.

Bauer, O.N. 1991. Spread of parasites and diseases of aquatic organisms by acclimatization: A short review. *Journal of Fish Biology* 39: 679-686.

Bauer, O.N., V.A. Musselius, V.M. Nikolayeva and Yu.A. Strelkov. 1977. *Ichthyopathology.* Pischchevaya Promyshlennost' Press. 432 pp. English Translation: Ansel Translations. 1980, by Teaneck, N.J.

Bennet, S.N. and M.L. Adamson. 2004. Prevalence, intensity, and differential development of *Pseudodelphis oligocotti* (Nematoda: Dracunculoidea) in sympatric fish hosts of the Northeastern Pacific coast. *Journal of Parasitology* 90: 678-684.

Benz, G.W. and S.A. Bullard. 2004. Metazoan parasites and associates of chondrichthyans with emphasis on taxa harmful to captive hosts. In: *The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and Their Relatives*, M. Smith, D. Warmolts, D. Thoney and R. Hueter (eds.). *Special Publication of the Ohio Biological Survey, Ohio Biological Survey, Columbus*, pp. 325-416.

Beregi, A., K. Molnár, L. Bekesi and C. Szekely. 1998. Radiodiagnostic method for studying swimbladder inflammation caused by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Diseases of Aquatic Organisms* 34: 155-160.

Berland, B. 1987. Helminth problems in sea-water aquaculture. In: *Parasites and Diseases in Natural Waters and Aquaculture in Nordic Countries*, E. Stenmark and G. Malmberg (eds.). Zoo-tax, Naturahistoriska Riksmuseet, Stockholm, Sweden, pp. 56-62.

Berland, B. 1989. Identification of larval nematodes from fish. In: *Nematode Problems in North Atlantic Fish*, H. Moeller (ed.). Report from a workshop in Kiel, 3-4 April, 1989, pp. 16-22.

Berland, B. and H-P. Fagerholm. 1994. Thirty years of anisakid research, and future trends. *Bulletin of the Scandinavian Society of Parasitology* 4: 40-46.

Black, G.A. 1984. Swimbladder lesions in lake trout (*Salvelinus namaycush*) associated with mature *Cystidicola stigmatura* (Nematoda). *Journal of Parasitology* 70: 441-443.

Bonnell, A.D. 1994. *Quality Assurance in Seafood Processing: A Practical Guide*. Chapman & Hall, New York.

Boon, J.H., V.M.H. Cannaerts, H. Augustijn, M.A.M. Machiels, D. DeCharleroy and F. Ollevier. 1990. The effect of different infection levels with infective larvae of *Anguillcola crassus* on haematological parameters of European eel (*Anguilla anguilla*). *Aquaculture* 87: 243-253.

Borucinska, J.D. and S. Frasca, Jr. 2002. Pancreatic fibrosis and ductal ectasia associated with the nematode *Pancreatonema americanum* sp. nov., in spiny dogfish, *Squalus acanthias* L., from the North-western Atlantic. *Journal of Fish Diseases* 25: 367-370.

Borucinska, J.D. and K. Heger. 1999. Disseminated granulomas associated with nematode larvae in a shortfin mako shark. *Journal of Wildlife Diseases* 35: 98-100.

Boshra, H., J. Li and J.O. Sunyer. 2006. Recent advances on the complement system of teleost fish. *Fish and Shellfish Immunology* 20: 239-262.

Bowen, W.D. (ed.). 1990. Population biology of the sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and seal hosts. Based on two workshops held in Halifax, Nova Scotia, April 1987 and June 1988. *Canadian Bulletin of Fisheries and Aquatic Sciences*, 222, Canadian Government Publishing Centre, Ottawa, 306 pp.

Brattey, J. and W.S. Dividson. 1996. Genetic variation within *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) from Canadian Atlantic marine fishes and seals: Characterization by RFLP analysis of genomic DNA. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 333-341.

Brocklebank. J.R., D. Groman and G. Conboy. 1996. Systemic nematodosis in farmed coho salmon in British Columbia. *The Canadian Veterinary Journal* (*La Revue Veterinaire Canadienne*) 37: 496-498.

Brugni, N. and G. Viozzi. 2006. *Pseudodelphis limnicola* sp. n. (Dracunculoidea: Guyanemidae) from the heart of *Percichthys trucha* (Perciformes: Percichthyidae) in Patagonian Andean lakes (Argentina). *Folia Parasitologica* 53: 134-138.

Bruno, D.W., B. Nowa and D.G. Elliott. 2006. Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. *Diseases of Aquatic Organisms* 70: 1-36.

Buchmann, K., L.O. Pedersen and J. Galmann. 1991. Humoral immune response of European eel *Anguilla anguilla* to a major antigen in *Anguillicola crassus* (Nematoda). *Diseases of Aquatic Organisms* 12: 55-57.

Bychovskaya-Pavolvskaya, I.E., A.V. Gusev, M.N. Dubinina, N.A. Izyumova, T.S. Smirnova, I.L. Sokolovskaya, G.A. Shtein, S.S. Shul'man and V.M. Epshtein. 1962. *Key to the Parasites of Freshwater Fishes of the U.S.S.R.* [English Translation of *Opredelitel' Parazitov Presnovodnykh Ryb SSSR*], Israel Program for Scientific Translations, Jerusalem, 919 pp.

Chai, J-Y., K. Darwin Murrell and A.J. Lymberry. 2005. Fish-borne parasitic zoonoses: Status and issues. *International Journal for Parasitology* 35: 1233-1254.

Cheung, P.J., R.F. Nigrelli and G.D. Ruggieri. 1984. *Philometra saltatrix* infecting the heart of the 0-class bluefish, *Pomatomus saltatrix* (L.), from the New York coast. In: *S.F. Snieszko. Commemoration Fish Disease Workshop*, Joint Workshop of Fish Health Section, AFS, and Midwest Disease Group, Little Rock, Arkansas, p. 27.

Choudhury, A. and T.A. Dick. 1996. Observations on the systematics and biogeography of the genus *Truttaedacnitis* (Nematoda: Cucullanidae). *Journal of Parasitology* 82: 965-976.

Choudhury, A., T.L. Hoffnagle and R.A. Cole. 2004. Parasites of native and nonnative fishes of the Little Colorado River, Grand Canyon, Arizona. *Journal of Parasitology* 90: 1042-1053.

Clarke, L.M., A.D.M. Dove and D.O. Conover. 2004. Prevalence, intensity, and effect of a nematode (*Philometra saltatrix*) in the ovaries of bluefish (*Pomatomus saltatrix*). *Fisheries Bulletin* 104: 118-124.

Conboy, G.A. and D.J. Speare. 2002. Dermal nematodosis in commercially captured rockfish (*Sebastes* spp.) from coastal British Columbia, Canada. *Journal of Comparative Pathology* 127: 211-213.

Coscia, M.R. and U. Oreste. 1998. Presence of antibodies specific for proteins of *Contracaecum osculatum* (Rudolphi, 1908) in plasma of several Antarctic teleosts. *Fish & Shellfish Immunology* 8: 295-302.

Coscia, M.R. and U. Oreste. 2000. Plasma and bile antibodies of the teleost *Trematomus bernacchii* specific for the nematode *Pseudoterranova decipiens*. *Diseases of Aquatic Organisms* 41: 37-42.

Coscia, M.R., A. Borriello, S. Amore and U. Oreste. 1997a. Immunological investigations on the Antarctic fish parasitism by *Pseudoterranova decipiens* nematode. *Immunology Letters* 56: 427.

Coscia, M.R., A. Borriello, S. Amore, S. Mattiucci and U. Oreste. 1997b. Antarctic fish antibodies specific for *Pseudoterranova decipiens* antigens. *Developmental and Comparative Immunology* 21: 247.

Coyner, D.F., S.R. Schaack, M.G. Spalding and D.J. Forrester. 2001. Altered predation susceptibilty of mosquitofish infected with *Eustrongylides ignotus*. *Journal of Wildlife Diseases* 37: 556-560.

Coyner, D.F., M.G. Spalding and D.J. Forrester. 2002. Epizootiology of *Eustrongylides ignotus* in Florida: Transmission and development of larvae in intermediate hosts. *Journal of Parasitology* 89: 290-298.

Crean, S.R., J.T.A. Dick, D.W. Evans, R.W. Elwood and R.S. Rosell. 2003. Anal redness in European eels as an indicator of infection by the swimbladder nematode, *Anguillicola crassus*. *Journal of Fish Biology* 62: 482-485.

Credille, K.M., L.K. Johnson and R. Reimschuessel. 1993. Parasitic meningoencephalitis in nurse sharks (*Ginglymostoma cirratum*). *Journal of Wildlife Diseases* 29: 502-506.

D'Amelio, S., K.D. Mathiopoulos, C.P. Santos, O.N. Pugachev, S.C. Webb, M. Picanco and L. Paggi. 2000. Genetic markers in ribosomal DNA for the identification of members of the genus *Anisakis* (Nematoda: Ascaridoidea) defined by polymerase-chain-reaction-based restriction length polymorphism. *International Journal of Parasitology* 30: 223-226.

Danilova, N., J. Bussmann, K. Jekosch and L.A. Steiner. 2005. The immunoglobulin heavy-chain locus in zebrafish: identification and expression of a previously unknown isotype, immunoglobulin Z. *Nature Immunology* 6: 295-302.

Dash, G., P. Nath and S. Saha. 2004. Prevalence of nematodes with zoonotic potential in Indian major carps in the freshwaters of West Bengal. *Journal of Environment and Sociobiology* 1: 55-60.

Deardorff, T.L. and R.M. Overstreet. 1980. Review of *Hysterothylacium* and *Iheringascaris* (both previously = *Thynnascaris*) (Nematoda: Anisakidae) from the Northern Gulf of Mexico. *Proceedings of the Biological Society of Washington* 93: 1035-1079.

Deardorff, T.L. and R.G. Stanton. 1983. Nematode induced abdominal distension in the Hawaiian pufferfish *Canthigaster jactator* (Jenkins). *Pacific Science* 37: 45-47.

Despres, L., M.L. Adamson and T.E. McDonald. 1995. Development of a diagnostic molecular marker for *Philonema* spp. (Nematoda; Dracunculoidea) infecting salmonids in British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 53 (Supplement 1): 129-133.

Dezfuli, B.S., E. Simoni, R. Rossi and M. Manera. 2000. Rodlet cells and other inflammatory cells of *Phoxinus phoxinus* infected with *Raphidascaris acus* (Nematoda). *Diseases of Aquatic Organisms* 43: 61-69.

Dick, T.A. 1987. The atrium of the fish heart as a site for *Contracaecum* spp. larvae. *Journal of Wildlife Diseases* 23: 328-330.

Dick, T.A. and A. Choudhury. 1995. Phylum Nematoda. In: *Fish Diseases and Disorders*, P.T.K. Woo (ed.). CABI Publishing, Wallingford, Oxon, U.K., Vol. 1, pp. 415-446.

Dunn, I.J., L.R. Russell and J.R. Adams. 1983. Cecal histopathology caused by *Truttaedacnitis truttae* (Nematoda: Cucullanidae) in rainbow trout, *Salmo gairdneri*. *International Journal for Parasitology* 13: 441-445.

Elarifi, A.O. 1982. The histopathology of larval anisakid nematode infections in the liver of whiting, *Merlangius merlangus* (L.), with some observations on the blood leucocytes of the fish. *Journal of Fish Diseases* 5: 411-419.

Eng, F. and J.H. Youson. 1992a. Morphology of the liver of the brook lamprey, *Lampetra lamottenii* before and during infection with the nematode, *Truttaedacnitis stelmoides*, hepatocytes, sinusoids and perisinosoid cells. *Tissue and Cell* 24: 575-592.

Eng, F. and J.H. Youson. 1992b. Morphology of the bile ducts of the brook lamprey, *Lampetra lamottenii* (Le Sueur) before and during infection with the nematode, *Truttaedacnitis stelmioides* (Vissichelli, 1910) (Nematoda: Cucullanidae). *Anatomical Record* 234: 201-214.

Eiras, J.C. and A.A. Rego. 1988. Histopatologia da parasitose de peixes do Rio Cuiaba (Mato Grosso) por larvas de *Eustrongylides* sp. (Nematoda: Dioctophymidae). *Revista Brasileira de Biologia* 48: 273-280.

Evans, D.W. and M.A. Matthews. 1999. *Anguillicola crassus* (Nematoda, Dracunculoidea); first documented record of this swimbladder parasite of eels in Ireland. *Journal of Fish Biology* 55: 665-668.

Fagerholm, H.-P. 1988. Incubation in rats of a nematodal larva from cod to establish its specific identity: *Contracaecum osculatum* (Rudolphi). *Parasitology Research* 75: 57-63.

Fischer, U., K. Utke, T. Somamoto, B. Kollner, M. Ototake and T. Nakanishi. 2006. Cytotoxic activities of fish leucocytes. *Fish & Shellfish Immunology* 20: 209-226.

Frantova, D. and F. Moravec. 2003. Ultrastructure of the body wall *of Cystidicoloides ephemeridarum* (Nematoda, Cystidicolidae) in relation to the histopathology of this nematode in salmonids. *Parasitology Research* 91: 100-108.

Garnick, E. and L. Margolis. 1990. Influence of four species of helminth parasites on orientation of seaward migrating sockeye salmon (*Oncorhynchus nerka*) smolts. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 2380-2389.

Garrido-Olvera, L., L. García-Prieto and G. Pérez-Ponce de León. 2006. Checklist of nematodes of freshwater fishes of Mexico. *Zootaxa* 1201: 1-45.

Geets, A., E.W. Liewes and F. Ollevier. 1992. Efficacy of some anthelmintics against the swimbladder nematode *Anguilliwla crassus* of eel *Anguilla anguilla* under saltwater conditions. *Diseases of Aquatic Organisms* 13: 123-128.

Ghittino, P. 1989. Anguillicolosis, a frequent parasitic aerocystitis of eels. *International Journal of Aquaculture and Fisheries Technology* 1: 64-68.

Gollock, M.J., C.R. Kennedy and J.A. Brown. 2005a. European eels, *Anguilla anguilla* (L.), infected with *Anguillicola crassus* exhibit a more pronounced stress response to severe hypoxia than uninfected eels. *Journal of Fish Diseases* 28: 429-436.

Gollock, M.J., C.R. Kennedy and J.A. Brown. 2005b. Physiological responses to acute temperature increase in European eels *Anguilla anguilla* infected with *Anguillicola crassus*. *Diseases of Aquatic Organisms* 64: 223-228.

Grabda, J. 1989. *Marine Fish Parasitology. An Outline*. Verlagsgesellschaft mbH, Weinheim.

Haenen, O.L.M., L. Grisez, D. de Charleroy, C. Belpaire and F. Ollevier. 1989. Experimentally induced infections of European eel *Anguilla anguilla* with *Anguillicola crassus* (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Diseases of Aquatic Organisms* 7: 97-101.

Haenen, O.L.M., P. van Banning and W. Dekker. 1994. Infection of eel *Anguilla anguilla* (L.) and smelt *Osmerus eperlanus* (L.) with *Anguillicola crassus* (Nematoda, Dracunculoidea) in the Netherlands from 1986 to 1992. *Aquaculture* 126: 219-229.

Haenen, O.L.M., T.A.M. van Wijngaarden, M.H.T. van der Heijden, J. Höglund, J.B. Cornelissen and L.A.M.G. van Leengoed. 1996. Effects of experimental infections with different doses of *Anguillicola crassus* (Nematoda, Dracunculoidea) on European eel (*Anguilla anguilla*). *Aquaculture* 141: 41-57.

Hafsteinsson, H. and S.S. Rizvi. 1987. A review of the sealworm problem: Biology, implications and solutions. *Journal of Food Protection* 50: 70-84.

Hartmann, F. 1989. Investigations on the effectiveness of Levamisol as a medication against the eel parasite *Anguillicola crassus* (Nematoda). *Diseases of Aquatic Organisms* 7: 185-190.

Hauck, A.K and E.B. May. 1977. Histopathologic alterations associated with *Anisakis* larvae in Pacific herring from Oregon. *Journal of Wildlife Diseases* 13: 290-293.

Hesp, S.A., R.P. Hobbs and I.C. Potter. 2002. Infection of the gonads of *Glaucosoma hebraicum* by the nematode *Philometra lateolabracis*: occurrence and host response. *Journal of Fish Biology* 60: 663-673.

Heupel, M.R. and M.B. Bennett. 1998. Infection of the epaulette shark, *Hemiscyllium ocellatum* (Bonnaterre), by the nematode parasite *Proleptus australis* Bayliss (Spirurida: Physalopteridae). *Journal of Fish Diseases* 21: 407-413.

Hine, P.M. and C.D. Anderson. 1982. Diseases of the gonads and kidneys of New Zealand snapper, *Chrysophrys auratus* Forster (F. Sparidae). In: *Wildlife Diseases of the Pacific Basin and Other Countries*, M.E. Fowler (ed.). Academic Press, London, pp. 166-170.

Hirose, H., T. Yabu, T. Aoki and I. Hirono. 1998. The phylogeny of *Anguillicola crassus* and *A. globiceps* based on partial 18S ribosomal RNA sequences. *Journal of Fish Diseases* 21: 265-271.

Hirshfield, M.F., R.P. Morin and D.J. Hepner. 1983. Increased prevalence of larval *Eustrongylides* (Nematoda) in the mummichog, *Fundulus heteroclitus* (L.) from the discharge canal of a power plant in the Chesapeake Bay. *Journal of Fish Biology* 23: 135-142.

Hoffman, G.L. 1975. Lesions due to internal helminths of freshwater fishes. In: *The Pathology of Fishes*, W.E. Ribelin and G. Migaki (eds.). University of Wisconsin Press, Madison, pp. 151-187.

Hoffman, G.L. 1999. *Parasites of Freshwater Fishes of North America*. Cornell University Press, Ithaca.

Höglund, J. and L. Pilström. 1994. Purification of adult *Anguillicola crassus* whole-worm extract antigens for detection of specific antibodies in serum from the European eel (*Anguilla anguilla*) by ELISA. *Fish and Shellfish Immunology* 4: 311-319.

Höglund, J. and L. Pilström. 1995. Mechanical isolation and characterization of antigens from adult *Anguillicola crassus*. *Fish and Shellfish Immunology* 5: 51-60.

Humason, G.L. 1979. *Animal Tissue Techniques*. 4th Edition. W.H. Freeman and Co., San Francisco.

Iglesias, L., A. Valero, R. Benitez and F.J. Adroher. 2001. *In vitro* cultivation of *Anisakis simplex*: Pepsin increases survival and moulting from fourth larval to adult stage. *Parasitology* 123: 285-291.

Iglesias, L., A. Valero, L. Galvez, R. Benitez and F.J. Adroher. 2002. *In vitro* cultivation of *Hysterothylacium aduncum* (Nematoda: Anisakidae) from 3rd-stage larvae to egg laying adults. *Parasitology* 125: 467-475.

Ivashkin, V.M. and L.A. Khromova. 1976. *Osnovy Nematodologii*. Vol. 27, Kukullanaty I Gnatostomataty Zhivotnykh I Cheloveka I vyzyvaemye imi zabolevniya. Izdatel'stvo "Nauka", Akademiya Nauk SSSR, Moscow.

Janiszewska, J. 1938. Studien über die Entwicklung und die Lebensweise der parasitischen Würmer in der Flunder (*Pleuronectes flesus* L). *Mémoires de l'Académie polonaise des Sciences et Lettres* Serie B1: 1-64.

Jilek, R. and J.L. Crites. 1982. Intestinal histopathology of the common bluegill, *Lepomis macrochirus* Rafinesque, infected with *Spinitectus carolini* Holl, 1928 (Spirurida: Nematoda). *Journal of Fish Diseases* 5: 75-77.

Justine, J-L. 1987. Three new species of *Huffmanella* Moravec, 1987 (Nematoda: Trichosomoididae) from the gills of marine fish off New Caledonia. *Systematic Parasitology* 59: 29-37.

Kahl, W. 1938a. Nematoden is Seefischen. I. Erhebungen ueber di durch Larven von *Porrocaecum decipiens* Krabbe in Fischwirten hervorgerufenen geweblichen Veranderungen und Kapselbildungen. *Zeitschrift für Parasitenkunde* 10: 415-431.

Kahl, W. 1938b. Nematoden in Seefischen. II. Erhebungen ueber den befall von Seefischen mit Larven von *Acanthocheilus rotundatus* (Rudolphi) und die durch diese Larven hervorgerufenen reaktionen des Wirtsgewebes. *Zeitschrift für Parasitenkunde* 10: 513-534.

Kåll, S., H.P. Fagerholm and J. Sarvala. 2004. Pathogenicity of the gill artery worm *Philometra obturans* (Nematoda) in northern pike (*Esox lucius*) in southwest Finland. *Journal of Parasitology* 90: 177-181.

Kelly, C.E., C.R. Kennedy and J.A. Brown. 2000. Physiological status of wild European eels (*Anguilla anguilla*) infected with the parasitic nematode, *Anguillicola crassus*. *Parasitology* 120: 195-202.

Kent, M.L., J.K. Bishop-Stewart, J.L. Matthews and J.M. Spitsbergen. 2002. *Pseudocapillaria tomentosa*, a nematode pathogen, and associated neoplasms of zebrafish (*Danio rerio*) kept in research colonies. *Comparative Medicine* 52: 354-358.

Kijewska, A., M. Slominska, G. Wegrzyn and J. Rokicki. 2000. A PCR-RFLP assay for identification of *Anisakis simplex* from different geographical regions. *Molecular and Cellular Probes* 14: 349-354.

Kijewska, A., J. Rokicki, J. Sitko and G. Wegrzyn. 2002. Ascaridoidea: A simple DNA assay for identification of 11 species infecting marine and freshwater fish, mammals, and fish-eating birds. *Experimental Parasitology* 101: 35.

Kim, D-H. and B. Austin. 2006. Innate immune responses in rainbow trout (*Oncorhynchus mykiss*, Walbaum) induced by probiotics. *Fish and Shellfish Immunology* 21: 513-524.

Kim, J., C.J. Hayward, S. Joh and G. Heo. 2002. Parasitic infections in live freshwater tropical fishes imported to korea. *Diseases of Aquatic Organisms* 52: 169-173.

Kirk, R.S. 2003. The impact of *Anguillicola crassus* on European eels. *Fisheries Management and Ecology* 10: 385-394.

Knopf, K., J. Wurtz, B. Sures and H. Taraschewski. 1998. Impact of low water temperature on the development of *Anguillicola crassus* in the final host *Anguilla anguilla*. *Diseases of Aquatic Organisms* 33: 143-149.

Knopf, K., K. Naser, M.H.T. van der Heijden and H. Taraschewski. 2000a. Humoral immune response of European eel *Anguilla anguilla* experimentally infected with *Anguillicola crassus*. *Diseases of Aquatic Organisms* 42: 61-69.

Knopf, K., K. Naser, M.H.T. van der Heijden and H. Taraschewski. 2000b. Evaluation of an ELISA and immunoblotting for studying the humoral immune response in *Anguillicola crassus* infected European eel *Anguilla anguilla*. *Diseases of Aquatic Organisms* 43: 39-48.

Koie, M. 1993. Aspects of the life cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). *Canadian Journal of Zoology* 71: 1289-1296.

Koie, M. and H-P. Fagerholm. 1993. Third-stage larvae emerge from eggs of *Contracaecum osculatum* (Nematoda: Anisakidae). *Journal of Parasitology* 79: 777-780.

Koie, M. and H-P. Fagerholm. 1995. The life cycle of *Contracaecum osculatum* (Rudolphi, 1802) sensu stricto (Nematoda, Ascaridoidea, Anisakidae) in view of experimental infections. *Parasitology Research* 81: 481-489.

Koponen, K. and M.S. Myers. 2000. Seasonal changes in intra- and interorgan occurrence of rodlet cells in freshwater bream. *Journal of Fish Biology* 56: 250-263.

Koubkova, B., V. Barus, M. Prokes and I. Dykova. 2004. *Raphidascaris acus* (Bloch, 1779) larvae infections of the stone loach, *Barbatula barbatula* (L.), from the River Hana, Czech Republic. *Journal of Fish Diseases* 27: 65-71.

Lankester, M.W. and J.D. Smith. 1980. Host specificity and distribution of the swim-bladder nematodes, *Cystidicola farionis* Fischer, 1798 and *C. cristivomeri* White, 1941 (Habronematoidea), in salmonid fishes of Ontario. *Canadian Journal of Zoology* 58: 1298-1305.

La Rosa, G., S. D'Amelio and E. Pozio. 2005. Molecular identification of nematode worms from seafood (*Anisakis* spp. and *Pseudoterranova* spp.) and meat (*Trichinella* app.). In: *Food-borne Pathogens: Methods and Protocols* C.C. Adley (ed.). Humana Press Inc., pp. 217-232.

Lefebvre, F. and A.J. Crivelli. 2004. Anguillicolosis: Dynamics of the infection over two decades. *Diseases of Aquatic Organisms* 62: 227-232.

Lefebvre, F., P. Contournet and A.J. Crivelli. 2002. The health state of the eel swimbladder as a measure of parasite pressure by *Anguillicola crassus*. *Parasitology* 124: 457-463.

Lefebvre, F., B. Mounaix, G. Poizat and A.J. Crivelli. 2004a. Impacts of the swimbladder nematode *Anguillicola crassus* on *Anguilla anguilla*: variations in liver and spleen masses. *Journal of Fish Biology* 64: 435-447.

Lefebvre, F., T. Schuster, M. Muenderle, M. Hine and R. Poulin. 2004b. Anguillicolosis in the short-finned eel *Anguilla australis*: Epidemiology and pathogenicity. *New Zealand Journal of Marine and Freshwater Research* 38: 577-583.

Li, J., D.R. Barreda, Y-A. Zhang, H. Boshra, A.E. Gelman, S. LaPatra, L. Tort and O. Sunyer. 2006. B-lymphocytes from early vertebrates have potent phagocytic and microbicidal properties. *Nature Immunology* 7: 1116-1124.

Likely, C.G. and M.D.B. Burt. 1989. Cultivation of *Pseudoterranova decipiens* (sealworm) from third-stage larvae to egg laying adults *in vitro*. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 347-348.

Likely, C.G. and M.D.B. Burt. 1992. *In vitro* cultivation of *Contracaecum osculatum* (Nematoda: Anisakidae) from third stage larvae to egg laying adults. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 1095-1096.

Lo Wing Yat, S. 1988. The control of *Capillaria* infections in *Symphysodon*. *Freshwater and Marine Aquarium* 11: 68-83.

Magnadottir, B. 2006. Innate immunity in fish (overview). *Fish and Shellfish Immunology* 20: 137-151.

Manfredi, M.T., F. Rosso, E. Zaffaroni, R. Rosa and G. Arlati. 1998. Diffusion of *Anguillicola crassus* in wild and cultured populations of European eels from North Italy. *Parasitologia* 40 (Supplement 1).

Marcogliese, D.J., L.G. Brambilla, F. Gagne and A.D. Gendron. 2005. Joint effects of parasitism and pollution on oxidative stress biomarkers in yellow perch *Perca flavescens*. *Diseases of Aquatic Organisms* 63: 77-84.

Margolis, L. 1970. Nematode diseases of marine fishes. In: *A Symposium on Diseases of Fishes and Shellfishes*, S.F. Snieszko (ed.). *Special Publication No. 5, American Fisheries Society*, pp. 190-208.

Marques, J.F., H.N. Cabral, M. Busi and S. D'Amelio. 2006. Molecular identification of *Anisakis* species from Pleuronectiformes off the Portugese coast. *Journal of Helminthology* 80: 47-51.

Martell, D.J. and G. McClelland. 1995. Transmission of *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) via benthic macrofauna to sympatric flatfishes (*Hippoglossoides platessoides*, *Pleuronectes ferrugineus*, *P. americanus*) on Sable Island, Canada. *Marine Biology* 122: 129-135.

Martins, M.L., M. Tavares-Dias, R.Y. Fujimota, E.M. Omaka and D.T. Nomura. 2004. Haematological alterations of *Leporinus macrocephalus* (Osteichthyes: Anostomidae) naturally infected by *Goezia leporini* (Nematoda: Anisakidae) in fish pond. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 56: 640-646.

Martín-Sánchez, J., M. Díaz, E.M. Artacho and A. Valero. 2003. Molecular arguments for considering *Hysterothylacium fabri* (Nematoda: Anisakidae) a complex of sibling species. *Parasitology Research* 89: 214-220.

Martín-Sánchez, J., M.E. Artacho-Reinoso, M. Diaz-Gavilan and A. Valero-Lopez. 2005. Structure of *Anisakis simplex* s.l. populations in a region sympatric for *A. pegreffi* and *A. simplex* s.s. Absence of reproductive isolation between both species. *Molecular and Biochemical Parasitology* 141: 155-162.

Mattiucci, S., S. D'Amelio, M. Di Paolo, L. Paggi, P. Orecchia and R. Cianchi. 1994. Genetic data supporting the existence of two sibling species within *Hysterothylacium corrugatum* (Ascaridida, Anisakidae) from the swordfish. *Parasitologia*, 36. (Supplement): 91.

Mattiucci, S., G. Nascetti, R. Cianchi, L. Paggi, P. Arduino and L. Margolis. 1997. Genetic and ecological data on the *Anisakis simplex* complex, with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). *Journal of Parasitology* 83: 401-416.

Mattiucci, S., L. Paggi, G. Nascetti, H. Ishikura, K. Kikuchi, N. Sato, R. Cianchi and L. Bullini. 1998. Allozyme and morphological identification of *Anisakis*, *Contracaecum* and *Pseudoterranova* from Japanese waters (Nematoda, Ascaridoidea). *Systematic Parasitology* 40: 81-92.

Mattiucci, S., P. Abaunza, L. Ramadori and G. Nascetti. 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. *Journal of Fish Biology* 65: 495-510.

Mattiucci, S., G. Nascetti, M. Dailey, S.C. Webb, N.B. Barros, R. Cianchi and L. Bullini. 2005. Evidence of a new species of *Anisakis* Dujardin, 1845: morphological description and genetic relationships between congeners (Nematoda: Anisakidae). *Systematic Parasitology* 61: 157-171.

McClelland, G. 2002. The trouble with sealworms (*Pseudoterranova decipiens* species complex, Nematoda): A review. *Parasitology* 124: 183-203.

McKinney, T., A.T. Robinson, D.W. Speas and R.S. Roberts. 2001. Health assessment, associated metrics, and nematode parasitism of rainbow trout in the Colorado River below Glen Canyon Dam, Arizona. *North American Journal of Fisheries Management* 21: 62-69.

McMinn, H. 1990. Effects of the nematode parasite *Camallanus cotti* on sexual and non-sexual behaviour in the guppy (*Poecilia reticulata*). *American Zoologist* 30: 245-249.

Measures, L. 1988. Epizootiology, pathology, and description of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in fish. *Canadian Journal of Zoology* 66: 2212-2222.

Meguid, M.A. and H.E. Eure. 1996. Pathobiology associated with the spiruroid nematodes *Camallanus oxycephalus* and *Spinitectus carolini* in the intestine of green sunfish, *Lepomis cyanellus*. *Journal of Parasitology* 82: 118-123.

Miyazaki, T., W.A. Rogers and K.J. Semmens. 1988. Gastro-intestinal histopathology of paddlefish *Polyodon spathula* (Walbaum) infected with larval *Hysterothylacium dollfusi* Schmidt, Leiby and Kritsky, 1974. *Journal of Fish Diseases* 11: 245-250.

Molnár, K. 1994. Formation of parasitic nodules in the swimbladder and intestinal walls of the eel *Anguilla anguilla* due to infections with larval stages *of Anguillicola crassus*. *Diseases of Aquatic Organisms* 20: 163-170.

Molnár, K., C. Szekely and F. Baska. 1991. Mass mortality of eel in Lake Balaton due to *Anguillicola crassus* infection. *Bulletin of the Europen Association of Fish Pathologists* 11: 211-212.

Molnár, K., F. Baska, G. Csaba, R. Glavits and C. Szekely. 1993. Pathological and histopathological studies of the swimbladder of eels *Anguilla anguilla* infected by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Diseases of Aquatic Organisms* 15: 41-50.

Molnár, K., C. Szekely and M. Perenyi. 1994. Dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) infection in eels of lake Balaton, Hungary. *Folia Parasitologica* 41: 193-202.

Molnár, K., J. Szakolczai and F. Vetesi. 1995. Histological changes in the swimbladder wall of eels due to abnormal location of adults and second stage larvae of *Anguillicola crassus*. *Acta Veterinaria Hungarica* 43: 125-137.

Moravec, F. 1994. *Parasitic Nematodes of Freshwater Fishes of Europe*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Moravec, F. 1996. Aquatic invertebrates (snails) as new paratenic hosts of *Anguillicola crassus* (Nematoda: Dracunculoidea) and the role of paratenic hosts in the life cycle of this parasite. *Diseases of Aquatic Organisms* 27: 237-239

Moravec, F. 1998. *Nematodes of Freshwater Fishes of the Neotropical Region*. Academia, Praha, Czech Republic.

Moravec, F. 2004. Some aspects of the taxonomy and biology of dracunculoid nematodes parasitic in fishes: A review. *Folia Parasitologica* 51: 1-13.

Moravec, F. and I. Dyková. 1978. On the biology of the nematode *Philometra obturans* (Prenant, 1886) in the fishpond system of Mácha Lake, Czechoslovakia. *Folia Parasitologica* 25: 231-240.

Moravec, F. and S. Cervinka. 2005. Female morphology and systematic status of *Philometroides cyprini* (Nematoda: Philometridae), a parasite of carp. *Diseases of Aquatic Organisms* 67: 105-109.

Moravec, F. and K. Nagasawa. 2004. Some anisakid nematodes from marine fishes of Japan and the North Pacific Ocean. *Journal of Natural History* 34: 1555-1574.

Moravec, F., K. Nagasawa and K. Ogawa. 1998. Observations of five species of philometrid nematodes from marine fishes in Japan. *Systematic Parasitology* 40: 67-80.

Moravec, F., J. Wolter and W. Korting. 1999. Some nematodes and acanthocephalans from exotic ornamental freshwater fishes imported into Germany. *Folia Parasitologica* 46: 269-310.

Moravec, F., J.B. Spanenberg and S. Frasca, Jr. 2001. *Syngnathinema californiense* n. gen., n. sp. (Nematoda: Dracunculoidea) from the circulatory system of the bay pipefish *Syngnathus leptorhynchus* in California. *Journal of Parasitology* 87: 1429-1432.

Moravec, F., K. Ogawa, M. Suzuki, K. Miyazaki and H. Donai. 2002. On two new species of *Philometra* (Nematoda: Philometridae) from the serranid fish *Epinephelus septemfasciatus* in Japan. *Acta Parasitologica* 47: 34-40.

Moravec, F., B. Glamuzina, G. Marino, P. Merella and D. Di Cave. 2003. Occurrence of *Philometra lateolabracis* (Nematoda: Philometridae) in the gonads of marine perciform fishes in the mediterranean region. *Diseases of Aquatic Organisms* 53: 267-269.

Moravec, F., K. Nagasawa and M. Miyakawa. 2005. First record of ostracods as natural intermediate hosts of *Anguillicola crassus*, a pathogenic swimbladder parasite of eels *Anguilla* spp. *Diseases of Aquatic Organisms* 66: 171-173.

Moser, M.L., W.S. Patrick and J.U. Crutchfiedl, Jr. 2001. Infection of American eels, *Anguilla rostrata*, by an introduced nematode parasite, *Anguillicola crassus*, in North Carolina. *Copeia* 2001: 848-853.

Muenderle, M., B. Sures and H. Taraschewski. 2004. Influence of *Anguillicola crassus* (Nematoda) and *Ichthyophthirius multifiliis* (Ciliophora) on swimming activity of European eel *Anguilla anguilla*. *Diseases of Aquatic Organisms* 60: 133-139.

Nagasawa, K. 1985. Prevalence of visceral adhesions in sockeye salmon, *Oncorhynchus nerka*, in central North Pacific Ocean. *Fish Pathology* 20: 313-322.

Nagasawa, K. 1989. Notes on parasites of aquatic organisms – 4. Visceral adhesions of high-seas sockeye salmon caused by the nematode *Philonema oncorhynchi*. *Aquabiology* 11: 320-321.

Natarajan, P. and P.S.B.R. James. 1977. A bibliography of parasites and diseases of marine and freshwater fishes of India. *Journal of Fish Biology* 10: 347-369.

Nielsen, M.E. 1999. An enhanced humoral immune response against the swimbladder nematode, *Anguillicola crassus*, in the Japanese eel, *Anguilla japonica*, compared with the European eel, *A. anguilla*. *Journal of Helminthology* 73: 227-232.

Nielsen, M.E. and M.D. Esteve-Gassent. 2006. The eel immune system: present knowledge and the need for research. *Journal of Fish Diseases* 29: 65-78.

Nimeth, K., P. Zwerger, J. Wurtz, W. Salvenmoser and B. Pelster. 2000. Infection of the glass-eel swimbladder with the nematode *Anguillicola crassus*. *Parasitology* 121: 75-83.

Ogawa, K. 1996. Marine parasitology with special reference to Japanese fisheries and mariculture. *Veterinary Parasitology* 64: 95-105.

Ooi, H., W. Wang, H. Chang, C. Wu, C. Lin and M. Hsieh. 1996. An epizootic of anguillicolosis in cultured American eels in Taiwan. *Journal of Aquatic Animal Health* 8: 163-166.

Otto, F. and W. Körting. 1973. Sektionsbericht zu einem Endoparasitenbefall bei Regenbogenforellen. *Veterinärisches Medizin Nachrichten* 2: 103-110.

Paggi, L., G. Nascetti, R. Cianchi, P. Orecchia, S. Mattiucci, S. D'Amelio, B. Berland, J.W. Smith and L. Bullini. 1991. Genetic evidence for three species within *Pseudoterranova decipiens* (Nematoda, Ascaridida, Ascaridoidea) in the North Atlantic and Norwegian and Barents Sea. *International Journal of Parasitology* 21: 195-212.

Paggi, L., S. Mattiucci, D.I. Gibson, B. Berland, G. Nascetti, R. Cianchi and L. Bullini. 2000. *Pseudoterranova decipiens* species A and B (Nematoda, Ascaridoidea): nomenclatural designation, morphological diagnostic characters and genetic markers. *Systematic Parasitology* 45: 185-197.

Paperna, I. 1974. Hosts, distribution and pathology of infections with larvae of *Eustrongylides* (Dioctophymidae, Nematoda) in fishes from East African lakes. *Journal of Fish Biology* 6: 67-76.

Paperna, I. 1991. Diseases casued by parasites in the aquaculture of warm water fish. *Annual Review of Fish Diseases* 1: 155-194.

Paperna, I. and D.E. Zwerner. 1976. Parasites and diseases of striped bass, *Morone saxatilis* (Walbaum), from the lower Chesapeake Bay. *Journal of Fish Biology* 9: 267-287.

Pena, N., A. Auro and H. Sumano. 1988. A comparative trial of garlic, its extract and ammonium-potassium tartrate as anthelmintics in carp. *Journal of Ethnopharmacology* 24: 199-203.

Perteguer, M.J., G. Ortiz, E. Garcia, M. Flores, E. Rodriguez and F.M. Ubeira. 2004. Application of the PCR-RFLP technique for the species-specific identification of nematodes involved in human anisakiasis. *Medicina Clinica* 122: 686-689.

Petersen, F., H. Palm, H. Moeller and M.A. Cuzi. 1993. Flesh parasites of fish from central Philippine waters. *Diseases of Aquatic Organisms* 15: 81-86.

Poole, B. and T.A. Dick. 1984. Liver pathology of the yellow perch, *Perca flavescens* (Mitchill) infected with the larvae of the nematode *Raphidascaris acus* (Bloch, 1779). *Journal of Wildlife Diseases* 20: 303-307.

Pontes, T., S. D'Amelio, G. Costa and L. Paggi. 2005. Molecular characterization of larval anisakid nematodes from marine fishes of Madeira by a PCR-based approach, with evidence for a new species. *Journal of Parasitology* 91: 1430-1434.

Priebe, K., C. Huber, E. Maertlbauer and G. Terplan. 1991. Nachweis von Antikoerpern gegen Larven von *Anisakis simplex* beim Seelachs *Polachius virens* mitttels ELISA. *Journal of Veterinary Medicine* B38: 209-214.

Ramachandran, P. 1975. *Philometra cephalus* sp. n. infecting the gonads of the striped mullet, *Mugil cephalus* L. from the Arabian coast of Kerala, India, with a note on its pathology. *Zoologischer Anzeiger* 194: 140-144.

Ramakrishna, N.R. and M.D.B. Burt. 1991. Tissue response of fish to invasion by larval *Pseudoterranova decipiens* (Nematoda: Ascaridoidea). *Canadian Journal of Fisheries and Aquatic Sciences* 48: 1623-1628.

Ramakrishna, N.R. and M.D.B. Burt. 1993. Cell-mediated immune response of rainbow trout (*Oncorhynchus mykiss*) to larval *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) following sensitization to live sealworm, sealworm extract, and non-homologous extracts. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 60-65.

Ramallo, G., H. Teran and E. Teisaire. 2000. Effects produced by *Spinitectus jamundensis* (Nematoda, Cystidicolidae) in the stomach of the shad, *Prochilodus lineatus* (Pisces, Prochilodidae). *Boletin Chileno de Parasitologia* 55: 36-38.

Reite, O.B. 1998. Mast cells/eosinophilic granule cells of teleostean fish: a review focusing on staining properties and functional responses. *Fish and Shellfish Immunology* 8: 489-513.

Reite, O.B. 2005. The rodlet cells of teleostean fish: their potential role in host defence in relation to the role of mast cells/eosinophilic granule cells. *Fish and Shellfish Immunology* 19: 253-267.

Reite, O.B. and Ø. Evensen. 2006. Inflammatory cells of teleostean fish: A review focusing on mast cells/eosinophilic granule cells and rodlet cells. *Fish and Shellfish Immunology* 20: 192-208.

Rigby, M. 1999. The Camallanidae (Nematoda) of Indo-Pacific fishes: taxonomy, ecology and host-parasite coevolution. In: *Proceedings of the 5th Indo-Pacific Fish Conference*, Noumea – New Caledonia, B. Seret and J.Y. Sire (eds.), pp. 633-644.

Robertsen, B. 2006. The interferon system in fish. *Fish and Shellfish Immunology* 20: 172-191.

Rohlwing, T., H.W. Palm and H. Rosenthal. 1998. Parasitation with *Pseudoterranova decipiens* (Nematoda) influences the survival rate of the European smelt *Osmerus eperlanus* retained by a screen wall of a nuclear power plant. *Diseases of Aquatic Organisms* 32: 233-236.

Rojas-Molina, N., S. Pedraza-Sanchez, B. Torres-Bibiano, H. Meza-Martinez and A. Escobar-Guiterrez. 1999. Gnathostomosis, an emerging foodborne zoonotic disease in Acapulco, Mexico. *Emerging Infectious Diseases* 5: 264-266.

Rosa-Molinar, E. and C.S. Williams. 1983. Larval nematodes (Philometridae) in granulomas in ovaries of black-tip sharks, *Carcharhinus limbatus* (Valenciennes). *Journal of Wildlife Diseases* 19: 275-277.

Sanmartin-Duran, M.L., P. Quinteiro and F.M. Ubeira. 1989. Nematode parasites of commercially important fish in NW Spain. *Diseases of Aquatic Organisms* 7: 75-77.

Santos, A.T., P. Sasal, O. Verneau and P. Lenfant. 2006. A method to detect the parasitic nematodes from the family Anisakidae, in *Sardina pilchardus*, using specific primers of 18S DNA gene. *European Food Research and Technology* 222: 71-77.

Saraiva, A.M., J.C. Eiras and C. Cruz. 1998. Fish histopathology caused by parasitic diseases. *Acta Parasitologica Portuguesa* 5: 67-72.

Scholz, T. 1999. Parasites in cultured and feral fish. *Veterinary Parasitology* 84: 317-335.

Schurmans-Stekhoven, J.H. and T.P.J. Botman. 1932. Zur ernährungsbiologie von *Proleptus obtusus* Duj. und die von diesem Parasiten hervorgerufenen reaktiven Änderungen des Wirtsgewebes. *Zeitschrift für Parasitenkunde* 4: 220-239.

Shih, H. 2004. Parasitic helminth fauna of the cutlass fish, *Trichiurus lepturus* L., and the differentiation of four anisakid nematode third-stage larvae by nuclear ribosomal DNA sequences. *Parasitology Research* 93: 188.

Shin, J. and C. Chen. 2000. Merthiolate-iodine-formalin stain method for diagnosis of cultured American eel anguillicolosis in Taiwan. *Journal of the Fisheries Society of Taiwan* 27: 129-138.

Silva, J.R.M.C., N.A. Staines, O.M. Parra and F.J. Hernandez-Blazquez. 1999. Experimental studies on the response of the fish (*Notothenia coriiceps* Richardson, 1844) to parasite (*Pseudoterranova decipiens* Krabbe, 1878) and other irritant stimuli at Antarctic temperatures. *Polar Biology* 22: 417-424.

Sima, A.R., F. Moravec and R.J. Gueemez. 1996. Pathogenic effects of the eggs of *Capillaria cichlasomae* (Nematoda: Capillariidea) in the liver of the cichlid *Cichlasoma urophthalmus* (Guenther) in Mexico. *Journal of Fish Diseases* 19: 175-176.

Sindermann, C.J. 1990. *Principal Diseases of Marine Fish and Shellfish*. 2nd Edition. Academic Press, London, Volume 1. 521 pp.

Sinha, A.K. and C. Sinha. 1988. Macrocytic hypochromic anaemia *in Heteropneustes fossilis* (Bl.) infected by the bloodsucker nematode *Procamallanus spiculogubernaculus*. *Indian Journal of Parasitology* 12: 93-94.

Smith, J.W. and R. Wootten. 1978. *Anisakis* and anisakiasis. *Advances in Parasitology* 16: 93-163.

Sood, M.L. 1988. *Fish Nematodes of South Asia*. Kalyani Publishers, New Delhi.

Sprengel, G. and H. Luechtenberg. 1991. Infection by endoparasites reduces maximum swimming speed of European smelt and European eel *Anguilla anguilla*. *Diseases of Aquatic Organisms* 11: 31-35.

Stoskopf, M. 1998. *Zoonotic Issues in Aquaculture*. World Aquaculture Society. 143 J.M. Parker Coliseum Louisiana State University Baton Rouge, LA 70803 U.S.A.

Sures, B. and K. Knopf. 2004a. Parasites as a threat to freshwater eels? *Science* 304: 209-211.

Sures, B. and K. Knopf. 2004b. Individual and combined effects of cadmium and 3,3',4,4',5-pentachlorobiphenyl (PCB 126) on the humoral immune response in european eel (*Anguilla anguilla*) experimentally infected with larvae *of Anguillicola crassus* (Nematoda). *Parasitology* 128: 445-454.

Sures, B., K. Knopf and W. Kloas. 2001. Induction of stress by the swimbladder nematode *Anguillicola crassus* in European eels *Anguilla anguilla*, after repeated experimental infection. *Parasitology* 123: 179-184.

Szalai, A.J. and T.A. Dick. 1991. Role of predation and parasitism in growth and mortality of yellow perch in Dauphin Lake, Manitoba. *Transactions of the American Fisheries Society* 120: 739-751.

Szekely, C. 1995. Dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) larval infection in paratenic host fishes of Lake Balaton, Hungary. *Acta Veterinaria Hungarica* 43: 401-422.

Szekely, C., J. Pazooki and K. Molnár. 1996. Host reaction in paratenic fish hosts against 3rd stage larvae of *Anguillicola crassus*. *Diseases of Aquatic Organisms* 26: 173-180.

Szekely, C., K. Molnár, T. Mueller, R. Romvari, C. Hancz and M. Bercsenyi. 2004. Comparative study of X-ray computerised tomography and conventional X-ray methods in diagnosis of swimbladder infection in eels caused by *Anguillicola crassus*. *Diseases of Aquatic Organisms* 58: 157-164.

Szekely, C., Molnár, K. and O.Z. Racz. 2005. Radiodiagnostic method for studying the dynamics of *Anguillicola crassus* (Nematoda: Dracunculoidea) infection and pathological status of the swimbladder in lake balaton eels. *Diseases of Aquatic Organisms* 64: 53-61.

Szostakowska, B., P. Myjak, J. Kur and T. Sywula. 2001. Molecular evaluation of *Hysterothylacium auctum* (Nematoda, Ascaridida, Raphidascarididae) taxonomy from fish of the southern Baltic. *Acta Parasitologica* 46: 194-201.

Szostakowska, B., P. Myjak and J. Kur. 2002. Identification of anisakid nematodes from the southern Baltic Sea using PCR-based methods. *Molecular and Cellular Probes* 16: 111-118.

Taraschewski, H., C. Renner and H. Melhorn. 1988. Treatment of fish parasites 3. Effects of levamisole HCl, metrifonate, febendazole, mebendazole, and ivermectin on *Anguillicola crassus* (nematodes) pathogenic in the air bladder of eels. *Parasitology Research* 74: 281-289.

Taraschewski, H., J. Boomker, K. Knopf and F. Moravec. 2005. *Anguillicola papernai* (Nematoda: Anguillicolidae) and other helminths parasitizing the African longfin eel *Anguilla mossambica*. *Diseases of Aquatic Organisms* 63: 185-195.

Thilakaratne, I.D.S.I.P., G. Rajapaksha, A. Hewakopara, R.P.V.J. Rajapakse and A.C.M. Faizal. 2003. Parasitic infections in freshwater ornamental fish in Sri Lanka. *Diseases of Aquatic Organisms* 54: 157-162.

Tonguthai, K. 1997. Control of freshwater fish parasites: A Southeast Asian perspective. *International Journal for Parasitology* 27: 1185-1191.

Uhazy, L.S. 1978. Lesions associated with *Philometroides huronensis* (Nematoda: Philometridae) in the white sucker (*Catostomus commersoni*). *Journal of Wildlife Diseases* 14: 401-408.

Ushikoshi, R., T. Inui, N. Mano and H. Hirose. 1999. A method for specific antibody detection from Japanese eel by indirect ELISA using cuticular antigen of *Anguillicola crassus*. *Fish Pathology* 34: 81-82.

Valter, E.D., T.I. Popova and M.A. Valovaya. 1982. Scanning electron microscopy study of four species of anisakid larvae (Nematoda: Anisakidae). *Helminthologia* 19: 195-209.

Valtonen, E.T., A. Haaparanta and R.W. Hoffmann. 1994. Occurrence and histological response of *Raphidascaris acus* (Nematoda: Ascarodoidea) in roach from four lakes differing in water quality. *International Journal of Parasitology* 24: 197-206.

Van Banning, P. and O. Haenen. 1990. Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel, *Anguilla anguilla*. In: *Pathology in Marine Science*, F.O. Perkins and T.C. Cheng (eds.). Academic Press, London, pp. 317-330.

Van den Thillart, G., S. Dufour, P. Elie, F. Volckaert and P. Sebert. 2005. Estimation of the reproduction capacity of European eel. Final Report Q5RS-2001-01836, Quality of and Management of Living Resources, European Union, Leiden University, 272 p. (www:Fishbiology.net/silvereel.html).

Van der Heijden, M.H.T., G.M. Helders, G.H.R. Booms, E.A. Huisman, J.H.W.M. Rombout and J.H. Boon. 1996. Influence of flumequine and oxytetracycline on the resistance of the European eel against the parasitic swimbladder nematode *Anguillicola crassus*. *Veterinary Immunology and Immunopathology* 52: 127-134.

Vázquez Núñez, V., J. Ramírez Lezamo, D. Osorio Sarabia, L.A. Chávez Suriano and F. Constantino Casas. 2004. Lesions caused by helminths of the digestive tract in estuarine fishes from the Tres Palos Lagoon, Guerrero, Mexico. *Veterinaria Mexicana* 35: 369-378.

Vismanis, K.O., V.V. Lomakin, V.D. Roitman, M.K. Semenova and V.Ya. Trofimenko. 1987. Klass Nematody, ili kruglye chervi – Nematoda. In: *Opredelitel' Parazitov Preznovodnykh Ryb fauny SSSR*, O.N. Bauer (ed.). Izdatel'stvo "NAUKA", Leningrad, pp. 199-310.

Wang, G.T. 2002. Seasonal dynamics and distribution of *Philometroides fulvidraconi* (Philometridae) in the bullhead catfish, *Pseudobagrus fulvidraco* (Richardson). *Journal of Fish Diseases* 25: 621-625.

Weerasooriya, M.V., T. Fujino, Y. Ishii and N. Kagei. 1986. The value of external morphology in the identification of larval anisakid nematodes: a scanning electron microscope study. *Zeitschrift für Parasitenkunde* 72: 765-778.

Williams, H.H. 1967. Helminth diseases of fish. *Helminthological Abstracts* 36: 261-295.

Williams, H.H. and A. Jones. 1994. *Parasitic Worms of Fish*. Taylor and Francis Ltd., London.

Willers, W.B., R.R. Dubielzig and L. Miller. 1991. Histopathology of the swimbladder of the cicso due to the presence of the nematode, *Cysticdicola farionis* Fischer. *Journal of Aquatic Animal Health* 3: 130-133.

Würtz, J. and H. Taraschewski. 2000. Histopathological changes in the swimbladder wall of the European eel *Anguilla anguilla* due to infections with *Anguillicola crassus*. *Diseases of Aquatic Organisms* 39: 121-134.

Yanong, R.P.E. 2002. Nematode (roundworm) infections in fish. *Circular 91, University of Florida, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences*, 9 pp.

Yu, Y., G.T. Wang, W. Feng, H.S. Wu and W.J. Wang. 1993. *Philometroides* of yellow catfish, *Pseudobagrus fulvidraco*: life cycle of the pathogen and its epidemiology. In: *Transactions of Researches on Fish Diseases*, No. 1 (Edited by Department of Fish Diseases, Institute of Hydrobiology, Chinese Academy of Sciences), pp. 116-122. Ocean Press, Beijing. [Original not seen, cited in Wang, 2002].

Zapata, A., B. Diez, T. Cejalvo, C. Guitérrez-de Frias and A. Cortés. 2006. Ontogeny of the immune system of fish. *Fish and Shellfish Immunology* 20: 126-136.

Zhu, X., R.B. Gasser, M. Podolska and N.B. Chilton.1998. Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. *International Journal for Parasitology* 28: 1911-1921.

Zhu, X.Q., S. D'Amelio, H.W. Palm, L. Paggi, M. George-Nascimento and R.B. Gasser. 2002. SSCP-based identification of members within the *Pseudoterranova decipiens* complex (Nematoda: Ascaridoidea: Anisakidae) using genetic markers in the internal transcribed spacers of ribosomal DNA. *Parasitology* 124: 615-623.

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CHAPTER

# 18

# Diseases Caused by Crustacea

**Wojciech Piasecki[1,*] and Annemarié Avenant-Oldewage[2]**

## INTRODUCTION

Crustaceans important to fish health can be assigned to five taxonomic groups (in the order of decreasing importance/pathogenicity):

(1) Copepoda
(2) Branchiura
(3) Isopoda
(4) Cirripedia
(5) Amphipoda

Due to the diversity of their morphology, biology, and pathogenic action on the fish they will be described individually in subsequent sections.

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*Note:* For convenience, all the figures are arranged on plates, hence the number skipped off in text.

## Classification

The classification of crustaceans parasitic on fishes is proposed below (based on Martin and Davis, 2001). Only the higher taxa accommodating parasitic species are shown.

Subphylum Crustacea Brünnich, 1772
- Class Maxillopoda Dahl, 1956
  - Subclass Thecostraca Gruvel, 1905
    - Infraclass Cirripedia Burmeister, 1834
      - Superorder Thoracica Darwin, 1854
        - Order Pedunculata Lamarck, 1818
          - Suborder Heteralepadomorpha Newman, 1987
            - Family Anelasmatidae Gruvel, 1905
  - Subclass Branchiura Thorell, 1864
    - Order Arguloida Yamaguti, 1963
      - Family Argulidae Leach, 1819
  - Subclass Copepoda Milne-Edwards, 1840
    - Infraclass Neocopepoda Huys et Boxshall, 1991
      - Superorder Podoplea Giesbrecht, 1882
        - Order Cyclopoida Burmeister, 1834
          - Family Lernaeidae Cobbold, 1879
        - Order Poecilostomatoida Thorell, 1859*
          - Family Bomolochidae Sumpf, 1871
          - Family Chondracanthidae Milne Edwards, 1840
          - Family Ergasilidae von Nordmann, 1832
          - Family Lernaeosoleidae Yamaguti, 1963
          - Family Philichthyidae Vogt, 1877
          - Family Shiinoidae Cressey, 1975
          - Family Taeniacanthidae Wilson, 1911
          - Family Tegobomolochidae Avdeev, 1978
          - Family Telsidae Ho, 1967
          - Family Tuccidae Vervoort, 1962
        - Order Siphonostomatoida Thorell, 1859
          - Family: Achidactylinidae Izawa, 1996
          - Family Caligidae Burmeister, 1834
          - Family Cecropidae Dana, 1849
          - Family Dichelesthiidae Milne Edwards, 1840

*According to Boxshall and Halsey (2004), the families of Poecilostomatoida should be included in Cyclopoida. Ho *et al.* (2006) provided evidence why poecilostomatoida should be retained as a separate order.

Family Dissonidae Yamaguti, 1963
Family Eudactylinidae Wilson, 1922
Family Euryphoridae Wilson, 1905*
Family Hatschekiidae Kabata, 1979
Family Hyponeoidae Heegaard, 1962
Family Kroyeriidae Kabata, 1979
Family Lernaeopodidae Milne Edwards, 1840
Family Lernanthropidae Kabata, 1979
Family Pandaridae Milne Edwards, 1840
Family Pennellidae Burmeister, 1834
Family Pseudocycnidae Wilson, 1922
Family Sphyriidae Wilson, 1919
Family Tanypleuridae Kabata, 1969
Family Trebiidae Wilson, 1905
Class Malacostraca Latreille, 1802
Subclass Eumalacostraca Grobben, 1892
Superorder Peracarida Calman, 1904
Order Amphipoda Latreille, 1816
Family Laphystiidae Sars, 1893
Family Lysianassidae Dana, 1849
Family Pardaliscidae Boeck, 1871
Family Trischizostomatidae Lilljeborg, 1865
Order Isopoda Latreille, 1817
Suborder Flabellifera Sars, 1882
Family Aegidae White, 1850
Family Cymothoidae Leach, 1814
Family Gnathiidae Leach, 1814

## COPEPODA

### Introduction

The subclass Copepoda is highly diversified ecologically. Humes (1994) estimated that there are 11,500 known copepod species. Copepods are commonly associated with microscopic components of marine- and freshwater zooplankton, but Boxshall (2005a) estimates that about half of

*According to Boxshall and Halsey (2004), Euryphoridae belong to Caligidae. It must be kept in mind however, that euryphorids differ substantially, in their early ontogeny, from all other caligids and the majority of siphonostomatoids in not having the frontal filament (a larval attachment organ) (Benz, 1989).

the known copepod species live in symbiotic associations, of which parasitic species are the majority. Copepods parasitize fishes, aquatic invertebrates, and a small number of them infect marine mammals. The parasitic species may reach 30 cm in length (Grabda, 1991). Their morphology, structure, and biology are highly diversified among families. Especially their diversified morphology deserves special attention of a parasitologist or a fish pathologist (Figs. 18.1–18.25).

In the course of host-parasite co-evolution, some of the copepods have assumed such astonishing shapes, that their affiliation to the subphylum Copepoda may seem questionable. The common features among some copepods have been a complete (or partial) loss of segmentation, extremely diversified shapes, and attainment of large sizes. In some cases, only the morphology of early larval stages can give evidence as to the identity of a species.

An important factor contributing to such extensive morphological diversity of parasitic copepods has been their need to attach to the host and to secure their position on (in) the hosts. Copepods pursued different ways in doing so and their attachment strategies defined not only their gross morphology but also some other aspects of their life.

The complexity of parasitic copepod's adaptations to the parasitic way of life is usually positively correlated with the duration of their evolutionary association with their hosts. Those parasitic copepods that are similar in size and shape to their free-living counterparts, have only slightly modified their morphology, retained their swimming ability (just in case), and their association with a fish is maintained during their adult period of life only. On the contrary, the well-adapted species modified their morphology and sizes very extensively as well as they did with their life cycles, biology, and physiology. They tend to limit the free-living portions of their life cycle and therefore their larvae attach to fishes as early as possible.

The attachment adaptations of copepods parasitic on fishes have been prompted by a number of distinct strategies. The simplest one was transformation of appendages into grasping limbs. For example, *Ergasilus sieboldi*, a small cyclopid-like parasite of a number of freshwater fishes,

**PLATE 1** Figs. 18.1–18.14. Morphological variability of Copepoda parasitic on fishes. Fig. 18.1. *Bomolochus solae*; male; dorsal. Fig. 18.2. *Ergasilus sieboldi*; female; dorsal. Fig. 18.3. *Ergasilus* sp.; female; lateral (attached to gill filament). Fig. 18.4. *Anthosoma crassum*; female; lateral. Fig. 18.5. *Dichelesthium oblongum*; female; ventral. Fig. 18.6. *Chondracanthus lophii*; female; lateral (with male attached to its posterior part). Fig. 18.7.

*Plate 1 Contd.*

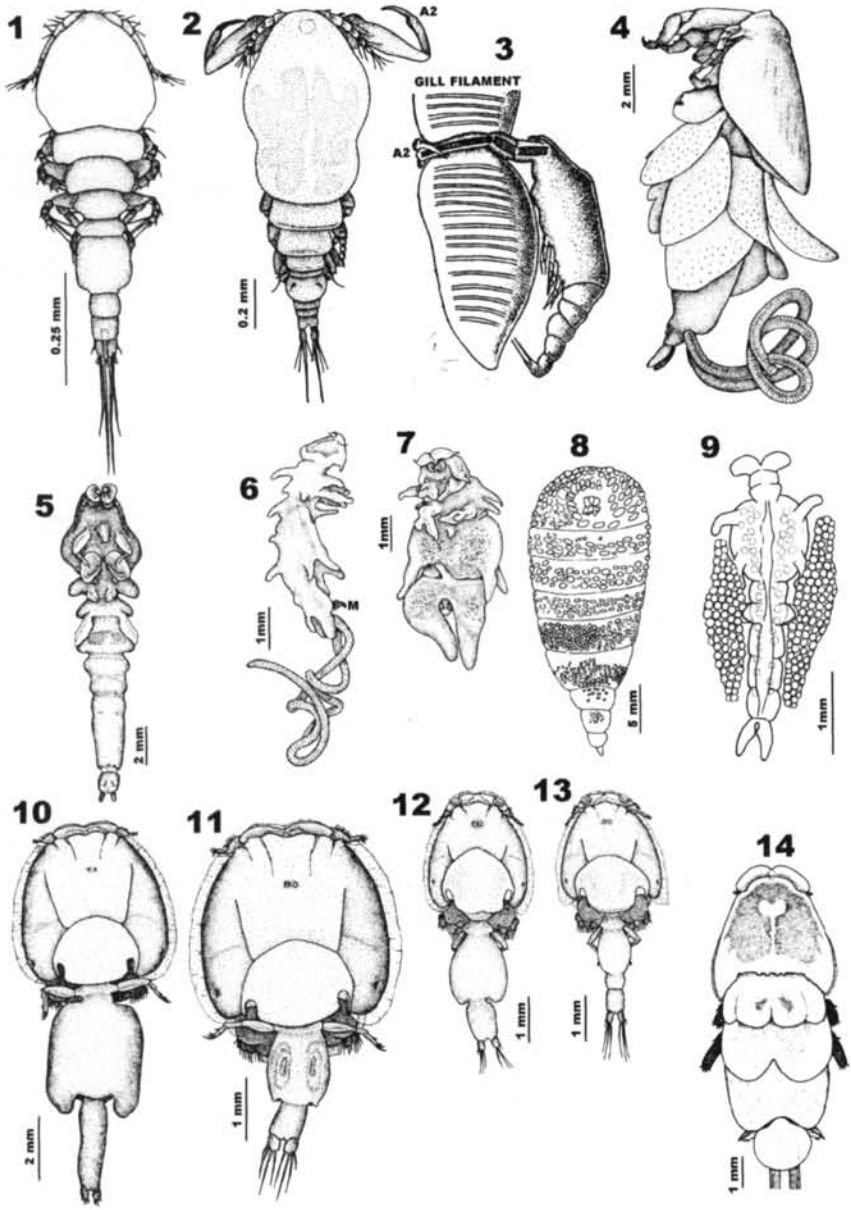


*Plate 1 Contd.*

Same; ventral. Fig. 18.8. *Sarcotaces namibiensis*; female; ventral. Fig. 18.9. *Colobomatus gymnoscopeli*; female; dorsal. Fig. 18.10. *Lepeophtheirus salmonis*; female; dorsal. Fig. 18.11. *L. salmonis*; male, dorsal. Fig. 18.12. *Caligus elongatus*; female; dorsal. Fig. 18.13. *C. elongatus*; male; dorsal. Fig. 18.14. *Pandarus bicolor*; female; dorsal. Labels: M, male. Source: Grabda and Linkowski, 1978 (Fig. 18.9); Kabata, 1979 (Figs. 18.1, 18.2, 18.4–18.7, 18.10–18.14); Markevič, 1956 (after Dogel' and Ahmerov) (Fig. 18.3); Reimer, 1991 (Fig. 18.8).

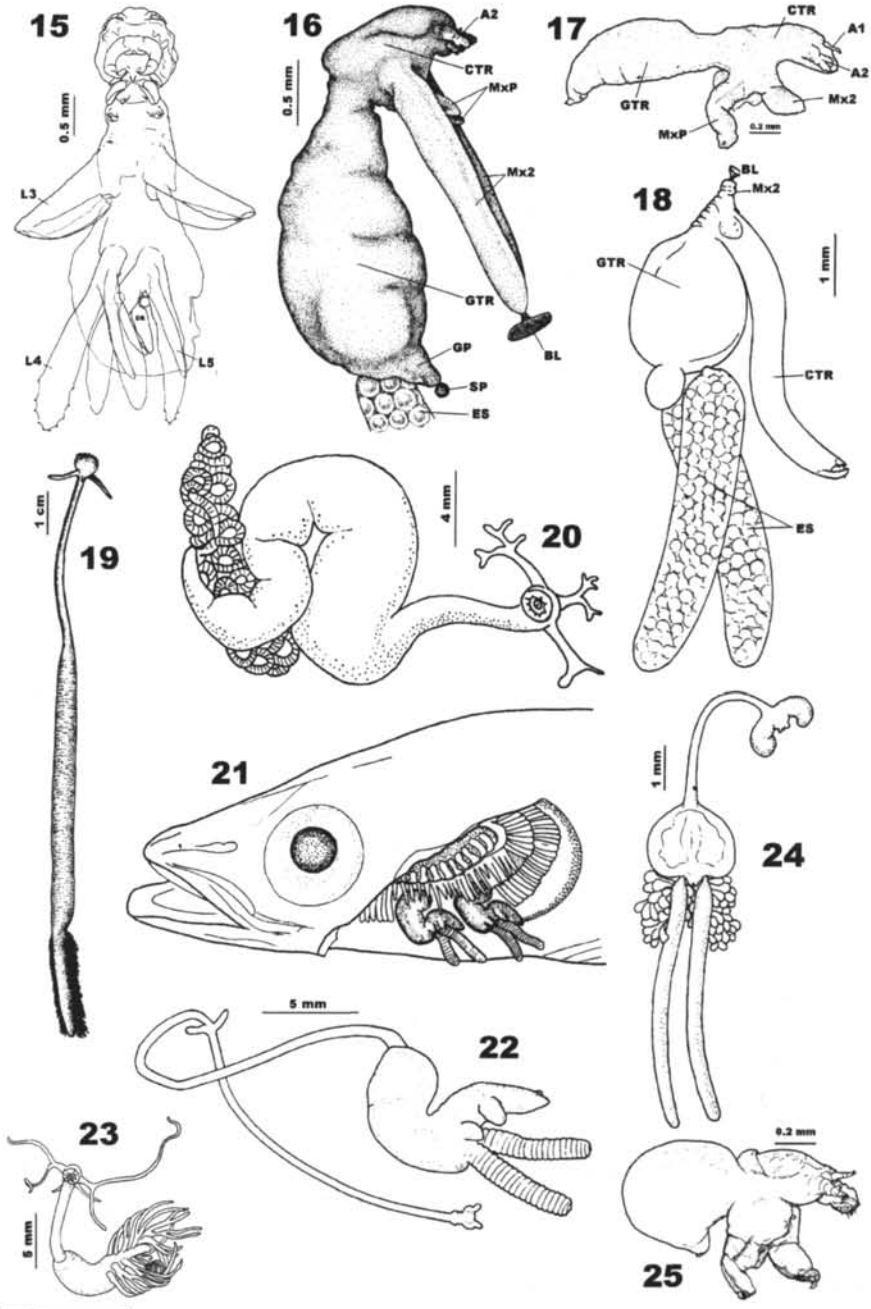


**PLATE 2** Figs. 18.15–18.25. Morphological variability of Copepoda parasitic on fishes. Fig. 18.15. *Lernanthropus polynemi*; female; ventral. Fig. 18.16. *Achtheres percarum*; female; lateral. Fig. 18.17. *A. percarum*; male; lateral. Fig. 18.18. *Clavella perfida*; female, lateral. Fig. 18.19. *Pennella filosa*; female. Fig. 18.20. *Lernaeocera branchialis*; female. Fig. 18.21. *Haemobaphes diceraus*; two adult ovigerous females on gills of Alaska pollock.

*Plate 2 Contd.*

transformed its second antennae into powerful arm-like structures, used for 'hugging' a gill filament (Figs. 18.2, 18.3). The majority of representatives of the family Ergasilidae maintain their contact with the host in the same way and this strategy is pursued by the species of Lernanthropidae (Fig. 18.15), and other gill-dwelling parasites.

Representatives of the family Lernaeopodidae modified their second antennae morphology very extensively, resulting in a total loss of segmentation and elaboration of a complex attachment system. The second maxillae were transformed into, more or less, powerful 'arms'. The frontal organ, hidden inside the female's cephalothorax, secretes a mushroom-shaped bulla (Figs. 18.16, 18.18, 18.42). The narrow part of the bulla is attached to distal ends of second maxillae, while its wider part is attached permanently to the host.

The Caligidae (Figs. 18.10–18.13) and related families opted for unrestrained mobility on the host's skin, combined with a firm attachment. The above assumption may seem impossible, but the caligids have managed to make it feasible. Their cephalothorax is flattened dorsoventrally and constitutes a single sucking cup. Anterior- and lateral margins of the cephalothorax are sealed with a membrane. The posterior margin is sealed by an 'apron', made of the third pair of transformed swimming legs. Intensive, correlated action of all legs propels the water from underneath the cephalothorax, thus creating negative pressure needed for attachment (Kabata, 1979). The entire parasite, like a small hovercraft, can now slide over the fish skin, covered by a thick layer of mucus. Additional attachment organs are claws of second antennae and second maxillae and cup-shaped lunulae—suckers, developed only by some caligids. The overall appearance of copepods of the family Pandaridae (Fig. 18.14) is similar to that of the Caligidae. The former, however, live on a rough skin surface of sharks and therefore their entire cephalothorax cannot act as a sucking cup. To remedy this problem pandarids have developed a set of unique attachment pads, suitable for sharkskin.

Some copepods of the family Philichthyidae become almost complete endoparasites. Very large females of *Sarcotaces* (Fig. 18.8) become totally

*Plate 2 Contd.*

Fig. 18.22. *H. diceraus*; adult ovigerous female. Fig. 18.23. *Lernaeolophus sultanus*; female (isolated from palate/eye socket of chub mackerel). Fig. 18.24. *Spyrion lumpi*; female. Fig. 18.25 *S. lumpi*; male; lateral. Labels: A1, first antenna; A2, second antenna; BL, bulla; CR, caudal rami; CTR, cephalothorax; ES, egg sac; GP, genital process; GTR, genital trunk; L3, leg 3; L4, leg 4; L5, leg 5; Mx2, second maxilla; MxP, maxilliped; SP, spermatophore. Source: Grabda, 1991 (Figs. 18.18, 18.20–18.24); Kabata, 1979 (Figs. 18.16, 18.19); Moran and Piasecki, 1994 (Fig. 18.25); Piasecki and Hayward, 2002 (Fig. 18.15); Piasecki *et al.*, 2006 (Fig. 18.17).

embedded in the muscle tissues of its host. A small aperture in the fish skin becomes open to maintain the contact between the parasite and the external environment. Other philichthyids are very small (Fig. 18.9) and they parasitize canals of the lateral line of fish.

A fairly large group of parasitic copepods maintain a permanent contact with the host, anchoring their head within fish tissue and at the same time keeping the rest of their body outside, in the water. The head of such parasites is equipped with, more or less extensively developed, 'holdfast' of cephalic outgrowths. Some species are anchored in the subdermal tissues only, while the others tend to penetrate deeper areas like muscles near the backbone, peritoneal cavity, eyeball, or heart. Kabata (1975, cited after Grabda, 1991) proposed the term of mesoparasite to denote species, which have the vast majority of their somites embedded in the host tissues. Species assigned to the families Pennellidae (Figs. 18.19–18.23) and Sphyriidae (Fig. 18.24) are typical examples of mesoparasites.

Copepods are not only diversified morphologically. Their adaptations to a parasitic way of living have also affected their life cycles and biology. Many species that extensively adapted their morphology to parasitism have also extensively modified their life cycle (e.g., Pennellidae or Lernaeopodidae—Fig. 18.42). On the other hand, some other extensively modified copepods have changed their life cycles only superficially (e.g., Lernaeidae—Figs. 18.26–18.41). To better understand the parasitic alteration of a life cycle, we should focus first on a typical life cycle of a free-living copepod. All copepods are dioecious and parasitism produced no hermaphroditic exceptions. Sperm transfer from male to female is through spermatophores (Kabata and Cousens, 1973). Female copepods carry their eggs in two egg sacs or egg strings (only some free-living copepods such as Harpacticoida, Calanoida have a single egg sac). The female carries her eggs throughout the entire period of embryonic development, until they hatch. Free-living copepods (e.g., *Cyclops* sp.) have a total of 10 larval stages: 5 nauplius instars and 5 copepodid instars. Copepods, like all arthropods, have to shed their cuticle to be able to increase their size. Therefore, each developmental stage is separated from

**PLATE 3** Figs. 18.26–18.41. Developmental stages of *Lernaea cyprinacea*. Fig. 18.26. Nauplius I. Fig. 18.27. Nauplius II. Fig. 18.28. Nauplius III. Fig. 18.29. Copepodid I. Fig. 18.30. Copepodid II. Fig. 18.31. Copepodid III. Fig. 18.32. Copepodid IV. Fig. 18.33. Copepodid V, male. Fig. 18.34. Copepodid V, female. Fig. 18.35. Cyclopoid male. Fig. 18.36. Cyclopoid female. Fig. 18.37. Juvenile, infective female. Fig. 18.38. Juvenile parasitic female during metamorphosis. Fig. 18.39. Juvenile parasitic female during

*Plate 3 Contd.*

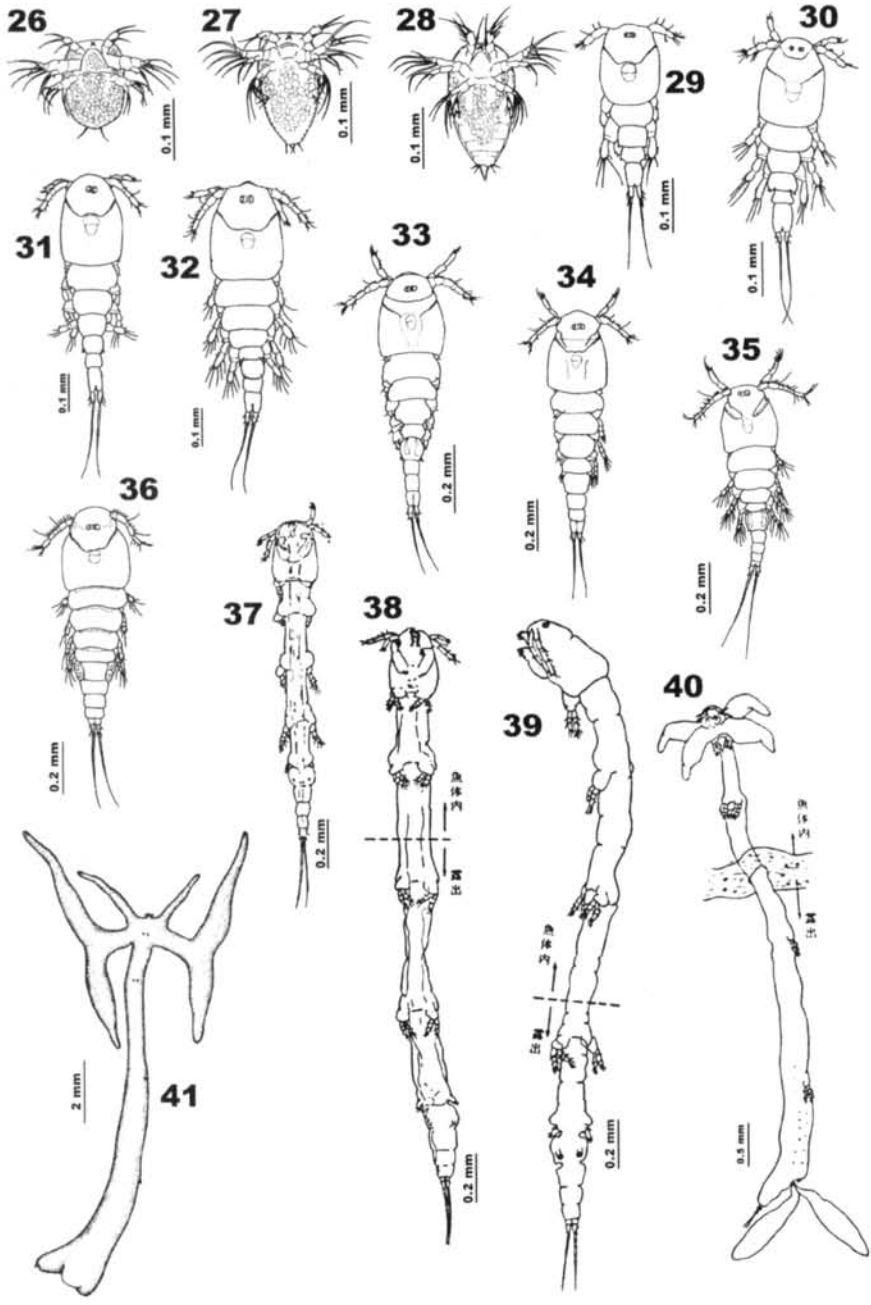


*Plate 3 Contd.*

metamorphosis (more advanced stage). Fig. 18.40. Adult parasitic female (metamorphosed). Fig. 18.41. Adult parasitic female (fully grown). Source: Kabata, 1979 (Fig. 18.41); Kabata, 1979 (redrawn from Grabda, 1963) (Figs. 18.26–18.36); Kasahara, 1962 (Figs. 18.37–18.40).

the other one by a moult. Nauplius stages are spherical or oval, have two or three pairs of functional limbs, and are generally poor swimmers. Copepodid stages—on the contrary—are elongate, streamline fast swimmers, equipped with efficient plumose swimming legs. Because of their qualities, only copepodids can become successful infective stages for a parasitic copepod. Nauplii, due to their body structure and poor swimming performance cannot act as infective stages of parasitic species. Their only positive role for a species is passive dispersal of the parasite to new areas. Therefore, parasitic copepods tend to eliminate the naupliar phase from their development. For example *Ergasilus*—a parasite resembling in its size and shape (Fig. 18.2) its free-living counterparts—has 6 naupliar stages and 5 copepodids (Abdelhalim *et al.*, 1991). Highly metamorphosed (but still a close relative of planktonic cyclopids) *Lernaea* has made some further progress in the reduction of unwanted nauplius stages and it has 3 nauplii and 5 copepodids in its life cycle (Grabda, 1963a; Kasahava, 1962). Copepods representing a typically parasitic order Siphonostomatoida seem to be more advanced in eliminating unwanted naupliar stages. Representatives of Caligidae and Pennellidae (Figs. 18.10, 18.19) have only 2 nauplius stages.

Retaining two nauplius stages by representatives of Pennellidae and Caligidae may also be perceived as an alternative strategy. Those copepods benefit from not shortening the naupliar stage completely, thereby achieving a substantial dispersal of their larvae while at the same time, increasing chances to find hosts in other, new areas.

The most successful in this evolutionary trend seem to be siphonostomes of the family Lernaeopodidae. They have only one nauplius stage (Fig. 18.42). Their nauplius, however, while hatching, is already at the pre-moult stage and consequently it quickly moults, becoming an infective copepodid. It is possible that newly hatched nauplii of some lernaeopodid species moult within seconds or minutes and the copepodid has a chance to attach to a host fish from the same school of fish. It is evident that that a reduced naupliar phase dramatically increases chances for acquiring the host. The parasites' dispersal is therefore dependant on the host fish migrations.

**PLATE 4** Fig. 18.42. Life cycle of *Tracheliastes maculatus* (Lernaeopodidae). INSET: Proximal part of frontal filament (lateral) of pre-moult chalimus IV female with elements added by consecutive chalimus stages; note exuvial claws or respective stages embedded into the filament base. Lables: Ch I, chalimus I; Ch II, chalimus II, Ch III, chalimus III, Ch IV, chalimus IV; AD, tip of second maxilla of adult male visible inside appendage of chalimus IV. Source: Piasecki, 1989.

*Plate 4 Contd.*

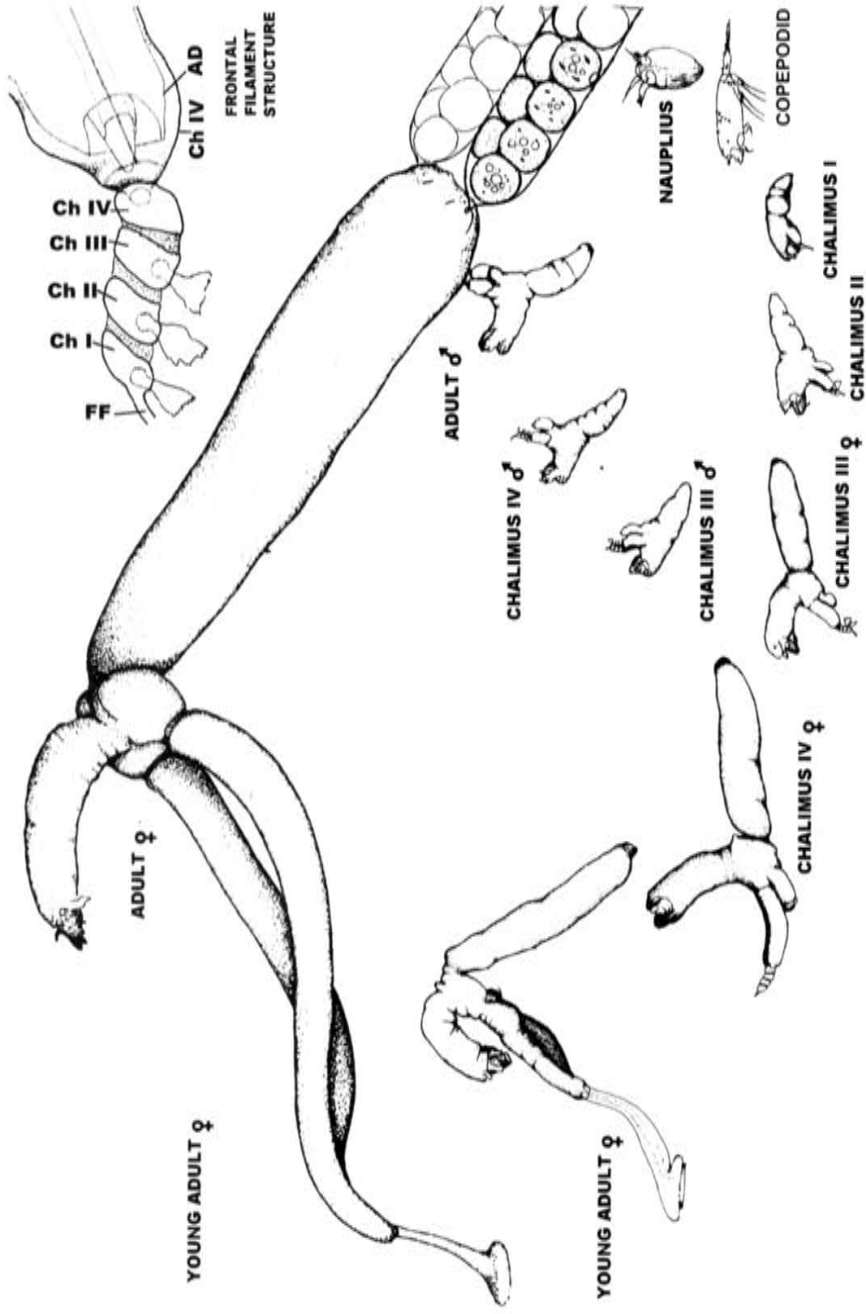
Ch IV AD
FRONTAL FILAMENT STRUCTURE
Ch IV
Ch III
Ch II
Ch I
FF
ADULT ♀
YOUNG ADULT ♀
YOUNG ADULT ♀
ADULT ♂
CHALIMUS IV ♂
CHALIMUS III ♂
CHALIMUS IV ♀
CHALIMUS III ♀
NAUPLIUS
COPEPODID
CHALIMUS I
CHALIMUS II

Plate 4

The original number of copepodid stages (5), seem to be 'well tolerated' by parasitic forms in their life cycles. The vast majority of parasitic copepods have 5 copepodids (post naupliar stages) in their life cycle. In many species (e.g., Ergasilidae), the copepodid stages are free living and the only parasitic form is the adult female. The copepodid phase is associated with the hosts in all representatives of siphonostome families. The first copepodid is infective to the fish host. The first copepodid stage in the majority of siphonostome families attaches permanently to the host by means of a frontal filament. The only exceptions are parasites of the family Lernanthropidae, Dichelesthiidae, and Euryphoridae (cf. Piasecki and MacKinnon, 1993), which are known to lack the filament. The frontal filament is formed by a frontal gland, situated inside the cephalothorax (Piasecki, 1989; Piasecki and MacKinnon, 1993). Copepodids attached to the host by means of a frontal filament are referred to as chalimus stages (singular chalimus, plural chalimi). Copepodid I is followed by chalimus I and, therefore, chalimus I is, in fact, copepodid II. Chalimi of the family Caligidae have the filament attached to their frontal area. Chalimi of the family Pennellidae hold the filament by means of their second antennae, while species of the family Lernaeopodidae attach to the filament with their second maxillae.

Very few copepods, in this number species of the genus *Lepeophtheirus*, increased the usual number of post-naupliar stages from 5 to 7. Their fourth chalimus (copepodid V) moults into preadult I, which is soon followed by preadult II (Fig. 18.43) (Johnson and Albright, 1991a, b; Schram, 1993).

The majority of copepods parasitic on fishes have a single host. Only representatives of the family Pennellidae are heteroxenous. Their intermediate hosts are some benthic fishes (flatfishes for species of *Lernaeocera;* cf. Sproston, 1942) or pelagic invertebrates (molluscs) (for species of the genus *Pennella;* cf. Perkins, 1983). All chalimus stages live on an intermediate host, while juvenile, pre-metamorphosis female becomes the infective stage for a definitive host.

Many copepods parasitic on fishes exhibit distinct sexual dimorphism. Often, only the female form is parasitic and the male dies after copulation. Therefore, the male form acquires no adaptations to parasitism in its adult life and consequently its development would not go far beyond the level attained by the last larval stage. Good examples of such developmental pattern are species of genera *Ergasilus* and *Lernaea*. Sexual dimorphism can also be observed in species, where larval stages are parasitic (Siphonostomatoida). Even though the larval males parasitize their hosts at the same intensity as the females do, it is generally not obligatory for a male to parasitize its host during the adult stage. As a result, the size and

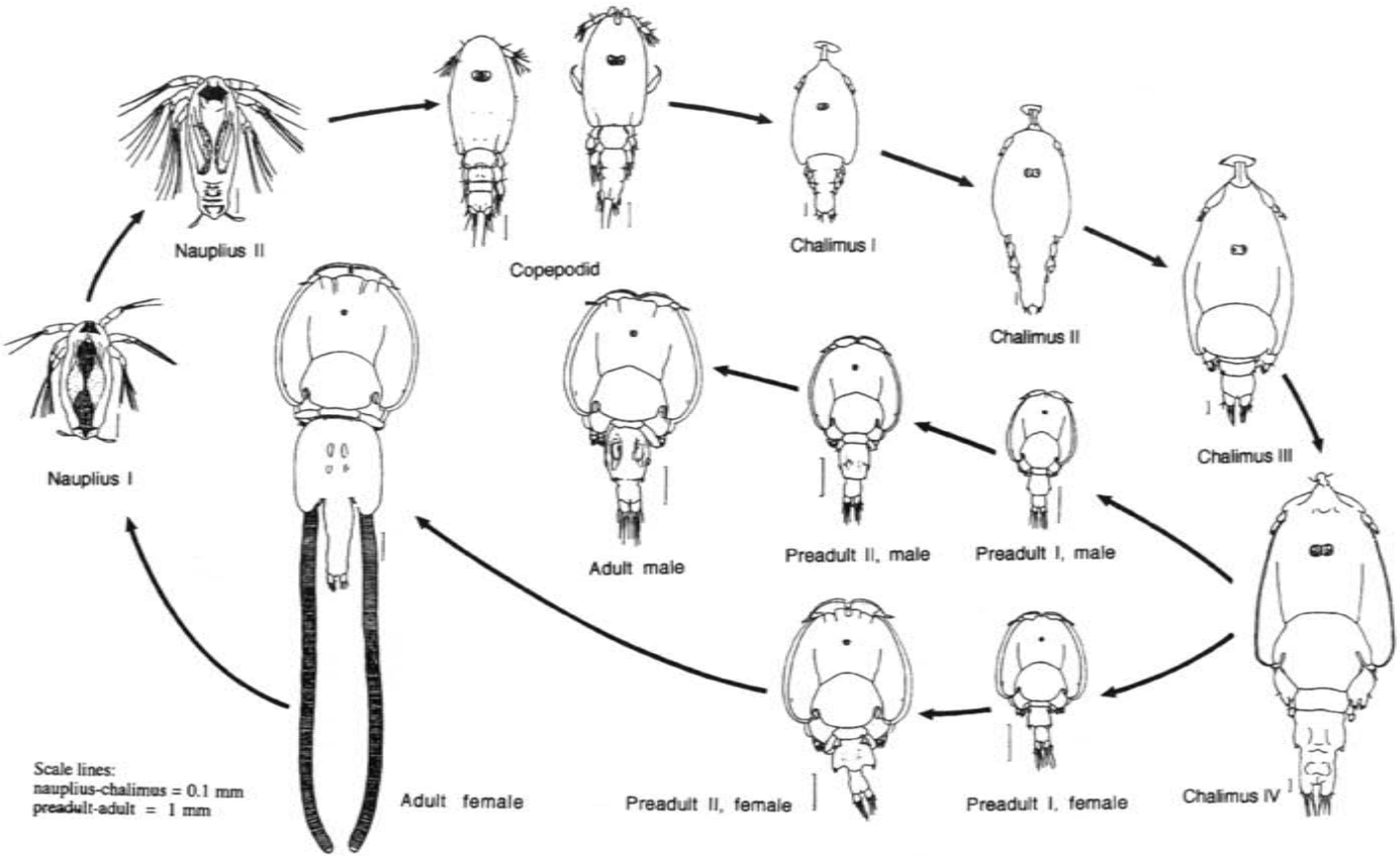


**PLATE 5** Fig. 18.43. Life cycle of *Lepeophtheirus salmonis* (Caligidae). Source: Schram, 1993.

structure of an adult male are usually similar to those of the last larval stage. Such a male is often referred to as 'dwarf', which is not true because the absolute dimensions do not decrease but slightly increase. The impression of alleged dwarfism is caused by very extensive growth of a post-moult female, and the attained difference in size makes a false impression that the males actually decrease their size. The occurrence of male 'dwarfism' among different higher taxa is not consistent with their: evolutionary advancement, overall morphological adaptations, taxonomic position, or life cycle pattern, although it is more likely for a highly metamorphosed copepod to have 'dwarf' males (Figs. 18.6, 18.17, 18.25). Extensive sexual dimorphism is equally characteristic for poecilostomes (e.g., Chondracanthidae; Fig. 18.6) and siphonostomes (e.g., Sphyriidae; Fig. 18.24, 18.25). Two different families of the same order of Siphonostomatoida (Caligidae and Pennellidae) show an extremely different approach to the relative size and structure of both sexes. Males representing the family Caligidae are almost equal in size to their female counterparts and their structure is almost identical, except for the size of the genital complex and some other minor details (Figs. 18.10–18.13, 18.43, 18.44). What is more important, caligid males are also parasitic and are capable of an independent life on the host.

Extensive differences in sexual dimorphism (and life strategy) can be found also among closely related species of the 'freshwater branch' of the family Lernaeopodidae. The male of *Tracheliastes maculatus* is very small in relation to female (Fig. 18.42). It probably dies soon after the copulation, what can be concluded from the fact that the males of *T. maculatus* are very rarely found during parasitic surveys. Males of *Achtheres percarum* (Fig. 18.17), on the other hand, are relatively bigger (in relation to females; Fig. 18.16); they can be quite abundant on the gills of the host fish, and they are well suited for independent parasitic life on the host (Piasecki *et al.* 2006).

The most recent review of copepods affecting freshwater aquaculture was published by Piasecki *et al.* (2004), while the most recent review of parasitic copepods in marine aquaculture was published by Johnson *et al.* (2004). Textbooks broadly covering health issues related to crustacean parasites of finfish are by Kabata (1985), Möller and Anders (1986), Grabda (1991), Rohde (2005). The most comprehensive overview of parasitic copepods (the famous 'Blue Book') was published by Kabata (1979).

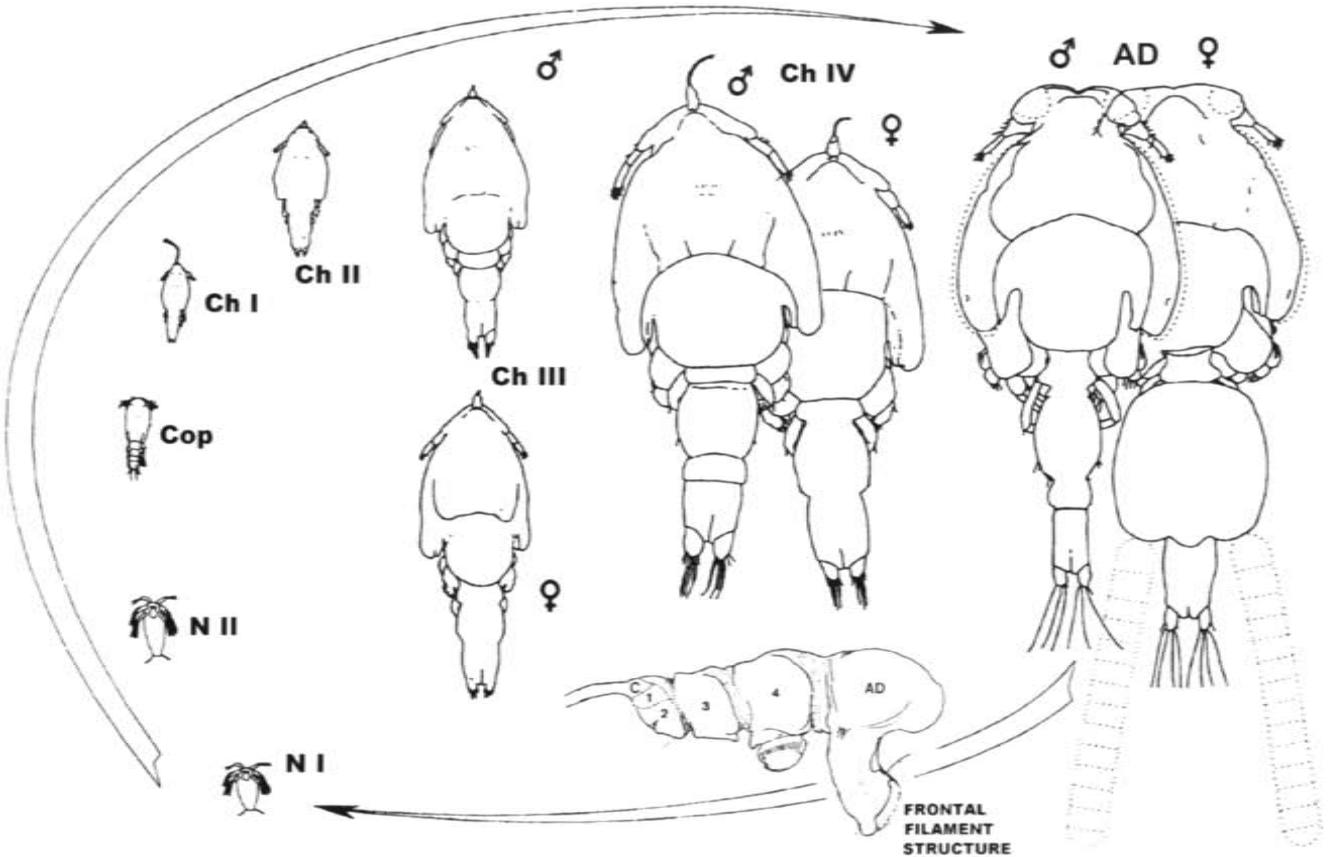


**PLATE 6** Fig. 18.44. Life cycle of *Caligus elongatus* (Caligidae). Inset: Proximal part of frontal filament (lateral) of juvenile adult female with elements added by consecutive stages. Labels: Ch I, chalimus I; Ch II, chalimus II; Ch III, chalimus III; Ch IV, chalimus IV; AD, juvenile adult female; N1, Nauplius 1; N2, Nauplius 2; Cop, Copepodid. Source: Piasecki; original.

## Important Pathogens

Nowadays, the easiest way to determine the importance of a parasite is to enter the parasite name into an Internet browser and record the number of 'hits'. The most important ones are those parasites that have importance to aquaculture, particularly marine aquaculture. The host species constituting the bulk of production define which parasite species are the best known and most important (if the number of the parasites translates into measurable losses for the industry).

The best-known and possibly the most important parasitic copepods of marine fishes are 'sea lice' of the genera *Lepeophtheirus*, and *Caligus* (54,100 and 54,700 'hits' on the Google browser in April 2007, respectively). Far less known are species of the genus *Lernaeocera* with 9480 'hits'. All other major parasitic copepods rarely exceeded 1000 hits. These are: *Lernaeenicus* (803), *Pennella* (751), *Haemobaphes* (625), *Neobrachiella* (600), *Sphyrion* (593), *Clavella* (552), *Brachiella* (540), *Chondracanthus* (539), *Bomolochus* (537), *Lernaeopoda* (440), *Sarcotaces* (290), *Pandarus* (214), *Phrixocephalus* (139), *Cecrops* (123), *Sarcotretes* (101), *Lophoura* (87), *Trifur* (49), and *Ichthyotaces* (21).

Among copepods parasitic on freshwater fishes the best known are: *Lernaea* (29,200), *Ergasilus* (22,800), *Salmincola* (15,700), *Lamproglena* (1090), *Achtheres* (685), *Tracheliastes* (538), *Caligus lacustris* (179), *Mugilicola* (66), and *Basanistes* (25).

**Genus *Lepeophtheirus*,** Salmon louse

**Important pathogen**: *Lepeophtheirus salmonis* (Krøyer, 1838)

**Disease:** salmon louse infection

**Pathogen overview:** Along with rapid development of marine aquaculture in the last three decades, it has become evident that some apparently harmless and sporadically encountered fish parasites of the subclass Copepoda have become a serious threat to cage-cultured fishes, Atlantic salmon in particular. In 1998, the world production of these fish amounted to 690,000 tons and the economic losses attributed to salmon lice constituted an important figure in the budgets of most fish farmers.

*L. salmonis* has been a leading parasitic copepod in terms of the number of papers reporting it from fish. The most comprehensive review (of all sea lice) was published by Pike and Wadsworth (1999).

**Morphology**: *Lepeophtheirus salmonis* is a fairly large caligid copepod. It is dark-brown in appearance and shows relatively distinct sexual dimorphism (Figs. 18.10, 18.11). Female (11.52 ± 0.07 mm in length) is much larger than male (6.22 ± 0.29 mm in length) (Schram, 1993). Female carry paired egg-strings, which are often longer than the female

itself. *L. salmonis*, like all its congeners does not have lunules (Kabata, 1979).

**Life cycle:** The life cycle of *L. salmonis* has been studied in detail by Johnson and Albright (1991a, b) and by Schram (1993) (Fig. 18.43). A single egg-string of the female may contain up to 300 eggs (Schram, 1993). There are five phases in the ontogeny on salmon louse: naupliar-, copepodid-, chalimus-, preadult-, and adult phase (and a total of ten stages). The naupliar phase consists of two instars. The second nauplius moults into the copepodid. The copepodid is the infective stage. It actively searches for the host fish, temporarily attaches to it with its grasping appendages, and permanently attaches to it with the frontal filament secreted by the frontal organ. (The structure of the filament was described by Gonzalez-Alanis *et al.*, 2001.) The next, chalimus phase consists of four instars. They are all attached to the hosts by means of a short, mushroom shaped filament. The two preadult stages are fully mobile, without the filament, restraining their movements on the host. The second preadult stage moults into adult forms (Johnson and Albright, 1991a, b). The adult forms are also freely moving on the host surface, maintaining contact with the hosts in the way typical for caligids (the strategy described earlier in this chapter). Sexual dimorphism becomes visible in preadult I stage (Schram, 1993). Both adult female and adult male are parasitic (Boxaspen, 2006). The generation time is 52 days at 10°C (=520 degree days) at ambient salinity (Johnson and Albright, 1991b).

**Disease symptoms:** Low-intensity infections may be asymptomatic. Salmon infected with higher numbers of lice are covered with distinctly visible parasites. They exhibit symptoms of anxiety and do not feed as intensively as healthy fish. Very high intensities of infection produce serious lesions, predominantly on the head. Intensive infections may result in fish mortality.

**Disease diagnostics:** Detecting distinctly brown, adult lice and their larvae on the skin (Fig. 18.45). The macroscopic detection should be followed by microscopic examination of the copepods found. *L. salmonis* can be distinguished from the smaller *Caligus elongatus* by its distinctly darker colouration and absence of lunules on their frontal plates. Also, microscopic methods should be applied for identification of larval forms (chalimi), permanently attached to hard structures, such as scales or fin rays. The chalimi are the most abundant on fins. Adult *L. salmonis* tend to concentrate in the perianal area and the dorsal surface behind the dorsal fin (Johnson, 1998). Intensively infected salmon may show skin lesions on the head.

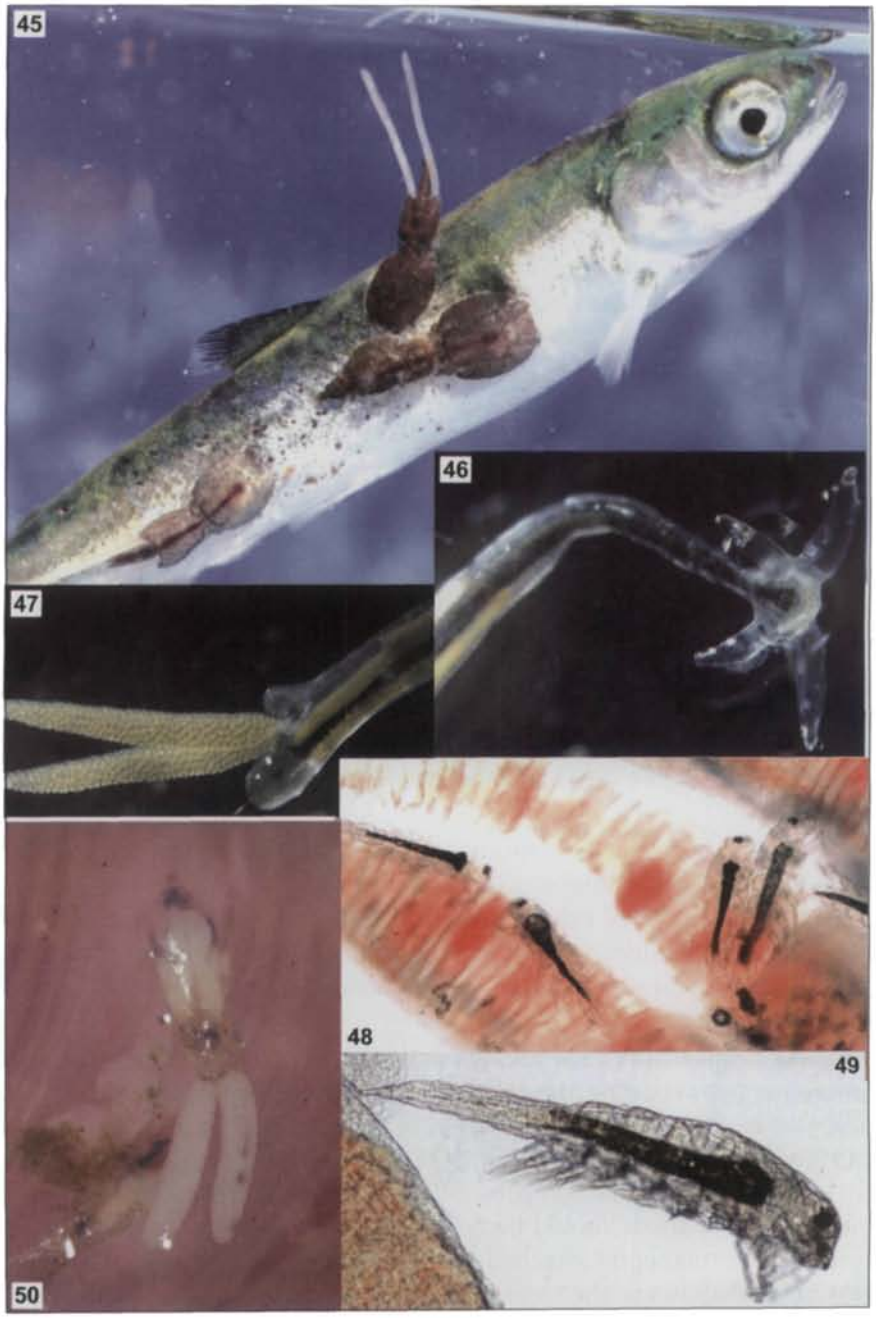


**PLATE 7** Figs. 18.45–18.50. Copepoda parasitic on fishes. Fig. 18.45. Juvenile pink salmon infected by *Lepeophtheirus salmonis* (note the lack of scale development on this young fish and the 'tracks' caused by gravid sea lice puncturing skin). Fig. 18.46. Adult,

*Plate 7 Contd.*

**Etiology/pathogenesis:** Larval lice (copepodid, chalimi) attach their frontal filaments to the host fish. By doing so, they damage the epithelium covering the scales or fin rays (Bron *et al.*, 1991). Adult lice damage the skin surface through temporary attachment with their uncinate appendages or through action of the mouth siphon and mandibles, used for feeding (Fig. 18.45). The copepods feed on host mucus, tissues, and blood (Brandal *et al.*, 1976). Mechanical activity of larvae and adults results in inflammation and hyperplasia (Jones *et al.*, 1990; Jónsdóttir *et al.*, 1992; Nolan *et al.*, 2000). Intensive feeding of numerous *L. salmonis* produces deep erosion areas on the dorsal side of the fish head and behind it. In the perianal area, distinct signs of erosion and sub-epidermal haemorrhage can be observed (Brandal and Egidius, 1979; Pike, 1989; Berland, 1993; Pike and Wadsworth, 1999). This characteristic localization of the lesions inflicted by salmon lice may be associated with abundant supply of mucus, provided by mucous cell-lined ducts in that region (Nolan *et al.*, 1999). The presence of lice on fish skin irritates the fish and increases the level of stress hormones in host organism, which in turn may increase the susceptibility of the fish to infectious diseases (Nolan *et al.*, 1999; Bowers et al., 2000; Fast *et al.*, 2006). Also the chalimi, when attached to the gills, may be responsible for fish mortalities, because of the respiratory impairment they cause (Johnson *et al.*, 2004). There have been a number of records of fish mortalities inflicted by *L. salmonis* (cf. Pike and Wadsworth, 1999; Finstad *et al.*, 2000; Holst *et al.*, 2003). Death results from secondary infections, fostered by skin erosion and stress, as well as from osmoregulatory failure (Brandal and Egidius, 1979; Wootten *et al.*, 1982; Bjorn and Finstad, 1997; Pike and Wadsworth; 1999; Bowers *et al.*, 2000; Finstad *et al.*, 2000; Johnson *et al.*, 2004). Morton *et al.* (2004) established that the lethal infection limit of juvenile pink- and chum salmon was 1.6 lice per 1 g of host mass. Short-term mortality in small wild fish (pink- and chum salmon), infected by numbers of larval stages is increased by infections of 1–3 adult sea lice (Morton and Routledge, 2005). Surprisingly, the Fulton's condition factor, as studied recently by Morton and Routledge (2006) cannot be a valid measure of sea lice impact on juvenile salmon.

*Plate 7 Contd.*

ovigerous female of *Lernaea* sp.; anterior part. Fig. 18.47. Adult, female of *Lernaea* sp.; posterior part. Fig. 18.48. Copepodids of *Lernaea* sp. on the gills of catfish. Fig. 18.49. Copepodid of *Lernaea* sp.; lateral. Fig. 18.50. Ovigerous female of *Ergasilus* sp. on the gills of catfish. Original photographs by: Alexandra Morton (Fig. 18.45); Andrew Goodwin (Figs. 18.46–18.50).

**Disease treatment and prophylaxis:** *Lepeophtheirus salmonis* is specific to salmonid fishes and, therefore, its mass transfer from other wild fishes seems to be unlikely. Hence, key prophylactic measures are aimed at limiting the likelihood of acquiring infection from cultured fish or from wild salmonids. Proper management of aquaculture operations is vital for maintaining low level of the planktonic stages of this copepod within the fish cages. An ideal site should have high flushing rates, caused by currents, winds, or tides. Intensively flowing water is likely to wash away the planktonic stages. The longer the fish are kept at a given site, the more infective stages of salmon lice are present in the water. Therefore, by the end of a culturing cycle the parasite's intensity may be very high. Coho salmon, *Oncorhynchus kisutch*, in Japan are reared for only one year prior to harvest and this is one of the factors contributing to low salmon lice intensities there (Ho and Nagasawa, 2001). In the case of Atlantic salmon—where the rearing time is much longer—a key management strategy helping to reduce natural burden of lice larvae in water has been by 'fallowing'. All cages on a farm should be stocked with the same year class of fish and, if possible, this rule should be extended to all fish farms in a given area. A fallowing period, applied post-harvest, to all cages in the area, should help to minimize the infection levels in newly stocked salmon (Bron *et al.*, 1993).

In some areas, the infection levels increase so much that the treatment is necessary. In some countries, there are official treatment thresholds as well. Treatment of salmon before restocking is also recommended (Johnson *et al.*, 2004). In Ireland, de-lousing must be performed when there are 0.3–0.5 ovigerous females per fish in the spring and 2 ovigerous females at other times of the year (McMahon, 2000). In Norway, the treatment threshold is 1–5 adult females per fish, depending on a number of additional conditions (Eithun, 2000). Some other countries have been trying to implement voluntary sea lice management strategies (Rae, 2002; Anonymous, 2003). A good example of an Integrated Pest Management strategy of sea lice in Canada was outlined by Anonymous (2003).

Treatment of sea lice is chiefly based on chemotherapeutants, but a strong effort has been made to implement alternative biological methods as well as fish immunization. The chemotherapeutic control of sea lice was reviewed by Costello (1993) and Roth *et al.* (1993). The chemical compounds can be applied either orally, or externally, in the form of bath- or dip treatments. Oral application of some drugs (e.g., ivermectin), although effective, (Smith *et al.*, 1993), it is unlikely to be licensed for aquaculture use because of certain health concerns (toxicity) (Costello, 1993). The other, well-known, orally administered chemotherapeutant is

diflubenzuron (marketed as Ektobann and Lepsidon), is especially effective against larval forms and preadult stages (Johnson, 1998). Chemical compounds used for salmon bath treatments include: dichlorvos (marketed as Nuvan 500EC or AquaGuard SLT), trichlorfon (marketed as Neguvon®), azamethiphos (marketed as Salmosan or Alfacron), cypermethrin, carbaryl, pyrethroids, and hydrogen peroxide (Costello, 1993). The chemotherapeutants listed above are generally effective only against adult and preadult stages of *L. salmonis*, leaving the attached chalimus stages alive. Therefore, it is suggested to apply the treatment later, when the chalimi reach maturity. Hydrogen peroxide proved to be toxic to fish under certain conditions. Moreover, the detached lice are able to recover and re-infect salmon (Johnson *et al.*, 1993). Therefore, the use of hydrogen peroxide has been discouraged (Johnson, 1998).

The side effects related to bath treatment include: fish stress and possible acquisition of some infectious diseases, and worsening of food conversion rates. Moreover, the toxicity of organophosphate compounds to the environment has been disputed, resulting in a formal ban of those chemotherapeutants in some countries.

The biological methods, alternative to chemical treatment, include: mechanical removal with a brush or comb (extremely heavy infections), cleaner fish (Bjordal, 1990; Tully *et al.*, 1996), or natural plant-origin substances (Costello, 1993).

A vaccine against salmon lice is not available, as yet. Antibody production against caligid copepods can be stimulated by injecting crude extracts of *L. salmonis* to fish (Grayson *et al.*, 1991, 1995).

**Epidemiology/ geographical distribution:** *L. salmonis* has a circumpolar distribution and it occurs commonly on the majority of salmonid fishes belonging to the genera *Salmon* and *Oncorhynchus*. The principal host of *L. salmonis* is Atlantic salmon, *Salmo salar* and the records from non-salmonids are sporadic (Kabata, 1979). It is a salt-water species and it cannot survive when salmon enter rivers to spawn (Bricknell *et al.*, 2006; Wells *et al.*, 2006). Johnson and Albright (1991b) determined that *L. salmonis* is not capable to reproduce successfully in the water below 30‰ salinity. *L. salmonis* is responsible for serious disease problems (including mortalities) in Atlantic salmon culture (Pike and Wadsworth, 1999). Even though the parasite is also present in Japan, on cultured Coho salmon, *Oncorhynchus kisutch*, it does not pose a serious problem. Apparently Coho salmon are more resistant to the infection than Atlantic salmon (Ho and Nagasawa, 2001; Johnson *et al.*, 2004). In some areas, the lice hosted by wild fish may spread the infection to salmonids cultured in net pens.

The reproductive potential of *L. salmonis* is high. The number of eggs per one egg-string is variable but as evident from Tully's (1989) observations one egg string of a female of the 'winter generation' (January) contains some 300 eggs while that of a 'summer generation' (August) contains some 100 eggs. Mean egg development time at 10°C is 8.6 days, while development from the egg to the adult male was 40 days and 52 days to the adult female (Johnson and Albright, 1991b). The generation times estimated from field data by Tully (1989) were approximately 93, 76, 56, and 48 days at water temperatures of 7.0, 8.0, 12.0, and 15.5°C, respectively. The reproductive potential of salmon lice has also been studied by many other authors, including Ritchie *et al.* (1993) and Revie *et al.*, 2005).

The numbers of infective stages of *L. salmonis*, present in the water, depend on many factors, such as: availability of wild fish serving as carriers, the number of salmon farms in a bay or fjord, exposure of the culture site to currents, winds, and tides, the magnitude of a tide ('flushing rate'), length of the culture cycle, and fallowing periods in a given area.

Adult and preadult *L. salmonis* can swim and are capable of transferring between host fishes, including feral fishes swimming around salmon cages (Bruno and Stone, 1990; Ritchie, 1997).

*L. salmonis* may act as vectors of some infectious diseases. Nylund *et al.* (1993) during their laboratory experiments demonstrated that those copepods might transmit viral agents responsible for infectious salmon anaemia (ISA). *Aeromonas salmonicida*, responsible for furunculosis has been found on the surface of *L. salmonis* (Nese and Enger, 1993). Viruses of infectious pancreatic necrosis (IPN) were also found on *L. salmonis* (cf. Johnson *et al.*, 2004, citing Jim Treasurer).

*L. salmonis* parasitizing salmon reared in sea cages pose a threat to wild salmonid fishes. Morton *et al.* (2004) noticed that 90% of wild juvenile pink- and chum salmon near salmon farms were infected with more than 1.6 lice per 1g of host body (a proposed lethal limit). According to Krkošek *et al.* (2007) the sea lice originating from salmon farms, in the Broughton Archipelago, induced 9%–95% mortality of a number sympatric wild juvenile pink- and chum salmon populations.

Heuch (2005) emphasized the epidemiological importance of salmon escaping from cages. The annual numbers of escaped fish in Norway alone are estimated to be some 500,000, with 10 lice per salmon.

## Economical Importance

*Lepeophtheirus salmonis* causes major losses in the Atlantic salmon aquaculture. In some countries, also *Caligus elongatus* contributes to the losses. The relevant statistics cover both species as the sea-lice. The losses due to sea lice infections are associated with: direct mortality of salmon, mortality to secondary infections, decreased growth rates, reduced value of fish carcass, and the costs associated with prophylaxis and treatment (Pike and Wadsworth, 1999). Atlantic salmon production has been a multi-million dollar business in Norway, Scotland, Ireland, Canada, Chile, and Australia. *L. salmonis* has been a major salmon pest in the northern hemisphere. Economic losses due to sea lice in Norway amount to $US67 million (Johnson *et al.*, 2004). Scotland sea-lice losses are between 0.18 and 0.45 US$ · $kg^{-1}$ of the salmon produced (Sinnott, 1999). In New Brunswick, Canada the losses were 0.08–0.11 US$ · $kg^{-1}$ (Mustafa *et al.*, 2001) with *Caligus elongatus* as the predominant sea lice species.

**Recent developments:** The life cycle of *L. salmonis* was described in the early 1990s by Johnson and Albright (1991a, b) and Schram (1993). Similarly, the majority of treatment methods were developed, tested, implemented, and optimized in the first half of the 1990s (Costello, 1993). Gustafson *et al.* (2006) and Telfer *et al.* (2006) studied the efficacy of a relatively new drug, emamectin benzoate (manufactured as SLICE), in sea lice treatment. Bricknell *et al.* (2006) studied the effect of different salinities on the *L. salmonis* settlement success. The most recent, and comprehensive, reviews of the sea lice issues in salmon aquaculture were published by Johnson *et al.* (2004), Boxaspen (2006), and Costello (2006). Prophylaxis and treatment trends of sea lice are directed into development of a comprehensive pest management strategy (Anonymous, 2003).

Alexandra Morton from Canada has recently been trying to focus attention of public opinion, governmental agencies, and scientists on the problem of serious threat posed by sea lice from salmon farms to declining wild salmon in British Columbia, Canada (Morton and Williams, 2003). In response to her activity, the British Columbia Ministry of Agriculture, Fisheries, and Food ordered (in 2002) salmon farm fallowing on the presumed route of wild juvenile pink- and chum salmon migration in British Columbia (Morton *et al.*, 2005).

## Other Related Species

There are about 109 valid species of the genus *Lepeophtheirus* (cf. Boxshall and Halsey, 2004). The following species of the genus *Lepeophtheirus* are frequently listed in the literature as important, or potentially important,

parasites of finfish reared in sea cages or important to fisheries: *L. cuneifer* Kabata, 1974 (on salmonid fishes), *L. pectoralis* Müller, 1776 (on flatfishes), and *L. hippoglossi* (Krøyer, 1837) (on halibut). *L. mugiloidis* has been recorded to parasitize cultured salmon in Chile (Carvajal *et al.*, 1998).

**Genus: *Caligus***, sea louse

**Important pathogens:** *Caligus elongatus* von Nordmann, 1832

**Disease:** sea lice infection

**Pathogen overview:** *Caligus elongatus* is the second most important ectoparasite of Atlantic salmon, following *L. salmonis*. The two species have been collectively known as the sea lice. *C. elongatus* is not host specific to salmonids and it has been recorded on at least, 80 fish species, representing 17 orders and 43 families (Kabata, 1979). Although in the majority of aquaculture operations, rearing Atlantic salmon, the principal pest is *Lepeophtheirus salmonis*, in some areas, the most prevalent sea lice is *C. elongatus*. In the early 1990s the cage sites on the coasts of New Brunswick, Canada (Bay of Fundy) were parasitized predominantly by *C. elongatus* (cf. Piasecki and MacKinnon, 1995). Hogans and Trudeau (1989a) estimated that *C. elongatus* constituted 97% of all sea lice in that area.

**Morphology:** *Caligus elongatus* is much smaller than *L. salmonis* and their sexual dimorphism is also less pronounced than that in the salmon louse. Both sexes are similar in size and shape (Figs. 18.12, 18.13) and the females differ only in having a larger genital complex and egg strings. The females (without egg strings) measure 4.93 ± 0.26 mm while the males measure 4.33 ± 0.25 mm (Piasecki, 1996). The egg strings measure from 0.9 to 6 mm and they are longer in the summer, than in winter. *C. elongatus* are poorly visible against the background of the dark parts of the salmon skin, because they are almost transparent, with only a delicate brownish coloration. *Caligus* females differ from females of *Lepeophtheirus* in having lunules—the cup-shaped attachment organs, situated on their frontal plates.

**Life cycle:** The life cycle of *C. elongatus* was described in detail by Piasecki and MacKinnon (1995) and Piasecki (1996) (Fig. 18.44). The cycle is different than previously thought (Hogans and Trudeau, 1989b). The preadult phase, known from *L. salmonis*, does not exist in *C. elongatus*. The identity of all stages is clearly marked by the structure of the proximal portion of the frontal filament (Piasecki and MacKinnon, 1993; Piasecki, 1996). Each consecutive stage, attached by the filament modifies its proximal end by adding a characteristic brick. The last such brick is added

by the post-moult young female (Fig. 18.44). A moult cannot be performed without temporary de-attaching the filament. Because ovigerous females, still attached by the filament, can occasionally be found it means that there is no preadult stage between chalimus IV and the adult. The filament in C. *elongatus* is relatively long and facilitates identification of the stage. For comparison, the filament in *L. salmonis*, is very short and shows no apparent stage identity (Gonzalez-Alanis *et al.*, 2001). Summing up, there are four phases and a total of 8 stages in the ontogeny on C. *elongatus*: naupliar-, copepodid-, chalimus-, and adult phase. The naupliar phase consists of two instars. The second nauplius moults into the copepodid. The copepodid is the infective stage. It actively searches for the host fish, attaches to it with its grasping appendages, and permanently attaches to it with the frontal filament. The subsequent, chalimus phase consists of four instars. They are all attached to the hosts by means of a long frontal filament. The adult forms, briefly re-attach to the filament after the last moult, but are freely moving on the host surface, maintaining contact with the hosts in the way typical for caligids (the strategy described earlier in this chapter). Sexual dimorphism becomes visible in chalimus III stage. Both adult female and adult male are parasitic. The generation time is 43.3 days at 10°C (=433 degree days) (Piasecki and MacKinnon, 1995).

**Disease symptoms:** Even moderately high infection intensities may be asymptomatic. In very high infections, lightly brownish, small sea lice are visible on ventral parts of salmon. The lice attached on the dorsal, dark parts of the body are poorly visible. The highly infected salmon exhibit symptoms of anxiety and do not feed as intensively as the healthy fish. Skin lesions, haemorrhages, or epithelial hyperaemia, even in very heavily infected fish, are rare.

**Disease diagnostics:** Small, light-brown C. *elongatus* are visible macroscopically on lightly coloured ventral parts of the skin and on fins. Those in darkly pigmented dorsal parts are usually poorly visible. C. *elongatus* can be easily distinguished from much bigger, darkly pigmented *Lepeophtheirus salmonis*. The latter species does not have lunules on its frontal plates. If microscopic methods are applied for identification of larval forms (chalimi), permanently attached to hard structures, such as scales or fin rays, it must be noted that chalimi of *Caligus* differ from chalimi of *Lepeophtheirus* by having long frontal filaments. Pathologic lesions of the skin, related to the infection are rare.

Taxonomic keys for identification of all other sea-lice species afftecting cultured fishes are provided by Schram (2004).

**Etiology/pathogenesis:** The pathogenicity of C. *elongatus* larvae is similar to the action of *L. salmonis* larvae. The epithelium is damaged at the point of attachment. A tiny drop of lymphatic exudate leaks out through the hole and clots around the attached portion of the filament. It can be seen as a tiny white spot on the skin of experimentally infected fish in aquarium. The feeding activities of adult C. *elongatus* are not as pathogenic as those of *L. salmonis*, chiefly because of the size differences. Adult C. *elongatus* feed mainly on the host's mucous. It is possible that the presence of lice on fish skin irritates the fish and may increase the level of stress hormones in the host organism, which in turn may increase the susceptibility of the fish to infectious diseases. Highly parasitized fish may be less competitive for food and show lower body mass increment.

**Disease treatment and prophylaxis:** Because of its wide host spectrum, C. *elongatus* can be easily transferred from wild fishes passing by the sea cages or simply being attracted to a cage site by unused salmon food. There is no way to deter the feral fishes, but the awareness of the possible infection source is essential for integrated pest management systems.

Other issues of the prophylaxis, rearing strategies, culture management, treatment thresholds, and treatment methods, described earlier, for *L. salmonis*, are also effective for C. *elongatus*.

**Epidemiology/geographical distribution:** C. *elongatus* has circumpolar distribution and, as mentioned earlier is not host specific and it has been reported from fishes representing as many as 43 families. Therefore, the likelihood of its transmission from wild fishes is much higher than in the case of *L. salmonis*.

The reproductive potential of C. *elongatus* is much lower than that of *L. salmonis*. The egg strings of *L. salmonis* may reach 20 mm in length while those of C. *elongatus* do not exceed 5–6 mm. The generation time of C. *elongatus* is shorter than that of the big salmon louse and it amounts to 433 degree days (=43.3 days at 10°C). Adult C. *elongatus* can swim and thus transfer from host to host. Revie *et al.* (2002) studied seasonality of C. *elongatus* infection within 1997–2000 in Scotland. They found some consistent patterns: (1) the infection levels rapidly increased in early July, (2) C. *elongatus* were more prevalent on salmon in the first year of production than in the second year and (3) fallowing had no effect on abundance.

**Economical importance:** *C. elongatus* is much smaller than *L. salmonis* and therefore definitely less pathogenic and its economical importance is lower than that of *L. salmonis*. The economical effect of *C. elongatus* has been assessed jointly with *L. salmonis*.

**Recent developments:** Most recent studies on *C. elongatus* focus on its population structure and identifying individual genotypes within the species (Øines and Heuch, 2007). Genotype identification may also help us to understand mechanisms of infecting cultured fish from wild fish (Øines *et al.*, 2006), especially when new alternative species, such as cod, are introduced into marine aquaculture.

Molecular biology methods can also help in detection and monitoring of the planktonic stages of sea lice within and outside the sea cages (McBeath *et al.*, 2006)

**Other related species:** The number of species assigned to the genus *Caligus* exceeds 250, as estimated by Boxsall and Halsey (2004). The vast majority of them infect marine fishes and only one is freshwater (*C. lacustris*). Salmonid fishes are sporadically parasitized by *Caligus clemensi* Parker et Margolis, 1964; *C. curtus* Muller, 1785; *C. orientalis* Gussev, 1951 (in the northern hemisphere); and *C. longicaudatus* Brady, 1899 (in the southern hemisphere). Cultured salmonids in Chile have been targeted by *C. rogercresseyi* Boxshall et Bravo, 2000; *C. teres* Wilson, 1905; and *C. cheilodactyli* Krøyer, 1963 (cf. Carvajal *et al.*, 1998; Boxshall and Bravo, 2000). *C. epidemicus* Hewitt, 1971 threatens tilapia cultured in Taiwan (Lin and Ho, 1993). The only freshwater representative of the genus *Caligus* has also shown its epizootic potential with mass infections of salmonids cultured in cages in the Bay of Puck (almost freshwater), Baltic Sea Poland (Rokicki, 1986).

**Pennellid gill worms** (genera: *Lernaeocera, Haemobaphes*)

**Important pathogens:** *Lernaeocera branchialis* (L.)

**Disease**: Lernaeocerosis

**Pathogen overview:** *L. branchialis* has been the mostly widely reported copepod, parasitic on marine fishes, at the time, before the intensive rearing of salmon has brought forward the problems with sea lice. This parasite is very pathogenic to its hosts and contributes to substantial mortalities, or weight loss, of the affected fish.

**Morphology:** *L. branchialis* is a large, highly metamorphosed copepod with an s-shaped appearance (Fig. 18.20). The length of adult female (measured in a straight line between anterior and posterior points) may

reach 60 mm (Kabata, 1979). The parasite is anchored in the host tissues by means of three major cephalic outgrowths ('antlers'), branching further at their ends. The genital trunk is bent at an angle of less than 90° or more than 90° (*L. branchialis* f. *branchialis* and *L. branchialis* f. *obtusa*, respectively; Kabata, 1979). The genital trunk protrudes into the branchial cavity of the host fish. The egg sacs are unseriate and curled elaborately.

**Life cycle:** The life cycle of *L. branchialis* is very complex (Sproston, 1942; Kabata, 1958) and it involves a flatfish (or other bottom-dwelling fish) as an intermediate host. The life cycle consists of 9 stages: two nauplius stages, a copepodid (infective stage), four chalimus stages attached to the intermediate hosts by their frontal filaments and the adult stages. A young, pre-metamorphosed female copulates with a male and attaches to its definitive host—a gadoid fish. Metamorphosis of a female occurs on a definitive host.

**Disease symptoms:** Infected fish are weaker and leaner than their non-infected counterparts. The principal symptom of the infection is the presence of large reddish worm-like parasites in the branchial chamber.

**Disease diagnostics:** *L. branchialis* can be distinguished from its congeners, living on fishes in the north Atlantic and the adjacent seas, mainly by its larger size and some details in the morphology (see Kabata, 1979). The size of adult *L. minuta* is 4–7.5 mm only. *L. lusci* is 6–15 mm long. The validity of the above mentioned species has been disputed and according to some authors they are conspecific with *L. branchialis*.

In the Pacific Ocean some fishes are infected with similar parasites, inflicting similar damage: *Haemobaphes diceraus* and *H. dispherocephalus*. *Haemobaphes* differs from *Lernaeocera* in having a much longer 'neck', smaller 'antlers' and more regularly coiled egg strings (Kabata, 1979).

**Etiology/pathogenesis:** There are very few papers focusing on abundance and pathogenicity of larval stages and juveniles of *L. branchialis* on its intermediate hosts (Van Damme, 1997). The pathogenicity of metamorphosed adult females, however, may be devastating an even lethal on its definitive host. Newly attached juvenile female holds the gill filament (proximal to the ventral end of gill arch) so tightly that constriction of the filament vessels is so severe that the entire gill filament is cut of from the blood supply. The infected filament assumes a blanched appearance and subsequently becomes necrotic and disintegrates (Kabata, 1958, 1970). With the growth of the parasite its cephalic area

progressively grows into the ventral aorta of the fish, reaching the bulbus arteriosus, or ventricle of the heart. The attachment of *L. branchialis* has serious consequences to the heart and the circulation. The bulbus becomes distorted and its walls become thicker, thus reducing the lumen of the vessel. The presence of the parasite's cephalic area in the proximity of fish heart contributes to substantial occlusion of the circulation, which increases chances for fish mortality. Because of its histological structure, the bulbus arteriosus is very plastic in its nature and can adjust to the growing size of the copepod's head (Kabata, 1970). The entire cardiac area undergoes vigorous histological changes with concurrent processes of change, breakdown, and reconstruction (Schuurmans Stekhoven, 1936; Kabata, 1970). Considering the magnitude of changes, it is obvious how devastating it can be in the action of the parasite. Multiple infections exceeding 2 *Lernaeocera* are probably lethal to young gadoid fish. The fish infected at a young age do not migrate, but stay close to the shore where they were infected.

**Disease treatment and prophylaxis:** Sea-ranched cod facilities should be located at sites where the cod could be separated from bottom-dwelling intermediate hosts of *Lernaeocera* such as flatfishes or lumpfish.

**Epidemiology/geographical distribution:** *L. branchialis* occurs in the North Atlantic and adjacent seas. Its principal hosts are fishes of the family Gadidae: cod, *Gadus morhua*, whiting, *Merlangius merlangus*, and haddock, *Melanogrammus aeglefinus*. Only females are parasitic and it is they who settle on fish gills. The infection intensity is usually low, not exceeding one or two specimens per fish, with a maximum of 11 *L. branchialis* (Kabata, 1979). The metamorphosed female attached to the ventral part of a gill arch penetrates the tissues with its head, reaching into the ventral aorta of the fish, or the bulbus arteriosus, or the ventricle of the heart. Intensities of about 700 juveniles on an intermediate host seem to cause no ill effects (Kabata (1979). Rosenthal (1967) noticed that under aquarium condition *Lernaeocera* larvae were capable of killing herring larvae.

**Economical importance:** Fish losses in wild fish, attributed to *Lernaeocera* can only be tentatively estimated. The losses include not only direct mortalities but also overall emaciation and carcass downgrading. Infection with juvenile *Lernaeocera* initially contributes to increase in haemoglobin levels in the blood and lipid levels in the liver. Also the condition factor slightly increases. Kabata (1958) attributes those changes

to the action of defence mechanisms fighting the infection. Further growth of the parasite results, however, in dramatic decrease of those parameters. Especially devastating are infections with two parasites (Kabata, 1958). Losses of the Scottish fisheries sector due to *Lernaeocera* were estimated to be almost 1000 tons of cod in 1955 (Kabata, 1958). Recently Jones-Megan and Taggart (1998) tried to use tagging for determining mortality of cod due to *Lernaeocera branchialis* infection.

The depletion of natural stocks of cod calls for alternative methods of acquiring this valuable fish. One of such method has been "sea ranching". Young cod, caught in the ocean, are reared in captivity, until they reach market size. Khan *et al.* (1990) studied long-term effects of *Lernaeocera* in the laboratory and in the field. They observed that 'Sixty-four percent (308) of 481 cod acquired infections in the laboratory and 33% (159) of the infected fish died over a 4-yr period. About 74% of the deaths occurred within 4 months of the infection'. The infected cod demonstrated reduced weight gain, lower liver somatic index, liver lipid, and blood parameter values than uninfected controls. It is evident that the devastating effects of *Lernaeocera* on cod could affect the success of the cod-ranching operation (Khan *et al.*, 1990). Lysne and Skorping (2002) have reported *L. branchialis* actually infecting cage-cultured cod in Norway.

**Recent developments:** A comprehensive account on the pathology of early stages of *L. branchialis* on cod was published by Smith *et al.* (2007). They observed that the juvenile parasite could actually migrate within the lumen of the afferent branchial artery and ventral aorta.

**Other related species:** The best known, similarly looking parasite is *Haemobaphes diceraus* Wilson, 1917 (Figs. 18.21–18.22). It occurs in the branchial cavity of Alaska pollock, *Theragra chalcogramma*, attaching to a gill arch between two rows of gill filaments. The parasite 'neck' grows very long, (much longer than that of *Lernaeocera*) and it gradually moves the head of the parasite along the branchial blood vessel towards the heart. The head of a fully grown female of *H. diceraus* reaches the bottom of the bulbus arteriosus. The head is equipped with two small cephalic outgrowths. The mid section of the 'neck' has two anchoring processes. The genital trunk is sigmoid and it has two prominent processes in its posterior part. The paired egg-strings are regularly coiled. The pathology caused by *H. diceraus* is similar to that described for *Lernaeocera* (cf. Grabda, 1975, 1977).

Another parasite of this genus, *Haemobaphes disphaerocephalus* Grabda, 1976, has been reported as an accidental parasite of seawater pen-

reared Atlantic salmon in British Columbia (Kent *et al.*, 1997). This parasite has been known to infect gills of eulachon, *Thaleichthys pacificus* (family Osmeridae) (cf., Grabda, 1976).

Members of the genus *Cardiodectes* (Pennellidae) (some 12 species) usually attach to the host fish between the pectoral fins, penetrate through the skin, and subdermal tissues, reaching the pericardium. Their branching cephalic holdfast grows through cardiac tissues (Noble and Collard, 1970; Grabda, 1991). The best know example is *C. medusaeus* (Wilson, 1908) anchored in the heart of a lanternfish, *Stenobrachius leucopsaurus* (cf., Kabata, 1981). Perkins (1983) described the life cycle of this parasite.

A number of pennellid parasites attack fish eyes. One of the most spectacular is *Phrixocephalus cincinnatus* Wilson, 1908. The juvenile female attaches to Pacific arrowhead flounder and enters the eye. There it completes its metamorphosis and again breaks through the cornea, protruding with its genital trunk outside, to the water to produce eggs. The parasite's length is less than 2 cm. Its genital trunk is elongate, while its holdfast is very elaborate, resembling the root system of a tree. The holdfast penetration of the ocular tissues and, later on, decomposition of a dead parasite leads to fish blindness (Kabata, 1969a).

Another well known, obligate eye-dwelling pennellid is *Lernaeenicus sprattae* (Sowerby, 1806). Its principal host is European sardine and sprat (and never herring). The juvenile female pierces the cornea and the point of entry coincides with the margin of the lens. It grows further, through the entire thickness of the eyeball and anchors the head in the fundus of the eye, causing a large haematoma (Kabata, 1970). Their life cycle was studied in detail by Schram (1979). He found that the 'intermediate host' of *L. sprattae* is also a sprat and a juvenile female has a brief free-swimming phase, while searching for the 'definitive host' sprat.

Among pennellids attacking the fish eye there are also: *Lernaeenicus longiventris* Wilson, 1917 attacking *Xenomelaniris brasiliensis* (cf. Carvalho, 1953; Möller and Anders, 1986) and *Lernaeolophus sultanus* (Milne Edwards, 1840) (similar in shape to *Lernaeocera*) targeting chub mackerel and penetrating through the mouth roof (Fig. 18.23) (Grabda, 1972).

Some pennellids are known to penetrate through the peritoneal cavity and attach within the internal organs of a fish. Good examples are *Sarcotretes scopeli* Jungersen, 1911, attacking myctophid fishes and *Lernaeenicus hemiramphi* Kirtisinghe, 1932, parasitizing red-tipped halfbeak, *Hyporhamphus xanthopterus* (cf., Natarajan and Balakrishnan Nair, 1972).

The largest mesoparasitic copepods of the families Pennellidae and Sphyriidae are known to anchor themselves within the muscle tissues of fish (and even marine mammals). *Pennella filosa* (L.), measuring up to 20 cm and 0.6 mm in diameter (Fig. 18.19) penetrates the skin and muscles of swordfish, *Xiphias gladius*, reaching with its head, as far as the backbone. The head is equipped with anchoring outgrowths, while the elongate neck is very thin. The slightly thicker genital trunk of the female parasite protrudes outside the fish to the water. A terminal portion of the trunk, covered with delicate hair-like outgrowths, looks like a brush. *P. instructa* Wilson, 1917 parasitizes sunfish, while *P. exoceti* (Holten, 1802) is commonly found on flying fish. *P. balenopterae* Koten et Danielsen, 1877—the largest parasitic copepod, measuring more than 30 cm—parasitizes marine mammals.

*Trifur tortuosus* Wilson, 1917 (similar in shape to *Lernaeocera*) is commonly found anchored in the muscles of *Merluccius hubbsi* from the Argentine hake on the Argentinean shelf. Their attachment is so firm that even mechanical filleting and skinning leaves the parasites almost untouched (Grabda, 1991). The mesoparasitic anchoring of all pennellids is very pathogenic and devastating or even lethal at some attachment sites.

*Sphyrion lumpi* (Krøyev, 1845), although representing another siphonostome family (Sphyriidae), represents a similar mesoparasitic anchoring strategy as *Lernaeocera* and all pennellids (Fig. 18.24). The parasite can be found anchored in the muscles of its hosts. The size of parasitic female is substantial, reaching 7 cm in length. The hammer-shape cephalic holdfast, embedded in the tissues of a redfish (*Sebastes marinus*, *S. mentella*, and rarely other fishes—a total of 28 species of 8 families) continues into the thin and very long neck, protruding outside the fish to the water and becoming a flat and circular genital trunk. The posterior portion of the genital trunk is equipped with grape-like outgrowths. Paired multiseriate egg-sacs 4–7 cm long may contain from 6000 to 30,000 eggs (Grabda, 1991). Male 1.12 mm long (Moran and Piasecki, 1994) closely resembles males of representatives of the family Lernaeopodidae. The parasite is very pathogenic to its host. The tissue surrounding the parasite's attachment site transforms into an abscess-like spherical tumour, measuring up to 5 cm in diameter. The fishermen must remove such changes manually and it can raise the costs of processing by 80%. If the infection rate is higher, sometimes the entire catch must be discarded (Grabda, 1991). Another better-known species is

*Sphyrion laevigatum* (Quoy et Gaimard, 1824). It can be found on *Genypterus* sp. off Chile and Uruguay, on *Merluccius hubbsi* near Argentina, on *Urophycis capensis* around Falklands, on *Genypterus capensis* off Namibia, and on *Genypterus blacodes* around New Zealand. Macrurid fishes in the Atlantic and Mediterranean seas are parasitized by a similarly looking parasite *Lophoura edwardsii* Kollikev, 1853. The latter is even more pathogenic, reaching with its holdfast the backbone or even internal organs.

In some species, their mesoparasitic attachment strategy, transformed them almost into endoparasites. The female of *Sarcotaces arcticus* Collett, 1874 (Cyclopoida; Family Philichthyidae), parasitizing blue ling, *Molva molva* is drop-shaped and its size approximates a small hen egg. Recenlty described *S. namibiensis* is smaller (Reimer, 1991) (Fig. 18.8). The body segmentation and the appendages are almost totally lost. The entire female is embedded in the host tissues and only a small aperture connects it with the surface of fish skin. The parasite, because of its location, is very pathogenic to the fish. Dark body fluid of the female copepod permanently stains fish flesh, when the parasite is accidentally cut during filleting, making the fish unmarketable (Möller and Anders, 1986). There are 7 nominal species of *Sarcotaces* in the Atlantic and in the Pacific oceans. The majority of other species of the family Philichthyidae are very small (less than 1 mm) and they live in the canals of the lateral line of fishes. A good example is *Colobomatus gymnoscopeli* described by Grabda and Linkowski (1978) (Fig. 18.9) and a number of new species of *Colobomatus* described by West (1992).

**Genus *Lernaea*, anchor worm**

**Important pathogens:** *Lernaea cyprinacea* (L.)

**Disease:** Lernaeosis, Anchor worm infection, *Lernaea* larvae infection

**Pathogen overview:** There has been a lot of confusion surrounding specific identity of individual species of this genus. According to (Kabata, 1979) there are about 37 valid species of *Lernaea*. Additionally, 7 nominal species described between 1980 and 2003, should be added (Anonymous, 2007). The number of valid *Lernaea* species should probably be reduced further because different hosts can extensively modify the morphology of *Lernaea* females. Poddubnaâ (1973) has demonstrated that the above statement is correct, through producing different phenotypes—resembling different, previously described, species of *Lernaea*—from a single maternal specimen. Also, Fryer (1968) noticed that a site selection on the hosts greatly affects the morphology of the holdfast—the principal identification character. Taking the above into consideration it is evident

that a thorough revision of the genus is needed and descriptions of new species should employ some molecular methods. The most common species is *Lernaea cyprinacea*.

**Morphology:** Female of *Lernaea cyprinacea* represents the anchoring, mesoparasitic attachment strategy (Figs. 18.40, 18.41). The shape of the juvenile, female resembles that of a free-living cyclopoid copepod. While the attachment to the host is established and the anterior part of the parasite embedded into the skin, the female begins its metamorphosis associated with extensive postecdysial allometric growth (Figs. 18.37–18.40). The segmentation becomes obliterated and the cephalic somites grow prominent holdfast outgrowths, permanently anchoring *Lernaea* in the subdermal tissues of the host fish (Fig. 18.46). There are four main holdfast outgrowths, showing additional host- or site-induced variability. Thoracic- and abdominal somites, jointly form an elongate genital trunk, protruding outside from the fish skin. Egg-sacs are multiseriate and moderately elongate (Fig. 18.47). Despite the extensive metamorphosis of the body, the female appendages retain their original, cyclopoid shape and structure in every detail. For example, microscopic, pinnate, swimming legs can be found evenly distributed on the entire stretch of the trunk and minute cephalic appendages are situated in the vicinity of the mouth. An adult, fully-grown female is 12–16 mm long, plus some 8 mm of egg sacs. An adult male is 1.1 mm without its caudal rami (Kabata, 1979)

**Life cycle:** The details of the parasite's biology have been studied by many authors but the most comprehensive ones were by Kasahara (1962) and Grabda (1963a). The adult male dies after the copulation. The embryonic development of eggs deposited in an egg sac lasts, at the very least, 4 days. There are three nauplius stages and five copepodid stages. The former are all free-living while the latter live on the host. The most typical host for *L. cyprinacea* is *Carassius carassius* (L.), or other cyprinid fishes, although a total of more than 100 fish species, representing different teleost families, have been recorded as suitable *Lernaea* hosts (Kabata, 1979). Copepodid stages, parasitic on a host, may inflict some damage to the tissues. According to Pan *et al.* (1979) the optimum temperature range for *L. cyprinacea* development is 20–25°C. The life cycle (generation time) is 25 days at 20°C, 20 days at 25°C, 17 days at 30°C and 14 days at 35°C (Sarig, 1971). At temperatures of 25–37°C, the life span of a female (adult phase) is some 20 days (Pan *et al.*, 1979). *L. cyprinacea* is a freshwater parasite. Salinities ranging from 16‰ to 20‰ are lethal to them (Shields and Sperber, 1974).

**Disease symptoms:** Parasites are visible on the skin surface or fins. The infected fish show anxiety, swim chaotically and may rub themselves against underwater objects (Jara and Chodyniecki, 1999). Intensive infections may translate into a serious weight loss. Some fish may experience respiratory difficulties, causing sluggishness (Kabata, 1985). Secondary fungal or bacterial infections may also occur. Affected fins may be seriously damaged. Mass mortalities of infected fish, particularly small ones, are not uncommon (reviewed in Piasecki *et al.*, 2004).

**Disease diagnostics:** Detecting anchor worms on the skin surface or the fins. As earlier mentioned specific differentiation of individual *Lernaea* species may be difficult. Adult females have their head with cephalic processes embedded into the subdermal tissues of a fish host. Identification of larvae may be aided by Grabda (1963a) or Kabata (1979).

**Etiology/pathogenesis:** Pathogenicity of adult *Lernaea* females is associated with its anchoring attachment strategy, with its feeding mode, and/or with possible secondary infections. The enlarging cephalic outgrowths progressively damage the subdermal muscle tissues making them hyperaemic and swollen. Parasite penetration may inflict haemorrhagic changes. An ulcer-like lesion is formed and the adjacent scales are damaged or lost altogether. Parts of the skin become necrotic. Necrotic areas are also observed in the deeper muscle tissues, surrounding the parasite's head. In the course of the host response, a connective-tissue capsule is formed around the parasite head. When the parasite is located on belly flaps of a small fish a fistula may be formed, penetrating the body wall through the peritoneal cavity. The associated peritonitis may kill the fish (Kabata, 1985). The parasite feeds on tissue debris and the blood. Sabatini *et al.* (1988) described the feeding and the gut structure of *Lernaea*. The food acquisition is aided by 'an independent proteolytic enzyme system' (Juhász *et al.*, 1980). Infection with *Lernaea* may also leave its mark on the internal organs. Occlusion of the renal tubules (with dislodged epithelial cells), deep epithelial lesions in the intestinal wall, focal disintegration of hepatocytes, regressive changes in bile ducts, and erythrocyte extravasations in liver were observed by Dzidziul (1973). The blood picture shows a significant increase in monocytes and polymorphonuclear agranulocytes. Development of gonads may be retarded (Kabata, 1985).

Not only adult females of *Lernaea* are pathogenic. In some cases the infective copepodid larvae may inflict a serious pathology (reviewed in

Piasecki *et al.*, 2004). As reported by Goodwin (1999), in June and July 1998 such problems were reported from at least 3 fish farms in Arkansas, polyculturing bighead carp (*Hypophthalmichthys nobilis*) with channel catfish (*Ictalurus punctatus*). In the course of *Lernaea* infection many catfish were lost. Adult *Lernaea* did not cause the mortality, as there were very few of them on the skin of catfish. It turned out that thousands of *Lernaea* copepodid larvae, settled on the gills of the catfish. There were up to 50 copepodids on each gill filament (Figs. 18.48, 18.49). The copepods, grazing on the gill tissue, caused gill damage including epithelial hyperplasia, telangiectasis, and haemorrhage. Also the skin surface of catfish was densely covered by copepodids. The bighed carp in the same ponds did not die because of the copepod infection, even though there were numerous adult *Lernaea* attached to their skin. The gills seemed not affected. Goodwin (1999) believed that the filter-feeding apparatus of the carp captured the copepodids, thus, preventing heavy infection of the gill filaments.

**Disease treatment and prophylaxis:** The most comprehensive review of *Lernaea* treatment methods was published by Kabata (1970, 1985). Shariff *et al.* (1986) overviewed *Lernaea* treatment in large aquarium systems. The prophylactic measures suggested against *Lernaea* infections are common for many other external parasites: (1) Provision of water free of parasite larvae, (2) Disinfection and quarantine of fishes to be stocked, (3) Disinfection of pond bottom before restocking and (4) Prevention of entry of possible carriers (fish, frogs, etc.).

The selection of chemotherapeutants tested on *Lernaea*-infected fish is wide. It includes traditional treatment compounds like common salt, calcium hypochloride, potassium permanganate, or formalin, or organic compounds such as DDT, benzene hexachloride (Lindane, etc.), trichlorfon (Dipterex, Dylox, etc.), dichlorvos (DDVP, etc.), chlorbromuron (Bromex), malathion etc. Usage of some compounds, especially organophosphate ones has been restricted in some countries, because of their adverse action on the aquatic environment.

Common salt (NaCl) is recommended against larval stages of *Lernaea* with concentrations of 0.8%–1.1% applied for 3 days. Prior to treatment, the sensitivity of fish (especially small ones) should be tested.

Potassium permanganate ($KMnO_4$) has been a common chemical to treat *Lernaea* infections, but has a number of disadvantages such as: low safety factor (1.7), rapid breakdown, and inability to destroy all parasite

stages. A 25 ppm solution is spread over the water surface. For details of application see Kabata, 1985). For treatment of aquaria fishes it is recommended to apply high-concentration $KMnO_4$ directly to female parasites with a brush.

Formalin can kill only larval stages of the parasite. The application is prolonged and time consuming. Concentrations of 250 ppm were used for 30–50 min baths.

Trichlorfon usage has been the most successful treatment in combating *Lernaea* infections, but at the same time criticised for its alleged action on the environment.

In Arkansas, USA, the most commonly used chemical for treating *Lernaea* infections in non-food fish has been diflubenzuron (Dimilin) (Piasecki *et al.*, 2004).

A selection of alternative, biological methods for treatment of this disease has been tested *in vitro* or traditionally have been applied in practise by fish growers. Kasahara (1962) observed that a free-living copepod *Mesocyclops* preys on free-swimming larvae of *Lernaea*.

*Lernaea* larvae are sensitive to abrupt environmental changes. There have been successful attempts in China to apply pig manure to fishponds with fish infected with *Lernaea*. A large proportion of *Lernaea* were killed, but this phenomenon should be further studied (Kabata, 1985).

Some plant-derived substances have been used for *Lernaea* treatment as well: teased cake or meal, bundles of *Gelsenium elegans* or *Acanthopanax spinosus*, with a potential of total eradication of the parasite (Kabata, 1985).

Tamuli and Shanbhogue (1995) described a promising *Lernaea*-treatment method, using tilapia.

**Epidemiology/geographical distribution:** Copepods of the genus *Lernaea* are ubiquitous parasites of freshwater fishes, occurring worldwide in temperate-, subtropical-, and tropical climate zones. Historically, *Lernaea* has not occurred in Australia, but it was unwillingly introduced to that continent with its host fishes. The optimum temperature for its development is above 20°C and higher temperatures can increase the pace of its development and shorten its generation time. According to Schäperclaus (1992) in some areas of the United States, no fewer than 10 generations can appear in the course of a year. The copepod intensities reached 40 parasites per fish. An interesting account of site selection by adult *L. cyprinacea* was published by Dorovskikh (1996). In recent years,

the numbers of *Lernaea* have drastically declined in Central Europe. In Poland, there has been no published record of *Lernaea* within the last 30 years, although more than 280 papers dealing with fish parasites were published during that period (Piasecki and Woliński, unpubl. data). Some authors speculate that lernaeid copepods are sensitive to water pollution (Avenant-Oldewage, 2003) and, thus, they cannot survive in the polluted waters of Europe.

**Economical importance; outlook:** The history of fish mortalities caused by *Lernaea* spp. dates back to 1880, when in one of the lakes of the Masurian Lake District (presently in Poland), lernaeosis almost wiped out an entire population of the crucian carp (Benecke, cited by Kocyłowski and Miączyński, 1960). According to Kocyłowski and Miączyński (1960) a mass mortality of crucian carp was also reported by Kozikowska in Karasiowe Lake, Poland and by Grabda in Wilczak Hatchery, Poland.

*Lernaea* also causes health problems for small baitfish and ornamental fish species. Those are mainly small fish below 5 cm in length and this type of business accounts for millions of dollars worldwide. In the U.S.A., *Lernaea* infections are common and a single copepod female is capable of killing the fish, especially when attached at some critical location. In ornamental aquarium fishes and koi the mortality threat is not so distinct, but the presence of external parasites can disfigure the fish lowering its market value or making it unsuitable for sale altogether (Piasecki *et al.*, 2004).

**Recent developments:** A number of new species of the genus *Lernaea* has been described in recent decades. The most recent one was *Lernaea multiobosa* described by Jafri and Mahar (2003). New treatment methods have been sought, including a resin of a pine tree (Tóro *et al.*, 2003). Lernaeid copepods have been used as indicators of environmental pollution (Avenant-Oldewage, 2003).

**Other related species:** As mentioned earlier, there are probably fewer than 50 valid species of the genus *Lernaea*. They are all quite similar in morphology, attachment strategy, pathogenesis and treatment. A taxonomic revision, supported by molecular methods, is needed to determine true identity of some species.

The genus *Lamproglena* is the second best known fish-parasite genus of the order Cyclopoida with some valid 37 species (Piasecki, 1993a). The attachment mode is, however, totally different, resembling that of the family Ergasilidae. Species of *Lamproglena* are gill dwellers with little

information available on their pathogenicity. Recently, however, Tsotetsi *et al.*, (2005) reported epithelial hyperplasia, resulting in fusion of gill lamellae and necrosis of the host tissue in the vicinity of the parasite's attachment site. They found no correlation between the infectation intensity and fish haematocrit values, altough they indicatd that the parasite feed on host tissue and blood.

**Species representing family Ergasilidae**

**Important pathogens:** *Ergasilus sieboldi* von Nordmann, 1832

**Disease:** Ergasilosis

**Pathogen overview:** The family Ergasilidae comprises 24 valid genera (Amado *et al.*, 1995) with some 280 nominal species (Anonymous, 2007). The overwhelming majority of species occurs in freshwater environments. The morphology of ergasilids largely resembles that of free-living cyclopoids (Figs. 18.2, 18.50), but some may be extensively transformed, mesoparasites, e.g., *Mugilicola* (cf. Piasecki *et al.*, 1991). The best known ergasilids are representatives of the genus *Ergasilus*, which contains 168 nominal species (Anonymous, 2007). Kabata (1985) estimated the number of valid species to be 80, but 50 new nomial species of this genus have been described ever since (Anonymous, 2007). The best known species is *Ergasilus sieboldi* (Fig. 18.2), which is 1.7 mm long and attaches to gill filaments of fishes using its 2nd antennae. The antennae 'hug' the gill filament tightly (Fig. 18.3), contributing to at times serious pathological effects.

The parasite feeds on gill epithelial cells (rarely on mucous cells) and to a high extent on blood (Einszporn, 1965a, b). This particular species attaches to the outside of the gill, allowing some of its congeners to explore the space between the gill filaments. In cases of extremely heavy infections of whitefish, the parasite attaches not only to gill filaments but also to the fins (Kozikowska, 1975). Molnár and Székely (1997) noticed that in small zander, *Sander lucioperca*, in Lake Balaton, *Ergasilus* usually attaches itself to the folds on the external surface of the operculum and on the base of the pectoral fins, and only a small number of those copepods cling to the gill filaments.

**Morphology:** Only female *Ergasilus* are found parasitic on fish. Their overall shape is similar to free-living cyclopoid copepods. Their total length may reach 1.7 mm. Their body segmentation is well pronounced and the morphology of the appendages is quite similar to that of free-living cyclopids. Their principal adaptation to a parasitic way of life are their

strongly enlarged and elongate second maxillae, resembling long arms, tipped with a big, only slightly bent claw. Those transformed "arms" are used for the attachment to the host. The egg sacs are paired, multiseriate and they contain up to 100 eggs each.

**Life cycle:** All larval stages are free-swimming and are often found in lacustrine plankton. Males are not parasitic and they die soon after copulation. A fertilized female settles on the host and produces eggs. The eggs are deposited into two egg sacs and they hatch after 10–12 days at 16°C or 6 days at 20°C (Bauer *et al.*, 1969). According to Abdelhalim *et al.* (1991), there are as many as 6 nauplius- and 5 copepodids stages in the life cycle of this copepod. In Central Europe, the first spring generation of *E. sieboldi* becomes sexually mature in mid-June. Their eggs hatch and the copepodids attack fish. They mature and produce a second generation in September. Sometimes, a third generation follows by the end of the season.

**Disease symptoms:** Although the host specificity of *E. sieboldi* is very wide, the most extensively infected are tench (*Tinca tinca*), apparently because of its sluggishness. The gills of fish are often covered with hundreds of adult females of *E. sieboldi*. Intensively infected fish are visibly weakened, may show signs of respiratory deficiency (faster pace of operculum, gulping for air) and even equilibrium disturbances (Prost, 1980). In summer, the disease may result in mass mortalities.

**Disease diagnostics:** The copepods, although very small and almost transparent are well visible on fish gills, especially in high numbers, because their digestive gland is darkly pigmented. Copepod females often carry two egg sacs, which, while pigmented are also well visible. The gills of infected fish become pale. Hypertrophied respiratory folds (secondary lamellae) and even entire neighbouring gill filaments fuse together. The parasites hold gill filaments tightly with their transformed second antennae. *E. sieboldi* should be differentiated from *E. briani*, similarly infecting a wide selection of freshwater host fishes. *E. briani*, reaching only 1 mm in length is much smaller that *E. sieboldi*, exceeding 1.5 mm. Moreover, *E. sieboldi* attaches to the outside of gill filaments while *E. briani* prefers the space between the two rows of the gill filaments. The third common European ergasilid *E. gibbus* is specific to eel, *Anguilla anguilla* and can be found on its gills. Ergasilids are also reported from North-American fish (Figs. 18.50–18.51) (Andrew Goodwin – pers. comm.).

**Etiology/pathogenesis:** The second antennae of *Ergasilus* sp., transformed into powerful hooks, hold the gill filaments tightly and can

cause tissue damage and obstruct blood flow (Fig. 18.3). Parasites feeding on the epithelial cells (Einszporn, 1965a, b) stimulate hypertrophy and consequently a coalescence of secondary gill lamellae (Kabata, 1970). This in turn, drastically reduces the surface available for gas exchange. Lesions on the gills are often affected by secondary pathogens such as bacteria and fungi. Fish mortalities may coincide with oxygen deficiencies, especially in summer. The blood picture of infected fish is also affected. Einszporn-Orecka (1973a, b) studying infected and non-infected tench observed that high intensity infection with *E. sieboldi* resulted in a decrease in the number of erythrocytes, a decrease in the levels of haemoglobin and haematocrit, and an increase in erythroblasts. The lymphocyte count increased, but many white blood cells showed signs of deterioration.

**Disease treatment and prophylaxis:** In pond culture, the water supply should be free from larval stages of *E. sieboldi* and the fish to be stocked should be examined for parasites. Reservoir hosts should be eliminated and spawners should be treated for *Ergasilus* by dusting with DDT or a similar substance (Kabata, 1985). A mixture of copper sulphate ($CuSO_4$) and ferric sulphate ($Fe_2SO_4$) is recommended as well as all other chemical substances endorsed for fighting *Lernaea*. It has been observed that a well-developed aquatic vegetation helps reduce infection levels. It apparently limits the movements of small bream that otherwise spread this parasite all over the lake (Schäperclaus, 1992).

**Epidemiology/geographical distribution:** One female of *Ergasilus sieboldi* can produce 200 offspring. Theoretically, the ensuing second generation can comprise 40,000 descendants and in the third, as many as 8 million (Schäperclaus, 1992). *Ergasilus sieboldi* is not host-specific and can infect a majority of freshwater fishes; however the tench, *Tinca tinca*, appears to be the most susceptible. This fact is attributed to the sluggishness of this fish, which may make it more vulnerable to copepod attack. Other less-infected fishes include pike (*Esox lucius*), bream (*Abramis brama*), whitefish (*Coregonus lavaretus*), vendace (*Coregonus albula*), carp (*Cyprinus carpio*), and roach (*Rutilus rutilus*) (reviewed in Piasecki *et al.*, 2004).

Schäperclaus (1954) described a case of a single, 36-cm-long tench that harboured some 3600 specimens of *E. sieboldi* on its gills. This heavily infected fish had a condition factor of only 0.88. Similarly, intensive infection of peled (*Coregonus peled*) was reported by Abrosov and Bauer

(1959, 1961) from Pskov Lake, Russia. Heinemann (1934) found 5431 specimens of *E. sieboldi* on a single tench that died of asphyxia. Severe infection with *E. sieboldi* can result in heavy losses in the yield of tench (reviewed in Piasecki *et al.*, 2004).

The second most common host of *E. sieboldi* is the pike. Pike deaths attributed to *E. sieboldi* infections were described from two Pomeranian lakes in Germany by Lehmann (1924) and Rumphorst (1924). Deaths of whitefish (*Coregonus wartmanni*) in Zugersee, Germany were described by Baumann (1913). A very extensive account on aspects of *E. sieboldi* occurrence in different hosts was published by Grabda (1963b). *Ergasilus sieboldi* causes mass fish kills not only of tench but also of perch, *Perca fluviatilis* in Włodawa Lake, Poland (Kocyłowski, 1954), whitefish in Cugersk Lake in the U.S.S.R., and bream in a number of lakes (Markevič, 1956).

**Economical importance:** *Ergasilus sieboldi* is the most important to its principal host, the tench, *Tinca tinca*. In heavy infections the fish become emaciated, loose weight and become more susceptible to infectious diseases. The inferior health state of the fish directly translates into economic losses of fishermen. For example, in Lake Scharmuzel, Germany, the yield of tench dropped from 5000 kg before the appearance of *E. sieboldi* to 350 kg after its unwanted introduction. In two other small German lakes, the yield of tench dropped from 31–47 to 16.5 kg · $ha^{-1}$ after the invasion of this parasite and with copepod prevalence of only 50%. In Lake Grimiz, Germany, the yield of tench between 1926 and 1931 declined from 4583 to 111 kg (Schäperclaus, 1992), again a reduction apparently related to *Ergasilus* infections (reviewed in Piasecki *et al.*, 2004).

**Recent developments:** Immune genes associated with the response to infection of grass carp, *Ctenopharyngodon idella* with *Sinergasilus major* have been identified (Chang *et al.*, 2005).

At least 26 new species of ergasilid copepods have been described within 2000–2005 (Anonymous, 2007).

**Other related species:** The genus *Ergasilus* comprises over 180 species (Boxshall and Halsey, 2004). In Europe, in addition to *E. sieboldi* also *E. briani* Markevich, 1933 parasitizes a wide variety of fish hosts (although it is much smaller and less frequent). *Ergasilus gibbus* von Nordmann, 1832 is specific to its host, European eel, *Anguilla anguilla*. The fish introduced to Europe from Asia also have their ergasilid parasites. *Sinergasilus lieni* Yin, 1949 infects gills of silver carp, *Hypophthalmichthys molitrix* and rarely

bighead carp, *Aristichthys nobilis*. *Sinergasilus major* infects gills of grass carp, *Ctenopharyngodon idella*. *Thersitina gasterostei* (Pagenstechev, 1861) can be found on the operculum of perch or stickleback (Prost, 1980).

In Southeast Asia, *Ergasilus* sp. occurs on the gills of cultured fishes including *Osteophilus hasselti*, *O. gouramy* (in Indonesia), *Ctenopharyngodon idella* (in Malaysia), and on *Oxyelotris marmoratus* (in Thailand). The parasite has the potential to adversely affect aquaculture in this region, but so far, no major outbreak attributed to *Ergasilus* sp. has been reported (Kabata, 1985). Another ergasilid, *Sinergasilus major* (Markevich, 1940), may have importance in Central Asian aquaculture (culture of *Ctenopharyngodon idella*), including the former Soviet republics and China (Bauer and Babaev, 1964). *Sinergasilus lieni* is another important parasite, affecting *Hypophthalmichthys molitrix* (cf., Musselius, 1973). In Japan, Nakajima and Egusa (1973) found *Pseudoergasilus zacconis* Yamaguti, 1936 on gills of cultured ayu, *Plecoglossus altivelis*. In spite of this record, Egusa (1992) saw no serious threat by ergasilids to Japanese freshwater aquaculture.

*Ergasilus lizae* Krøyev, 1863 caused mortalities in grey mullet, *Mugil cephalus*, cultured in brackish water ponds in Israel (reviewed in Paperna, 1975). Lin and Ho (1998) reported outbreaks of disease in brackish-water aquaculture operations in Taiwan, caused by *Ergasilus lobus* Lin et Ho, 1998 infecting Malabar reef cod (*Epinephelus malabaricus*) and *Diergasilus kasaharai* Do, 1981, attacking milkfish (*Chanos chanos*) and Borneo mullet (*Liza macrolepis*). Pathogenic effects of *Ergasilus labracis* Krøyev, 1863, on Atlantic salmon in New Brunswick, Canada, were described by Hogans (1989). Recently, intensive infections of ergasilid copepods have been reported from catfish cultured in Arkansas (Fig. 18.51) (Andrew Goodwin, pers. comm.).

**Genera *Salmincola* and *Achtheres*; gill maggots**

**Important pathogens:** *Salmincola californiensis* (Dana, 1852)

**Disease:** Gill-maggot infection

**Pathogen overview:** Gill-dwelling copepods representing the genus *Salmincola* are probably the most characteristic gill parasites (also fin parasites) of salmonid fishes in Eurasia and North America. The genus *Salmincola* consists of at least 16 valid species (Kabata, 1969b). The vernacular name for *Salmincola* spp. (and other gill lernaeopodids) is the gill maggot. Gill maggots may occur at high intensities and create serious problems for fish kept at high densities in cages and other aquaculture facilities (Kabata, 1970; Vaughan and Coble, 1975; Kabata and Cousens,

1977; Sutherland and Wittrock, 1985). In natural fish populations, the prevalence and intensity of infection are usually low and have little impact on fish (Black, 1982; Black *et al.*, 1983; Bowen and Stedman, 1990; Amundsen *et al.*, 1997).

**Morphology:** *Salmincola* is a typical lernaeopodid copepod with distinct sexual dimorphism. Female attachment strategy involves usage of transformed, arm-like second maxillae and bulla. The bulla attaches the parasite to its host. *Salmincola* have relatively long second maxillae and a relatively short cephalothorax tipped with cephalic appendages. The overall length of a female is between 3 and 5 mm (plus egg sacs). The egg sacs are multiseriate.

**Life cycle:** Kabata and Cousens (1973) described the life cycle of the gill maggot, *S. californiensis*. It consists of 6 stages: the copepodid (free-swimming infective stage), 4 chalimus stages (which attach to a host by the frontal filament), and adults (the adult female attaches to a host by a bulla, while the male remains mobile).

**Disease symptoms:** No particular changes in fish behaviour are associated with moderate level infections of *S. californiensis* and other freshwater lernaeopodids. Females of this copepod attached to gill arches are well visible on the bottom of the mouth cavity (branchial cavity). In heavy infections, fish with extensive hyperplasia of the epithelium may exhibit symptoms of respiratory deficiency. Hypertrophied gill filaments, as well as nerotic erosion of some filaments can be easily distinguished without a microscope.

**Disease diagnostics:** Representatives of the family Lernaeopodidae are very characteristic, because of the mode of attachment with bulla and the transformed, elongate, arm-like second maxillae. Gills of freshwater fishes can be infected only by representatives of three genera: *Salmincola*, *Achtheres*, and *Basanistes*. They attach in the mouth cavity, gill chamber (Figs. 18.52, 18.53), mostly on gill arches and rarely on gill filaments. Females of all three genera have a similar overall body structure and similar size of 3–5 mm. They have a pyriform or oval genital trunk, elongate 'arms' and a relatively short cephalothorax. Females of the genus

**PLATE 8** Figs. 18.51–18.56. Copepoda parasitic on fishes. Fig. 18.51. Numerous females of *Ergasilus* sp. on the gills of catfish. Fig. 18.52. Ovigerous female of *Achtheres* sp. from smallmouth bass; dorsal. Fig. 18.53. Numerous ovigerous females of *Achtheres* sp. in the branchial cavity of stripped bass. Fig. 18.54. Adult female of *Tracheliastes maculatus* attached to a scale. Note the scale perforation caused by the parasite. Fig. 18.55. Numerous adult, ovigerous females of *Tracheliastes maculatus* and the haemorhagic lesions inflicted by them, on the skin of common bream. Fig. 18.56. *Ommatokoita elongata* attached to the eye of Greenland shark. Source: Andrew Goodwin

*Plate 8 Contd.*

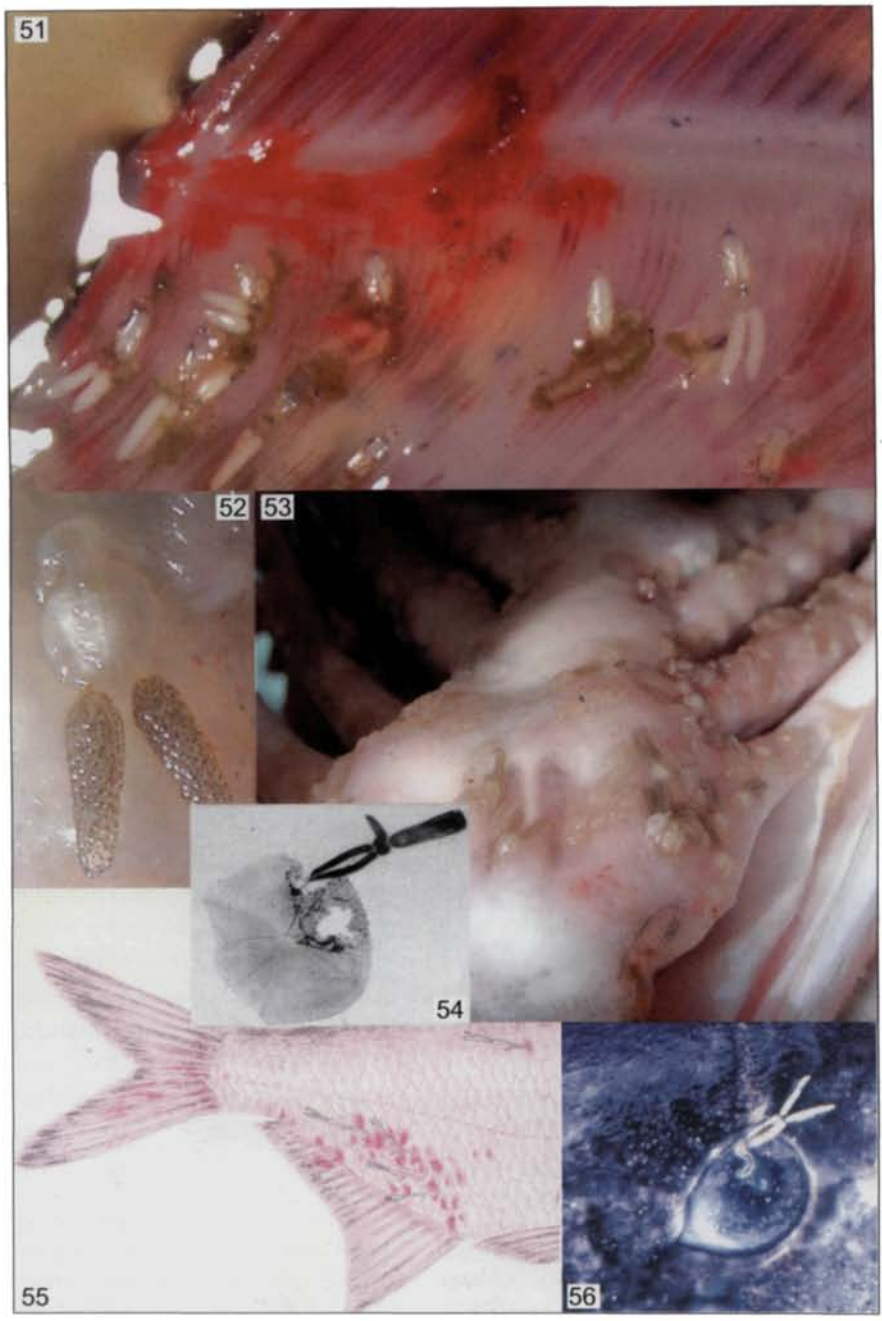


*Plate 8 Contd.*

(Fig. 18.51); Jim Negus (Figs. 18.52, 18.53); Krystyn Wierzbicki (Fig. 18.54); Jadwiga Wierzbicka (Fig. 18.55); George Benz (Fig. 18.56).

*Achtheres* differ from those of *Salmincola* in having a prominent genital process in the posterior part of their genital trunk (Fig. 18.16), between the egg sacs. Females of *Basanistes* have a number of distinct tubercles on their genital trunk (either lateral and/or dorsal). Identification of *Salmincola* species can be aided by Kabata (1969b, 1988).

**Etiology/pathogenesis:** Infections were associated with hyperplasia of gill filaments caused by female copepods and atrophy or growth inhibition of affected gill filaments. Sutherland and Wittrock (1985) have also noticed a harmful effect of chalimus larvae, such as hyperplasia and sometimes fusion of the basal elements of adjacent gill filaments. Gall *et al.* (1972) found evidence of the influence of *S. californiensis* on the reproductive performance of a domesticated strain of rainbow trout.

In zander, *Sander lucioperca*, infected with a related gill-maggot species *Achtheres sandrae* entire sections of the gills filaments were missing, below the point of attachment of those parasites (Kozikowska *et al.*, 1956).

**Disease treatment and prophylaxis:** Roberts *et al.* (2004) compared oral ivermectin treatment of rainbow trout heavily infected with *S. californiensis* with manual removal of the gill maggots with forceps. The former treatment proved to be equally effective and it produced fewer side effects (pathologic changes).

**Epidemiology/geographical distribution:** *S. californiensis* was originally confined to salmonid fishes of the genus *Oncorhynchus* in streams emptying into the northern Pacific Ocean, but has recently moved eastwards in the US, e.g., to Missouri and Arkansas (Hoffman, 1984) and subsequently to Iowa, New Jersey, and West Virginia (Sutherland and Wittrock, 1985). Sutherland and Wittrock (1985) reported the parasite from an aquaculture site in Iowa, stating a prevalence of 83% and a mean intensity of 4.6 female copepods. Johnson and Heindel (2001) reported problems with high numbers of *S. californiensis* in broodfish of Chinook salmon, *O. tshawytscha*, and they described a method for their manual removal.

**Economical importance:** No comprehensive reviews available.

**Recent developments:** Two new species of the genus *Salmincola* were described in Russia (Shed'ko, 2004, 2006).

**Other related species:** Another significant species from the same genus is *Salmincola edwardsii* (Olsson, 1869), a parasite specific to Arctic charr, *Salvelinus alpinus*, and brook trout, *Salvelinus fontinalis*. This parasite inflicted health problems in fishes cultured in Quebec and New Brunswick, Canada (Parissa Irani-Bunin, pers. comm.). *Salmincola salmoneus* (L.) is specific for Atlantic salmon, *Salmo salar*. Heavy infections with this parasite of salmon broodstock kept in hatcheries in the Maritime

Provinces of Canada were reported by McGladdery and Johnston (1988). *Salmincola carpionis* (Krøyev, 1837) was reported from central Japan infecting cultured brook trout and whitespotted charr, *Salvelinus leucomaenis* (cf. Nagasawa *et al.*, 1997, 1998). *Salmincola stellatus* Markevich, 1936 were recently reported in Hokkaido, Japan in the buccal cavity of cultured taimen, *Hucho perryi*, with intensities ranging from 10 to 50 individuals per fish (Nagasawa *et al.*, 1994) (reviewed in Piasecki *et al.*, 2004).

The closest relatives of *Salmincola* spp. are representatives of the genus *Achtheres*. The latter genus differs from the former one in having a prominent genital process. The genus *Achtheres* needs a comprehensive revision before the number of valid species is determined. Kabata (1969b) postulated that there were 6 valid species of this genus. In his more recent work Kabata (1988) suggested that there are only three valid species. Recently, Kempter *et al.* (2006) and Piasecki *et al.* (2006) proved the separate identity of *Achtheres* parasitizing European perch and zander, which were earlier indicated by Kozikowska *et al.* (1956). *A. percarum* von Nordmann, 1832 occurs on perch (*Perca fluviatilis*). *A. sandrae* Gadd, 1901 parasitizes zander (*Sander lucioperca*) and can be pathogenic to it. Because of its attachment to gill filaments, it can trigger epithelial hyperplasia, which fuses gill lamellae, and cause partial or total loss of gill filaments. The prevalence often reaches 100%, and the number of *Achtheres* females can amount to 40 per fish (Kozikowska *et al.*, 1956). The highest observed number of all stages (including the larvae) of the parasite in zander was 117 (Piasecki, 1993b) and 81 individuals in perch (Piasecki and Wołoszyn, 1991). The life cycle of *A. percarum* includes 7 stages: nauplius, copepodid, 4 chalimus stages, and adults (Piasecki and Kuźmińska, 2007) (reviewed in Piasecki *et al.*, 2004).

*Basanistes huhonis* (Sohrank, 1786) is another 'cousin' of *Salmincola*. It looks similar to *Salmincola* spp. but is covered with 12 prominent humps or outgrowths. It infects Danube salmon, *Hucho hucho*, and broodstock may host very high numbers of this parasite (Ivaška, 1951; Witkowski and Błachuta, 1980). Three other *Basanistes* species were described by Markevič (1956) from fishes of the USSR.

### *Tracheliastes maculatus* Kollar, 1835

The least known freshwater lernaeopodid genus is *Tracheliastes*, and the most-pathogenic species within this genus is *T. maculatus*. *Tracheliastes* parasites are often confused with *Lernaea*, despite their attachment through bulla and second maxillae and not anchoring cephalic outgrowths. This copepod has a very slim appearance, it is slightly longer

than *Lernaea*, and its bulla attaches permanently to the scales of common bream, *Abramis brama* (Figs. 18.54, 18.55). Its presence is initially associated with focal inflammation around its feeding area, with pronounced congestion of the skin and extravasations of blood (Fig. 18.55). With heavy infections, diffuse inflammation of the skin can be observed and even extensive perforation of the scales (Fig. 18.54) to which the parasites' bullae are attached. There are two documented cases of extreme infection levels of this parasite on bream. The first was in Jamno Lake, Poland and was associated with fish mortalities. The intensity reached 56 individuals per fish (Grabda and Grabda, 1958). The other mass infection occurred in Pierzchały Lake, Poland with an intensity of 61 individuals per fish (Piasecki, 1991). The life cycle of *T. maculatus* consists of 7 stages: pre-moult nauplius, infective copepodid, 4 chalimus stages, and adults (Fig. 18.42). The filament is 'inherited' by all subsequent chalimus stages. They must reattach to the proximal part of the filament after each moult. (Piasecki, 1989) (reviewed in Piasecki *et al.*, 2004). Another species, *T. polycolpus* von Nordmann, 1832, attaches to fins of a number of small cyprinid fishes, in Europe without apparent signs of pathology.

## Marine Lernaeopodids

The genus *Clavella* consists of, at least 93 nominal species. One of the better-known species is *Clavella adunca* (Strøm, 1762) (=*Clavella uncinata*). This copepod is very similar to freshwater *Achtheres* in its size and overall shape. The principal difference between *Clavella* and *Achtheres* is in the relative proportions of the second maxillae and the cephalothorax. In *Clavella*, the cephalothorax is extremely long and it looks like an elephant's trunk, while the second maxillae are extremely short and fused with the opposite appendage. *C. adunca* (some 6 mm long) commonly infects gadid fishes in the north Atlantic and the adjacent seas. It attaches to gill filaments, mouth, fins, and perianal area. "Dwarf" males can be found attached to females.

*Clavella perfida* Wilson, 1915 (Fig. 18.18) is a specific parasite of the Alaska pollock, *Theragra chalcogramma*. In the 1970s about 30% of pollock in the Bering Sea were infected with up to 30 specimens on the gills of a single fish (Grabda, 1977).

Over 645 nominal species of the family Lernaeopodidae have been assigned to 40 valid genera. The best known are: *Lernaeopoda, Clavellotis, Brachiella, Parabrachiella* (senior synonym of *Neobrachiella*; according to

Boxshall and Halsey, 2004), *Charopinus*, *Thysanote*, *Ommatokoita*, *Clavellistes*, *Clavellisa*, *Clavellopsis*, *Eubrachiella*, and *Clavellodes*.

*Eubrachiella antarctica* (Quidov, 1906) is the best-known parasitic copepod of many species of Antarctic fishes (Kock and Möller, 1977). *Ommatokoita elongata* (Grant, 1827) attaches to the eye of the Greenland shark (Fig. 18.56) (Benz *et al.*, 2002).

## Other Lesser-known Copepod Parasites

*Anthosoma crassum* Abildgaard, 1794 (family Dichelesthiidae) (Fig. 18.4) occurs on the skin and in the mouth cavity of sharks (*Lamna cornubica*, *Isurus oxyrhynchus*, *Selache maxima*, etc.). It penetrates the host tissue so deeply that only the egg sacs protrude outside from the 'pit'. The female is 8.5–15 mm long while the male is 7–10 mm. The body of *A. crassum* is covered with characteristic overlapping plates.

*Dichelesthium oblongum* Abildgaard, 1794 (family Dichelesthiidae) (Fig. 18.5) parasitizes in the gill cavity of acipenserid fishes in Eurasia and North America. The female is 17–27 mm; the male is 13 mm (Kabata, 1979).

*Cecrops latreilli* Leach, 1816 (family Cecropidae) is the largest representative of its family and (female 25–30 mm; male 14–17 mm) it attaches to the gills of sunfish, *Mola mola*. The copepod is very common occurring on its host all over the world.

*Pandarus bicolor* Leach, 1816 (family Pandaridae) (Fig. 18.14) is a common skin parasite of sharks in the Atlantic. The female reaches 8–10 mm, while the male some 6 mm. It is dorsoventrally flattened like representatives of Caligidae, but because of the rough sharkskin it developed special attachment pads.

*Chondracanthus lophii* Johnston, 1836 (family Chondracanthidae) (Figs. 18.6, 18.7) is characterised by obliterated segmentation and many irregular processes on their body. Dwarf males are attached to the female's body (Fig. 18.6). A female has its head embedded into soft tissues of the mouth cavity of host (*Lophius piscatorius*).

*Chondracanthus goldsmidi* Tang, Andrews et Cobcroft, 2007 was recently described from cultured striped trumpeter, *Latris lineata*, cultured in Tasmania. This was the first record of a chondracanthid copepod to infect cultured finfish (Tang *et al.*, 2007)

Copepods of the family Bomolochidae, are small (some 2 mm) parasites, dwelling in the nasal cavities of marine fishes (genera: *Bomolochus* (Fig. 18.1), *Holobomolochus*, *Naricolax*, etc.) without any

apparent pathology. *Naricolax chrysophryenus* Hutson et Tang 2007 was recently found on farmed yellowtail kingfish, *Seriola lalandi*, in southern and eastern Australian waters (Hutson and Tang, 2007).

Copepods can be also harmful to chondrichthyans aspecially those held in captivite in marine aquaria (Benz and Bullard, 2004). Infection levels of wild sharks with copepods can be very high. Benz and Dupre (1987) estimated that a single wild-caught blue shark, *Prionace glauca* harboured over 1200 hematophagous *Kroyeria carchariaeglauci* on its gills.

## BRANCHIURA

The first report on the Branchiura is probably in the *Wu lei hsiang chih* (Account of the mutual influence of things), a Chinese work by the monk (Kao) Tsan-ning (918–999), who wrote that if goldfish eat the wastes from olives or soapy water, they will die but when they eat the bark *of poplar trees they will not breed lice* (Fryer, 1982).

The Branchiura is a small group of ectoparasites, comprising a single family, the Argulidae, with only four genera, i.e., *Argulus*, *Dolops*, *Chonopeltis*, and *Dipteropeltis*. There are approximately 210 species (Møller *et al.*, 2007). They are all parasites of fishes but on rare occasion have been reported from alligators (Ringuelet, 1943), tadpoles (Stuhlmann, 1891; Wolfe *et al.*, 2001), and salamanders (Carvalho, 1939; Poly, 2003). Overstreet *et al.* (1992) considered *Dipteropeltis* as belonging to a separate family the Dipteropeltidae but this opinion has not received wide recognition. *Dipteropeltis* was described in 1912 (Calman, 1912) and since its description, a limited number of reports appeared sporadically. Information on this genus is therefore very limited. The adults range between 5 and 15 mm in size although *Dipteropeltis* may reach up to 30 mm. Live branchiuran specimens are transparent to milky white and *Dolops* contains melanin giving it a brown to green appearance whereas ethanol or formalin fixed specimens are grey. The eyes are dark brown to black and their presence facilitates finding of specimens on the host.

Branchiurans are commonly known as fish lice and are characterized by their dorso-ventrally flattened bodies, which are covered by a horseshoe-shaped dorsal cephalic shield formed by the carapace lobes. Its posterolateral lobes extend over the four pairs of thoracic biramous legs to a greater or lesser extend depending on the species (Figs. 18.57–18.63). Five pairs of cephalic appendages occur ventrally, i.e., the antennulae (absent or reduced in *Chonopeltis*), antennae, mandibles, maxillulae, and maxillae. The antennulae bear a small hook terminally. The maxillulae are

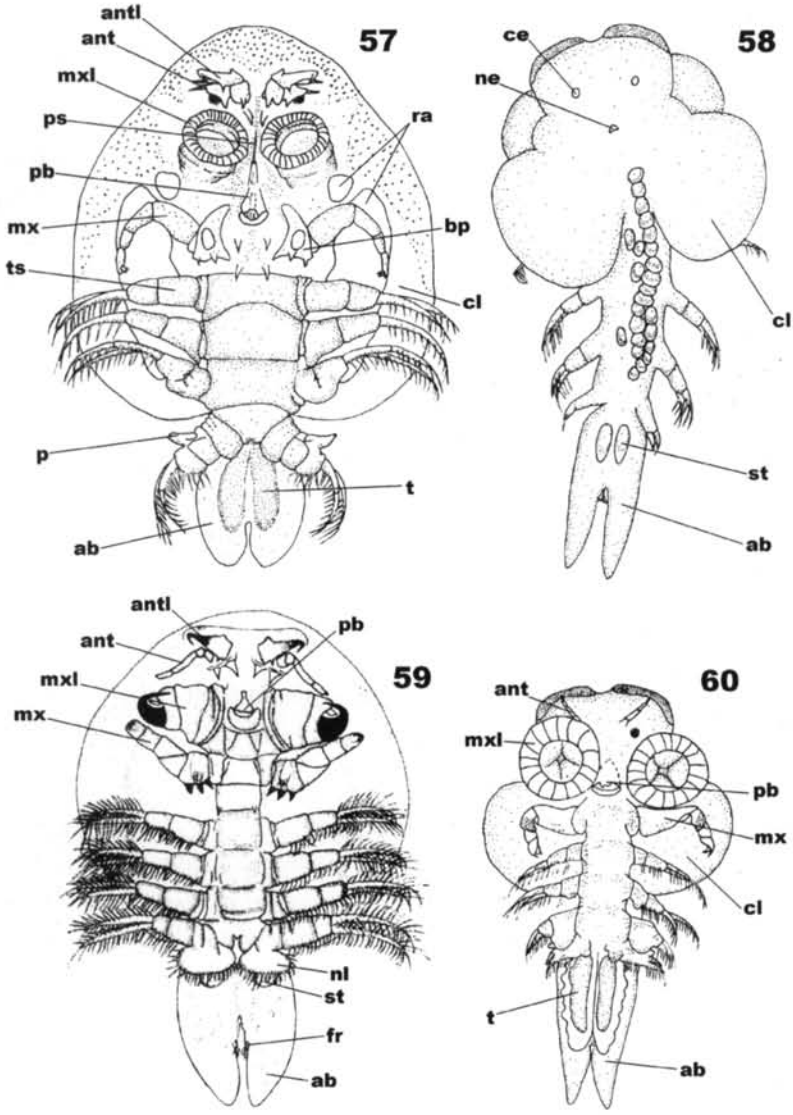


**PLATE 9** Figs. 18.57–18.60. Schematic representations of branchiurans. Fig. 18.57. *Argulus japonicus*; male; ventral. Fig. 18.58. *Chonopeltis australis*; female; dorsal. Fig. 18.59. *Dolops ranarum*; female, ventral. Fig. 18.60. *Chonopeltis australis*; male; ventral. Labels: ab, abdomen; ant, antenna; antl, antennule; bp, basal plate; ce, compound eye; cl, carapace lobe; fr, furcal ramus; mx, maxilla; mxl, maxillule; nl, natatory lobe; p, peg; pb, proboscis; ps, pre-oral spine; st, spermatheca; t, testis; tl, thoracic leg; ts, thoracic spine. Source: Avenant *et al.*, 1988.

transformed into powerful suckers in the adults and are utilized to attach to their hosts except in *Dolops*, where the maxillulae bear strong hooks that penetrate the host for attachment. The suckers bear supporting

sclerites on the brim. The suckers are situated on stalks and are provided with apparent musculature. Posterior to them are the uniramous maxillae. A small bilobed abdomen occurs; its shape is characteristic of the species and the shape differs slightly between the sexes in the same species. Sexual dimorphism is limited to the presence of testes or spermathecae, respectively, which are situated in the abdomen as well as the occurrence of accessory copulatory structures on the legs of males (Figs. 18.57, 18.62, 18.63, 18.66). Attachment to the host is facilitated by suckers or hooks on the maxillulae as well as the hooks on the antennulae. In genera with suckers they are used to 'walk' across the host by attaching and releasing the suckers alternately.

The sickle-shaped mandibles are housed in a proboscis (which is very short in *Dolops* and *Chonopeltis*). The mandibles are armed with rows of tiny denticles on their distal perimeter (Figs. 18.67, 18.69). *Argulus* has a retractable pre-oral spine also referred to as a sting, or 'giftstachel' (poison sting), which is situated midventrally between the suckers. Two hollow tubes in the terminal opening are connected via ducts to gland cells in the carapace lobes (Swanepoel and Avenant-Oldewage, 1992). A pre-oral spine is absent in *Chonopeltis* and *Dolops* and a pre-oral papilla has been reported in *Dipteropeltis* (cf. Calman, 1912). In *Argulus*, two labial spines are conspicuously positioned in the opening to the buccal cavity.

Scales, setae, and spines may occur on the ventral surface of the thorax, abdomen and appendages. In *Argulus* and *Dolops*, the most conspicuous spines are on the basal plates of the maxillae, the shape of these is species specific. Two pairs of thoracic spines are situated between the maxillae and the bases of the first legs. Additional scales and spines are organized into plates on the appendages and scattered across the cephalic lobes to create rough surfaces, which aids the parasites from slipping of the host surface (Fig. 18.66). Respiratory areas occur on the ventral surface of the carapace lobes in *Argulus*. These areas are devoid of scales or spines and possess a thin cuticle, which facilitates diffusion of oxygen into the haemocoel. Their shape is species characteristic.

Argulids have two, well developed, compound eyes and a median naupliar eye. Meyer-Rochow *et al.* (2001) studied the eyes and reported that, considering the complexity, the eyes should possess a considerable absolute sensitivity which would enable perception of moving objects at

**PLATE 10** Figs. 18.61–18.65. Branchiura. Fig. 18.61. *Chonopeltis australis*; female; ventral. Fig. 18.62. *Argulus japonicus*; male; ventral. Fig. 18.63. *Chonopeltis victori*; male; ventral. Fig. 18.64. *Dolops ranarum* on *Oreochromis mossambicus*. Fig. 18.65. *Argulus japonicus*; egg string. (Two eggs show larvae and the remainder empty eggshells after

*Plate 10 Contd.*

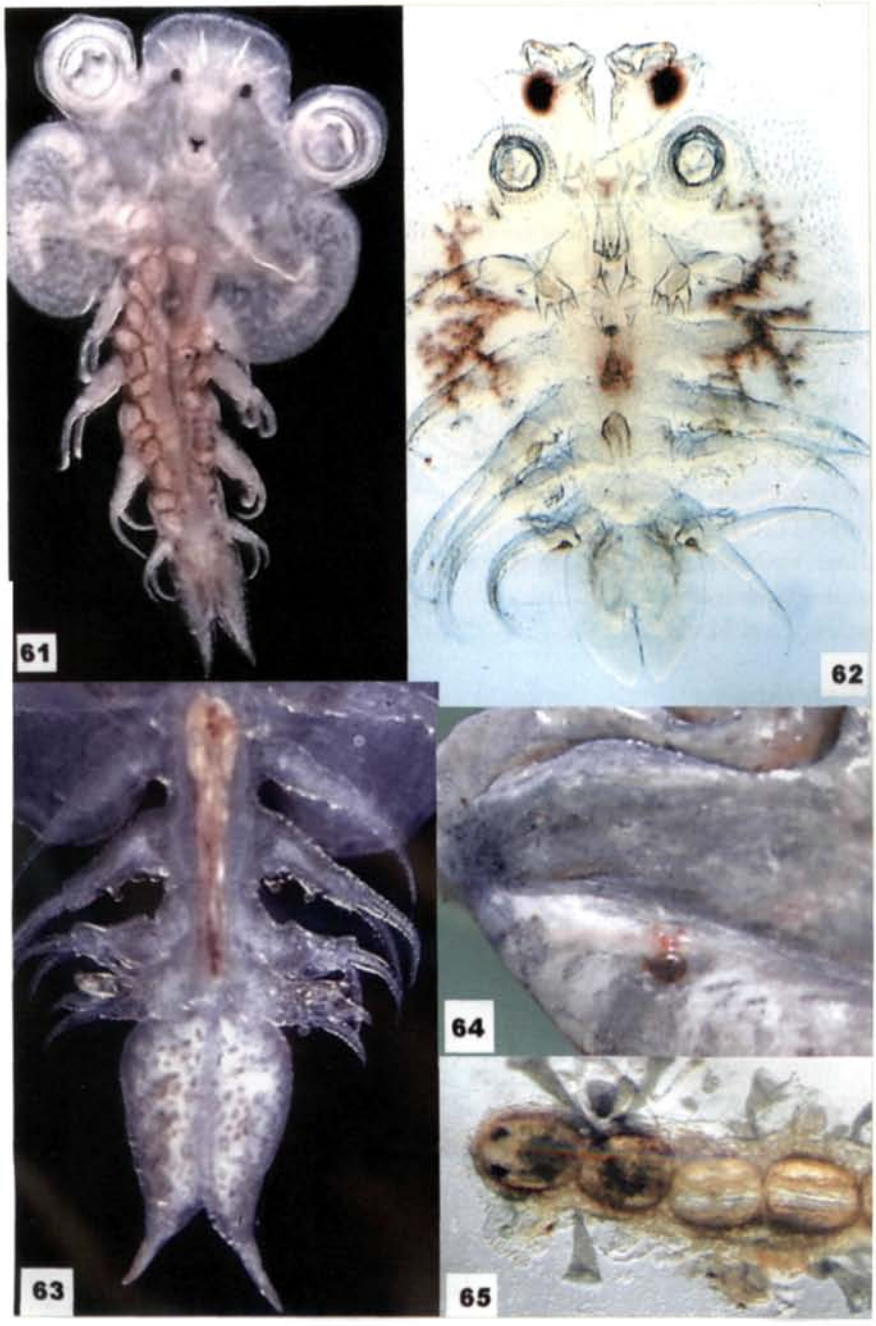


*Plate 10 Contd.*

hatching. Opportunistic sessile ciliates and rotiferans attached to the eggshells.) Source: Avenant-Oldewage, original (Figs. 18.61-18.63); J. Thevon (Fig. 18.64), Q. Tam (Fig. 18.65).

least 7–9° of arc and that the eyes have the capacity to distinguish the degree to which light is polarized. They arrived at the conclusion that this ability should aid argulids to detect a potential host against a background of flickering light from the water surface.

*Argulus* and *Dolops* are ardent swimmers and propel themselves in the water column utilizing the four pairs of thoracic legs. The legs are adorned with rows of setae that increase the contact surface with the surrounding water when the legs are moved. *Chonopeltis*, on the other hand, is a very feeble swimmer. All genera leave their hosts for oviposition and the copulation takes place on- or off the host.

## Systematics

Several reviews are available for identification of species. The most recent for *Chonopeltis* are Avenant-Oldewage and Knight (1994) and van As and van As (2001). *Argulus* species in the U.K. were reviewed by Fryer (1982), those in North America by Kabata (1988) and Poly (1997, 1998a, b), while those in Africa by Rushton-Mellor (1994a, b). *Dolops ranarum* was redescribed by Avenant *et al.* (1989a).

## Distribution

Branchiura have been reported from marine, fresh-, and brackish water, although the majority live in fresh water. A total of 11 species of *Dolops* have been described, of which nine occur in South America, one species in Tasmania, and a single species in Africa, respectively. The African species, *D. ranarum*, occurs from Egypt to South Africa, mainly on the silurids and cichlids (Avenant and van As, 1985).

*Argulus* has a cosmopolitan distribution with more than 140 species reported and their host preference varies from opportunistic, as in *Argulus japonicus* (cf., La Marre and Cochran, 1992; Shafir and Oldewage, 1992), to more restricted as in *Argulus coregoni* (cf., Pasternak *et al.*, 2004), depending on the species, but the genus, in general, shows low specificity (Walker *et al.*, 2004). The host selection depends on a combination of several factors such as host species available, density of the population, and environmental conditions such as availability of a vulnerable host species (Walker, 2002). The opportunistic nature of a species such as *Argulus japonicus* has hastened its spread through southern Africa (Kruger *et al.*, 1983; Avenant-Oldewage, 2001) after its importation. It was also introduced into the U.K. (Rushton-Mellor, 1992).

*Argulus coregoni* is a common pest on fish farms in the U.K. and is responsible for fish kills and also in changing the appearance and

catchability of fish, resulting in a reduction in the number of anglers, visiting areas where the epizootics occur (Taylor *et al.*, 2006). Parasites exhibit an aggregated distribution and it was shown that the trout that was previously exposed to *Argulus coregoni* did not develop protective resistance. Moreover, they were even more susceptible to reinfection than the naïve fish. Bandilla *et al.* (2005) showed that the naïve fish acquired infection at the same rate as previously infected fish, and that aggregation was not the result of immunosuppressive mechanisms exhibited by the parasite towards the fish. They came to the conclusion that the variance in intensity was related to differential exposure and that this was responsible for aggregation.

*Chonopeltis* is endemic to Africa and 14 species have been described, nine of which from southern Africa. Representatives of this genus exhibit narrow host specificity (Fryer, 1968; Paperna, 1996), occurring on cyprinids and silurids (Avenant-Oldewage and Knight, 1994).

*Dipteropeltis* was only reported from South America where its representatives occur on various piranha species (Characidae) as well as on siluriformes such as the tiger catfish (Carvalho *et al.*, 2004; Mamani *et al.*, 2004).

## Life Cycle and Biology

Specimens of *Argulus* and *Dolops* are parasitic throughout life, leaving their hosts only for oviposition. *Argulus* may survive for periods of up to 15 days without a host (Hoffman, 1977), and the author's (A.A.-O.) personal observations show that *Dolops* have the same ability, wheras *Chonopeltis* succumb after two to three days without a host. The sperm transfer in *Dolops* is aided by a spermatophore (Fryer, 1958, 1960) but a direct copulation was observed in the other genera. The elongate, motile sperm (Wingstrand, 1972) is stored in the spermathecae, which are not linked to the reproductive system and are situated in the abdomen (Avenant-Oldewage and Swanepoel, 1993). The eggs are cemented to the substrate by means of a sticky substance secreted with the eggs (Avenant *et al.*, 1989b). When branchiuran females leave the host to deposit eggs they may not be able to relocate a host resulting in a male-biased population on the host in *Argulus* (cf., Poulin and FitzGerald, 1989; Shafir and Oldewage, 1992; Halakati and Valtonen, 2003).

In *Argulus*, the eggs are deposited in untidy rows (Fig. 18.65) and the number of eggs varies in total (20–1200) as well as per batch (Fryer, 1968;

Hoffman, 1977; Shafir and van As, 1986; Boxshall, 2005b). In *A. japonicus*, the egg strings contain 5–266 eggs and each female lays one to nine strings (Shafir and van As, 1986). Sundara-Bai *et al.* (1988) showed in *Argulus sianemensis* var *sianemensis* and *Argulus sianemensis* var. *hessarghattaris* that the number of eggs, deposited by individual females, depends upon the size of the female involved. The eggs are deposited on hard structures in the water and hatch within a number of days, depending on the temperature. In *A. japonicus*, they hatched from 10 days at 35°C to 61 days at 10°C, whereas in *A. americanus*, the eggs hatched after 35–37 days at 15–16°C (Shimura and Asai, 1984) and *A. varians* in 25 days at 25°C (Bouchet, 1985). Harrison *et al.* (2006) showed that *A. foliaceus* deposited eggs closer to the surface (1 m) during summer but in deeper water (8.5 m) during the colder months. Hakalahti *et al.* (2004c) reported that most *A. coregoni* eggs were deposited in locations in shadow and in the deepest water in a 2-m-deep farming canal. Furthermore, more eggs were deposited on bottom stones situated near artificial egg-laying plates, indicating a preference for irregular stones.

New cohorts of larvae were detected throughout spring and summer but not in autumn and winter (Shafir and Oldewage, 1992) and overwintered eggs provide a source of infection in spring (Shafir and Oldewage, 1992; Mikheev *et al.*, 2001). Eggs hatch in an unsynchronized manner which is unrelated to their position in the egg string (Shafir and van As, 1986). Hakalahti *et al.* (2004a), as well as Fenton *et al.* (2006), indicated a flexible hatching dynamics among *A. coregoni* eggs and showed that a single egg clutch hatch throughout an extended period of seven months on average. Furthermore, it was shown that longer (two weeks) cold spells prompted hatching, which would deliver larvae to the system long after the parents have died. Host availability seems to drive the egg production and extended oviposition periods were reported in a lake where fewer hosts were available, compared to farm conditions (Pasternak *et al.*, 2000). Newly hatched larvae are free swimming and their cephalic appendages are not fully developed.

The life cycle of *Argulus foliaceus* was described by Rahman (1995) and Rushton-Mellor and Boxshall (1994), while that of *A. japonicus* by Stammer (1959). The antennula is prominent and bears a terminal hook. The antennae are biramous and in newly hatched larvae the exopod of the antennae and mandibular palps are used for propulsion (Gresty *et al.*, 1993; Lutsch and Avenant-Oldewage, 1995; Møller *et al.*, 2007). The maxillulae bear a terminal hook and the basal podomeres of this

appendage change gradually to suckers during the ontogeny. Only the first pair of thoracic appendages is fully developed, the remainder is rudimentary. In the second stage, the larva is propelled by the thoracic appendages, which are then fully developed (Møller *et al.*, 2007). In newly hatched larvae, the digestive system resembles that of adults and they are able to consume mucus soon after hatching (Tam and Avenant-Oldewage, 2006). The eyes are developed to a similar level of complexity than that of adults and it is possible that even the smallest larvae can locate their hosts by vision (Keshkinen and Meyer-Rochow, 2004). In adult and sub-adult individuals, the vision is complimented by olfaction (Mikheev *et al.*, 1998, 2004). According to Mikheev *et al.* (2000), the host searching strategy of *Argulus foliaceus* varies depending upon the light conditions and during daylight a hover and wait ambush is engaged as compared to a cruising strategy during the dark.

In *D. ranarum*, the males produce a spermatophore that is deposited onto the female spermathecal spines during copulation. Sperm enter into the spermathecae via the needle-shaped spermathecal spines (Avenant-Oldewage and Swanepoel, 1993). As the spermatophore casing is initially soft, it flows into the space between the natatory lobes and the thorax, thereby obstructing the genital opening of the female (Fryer, 1960). Therefore, ecdysis, effecting shedding of the empty spermatophore and exoskeleton occurs before the female deposits up to 566 eggs (Fryer, 1959). Each shelled egg is inseminated individually while it exits the body of the female when a spermathecal spine enters it and sperm is injected into it (Avenant *et al.*, 1990). The eggs hatch in 25–35 days at 24°C (Fryer, 1964) but at 20°C hatching is delayed to 57 days. Newly hatched larvae resemble juvenile adults (Avenant *et al.*, 1989) apart from the shape of the maxillulae and mandibles that change to the adult shape during larval ontogeny. The larvae swim actively propelled by their thoracic appendages, aided by the mandibular and maxillular palps, immediately after hatching.

*Chonopeltis* larvae bear resemblance to *Argulus* larvae. The maxillulae terminate in an elongate structure with two serrated parts that are driven into the host for attachment. During development the second segment enlarges continuously while retaining the serrated tips and eventually forms the sucker (Fryer, 1961; Van Niekerk and Kok, 1989; Van As and van As, 1996). The thoracic legs are reduced in size and the larvae are not able to swim. In later stages, the suckers are used for shuffling movements on the host. Host change has been reported by Fryer (1961), where

*C. brevis* larvae are present on *Amphilius* and *Garra* species but the adults are found on *Labeo* and *Barbus* species. Similarly, Van Niekerk and Kok (1989) reported *C. australis* larvae form *Labeobarbus aeneus* and the adults from *Labeo capensis*. It is not clear how the host change is facilitated, given the fact that representatives of this genus cannot swim and at most will be able to float to a different host.

## Pathology

*Argulus* is considered a pest in aquaculture conditions (Landsberg, 1989; Walker *et al.*, 2004; Taylor *et al.*, 2006) and it has also caused havoc in natural impoundments. An introduction of *A. foliaceus* into Logoa das Sete Cidades, Azores resulted in mass mortalities in the natural fish population (Menezes *et al.*, 1990) and Kruger *et al.* (1983) reported a similar scenario with up to 250 parasites per fish after the introduction of *A. japonicus* into Bloemhof Dam and Baberspan in South Africa. Some fish are more sensitive to infection and are stunned by a single puncture (Kroger and Guthrie, 1972). Mortalities are usually associated with hundreds of parasites per host and are probably the result of a breakdown of the epithelial integrity.

Infected fish are lethargic and in a weakened condition. *Argulus* feeding and attachment causes severe localized damage to the integument of their hosts (Paperna, 1996; Rahman, 1996). Their presence may also lead to suppression of the appetite with resulting anorexia and cessation of growth (Kabata, 1985) or at least ratarded growth (Singhal *et al.*, 1990). Behavioural changes in parasitized fish include erratic swimming and flashing (Yldz and Kumantas, 2002). The feeding sites are marked by haemorrhagic spots with damage to the epidermis (Fig. 18.68) or in some cases even the dermis and stratum spongiosum. In terminal cases the epidermis over the whole fish becomes thin and may be missing in some parts of the body and fins (Stammer, 1959). Furthermore, the parasitized fishes display a greater number of monocytes and special granulocytes in the peripheral blood than unparasitized fish (Tavares-Dias *et al.*, 1999). The plasma glucose concentration increases until after 10 days of infection, when the erythrocyte and leucocyte counts, haemoglobin concentration, heamatocrit value, plasma total protein, total cholesterol, and calcium concentration decrease and the number of immature erythrocytes increases in some infected fish (Shimura *et al.*, 1983). Thrombocyte numbers are also lower (Tavares-Dias *et al.*, 1999). It was

shown that the fish infected with *Argulus canadensis* tend to form larger, denser schools than uninfected schools (Poulin and FitzGerald, 1988; Northcott, 1997). Furthermore, Dugatkin *et al.* (1994) showed, under experimental conditions, that uninfected fish would avoid schools of conspecific, parasitized fish. The mechanism that induces this behavior in fish is, as yet, unclear.

Moreover, the parasite-inflicted wounds may become secondarily infected by bacteria and fungi such as *Saprolegnia* (cf., Singhal *et al.*, 1990). An *A. coregoni* infection in salmonids was followed by furunculosis caused by *Aeromonas salmonicida* (cf., Shimura *et al.*, 1983). *Argulus* was also reported to transmit viral infections such as *Rhabdovirus carpio* (cf., Pfeil-Putzien and Baath, 1978) and spring viraemia rhabdovirus (Pfeil-Putzien, 1977; Ahne, 1985). Rudomëtova (1974), Tihomirova (1983), Molnár and Moravec (1997), and Molnár and Székely (1998) reported the presence of larval Skrjabillanidae nematodes in *Argulus* and the transmission of *Molnaria erythrophthalmi* were found in 3 of 79 *Argulus foliaceus*, from various fish in the Elbe River basin in what was then Czechoslovakia (Moravec, 1978). Moravec *et al.* (1999) reported transmission of the daniconemrid nematode, *Mexiconema cichlasomae*, by an unidentified *Argulus* sp. in Mexico.

In a study conducted on the survival of rainbow trout, *Oncorhynchus mykiss*, concurrently infected with *A. coregoni* and *Flavobacterium columnare*, compared to fish infected with either the parasite or the bacterium alone, the mortality of fish, challenged with *A. coregoni*, was negligible wheras infection with *F. columnare* alone led to a significantly lower survival. However, compared to fish with single infections, the mortality was significantly higher and the onset of disease condition was earlier among fish, which were concomitantly infected by *A. coregoni* and *F. columnare*, indicating that the presence of *Argulus* increases the susceptibility of fish to this bacterial pathogen (Bandilla *et al.*, 2006).

*Dolops* attaches to its host by means of the powerful hooks on the maxillulae. These are driven into the dermis of the host and result in bleeding lesions, surrounded by a haemorrhagic area (Fig. 18.64). The dermal layer is compressed and torn, disrupting the stratum spongiosum while they feed by rasping the host tissue away with the mandibles, to expose a blood vessel. Bacteria are frequently present in the area, implying infection by opportunistic bacteria following the opening in the skin by the parasite. The parasite moves around, reattaching and it results in a trail

of lesions (Avenant-Oldewage, 1994). *Dolops* infection has not been reported from culture situations. However, Paperna (1996) reported over 30 *D. ranarum* individuals per fish with 78% prevalence from *Bagrus docmac*, collected in Lake Victoria. In the temperate water of southern Africa *Dolops* never reach such a high prevalence or mean intensity and only in a single case, 35 specimens were removed from a large African catfish (Avenant and van As, 1986). Sazima and Machado (1990)—in a study in a small pond—observed piranha (*Serrasalmus marginatus*) removing *Dolops* sp. in, what they described as, a cleaning action. Similarly, in aquaria, African catfish (*Clarias gariepinus*) shorter than 20 cm cleaned each other of *D. ranarum* by eating them (Avenant-Oldewage, unpubl.).

*Chonopeltis* exhibits narrow host specificity and it has been reported to change their host during their life cycle. Van Niekerk (1984) recorded localized inflammation and destruction of the epithelium in the area of attachment. Collagen fibres in the underlaying dermis were damaged and lethargic behaviour of the host was induced. According to Swanepoel and Avenant-Oldewage (1993) and Avenant-Oldewage *et al.* (1994), the morphology of the digestive tract and mandibles in *C. australis* is similar to that of *Argulus* and *Dolops* suggesting that they are able to rasp food from the host with the mandibles and feed in a similar manner. However, the prevalence and mean intensity is always very low (Van Niekerk and Kok, 1989; Avenant-Oldewage, 2008) and *Chonopeltis* has never been reported as a pathogen.

Carvalho *et al.* (2003) reported a prevalence of 73% in *Dipteropeltis hirundo* on 15 *Pseudoplatystoma fasciatum* with a mean intensity of 2.7 and analysis of their data revealed aggregation. The type specimens of *D. hirundo* are 30 mm long and it can, therefore, be imagined that they should have an effect on their host, although no reports to this effect are available.

## Treatment

Since only *Argulus* species have been reported as pests in aquaculture, the treatment information is limited to this genus.

Benz *et al.* (2001) suggest removal of *Argulus* with a forceps from individual fish. This method is intended only for individual fish and the disadvantage is that the tiny larvae may be missed. Under aquaculture conditions organohalogen- and organophosphate insecticides have been

used. Futhermore, Pyrethrum (20–100 ppm for 10–20 min); malathion (0.25 ppm for 6 hours) for short treatments or for long treatments. Furhtermore the antimalarial drug quinine hydrochloride (13.5 ppm) or Atebine (10 ppm) for several days is effective against *Argulus* infection (Kabata, 1985). Puffer and Beal (1981) used DTHP or 0.0-dimethyl 2,2,2, trichloro-1-hydroxethylphosphonate at 2.5 mg $L^{-1}$. Padmavathi and Prasad (1998) reported that they were able to effectively kill *Argulus japonicus* with 25% quinalphos in combination with 0.0025 ppm common salt but that the parasites died only after laying eggs which were resistant to the pesticide. By repeating the treatment with weekly intervals the infection was completely controlled. The egg-laying capacity of the parasite was also reduced. It had a minimal deleterious effect on plankton and fish.

Cortisol-fed fish had reduced numbers of *A. japonicus*, but since the treatment caused a change in the skin structure, this was the most likely the explanation for the reduction in parasite numbers (Haond *et al.*, 2003). Hakalahti *et al.* (2004b) treated rainbow trout against *A. coregoni* with 50 mg Emamectin benzoate per kg per day for seven days. After three days, the infection level was lower than in control fish with a 100% efficacy against newly hatched larvae and 80% against juveniles. The treatment remained effective for 9 weeks.

Consumer resistance and the damaging effect on the environment are responsible for a change in the approach to treatment and alternative methods received attention lately. One such method is the use of a chitin inhibitor that was evaluated by Williams (1997) who showed that diflubenzuron had a potential to control fish lice. Administering the treatment agent with the fish food in a palatable manner still presents a challenge. Kabata (1985) suggested that hard objects should be removed from ponds and replaced with wooden slats to which the eggs could be attached; these should then be removed and destroyed. Similarly, Hakalahti *et al.* (2004a) exploited the preference of *A. coregoni* to deposit eggs on irregular stones in deeper water by supplying egg traps 20 cm above the bottom for females to deposit their eggs on. These are then removed and destroyed. They urge that for the method to be effective it is important that the pond should not contain other stones or hard substrate. In still waters, trout farm egg-laying boards were placed into the ponds. The parasites deposited eggs on the boards and the boards were removed continuously to reduce the number of available eggs thereby reducing the infection (Gault *et al.*, 2002). The present author observed,

under laboratory conditions, that, *A. japonicus* deposits eggs on the smooth sides of glass aquaria and the removal method is therefore not effective in this species. It was shown that *Gambusia*, angelfish, and stickleback feed on *Argulus* (cf., Kabata, 1985; Poulin and FitzGerald, 1987). All of these methods show potential and further results will probably become available soon. Taking water from a fish-free source or filtering the water can prevent the introduction of argulids. Quarantine of new fishes to be stocked, combined with treatment, when necessary, will also prevent unwanted pest introduction (Lester and Roubal, 1995).

### *Hyperparasitism*

Specimens of *D. ranarum*, collected from fishes in natural ponds and rivers, are frequently infected by *Epistylis nympaharum* (cf., Van As and Viljoen, 1984) and the infestation is frequently high enough to be visible with the naked eye. Sutherland and Wittrock (1986) reported a similar occurrence in *Argulus appendiculosus*.

## ISOPODA

Isopods cannot even match the diversity of parasitic copepods. The number of species, parasitizing fishes, is also relatively low, although it is much higher than that of branchiurans. Isopods, similarly as copepods are ecologically diversified and only some species are parasitic while the rest are free living. There are about of 450 species occurring on their hosts in various environments (Möller and Anders, 1986). The parasitic isopods belong to the suborder Flabellifera. The majority of parasitic isopods are marine species and only few of them attack freshwater fishes.

The body of a flabelliferid isopod is dorsoventrally flattened, consisting of seven peraeonal somites and six pleonal somites, including the pleotelson. There are seven pairs of pareopods and five pairs of pleopods (Rafi, 1988). There is no carapax. Paired, compound eyes consist of numerous ommatidia (Figs. 18.70–18.72).

The majority of parasitic isopods (families: Cymothoidae and Aegidae) tend to be associated with their hosts through their entire life, while representatives of the family Gnathiidae are parasites only at their larval phase of life. Adult males and females are free-living.

**PLATE 11** Figs. 18.66–18.76. Branchiura, Isopoda, and Cirripedia parasitic on fishes. Fig. 18.66. *Argulus japonicus*; male; SEM. Fig 18.67. *Argulus japonicus*; mandible. Fig. 18.68. Host epithelium following attachment by *Argulus japonicus*; bacteria are visible on

*Plate 11 Contd.*

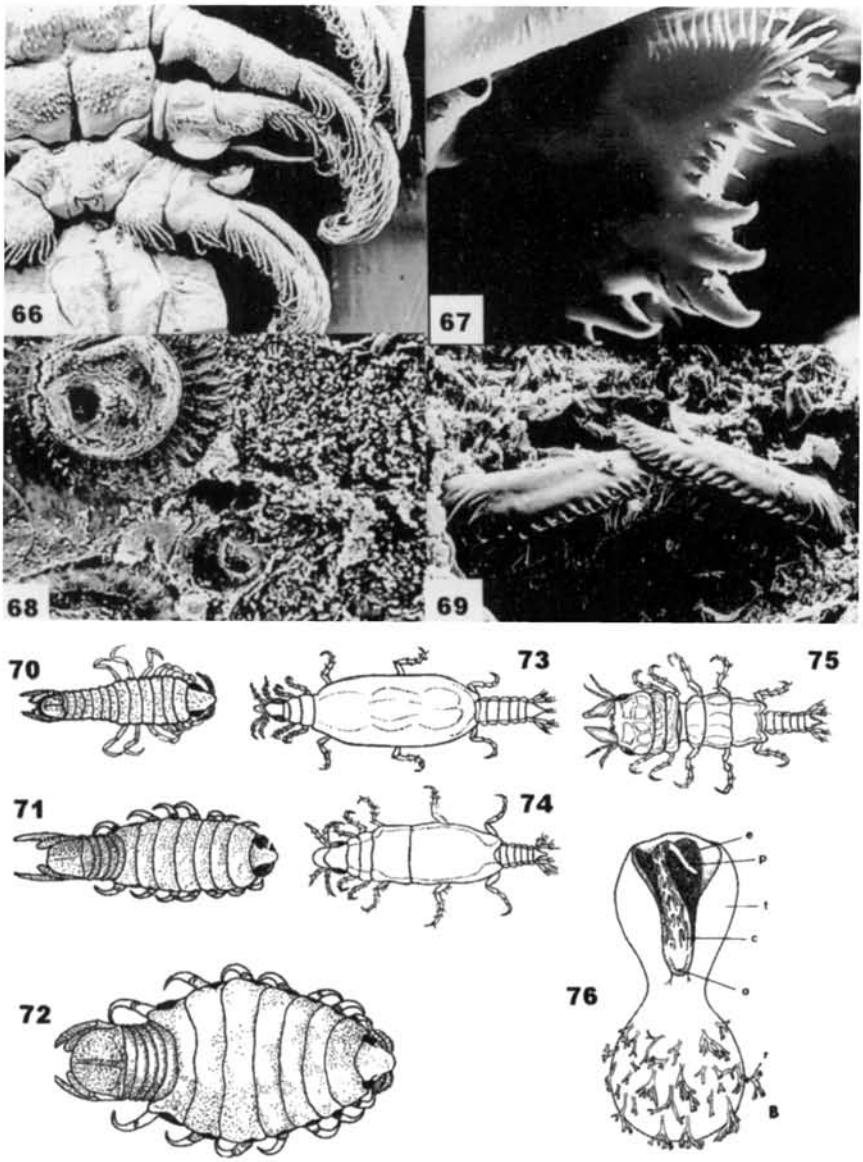


*Plate 11 Contd.*

partly destroyed epithelium and evidence of penetration by proboscis. Fig. 18.69. *Dolops ranarum*; mandibles. Fig. 18.70. *Anilocra frontalis* (Isopoda, Cymothoidae); larva pullus 2; dorsal. Fig. 18.71. *A. frontalis*; male; dorsal. Fig. 18.72. *A. frontalis*; female; dorsal. Fig. 18.73. *Gnathia maxillaris* (Isopoda; Gnathiidae); larva praniza; dorsal. Fig. 18.74. *G. maxillaris*; female; dorsal. Fig. 18.75. *G. maxillaris*; male; dorsal. Fig. 18.76. *Anelasma squalicola* (Cirripedia). Labels: e, egg sac; p, penis; t, mantle; c, biramous appendage; o, mouth. Source: Avenant-Oldewage – original (Figs. 18.66–18.69); Grabda, 1991 (redrawn from Trilles) (Figs. 18.70–18.72); Grabda, 1991 (redrawn from Sars) (Figs. 18.73–18.75); Lester and Roubal, 1995 (Fig. 18.76).

## Family Cymothoidae

This family covers more than 200 species, all very well adapted to parasitism (Grabda, 1991). They are all protandrous hermaphrodites, which means that they first develop as a male (Fig. 18.71) and then develop as a female (Fig. 18.72). The female carries eggs in a brood chamber, formed on the ventral side of the body by special plates, also called oostegites. The eggs hatch in the brood chamber as pullus I larvae (Fig. 18.70), soon becoming pullus II. The entire larval development takes place in the brood chamber (Grabda, 1991). Cymothoids are fairly large, and their length is measured in cm (1–6 cm) and they attach at different sites on the fish body such as: skin, fins, buccal chamber, and gill chamber. Selected species are reviewed in Grabda (1991). Cymothoids parasitize teleost fishes and few records from elasmobranchs were probably accidental (Lester and Roubal, 1995).

*Anilocra frontalis* (around 3 cm in length) parasitizes fishes of the family Labridae in the Mediterranean Sea, Adriatic and Atlantic. They attach to the skin of fish, behind the eye; the male slightly below the female.

*A. physodes* prefers fishes of the families Sparidae and Centrarchidae, with a slightly different location on the fish skin.

*Livoneca amurensis* (32 mm long), a freshwater representative of the family, settles as a juvenile in a skin depression behind the pectoral fin of *Leuciscus waleckii*, in the Amur River drainage basin. As the size of the parasite increases, it becomes increasingly deeper embedded in a 'crypt' formed by invaginated skin of the initial depression. As a consequence, the isopod becomes trapped for life in the crypt and presumably can feed on fish blood. The parasite causes growth retardation of the fish, its emaciation, and even mortality. *L. pontica* infects clupeid fishes in the Black Sea, inflicting serious pathology of the gills. *L. puhi* can be found on 15%–70% of *Gymnothorax eurostus* off Hawaii.

*Caratothoa gaudichaudii* and *Ceratothoa* f. *imbricata* commonly infect the buccal cavity of salmon reared in Chile and Australia (Schafer *et al.*, 1989 cited by Lester and Roubal, 1995).

*Nerocila orbignyi* attached to the skin of cultured *Dicentrarchus labrax* in the Mediterranean was responsible for poor fish growth, while gill parasite *Mothocya parvostis* had been associated with decreased growth rates and mortalities of *Girella punctata* cultured in Japan (reviewed in Lester and Roubal, 1995).

### Family Aegidae

Aegids are temporary parasites and there are more than 130 species. The adults feed on blood of fishes. When fully engorged, they dislodge form the host and sit on the bottom to digest their meal.

In the northwest Atlantic, a number of aegids have been reported to attack cod, *Gadus morhua*. These were: *Aega psora*, *A. entricosa*, *A. stroemi*, to name just a few (Rokicki, 1976).

### Family Gnathiidae

This family is very small with only some 50 species infecting teleost fishes. Only the larvae are parasites while the adults are free living (Figs. 18.73–18.75). The adults show very distinct sexual dimorphism (Figs. 18.74, 18.75). The life cycle of *Gnathia africana* was recently described by Smit *et al.* (2003). There are three larval stages separated by moult (stage 1, stage 2, and stage 3), each of them constituting of 2 functionally different phases (unfed zuphea and praniza–engorged with host blood). Newly hatched zuphea 1 larva is free-living and the following praniza stage—a hematophagous parasite of fish gills (Fig. 18.73). The thoracic somites of praniza larva are very elastic and, therefore, the larva can drink enormous quantities of the host's blood. The isopod moults after each blood meal (outside the host), thereby reaching a higher developmental stage (stage 2: zuphea 2 + praniza 2; and stage 3: zuphea 3 + praniza 3). Praniza 3 moults, becoming a free-living adult.

It was shown that these parasites transmit haemogregarine blood parasites (Davies and Smit, 2001)

## CIRRIPEDIA – BARNACLES

A number of free-living barnacles attach to different inanimate objects in the sea as well as to various invertebrates and vertebrates. These include *Conchoderma virgatum* and *C. auritum*. They are not considered parasites in any way, though.

The only true fish-parasite barnacle is *Anelasma squalicola*. It can be found embedded into the subdermal tissues in the dorsal fin area of a small bathypelagic shark, *Etmopterus spinax*. The infection rates in the only published survey, carried out west of Ireland were only 5% (Hickling, 1963).

Like most barnacles, it is hermaphroditic and sits upside-down on its hosts. It resembles other lepadid barnacles in overall shape and its leathery

structure, but its anterior half is deeply embedded into the tissues of the shark. The anterior 'peduncle' is equipped with numerous root-like outgrowths, penetrating the host's tissues (Fig. 18.76). The posterior part, protruding from the sharkskin is purple brownish.

The affected hosts experienced retarded sexual maturation and their livers were by 10% lighter that those of uninfected fish of the same size class (Hickling, 1963).

The distinct effect on the host, the structure of the branching "root system" and dysfunctional cirriped filtrating system suggest that this parasite acquires food directly from its host through the roots, despite having a functional mouth and intestine (Lester and Roubal, 1995).

## AMPHIPODA

Amphipods are laterally flattened crustaceans and they can be easily differentiated from isopods. Bousfield and Kabata (1988) reviewed amphipods occurring on Canadian fishes. Amphipods tend to attach to slowly moving marine fishes such as benthic sharks or benthic teleosts acting as 'ambush predators'. Quite often they can be found feeding on fish entangled into gill nets. They are not specifically adapted to parasitism and they are treated either as facultative parasites or opportunistic scavengers. Some species occasionally found on fish are simply 'hitchhikers'. No comprehensive accounts on the pathogenicity of amphipods are available.

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## References


Abdelhalim, A.I., J.W. Lewis and G.A. Boxshall. 1991. The life-cycle of *Ergasilus sieboldi* Nordmann (Copepoda: Poecilostomatoida), parasitic on British freshwater fish. *Journal of Natural History* 25: 559-582.

Abrosov, V.N. and O.N. Bauer. 1959. Ergasilos peladi v ozerah Pskovskoj oblasti. (Ergasilose des Peleds (*Coregonus peled*) in den Seen des Pskowsker Gebiets.) *Izvestâ gosudarstviennogo naučno-issledovatel'skogo Instituta ozer'nogo i rečnogo rybnogo hozâistva* 49: 213-216. (In Russian).

Abrosov, V.N. and O.N. Bauer. 1961. Die Peladmarane—ein neues Object der Fischwirtschaft. *Zeitschrift für Fisherei* 10: 299-307. (In German).

Ahne, W. 1985. *Argulus foliaceus* L. and *Piscicola geometra* L. as mechanical vectors of spring viraemia of carp virus. *Journal of Fish Diseases* 8: 241-242.

Amado, M.A.P.M., J.-s. Ho and C.E.F. Rocha. 1995. Phylogeny and biogeography of the Ergasilidae (Copepoda, Poecilostomatoida), with reconsideration of the taxonomic status of the Vaigamidae. *Contributions to Zoology* 65: 233-243.

Amundsen, P.A., R. Kristoffersen, R. Knudsen and A. Klemetsen. 1997. Infection of *Salmincola edwardsii* (Copepoda: Lernaeopodidae) in an age-structured population of Arctic charr: A long-term study. *Journal of Fish Biology* 51: 1033-1046.

Anonymous. 2003. *Integrated Pest Management of Sea Lice in Salmon.* Aquaculture. Health Canada. (Available at: http://www.pmra-arla.gc.ca/english/pdf/spm/spm2003-e.pdf)

Anonymous. 2007. *World of Copepods.* (Available at: http://www.nmnh.si.edu/iz/copepod/).

Avenant, A. and J.G. van As. 1985. Occurrence and distribution of *Dolops ranarum* (Stuhlmann), ectoparasite of freshwater fish in the Transvaal, South Africa. *Journal of Fish Biology* 27: 403-416.

Avenant, A. and J.G. van As. 1986. Observations on the seasonal occurrence of the ectoparasite *Dolops ranarum* (Stuhlmann, 1891) (Crustacea: Branchiura) in the Transvaal. *South African Journal of Wildlife Research* 16: 62-64.

Avenant, A. and J.G. van As. 1990. On the reproductive system of *Dolops ranarum* (Stuhlmann, 1891) (Crustacea: Branchiura). *South African Journal of Zoology* 25: 67-71.

Avenant, A., J.G. van As and G.C. Loots. 1988. Verklarende woordelys vir die orde Branchiura. [List of explanatory terms for the order Branchiura.] *Suid Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie* 7: 91-97.

Avenant, A., J.G. van As and G.C. Loots. 1989a. On the hatching and morphology of *Dolops ranarum* larvae (Crustacea: Branchiura). *Journal of Zoology (London)* 217: 511-519.

Avenant, A., G.C. Loots and J.G. van As. 1989b. A redescription of *Dolops ranarum* (Stuhlmann, 1891) (Crustacea: Branchiura). *Systematic Parasitology* 13: 141-151.

Avenant-Oldewage, A. 1994. Integumental damage caused by *Dolops ranarum* (Stuhlmann, 1891) (Crustacea: Branchiura) to *Clarias gariepinus* (Burchell), with reference to normal histology and wound-inflicting structures. *Journal of Fish Diseases* 17: 641-647.

Avenant-Oldewage, A. 2001. *Argulus japonicus* in the Olifants River system—Possible conservation threat? *South African Journal of Wildlife Research* 31: 59-63.

Avenant-Oldewage, A. 2003. *Lamproglena* and *Lernaea* as possible indicators of environmental deterioration in a river in South Africa. *Journal of the South African Veterinary Association* 74: 96.

Avenant-Oldewage, A. and E. Knight. 1994. A diagnostic species compendium of the genus *Chonopeltis* Thiele, 1900 (Crustacea: Branchiura) with notes on its geographical distribution. *Koedoe* 37: 41-56.

Avenant-Oldewage, A. and E. Knight. 2008. Aspects of the ecology of *Chonopelti australis* Boxshall, 1976 in Boskop Dam, North West Province. *South African Journal of Wildlife Research* 38: 28-34.

Avenant-Oldewage, A. and J.H. Swanepoel. 1993. The male reproductive system and mechanism of sperm transfer in *Argulus japonicus* (Crustacea: Branchiura). *Journal of Morphology* 215: 51-63.

Avenant-Oldewage, A., J.H. Swanepoel and E. Knight. 1994. Histomorphology of the digestive tract of *Chonopeltis australis* (Crustacea: Branchiura). *South African Journal of Zoology* 29: 74-81.

Bandilla, M., T. Hakalahti, P. J. Hudson and E.T. Valtonen. 2005. Aggregation of *Argulus coregoni* (Crustacea: Branchiura) on rainbow trout (*Oncorhynchus mykiss*): A consequence of host susceptibility or exposure? *Parasitology* 130: 169-176.

Bandilla, M., E.T. Valtonen, L.R. Suomalainen, P.J. Aphalo and T. Hakalahti. 2006. A link between ectoparasite infection and susceptibility to bacterial disease in rainbow trout. *International Journal for Parasitology* 36: 987-991.

Bauer, O.N. and B. Babaev. 1964. *Sinergasilus major* (Markewitsch, 1940), ego biologiâ i patogennoe značenie. [*Sinergasilus major* (Markewitsch, 1940), its biology and pathogenic significance.] *Izvestâ Akademii Nauk Turkmen'skoj SSR, Biologiâ* 3: 63-67. (In Russian).

Bauer, O.N., W.A. Musselius and Û. Strielkov. 1969. *Bolezni prudovyh ryb.* [*Diseases of Pond Fishes.*] Izdatel'stvo Kolos, Moskva. (In Russian).

Baumann, F. 1913. Parasitische Copepoden auf Coregonen. *Revue Suisse de Zoologie* 21: 147-178. (In German).

Benz, G.W. 1989. Developmental stages of *Alebion lobatus* Cressey, 1970 (Copepoda): Euryphoridae) found parasitic on the sandbar shark (*Carcharhinus plumbeus* (Nardo, 1827)) in the western north Atlantic, and a phylogenetic analysis of the genus *Alebion* Krøyer, 1863. *Canadian Journal of Zoology* 67: 1578-1598.

Benz, G.W. and S.A. Bullard. 2004. Metazoan parasites and associates of chondrichthyans with emphasis on taxa harmful to captive hosts. In: *The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and Their Relatives*, M. Smith, D. Warmolts, D. Thoney and R. Hueter (eds.). *Special Publication, Ohio Biological Survey, Columbus*, OH, pp. 325-416.

Benz, G.W. and K.S. Dupre. 1987. Spatial distribution of the parasite *Kroyeria carchariaeglauci* Hesse, 1979 (Copepoda: Siphonostomatoida: Kroyeriidae) on gills of the blue shark (*Prionace glauca* L., 1758)). *Canadian Journal of Zoology* 65: 1275-1281.

Benz, G.W., S.A. Bullard and A.D.M. Dove. 2001. Metazoan parasites of fishes: Synoptic information and portal to the literature for aquarists. In: *Regional Conference Proceedings 2001*, Anonymous (ed.). American Zoo and Aquarium Association, Silver Spring, MO, 160 pp.

Benz, G.W., J.D. Borucinska, L.F. Lowry and H.E. Whiteley. 2002. Ocular lesions associated with attachment of the copepod *Ommatokoita elongata* (Lernaeopodidae: Siphonostomatoida) to corneas of Pacific sleeper sharks *Somniosus Pacificus* captured off Alaska in Prince William Sound. *Journal of Parasitology* 88: 474-481.

Berland, B. 1993. Salmon lice on wild salmon (*Salmo salar* L.) in western Norway. In: *Pathogens of Wild and Farmed Fish: Sea Lice*, G.A. Boxshall and D. Defaye (eds.). Ellis Horwood, Chichester, pp. 179-187.

Bjordal, A. 1990. Sea lice infestation on farmed salmon: possible use of cleaner-fish as an alternative method for de-lousing. *Canadian Technical Report of Fisheries and Aquatic Sciences* 1761: 85-89.

Bjorn, P.A. and B. Finstad. 1997. The physiological effects of salmon lice infection on sea trout post smolts. *Nordic Journal of Freshwater Research* 73: 60-72.

Black, G.A. 1982. Gills as an attachment site for *Salmincola edwardsii* (Copepoda: Lernaeopodidae). *Journal of Parasitology* 68: 1172-1173.

Black, G.A., W.L. Montgomery and F.G. Whoriskey. 1983. Abundance and distribution of *Salmincola edwardsii* (Copepoda) on anadromous brook trout, *Salvelinus fontinalis* (Mitchill) in the Moisie River system, Quebec. *Journal of Fish Biology* 22: 567-575.

Bouchet, G.C. 1985. Redescrption of *Argulus varians* Bere, 1936 (Branchiura: Argulidae) including a description of its early development and first larval stage. *Crustaceana* 49: 30-35.

Bousfield, E.L. and Z. Kabata. 1988. Amphipoda. In: *Guide to the Parasites of Fishes of Canada. Part II–Crustacea*, L. Margolis and Z. Kabata (eds.). *Canadian Special Publication of Fisheries and Aquatic Sciences* 1988(101): 149-163.

Bowen, C.A. II, and R.M. Stedman. 1990. Host-parasite relationship and geographic distribution of *Salmincola corpulentus* (Copepoda: Lernaeopodidae) on bloater (*Coregonus hoyi*) stocks in Lake Huron. *Canadian Journal of Zoology* 68: 1988-1994.

Bowers, J., A. Mustafa, J. Burka, D. Speare, D. Sims and G. Conboy. 2000. The effects of sea lice, *Lepeophtheirus salmonis* on the stress response of Atlantic salmon, *Salmo salar*. *Journal of Fish Diseases* 23: 165-172.

Boxaspen, K. 2006. A review of the biology and genetics of sea lice. *International Council for Exploration of the Sea. Journal of Marine Science* 63: 1304-1316.

Boxshall, G. 2005a. Copepoda (copepods). In: *Marine Parasitology*, K. Rohde (ed.). CABI Publishing, Wallingford, Oxon, U.K., pp. 123-138.

Boxshall, G. 2005b. Branchiura (fish lice). In: *Marine Parasitology*, K. Rohde (ed.). CABI Publishing, Wallingford, Oxon, U.K., pp. 145-147.

Boxshall, G.A. and S. Bravo. 2000. On the identity of the common *Caligus* (Copepoda: Siphonostomatoida: Caligidae) from salmonid netpen systems in southern Chile. *Contributions to Zoology* 69: 137-146.

Boxshall, G.A. and S.H. Halsey 2004. *An Introduction to Copepod Diversity*. The Ray Society, London.

Brandal, P.O. and E. Egidius. 1979. Treatment of salmon lice (*Lepeophtheirus salmonis* Krøyer, 1838) with Neguvon®—description of method and equipment. *Aquaculture* 18: 183-188.

Brandal, P.O., E. Egidius and I. Romslo. 1976. Host blood: a major food component for the parasitic copepod *Lepeophtheirus salmonis* Kröyer, 1838 (Crustacea: Caligidae). *Norwegian Journal of Zoology* 24: 341-343.

Bricknell, I.R., S.J. Dalesman, B. O'Shea, C.C. Pert and A.J. Mordue-Luntz. 2006. The effect of environmental salinity on sea lice *Lepeophtheirus salmonis* settlement success. *Diseases of Aquatic Organisms* 71: 201-212.

Bron, J.E., C. Sommerville, M. Jones and G.H. Rae. 1991. The settlement and attachment of early stages of the salmon louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae) on the salmon host, *Salmo salar*. *Journal of Zoology (London)* 224: 201-212.

Bron, J.E., C. Sommerville, R. Wootten and G.H. Rae. 1993. Fallowing of marine Atlantic salmon, *Salmo salar* L. farms as a method for the control of sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837). *Journal of Fish Diseases* 16: 487-493.

Bruno, D.W. and J. Stone. 1990. The role of saithe, *Pollachius virens* L., as a host for the sea lice, *Lepeophtheirus salmonis* Krøyer and *Caligus elongatus* Nordmann. *Aquaculture* 89: 201-207.

Calman, W.T. 1912. On *Dipteropeltis*, a new genus of the crustacean order Branchiura. *Proceedings of the Zoological Society of London* 1912: 763-766.

Carvajal, J., L. Gonzalez and M. George-Nascimento. 1998. Native sea lice (Copepoda: Caligidae) infestation of salmonids reared in net pen systems in southern Chile. *Aquaculture* 166: 241-246.

Carvalho, L.N., K. Del-Claro and R.M. Takemoto. 2003. Host-parasite interaction between branchiurans (Crustacea: Argulidae) and piranhas (Osteichtyes: Serrasalminae) in the Pantanal wetland of Brazil. *Environmental Biology of Fishes* 67: 289-296.

Carvalho, L.N., R. Arruda and K. Del-Claro. 2004. Host-parasite interactions between the piranha *Pygocentrus nattereri* (Characiformes: Characidae) and isopods and branchiurans (Crustacea) in the Rio Araguaia basin, Brazil. *Neotropical Ichthyology* 2: 93-98.

Carvalho de Paiva, J. 1939. Sôbre dois parasitos do gênero *Dolops*, encontrados em peixes de agua dôce. *Revista de Indústria Animal, Nova Série* 2: 109-116.

Carvalho de Paiva, J. 1953. Nota sôbre *Lernaeenicus longiventris* Wilson e sua ocorrência em *Xenomelaniris brasiliensis* (Quoy & Gaimard) (Crustacea, Copepoda – Pisces, Atherinidae). *Boletim do Instituto Oceanográfico, Sao Paulo* 4: 181-190.

Chang, M.X., P. Nie, G.Y. Liu, Y. Song and Q. Gao. 2005. Identification of immune genes in grass carp *Ctenopharyngodon idella* in response to infection of the parasitic copepod *Sinergasilus major*. *Parasitology Research* 96: 224-229.

Costello, M.J. 1993. Review of methods to control sea lice (Caligidae Crustacea) infestations on salmon (*Salmo salar*) farms. In: *Pathogens of Wild and Farmed Fish: Sea Lice*, G.A. Boxshall and D. Defaye (eds.). Ellis Horwood, Chichester, pp. 219-252.

Costello, M.J. 2006. Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology* 22: 475-483.

Davies, A.J. and N.J. Smit. 2001. The life cycle of *Haemogregarina bigemina* (Adeleina: Haemogregarinidae) in South African hosts. *Folia Parasitologica* 48: 169-177.

Dorovskikh, G.N. [Dorovskih, G.N.] 1996. Lokalizacjâ *Lernaea cyprinacea* (Copepoda: Lernaeidae) na tele karasâ [Location of *Lernaea cyprinacea* (Copepoda: Lernaeidae) on the body of crucian.] *Parazitologiâ* 30: 540-544. (In Russian).

Dugatkin, L.A., G.J. FitzGerald and J. Lavoie. 1994. Juvenile three-spined sticklebacks avoid parasitoid conspecifics. *Environmental Biology of Fishes* 39: 215-218.

Dzidziul, A. 1973. The pathogenicity of *Lernaea cyprinacea* L. in the cases of heavy infestation, in *Carassius carassius* (L.). *Acta Parasitologica Polonica* 21: 281-288.

Egusa, S. 1992. *Infectious Diseases of Fish*. A.A. Balkema, Rotterdam.

Einszporn, T. 1965a. Nutrition of *Ergasilus sieboldi* Nordmann. I. Histological structure of the alimentary canal. *Acta Parasitologica Polonica* 13: 151-160.

Einszporn, T. 1965b. Nutrition of *Ergasilus sieboldi* Nordmann. II. The uptake of food and the food material. *Acta Parasitologica Polonica* 13: 373-382.

Einszporn-Orecka, T. 1973a. Changes in the picture of peripheral blood of tench *Tinca tinca* (L.) under the influence of *Ergasilus sieboldi* Nordm. 1. Composition of morphotic blood constituents of uninfected fish in annual cycle. *Acta Parasitologica Polonica* 21: 393-411.

Einszporn-Orecka, T. 1973b. Changes in the picture of peripheral blood of tench *Tinca tinca* (L.) under the influence of *Ergasilus sieboldi* Nordm. 2. Changes in the leucotic system. *Acta Parasitologica Polonica* 21: 485-499.

Eithun, I. 2000. Measures to control sea lice in Norwegian fish farms. *Caligus* 6 (Available at http://www.ecoserve.ie/projects/sealice/caligus6.html).

Fast, M.D., D.M. Muise, R.E. Easy, N.W. Ross and S.C. Johnson. 2006. The effects of *Lepeophtheirus salmonis* infections on the stress response and immunological status of Atlantic salmon (*Salmo salar*). *Fish and Shellfish Immunology* 21: 228-241.

Fenton, A., T. Hakalahti, M. Bandilla and E.T. Valtonen. 2006. The impact of variable hatching rates on parasite control: A model of an aquatic ectoparasite in a Finnish fish farm. *Journal of Applied Ecology* 43: 660-668.

Finstad, B., P. Bjorn, A. Grimnes and N. Hvidsten. 2000. Laboratory and field investigations of salmon lice [*Lepeophtheirus salmonis* (Krøyer)] infestation on Atlantic salmon (*Salmo salar* L.) post-smolts. *Aquaculture Research* 31: 795-803.

Fryer, G. 1958. Occurrence of spermatophores in the genus *Dolops* (Crustacea: Branchiura). *Nature (London)* 181: 1011-1012.

Fryer, G. 1959. A report on the parasitic Copepoda and Branchiura of the fishes of Lake Bangweulu (Northern Rhodesia). *Proceedings of the Zoological Society of London* 132: 517-550.

Fryer, G. 1960. The spermatophore of *Dolops ranarum* (Crustacea: Branchiura): Their structure, formation and transfer. *Quarterly Journal of Microscopic Science* 101: 407-432.

Fryer, G. 1961. Larval development in the genus *Chonopeltis* (Crustacea: Branchiura). *Proceedings of the Zoological Society of London* 137: 61-70.

Fryer, G. 1964. Further studies on the parasitic Crustacea of African freshwater fishes. *Proceedings of the Zoological Society of London* 143: 79-102.

Fryer, G. 1968. The parasitic Crustacea of African freshwater fishes: Their biology and distribution. *Journal of Zoology (London)* 156: 45-95.

Fryer, G. 1982. *The Parasitic Copepoda and Branchiura of British Freshwater Fishes. Freshwater Biological Association Scientific Publication* 46, *Windermere*, 87 pp.

Gall, G.A.E., E.L. McClendon and W.E. Schafer. 1972. Evidence on the influence of the copepod (*Salmincola californiensis*) on the reproductive performance of a domesticated strain of rainbow trout (*Salmo gairdneri*). *Transactions of the American Fisheries Society* 101: 345-346.

Gault, N.F.S., D.J. Kilpatrick and M.T. Stewart. 2002. Biological control of the fish louse in a rainbow trout fishery. *Journal of Fish Biology* 60: 226-237.

Gonzalez-Alanis, P., G.M. Wright, S.C. Johnson and J.F. Burka. 2001. Frontal filament morphogenesis in the salmon louse *Lepeophtheirus salmonis*. *Journal of Parasitology* 87: 561-574.

Goodwin, A.E. 1999. Massive *Lernaea cyprinacea* infestations damaging the gills of channel catfish polycultured with bighead carp. *Journal of Aquatic Animal Health* 11: 406-408.

Grabda, E. and J. Grabda. 1958. Tracheliastosis in the common bream, *Abramis brama* L. in Lake Jamno. *Zoologica Poloniae* 8: 325-334.

Grabda, J. 1963a. Life cycle and morphogenesis of *Lernaea cyprinacea* L. *Acta Parasitologica Polonica* 11: 169-198.

Grabda, J. 1963b. On the invasiology of *Ergasilus sieboldi* Nordm. in Poland. *Acta Hydrobiologica* 5: 245-259. (In Polish).

Grabda, J. 1972. Observations on penetration of *Lernaeolophus sultanus* (Milne Edwards, 1840) (Lernaeoceridae) in organs of *Pneumatophorus colias* (Gmelin, 1788). *Acta Ichthyologica et Piscatoria* 2(1): 115-125.

Grabda, J. 1975. Observations on the localization and pathogenicity of *Haemobaphes diceraus* Wilson; 1917 (Copepoda: Lernaeoceridae) in the gills of *Theragra chalcogramma* (Pallas). *Acta Ichthyologica et Piscatoria* 5(2): 13-23.

Grabda, J. 1976. *Haemobaphes disphaerocephalus* sp.n. (Copepoda: Lernaeoceridae) from the gill cavity of *Thaleichthys pacificus* (Richardson, 1836) (Osmeridae). *Acta Ichthyologica et Piscatoria* 6(1): 23-33.

Grabda, J. 1977. Studies on parasitisation and consumability of Alaska pollack, *Theragra chalcogramma* (Pall.). *Acta Ichthyologica et Piscatoria* 7(2): 15-34.

Grabda, J. 1991. *Marine Fish Parasitology: An Outline*. PWN, Warszawa and VCH, Weinheim.

Grabda, J. and T. Linkowski. 1978. *Colobomatus gymnoscopeli* n. sp. (Copepoda: Philichthyidae), a parasite of lateral line sensory canals of *Gymnoscopelus aphya* Günther, 1873 (Myctophidae) from the antarctic waters. *Acta Ichthyologica et Piscatoria* 8(2): 91-110.

Grayson, T.H., P.G. Jenkins, A.B. Wrathmell and J.E. Harris. 1991. Serum responses to the salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1838) in naturally infected salmonids and immunised rainbow trout *Oncorhynchus mykiss* (Walbaum) and rabbits. *Fish and Shellfish Immunology* 1: 141-156.

Grayson, T.H., R.J. John, S. Wadsworth, K. Greaves, D. Cox, J. Roper, A.B. Wrathmell, M.L. Gilpin and J.E. Harris. 1995. Immunization of Atlantic salmon against the salmon louse: Identification of antigens and effects on louse fecundity. *Journal of Fish Biology* A47: 85-94.

Gresty, K.A., G.A. Boxshall and K. Nagasawa. 1993. The fine structure and function of the cephalic appendages of the branchiuran parasite, *Argulus japonicus* Thiele. *Philosophical Transactions of the Royal Society of London B*, 339: 119-135.

Gustafson, L., S. Ellis, T. Robinson, F. Marenghi and R. Endris. 2006. Efficacy of emamectin benzoate against sea lice infestations of Atlantic salmon, *Salmo salar* L.: Evaluation in the absence of an untreated contemporary control. *Journal of Fish Diseases* 29: 621-627.

Hakalahti, T. and E.T. Valtonen. 2003. Population structure and recruitment of the ectoparasite *Argulus coregoni* Thorell (Crustacea: Branchiura) on a fish farm. *Parasitology* 127: 79-85.

Hakalahti, T., H. Hakkinen and E.T. Valtonen 2004a. Ectoparasitic *Argulus coregoni* (Crustacea: Branchiura) hedge their bets—Studies on egg hatching dynamics. *Oikos* 107: 295-302.

Hakalahti, T., Y. Lankinen and E.T. Valtonen. 2004b. Efficacy of emamectin benzoate in the control of *Argulus coregoni* (Crustacea: Branchiura) on rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 60: 197-204.

Hakalahti, T., A.F. Pasternak and E.T. Valtonen. 2004c. Seasonal dynamics of egg laying and egg-laying strategy of the ectoparasite *Argulus coregoni* (Crustacea: Branchiura). *Parasitology* 128: 655-660.

Haond, C., D.T. Nolan, N.M. Ruane, J. Rotlland and S.E. Wendelaar Bonga. 2003. Cortisol influences the host-parasite interaction between the rainbow trout (*Oncorhynchus mykiss*) and the crustacean ectoparasite *Argulus japonicus*. *Parasitology* 127: 551-560.

Harrison, A.J., N.F.S. Gault and J.T.A. Dick. 2006. Seasonal and vertical patterns of egg-laying by the freshwater fish louse *Argulus foliaceus* (Crustacea: Branchiura). *Diseases of Aquatic Organisms* 68: 167-173.

Heinemann, E. 1934. Gross-Schadliche. Der Kiemenkrebs (*Ergasilus sieboldi* Nordm.). *Biologia* 3: 284-287. (In German).

Heuch, P.A. 2005. Effects of salmon lice on Atlantic salmon. In: *Marine Parasitology*, K. Rohde (ed.). CABI Publishing, Wallingford, Oxon, U.K., pp. 374-378.

Hickling, C.F. 1963. On the small deep-sea shark *Etmopterus. spinax* and its cirripede parasite *Anelasma squalicola* (Loven). *Journal of the Linnean Society of London, Zoology* 45: 17-24.

Ho, J.-s. and K. Nagasawa. 2001. Why infestation by *Lepeophtheirus salmonis* (Copepoda: Caligidae) is not a problem in the coho salmon farming industry in Japan. *Journal of Crustacean Biology* 21: 954-960.

Ho, J.-s., S. Ohtsuka and N. Nakadachi. 2006. A new family of poecilostomatoid capepods (Umazuracolidae) based on specimens parasitic on the black scraper (*Thamnaconus modestus*) in Japan. *Zoological Science* 23: 483-496.

Hoffman, G.L. 1977. *Argulus*, a branchiuran parasite of freshwater fish. *United States Department of the Interior, Fish Disease Leaflet* 49.

Hoffman, G.L. 1984. *Salmincola californiensis* continues the march eastward. *American Fisheries Society Newsletter* 12: 4.

Hogans, W.E. 1989. Mortality of cultured Atlantic salmon, *Salmo salar* L., caused by an infection of *Ergasilus labracis* (Copepoda: Poecilostomatoida) in the lower Saint John River, New Brunswick, Canada. *Journal of Fish Diseases* 12: 529-531.

Hogans, W.E. and D.J. Trudeau. 1989a. *Caligus elongatus* (Copepoda: Caligdae) from Atlantic salmon (*Salmo salar*) cultured in marine waters of the lower Bay of Fundy. *Canadian Journal of Zoology* 67: 1080-1082.

Hogans, W.E. and D.J. Trudeau. 1989b. Preliminary studies on the biology of sea lice, *Caligus elongatus*, *Caligus curtus*, and *Lepeophtheirus salmonis* (Copepoda: Caligoida) parasitic on cage-cultured salmonids in the Lower Bay of Fundy. *Canadian Technical Report of Fisheries and Aquatic Sciences* 1715: 1-14.

Holst, J.C., P. Jakobsen, F. Nilsen, M. Holm, L. Asplin and J. Aure. 2003. Mortality of seaward migrating post-smolts of Atlantic salmon due to salmon lice infection in Norwegian salmon stocks. In: *Salmon at the Edge. Proceedings of the 6th International Atlantic Salmon Symposium 'Salmon on the Edge'*, D. Mills (ed.). *16–18 July* 2002, *Edinburgh, U.K.*, Atlantic Salmon Trust/Atlantic Salmon Federation, Blackwell Publishing, Oxford, pp. 136-138.

Humes, A.G. 1994. How many copepods? *Hydrobiologia* 292/293: 1-7.

Hutson, K.S. and D. Tang. 2007. *Naricolax hoi* n. sp. (Cyclopida: Bomolochidae) from *Arius maculatus* (Siluriformes: Ariidae) off Taiwan and a redescription of *N. chrysophryenus* (Roubal, Armitage & Rohde, 1983) from a new host *Serola lalandi* (Perciformes: Carangidae) in Australian waters. *Systematic Parasitology* 68: 97-113.

Ivaška, S. 1951. *Hlavátka (Jej lov a umelý chov). Danube salmon (Its Capture and Proper Culture).*] Tatran, Bratislava. (In Slovak).

Jafri, S.I.H. and M.A. Mahar. 2003. Parasitic copepod, *Lernaea multiobosa* sp. nov. (Family: Lernaeidae), from a cyprinid fish of Manchhar lake (Sindh), Pakistan. *Pakistan Journal of Zoology* 35: 29-34.

Jara, Z. and A. Chodyniecki. 1999. *Ichtiopatologia* [*Ichthyopathology.*] Wydawnictwo Akademii Rolniczej we Wrocławiu. (In Polish).

Johnson, S.C. 1998. Parasitic Crustaceans. In: *Diseases of Seawater Netpen-Reared Salmonid Fishes*, M.L. Kent and T.T. Poppe (eds.). Pacific Biological Station, Nanaimo, Canada, pp. 80-90.

Johnson, S.C. and L.J. Albright. 1991a. The developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). *Canadian Journal of Zoology* 69: 929-950.

Johnson, S.C. and L.J. Albright. 1991b. Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. *Journal of the Marine Biological Association of the United Kingdom, Plymouth* 71: 425-436.

Johnson, K.A. and J.A. Heindel. 2001. Efficacy of manual removal and ivermectin gavage for control of *Salmincola californiensis* (Wilson) infestation of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), captive broodstocks. *Journal of Fish Diseases* 24: 197-203.

Johnson, S.C., J.N.C. White and L. Margolis. 1993. The efficacy of hydrogen peroxide against the salmon louse *Lepeophtheirus salmonis*, its toxicological effects on Atlantic and chinook salmon, its stability in sea water, and its toxic effects on some non-target species. *Aquaculture Update* 63: 1-6.

Johnson, S.C., J.W. Treasurer, S. Bravo, K. Nagasawa and Z. Kabata. 2004. A review of the impact of parasitic copepods on marine aquaculture. *Zoological Studies* 43: 229-243.

Jones, M.W., C. Sommerville and J. Bron. 1990. The histopathology associated with the juvenile stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 13: 303-310.

Jones-Megan, E.B. and C.T. Taggart. 1998. Distribution of gill parasite (*Lernaeocera branchialis*) infection in Northwest Atlantic cod (*Gadus morhua*) and parasite-induced host mortality: Inferences from tagging data. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 364-375.

Jónsdóttir, H., J.E. Bron, R. Wootten and J.F. Turnbull. 1992. The histopathology associated with the pre-adult and adult stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 15: 521-527.

Juhász, S., P. Gálfi and K. Molnár. 1980. Data on the proteolytic enzyme system of *Lernaea cyprinacea*. *Acta Veterinaria Academiae Scientiarum Hungaricae* 28: 57-69.

Kabata, Z. 1958. *Lernaeocera obtusa* n. sp., its biology and its effects on the haddock. *Marine Research* 1958(3): 1-26.

Kabata, Z. 1969a. *Phrixocephalus cincinnatus* Wilson, 1908 (Copepoda: Lernaeoceridae): morphology, metamorphosis, and host-parasite relationship. *Journal of the Fisheries Research Board of Canada* 26: 921-934.

Kabata, Z. 1969b. Revision of the genus *Salmincola* Wilson, 1915 (Copepoda: Lernaeopodidae). *Journal of the Fisheries Research Board of Canada* 26: 2987-3041.

Kabata, Z. 1970. *Crustacea as Enemies of Fishes*. Book 1. In: *Diseases of Fishes*, S. Snieszko (ed.). T.F.H. Publications, Neptune City, NJ.

Kabata, Z. 1975. A rational look at parasitic Copepoda. *Third International Wildlife Disease Conference*, Munich; Abstracts. (Cited after Grabda, 1991).

Kabata, Z. 1979. *Parasitic Copepoda of British Fishes*. The Ray Society, London.

Kabata, Z. 1981. Copepoda (Crustacea) parasitic on fishes: Problems and perspectives. *Advances in Parasitology* 19: 1-71.

Kabata, Z. 1985. *Parasites and Diseases of Fish Cultured in the Tropics*. Taylor and Francis, London.

Kabata, Z. 1988. Copepoda and Branchiura. In: *Guide to the Parasites of Fishes of Canada. Part II – Crustacea*, L. Margolis and Z. Kabata (eds.). *Canadian Special Publication of Fisheries and Aquatic Sciences* 101, pp. 3-127.

Kabata, Z. and B. Cousens. 1973. Life cycle of *Salmincola californiensis* (Dana, 1852) (Copepoda: Lernaeopodidae). *Journal of the Fisheries Research Board of Canada* 30: 881-903.

Kabata, Z. and B. Cousens. 1977. Host-parasite relationship between sockeye salmon, *Oncorhynchus nerka* and *Salmincola californiensis* (Copepoda: Lernaeopodidae). *Journal of the Fisheries Research Board of Canada* 34: 191-202.

Kasahara, S. 1962. Studies on the biology of the parasitic copepod *Lernaea cyprinacea* Linnaeus and the method for controlling this parasite in fish-culture ponds. *Contributions of the Fisheries Laboratory, Faculty of Agriculture University of Tokyo* 3: 103-196.

Kempter, J., W. Piasecki , K. Więski and B. Krawczyk. 2006. Systematic position of copepods of the genus *Achtheres* (Crustacea: Copepoda: Siphonostomatoida) parasitizing perch, *Perca fluviatilis* L., and zander, *Sander lucioperca* (L.). *Journal of Fish Diseases* 29: 103-113.

Kent, M.L., D.J. Whitaker, J.D.W. Moran and Z. Kabata. 1997. *Haemobaphes disphaerocephalus*, an accidental parasite of seawater pen-reared Atlantic salmon. *Canadian Veterinary Journal* 38: 110-111.

Keshkinen, E. and V.B. Meyer-Rochow. 2004. Postembryonic photoreceptor development in the fishlouse *Argulus coregoni* Thorell, 1865 (Crustacea, Branchiura). *Acta Parasitologica* 49: 336-347.

Khan, R.A., E.M. Lee and D. Barker. 1990. *Lernaeocera branchialis*: a potential pathogen to cod ranching. *Journal of Parasitology* 76: 913-917.

Kock, K.H. and H. Möller. 1977. On the occurrence of the parasitic copepod *Eubrachiella antarctica* on some Antarctic fish. *Archiv für Fischereiwissenschaft* 28: 149-156.

Kocyłowski, B. 1954. Ergasilosis. *Gospodarka Rybna* 6: 16-17. (In Polish).

Kocyłowski, B. and T. Miączyński. 1960. *Choroby ryb i raków* [*Diseases of Fish and Crayfish.*] PWRiL, Warszawa. (In Polish).

Kozikowska, Z. 1975. *Ergasilus sieboldi* Nordm., przystosowanie pasożyta do morfologii i biologii żywicieli oraz uzupełnienie danych o występowaniu innych gatunków pasożytniczych skorupiaków na terenie Mazur i Warmii. [*Ergasilus sieboldi* Nordm., adaptations of the parasite to the morphology and biology of hosts and additional data on the occurrence of other species of parasitic crustaceans in the area of Masuria and Warmia.] *Acta Universitatis Wratislaviensis, Prace Zoologiczne* 7: 3-40. (In Polish).

Kozikowska, Z., Z. Jara and E. Grabda. 1956. *Achtheres percarum* Nordm. sur la perche et la sandre. (Essai d'explication de la relation mutuelle des formes *percarum* et *sandrae*). *Zoologica Poloniae* 7: 219-267. (In Polish).

Krkošek, M., M.A. Lewis, A. Morton, L.N. Frazer and J.P. Volpe. 2006. Epizootics of wild fish induced by farm fish. *Proceedings of the National Academy of Sciences of the United States of America* 103: 15506-5510.

Kroger, R.L. and J.F. Guthrie. 1972. Occurrence of the parasitic branchiuran *Argulus alosae*, on dying Atlantic menhaden, *Brevoortia tyrannnus*, in the Connecticut River. *Transactions of the American Fisheries Society* 101: 559-560.

Kruger, I., J.G. van As and J.E. Saayman. 1983. Observations on the occurrence of the fish louse *Argulus japonicus* Thiele, 1900 in the western Transvaal. *South African Journal of Zoology* 18: 408-410.

La Marre, E. and P.A. Cochran. 1992. Lack of host species selection by the exotic parasitic crustacean, *Argulus japonicus*. *Journal of Freshwater Ecology* 7: 77-80.

Landsberg, J.H. 1989. Parasites and associated diseases of fish in warm water culture with special emphasis on intensification. In: *Fish Culture in Warm Water Systems: Problems and Trends*, M. Shilo and S. Sarig (eds.). CRC Press, Boca Raton, pp. 195-252.

Lehmann, C. 1924. Das Hechtsterben in Jamunder See bei Köslin. Mitteilungen Fischerei Vereine Provinzen Brandenburg, Ostpreußen, *Pommern Grenzmark* 6: 261-263. (In German).

Lester, R.J.G. and F.R. Roubal. 1995. Phylum Arthropoda. In: *Fish Diseases and Disorders*, P.T.K. Woo (ed.). CABI Publishing, Wallingford, Oxon, U.K., Vol. 1, pp. 475-598.

Lin, C.L. and J.-s. Ho. 1993. Life history of *Caligus epidemicus* Hewitt, parasitic on tilapia (*Oreochromis mossambicus*) cultured in brackish water. In: *Pathogens of Wild and Farmed Fish: Sea Lice*, G.A. Boxshall and D. Defaye (eds.). Ellis Horwood, Chichester, pp. 5-15.

Lin, C.L. and J.-s. Ho. 1998. Two new species of ergasilid copepods parasitic on fishes cultured in brackish water in Taiwan. *Proceedings of the Biological Society of Washington* 111: 15-27.

Lutsch, E. and A. Avenant-Oldewage. 1995. The ultra structure of the newly hatched *Argulus japonicus* Thiele, 1900 larvae (Branchiura). *Crustaceana* 68: 229-340.

Lysne, D.A. and A. Skorping. 2002. The parasite *Lernaeocera branchialis* on caged cod: Infection pattern is caused by differences in host susceptibility. *Parasitology* 124: 69-76.

Mamani, M., C. Hamel and P.A. van Damme. 2004. Ectoparasites (Crustacea: Branchiura) of *Pseudoplatystoma fasciatum* (surubí) and *P. tigrinum* (chuncuina) in Bolivian white-water floodplains. *Ecologia en Bolivia* 39(2): 9-20.

McGladdery, S.E. and C.E. Johnston. 1988. Egg development and control of the gill parasite *Salmincola salmoneus*, on Atlantic salmon kelts (*Salmo salar*) exposed to four different regimes of temperature and photoperiod. *Aquaculture* 68: 193-202.

Markevich, A.P. 1956. Parazitičeskie veslonogie ryb SSSR. [*Parasitic Copepods of Fishes of the USSR*] *Izdatel'stvo Akademii Nauk Ukrainskoj SSR*, Kiev. (In Russian).

Martin, J.W. and G.E. Davis. 2001. An updated classification of the recent Crustacea. *Natural History Museum of Los Angeles County, Science Series* 39: 1-124.

McBeath, A.J.A., M.J. Penston, M. Snow, P.F. Cook, I.R. Bricknell and C.O. Cunningham. 2006. Development and application of real-time PCR for the specific detection of *Lepeophtheirus salmonis* and *Caligus elongatus* larvae in Scottish plankton samples. *Diseases of Aquatic Organisms* 73: 141-150.

McMahon, T. 2000. Regulation and monitoring of marine aquaculture in Ireland. *Journal of Applied Ichthyology* 16: 177-181.

Menezes, J., M.A. Ramos, T.G. Pereira and A. Moreira da Silva. 1990. Rainbow trout culture failure in a small lake as a result of massive parasitosis related to careless fish introductions. *Aquaculture* 89: 123-126.

Meyer-Rochow, V.B. and E. Keskinen. 2001. Photoreception in fishlice (Branchiura): the eyes of *Argulus foliaceus* Linne, 1758 and *A. coregoni* Thorell, 1865. *Acta Parasitologica* 46: 321-331.

Mikheev, V.N., E.T. Valtonen and P. Rintamaki-Kinnunen. 1998. Host searching in *Argulus foliaceus* L. (Crustacea: Branchiura): The role of vision and selectivity. *Parasitology* 116: 425-430.

Mikheev, V.N., A.V. Mikheev, A.F. Pasternak and E.T. Valtonen. 2000. Light-mediated host searching strategies in a fish ectoparasite, *Argulus foliaceus* L. (Crustacea: Branchiura). *Parasitology* 120: 409-416.

Mikheev, V.N., A.F. Pasternak, E.T. Valtonen and Y. Lankien. 2001. Spatial distribution and hatching of overwintered eggs of a fish ectoparasite, *Argulus coregoni* (Crustacea: Branchiura). *Diseases of Aquatic Organisms* 46: 123-128.

Mikheev, V.N., A.F. Pasternak and E.T. Valtonen. 2004. Tuning host specificity during the ontogeny of a fish ectoparasite: Behavioural responses to host-induced cues. *Parasitology Research* 92: 220-224.

Molnár, K. and C. Székely. 1997. An unusual location for *Ergasilus sieboldi* Nordmann (Copepoda, Ergasilidae) on the operculum and base of pectoral fins of the pikeperch (*Stizostedion lucioperca* L.). *Acta Veterinaria Hungarica* 45: 165-175.

Molnár, K. and F. Moravec. 1997. *Skrjabillanus cyprini* n.sp. (Nematoda: Dracunculoidea) from the scales of common carp *Cyprinus carpio* (Pisces) from Hungary. *Systematic Parasitology* 38: 147-151.

Molnár, K. and C. Székely. 1998. Occurrence of skrjabillanid nematodes in fishes of Hungary and in the intermediate host, *Argulus foliaceus* L. *Acta Veterinaria Hungarica* 46: 451-463.

Möller, H. and K. Anders. 1986. *Diseases and Parasites of Marine Fishes*. Verlag Heino Moller, Kiel.

Møller, O.S., J. Olesen and D. Waloszek. 2007. Swimming and cleaning in the free-swimming phase of *Argulus larvae* (Crustacea: Branchiura) – Appendage adaptation and functional morphology. *Journal of Morphology* 268: 1-11.

Moran, J.D.W. and W. Piasecki. 1994. External morphology of the male and female of *Sphyrion lumpi* (Krøyer, 1845) (Copepoda; Siphonostomatoida; Sphyriidae). *Hydrobiologia* 292/293: 171-178.

Moravec, F. 1978. First record of *Molnaria erythrophthalmi* larvae in the intermediate host in Czechoslovakia. *Folia Parasitologica* 25: 141-142.

Moravec, F., V. Vidal-Martinez and L. Aguirre-Macedo. 1999. Branchiurids (*Argulus*) as intermediate hosts of the daniconematid nematode *Mexiconema cichlasomae*. *Folia Parasitologica* 46: 79.

Morton, A. and R. Williams. 2003. First report of the sea louse, *Lepeophtheirus salmonis*, infestation on juvenile pink salmon, *Oncorhynchus gorbuscha*, in nearshore habitat. *Canadian Field-Naturalist* 117: 634-641.

Morton, A. and R.D. Routledge. 2005. Mortality rates for juvenile pink *Oncorhynchus gorbuscha* and chum *O. keta* salmon infested with sea lice *Lepeophtheirus salmonis* in the Broughton Archipelago. *Alaska Fishery Research Bulletin* 11: 146-152.

Morton, A. and R.D. Routledge. 2006. Fulton's condition factor: Is it a valid measure of sea lice impact on juvenile salmon? *North American Journal of Fisheries Management* 26: 56-62.

Morton, A., R. Routledge, C. Peet and A. Ladwig. 2004. Sea lice (*Lepeophtheirus salmonis*) infection rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the nearshore marine environment of British Columbia, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* 61: 147-157.

Morton, A., R. Routledge and R. Williams. 2005. Temporal patterns of sea louse infestation on wild Pacific salmon in relation to the fallowing of Atlantic salmon farms. *North American Journal of Fisheries Management* 25: 811-821.

Mustafa, A., W. Rankaduwa and P. Campbell. 2001. Estimating the cost of sea lice to salmon aquaculture in eastern Canada. *Canadian Veterinary Journal* 42: 54-56.

Musselius, V.A. 1973. Parazity i bolezni rastitel'noâdnyh ryb dal'nevostočnogo kompleksa vod prudovyh SSSR [Parasites and diseases of phytophagous fishes of the far-east complex in pond farms of the USSR]. *Trudy vsesoûznogo naučno-issledovatel'skogo Instituta prudovogo rybnogo hozâjstva* 22: 4-129. (In Russian).

Nagasawa, K., J.R. Watanabe, S. Kimura and A. Hara. 1994. Infection of *Salmincola stellatus* (Copepoda: Lernaeopodidae) on Sakhalin taimen *Hucho perryi* reared in Hokkaido. *Bulletin of the Faculty of Fisheries, Hokkaido University* 45: 109-112.

Nagasawa, K., K. Ikuta and S. Kitamura. 1997. Distribution of *Salmincola carpionis* (Copepoda: Lernaeopodidae) in the buccal cavity of salmonids. *Bulletin of the National Research Institute of Aquaculture* 26: 35-39.

Nagasawa, K., K. Ikuta, H. Nakamura, T. Shikama and S. Kitamura. 1998. Occurrence and effects of the parasitic copepod *Salmincola carpionis* on salmonids in the Nikko District, central Japan. *Journal of Marine Systems* 15: 269-272.

Nakajima, K. and S. Egusa. 1973. Parasitic copepod, *Pseudoergasilus zacconis* Yamaguti, found on the gills of cultured ayu, *Plecoglossus altivelis*. I. Morphology. *Fish Pathology (Gyobyu Kenkyo)* 8: 106-110.

Natarajan, P. and N. Balakrishnan Nair. 1976. Effects of infestation by *Lernaeenicus hemirhamphi* Kirtisinghe on the biochemical composition of the host fish

*Hemirhamphus xanthopterus* (Val.). *Journal of Animal Morphology and Physiology* 23: 25-31.

Nese, L. and O. Enger. 1993. Isolation of *Aeromonas salmonicida* from salmon lice *Lepeophtheirus salmonis* and marine plankton. *Diseases of Aquatic Organisms* 16: 79-81.

Noble, E.R. and S.B. Collard. 1970. The parasites of midwater fishes. In: *A Symposium on Diseases of Fishes and Shellfishes*, S.F. Snieszko (ed.). *American Fisheries Society, Washington, D.C., Special Publication No.* 5, pp. 57-68.

Nolan, D.T., P. Reilly and S. Wendelaar Bonga. 1999. Infection with low numbers of the sea louse *Lepeophtheirus salmonis* induces stress-related effects in post-smolt Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 56: 947-959.

Nolan, D.T., N.M. Ruane, Y. van der Heijden, E.S. Quabius, J. Costelloe and S.E. Wendelaar Bonga. 2000. Juvenile *Lepeophtheirus salmonis* (Krøyer) affect the skin and gills of rainbow trout *Oncorhynchus mykiss* (Walbaum) and the host response to a handling procedure. *Aquaculture Research* 31: 823-833.

Northcott, S.J. 1997. An outbreak of freshwater fish lice *Argulus japonicus* L., seriously affecting a Scottish Stillwater fishery. *Fisheries Management Ecology* 4: 73-75.

Nylund, A., C. Wallace and T. Hovland. 1993. The possible role of *Lepeophtheirus salmonis* (Krøyer) in the transmission of infectious salmon anemia. In: *Pathogens of Wild and Farmed Fish: Sea Lice*, G.A. Boxshall and D. Defaye (eds.). Ellis Horwood, Chichester, pp. 367-373.

Overstreet, R.M., I. Dykova and W.E. Hawkins. 1992. Branchiura. In: *Microscopic Anatomy of Invertebrates, Crustacea*, F.W. Harrison and A.G. Humes (eds.). Wiley-Liss Inc., Wilmington, Vol. 9, pp. 385-413.

Øines, Ø. and P.A. Heuch. 2007. *Caligus elongatus* Nordmann genotypes on wild and farmed fish. *Journal of Fish Diseases* 30: 81-91.

Øines, Ø., J.H. Simonsen, J.A. Knutsen and P.A. Heuch. 2006. Host preference of adult *Caligus elongatus* Nordmann in the laboratory and its implications for Atlantic cod aquaculture. *Journal of Fish Diseases* 29: 167-174.

Padmavathi, P. and M.K.D. Prasad. 1998. An effective and economically feasible treatment of organophosphate pesticide and common salt to eradicate the fish ectoparasite, *Argulus japonicus* Thiele in carp culture ponds. *Journal of Environmental Biology* 19: 193-203.

Pan, J., T. Yang and G. Xu. 1979. Studies on the biology of the parasitic copepod *Lernaea polymorpha* of silver carp (*Hypophthalmichthys molitrix*) and big-head carp (*Aristichthys nobilis*) and its control. *Acta Hyrobiologica Sinica* 6: 377-391. (In Chinese with English Summary).

Paperna, I. 1975. Parasites and disease of the grey mullet (Muglidae) with special reference to the seas of the near east. *Aquaculture* 5: 65-80.

Paperna, I. 1996. Parasites, infections and diseases of fishes in Africa—An update. *FAO, Rome, CIFA Technical Paper* 31.

Pasternak, A.F., V.N. Mikheev and E.T. Valtonen. 2000. Life history characteristics of *Argulus foliaceus* L. (Crustacea: Branchiura) populations in Central Finland. *Annales Zoologici Fennici* 37: 25-35.

Pasternak, A.F., V. Mikheev and E.T. Valtonen. 2004. Growth and development of *Argulus coregoni* (Crustacea: Branchiura) on salmonid and cyprinid hosts. *Diseases of Aquatic Organisms* 58: 203-207.

Perkins, P.S. 1983. The life history of *Cardiodectes medusaeus* (Wilson), a copepod parasite of lanternfishes (Myctophidae). *Journal of Crustacean Biology* 3: 70-87.

Pfeil-Putzien, C. 1977. New results in the diagnosis of spring viraemia of carp caused by experimental transmission of *Rhabdovirus carpio* with carp louse (*Argulus foliaceus*). *Bulletin de l'Office International des Epizooties* 87: 457.

Pfeil-Putzien, C. and C. Baath. 1978. Demonstration of *Rhabdovirus carpio* infection among carp in the autumn. *Berliner und Münchener Tierarztliche Wochenschrift* 91: 445-447.

Piasecki, W. 1989. Life cycle of *Tracheliastes maculatus* Kollar, 1835 (Copepoda, Siphonostomatoida, Lernaeopodidae). *Wiadomoœci Parazytologiczne* 35: 187-245.

Piasecki, W. 1991. Tracheliastoza leszcza *Abramis brama* (L.) w jeziorze Pierzchalskim koło braniewa. [Tracheliastosis of bream, *Abramis brama* (L.) in Pierzchalskie Lake near Braniewo.] Materiały *XVI Zjazdu, Polskie Towarzystwo Parazytologiczne, Poznań, Poland,* 12–13 September 1991, p. 28. (In Polish).

Piasecki, W. 1993a. Comparative morphology of the three species of *Lamproglena* (Copepoda, Cyclopoida, Lernaeidae) described by von Nordmann, based on re-examination of the types. *Mitteilungen aus dem Zoologischen Museum in Berlin* 69: 307-315.

Piasecki, W. 1993b. Crustacean parasites from three fish species from the Odra River estuary, Poland. *Acta Ichthyologica et Piscatoria* 23: 77-87.

Piasecki, W. 1996. The developmental stages of *Caligus elongatus* von Nordmann, 1832 (Copepoda, Siphonostomatoida, Caligidae). *Canadian Journal of Zoology* 74: 1459-1478.

Piasecki, W. and E. Kuźmińska. 2007. Developmental stages of *Achtheres percarum* (Crustacea: Copepoda), parasitic on European perch, *Perca fluviatilis* (Actinopterygii: Perciformes). *Acta Ichthyologica et Piscatoria* 37: 117-128.

Piasecki, W. and B.M. MacKinnon. 1993. Changes in structure of the frontal filament in sequential developmental stages of *Caligus elongatus* von Nordmann, 1832 (Crustacea: Copepoda: Siphonostomatoida). *Canadian Journal of Zoology* 71: 889-895.

Piasecki, W. and B.M. MacKinnon. 1995. Life cycle of the sea louse, *Caligus elongatus* von Nordmann, 1832 (Copepoda, Siphonostomatoida, Caligidae). *Canadian Journal of Zoology* 73: 74-82.

Piasecki, W. and A. Wołoszyn. 1991. Masowa inwazja *Achtheres percarum* von Nordmann, 1832 (Copepoda, Siphonostomatoida, Lernaeopodidae) u okonia *Perca fluviatilis* L. w wodach ujœciowych Odry. [A mass infection of *Achtheres percarum* von Nordmann, 1832 (Copepoda, Siphonostomatoida, Lernaeopodidae) in perch *Perca fluviatilis* in the estuary waters of the Odra River.] *Materiały XVI Zjazdu, Polskie Towarzystwo Parazytologiczne,* Poznań, Poland, 12–13 September 1991, pp. 27. (In Polish).

Piasecki, W., N.R. Khamees and F.T. Mhaisen. 1991. A new species of *Mugilicola* Tripathi, 1960 (Crustacea, Copepoda, Therodamasidae) parasitic on Iraqi fish. *Acta Ichthyologica et Piscatoria* 21(2): 143-151.

Piasecki, W. and C.J. Hayward. 2002. Redescription of the fish parasite *Lernanthropus polynemi* Richiardi, 1881 (Copepoda: Siphonostomatoida) and relegation of two congeners to synonymy. *Systematic Parasitology* 52: 137-144.

Piasecki, W., A.E. Goodwin, J.C. Eiras and B.F. Nowak. 2004. Importance of copepoda in freshwater aquaculture. *Zoological Studies* 43: 193-205.

Piasecki, W., A. Sękowska-Jakubowska and E. Sobecka. 2006. Comparative description of males of two species of *Achtheres* von Nordmann, 1832 (Copepoda: Siphonostomatoida: Lernaeopodidae) infecting zander and European perch. *Folia Parasitologica* 53: 211-216.

Pike, A.W. 1989. Sea lice—major pathogens of farmed Atlantic salmon. *Parasitology Today* 5: 291-297.

Pike, A.W. and S.L. Wadsworth. 1999. Sea lice on salmonids: their biology and control. *Advances in Parasitology* 44: 233-337.

Poddubnaâ, A.V. [Poddubnaya, A.V.] 1973. Izmenčivost' i specifičnost' lernej prudovyh ryb. [Variability and specificity of *Lernaea* from pond fishes.] *Trudy Vsesoûznogo naučno-issledovatel'skogo instituta prudovogo rybnogo hozâjstva* 22: 159-173. (In Russian).

Poly, W.J. 1997. Host and locality records of the fish ectoparasite, *Argulus* (Branchiura), from Ohio (USA). *Crustaceana* 70: 867-874.

Poly, W.J. 1998a. New state, host, and distribution records of the fish ectoparasite, *Argulus* (Branchiura), from Illinois (USA). *Crustaceana* 71: 1-8.

Poly, W.J. 1998b. *Argulus purpureus* (Risso, 1827), a junior synonym of *Argulus vittatus* (Rafinesque-Schmaltz, 1814) (Branchiura, Arguloida). *Crustaceana* 71: 628-631.

Poly, W.J. 2003. *Argulus ambystoma*, a new species parasitic on the salamander *Ambystoma dumerilii* from Mexico (Crustacea: Branchiura: Argulidae). *Ohio Journal of Science* 103: 52-61.

Poulin, R. and G.J. FitzGerald. 1987. The potential of parasitism in the structuring of a salt marsh stickleback community. *Canadian Journal of Zoology* 65: 2793-2798.

Poulin, R. and G.J. FitzGerald. 1988. Water temperature, vertical distribution, and risk of ectoparasitism in juvenile sticklebacks. *Canadian Journal of Zoology* 66: 2002-2005.

Poulin, R. and G.J. FitzGerald. 1989. Male-biased sex ratio in *Argulus canadensis* Wilson, 1916 (Crustacea: Branchiura) ectoparasitic on sticklebacks. *Canadian Journal of Zoology* 67: 2078-2080.

Prost, M. 1980. *Choroby ryb. [Fish Diseases]* PWRiL, Warszawa. (In Polish).

Puffer, H.W. and M.L. Beal. 1981. Control of parasitic infestations in killifish (*Fundulus parvipinnis*). *Laboratory Animal Science* 31: 200-201.

Reimer, L.W. 1991. *Sarcotaces namibiensis* sp. nov., the first evidence of the genus *Sarcotaces* Olsson, 1872, in the South Atlantic (Copepoda). *Crustaceana* 60: 141-144.

Roberts, R.J., K.A. Johnson and M.T. Casten. 2004. Control of *Salmincola californiensis* (Copepoda: Lernaeapodidae [sic]) in rainbow trout, *Oncorhynchus mykiss* (Walbaum): A clinical and histopathological study. *Journal of Fish Diseases* 27: 73-79.

Rae, G.H. 2002. Sea louse control in Scotland, past and present. *Pest Management Science* 58: 515-520.

Rafi, F. 1988. Isopoda. In: *Guide to the Parasites of Fishes of Canada. Part II – Crustacea*, L. Margolis and Z. Kabata (eds.). *Canadian Special Publication of Fisheries and Aquatic Sciences* 101, pp. 129-148.

Rahman, M.M. 1995. Some aspects of the biology of a freshwater fish parasite *Argulus foliaceus* (L.) (Argulidae, Branchiura, Crustacea). *Bangladesh Journal of Zoology* 23: 77-86.

Rahman, M.M. 1996. Effects of a freshwater fish parasite, *Argulus foliaceus* Linn. infection on common carp, *Cyprinus carpio* Linn. *Bangladesh Journal of Zoology* 24: 57-63.

Revie, C.W., G. Gettinby, J.W. Treasurer and G.H. Rae. 2002. The epidemiology of the sea lice, *Caligus elongatus* Nordmann, in marine aquaculture of Atlantic salmon, *Salmo salar* L., in Scotland. *Journal of Fish Diseases* 25: 391-399.

Revie, C.W., C. Robbins, G. Gettinby, L. Kelly and J.W. Treasurer. 2005. A mathematical model of the growth of sea lice, *Lepeophtheirus salmonis*, populations on farmed Atlantic salmon, *Salmo salar* L., in Scotland and its use in the assessment of treatment strategies. *Journal of Fish Diseases* 28: 603-613.

Ringuelet, R. 1943. Revision de los Argúlidos Argentinos (Crustácea: Branchiura) con el catallogo de las espesies neotropicales. *Revta Musee La Plata (Seccion Zoología)* 3: 43-99.

Ritchie, G. 1997. The host transfer ability of *Lepeophtheirus salmonis* (Copepoda: Caligidae) from farmed Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 20: 153-157.

Ritchie, G., A.J. Mordue (Luntz), A.W. Pike and G.H. Rae. 1993. The reproductive output of *Lepeophtheirus salmonis* adult females in relation to seasonal variability of temparature and photoperiod. In: *Pathogens of Wild and Farmed Fish: Sea Lice*, G.A. Boxshall and D. Defaye (eds.). Ellis Horwood, Chichester, pp. 153-165.

Rohde, K. (ed.) 2005. *Marine Parasitology*. CABI Publishing, Wallingford, Oxon, U.K.

Rokicki, J. 1976. Aegidae dorsza (*Gadus morhua*) na obszarze ICNAF (Północno zachodni Atlantyk). [Aegidae of cod (*Gadus morhua*) within the ICNAF area (North-western Atlantic).] *Wiadomości Parazytologiczne* 22: 303-307. (In Polish).

Rokicki, J. 1986. Widłonogi ryb łososiowatych Zatoki Puckiej. [Copepods of the salmonid fishes of the Gulf of Puck.] *Wiadomości Parazytologiczne* 32: 509–510. (In Polish).

Rosenthal, H. 1967. Parasites in larvae of the herring (*Clupea harengus* L.) fed with wild plankton. *Marine Biology* 1: 10-15.

Roth, M., G. Rae, A.S. McGill and K.W. Young. 1993. Current practices in the chemotherapeutic control of sea lice infections in aquaculture: A review. *Journal of Fish Diseases* 16: 1-26.

Rudomëtova, N.K. 1974. K biologii *Skrjabillanus amuri* Garkawi, 1972 (Cammallanata: Skrabillanidae)—parazita belogo amura (*Ctenopharyngodon idella*). [The biology of *Skrjabillanus amuri* Garkawi, 1972 (Camallanata: Skrjabillanidae), a parasite of *Ctenopharyngodon idella*.] *Sbornik naučnyh trudov Vsesoûznogo naučno-issledovatel'skogo instituta prudovogo rybnogo hozâjstva* 1974 (11): 298-305. (In Russian with English abstract).

Rumphorst, H. 1924. Über Ergasiliden. (Schmarotzer-Krebschen). Mitteilungen Fischerei Vereine Provinzen Brandenburg, Ostpreußen, *Pommern Grenzmark* 16: 305-308. (In German).

Rushton-Mellor, S.K. 1992. Discovery of the fish louse, *Argulus japonicus* Thiele (Crustacea: Branchiura), in Britain. *Aquaculture and Fisheries Management* 23: 269-271.

Rushton-Mellor, S.K. 1994a. The genus *Argulus* (Crustacea: Branchiura) in Africa: Identification keys. *Systematic Parasitology* 28: 51-63.

Rushton-Mellor, S.K. 1994b. The genus *Argulus* (Crustacea: Branchiura) in Africa: redescriptions of type-material collected by W.A. Cunnington during the Lake Tanganyika Expedition in 1913, with notes on A. *giganteus* Lucas and A. *arcassonensis* Cuenot. *Systematic Parasitology* 28: 33-49.

Rushton-Mellor, S.K. and G.A. Boxshall. 1994. The developmental sequence of *Argulus foliaceus* (Crustacea: Branchiura). *Journal of Natural History* 28: 763-785.

Sabatini, M.A., M. Marini, B. Fratello, C. Gelosini, L. Mola and I. Benedetti. 1988. The digestive apparatus of the parasitic copepod *Lernaea cyprinacea* L. during development and in the adults. *Acta embryologiae et morphologiae experimentalis* 8: 389-409.

Sarig, S. 1971. *Diseases of Fishes. Book 3: The Prevention and Treatment of Diseases of Warmwater Fishes under Subtropical Conditions, with Special Emphasis on Intensive Fish Farming*. T.F.H. Publications, Inc. Neptune City, N.J.

Sazima, I. and F.A. Machado. 1990. Underwater observations of piranhas in Western Brazil. *Environmental Biology of Fishes* 28: 17-31.

Schäperclaus, W. 1954. *Fischkrankheiten*. 3 Auflage. Berlin: Akademie-Verlag. (In German).

Schäperclaus, W. 1992. *Fish Diseases* (English translation of 'Fischkrankheiten', 5th corrected and substantially enlarged edition 1986). A.A. Balkema, Rotterdam.

Schram, T.A. 1979. The life history of the eye-maggot of the sprat, *Lernaeenicus sprattae* (Sowerby) (Copepoda, Lernaeoceridae). *Sarsia* 64: 279-316.

Schram, T.A. 1993. Supplementary descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). In: *Pathogens of Wild and Farmed Fish: Sea Lice*, G.A. Boxshall and D. Defaye (eds.). Ellis Horwood, Chichester, pp. 30-47.

Schram, T.A. 2004. Practical identification of pelagic sea lice larvae. *Journal of the Marine Biological Association of the United Kingdom, Plymouth* 84: 103-110.

Schuurmans Stekhoven, J.H. 1936. De biologie van *Lernaeocera branchialis*. [The biology of *Lernaeocera branchialis*.] *Natuurwetenschappelijk Tijdschrift* 18: 157-161. (In Dutch).

Shafir, A. and J.G. van As. 1986. Laying, development and hatching of eggs of the ectoparasite *Argulus japonicus* (Crustacea: Branchiura). *Journal of Zoology (London)* 210: 401-414.

Shafir, A. and W.H. Oldewage. 1992. Dynamics of a fish ectoparasite population: Opportunistic parasitism in *Argulus japonicus* (Branchiura). *Crustaceana* 62: 50-64.

Shariff, M., Z. Kabata and C. Sommerville. 1986. Host susceptibility to *Lernaea cyprinacea* L. and its treatment in a large aquarium system. *Journal of Fish Diseases* 9: 393-401.

Shed'ko, M.B. 2004. Novyj vid parazitičeskoj kopepody *Salmincola mica* sp. n. (Lernaeopodidae) s obyknovennogo valka, *Prosopium cylindraceum* (Coregonidae), iz reki Anadyr'. [New species of the parasitic copepod *Salmincola mica* sp. n. (Lernaeopodidae) from the round whitefish *Prosopium cylindraceum* (Coregonidae), of the Anadyr River.] *Vestnik Zoologii* 38: 39-45. (In Russian).

Shed'ko, M.B. 2006. *Salmincola germani* – novyj dlâ nauki vid parazitičeskoj kopepody (Copepoda, Lernaeopodidae) ryb roda *Thymmalus* (Salmoniformes, Thymmallidae) iz ûga Dal'nego Vostoka Rossii. [*Salmincola germani* is a new parasitic copepod (Copepoda, Lernaeopodidae) species of fishes of the genus *Thymallus*

(Salmoniformes, Thymallidae) from the southern Russian Far East.] *Zoologičeskij Žurnal* 85: 435-440. (In Russian).

Shields, R.J. and R.G. Sperber. 1974. Osmotic relationships of *Lernaea cyprinacea* L. *Crustaceana* 26: 157-161.

Shimura, S. and M. Asai. 1984. *Argulus americanus* (Crustacea: Branchiura) parasitic on the bowfin, *Amia calva*, imported from North America. *Fish Pathology* 18: 199-203.

Shimura, S., K. Inoue, K. Kasai and M. Saito. 1983. Hematological changes of *Oncorhynchus masou* (Salmonidae) caused by the infection of *Argulus coregoni* (Crustacea: Branchiura). *Fish Pathology* 18: 157-162.

Singhal, R.N., S. Jeet and R.W. Davies. 1990. The effects of argulosis-saprolegniasis on the growth and production of *Cyprinus carpio*. *Hydrobiologia* 202: 27-31.

Sinnott, R. 1999. Cost of sea lice to Scottish salmon farmers. *Trouw Outlook* 11: 8-10.

Smit, N.J., L. Basson and J.G. van As. 2003. Life cycle of the temporary fish parasite, *Gnathia africana* (Crustacea: Isopoda: Gnathiidae). *Folia Parasitologica* 50: 135-142.

Smith, J.L., R. Wootten and C. Sommerville. 2007. The pathology of the early stages of the crustacean parasite, *Lernaeocera branchialis* (L.), on Atlantic cod, *Gadus morhua* L. *Journal of Fish Diseases* 30: 1-11.

Smith, P.R., M. Moloney, A. McElligott, S. Clarke, R. Palmer, J. O'Kelley and F. O'Brien. 1993. The efficiency of oral ivermectin in the control of sea lice infestations of framed Atlantic salmon. In: *Pathogens of Wild and Farmed Fish: Sea Lice*, G.A. Boxshall and D. Defaye (eds.). Ellis Horwood, Chichester, pp. 296-310.

Sproston, N.G. 1942. The developmental stages of *Lernaeocera branchialis* (Linn.). *Journal of the Marine Biological Association of the United Kingdom, Plymouth* 25: 441-466.

Stammer, J. 1959. Beiträge zur Morphologie, Biologie und Bekämpfung der Karfenläuse. *Zeitschrift für Parasitenkunde* 19: 135-208.

Stuhlmann, F. 1891. Zur kenntniss der Fauna central-afrikanischer Seen II. Ueber eine neue Art der Arguliden-Gattung *Gyropeltis*. *Zoologische Jahrbucher, Abteilung für Systematik* 5: 152-154.

Sundara-Bai, A., D. Seeneappa and K.V. Devaraj. 1988. Occurrence of fish lice in freshwater fishes. *Current Research (University of Agricultural Sciences, Bangalore)* 17(10): 137-138.

Sutherland, D.R. and D.D. Wittrock. 1986. Surface topography of the branchiuran *Argulus appendiculosus* Wilson, 1907 as revealed by scanning electron microscopy. *Zeitschrift für Parasitenkunde* 72: 405-415.

Swanepoel, J.H. and A. Avenant-Oldewage. 1992. Comments on the morphology of the pre-oral spine in *Argulus* (Crustacea: Branchiura). *Journal of Morphology* 212: 155-162.

Swanepoel, J.H. and A. Avenant-Oldewage. 1993. Functional morphology of the foregut of *Chonopeltis australis* Boxshall (Branchiura). *Journal of Crustacean Biology* 13: 656-666.

Tam, Q. and A. Avenant-Oldewage. 2006. The digestive system of larval *Argulus japonicus* (Branchiura). *Journal of Crustacean Biology* 26: 447-454.

Tamuli, K.K. and S.L. Shanbhogue. 1995. Biological control of *Lernaea* L. infection employing *Oreochromis mossambica*, Peters. *Journal of the Assam Science Society* 37: 123-128.

Tang, D., M. Andrews and J.M. Cobcroft. 2007. The first chondracanthid (Copepoda: Cyclopoida) reported from cultured finfish, with a revised key to the species of *Chondracanthus*. *Journal of Parasitology* 93: 788-795.

Tavares-Dias, M., M.L. Martins and S. do N. Kronka. 1999. Evaluation of the haematological parameters in *Piaractus mesopotamicus* Holmberg (Osteichthyes, Characidae) with *Argulus* sp. (Crustacea, Branchiura) infestation and treatment with organophosphate. *Revista Brasileira de Zoologia* 16: 553-555.

Taylor, N.G.H., C. Sommerville and R. Wootten. 2006. The epidemiology of *Argulus* spp. (Crustacea: Branchiura) infections in stillwater trout fisheries. *Journal of Fish Diseases* 29: 193-200.

Telfer, T.C., D.J. Baird, J.G. McHenery, J. Stone, I. Sutherland and P. Wislocki. 2006. Environmental effects of the anti-sea lice (Copepoda: Caligidae) therapeutant emamectin benzoate under commercial use conditions in the marine environment. *Aquaculture* 260: 163-180.

Tihomirova, V.A. [Tikhomirova, V.A.] 1983. Karpoedy—promežutočnye hozâeva nematod sem. Skrjabillanidae. [Carp lice—intermediate hosts of nematodes of the family Skrjabillanidae.] In: *Vliânie antropogennyh faktorov na strukturu i funkcionirovanie èkosistem*. [*The effect of antropogenic factors on the structure and functioning of ecosystems*.] KGU, Kalinin, pp. 104-109. (In Russian).

Tóro, R.M., A.A.F. Gessner, N.A.J.C. Furtado, P.S. Ceccarelli, S. Albuquerque and J.K. Bastos. 2003. Activity of the *Pinus elliottii* resin compounds against *Lernaea cyprinacea* in vitro. *Veterinary Parasitology* 118: 143-149.

Tsotetsi, A.M., A. Avenant-Oldewage and S.N. Mashego. 2005. Aspects of the pathology of *Lamproglena clariae* (Copepoda: Lernaeidae) on gills of *Clarias gariepinus* from the Vaal River system, South Africa. *African Zoology* 40: 169-178.

Tully, O. 1989. The succession of generations and growth of the caligid copepod *Caligus elongatus* and *Lepeophtheirus salmonis* parasitising farmed Atlantic salmon smolts (*Salmo salar* L.). *Journal of the Marine Biological Association of the United Kingdom, Plymouth* 69: 279-287.

Tully, O., P. Daly, S. Lysaght, S. Deady and S.J.A. Varian. 1996. Use of cleaner-wrasse (*Centrolabrus exoletus* (L.) and *Ctenolabrus rupestris* (L.)) to control infestations of *Caligus elongatus* Nordmann on farmed Atlantic salmon. *Aquaculture* 142: 11-24.

Van As, J.G. and S.A. Viljoen. 1984. A taxonomic study of sessile peritrichs (Ciliophora: Peritricha) associated with crustacean fish ectoparasites in South Africa. *South African Journal of Zoology* 19: 275-279.

Van As, J.G. and L.L. van As. 2001. Branchiura. In: *Guides to the Freshwater Invertebrates of Southern Africa, Volume 3, Crustacea II*. J.A. Day, I.J. De Moor, B.A. Stewart and A.E. Louw (eds.). *Water Research Commission of South Africa, Pretoria, Publ TT148/01*, pp. 124-159.

Van As, L.L. and J.G. van As. 1996. A new species of *Chonopeltis* (Crustacea: Branchiura) from the southern Rift Valley, with notes on larval development. *Systematic Parasitology* 35: 69-77.

Van Damme, P.A., A. Geets, O. Hamerlynck and F. Ollevier. 1997. The suprapopulation dynamics of *Lernaeocera branchialis* and *L. lusci* in the Oosterschelde: Seasonal abundance on three definitive host species. *ICES (International Council for the Exploration of the Sea) Journal of Marine Science* 54: 24-31.

Van Niekerk, J.P. 1984. *Die Biologie van die genus Chonopeltis Thiele, 1900 (Crustacea: Branchiura) uit die Oranje-Vaal opvanggebied. [The biology of the genus Chonopeltis Thiele, 1900 (Crustacea: Branchiura) from the Orange-Vaal catchment area.]* Ph.D. Thesis, University of the Orange Free State, Bloemfontein, South Africa. (In Afrikaans).

Van Niekerk, J.P. and D.J. Kok. 1989. *Chonopeltis australis* (Branchiura): Structural, developmental and functional aspects of the trophic appendages. *Crustaceana* 57: 52-56.

Vaughan, G.E. and D.W. Coble. 1975. Sublethal effects of three ectoparasites on fish. *Journal of Fish Biology* 7: 283-294.

Walker, P.D. 2002. An investigation into the population structure, distribution, host selection and effects of abiotic factors on the off-host longevity of the crustacean ectoparasite of fish, *Argulus foliaceus* L. (Crustacea: Branchiura). MRes Thesis. University of Plymouth, U.K.

Walker, P.D., G. Flik and S.J. Wendelaar Bonga. 2004. The biology of parasites from the genus *Argulus* and a review of the interactions with its host. In: *Host-Parasite Interactions*, G.F. Wiegertjes and G. Flik (eds.). Garland Science/BOIS Scientific Publishers, Hampshire, pp. 107-129.

Wells, A., C.E. Grierson, M. MacKenzie, I.J. Russon, H. Reinardy, C. Middlemiss, P.A. Bjørn, B. Finstad, S.E. Wendelaar Bonga, C.D. Todd and N. Hazon. 2006. Physiological effects of simultaneous, abrupt seawater entry and sea lice (*Lepeophtheirus salmonis*) infestation of wild, sea-run brown trout (*Salmo trutta*) smolts. *Journal of Fisheries and Aquatic Sciences* 63: 2809-2821.

West, G.A. 1992. Eleven new *Colobomatus* species (Copepoda: Philichthyidae) from marine fishes. *Systematic Parasitology* 23: 81-133.

Williams, C. 1997. Investigation of difluorobenzuron in the control of *Argulus foliaceus* L. in relation to management strategies. M.Sc. Thesis. University of Plymouth, Plymouth, U.K.

Wingstrand, K.G. 1972. Comparative spermatology of a pentastomid, *Raillietiella hemidactyli* and a branchiuran crustacean *Argulus foliaceus* with a discussion of pentastomid relationships. *Det Kongelige Danske Videnskabernes Selskab, Biologiske Skrifter* 19: 3-72.

Witkowski, A. and J. Błachuta. 1980. Występowanie *Basanistes huchonis* (Schrank, 1786) (Copepoda parasitica, Lernaeopodidae) na glowacicy *Hucho hucho* (L.) w Dunajcu. [Occurrence of *Basanistes huchonis* (Schrank, 1786) (Copepoda parasitica, Lernaeopodidae) on the Danube salmon, *Hucho hucho* (L.) in the Dunajec River.] *Przegląd Zoologiczny* 24: 463-467. (In Polish).

Wolfe, B.A., C.A. Harms, J.D. Groves and M.R. Loomis. 2001. Treatment of *Argulus* sp. infestation of river frogs. *Contemporary Topics in Laboratory Animal Science* 40(6): 35-36.

Wootten, R., J.W. Smith and E.A. Needham. 1982. Aspects of the biology of the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. *Proceedings of the Royal Society of Edinburgh* B 81: 185-197.

Yldz, K. and A. Kumantas. 2002. *Argulus foliaceus* infection in a goldfish (*Carassius auratus*). *Israel Journal of Veterinary Medicine* 57: 118-120.

CHAPTER

19

# Non-infectious Diseases: Nutritional Factors

**Delbert M. Gatlin III**

## INTRODUCTION

Aquacultural production of various fish and crustacean species has developed into major industries in many countries, and further development is expected as the demand for fisheries products continues to increase and supply from natural sources becomes more limited. As in more traditional forms of animal production, nutrition plays a critical role in intensive aquaculture because it influences not only production costs but also fish growth, health and waste generation. Development of nutritious and cost-effective diets is dependent on knowing a species' nutritional requirements and meeting those requirements with balanced diet formulations and appropriate feeding practices (Gatlin, 2002). Information concerning the nutritional requirements of cultured fishes has increased rapidly over the last decade as a result of heightened research efforts to support aquacultural production of established and

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emerging species throughout the world. Deficiency signs associated with the lack of specific nutrients have been characterized in many of these requirement studies. Additional information on anti-nutritional factors and various toxic compounds that may be present in feedstuffs or prepared diets has also continued to accumulate. This chapter describes the various pathological conditions associated with deficiencies of specific nutrients due to inadequate dietary supplementation or reduced bioavailability in various fish species. Some general information concerning research methodology will also be discussed briefly before considering each of the major nutrient groups and the pathologies associated with deficiencies. In addition, the potential adverse effects of various toxins or other constituents of dietary origin will be considered.

## NUTRIENT REQUIREMENT METHODOLOGY

General methods for determining nutrient requirements of fish and crustaceans have been described in several publications (e.g., Lovell, 1989; D'Abramo and Castell, 1997). These methods generally entail feeding graded levels of the nutrient under investigation to young, rapidly growing animals under controlled conditions. Measured responses typically include weight gain, feed efficiency and survival, which may be augmented by additional responses such as body composition, biochemical indices or tissue levels of the nutrient under investigation. Minimum dietary requirement estimates are generally obtained by regression analysis of the dose-response curves. Specific deficiency signs associated with inadequate intake of a given nutrient are often characterized in these requirement studies. This information will be described below for the various nutrients.

## ENERGY YIELDING NUTRIENTS

### Proteins and Amino Acids

Fish represent the most diverse group of vertebrates, and considerable variation has been noted in their natural feeding habits. These organisms are typically classified as herbivorous, omnivorous or carnivorous, based on their natural feeding habits. A relationship between natural feeding habits and dietary protein requirements has generally been noted, in which herbivorous and omnivorous species require lower concentrations of dietary protein while carnivorous species require higher concentrations

(NRC, 1993; D'Abramo and Castell, 1997). This appears to be related to the limited use of carbohydrate for energy by carnivorous species which, in turn, are very proficient at using dietary protein for energy. The efficient use of protein for energy is largely attributed to the way in which ammonia from deaminated protein is excreted via the gills with limited energy expenditure.

Determining an animal's minimum dietary requirement for protein or a balanced mixture of amino acids is critical because satisfying these requirements is necessary in order to ensure adequate growth and health of the fish. Providing excessive levels of dietary protein is both economically and environmentally unsound because protein is the most expensive dietary component and levels above that needed to satisfy requirements will result in elevated nitrogenous waste excretion into the receiving waters. Most herbivorous and omnivorous fish and crustaceans evaluated to date have been determined to require crude protein at 25 to 35% of diet, whereas carnivorous species may require crude protein at 40 to 50% (NRC, 1993). Determination of individual amino acid requirements of a species will provide even more specific information about its protein needs. Quantitative dietary requirements for the 10 indispensable amino acids have been determined for several fish species (NRC, 1993; Cowey, 1994). Inadequate intake of protein or consumption of a diet deficient in one of more indispensable amino acids results in a rather prompt reduction in weight gain and feed efficiency. The severity of such deficiency signs is directly proportional to the degree of nutrient deficiency. If such a deficiency is not corrected within a relatively short period (weeks), the fish's performance will continue to be reduced and ultimately death may occur.

A deficiency of each of the 10 indispensable amino acids will result in impaired growth due to their fundamental role in protein synthesis. However, some specific deficiency signs have also been associated with certain amino acids. In particular, a deficiency of tryptophan has been reported to cause various spinal deformities in salmonids such as scoliosis and lordosis in sockeye salmon *Oncorhynchus nerka* (Halver and Shanks, 1960), rainbow trout *Oncorhynchus mykiss* and chum salmon *Oncorhynchus keta* (Akiyama *et al.*, 1985). These deformities appear to be related to a depletion of 5-hydroxytryptophan in the body or brain (Akiyama *et al.*, 1986). Several other pathologies associated with tryptophan deficiency in rainbow trout include renal calcinosis (Kloppel

and Post, 1975) as well as caudal fin erosion, cataracts and short gill opercula (Poston and Rumsey, 1983). Methionine is another indispensable amino acid which has been reported to cause bilateral cataracts in the eye lens of some fish species such as rainbow trout (Walton *et al.*, 1982; Rumsey *et al.*, 1983; Poston, 1986; Cowey *et al.*, 1992), Arctic char *Salvelinus alpinus* (Simmons *et al.*, 1999) and hybrid striped bass *Morone chysops* × *M. saxatilis* (Keembiyehetty and Gatlin, 1993). However, cataracts have not been consistently observed in all fish species suffering from a deficiency in total sulfur amino acids (NRC, 1993).

## Carbohydrates

Fish do not have a specific dietary requirement for carbohydrates, but the presence of these compounds in diets may provide a rather inexpensive source of energy. However, the ability of fish to utilize dietary carbohydrate for energy varies considerably, with most carnivorous species having more limited ability (Wilson, 1994). The amount of soluble carbohydrate included in prepared diets for carnivorous species is generally less than 20% while diets for omnivorous species generally contain from 25 to 45% soluble carbohydrate. In rainbow trout, high levels of soluble carbohydrate have been shown to increase glycogen accumulation in the liver to such an extent that it impairs hepatic function (Cowey *et al.*, 1977; Hilton and Hodson, 1983).

Fibrous carbohydrate, primarily in the form of cellulose, is essentially indigestible by fish and crustaceans; thus, it does not make a positive contribution to their nutrition. In fact, the level of crude fiber in fish feeds is typically restricted to less than 7% of diet to limit the amount of undigested material entering the culture system.

## Lipids

This nutrient group consists of several different compounds that may contribute to the nutrition of fish and crustaceans. Neutral lipid in the form of triglycerides is important because it provides a concentrated source of energy that is typically well utilized by aquatic species. In addition, dietary lipid supplies essential fatty acids which can not be synthesized by the organism (Sargent *et al.*, 1995). Fatty acids of the linolenic acid (n-3) family are generally predominant over those of the linolenic acid (n-6) family in terms of essentiality to various aquatic

organisms. Many freshwater fish species possess the ability to elongate and desaturate linolenic and linolenic acids to longer chain (20 and 22 carbons), more highly unsaturated fatty acids (HUFA). In contrast, most marine fish and crustaceans require HUFA preformed in the diet. These HUFA are components of cell membrane bilayers in the form of phosphoglycerides, especially in neural tissues of the brain and eye. In addition, they also serve as precursors of steroid hormones and the highly active eicosanoids produced from 20-carbon HUFA (Sargent *et al.*, 2002). Such eicosanoid compounds are autocrine in nature and include cyclic molecules such as prostaglandins, prostacyclins and thromboxanes produced by the action of cyclo-oxygenase, and linear compounds such as leukotrienes and lipoxins initially formed by lipoxygenase enzymes. Eicosanoids are responsible for numerous physiological actions including blood clotting, immunological and inflammatory responses as well as renal function, cardiovascular tone and neural function. However, excess eicosanoid production also occurs in pathological conditions.

As with other macronutrients, a dietary deficiency of essential fatty acids initially results in reduced weight gain but usually after an extended period due to mobilization of essential fatty acids from endogenous tissue lipids (Lee *et al.* 1967). Overt deficiency signs may eventually occur and include various pathologies such as mitochondrial swelling, edema—including increased muscle water content—and degeneration of heart tissue due to impaired membrane function (Castell *et al.*, 1972). Similar pathological conditions have been observed when HUFA in cell membranes undergo peroxidation by oxygen and other organic radicals. Such oxidative damage impairs function and fluidity of cell membranes and may potentially have pathological effects on cells and tissues (Sies, 1991). Fish have an efficient antioxidant system to protect against oxidative damage to tissues containing high levels of n-3 HUFA. However, inadequate intake of antioxidant nutrients (particularly vitamin C, vitamin E and selenium as reviewed in subsequent sections) or consumption of oxidized lipid (also reviewed in a subsequent section) may result in various pathological conditions such as exudative diathesis, erythrocyte fragility, anemia, myopathy of skeletal muscle and lipoid liver degeneration with accumulation of ceroid pigments (review by Sargent *et al.*, 2002).

In recent years, there has been a trend to increase dietary energy density by supplementing more lipids in the diet, especially for some

carnivorous species such as salmon. This dietary manipulation is intended to spare protein for tissue synthesis but may also result in extensive lipid storage in the adipose tissue and in the liver. This hepatic lipid storage caused by energy rich or lipid-rich feeding should be differentiated from pathologically or toxicologically induced lipid deposition/steatosis as it typically does not impair liver function (Craig *et al.*, 1999).

## MICRONUTRIENTS

### Minerals

This nutrient group consists of inorganic elements that are required in the body for various purposes. It has been established that fish require the same minerals as terrestrial animals for tissue formation and other metabolic functions, including osmoregulation (NRC, 1993; Schwarz, 1995). However, dissolved minerals in the aquatic environment may contribute to satisfying the metabolic requirements of fish and interact with dietary requirements.

Minerals are typically classified as either macro- or microminerals, based on the quantities required in the diet and stored in the body. Macrominerals consist of calcium, phosphorus, magnesium, chloride, sodium, potassium, and sulfur. Dietary deficiencies of most macrominerals such as calcium have been generally difficult to produce with fish species because of the presence of these ions in the water. However, supplementation of phosphorus in fish diets is usually most critical since its presence in the water and utilization by fish is limited. The influence of excreted phosphorus on eutrophication of receiving waters has resulted in a considerable amount of research being focused on phosphorus nutrition in recent years with the aim of minimizing phosphorus excretion. Such efforts have increased the potential for marginal phosphorus deficiency in some aquacultured species. Phosphorus is not only a major constituent of hard tissues such as bone and scales, it is also present in various biochemicals. Impaired growth and feed efficiency are common deficiency signs exhibited by various fish species when fed diets deficient in phosphorus. Other more specific deficiency signs include reduced tissue mineralization and impair skeletal formation in juvenile fish (NRC, 1993).

Chloride, sodium and potassium are important electrolytes involved in osmoregulation (Lall, 2002). These minerals are typically abundant in

water and feedstuffs and so, metabolic deficiencies have rarely been observed. Magnesium is another macromineral abundant in most feedstuffs, but various deficiency signs have been reported for numerous freshwater fish species fed semi-purified diets without supplemental magnesium. These deficiency signs include anorexia, growth retardation, nephrocalcinosis (calcium accumulation in the kidney), reduced bone and whole-body magnesium concentration, as well as vertebral deformity and degeneration of muscle fibers (Gatlin *et al.*, 1982; Lall, 2002).

Dietary deficiencies of most microminerals (also known as trace minerals) have been produced in some species by feeding purified or practical diets for extended periods of time. These microminerals include cobalt, chromium, copper, iodine, iron, manganese, selenium and zinc. In contrast to other nutrients, reduced growth and feed efficiency are not readily induced with micromineral deficiencies but may occur after an extended period of time. However, reduced growth and feed efficiency may also appear as signs of toxicity when excessive levels of some microminerals are included in the diet. Specific deficiency signs associated with structural or metabolic functions of the various microminerals have been observed. For example, copper deficiency of channel catfish *Ictalurus punctatus* has resulted in reduced activity of various copper-containing metalloenzymes including cytochrome c oxidase and copper-zinc superoxide dismutase (Gatlin and Wilson, 1986a). This mineral is a component of several different enzymes involved in various other metabolic processes including brain neurotransmission, collagen synthesis and melanin production (Lall, 2002). Reduced growth and lens cataracts were reported in common carp *Cyprinus carpio* (Satoh *et al.*, 1983) fed copper-deficient diets although more overt signs have been difficult to demonstrate in other fish species (Lall, 2002). Toxicity to water-borne copper in pollutants has been demonstrated to adversely affect the gills, liver and kidney of fish. However, relatively high levels of dietary copper (500 Cu/kg) did not cause overt toxicity signs in rainbow trout (Knox *et al.*, 1984) or channel catfish (Gatlin *et al.*, 1989).

Iron is another micromineral found in the body complexed to various heme and non-heme compounds. As a constituent of hemoglobin, a dietary deficiency has been shown to produce a microcytic anemia in various fish species (Gatlin and Wilson, 1986b; Lall, 2002). Iron toxicity has also been reported in rainbow trout fed more than 1380 mg Fe/kg diet (Desjardins *et al.*, 1987).

Iodine is a constituent of the thyroid hormones and, thus, may influence thyroid function. Fish primarily obtain iodine from the water, although dietary iodine was demonstrated to cure goiter in salmonids in the early 1900s (Lall, 2002). However, dietary iodine supplementation is typically not required for various fish species.

Manganese is a cofactor for numerous enzymes involved in protein, lipid and carbohydrate metabolism. Reduced activity of some of these enzymes have been noted in a few fish species fed with low levels of manganese, while reduced growth and skeletal abnormalities were reported for rainbow trout and common carp (Lall, 2002). Other fish species such as the channel catfish did not show overt deficiency signs when fed semi-purified diets containing low levels of manganese over an extended period of time (Gatlin and Wilson, 1984a).

Selenium is a constituent of glutathione peroxidase which serves as a cytosolic antioxidant. The function of this enzyme is complementary to that of vitamin E, which is a lipid-soluble antioxidant. A dietary deficiency of selenium has been generally reported to result in reduced activity of glutathione peroxidase as well as growth reduction. However, a combined deficiency of selenium and the fat-soluble antioxidant vitamin E was required to produce more overt deficiency signs such as nutritional muscular dystrophy and exudative diathesis in channel catfish (Gatlin and Wilson, 1984b; Lall, 2002) (Fig. 19.1). The toxicity of selenium to various animals was well established before its dietary essentiality. Selenium toxicity has been demonstrated in several fish species and includes reduced growth and elevated mortality (Hilton *et al.*, 1980; Gatlin and Wilson, 1984b) as well as renal calcinosis (Hilton and Hodson, 1983).

Zinc is a cofactor of several enzymes and an integral component of other metalloenzymes (Lall, 2002). As such, adequate zinc nutrient is critical to maintain various functions in intermediary metabolism. One of the most obvious deficiency signs reported for fish with zinc deficiency is lens cataracts (Lall, 2002), although this pathology has not been consistently observed for all species such as the channel catfish (Gatlin and Wilson, 1983). This deficiency sign has also been noted in fish fed diets containing adequate levels of zinc, but with reduced bioavailability from antagonistic interactions with other dietary components including calcium, phosphorus and phytate (Hardy and Shearer, 1985; Richardson *et al.*, 1985; McClain and Gatlin, 1988; Gatlin and Phillips, 1989; Satoh *et al.*, 1989). Thus, supplementation of zinc at levels approximately one

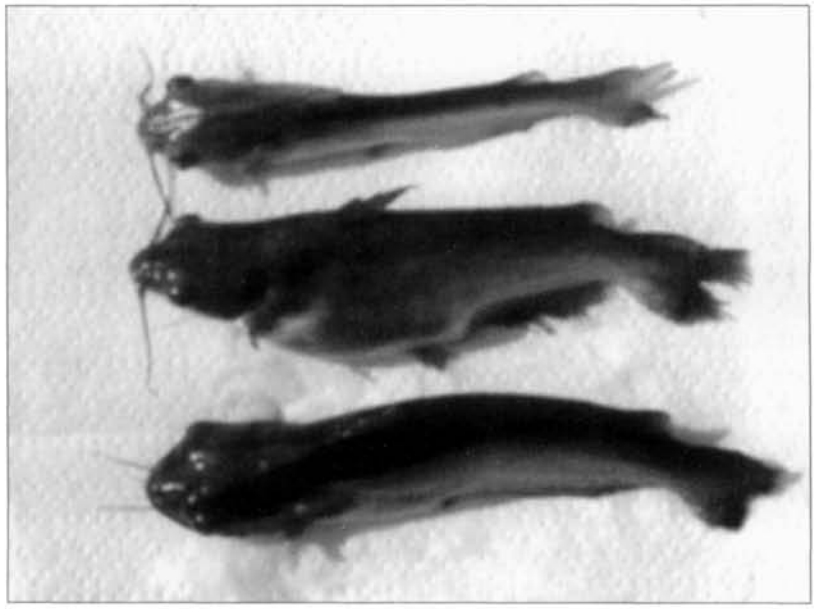

**Fig. 19.1** Overt signs of a combined deficiency of selenium and vitamin E in channel catfish including exudative diathesis (upper fish), nutritional muscular dystrophy (center fish) and control (bottom fish).

order of magnitude higher than the minimum dietary requirement has been recommended for various fish species to compensate for reduced bioavailability in practical diets (Gatlin and Wilson, 1984c; NRC, 1993).

Of all the microminerals, copper, iron, manganese, selenium and zinc have been demonstrated in some fish species to be most important to supplement in diets due to low levels in practical feedstuffs and/or interactions with other dietary components that may reduce bioavailability (Watanabe *et al.*, 1997). Although supplementation of practical diets with other microminerals has not been shown to be essential in most instances, an inexpensive trace mineral premix is typically added to most nutritionally complete diets in order to ensure adequacy.

## Vitamins

There are 15 vitamins established as being essential for terrestrial animals as well as for several fish species that have been examined to date (Woodward, 1994). These nutrients are characterized as being organic

compounds distinct from carbohydrates, protein and lipid that are required in relatively small concentrations to support specific structural or metabolic functions (Halver, 2002). Vitamins are divided into two groups based on solubility, with the fat-soluble vitamins including vitamin A (retinol), vitamin D (cholecalciferol), vitamin E (alpha-tocopherol) and vitamin K. Due to the deposition and storage of these vitamins in body lipids, extended periods without a dietary supply are needed before overt deficiency signs are manifested. Also, there is the potential for toxicity of these fat-soluble vitamins by feeding excessive levels (NRC, 1993). In contrast, the water-soluble vitamins including vitamin C (ascorbic acid), biotin, choline, folic acid, inositol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamin, and vitamin $B_{12}$ are not stored in appreciable amounts in the body in such a way that deficiency signs will be generally manifested within weeks in young, rapidly-growing fish fed a diet deficient in one or more of these vitamins. Most vitamin deficiency signs include rather general responses such as anorexia, reduced weight gain and impaired feed efficiency, although some distinct deficiency signs have been associated with certain vitamins in some fish species. These include clubbed gills in fish with pantothenic acid deficiency, convulsions due to pantothenic acid deficiency, lens cataracts due to riboflavin deficiency, hyperirritability and convulsions from lack of thiamin, vision problems from lack of vitamin A, reduced bone calcification from lack of adequate vitamin D, anemia and membrane fragility due to vitamin E deficiency, and prolonged blood clotting due to vitamin K deficiency (NRC, 1993; Hardy, 2001). One of the most widely observed and documented (Halver 2002) vitamin deficiencies has been associated with vitamin C, in which various pathological conditions including scoliosis and/or lordosis, 'broken back' syndrome and other skeletal abnormalities have been observed due to impaired collagen synthesis (Fig. 19.2). Based on the quantified dietary requirements for these various vitamins, premixes have been developed to include in prepared diets so as to provide adequate levels of each vitamin independent of levels in dietary ingredients and also provide a margin of safety for losses associated with processing and storage. The stability of vitamins during feed manufacture and storage has been improved over the years with protective coatings and/or chemical modifications. This is particularly evident in the development of various stabilized forms of the labile ascorbic acid (Halver, 2002). As such, vitamin deficiencies are not commonly observed in commercial production unless the vitamin premix

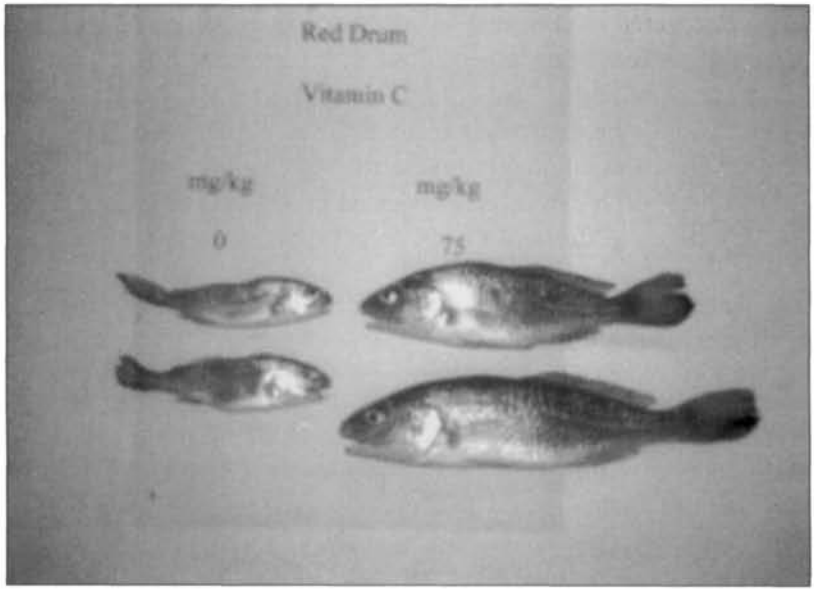


**Fig. 19.2** Vitamin C deficiency in red drum (*Sciaenops ocellatus*) showing reduced weight gain and cranial deformation resulting from impaired collegen synthesis as compared to fish fed adequate vitamin C (right).

is inadvertently omitted or there are antagonistic interactions with other dietary constituents that reduce available vitamin levels (Hardy, 2001).

## ANTI-NUTRITIONAL FACTORS

Prepared diets for fish may contain feedstuffs that contribute various constituents that impart negative effects on the target organism either by directly affecting its metabolism or by interfering with the availability of specific nutrients in the diet. Such constituents are commonly called anti-nutritional factors. Many of the established anti-nutritional factors are present in feedstuffs of plant origin, although a few are associated with feedstuffs of animal origin. Thus, the effects of these anti-nutritional factors on various fish species have generally become apparent when substituting alternative feedstuffs for fish meal which is the most nutritious protein feedstuff for aquafeeds due to its general lack of anti-nutrients, high protein concentration with balanced amino acid profile, as well as high nutrient digestibility. However, fish meal is one of the most expensive feedstuffs with a limited supply and increasing demand so that

its cost is expected to continue to increase. Therefore, a greater reliance on alternative feedstuffs is expected in the future. Such alternative feedstuffs of plant and animal origin may contain certain anti-nutritional factors that must be controlled to limit adverse effects on the cultured organism. The most common anti-nutritional factors that may adversely affect fish have been extensively reviewed in various publications (NRC, 1993; Hendricks, 2002) and thus will be briefly summarized here.

## Seed Proteins including Antigenic Compounds, Protease Inhibitors and Lectins

Various feedstuffs of plant origin, most commonly soybean meal (SBM), contain various anti-metabolic proteins such as lipoxygenase, lectins, urease and trypsin inhibitors as well as antigenic compounds that may induce immunological-based food intolerances. The degree to which various fish species are sensitive to such compounds varies considerably with some species such as rainbow trout and Atlantic salmon being much more sensitive than other species such as channel catfish and common carp. For example, Atlantic salmon fed on diets containing high levels of SBM may exhibit skin lesions, gastrointestinal tract alterations and excess mucus excretion in the feces which mimics a food allergy (Bakke McKellep *et al.*, 2000). Continued exposure has been shown to result in intestinal morphology changes in the form of enteritis which may lead to autoimmune disease.

### *Antigenic Proteins*

Allergenic responses to various seeds almost always involve sensitivity to multiple and varied proteins such that seed protein intolerance is often not restricted to a single sensitizing crop species but may be manifested as broader intolerance extending across species. The large majority of seed protein content results from the expression of gene families including the legumin and vicilin storage proteins, prolamine storage proteins, lectins, trypsin inhibitors and oleosin, a protein associated with seed oil storage. The genes that encode these proteins from diverse plant species are generally similar in sequence and thus the resulting protein products have similar three-dimensional structure and sequence (Herman, 2004). Globular storage proteins in soybeans such as glycinin and β-conglycinin or their breakdown products have been identified as causing allergic reactions in various animals although the severity of such reactions may

vary considerably (Herman, 2004). Such responses in fish have not been well characterized to date.

### Protease Inhibitors

Protease inhibitors are most commonly associated with legume seeds which are equipped with one or more protease inhibitors that inhibit proteolytic enzymes in the gastrointestinal tract of pests that attack these seeds (Liener, 2000). These plant protease inhibitors such as the trypsin inhibitors also inhibit proteases in the gastrointestinal tract of monogastric animals, including fish (NRC, 1993; Hendricks, 2002). Heat treatment will inactivate protease inhibitors to a large extent; however, the heat needed for total inactivation of the inhibitors may reduce protein quality through oxidation and/or fusion with other components of the soybean. Thus, a compromise is generally made between heat inactivation and deterioration of protein quality which may leave some inhibitors active. Although without compromising growth and protein digestibility, low levels of protease inhibitors in salmonid diets have been observed to trigger increased secretion of pancreatic proteases, indicating increased use of metabolic resources; whereas, at higher levels both protein digestibility and growth are compromised (Olli *et al.*, 1994). Channel catfish have also been shown to be adversely affected by high levels of trypsin inhibitor (Wilson and Poe, 1985), but the normal heating that commercial solvent-extracted SBM undergoes is generally sufficient to limit adverse effects of these protease inhibitors.

### Lectins

These anti-nutritional factors are glycoproteins found in soybeans and other plant feedstuffs (Grant, 1991). Because of their ability to agglutinate red blood cells, these compounds are also referred to as agglutinins or specifically for soybeans, as soybean agglutinins (SBA). These lectins possess the ability to bind reversibly and specifically to carbohydrates and glycoconjugates which results in their numerous physiological effects. For example, lectins may bind with intestinal glycoproteins on the epithelial surface and interfere with nutrient absorption. They may easily avoid digestion and then pass into the intestine and bind with the epithelium. Some lectins may result in the disruption of membrane integrity and the initiation of a cascade of immune and autoimmune events that ultimately

lead to cell death. In addition, lectins also may alter gut permeability and gain entry into circulation and may bind to organs. They may also increase permeability of membranes to other proteins increasing incidence of allergic reactions. Lectins are resistant to proteolytic enzymes, but are either inactivated or destroyed by moist heat treatment (Francis *et al.*, 2001). The influences of lectins and antigenic proteins in plant feedstuffs such as SBM need to be characterized more specifically in various fish species.

## Anti-nutritional Factors Associated with Carbohydrates

### *Non-starch Polysaccharides*

Soybeans are characterized by a high content of non-starch polysaccharides (NSPs) and negligible starch. This fraction provides marginal energy for the fish due to limited microbial fermentation, and may negatively affect nutrient utilization and reduce feed efficiency. A review of soy products indicates that raw soybeans contain in the range of 20% NSPs, approximately one-third of which are soluble and another fraction is highly viscous (Storebakken *et al.*, 2000). Elevated fecal water content has been reported in salmonids when fed diets with SBM (Olli *et al.*, 1994; Refstie *et al.*, 1998; Storebakken *et al.*, 1998), which, probably in part, is related to its content of NSPs. Refstie *et al.* (1999) also attributed a reduced digestibility of dietary fat and protein in both Atlantic salmon and broiler chickens to increased intestinal viscosity due to soybean NSPs. In some fish, however, soybean NSPs have been shown to increase intestinal and fecal water contents without affecting the intestinal viscosity. In spite of this, the anti-nutritional effects were the same including reduced digestibility of fat and nitrogen with soy products containing the greatest amount of NSPs. Similar but more severe effects on nutrient absorption and fecal water content were reported in rainbow trout when using NSPs such as guar gum and alginates as binders in aquafeeds (Davies, 1985; Storebakken, 1985; Storebakken and Austreng, 1987).

The antinutritional actions of soluble NSPs are yet to be fully understood. Increased viscosity of the intestinal contents may subsequently impair diffusion and convective transport of digestive enzymes and substrates within the gastrointestinal contents. However, antinutritional effects are also probably associated with binding or trapping of and subsequent increased fecal excretion of steroids. As NSPs

do not appear to affect intestinal viscosity in fish, this latter effect may be responsible for the reduced nutrient absorption with NSP-rich diets. NSPs also may have similar effects as do oligosaccharides on water balance in saltwater fish; these effects could be additive when feeding diets with oligosaccharide-containing soy meals. Increased drinking has been indicated by an increase in fecal output of sodium in Atlantic salmon fed diets with high amounts of oligosaccharide-free soy protein concentrate, independent of dietary phytate concentration (Storebakken *et al.*, 1998).

### *Oligosaccharides*

SBM contains oligosaccharide levels of up to 15% while the oligosaccharide levels in soy protein concentrate are ~3% (Russett, 2002). Stachyose and raffinose are the two most predominant oligosaccharides in SBM. Oligosaccharides commonly found in SBM have been reported to be indigestible to fish (Refstie *et al.*, 1998). They have also been reported to increase the viscosity of the chime in the digestive tract and interfere with the uptake of nutrients, particularly lipids and minerals, in salmonid species (Refstie *et al.*, 1998, 2005). The oligosaccharide component of SBM has also been linked with reduced growth performance (Refstie *et al.*, 1998) and the occurrence of SBM-induced enteritis in several salmonid species (van den Ingh *et al.*, 1991, 1996; Bureau *et al.*, 1998). In Atlantic salmon, the enteritis develops rapidly during the first weeks of exposure even at SBM inclusion levels as low as 10% of diet and regresses to apparently normal histology three weeks after introduction of a SBM-free diet (Bæverfjord and Krogdahl, 1996). van den Ingh *et al.* (1996) fed Atlantic salmon a series of diets containing 26% SBM that had been exposed to a range of heat treatments to denature trypsin inhibitor as well as a fish meal-based diet in which the alcohol-soluble components of SBM were added back, and two control fish meal diets containing no soy products. Control diets failed to induce any morphological changes in the intestinal tract; whereas, morphological changes were evident in all fish fed diets containing SBM and the diet containing the alcohol-soluble extract which contained oligosaccharides, low levels of saponins, and possibly low levels of other antinutrients. van den Ingh *et al.* (1996) suggested that the anti-nutritional factor was in the alcohol-soluble component of the SBM and concluded that either saponins or oligosaccharides were possible candidates. However, in a trial screening with isolated soybean antinutrients, neither oligosaccharides, trypsin inhibitors, saponins nor lectins showed any enteritis-inducing effect (Krogdahl, 1996).

### *Phytic Acid*

This compound is chemically known as the hexaphosphate of myo-inositol. Approximately two-thirds of the total phosphorus in oilseed meals such as soybean, rapeseed or canola and cottonseed as well as in various cereal grains is present as phytic acid or phytate. The bioavailability of phytate-phosphorus to fish and virtually all monogastric animals is only approximately 30%. Further, phytic acid lowers the availability of certain divalent cations, notably zinc, to carnivorous species such as trout and salmon as well as omnivorous species such as channel catfish as noted previously. The only effective strategies to reduce the effects of phytic acid in SBM or other seed-derived products is to remove phytic acid by processing, hydrolyze it using the enzyme phytase, or to utilize single-gene mutant varieties of grains and oilseeds in which a smaller percentage of total phosphorus in the seed is stored as phytate-phosphorus. Low-phytate cultivars of corn, barley, wheat and soybeans have been developed but are not widely available presently. Microbial phytase is more effective at increasing phosphorus bioavailability in soybean and canola meals, as well as in wheat middlings, compared to whole grains, such as wheat or barley (Cheng and Hardy, 2002). However, there are issues related to the heat sensitivity of phytase and its temperature of activity that must be resolved before phytase is regularly used in aquafeeds.

### *Other Anti-nutritional Factors in Soybean Products*

Various other compounds in soybean products that are suspected of having anti-nutritional effects on fish include saponins and estrogenic compounds. Much more research is needed to characterize the effects of these compounds.

## Anti-nutritional Factors in Rapeseed/Canola Meals

### *Glucosinolates*

These compounds are found in rapeseed/canola meal and their hydrolysis products are antithyroid compounds (Hendricks *et al.*, 2002). Goitrin is the most potent of the hydrolysis products and acts by inhibiting the organic binding of iodine, whereas another hydrolysis product, thiocyanate, lowers iodine uptake by the thyroid. Thus, glucosinolates are

not themselves harmful substances, as are their hydrolysis products. Supplementing additional iodine to the diet does not prevent thyroid insufficiency in fish fed diets containing rapeseed meal. Canola meal is produced from cultivars of rapeseed that have been bred to contain low levels of glucosinolates relative to levels found in rapeseed meal. Rapeseed meals contain from 3-8% glucosinolates; whereas, canola contains less than 2 mg/g, a level below that causing physiological effects in fish.

### *Erucic Acid*

Erucic acid (C22:1, n-9) is a normal constituent of rapeseed oil, and levels in oil from standard varieties of rapeseed range from 20-55% of the oil. This fatty acid is valuable for production of industrial lubricants from rapeseed oil, but deleterious in oils used for livestock and fish diets because erucic acid is cardiotoxic, causing cardiac lesions in rats, even at low levels of 1-2% of the lipid fraction. Varieties of rapeseed have been developed that contain reduced levels of erucic acid, and solvent extraction of rapeseed/canola following mechanical pressing of the seeds reduces the residual amount of erucic acid in the meal to levels below that required to produce pathological effects in fish.

## Anti-nutritional Factors in Cottonseed Meal

### *Gossypol*

This is a pigment found in cottonseed meal (CSM) which is recognized for its toxicity to animals, especially in regard to reproduction. Free gossypol is toxic to animals; whereas, bound gossypol is generally not toxic (Hendricks, 2002). The commercial processing of CSM typically results in 80 to 99% of the gossypol to be present in the bound form. Therefore, CSM can typically be included in fish diets at 15 to 20% with causing adverse effects such as growth suppression.

### *Cyclopropene Fatty Acids*

Sterculic and malvalic acids are cyclopropene fatty acids present in cottonseed oil and the oil residue in CSM. These fatty acids have been shown to cause growth depression and altered lipid metabolism in various animals including rainbow trout which also exhibited hepatic neoplasms (Hendricks, 2002).

## Anti-nutritional Factors in Lupins/Peas

### *Alkaloids*

The predominant anti-nutrients in lupins are the quinolizidine alkaloids (Duke, 1981). There are over 300 species of the genus *Lupinus*, of which many contain high levels of alkaloids ('bitter' varieties). These alkaloids, a class of bitter-tasting compounds, make the seed unpalatable and sometimes toxic to animals.

## Anti-nutritional Factors of Animal Origin

### *Oxidized Lipid*

Marine lipids contain large quantities of polyunsaturated fatty acids which are quite susceptible to auto-oxidation. The oxidation products of these lipids include aldehydes and ketones as well as free radicals which increase the demand for antioxidants including vitamins C and E. Thus, the presence of oxidized lipid products in the diet may directly affect fish and/or exacerbate deficiencies of antioxidant vitamins, resulting in various pathological conditions including liver degeneration, spleen abnormalities and anemia (NRC, 1993; Hardy, 2001). Synthetic antioxidants such as ethoxyquin are typically added to marine lipids to ensure they are properly protected from oxidation.

### *Thiaminase*

Some raw fish preparations, particular from freshwater species, may contain elevated levels of the enzyme thiaminase. This enzyme destroys the vitamin thiamin after contact for some period of time; therefore, feeding fresh fish or supplemental thiamin will prevent a thiamin deficiency.

### *Biogenic Amines*

Various derivatives of amino acids such as histamine and gizzerosine may be found in high concentrations in fish meals produced from scombroid fishes due to microbial metabolism (Hardy, 2001; Hendricks, 2002). The toxicity of these compounds to fish such as rainbow trout appears to be more limited that observed in poultry (Hardy, 2001).

## DIETARY TOXINS

### Microbial Toxins

Mycotoxins represent a diverse group of toxic compounds that are produced by a variety of fungal organisms or molds that may contaminate feedstuffs and/or prepared diets (NRC, 1993; Manning, 2001). The molds that produce mycotoxins are often present on most grains and oilseeds during harvest and their growth during storage can be inhibited primarily by maintaining moisture levels below 12%. The most common fungal organisms that may contaminate feedstuffs used in the preparation of aquafeeds include those of the genera *Aspergillus, Penicillium* and *Fusarium*. Four different aflatoxins may be produced by *Aspergillus* molds and aflatoxin $B_1$ was reported over 30 years ago to be extremely toxic to rainbow trout by producing liver carcinomas when fed at extremely low concentrations (0.4 to 20 ug/kg) (Halver, 1969). Rainbow trout were more susceptible to aflatoxin $B_1$ compared to other salmonids, and much more sensitive than warm-water species such as channel catfish and tilapia (Manning, 2001).

Other mycotoxins produced by *Aspergillus* molds include cyclopiazonic acid, which is a neurotoxin, and ochratoxin A which adversely affects the kidney and liver. These mycotoxins also may be produced by certain *Penicillium* molds. Various other mycotoxins are produced by *Fusarium* molds including fumonisins, moniliforme, tricothecenes (T-2 toxin), deoxynivalenol and zearalenone which have been documented to have numerous adverse effects on animals including fish. Considerable information on the effects of these diverse compounds on warm-water species such as channel catfish has accumulated in recent years and is thoroughly reviewed by Manning (2001).

Proper storage of feedstuffs and finished diets is critical to prevent contamination with mycotoxins. These conditions include low moisture (< 12%), low relatively humidity and limited oxygen. Additional precautions are generally taken such as supplementing mold inhibitors such as proprionic acid to prepared diets.

### Anthropogenic Toxins

There are a variety of compounds, both of natural and industrial origin that may contaminate feedstuffs and/or prepared diets for fish. Examples of such compounds include polychlorinated biphenyls (PCBs), pesticides and heavy metals such as arsenic, cadmium and mercury (Hendricks,

2002). The adverse effects of these compounds may include reduced growth, tissue lesions and even mortality, but the severity of these conditions vary considerably depending on dietary concentration of the contaminant as well as the fish species. Care in monitoring the quality of feedstuffs to be used in aquafeeds will generally limit the potential adverse effects of these contaminants.

## Conclusions

A considerable amount of information concerning nutrition and feeding of fish has been generated in the past two decades. Reliable estimates of nutrient requirements have been established for the predominant fish species produced in aquaculture, and these estimates are rather similar among species whose natural feeding habits and environmental requirements are comparable. Many studies designed to quantify nutrient requirements of fish have also characterized deficiency signs associated with inadequate intake of specific nutrients. Information has also been generated on the suitability of common feedstuffs of plant and animal origin for use in aquafeeds, including the potential adverse effects of certain anti-nutritional factors in these feedstuffs. Additional studies have characterized the effects of various microbial and anthropogenic toxins that may be present in the diet on numerous fish species. Such information has significantly contributed to our understanding of fish nutrition for the advancement of aquaculture and has guided diet formulation and feed management practices that promote efficient and cost-effective production while maintaining the health of the cultured organism.

## References

Akiyama, T., S. Aria, T. Murai and T. Nose. 1985. Tryptophan requirement of chum salmon fry. *Bulletin of the Japanese Society of Scientific Fisheries* 51: 1005-1008.

Akiyama, T., S.T. Murai and K. Mori. 1986. Role of tryptophan metabolites in inhibition of spinal deformity of chum salmon fry caused by tryptophan deficiency. *Bulletin of the Japanese Society of Scientific Fisheries* 52: 1225-1259.

Bakke McKellep, A.M., C.M. Press, G. Baeverfjord, K. Krogdahl and T. Landsverk. 2000. Changes in immune and enzyme histochemical phenotypes of cells of the intestinal mucosa of Atlantic salmon (*Salmo salar* L.) with soybean-meal induced enteritis. *Journal of Fish Diseases* 23: 115-127.

Bæverfjord, G. and Å. Krogdahl. 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon distal intestine. A comparison with the intestines of fasted fish. *Journal of Fish Diseases* 19: 375-387.

Bureau, D.P., A.M. Harris and C.Y. Cho. 1998. The effects of purified alcohol extracts from soy products on the feed intake and growth of Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 16: 27-43.

Castell, J.D., R.O. Sinnhuber, D.J. Lee and J.H. Wales. 1972. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): Physiological symptoms of EFA deficiency. *Journal of Nutrition* 102: 87-92.

Cheng, Z.J. and R.W. Hardy. 2002. Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measured *in vivo* using rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition* 8: 271-277.

Cowey, C.B. 1994. Amino acid requirements of fish: A critical appraisal of present values. *Aquaculture* 124: 1-11.

Cowey, C.B., M. de la Higuera and J.W. Adron. 1977. The effect of dietary compositon and of insulin on gluconeogenesis in rainbow trout (*Salmo gairdneri*). *British Journal of Nutrition* 38: 385-395.

Cowey, C.B., C.Y. Cho, J.G. Sivak, J.A. Weerheim and D.D. Stuart. 1992. Methionine intake in rainbow trout, relationship to cataract formation and the metabolism of methionine. *Journal of Nutrition* 122: 1154-1163.

Craig, S.R., B.S. Washburn and D.M. Gatlin III. 1999. Effects of dietary lipids on body composition and liver function in juvenile red drum, *Sciaenops ocellatus*. *Fish Physiology and Biochemistry* 21: 249-255.

D'Abramo, L.R. and J.D. Castell. 1997. Research methodology. In: *Crustacean Nutrition*, L.R. D'Abramo, D.E. Conklin and D.M. Akiyama (eds.). The World Aquaculture Society, Baton Rouge, Louisiana, pp. 3-25.

Davies, S.J. 1985. The role of dietary fibre in fish nutrition. In: *Recent Advances in Aquaculture*, J.F. Muir and R.J. Roberts (eds.). Westview Press, Boulder, Vol. 2, pp. 219-249.

Desjardins, L.M., B.D. Hicks and J.W. Hilton. 1987. Iron catalyzed oxidation of trout diets and its effect on the growth and physiological response of rainbow trout (*Salmo gairdneri*). *Fish Physiology and Biochemistry* 3: 173-182.

Duke, J.A. 1981. *Handbook of Legumes of World Economic Importance*. Plenum Press, New York.

Francis, G., H.R.S. Makkar and K. Becker. 2001. Antinutritional factors present in plant-derived alternative fish feed ingredients and their effects in fish. *Aquaculture* 199: 197-227.

Gatlin, D.M., III, 2002. Nutrition and fish health. In: *Fish Nutrition*, J.E. Halver and R.W. Hardy (eds.). 3rd Edition. Academic Press, London, pp. 671-702.

Gatlin, D.M., III and R.P. Wilson. 1983. Dietary zinc requirement of fingerling channel catfish. *The Journal of Nutrition* 113: 630-635.

Gatlin, D.M., III and R.P. Wilson. 1984a. Studies on the manganese requirement of fingerling channel catfish. *Aquaculture* 41: 85-92.

Gatlin, D.M., III and R.P. Wilson. 1984b. Dietary selenium requirement of fingerling channel catfish. *Journal of Nutrition* 114: 627-633.

Gatlin, D.M., III and R.P. Wilson. 1984c. Zinc supplementation of practical channel catfish diets. *Aquaculture* 41: 31-36.

Gatlin, D.M., III and R.P. Wilson. 1986a. Dietary copper requirement of fingerling channel catfish. *Aquaculture* 54: 277-285.

Gatlin, D.M., III and R.P. Wilson. 1986b. Characterization of iron deficiency and the dietary iron requirement of fingerling channel catfish. *Aquaculture* 52: 191-198.

Gatlin, D.M., III and H.F. Phillips. 1989. Dietary calcium, phytate and zinc interactions in channel catfish. *Aquaculture* 79: 259-266.

Gatlin, D.M., III, E.H. Robinson, W.E. Poe and R.P. Wilson. 1982. Magnesium requirement of fingerling channel catfish and signs of magnesium deficiency. *Journal of Nutrition* 112: 1182-1187.

Gatlin, D.M., III, H.F. Phillips and E.L. Torrans. 1989. Effects of various levels of dietary copper and zinc on channel catfish. *Aquaculture* 76: 127-134.

Grant, G. 1991. Lectins. In: *Toxic Substances in Crop Plants*, F.J.P. D'Mello, C.M. Duffas and J.H. Duffas (eds.). The Royal Society of Chemistry, Thomas Graham House, Cambridge, pp. 49-67.

Halver, J.E. 2002. The vitamins. In: *Fish Nutrition*, J.E. Halver and R.W. Hardy (eds.). 3rd Edition. Academic Press, London, pp. 61-141.

Halver, J.E. and W.E. Shanks. 1960. Nutrition of salmonid fishes. 8. Indispensable amino acids for sockeye salmon. *Journal of Nutrition* 72: 340-346.

Hardy, R.W. 2001. Nutritional deficiencies in commercial aquaculture: likelihood, onset, and identification. In: *Nutrition and Fish Health*, C. Lim and C.D. Webster (eds.). The Haworth Press, Inc., New York, pp. 131-147.

Hardy, R.W. and K.D. Shearer. 1985. Effect of dietary calcium phosphate and zinc supplementation on whole body zinc concentration of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* 42: 181-184.

Hendricks, J.D. 2002. Adventitious Toxins. In: *Fish Nutrition*, J.E. Halver and R.W. Hardy (eds.). 3rd Edition. Academic Press, London, pp. 601-649.

Herman, E.M. 2004. Allergenic responses to legume proteins. In: *Genomics for Legume Crops*, E.C. Brummer and R. Wilson (eds.). AOCS Press, Urbana, Illinois, pp. 326-347.

Hilton, J.W. and P.V. Hodson. 1983. Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdneri*). *Journal of Nutrition* 113: 1241-1248.

Hilton, J.W., P.V. Hodson and S.J. Slinger. 1980. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). *Journal of Nutrition* 110: 2527-2535.

Keembiyehetty, C.N. and D.M. Gatlin, III. 1993. Total sulfur amino acid requirement of juvenile hybrid striped bass, *Morone chrysops* × *M. saxatilis*. *Aquaculture* 110: 331-339.

Kloppel, T.M. and F. Post. 1975. Histological alterations in tryptophan-deficient rainbow trout. *Journal of Nutrition* 105: 861-866.

Knox, D., C.B. Cowey and J.W. Adron. 1984. Effects of dietary zinc intake upon copper metabolism in rainbow trout (*Salmo gairdneri*). *Aquaculture* 40: 199-207.

Krogdahl, Å. 1996. Effects of soybean antinutrients on intestinal environment, lipid hydrolysis and utilization on Atlantic salmon (*Salmo salar* L.). In: *Effects of Antinutrient on the Nutritional Value of Legume Diets*, S. Bardocs, F.V. Nekrep, Og A. Pusztai (eds.). European Commission, Directorate-General XII, Science Research Development, Luxembourg, Vol. 3, pp. 74-81.

Lall, S.P. 2002. The minerals. In: *Fish Nutrition*, J.E. Halver and R.W. Hardy (eds.). 3rd Edition. Academic Press, London, pp. 61-141.

Lee, D.J., N. Roehm, T.C. Yu and R.O. Sinnhuber. 1967. Effect of omega 3 fatty acids on the growth rate of rainbow trout, *Salmo gairdnerii*. *Journal of Nutrition* 92: 93-98.

Liener, I.E. 2000. Non-nutritive factors and bioactive compounds in soy. In: *Soy in Animal Nutrition*, J.K. Drackley (ed.). Federation of. Animal Science Society, Savoy, IL, USA, pp. 127-170.

Lovell, R.T. 1989. Fish feeding experiments. In: *Nutrition and Feeding of Fish*, Van Nostrand Reinhold, New York, pp. 129-144.

NRC (National Research Council). 1993. *Nutrient Requirements of Fish*. National Academy Press, Washington.

Manning, B.B. 2001. Mycotoxins in fish feeds. In: *Nutrition and Fish Health*, C. Lim and C.D. Webster (eds.). The Haworth Press, New York, pp. 267-287.

McClain, W.R. and D.M. Gatlin, III. 1988. Dietary zinc requirement of *Oreochromis aureus* and effects of dietary calcium and phytate on zinc bioavailability. *Journal of the World Aquaculture Society* 19: 103-108.

Olli, J.J., K. Hjelmeland and Å. Krogdahl. 1994. Soybean trypsin inhibitors in diets for Atlantic salmon (*Salmo salar* L.): effects on nutrient digestibilities and trypsin in pyloric caeca homogenate and intestinal content. *Comparative Biochemistry and Physiolology* A109: 923-928.

Poston, H.A. 1986. Response of rainbow trout to source and level of supplemental dietary methionine. *Comparative Biochemistry and Physiology* A83: 739-744.

Poston, H.A. and G.L. Rumsey. 1983. Factors affecting dietary requirement and deficiency of signs of L-tryptophan in rainbow trout. *Journal of Nutrition* 113: 2568-2577.

Refstie, S., T. Storebakken and A.J. Roem. 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soy antigens. *Aquaculture* 162: 301-312.

Refstie, S., B. Svihus, K.D. Shearer and T. Storebakken. 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soybean products. *Animal Feed Science and Technology* 79: 331-345.

Refstie, S., S. Sahlstrom, E. Brathen, G. Baeverfjord and P. Krogedal. 2005. Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). *Aquaculture* 246: 331-345.

Richardson, N.L., D.A. Higgs, R.M. Beames and J.R. McBride. 1985. Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Nutrition* 115: 553-567.

Rumsey, G.L., J.W. Page and M.L. Scott. 1983. Methionine and cystine requirements of rainbow trout. *Progressive Fish-Culturist* 45: 139-143.

Russett, C. 2002. Soy protein concentrates for animal feeds. *Animal Nutrition Research Notes*. The Solae Company. SPC-T-47, 12 pp.

Sargent, J.R., J.G. Bell, M.V. Bell, R.J. Henderson and D.R. Tocher. 1995. Requirement criteria for essential fatty acids. *Journal of Applied Ichthyology* 11: 183-198.

Satoh, S., W.E. Poe and R.P. Wilson. 1989. Effects of supplemental phytate and/or tricalcium phosphate on weight gain, feed efficiency and zinc content in vertebrae of channel catfish. *Aquaculture* 80: 155-161.

Scharz, F.J. 1995. Determination of mineral requirements of fish. *Journal of Applied Ichthyology* 11: 164-174.

Sies, H. 1991. *Oxidative Stress: Oxidants and Antioxidants*. Academic Press, New York.

Simmons, L., R.D. Moccia, D.P. Bureau, J.G. Sivak and K. Herbert. 1999. Dietary methionine requirement of juvenile Arctic charr *Salvelinus alpinus* (L.). *Aquaculture Nutrition* 5: 93-100.

Storebakken, T. 1985. Binders in fish feeds I. Effect of alginate and guar gum on growth, digestibility, feed intake and passage through the gastrointestinal tract of rainbow trout. *Aquaculture* 47: 11-26.

Storebakken, T. and E. Austreng. 1987. Binders in fish feeds II. Effect of different alginates on the digestibility of macronutrients in rainbow trout. *Aquaculture* 60: 121-131.

Storebakken, T., I.S. Kvien, K.D. Shearer, B. Grisdale-Helland, S.J. Helland and G.M. Berge. 1998. The apparent digestibility of diets containing fish meal, soybean meal or bacterial meal fed to Atlantic salmon (*Salmo salar*): Evaluation of different faecal collection methods. *Aquaculture* 169: 195-210.

Storebakken, T., S. Refstie and B. Ruyter. 2000. Soy products as fat and protein sources in fish feeds for intensive aquaculture. In: *Soy in Animal Nutrition*, J.K. Drackley (ed.). Federation of Animal Science Society, Savoy, IL, U.S.A., pp. 127-170.

van den Ingh, T.S.G.A.M., Å. Krogdahl, J.J. Olli, H.G.C.J.M. Hendrix and J.G.J.F Koninkx. 1991. Effects of soybean containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): A morphological study. *Aquaculture* 94: 297-305.

van den Ingh, T.S.G.A.M., J.J. Olli and Å. Krogdahl. 1996. Alcohol-soluble components in soybeans cause morphological changes in the distal intestine of the Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 19: 47-53.

Watanable, T., T. Takeuchi, S. Satoh, K. Tozama and M. Okuzumi. 1987. Effect of dietary histadine or histamine on growth and development of stomach erosion in rainbow trout. *Nippon Suisan Gakkaishi* 53: 1207-1214.

Walton, M.J., C.B. Cowey and J.W. Adron. 1982. Methionine metabolism in rainbow trout fed diets of differing methionine and cystine content. *Journal of Nutrition* 112: 1525-1535.

Watanabe, T., V. Kiron and S. Satoh. 1997. Trace minerals in fish nutrition. *Aquaculture* 151: 185-207.

Wilson, R.P. 1994. Utilization of dietary carbohydrate by fish. *Aquaculture* 124: 67-80.

Wilson, R.P. and W.E. Poe. 1985. Effects of feeding soybean meal with varying trypsin inhibitor activities on growth of fingerling channel catfish. *Aquaculture* 46: 19-25.

Woodward, B. 1994. Dietary vitamin requirement of cultured young fish, with emphasis on quantitative estimates for salmonids. *Aquaculture* 124: 133-168.

CHAPTER

20

# Environmental Diseases of Intensively Reared Fish

**David J. Speare**

## INTRODUCTION

With the global expansion of finfish aquaculture, a number of common disease scenarios have emerged which directly reflect the interaction of production fish with their culture conditions. Often referred to as *production diseases*, and generally overlooked when compared to diseases caused by pathogens, this subgroup of disease conditions often exerts significant economic constraints either by direct losses, or by indirect influences on infectious disease pathogenesis. The purpose of this chapter is to closely examine the range of environmental diseases which emerge during intensive culture of fish, utilizing the salmonids (particularly the Atlantic salmon *Salmo salar*) as representative and the life-stages of salmonids as an influencing parameter. General concepts, however, apply broadly to other fish species under intensive cultivation, although in many

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situations, the breadth of published research is strikingly less than for salmonids. Secondarily, where the research permits review, this chapter will also serve to illustrate the manner in which environmental diseases may set the stage, or act as a co-factor, for the establishment of infectious diseases. Diseases caused by nutritional factors are extremely pertinent to intensive aquaculture, but this topic is largely covered elsewhere in this text, and thus the discussion on nutrition herein will focus on some relatively unique scenarios within the salmon industry.

The term *intensive* production has no strict definition since the continuum between extensive, semi-intensive, and intensive must include several independent and dependent variables. Although the term is generally used to denote a relationship between fish biomass and tank size (for example, rainbow trout tank culture where stocking density exceeds 40 kg of fish per cubic meter of water), it is also used to indicate situations where rates of water exchange to a tank are extended; the latter includes most situations of recirculation. In the context of this chapter, the term 'environmental' does not extend to the microbes and parasites which are a part of the environment as these will be dealt with elsewhere.

To understand the manner in which the life-stages of an intensively reared fish can influence the range of environmental diseases, it is worth addressing the production cycle of Atlantic salmon as an example. Based on current practices, the salmon production cycle extends over a 36-month period during which time fish are first exposed to an intensive phase of freshwater rearing, followed by transport to marine sea cages where, based on stocking density, the term semi-intensive is likely the best descriptive adjective. Compared to other food animal species, the prolonged production cycle and diversity of the environment (freshwater vs. saltwater) raises many possibilities for environmental disorders. Although culture during the freshwater phase is potentially highly controllable, the nature of the marine sea cages is such that there is little (other than mesh netting) separating the fish from the direct influences of ocean variables.

In the northern hemisphere, eggs and milt are taken from broodstock in late autumn; rearing of fertilized ova in hatcheries supplied with freshwater (from lakes, rivers, or wells) occurs from November through to March, when the fry begin to accept feed. Eggs are prone to damage, particularly prior to the point where *eye spots* are visible. During hatching, as much as 70% of the embryo's mass is composed of the nutrient-rich external yolk sac which is sensitive to abrasion. Following first feeding, and

with growth into a fingerling (*parr*) stage, the juveniles are moved into a smolt production unit. Here, with further growth, the parr undergo transformation into *smolt*, which are capable of making the transition to the marine environment.

Smolt typically weigh in excess of 100 g when they are moved to seawater, and this most often occurs in the spring, one year after they have reached first feeding. In practice, although the benefits of a gradual entrance to the marine environment are known, it is common for smolt to be moved directly to marine sites where seawater is full strength. Rarely may a lens of freshwater be available near a production site and, in those areas, smolt may be placed into these lensing sites, thus allowing the smolt to control their own final entrance into the deeper and more saline water. During the ensuing adaptation period, it may take up to 2 months before normal feeding begins and significant loss of fat reserves occurs (Jobling *et al.*, 2002). Once established, feeding rates result in spectacular growth rates, concluding in a harvestable fish (3-5 kg) in 12-15 months. Broodstock are selected from stocks which have shown the tendency to smoltify quickly, grow rapidly, and have reduced tendency to undergo early sexual maturation. With this simplified production in mind, environmental diseases which emerge during each phase will be introduced in order.

## ENVIRONMENTAL DISEASES OF EGGS AND EARLY FRY

Eggs are highly prone to environmental factors, including gas bubble disease, high levels of iron which can precipitate on egg surfaces, oxygen deficiency, and even spectral irradiance. Iodophor baths used to block the congenital transmission of several pathogens can also reduce egg hatch rates. Issues attending to egg damage also include increased susceptibility to zoospores of water molds, and even low numbers of affected eggs can serve as a source of a high number of zoospores which may then colonize other healthy eggs although some work suggests that genetic selection for enhanced resistance to water molds may be possible (Hanke *et al.*, 1991; Nilsson, 1992).

The yolk sack is a delicate structure with a thin layer of epidermis overlying a reduced dermis with no stratum compactum. Spongiotic dermatitis presenting as multiple white spots can develop following abrasion or after exposure of the fry to treatment chemicals. If damage is sufficient to allow water to pass through the skin, tissue around the yolk

and the yolk itself can become edematous and this imparts a bluish tinge when observed grossly, and hence the term *blue-sack disease*. Conformational changes to the yolk sack can also occur, giving rise to such terms as *pinched yolk sack* and *dumb-bell sack* (Fig. 20.1). A significant risk factor for these latter two conditions results from excess movement of sack fry on egg tray substrates. Fry movement increases if the egg trays are exposed to excess lighting. Yolk sack changes may become lethal if the juvenile has restricted access to the protein content contained in the sack, or more commonly when the damaged sack becomes secondarily infected with fungus. Saprolegnial peritonitis with extension to hepatitis is a common sequel to primary colonization of the yolk sac; less commonly mycotic myocarditis can be established through embolic distribution (Fig. 20.2) yielding unusual disease conditions later on during fry cultivation.

Hatchery managers of salmon fry pay critical attention to the so-called *first feeding window*. Determining the best time to begin feeding using degree-day criteria (Koss and Bromage, 1990), and encouraging the fry to begin looking for exogenous food is critical to hatchery success, and fry which do not find food when they begin looking for it frequently become non-feeders and die (Peterson and Martin-Robichaud, 1989). Non-feeding fry may look healthy for several days as they sustain themselves on

**Fig. 20.1** Sack fry with damaged 'pinched' yolk sack. The terminal section is often prone to mycotic infection.

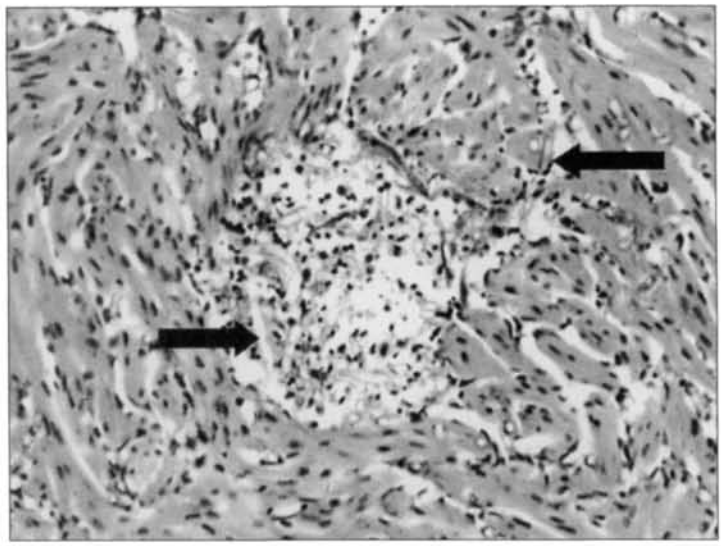

**Fig. 20.2** Mycotic infection (arrows) that has extended into the heart from the liver. Cardiac failures can result from this leading to passive congestion and abdominal distension.

portions of the yolk sack which are within the peritoneal cavity. However, they become progressively thinner and die. The term *pinhead* is used to describe these failing fry. Their heads appear disproportionately large as the remainder of the body shrinks.

During the critical first feeding window, which is up to two weeks long, much of the food which is offered to the fry remains uneaten, and this becomes a risk factor for bacterial and fungal overgrowth. Fungal-laden particles of feed may become eaten and lodge either in the esophagus or branchial cavity where they can lead to necrotizing esophagitis or bronchitis, respectively (Hanke *et al.*, 1991).

## GAS BUBBLE DISEASE

Uncompensated hyperbaric total dissolved gas pressure is a significant problem within hatcheries. Although all the life stages of fish—and indeed other groups of aquatic animals—can be susceptible, the most prone appear to be the youngest (Bouck *et al.*, 1980; Weitkamp and Katz, 1980) and gas bubbles readily appear in the perivascular compartments of the yolk sack and nearby abdominal cavity causing the fish to float tail up, or

belly-up depending on the distribution of the bubbles. Although much of the earlier work on GBD dealt with the syndrome in native or feral fish downstream from hydroelectrical projects—where atmospheric gas becomes entrained—more recent work has examined GBD either as an *in vivo* model for hyperbaric medical emergencies, or as a management disease within aquaculture.

Gas solubility relates inversely to water temperature and directly to hydrostatic pressure; nitrogen, oxygen and argon are the three most abundant gases in water and, almost exclusively, GBD relates to elevated nitrogen levels. Within aquaculture, air injection systems, accidental entrainment of air through leaky fittings, or the use of groundwater are the scenarios most commonly linked to GBD problems. Multi-gas transfer models show that aerating systems with high transfer efficiency for oxygen also have high transfer efficiency for nitrogen (Colt and Westers, 1982). Edsall and Smith (1991) have also shown that oxygen injection systems could cause GBD directly through supersaturation of water with oxygen alone, although this challenges the assumption that an inert gas is necessary for GBD, and it is possible that the oxygen emboli may not be as persistent as nitrogen emboli. Delivery of groundwater through closed pipes does not allow the water to depressurize when pumped to the surface. The problem is further compounded if the water is subsequently heated; chronic exposure to a total gas pressure at 102% of saturation is typically sufficient to induce GBD in at least some fish in a population.

Symptoms of GBD are protean. In sack fry, gas emboli form between the yolk and perivitelline membranes, as well as the abdominal cavity, fins and cranium (Cornacchia and Colt, 1984). In older juveniles, symptoms include loss of buoyancy, side swimming, and spasmodic convulsions (Machado *et al.*, 1987) and exophthalmia. The mechanism of gas embolus formation begins with initial formation of an intravascular gas bubble and this requires either a stable nucleus such as a pre-existing small stable pocket of gas, or a zone of decreased surface tension (Strauss, 1979). Bubble growth requires that the total gas pressure inside the bubble exceeds the combination of forces restricting its growth and, thus, is affected by perfusion and clearance rates of gases from surrounding tissue.

Large gas bubbles (when comprised of inert gases) may completely inhibit blood flow, leading to anoxic injury to the subjacent endothelium; endothelial cells undergo degeneration with marked exocytotic vesiculation and pitting of the apical membrane. Endothelial damage and

exposure of subendothelial connective tissue, with the consequent formation of cellular thrombi, activation of clotting mechanisms and perivascular leukocytic response is the pivotal pathophysiological event driving GBD (Casillas *et al.*,1975; Speare 1990, 1991).

Ocular damage during GBD can involve many parts of the eye; primary damage to the uvea from the emboli triggers secondary responses in other parts of the eye. Sequential pathology studies have shown that early changes include a mild exophthalmia stemming from bubbles in the choroid gland of the posterior uvea; the retina and choroid are displaced anteriorly into the vitreous cavity. This progresses to a more dramatic expansion of the equatorial anatomical axis, and larger retrobulbar bubbles develop; as the orbit compresses along its anterior-posterior diameter (Figs. 20.3a, b), the iris forms synechia with the corneal endothelium. Lenticular cataracts are common (Fig. 20.4) along with suppurative panuveitis. In more severe or protracted cases, there can be marked attenuation of the optic nerve, retinal artery and retinal vein; combined with intense uveitis, phthisis bulbi (Fig. 20.5) is a not uncommon outcome (Speare, 1990)

Within the gill, emboli are most often noted in the afferent or efferent filament arteries, and also within the central venous sinusoid; a diagnosis of GBD can often be made using whole-mounts of gill tissue. Damage to the skin arises due to thrombi within dermal vessels; epithelial erosion is common in areas overlying damaged vessels and this may help provide a link between the frequency of skin disease outbreaks following GBD episodes (Speare, 1991).

## PREDATION

Fish farms are an abundant source of food for many opportunistic predators. Predatory bird are a particular problem for land-based farms, and economic losses stem not only from direct losses, but also indirectly through injury and stress. Opportunistic infections of deep wounds inflicted by beaks and talons are common. At marine sites, seal attacks are often at their peak during cold weather when cold water temperature delays wound healing. Additionally, seal attacks can damage nets and stress fish. Although site selection for fish farms takes into account the local presence of seal haul-out sites and rookeries, seal populations nonetheless respond quickly to the presence of a farm site and remain a problem despite use of acoustic deterrents.

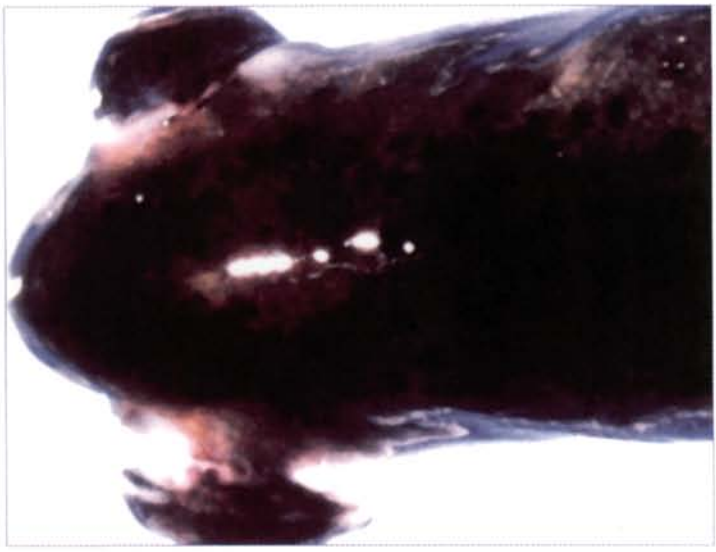

**Fig. 20.3a** Gross appearance of trout with severe chronic gas bubble disease and associated exophthalmia. Exophthalmia is the result of retro-orbital gas-filled lesions.

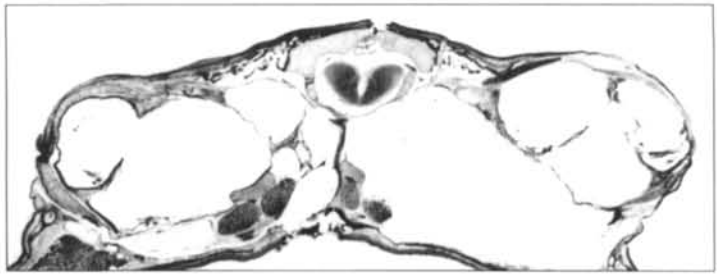

**Fig. 20.3b** Section through cranium of trout with severe gas bubble disease; large space occupying lesions are present retro-orbitally and have displaced the eyes and stretched the optic nerve and artery.

## TERRITORIAL BEHAVIOR

Antagonistic behavior of salmonids is most commonly detected by the presence of bite wounds on the tail fin, peduncle region, anal fin, vent, and cornea; bite wounds can easily become infected with opportunistic pathogens (Fig. 20.6). Although it is intuitive to think that high stocking densities would lead to more fighting, the opposite is true; densities as high as 229 kg per meter had no appreciable effect (Soderberg *et al.*, 1993),

**Fig. 20.4** Histology section of lens showing subcapsular globules characteristic of some forms of lens cataract. Lens defects such as this can remain as persistent markers in fish which recover from GBD.

whereas at low stocking densities, as might be used in laboratory studies, interactions between dominant and subdominant fish increased. Juvenile Atlantic salmon have strong territorial tendencies which are suppressed with increased stocking density.

*Eye-snapping* is a peculiar problem relating to situations where shafts of light enter the tank and reflect from the fish's eyes. The resulting flash evokes attacks from tank mates and this often results in a peculiar pattern of unilaterial periocular dermatitis, corneal ulceration and sometimes enucleation. The angle of light, combined with the distribution of fish in the tank can favor damage to either the left or right eye.

**Fig. 20.5** Irreversible ocular damage (phthisis bulbi) resulting from gas bubble disease. Such eyes are often completely lost, setting the stage for establishment of bacterial infections which move retrograde along the perineurium of the optic nerve to the base of the brain.

**Fig. 20.6** Bite wounds on these rainbow trout have resulted in opportunistic bacterial dermatitis. Tail bites are most common in subdominant fish within a population.

## DISEASES ASSOCIATED WITH WATER RECIRCULATION SYSTEMS AND AUTOINTOXICATION

Recirculation systems, wherein water exiting a production tank is treated and returned to the tank, are becoming very popular as a means of conserving water and thermal resources. Use of supplemental oxygen is a key feature of these systems; low dissolved oxygen is a critical primary rule-out during investigations of *Respiratory Distress Syndrome*. The pathology of RDS can include acute hepatocellular necrosis (Fig. 20.7) with a distribution favoring cells near the equivalent of central veins, thus differentiating it from toxic hepatitis.

Biofiltration to remove ammonia, by converting it first to nitrite and then to nitrate, is a key feature of recirculation systems, and disease associated with elevated ammonia or its nitrification by-products is common. Biofiltration efficiency, specifically the metabolic rate of bacteria, can be affected by sudden drops in water temperature, reduced oxygen levels, or exposure to treatment chemicals.

Despite years of research, the physiological effects of ammonia are uncertain. Although gill lesions are commonly assumed to arise from

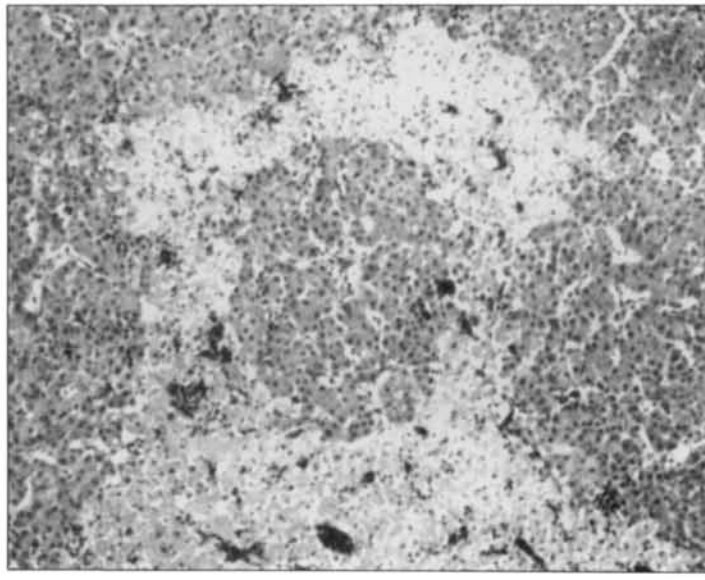

**Fig. 20.7** Histology section showing severe acute hepatocellular necrosis stemming from prolonged exposure of salmon to low levels of oxygen. Note the lack of inflammatory response at this stage.

ammonia toxicity, no exact model of this damage has been developed, and indeed even high levels of unionized ammonia often fail to elicit any degree of gill damage (Lang *et al.*, 1987). Eels under intensive culture exhibit increased oxygen consumption rates and growth suppression in systems with high ambient ammonia concentrations (Knights, 1989), and in rainbow trout growth suppression is linked to reduced appetite (Lang *et al.*, 1987). A remarkable finding is the ability of fish to adapt to elevated ammonia levels (Knoph, 1992) and this may partially explain discrepancies between studies.

Methemoglobin production, arising from the interaction of nitrite as a chemical oxidizer of haeme iron, leads to hypoxia in affected fish (Lewis and Morris, 1986). Additionally, the methemoglobin fraction can also affect the oxygen dissociation of residual unaffected hemoglobin. Nitrite toxicity also leads to vasodilation, through the chemical's direct effects as a vasodilator. Outbreaks of infectious disease following nitrite exposure (Hanson and Grizzle, 1985; Speare and Backman, 1988) may relate to the toxic effect of the chemical on leukocytes.

Reuse of water promotes elevated carbon dioxide concentrations, which is often not effectively dealt with and leads to a depressed pH. Fish in recirculation systems often have an increased incidence of renal tubular mineralization and degeneration termed *nephrocalcinosis*, which is rarely a clinical problem, but can give rise to downgrading at the time of marketing. This not only occurs in salmon, but also other species in recirculation systems (Chen *et al.*, 2001), and can be managed through the addition of sodium bicarbonate to maintain water alkalinity. *Swimbladder Dilation Syndrome* (Fig. 20.8) is more common in fish in recirculation

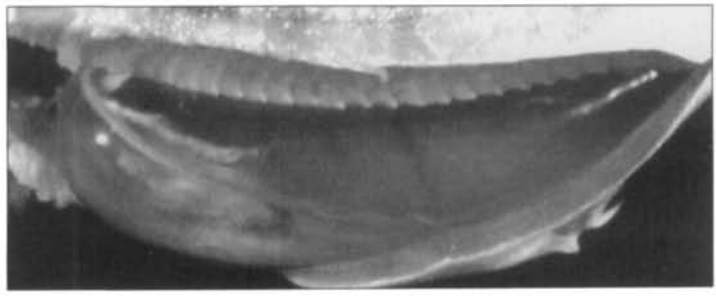

**Fig. 20.8** Gross dissection revealing a severely distended swimbladder. Typically, the affected structure shows no histopathological changes.

systems than in flow-through systems and it is suspected to be related to carbon dioxide levels although definitive studies are lacking. Fish with SDS can survive for protracted periods, can be manually deflated by the use of hypodermic needles, and are prone to sunburn and predation as they struggle at the water surface. Although dilated, histological changes affecting the wall of the swimbladder are typically lacking.

The term *Autointoxication* applies to situations where cage-cultured fish are affected by products released from benthic degradation of waste materials accumulating below the cage site. Sites at risk are those where currents are not strong, and the distance between the cage bottom and the ocean bottom is minimal such that waste material deposits directly below the cage itself. As anoxic conditions develop within the debris pile, production of hydrogen sulphide can occur and when this off-gasses into the water, it rises directly up into the cage of fish (Papoutsoglou *et al.*, 1996), causing the condition known as *site souring*. Hydrogen sulphide, with action similar to that of nitrite, can affect the kinetics of red blood cell oxygenation; Kiemer *et al.* (1995) have shown that hydrogen sulphide is also directly damaging to gill tissue.

## SEAWATER ACCLIMATION

Up to 3% of Atlantic salmon smolt which are moved to marine cage sites may die from their inability to cope with the marine environment. Post-transfer mortality spikes may occur shortly after fish are introduced to seawater, and some of this may be attributed to mechanical injuries which fish have received during shipment. Protracted patterns of low level mortality which persist are more typically the result of inadequate seawater readiness; fish can persist for several weeks before dying and, overall, the problem is greater with fall transfers as compared to spring transfers. These fish, sometimes referred to as 'slinks' develop reduced body condition, show atrophy of many visceral organs, have a distended gall bladder, serous atrophy of fat stores, and often have increased amounts of melanin and lipofuscin within splenic and renal macrophages.

Treatment of fish with chemicals near the time of smoltification may have the unexpected effect of rendering the fish less tolerant to seawater, and this is particularly the case for treatment chemicals that are capable of damaging chloride cells (Bouck and Johnson, 1979), although more recent work has failed to confirm this (Powell *et al.*, 1996). This problem

is accentuated by the pre-smoltification process, during which, the apical surface of chloride cells becomes enlarged and accessory cells with ion-permeable junctions develop. Copper's effects on cellular tight junctions and the specific effect on sodium-potassium ATPase enzymes strongly suggest avoiding copper sulfate prior to smolt transfer (Lauren and McDonald, 1986).

Strong currents at marine net sites can be damaging to recently introduced smolt. Exertional muscle necrosis has been noted, and lesions such as myofibrillar necrosis, interfibrillar fibrosis, and regeneration of myotubes, can persist in the muscle for months. Furthermore, Rodger *et al.* (1991) have suggested that *Sudden Death Syndrome* in farms with a history of *Pancreas Disease* (see elsewhere in this text), may relate to the effects of acute necrotizing exertional myopathy.

## SPECIFIC EXAMPLES OF DISEASES RELATING TO NUTRITION

Although modern methods of feed manufacture have reduced the problems inherent to ingredient stability, polyunsaturated fatty acid stability remains an issue (Winston, 1991). There are many patterns of clinical disease associated with production of malonaldehyde (a product developing when PUFAs are exposed to oxygen) within feed, and the common link is exhaustion of antioxidant defenses within the fish. There are quantitative differences between species for the major antioxidant enzymes which may also contribute to differences noted in susceptibility, and even the target organs affected when rancid feeds are accidentally used (Winston, 1991). Other dietary components can also modify the type or severity of clinical disease. For example, Frischknecht *et al.* (1994) demonstrated the sparing effects brought about by dietary levels of vitamins C and E when rancid fats were used. Furthermore, environmental stresses may accentuate other sources of oxidative stress (Welker and Congleton, 2004). Although lipoid liver disease is the most common manifestation of rancid feed use, the overall syndrome includes depressed growth, anaemia due to red blood cell lysis, muscle necrosis, and haemosiderin and ceroid deposits in the spleen, kidney and liver. Severe granulomatous hepatitis, centered on areas of marked ceroid deposition (Fig. 20.9) has been noted in haddock fed rancid diets; a condition of fat cell necrosis affecting subdermal adipose tissue (Fig. 20.10) has been noted specifically in Atlantic halibut (Bricknell *et al.*, 1996) and can be

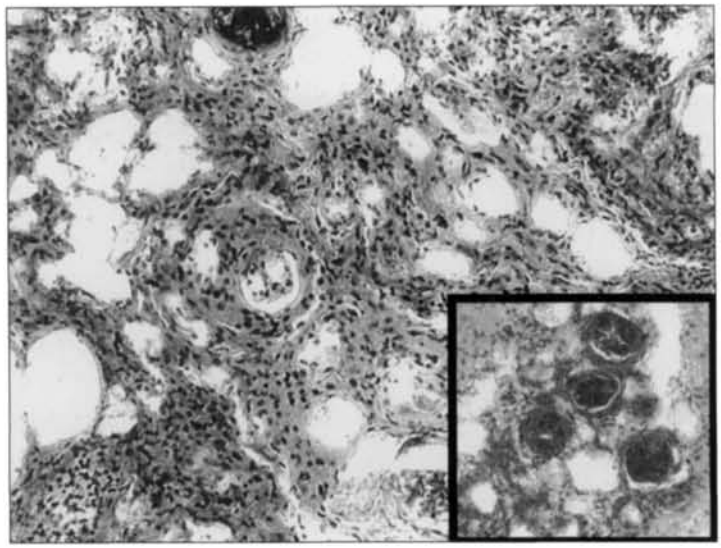

**Fig. 20.9** Multifocal granulomatous hepatitis in a haddock fed oxidized oils. Inset reveals PAS positive reaction of the ceroid deposits at the centre of the tissue reaction. Material courtesy of Dr Santosh Lall.

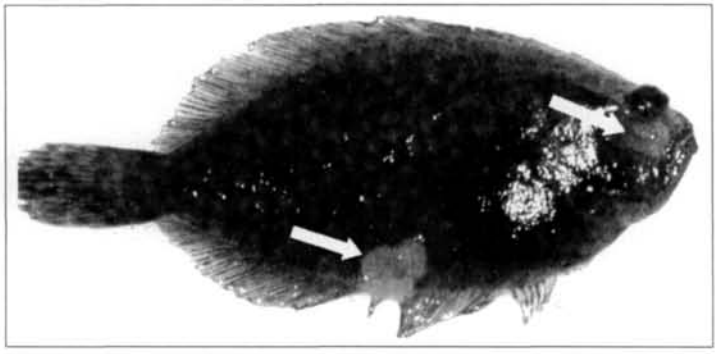

**Fig. 20.10** Juvenile summer flounder with ulcerative dermatitis (arrows) overlying regions of necrotizing panniculitis. Material courtesy of Dr Greg Goff.

accompanied by severe granulomatous pericarditis with extension of the inflammatory process into the heart muscle (Fig. 20.11).

Oxidized lipids in diet can also serve as a co-factor for infectious disease. For example, *Rainbow Trout Fry Syndrome* resulting from the bacterium *Flavobacterium psychrophilum* is more common in fish fed

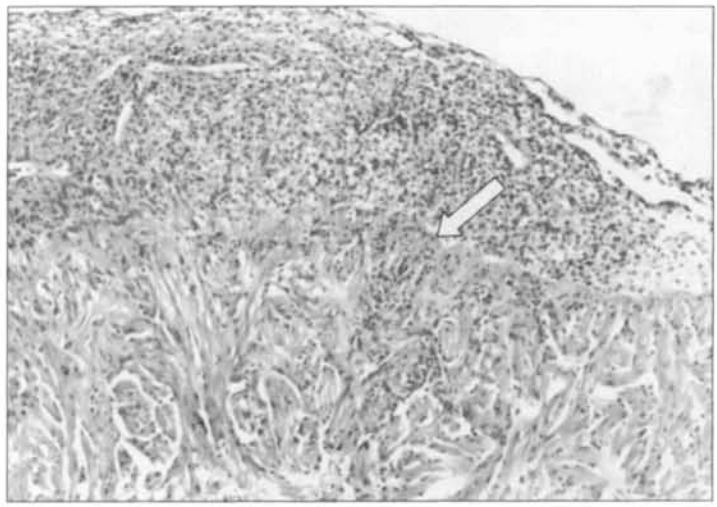

**Fig. 20.11** Histology of granulomatous epicarditis in a halibut; the lesion has extended through the compact myocardium and in invading the trabecular portion of the ventricle (arrow). Curiously, affected fish may not show any clinical symptoms of heart disease. Material courtesy of Dr Roland Cusack.

oxidized lips in feed than in controls (Daskalov *et al.*, 2000). The so-called *Cardiomyopathy Syndrome* or CMS is an unusual condition noted in fat fast-growing salmon that have spent at least one winter in marine cages in which the best appearing fish often die during feeding due to a ruptured atrium (Grotmol *et al.*, 1997). Whether the disease reflects the effects of primary oxidant damage, or an endotheliotropic nodavirus, or a combination of a nutritional and viral etiology has yet to be determined. Of interest, previous work has shown that Atlantic salmon, during periods of stress, will accumulate adrenaline in atrial tissues and this can be cardiotoxic, resulting in necrosis of spongy myocardium (Carlsten *et al.*, 1983; Floysand *et al.*, 1992). Although initially regarded as an unusual disease characterized solely by sporadic mortality spikes, recent work has shown that CMS can also be a chronic disease with debilitating economic effects (Brun *et al.*, 2003).

Gastrointestinal impaction, also known as *Water Belly Syndrome* is a problem of increasing concern in some parts of the world where salmon are cultured, particularly where salinity is high and water temperatures are low (Staurnes *et al.*, 1990). Fish with WBS show gross distension of the abdomen, and upon dissection a fluid-filled stomach is revealed. The tunica muscularis and serosa are remarkably thinned. The incidence of

WBS has also been linked to changes in feeding programs, specifically a shift to a more intensive feeding rate using extruded pellets with a higher fat and digestible carbohydrate composition (Staurnes *et al.*, 1990); chilled water may affect the mechanisms of ion transport (Rorvik *et al.*, 2000). Miller and Black (1992) documented a disease with similar gross signs, but relating to fish consuming wood chip debris leading to impaction. Related to WBS, Lumsden *et al.* (2002) have also found epithelial hyperplasia and inflammation of the submucosa of the airbladder in fish with WBS, thus giving rise to the new term *Gastric Dilation Air Sacculitis Syndrome.*

Accelerated growth rates are implicated as a factor contributing to the high incidence of lens cataracts in farmed fish (Ersdal *et al.*, 2001) and it is proposed that because the outer cortical layers of the lens have the highest metabolic activity, this layer may suffer a relative lack of some essential nutrients during rapid growth. High dietary levels of antioxidants proves protective, whereas high levels of iron, lipids, or manganese promoted the incidence of cataracts (Waagbo *et al.*, 2003).

## DISEASES RELATING TO WEATHER CONDITIONS

*Superchill Disease* characterized by extreme rates of mortality, sometimes affecting entire farms occurs in those areas of salmon culture where water temperatures fall below the blood plasma freezing temperatures of fish (Fletcher *et al.*, 1998). The pathogenesis of superchill involves propagation of ice nuclei from the environment and across the epidermis. Survivors may have long-term health problems, including increased rates of lenticular cataract caused by denaturating of temperature sensitive lens proteins known as crystallins (Bjerkas *et al.*, 2001).

Ultraviolet-B radiation can be a cause of necrotizing dermatitis (Fig. 20.12) in both land-based and sea cage operations. It can be especially problematic shortly after release of smolt in netpens, particularly when the smolt have been previously sheltered from UV at the tank farm site, and when the sea cage nets have been made shallow for smolt entry and when the water is clear (Rodger, 1991). Additionally, it has been shown that in young fish with rapidly growing epidermis, UV exposure can be even more of a problem than expected and can also delay wound healing by interfering with epithelial cell migration across defects (Bullock and Roberts, 1992).

*Screamer Disease*, a condition in which salmon develop a permanently gaping mouth and flared operculae, may be related to situations where

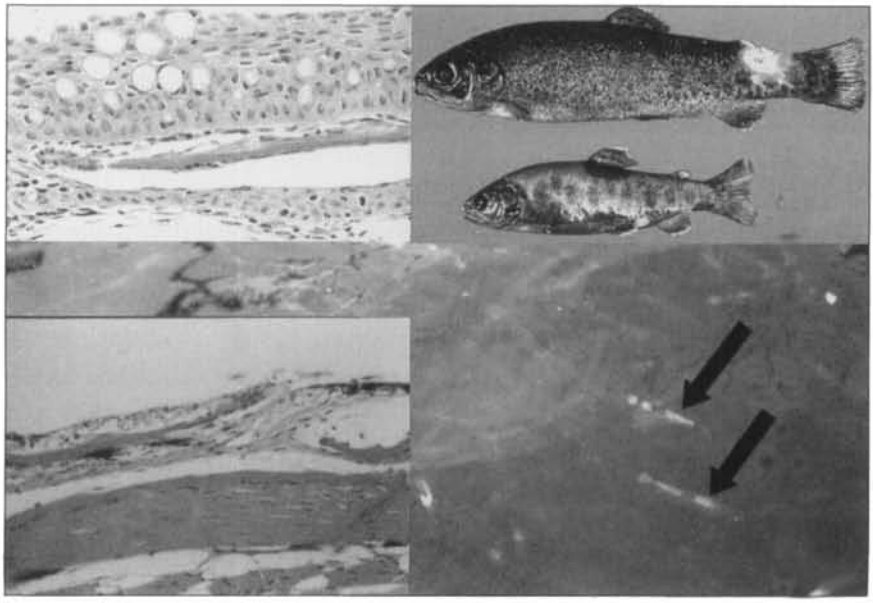

**Fig. 20.12** Lower right panel shows fish with dorsal sunburn lesions in an uncovered raceway. Top right panel shows two fish with UV damage; top left illustrates typical normal trout skin; bottom left reveals the extensive epithelial necrosis typical for severe sunburn.

smolt experience a temperature change when moved from the smolt unit to netpen sites (Roberts *et al.*, 2001). Chilean salmon afflicted with this condition have been shown to exhibit ankylosis of mandibular articulations suggestive of a nutritional etiology, although this is yet to be confirmed. Warped gill opercula, fin and jaw abnormalities have also been linked to elevated water temperature during egg incubation (Ornsrud *et al.*, 2004). Similarly, elevated water temperature during egg incubation has been cited as the cause for ventricular hypoplasia in Atlantic salmon. This cardiac malformation involves a reduced size of the ventricle, poor development of the outer compact myocardium, abundant fat in the epicardium, and inflammation at the interface between compact and spongy myocardium (Poppe and Taksdal, 2000).

Blooms of diatom algae can have disastrous effects on marine fish farms; conditions for diatom blooms are usually brought about from upwelling currents which cause nutrient-rich water to mix with warm water, thus, facilitating bloom dynamics. Although sentinel programs are in place in many parts of the world where diatom blooms are a common

occurrence, the fixed nature of large sea cage platforms makes it difficult to consider moving a farm from the path of a bloom. However, management practices such as not feeding fish, and keeping workers off the farm site can help in keeping the salmon in the lower parts of the nets where diatom concentrations may be lower. Some species of salmon (for example, chinook as compared to Atlantic) are more responsive to diatom blooms and may seek the bottom of nets as a natural behavioral-avoidance reaction. The use of skirting curtains to limit diatoms from entering cages usually has limited effects; addition of supplemental oxygen to a farm site during and after bloom exposure can help mitigate against the respiratory compromise, which is a shared feature of the pathophysiological sequalae of exposure to diatoms. The long spiny setae of several *Chaetocerous* and *Corethron* spp. (Speare *et al.*, 1989) cause these diatoms to become trapped between gill lamellae (Figs. 20.13 and 20.14). Chronic reactions in the gill are similar to silicosis in the mammalian lung and giant cell macrophages and progressive granulomatous branchitis can remain as a persistent marker of diatom exposure long after the bloom has subsided. Diatoms which do not possess setae—but form chains—are also implicated as

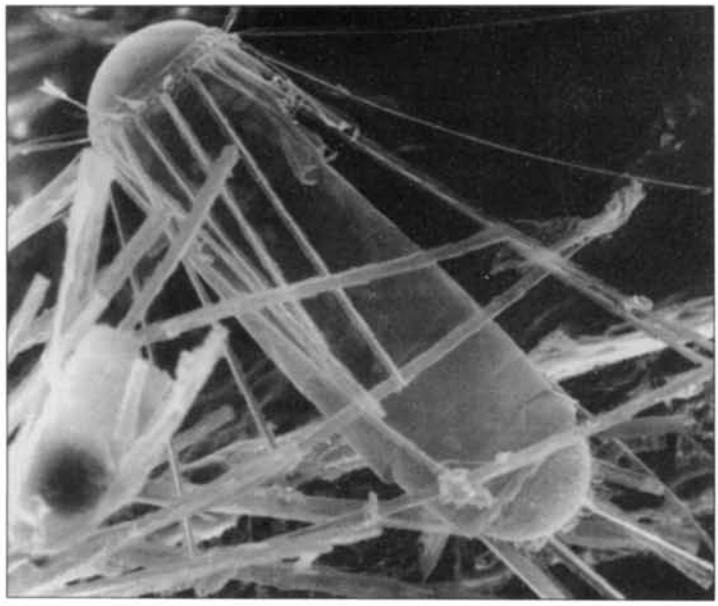

**Fig. 20.13** SEM of *Corethron* sp. diatom showing the extensive array of setae emerging from the characteristic corona of this non chain-forming genus.

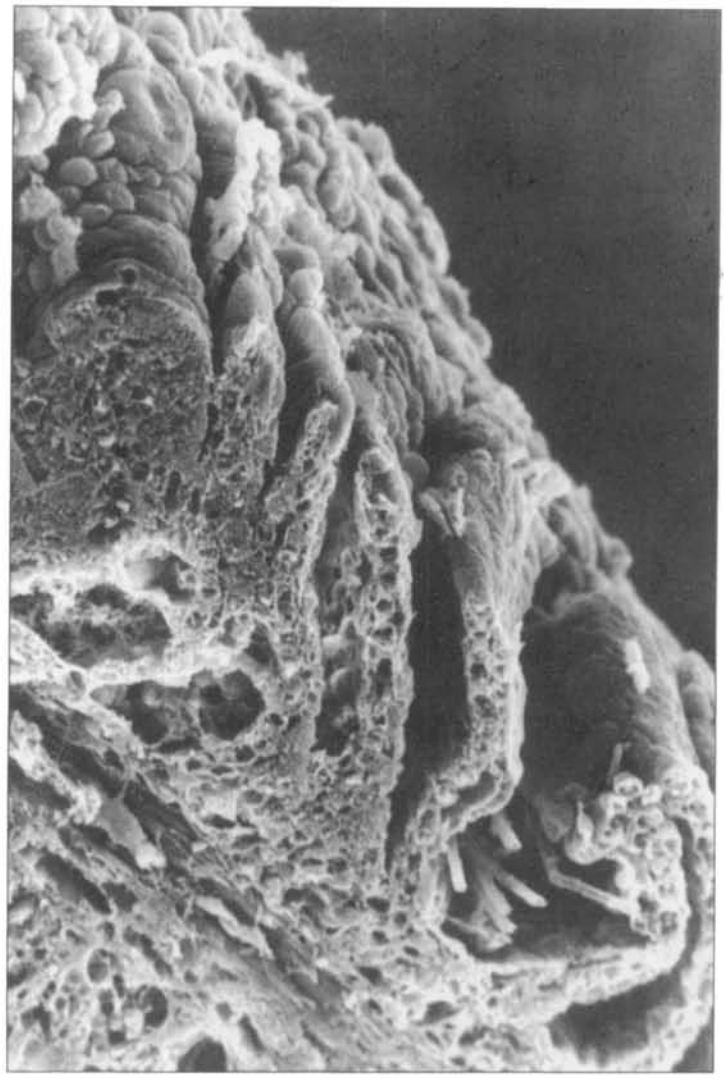

**Fig. 20.14** Freeze fracture SEM revealing extensive lamellar fusion trapping diatom setae. Such trapped material stimulates persistent branchitis and may be found within gill tissue several months after the diatom bloom has subsided.

harmful diatoms. Kent *et al.* (1995) demonstrated severe gill lesions caused by *Skeletonema costatum* and two species of *Thalassiosira*. Other diatoms exert an influence on fish health through release of toxins. *Heterosigma carterae (akashiwo)* possess a neurotoxic brevitoxin that

causes exposed fish to appear anesthetized rather than oxygen starved (Black *et al.*, 1991; Khan *et al.*, 1997); *Chrysochromulina polylepsis* appears to have a toxin affecting the permeability of gill epithelial tight junctions (Underdal *et al.*, 1989). A unique scenario attributable to toxic fractions (microcystin) of blue-green algae has been noted, in which hepatic necrosis and production of characteristic megalocytic hepatocytes (Fig. 20.15) develops (Kent *et al.*, 1988; Stephen *et al.*, 1993). This condition, called *Net Pen Liver Disease* is most typical when it affects Atlantic salmon

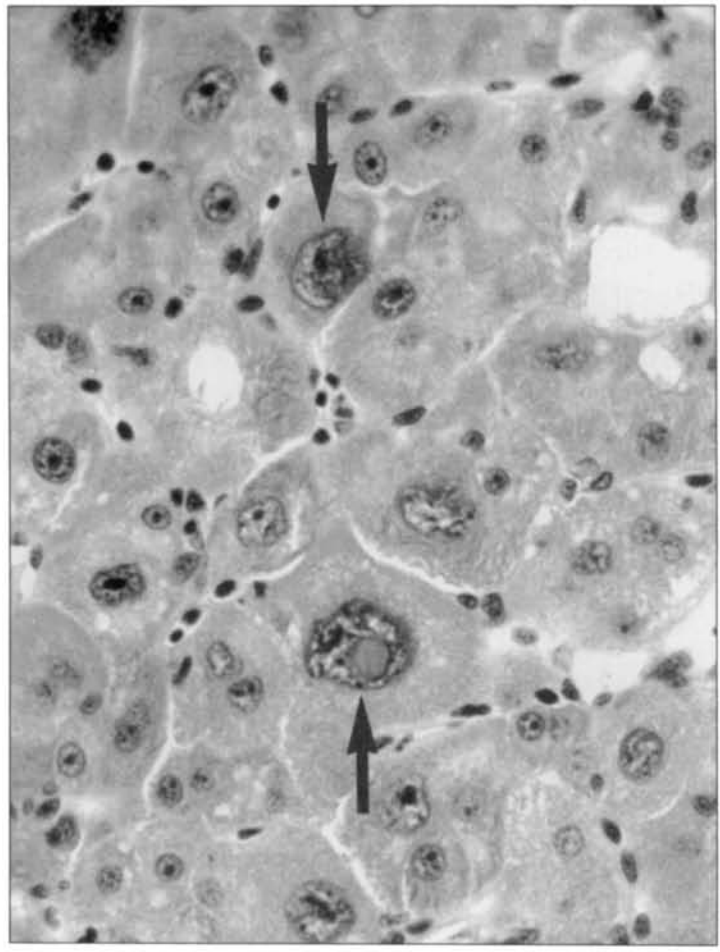

**Fig. 20.15** Histology from a case of Net Pen Liver Disease, arrows show megalocytic hepatocytes. Material courtesy of Dr M.L. Kent.

during their first year in seawater. In some situations, cumulative mortality can reach 90% (Kent, 1990).

## IATROGENIC CONDITIONS

Treatment and vaccination, in addition to their benefits, can have unexpected untoward effects. Although anecdotal evidence is common, published reports are relatively few. Bath delivery of treatment chemicals to control external gill and skin diseases is very common in hatcheries. Chemicals may be used as treatment, as prophylaxis, or as metaphylaxis and dose and exposure times vary accordingly. Outright errors in chemical use—as may come about through incorrect calculations—incorrect target dose, errors in chemical contact time with fish, and water chemistry or quality variables which increase the chemicals toxicity are not uncommon (Byrne *et al.* 1989; Speare and Ferguson, 1989; Newbound *et al.*, 1993). The addition of oxygen during treatment periods is often used to counteract effects which chemical may directly have on oxygen levels (chemical oxygen demand), or changes in oxygen uptake and consumption by the fish during the stress of treatment (biological oxygen demand). Although intuitive, this practice may by contra-indicated as demonstrated by Powell and Harris (2004) who showed that the toxicity of chloramine-T is enhanced when treatment water received supplemental oxygen. Additionally, drug and chemical target-animal safety data is typically collected from trials on healthy fish, and little regard has been placed on the potential effect of pre-existing disease on chemical tolerance. For example, fish with chronic branchitis—as can develop during Nodular Gill Disease of salmon—are highly prone to formalin treatment and often die even when relatively low doses, typically well tolerated by other fish, are used (Speare and Ferguson, 1989). Water characteristics are also very important. Whereas water rich in divalent cations renders chloramine-T (sodium para-toluene-sulphonchloramide) relatively safe, great care and lower doses must be used in softer waters. For other chemicals, the margin of safety is slim; quaternary ammonia compounds are capable of eliciting significant gill damage even when used according to widely published dose rates (Bryne *et al.*, 1989). Furthermore, quaternary ammonia compounds are selectively attracted to the negatively charge mucopolysaccharide coat of the gill (Knezovich *et al.*, 1989), suggesting that a pre-existing branchial mucous cell metaplasia (Fig. 20.16) and mucous hypersecretion scenario would increase the odds of this type of treatment damaging the gills (Fig. 20.17).

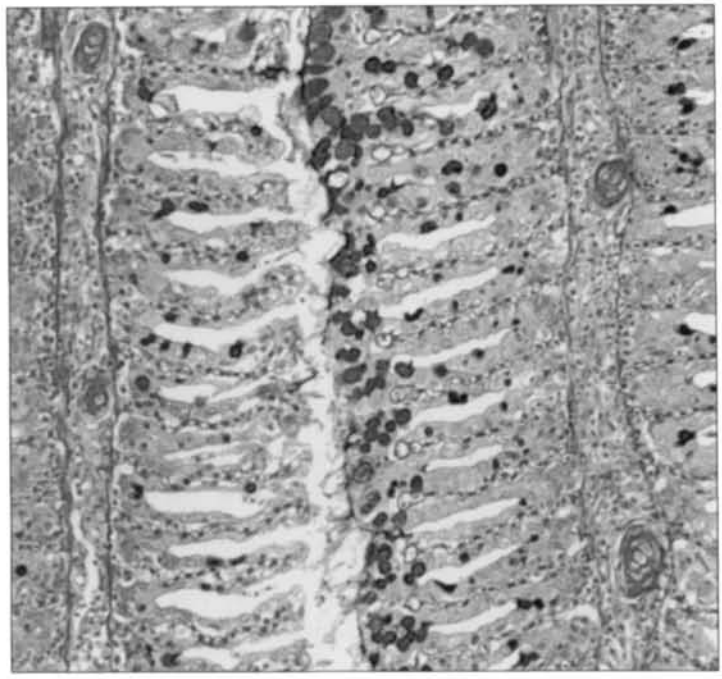

**Fig. 20.16** Diffuse mucous cell metaplasia on the gill of an Atlantic salmon.

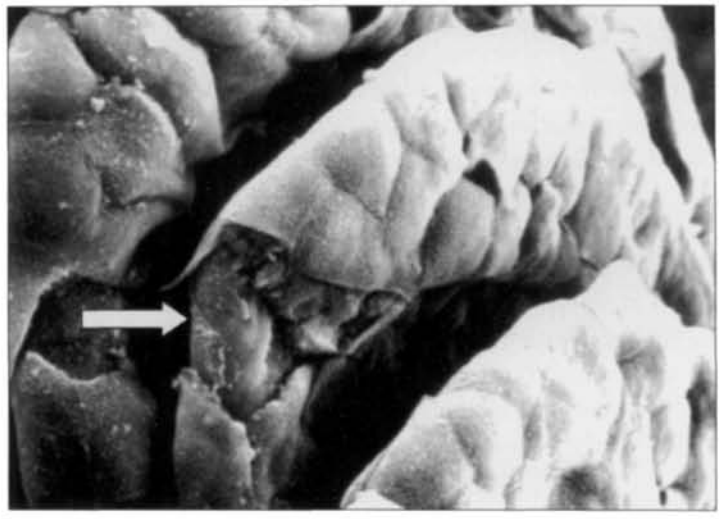

**Fig. 20.17** SEM from the gill of a trout exposed to a quaternary ammonia treatment compound. Arrow denotes region of the lamella where epithelial necrosis has occurred. Material courtesy of Dr Phil Byrne.

Subclinical effects of treatments are also now becoming recognized. Growth rate reductions due to inhibited feed intake have been shown to occur when formalin in used as a prophylactic treatment (Speare *et al.*, 1997), and Yacob *et al.* (2002) have shown the mechanism behind this to be a suppression of gustatory neural pathways, specifically oxidation of ion channels which then affects their gating kinetics such that taste transduction is reduced. Formalin also exerts effects on red blood cells; in olive flounder (*Paralichthys olivaceus*), formalin causes haemolysis and methaemoglobin production and elevated levels of released liver enzymes (Jung *et al.*, 2002).

Vaccination of fish with preparations containing oil-based adjuvants can result in chronic granulomatous peritonitis with persistent adhesions, melanosis, and focal muscle degeneration and necrosis affecting white muscle of the fillet, leading to downgrading at the time of marketing (Poppe and Breck, 1997; Koppang *et al.*, 2005). Growth of fish is also affected by this exuberant tissue reaction and potential effects on the future reproductive capability of broodstock has been highlighted (Colquhoun *et al.*, 1998; Pylkkö *et al.*, 2000). Other organs, distant from the vaccination site, may also be affected; granulomatous uveitis has been documented in Atlantic salmon following vaccination and possibly resulted in glaucoma (Koppang *et al.*, 2004).

## Summary

Competition within the aquaculture industry continues to favor those farms which can effectively manage their costs of production. Increasing the degree of intensification, water reuse, accelerated growth rates, and—with respect to salmon—early introduction to seawater grow-out sites, have become necessary steps in order to achieve a profit. Trade-offs and balances between production efficiency and animal health are encountered in all types of agriculture; in fish farming this is coupled with the vulnerability of fish stocks to environmental indices which are often out of the control of the farm manager.

## References

Bjerkas, E., E. Bjørnstad, O. Breck and R. Waagbø. 2001. Water temperature regimes affect cataract development in smolting Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 24: 281-291.

Black, E.A., J.N.C. White, J.W. Bagshaw and N.G. Ginther. 1991. The effects of *Heterosigma akashiwo* on juvenile *Oncorhynchus tshawytscha* and its implications for fish culture. *Journal of Applied Ichthyology* 7: 168-175.

Bouck, G.R. and D.A. Johnson. 1979. Medication affects tolerance to seawater in coho salmon smolts. *Transactions of the American Fisheries Society* 108: 63-66.

Bouck, G.R., B. D'Aoust, W.J. Ebel and R. Rulifson. 1980. Atmospheric gas supersaturation: Educational and research needs. *Transactions of the American Fisheries Society* 109: 769-771.

Bricknell, I.R., D.W. Bruno, T.J. Bowden and P. Smith. 1996. Fat cell necrosis syndrome in Atlantic halibut, *Hippoglossus hippoglossus* L. *Aquaculture* 144: 65-69.

Brun, E., T. Poppe, A. Skrudland and J. Jarp. 2003. Cardiomyopathy syndrome in farmed Atlantic salmon *Salmo salar*: Occurrence and direct financial losses for Norwegian aquaculture. *Diseases of Aquatic Organisms* 56: 241-247.

Bullock, A.M. and R.J. Roberts. 1992. The influence of ultraviolet-B radiation on the mechanism of wound repair in the skin of the Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 15: 143-152.

Byrne, P., D.J. Speare and H.W. Ferguson. 1989. Effects of a cationic detergent on the gills and blood chemistry of rainbow trout *Salmo gairdneri*. *Diseases of Aquatic Organisms* 6: 185-196.

Carlsten, A., O. Poupa and R. Volkmann. 1983. Cardiac lesions in poikilotherms by catecholamines. *Comparative Biochemistry and Physiology* A76, 567-581.

Casillas, E., S.E. Miller, L.S. Smith and B.G. D'Aoust. 1975. Changes in hemostatic parameters in fish following rapid decompression. *Undersea Biomedical Research* 2: 267-276.

Chen, C.Y., G.A. Wooster, R.G. Getchell and P.R. Bowser. 2001. Nephrocalcinosis in Nile Tilapia from a recirculation aquaculture system: A case report. *Journal of Aquatic Animal Health* 13: 368-372.

Colt, J. and H. Westers. 1982. Production of gas supersaturation by aeration. *Transactions of the American Society* 111: 342-360.

Cornacchia, J.W. and J.E. Colt. 1984. The effects of dissolved gas supersaturation on larval striped bass *Morone saxatilis* (Walbaum). *Journal of Fish Diseases* 7: 15-27.

Daskalov, H., P.A.W. Robertson and B. Austin. 2000. Influence of oxidized lipids in diets on the development of rainbow trout fry syndrome. *Journal of Fish Diseases* 23: 7-14.

Edsall, D.A. and C.E. Smith. 1991. Oxygen-induced gas bubble disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture and Fisheries Management* 22: 135-140.

Ersdal, C., P.J. Midtlyng and J. Jarp. 2001. An epidemiological study of cataracts in seawater farmed Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 45: 229-236.

Ferguson, H.W., T. Poppe and D.J. Speare. 1990. Cardiomyopathy in farmed Norwegian salmon. *Diseases of Aquatic Organisms* 8: 225-231.

Fletcher, G.L., M.H. Kao and J.B. Dempson. 1998. Lethal freezing temperatures of arctic char and other salmonids in the presence of ice. *Aquaculture* 71: 369-378.

Fløysand, R., J.A. Ask, G. Serck-Hanssen and K.B. Helle. 1992. Plasma catecholamines and accumulation of adrenaline in the atrial cardiac tissue of aquaculture Atlantic salmon (*Salmo salar*) during stress. *Journal of Fish Biology* 41: 103-111.

Frischknecht, R., T. Wahli and W. Meier. 1994. Comparison of pathological changes due to deficiency of vitamin C, vitamin E and combinations of vitamins C and E in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 17: 30-45.

Grotmol, S., G.K. Totlund and H. Kryvi. 1997. Detection of a nodavirus-like agent in heart tissue from reared Atlantic salmon *Salmo salar* suffering from cardiac myopathy syndrome (CMS). *Diseases of Aquatic Organisms* 29: 79-84.

Hanke, A.R., S. Backman, D.J. Speare and G.W. Friars. 1991. An uncommon presentation of fungal infection in Atlantic salmon fry. *Journal of Aquatic Animal Health* 3: 192-197.

Hanson, L.A. and J.M. Grizzle. 1985. Nitrite-induced predisposition of channel catfish to bacterial diseases. *Progressive Fish-Culturist* 47: 98-101.

Jobling, M., B. Andreassen, A.V. Larsen and R.L. Olson. 2002. Fat dynamics of Atlantic salmon *Salmo salar* L. smolt during early seawater growth. *Aquaculture Research* 33: 739-745.

Jung, S.H, D.S. Sim, M.S. Park, Q. Jo and Y. Kim. 2003. Effects of formalin on haematological and blood chemistry in olive flounder, *Paralichthys olivaceus* (Temminck et Schlegel). *Aquaculture Research* 34: 1269-1275.

Kent, M.L. 1990. Netpen liver disease (NLD) of salmonid fishes reared in seawater: Species susceptibility, recovery, and probable cause. *Diseases of Aquatic Organisms* 8: 21-28.

Kent, M.L., M.S. Myers, D.E. Hinton, W.D. Eaton and R.A. Elston. 1988. Suspected toxicopathic hepatic necrosis and megalocytosis in pen-reared Atlantic salmon *Salmo salar* in Puget Sound, Washington, USA. *Diseases of Aquatic Organisms* 4: 91-100.

Kent, M.L., J.N.C. Whyte and C. LaTrace. 1995. Gill lesions and mortality in seawater pen-reared Atlantic salmon *Salmo salar* associated with a dense bloom of *Skeletonema costatum* and *Thalassiosira* species. *Diseases of Aquatic Organisms* 22: 77-81.

Khan, S., O. Arakawa and Y. Onoue. 1997. Neurotoxins in a toxic red tide of *Heterosigma akashiwo* (Raphidophyceae) in Kagoshima Bay, Japan. *Aquaculture Research* 28: 9-14.

Kiemer, M.C.B., K.D. Black, D. Lussot, A.M. Bullock and I. Ezzi. 1995. The effects of chronic and acute exposure to hydrogen sulphide on Atlantic salmon (*Salmo salar* L.). *Aquaculture* 135: 311-327.

Knezovich, J.P., M.P. Lawton and L.S. Inouye. 1989. Bioaccumulation and tissue distribution of a quaternary ammonium surfactant in three aquatic species. *Bulletin of Environmental Contamination and Toxicology* 42: 87-93.

Knights, B. 1989. Effects of ammonia accumulation on metabolic rates and growth of European eel, *Anguilla anguilla* L., in relation to warm water aquaculture. *Aquaculture and Fisheries Management* 20: 111-117.

Knoph, M.B. 1992. Acute toxicity of ammonia to Atlantic salmon (*Salmo salar*) parr. *Comparative Biochemistry and Physiology* 101: 275-282.

Koss, D.R. and N.R. Bromage. 1990. Influence of the timing of initial feeding on the survival and growth of hatchery reared Atlantic salmon (*Salmo salar* L.). *Aquaculture* 89: 149-164.

Koppang, E.O., E. Haugarvoll, I. Hordvik, T.T. Poppe and I. Bjerkås. 2004. Granulomatous Uveitis associated with vaccination in the Atlantic salmon. *Veterinary Pathology* 41: 122-130.

Koppang, E.O., E. Haugarvoll, I. Hordvik, L. Aune and T.T. Poppe. 2005. Vaccine-associated granulomatous inflammation and melanin accumulation in Atlantic salmon, *Salmo salar* L., white muscle. *Journal of Fish Diseases* 28: 13-22.

Lang, T., G. Peters, R. Hoffman and E. Meyer. 1987. Experimental investigations on the toxicity of ammonia effects on ventilation frequency, growth, epidermal mucous cells, and gill structure of rainbow tout, *Salmo gairdneri*. *Diseases of Aquatic Organisms* 3: 159-165.

Lauren, D.J. and D.G. McDonald. 1986. Influence of water hardness, pH and alkalinity on the mechanisms of copper toxicity in juvenile rainbow trout, *Salmo gairdneri*. *Canadian Journal of Fisheries and Aquatic Sciences* 43: 1488-1496.

Lewis, W.M. and D.P. Morris. 1986. Toxicity of nitrite to fish: A review. *Transactions of the American Fisheries Society* 115: 183-195

Lumsden, J.S., P. Clark, S. Hawthorn, M. Minamikawa, S.G. Fenwick, M. Haycock and B. Wybourne. 2002. Gastric dilation and air sacculitis in farmed chinook salmon *Oncorhynchus tshawytscha* (Walbaum). *Journal of Fish Diseases* 25: 155-163.

Machado, J.P., D.L. Garling, N.R. Kevern, A.L. Trapp and T.G. Bell. 1987. Histopathology and pathogenesis of embolism (gas bubble disease) in rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* 44: 1985-1994.

Miller, H.J. and E.A. Black. 1992. The effect of consumption of woody debris on the health of marine cage-reared salmon. *Bulletin of the Aquaculture Association of Canada* 92-3: 76-78.

Newbound, G.W., D.J. Speare, K.L. Hammell, M.L. Kent, V.E. Ostland and G.S. Traxler. 1993. Chehalis River disease: A unique gill disease of salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1092-1100.

Nilsson, J. 1992. Genetic variation in resistence of arctic char to fungal infection. *Journal of Aquatic Animal Health* 4: 126-128.

Ørnsrud, R., L. Gil and R. Waagbø. 2004. Teratogenicity of elevated egg incubation temperature and egg vitamin A status in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 27: 213-223.

Papoutsoglou, S., M.J. Costello, E. Stamou and G. Tziha. 1996. Environmental conditions at sea-cages, and ectoparasites on farmed European sea bass, *Dicentrarchus labrax* (L.), and gilt-head seabream, *Sparus aurata* L., at two farms in Greece. *Aquaculture Research* 2: 25-34.

Peterson, R.H. and D.J. Martin-Robichaud. 1989. First feeding of Atlantic salmon (*Salmo salar* L.) as influenced by temperature regime. *Aquaculture 78: 35-53.*

Poppe, T.T. and O. Breck. 1997. Pathology of Atlantic salmon *Salmo salar* intraperitoneally immunized with oil-adjuvanted vaccine. A case report. *Diseases of Aquatic Organisms* 29: 219-226.

Poppe, T.T and T. Taksdal. 2000. Ventricular hypoplasia in farmed Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 42: 25-40.

Powell, M. and J. Harris. 2004. Influence of oxygen on the toxicity of Chloramine-T to Atlantic salmon smolts in freshwater and seawater. *Journal of Aquatic Animal Health* 16: 83-92.

Powell, M., D.J. Speare, A.E. Fulton and G.W. Friars. 1996. Effects of intermittent formalin treatment of Atlantic salmon juveniles on growth, condition factor, plasma electrolytes, and hematocrit in freshwater and after transfer to seawater. *Journal of Aquatic Animal Health* 8: 64-69.

Pylkkö, P., T. Lyytikäinen, O. Ritola and S. Pelkonen. 2000. Vaccination influences growth of Arctic charr. *Diseases of Aquatic Organisms* 43: 77-80.

Roberts, R.J., R.W. Hardy and S.H. Sugiura. 2001. Screamer disease in Atlantic salmon, *Salmo salar* L., in Chile. *Journal of Fish Diseases* 24: 543-550.

Rodger, H.D. 1991. Summer lesion syndrome in salmon: A retrospective study. *Veterinary Record* 129: 237-239.

Rodger, H.D., T.M. Murphy, E.M. Drinnan and D.A. Rice. 1991. Acute skeletal myopathy in farmed Atlantic salmon, *Salmo salar*. *Diseases of Aquatic Organisms* 12: 17-23.

Rorvik, K.A., P.O. Skjervold, S.O. Fjaera and S.H. Steien. 2000. Distended water-filled stomach in seawater farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), provoked experimentally by osmoregulatory stress. *Journal of Fish Diseases* 23: 15-18.

Soderberg, R.W., J.W. Meade and L.A. Redell. 1993. Fin condition of Atlantic salmon reared at high densities in heated water. *Journal of Aquatic Animal Health* 5: 77-79.

Speare, D.J. 1990. Histopathology and ultrastructure of ocular lesions associated with gas bubble disease in salmonids. *Journal of Comparative Pathology* 103: 421-432.

Speare, D.J. 1991. Endothelial lesions associated with gas bubble disease in fish. *Journal of Comparative Pathology* 104: 327-335.

Speare, D.J. and S. Backman. 1988. Ammonia and nitrite waterborne toxicity of commercial rainbow trout. *Canadian Veterinary Journal* 29: 666.

Speare, D.J., J. Brackett and H.W. Ferguson. 1989. Sequential pathology of the gills of coho salmon with a combined diatom and microsporidian gill infection. *Canadian Veterinary Journal* 30: 571-575.

Speare, D.J., G. Arsenault, N. MacNair and M.D. Powell. 1997. Branchial lesions associated with intermittent formalin bath treatment of Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 20: 27-33.

Staurnes, M., G. Andorsdottir and A. Sundby. 1990. Distended water filled stomachs in sea-farmed rainbow trout. *Aquaculture* 90: 333-343.

Stefansson, S.O., R. Nortvedt, T.J. Hansen and G.L. Taranger. 1990. First feeding of Atlantic salmon, *Salmo salar* L., under different photoperiods and light intensities. *Aquaculture and Fisheries Management* 21: 435-441.

Stephen, C., M.L. Kent and S.C. Dawe. 1993. Hepatic megalocytosis in wild and farmed chinook salmon *Oncorhynchus tshawytscha* in British Columbia, Canada. *Diseases of Aquatic Organisms* 16: 35-39.

Strauss, R.H. 1979. Diving medicine. *American Review of Respiratory Disease* 119: 1001-1023.

Underdal, B., O.M. Skulberg, E. Dahl and T. Aune. 1989. Disastrous bloom of *Chrysochromulina polylepis* (Prymnesiophyceae) in Norwegian coastal water 1988: Mortality in marine biota. *Ambio* 18: 265-270.

Waagbø, R., K. Hamre, E. Bjerkås, R. Berge, E. Wathne, Ø. Lie and B. Torstensen. 2003. Cataract formation in Atlantic salmon, *Salmo salar* L., smolt relative to dietary pro- and antioxidants and lipid level. *Journal of Fish Diseases* 26: 213-229.

Weitkamp, D.E. and M. Katz. 1980. A review of dissolved gas supersaturation literature. *Transactions of the American Fisheries Society* 109: 659-702.

Welker, T.L. and J.L. Congleton. 2004. Oxidative stress in juvenile chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). *Aquaculture Research* 35: 881-887.

Winston, G.W. 1991. Oxidant and antioxidants in aquatic animals. *Comparative Biochemistry and Physiology* 100: 173-176.

Yacob, S.Y., K. Anraku, M.V. Archdale, T. Matsuoka and S. Kiyohara. 2002. Exposure of taste buds to potassium permanganate and formalin suppresses the gustatory neural response. *Aquaculture Research* 33: 445-454.

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CHAPTER

# 21

# A Guide for Aquaculture Veterinarians: What should be done at a Farm Visit

**H.J. Schlotfeldt**

## INTRODUCTION

To assess the health status of fish in rearing and producing facilities, it is necessary that the aquaculture veterinarian looks for the presence or absence of infectious pathogens, but it is equally important that he considers the overall environmental conditions since they are major determinants for the health of the cultured fish and the resistance to pathogens.

Fish are 'poikilothermic' (cold-blooded) animals. This means that their whole metabolism, including the immune response, is dependant on the water temperature, which is essential for fish condition. Compared to air, the water medium is biologically and physico-chemically much more

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complex and the oxygen availability lower. The environmental influence on fish is fundamental and more important than for air-breathing animals.

The fish health depends strongly on water quality, temperature range optimal for each fish species, nutrition, husbandry and management policies. The better these factors, the better the fish condition and, therefore, their resistance to disease. The deterioration of one or more of these factors leads to stress. Stress leads to lowering of fish condition and as a consequence to a higher predisposition to disease. The avoidance of stress is an ultimate essential to be considered by the attending field veterinary practitioner in pond farming and fish rearing.

One of the main interesting and often amazing features of fish physiological behaviour is that fish can 'live in harmony' and remain healthy even in the presence of many specific or facultative fish pathogens, provided the environmental parameters are correct and, therefore, the fish condition is optimal, despite the fact that water is 100% more 'efficient' in the transmission of pathogens than air. This is another basic difference to air-breathing and body-temperature constant ('homeothermic') animals. The relationship between host, pathogen and environment is illustrated in Fig. 21.1 and shows disease: (a) as a result of the interaction between host, pathogen and environment; and (b) the influence of the environment (water quality) on the fish.

The following paragraphs give a short description on points to be considered by a veterinarian or a Competent Veterinary Authority visiting a fish farm for control reasons or in the framework of a care programme. These proposed steps coincide in many aspects with the current EU legislation and other national or international legislations in place in non-EU countries.

**A schedule** for a private field aquaculture and fish disease veterinarian or for a Competent Veterinary Authority planning a control and care programme for a fish rearing and producing facility should consider and record the following points:

## FARM HISTORY

A brief history of the facility, in operation since when, history of eventual pathologies, responsible management and other relevant aspects should be asked to the operator and a summary of all these factors put in record.

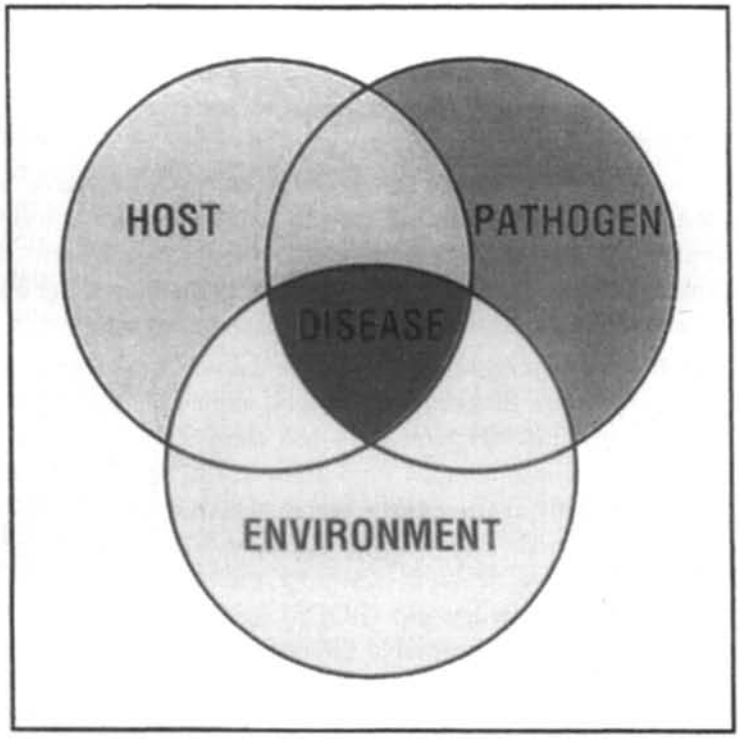


**Fig. 21.1** Relation between host, pathogen and environment in regard to the occurrence of fish diseases (after Sniezko, 1974).

## FARM LAYOUT

The design of the farm, how many ponds or raceways or whatever other fish holding units are present, sizes of them, quality of the ground (natural – earthen, gravel – or concrete), quality of the water supply and water interconnections among the fish holding units should be examined.

This aspect can be considerably shortened if for example the farm is an EU-approved one according to EU Directive 2006/88/EC (freedom of 'Annex A diseases' – Viral Haemorrhagic Septicaemia/VHS and Infectious Haematopoietic Necrosis/IHN) (Anonymous, 2006).

The application for such a status includes a detailed map of the facility in question. Regular updates of the approved zones and farms in the EU are published in the **OJ** (Official Journal of the European Communities).

## WATER SUPPLY AND QUALITY, POND HYGIENE

A fundamental aspect is the water supply. The amount of water available for the farm, water source, watercourse and water quality are basic essentials for the health of the fish stock.

If the water quality parameters are not known, they have to be determined. For this, practical and easy to handle electronic tools are widely available and should be part of the standard equipment of an aquaculture vet's service car, or at least available in the next water quality laboratory cooperating with the Competent Veterinary Authority.

The main parameters which should be known are: temperature, oxygen content, carbon dioxide content, pH, ammonia ($NH_3$) for pH's above 8,5, ammonium ($NH_4$) for pH's less than 8,5, nitrite content, nitrate content, total iron content (important in the brood house where egg incubation and fry rearing takes place). Further values should be additionally determined: acid binding capacity, inorganic phosphate, potassium permanganate demand ($KMnO_4$), chemical oxygen demand (COD), biological oxygen demand (BOD5), oxygen demand (BOD2), chlorine ($Cl^{2-}$) and $H_2S$. Acceptable limits for these factors are highly variable among fish species and, thus, cannot be generalized. Numerous data are available in the pertinent literature (Roberts and Schlotfeldt, 1985; Schaeperclaus, 1990; Schlotfeldt and Alderman 1995; Baur and Rapp, 2003).

Pond hygiene is essential for fish health. The yearly lime ($CaCO_3$, 1 kg/$m^2$) disinfection of earthen ponds (Fig. 21.2 a, b) and with formalin 2-3% or other disinfectants profusely used in veterinary medicine of concrete ponds and raceways is highly advisable.

## NECESSARY DATA REGARDING THE FARM'S FISH POPULATION

### Fish Species and Population Structure

Basic information on the fish population present should be noted, including species reared, age-classes, average of live weight/$m^3$ of water.

### Source of Fish

What is the source of the fish population on the site: own spawners present or foreign source of gametes required. If foreign sources are

**Fig. 21.2** Lime disinfection of earthen ponds. Blowing lime onto the surface of the emptied pond (a) to cover the whole surface of the pond (b).

involved, a careful investigation regarding the health quality and certification of the source is essential. The same applies to foreign sources of restocking and other sizes of fish eventually introduced into the farm. In case that the farm is located in an approved zone guarantees for freedom of those diseases, the zone is approved for are present and an investigation for these diseases is, therefore, not necessary.

### Breeding Facility

If own spawners and own egg incubation and production of fry and restocking sizes take place, the quality of the broodhouse, its water supply and fry holding facilities should be evaluated.

### Production Purpose

The main focus of the production on the respective site should be noted: production and selling of gametes, fry, restocking fish, 'portion' fish (average 300 g) or larger fish (e.g., in the Central Europe so-called 'Salmon trout', average > 1.5 kg).

## DETECTION AND TRACING OF POSSIBLE PATHOLOGIES

### Inspection of Ponds

The vet should walk slowly around the ponds, 1-2 m far from the pond's edges and carefully study the behaviour of the stock. The degree of excitation or the opposite—phlegmatic behaviour—are an important indicator for the fish health as well as other anomalies like aberrant swimming or apathetic marginal staying of fish along the edges of ponds or raceways. Those fish showing abnormal behaviour are the target fish ('negative selection') for sampling, necropsy and—if clinical symptoms are present—diagnostic examination in the laboratory. If suspicion of viral infections relevant for Annex IV/Part II of Directive 2006/88/EC (Anonymous, 2006) arises, sampling schemes and sampling procedures according to Part I of Commission Decision 2001/183 (Anonymous, 2001) must be taken into consideration. This reference is especially important if the facility is involved in a program for EU-approval for freedom of the 'Annex IV/Part II ' viral diseases VHS (Viral Haemorrhagic Septicaemia) and IHN (Infectious Haematopoietic Necrosis) (Anonymous, 2006).

Even if the farm is not foreseen to join a programme as to reach the approved status according to the pertinent legislation of the European Union (EU) (Anonymous, 2001, 2006) but based on clinical findings a sampling shows necessary, it is advisable to follow the sampling schemes of the Commission Decision 1002/183 (Anonymous, 2001) mentioned above. This applies also if a suspicion of bacterial infection becomes evident.

## Necropsy

Wherever possible, a necropsy should be carried out on site, if suitable facilities (electricity, table for microscopy, hygiene conditions, washing devices, etc.) are available.

If this is not the case, live fish samples should be taken to the facilities of the private field vet—if available—or to a laboratory of the Competent Veterinary Authority. Today, the transport of live fish of any size in plastic bags with one third of water and two thirds of pure oxygen is an optimal tool. The bags should lie as to maximally enlarge the surface contact between the gas (oxygen) and the water. They should not stay very long motionless—movement enhances the gas/water interchange (Fig. 21.3).

The necropsy procedures are widely described in the fish pathology literature (Roberts and Schlotfeldt, 1985; Schaeperclaus, 1990; Baur and Rapp, 2003).

The gill is the most exposed fish organ to the environment. Its sensitive structure (only a single cell layer, the respiratory epithelium) is in **direct** contact with the aquatic environment and all possible pollutants in the water. Gills are the **'mirror of fish health'** and must be examined at first priority in view of the fact that even five minutes after death of the fish, its sensitive structure becomes unsuitable for an adequate examination allowing a reliable clinical statement.

Important is the optimal hygiene if taking samples for further virological and/or bacteriological laboratory examinations becomes necessary. A brief flaming with ethanol of the whole fish body before opening is advisable, so as to ensure an optimal sterility regarding the sampling of target organs for virological and/or bacteriological examinations.

The sampling for virological examination purposes, the preparation and transport of them as well as the details of the virological examination and virus identification is described in Part I of Commission Decision 2001/183 (Anonymous, 2001).

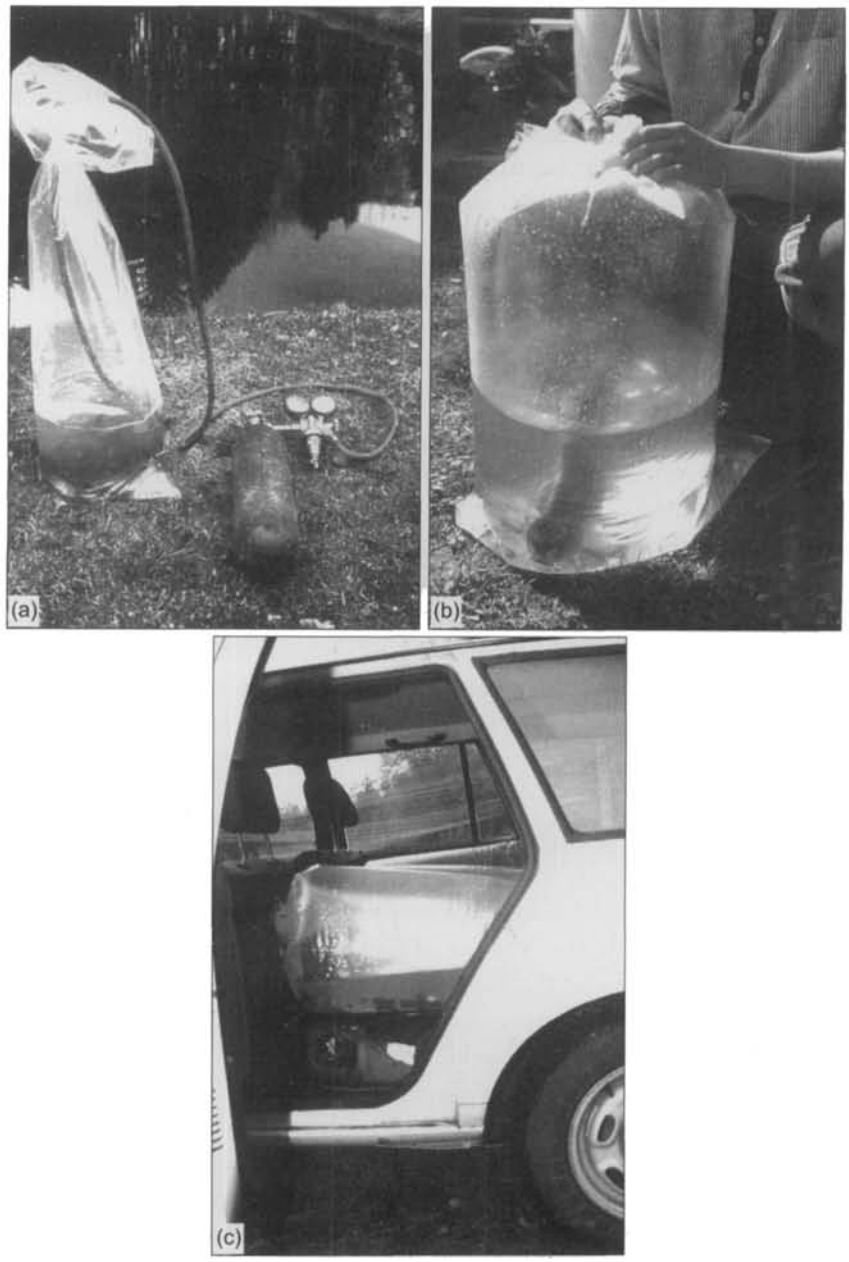


**Fig. 21.3** Transport of live fish: handling of water/oxygen bags. The smaller the fish the more can be charged into the bag. Bags should be filled to one third with water and two thirds with oxygen (a), the blown-up bag should be carefully sealed (b) and bags should lie during transport (c).

In case of suspicion of KHV (Koi Herpes Virus) gill and gut samples should be additionally taken (Haenen and Hedrick, 2006).

The target organs for bacteriological examinations are kidney, spleen, liver (small pieces, no more than 1-2 cubic mm) and, in some cases, the brain as well as skin pieces in case of dermo-ulcerative diseases. Tissue samples for histology or immunohistochemistry are also generally taken from these target organs.

The inspection frequency in case of EU-approval programmes of freedom of VHS and IHN is presented in the Tables 1A, 1B and 1C of Part I of Commission Decision 2001/183 (Anonymous, 2001).

## Recommendations for Therapy

If a certain treatable disease is confirmed, a schedule for a suitable **therapy** should be discussed with the concerned responsible of the farm. But, unfortunately, the outworking of such a schedule is nowadays seriously hindered and restricted at least in the EU due to the limited amount of medicines officially licensed for therapies in fish for human consumption. The number and type of available therapeutics varies from one Member State to another and, of course, among countries outside the EU, but in average the number of licensed products became smaller and smaller during the last 10 years (Schlotfeldt, 1992). An improvement of this situation as well as the aimed EU-wide harmonization of the licensing of drugs used in veterinary medicine has occurred very slowly. Therefore, no concrete therapy advices for EU-Member States can be given here (Schlotfeldt and Alderman, 1995).

In other countries outside the EU or the U.S.A., worldwide in which aquaculture plays an economic role, fish therapy possibilities may be more liberal. But restrictions are increasing, especially if fish for consumption are exported into the areas of the EU, U.S.A., and possibly other regions with strict controls for residues of the fish as food.

## Prophylaxis

More important than drug-based therapies is the **prevention**, the avoidance of the outbreak of a disease through **vaccination**. 'It is far better to prevent than to treat' is an old and well-known wisdom in medicine.

Vaccination for the protection against bacterial diseases made great progress during the last 10 years, especially in Norway, where the delivery of antibiotics for use in aquaculture lowered from the highest amount of about 70,000 kg in 1986/88 to less than 1000 kg in 1996.

Today, more or less satisfactorily working vaccines (mainly bacterines) against the main bacterial diseases are commercially available. This is not yet the case regarding viral diseases (Schlotfeldt *et al.*, 1985, 1989; Schlotfeldt, 2004).

The most common way of delivery of vaccines is by: (a) immersion (bath) (Schlotfeldt *et al.*, 1985; Schlotfeldt, 1991, 2004), (b) spray (Schlotfeldt, 1992, 1993) and (c) injection. Oral vaccines are increasingly available, but for the moment, mainly as boosters after a first application by bath or spray. Injection is somewhat labour- and time-consuming, and economic mainly in big fish of high individual value.

In the field practice of vaccination, it is very important to strictly follow the directions for use prescribed by the vaccine manufacturers. They still often differ considerably from one product to another. As an example, the previewed weight of fish batches in the case of immersion vaccines must be followed in most accurate way. A simple 'optical impression' of an 'approximate weight' proves inaccurate; each batch must be weighed individually (Fig. 21.4). The fish must swim freely in the

**Fig. 21.4** Vaccination by immersion. Each fish batch should be weighed individually.

vaccines dilution (Fig. 21.5) as to ensure a whole body contact (gills, skin) with the antigen. Also, in the case of spray vaccination, the manufacturer's directives regarding, e.g., the diameter of the spray – device's head must be strictly followed (Fig. 21.6). Otherwise 'holes' in

**Fig. 21.5** Fish must swim freely in the vaccine bath.

**Fig. 21.6** Spray vaccination. Diameter of the spraying valve, spraying time and amount of meandering-lines must follow strictly the manufacturer's advice.

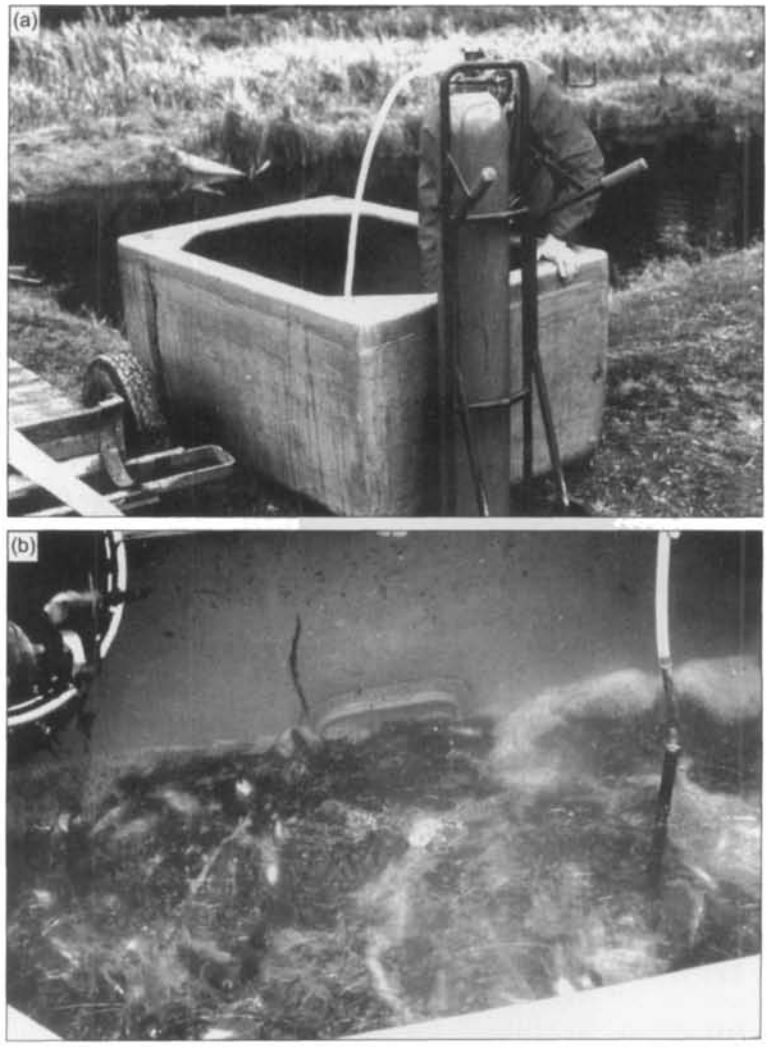


**Fig. 21.7** Stamping out measures: careful and animal welfare rules-agreed for fish killing by blowing in carbon dioxide ($CO_2$). Preparation of tank (a) for exposing fish to carbon dioxide (b).

immunity arise and in practice the operator complains, 'the vaccine is not effective'.

In EU-approved areas free of VHS and IHN (Anonymous, 2006), the vaccination against these viral diseases is forbidden as well as the use of live vaccines (in which the pathological agent is attenuated but alive) is not allowed or severely restricted in the EU.

Also, **fish killing** in agreement with the **animal welfare** legislation in case of stamping out measures due to 'Annex IV/Part II'/Directive 2006/88/EC (VHS & IHN) (Anonymous, 2006) outbreaks is an issue. Some examples: for trout in amounts up to 1 t, about 70-80 kg of carbon dioxide ($CO_2$) are necessary. Fish are quickly anaesthetized and die within 2-3 minutes (Fig. 21.7). For larger lots, overdoses of anaesthetics are advised. The drugs tolerated for this purpose vary largely among the Member States of the EU and aquaculture countries worldwide. Therefore, no product names can be given here (Roberts and Schlotfeldt, 1985).

Finally, the examining veterinarian should sit down with the farm responsible(s) and consider on a step-by-step basis the results of the examinations exposed here in order to identify what and where avoidance of stress can be optimized. In practice, even rather small details, for e.g., that visitors and the person responsible for daily feeding (by hand) of ponds should wear clothes of the same colour (preferably a flashy one) as to reduce superfluous agitation of fish. The fish will adapt to these colours and within less than a week, they will stay totally calm even if surrounded by foreign visitors around the pond wearing clothes with the respective colours. Such an apparently negligible measure results in one little step towards avoiding stress.

## References

Anonymous. 1991. COUNCIL DIRECTIVE 91/67/EEC of 28.01.1991 concerning the animal health conditions governing the placing on the market of aquaculture animals and products. *Official Journal of the European Communities* L 46 February 19th: 1. http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31993L0067&model=guichett

Anonymous. 2006. COUNCIL DIRECTIVE 2006/88/EC of 24 October 2006 on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases of aquatic animals. Official Journal of the European Union of Nov. 24th 2006. lex.europa.eu/LexUriServ/site/en/oj/2006/l_328/l_3280061124en00140056.pdf

Schlotfeldt, H.J. and D. Alderman. 1995. What should I do – A Practical Guide for the Fresh Water Fish Farmer. Published by the European Association of Fish Pathologists/ EAFP as a *Supplement to the Bulletin of The European Association of Fish Pathologists* 15, 60 pp.

Roberts, R.J. and H.J. Schlotfeldt. 1985. Grundlagen der Fischpathologie, mit einer Einführung in die Anatomie, Physiologie, Pathophysiologie und Immunologie sowie in den aquatischen Lebensraum der Knochenfische. Paul Parey, Berlin.

Schaeperclaus, W. 1990. Fischkrankheiten. Akademie-Verlag Berlin.

Baur, W.H. and J. Rapp. 2003. Gesunde Fische. Praktische Anleitung zum Vorbeugen, Erkennen und Behandeln von Fischkrankheiten. Blackwell Verlag, Berlin.

Haenen, O. and R. Hedrick. 2006. Koi herpes virus workshop. Held at the XII. Int. Conference of the EAFP, Copenhagen Sept. 11-16th 2005. *Bulletin of the European Association of Fish Pathologists* 26: 26-37.

Schlotfeldt, H.J. 1991. Frühjahr 1991: Zeit zur Schutzimpfung gegen die Rotmaulseuche/ ERM und jetzt auch gegen die Furunkulose der Salmoniden und gegebenfalls gegen die 'Hautrotseuche'/'Karpfen-Furunkulose' oder Erythrodermatitis (ED). *Fischer und Teichwirt* 42: 195-197.

Schlotfeldt, H.J. 1992a. Sprühvakzine 'PIYERSIVAC' zum Schutz gegen die ERM/ 'Rotmaulseuche' zugelassen. *Fischer und Teichwirt* 43: 334-336.

Schlotfeldt, H.J. 1992b. Current Practices of Chemotherapy in Fish Culture. Office Internationale des Epizooties/OIE: Chemotherapy in Aquaculture: From theory to reality. Symposium, March 12-15, 1992, Paris, pp. 25-38.

Schlotfeldt, H.J. 1993. Frühjahr 1993: Zeit zur Fisch-Vakzinierung. Hinweise zur Anwendung der Sprühvakzine 'Piyersivac' zum Schutz gegen die ERM/ 'Rotmaulseuche'. *Fischer und Teichwirt* 44: 124-125.

Schlotfeldt, H.J. 2004. Impfung bei Fischen – Grundlagen und Hinweise. *Aquakultur und Fischereiinmformationen des Landes Baden-Württemberg* 2: 7-11.

Schlotfeldt, H.J., V. Alvarado, S. Stanislawski and K.H. Böhm. 1989. Schutz-Vakzinierung gegen die Furunkulose der Forellen und geschwürige 'Karpfen-Furunkulose' (Erythrodermatitis/ED). *Fischer und Teichwirt* 27: 12.

Schlotfeldt, H.J., K.H. Böhm, F. Pfortmüller and K. Pfortmüller 1985. Rotmaulseuche / ERM (Enteric Redmouth Disease) der Forellen und anderer Nutzfische in Nordwestdeutschland – Vorkommen, Therapie und Vakzinierungsergebnisse. *Tierärztliche Umschau* 40: 985-995.

# Index


18SS rRNA 454, 479
2-amino-5-nitrothiazol 475, 476

A
*A. caviae* 247
*A. ocellatum* 426, 427, 430-433, 437-439
*A. sobria* 247
*A. veronii* 247
*Abra abra* 900
*Abramis brama* 100, 119, 144, 361, 372, 483, 881, 1083, 1155, 1162
Abscess 32, 1083, 1146
*Acanthamoeba* 401, 408, 409, 411, 413
*Acanthamoeba* sp. 408
*Acanthamoeba* strains 409
Acanthella 1039
Acanthellae 1040
Acanthocephala 1025, 1030
Acanthocheilidae 1065
*Acanthocheilus nidifex* 1086
*Acanthocheilus rotundatus* 1066, 1083
*Acanthocybium solandri* 818, 936
*Acanthogobius flavimanus* 649
*Acanthopanax spinosus* 1149
Acanthor 1038
*Acanthostomum spiniceps* 839
*Acartia clausi* 834
Accumulation capacity 1056, 1059
–indication 1055, 1059
–indicator(s) 1029, 1056-1058
Acetabula 979
*Acetodextra ameirui* 821
*Acetodextra* spp. 890
*Achlya* 286, 287, 316
*Achromogenes* 244
*Achtheres percarum* 1120
*Achtheres sandrae* 1160
Acid binding capacity 1258
Acquired immunity 438, 654, 712, 713, 716, 915, 941, 1010, 1015
–protection 715, 747, 748
Acrasida 398, 401, 411
Acrasidae 398
Acriflavin 430, 435, 595
*Acrocheilus alutaceus* 893
Actinosporea 614
Acute response proteins 472
Adeleorine apicomplexans 547, 550
Adhesive secretions 693, 694, 795
*Aega entricosa* 1179
*Aega psora* 1179
*Aega stroemi* 1179
Aegidae 1117, 1176, 1179
*Aeromonas hydrophila* 152, 247, 586
*Aeromonas salmonicida* 27, 55, 88, 239-245, 1136, 1173

*Aeromonas salmonicida* subspecies *salmonicida* 239
Aflatoxin $B_1$ 1219
Agglutinin 63, 1213
AIDS 352
Alaska pollock 1120, 1162
Albendazole 449
*Alburnus alburnus* 119, 372
*Alepisaurus ferox* 818
Alewife 27
Alkaloids 1218
Alligators 1164
*Allocreadium isoporum* 839
*Alosa chrysochloris* 841
Alpha-tocopherol 1210
Alpha-tubulin 452, 453
Alpha-tubulin gene 452, 453
*Alphavirus* 68-70
Aluminium 766
*Alvelinus alpinus* 61, 66, 245, 250, 444, 446, 450-453, 625, 1010, 1160, 1204
Alveolae 422
Alveolata 422
Alveolates 570
Amastigotes 481, 482
*Amazonspora hassar* 385
Amberjacks 923, 924
*Ambiphrya* 573, 586, 587
*Ameirus [Ictalurus] melas* 88
*Ameiurus melas* 877
Ammonia toxicity 1236
Ammonium ($NH_3$) 723, 753, 765, 1096, 1258
*Amnicola limosa* 893
Amoebic gill disease 402
Amoebozoa 398
Amphilinidea 978
Amphipoda 1115, 1117
*Amphiprion frenatus* 438
*Amyllodinium* cf. *ocellatum* 426
*Amyllodinium ocellatum* 437
Amyloodiniosis 426, 427, 430, 431, 433, 434, 438
*Amyloodinium* 422, 425, 427-435, 438, 439
*Amyloodinium ocellatum* 425, 428, 429, 438, 439
*Amyloodinium ocellatum* antibodies 438
*Amyloodinium* sp. 431, 432
*Amyloodinium* trophonts 438
Amyloodinium-like 431
Anaesthetics 1267
*Anaporrhutum albidium* 839
*Anas platyrhynchos* 883
*Anchoa mitchilli* 836, 840, 929
*Anchoa* spp. 930
*Anelasma squalicola* 1179
Angel fish 450
*Anguilla* 51, 192, 257, 459, 483, 485, 489, 548, 598, 645
*Anguilla anguilla* 459, 485
*Anguilla australis* 913, 1089
*Anguilla dieffenbachi* 913
*Anguilla japonica* 895
*Anguillicola crassus* 485, 1065, 1067, 1080, 1085, 1090, 1095
*Anguillicola novazaelandea* 1089
*Anguillicola globiceps* 1085
Anguillids 458, 1053
Anguilliformes 483
*Anilocra frontalis* 1177
Animal welfare 247, 249, 1267
*Anisakis* 1065, 1066, 1073, 1075, 1077-1079, 1082, 1086
*Anodonta piscinalis* 907
Anostomidae 436
Antagonistic behavior 1232
Antarctic 546, 557, 1026, 1056, 1058, 1163

Antheridium 291
*Anthosoma crassum* 1163
Antibiotics 289-291, 438, 471, 543, 1264
Antibody(-ies) 46-55, 57, 60, 68, 70, 71, 88, 94, 96, 98, 103, 110, 114, 118, 124, 128, 135-138, 200, 202, 214, 244, 260, 318, 427, 438, 447, 466, 467, 469, 473, 484, 493, 536, 581, 651, 652, 654, 656, 714, 715, 743, 747, 748, 781, 871, 983, 907, 1005, 1006, 1008-1010, 1037, 1046, 1079, 1080
Antigens 46, 57, 124, 157, 192, 200, 263, 465, 468, 469, 485, 494, 582, 654, 713, 856, 871, 915, 1008-1010, 1015, 1080
Antioxidant 1205, 1208, 1218
Anti-nitrational factors 1211, 1212, 1216-1218, 1220
*Anylocra physodes* 1178
*Apatemon gracilis* 833, 884
*Aphanius dispar* 432
*Aphanomyces* 280-283, 286, 287, 289, 290, 305, 309, 311-318, 320-323, 337
*Aphanomyces astaci* 281-283, 289, 290, 305, 316, 322
*Aphanomyces invadans* 281-283, 289, 309, 312-318, 320-323, 337
Apical complex 517-519, 525, 528, 558
Apical organs 979
Apicomplexa 27, 475, 517-522, 525, 543, 545, 558, 570
Apicomplexan 518-522, 528, 531, 545-547, 549, 550, 552, 553, 555, 558
*Apiosoma* 573, 578, 586
*Apocreadium mexicanum* 839
Apolytic 979
*Apophallus* 642, 838, 890, 893
*Apophallus brevis* 838, 890, 893
*Apophallus imperator* 893
Apoptosis 5, 7
*Aporocotyle simplex* 911, 913, 920
Approved zone 1257, 1260
Aquabirnaviruses 143, 144
Aquaculture 17, 55, 65, 93, 96, 98, 116, 122, 123, 126, 139, 186, 203, 205, 209, 211, 240, 242, 243, 246, 249-252, 258, 264, 265, 287, 290, 295, 303, 308, 328, 334, 335, 352, 368, 389, 426, 437, 463, 478, 486, 613, 623-625, 651, 653-656, 689-691, 706-710, 727, 729, 733, 735, 749, 750, 754, 778, 783-785, 789, 791, 792, 796, 798, 823, 843, 848, 849, 851, 854, 857, 863, 865-868, 886, 889, 910, 917, 922, 928, 933, 938, 941-944, 977, 985, 1012-1016, 1064, 1076, 1090, 1093, 1094, 1097, 1128, 1130, 1134, 1137, 1138, 1141, 1157, 1160, 1172, 1174, 1201, 1206, 1220, 1225, 1226, 1230, 1248, 1255, 1256, 1258, 1263, 1264, 1267
Aquaculture veterinarian 1255
*Aquareovirus* 133-135, 137, 140, 149, 150, 159, 216-219
Aquaria 426, 430, 558, 582, 590, 593, 708, 727, 733, 741, 742, 748, 749, 771, 772, 777, 791, 793, 797, 855, 1032
Aquarium 332, 334, 368, 409, 430, 434, 435, 437, 445, 446, 450, 458, 459, 479, 691, 706, 708-710, 727, 729, 742, 748, 749, 754, 772, 776, 777, 791, 797, 892, 1064, 1095, 1150, 1152
Arctic charr 245, 250, 263, 452, 625, 628, 1160
*Arctic grayling* 327, 873
*Ardea alba* 892
*Ardea herodias* 887
Argulidae 1116
*Argulus* 1164, 1166, 1168-1176
–larvae 1171
–life cycle 1169
–morphology 1165
–pathology 1172
–treatment 1174
*Argulus americanus* 1170
*Argulus coregoni* 1168, 1169
*Argulus foliaceus* 99, 1170, 1171, 1173
*Argulus japonicus* 1165, 1166, 1168, 1175, 1176
*Argulus sianemis* 327, 873

*Argulus varians* 1170
*Aristichthys nobilis* 89, 436, 639, 926, 1156
Arsenic 1219
*Artacama proboscidea* 911, 913
*Artemia salina* 430
Ascarida 1065, 1068
*Ascocotyle angrense* 835
*Ascocotyle gemina* 836, 891
*Ascocotyle pachoystis* 847, 894
*Ascocotyle tenuicollis* 894
Ascomycetes 279, 280
Ascorbic acid 1210
*Atherina boyeri* 373, 903
Atlantic bigeye 931
Atlantic cod 44, 188, 193, 211, 212, 241, 245, 248, 250, 260, 450, 482, 487
Atlantic croaker 940
Atlantic cutlassfish 841
Atlantic halibut aquareovirus disease 218
Atlantic herring 44, 895
Atlantic menhaden 29, 283, 313, 314, 318, 321, 322, 648
Atlantic salmon 35, 37, 42, 44, 51, 55, 56, 60-63, 66, 217, 242, 245-248, 250, 252, 256, 261-263, 299, 305, 330, 399, 402, 406, 407, 445, 446, 449-453, 459, 623-625, 627, 630, 631, 633, 652, 703, 710, 762, 763, 765-767, 1130, 1134, 1135, 1137, 1138, 1144, 1157, 1160, 1212, 1214, 1215, 1225, 1226, 1233, 1237, 1240, 1242, 1245, 1247, 1248
Atrophy 69, 71, 361, 373, 375, 382, 534, 615, 631, 634, 639, 642-645, 650, 993, 941, 1084, 1089, 1160, 1237
Attachment 29, 404, 426-428, 434, 445, 538, 555, 586, 594, 684-686, 701, 712, 715, 717, 719, 723, 734, 737, 743, 744, 746, 748, 752, 757, 759, 764, 767, 773, 780, 781, 784, 786-788, 792, 795, 819, 992, 877, 1002, 1003, 1006, 1015, 1033, 1042-1049, 1088, 1117, 1176
Attenuated vaccine 97, 468, 492
Atypical A. *salmonicida* 243, 244, 246
*Aulonocara stuartgranti* 476
Austria 97, 98, 114
*Austrodiplostomum compactum* 833, 840
Autointoxication 1237
Autolysis 7, 846, 1087
Ayu 251, 263, 311, 314, 326, 858, 889, 898
*Azygia micropteri* 839

B

*B. graellsi* 144
*B. schwanenfeldi* 144
*Babesiosoma* 521, 547-549, 556
*Bacciger* 833
Bacteria 9, 13, 17, 27, 29, 32, 33, 35, 37, 52, 55, 101, 107, 126, 141, 151-153, 208, 216, 240, 241, 243, 246-249, 252, 254, 255, 259, 261, 262, 264-266, 288, 289, 312, 318, 320, 337, 412, 413, 415, 435, 454, 475, 543, 570, 579, 585, 588, 589, 595, 648, 697, 717, 736, 744, 759, 769, 790, 792, 850, 853, 857, 874, 905, 936, 937, 988, 1008, 1050, 1095, 1149, 1155, 1173, 1176, 1229, 1234, 1235, 1261, 1264
Bacterial kidney disease 263
*Balamuthia mandrillaris* 411
Balantidiids 599
*Balantidium* 577, 597, 599, 600
Barb 66, 119, 144, 155, 372, 431, 450, 484, 546, 551, 877, 987, 989, 990, 1029, 1031, 1083, 1172
*Barbatula barbatula* 1083
Barbel 119, 144
*Barbus barbus* 119, 144, 450, 1029
*Barbus cohnus* 484
*Barbus graellsi* 66, 155
Barfin flounder nervous necrosis virus-type 187
*Barilius bendelisis* 487
*Barisomum candidulum* 839
Barnacles 930, 1179
Barramundi 330, 928

*Basanistes* 1158, 1160, 1161
*Basanistes huhonis* 1161
Basidiomycetes 279, 280
Basking shark 913
Bass 250, 255, 256, 259, 260, 262, 835, 855, 880, 882, 887, 892, 895, 903, 917, 1046
*Batracobdelloides tricarinata* 486, 548, 551
Bay anchovie 929
Belarus 98
Belgium 114
Belontid 486
*Benedenia seriolae* 686, 687, 689, 704, 707, 716, 726, 731, 733, 734, 741, 743, 746, 797
Ben imidaole 728
Benzene hexachloride 1150
Benzimidazole 458, 728
*Betanodavirus* 186-188, 192
*Betta splendens* 436
BF-2 47, 49, 59, 102, 111, 124, 130, 145, 149-151, 157, 158, 205, 206, 215, 218
BF-2 cells 130, 205, 206, 218
BFNNV 187, 188
*Bianium* 840
Bighead carp 89, 105, 132, 137, 150, 154, 639, 854, 926, 1150, 1156
Bignose unicornfish 937
Big-scale sand smelt 903, 904
Bile salts 1002, 1059
Bio-indication 1054, 1055
Bioindicators 1014, 1015, 1054
Biological control 725
Biological oxygen demand (BOD5) 1258
Biomarkers 1054, 1055
Biotin 651, 1210
Birnaviridae 66, 67
Birnavirus 65, 66, 143, 144, 146
Bithionol 458, 731, 794
*Bivesicula claviformis* 839
Black bullhead 88
Black carp 132, 144, 854
Black drum 915
Black grub disease 882
Black-spot seabream 477
Blacktip shark 823
Blastodiniales 422, 425
Blastodiniphyceae 422, 438
Blastomycetes 323
Bleak 119
*Blicca bjoerkna* 100, 144, 371, 410, 530, 881, 901
Bludgeon 144
Blue mackerel 929
Blue shark 984, 992, 997, 1000, 1002
Bluefin tuna 920-922, 924, 928
Bluegill sunfish 860, 863, 869, 880-883, 892
Blue-sack disease 1228
Bluntnose pufferfish 840
Bodo 424
Bodonid(s) 424, 454, 463, 479, 480
Bodonidae 454, 455
Bodonina 454, 455
Bodoninae 454, 455
Bogue 923
*Bolbophorus damnificus* 840, 875, 939
*Bolbophorus levantinus* 855
Bomolochidae 1116, 1163
*Bomolochus* 1118, 1130
*Boops boops* 923
Boosters 1264
Borneo mullet 1157
Bosnia and Herzegovina 97, 98
Bothria 979, 980, 984, 997
Bothridia 979, 980, 995
*Bothriocephalus acheilognathi* 1006, 1014, 1052, 1053
*Botulus microporus* 818
Boxfish 31
*Brachiella* 1130, 1162
*Brachydanio rerio* 66, 144, 938
*Brachyphallus crenatus* 937

Brain 45, 46, 70, 71, 89, 94, 96, 102, 107, 110-112, 121, 124, 130, 148, 152, 185, 187, 190, 192, 197, 198, 251, 260, 318, 330, 445, 475, 620, 634, 637, 643, 644, 646-648, 820, 833, 847, 902, 903, 905, 942, 1234, 1263
*Branchiomyces* 279, 280
Branchiura 636, 1115, 1116, 1166, 1176
Brazil 98, 433, 436, 487, 488, 551, 557
Bream 66, 100-102, 119, 132, 136, 144-246, 250, 255, 256, 530, 757, 901, 904, 1083, 1155, 1156, 1158, 1162
*Brochis splendens* 436
Bromex 1150
Bronopol 336, 458
Broodhouse 1260
Brook charr 469
Brook stickleback 870, 873, 876, 883
Brook trout 66, 299, 548, 623, 633, 859, 878, 893, 925, 926, 1160, 1161
*Brooklynella hostilis* 594, 596
Brown bullhead 31, 124
Brown ring disease 254
Brown shark 31
Brown trout 100, 102, 263, 926
*Bucephaloides gracilescens* 823
*Bucephalopsis kweiyangensis* 900
*Bucephalus* 839, 860, 899, 903, 904
*Bucephalus haimeanus* 903
*Bucephalus minimus* 838, 903
*Bucephalus polymorphus* 839, 860
*Bucephalus* sp. 944
Buddenbrokia 614
*Buddleja cordata* 458
*Bufo americanus* 876
Bulgaria 97-98, 127
Bullseye puffer fish 430
*Bursacetabulus pelecanus* 840, 869, 939
*Butorides virescens* 892
Butterflyfishes 912

C

*C. branchialis* 477-479
*C. bullocki* 479-480, 491
*C. carassius* 89, 119, 144, 158
*C. catostomi* 479, 480, 491
*C. eilatica* 477, 478
*C. iubilans* 475, 476
*C. minus* 410
*C. minus Page*, 1976 410
*C. nigrofasciatum* 476
*C. octofasciatum* 476
*C. salmositica* 464, 466-469, 471, 474, 479, 480, 493, 494
*C. salmositica* vaccine 468
*Calbellerotrema brasiliense* 839
Cadmium 1219
*Caecincola parvulus* 830
*Calcareous corpuscles* 982
Calcification 2, 7, 8, 926, 1210
Calcium 1150, 1172, 1206-1208
Calcium hypochloride 852, 1150
Caligidae 791, 1117, 1127, 1129, 1163
*Caligus elongatus* 791, 1119, 1129, 1131, 1137, 1138
*Caliobdella vivida* 474
Callichthydae 436
*Calliobdella nodulifera* 488
*Calliobdella vivida* 473
*Calyptospora funduli* 523, 525, 527, 530-532, 535, 541, 543-845
Camallanidae 1065
*Camallanus oxycephalus* 1067, 1085
Canada balsam 723
*Candacia pachydactyla* 834
Canola meal 1216, 1217
*Capillaria* 475, 1082, 1085, 1093, 1096
*Capillaria cichlasomae* 1082
*Capillaria eupomitis* 1082
*Capriniana piscium* 585

Capsalidae 686, 687, 689, 691, 698, 704, 707, 713, 714, 716, 725, 726, 729, 731-733, 739, 744, 746, 773, 793, 794, 797
Carangidae 66, 195, 431, 689, 704, 707, 710, 716, 726, 728, 731, 734, 746, 750, 784, 797, 917
*Carassius auratus* 66, 89, 119, 144, 370, 436, 471, 483, 640, 641, 728, 751, 870
*Carassius auratus gibelio* 483, 640
*Carassius carassius* 100, 435, 471, 482, 636, 1148
Carbon dioxide content 1236, 1258
*Caratothoa gaudichaudii* 1178
Carbohydrate 485, 494, 543, 838, 1203, 1204, 1208, 1241
*Carcharhinus limbatus* 769, 823, 1084
*Carchesium* 573, 586
*Cardicola currani* 915, 916
*Cardicola forsteri* 920, 924, 928
*Cardicola palmeri* 915
*Cardiodectes* 1145
*Cardiodectes medusaeus* 1145
Cardiomyopathy syndrome 1240
Carp(s) 66, 87-102, 104-106, 108, 110-124, 126-128, 131-139, 141, 143-146, 148-155, 157-160, 210, 217, 255, 322, 327, 450, 470-473, 477, 478, 492, 524, 529, 532-536, 544, 599, 617, 618, 635-641, 653, 654, 702, 715, 751-754, 979, 988, 854, 877, 881, 904, 913, 914, 917, 924, 926, 1006, 1008-1010, 1014, 1052, 1067, 1096, 1150, 1152, 1155, 1156
Carp bream 904
Carp coronavirus 151
Carp edema 146, 147
Carp louse 99
Carp pox 108, 118, 120, 146
Catfish (*Clarias angolensis*) 17, 29, 31, 37, 57, 89, 105, 109, 140, 142, 149, 158, 192, 196, 203, 204, 217, 258, 303, 304, 306, 317, 329, 331, 458, 463, 477, 478, 486, 487, 489, 581, 583, 585, 821, 840, 849, 852-855, 865, 868, 869, 874, 875, 878, 887, 895, 906, 917, 939, 1090, 1133, 1150, 1157, 1158, 1169, 1174, 1209
*Catla catla* 882
*Catostomus commersonii* 467, 484, 870, 1081
Caudal cilium 579, 584, 592, 595, 597
CCIV 153, 154
*Cecrops latreilli* 1163
*Centrocestus* cf. *formosanus* 892
*Cephaloporus monacanthi* 839
*Cerastoderma edule* 944
*Ceratomyxa* 613, 621, 628, 641, 646, 650, 651
*Ceratomyxa shasta* 613, 628, 641
*Ceratomyxa sparusaurati* 646
Ceratomyxosis 613, 628, 646
*Ceratothoa* f. *imbricata* 1178
*Cercaria bessiae* 863
*Cercaria bucephalopsis* 944
*Cercaria* C 877
*Cercaria caribbea* 830
*Cercaria* X 870
Ceroid 915, 916, 1205
Cestodaria 978
*Cetorhinus maximus* 913
*Chaetocerous* sp. 1243
*Chaetodipterus faber* 461
Chalimus 1124, 1126, 1129, 1131, 1135, 1139, 1142, 1158, 1160, 1161, 1162
Chanidae 432
*Channa punctatus* 486
Channel catfish 105, 109, 142, 258, 317, 331, 463, 585, 634, 840, 849, 852-855, 865, 868, 869, 874, 875, 878, 887, 895, 939, 1150, 1207-1209, 1212, 1216, 1219
*Chanos chanos* 432, 1157
Characidae 436, 450, 456, 459, 477, 1169
Characiformes 436
*Charopinus* 1162
Charr resistant 468

Chemical control 725
Chemical oxygen demand (COD) 1258
*Chilodonella* 107, 576, 578, 582, 583, 589, 602
China 96, 98, 115, 122, 126, 128, 139, 193, 311, 318, 321, 411, 450, 476, 640, 646, 1090, 1151, 1157
China (Hong Kong) 115
Chinook salmon 44, 59, 102, 212, 217, 242, 262, 263, 302, 326-328, 336, 446, 447, 452, 453, 466, 625, 855, 987, 1160
*Chlamydia* 27
Chloramine-T 1246
Chlorbromuron 1150
Chloride 1206
Chlorine ($Cl_2^-$) 1258
*Chloriridovirus* 202
*Chloromyxum* 632-633, 651
*Chloromyxum schurovi* 632, 633
*Chloromyxum truttae* 633
Chloroplasts 425, 434, 437
Cholangiofibrosis 650
Cholecalciferol 1210
Cholestasis 650
Cholin 902, 1210
*Chondracanthidae* 1116, 1128, 1163
*Chondracanthus goldsmidi* 1163
*Chondracanthus lophii* 1118, 1163
*Chondrostoma nasus* 144, 450
*Chonopeltis* 1165, 1166
–larvae 1171
–morphology 1165
–pathology 1174
*Chromalveolata* 422, 425
Chromista 280, 281
Chromium 1207
*Chrysichthys auratus* 486
*Chrysochromulina polylepsis* 1245
Chub 119, 149, 217
Chum salmon 51, 124, 212, 262, 457, 459, 631, 1133, 1136, 1137, 1203
*Cichlasoma* 476, 487, 1082
*Cichlasoma managuense* 487
*Cichlasoma meeki* 476
Cichlid(s) 27, 66, 435, 445, 450, 458, 459, 475, 476, 527-530, 532, 541, 556, 583, 584, 888, 892, 895, 1088, 1095, 1096, 1168
Cichlid fish 475
Cichlid *Symphysodon aequifasciata* 458
Cichlidae 66, 432, 436, 450, 487
Ciliophora 27, 569, 570, 572
Cimetidine 731
Circadian rhythms 590, 592
Cirripedia 1115, 1116, 1176, 1177
CKX 633
*Cladosporium* 327
*Clarias dussunieri* 487
*Clarias gariepinus* 486, 1174
*Clarias lazera* 486
*Clarias mossambicus* 486
*Clavella* 1120, 1130, 1162
*Clavella adunca* 1162
*Clavella perfida* 1120, 1162
*Clavella uncinata* 1162
*Clavellisa* 1163
*Clavellistes* 1163
*Clavellodes* 1163
*Clavellopsis* 1163
*Clavellotis* 1162
*Clinostomum attenuatum* 887
*Clinostomum complanatum* 835, 886-889
*Clinostomum cutaneum* 888, 889
*Clinostomum marginatum* 833, 869, 886, 888
*Clinostomum tilapiae* 888-889
*Clinostomum vanderhorsti* 888
*Clonorchis sinensis* 941
*Clupea harengus* 44, 61, 245, 542, 648, 895, 1087
Clupeiformes 459
Cobalt 1207
Coccidiosis 519

Cochliopodidae 399
*Cochliopodium minus* 408
*Cochliopodium* sp. 401
Cod (*Gadus morhua*) 4, 44, 46, 47, 52, 56, 60-63, 92, 109, 139, 187, 188, 192, 193, 195, 201, 202, 211, 212, 214, 240, 241, 244-246, 248-251, 253, 260, 261, 330, 332, 423, 450, 452, 460, 480, 482, 487-489, 491, 550, 557, 614, 621, 638, 649, 794, 821, 837, 856, 898, 899, 933, 1038, 1066, 1068, 1072, 1074, 1078, 1079, 1082, 1089, 1091, 1094, 1141, 1143, 1144, 1147, 1157, 1179, 1212
Coelomycetes 323, 324
Cohabitation 70, 158, 191, 198, 321, 380, 384, 598, 646, 647
Coho salmon (*Oncorhynchus kisutch*) 44, 51, 65, 140, 212, 217, 242, 245, 250, 257, 262, 327, 328, 402, 464, 467, 469, 625, 630, 631, 633, 836, 1090, 1134, 1135
Coldwater vibriosis 253
*Colisa fasciatus* 486
*Colisa* sp. 436
Collagen fibers 15, 1041, 1044, 1049, 1050
*Colobomatus gymnoscopeli* 1119
Colonization 265, 266, 545, 625, 653, 1051, 1052, 1228
Colonization success 1052
*Colossoma macropomum* 436, 477
Columnaris disease 258
Common bream 1158
Common carp 89, 93, 100-102, 105, 110, 113, 115-117, 119, 122, 123, 126, 128, 132, 144, 149-152, 155, 157, 158, 160, 470, 472, 529, 532, 617, 635, 636, 641, 702, 751, 754, 877, 904, 913, 914, 917, 1207, 1208, 1212
Common cockle 944
Common dace 860
Common loon 893
Common merganser 884
Common salt 1150, 1175
Common shiner 531, 644, 869
Common sole 196, 937
Competent Veterinary Authority 1256, 1258, 1261
Complement 31, 95, 244, 421, 452, 465, 468, 469, 472, 473, 485, 493, 494, 714, 849, 863, 1005-1009, 1096, 1208
*Conchoderma auritum* 1179
*Conchoderma virgatum* 1179
Condition factor 787, 904, 1051, 1082, 1091, 1133, 1143, 1155
*Conger conger* 903
Congestion 21, 23, 56, 101, 133, 446, 471, 648, 905, 1000, 1229
Connective tissue 11, 13, 15, 18, 21, 23, 31, 33, 35, 37, 119, 120, 151, 359, 361, 362, 372, 373, 376, 378, 382, 530, 598, 632, 642, 643, 645, 826, 829, 834, 837, 872, 873, 880, 888, 896, 897, 902, 905, 915, 933, 936, 991, 1003, 1034, 1042, 1044, 1045, 1049, 1086, 1088, 1231
*Contracaecum* 1065, 1066, 1069, 1072, 1073, 1075, 1077, 1079, 1080, 1082, 1087-1089, 1094
*Contracaecum microcephalum* 1082, 1088
*Contracaecum multipapillatum* 939
Copepoda 793, 1115-1118, 1120, 1130, 1132, 1158
Copepodid 1122, 1124, 1126, 1131, 1139, 1142, 1148-1150, 1158, 1161, 1162
Copper 336, 571, 727, 852, 1207
Copper sulphate 336, 427, 430, 458, 727, 771, 852, 1155
Copulatory cap 1034
Coracidium 983
*Corallomyxa* 409
*Coregonus albula* 1155
*Coregonus lavaretus* 1155
*Coregonus wartmanni* 1156
*Corethron* spp. 1243
*Coronaviridae* 150
*Coronavirus cyprini* 151
*Corydoras* spp. 436
*Coryphaena* spp. 937

*Corythionella* sp. 407
Cosmocercoidea 1065
*Costia* 455, 456, 461
*Costia necatrix* 456
Costiasis 461
Cottonseed meal 1217
Cotton-wool disease 258
*Cottus rhotheus* 467
*Cotylurus pileatus* 861, 873, 884
*Crassicutis archosargi* 819
*Crassicutis cichlasomae* 839
*Crassostrea gigas* 898
Creek chub 132, 139, 140, 869, 870
*Crepidoodinium* 422, 425, 437, 439
*Crepidoodinium australe* 437
*Crepidoodinium cyprinodontum* 437
*Crepidostomum farionis* 858
Crhysops 431
*Crithidia* 424
Croacker 474
Croatia 97-98, 762
Crowding effect 1045
Crucian carp 89, 100, 119, 144, 158, 470, 471
Crucian carp haematopoietic necrosis virus 158
*Cruoricola lates* 922, 928
*Cryptobia* 107, 424, 448, 450, 463, 464, 466-469, 472-480, 490-494
*Cryptobia branchialis* 476, 494
*Cryptobia eilatica* 477, 478
*Cryptobia helicis* 480, 494
*Cryptobia iubilans* 450, 475, 479
*Cryptobia kahibulaewi* 469
*Cryptobia neghmei* 474
*Cryptobia salmositica* 448, 464, 467-469, 472, 473, 479, 491-494
*Cryptobia* sp. 107, 448, 466, 474-479
Cryptobia-resistant 469
Cryptobiidae 454
Cryptobiinae 454
Cryptobiosis 464, 465, 469, 475, 479, 493
*Cryptocaryon irritans* 570, 572, 590, 591, 593, 594, 601
*Cryptocotyle lingua* 828, 833, 890, 895, 896
Cryptomitosis in microsporidia 355
*Cryptosporidium* 475, 521, 527-530, 534, 537-539, 544
*Cryptosporidium molnari* 529, 530, 534, 538, 539, 544
Crypts of the outer membrane 1035, 1037, 1041
*Ctenopharyngodon idella* 89, 100, 158, 436, 450, 854, 1053, 1156, 1157
*Cucullanellus* 1085
*Cucullanidae* 1065
*Cucullanus* 1068, 1085, 1086
*Cucullanus minutus* 1086
*Culaea inconstans* 1088
*Cuprinids* 143
Cutthroat trout 66, 623, 924, 926
Cyclopiazonic acid 1219
Cyclopropene fatty acid 1217
CyHV-1 108-110, 112, 121, 122, 124, 125, 128, 129
CyHV-2 108, 109, 122, 129
CyHV-3 108
Cymothoidae 1117, 1176-1178
*Cynoscion nebulosus* 835
*Cyphotilapia frontosa* 476
Cyprinid coronavirus 1 151
Cyprinidae 436, 702, 715, 728, 750, 751, 917
Cyprinids 87-89, 100, 104, 118, 127, 128, 143, 146, 149, 151, 246, 257, 372, 435, 445, 446, 450, 458, 459, 467, 469-471, 482, 483, 542, 583, 585, 599, 639, 642, 860, 861, 880, 881, 885, 904, 905, 917, 1006, 1010, 1011, 1053, 1067, 1169
Cypriniformes 436, 476
*Cyprinodon variegatus* 437, 835, 836, 840, 847, 892
Cyprinodontid 437

Cyprinodontidae 432, 879
Cyprinodontiformes 196, 430, 432
*Cyprinus carpio* 66, 89, 119, 144, 153, 255, 370, 411, 436, 450, 471, 483, 518, 524, 526, 529, 532, 617, 638, 641, 702, 715, 751, 877, 979, 1006, 1053, 1155, 1207
*Cyprinus carpio* iridovirus 153
Cyrilia 521, 547, 549, 550, 551
Cyst 21, 27, 29, 37, 38, 202-208, 210, 211, 260, 280, 285, 286, 301, 304-307, 315, 364, 369, 370, 371, 379, 380, 384, 385, 398, 408-410, 422, 425-427, 434, 440, 441, 467, 494, 519, 520, 522-544, 547-552, 579, 580, 583-585, 588, 591-593, 597, 601, 616, 631, 634, 636-640, 643, 646-649, 759, 786, 787, 790, 794, 823, 825, 826, 828-838, 840, 841, 844, 847, 859, 861, 863, 865-868, 872, 874, 875, 877, 879-908, 910-912, 924, 929, 931, 932, 937
Cystacanth 1032, 1039, 1040, 1043, 1045, 1058
*Cystidicola farionis* 1084
*Cystidicola stigmatura* 1084
Cystidicolidae 1065
*Cystidicoloides ephimeridarum* 1086
*Cystoopsis acipenseris* 1081
Cytokines 15, 472, 714, 536, 1004, 1005, 1015
Cytopathic effect 94, 136, 154, 199
Cytoplasm 207, 554
Cytostome 423, 424, 439-441, 456, 481
Czech Republic 97, 98, 351, 389, 397, 411, 415, 557, 643

## D

D, L-alpha-difluoromethylornithine (DFMO) 486
*D. dendriticum* 978, 1014
*D. labrax* 477, 645, 757, 762
Dab 44, 206, 455, 902, 913, 937
Dactylogyridae 686, 687, 691, 707, 713, 715, 722, 728, 733, 750, 751, 756, 757, 760
*Dactylogyrus vastator* 733, 750-753
Dactylopteriformes 459
*Dactylosoma* 521, 547-549
Dactylosomatids 521, 545-547, 549, 550, 553
*Danio malabaricus* 484
*Danio rerio* 88, 938
Dasyatidae 433, 729, 776, 777
*Dasyatis americana* 433, 816
*Davisia longibrachia* 641
DDT 1150, 1155
DDVP 1150
Deep sea 1026
Defence 123, 359, 472, 1030, 1032, 1037, 1143
Deficiency 1154, 1158, 1202-1208, 1210, 1218, 1220
Degenerative changes 4, 5, 247, 628
*Dendromonocotyle* species 685, 695, 696, 733, 772-775, 777, 793
Denmark 46, 57, 98, 114, 449, 485, 710, 766, 797
Deoxynivalenol 1219
Dermocystidium 280
*Dermophthirius* species 733, 767, 768, 793
*Desseria* 521, 547, 549, 551, 552, 557
Detarox 458
Deuteromycotina 323
Development of microsporidia 354-359
DFMO 486
Diatom blooms 1242, 1243
*Dicentrarchus labrax* 44, 187-190, 193, 197, 212, 255, 259, 262, 400, 428, 430, 431, 460, 461, 477, 532, 538, 539, 587, 598, 645, 757, 759-762, 903, 1178
Dichelesthiidae 1116, 1126, 1163
*Dichelesthium oblongum* 1118, 1163
Dichlorvos 1135, 1150
Diclidophoridae 687, 691, 714, 715, 724, 733, 778, 793
*Dictyuchus* 287
*Didelphis virginiana* 892

*Didymocystis scomberomori* 931
*Didymozoon branchiale* 839
*Diergasilus kasaharai* 1157
Diflubenzuron 1134, 1151, 1175
*Dikerogammarus villosus* 1053
Dilation 24, 635, 637, 648, 1082, 1086, 1236
*Dimastigiella* 424
Dimetridazole 449, 475, 476
*Dimidiochromis compressiceps* 476
Dimilin 1151
Dinoflagellata 422, 425
Dinoflagellates 421-423, 425, 436-439
Dinokaryon 422, 425
Dinospores 425-427, 429
Dinozoa 425
*Dipartiella* 576, 588
*Diphtherostomum* sp. 940
*Diphyllobothrium latum* 978, 986
*Diplanopharyngotrema laterolabracis* 839
Diplectanidae 687, 691-693, 701, 702, 722, 751
Diplectanosis 760
*Diplectanum aequans* 587, 687, 733, 751, 757-762
*Diplodus noct* 477
*Diplodus puntazzo* 209, 250, 430, 431, 461, 477
Diplogonoporiasis 978
Diplomonad(s) 421-423, 439, 440, 443-446, 450-453
Diplomonad flagellates 443, 450
Diplomonadida 423, 439
Diplomonadosis 443
*Diplostomum* 833, 855, 859-862, 869-873, 875-877, 879, 905
*Diplostomum adamsi* 870, 875
*Diplostomum baeri eucaliae* 870, 873, 876
*Diplostomum flexicaudum* 870, 872, 876
*Diplostomum gasterostei* 862, 871
*Diplostomum gigas* 879
*Diplostomum huronense* 879
*Diplostomum phoxini* 877
*Diplostomum pusillum* 855
*Diplostomum scudderi* 875, 876
*Diplostomum spathaceum* 833, 855, 859, 861, 862, 869-871, 873, 877, 905
Diplozoic 439, 440
Diplozoic forms 439, 440
Dipterex 729, 753, 1150
Dipterex® 729, 753
*Dipteropeltis*–morphology 1166
Discus 450, 476
Disease symptoms 51, 56, 58, 61, 66, 68, 89, 101, 106, 119, 128, 132, 140, 144, 146, 386, 402, 426, 434, 443, 456, 465, 470, 473, 475, 477, 483, 636, 986, 1131, 1139, 1142
DNA editing 494
–markers 491
–vaccine 55, 99, 118, 202, 215
Doctorfish 23
*Dollfustrema vaneyi* 906
Dolops 1164-1166, 1168, 1169, 1173, 1174, 1177
–egg 1169, 1171
–hyperparasitism 1176
–larvae 1171
–life cycle 1169
–morphology 1165
–pathology 1173, 1174
–spermatophore 1169, 1171
*Dolops ranarum* 1165, 1166, 1168, 1177
Dolphinfishes 937
Dracunculoidea 1065, 1081
Drain of energy 1047
*Dreissena polymorpha* 901, 904, 1055
Drug development 796
Drug targets 796
Dylox 770, 1150
Dylox® 729, 770
Dysplasia 17, 893, 992, 1003, 1004

E
*E. ctenopharyngodoni* 398
*E. gadi* 398
*E. molae* 398
*E. salmonis* 330-332, 386
*E. salpae* 398
E-11 cells 199
*Echinamoeba* 411
*Echinochasmus japonicus* 941, 942
*Echinochasmus liliputanus* 942
*Echinostoma cinetorchis* 942
*Echinostoma hortense* 942
*Echinostoma* spp. 941
Ecological sink 1031, 1040, 1041
Ectoparasitic flukes 683
Ectopic 38, 823, 941
Edema 13, 19, 23, 31, 46, 51, 56, 61, 90, 146-148, 151-153, 318, 329, 404, 446, 456, 465, 474, 899, 917, 926, 941, 995, 1000, 1009, 1080, 1081, 1083-1085, 1205, 1228
*Edwardiella tarda* 29
*Edwardsiella ictaluri* 853, 874
Eels 17, 37, 42, 44, 51, 52, 95, 124, 192, 193, 245, 246, 250, 251, 254, 255, 257, 435, 445, 448, 485, 548, 551, 641, 699, 710, 779, 793, 899, 913, 937, 1053, 1057, 1067, 1073, 1089, 1092, 1094, 1096, 1097, 1154, 1156
Eel *Anguilla anguilla* 448, 489
EF-1α 452
Effect indicators 1055
Egg 54, 55, 67, 68, 122, 142, 145, 192, 198, 201, 202, 282, 287, 288, 294, 300, 308, 323, 329, 334, 336, 352, 373, 381, 586, 689, 698, 707, 712, 718-720, 724, 725, 730, 732, 734-740, 743, 744, 747, 751-755, 758, 761, 766, 768-771, 774-776, 779-786, 788, 789, 792-794, 797, 817, 819, 823, 825, 827, 828, 832, 834, 842, 844, 850, 851, 859, 865, 866, 871, 875, 884, 886-888, 891, 900, 902, 1051, 1071, 1072-1075, 1079, 1081, 1093, 1095, 1121, 1122, 1130, 1131, 1135, 1136, 1138, 1140, 1142, 1144-1148, 1166, 1167, 1177, 1226-1228, 1242, 1258, 1260
Egg incubation 1242, 1258, 1260
*Egretta rufescens* 892
*Egretta thula* 887
Egtved virus 42, 46
EHN 55-60
EHNV 56-60, 203, 204
Eicosanoids 1205
eIF-2γ 452
*Eimeria* 27, 521, 524, 525, 528-532, 535, 537-544
*Eimeria* (s.l.) *vanasi* 524, 525, 529, 542
*Elaphrobates euzeti* 910, 918
Elasmobranchii 433
Elasmobranchs 430, 480, 542, 721, 725, 726, 730, 742, 770, 775-777, 793, 1178
ELISA 48, 49, 57, 59, 67, 68, 192, 200, 210, 427, 438, 447, 466, 652, 1009, 1079
Elongation factor-1α (EF-1α) 452
Embolism 23
Enapolytic 979
Endodyogony 523, 524
Energy 1047, 1050, 1051, 1203
Entamoeba 398
Enteric redmouth disease 255
Enteritic 534-537, 541, 542
Enteritis 535, 599, 988, 993, 1001-1002, 1004, 1212, 1215
Enteromonad 453
*Enteromyxum* 614, 632, 646, 647
*Enteromyxum fugu* 647
*Enteromyxum leei* 614, 632, 646
Environment 42, 45, 52, 58, 127, 186, 191, 192, 201, 202, 207, 240, 241, 246, 250, 252, 254-258, 264, 265, 281, 286, 289, 316, 328, 334-336, 402, 409, 410, 425, 429, 522, 524, 527, 544, 546, 823, 827, 842, 978, 980, 985, 986, 1029, 1030, 1047, 1050, 1054-1058, 1063-1065, 1067, 1071, 1074, 1077, 1091, 1094, 1150-1153, 1168, 1175, 1176, 1225-1227

Environmental hazard 1055
Environmental Parasitology 1054
Environmental pollution 1029, 1055, 1058
Enzyme linked immunosorbent assay 1079
Eoacanthocephala 1030, 1034, 1035, 1038, 1040
Eopharyngia 423, 439
Eosinophilic granulocytes 994, 996, 997, 1048
EPC 49, 54, 59, 94, 102, 111, 124, 130, 136, 142, 145, 149-155, 157, 159, 160, 215, 217-219
Ephippidae 461
*Epieimeria* 521, 529, 538-540
Epimastigotes 481-483
*Epinephelus malabaricus* 214, 255, 648, 1157
*Epinephelus mystacinus* 934
*Epinephelus niveatus* 931, 934
*Epistylis* 573, 586, 587, 1176
Epithelioma papulosum cyprini (EPC) 215
Epitheloid 362, 1044, 1046
Epitheloid cells 1044, 1046
Eepizootic 42, 45, 56, 57, 144, 185, 205, 212, 239, 241, 242, 249, 250, 252, 261, 282, 311, 312, 318, 329, 426, 430, 706, 1169
Epizootic haematopoietic necrosis 55, 204
*Epizootic haematopoietic necrosis virus* 203, 204
Epizootic necrosis syndrome 282
Epizootic ulcerative syndrome 309
Ergasilosis 1153
*Ergasilus briani* 1156
*Ergasilus gibbus* 1156
*Ergasilus japonicas* 1053
*Ergasilus labracis* 1157
*Ergasilus lizae* 1157
*Ergasilus lobus* 1157
*Ergasilus sieboldi* 1118, 1153, 1155, 1156
ERM 255
Erucic acid 1217
*Esomus danricus* 882
*Esox lucius* 44, 89, 370, 372, 626, 1004, 1012, 1066, 1070, 1087, 1088, 1155
*Esox* spp. 1013
Essential elements 1047, 1054, 1059
Essential fatty acids 1204, 1205
*Etheosoma caeruleum* 484
*Etheostoma fonticola* 860, 891
*Etmopterus spinax* 1179
EU legislation 1256
Eubodonida 479
*Eubrachiella* 1163
*Eubrachiella antarctica* 1163
Eucestoda 978
*Euclinostomum heterostomum* 888, 889
*Euclinostomum indicum* 833
Euglenozoa 424, 454, 490
Euglyphidae 407
*Euhaplorchis* 833, 836, 847, 896
*Euhaplorchis californiensis* 833, 896
Eulachon 1145
*Eumycota* 280, 323
*Euplotes* 572, 598
*Eupomotis gibbosus* 863
Eurasian minnow 877
European catfish 57, 89, 203-204
European catfish virus (ECV) 57, 203, 204
European chub 987, 904
European conger 903
European eel 485, 1053, 1156
European perch 904, 905, 1074, 1161
European plaice 896
European seabass 587, 903
Euryphoridae 1117, 1126
*Euryphorus brachypterus* 850
*Eustrongylides* 1063, 1065-1069, 1072, 1073, 1081, 1084, 1091, 1094
*Eustrongylides ignotus* 1072, 1073
*Euthynnus affinis* 933
*Euthynnus pelamys* 933
Excavata 398, 422-424

*Exophiala* 327, 330-333
Exophthalmia 1081, 1232
Extra-intestinal sites 531, 821, 827, 1074, 1080
Extrasporogonic stages 640
Extrusion apparatus 351, 353, 356
Eye-snapping 1233
Eye-spot barb 144

F

*F. culmorium* 327
*F. philomiragia* 260
*F. solani* 328
False harlequin 100
Farm layout 1257
*Fasciola* 851, 855
Fathead minnow 59, 94, 105, 132, 133, 139, 157, 158, 643, 860, 869, 870, 872, 873, 881, 895
Fathead minnow rhabdovirus 157
Fatty acids 8, 1035, 1059, 1204, 1205, 1217, 1218
Fatty change 8
Febendazole 449, 1095
Fecundity 699, 1051
*Fellodistomum agnotum* 839
*Ferosagitta hispida* 834
Ferric sulphate 1155
Fibrosis 446, 628, 633, 645, 649, 650, 881, 889, 991-993, 995, 1001, 1002, 1004, 1082-1087
*Filamoeba* Page, 1967. 411
*Filamoeba sinensis* 408
Final host 892, 939, 988, 1011, 1013, 1025, 1031, 1032, 1053, 1058, 1071, 1073, 1074
Finland 58, 127, 303, 710, 766, 903, 1097
First feeding window 1228, 1229
Fish condition 787, 1255, 1256
Fish culture 256, 352, 378, 705, 851, 941
Fish eggs 287
Fish farm 58, 62, 68, 89, 96, 104, 145, 213, 240, 241, 253, 257, 258, 334, 385, 397, 401, 436, 544, 558, 709, 736, 739, 761, 766, 849, 853, 855, 881, 936, 1130, 1134, 1150, 1168, 1231, 1242, 1248, 1256
Fish lice 1164
Fish species 89, 100, 245, 793, 1258
Fitness 1028, 1051, 1052
*Flabellula* 407, 408, 411
*Flabellula* spp. 408
Flagella 27, 247, 251, 253, 280, 283-285, 314, 421-425, 444, 446, 448-452, 454-460, 463-470, 473-476, 480, 481, 483-486, 489, 490, 492, 493, 519, 522, 526, 527, 538, 570
Flagellates 27, 421-423, 425, 437-439, 443, 446, 448-451, 454, 457-459, 464, 473, 475, 476, 480, 481, 486, 490, 492, 493, 570
Flagellum 422-424, 426, 429, 439, 441, 442, 455, 464, 469, 473, 475, 476, 478, 480, 481, 527, 592
Flashlight fish 37
Flatfish 368, 375, 460, 473, 649, 782, 783, 836, 847, 911, 920, 1126, 1138, 1142, 1143
Flathead mullet 838, 898, 916, 919, 939
*Flavobacterium* 33, 52, 257, 258, 1239
*Flavobacterium columnare* 258
*Flavobacterium psychrophilum* 52, 257
Flexibacteriosis 255
Florida pompano 841
Flounder 42, 44, 47, 66, 187, 188, 195, 196, 206, 209-211, 243, 245, 250, 376, 459, 460, 463, 473, 474, 487, 557, 598, 647, 701, 716, 743, 747, 750, 778, 780-784, 913, 936, 1066, 1145, 1239, 1248
*Fluminicola fusca* 926
Flying barb 882
Formalin 11, 31, 126, 137, 160, 202, 215, 336, 430, 457, 458, 581, 583, 585, 586, 588, 589, 595, 718, 720-722, 727, 738, 739, 745, 755, 761, 765, 767, 770, 775,

776, 788, 845, 848, 1076, 1079, 1150, 1151, 1164, 1246, 1248, 1258
Fornicata 423, 439
Fountain darter 860, 891, 894, 895
France 46, 57, 69, 71, 98, 114, 145, 193, 195, 402, 431, 441, 442, 461, 463, 471, 477, 478, 860, 905
France 98, 114, 145
*Francisella* sp. 260, 261
Francisellosis 261
*Fredericella sultana* 625, 626
Freshwater minnow 907, 908
Frontal filament 1117, 1124, 1126, 1129, 1131, 1133, 1138, 1139, 1142
Fumagillin 365, 654
*Fundulus granids* 523, 530, 835, 836, 841, 847
*Fundulus parvipinnis* 892
*Fundulus pulvereus* 892
*Fundulus similis* 892
*Fundulus* sp. 437
Furanace 430
Furazolidone 449
*Furnestinia echeneis* 928
Furunculosis 239, 241, 243, 252, 1136, 1173
*Fusarium* 327, 328, 333, 1219

G

G. *carpelli* 525, 527, 530-532, 534, 536, 540, 541
G. *cichlidarum* 527, 529, 530
G. *laureleus* 525
G. *subepithelialis* 527, 530, 531, 534, 536, 541
Gadidae 193, 462, 1082, 1143
Gadiformes 193, 459, 462
Gadoid 402, 451, 460, 540, 542, 621, 622, 649, 902, 1142, 1143
Gadoid cod 460
GADPH gene 491
*Gadus morhua* 44, 61, 62, 193, 211, 241, 245, 248, 250, 253, 332, 378, 450, 462, 487, 621, 622, 821, 1081, 1083, 1086, 1143, 1179
*Gallus gallus domesticus* 869
*Gambusia affinis* 56, 836, 840, 847, 869, 1014, 1091
Gametogony 520
GAPDH 452, 489
Gas Bubble Disease 1229
Gasterosteiformes 459
*Gasterosteus aculeatus* 467
Gasterosteus *aculeatus aculeatus* 860
Gastric dilation air saccultis syndrome 1241
*Gavia immer* 893
GCHD 126, 133
GCRV 131, 132, 134-139, 217
*Gelsenium elegans* 1151
*Genarchella genarchella* 830
Generation time 699, 724, 752, 754, 792, 793, 1131, 1136, 1139, 1140, 1148
Genome(s) 46, 48, 52, 57, 62, 63, 69, 92, 108, 109, 122, 134, 187, 191, 192, 198, 200, 202, 206, 213, 216, 303, 351, 453, 491, 570, 796, 1078
Genotypes 47, 49, 51, 137, 187, 486, 624, 883
*Genypterus capensis* 1147
Genus *Amazonspora* – definition 385
Genus *Glugea* – definition 373
Genus *Heterosporis* – definition 369
Genus *Ichthyosporidium* – definition 381
Genus *Kabatana* – definition 384
Genus *Loma* – definition 376
Genus *Microgemma* – definition 384
Genus *Nucleospora* – definition 385
Genus *Ovipleistophora* – definition 370
Genus *Pleistophora* – definition 367
Genus *Pseudoloma* – definition 381
Genus *Spraguea* – definition 381
Genus *Tetramicra* – definition 382

Georgia 98
Germany 41, 57, 71, 98, 103, 104, 114, 115, 116, 127, 145, 435, 517, 622, 625, 637, 642, 643, 710, 766, 860, 1025, 1053, 1057, 1156, 1255
GFV-1 152
GFV-2 152
GHSBL-1 206, 210
GHSBL-1 cells 210
Giant cells 4, 15, 33, 35, 37, 330-332, 991, 1001
*Giardia* 452, 453
*Giardia lamblia* 453
Gill lamellae 146-148, 153, 641, 686, 694, 696, 701, 705, 714, 726, 750, 751, 753-756, 758, 759, 761, 778-780, 782, 784-786, 929, 1243
Gill maggot 1157, 1158, 1160
Gilling 717, 752, 786
Gilt-head seabream (*Sparus aurata*) 259, 429, 448, 477, 478, 544-646
*Girella punctata* 1178
Gizzerosine 1218
Global change 1028, 1029, 1051
*Glossatella* 573, 586
Glucosinolate 1216, 1217
*Glugea* 27, 353, 357-361, 363, 366, 367, 373, 374, 893
*Glugea anomala* 353, 358, 361, 363, 373, 374
*Glugea atherinae* 373
*Glugea cepedianae* 375
*Glugea hertwigi* 361, 375
*Glugea atherinae* 373
*Glugea cepedianae* 375
*Glugea* luciopercae 375
*Glugea plecoglossi* 360, 375
*Glugea* sp. 893
*Glugea stephani* 360
Glyceraldehyde 3-phosphate dehydrogenase 489
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene 452
Glycocalyx 409, 983, 1035, 1037
Glycosomes 454, 480
Glycosyl-phosphatidylinositol (GPI) 494
Gnathiidae 1117, 1176, 1177, 1179
*Gobio gobio* 144, 410, 471
*Gobiocypris rarus* 132
*Gobiusculus flavescens* 904
Goblet cell hyperplasia 1047
Goezia leporini 1087, 1092
*Goezia pelagia* 1087
*Goezia sinamora* 1087
Golden ide 121, 150
Golden shiner 88, 105, 124, 132, 134, 135, 139-141, 143, 158, 159, 895
Golden shiner virus 139
Goldfish 7, 11, 13, 15, 21, 23, 29, 66, 89, 98, 99, 105, 108, 119, 124, 128-132, 114, 144, 145, 150, 152-154, 158, 368, 371, 401, 463, 470, 471, 477, 478, 483, 484, 636, 640, 641, 708, 728, 751, 754, 870, 877, 1034, 1058, 1164
Goldfish virus 1 152
Goldfish virus 2, 152
Gonadosomatic index 1051
*Gonocerca macroformis* 821
Gonorhynchiformes 430, 432
Gossypol 1217
*Goussia iroquoina* 522, 525, 527, 530, 543
Granulation tissue 4, 15, 362, 363, 644, 981, 982, 994-996, 1001
Granulocytes 13, 31, 35, 468, 472, 533, 780, 838, 928, 994-997, 1000, 1005, 1007, 1010, 1038, 1046, 1048, 1086, 1089, 1092, 1095, 1149, 1172
Granuloma 4, 32, 33, 363, 364
Grass carp 92, 93, 101, 102, 104, 105, 118, 131-139, 141, 149, 150, 154, 155, 157-159, 210, 450, 599, 854, 926, 1052, 1156
Grass carp haemorrhage disease 131
Grayling 44, 327
Great blue heron 887
Great Britain 98

Great egret 892
*Greater amberjack* 195, 377, 313, 923, 924
Green frog 876
Green heron 891, 892
Green moray eel 17, 37
Greenland shark 1158, 1163
Grey snapper 910
Grouper 648, 757, 912, 915, 931, 933, 934, 944
Growth changes 2, 4, 13
Gruberellidae 398
Grunt fin (GF) 213, 215
Gudgeon 144
Gulf killifish 835, 836, 841, 847, 892
Gulls 860, 869
Guppies 437, 584, 710, 711, 859, 878, 1091
*Gymnocephalus cernuus* 885
*Gymnocephalus* spp. 885
Gymnodiniphycidae 439
*Gymnophalloides seoi* 898
Gymnospores 434
*Gymnothorax eurostus* 1178
*Gyraulus parvus* 869
Gyrinocheilidae 476
*Gyrinocheilus aymonieri* 476
Gyrocotylidea 978
Gyrodactylidae 686, 687, 690, 691, 703, 712, 714, 722, 728, 733, 762, 764, 792, 796
Gyrodactylosis 762
*Gyrodactylus salaris* 687, 690, 703, 710, 712, 733, 762-766, 792, 797

## H

*H. salmonis* 443, 451
$H_2S$ 1258
Haddock 450, 621, 1143, 1238, 1239
Haematocrit centrifuge technique (HCT) 483
Haematozoan species 480
Haematozoic *cryptobia* 466, 474, 475
Haematozoic species 463, 464, 473, 479
*Haemiclepsis marignata* 470
*Haemobaphes* 1121, 1130, 1141, 1142, 1144
*Haemobaphes diceraus* 1121, 1142, 1144
*Haemobaphes disphaerocephalus* 1144
*Haemogregarina* 521, 547, 549-551, 554, 557, 558
Haemogregarines 521, 545-547, 549-555, 558
Haemolysin 247, 465, 483
Haemolysis 465, 493
Haemorrhagic 42, 44, 47, 53, 55, 61, 64, 88, 89, 95, 133, 151, 155, 157, 202, 324, 333, 630, 636, 653, 717, 718, 775, 1149, 1172, 1173
Haemorrhagic septicaemia 42, 44, 47, 55, 247, 249
Halibut 44, 66, 188-190, 195, 197, 198, 201, 202, 218, 240, 245, 248-250, 253, 254, 257, 460, 650, 732, 748, 1238, 1240
*Haliotrema abaddon* 687, 707, 733, 751, 797
*Hampala dispar* 144
Hamuli 684-686, 692-694, 700-702, 734, 736, 737, 739, 743, 750, 751, 756-759, 763, 764, 767, 773
*Haplochromis macula* 476
*Haploporus lateralis* 839
*Haplorchis* 833, 855, 890, 896, 897, 941
*Haplorchis pumilio* 833
*Haplosplanchnus pachysomus* 839
Haptor 29, 684-686, 698, 719, 720, 734, 737, 739, 743, 745, 746, 750, 752, 756-758, 763-765, 767-773, 778-782, 785, 796
*Hartmannella vermiformis* 408, 411
HC 1001, 1009
HCT 447, 466, 483
Heat shock protein 1054
Heavy metals 1054-1056, 1059, 1219
*Helicostoma* 572, 598

*Helisoma anceps* 869
Hematocrit centrifuge technique (HCT) 447
*Hemiclepsis marginata* 483, 485, 487
*Hemiculter bleekeri* 132
*Hemiramphus brasiliensis* 840
*Hemitrichodina* 576, 588
*Hemiurus appendiculatus* 839
*Hemiurus communis* 834
Hemodynamic change 2, 4, 19
Hemorrhage 11, 19, 23, 24, 27, 38, 899, 904-907, 1000, 1086, 1087
Hemp seed 289, 291, 308
*Henneguya* 615, 631, 632, 634, 643, 644, 648
*Henneguya creplini* 643
*Henneguya exilis* 634
*Henneguya ictaluri* 634
*Henneguya psorospermica* 643
*Henneguya salminicola* 631
*Herichthys cyanoguttatus* 476
Heron 66, 643, 840, 869, 882, 884, 886-888, 891, 892, 899
Herpesviral epidermal hyperplasia 118, 120
Herpesviral haematopoietic necrosis (HVHN) 128
*Herpesvirus cyprini* 121
Heteraxinidae 685, 687, 689, 692, 698, 704, 714, 728, 732, 733, 738, 784
*Heterobothrium okamatoi* 784
Heterolobosea 398
Heteronchoinea 688
*Heterophyes heterophyes* 855
*Heterophyes nocens* 898
*Heterophyes* spp. 890, 897, 941
*Heterophysopsis continua* 941
*Heteropneustes fossilis* 487, 1085
*Heterosigma carterae (akashiwo)* 1244
*Heterosporis anguillarum* 356, 365, 370
*Heterosporis schuberti* 353, 371
*Heterosporis* sp. 370
Heteroxenous 520, 522, 524, 537, 539, 541, 547, 1126
Hexacanth 983
*Hexamita* 423, 440, 441, 443-445, 449-453, 475
*Hexamita salmonis* 443-445, 449, 451
Hexamitiasis 443
*Hexamitids* 445, 447, 450, 451, 475
Hexamitinae 423, 440
Hexamitosis 440, 443-446, 449, 451, 453
Hexamitosis/spironucleosis 445, 446, 451, 453
*Hexastoma thynni* 850
Highly unsaturated fatty acid 1205
*Hiodon tergisus* 821
*Hippoglossoides platessoides* 245, 368, 911
*Hippoglossus Hippoglossus* 190, 195, 197, 198, 218, 248, 253, 257, 461
*Hirudinella ventricosa* 818, 937
Histamine 1218
Histone-like proteins 438
Histones 422
Hitra disease 253
Hoferellosis 640
*Hoferellus carassii* 641
Hogchoker 474
Hole-in-the-head disease 445, 450
*Holobomolochus* 1163
Homeothermic 536, 545, 620, 1256
Homoxenous life cycle 520, 522, 530, 539
Hooks 29, 304-307, 684, 685, 692-694, 698, 700, 701, 703, 734, 736, 743, 745, 750, 752, 756, 758, 763-765, 767, 979, 980, 990, 993-995, 864, 1026, 1028, 1033, 1037, 1038, 1041, 1042, 1044, 1046, 1048, 1154, 1164-1166, 1170 1173
Horizontal transmission 186, 191, 207
Host immunity 712, 713, 1004
Host specificity 388, 488, 597, 690, 711, 733, 742, 748, 749, 822, 823, 850, 885, 892, 900, 1007, 1013, 1030, 1052

Host-parasite interface 846, 1026, 1029, 1037
Hsp90 479
*Hucho perryi* 1161
*Huffmanella* 1081, 1093
Humoral 55, 468, 581, 714, 715, 907, 914, 1005, 1008, 1015, 1095
Humoral specific immunity in fish 1008
Humpnose unicornfish 937
Hungary 98, 127, 158, 450, 483, 640, 643, 1090
Husbandry 57, 59, 60, 64, 65, 68, 103, 143, 334, 414, 535, 791, 792, 797, 986, 1011, 1016, 1094
Hyaline 7, 9, 133, 207, 323, 324, 837
Hyaluronidase 580, 592
Hydrogen peroxide 336, 458, 727, 789
Hydropic swelling 11
Hyperapolytic 979
*Hyperandrotrema cetorhini* 913
Hyperplasia 4, 17, 19, 29, 56, 87, 107, 108, 118-123, 126, 127, 129, 146, 148-150, 154, 380, 404, 427, 428, 435, 447, 457, 471, 570, 579, 582, 583, 585, 589, 590, 594, 628, 635, 636, 640, 702, 707, 718, 752, 755, 759, 786, 858, 895, 923, 926, 928, 994, 1001, 1003, 1004, 1006, 1008, 1009, 1047, 1083, 1085, 1086, 1133, 1150, 1153, 1158, 1160, 1161, 1241
Hypertrophy 27, 107, 133, 203, 206, 351, 357, 362, 372, 404, 626, 627, 629, 635, 636, 640, 642, 644-646, 648, 649, 759, 894, 921, 991, 993, 1001, 1003, 1155
Hyperventilation 717
Hyphomycetes 323, 327
*Hypophthalmichthys molitrix* 89, 639, 854, 1156, 1157
*Hypophthalmichthys nobilis* 450, 854, 1150
*Hyporhamphus xanthopterus* 1145
*Hysterothylacium* 1066, 1075, 1077, 1078, 1082, 1087, 1089
*Hysterothylacium aduncum* 1082, 1087, 1089
*Hysterothylaceum bidentatum* 1087
*Hysterothylacium dollfusi* 1087

## I

*I. necator* 456, 458-460, 462, 490, 491
*Ichthyobodo* 107, 414, 422, 424, 448, 454-457, 459-463, 490, 491
*Ichthyobodo necator* 414, 456, 458, 460
*Ichthyobodo* sp. 107, 448, 455, 459, 461, 462, 490, 491
*Ichthyobodo*-like 461
Ichthyobodosis 456, 458, 459
*Ichthyophonus* 279, 280
*Ichthyophthirius* spp. 571
*Ichthyophthirius multifiliis* 570, 571, 578, 580, 601
*Ichthyosporidium giganteum* 362, 379, 380
Ichthyobodo spp. 455
*Ictalurus nebulosus* 484
*Ictalurus punctatus* 105, 634, 840, 875, 1150
Ide 89, 100, 119
IFAT 48, 49, 57, 192, 199, 213, 214, 260, 316, 403, 466
IHN 51
Il-1β 472, 492
Immobilization antigens (i-antigens) 582
Immune evasion 1010
Immunization 484
Immunofluorescence 96, 112, 192, 199, 264
Immunohistochemistry 68, 199, 240, 260, 1263
Immunology 690, 711, 793, 796, 1097
Immunoprophylactic strategies 466, 468
Immunosuppression 110, 535, 716, 986, 1014, 1091
*In vitro* 366, 437, 449, 451, 466, 468, 471, 472, 484-486, 490, 493

*In vivo* 97, 244, 308, 439, 472, 473, 700, 718, 719, 735, 744, 748, 751, 758, 780, 784, 785, 793, 795, 871, 1230

Inclusion bodies 27, 59, 120, 121, 207, 210, 212, 213

Indispensable amino acids 1203

*Indodidymozoon pearsoni* 931

*Indodidymozoon suttiei* 929

Indonesia 105, 115-117, 193, 194, 450, 889, 1157

Infection 4, 5, 13, 17, 23, 25, 27, 29, 31, 33, 35, 37, 42, 45-50, 52-56, 58, 88, 94, 96-102, 104, 105, 110-113, 115, 116, 120-124, 126-128, 130, 185, 186, 190, 192, 193, 197-202, 205-208, 210-216, 218, 219, 280, 282, 287, 289, 292, 295, 296-299, 303, 308, 311-313, 317, 318, 320-322, 324, 326-334, 337, 352, 356, 357, 359, 360, 362-366, 368-370, 372, 375-378, 380-386, 388-402, 404, 406, 409, 410, 412, 426, 427, 430, 434, 435, 438, 443-447, 483-488, 493, 494, 522, 523, 525, 529-536, 538-545, 548, 552-555, 557, 558, 570, 571, 579, 581-585, 590, 594, 595, 598, 614, 616, 618, 620-624, 627-629, 630-639, 642-656, 691, 694, 697, 699, 700, 704, 705, 707, 708, 710, 712, 713, 715-718, 724-726, 728-732, 734-736, 738, 740, 741, 743-750, 753, 754, 755, 757, 759-761, 764-767, 769-772, 774, 775, 777-781, 783, 784, 786-793, 795-797, 835, 847, 944, 1003, 1005-1007, 1010-1013, 1015, 1016, 1028, 1040, 1042, 1044, 1047, 1048, 1050, 1058, 1064, 1071, 1072, 1073, 1077, 1079, 1080, 1083, 1089-1092, 1095-1097, 1130, 1131, 1133-1135, 1137-1144, 1146, 1147, 1228, 1229, 1231, 1234, 1259

Infectious haematopoietic necrosis (IHN) 51, 1257, 1260

Infectious pancreatic necrosis 65, 143, 1136

Infectious salmon anaemia 60, 61

Inflammation 4, 5, 9, 11, 13, 23, 25, 27, 31-33, 35, 37, 90, 107, 255, 257, 317, 361, 362, 404, 406, 427, 446, 628, 635, 637, 643, 645, 646, 649, 759, 780, 838, 858, 889, 898, 919, 921, 942, 990, 995, 1000, 1001, 1003, 1004, 1010, 1032, 1037, 1040, 1048, 1079, 1087, 1133, 1162, 1174, 1241, 1242

Inflammatory 13, 17, 19, 21, 23, 25, 29, 31-33, 35, 120, 152, 320, 326, 372, 404, 406, 451, 465, 483, 535, 620, 635, 637, 642, 647, 649, 714, 717, 780, 847, 906, 915, 916, 919, 921, 922, 924, 928, 937, 1004, 1005, 1046, 1049, 1088, 1095, 1096, 1205, 1235, 1239

–change 404, 406

–tissue 647, 649, 1044, 1045, 1046, 1049

Innate 308, 438, 628, 706, 783, 1005, 1007, 1015, 1016, 1096, 1097

Innate immunity 438, 469, 1005, 1007, 1096

Inorganic phosphate T258

Inositol 485, 494, 1210, 1216

Inspection frequency 1263

Intermediate hosts 470, 485, 487, 520, 522, 530, 531, 543, 547, 601, 688, 690, 817-820, 822, 825-828, 831-834, 837, 844, 850, 858-861, 863, 865-867, 870, 871, 884, 886, 887, 891-895, 899, 900, 903, 905, 907, 908, 910, 913, 915, 917, 921, 922, 925, 927, 930, 932, 933, 938-940, 983, 985, 1012, 1013, 1016, 1031, 1038, 1040, 1041, 1053-1055, 1058, 1064, 1066, 1067, 1071-1075, 1093, 1094, 1126, 1142, 1143, 1145

Invasion 25, 46, 445, 519, 529, 545, 752, 768, 774, 793, 1007, 1052

Iodine 97, 1079, 1207

IPN 65-67, 143

IPNV 65-68, 143

*Iridoviridae* 57, 152, 202, 203, 206, 211

Iridovirus(es) 27, 55-57, 152, 153, 202, 203, 213, 214

Iron 17, 18, 25, 29, 37, 38, 42, 45, 52, 58, 1063-1065, 1067, 1071, 1074, 1077, 1091, 1094, 1122, 1135, 1150, 1151-1153, 1168, 1175, 1176, 1203, 1206,

1207, 1209, 1220, 1225-1227, 1236-1238, 1241, 1248, 1255-1258, 1261
ISA 48, 49, 57, 59-65, 67, 68, 95, 96, 103, 110, 112, 124, 132, 134, 136, 192, 200, 210, 260, 263, 264, 427, 438, 447, 466, 652, 1009, 1079, 1136
*Isavirus* 62
*Isoachlya* 287
Isoenzyme 488
Isometamidium chloride 466
Israel 115, 193-195, 635, 777, 855, 895, 896, 1157
*Isurus oxyrhynchus* 1163
Italy 71, 98, 115, 145, 207, 209, 218, 645, 761, 762
ITS rRNA 463

J

*Janickina* spp. 414
Japan 42, 47, 50, 51, 66, 112, 115, 117, 121, 124, 146, 201, 205, 209-211, 213, 214, 246, 256, 259, 260, 282, 459, 460, 462, 463, 622, 631, 640, 646-649, 651, 652, 701, 710, 716, 726, 727, 729, 731, 777-784, 789, 790, 793, 1052, 1079
Japanese eels 895, 899, 1052
Japanese flounder 42, 47, 459, 463, 647, 716, 743, 747, 750, 781
*Johanssonia arctica* 487, 551

K

*Kabatana arthuri* 353, 383
*Kabatana takedai* 355, 356, 360, 361
*Kamegaia kawakawa* 933
Karyomastigonts 439
*Katsuwonus pelamis* 933
kDNA 454, 480, 489, 494, 495
KED 4, 29, 46, 48, 54, 65, 95, 361, 362, 365, 366, 369, 398, 399, 411, 457, 471, 474, 495, 534, 550, 585, 586, 590, 600, 601, 622, 623, 627, 635, 636, 640, 642, 644, 652, 777, 781, 787, 846, 849, 853, 857, 862, 878, 879, 885, 890, 895, 897-900, 905, 907, 913, 916, 921, 924, 937, 942, 943, 1003, 1052, 1059, 1067, 1079, 1084, 1150, 1155, 1169, 1172, 1176, 1215, 1225, 1230, 1231
Khanka gudgeons 907, 908
*Khawia sinensis* 1053
KHV 104, 105, 109-117, 124, 128, 129, 155, 159, 160, 1263
KHV (Koi Herpes Virus) 1263
Kidney 51, 53, 111, 157, 1263
Kidney bloater 640
Kidney enlargement disease 641
Kinetid(s) 423
Kinetoplast 422, 424, 454-456, 464, 478, 480, 481, 489, 491, 494, 495
Kinetoplastida 424
Kinetoplastea 422, 424, 454
Kinetoplastid(s) 414, 421-424, 454, 479, 480, 489-491, 494, 495
Kinetosoma 481
Kinetosomes 423, 424, 441, 442, 481, 569
Kingfisher 882
Koi 29, 35, 96, 98, 99, 104-108, 110, 111, 114-117, 120, 122-124, 146-151, 154, 155, 159, 160, 463, 485, 621, 636, 942, 1152, 1158, 1162, 1163
Koi carp 98, 99, 105, 106, 108, 110, 111, 114-117, 120, 122, 123, 146, 148-150, 154, 159, 636
Koi herpesvirus 104, 105, 115, 116
Koi pla 942
Koi sleepy disease 146, 147
*Kollikeria filicollis* 931
Korea 109, 115, 128, 159, 194-196, 205, 214, 215, 598, 783, 784, 898, 941, 942
KRE-3 213
*Kroyeria carchariaeglauci* 1164
*Kudoa* 619, 620, 631, 646-650, 652, 656
*Kudoa clupeidae* 648
*Kudoa thyrsites* 631, 641, 652

L

*L. (Penaeus) vannamei* 88
*Labeo* 476, 548, 882, 933, 1172

*Labeotropheus fuelleborni* 476
*Labratrema lamirandi* 903
*Labyrinthula* cienkowski, 1867 414
Lake trout 66, 763, 870, 893
*Lamna cornubica* 1163
*Lamproglena* 1152
*Lanassa nordenskioeldi* 911
Lao PDR 115, 145
Largemouth bass 17, 23, 204, 880, 882, 892, 1032, 1046
Lasalocid 430
Laser capture microdissection 653
Lateral gene transfer 453
*Lates calcarifer* 188, 190, 192, 193, 197, 209, 432, 750, 757, 922, 928
Latidae 193, 432, 917
*Latris lineata* 195, 646
LCDV-1 203, 204, 206
LCDV-2 204, 206
LCMD 653
*Lebeo calbasu* 882
*Lebistes reticulatus* 878
*Lecithaster confusus* 940
*Lecithochirium* 834, 940
*Lecithocladium chingi* 937
*Lecithocladium invasor* 937
Lectins 651, 714, 1213
Leech(es) 99, 206, 413, 464, 465, 467, 470, 473, 474, 481-483, 485-488, 520, 547-552, 558, 693, 831
*Leishmania* 424, 480
*Lepeophtheirus cuneifer* 1138
*Lepeophtheirus hippoglossi* 1138
*Lepeophtheirus mugiloidis* 1138
*Lepeophtheirus pectoralis* 1138
*Lepeophtheirus salmonis* 63, 791, 1119, 1127, 1130, 1132, 1133, 1137-1139
Lepidex® 729, 776
*Lepocreadium album* 830
*Lepocreadium bimarinum* 839
*Lepomis gibbosus* 484, 1082
*Lepomis humilis* 892
*Lepomis macrochirus* 206, 860, 991, 1051, 1086
*Lepomis* spp. 887
*Leporinus macrocephalus* 436, 1087
*Leptobarbus hoevenii* 436
*Leptotheca* 621, 646, 647
*Leptotheca sparidarum* 646
*Leptoxis carinata* 926
*Lernaea* 1053, 1122, 1124, 1126, 1130, 1133, 1147-1152, 1155, 1161
*Lernaea cyprinacea* 1053, 1122, 1147, 1148,
*Lernaea multiobosa* 1152
*Lernaeenicus* 1130, 1145
*Lernaeenicus hemiramphi* 1145
*Lernaeenicus longiventris* 1145
*Lernaeenicus sprattae* 1145
Lernaeidae 1116, 1122
*Lernaeocera* 1120, 1126, 1130, 1141-1146
*Lernaeocera branchialis* 1120, 1141, 1144
*Lernaeolophus sultanus* 1121, 1145
*Lernaeopoda* 1130, 1162
Lernaeopodidae 1124, 1158, 1162
Lernanthropidae 1117, 1121, 1126
Lesions 56, 59, 61, 69-71, 118, 128, 140, 148, 151, 152, 159, 160, 189, 239, 244, 245, 247-249, 254, 256-259, 261, 263, 384, 385, 398, 399, 401, 402, 404-406, 412, 427, 445, 451, 456, 457, 465, 474, 486, 554, 555, 558, 620, 624, 627, 628, 636, 645, 648, 704, 717, 718, 736, 744, 759, 765, 767, 769, 771, 775, 1026, 1046, 1083, 1085-1087, 1149, 1158, 1173, 1174, 1212, 1217, 1220, 1235, 1238, 1242, 1244
*Leuciscus cephalus* 119, 372, 642, 904, 1039, 1054
*Leuciscus idus* 89, 100, 450, 471
*Leuciscus idus melanotus* 150
*Leuciscus leuciscus* 860, 1012
*Leuciscus souffia* 144
*Leuciscus waleckii* 1178

Leukocytes 51, 53, 529, 535, 536, 540, 546, 551, 553, 554, 780, 1236
Levamisole 406, 1095, 1096
*Licnophora* 572, 597, 599
Life cycle 388, 422, 425, 426, 434, 438, 454, 456, 463-465, 469, 470, 473, 480, 481, 483, 487, 491, 520, 522, 530, 536, 537, 539-541, 547, 570, 580, 581, 590, 591, 593, 601, 613, 614, 623, 634, 640, 655, 688, 690, 694, 699, 700, 705, 706, 708, 709, 711, 736, 751, 753, 771, 786, 791, 792, 817-820, 824-826, 829, 830, 900, 904, 910, 913, 914, 923, 927, 930, 932, 938, 939, 1025, 1029, 1031, 1038, 1040, 1053, 1071-1075, 1093, 1094, 1096, 1154, 1158, 1161, 1162, 1170, 1174, 1179
*Limanda limanda* 206, 245, 375, 455, 457, 902, 911
Lime ($CaCO_3$) 1258
Lime disinfection 1259
Lindane 1150
*Liopsetta putnami* 474
Lipid 90, 478, 525, 617, 838, 1037-1039, 1048, 1091, 1204-1206, 1208, 1210, 1215, 1217, 1218
Lipoid liver disease 1238
*Lipophrys pholis* 553, 557, 902
Listonella anguillarum 251, 253
Lithuania 98
*Litopenaeus* 88
*Littoridinops monroensis* 891
*Littorina littorea* 828
Live vaccines 1266
Liver 5, 530, 992, 1245, 1263
*Livoneca amurensis* 1178
*Livoneca pontica* 1178
*Livoneca puhi* 1178
*Liza macrolepis* 1157
*Liza ramado* 838
*Lobatostoma ringens* 841
*Lockenloia sanguinis* 1092
*Loma acerinae* 355, 358, 363, 374, 377, 380
*Loma branchialis* 353, 379
*Loma camerounensis* 378
*Loma salmonae* 357-360, 365, 366
Longevity 699, 736, 754, 779, 780, 792, 793, 1043
Longnose dace 925
Longnose killifish 892
Longnose lancetfish 818, 944
Longspined bullhead 902
Loop-mediated isothermal amplification (LAMP) 112, 215
*Lophius piscatorius* 363, 379, 382, 900, 1163
*Lophoura edwardsii* 1147
*Lota lota* 450, 1083
*Lucania parva* 437
*Lunulae* 1121
Lupin 1218
Lutjanidae 195, 432, 912, 918
*Lutjanus argentimaculatus* 432
*Lutjanus campechanus* 841, 850, 918
*Lutjanus griseus* 432
Luxembourg 114, 115
*Luxilus cornutus* 869
*Lymnaea peregra* 869, 871, 883, 884
*Lymnaea stagnalis* 869, 871
Lymphocystis 29, 203-205
Lymphocystis disease 205
Lymphocysts 208, 210
*Lymphocystivirus* 202-204, 206
Lymphocytes 5, 32, 33, 35, 37, 120, 208, 210, 357, 366, 451, 472, 473, 487, 519, 531, 554, 557, 624, 628, 714, 781, 906, 914, 915, 928, 934, 937, 987, 991, 1001, 1005-1007, 1009, 1010, 1046, 1085, 1086, 1089, 1092, 1095, 1155

M

*M. crhysops* 431
mAb 467, 469
Macrophages 11, 13, 15, 23, 32, 33, 35, 37,

38, 218, 357, 359, 377, 383-385, 451, 468, 472, 484, 492, 534, 542, 554, 624, 637, 649, 714, 837, 906, 914-916, 922, 928, 937, 990, 991, 995, 1001, 1005-1007, 1034, 1044-1046, 1086, 1087, 1089, 1096, 1237, 1243
Magnesium 449, 748, 1206, 1207
Magnesium sulphate 449
*Makaira audax* 933
Malabar reef cod 1157
Malachite green 430, 334, 654, 458
Malacosporea 614, 619, 620
Malathion 1150, 1175
Malaysia 115, 193, 194, 436
Manganese 1207-1209, 1241
*Marcusenius macrolepidotus* 888
Mariculture 426, 430, 437, 754
Mastigonemes 424
Mebendazole 449, 728, 794, 1095
Mechanical control 724
Medaka 21
Mediterranean horse mackerel 923
Mediterranean sea bass 438, 478
*Megaceryle* spp. 882
*Megacreadium tetrodontis* 839
mega-kDNA 494
*Megalobatrachus japonicus* 900
*Megalocytivirus* 202-204, 213
*Meganyctiphanes* spp. 930
*Melanogrammus aeglefinus* 44, 193, 245, 379, 450, 621, 1143
*Melanoides tuberculatus* 891, 892, 895
Melano-macrophage(s) 534, 928, 937, 1034, 1045, 1046
Mercury 1219
*Mergus merganser* 884
*Merlangius merlangus* 44, 638, 649, 902, 1082, 1143
*Merluccius hubbsi* 1146, 1147
Merogony 354, 356-358, 366, 369, 380, 518, 520, 523, 524, 529, 531, 533, 535, 546-553, 556
*Mesocricetus auratus* 892
*Mesocyclops* 1151
Mesoparasite 1122, 1153
*Mesostephanus appendiculoides* 939
Metacyclic 470, 481, 482
Metacyclic forms 465
Metacyclic trypanomastigotes 482
*Metagonimus yokogawai* 898
Metakinetoplastina 422, 424, 463
Metakinoplastina 454
Metallopretase 468
Metalloprotease 466, 467, 493, 597
Metallothionein 1055
Metaplasia 17, 18, 29, 991, 992, 1003, 1246, 1247
Methemoglobin 1236
*Metorchis* spp. 941
Metronidazole 449, 458
*Miamiensis avidus* 598
Microbothriidae 686, 687, 692, 693, 702, 707, 727, 730, 733, 767, 768, 771, 793
*Microcotyle chrysophrii* 928
Microcytic anemia 1207
*Microgemma hepaticus* 384
*Microphallus opacus* 830, 839
*Micropogonias undulatus* 474, 940
*Micropterus salmoides* 880, 1032, 1046
*Micropterus* spp. 887
Microscopic immuno-substrate-enzyme technique 466
Microsporidia 353, 354, 358, 362, 364-367, 373, 375, 378, 381, 382, 385, 388, 389, 517, 552
Microtriches 981, 983, 984, 989-991, 997, 1006, 1007
Microtubules 446, 478, 518, 569
Milkfish 322, 1157
Mineralized necrotic tissues (MM) 999

Minerals 1206, 1207, 1209, 1215
Minnow 132, 144
MISET 466
*Misgurnus anguillicaudatus* 66, 132, 889
Mixed infections 450, 457, 475, 477, 479
Mode of attachment 1031, 1044, 1045, 1049, 1158
*Mola mola* 819, 1163
Moldova 93, 98
Moniliforme 1219
Molts 62, 1071, 1072, 1075
*Molva molva* 1147
Monoclonal antibody(-ies) 52, 60, 96, 98, 103, 214, 318, 466, 467, 469, 484, 493, 652
Monocotylidae 685-687, 692, 698, 707, 773, 790, 793
Monocytes 32, 357, 487, 519, 554, 780, 906, 1044, 1085, 1149, 1172
Monogenea 683-685, 687, 688, 690-692, 694, 696-699, 700, 705-722, 724, 726, 730, 732, 733, 739, 742, 747, 749, 751, 755, 765, 770, 773, 775, 776, 783, 787, 790-797, 817, 819
Monogeneans 477, 685, 686, 688, 692, 695-697, 700, 701, 709, 712-723, 725-730, 732, 739, 742, 747, 753, 755, 756, 763, 767, 773, 775-777, 787, 788, 791, 793, 795, 797, 855, 921, 928, 1053, 1055, 1093
Monogenoidea 688, 817
Mononuclear cells 21, 32, 33, 35, 37, 984, 997, 1001
Monopisthocotylea 684-703, 705-710, 712-717, 725-729, 731, 732, 746, 751, 764, 768, 773, 790, 792-795, 797
Monopisthocotyleans 691-693, 701, 737, 767
*Monorchis monorchis* 839
*Moritella viscosa* 64, 247, 248
Mormyrid bulldog 888
*Morone* 250, 259, 431, 757, 835, 855, 903, 1083, 1084, 1087, 1204
*Morone chrysops* 855
*Morone saxatilis* 250, 431, 855, 1083, 1084, 1087
Moronidae 66, 193, 431, 461, 757
Morphology 425, 434, 456, 464, 469, 473, 481, 487, 614, 684, 1032, 1130, 1138, 1141, 1148
Mortality 1, 45-48, 51, 52, 57, 62, 66, 67, 69, 70, 103-105, 109, 110, 114, 115, 122, 123, 126-129, 157, 190, 197, 203, 208, 212, 214-216, 218, 219, 257, 331, 376, 380, 385, 406, 435, 436, 444, 456, 457, 459, 465, 467, 470, 473-475, 479, 541, 624, 629, 630, 640, 645, 646, 728, 786, 904, 906, 977, 986-988, 917, 921, 923-927, 933, 1011, 1013, 1015, 1028, 1041, 1049, 1051, 1064, 1082, 1087, 1089, 1090, 1150, 1152, 1173, 1178, 1208, 1220, 1237, 1240, 1241, 1246
*Mothocya parvostis* 1178
Motile aeromonads 246, 247
Motile aeromonas septicaemia 247
Mouth fungus 258
*Mozambique tilapia* 192
mRNAs 472, 492
Mucin-like 485, 489, 494
Mucins 489, 490, 1037
Mucus 106, 110, 240, 456, 457, 464, 477, 478, 536, 688, 692, 693, 697, 720, 737, 743, 744, 752-754, 759, 760, 765, 769, 770, 775, 786, 793, 829, 1003, 1005, 1006, 1008, 1121, 1133
*Mugil cephalus* 195, 214, 311, 431, 646, 838, 919, 1084, 1157
*Mugil platanus* 460
*Mugilicola* 1130, 1153
Mugilidae 195, 431, 703, 706, 733, 917
Mullets (*Mugil platanus*) 430, 431, 460, 488, 650, 897
Mummichog 7
*Mycobacterium* 25, 33
Mycotic granulomatosis 282, 311
Mycotoxin 1219
*Mylocheilus caurinus* 893
*Mylopharyngodon piceus* 132, 144, 854

Myoliquefaction 641, 648
*Mystus malabaricus* 487
*Myxidium* 621, 626, 632, 633, 641, 642, 644-647, 650, 655
*Myxidium giardi* 641, 645
*Myxidium lieberkuehni* 626, 644
*Myxidium rhodei* 632, 642
*Myxidium truttae* 632, 633
*Myxobilatus* 615
*Myxobolus* 27, 613, 614, 622, 630, 631, 633, 636-640, 642-644, 647, 649, 654
*Myxobolus aeglifini* 638
*Myxobolus arcticus* 630
*Myxobolus artus* 638
*Myxobolus buckei* 642
*Myxobolus cerebralis* 613, 614, 622
*Myxobolus encephalicus* 637
*Myxobolus neurobius* 631
*Myxobolus pfeifferi* 639

N

*N. australiensis* 410
*N. branchiphila* 399, 400
*N. clarki* 410
*N. pemaquidensis* 399, 400, 402, 405
*Naegleria australiensis* 401
*Naegleria* sp. 408, 410
*Naegleria* strains 410
*Nagmia floridensis* 818
*Nanophyetus salmincola* 941, 942
*Naricolax* 1163
*Naricolax chrysophryenus* 1163
Nase 144
*Naso annulatus* 937
*Naso tuberosus* 937
*Naso vlamingii* 937
Nauplius 1122, 1124, 1131, 1139, 1142, 1148, 1154, 1161, 1162
*Neascus* 836, 868, 880
Necator 414, 456, 458, 459, 460, 462, 490, 491
Neck 979, 980, 983, 995, 997, 1026, 1027, 1031-1033, 1036, 1039, 1042, 1043, 1046, 1048, 1142, 1144, 1146, 1152
Necropsy 1-3, 32, 465, 487, 846, 861, 864, 978, 1260, 1261
Necrotic tissue 106, 332, 981, 982, 991, 999, 1034, 1037, 1039, 1044, 1046
Negative selection 1260
Neguvon® 729, 760, 761, 1135
*Nematobibothrioides histoidii* 819, 929
*Nematobothrium labeonis* 933
*Nematobothrium spinneri* 936, 943
Nematode 475, 1063-1069, 1071-1080, 1084, 1089-1097
*Neobenedenia girellae* 710, 716, 731, 732, 793
*Neobenedenia melleni* 690, 708, 709, 713, 725, 742
*Neobenedenia* species 733, 737, 741-746, 748-750
*Neobrachiella* 1130, 1162
*Neodidymozoon macrostoma* 933
*Neodiplotrema pelamydis* 933
*Neogogatea kentuckiensis* 872, 873
*Neoheterobothrium affine* 793
*Neoheterobothrium hirame* 687, 733, 778, 779, 783, 784
*Neometadidymozoon helicus* 933
*Neometadidymozoon polymorphis* 931
*Neometadidymozoon tuberosum* 931
*Neoparamoeba pemaquidensis* 399, 405, 406
*Neoparamoeba* spp. 403, 412, 415
Neoplasia 15, 386
Neoplasm 4, 15, 18, 21, 1217
*Nephrocalcinosis* 1207, 1236
*Nerocila orbignyi* 1178
Nervous tissue lesions 197
Net pen liver disease 1245
Neutralization antibody titres 202
Neutrophil 13, 31, 357, 359, 374, 472, 536, 554, 714, 914, 915, 937, 1005-1007, 1089, 1092

New Zealand longfin eel 913
Niacin 1210
Nitrate 1235, 1258
Nitrite 126, 1258
Nitrofurazone 430
Nitroimidazoles 449
*Nocardia* 33
Nodaviridae 186, 187
Nodavirus infection 185, 186, 192, 197, 200
Nodaviruses 185
Non-perforating species 1031, 1042, 1048-1050
Non-perforating acanthocephalans 1049, 1050
Non-perforating species 1042
Non-Salivarian 485, 493, 494
Non-Salivarian trypanosomes 485, 493
Non-starch polysaccharides 1214
Northern Ireland 101, 103, 104, 449
Northern leopard frog 876
Northern pikeminnow 893
Northern puffer 7, 17, 23
Norway 44, 65, 69, 127, 195-197, 445, 462, 791, 793, 797
*Nosema* 358, 365, 379, 388
*Notemigonus crysoleucas* 88, 372, 643, 895
*Notropis cornutus* 484
*Noturus miurus* 888
Nova 89, 93, 98, 244, 469, 796, 1066, 1068, 1072, 1075, 1077-1079, 1081, 1089, 1090
*Novirhabdovirus* 42, 46, 52
*Nuclearia cienkowski* 1865 411
*Nuclearia pattersoni* 411, 413
Nucleospora 352, 355, 356, 364-367, 385-387
*Nucleospora salmonis* 386
*Nucleospora secunda* 355, 386, 387
Nutrient uptake 989, 1035, 1037
Nutrition 208, 449, 475, 479, 795, 797, 1032, 1238, 1240, 1242, 1256
*Nycticorax nycticorax* 887

O

*O. masou* 329, 378, 385, 459
*O. nerka* 469
Ocean sunfish 819, 929
Ochratoxin A 1219
*Ochroconis* 327-330, 333
*Octomitus* 440, 443, 449
*Octomitus salmonis* 443, 449
*Odontesthes bonariensis* 879
Office International des Epizooties 185
OIE 94, 95, 97, 117, 762, 792
OJ (Official Journal of the European Communities) 1257
Oligosaccharides 1215
Olive 460, 778
Olive flounder 460, 598, 778, 1248
*Ommatokoita* 1158, 1162, 1163
*Ommatokoita elongata* 1158, 1163
Oncomiracidia 692, 693, 697, 698, 700, 706, 708, 711, 715, 724, 725, 735, 738, 743, 748, 751, 758, 761, 768, 769, 774, 779, 785, 793, 795
Oncomiracidium 688, 689, 692, 698-700, 754, 758
*Oncorhynchus* 44, 51, 56, 66, 68, 89, 100, 242, 245, 248, 250, 255, 263, 299, 327, 328, 332, 360, 449, 450, 457, 459, 464, 465, 467, 469, 484, 618, 626, 633, 641, 714, 715, 728, 763, 836, 855, 870, 884, 924, 987, 1014, 1070, 1080-1083, 1085-1087, 1134, 1135, 1160, 1173, 1203
*Oncorhynchus clarkii clarkii* 924
*Oncorhynchus keta* 242, 245, 457, 459, 1203
*Oncorhynchus kisutch* 242, 245, 250, 402, 464, 467, 469, 836, 1083, 1134, 1135
*Oncorhynchus mykiss* 89, 100, 332, 360, 361, 366, 370, 375, 378, 398, 401, 410, 448, 449, 459, 484, 618, 626, 714, 715, 728, 763, 870, 884, 1070, 1081-1083, 1085-1087, 1173, 1203

*Oncorhynchus tshawytscha* 44, 242, 327, 328, 365, 386, 445, 447, 450, 451, 855, 987,
*Onus mustelus* 902
Oocysts 520, 522, 523, 527-530, 532-537, 539-543, 547, 549, 551, 552
Oodinaceae 425
*Oodinium cyprinodontum* 437
*Oodinium limneticum* 434
*Oodinium pillularis* 434, 435
*Oodinium* sp 431
Oogonium 291
Oomycetes 280, 281, 289, 290, 322, 335
Oospheres 291
*Opecoeloides fimbriatus* 940
*Opecoelus sphaericus* 839
*Opisthorchis parasiluri* 839
*Opisthorchis viverrini* 941
Oral treatments 731
Oral vaccines 1264
Orange spotted sunfish 892
Orange-fin labeo 882
*Oreochromis* 105, 192, 378, 411, 428, 432, 438, 458, 529, 744, 888, 889, 896, 1166
*Oreochromis aurea* 438, 529, 896
*Oreochromis aureus* 438
*Oreochromis mossambicus* 192, 428, 432, 477, 744, 889, 1166
*Oreochromis nilotica* 896
*Oreochromis* spp. 888, 896
Orfe 89, 100, 119
Organomettalic complexes 1059
*Oriental weatherfish* 132
*Ornithodiplostomum ptychocheilus* 860, 869, 873
Orthomyxoviridae 62, 154
Orthomyxovirus 60, 61
Osphronaemidae 436
*Osteodidymocodium kawakawa* 933
*Osteophilus hasselti* 1157
Oviparity 688
*Ovipleistophora mirandellae* 352, 353, 361, 371, 372
*Ovipleistophora ovariae* 354, 359, 366, 372
*Oxyelotris marmoratus* 1157
Oxygen content 1258
Oxygen demand (BOD2) 1258

P

*P. aestuarina* 399
*P. axelrodi* 450
*P. elongatus* 476
*P. mesopotamicus* 436
*P. pillulare* 434-436
*P. sphenops* 458
Pacific herring 44, 47, 212, 245
*Paecilomyces* 333
Palaeacanthocephala 1030, 1035, 1040, 1041
pan-kDNA 494
*Pancreatonema americanum* 1083
Pandaridae 1117, 1121, 1163
*Pandarus bicolor* 1119, 1163
Pantothenic acid 1210
*Parabodo* 424, 454, 463, 479
Parabodonida 424, 454, 463, 479
*Parabrachiella* 1162
*Parabucephalopsis* spp. 907
*Paracardicoloides yamagutii* 913
*Paracheirodon innesi* 450
*Parachromis managuensis* 487
*Paradeontacylix* 823, 913, 923, 924, 928
*Paradeontacylix grandispinus* 913, 923, 928
*Paradeontacylix kampachi* 913, 928
*Paragonomus* spp. 930
*Parahemiurus merus* 834
Paralichthydae 196, 433, 461
Paralichthys 44, 66, 196, 209, 245, 433, 459, 462, 474, 557, 598, 647, 701, 716, 778, 780, 782, 783, 793, 1248
*Paralichthys dentatus* 66, 433, 474, 782, 793

*Paralichthys dentostigma* 474
*Paralichthys lethostigma* 66, 936
*Paralichthys olivaceus* 44, 196, 245, 459, 462, 598, 647, 743, 747, 750, 778, 780, 783
*Paralichthys orbygnyanus* 433
Paramoeba 399
*Paramoeba pemaquidensis* 399
Paramyxoviridae 154
*Parasanguinicola vastispina* 922, 928
Parasite escape hypothesis 1052
Paratenic hosts 359, 531, 532, 537, 826, 834, 844, 930, 932, 1025, 1031, 1039, 1040
*Paratrichodina* 576, 588
*Paravortex* 819
*Parvicapsula* 629, 630, 649
*Parvicapsula minibicornis* 629
*Parvicapsula pseudobranchicola* 630
PAS reaction 364
Passive vectors 206
Pasteurellosis 259
Pathogenesis 197, 206, 247, 404, 553, 987
Pathogens 25, 687, 708, 718, 726, 732, 733, 791, 823, 834
*Paurorhynchus hiodontis* 821
PCR 48, 49, 59, 61, 64, 68, 71, 134, 136, 137, 146, 148, 160, 191, 192, 199, 200, 201, 210, 214, 215, 403, 410, 427, 439, 492, 652, 1079
PCR diagnostic 492
PCR-RFLP 1079
*Pearsonellum corventum* 915
*Pecten fumatus* 944
*Pelecanus erythrorhynchos* 849, 875
*Pelecanus* spp. 886
*Pelichnibothrium speciosum* 818
*Pelteobagrus fulvidraco* 906
*Pelvicachromis teniatus* 436
Penaeid shrimp 88, 940
*Penaeus* 88
*Pennella balenopterae* 1146
*Pennella exoceti* 1146
*Pennella filosa* 1120, 1146
*Pennella instructa* 1146
Pennellidae 1117, 1122, 1124, 1126, 1128, 1145
Peracetic acid 458
*Perca flavescens* 370, 699, 838
*Perca fluviatilis* 56, 66, 370, 410, 411, 413, 459, 484, 557, 643, 904, 1054, 1059, 1074, 1156, 1161
Perch(s) (*Perca fluviatilis*) 44, 56-58, 60, 66, 105, 149, 203, 209, 213, 246, 255, 259, 337, 375, 410, 458, 459, 530, 557, 585, 643, 699, 700, 904, 905, 985, 1033, 1041, 1054, 1056, 1059, 1074, 1081, 1091, 1161, 1241
Perciform 488, 1091
Perciformes 193-195, 430, 431, 436, 459, 461
*Percopsis omiscomaycus* 879
Perforating acanthocephalan 1037, 1046-1049, 1050
Perforating archiacanthocephalans 1049
Perforating species 1031, 1039-1042, 1044, 1045, 1058
Perforatorium 527, 579
Pericyte 627
Peritrichs 571, 583, 585, 586, 588
*Perkinsiella* 400, 403, 414, 422, 424, 460
Perkinsiella amoebae 400, 403, 414
*Perkinsiella amoebae* Hollande, 1980 414
*Perkinsiella amoebae*-like organism 400, 414
*Perkinsiella* cf. *amoebae* 460
*Pfiesteria psicicida* 314, 439
PGD 401, 634
pH 142, 519, 709, 727, 793, 852, 857, 1002, 1236, 1258
PFRV 88, 92, 157, 158
*Phagicola* 836, 837, 890, 892, 897
*Phagicola diminuta* 836
*Phagicola longus* 939
*Phagicola nana* 837

*Phalacrocorax* 869
Pharyngodonidae 1065
*Philasterides dicentrarchi* 598
Philichthyidae 1116, 1121, 1147
*Philometra* 1063, 1069, 1070, 1074, 1081, 1084, 1088, 1089, 1092
*Philometra cephalus* 1084
*Philometra lateolabracis* 1089
*Philometra lusiana* 1084, 1085
*Philometra obturans* 1070, 1088, 1092
*Philometra rubra* 1084
*Philometra saltatrix* 1088, 1092
*Philometridae* 1084, 1090
*Philometroides cyprini* 1067
*Philometroides fulvidraconi* 1090
*Philometroides huronensis* 1081
*Philometroides lusiana* 1085
*Philometroides oken* 1081
*Philonema agubernaculum* 1080, 1084, 1090
*Philonema americana* 1081
*Philonema oncorhynchi* 1079, 1083, 1090
*Philonema opsalichthydis* 1083
*Philonema rubra* 1083, 1084
*Philonema saltatrix* 1084
*Phlyctainophora lamnae* 1081
*Phoma herbarum* 324-326
phosphatidylinositol 485, 494
Phosphorous 1206
*Photobacterium damselae* ssp. *piscicida* 259
*Phoxinus phoxinus* 66, 144, 471, 877
*Phrixocephalus cincinnatus* 1145
*Phyllodistomum staffordi* 830
Phylogenetic 63, 187, 255, 351, 367, 409-411, 413, 414, 421, 438, 439, 453, 454, 463, 479, 480, 489-491, 532, 537, 539, 541, 545, 600, 602, 614, 619, 625, 796, 822, 826, 828, 831, 832, 910, 938, 1096
Phylogeny(-ies) 388, 398, 422, 424, 453, 479, 488, 489, 491, 520, 547, 655, 848
Phylum Nematoda 1063
Phylum Platyhelminthes 683, 978
*Physa anatina* 1084
*Physella gyrina* 869
Phytate 1208, 1215, 1216
Phytic acid 1216
*Phytomonas* 480
*Piaractus mesopotamicus* 436, 456, 459, 477, 643
Picornaviridae 155
Pigment 15, 21, 37, 38, 240, 244, 324, 425, 547, 549, 552, 554, 644, 684, 688, 695, 725, 737, 745, 774, 775, 828, 829, 835, 837, 876, 885, 895, 896, 915, 916, 918, 1007, 1139, 1154, 1205, 1217
Pike 41, 42, 44, 50, 54, 66, 88, 89, 99, 102, 149, 152, 155, 159, 203, 255, 375, 530, 625, 626, 643, 644, 649, 791, 838, 882, 893, 898, 1004, 1012, 1013, 1066, 1070, 1130, 1133, 1135, 1137, 1155, 1156
Pike fry rhabdovirus 88
*Pimephales promelas* 105, 372, 522, 643, 860, 1088
Pinhead 317, 542, 590, 1229
Pink salmon 1132
Pipefish 31
Piperazine 1095, 1096
Piping 717
Piranha 1169, 1174
*Piscicola geometra* 99, 470, 483
*Piscicola salmositica* 464
*Piscinoodinium* 422, 425, 434-437, 439
*Piscinoodinium pillulare* 434, 436, 437
*Piscirickettsia salmonis* 261, 262
Piscirickettsiosis 261
PKX 618
*Planorbella trivolvis* 869, 871, 875, 882, 887
Plasma cells 32, 33, 628, 906, 991, 1005, 1008, 1046, 1085
Plasmotomy 384, 615, 627
*Platichthys flesus* 44, 206, 243, 245, 913
Platinum group metals 1058

*Platyamoeba* 407
Platyamoeba Page, 1969 409
*Platycephalus fuscus* 933
Platyfish 458
Platyhelminthes 27, 683, 817, 825
*Plecoglossus altivelis altivelis* 257, 263, 326, 352, 375, 889, 1157
*Plectropomus leopardus* 915
*Pleistophora ehrenbaumi* 368
*Pleistophora hippoglossoides* 368
*Pleistophora hyphessobryconis* 359, 363
*Pleistophora macrozoarcidis* 362, 363, 369
*Pleistophora typicalis* 368, 371
Pleomorphic species 485, 488
Pleomorphism 481, 485
Plerocercus 983
*Plethorchis acanthus* 916, 919
*Pleuronectes platessa* 44, 245, 248, 598, 621, 896, 911
Pleuronectiforme 195, 196, 430, 433, 459, 461, 488
*Plumatella emarginata* 625
*Poecilia latipinna* 892
*Poecilia reticulata* 192, 196, 437, 458, 539, 859
Poecilids 458
*Poeciliopsis monacha* 883
*Pogonias cromis* 915
Poikilothermic 211, 531, 1255
*Polachius virens* 241, 250, 450
Poland 98, 115, 127, 483, 485, 905, 1115, 1152, 1156, 1162
Pollution 208, 706, 857, 1007, 1014, 1015, 1029, 1053-1056, 1058, 1091
Polyacanthocephala 1030
Polychlorinated biphenyls (PCBs) 1219
Polyclonal antibodies 46, 466, 651
*Polydactylus sexfilis* 432
Polykinetoplastic 424, 455
Polymorphic 63, 302, 316, 489, 491
Polymorphism 63, 303, 407, 473, 1079
Polymorphonuclear cells 31, 876
Polynaemidae 432
Polyonchoinea 688
Polyopisthocotylea 684, 685, 687-691, 693-698, 700-702, 704, 705, 713-715, 718, 724, 727-733, 738, 778, 793-794
Polyopisthocotyleans 688, 692-695, 705, 778, 781, 784
Polysporoplasma 615, 645
*Polysporoplasma sparis* 645
Polyunsaturated fatty acid 1218, 1238
*Pomatoschistus minutus* 902
*Pomphorhynchus laevis* 1027, 1029-1031, 1037, 1039, 1050, 1051, 1054, 1055, 1057-1059
Pond hygiene 1258
*Porpostoma notatum* 598
Portion fish 1260
Post-cyclic transmission 1025, 1039, 1040
*Posthodiplostomum centrarchi* 869, 880
*Posthodiplostomum cuticola* 861, 881
*Posthodiplostomum minimum* 837
Potassium 290, 435, 581, 852, 1206, 1258
Potassium permanganate 435, 852, 1258
Potassium permanganate demand ($KMnO_4$) 1258
Praniza 1177, 1179
Praziquantel 729, 739, 1011
Preadult 1126, 1131, 1134-1136, 1138, 1139
Predation 1231
Prevention 50, 322, 544, 716, 866, 1011, 1094, 1095, 1263
*Priacanthus arenatus* 931
Prionace glauca 992, 1000, 1002, 1164
Probiotic bacteria 265
Probiotics 265, 266
Proboscis 456, 470, 481, 845, 1026-1028, 1030, 1032-1034, 1036-1038, 1041, 1042, 1044, 1046-1050, 1165, 1166, 1177

*Procamallanus spiculogubernaculus* 1085
Procercoid 983
*Procerovum cheni* 899
*Procerovum varium* 941
Prochilodontidae 477
*Prochilodus lineatus* 477, 1086
*Procryptobia* 424
*Procyon lotor* 892
Proglottids 979, 985
*Prohemistomulum* 867
Prokinetoplastida 454
Prokinetoplastina 422, 424, 454
*Proleptus obtusus* 1087, 1088
Proliferative gill disease 634
Proliferative kidney disease 624
Promastigotes 481
*Propyxidium* 574, 586
*Prosopium williamsoni* 467
*Prosorhynchus* 900, 902
*Prosorhynchus crucibulum* 900, 902
*Protacanthamoeba bohemica* 408, 411
Protease inhibitor 1212, 1213
Protein 7, 9, 41, 63, 187, 202, 244, 320, 491, 493, 534, 761, 827, 880, 939, 987, 1009, 1202, 1203, 1206, 1210-1215, 1228
Protein requirements 1202
Proteinase 494, 1037
*Proteocephalus* 881
Protomont 580, 591-593
*Psetta maxima* 399, 400, 404, 405, 407, 414, 433, 902
*Psettarium* 823, 928
*Pseudanisakis rotundata* 1087
*Pseudodactylogyrus* 793, 1053
*Pseudodelphis* 1092
*Pseudodelphis limnicola* 1092
*Pseudoergasilus zacconis* 1157
*Pseudoloma neurophilia* 381
*Pseudoparamoeba* sp. 407
*Pseudoplasmodia* 615
*Pseudopleuronectes americanus* 250, 360, 375, 474
*Pseudorasbora parva* 100, 132
*Pseudoterranova* 1066, 1068, 1072, 1075, 1077-1079, 1081, 1090
*Pseudoterranova decipiens* 1066, 1072, 1078, 1081, 1090
*Pseudotropheus zebra* 476
Pseudotuberculosis 259
*Psorospermium* 280
*Pterophyllum scalare* 369, 445, 450
*Ptychocheilus oregonensis* 893
*Ptychogonimus megastomus* 839
Pullus 1177
*Puntius gonionotus* 435-437
*Pygidiopsis* spp. 890
*Pygidiopsis summa* 898, 941
Pyknosis 7, 46, 190
Pyridoxine 1210

Q

Quarternary ammonia compounds 1246

R

Rachycentridae 195, 432, 749, 917
*Rachycentron canadum* 195, 214, 432, 749, 917
Rainbow trout 42-44, 47, 48, 56-58, 60, 61, 63, 65, 66, 68-71, 88, 89, 100, 102, 145, 146, 149, 150, 154, 158, 218, 241, 242, 245, 248, 250, 255, 257, 262, 263, 298, 299, 309, 310, 314, 325, 326, 329, 332, 336, 378, 401, 410, 444, 447-449, 451, 458, 463, 465, 466, 585, 618, 623-626, 628, 654, 715, 728, 763, 765, 870, 872, 873, 878, 879, 883, 884, 893, 895, 1007, 1038-1050, 1052, 1070, 1073, 1074, 1089, 1090, 1160, 1173, 1175, 1219, 1226, 1234, 1236
Rainbow trout fry syndrome 1239
Rajiformes 433

*Rallus longirostris* 892
*Rana clamitans* 876
*Rana pipiens* 876
*Ranavirus* 55-57, 202-204, 213
Random amplification of polymorphic DNA 302, 316, 489
RAPD 302, 303, 306, 489
Rapeseed meal 1217
Rapeseed oil 1217
*Raphidascaris acus* 1066, 1072, 1073, 1075, 1083, 1087, 1090, 1091
Rare minnow 132, 133, 135
Recombinant cystein protease 467
Recombinant vaccine 202, 492
Red drum 209, 438, 915, 916, 1211
Red grouper 648
Red pest 247
Red seabream iridovirus disease (RSIVD) 213
Red snapper 646, 850, 918
Red spot disease 282, 311
Red spotted grouper nervous necrosis virus-type (RGNNV) 187
Redbelly tilapia 889
Reddish egret 892
Redear sunfish 892
Redsore disease 247
Regeneration 19, 362, 1001, 1238
Regulation of wild fish populations 1028
*Renibacterium salmoninarum* 263
*Renicola buchanani* 896
Repair 11, 15, 19, 29, 362, 570, 736, 991, 1001
Reproduction 280, 284, 287, 291, 300, 302, 307, 479, 491, 570, 601, 792, 1050, 1217
Reproductive success 1028, 1051
Required host 244, 291, 299, 447, 458, 614, 618, 644, 655, 656, 702, 753-756, 769, 782, 788, 920, 1031, 1058, 1059, 1072, 1206, 1208, 1210, 1217
Resistance 191, 308, 438, 469, 473, 485, 581, 629, 712, 713, 724, 729, 783, 791, 792, 794, 795, 1007, 1009, 1013, 1015, 1016, 1227, 1255, 1256
Resistant 139, 290, 322, 438, 468, 469, 473, 623, 624, 628, 636, 654, 713, 753, 782, 795, 1015, 1016, 1095
Respiratory distress syndrome 1235
Responder 712, 713
Restocking 264, 411, 1260
Retinol 1210
Reverse transcriptase-polymerase chain reaction 199
Rhabdochonidae 1065
Rhabdoviridae 155
*Rhinichthys cataractae* 467
*Rhipidocotyle* 835, 899, 903, 906, 907
*Rhipidocotyle fennica* 903, 907
*Rhipidocotyle johnstonei* 906
*Rhipidocotyle* spp. 906
Rhizocysts 434
Rhizoids 425-427
*Rhopalotrema elusive* 931
*Rhopalotrema pterygionastes* 931
*Rhynchobodo* 424
Riboflavin 1210
*Riboscyphidia* 574, 586
Ribosomal gene 427, 438
*Rickettsia* 27, 413
*Rickettsia* sp. 413
RNA editing 480, 494, 495
Roach 1, 4, 89, 99-102, 126, 127, 137, 144, 145, 149, 200, 255, 295, 301-303, 337, 366, 411, 415, 421, 422, 427, 492, 493, 531, 600, 601, 622, 630, 632, 639, 642, 653, 654, 656, 690, 691, 725, 791, 792, 796, 881, 904, 907, 943, 987, 988, 1009, 1015, 1066, 1083, 1128, 1155, 1175
*Roccus saxatilis* 880
Rockling 902
*Rosculus ithacus* 401
Rosette agent 280
*Rossicotrema donicum* 893

Round sardinella 923
rRNA gene 386, 388, 403, 409-411, 414, 422, 424, 439, 449, 452-454, 463, 479, 488, 489, 491, 600, 601, 1078
RTG-2 47, 136, 142, 145, 149-152, 155, 157, 158, 160, 218
Rudd 119, 144, 878, 881, 884
Ruffe 885
Rumania 98
Russian Federation 98, 763
*Rutilus rutilus* 89, 100, 119, 144, 255, 295, 411, 413, 471, 483, 632, 642, 881, 987, 1083, 1155
Rutilus spp. 885

S

*S. aequifasciatus* 476
*S. aurata* 477, 646
*S. barkhanus* 443, 445, 447, 449-453
*S. diclina* 299, 301-304
*S. elegans* 443
*S. limax* 411
*S. parasitica* 294, 299-304, 306, 308, 317, 322
*S. salar* 411, 445, 631, 633, 710, 712
*S. salmonis* 443, 447, 449
*S. torosa* 443, 450, 451
*S. vortens* 443, 445, 447, 449-451, 453
Sac 1228
*Saccamoeba* spp. 411
*Saccobranchus (=Heteropneustes) fossilis* 483
*Saccocoelioides sogandaresi* 830
Saddleback disease 258
Sailfin molly 892
Salamanders 57, 1164
Salivaria 485, 493, 494
*Salmincola californiensis* 1157
*Salmincola carpionis* 1161
*Salmincola edwardsii* 1160
*Salmincola salmoneus* 1160
*Salmincola stellatus* 1161
Salmo salar 44, 51, 56, 60, 66, 69, 242, 245, 247, 248, 250, 253, 256, 330, 331, 399, 400, 405, 407, 411, 445, 447, 449, 459, 462, 469, 630, 690, 703, 710, 762, 1084, 1135, 1160, 1225
*Salmo trutta* 100, 378, 449, 458, 459, 467, 622, 631, 987, 1005
*Salmo trutta trutta* 870
Salmon louse 1130
*Salmon pancreas disease* 69
*Salmonicida* 27, 55, 239-246, 252, 253, 443, 449
Salmonid(s) 42, 51-53, 55, 65, 68, 70, 241-243, 246, 250, 251, 255, 257, 258, 262, 264, 282, 287, 288, 294, 295, 298, 299, 302, 304, 308, 322-324, 326, 399, 401, 402, 445, 451, 457-461, 467, 479, 493, 712, 713, 858, 885, 926, 942, 1052, 1225, 1226, 1232
Salmonid cryptobiosis 493
Salmonidae 462, 714, 715, 728, 917
Salmoniformes 459, 462
*Salvelinus alpinus* 61, 66, 245, 250, 444, 446, 450-453, 625, 1010, 1204
*Salvelinus fontinalis* 44, 66, 242, 299, 378, 398, 410, 449, 459, 465, 469, 548, 623, 763, 860, 925, 1160
*Salvelinus leucomaenis* 242, 1161
*Salvelinus namaycush* 44, 66, 242, 449, 1084
Samorin 466
Sand goby 902
Sand grain grub 890
Sandbar shark 13, 638
*Sander lucioperca* 530, 901, 904, 1153, 1160, 1161
*Sanguinicola* 858, 910, 913, 924-926
*Sanguinicola alsae* 926
*Sanguinicola davisi* 926
*Sanguinicola fontinalis* 925
*Sanguinicola idahoensis* 926
*Sanguinicola inermis* 910, 913, 925
*Sanguinicola klamathensis* 924

*Sanguinicola lophophora* 924
Sanitary function 1059
*Santee-cooper ranavirus* 57, 203
Saponin(s) 1215, 1216
*Saprolegnia* 280-289, 291-295, 298-310, 316, 320, 322, 323, 1228
*Saprolegnia parasitica* 281-285, 288, 289, 293, 295, 300, 302, 304-308, 322, 323
Saprolegniaceae 281, 284, 291, 300
Saprolegniales 281, 282, 284, 294
Saprolegniosis 295
*Sarcotaces* 1119, 1121, 1130, 1147
*Sarcotaces arcticus* 1147
*Sarcotaces namibiensis* 1119
*Sarcotretes scopeli* 1145
*Sardinella aurita* 923
*Scaphanocephalus* 840
*Scardinius erythrophthalmus* 119, 144, 463, 471, 878
*Scarthychthyis viridis* 474, 475
*Schizamoeba salmonis* 398
Schwanenfeld's barb 144
Sciaenidae 432, 917
*Sciaenops ocellatus* 195, 209, 432, 438, 649, 915, 1211
Scolex 979, 1002, 1008, 1009
*Scolex amorphous* 979
Scoliosis 647
*Scomber australasicus* 929
*Scomberomorus maculatus* 931, 935
*Scomberomorus regalis* 931
*Scophthalmus maximus* 399, 462, 554, 645, 647
*Scophthalmus maximus* 190, 196, 197, 462, 902
*Scophthalmus rhombus* 902
*Scorpaenichthys marmoratus* 474
Screamer disease 1241
Sculpins 465, 467
Scuticociliates 595, 596, 598
*Scyphidia* 574, 586
SD 68, 852
Sea bass 187, 189, 193, 197, 438, 477, 478
Seabream 195, 203, 207-210, 213-216, 218, 250, 259, 330, 429, 448, 477, 478, 544, 554, 591, 598, 632, 645, 646, 648, 654, 750, 757, 854, 927, 928
Seabream aquareovirus disease 218
*Seawater acclimation* 1237
*Sebastes marinus* 1146
*Sebastes mentella* 1146
Secnidazole 458
*Selache maxima* 1163
*Selachohemecus benzi* 822
Selenium 1205, 1207-1209
*Semotilus atromaculatus* 869
Senescence 582
Sentinels 938, 1054-1056, 1058
Serbia 98
*Seriola* 66, 195, 209, 213, 250, 256, 385, 430, 431, 647, 689, 704, 707, 710, 716, 726, 728, 731, 732, 734, 736-738, 740, 741, 746, 750, 784-786, 789, 790, 797, 928, 1164
*Seriola dumerili* 195, 430, 431, 710, 913
*Seriola lalandi* 66, 704, 716, 728, 732, 746, 797, 1164
*Seriola purpurescens* 913
Sexual maturity 699, 780, 785, 786, 792, 793
Shaite 450
Shanny 553, 557, 902
Sheatfish 57, 89, 203, 204
Shedding 98, 122, 1010, 1012, 1013
Sheepshead minnow 835, 836, 840, 847, 892, 894
Shortfin eel 913
*Sillago ciliata* 437
Silurids 1168, 1169
Siluriformes 196, 436, 483, 486, 917
Siluroid catfish 487
*Silurus glanis* 57, 89, 410, 413
Silver bream 144
Silver carp 89, 105, 132, 137, 150, 154, 639, 640, 854, 1156
*Sinergasilus lieni* 1156, 1157

*Sinergasilus major* 1156, 1157
Singapore 115, 193, 194, 196, 204, 205, 450, 460, 476
*Sinoichthyonema amuri* 1067
Site souring 1237
SJNNV 187, 188, 199
Skeletal deformation 1048
*Skeletonema costatum* 1244
Skin 8, 11, 13, 15, 21, 23, 25, 29, 31, 43, 45, 46, 51, 53, 57, 59, 61, 66, 87, 89, 101, 106, 111, 119, 121, 124, 126, 146-148, 150, 151, 157, 203, 205-209, 215, 243, 247, 249, 255-258, 261, 263, 282, 283, 287, 289, 295, 296, 298, 299, 308, 317, 322, 327, 329, 359, 360, 368-370, 378, 382, 385, 426-428, 439, 445, 456, 457, 554, 570, 571, 582-584, 586, 588-591, 594, 597, 598, 615, 618, 624, 627, 633, 636, 637, 639, 640, 647, 683, 684, 689, 693, 695, 696, 698, 703-705, 707, 713, 716, 717-720, 725-734, 736, 737, 742, 744, 746, 747, 752, 759, 763-765, 767-769, 771-777, 786, 794, 819, 820, 837, 840, 983, 857, 864, 872, 873, 881, 889, 896, 898, 899, 901, 903, 905, 911, 913, 931, 933, 938, 1015, 1063, 1064, 1074, 1121, 1122, 1131, 1133, 1138-1140, 1242, 1246, 1263, 1265
Skipjack herring 841
Skipjack tuna 933
*Skrjabillanus schigini* 1067
Sleeping disease 68
SLICE 1137
Slovakia 98
Small subunit rRNA gene 410, 479
Smooth flounder 474
Snail carp 854
Snapper 195, 646, 841, 850, 910, 912, 916, 918
Snowy egret 887
Snowy grouper 931, 934
Sockeye salmon 51, 124, 469, 622, 629, 630, 1014, 1203
Sodium 58, 113, 137, 458, 534, 543, 581, 583, 588, 589, 593, 726, 728, 782, 852, 1206, 1215, 1236, 1238, 1246
Sodium chloride 458
*Solea solea* 196, 256, 368, 598, 696, 937
Source of the fish population 1258
South Africa 115
Southern bluefin tuna 597, 850, 920-922, 928
Southern flounder 66, 474, 936
Southern stingray 818
Soybean meal 1212
Spain 69, 98, 145, 155, 194, 303, 402, 421, 428, 431, 461, 485, 597, 598, 645, 762, 786, 790, 927
Spanish mackerel 931, 935
Sparidae 195, 431, 461, 700, 702, 727, 729, 750, 757, 1178
*Sparus* 195, 207-209, 218, 250, 255, 256, 259, 352, 428-431, 448, 461, 477, 532, 538, 554, 591, 598, 632, 645, 757, 927
*Sparus aurata* 195, 207-209, 218, 250, 255, 256, 259, 352, 428-431, 448, 461, 477, 532, 538, 554, 591, 598, 632, 645, 757, 927
Specific antibodies 71, 427, 465, 466, 468, 472, 473, 781, 1008, 1079
Specific cellular immunity 1007
Spermatophore 1121, 1122, 1169, 1171
Sphaeromastigote 481, 482
*Sphaerospora* 621, 631, 633, 635, 641-643, 645, 648, 650, 651
*Sphaerospora epinepheli* 648
*Sphaerospora renicola* 635
*Sphaerospora testicularis* 645
*Sphaerospora tincae* 631, 642
*Sphaerospora truttae* 631
*Sphoeroides annulatus* 430, 433, 649, 778
*Sphoeroides pachygaster* 840
Sphyriidae 1117, 1122, 1128, 1146
*Sphyrion laevigatum* 1146
*Sphyrion lumpi* 1146

*Spicara maena* 923
*Spinitectus* 1068, 1069, 1072, 1086
*Spinitectus carolini* 1086
*Spinitectus Jamundensis* 108
Spironucleosis 440, 443, 445, 446, 450-452
*Spironucleus* 423, 440-446, 448-453, 475
*Spironucleus barkhanus* 444-449, 451, 453
*Spironucleus elegans* 450
*Spironucleus salmonis* 443, 449
*Spironucleus vortens* 444, 449-453
Spirurida 1065, 1068
Spleen 13, 23, 29, 38, 45, 46, 51, 53, 56, 58, 59, 63, 142, 148, 150-152, 154, 155, 158, 159, 203, 204, 208, 213-215, 240, 247, 259, 264, 486, 487, 531, 554, 786, 922, 927, 1009, 1263
Spore structure 354
Sporocyst 520, 523-526, 528, 531, 532, 536-541, 543, 616, 825, 828, 829, 844, 875, 886, 887, 901, 911, 925
Sporogony 354, 356, 357, 369-371, 373, 381, 382, 384, 388, 520, 522, 529, 540, 547, 548, 550, 551, 615, 618, 628, 633, 640
Sporophorous vesicle–SPV 356
Sporoplasm 615, 624
Sporoplasmosomes 615, 617, 627
Sporozoa 517, 520
Sporozoites 520, 522-524, 526, 528-531, 536, 538-541, 543, 544, 547-552
Spotted weakfish 835
*Spraguea lophii* 358, 362, 363, 379, 382
Spray 326, 327, 1264, 1265
Spring viraemia of carp 88, 91
*Squalidus chankaensis chankaensis* 907, 908
Squirrelfish 29
SS rRNA 439, 453, 454, 479
SS rRNA gene 439, 453
SSN-1 188, 199, 200
SSU 351, 365, 367, 409-411, 414, 427, 438, 439, 488, 489, 491, 600, 601
SSU rDNA gene 439
SSU ribosomal gene 427
SSU rRNA 388, 409-411, 414, 449, 452, 463, 488, 489, 491, 600, 601
SSU rRNA genes 409-411, 414, 449, 452, 488, 491
*Stagnicola palustris* 869, 870
*Stagnicola palustris elodes* 870
*Stamping out measures* 1266
*Steatosis* 1206
*Stellantchasmus falcatus* 898
*Stenobrachius leucopsaurus* 1145
*Stephanochasmus baccatus* 937
*Stephanostomum tenue* 830, 854, 936
Stickleback 44, 373, 374, 467, 860, 870, 873, 876, 877, 883, 884, 987, 990, 1007, 1014, 1051, 1157, 1176
*Stictodora* 833, 898, 941
*Stictodora lari* 898
*Stictodora tridactyla* 833
Stomopode 426, 428, 434, 437
Stone loach 877, 883, 884, 1083
Strategic 739, 755, 761, 788, 791
Stress 17, 29, 48, 50, 55, 89, 99, 127, 198, 208, 243, 246, 256, 258, 261, 295, 299, 302, 308, 335, 352, 359, 375, 384, 440, 444, 570, 579, 583, 584, 589, 590, 594, 597, 653, 699, 706, 708, 709, 712, 713, 719, 723, 724, 726, 727, 731, 776, 777, 792-795, 797, 844, 856, 859, 893, 894, 921, 940, 1007, 1011, 1013, 1014, 1016, 1054, 1091, 1095, 1096, 1133, 1135, 1140, 1231, 1235, 1238, 1240, 1246, 1256, 1267
Stress hormone 1054
Striped bass 137, 140, 259, 260, 438, 855, 880, 1158, 1204
Striped jack nervous necrosis virus-type 187
Striped marlin 933
Strobila 978, 1006, 1008, 1013
*Stylirostris* 88

Sucker 66, 467, 548, 734, 739, 1121, 1164-1166, 1171
Sudden death syndrome 1238
Suitable host 570, 579, 1032
Sulfadimethoxine 475, 476
Sulfur 1204, 1206
Summer flounders 66, 473, 474, 782
Sunfish 819, 860, 863, 869-883, 887, 892, 929, 1032, 1045, 1051, 1146
Superchill disease 1241
Suramin 493
Susceptibility 45, 47, 49, 55, 63, 124, 126, 136, 137, 435, 438, 467, 469, 473, 493, 623, 628, 1005, 1012, 1014, 1133, 1140, 1173, 1238
Susceptible 42, 45, 48, 53-56, 58, 64, 66, 87, 88, 93, 97, 98, 100, 109, 119, 122, 126, 188, 197, 199, 205, 207, 208, 212, 213, 215, 465, 473, 538, 580, 594, 623-625, 628, 630, 634, 636, 639, 640, 642, 644, 646, 653, 713, 741, 747, 769, 791, 793, 921, 927, 1014, 1052, 1053, 1091
SVC 88, 89, 155, 157, 158, 160
SVCV 88-90, 92-104, 155, 157, 158, 160
Swimbladder dilation syndro2me 1236
Swimbladder inflammation 90, 635, 1079
Switzerland 98, 114, 115, 627
Swordfish 648, 1146
*Symphysodon* 66, 1085, 1096
*Symphysodon discus* 66, 445, 450, 476
*Syncoelium* 834, 839
*Syncoelium ragazzii* 839
Syndermata 1030
*Syngnathinema californiense* 1092
Synonymy 443, 463, 464, 479, 480, 491, 782

T

*T-2 toxin* 1219
*T. borreli* 470, 471-473, 492, 494
*T. brucei* 484, 489
T. bullocki 473, 474
*T. carassii* 469, 482-485, 489, 492, 494
*T. cruzi* 485, 494, 495
*T. danilewski* 482
*T. granulosum* 485, 486, 489, 491
*T. hilla* 409
*T. keysselitzi* 469
*T. markevitschi* 486
*T. mukasai* 486, 489
*T. mukundi* 487
*T. murmanense* 487, 488
*T. ovolyticum* 257
*T. punctati* 486
*T. tincae* 469, 482
*T. trichogasteri* 486
*T. wincheniense* 482
Tadpoles 5, 579, 589, 870, 876, 877, 1164
Taimen 1161
Taiwan 115, 116, 131, 135, 145, 193-196, 203-205, 212, 214, 431-433, 487, 622, 646, 651, 749, 889, 899, 941, 1090, 1141, 1157
*Takifugu rubripes* 196, 433, 646, 696, 714, 715, 724, 732, 750, 784, 927
Tapeworms 817, 844, 978-980, 982, 983, 985, 988, 989, 993-995, 997-999, 1002, 1006, 1010-1013, 1035, 1056
Target cell for microsporidia 357
*Taurulus bubalis* 902
*Tegument* 982
Telangiectasia 27
*Telolicithus pugetensis* 830
Telotrochs 586
*Tenacibaculum maritimum* 255, 256
Tench 88, 89, 92, 100, 102, 105, 119, 140, 149, 150, 217, 459, 470, 471, 642, 877, 904, 1154-1156
Tench rhabdovirus 88, 92, 100
Tentacles (T) 585, 819, 828, 845, 899, 979-982, 991, 992, 995, 996
Tepozán 458

TenRV 88, 92, 100, 157
*Tetracapsuloides bryosalmonae* 613, 624, 626
*Tetracotyle* 867, 883, 885
*Tetracotyle intermedia* 885
*Tetrahymena* 572, 578, 584, 585, 602
Tetrahymenids 584, 585
*Tetramicra brevifilum* 357, 363, 379, 382, 383
Tetraodontidae 430, 433, 696, 714, 715, 725, 732, 750, 778, 784
Tetraodontiformes 196, 430, 433, 459
*Tetrapturus audax* 933
Thailand 105, 115, 193, 194, 205, 214, 401, 432, 648, 941, 942
*Thaleichthys pacificus* 44, 1145
The Netherlands 102, 103, 114, 115, 1053
Theca 287, 425, 426, 429, 434, 621, 646, 647, 1032, 1051, 1165, 1166, 1169, 1171
*Thecamoeba hilla* 408, 414
*Thecamoeba hilla* schaeffer, 1926 414
*Thecamoeba hoffmani* 399, 401
*Thecamoeba* sp. 409
*Thelohanellus* 636, 637, 640
*Theohanellus hovorkai* 636
*Thelohanellus nikolskii* 636
*Theragra chalcogramma* 44, 1144, 1162
Therapeutants 335, 654, 655, 729, 760, 795, 1134, 1135, 1150
Therapy 334, 1263
Theront 579, 580, 582, 591-594
*Thersitina gasterostei* 1157
Thiamin 1210, 1218
Thiaminase 1218
Thinlip mullet 838, 897
*Thraustotheca* 287
Three-spined sticklebacks 860, 870, 876, 987
Thrombus 11, 23, 27
*Thunnus albacores* 818, 931, 935
*Thunnus maccoyii* 597, 920, 928
*Thunnus thynnus* 920
*Thymallus arcticus* 327, 873
*Thymallus thymallus* 44, 66, 263, 449, 451, 631, 690, 763, 1031, 1040
*Thysanote* 1162
Tiger catfish 1169
Tiger puffer 187, 646, 696, 750, 778, 779, 784, 927
Tiger puffer nervous necrosis virus-type 187
Tilapia 105, 130, 192, 262, 322, 333, 378, 428, 458, 476, 478, 576, 586, 725, 744, 747, 749, 750, 855, 888, 889, 896, 1089, 1141, 1219
*Tilapia zilli* 643, 889
*Tinca tinca* (Cypriniformes) 89, 100, 119, 411, 471, 483, 642, 877, 1154-1156
Tissue reaction to microsporidia 361-363
TNFα 472, 492
TNP-470 365, 654
Toadfish 7
Togaviridae 69
Toltrazuril 654
Tomites 434, 578-580, 584, 592
Tomont(s) 426, 427, 430, 434, 578-580, 591-593
Topmouth gudgeon 132
*Tormopsolus orientalis* 839
Torrent sculpin 467
TPNNV 187, 188
*Tracheliastes maculatus* 1124, 1128, 1158
*Tracheliastes polycolpus* 1162
*Trachinotus carolinus* 841
*Trachurus mediterraneus* 923
Translation initiation factor-2 452
Transmission 45, 58, 64, 65, 69, 70, 98, 99, 102, 103, 105, 116, 123, 147, 150, 186, 191, 192, 198, 201, 206, 207, 209, 211, 213, 219, 352, 359, 360, 365, 381, 400, 403, 413, 415, 426, 440, 443, 464, 467, 471, 476, 487, 488, 490, 491, 518, 530-532, 541, 547, 548, 552, 555, 618, 631,

640, 646, 699, 706, 826, 839, 1015, 1025, 1039, 1040, 1067, 1072, 1073, 1075, 1093, 1140, 1173, 1227, 1256
Transport cell for microsporidia 358
Transport of live fish 1262
Trans-sialidase-like 489
*Transversotrema* 826, 830, 839, 938
*Transversotrema haasi* 839
*Transversotrema laruei* 830
Treatment and prophylaxis 427, 435, 653, 1011
*Trepomonas* 423
*Triactinomyxon* 616, 620
*Triangula percae* 644
Tricaine 457
*Trichiurus lepturus* 841
Trichlorfon 1135, 1150
Trichlorphon 729
*Trichodina* 107, 574, 576, 587-589, 602
*Trichodinella* 576, 588
*Trichodinids* 572, 583, 588, 589, 600
Trichosomoididae 1093
Triclabendazole 458
Tricothecenes (T-2 toxin) 1219
*Trifur tortuosus* 1146
Triglycerides 1204
*Trinectes maculatus* 474
*Tripartiella* 576, 588
Trophont(s) 425-429, 578-581, 590-594
Trophozoite 403, 413, 629, 633
Trout 7, 11, 15, 27, 35, 42-44, 47, 48, 50, 51, 56-58, 60-63, 65-71, 88, 89, 100, 102, 145, 146, 149-151, 154, 158, 212, 218, 241, 242, 245, 248, 250, 255, 257, 262, 263, 297-299, 303, 305, 306, 309, 310, 314, 325, 326, 329, 332, 336, 378, 401, 410, 435, 444, 447-449, 451, 458, 463, 465, 466, 473, 544, 548, 585, 618, 622-626, 628, 631-633, 654, 656, 763, 765, 884, 924-926, 936, 987, 1005, 1007, 1009, 1012, 1038, 1047-1050, 1052, 1066, 1067, 1070, 1073, 1074, 1089, 1090, 1094, 1160, 1161, 1169, 1173, 1175, 1203, 1204, 1207, 1208, 1212, 1214, 1216-1219, 1226, 1232, 1234, 1236, 1239, 1242, 1247, 1260, 1267
Trout-perch 879
*Truttaedacnitis* 1066
*Truttaedacnitis pybusae* 1073, 1082
*Truttaedacnitis truttae* 1067, 1069, 1070, 1086, 1090, 1094
Tryanoplasma 463
Trypanocidal drugs 493
*Trypanoplasma* 424, 463, 464, 469, 470, 473, 474, 479, 480, 490-494
*Trypanoplasma beckeri* 474
*Trypanoplasma borreli* 469, 470, 479, 480, 491-494
*Trypanoplasma bullocki* 473
*Trypanoplasma cyprini* 469
*Trypanoplasma lynchi* 464
Trypanoplasms 470
*Trypanosoma* 424, 454, 480-482, 485-492, 495
*Trypanosoma brucei* 495
*Trypanosoma carassii* 482, 491, 492
*Trypanosoma granulosum* 485
*Trypanosoma mukasai* 486
*Trypanosoma murmanense* 481, 482, 487
Trypanosomatid 480, 489, 491, 494
Trypanosomatida 424, 454, 480
Trypanosomatidae 480
Trypanosomatids 480, 489, 494
Trypanosomatina 480
Trypanosomes 480, 481, 485-491, 493, 494
Trypanosomosis 482, 486, 487
Trypomastigotes 481, 483
Trypsin inhibitor 1212, 1213, 1215
Tryptophan deficiency 1203
Turbot 42, 47, 57, 91, 240, 241, 248, 255, 256, 399, 400, 402, 404-407, 409, 414, 460, 554, 558, 597, 598, 645, 647, 654, 902, 937

Turbot aquareovirus disease 216
Turbot iridovirus disease 215
Turkey 127, 128, 209, 431
*Tylodelphys calvata* 862
*Tylodelphys destructor* 879

U
Ukraine 93, 98
Ulcer disease 243
Ulcerative dermal necrosis 295
Ulcerative mycosis 282, 311, 318
*Unikaryon* 388
United Kingdom (UK) 65, 104, 115, 127, 131, 145, 193, 196, 336, 618
*Unitubulotestis* 936
Unsuitable host 720, 721, 756, 1031, 1043, 1152, 1261
Uptake of nutrients 1037, 1215
*Uronema* 27, 572, 595, 597, 598, 602
*Uronema marinum* 595, 597, 598
*Urophycis capensis* 1147
USA 69, 93, 95, 96, 98, 99, 115, 128, 129, 131, 132, 134, 135, 139, 141-143, 152, 155, 157, 159, 160, 193, 195, 256, 259, 372, 375, 433, 477, 555, 734, 749, 783, 784, 860, 861, 1151
UV irradiation 544
*Uvulifer ambloplitis* 833, 837, 860, 863, 871, 882

V
*V. expectata* 410
*V. fischeri* 248, 254
*V. logei* 248, 254
*V. ordalii* 254
*V. pelagius* 254
V. splendidus 254
*V. wodanis* 248
Vaccination 64, 138, 252, 1248, 1264
Vaccines 50, 55, 60, 64, 68, 97, 99, 103, 105, 118, 137, 138, 186, 202, 211, 215, 241-243, 249, 252, 261, 438, 466-469, 492, 493, 581, 582, 594, 654, 655, 713, 715, 793, 796, 1011, 1015, 1096, 1135, 1264-1266
Vacuolar degeneration 11, 15, 133
Vahlkampfiidae 398
*Vannella* 407, 409
Vannella Bovee, 1965 409
Vannellidae 407
Variant surface glycoproteins 493
Varione 144
*Vauchomia* 575, 588
Velvet disease 426
Vendace 1155
VEN 211
VER 186, 193
Verbascoside 458
Vertical transmission 58, 64, 98, 186, 192, 198, 201, 219, 263, 381, 618
*Vexillifera* 411
*Vexillifera bacillipedes* 410
*Vexillifera* species 410
VHS 42
VHSV 42, 45-51, 54, 89, 95, 155, 158, 202
*Vibrio alginolyticus* 254, 255
*Vibrio harveyi* 254
Vibrio salmonicida 252
*Vibrio tapetis* 254
*Vibrio vulnificus* 254
*Vibrio vulnificus* serovar E 254
Vibriosis 249, 250, 252
Vietnam 139, 941, 942
Viraemia-associated ana-aki-byo 151
Viral encephalopathy and retinopathy 186
Viral erythrocytic necrosis 211
Viral haemorrhagic septicaemia 42, 47, 155
Viral haemorrhagic septicaemia virus 47, 155

Viral Haemorrhagic Septicaemia/VHS 1257
Viral nervous necrosis 186
Virginia opossum 892
Virulence 52, 67, 93, 114, 197, 244, 247, 282, 366, 453, 468, 469, 492, 493, 749, 850, 874, 1089
Vitamin A (retinol) 1037, 1210
Vitamin $B_{12}$ 978, 1210
Vitamin C (ascorbic acid) 1210
Vitamin D (cholecalciferol) 1210
Vitamin E (alpha-tocopherol) 1205, 1208-1210
Vitamin K 1210
Viviparity 697, 699, 764, 792
VNN 186
*Vorticella* 573, 586, 588
VSGs 493

W
Warm springs virus 155
Water belly syndrome 1240
Water quality 17, 59, 60, 153, 337, 709, 724, 755, 756, 1007, 1016, 1256
Water supply 544, 1094, 1257, 1258, 1260
Water temperature 48, 52, 53, 67, 159, 256, 257, 260, 264, 336, 426, 458, 583, 592, 598, 627, 653, 699, 706, 734-736, 738, 739, 751, 752, 758, 770, 777, 779, 780, 785, 786, 904, 922, 1136, 1235, 1240, 1241, 1242, 1255
Wels 89
Whirling disease 613, 622
White amur 89, 100
White grub 880
White bream 100, 102, 144, 530, 881, 901, 904
White mouse 892
White spot disease 571, 578, 590, 591
White sucker 66, 870, 879
Whitefish 66, 263, 631, 1013, 1153, 1155, 1156
Whitemargin unicornfish 937
Whitespotted charr 1161
Whiting 44, 535, 638, 649, 902, 1143
Winkle 828
Winter flounder 474, 487
Winter ulcer 64, 247
Wolffish 27, 192, 332
World Organization for Animal Health 94, 762
Wound healing 15, 19, 1231, 1241

X
X-cells 402, 457
Xenoma 357-359, 360-362, 373-384, 386-388, 626, 644, 893
*Xenomelaniris brasiliensis* 1145
*Xiphias gladius* 648, 1146
*Xiphophorus helleri* 458

Y
Yeasts 323
Yellow bodies 533, 535
Yellow catfish 906
Yellow grub 887
Yellow perch 838, 870, 875, 888, 890, 893, 1091
Yellowfin goby 647, 898
Yellowfin tuna 818, 944
Yellowtail 66, 652, 704, 728, 784, 913, 928, 1164
Yellowtail kingfish 704, 728, 784, 1164
*Yersinia ruckeri* 255
Yolk sac 63, 96, 198, 240, 257, 1226-1229
Yugoslavia 98, 127, 128

Z
*Zacco platypus* 907
*Zander* 901, 904, 905, 1153, 1160, 1161
*Zanobatus atlanticus* 489
Zearalenone 1219
Zebra danio 938
Zebra fish 66, 88, 144, 145, 381

Zebra mussel 901, 1055, 1056
*Zeuxapta seriolae* 685, 687, 689, 698, 704, 733, 784, 789
Zeuxaptosis 784, 785
Zinc 766, 848, 1207-1209, 1216
*Zoogonus rubellus* 839
Zoonotic 890
Zoosporangium 284-287
Zoosporulation 291
*Zoothamnium* 573, 586
*Zschokkella* 622, 645, 650
Zuphea 1179