

FISH PARASITES

Pathobiology and Protection

Edited by Patrick T.K. Woo and Kurt Buchmann



Fish Parasites

Pathobiology and Protection



This page intentionally left blank

Fish Parasites

Pathobiology and Protection

Edited by

Patrick T.K. Woo

University of Guelph, Canada

and

Kurt Buchmann

University of Copenhagen, Denmark



CABI is a trading name of CAB International

CABI
Nosworthy Way
Wallingford
Oxfordshire OX10 8DE
UK

Tel: +44 (0)1491 832111
Fax: +44 (0)1491 833508
E-mail: cabi@cabi.org
Website: www.cabi.org

CABI
875 Massachusetts Avenue
7th Floor
Cambridge, MA 02139
USA

Tel: +1 617 395 4056
Fax: +1 617 354 6875
E-mail: cabi-nao@cabi.org

© CAB International 2012. All rights reserved. No part of this publication may be reproduced in any form or by any means, electronically, mechanically, by photocopying, recording or otherwise, without the prior permission of the copyright owners.

A catalogue record for this book is available from the British Library,
London, UK.

Library of Congress Cataloging-in-Publication Data

Patrick T.K. Woo, Kurt Buchmann

Fish parasites : pathobiology and protection / edited by Patrick T.K. Woo, Kurt Buchmann.
p. cm.

Includes bibliographical references and index.

ISBN 978-1-84593-806-2 (alk. paper)

1. Fishes--Parasites. I. Woo, P. T. K. II. Buchmann, Kurt. III. Title.

SH175.F57 2012
333.95'6--dc23

2011028630

ISBN-13: 978 1 84593 806 2

Commissioning editor: Rachel Cutts
Editorial assistant: Gwenan Spearing
Production editor: Shankari Wilford

Typeset by AMA Dataset, Preston, UK.
Printed and bound in the UK by CPI Group (UK) Ltd, Croydon, CR0 4YY.

Contents

Contributors	vii
Preface	ix
1 <i>Neoparamoeba perurans</i> Barbara F. Nowak	1
2 <i>Amyloodinium ocellatum</i> Edward J. Noga	19
3 <i>Cryptobia (Trypanoplasma) salmositica</i> Patrick T.K. Woo	30
4 <i>Ichthyophthirius multifiliis</i> Harry W. Dickerson	55
5 <i>Miamiensis avidus</i> and Related Species Sung-Ju Jung and Patrick T.K. Woo	73
6 <i>Perkinsus marinus</i> and <i>Haplosporidium nelsoni</i> Ryan B. Carnegie and Eugene M. Bureson	92
7 <i>Loma salmonae</i> and Related Species David J. Speare and Jan Lovy	109
8 <i>Myxobolus cerebralis</i> and <i>Ceratomyxa shasta</i> Sascha L. Hallett and Jerri L. Bartholomew	131
9 <i>Enteromyxum</i> Species Ariadna Sitjà-Bobadilla and Oswaldo Palenzuela	163
10 <i>Henneguya ictaluri</i> Linda M.W. Pote, Lester Khoo and Matt Griffin	177

11	<i>Gyrodactylus salaris</i> and <i>Gyrodactylus derjavinoidea</i> <i>Kurt Buchmann</i>	193
12	<i>Pseudodactylogyrus anguillae</i> and <i>Pseudodactylogyrus bini</i> <i>Kurt Buchmann</i>	209
13	<i>Benedenia seriola</i> and <i>Neobenedenia</i> Species <i>Ian D. Whittington</i>	225
14	<i>Heterobothrium okamotoi</i> and <i>Neoheterobothrium hirame</i> <i>Kazuo Ogawa</i>	245
15	<i>Diplostomum spathaceum</i> and Related Species <i>Anssi Karvonen</i>	260
16	<i>Sanguinicola inermis</i> and Related Species <i>Ruth S. Kirk</i>	270
17	<i>Bothriocephalus acheilognathi</i> <i>Tomáš Scholz, Roman Kuchta and Chris Williams</i>	282
18	<i>Anisakis</i> Species <i>Arne Levsen and Bjørn Berland</i>	298
19	<i>Anguillicoloides crassus</i> <i>François Lefebvre, Géraldine Fazio and Alain J. Crivelli</i>	310
20	<i>Argulus foliaceus</i> <i>Ole Sten Møller</i>	327
21	<i>Lernaea cyprinacea</i> and Related Species <i>Annemarië Avenant-Oldewage</i>	337
22	<i>Lepeophtheirus salmonis</i> and <i>Caligus rogercresseyi</i> <i>John F. Burka, Mark D. Fast and Crawford W. Revie</i>	350
	Index	371

The colour plates can be found following p. 294

Contributors

- Annemarië Avenant-Oldewage**, Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, Johannesburg, South Africa. E-mail: aoldewage@uj.ac.za
- Jerri L. Bartholomew**, Department of Microbiology, Oregon State University, Corvallis, Oregon 97331, USA.
- Bjørn Berland**, Department of Biology, University of Bergen, PO Box 7800, N-5020 Bergen, Norway. E-mail: bjoern.berland@zoo.uib.no
- Kurt Buchmann**, Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Denmark. E-mail: kub@life.ku.dk
- John F. Burka**, Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island, Canada C1A 4P3. E-mail: burka@upei.ca
- Eugene M. Bureson**, Virginia Institute of Marine Science, College of William & Mary, PO Box 1346, Gloucester Point, Virginia 23062, USA. E-mail: gene@vims.edu
- Ryan B. Carnegie**, Virginia Institute of Marine Science, College of William & Mary, PO Box 1346, Gloucester Point, Virginia 23062, USA. E-mail: carnegie@vims.edu
- Alain J. Crivelli**, Station Biologique de la Tour du Valat, Arles, France.
- Harry W. Dickerson**, Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA. E-mail: hwd@uga.edu
- Mark D. Fast**, Novartis Research Chair in Fish Health, Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island, Canada C1A 4P3. E-mail: mfast@upei.ca
- Géraldine Fazio**, Institute of Integrative and Comparative Biology, University of Leeds, Leeds, UK.
- Matt Griffin**, Thad Cochran National Warmwater Aquaculture Center, College of Veterinary Medicine and Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Stoneville, Mississippi 38756, USA. E-mail: griffin@cvm.msstate.edu
- Sascha L. Hallett**, Department of Microbiology, Oregon State University, Corvallis, Oregon 97331, USA.
- Sung-Ju Jung**, Department of Aqualife Medicine, Chonnam National University, Dunduck Dong, Yeosu, Chonnam 550-749, Republic of Korea.
- Anssi Karvonen**, Department of Biological and Environmental Science, Centre of Excellence in Evolutionary Research, University of Jyväskylä, PO Box 35, FI-40010 Jyväskylä, Finland. E-mail: anssi.t.karvonen@jyu.fi

- Lester Khoo**, Director Aquatic Diagnostic Laboratory, Thad Cochran National Warmwater Aquaculture Center, College of Veterinary Medicine, Mississippi State University, Stoneville, Mississippi 38756, USA. E-mail: khoo@cvm.msstate.edu
- Ruth S. Kirk**, School of Life Sciences, Kingston University, Kingston upon Thames, Surrey KT1 2EE, UK.
- Roman Kuchta**, Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic. E-mail: krttek@yahoo.com
- François Lefebvre** (scientific associate with the Natural History Museum of London, UK; and the Station Biologique de la Tour du Valat, Arles, France), 47 rue des TroisRois, 86000 Poitiers, France. E-mail: f.lefebvre@nhm.ac.uk
- Arne Levsen**, National Institute of Nutrition and Seafood Research, PO Box 2029, Nordnes, N-5817 Bergen, Norway. E-mail: arne.levsen@nifes.no
- Jan Lovy**, Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Canada C1A 4P4.
- Ole Sten Møller**, Allgemeine und Spezielle Zoologie, Institute of Biosciences, University of Rostock, Universitätsplatz 2, D-18055 Rostock, Germany. E-mail: ole.moeller@uni-rostock.de
- Edward J. Noga**, Department of Clinical Sciences, North Carolina State University College of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, North Carolina 27606, USA. E-mail: sagonje@gmail.com
- Barbara F. Nowak**, National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Locked Bag 1370, Launceston 7250 Tasmania, Australia. E-mail: B.Nowak@utas.edu.au
- Kazuo Ogawa**, Laboratory of Fish Diseases, Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo 113-8657, Japan. E-mail: aogawak@mail.ecc.u-tokyo.ac.jp
- Oswaldo Palenzuela**, Instituto de Acuicultura de Torre de la Sal, Consejo Superior de Investigaciones Científicas, Torre de la Sal, s/n, 12595 Ribera de Cabanes, Castellón, Spain.
- Linda M.W. Pote**, Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi 39759, USA. E-mail: lpote@cvm.msstate.edu
- Crawford W. Revie**, Canada Research Chair – Population Health: Epi-Informatics, Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island, Canada C1A 4P3. E-mail: crevie@upei.ca
- Tomáš Scholz**, Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic. E-mail: tscholz@paru.cas.cz
- Ariadna Sitjà-Bobadilla**, Instituto de Acuicultura de Torre de la Sal, Consejo Superior de Investigaciones Científicas, Torre de la Sal, s/n, 12595 Ribera de Cabanes, Castellón, Spain. E-mail: ariadna@iats.csic.es
- David J. Speare**, Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Canada C1A 4P4. E-mail: speare@upei.ca
- Ian D. Whittington**, Monogenean Research Laboratory, Parasitology Section, The South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia; Marine Parasitology Laboratory, School of Earth and Environmental Sciences (DX 650 418), The University of Adelaide, North Terrace, Adelaide, South Australia 5005, Australia; Australian Centre for Evolutionary Biology and Biodiversity, The University of Adelaide, North Terrace, Adelaide, South Australia 5005, Australia. E-mail: ian.whittington@samuseum.sa.gov.au
- Chris Williams**, Environment Agency, Bromholme Lane, Brompton, Cambridgeshire, PE28 4NE, UK. E-mail: chris.williams@environment-agency.gov.uk
- Patrick T.K. Woo**, Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. E-mail: pwoo@uoguelph.ca

Preface

Fish Parasites: Pathobiology and Protection (FPPP) covers protozoan and metazoan parasites that cause disease and/or mortality in economically important fishes. In this respect FPPP is similar to *Fish Diseases and Disorders, Vol. 1: Protozoan and Metazoan Infections* 2nd edition (FDD1.2). However, the two books are different in that FPPP is concise and focuses on specific pathogens while FDD1.2 covers parasites that are known to be associated with morbidity and mortality in fish. Also, FDD1.2 is more encyclopaedic as it includes parasite systematics, evolution, molecular biology, *in vitro* culture, and ultrastructure; however, these areas are not addressed in FPPP. Finally, FPPP has much more recent information than FDD1.2, which was published in 2006.

All chapters in FPPP are written by scientists who have considerable experience and expertise on the parasite(s). The selection of pathogens for inclusion in the book has been made by the editors, and it is based on numerous criteria, which include those parasites that (i) have not been discussed (e.g. *Argulus foliaceus*, *Neoheterobothrium hirame*) in FDD1.2, or (ii) are relatively well-studied fish pathogens (e.g. *Cryptobia salmositica*, *Ichthyophthirius multifiliis*) which may serve as disease models for studies on other parasites, or (iii) cause considerable financial problems/hardships to certain sectors of the aquaculture industry (e.g. marine cage/net culture of salmonids – *Lepeophtheirus salmonis* in Norway and *Caligus rogercresseyi* in Chile), or (iv) have been accidentally introduced to new geographical regions through the transportation of infected fish (e.g. *Gyrodactylus salaris* in Norway, *Anguillicoloides crassus* in Europe) and subsequently have become significant threats to local fish populations, or (v) are disease agents to specific groups of fishes (e.g. *Myxobolus cerebralis* to salmonids, *Henneguya ictaluri* to catfish) and adversely affect fish production, or (vi) are not host-specific, and have worldwide distributions (e.g. *Amyloodinium ocellatum*, *Bothriocephalus acheilognathi*), or (vii) are facultative parasites which under certain conditions are emerging as important pathogens (e.g. *Miamiensis avidus* to flatfishes).

Numerous other groups of pathogenic parasites (e.g. Trichodinidae, Caryophyllidea) are not included in the book because not much is known about their pathobiology and/or protective strategies against them. We are hopeful this book will stimulate research on some of these 'neglected' parasites in the near future. The present volume also points out obvious gaps in our knowledge even on the selected parasites, and we hope these will be rectified with further research.

As with the trilogy on *Fish Diseases and Disorders* (1st and 2nd editions) the principal audience for FPPP are research scientists in the aquaculture industry and universities, and fish health consultants/managers of private or government fish health laboratories. Also, the present volume is appropriate for the training of fish health specialists, and for senior undergraduate/graduate students who are conducting research on diseases of fishes. FPPP may be a useful reference book for university courses on infectious diseases, general parasitology, and on impacts of diseases to the aquaculture industry.

Patrick T.K. Woo and Kurt Buchmann

1 *Neoparamoeba perurans*

Barbara F. Nowak

National Centre for Marine Conservation and Resource Sustainability,
University of Tasmania, Australia

1.1. Introduction

Neoparamoeba perurans Young, Crosbie, Adams, Nowak et Morrison, 2007 is a marine amoeba (Amebozoa, Dactylopodida) which colonizes fish gills resulting in outbreaks of amoebic gill disease (AGD) in fish farmed in the marine environment (Young *et al.*, 2007, 2008a). The transmission is horizontal. Experimental AGD infections are achieved either by cohabitation with infected fish or by exposure to amoebae isolated from the gills of fish affected by AGD. As few as 10 amoebae/l of water cause AGD in naïve Atlantic salmon (*Salmo salar*) (Morrison *et al.*, 2004). There is a positive correlation between the number of amoebae in the water and the severity of the lesions (Zilberg *et al.*, 2001; Morrison *et al.*, 2004). Other members of this genus are free-living amoebae, ubiquitous in the marine environment (Page, 1974, 1983) and have been cultured from marine sediments, water and marine invertebrates both from fish-farming and non-farming areas, ranging from polar to subtropical climate zones (Page, 1973; Crosbie *et al.*, 2003, 2005; Mullen *et al.*, 2005, Dyková *et al.*, 2007; Moran *et al.*, 2007). Massive mortality of American lobster (*Homarus americanus*) in Western Long Island Sound, which resulted in the collapse of the fishery, was partly attributed to *Neoparamoeba pemaquidensis*, which was identified on the basis of

small-subunit ribosomal RNA (SSU rRNA) fragments having 98% identity with *N. pemaquidensis* from the gills of Atlantic salmon (Mullen *et al.*, 2005). It was also proposed that *Paramoeba invadens*, which is a pathogen of sea urchins (Jones and Scheibling, 1985), is a junior synonym of *N. pemaquidensis* (see Mullen *et al.*, 2005).

There is little information about the biology of *N. perurans*. Using PCR tests, *N. perurans* has been detected in water from cages containing farmed Atlantic salmon affected by AGD in Tasmania and from fresh water used to bathe fish on the same farm (Bridle *et al.*, 2010). It was not detected in water from another salmon farm that was not affected by AGD at the sampling time, or in other areas further away from salmon farms (Bridle *et al.*, 2010). Negative results may have been due to the low sensitivity of the technique as small volumes of water were used (50 ml). Further research is needed to determine the environmental distribution of *N. perurans*.

AGD was first reported more than 20 years ago in coho salmon (*Oncorhynchus kisutch*) farmed in Washington State USA and *Paramoeba pemaquidensis* was proposed as the disease agent (Kent *et al.*, 1988). This species was transferred (together with *Paramoeba aestuarina*) to genus *Neoparamoeba* due to the absence of microscales on the surface of the

trophozoites (Page, 1987; Dyková *et al.*, 2000). *N. pemaquidensis* was repetitively isolated by *in vitro* culture from gills of infected coho salmon and Atlantic salmon from different locations, including USA and Australia (Kent *et al.*, 1988; Dyková *et al.*, 1998). Another species, *Neoparamoeba branchiphila*, was described based on cultures from the gills of AGD-affected Atlantic salmon in Tasmania (Dyková *et al.*, 2005). A recent molecular study that was to determine if both or one of these species caused AGD resulted in the description of *N. perurans* (see Young *et al.*, 2007).

N. perurans (Fig. 1.1) is the only species associated with AGD lesions on the gills of fish (Young *et al.*, 2008a; Crosbie *et al.*, 2010a; Bustos *et al.*, 2010). The other two species of *Neoparamoeba* have not been found (using *in situ* hybridization) in histological sections of gills of fish affected by AGD. It is possible that *in vitro* culture conditions used for isolations of amoebae from fish gills which initially suggested *N. pemaquidensis* and *N. branchiphila* as the causative species are more suitable for these species than for *N. perurans* which is the only species that is clearly associated with the gill pathology and AGD. It is also possible, but less likely, that the histological fixation or processing may select for *N. perurans*. While experimental exposure to *N. perurans* isolated from the gills of affected salmon causes AGD in naïve Atlantic salmon (Young *et al.*, 2007;

Crosbie *et al.*, 2010a), cultured *N. pemaquidensis* or *N. branchiphila* did not (Morrison *et al.*, 2005; Vincent *et al.*, 2007). As stated earlier, efforts to culture *N. perurans* have not yet been successful.

AGD was reported during the 1980s from farmed coho salmon in Washington State in the USA (Kent *et al.*, 1988) and from Atlantic salmon in Tasmania Australia (Munday, 1986; Munday *et al.*, 1990). The disease affects fishes farmed in the marine environment (Kent *et al.*, 1988; Dyková *et al.*, 1998; Young *et al.*, 2007, 2008a; Crosbie *et al.*, 2010a), and they include coho salmon (*O. kisutch*), Atlantic salmon (*S. salar*), rainbow trout (*O. mykiss*), chinook salmon (*Oncorhynchus tshawytscha*), turbot (*Psetta maxima*), sea bass (*Dicentrarchus labrax*) and ayu (*Plecoglossus altivelis*). It has been suggested that some salmonids may be more resistant to AGD than others (Munday *et al.*, 2001), however it is difficult to resolve given the difficulty of running experimental infections in exactly the same environmental conditions and using comparable fish from different species. Despite surveys of large numbers of wild fishes near salmon farms affected by AGD in Tasmania (Nowak *et al.*, 2004), only one individual wild fish has ever been found with *Neoparamoeba* sp. on its gills (Adams *et al.*, 2008). This fish, a blue warehou (*Seriolella brama*) was from a cage containing infected

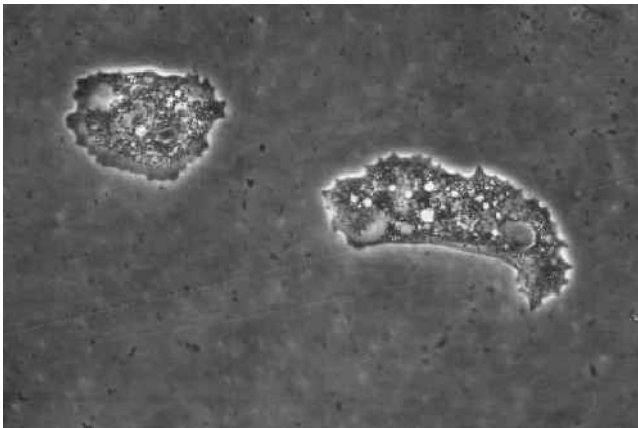


Fig. 1.1. Amoebae isolated from the gills of Atlantic salmon affected by AGD. The amoebae were later confirmed to be *Neoparamoeba perurans* using PCR. Photo, Dr Philip Crosbie.

Atlantic salmon (Adams *et al.*, 2008). The geographic distribution of *N. perurans* includes the west coast of USA, Australia, Chile, New Zealand, Japan, South Africa, Ireland, Scotland and Norway (Young *et al.*, 2007; Nylund *et al.*, 2008; Steinum *et al.*, 2008; Bustos *et al.*, 2010; Crosbie *et al.*, 2010a; A. Mouton, P.B.B. Crosbie and B.F. Nowak unpublished; P.B.B. Crosbie and B.F. Nowak unpublished).

If the infected fish are not treated, AGD can cause mortalities of over 50% affected fish (Munday *et al.*, 1990). Mortalities have been reported in farmed fish in USA, Tasmania, Ireland, Scotland, Norway, Japan and Chile (Kent *et al.*, 1988; Rodger and McArdle, 1996; Palmer *et al.*, 1997; Nylund *et al.*, 2008; Steinum *et al.*, 2008; Bustos *et al.*, 2010; Crosbie *et al.*, 2010a). All salmon-producing countries except Canada are affected or have been affected by AGD. While the outbreaks in many of these locations have been sporadic (for example in Norway or Scotland) AGD is the most significant health problem in Atlantic salmon farmed in Tasmania where it contributes up to 20% of production costs (Munday *et al.*, 2001), and this was mostly due to the cost of freshwater bathing. AGD has also been reported regularly from the USA and Chile, where it can contribute to significant mortalities of Atlantic salmon (Douglas-Helders *et al.*, 2001a; Bustos *et al.*, 2010; Nowak *et al.*, 2010).

One of the main risk factors for the disease outbreaks is high salinity (Munday *et al.*, 1990; Clark and Nowak, 1999; Nowak, 2001; Adams and Nowak, 2003; Bustos *et al.*, 2010). Outbreaks in Ireland (Palmer *et al.*, 1997) and Chile (Bustos *et al.*, 2010) have occurred in years with unusually low rainfall. In experimental AGD infections mortalities are greater at salinities of 37–40 ppt than 35 ppt and below (Nowak, 2001). In Tasmania, salmon farmed at sites with a strong influx of fresh water following heavy rain were less affected by AGD (Munday *et al.*, 1993). This may be due to the sensitivity of the amoeba to low salinity as it is a marine species. There was a reduced survival of amoebae isolated from the gills of AGD-affected salmon when the amoebae were exposed for 6 days to 15 ppt salinity compared to survival at 27 or 38 ppt (Douglas-Helders *et al.*, 2005).

1.2. Diagnosis of the Infection: Clinical Signs of the Disease

While respiratory distress and lethargy have been reported in AGD-affected fish, behavioural changes are not used to diagnose infection. Salmon farmers in Tasmania determine the severity of AGD by the presence of white gross lesions on the gills (Fig. 1.2) as they are a good indicator of AGD in fish farmed in areas enzootic for AGD (Adams *et al.*, 2004) when gill checks are done by an experienced person (Clark and Nowak, 1999). The gill patches represent hyperplastic lesions (Fig. 1.3), which can lead to lamellar fusion, often affecting whole filaments (Adams *et al.*, 2004). Amoebae are usually present in the histological sections (Adams and Nowak, 2003; Dyková *et al.*, 2003, 2008). The parasite can be distinguished as a member of one of the two genera *Paramoeba* or *Neoparamoeba* on the basis of the presence of endosymbionts (Dyková *et al.*, 2003; Adl *et al.*, 2005); however, more detailed identification (to genus and species level) requires either PCR or *in situ* hybridization (Fig. 1.4; Young *et al.*, 2007, 2008a, b). This is due to the lack of morphological differences (even ultrastructural) between species of *Neoparamoeba* (see Dyková *et al.*, 2005; Young *et al.*, 2007). While immunofluorescence antibody test and immune-dot-blot were used to confirm the presence of the parasite (Howard *et al.*, 1993; Douglas-Helders *et al.*, 2001b), the polyclonal antibodies used were not species specific (Morrison *et al.*, 2004). PCR of gill swabs has been developed and validated (Young *et al.*, 2008b; Bridle *et al.*, 2010). The advantages of this method are high sensitivity and specificity for the parasite and non-terminal sampling (Young *et al.*, 2008b). There was a positive correlation between the severity of the gross gill lesions and quantitative real time PCR (qPCR) of gill swabs for *N. perurans* (see Bridle *et al.*, 2010) which further validates it as a diagnostic method.

Paramoeba and *Neoparamoeba* have eukaryotic endosymbionts (parasomes) in the trophozoites when examined under the light microscope (Fig. 1.3; Adl *et al.*, 2005). These endosymbionts, *Perkinsela amoebae*-like organisms (PLOs), are members of the order



Fig. 1.2. Gross gill lesions characteristic of Atlantic salmon affected by AGD. Photo, Dr Benita Vincent.

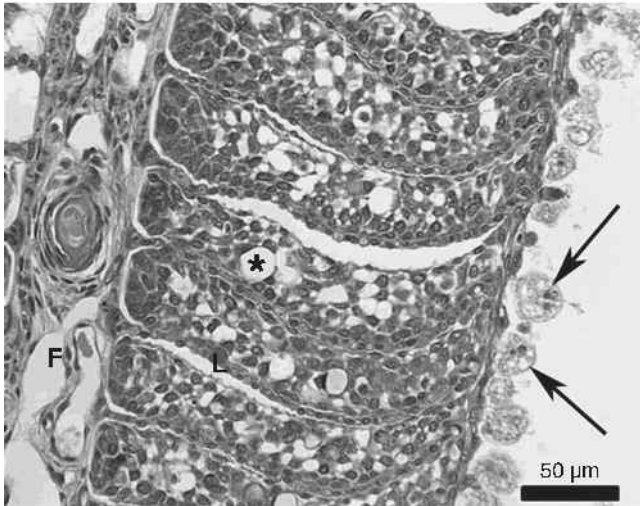


Fig. 1.3. Gill lesions typical of AGD, showing hyperplasia of epithelial and mucous cells leading to lamellar fusion. Numerous amoebae are present between gill filaments. Arrows indicate two examples of amoebae showing nucleus and endosymbiont; F, filament; L, lamella; *, mucous cell. Photo, Karine Cadoret.

Kinetoplastida and are closely related to the fish parasite, *Ichthyobodo necator*, based on SSU rRNA gene sequence from different strains of *Neoparamoeba* (see Dyková *et al.*, 2003). The endosymbionts can be easily seen in smears (Zilberg *et al.*, 1999) and histological sections (Dyková and Novoa, 2001). The

diagnosis of AGD is based on gill histopathology when amoebae possessing one or more endosymbiotic PLOs are detected in close association with hyperplastic epithelial-like cells (Fig. 1.3; Dyková and Novoa, 2001; Adams and Nowak 2003; Dyková *et al.*, 2003, 2008).

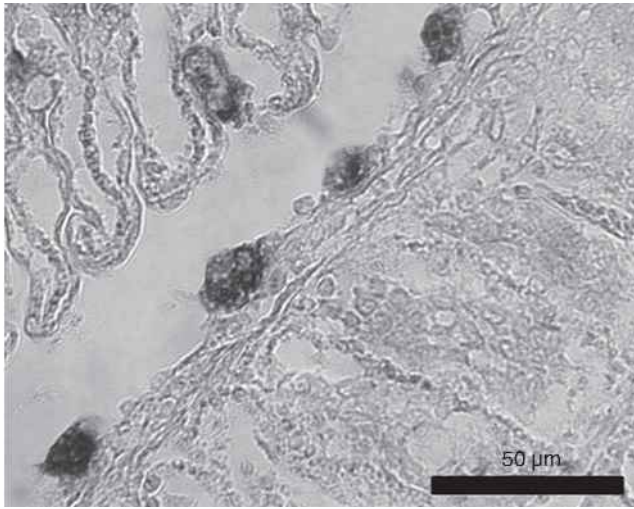


Fig. 1.4. *In situ* hybridization showing that all amoebae in the field of view are positive for *N. perurans*. Photo, Karine Cadoret.

1.3. External/Internal Lesions

Gills are the only organ affected and most fish species develop white raised lesions on their gills (Fig. 1.2). The lesions usually start from the base of filaments, spread through the gill arch and often coalesce into a big lesion. In Atlantic salmon the dorsal area of the gills is usually more affected than the ventral area (Adams and Nowak, 2001). Macroscopic lesions in Atlantic salmon show good agreement with histological changes during the progression of AGD (Adams *et al.*, 2004).

In Atlantic salmon farmed in Tasmania, AGD was detected in histological sections at 13 weeks post-transfer to the marine environment, while gross signs were not detected until a week later. Increased intensity of lesions was associated with increased salinity (cessation of halocline) and higher water temperatures (Adams and Nowak, 2003). Natural infections in farmed Atlantic salmon start with colonization of gills by amoeba and localized cellular changes, including epithelial desquamation and oedema. This is followed by initial focal epithelial hyperplasia and finally squamation-stratification of

epithelium and an increase in the numbers of mucous cells within the lesions (Adams and Nowak, 2003). Formation of fully enclosed interlamellar vesicles in the advanced lesion is most likely a result of the proliferative character of this disease and may help with trapping and killing of amoebae (Adams and Nowak, 2001). Reinfection of salmon on the farm is evident 2 weeks after commercial freshwater bathing with the severity of the lesions increasing 4 weeks post-bathing when gross pathology appears (Adams and Nowak, 2004). The lesion development is identical to the initial infection of the naïve fish (Adams and Nowak, 2004). Lesion characteristics and disease progression are the same in the laboratory challenges as that on farms. The disease usually progresses faster in a laboratory challenge, particularly when gill-isolated amoebae are added directly to the water in the tank containing naïve salmon, with morbidity occurring within 4 weeks at 15°C (Crosbie *et al.*, 2010b).

Reduced numbers of chloride cells and increased numbers of mucous cells (Munday *et al.*, 1990; Nowak and Munday, 1994; Zilberg and Munday, 2000; Powell *et al.*, 2001; Adams

and Nowak, 2003; Roberts and Powell 2003, 2005) and formation of fully enclosed interlamellar vesicles (Adams and Nowak, 2001) are reported within AGD lesions. Inflammatory cells, identified on the basis of their morphology as neutrophils and macrophages are present in the interlamellar cysts (Adams and Nowak, 2001). Cells positive for major histocompatibility complex (MHC) class II were present in higher numbers in AGD lesions (Morrison *et al.*, 2006a), while Ig-positive cells occurred in low numbers similar to those in uninfected Atlantic salmon (Gross, 2007). While eosinophils were claimed to be the primary infiltrating cells in AGD lesions (Lovv *et al.*, 2007), there was no evidence of eosinophilia at the transcriptional level (Young *et al.*, 2008c). The eosinophilia might have been due to the moribund state of salmon used for the ultrastructural study (Lovv *et al.*, 2007) and not AGD.

1.4. Pathophysiology

The behaviour of fish dying of AGD and the fact that the disease causes severe gill lesions suggest that fish respiration would be affected (Kent *et al.*, 1988; Munday *et al.*, 1990; Rodger and McArdle, 1996). However, this was not supported in physiological studies (Powell *et al.*, 2000; Fisk *et al.*, 2002; Leef *et al.*, 2005a, 2007). There were no differences in the rate of oxygen uptake between infected and control fish (Powell *et al.*, 2000). Arterial PO_2 and pH were significantly lower in the infected fish whereas PCO_2 was significantly higher in infected fish compared with controls prior to hypoxia (Powell *et al.*, 2000). The respiratory acidosis could have been due to increased mucus secretion observed during AGD (Powell *et al.*, 2000). Despite respiratory acidosis in AGD-affected fish, environmental hypoxia down to 25% of oxygen saturation did not result in respiratory failure in those fish (Powell *et al.*, 2000). Atlantic salmon with clinical AGD showed increased amplitude and rate of opercular movements (Fisk *et al.*, 2002).

This discrepancy between the presence of gill lesions and apparent lack of effects on respiration could be at least partly due to the fact that

survival in AGD-affected Atlantic salmon following even minor surgical procedures such as dorsal aorta cannulation is relatively poor (Leef *et al.*, 2005a, b). The lack of AGD effect on fish respiration could also be explained by cardiovascular or respiratory adjustments that can compensate for the reduction in gill surface area (Powell *et al.*, 2008).

Changes in heart morphology in AGD-affected fish were reported (Powell *et al.*, 2002), however there were no changes in lactate dehydrogenase activity in the ventricle suggesting that at least some of the heart functions were not affected. However, there was an overall thickening of the muscularis compactum in the ventricle of fish that had a history of heavy AGD (Powell *et al.*, 2002). AGD-affected Atlantic salmon had lower cardiac output and higher systemic vascular resistance than control fish (Leef *et al.*, 2005a, b, 2007). AGD-associated cardiac dysfunction appeared to be specific to Atlantic salmon which would explain the higher susceptibility of this species compared with both brown and rainbow trout (Leef *et al.*, 2005b). While Atlantic salmon, brown trout (*Salmo trutta*) and rainbow trout had similar dorsal aortic pressure, cardiac output and systemic vascular resistance values, only AGD-affected salmon had significantly elevated systemic vascular resistance compared with the non-affected controls (Leef *et al.*, 2005a, b). Cardiac output was also approximately 35% lower in affected fish (Leef *et al.*, 2005a, b).

Numbers of chloride cells were reduced in the lesions (Adams and Nowak, 2001), suggesting that osmoregulation might be affected. This is further reflected by reduced succinate dehydrogenase activity and greater whole body net efflux of ions (Powell *et al.*, 2001; Roberts and Powell, 2003). While there is some evidence of osmoregulatory problems in fish with AGD (Munday *et al.*, 2001; Powell *et al.*, 2005), it occurs only in severely affected fish, most likely those that are becoming moribund (Powell *et al.*, 2008). Osmoregulatory problems in AGD-affected fish may be because of the fish dying and not a cause of mortality due to AGD.

One of the main responses in AGD lesions is epithelial hyperplasia (Adams and Nowak, 2001). This morphological change is

confirmed by an increase of proliferating cell nuclear antigen (PCNA) and interleukin-1 beta in the gill epithelium (Adams and Nowak, 2003; Bridle *et al.*, 2006a) and down-regulation of the p53 tumour suppressor gene in the gills of Atlantic salmon experimentally infected with *N. perurans* (see Morrison *et al.*, 2006b). Other gene expression changes observed in the gills of infected fish may be due to changes in the types and ratios of cell populations in lesions. Despite different experimental conditions, including duration of infection and controls used, some of the changes in gene regulation were consistent in two experimental AGD infections (Table 1.1). The upregulation of anterior gradient 2-like protein could be a result of an increased number of mucous cells in lesions (Morrison and Nowak, 2005). Similarly, the downregulation of Na/K ATPase in AGD-affected fish or AGD lesions could reflect the reduction in numbers of chloride cells in AGD lesions (Adams and Nowak, 2001). Significant downregulation of immune genes was observed in the gills, and particularly in the gill lesions, of AGD-affected Atlantic salmon (Young *et al.*, 2008c). However, AGD had no effect on gene expression in other

organs (Bridle *et al.*, 2006a, b) confirming that AGD is a gill disease.

Haemoglobin subunit beta was down-regulated both at gene (36 days post-infection, Young *et al.*, 2008c) and protein (21 days post-infection, E. Lowe and B.F. Nowak unpublished) levels in AGD-affected Atlantic salmon. This might be due directly to respiratory changes, or alternatively it could be related to changes in the level of antimicrobial peptides derived from beta subunit of haemoglobin, which have been described from channel catfish (*Ictalurus punctatus*) infected with *Ichthyophthirius multifiliis* (see Ullal *et al.*, 2008). These peptides were reported to have parasitocidal properties against *I. multifiliis*, *Tetrahymena pyriformis* and *Amyloodinium ocellatum* (see Ullal *et al.*, 2008; Ullal and Noga, 2010).

An increase in standard and metabolic rates has been reported in AGD-affected fish (Powell *et al.*, 2008). This effect was related to the severity of infection. AGD can affect swimming performance of Atlantic salmon, particularly in repeated tests, possibly due to the inability of the infected salmon to recover from the previous test (Powell *et al.*, 2008).

Table 1.1. Consistent changes in gene expression in Atlantic salmon from two separate experimental infections shown as fold change.

Genes	Fold change	
	Whole gill versus infected naïve fish up to 8 days post-infection (hours post-infection in parentheses) (Morrison <i>et al.</i> , 2006b)	Lesion area versus normal gill area of the same individual 36 days post-infection (Young <i>et al.</i> , 2008c)
Upregulated genes		
Differentially regulated trout protein	2.31 (114–189)	2.82
Anterior gradient 2-like protein ^a	2.0–2.57 (0–189)	2.15–2.52
Downregulated genes		
TIMP-2 (tissue inhibitor of metalloproteinases)	7.67 (189)	2.32
Brain protein 44	2.36 (189)	2.12
Guanine-nucleotide binding protein	2.15 (189)	2.63–3.57
Beta-2-microglobulin	3.08 (114)	2.06–2.56
Na/K ATPase	2.32 (44)	3.12–6.10

^a Anterior gradient 2 expression was confirmed by qPCR (Morrison *et al.*, 2006b).

1.5. Protective/Control Strategies

Freshwater bathing (Fig. 1.5) has been used by the salmon industry in Tasmania on a regular basis with frequency depending on severity of AGD as determined by gross gill checks. In the past, three to four freshwater baths during the full marine salmon production cycle were used (Clark and Nowak, 1999). More recently the bathing frequency at least doubled, possibly partly due to an increased biomass of salmon in sea cages. Bathing frequency is driven by infection intensity; however now it is conducted at a lower gill score than previously as the infection proceeds more rapidly and hence requires earlier treatment. The salmon industry in Washington State also uses freshwater bathing when AGD becomes a problem. Freshwater bathing involves moving affected fish to an empty production cage with a liner filled with oxygenated fresh water (usually hyperoxic, at least at the beginning of the bath). The bath takes approximately 2–3 h from the time when the last fish entered the liner, but duration depends on the fish size with the larger salmon (over 3 kg) bathed for a shorter time. At the end of the bath the liner is pulled out and the fish are released into the production cage. AGD in turbot has also been treated with freshwater bathing (Nowak

et al., 2002). The life cycle of ayu requires the fish to be moved from the marine hatchery to freshwater grow-out during the production cycle, which resolves AGD in the surviving fish (Crosbie *et al.*, 2010a).

Freshwater treatment is successful in removing most of the amoebae from the gills of infected fish, however, reinfection can occur within a few weeks, particularly in summer when the water temperature is high (Parsons *et al.*, 2001; Adams and Nowak, 2004). Additionally, limited access to fresh water in some salmon farming areas and a high number of cages requiring bathing can restrict salmon production. Even very low salinity of the bath water can affect bathing efficacy. Bathing in soft water (19.3–37.4 mg/l CaCO_3) is more beneficial than bathing in hard water (173–236.3 mg/l CaCO_3) (Roberts and Powell, 2003). Freshwater bathing (up to 2 h hyperoxic bath) has no demonstrable adverse effects on Atlantic salmon, including no significant effect on blood plasma ions, acid–base and respiratory variables (Powell *et al.*, 2001). Alterations in bathing procedure or an alternative treatment may be required to achieve the total removal of the amoebae from the gills of fish (Parsons *et al.*, 2001).

While freshwater bathing is effective; it is however a short-term solution that is labour intensive, expensive and requires access to



Fig. 1.5. Freshwater bathing on an Atlantic salmon farm in Tasmania. Note liner inside the mesh cage.

fresh water. A range of alternative experimental treatments were tested. Bath treatments ranged from using disinfectants (hydrogen peroxide, chlorine dioxide and chloramine T) to parasiticides such as levamisole and bithionol (Clark and Nowak, 1999; Zilberg *et al.*, 2000; Munday and Zilberg, 2003; Harris *et al.*, 2004, 2005; Powell *et al.*, 2005; Florent *et al.*, 2007a). In some trials, chemicals were added to the freshwater bath. Generally new treatments would be more useful if they could be applied directly to fish in sea water so that there would no longer be need for freshwater bathing. Some experimental results suggested that a treatment should work well, but the field studies based on the experimental results did not confirm this. For example, 1.25 mg/l of levamisole added to the freshwater bath reduced mortality of AGD-affected Atlantic salmon under laboratory conditions (Zilberg *et al.*, 2000) but 2.5–5.0 mg/l did not have any effect on: (i) the time between bathings; (ii) the number of lesions; or (iii) the number of amoebae in histological lesions (Clark and Nowak, 1999). Levamisole was ineffective in a seawater bath at concentrations below 50 mg/l. At the effective concentration (results comparable to freshwater bath) it caused high fish mortality (Munday and Zilberg, 2003). Oral treatments included bithionol and mucolytic agents (Roberts and Powell, 2005; Florent *et al.*, 2007b, 2009). While some of these treatments gave promising results in laboratory challenges, particularly L-cysteine (a mucolytic agent) and bithionol (Roberts and Powell, 2005; Florent *et al.*, 2007a, b), they are not used commercially possibly due to their higher costs.

The innate immune response appears to be suppressed in infected fish. Atlantic salmon kidney phagocyte respiratory burst was suppressed 8 and 11 days post-infection in a laboratory challenge (Gross *et al.*, 2004a, 2005). Innate immunity is considered important for protection against AGD (Findlay and Munday, 1998) and thus immunostimulants should have a role in reducing the impact of AGD on the salmon industry. Experimental injection with CpGs (DNA motifs characteristic for bacteria) increased protection against AGD by 38% (Bridle *et al.*, 2003). This suggested that immunostimulants could contribute to the successful management of AGD.

However, there were no consistent effects detected in laboratory or field experiments involving Atlantic salmon fed beta glucans or other commercially available immunostimulants (Zilberg *et al.*, 2000; Nowak *et al.*, 2004; Bridle *et al.*, 2005).

Both increased survival and reduced gill pathology have been used to measure resistance to AGD in experimental studies. Resistance to AGD was described in Atlantic salmon as a result of previous exposure (Table 1.2) or prolonged exposure (Bridle *et al.*, 2005; Vincent *et al.*, 2008) at low water temperatures. This resistance to subsequent infections suggests vaccination may be a successful way to manage AGD. Experimental vaccines tested ranged from live or killed amoebae (with or without adjuvant) to DNA vaccine (Zilberg and Munday, 2001; Morrison and Nowak, 2005; Cook *et al.*, 2008). The live or killed vaccines were applied by bath (Morrison and Nowak, 2005) or anal intubation or intraperitoneal injection (Zilberg and Munday, 2001). DNA vaccine was injected intramuscularly (Cook *et al.*, 2008). None of the experimental vaccinations provided significant and consistent protection against infection (Zilberg and Munday, 2001; Morrison and Nowak, 2005; Cook *et al.*, 2008).

So far there is no evidence of an effective innate (Bridle *et al.*, 2006a, b; Morrison *et al.*, 2007) or acquired (Findlay and Munday, 1998; Gross *et al.*, 2004b; Morrison *et al.*, 2006b; Vincent *et al.*, 2006, 2009) immune response to AGD. Based on a transcriptional response study of AGD-affected Atlantic salmon it was suggested that *N. perurans* can evade the host immune response by disrupting the molecular mechanisms essential for activation of effector T-cell mediated responses (Young *et al.*, 2008c). However the mechanism of this disruption is still unclear.

Selective breeding for AGD resistance has been one of the components of Atlantic salmon industry selective breeding programmes in Tasmania. Knowledge of the actual resistance mechanism is not essential for the success of selection for resistance (Guy *et al.*, 2006). A significant heritable component in AGD resistance, measurable through gross gill scores, was demonstrated in an Atlantic salmon population in Tasmania (Taylor *et al.*, 2007, 2009a, b).

Table 1.2. Experimental evidence for resistance to subsequent AGD infections following previous exposures (adapted from Gross, 2007 and Vincent, 2008).

	Findlay <i>et al.</i> (1995)	Findlay and Munday (1998)		Gross <i>et al.</i> (2004a)	Vincent <i>et al.</i> (2006)
		Trial 1	Trial 2		
Treatment groups	FW ^a maintained ^b FW bathed/SW maintained; naïve	FW bathed; ^b naïve	FW maintained ×2 FW bath, ×1 FW bath; naïve	FW bathed/SW maintained ^b FW maintained; naïve	FW bathed; ^b naïve
Infection method	Cohabitation	Cohabitation	Cohabitation	Inoculation (3300 cells/l)	Inoculation (500 cells/l)
Salinity	Unknown	Unknown	Unknown	36 ppt	35 ppt
Temperature	14°C	14°C	14°C	17°C	12°/16°C
First exposure (weeks)	4	4	4	2	4
FW bath (h)	None	2	2	4	24
Resolution (weeks)	4	4	4	4	5
Second exposure (weeks)	4	4	4	4	5
Assessment of infection	Gross gill score	Gross gill score	Gross gill score	Cumulative mortality, histology	Cumulative mortality, histology

^a FW, Fresh water; SW, sea water.^b Treatment protected from subsequent infection.

The selection trait for AGD resistance utilized in the Tasmanian Atlantic salmon industry breeding programme is gill score at the population average freshwater bathing threshold (Taylor, 2010). There is no relationship between resistance to AGD and specific anti-*Neoparamoeba* antibody titre in both natural and experimental infections (Vincent *et al.*, 2008; Taylor *et al.*, 2009a, b, 2010; Villavedra *et al.*, 2010). It therefore appears that resistance to AGD in Atlantic salmon is most likely multifactorial and under polygenic control (Taylor, 2010).

Other health management strategies used on salmon farms can include: (i) reducing stocking density; (ii) frequent removal of mortalities; (iii) net fouling management; and (iv) fallowing of sites. Lower Atlantic salmon stocking density significantly improved survival of the fish in an experimental AGD challenge, with morbidity starting after 23 days for salmon stocked at 5.0 kg/m³ and after 29 days for salmon stocked at 1.7 kg/m³ (Crosbie *et al.*, 2010b). AGD prevalence was greater in Atlantic salmon farmed in 60 m cages (stocked at 1.7 kg/m³) than 80 m cages (stocked at 0.7 kg/m³) at the beginning of a field experiment (Douglas-Helders *et al.*, 2004). This is consistent with anecdotal information from salmon farms in Tasmania where cages with lower stocking densities require less frequent freshwater bathing (Nowak, 2001). One salmon company in Tasmania uses reduced stocking density in summer (summer average 5–6 kg/m³ with summer maximum at 8 kg/m³; and winter average 7–8 kg/m³ with winter maximum at 12 kg/m³). Removal of dead fish can contribute to reduction of the risks of AGD outbreaks. The amoebae can not only survive on the gills of dead fish for up to 30 h but also colonize salmon gills post-mortem, therefore dead salmon can be a reservoir of the pathogen (Douglas-Helders *et al.*, 2000).

Cage netting and associated fouling were suggested to be reservoirs of amoebae (Nowak, 2001; Tan *et al.*, 2002). There was a negative relationship between the number of net changes and the prevalence of AGD infection (Clark and Nowak, 1999). However, Atlantic salmon in cages treated with copper-based antifouling paint had significantly greater prevalence of AGD infection (Douglas-Helders *et al.*, 2003a, b). This is in contrast to

the results of *in vitro* toxicity tests. Six day exposure to copper sulfate concentrations (ranging from 10 to 100,000 µM) at 20°C significantly reduced survival of gill-isolated amoebae under *in vitro* conditions (Douglas-Helders *et al.*, 2005). This discrepancy could be due to the antifouling paint affecting AGD prevalence through other mechanisms than its toxicity to the amoeba. So far the results of *N. perurans*-specific PCR tests of net fouling have been negative (L. Gonzalez, P.B.B. Crosbie, A.R. Bridle and B.F. Nowak, unpublished) and it is possible that the effects of net fouling on AGD may be site specific (Nowak, 2001).

Fallowing has not been fully investigated as a management strategy. Atlantic salmon from cages which were rotated to other farm sites fallowed for 4–97 days needed fewer freshwater baths, and had greater biomass at the end of the trial than fish grown in stationary cages (Douglas-Helders *et al.*, 2004). While towing cages was considered by the industry as a potential way to reduce infection through increased water flow, a short-term towing experiment did not show any effect on AGD prevalence (Douglas-Helders *et al.*, 2004).

Most experimental studies on AGD are based on mixed-sex diploid Atlantic salmon. However, salmon industries increasingly rely on all female stock and triploid fish to provide whole-year market supply and avoid early maturation. Triploid Atlantic salmon appeared to be more sensitive to AGD on the farms (Nowak, 2001). In an experimental infection the survival of triploid fish was significantly lower and mortality occurred earlier than in diploid Atlantic salmon (Powell *et al.*, 2008). However, this difference was not related to the severity of gill lesions as on day 28 post-infection the triploid fish had a lower percentage of gill filaments affected by AGD than diploid fish (Powell *et al.*, 2008).

1.6. Conclusions and Suggestions for Future Studies

While AGD has been continuously affecting Tasmanian salmon producers, it now appears to be an emerging disease on a global scale. There are increased reports of new geographic

locations and hosts for AGD. This may be related to the intensification of aquaculture (Nowak, 2007) or global climate change (Nowak *et al.*, 2010), or an increased awareness of the disease and improved diagnostic tests. *N. perurans* is a cosmopolitan species and since it has been recently described (Young *et al.*, 2007) very little is known about its biology. Currently our understanding of *N. perurans* is mostly based on extrapolations from our knowledge about other amoebae from the same genus and we do not yet have any evidence that *N. perurans* is free living. On the basis of other species from the same genus and our experience with maintaining *N. perurans* alive *in vitro* over a few weeks (P. Crosbie unpublished), we expect that this species is free living, but this remains to be proven.

The presence of the eukaryotic endosymbiont is one of the characteristics of this species and the genus, as well as for the members of the genus *Paramoeba*. SSU rRNA gene phylogenies of *Neoparamoeba* sp. and its endosymbiont (PLO) strongly supported co-evolution of the amoeba and the endosymbiont (Dyková *et al.*, 2008). However, the role of the endosymbiont, in particular its contribution to pathogenicity of different isolates, is unclear and warrants further investigation.

Co-infections with other parasites were described in some AGD outbreaks (Bustos *et al.*, 2010; Dyková *et al.*, 2010; Nowak *et al.*, 2010), however their significance is unclear. *Uronema marinum* were isolated from gills of a salmon affected by AGD and on rare occasions were seen in histological sections from AGD-affected salmon gills, however its contribution to the gill pathology is unknown (Dyková *et al.*, 2010). Ectoparasites such as sea lice *Lepeophtheirus salmonis* were suggested to be involved in the AGD infection of farmed Atlantic salmon in the USA (Nowak *et al.*, 2010) and co-infection of *N. perurans* and *Caligus rogercresseyi* was reported in Atlantic

salmon in Chile (Bustos *et al.*, 2010). The role of bacteria was evaluated in experimental challenges and in the field (Bowman and Nowak, 2004; Embar-Gopinath *et al.*, 2005, 2006). Exposure to bacteria *Winogradskyella* sp. before exposure to *N. perurans* significantly increased the percentage of affected gill filaments, but the salmon exposed to the amoeba alone still got infected (Embar-Gopinath *et al.*, 2006). Improved understanding of the relationship between the amoeba and other organisms may improve management of this disease. However, numerous experimental challenges showed that *N. perurans* by itself causes AGD (Young *et al.*, 2007; Crosbie *et al.*, 2010b).

While our knowledge of *N. perurans* and AGD has significantly increased during the last 10 years there are still many unanswered questions about the pathogen and the disease. As the disease is increasingly affecting fish farmed in the marine environment, and is one of the more significant emerging diseases in mariculture, further research is necessary to improve our ability to manage AGD.

Acknowledgements

I am grateful to my research students (Honours, Masters and PhD) as well as research and technical staff who all significantly contributed to our knowledge and understanding of AGD. I would like to thank Dr Phil Crosbie, Dr Mark Adams, Dr Benita Vincent, Dr Andrew Bridle, Dr Dina Zilberg and Dr Melanie Leef for their helpful comments on drafts of this chapter. I am also grateful to the salmon industry for providing information on current management strategies. Thanks to Dr Benita Vincent, Dr Philip Crosbie and Karine Cadoret for providing photographs used in this chapter. Financial support was provided by the ARC/NHMRC Network for Parasitology and Australian Academy of Science.

References

- Adams, M.B. and Nowak, B.F. (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 Nowak, B.F. (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 Nowak, B.F. (2004) Experimental amoebic gill disease of Atlantic salmon, *Salmo salar* L.: further evidence for the primary pathogenic role of *Neoparamoeba* sp., (Page, 1987). *Journal of Fish Diseases* 27, 105–113.
- Adams, M.B., Ellard, K. and Nowak, B.F. (2004) Gross pathology and its relationship with histopathology of amoebic gill disease (AGD) in farmed Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 27, 151–161.
- Adams, M.B., Villavedra, M. and Nowak, B.F. (2008) An opportunistic detection of amoebic gill disease (AGD) in blue warehou (*Seriola lalandi* Günther) collected from an Atlantic salmon (*Salmo salar* L.) production cage in south eastern Tasmania. *Journal of Fish Diseases* 31, 713–717.
- Adl, S.M., Simpson, A.G.B., Farmer, M.A., Anderson, R.A., Anderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G., Fensome, R.A., Frederico, S., James, T.Y., Karpov, S., Kugrens, P., Krug, J., Lane, C.E., Lewis, L.A., Lodge, J., Lynn, D.H., Mann, D.G., McCourt, R.M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S.E., Nerad, T.A., Shearer, C.A., Smirnov, A.V., Spiegel, F.W. and Taylor, M.F.J.R. (2005) The new higher level classification of Eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology* 52, 399–455.
- Bowman, J. and Nowak, B. (2004) Salmonid gill bacteria and their relationship to amoebic gill disease (AGD). *Journal of Fish Diseases* 27:483–492.
- Bridle, A.R., Butler, R. and Nowak, B.F. (2003) Immunostimulatory CpG oligodeoxynucleotides increase resistance against amoebic gill disease in Atlantic salmon, *Salmo salar* L.. *Journal of Fish Diseases* 26, 367–371.
- Bridle, A.R., Carter, C.G., Morrison, R.N. and Nowak, B.F. (2005) The effects of beta-glucan administration on macrophage respiratory burst activity and Atlantic salmon (*Salmo salar* L.) challenged with amoebic gill disease (AGD) – evidence of inherent resistance. *Journal of Fish Diseases* 28, 347–356.
- Bridle, A.R., Morrison, R.N. and Nowak, B.F. (2006a) The expression of immune-regulatory genes in rainbow trout, *Oncorhynchus mykiss*, during an amoebic gill disease (AGD) infection. *Fish and Shellfish Immunology* 20, 346–364.
- Bridle, A., Morrison, R., Cupit Cunningham, P.M. and Nowak, B. (2006b) Quantitation of immune response gene expression and cellular localisation of interleukin-1 β mRNA in Atlantic salmon, *Salmo salar* L., affected by amoebic gill disease (AGD). *Veterinary Immunology and Immunopathology* 114, 121–134.
- Bridle, A.R., Crosbie, P.B.B., Cadoret, K. and Nowak, B.F. (2010) Rapid detection and quantification of *Neoparamoeba perurans* in the marine environment. *Aquaculture* 301, 56–61.
- Bustos, P.A., Young, N.D., Rozas, M.A., Bohle, H.M., Ildefonso, R.S., Morrison, R.N. and Nowak, B.F. (2010) Amoebic gill disease (AGD) in Atlantic salmon (*Salmo salar*) farmed in Chile. *Aquaculture* 310, 281–288.
- Clark, A. and Nowak, B.F. (1999) Field investigations of amoebic gill disease in Atlantic salmon, *Salmo salar* L., in Tasmania. *Journal of Fish Diseases* 22, 433–443.
- Cook, M., Elliott, N., Campbell, G., Patil, J., Holmes, B., Lim, V. and Prideaux, C. (2008) *Amoebic Gill Disease (AGD) Vaccine Development Phase II – Molecular Basis of Host–Pathogen Interactions in Amoebic Gill Disease*. Aquafin Cooperative Research Centre for Sustainable Aquaculture of Finfish project 3.4.4 (2) (Fisheries Research and Development Corporation project 2004/217). Commonwealth Scientific and Industrial Research Organisation (CSIRO), Hobart, Tasmania, Australia, 85 pp.
- Crosbie, P.B.B., Nowak, B.F. and Carson, J. (2003) Isolation of *Neoparamoeba pemaquidensis* Page, 1987 from marine and estuarine sediments in Tasmania. *Bulletin of European Association of Fish Pathologists* 23, 241–244.
- Crosbie, P.B.B., Macload, C., Forbes, S. and Nowak B. (2005) Distribution of *Neoparamoeba* sp. in sediments around marine finfish farming sites in Tasmania. *Diseases of Aquatic Organisms* 67, 61–66.
- Crosbie, P.B.B., Ogawa, K., Nakano, D. and Nowak, B.F. (2010a) Amoebic gill disease in hatchery-reared ayu, *Plecoglossus altivelis* (Temminck and Schlegel), in Japan is caused by *Neoparamoeba perurans*. *Journal of Fish Diseases* 33, 455–458.
- Crosbie, P.B.B., Bridle, A.R., Leef, M.J. and Nowak, B.F. (2010b) Effects of different batches of *Neoparamoeba perurans* and fish stocking densities on the severity of amoebic gill disease in Atlantic salmon, *Salmo salar* L. 41, e505–e516.
- Douglas-Helders, M., Nowak, B., Zilberg, D. and Carson, J. (2000) Survival of *Paramoeba pemaquidensis* on dead salmon: implications for management of cage hygiene. *Bulletin of European Association of Fish Pathologists* 20, 167–169.
- Douglas-Helders, M., Saksida, S., Raverty, S. and Nowak, B.F. (2001a) Temperature as a risk factor for outbreaks of amoebic gill disease in farmed Atlantic salmon (*Salmo salar*). *Bulletin of European Association of Fish Pathologists* 21, 114–116.

- Douglas-Helders, M., Carson, J., Howard, T. and Nowak, B. (2001b) 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., Tan, C.F.K., Carson, J. and Nowak, B.F. (2003a) Effects of copper-based antifouling treatment on the presence of *Neoparamoeba pemaquidensis* Page 1987 on nets and gills of reared Atlantic salmon (*Salmo salar*). *Aquaculture* 221, 13–22.
- Douglas-Helders, M., O'Brien, D.P., McCorkell, B.E., Zilberg, D., Gross, A., Carson, J. and Nowak, B. (2003b) Temporal and spatial distribution of paramoebae in the water column – a pilot study. *Journal of Fish Diseases* 26, 231–240.
- Douglas-Helders, G.M., Weir, I.J., O'Brien, D.P., Carson, J. and Nowak, B.F. (2004) Effects of husbandry on prevalence of amoebic gill disease and performance of reared Atlantic salmon (*Salmo salar* L.). *Aquaculture* 241, 21–30.
- Douglas-Helders, M., Nowak, B. and Butler, R. (2005) The effect of environmental factors on the distribution of *Neoparamoeba pemaquidensis* in Tasmania. *Journal of Fish Diseases* 28, 583–592.
- Dyková, I. and Novoa, B. (2001) Comments on diagnosis of amoebic gill in turbot (*Scophthalmus maximus*). *Bulletin of the European Association of Fish Pathologists* 21, 40–44.
- Dyková, I., Figueras, A., Novoa, B. and Casal, J.F. (1998) *Paramoeba* sp., an agent of amoebic gill disease of turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms* 33, 137–141.
- Dyková, I., Figueras, A. and Peric, Z. (2000) *Neoparamoeba* Page 1987: light and electron microscopic observations on six strains of different origin. *Diseases of Aquatic Organisms* 43, 217–223.
- Dyková, I., Fiala, I., Lom, J. and Lukeš, J. (2003) *Perkinsiella amoebae*-like endosymbionts of *Neoparamoeba* spp., relatives of the kinetoplastid *Ichthyobodo*. *European Journal of Protistology* 39, 37–52.
- Dyková, I., Nowak, B.F., Crosbie, P.B.B., Fiala, I., Pecková, H., Adams, M., Macháčkova, B. and Dvořáková, H. (2005) *Neoparamoeba branchiphila* n. sp. and related species of genus *Neoparamoeba* Page, 1987: morphological and molecular characterisation of selected strains. *Journal of Fish Diseases* 28, 49–64.
- Dyková, I., Nowak, B., Pecková, H., Fiala, I., Crosbie, P. and Dvořáková, H. (2007) Phylogeny of *Neoparamoeba* strains isolated from marine fish and invertebrates as inferred from SSU rDNA sequences. *Diseases of Aquatic Organisms* 74, 57–65.
- Dyková, I., Fiala, I. and Pecková, H. (2008) *Neoparamoeba* spp. and their eukaryotic endosymbionts similar to *Perkinsella amoebae* (Hollande, 1980): coevolution demonstrated by SSU rRNA gene phylogenies. *European Journal of Protistology* 44, 269–277.
- Dyková, I., Tým, T., Kostka, M. and Pecková, H. (2010) Strains of *Uronema marinum* (Scuticociliatia) co-isolated with amoebae of the genus *Neoparamoeba*. *Diseases of Aquatic Organisms* 89, 71–77.
- Embar-Gopinath, S., Butler, R. and Nowak, B. (2005) Influence of salmonid gill bacteria on development and severity of amoebic gill disease. *Diseases of Aquatic Organisms* 67, 55–60.
- Embar-Gopinath, S., Crosbie, P. and Nowak, B.F. (2006) Concentration effects of *Winogradskyella* sp. on the incidence and severity of amoebic gill disease. *Diseases of Aquatic Organisms* 73, 43–47.
- Findlay, V.L. and Munday, B.L. (1998) Further studies on acquired resistance to amoebic gill disease (AGD) in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 21, 121–125.
- Findlay, V., Helders, M., Munday, B.L. and Gurney, R. (1995) Demonstration of resistance to reinfection with *Paramoeba* sp. by Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 18, 639–642.
- Fisk, D.M., Powell, M.D. and Nowak, B.F. (2002) The effect of amoebic gill disease and hypoxia on survival and metabolic rate of Atlantic salmon (*Salmo salar*). *Bulletin of European Association of Fish Pathologists* 22, 190–194.
- Florent, R.L., Becker, J. and Powell, M.D. (2007a) Evaluation of bithionol as a bath treatment for amoebic gill disease caused by *Neoparamoeba* spp. *Veterinary Parasitology* 144, 197–207.
- Florent, R.L., Becker, J. and Powell, M.D. (2007b) Efficacy of bithionol as an oral treatment for amoebic gill disease in Atlantic salmon *Salmo salar* (L.). *Aquaculture* 270, 15–22.
- Florent, R.L., Becker, J. and Powell, M.D. (2009) Further development of bithionol therapy as a treatment for amoebic gill disease in Atlantic salmon, *Salmo salar*. *Journal of Fish Diseases* 32, 391–400.
- Gross, K.A. (2007) Interactions between *Neoparamoeba* spp. and Atlantic salmon (*Salmo salar* L.) immune system components. PhD thesis, University of Tasmania, Launceston, Tasmania, Australia.
- Gross, K., Morrison, R.N., Butler, R. and Nowak, B.F. (2004a) Atlantic salmon (*Salmo salar* L.) previously infected with *Neoparamoeba* sp. are not resistant to re-infection and have suppressed macrophage function. *Journal of Fish Diseases* 27, 47–56.
- Gross, K., Carson, J. and Nowak, B.F. (2004b) The presence of anti-*Neoparamoeba* sp. antibodies in Tasmanian cultured Atlantic salmon (*Salmo salar* L.). *Journal of Fish Diseases* 27, 81–88.

- Gross, K.A., Powell, M.D., Butler, R., Morrison, R.N. and Nowak, B.F. (2005) Changes in the innate immune response of Atlantic salmon (*Salmo salar*) exposed to experimental infection with *Neoparamoeba* sp. *Journal of Fish Diseases* 28, 293–299.
- Guy, D.R., Bishop, S.C., Brotherstone, S., Hamilton, A., Roberts, R.J., McAndrew, B.J. and Woolliams, J.A. (2006) Analysis of the incidence of infectious pancreatic necrosis mortality in pedigreed Atlantic salmon, *Salmo salar* L., populations. *Journal of Fish Diseases* 29, 637–647.
- Harris, J.O., Powell, M.D., Attard, M. and Green, T.J. (2004) Efficacy of chloramines-T as a treatment for amoebic gill disease (AGD) in marine Atlantic salmon (*Salmo salar* L.) *Aquaculture Research* 35, 1448–1456.
- Harris, J.O., Powell, M.D., Attard, M.G. and Dehayr, L. (2005) Clinical assessment of chloramines-T and freshwater treatments for the control of gill amoebae in Atlantic salmon, *Salmo salar* L. *Aquaculture Research* 36, 776–784.
- Howard, T.S., Carson, J. and Lewis, T. (1993) Development of a model of infection for amoebic gill disease. In: Valentine, P. (ed.) *Salmon Enterprises of Tasmania (SALTAS) Research and Development Seminar*. SALTAS, Hobart, Tasmania, pp. 103–111.
- Jones, G.M. and Scheibling, R.E. (1985) *Paramoeba* sp. (Amebida, Paramoebidae) as the possible causative agent of sea-urchin mass mortality in Nova Scotia. *Journal of Parasitology* 71, 559–565.
- Kent, M.L., Sawyer, T.K. and Hedrick, R.P. (1988) *Paramoeba pemaquidensis* (Sarcocystidophora: Paramoebidae) infestation of the gills of coho salmon *Oncorhynchus kisutch* reared in sea water. *Diseases of Aquatic Organisms* 5, 163–169.
- Leef, M.J., Harris, J.O. and Powell, M.D. (2005a) Respiratory pathogenesis of amoebic gill disease (AGD) in experimentally infected Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 66, 205–213.
- Leef, M.J., Harris, J.O., Hill, J. and Powell, M.D. (2005b) Cardiovascular responses of three salmonid species affected with amoebic gill disease (AGD). *Journal of Comparative Physiology B – Biochemical Systemic and Environmental Physiology* 175, 523–532.
- Leef, M.J., Harris, J.O. and Powell, M.D. (2007) Metabolic effects of amoebic gill disease (AGD) and chloramine-T exposure in seawater-acclimated Atlantic salmon *Salmo salar*. *Disease of Aquatic Organisms* 78, 37–44.
- Lovy, J., Becker, J.A., Speare, D.J., Wadowska, D.W., Wright, G.M. and Powell, M.D. (2007) Ultrastructural examination of the host cellular response in the gills of Atlantic salmon, *Salmo salar*, with amoebic gill disease. *Veterinary Pathology* 44, 663–671.
- Moran, D.M., Anderson, O.R., Dennett, M.R., Caron, D.A. and Gast, R.J. (2007) A description of seven Antarctic marine Gymnamoebae including a new subspecies, two new species and a new genus: *Neoparamoeba aestuarina antarctica* n. subsp., *Platyamoeba oblongata* n. sp., *Platyamoeba contorta* n. sp. and *Vermistella antarctica* n. gen. n. sp. *Journal of Eukaryotic Microbiology* 54, 169–183.
- Morrison, R.N. and Nowak, B.F. (2005) Bath treatment of Atlantic salmon (*Salmo salar*) with amoebae antigens fails to affect survival to subsequent amoebic gill disease (AGD) challenge. *Bulletin of European Association of Fish Pathologists* 25, 155–160.
- Morrison, R.N., Crosbie, P.B.B. and Nowak, B.F. (2004) The induction of laboratory-based amoebic gill disease (AGD) revisited. *Journal of Fish Diseases* 27, 445–449.
- Morrison, R.N., Crosbie, P., Adams, M.B., Cook M.T. and Nowak, B.F. (2005) Cultured gill derived *Neoparamoeba pemaquidensis* fail to elicit AGD in Atlantic salmon (*Salmo salar*). *Diseases of Aquatic Organisms* 66, 135–144.
- Morrison, R.N., Koppang, E.O., Hordvik, I. and Nowak, B.F. (2006a) MHC class II⁺ cells in the gills of salmon experimentally infected with amoebic gill disease. *Veterinary Immunology and Immunopathology* 109, 297–303.
- Morrison, R.N., Cooper, G.A., Koop, B.F., Rise, M.L., Bridle, A.R., Adams, M.B. and Nowak, B.F. (2006b) Transcriptome profiling of the gills of amoebic gill disease (AGD)-affected Atlantic salmon (*Salmo salar* L.) – a role for the tumor suppressor protein p53 in AGD-pathogenesis? *Physiological Genomics* 26, 15–34.
- Morrison, R.N., Zou, J., Secombes, C.J., Scapigliatti, G., Adams, M.B. and Nowak, B.F. (2007) Molecular cloning and expression analysis of tumor necrosis factor- α in amoebic gill disease (AGD)-affected Atlantic salmon (*Salmo salar* L.). *Fish and Shellfish Immunology* 23, 1015–1031.
- Mullen, T.E., Nevis, K.R., O'Kelly, C.J., Gast, R.J. and Frasca, S. (2005) Nuclear small-subunit ribosomal RNA gene-based characterisation, molecular phylogeny and PCR detection of the *Neoparamoeba* from western Long Island Sound lobster. *Journal of Shellfish Research* 24, 719–731.

- Munday, B.L. (1986) Diseases of salmonids. In: Humphrey, J.D. and Langdon, J.S. (eds) *Proceedings of the Workshop on Diseases of Australian Fish and Shellfish*. Department of Agriculture and Rural Affairs, Benalla, Victoria, Australia, pp. 127–141.
- Munday, B.L. and Zilberg, D. (2003) Efficacy of, and toxicity associated with, the use of levamisole in sea-water to treat amoebic gill disease. *Bulletin of the European Association of Fish Pathologists* 23, 3–6.
- Munday, B.L., Foster, C.K., Roubal, F.R. and Lester, R.J.G. (1990) Paramoebic gill infection and associated pathology of Atlantic salmon, *Salmo salar*, and rainbow trout, *Salmo gairdneri*, in Tasmania. In: Perkins, F.O. and Cheng, T.C. (eds) *Pathology in Marine Science*. Academic Press, London, pp. 215–222.
- Munday, B.L., Lange, K., Foster, C., Lester, R.J.G. and Handler, J. (1993) Amoebic gill disease of sea-caged salmonids in Tasmanian waters. *Tasmanian Fisheries Research* 28, 14–19.
- Munday, B.L., Zilberg, D. and Finlay, V. (2001) Gill disease of marine fish caused by infection with *Neoparamoeba pemaquidensis*. *Journal of Fish Diseases* 24, 497–507.
- Nowak, B. (2001) Qualitative evaluation of risk factors for amoebic gill disease in cultured Atlantic salmon. In: Rodgers, C.J. (ed.) *Risk Analysis in Aquatic Animal Health*. World Organisation for Animal Health, Paris, France, pp. 158–154.
- Nowak, B.F. (2007) Parasitic diseases in marine cage culture – an example of experimental evolution of parasites? *International Journal for Parasitology* 37, 581–588.
- Nowak, B.F. and Munday, B.L. (1994) Histology of gills of Atlantic salmon during the first few months following transfer to sea water. *Bulletin of European Association of Fish Pathologists* 14(3), 77–81.
- Nowak, B.F., Powell, M.D., Carson, J. and Dykova, I. (2002) Amoebic gill disease in the marine environment. *Bulletin of European Association of Fish Pathologists* 22, 144–147.
- Nowak, B.F., Dawson, D., Basson, L., Deveney, M. and Powell, M.D. (2004) Gill histopathology of wild marine fish in Tasmania – potential interactions with gill health of cultured Atlantic salmon (*Salmo salar* L.). *Journal of Fish Diseases* 27, 709–717.
- Nowak, B.F., Bryan, J. and Jones, S. (2010) A role of sea lice *Lepeophtheirus salmonis* in the epidemiology of amoebic gill disease caused by *Neoparamoeba perurans*? *Journal of Fish Diseases* 33, 683–687.
- Nylund, A., Watanabe, K., Nylund, S., Karlsen, M., Sæther, P.A., Arnesen, C.E. and Karlsbakk, E. (2008) Morphogenesis of salmonid gill poxvirus associated with proliferative gill disease in farmed Atlantic salmon (*Salmo salar*) in Norway. *Archives of Virology* 153, 1299–1309.
- Page, F.C. (1973) *Paramoeba*: a common marine genus. *Hydrobiologia* 41, 183–188.
- Page, F.C. (1974) *Rosculus ithacus* Hawes, 1963, Amoebeida, Flabellulidea and the amphizoic tendency in amoebae. *Acta Protozoologica* 13, 143–154.
- Page, F.C. (1983) *Marine Gymnamoebae*. Institute of Terrestrial Ecology, Culture Centre of Algae and Protozoa, Cambridge, UK, 54 pp.
- Page, F.C. (1987) The classification of ‘naked’ amoebae of phylum Rhizopoda. *Archives of Protistenkd* 133, 199–217.
- Palmer, R., Carson, J., Rutledge, M., Drinan, E. and Wagner, T. (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., Powell, M., Fisk, D. and Nowak, B. (2001) Effectiveness of commercial freshwater bathing as a treatment against amoebic gill disease in Atlantic salmon. *Aquaculture* 195, 205–210.
- Powell, M., Fisk, D. and Nowak, B. (2000) Effects of graded hypoxia on Atlantic salmon (*Salmo salar* L.) infected with amoebic gill disease (AGD). *Journal of Fish Biology* 57, 1047–1057.
- Powell, M.D., Parsons, H.J. and Nowak, B.F. (2001) Physiological effects of freshwater bathing of Atlantic salmon (*Salmo salar*) as a treatment for amoebic gill disease. *Aquaculture* 199, 259–266.
- Powell, M.D., Nowak, B.F. and Adams, M. (2002) Cardiac morphology in relation to amoebic gill disease history in Atlantic salmon (*Salmo salar* L.). *Journal of Fish Disease* 25, 209–215.
- Powell, M.D., Attard, M., Harris, J., Roberts, S.D. and Leef, M.J. (2005) Why fish die – treatment and pathophysiology of AGD. University of Tasmania, Launceston, Tasmania, Australia (ISBN 1 86295 259 0).
- Powell, M.D., Leef, M.J., Roberts, S.D. and Jones, M.A. (2008) Neoparamoebic gill infections: host response and physiology of salmonids. *Journal of Fish Biology* 73, 2161–2183.
- Roberts, S.D. and Powell, M.D. (2003) Reduced total hardness of fresh water enhanced the efficacy of bathing as a treatment against amoebic gill disease in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 26, 591–599.
- Roberts, S.D. and Powell, M.D. (2005) Oral L-cysteine ethyl ester (LCEE) reduces amoebic gill disease (AGD) in Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 66, 21–28.

- Rodger, H.D. and McArdle, J.F. (1996) An outbreak of amoebic gill disease in Ireland. *Veterinary Record* 139, 348–349.
- Steinum, T., Kvellstad, A., Rønneberg, L.B., Nilsen, H., Asheim, A., Fjell, K., Nygård, S.M.R., Olsen, A.B. and Dale, O.B. (2008) First case of amoebic gill disease (AGD) in Norwegian seawater farmed Atlantic salmon, *Salmo salar* L., and phylogeny of the causative amoeba using 18S cDNA sequences. *Journal of Fish Diseases* 31, 205–214.
- Tan, C., Nowak, B.F. and Hodson, S.L. (2002) Biofouling as a reservoir of *Neoparamoeba pemaquidensis* (Page 1970), the causative agent of amoebic gill disease in Atlantic salmon. *Aquaculture* 210, 49–58.
- Taylor, R.S. (2010) Assessment of resistance to amoebic gill disease in the Tasmanian Atlantic salmon selective breeding population. PhD thesis, University of Tasmania, Launceston, Tasmania, Australia.
- Taylor, R.S., Wynne, J.W., Kube, P.D. and Elliott, N.G. (2007) Genetic variation of resistance to amoebic gill disease in Atlantic salmon (*Salmo salar*) assessed in a challenge system. *Aquaculture* 272, S94–S99.
- Taylor, R.S., Kube, P.D., Muller, W.J. and Elliott, N.G. (2009a) Genetic variation of gross gill pathology and survival of Atlantic salmon (*Salmo salar* L.) during natural amoebic gill disease challenge. *Aquaculture* 294, 172–179.
- Taylor, R.S., Muller, W.J., Cook, M.T., Kube, P.D. and Elliott, N.G. (2009b) Gill observations in Atlantic salmon (*Salmo salar*, L.) during repeated amoebic gill disease (AGD) field exposure and survival challenge. *Aquaculture* 290, 1–8.
- Taylor, R.S., Crosbie, P.B. and Cook, M.T. (2010) Amoebic gill disease resistance is not related to the systemic antibody response of Atlantic salmon (*Salmo salar*, L.). *Journal of Fish Diseases* 33, 1–14.
- Ullal, A.J. and Noga, E.J. (2010) Antiparasitic activity of the antimicrobial peptide Hb beta P-1, a member of the beta-haemoglobin peptide family. *Journal of Fish Diseases* 33, 657–664.
- Ullal, A.J., Litaker, R.W. and Noga, E.J. (2008) Antimicrobial peptides derived from hemoglobin are expressed in epithelium of channel catfish (*Ictalurus punctatus*, Rafinesque). *Developmental and Comparative Immunology* 32, 1301–1312.
- Villavedra, M., To, J., Lemke, S., Birch, D., Crosbie, P., Adams, M., Broady, K., Nowak, B., Raison, R.L. and Wallach, M. (2010) Characterisation of an immunodominant, high molecular weight glycoprotein on the surface of infectious *Neoparamoeba* spp., causative agent of amoebic gill disease (AGD) in Atlantic salmon. *Fish and Shellfish Immunology* 29, 946–955.
- Vincent, B.N. (2008) Amoebic gill disease of Atlantic salmon: resistance, serum antibody response and factors that may affect disease severity. PhD thesis, University of Tasmania, Launceston, Tasmania, Australia.
- Vincent, B.N., Morrison, R.N. and Nowak, B.F. (2006) Amoebic gill disease (AGD)-affected Atlantic salmon *Salmo salar* L. are resistant to subsequent AGD challenge. *Journal of Fish Diseases* 29, 549–559.
- Vincent, B.N., Adams, M.B., Crosbie, P.B.B., Nowak, B.F. and Morrison, R.N. (2007) Atlantic salmon (*Salmo salar* L.) exposed to cultured gill-derived *Neoparamoeba branchiphila* fail to develop amoebic gill disease (AGD). *Bulletin of the European Association of Fish Pathologists* 27, 112–115.
- Vincent, B.N., Nowak, B.F. and Morrison, R.N. (2008) Detection of serum anti-*Neoparamoeba* spp. antibodies in amoebic gill disease affected Atlantic salmon. *Journal of Fish Biology* 73, 429–435.
- Vincent, B.N., Adams, M.B., Nowak, B.F. and Morrison, R.N. (2009) Cell surface carbohydrate antigen(s) of wild type *Neoparamoeba* spp are immunodominant in sea-cage cultured Atlantic salmon (*Salmo salar* L.) affected by amoebic gill disease (AGD). *Aquaculture* 288, 153–158.
- Young, N.D., Crosbie, P.B.B., Adams, M.B., Nowak, B.F. and Morrison, R.N. (2007) *Neoparamoebae perurans* n. sp., an agent of amoebic gill disease of Atlantic salmon (*Salmo salar*). *International Journal of Parasitology* 37, 1469–1481.
- Young, N.D., Dyková, I., Snekvik, K., Nowak, B.F. and Morrison, R.N. (2008a) *Neoparamoeba perurans* is a cosmopolitan aetiological agent of amoebic gill disease. *Diseases of Aquatic Organisms* 78, 217–223.
- Young, N.D., Dyková, I., Nowak, B.F. and Morrison, R.N. (2008b) Development of a diagnostic PCR to detect *Neoparamoeba perurans*, agent of amoebic gill disease (AGD). *Journal of Fish Diseases* 31, 285–295.
- Young, N.D., Cooper, G.A., Nowak, B.F., Koop, B.F. and Morrison, R.N. (2008c) Coordinated down-regulation of the antigen processing machinery in the gills of amoebic gill disease-affected Atlantic salmon (*Salmo salar*). *Molecular Immunology* 45, 1469–1481.
- Zilberg, D. and Munday, B.L. (2000) Pathology of experimental amoebic gill disease in Atlantic salmon (*Salmo salar* L.) and the effect of pre-maintenance of fish in seawater on the infection. *Journal of Fish Diseases* 23, 401–407.
- Zilberg, D. and Munday, B.L. (2001) Response of Atlantic salmon (*Salmo salar* L.) to *Paramoeba* antigens administered by a variety of routes. *Journal of Fish Diseases* 24, 181–183.

- Zilberg, D., Nowak, B., Carson, J. and Wagner, T. (1999) Simple gill smear staining for diagnosis of amoebic gill disease. *Bulletin of European Association of Fish Pathologists* 19, 186–189.
- Zilberg, D., Findlay, V.L., Girling, P. and Munday, B.L. (2000) Effects of treatment with levamisole and glucans on mortality rates in Atlantic salmon (*Salmo salar* L.) suffering from amoebic gill disease. *Bulletin of the European Association of Fish Pathologists* 20, 23–27.
- Zilberg, D., Gross, A. and Munday, B.L. (2001) Production of salmonid amoebic gill disease by exposure to *Paramoeba* sp. harvested from the gills of infected fish. *Journal of Fish Diseases* 24, 79–82.

2 *Amyloodinium ocellatum*

Edward J. Noga

South Eastern Aquatechnologies, Inc., Marathon, Florida, USA

2.1. Introduction

Amyloodinium ocellatum is a dinoflagellate, and the great majority of dinoflagellates are primary producers and consumers in aquatic food webs. A few are endosymbionts in invertebrates (Fensome *et al.*, 1993), while others produce ichthyotoxins, which may kill fish (Rensel and Whyte, 2003). Some are parasites, mainly of invertebrates (Coats, 1999), but only six or so genera are fish parasites. Of these, the monospecific genus *Amyloodinium* is by far the most important member (Noga and Levy, 2006).

Amyloodinium has a direct, but triphasic life cycle. The parasites feed as stationary trophozoites (trophonts) on the epithelial surfaces of the skin and gills. Trophonts remain attached to the fish by root-like structures (rhezoids) that firmly anchor the parasite to the epithelium. After reaching maturity, the trophont detaches from the host, forming a reproductive 'cyst' or tomonet in the substrate. This tomonet divides, forming up to several dozen free-swimming individuals (dinospores) that can then infest a new host (Noga, 1987).

A. ocellatum (Fig. 2.1) causes serious morbidity and mortality in both brackish and marine warm-water food fishes at aquaculture facilities worldwide (Noga and Levy, 2006) and is often considered the most

consequential pathogen of marine fish (Paperna *et al.*, 1981). Outbreaks can occur extremely rapidly, resulting in 100% mortality within a few days. *A. ocellatum* is also a major problem in aquarium fish (Lawler, 1977b), including both public aquaria and hobbyist tanks. It rarely causes natural epidemics; the best documented outbreak was in fish in a hypersaline inland lake (Salton Sea) in eastern California, USA (Kuperman *et al.*, 2001). Almost all fish (more than 100 species) that live within the ecological range of *Amyloodinium* are susceptible to infestation. It is one of the few fish parasites that can infest both elasmobranchs and teleosts (Lawler, 1980).

2.2. Diagnosis of the Infection

For classical diagnosis of *Amyloodinium*, parasites are visualized on infested tissues under a microscope. Fish are best examined while still living or immediately after death, as parasites often detach shortly after host death. At diagnosis it is important to obtain an accurate estimate of the severity of infestation. Gross skin infestations are most easily seen on dark-coloured fish. With the naked eye, parasites are best observed using indirect illumination, such as by shining a flashlight on top of the fish in a darkened room. Observing fish against a dark background also helps. While



Fig. 2.1. *Amyloodinium* trophonts (arrows) on a damselfish (*Dacyllus* sp.) fin.

presumptive diagnosis of infestation may sometimes be made from the gross clinical appearance (e.g. 'velvet'), microscopic identification of trophonts or tomonts is required for definitive diagnosis. If fish are small, they can be restrained in a dish of water, and eyes, skin and fins examined under a dissecting microscope. Lifting the operculum allows examination of the gills. Trophonts can be removed by gently brushing or scraping the skin or gills, followed by microscopic examination of the sediment, which contains detached parasites. However, it is best to observe trophonts in their diagnostic attachment to epithelium (Fig. 2.1). Snips of gill are also removed from living or recently dead fish for examination (Lawler, 1977b, 1980; Noga, 2010). Staining the skin or gill tissue with dilute Lugol's iodine also helps to visualize the parasites, since the iodine reacts with the starch-containing parasites.

A freshwater bath will dislodge *Amyloodinium* and is especially useful for small fish. Fish are placed in a beaker of fresh water for 1–3 min. After 15–20 min, tomonts settle to the bottom of the beaker. Trophonts can be detected using a dissecting or inverted microscope (Bower *et al.*, 1987). Sometimes *Amyloodinium* tomonts are sensitive to fresh water and may begin to lyse (E. Noga, unpublished data), so samples should be examined quickly after the bath. Interestingly, the kinetoplastid flagellate parasite *Ichthyobodo* is detached from fish by treatment with tricaine anesthetic in poorly buffered water (Callahan and Noga, 2002). Whether tricaine has the same effect on ectoparasitic dinoflagellates is unknown. Thus, while histopathology can be used for diagnosis (Fig. 2.2), some and possibly many trophonts will dislodge during fixation, making it difficult to gauge the severity of infestation.

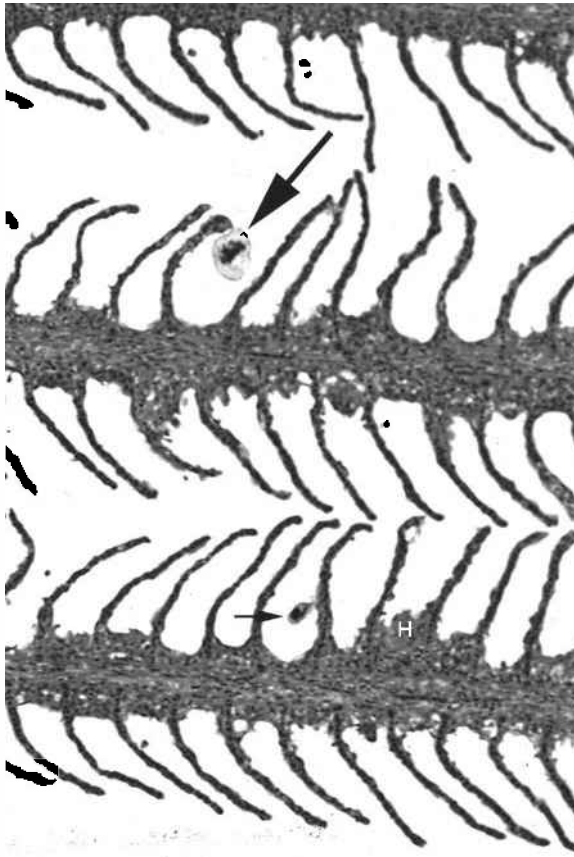


Fig. 2.2. Histological section of gill infested with *Amyloodinium*. Note the variably-sized trophonts (arrows), probably due to individual parasites having infested the host at different points in time. Note also that the larger trophont (large arrow) does not appear to be attached to the gill, but this is an artefact because the attachment site was not cut in the histological section. There is some lamellar epithelial hyperplasia (H) between the secondary lamellae.

Sequencing of the small-subunit ribosomal RNA (SSU rRNA) genes from three geographic isolates of *A. ocellatum* (DC-1, Gulf of Mexico (Florida) and Red Sea) revealed very high sequence identity (Levy *et al.*, 2007). Consensus *Amyloodinium*-specific oligonucleotide primers in a PCR assay could detect as few as ten dinospores/ml of water. This method potentially allows for highly sensitive monitoring of pathogen load in susceptible fish populations. Another attempt has been made to monitor dinospore concentrations during a spontaneous epidemic (Abreu *et al.*, 2005). High concentrations of what were presumed to be *Amyloodinium*

dinospores (as high as 7000/l) were observed in tanks having infested fish. However, since only Lugol's iodine-stained specimens were examined using routine light microscopy, and no molecular probes were used for definitive identification, these findings require confirmation.

Fish that are recovering from spontaneous *Amyloodinium* infestation or that have been experimentally exposed to parasite antigen may produce detectable serum antibody (Smith *et al.*, 1992; Cobb *et al.*, 1998a, b; Cecchini *et al.*, 2001), which might be useful for monitoring levels of protection in susceptible populations, since elevated antibody titres

have been associated with resistance (Cobb *et al.*, 1998a, b).

2.3. External/Internal Lesions

Clinical signs of amyloodiniosis include anorexia, depression, dyspnea and pruritis (Lawler, 1977a, b; Noga, 2010). The gills are usually the primary site of infestation, but heavy infestations may also involve the skin, fins and eyes. Heavily infested skin may have a dusty appearance consequently the disease is sometimes called 'velvet disease', but this is an uncommon finding and fish often die without obvious gross skin lesions. Young fish appear to be most susceptible, although there is little hard data in this area. Trophonts may also occur on the pseudobranch, branchial cavity and nasal passages (Lawler, 1980).

Mild infestations (e.g. one or two trophonts per gill filament) cause little pathology. However, heavy infestations (up to 200 trophonts per gill filament) cause serious gill hyperplasia (Fig. 2.2), inflammation, haemorrhage and necrosis. Death is usually attributed to anoxia and can occur within 12 h with an especially heavy infestation (Lawler, 1980). In contrast, acute mortalities are sometimes associated with apparently mild infestations suggesting that hypoxia may not always be the cause of death. Osmoregulatory impairment and secondary microbial infections due to severe epithelial damage may also be important causes of debilitation and death.

2.4. Pathophysiology

The key lesion caused by *Amyloodinium* is destruction of epithelial cells of the skin and gills (Noga, 1987) and clinical signs are directly proportional to epithelial damage. A single trophont can feed on multiple epithelial cells simultaneously (Paperna, 1980; Noga, 1987). While it has not been documented, the cause of death is probably osmotic imbalance in most cases, although secondary infections may also occur.

Temperature and salinity are the primary environmental modulators of *Amyloodinium*

pathogenicity, with greater virulence at higher temperatures (Paperna, 1980; Kuperman *et al.*, 2001); thus, in more temperate regions, it is only a problem in warmer months (Noga *et al.*, 1991; Kuperman and Matey, 1999). Optimal temperature has not been determined for most isolates but it probably ranges from about 23 to 28°C. Reproduction stops at about 15–17°C. Geographic isolates vary greatly in salinity tolerance, with tolerance appearing to reflect the ambient environmental conditions. For example, Red Sea isolates (a high salinity sea) can sporulate at up to 50 ppt salinity, but cannot reproduce at <12 ppt salinity (Paperna, 1984). In contrast, isolates from the estuarine regions in the Gulf of Mexico can cause epidemics at 3 ppt salinity (Lawler, 1977b). Temperature can affect salinity tolerance, which usually narrows as one deviates further from the optimal temperature range. However, *Amyloodinium* in the Salton Sea was most pathogenic when temperatures were very high (39–41°C), even though the salinity was also very high (46 ppt) (Kuperman and Matey, 1999; Kuperman *et al.*, 2001). Other risk factors have not been well studied, although low dissolved oxygen has been associated with outbreaks of some epidemics (Sandifer *et al.*, 1993; Kuperman *et al.*, 2001). Anecdotal observations have also suggested that parasite prevalence in wild fish populations increased after the stressful event of a hurricane (Overstreet, 2007).

2.5. Protective/Control Strategies

2.5.1. Medical treatments

The economic importance of warm-water mariculture has created an effort towards development of methods for the prophylaxis and treatment of amyloodiniosis (Table 2.1). As indicated earlier *Amyloodinium* has a very rapid reproductive rate and can complete its life cycle in less than 1 week under optimal conditions; thus, prompt treatment is imperative to prevent the disease from quickly overwhelming a susceptible fish population. The free-swimming dinospore is susceptible to

Table 2.1. Treatments reported to be effective for treating *Amyloodinium ocellatum*. Note that no treatment has been shown to unequivocally cure fish of the infestation (i.e. not eliminate the latent carrier state), but rather only control the disease.

Treatment	Dosage and time	Comments	References
Copper	0.12–0.15 mg/l for 10–14 days	Must maintain within this range	Noga (2010)
Chloroquine	5–10 mg/l for 10 days	Single dose exposure	C. Bower (unpublished data)
Hyposalinity	0–10 ppt salinity for 10–14 days	Isolates vary in salinity tolerance, but fresh water is usually needed to treat	Paperna (1984), Barbaro and Francescon (1985), Noga (2010)
	0 ppt salinity for 5 min; repeat every 3 days × three times	Must remove fish to uncontaminated system after each treatment to prevent reinfestation by detached trophonts	Ostrowski and Molnar (1998)
	0 ppt salinity for up to 5 min	Must remove fish to uncontaminated system to prevent reinfestation by detached trophonts	Kingsford (1975), Lawler (1977b)
Hydrogen peroxide	75 mg/l for 30 min; repeat after 6 days and then transfer to uncontaminated tank	Must remove fish to uncontaminated system within 24 h after last treatment to prevent reinfestation by detached trophonts; no trophonts detected after 2 weeks	Montgomery-Brock <i>et al.</i> (2001)
Formalin	100–200 mg/l for 6–9 h	Must place fish in an uncontaminated system to prevent reinfestation by detached tomonts	Paperna (1984)
	50 mg/l for 1 h; repeat after 15 days	Reported to control a natural outbreak	Fajer-Ávila <i>et al.</i> (2003)
	4 mg/l for 7 h; repeat after 15 days		Fajer-Ávila <i>et al.</i> (2003)
Lasalocid	1 mg/l for 24 h	Requires water-soluble form (not commercially available)	Oestmann and Lewis (1996)
Hypothermia	Temperature <15°C	Isolates vary in temperature tolerance	Paperna (1984)

certain drugs (Lawler, 1980; Paperna, 1984), but trophonts and tomonts are relatively resistant, making eradication difficult. Even when tomonts are inhibited from dividing, they can often resume dividing when returned to untreated water (Paperna *et al.*, 1981). Thus, periodic examination of fish for reinfestation after treatment is advisable.

Copper is the most widely used drug (Noga, 2010). The free copper ion is the active component and free copper must be maintained at 0.12–0.15 mg/l for 10–14 days to control epidemics. Higher concentrations of free copper should be avoided because it is also toxic to fish. Copper levels needed to treat amyloodiniosis are also toxic to most invertebrates and algae. The free copper ion is unstable in sea water and thus copper levels should be monitored closely with a copper test kit, and levels adjusted as needed. Copper that is chelated (e.g. with citrate or EDTA) has increased stability in water but it can be more difficult to monitor (Noga, 2010).

Carol Bower discovered that the antimalarial chloroquine diphosphate is very safe and effective in treating amyloodiniosis. Experimentally infested clownfish (*Amphiprion ocellaris*) were free of *A. ocellatum* infestation after a 10-day exposure to a single water-borne treatment of 5–10 mg/l chloroquine diphosphate. Chloroquine has no effect on tomont division, but kills dinospores immediately upon their excystment. This concentration is non-toxic to fish, but is highly toxic to micro- and macroalgae and to various invertebrates (C.E. Bower, Connecticut, personal communication, 1987) and thus cannot be used in reef aquaria, at least as a water-borne formulation. The pharmacokinetics of orally administered chloroquine in cultured red drum (*Sciaenops ocellata*) suggested promise as an oral medication (Lewis *et al.*, 1988), but no dosing has yet been developed. Despite its efficacy, chloroquine is very expensive and is not legally approved for use in fish. None the less, it appears to be in widespread use in the marine aquarium fish industry (unpublished data).

For many water-borne treatments, the most successful approach has involved repeated treatments, often followed by removal of the fish to a clean (uncontaminated)

environment. For example, treatment of juvenile bullseye puffers (*Sphoeroides annulatus*) in sea water with 51 mg/l formalin for 1 h or 4 mg/l formalin for 7 h significantly reduced *Amyloodinium* loads on the skin and gills. Reinfestation occurred after 15 days but it was supposedly controlled by repeating the treatment (Fajer-Ávila *et al.*, 2003). Flush treatment with 100–200 mg/l formalin for 6–9 h causes trophonts to detach from the gilthead sea bream (*Sparus aurata*), but tomonts resume division after removal of formalin (Paperna, 1984), requiring that the fish be immediately moved to an uncontaminated culture system after treatment. Another promising antiseptic is hydrogen peroxide (H_2O_2). In a field trial with Pacific threadfin (*Polydactylus sexfilis*), two treatments of 75 mg H_2O_2 /l 6 days apart followed by moving the fish to a clean tank, reduced parasite numbers to undetectable levels for at least 14 days (Montgomery-Brock *et al.*, 2001). However, fish treated with a 300 mg/l dose died, suggesting that this drug has a relatively low therapeutic index. It is likely that repeatedly reducing the parasite burden with sequential treatments is allowing time for an acquired immune response to develop, that helps to clear the infection (see '2.5.4 Acquired resistance' below).

The polyether ionophorous antibiotic, 3,N-methylglucamine lasalocid, experimentally cures red drum (*S. ocellata*) fry, but this drug is not commercially available. Many other agents have shown limited or no success against amyloodiniosis, and these include chlorotetracycline, tetracycline, aureomycin, nitrofurazone, nifurpirinol, acriflavin, malachite green, simazine, endothall or diuron (Lawler, 1977a; Johnson, 1984; Paperna, 1984). In addition, it is important to realize that even treatments that have shown efficacy (Table 2.1) are not always approved for use in food fish and whether they are approved varies considerably from one country to another.

2.5.2. Environmental treatments

Amyloodinium tolerates wide temperature and salinity ranges, making environmental control difficult. Lowering the temperature to 15°C arrests the disease process, but this is

almost never feasible. Lowering salinity delays but does not prevent infestations (Barbaro and Francescon, 1985), unless fish are placed in fresh water. A short freshwater bath of up to 5 min dislodges most but not all trophonts (Lawler, 1977b). However, treatment of Pacific threadfin with a 5 min freshwater bath followed by transfer to a clean tank, repeated three times every 3 days, is effective (Ostrowski and Molnar, 1998).

The risk of introducing infectious dinospores into an aquaculture system may be reduced by disinfection (e.g. using ultraviolet irradiation, ozonation or chlorination) of incoming water (Lawler, 1977b). Ageing of water beyond the survival time of dinospores, and quarantine of new fish for at least 20 days are additional measures that may reduce, but not eliminate, the risk of parasite introduction. Dinospores remain infective for at least 6 days at 26°C (Bower *et al.*, 1987) and one must allow time for all tomonts to sporulate. To reduce epidemics in wild-caught southern flounder (*Paralichthys lethostigma*), fish were treated prophylactically and also placed in low salinity (0–1 ppt) water for at least 1 week (Smith *et al.*, 1999). Systems in close proximity to *Amyloodinium*-infested waters must also be protected (e.g. use of tight-fitting lids on aquaria) from aerosol transfer of parasites (Roberts-Thomson *et al.*, 2006).

Large, repeated water changes might control some infestations by diluting out the motile dinospores as they emerge from tomonts (Schwartz and Smith, 1998). Even 150% daily multiple water changes may not be sufficient (Abreu *et al.*, 2005). However, removal of tomonts that have settled on the bottom of a tank (by scrubbing the tank surface with an acid solution) has, as expected, been associated with a significant decrease in parasite load and prevalence of clinical disease, at least until parasite levels rebound (Abreu *et al.*, 2005).

2.5.3. Innate resistance

Some fish species are naturally resistant to infestation, such as killifish (*Fundulus grandis*), American eel (*Anguilla rostrata*) and molly (*Poecilia latipinna*), among others. Resistant

species are generally those which produce thick mucus or can tolerate low oxygen levels, presumably due to their greater ability to withstand attack or feeding on gill tissue by the parasite (Lawler, 1977b). Host factors must play an important role in host–parasite interactions. Fish parasitic oodinids feed exclusively on or within the epithelial tissues of the skin or gills. Thus, all host–parasite interactions (i.e. host recognition, defensive mechanisms responsible for protecting against these pathogens, etc.) are located in the mucus, or on/in epithelial cells and extracellular fluid of the epithelium.

In vitro data suggests that serum can have strong anti-*Amyloodinium* activity (Landsberg *et al.*, 1992). *Amyloodinium* is also highly sensitive to natural, host-produced antibiotics, known as antimicrobial polypeptides (AMPPs). One type of AMPP, the histone-like proteins (HLPs) are present in high concentrations in the skin and gills of hybrid striped bass (*Morone saxatilis* × *Morone chrysops*) and other fish (Noga *et al.*, 2001). The concentrations at which HLPs were lethal to *Amyloodinium* were well within the range that these compounds are present in fish tissues; thus, they probably play an important role in protecting fish against this parasite. Interestingly, HLPs are highly lethal to trophonts but have no effect on dinospores (Noga *et al.*, 2001). Recent studies have also shown that another group of antimicrobial polypeptides, the piscidins, are also highly toxic to both dinospores and trophonts (Colorni *et al.*, 2008). Piscidins are present in high concentration in epithelial tissues including skin and gills (Noga *et al.*, 2009). They are widespread in higher teleosts, especially perciform fish (Silphaduang *et al.*, 2006; Noga *et al.*, 2011) and thus this defence may play an important role in resistance to amyloodiniosis.

In terms of upregulated defences, recent data shows that a natural AMPP response in fish, consisting of a suite of AMPPs, some if not all of which are derived from the β -chain of the respiratory protein haemoglobin (Hb β), can be strongly upregulated *in vivo* to levels that are highly lethal to important pathogens (Ullal *et al.*, 2008), including *Amyloodinium* (Ullal and Noga, 2010; Noga *et al.*, 2011). These AMPPs originate not in the blood but

rather in the skin and gill epithelium, the target tissue of *Amyloodinium*. Most importantly, the Hb AMP concentrations expressed *in vivo* appear to be well within the antiparasitic concentrations measured *in vitro* (Ullal *et al.*, 2008). These data provide encouragement that mechanisms exist to increase at least some AMPP responses to highly tidal levels *in vivo*, thus providing the opportunity to use this as a direct protective tool. In this regard, feeding leopard grouper (*Mycteroperca rosacea*) a diet having the live probiotic yeast *Debaryomyces hansenii* resulted in a significantly greater resistance to experimental challenge with *Amyloodinium*, compared with fish fed the same diet without the probiotic. Probiotic-fed fish recovering from the infection also had higher serum antibody levels (Reyes-Becerril *et al.*, 2008). However, red drum fed a diet containing brewer's yeast and nucleotides, either alone or in combination, had no effect on resistance to amyloodiniosis (Li *et al.*, 2005).

2.5.4. Acquired resistance

At present, there are no commercial vaccines available for treatment of any fish-parasitic protozoa, including dinoflagellates (Woo, 2007). However, recent studies have identified important defensive mechanisms that might be used to specifically enhance protection to these pathogens. Early studies provided anecdotal evidence that fish recovering from amyloodiniosis were resistant to reinfection (Lawler, 1977b, 1980; Paperna, 1980). Smith *et al.* (1993) then showed that serum from fish immunized with dinospores could agglutinate living dinospores and kill *Amyloodinium* in cell culture. Immunized fish also mounted an antibody response that was detectable via ELISA (enzyme-linked immunosorbent assay). Using DC-1 isolate dinospores as the capture antigen, this ELISA detected anti-*Amyloodinium* serum antibody in blue tilapia (*Oreochromis aureus*) immunized with the DC-1 isolate (Smith *et al.*, 1993) and in sea bream immunized with a Red Sea *Amyloodinium* isolate (Noga *et al.*, 1992). Using this same ELISA, anti-*Amyloodinium* serum antibody was also detected in hybrid striped bass that had

recovered from a spontaneous amyloodiniosis outbreak on a North Carolina fish farm (Smith *et al.*, 1994). These data suggest that fish can mount a significant antibody response with both experimental and natural challenges and that there was considerable cross-reactivity between these three *Amyloodinium* isolates. The latter supports the molecular data indicating that *Amyloodinium* might be a very homogeneous taxon (Levy *et al.*, 2007). Using tomont antigen, Cecchini *et al.* (2001) also detected anti-*Amyloodinium* antibody in cultured European sea bass (*Dicentrarchus labrax*) that had recovered from an amyloodiniosis outbreak.

Subsequent studies have shown that *Amyloodinium*-infested fish can develop protective resistance following experimental challenge. Following weekly sub-lethal challenges with *Amyloodinium*, tomato clownfish (*Amphiprion frenatus*) developed significant immunity to infection in about 1 month. This protection was long lived (at least 6 months) and appeared to be directed against the trophont (Cobb *et al.*, 1998a). Protection was associated with an antibody response as measured by ELISA. The reaction of immune fish serum against dinospore and trophont antigens in Western blots suggested the presence of both shared and stage-specific antigens (Cobb *et al.*, 1998b). Immune serum also reacted with trophonts and dinospores in an indirect fluorescent antibody test. There was a suggestion that immunity could also be passively transferred to naïve fish (Cobb *et al.*, 1998b). Local antibody was hypothesized to be more important than serum antibody in protection since protection lasted long after serum antibody was undetectable via ELISA. Probiotic-fed leopard grouper, which were more resistant to *Amyloodinium* challenge than fish fed a control diet, also had higher convalescent serum antibody titres than previously challenged fish not fed the probiotic (Reyes-Becerril *et al.*, 2008).

2.6. Conclusions and Suggestions for Future Studies

Within the stressful confines of aquaculture, parasites like *Amyloodinium* exert their greatest impact. It is very difficult to eliminate the

infestation, and with the increasing regulations on the use of drugs in aquaculture, it is necessary to optimize the application of currently approved drugs, as well as try other approaches. One alternative to drugs is environmental manipulation, but a better understanding of environmental conditions that affect parasite growth and survival is needed, as well as means to feasibly utilize these data in commercial applications. The identification of potent non-specific defences has the potential to allow broad-spectrum protection. Likewise, the strong evidence for a protective

immune response against *Amyloodinium* holds promise for eventual development of protective vaccines.

More molecular studies are needed to further clarify the taxonomic relationships among various *Amyloodinium* isolates, so that highly specific and sensitive tests can be developed for effective biosecurity (especially excluding exotic isolates) and management of infestations/infections in culture. This tool would be especially useful for detecting latent carriers, which are probably a major source of parasite introduction.

References

- Abreu, P.C., Robaldo, R.B., Sampiao, L.A., Ianchini, A. and Debrecht, C. (2005) Recurrent amyloodiniosis on broodstock of the Brazilian flounder *Purulichthys orbignyus*: dinospore monitoring and prophylactic measures. *Journal of the World Aquaculture Society* 36, 42–50.
- Barbaro, A. and Francescon, A. (1985) Parassitosi da *Amyloodinium ocellatum* (Dinophyceae) su larve di *Sparus aurata* allevate in un impianto di riproduzione artificiale. *Oebalia* 11, 745–752.
- Bower, C.E., Turner, D.T. and Biever, R.C. (1987) A standardized method of propagating the marine fish parasite, *Amyloodinium ocellatum*. *Journal of Parasitology* 73, 85–88.
- Callahan, H.C. and Noga, E.J. (2002) Tricaine dramatically reduces the ability to diagnose protozoan ectoparasite (*Ichthyobodo necator*) infections. *Journal of Fish Diseases* 25, 433–437.
- Cecchini, S., Saroglia, M., Tero, G. and Albanesi, F. (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.
- Coats, D.W. (1999) Parasitic life styles of marine dinoflagellates. *Journal of Eukaryotic Microbiology* 46, 402–409.
- Cobb, C.S., Levy, M.G. and Noga, E.J. (1998a) Development of immunity by the tomato clownfish *Amphiprion frenatus* to the dinoflagellate parasite *Amyloodinium ocellatum*. *Journal of Aquatic Animal Health* 10, 259–263.
- Cobb, C.S., Levy, M.G. and Noga, E.J. (1998b) Acquired immunity to amyloodiniosis is associated with an antibody response. *Diseases of Aquatic Organisms* 34, 125–133.
- Colorni, A., Ullal, A., Heinisch, G. and Noga, E.J. (2008) Activity of the antimicrobial polypeptide piscidin 2 against fish ectoparasites. *Journal of Fish Diseases* 31, 423–432.
- Fajér-Ávila, E.J., Abdo-de la Parra, I., Aguilar-Zarate, G., Contreras-Arce, R., Zaldivar-Ramirez, J. and Betancourt-Lozano, M. (2003) Toxicity of formalin to bullseye puffer fish (*Sphoeroides annulatus* Jenyns) and its effectiveness to control ectoparasites. *Aquaculture* 223, 41–50.
- Fensome, R.A., Taylor, F.J.R., Norris, G., Sarjeant, W.A.S., Wharton, D.I. and Williams, G.L. (1993) A classification of living and fossil dinoflagellates. *Micropaleontological Species Publication* 7, 351.
- Johnson, S.K. (1984) *Evaluation of Several Chemicals for Control of Amyloodinium ocellatum, a Parasite of Marine Fishes*. Texas A & M University FDDL-M5, College Station, Texas, 4 pp.
- Kingsford, E. (1975) *Treatment of Exotic Marine Fish Diseases*. Palmetto Publishing Co., St Petersburg, Florida, 90 pp.
- Kuperman, B.L. and Matey, V.E. (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.L., Matey, V.E. and Hurlbert, S.H. (2001) Parasites of fish from the Salton Sea, California, USA. *Hydrobiologia* 466, 195–208.
- Landsberg, J.H., Smith, S.A., Noga, E.J. and Richards, S.A. (1992) Effect of serum and mucus of blue tilapia, *Oreochromis aureus* on infectivity of the parasitic dinoflagellate, *Amyloodinium ocellatum* in cell culture. *Fish Pathology* 27, 163–169.

- Lawler, A.R. (1977a) The parasitic dinoflagellate *Amyloodinium ocellatum* in marine aquaria. *Drum and Croaker* 17, 17–20.
- Lawler, A.R. (1977b) Dinoflagellate (*Amyloodinium*) infestation of pompano. In: Sindermann, C.J. (ed.) *Disease Diagnosis and Control in North American Marine Aquaculture*. Elsevier, Amsterdam, pp. 257–264.
- Lawler, A.R. (1980) Studies on *Amyloodinium ocellatum* (Dinoflagellata) in Mississippi Sound: natural and experimental hosts. *Gulf Research Reports* 6, 403–413.
- Levy, M.G., Poore, M.F., Colorni, A., Noga, E.J. and Litaker, R.W. (2007) A PCR assay for detection of *Amyloodinium ocellatum*. *Diseases of Aquatic Organisms* 73, 219–226.
- Lewis, D.H., Wenxing W., Ayers, A. and Arnold, C.R. (1988) Preliminary studies on the use of chloroquine as a systemic chemotherapeutic agent for amyloodinosis in red drum (*Sciaenops ocellatus*). *Contributions in Marine Science* 30 (Supplement), 183–189.
- Li, P., Burr, G.S., Go, J., Whiteman, K.W., Davis, K.B., Vega, R.R., Neill, W.H. and Gatlin III, D.M. (2005) A preliminary study on the effects of dietary supplementation of brewers yeast and nucleotides, singularly or in combination, on juvenile red drum (*Sciaenops ocellatus*). *Aquaculture Research* 36, 1120–1127.
- Montgomery-Brock, D., Sato, V.T., Brock, J.A. and Tamaru, C.S. (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.
- Noga, E.J. (1987) Propagation in cell culture of the dinoflagellate *Amyloodinium*, an ectoparasite of marine fishes. *Science* 236, 1302–1304.
- Noga, E.J. (2010) *Fish Disease: Diagnosis and Treatment*, 2nd edn. Wiley-Blackwell, Ames, Iowa, 519 pp.
- Noga, E.J. and Levy, M.G. (2006) Phylum Dinoflagellata. In: Woo, P.T.K. (ed.) *Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections*, 2nd edition. CABI Publishing, Wallingford, Oxon, UK, pp. 16–45.
- Noga, E.J., Smith, S.A. and Landsberg, J.H. (1991) Amyloodinosis in cultured hybrid striped bass (*Morone saxatilis* × *M. chrysops*) in North Carolina. *Journal of Aquatic Animal Health* 3, 294–297.
- Noga, E.J., Colorni, A., Levy, M.G., Diamant, A., Smith, S.A., Landsberg, J.H. and Avtalion, R. (1992) *The Immune Response of Fish to Amyloodinium: a Model for the Protozoan Ectoparasites*. Final Report to Binational US-Israel Agricultural Research and Development (BARD) Program Project. BARD, Bet Dagan, Israel, 90 pp.
- Noga, E.J., Fan, Z. and Silphaduang, U. (2001) Histone-like proteins from fish are lethal to the parasitic dinoflagellate *Amyloodinium ocellatum*. *Parasitology* 123, 57–65.
- Noga, E.J., Silphaduang, U., Park, N.G., Seo, J.-K., Stephenson, J. and Kozłowicz, S. (2009) Piscidin 4, a novel member of the piscidin family of antimicrobial peptides. *Comparative Biochemistry and Physiology B* 152, 299–305.
- Noga, E.J., Ullal, A.J., Corrales, J. and Fernandes, J.M.O. (2011) Application of antimicrobial polypeptide host defenses to aquaculture: exploitation of downregulation and upregulation responses. *Comparative Biochemistry and Physiology D* 6, 44–54.
- Oestmann, D.J. and Lewis, D.H. (1996) Effects of 3,N-methylglucamine lasalocid on *Amyloodinium ocellatum*. *Diseases of Aquatic Organisms* 24, 179–184.
- Ostrowski, A.C. and Molnar, A. (1998) *Pacific Threadfin Polydactylus sexfilis (moi) Hatchery Manual*. Center for Tropical and Subtropical Aquaculture Publication #132. Center for Tropical and Subtropical Aquaculture, Waimanalo, Hawaii, USA.
- Overstreet, R.M. (2007) Effects of a hurricane on fish parasites. *Parassitologia* 49, 161–168.
- 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. (1984) Reproduction cycle and tolerance to temperature and salinity of *Amyloodinium ocellatum* (Brown 1931) (Dinoflagellida). *Annales de Parasitologie Humaine et Comparée* 59, 7–30.
- Paperna, I., Colorni, A., Ross, B. and Colorni, B. (1981) Diseases of marine fish cultured in Eilat mariculture project based at the Gulf of Aqaba, Red Sea. *European Mariculture Society Special Publication* 6, 81–91.
- Rensel, J.E. and Whyte, J.N.C. (2003). Finfish mariculture and harmful algal blooms. In: Hallegraeff, G.M., Anderson, D.M. and Cembella, A.D. (eds) *Manual on Harmful Marine Microalgae*. UNESCO Publishing, Paris, pp. 693–722.
- Reyes-Becerril, M., Tovar-Ramírez, D., Ascencio-Valle, F., Civera-Cerecedo, R., Gracia-López V. and Barbosa-Solomieu, V. (2008) Effects of dietary live yeast *Debaryomyces hansenii* on the immune and

- antioxidant system in juvenile leopard grouper *Mycteroperca rosacea* exposed to stress. *Aquaculture* 280, 39–44.
- Roberts-Thomson, A., Barnes, A., Fielder, D.S., Lester, R.J.G. and Adlard, R.D. (2006) Aerosol dispersal of the fish pathogen, *Amyloodinium ocellatum*. *Aquaculture* 257, 118–123.
- Sandifer, P.A., Hopkins, J.S., Stokes, A.D. and Smiley, R.D. (1993) Experimental pond grow-out of red drum, *Sciaenops ocellatus*, in South Carolina. *Aquaculture* 118, 217–228.
- Schwartz, M.H. and Smith, S.A. (1998) *Getting Acquainted with Amyloodinium ocellatum*. *Commercial Fish and Shellfish Technology*. Virginia Cooperative Extension Fact Sheet Publication 600-200. Virginia Cooperative Extension, Blacksburg, Virginia, USA.
- Silphaduang, U., Colorni, A. and Noga, E.J. (2006) Evidence for the widespread distribution of piscidin antimicrobial peptides in teleost fish. *Diseases of Aquatic Organisms* 72, 241–252.
- Smith, S.A., Levy, M.G. and Noga, E.J. (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., Noga, E.J., Levy, M.G. and Gerig, T.M. (1993) Effect of serum from tilapia *Oreochromis aureus*, immunized with dinospores of *Amyloodinium ocellatum*, on the motility, infectivity and growth of the parasite in cell culture. *Diseases of Aquatic Organisms* 15, 73–80.
- Smith, S.A., Levy, M.G. and Noga, E.J. (1994) Detection of anti-*Amyloodinium ocellatum* antibody from cultured hybrid striped bass (*Morone saxatilis* × *Morone chrysops*) during an epizootic of amyloodiniosis. *Journal of Aquatic Animal Health* 6, 79–81.
- Smith, T.I.J., McVey, D.C., Jenkins, W.E., Denson, M.R., Heyward, L.D., Sullivan, C.V. and Berlinsky, B.L. (1999) Broodstock management and spawning of southern flounder, *Paralichthys lethostigma*. *Aquaculture* 176, 87–99.
- Ullal, A.J. and Noga, E.J. (2010) Antiparasitic activity of the antimicrobial peptide HbβP-1, a member of the β-hemoglobin peptide family. *Journal of Fish Diseases* 33, 657–664.
- Ullal, A.J., Litaker, R.W. and Noga, E.J. (2008) Antimicrobial peptides derived from hemoglobin are expressed in epithelium of channel catfish (*Ictalurus punctatus*, Rafinesque). *Developmental and Comparative Immunology* 32, 1301–1312.
- Woo, P.T.K. (2007) Protective immunity in fish against protozoan diseases. *Parassitologia* 49, 185–191.

3 *Cryptobia (Trypanoplasma) salmositica*

Patrick T.K. Woo
University of Guelph, Guelph, Ontario, Canada

3.1. Introduction

3.1.1. The parasite

Cryptobia spp. are parasitic flagellates (class Kinetoplastea, subclass Metakinetoplastina, order Parabodonida) of invertebrates and vertebrates, and they have worldwide distribution. There are at least 52 nominal species, and most species are not known to cause disease. The pathogenic species are relatively well studied as they cause disease and/or mortality in marine and freshwater fishes. They are either haematozoic (normally associated with the blood system) or non-haematozoic (on gills or in the digestive system) parasites. Scientists in Europe prefer to assign the haematozoic species to the genus *Trypanoplasma* and the non-haematozoic species to the genus *Cryptobia*; however, many North American workers do not believe there is sufficient evidence for the separation into two genera and this has been discussed extensively earlier (Woo, 1994, 2006). Briefly, Woo (1987a, 1994) agrees there are similarities and differences between haematozoic and non-haematozoic species, and a close relationship between the two groups. Consequently, he divided the genus *Cryptobia* into two subgenera: (i) sub-genus *Trypanoplasma* for the haematozoic species; and (ii) sub-genus *Cryptobia* for the non-haematozoic species.

The pathogenic haematozoic species include: *Cryptobia (Trypanoplasma) bullock* (in flatfishes), *Cryptobia (Trypanoplasma) salmositica* (in salmonids), and *Cryptobia (Trypanoplasma) borreli* (in cyprinids); while the non-haematozoic species that cause disease in fishes are *Cryptobia (Cryptobia) iubilans* (in the digestive system) and *Cryptobia (Cryptobia) branchialis* (on the gills). In general, non-haematozoic *Cryptobia* spp. of fishes are transmitted directly between hosts while blood sucking invertebrates are vectors of haematozoic species.

The current review is on *Cryptobia (T.) salmositica* and includes especially relevant information on its biology (section 3.1) as it relates to the pathobiology of the parasite which includes the disease mechanism (sections 3.2, 3.3 and 3.4), and strategies against cryptobiosis (section 3.5). Suggestions for further research are also included in many of the sections.

3.1.2. *Cryptobia (T.) salmositica*

The parasite (Fig. 3.1) was first described from coho salmon (*Oncorhynchus kisutch*) in Washington State, USA (Katz, 1951). It is not a very host-specific parasite and it has since been reported in all species of Pacific salmon

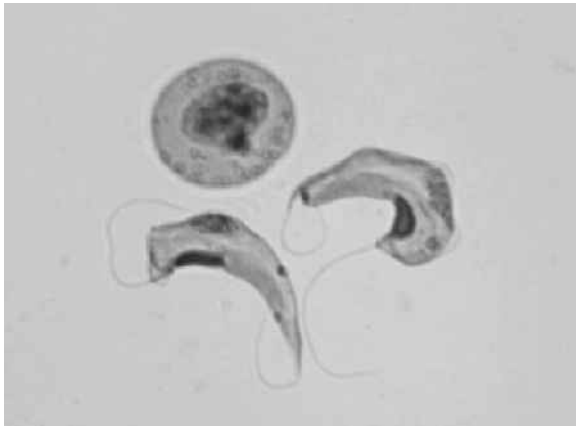


Fig. 3.1. *Cryptobia salmositica* with a red blood cell from a fish with microcytic and hypochromic anaemia; note the red blood cell is not oval and there is reduced haemoglobin ($\times 1150$) (from Woo, 1987a; courtesy of *Advances in Parasitology*).

(*Oncorhynchus* spp.) and in seven species of sculpins (*Cottus* spp.) which inhabit freshwater streams/rivers from California (USA) to British Columbia (Canada) and to south-western Alaska. Although it causes cryptobiosis in most species of salmonids it is not known to cause disease in sculpins in the wild, and they are considered the natural reservoir hosts of the pathogen (Woo, 2003). Further studies with experimentally infected laboratory raised sculpins are needed to confirm there is no disease and the mechanism of protection as in *Cryptobia*-tolerant brook charr (*Salvelinus fontinalis*) (section 3.5.2). Based on laboratory studies Ardelli *et al.* (1994) suggested that *Cryptobia*-tolerant brook charr might also be potential reservoirs in parts of the west coast.

Cryptobia salmositica is an elongated organism which is slightly longer than a red blood cell. Its body measurements are based on air-dried blood smears stained with Giemsa's stain: (i) body length 14.9 (6.0–25.0) μm ; (ii) body width 2.5 (1.3–4.0) μm ; (iii) anterior flagellum 16.1 (6.5–27.0) μm ; (iv) posterior free flagellum 9.0 (4.0–17.0) μm ; (v) kinetoplast length 2.0–9.0 μm ; (vi) kinetoplast width 0.5–2.0 μm ; (vii) nucleus length 1.5–3.5 μm ; (viii) nucleus width 1.0–2.5 μm ; (ix) ratio of anterior flagellum to body length 1.07 (0.40–1.95); (x) ratio of posterior flagellum to body

length 0.61 (0.25–1.15); and (xi) ratio of anterior flagellum to posterior free flagellum 1.97 (0.6–3.7) (Katz, 1951).

In general, the ultrastructure of *C. salmositica* is similar to those of other *Cryptobia* spp. However, the blood form of *C. salmositica* has a functional contractile vacuole (with systole and diastole stages) and its lumen has electron-dense filamentous materials (Paterson and Woo, 1983). Contractile vacuoles have not been found in other haematozoic species (Vickerman, 1971; Brugerolle *et al.*, 1979). In *C. salmositica*, the vacuole is located at the base of the flagellar pocket and is associated with the postflagellar pit (Paterson and Woo, 1983).

As indicated earlier *C. salmositica* causes cryptobiosis in salmonids and the pathogen has been reported from all species of Pacific *Oncorhynchus* spp. Outbreaks of cryptobiosis with high fish mortalities have occurred in freshwater hatcheries and in sea-cage cultures on the west coast of North America (section 3.1.4). In the wild the parasite is normally transmitted indirectly by blood-sucking leeches (section 3.1.3) which occur in freshwater streams and rivers; however, it can also be transmitted directly between fish that are in close proximity to each other and under certain aquaculture conditions (section 3.1.3).

3.1.3. Transmission

Indirect transmission

In general, after an infective blood meal the haematozoic *Cryptobia* multiplies rapidly in the crop of the blood-sucking leech. The dividing parasite accumulates in large numbers either in the crop and/or in the proboscis sheath of the leech, and they are transmitted to fish when the infected leech feeds again (Woo, 1994).

The freshwater leech, *Piscicola salmositica* is the only known natural vector for *C. salmositica* and it occurs in streams and rivers on the west coast of North America. Leeches hatch in late summer/early autumn from cocoons before the salmon return to fresh water from the sea (Becker and Katz, 1965b, 1966; Bower and Margolis, 1984b). It is assumed they initially pick up the *Cryptobia* by feeding on infected resident fishes (e.g. sculpins). Briefly, once the parasite is ingested by the leech it multiplies and large numbers of dividing parasites are in the crop of the leech 7–8 days after an infective blood meal. As far as is known the flagellate is always infective to fish and there are no indications the flagellate is in the proboscis sheath of the infected leech (Becker and Katz, 1965a). Further studies on its development in the leech are suggested as S. Li and P.T.K. Woo (unpublished) had found numerous *Cryptobia* in the proboscis sheaths of *P. salmositica* removed from infected salmon. The isolates of *Cryptobia* from the proboscis sheaths were infective and caused disease when inoculated into rainbow trout (*Oncorhynchus mykiss*).

Direct transmission

In experimentally infected fish the parasite first multiplied in the blood of infected sockeye salmon fry (*Oncorhynchus nerka*) and later in the infection it was also found on the body surface. It was suggested the parasite passed through blisters caused by the dissociation of connective tissues near the abdominal pore. If heavily infected fish and uninfected fish were held together in dip

nets for brief periods out of water the eventual fish mortality due to cryptobiosis ranged from 64 to 89% in fish maintained in fresh water, and was 94% in fish maintained in sea water (Bower and Margolis, 1983). This mode of transmission may occur in hatcheries, for example when fish are periodically brought together during grading and/or weighing or when fish are transferred from pond to pond.

Woo and Wehnert (1983) demonstrated the parasite was in the mucus on the body surface of adult rainbow trout about 6 weeks after experimental infection. These ecto-parasitic flagellates were infective when they were inoculated into fish. Also, 67–80% of uninfected trout became infected in about 20 weeks if they were allowed to mix freely with infected trout in the same tank. The percentage of infected trout was lower if the two groups of fish were separated by a wire screen with the water flowing from the infected to uninfected fish.

C. salmositica has at least two proteases: (i) a cysteine protease (49, 60, 66 and 97 kDa); and (ii) a metalloprotease (200 kDa). These have been isolated and purified (Fig. 3.2). The 200 kDa metalloprotease is a histolytic enzyme (Fig. 3.3) and it is an important virulent factor (Zuo and Woo, 1997a, d, 1998a). It is likely involved in direct transmission of the parasite between fish; initially by causing lesions in the skin so that the parasite can become ecto-parasitic and in entry of the parasite (e.g. via the mucous membrane) in uninfected fish. As indicated earlier (section 3.1.2) the parasite has a functional contractile vacuole (Paterson and Woo, 1983) which will allow the ecto-parasitic parasite to osmoregulate when fish are in hypo-osmotic environments (e.g. in fresh water). Also, copious amount of mucous is secreted by the infected fish and this may also help the parasite to survive in the hypo-osmotic environment. Woo and Wehnert (1983) suggested the ecto-parasitic form was carried in mucous strands in the water column, and the parasite entered the uninfected recipient fish either through lesions on the body surface or it penetrated (with the help of the metalloprotease) the mucous membrane of the gills and/or the oral cavity.

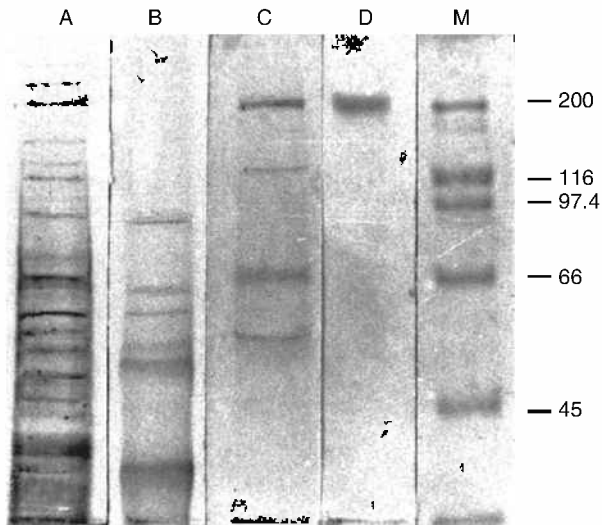


Fig. 3.2. Purified cysteine protease and metalloprotease from *C. salmositica*. Lane A, crude parasite lysate; B, partially purified cysteine protease from diethylaminoethyl (DEAE)-agarose column; C, metalloprotease from DEAE-agarose column; D, purified metalloprotease from Sephacryl S-300 column; M, molecular weight markers (kDa) (from Woo, 2003, which was modified from Zuo and Woo, 1997d; courtesy of *International Journal for Parasitology*).

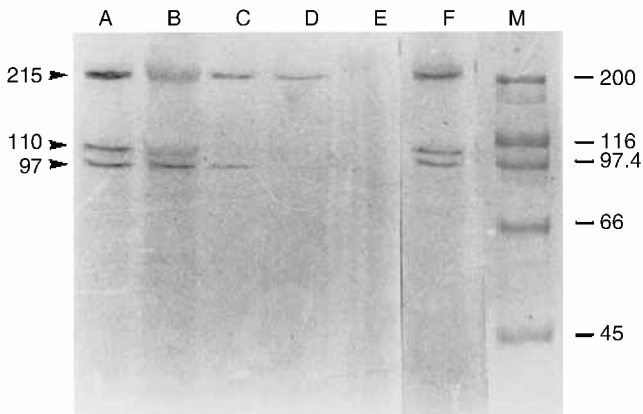


Fig. 3.3. *In vitro* degradation of collagen type V by purified metalloprotease from *C. salmositica*. Lanes A–E, collagen incubated with metalloprotease for 0, 2, 5, 6 and 8 h, respectively; Lane F, collagen + phosphate buffered saline at 8 h; Lane M, molecular weight markers (kDa) (from Zuo and Woo, 1997d; courtesy of *Diseases of Aquatic Organisms*).

3.1.4. Impacts of cryptobiosis

In the Fraser River drainage, British Columbia, Canada, the prevalence of *C. salmositica* in salmon returning to fresh water to spawn was low in September but it increased to about

100% by December and January. Also, returning salmon had detectable infections within 5 days in fresh water and the longer the fish were in fresh water the higher were their parasitaemias (Bower and Margolis, 1984b). These increases were related to increased numbers of

infected leeches in the streams in November (Becker and Katz, 1965b, 1966; Bower and Margolis, 1984b).

Putz (1972) indicated that *C. salmositica* was more pathogenic to coho salmon (*O. kisutch* with 100% mortality) than to chinook salmon (*Oncorhynchus tshawytscha*) in the USA. However, Bower and Margolis (1985) found 100% mortality in chinook salmon while coho salmon from some stocks in Canada did not die from the infection (0% mortality). The difference is probably due to the genetics of the fish. For example, sockeye salmon (*O. nerka*) from the Fulton River stock (British Columbia, Canada) suffered high mortality when injected with a low dose (about 100 parasites per fish), while the Weaver Creek stock of sockeye salmon (British Columbia) had light mortality even when injected with about a million parasites per fish (Bower and Margolis, 1984a). Mortality of infected sockeye salmon is consistent within the same fish stock and to different parasite isolates (Bower and Margolis, 1985).

The parasite is considered a lethal pathogen of salmon in many semi-natural and intensive salmon culture facilities on the Pacific coast of North America (Bower and Thompson, 1987). In the USA, the prevalence of the parasite in down-stream migrants of salmon ranged from 3 to 21% in some streams (Becker and Katz, 1966). It is most likely the prevalence would be lower if more fish were examined and from more streams. Experimental studies showed that pre-smolt salmon infected in fresh water retained their infection and with no reduction in mortality after they were transferred to salt water (Bower and Margolis, 1985). Consequently the disease may be quite a significant cause of fish loss due to mortality in the sea; however, no field studies had been conducted to test the validity of this suggestion.

There had been a few reported serious outbreaks of the disease in juvenile salmon held in freshwater hatcheries in Washington State, USA. These include three outbreaks in chinook salmon in three localities between 1972 and 1973 (Wood, 1979). Also, P.F. Chapman (Department of Fisheries, State of Washington, USA, personal communication, 1993) described outbreaks of the disease in chinook

salmon in hatcheries in Washington State which began in December 1992 and peaked in February 1993. Infected fish had the typical clinical signs (anaemia, splenomegaly and ascites) of the disease (section 3.2.1), and 65,000 fish were involved with peak mortality of 0.1%/day in February. In one hatchery the mortality of adult chinook brood stock brought into the hatchery (for breeding purposes) was about 50%. He noted that cryptobiosis had occurred in the same hatcheries in the past but they were not as severe.

Outbreaks of the disease had also occurred in fish held in sea cages. In 1997 the parasite caused significant morbidity/mortality in smolts and pre-harvest chinook salmon in a hatchery on Vancouver Island, Canada. There was a small mortality spike (about 1%) in post-smolts in the first 10–15 weeks after fish were transferred to salt water. However, re-emergence of the disease with significant morbidity and mortality occurred later in the pre-harvest fish. Another outbreak in the pre-harvest chinook salmon occurred in the same hatchery in 2001. Large numbers of *Cryptobia* were in the blood and ascites fluid of moribund fish, and clinical signs (section 3.2.1) were evident in many fish. Fish mortality varied between cages and it ranged from 3.3 to 24.9%. Briefly, the parasite was detected in the blood of some fish (while in fresh water in the hatchery) before they were transferred to sea cages in August–September 1999. Parasites from moribund fish in sea cages were morphologically similar to *C. salmositica*, and caused clinical disease in experimentally infected rainbow trout (Woo, 2006).

Significant mortalities due to cryptobiosis were also associated with post-spawning rainbow trout in a hatchery in California, USA (Wales and Wolf, 1955). The authors suggested that many of the post-spawning trout died from a combined effect of *Cryptobia* and *Saprolegnia parasitica*. In field studies conducted in the 1980s adult salmon returning from the Pacific Ocean had detectable infections as early as 5 days after they returned to fresh water in the Fraser River, British Columbia, Canada. The parasitaemias were very high in many fish at spawning, and numerous fish had died before they spawned (Bower and Margolis, 1984b). In the Soleduck

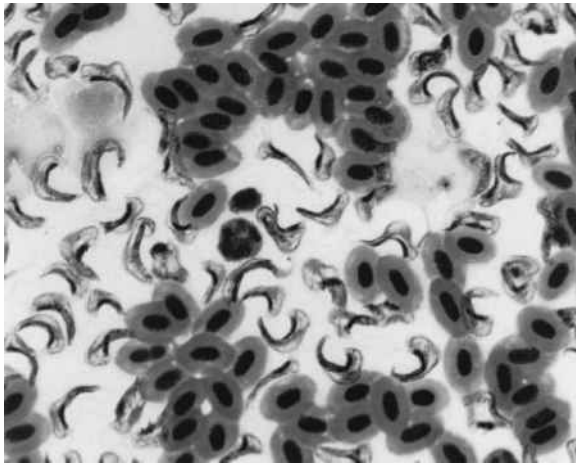


Fig. 3.4. Blood smear from a sexually mature spring chinook salmon naturally infected with *C. salmositica* (courtesy of Craig Banner, Oregon Department of Fish and Wildlife, USA).

hatchery (Washington State, USA) about 50% of spring chinook salmon bloodstock brought in from streams annually died from cryptobiosis (L. Peck, Department of Fisheries, State of Washington, USA, personal communication, 1994). Outbreak of the disease in brood stock had occurred in a hatchery on the Rogue River in Oregon, USA (C. Banner, Oregon Department of Fisheries and Wildlife, Oregon State, USA, personal communication, 2004). Mortality was over 50% and moribund fish had massive number of parasites in the blood (Fig. 3.4). No leeches were found on infected fish and total mortality was similar in both male and female fish.

Currie and Woo (2007, 2008) conducted experimental studies on the infection in sexually mature rainbow trout. They showed infected sexually mature females were more susceptible than sexually mature males. All infected females had exophthalmia (section 3.2.1) while none of the males showed this clinical sign although both males and females were anaemic. Most infected females with eggs died before or shortly after spawning and none of the infected males died. Infected males initially increased milt production and sperm concentration after infection; however this declined as the disease progressed. Also, parasitaemias were higher in females than in males. Fresh plasma from both sexually mature females

and males significantly increased the *in vitro* multiplication of the parasite, and plasma from females was better than plasma from males. The addition of 17 β -estradiol (at physiological level or higher) did not enhance *in vitro* multiplication of the *Cryptobia*. Further studies are needed to determine and isolate the 'factor(s)' in plasma in sexually mature fish that promotes parasite multiplication.

3.2. Clinical Signs and Diagnosis of Salmonid Cryptobiosis

3.2.1. Clinical signs

C. salmositica is in the blood soon after infection and it multiplies readily by longitudinal binary fission (Woo, 1978). The severity of the disease, peak parasitaemia, appearance of the clinical signs and mortality are related to numerous abiotic and biotic factors which include: (i) the size of the parasite inoculum; (ii) water temperature; (iii) fish diets; (iv) size and strain/species of the fish; and (v) the genetics and sexual maturity of the fish (e.g. Woo, 1979; Woo *et al.*, 1983; Bower and Margolis, 1985; Thomas and Woo, 1990b; Li and Woo, 1991; Li *et al.*, 1996; Chin *et al.*, 2002, 2004a; Currie and Woo, 2008).



Fig. 3.5. Dorsal view of obvious exophthalmia in a rainbow trout experimentally infected with *C. salmositica* (from Woo and Poynton, 1995; courtesy of CAB International).

Infected fish produce copious amounts of mucus on the body surface, are lethargic, and they remain at the bottom of the tank during the acute phase of the disease. Briefly, the parasitaemia peaks at about 4–8 weeks after infection (acute phase of the disease), and as indicated earlier this is dependent on the dosage of the inoculum and size and genetics of the fish. The severity of the anaemia (microcytic and hypochromic) in trout is directly related to the parasitaemia (Woo, 1979). Another consistent clinical sign is anorexia; its onset is at 3–5 weeks post-infection (pi) and this is also partly influenced by water temperature (Thomas and Woo, 1992; Chin *et al.*, 2004b). The most consistent clinical signs of the disease are the anaemia and anorexia and both are most evident at peak parasitaemia. Other clinical signs of the disease include: (i) exophthalmia (Fig. 3.5); (ii) general oedema; (iii) abdominal distension with ascites; and (iv) a positive anti-globulin reaction (or positive Coombs' test) of red cells (e.g. Woo, 1979; Li and Woo, 1995; Thomas and Woo, 1988).

3.2.2. Diagnosis of infection

Parasitological techniques

Clinical signs can be used for preliminary diagnosis of the disease and the infection can be confirmed by examination of blood and/or

ascites under a microscope. During the acute phase of the disease parasites are easily detected when freshly collected blood or ascites is examined under the compound microscopic (wet mount technique). The identity and morphology of the parasite can be confirmed by examination of air-dried Giemsa-stained smears (Woo, 1969) and/or by using a DNA probe which is specific for *C. salmositica* (Li and Woo, 1996).

The haematocrit centrifuge technique (HCT; Woo, 1969) which was initially described to detect low numbers of trypanosomes in animals including humans (Woo, 1970) was modified to detect *Cryptobia* infections (Woo and Wehnert, 1983). It is used routinely to detect *Cryptobia* in the blood either before the acute phase of the disease or during the chronic phase of the infection as it is much more sensitive and less time consuming than the wet mount technique. Briefly, haematocrit tubes with freshly collected blood are centrifuged cold (5–10°C) for about 5 min at 13,000 g (Woo and Wehnert, 1983; Bower and Margolis, 1984a). The parasite becomes very sluggish and dies if the blood is not kept cold (about 10°C) at all times. After centrifugation, the junction of the plasma and packed red cell is examined under a compound microscope (Woo, 1969). The sensitivity of the technique is relatively high, detecting infections when there are about 75 *Cryptobia*/ml of blood (Bower and Margolis,

1984a). However, its sensitivity could be increased if more than one capillary tube of centrifuged blood was examined as was shown with pathogenic mammalian trypanosomes (Woo and Rogers, 1974).

Immunological techniques

Both cell-mediated and humoral assays have been developed to detect *C. salmositica* infections and the techniques are quite sensitive.

Cell-mediated techniques include: (i) delayed-type hypersensitivity (DTH – skin test) reactions; (ii) macrophage migration inhibition (MMI – head kidney cells) assay (Thomas and Woo, 1990a); and (iii) the respiratory burst (RB – head kidney cells) assay (Mehta and Woo, 2002). Both MMI and RB cannot be used routinely as they require killing the fish; however DTH is a non-lethal technique and it only involves intradermal injection of sonicated parasite antigen and determining the increase in skin thickness 72 h later; it is relatively sensitive and is positive at 2 weeks pi.

Serological (detection of either antibodies or parasite antigens in the blood) assays include using the complement fixing antibody test (*in vitro* immune lysis test on live *Cryptobia*) and the indirect haemagglutination test using sonicated parasite antigens (Jones and Woo, 1987), and the microscopic immune-substrate-enzyme technique (MISSET) and the immunofluorescent antibody technique (IFAT) on whole parasites (Woo, 1990). Both MISSET and IFAT are equally sensitive (about 1–2 weeks pi) in detecting specific antibodies against *C. salmositica*; however, MISSET is preferred because it does not require a fluorescent microscope, and the slide can be stored for extended periods after it has been examined.

An antibody-capture ELISA (enzyme-linked immunosorbent assay) is also available to detect *C. salmositica* infections in salmonids. It is sensitive and can also be used on blood blotted on to filter paper and stored at –20°C (Sitja-Bobadilla and Woo, 1994). Finally, Verity and Woo (1996) developed an antigen-capture ELISA using a monoclonal antibody. The antibody (designated mAb-007) was produced against a major *C. salmositica* surface polypeptide (47 kDa) and

the technique detected infections in experimentally infected rainbow trout (inoculated with either the virulent or avirulent strains of *C. salmositica*) as early as 1 week pi. This antigen-capture ELISA can detect as little as 0.5 µg/ml of *C. salmositica* antigen in a cell lysate. However, the technique is not species specific as it reacts with the 47 kDa polypeptide from *C. bullock* and *Cryptobia catostomi*. The technique may be useful for detection of *Cryptobia* infections in naturally infected fishes.

3.3. Pathology

The anaemia is a very consistent clinical sign, and its severity is directly related to parasitaemia (Woo, 1979); consequently it is most severe at peak parasitaemia (acute phase of the disease) which is usually 4–8 weeks pi and this period depends on numerous abiotic and biotic factors (section 3.2.1). There are obvious lesions in haemopoietic tissues during the acute phase in rainbow trout. Severity of the anaemia and histopathological lesions (see below) are directly related to the parasitaemia in the blood and to the extravascular localization of the parasite (Bahmanrokh and Woo, 2001). If an infected fish survives the disease the blood values return to near pre-infection levels during the chronic phase of the infection (Li and Woo, 1991). The other contributing factors to the anaemia include: (i) haemodilution; (ii) splenomegaly; and (iii) haemolysis (Woo, 1979; Laidley *et al.*, 1988; Thomas and Woo, 1988).

Haemolysis is a major contributing cause of the anaemia; it is the result of secretion of a 'haemolysin' by the parasite and it is also released when the parasite is lysed by complement fixing antibodies produced by the host. The 'haemolysin' lyses red cells directly while the other released antigens (from lysed parasites) form immune complexes with antibodies to coat red blood cells. These result in intravascular and/or extravascular haemolysis (Thomas and Woo, 1988). Red blood cells from infected trout are anti-globulin positive (or Coombs' positive), and these red cells are lysed when incubated with fresh trout

complement. However, in infected fish haemolytic activity of serum complement is significantly lowered (Thomas and Woo, 1989b). The antigen(s) is also secreted by the pathogenic strain when it is cultured (Thomas and Woo, 1989a; Woo and Thomas, 1992).

A 200 kDa metalloprotease (glycoprotein) has been identified, isolated and purified (Fig. 3.2) from the pathogenic *C. salmositica*. The optimal activity of the purified enzyme is pH 7.0 (Zuo and Woo, 1997a, d, 1998a). It has high proteolytic activities against azocasein, haemoglobin, fibrinogen and gelatin, but low activity against albumin. It is inhibited by metal-chelating agents and zinc ions, but it is activated by calcium ions (Zuo and Woo, 1997d, 1998a). The purified enzyme also lyses red blood cells under *in vitro* conditions (Zuo and Woo, 2000) by digesting proteins in erythrocyte membranes (Zuo and Woo, 1997d). Hence, it is an important contributing factor to the anaemia, and is the 'haemolysin' that was identified earlier as an important cause of the anaemia (Thomas and Woo, 1988, 1989a). The metalloprotease also digests collagen (Fig. 3.3), and it readily degrades different collagens (types I, IV and V) and laminin (Zuo and Woo, 1997d). The protease is secreted by the parasite during an infection (Zuo and Woo, 1997c) and in culture (Zuo and Woo, 1998a). In cultures, its secretion is significantly increased in the presence of either type I or IV collagen and/or their breakdown products (Zuo and Woo, 1998b). Since the metalloprotease is secreted by the pathogen it contributes to the development of the disease and histopathological lesions (see below) in infected fish. This confirms that the severity of the disease in *Oncorhynchus* spp. is directly related to the parasitaemia (Woo, 1979).

The histopathology includes: (i) focal haemorrhages; (ii) congestion of blood vessels; (iii) occlusion of capillaries with parasites; and (iv) changes in kidney glomeruli (Putz, 1972). Bahmanrokh and Woo (2001) conducted a sequential study in experimentally infected juvenile rainbow trout and showed the histopathology was a generalized inflammatory reaction, and lesions were in connective tissues and the reticulo-endothelial systems. Lesions were seen first in the liver,

gills and spleen at about 1–2 weeks pi. Endo-vasculitis and mononuclear infiltration occurred at 3 weeks pi and these were followed by tissue necrosis and extravascular localization of parasites at 4 weeks pi. Extensive necrosis of tissues was related directly to high parasitaemias and extravascular localization of flagellates. Necrosis in the liver and kidney, depletion of haematopoietic tissues, and anaemia were probably responsible for mortality of fish during the acute phase of the disease. In some fish regeneration and replacement of necrotic tissues with normal structures were noticeable in haematopoietic and reticular tissues at 7–9 weeks pi and these were associated with reduced parasitaemias in the blood (recovery or chronic phase of the disease).

3.4. Pathophysiology

During the acute phase of the disease the haemolytic activity of serum complement is significantly lowered in infected trout (Thomas and Woo, 1989b), and this would contribute to the immunodepression and increased susceptibility to secondary infections (Jones *et al.*, 1986; Thomas and Woo, 1992). In infected rainbow trout the haemolytic levels of complement are about 20% of pre-infected levels and this persists throughout the infection (Thomas and Woo, 1989b). Low complement decreases phagocytic activity and antigen presentation by macrophages and hence contributes to immunodepression. Anorexia also contributes to the immunodepression (Thomas and Woo, 1992) and is also correlated with the anaemia during acute disease (MacDonald, 2007). Further studies are needed to elucidate other factors that contribute to it.

In addition, plasma thyroxine (T3 and T4), protein and glucose are reduced along with depletion of liver glycogen (Laidly *et al.*, 1988). The metabolism and swimming performance of infected juvenile rainbow trout are also significantly reduced especially during the acute phase of the disease (Kumaraguru *et al.*, 1995), and the bioenergetic cost of the disease in juvenile fish is considerable.

These are contributing factors to the retarded growth of juvenile fish as there are significant reductions in food consumption, dry weight and energy gained, energy concentration and gross conversion efficiency. However, the attenuated vaccine strain (section 3.5.3) has no detectable bioenergetic cost to juvenile fish (Beamish *et al.*, 1996). It would be productive to examine in greater detail the effects the disease has on the endocrine system.

The anaemia and large numbers of parasites occluding small blood vessels would combine to reduce oxygen delivery to tissues and vital organs. Part of this is manifested as an increase in susceptibility of infected fish to environmental hypoxia. This would be an important contributing factor to fish mortality under some conditions, especially when dissolved oxygen is reduced as a result of overcrowding, or slow water flow or during algal blooms (Woo and Wehnert, 1986).

Anorexia is another consistent clinical sign of the disease (section 3.2.1) and it contributes to the immunodepression in infected fish during acute disease (Thomas and Woo, 1992); however, it is also beneficial to the host because anorexia decreases plasma protein by reducing protein intake (Li and Woo, 1991). Reduction in plasma protein lowers parasite multiplication which in turn reduces the parasitaemia, and a lower parasitaemia decreases the severity of the disease and fish mortality. As the infection progresses anorexia strengthens the feeding hierarchy within groups of fish; that is it exacerbates the difference between dominant and subordinate fish (Chin *et al.*, 2004). It is also positively correlated with reduction in oxygen carrying capacity during acute disease. There are also increases in corticotrophin-releasing factor (CRF) and urotensin 1 (U1) mRNA but these are not correlated with food intake. Interleukin-1 beta mRNA levels in the head kidney and spleen are significantly reduced in infected fish. Lastly, neither plasma cortisol nor adrenocorticotropin hormone (ACTH) levels are affected. Consequently, it is suggested that hypoxaemia is probably a mediator of anorexia in cryptobiosis (MacDonald, 2007).

As indicated earlier (section 3.2.1) anorexia is most evident during the acute phase of the disease and it contributes to the

immunodepression in cryptobiosis (Thomas and Woo, 1992). As a result of the immunodepression fish are more susceptible to secondary infections and their ability to mount a protective response (via vaccination) is also significantly reduced (Jones *et al.*, 1986). If the infected fish survive the acute disease the anaemia and anorexia gradually subside as the parasitaemia is reduced (chronic phase of the disease) due to the development of protective humoral (e.g. Jones and Woo, 1987; Li and Woo, 1995; Feng and Woo, 1997a) and cell-mediated (e.g. Thomas and Woo, 1990a; Feng and Woo, 1996a; Mehta and Woo, 2002) immune responses to the pathogen.

3.5. Protective and Control Strategies

Most parasitic (protozoan and metazoan) infections in fishes are usually controlled using chemotherapy. There is no doubt chemical treatments are necessary under certain circumstances (e.g. during disease outbreaks); however, the use of chemicals is becoming more restrictive due to many factors which include increasing concerns over food safety and environmental pollution. Woo (e.g. 1987b, 1992, 2001, 2007) has always been interested in the immune response of fish to parasites and exploiting it (both innate and adaptive components) as part of an overall control strategy against parasites. The following section shows that this approach is possible and it is hoped that these proof-of-concept strategies developed using *C. salmositica* are considered and modified/refined for use on other piscine parasites.

3.5.1. Serological

Fish that have recovered from cryptobiosis are protected from the pathogen and their antisera have high titres of agglutinating, neutralizing and complement-fixing antibodies (e.g. Jones and Woo, 1987; Thomas and Woo, 1990b; Sitja-Bobadilla and Woo, 1994; Li and Woo, 1995; Feng and Woo, 1997a; Ardelli and Woo, 2002; Mehta and Woo, 2002). Also, intraperitoneal implantation of cortisol lowers

antibody production and this increases parasitaemia in rainbow trout. The mortality of infected cortisol-implanted fish is higher than in infected fish or uninfected cortisol-implanted fish (Woo *et al.*, 1987). Also, both antisera from recovered fish and the monoclonal antibody mAb-001 (see below) are therapeutic and prophylactic against the parasite in fish (Feng and Woo, 1997b). Titres of complement-fixing antibodies in recovered and vaccinated fish rise significantly after *C. salmositica* challenge (e.g. Li and Woo, 1995; Ardelli and Woo, 1997, 2002; Feng and Woo, 1998c; Mehta and Woo, 2002), and this classical anamnestic response also confirms that the protection is in part due to humoral response in recovered and vaccinated fish.

A murine IgG1 monoclonal antibody (mAb-001) was produced against the 200 kDa glycoprotein (Feng and Woo, 1996b). The epitope (designated Cs-gp200) consists of carbohydrate determinants and conformational polypeptide with internal disulfide bonds. It is hydrophilic and is secreted by the parasite (Feng and Woo, 1998a). Cs-gp200 has its asparagine-bound N-glycosidically linked hybrid-type carbohydrate chain with the minimum length of a chitobiose core unit. It has a phosphatidylinositol residue which anchors the conformational polypeptide (with disulfide bonds) to the surface of the pathogen. The molecule is extensively post-translationally modified (Feng and Woo, 1998b). 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 (Feng and Woo, 2001).

The antibody mAb-001 is therapeutic when injected intraperitoneally into infected fish – it significantly lowered the parasitaemias in fish and this was similar to the effects of the inoculation of antisera from fish that had recovered from cryptobiosis (Fig. 3.6). Also, the antibody was prophylactic against *C. salmositica* (Feng and Woo, 1997b); however, it did not fix complement to lyse the parasite but agglutinated it. *In vitro* exposure of the parasite to mAb-001 reduced its survival and infectivity when inoculated back into fish (Feng and Woo, 1996b). Under *in vitro* conditions the parasite normally consumed oxygen, however, its aerobic respirations and mobility

were inhibited when it was exposed to sodium azide. These activities were restored on washing even after prolonged exposure (24 h) to the metabolic poison; attempts to show it had glycolytic enzymes were not successful (Thomas *et al.*, 1992). Using more refined techniques it was later shown that the parasite indeed had glycolytic enzymes sequestered in microbodies called glycosomes (Ardelli *et al.*, 2000). In another metabolic study it was confirmed that parasite multiplication and aerobic respiration were totally inhibited in the presence of mAb-001 (Hontzeas *et al.*, 2001).

Also, the antibody neutralizes 100% of the histolytic activities of the metalloprotease and about 80% of the enzymatic activities of the cysteine protease under *in vitro* conditions (Zuo *et al.*, 1997). Consequently, mAb-001 has two main effects in cryptobiosis: (i) the neutralization of the metalloprotease which forms the basis of the metalloprotease-DNA vaccine (section 3.5.3); and (ii) inhibition of the metabolism of the parasite which is manifested in inhibition of aerobic respiration and parasite multiplication. These inhibitory effects would contribute to its therapeutic and prophylactic properties in fish against the pathogen (Feng and Woo, 1997b); however, further studies are needed to confirm this suggestion.

3.5.2. Innate (natural) immunity

Two forms of natural immunity have been shown to occur in fish against *C. salmositica* and both are humoral related: (i) resistance to infection by a fish (pathogen-resistant fish); and (ii) the absence of disease in an infected fish (pathogen-tolerant fish). Besides these humoral immune responses there is also innate cell-mediated response and this is evident soon after infection (Chin and Woo, 2005). The nitroblue tetrazolium slide assay (Anderson *et al.*, 1992) was used to detect activated peripheral phagocytes in the blood of experimentally infected Atlantic salmon (Chin and Woo, 2005). However, the importance of peripheral phagocytes in innate protection against *Cryptobia* needs further studies and elucidation.

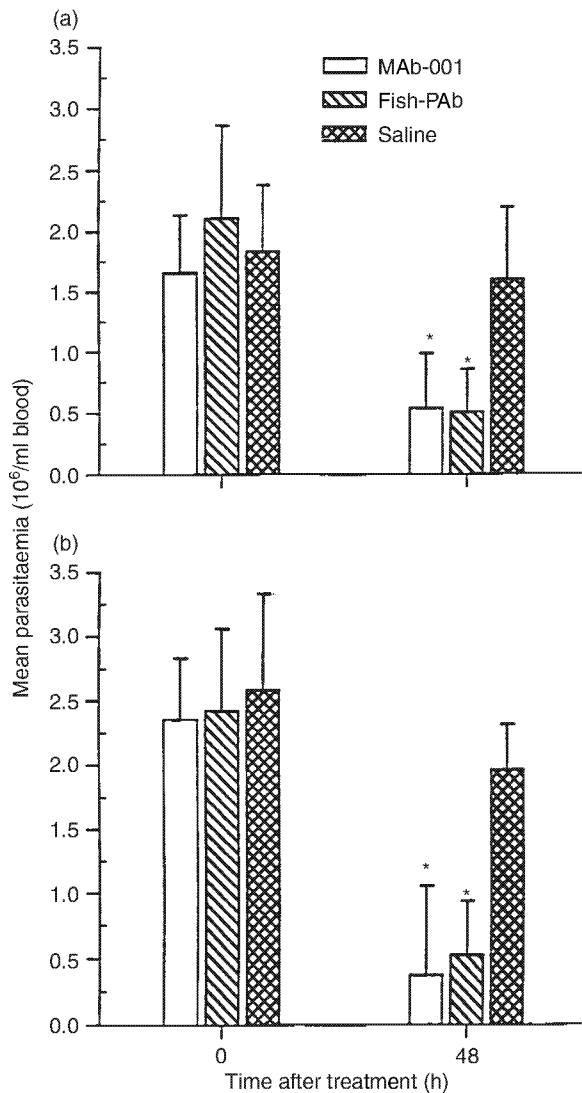


Fig. 3.6. Parasitaemias in rainbow trout 48 h after injection of antibodies: (a) fish infected with 2000 parasites; (b) fish infected with 20,000 parasites. MAb-001, fish injected with monoclonal antibody (mAb-001); Fish-PAb, fish injected with antiserum from a recovered fish; Saline, fish injected with cold-blooded vertebrate Ringer's saline; *, significantly lower ($P < 0.05$) than prior to injection of antibodies (from Feng and Woo, 1997b; courtesy of *Diseases of Aquatic Organisms*).

Cryptobia-resistant fish

Some hatchery-raised brook charr (*S. fontinalis*) with no prior exposure to the parasite or its antigens cannot be infected with *C. salmositica*; this is innate resistance to infection. The resistance is inherited by progeny and it is

controlled by a single dominant Mendelian locus. Briefly, *Cryptobia*-infected brook charr are homozygous recessive while the *Cryptobia*-resistant fish are either homozygous or heterozygous dominant for the locus (Forward *et al.*, 1995). Fresh plasma from *Cryptobia*-resistant brook charr lyse the parasite via the alternative

pathway of complement activation under *in vitro* conditions (Forward and Woo, 1996). Consequently, we can now pre-select and breed *Cryptobia*-resistant brook charr by testing the freshly collected plasma from the brood fish for cryptobiocidal effects. There is no detectable difference in the immune responses of both *Cryptobia*-tolerant and *Cryptobia*-resistant brook charr to antigenic stimulations including to a commercially available bacterial vaccine (Ardelli and Woo, 1995).

Cryptobia-tolerant fish

Parasitaemias in some infected brook charr are just as high as those in *Oncorhynchus* spp., however, they do not have clinical signs (e.g. anaemia) associated with cryptobiosis – these are *Cryptobia*-tolerant fish. These brook charr do not have the disease because the metalloprotease secreted by *C. salmositica* is neutralized by the alpha2 ($\alpha 2$) macroglobulin (a natural anti-protease) in the blood. The amount of $\alpha 2$ macroglobulin is higher in brook charr than in rainbow trout prior to infection and it remains relatively high (about 40%) even at peak parasitaemia while that in trout drops to about 12% (Zuo and Woo, 1997a, b). Parasitaemias in infected rainbow trout and brook charr peak at 4–6 weeks pi and as antibodies are produced they decline; however, the parasitaemia fluctuates in rainbow trout (e.g. Woo, 1979) while that in infected *Cryptobia*-tolerant charr it rapidly declines after peak parasitaemia. Since *Cryptobia*-tolerant charr do not suffer from clinical disease, the immune system readily controls the infection and charr recover more rapidly than trout from the infection (Ardelli and Woo, 1995).

An obvious option to control cryptobiosis is to consider producing transgenic *Cryptobia*-tolerant salmon. It is expected the transgenic salmon will maintain high levels of $\alpha 2$ macroglobulin in their blood to neutralize the metalloprotease secreted by the pathogen – this will eliminate or at least reduce the severity of the disease. Since the disease is absent or less severe the fish immune system can more effectively control the infection. This proposal is a novel approach to the management of an infectious disease and it perhaps

needs further research and discussions. Obvious important ‘downsides’ to consider would include the acceptance of transgenic fish for human consumption. Also, should the transgenic fish escape from hatcheries they would breed with the ‘wild’ population which would not be acceptable. However, the latter point could be overcome with further research. One important advantage is that no further human intervention (e.g. vaccination, chemotherapy) is required once the transgenic animal is produced.

3.5.3. Adaptive (acquired) immunity

Two distinctly different experimental vaccines (a live attenuated vaccine and a metalloprotease-DNA vaccine) have been developed to protect fish from the pathogen and disease. Susceptible fish inoculated with the attenuated *Cryptobia* vaccine are protected from infection when challenged with the pathogen. The second experimental vaccine (metalloprotease-DNA vaccine) does not prevent infection in vaccinated fish after they are challenged with the pathogen. However, antibodies produced in vaccinated fish neutralize the disease-causing factor secreted by the pathogen so that the DNA-vaccinated fish does not suffer from cryptobiosis. The vaccinated fish essentially turns the pathogenic *Cryptobia* into a non-pathogenic flagellate as in the case of the *Cryptobia*-tolerant brook charr (section 3.5.2) and the antibody also (like mAb-001) inhibits parasite respiration and multiplication (section 3.5.1).

Live vaccine

The pathogen was attenuated after prolonged *in vitro* culture and the strain has been cloned. It is maintained in tissue culture medium and it has remained avirulent since its attenuation in 1990. The strain produces a low infection in rainbow trout, does not cause disease, circulates in the blood for at least 6 months and is protective when the fish is challenged with the pathogen (Woo and Li, 1990). A single injection of the vaccine protects fish and all vaccinated fish are protected from disease when challenged. Consequently the strain is

also used routinely as an experimental vaccine to study the development and mechanism of protective immunity in salmonids and the pathobiology of the disease. As indicated earlier (section 3.4) the vaccine has no detectable bioenergetic cost to juvenile trout, and it protects various species of juvenile and adult salmonids from the pathogen (e.g. Sitja-Babodilla and Woo, 1994; Li and Woo, 1995; Ardelli and Woo, 1997, 2002; Feng and Woo, 1997a, 1998c; Mehta and Woo, 2002; Chin *et al.*, 2004).

Rainbow trout vaccinated while in fresh water and transferred to sea water are also protected on challenge (Li and Woo, 1997), and a single dose of the vaccine protects them for at least 24 months (Li and Woo, 1995). Vaccinated fish are only partially protected if they are challenged at 2 weeks post-vaccination (pv) while all vaccinated fish are protected at 3 weeks pv (Fig. 3.7). Protection is via the production of complement fixing antibodies (Fig. 3.8), and under *in vitro* conditions activated macrophages from

head kidneys of vaccinated fish have enhanced phagocytosis, and show antibody-independent and antibody-dependent cytotoxicity. Also, in the presence of antiserum macrophages are very efficient in engulfing living parasites (Fig. 3.9). Also, the complement fixing antibody titres (e.g. Li and Woo, 1995) and cell-mediated response (e.g. Mehta and Woo, 2002) in vaccinated fish rise significantly soon after parasite challenge (classical secondary responses). Humoral and cell-mediated immunity are involved in the protective mechanism in both vaccinated and recovered fish (e.g. Li and Woo, 1995; Ardelli and Woo, 1997, 2002; Mehta and Woo, 2002; Feng and Woo, 1996a).

Metalloprotease-DNA vaccine

As discussed earlier the metalloprotease is the main disease-causing agent and this 200 kDa glycoprotein can be neutralized either by a natural anti-protease ($\alpha 2$ macroglobulin) in *Cryptobia*-tolerant brook charr (Zuo and Woo,

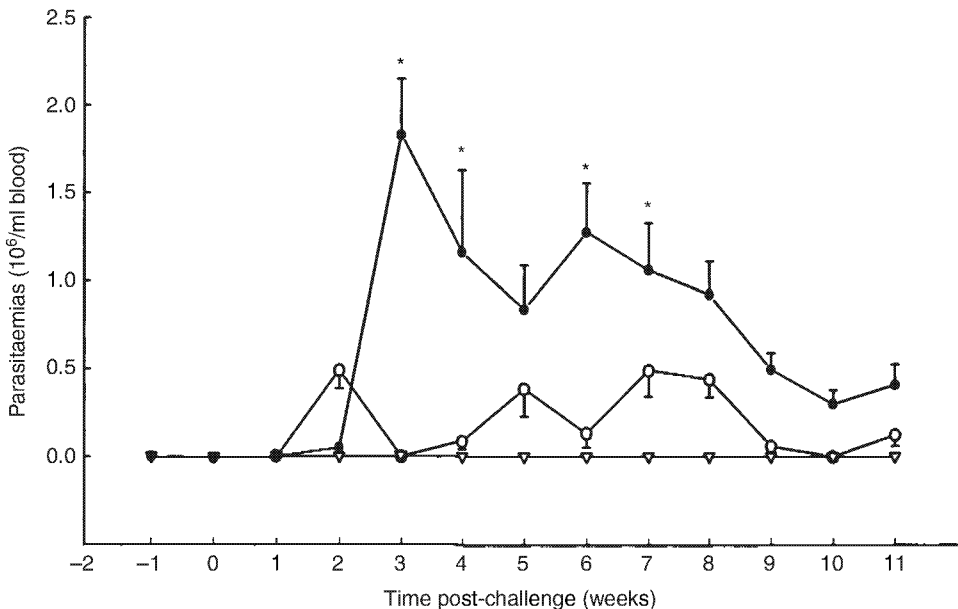


Fig. 3.7. Parasitaemias (mean $\pm$ SE) in vaccinated (10,000 attenuated *C. salmositica*) rainbow trout experimentally challenged with the pathogenic *C. salmositica*. Open triangles, vaccinated fish challenged at 3 weeks post-vaccination (pv); open circles, vaccinated fish challenged at 2 weeks pv; solid circle, control fish challenged at 3 weeks after inoculation with Ringer's saline; *, significantly higher at $P \leq 0.05$ (redrawn from Li and Woo, 1995; courtesy of *Veterinary Immunology and Immunopathology*).

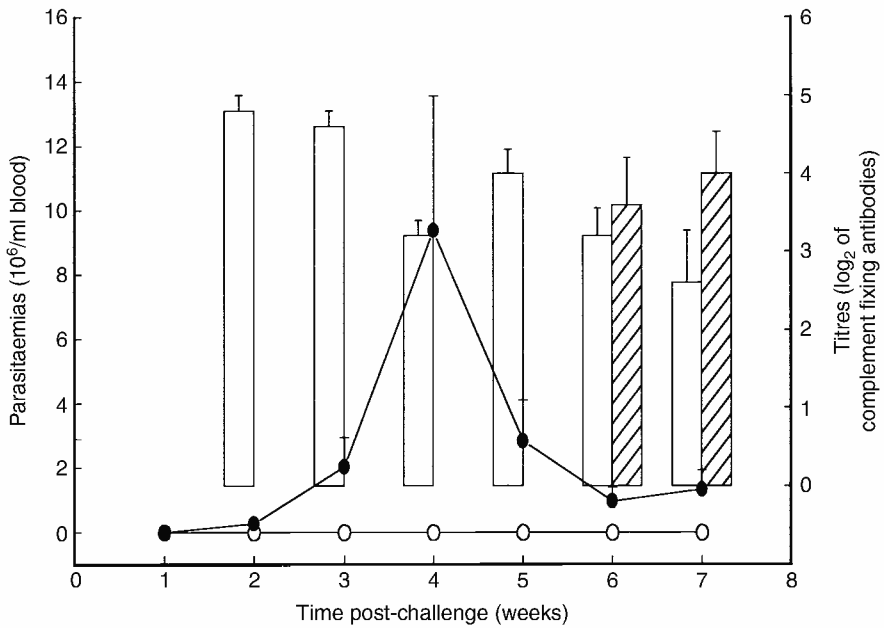


Fig. 3.8. Parasitaemias (line graph; mean $\pm$ SE) and complement fixing antibodies (bar graph; mean $\pm$ SE) in infected controls (solid circles and bars with diagonal lines) and in vaccinated rainbow trout (open circles and open bars) challenged with 100,000 pathogenic *C. salmositica* at 4 weeks after vaccination (from Li and Woo, 1995; courtesy of *Veterinary Immunology and Immunopathology*).

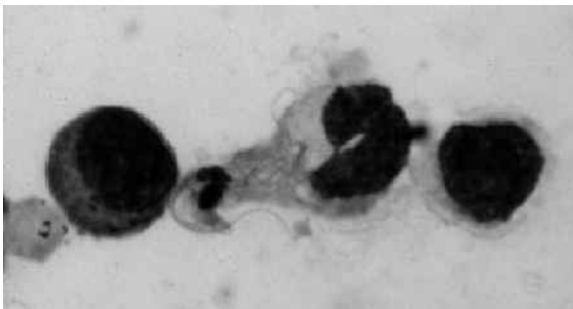


Fig. 3.9. Peritoneal macrophage in ascites of an experimentally infected rainbow trout. *C. salmositica* in the process of being engulfed (from Woo, 1979; courtesy of *Experimental Parasitology*).

1997a, b, c) or by an antibody (mAb-001) against the 200 kDa glycoprotein (Zuo *et al.*, 1997). The monoclonal antibody mAb-001 agglutinates the parasite and reduces its survival and infectivity (Feng and Woo, 1996b). Neutralization of the metalloprotease secreted by the pathogen, and inhibition of its multiplication and metabolic activities (section 3.5.1) by specific antibodies are the main functions of the DNA vaccine.

Briefly, the metalloprotease (histolytic enzyme) and cysteine protease (metabolic

enzyme) genes of *C. salmositica* were sequenced (Jesudhasan *et al.*, 2007a, b) and inserted into plasmid vectors (pEGFP-N) to produce a metalloprotease-plasmid vaccine and a cysteine-plasmid vaccine (Tan *et al.*, 2008). Rainbow trout and Atlantic salmon injected intramuscularly with the metalloprotease-plasmid vaccine consistently had lower packed cell volume (as metalloprotease was secreted into the blood) than control fishes (fishes inoculated either with plasmid alone or with the cysteine-plasmid vaccine) at 2–4

weeks pv. However, the packed cell volume in metalloprotease-vaccinated fishes returned to normal by 5 weeks pv – this was because the metalloprotease (secreted by the vaccine) was neutralized as antibodies were produced by the fish. Agglutinating antibodies against *C. salmositica* were detected 5–7 weeks pv in the blood (but not before 5 weeks pv) in metalloprotease-vaccinated trout. However, no agglutinating antibodies were detected in fish injected with either the cysteine-plasmid-injected or plasmid-alone-injected fish. On challenge with the pathogen the metalloprotease-vaccinated trout had: (i) lower parasitaemia; (ii) delayed peak parasitaemia; and (iii) faster recovery than control infected fish. Further studies are needed to refine this very promising approach and this would include determination of dosages and longevity of protection. In a review on the use of DNA vaccines in aquatic organisms Kurath (2008) confirms that this is the ‘first published demonstration of protective effects of a fish parasite DNA vaccine in fish’.

3.5.4. Chemotherapy and immunochemotherapy

Chemotherapy is essentially differential toxicity of the administered chemical; that is, the drug is more toxic to the target organism than it is to host tissues. Severity of the side effects of chemotherapy is dependent partly on tissue damage and adverse reactions by the host to the chemical. However, the drug can be directed more specifically to the pathogen if it is conjugated to an antibody specific for the target organism (immunochemotherapy). This will obviously increase the cost of treatments and it is generally not meant for routine use. It may, however, be a useful tool under certain circumstances as it reduces the drug dosage and its side effects. In cryptobiosis, it can be used to treat infected brood fish as about 50% of brood fish annually die from cryptobiosis in some hatcheries on the west coast of North America (section 3.1.4). It is expected side effects and accumulation of drug residues in host tissues are reduced in immunochemotherapy, and this may also

lower the risk of the development of drug resistance by the pathogen. Reduction in drug residue in host tissues is also an important consideration if treated animals are for human consumption.

Chemotherapy

A combination of antibiotics (Penicillin, Streptomycin and Amphotericin B) affects the viability of the parasite under *in vitro* conditions; however this combination does not modulate the infection in infected fish (Thomas and Woo, 1991). According to Chapman (1994) the main cryptobiacidal factor in the combination is Amphotericin B. Crystal violet, a triphenylmethane dye is also cryptobiacidal and under *in vitro* conditions low concentrations of the dye inhibits parasite multiplication, reduces its infectivity to fish, and causes ultrastructural lesions on mitochondrial and nuclear membranes (Ardelli and Woo, 1998). However, its therapeutic dose is too toxic (74% mortality) in juvenile trout (B.F. Ardelli and P.T.K. Woo, unpublished).

Isometamidium chloride (Samorin) is widely used against trypanosomiasis in domestic animals in tropical Africa (e.g. Kinabo *et al.*, 1989), and it is also used as a prophylactic drug against bovine trypanosomiasis (e.g. Kinabo and Bogan, 1987). In fish, Samorin (1.0 mg/kg body weight) peaks in the blood 2–3 weeks after intramuscular injection (Ardelli and Woo, 2000). The drug is therapeutic against *C. salmositica* in rainbow trout during pre- and post-clinical phases of the disease. However, it is not effective during acute disease partly as we believe the drug ‘modifies’ surface epitopes of the parasite so that parasites are not lysed by complement fixing antibodies (Ardelli and Woo, 1999). The drug is more effective in infected Atlantic salmon, and at a higher dose (2.5 mg/kg) the infection is eliminated in about 30% of adult fish and significantly reduces the parasitaemias in remaining fish. Also all infected juvenile chinook salmon treated with isometamidium chloride (1.0 mg/kg) survived the disease while 100% of untreated infected fish died with massive parasitaemias. The drug also has prophylactic value,

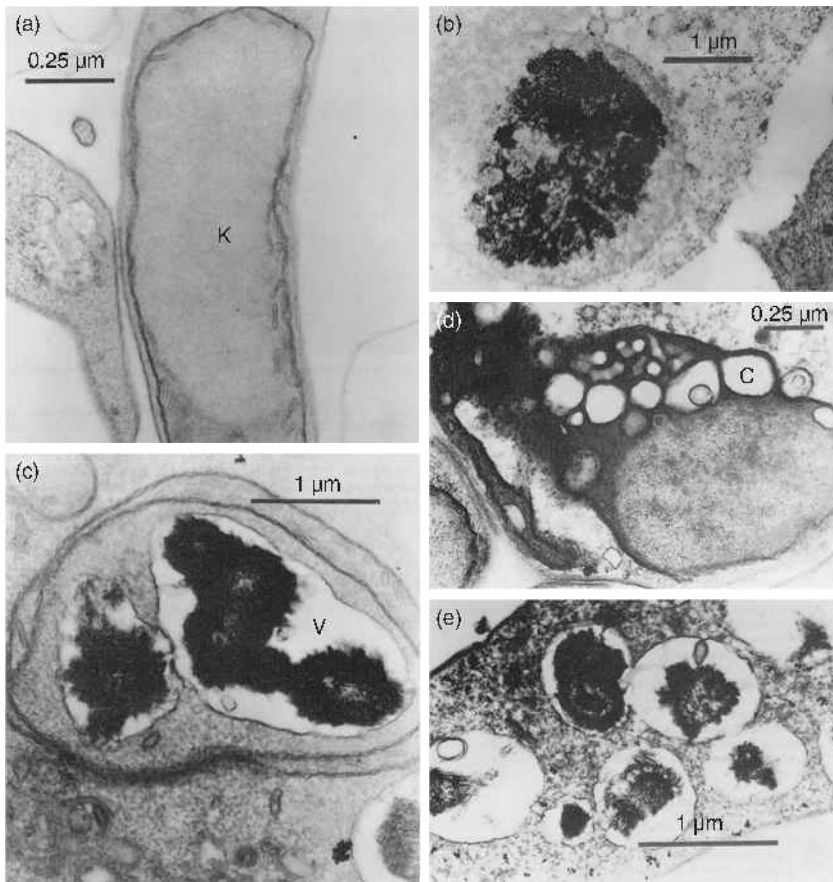


Fig. 3.10. Micro-lesions in *C. salmositica* (transmission electron microscopy) after *in vitro* exposure to isometamidium chloride. (a) Normal kinetoplast – not exposed to Samorin; (b) condensation of DNA in kinetoplast after exposure to Samorin; (c) vacuole formation in kinetoplast after drug exposure; (d) swelling of cristae after exposure to Samorin; (e) vacuole formation in cytoplasm after drug exposure (C, cristae; K, kinetoplast; V, vacuole) (from Ardelli and Woo, 2001a; courtesy of *Journal of Parasitology*).

and there is no evidence the drug affects fish growth, food consumption, blood complement levels or haematocrit values (Ardelli and Woo, 2001b).

Samorin accumulates rapidly in the kinetoplast of the parasite (Ardelli and Woo, 2001a) and causes condensation of its kinetoplast DNA, formation of vacuoles and swelling of the mitochondrial cristae (Fig. 3.10). The parasite normally undergoes aerobic respiration (Thomas *et al.*, 1992; Hontzeas *et al.*, 2001); however, it also has glycolytic enzymes sequestered in microbodies called glycosomes (Ardelli *et al.*, 2000). The *in vitro* oxygen

consumption and carbon dioxide production by the parasite decrease significantly after drug exposure, and there are very significant increases in secretion of glycolytic products (lactate and pyruvate) as the parasite switches from aerobic respiration to glycolysis after its mitochondrion is damaged by the drug (Ardelli and Woo, 2001a). Also, *in vitro* exposure to sub-lethal dosages of the drug reduces infectivity of the parasite to fish, and changes the surface glycoprotein antibody-receptor sites of the parasite (Ardelli and Woo, 1999). This alteration of surface epitopes by the drug would explain the protection of some

Table 3.1. Infectivity of cultured *C. salmositica* to chinook salmon after *in vitro* exposure to isometamidium conjugated to anti-*C. salmositica* polyclonal antibodies.

Time after infection (weeks)	Group 1	Group 2	Group 3	Group 4	Group 5
	PAIC ^a	Isometamidium	PAI ^a	Antibody	Untreated controls
1	1/10 ^b 0.30 ± 0.95 ^c	7/10 1.80 ± 2.78	0/10 0	0/10 0	8/10 6.60 ± 4.72
2	1/10 0.30 ± 0.95	6/10 8.10 ± 5.28	2/10 1.80 ± 3.91	0/10 0	10/10 48 750 ± 45 814
3	2/10 0.50 ± 1.27	7/10 33 250 ± 38 207	2/10 1950 ± 4310	0/10 0	10/10 35 625 ± 29 978
4	2/10 0.30 ± 0.675	8/10 302 000 ± 254 142	3/10 2500 ± 5270	3/10 5558 ± 16 665	10/10 5 487 500 ± 5 439 838
5	2/10 0.60 ± 1.58	10/10 9 395 000 ± 16 925 911	4/10 54 740 ± 112 499	4/9 556 944 ± 1 500 001	10/10 3 175 000 ± 3 639 196
6	1/10 3750 ± 11 858	10/10 13 475 000 ± 15 298 624	4/10 503 750 ± 1 030 810	4/10 2 600 000 ± 5 577 465	10/10 3 243 750 ± 5 416 196
7	1/10 11 250 ± 35 575	10/10 18 305 000 ± 52 500 000	4/10 928 860 ± 1 326 575	4/10 44 383 334 ± 129 150 260	10/10 27 051 250 ± 56 209 714

^a PAIC, Polyclonal antibodies-conjugated drug; PAI, drug plus polyclonal antibodies without conjugation.^b Number of infected fish/number of fish inoculated.^c Mean parasitaemia ±SD; determined by HCT or haemocytometer.

parasites from lysis by complement fixing antibodies when the treatment was administered to rainbow trout with acute infections.

Immunochemotherapy

Isometamidium chloride was conjugated to polyclonal antibodies (from 'recovered' fish) and to the monoclonal antibody (mAb-001). On *in vitro* exposure the conjugated drug was on the entire parasite while the unconjugated drug accumulated only in the kinetoplast (Ardelli and Woo, 2001c). Before drug conjugation both antibodies agglutinated living parasites but the antibodies reacted differently after drug conjugation. Polyclonal antibodies-conjugated drug (PAIC) lysed most of the parasite under *in vitro* conditions and it no longer agglutinated the parasite. In contrast, the mAb-001-conjugated drug did not lyse *C. salmositica* but agglutinated it. After *in vitro* exposure to PAIC the infectivity of the parasite was significantly lowered. The number of infected juvenile chinook salmon and their parasitaemias at 7 weeks pi were significantly lower in fish injected with PAIC-exposed parasites than in those exposed to drug alone or to polyclonal antibodies alone or to drug plus polyclonal antibody without conjugation (Table 3.1). Also, fish survival in the PAIC group was higher than in the other groups. Preliminary studies indicate the drug-antibody conjugate is also effective when injected into infected fish. These results are encouraging and further studies are needed (e.g. to determine dosages needed for effective treatment, and refinement of the approach which would include stage of infection and species of salmonids).

3.5.5. Environmental modification and vector control

Water temperature

The parasite becomes sluggish and does not survive if infected blood is left at room temperature (about 20°C) for any length of time. Experimentally infected juvenile rainbow trout lost their infections or there was no fish mortality when the water temperature was

slowly raised from about 10°C to 20°C (Woo *et al.*, 1983; Bower and Margolis, 1985). Consequently Woo (1987a) suggested that modification(s) of this approach might be useful approach to protecting fish under certain circumstances. Bower and Evelyn (1988) confirmed that infected juvenile sockeye salmon acclimated to 20°C survived while all infected fish maintained at 10°C died from the disease. Also, 60 of temperature-acclimated infected fish survived a parasite challenge at 10°C while 95 of infected non-temperature-acclimated fish died.

Vector control

As indicated earlier *P. salmositica* is the only known vector of the pathogen. This freshwater leech is not host specific and is in cold, fast-flowing rivers and streams (Becker and Katz, 1965a, c). Since leech cocoons and adult *P. salmositica* are susceptible to drying and freezing, draining areas is a method to control leeches in hatcheries where cocoons are deposited or where large numbers of leeches are present (Bower and Thompson, 1987). Also, adult leeches are susceptible to chlorine (Bower *et al.*, 1985), hence the chemical can be considered for controlling leeches under certain conditions.

3.6. Conclusions

C. salmositica infects all species of Pacific salmon and sculpins on the west coast of North America. It is normally transmitted indirectly by the freshwater leech, *P. salmositica* in streams and rivers; however direct transmission between fish can occur under certain aquaculture conditions. Although the parasite is pathogenic to salmon it is not known to cause disease in naturally infected sculpins.

Outbreaks of cryptobiosis in juvenile and adult salmon with high fish mortalities have occurred both in freshwater hatcheries and in sea cages. Numerous factors contribute to severity of the disease and they include: (i) the size of the parasite inoculums; (ii) water temperature; (iii) fish diets; (iv) size and strain/species of the fish; and (v) the genetics

and sexual maturity of the fish. Clinical signs of the disease include anaemia, anorexia, abdominal distension with ascites and exophthalmia. Severity of cryptobiosis, appearance of the clinical signs and mortality are related to parasitaemias, and the disease-causing factor is a secreted 200 kDa metalloprotease which is a histolytic enzyme. The histopathology indicates the disease is a generalized inflammatory reaction, and lesions are in connective tissues and the reticulo-endothelial systems. During acute infections the immune system is depressed, fish are more susceptible to secondary infections and they do not respond to vaccination. The bioenergetic cost of the disease is tremendous and in juvenile fish it retards growth, metabolism and swimming performance.

Salmonids that survived the infection are protected from the pathogen. Humoral (neutralizing antibodies, complement fixing

antibodies, etc.) and cell-mediated (cell-mediated cytotoxicity, enhanced phagocytosis, etc.) immunity are involved in the protection against cryptobiosis. Several protective strategies have been developed to protect salmonids and these proof-of-concept strategies include the exploitation of innate (breeding of *Cryptobia*-resistant brook charr and the possibility of a transgenic *Cryptobia*-tolerant salmon) and adaptive (an attenuated live vaccine, a metalloprotease-DNA vaccine and immunochemotherapy) components of the piscine immune system. Since this is research-in-progress more studies will have to be conducted in the future to refine and to further test many of these proof-of-concept strategies, especially under field conditions. It is also hoped that some of these strategies would be considered and could be adapted against other pathogenic parasites in fishes.

References

- Anderson, D.P., Moritamo, T. and Grooth, R. (1992) Neutrophil, glass-adherent, nitroblue tetrazolium assay gives early indication of immunization effectiveness in rainbow trout. *Veterinary Immunology and Immunopathology* 30, 419–429.
- Ardelli, B.F. and Woo, P.T.K. (1995) Immune response of *Cryptobia*-resistant and *Cryptobia*-susceptible *Salvelinus fontinalis* to an *Aeromonas salmonicida* vaccine. *Diseases of Aquatic Organisms* 23, 33–38.
- Ardelli, B.F. and Woo, P.T.K. (1997) Protective antibodies and anamnestic response in *Salvelinus fontinalis* to *Cryptobia salmositica* and innate resistance of *Salvelinus namaycush* to the haemoflagellate. *Journal of Parasitology* 83, 943–946.
- Ardelli, B.F. and Woo, P.T.K. (1998) The *in vitro* effects of crystal violet on the pathogenic haemoflagellate *Cryptobia salmositica* Katz 1951. *Parasite* 5, 27–36.
- Ardelli, B.F. and Woo, P.T.K. (1999) The therapeutic use of isometamidium chloride against *Cryptobia salmositica* in rainbow trout (*Oncorhynchus mykiss*). *Diseases of Aquatic Organisms* 37, 195–203.
- Ardelli, B.F. and Woo, P.T.K. (2000) An antigen-capture enzyme-linked immunosorbent assay (ELISA) to detect isometamidium chloride in *Oncorhynchus* spp. *Diseases of Aquatic Organisms* 39, 231–236.
- Ardelli, B.F. and Woo, P.T.K. (2001a) The *in vitro* effects of isometamidium chloride (Samorin) on the piscine haemoflagellate *Cryptobia salmositica* (Kinetoplastida, Bodonina). *Journal of Parasitology* 87, 194–202.
- Ardelli, B.F. and Woo, P.T.K. (2001b) Therapeutic and prophylactic effects of isometamidium chloride (Samorin) against the haemoflagellate *Cryptobia salmositica* in chinook salmon (*Oncorhynchus tshawytscha*). *Parasitology Research* 87, 18–26.
- Ardelli, B.F. and Woo, P.T.K. (2001c) Conjugation of isometamidium chloride to antibodies and the use of the conjugate against the haemoflagellate, *Cryptobia salmositica*: an immuno-chemotherapeutic strategy. *Journal of Fish Diseases* 24, 439–451.
- Ardelli, B.F. and Woo, P.T.K. (2002) Experimental *Cryptobia salmositica* (Kinetoplastida) infections in Atlantic salmon, *Salmo salar* L.: cell-mediated and humoral immune responses against the pathogenic and vaccine strains of the parasite. *Journal of Fish Diseases* 25, 265–274.

- Ardelli, B.F., Forward, G.M. and Woo, P.T.K. (1994) Brook charr (*Salvelinus fontinalis*) and cryptobiosis: a potential salmonid reservoir host for *Cryptobia salmositica* Katz 1951. *Journal of Fish Diseases* 17, 567–577.
- Ardelli, B.F., Witt, J.D.S. and Woo, P.T.K. (2000) The identification of glycosomes and metabolic end products in pathogenic and nonpathogenic strains of *Cryptobia salmositica* (Kinetoplastida). *Diseases of Aquatic Organisms* 42, 41–51.
- Bahmanrokh, M. and Woo, P.T.K. (2001) Relationships between histopathology and parasitaemias in *Oncorhynchus mykiss* infected with *Cryptobia salmositica*, a pathogenic haemoflagellate. *Diseases of Aquatic Organisms* 46, 41–45.
- Beamish, F.W.H., Sitja-Bobadilla, A., Jebbink, J.A. and Woo, P.T.K. (1996) Bioenergetic cost of cryptobiosis in fish: rainbow trout (*Oncorhynchus mykiss*) infected with *Cryptobia salmositica* and with an attenuated live vaccine. *Diseases of Aquatic Organisms* 25, 1–8.
- Becker, C.D. and Katz, M. (1965a) Transmission of the haemoflagellate *Cryptobia salmositica* Katz 1951, by a rhynchobdellid leech vector. *Journal of Parasitology* 51, 95–99.
- Becker, C.D. and Katz, M. (1965b) Infections of the haemoflagellate *Cryptobia salmositica* Katz 1951, in freshwater teleosts of the Pacific coast. *Transactions of the American Fisheries Society* 94, 327–333.
- Becker, C.D. and Katz, M. (1965c) Distribution, ecology and biology of the salmonid leech, *Piscicola salmositica* Meyer 1946 (Rhynchobdella, Piscicolidae). *Journal of the Fisheries Research Board of Canada* 22, 1175–1195.
- Becker, C.D. and Katz, M. (1966) Host relationships of *Cryptobia salmositica* (Protozoa, Mastigophora) in Washington hatchery stream. *Transactions of the American Fisheries Society* 95, 196–202.
- Bower, S.M. and Evelyn, T.P.T. (1988) Acquired and innate resistance to the haemoflagellate *Cryptobia salmositica* in sockeye salmon (*Oncorhynchus nerka*). *Developmental and Comparative Immunology* 12, 749–760.
- Bower, S.M. and Margolis, L. (1983) Direct transmission of the haemoflagellate *Cryptobia salmositica* among Pacific salmon (*Oncorhynchus* spp.). *Canadian Journal of Zoology* 61, 1242–1250.
- Bower, S.M. and Margolis, L. (1984a) Detection of infection and susceptibility of different Pacific salmon stocks (*Oncorhynchus* spp.) to the haemoflagellate *Cryptobia salmositica*. *Journal of Parasitology* 70, 273–278.
- Bower, S.M. and Margolis, L. (1984b) Distribution of *Cryptobia salmositica*, a haemoflagellate of fishes in British Columbia and the seasonal pattern of infection in a coastal river. *Canadian Journal of Zoology* 62, 2512–2518.
- Bower, S.M. and Margolis, L. (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. and Thompson, A.B. (1987) Hatching of the Pacific salmon leech (*Piscicola salmositica*) from cocoons exposed to various treatments. *Aquaculture* 66, 1–8.
- Bower, S.M., Margolis, L. and MacKay, R.J. (1985) Potential usefulness of chlorine for controlling Pacific salmon leeches, *Piscicola salmositica* in hatcheries. *Canadian Journal of Fisheries and Aquatic Sciences* 42, 1986–1993.
- Brugerolle, C., Lom, J., Nohynkova, E. and Joyon, L. (1979) Comparaison et evolution des structures cellulaires chez plusieurs especes de bodonides et cryptobiides appartenant aux genres *Bodo*, *Cryptobia* et *Trypanoplasma* (Kinetoplastida, Mastigophora). *Protistologica* 15, 197–221.
- Chapman, P.F. (1994) Effects of Amphotericin B, Penicillin, and Streptomycin on cultures of *Cryptobia salmositica*. *Journal of Aquatic Animal Health* 6, 215–219.
- Chin, A. and Woo, P.T.K. (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., Eldridge, M., Glebe, B.D. and Woo, P.T.K. (2002) *Salmo salar* and *Cryptobia salmositica*: variations in susceptibility and humoral response in families of Atlantic salmon to the pathogen. *AquaNet II, Annual General Meeting*, 2002, Moncton, New Brunswick, Canada (Abstract).
- Chin, A., Glebe, B. and Woo, P.T.K. (2004a) Humoral response and susceptibility of five full-sib families of Atlantic salmon (*Salmo salar* L.) to the haemoflagellate, *Cryptobia salmositica* Katz 1951. *Journal of Fish Diseases* 27, 471–481.
- Chin, A., Guo, F.C., Bernier, N.J. and Woo, P.T.K. (2004b) Effect of *Cryptobia salmositica*-induced anorexia on feeding behavior and immune response in juvenile rainbow trout, *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 58, 17–26.

- Currie, J.L.M. and Woo, P.T.K. (2007) Susceptibility of sexually mature rainbow trout, *Oncorhynchus mykiss* to experimental cryptobiosis caused by *Cryptobia salmositica*. *Parasitology Research* 101, 1057–1067.
- Currie, J.L.M. and Woo, P.T.K. (2008) Effects of the pathogenic haemoflagellate, *Cryptobia salmositica* on brood fish, *Oncorhynchus mykiss*. *Environmental Biology of Fishes* 83, 355–365.
- Feng, S. and Woo, P.T.K. (1996a) Cell-mediated immune response and T-like cells in thymectomized *Oncorhynchus mykiss* (Walbaum) infected with or vaccinated against the pathogenic hemoflagellate *Cryptobia salmositica* Katz 1951. *Parasitology Research* 82, 604–611.
- Feng, S. and Woo, P.T.K. (1996b) Biological characterization of a monoclonal antibody against a surface membrane antigen on *Cryptobia salmositica* Katz 1951. *Journal of Fish Diseases* 19, 137–143.
- Feng, S. and Woo, P.T.K. (1997a) Complement fixing antibody production in thymectomized *Oncorhynchus mykiss* (Walbaum), vaccinated against or infected with the pathogenic haemoflagellate *Cryptobia salmositica*. *Folia Parasitology* 44, 188–194.
- Feng, S. and Woo, P.T.K. (1997b) The therapeutic and prophylactic effects of a protective monoclonal antibody (Mab-001) against the pathogenic haemoflagellate *Cryptobia salmositica* Katz 1951. *Diseases of Aquatic Organisms* 28, 211–219.
- Feng, S. and Woo, P.T.K. (1998a) Characterization of a 200 kDa glycoprotein (Cs-gp2000) on the pathogenic piscine haemoflagellate *Cryptobia salmositica*. *Diseases of Aquatic Organisms* 32, 41–48.
- Feng, S. and Woo, P.T.K. (1998b) Biochemical characterization of an epitope on the surface membrane antigen (Cs-gp200) of the pathogenic piscine hemoflagellate *Cryptobia salmositica* Katz 1951. *Experimental Parasitology* 88, 3–10.
- Feng, S. and Woo, P.T.K. (1998c) The *in vitro* and *in vivo* effects of rabbit anti-thymocyte serum on circulating leucocytes and production of complement fixing antibodies in thymectomized *Oncorhynchus mykiss* (Walbaum) infected with *Cryptobia salmositica* Katz 1951. *Journal of Fish Diseases* 21, 241–248.
- Feng, S. and Woo, P.T.K. (2001) Cell membrane glycoconjugates on virulent and avirulent strains of the pathogenic haemoflagellate *Cryptobia salmositica* Katz. *Journal of Fish Diseases* 24, 23–32.
- Forward, G.M. and Woo, P.T.K. (1996) An *in vitro* study on the mechanism of innate immunity in *Cryptobia*-resistant brook charr (*Salvelinus fontinalis*) against *Cryptobia salmositica*. *Parasitology Research* 82, 238–241.
- Forward, G.M., Ferguson, M.M. and Woo, P.T.K. (1995) Susceptibility of brook charr, *Salvelinus fontinalis* to the pathogenic haemoflagellate, *Cryptobia salmositica*, and the inheritance of innate resistance by progenies of resistant fish. *Parasitology* 111, 337–345.
- Hontzeas, N., Feng, S. and Woo, P.T.K. (2001) Inhibitory effects of a monoclonal antibody (Mab-001) on *in vitro* oxygen consumption and multiplication of the pathogenic haemoflagellate, *Cryptobia salmositica* Katz. *Journal of Fish Diseases* 24, 391–398.
- Jesudhasan, P.R.R., Tan, C.W. and Woo, P.T.K. (2007a) A metalloproteinase gene from the pathogenic piscine hemoflagellate, *Cryptobia salmositica*. *Parasitology Research* 100: 899–904.
- Jesudhasan, P.R.R., Tan, C.W., Hontzeas, N. and Woo, P.T.K. (2007b) A cathepsin L-like cysteine proteinase gene from the pathogenic piscine hemoflagellate, *Cryptobia salmositica*. *Parasitology Research* 100, 881–886.
- Jones, S.R.M. and Woo, P.T.K. (1987) The immune response of rainbow trout *Salmo gairdneri* Richardson to the haemoflagellate *Cryptobia salmositica* Katz 1951. *Journal of Fish Diseases* 10, 395–402.
- Jones, S.R.M., Woo, P.T.K. and Stevenson, R.M.W. (1986) Immunosuppression in *Salmo gairdneri* Richardson caused by the haemoflagellate, *Cryptobia salmositica* (Katz, 1951). *Journal of Fish Diseases* 9, 431–438.
- Katz, M. (1951) Two new hemoflagellates (genus *Cryptobia*) from some western Washington teleosts. *Journal of Parasitology* 37, 245–250.
- Kinabo, L.D.B. and Bogan, J.A. (1987) Binding of isometamidium to calf thymus DNA and lipids: pharmacological implications. *Journal of Veterinary Pharmacology and Therapeutics* 10, 357–362.
- Kinabo, L.D.B., Bogan, J.A., McKellar, Q.A. and Murray, M. (1989) Relay bioavailability and toxicity of isometamidium residues: a model for human risk assessment. *Veterinary and Human Toxicology* 31, 417–421.
- Kumaraguru, A.K., Beamish, F.W.H. and Woo, P.T.K. (1995) Impact of a pathogenic haemoflagellate, *Cryptobia salmositica* on the metabolism and swimming performance of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 18, 297–305.
- Kurath, G. (2008) Biotechnology and DNA vaccines for aquatic animals. *Revue Scientifique et Technique* 27, 175–196.

- Laidley, C.W., Woo, P.T.K. and Leatherland, J.F. (1988) The stress response of rainbow trout to experimental infection with the blood parasite, *Cryptobia salmositica* Katz, 1951. *Journal of Fish Biology* 32, 253–261.
- Li, S. and Woo, P.T.K. (1991) Anorexia reduces the severity of cryptobiosis in *Oncorhynchus mykiss*. *Journal of Parasitology* 77, 467–471.
- Li, S. and Woo, P.T.K. (1995) Efficacy of a live *Cryptobia salmositica* vaccine, and the mechanism of protection in vaccinated *Oncorhynchus mykiss* (Walbaum) against cryptobiosis. *Veterinary Immunology and Immunopathology* 48, 343–353.
- Li, S. and Woo, P.T.K. (1996) A species specific *Cryptobia salmositica* (Kinetoplastida) DNA probe and its uses in salmonid cryptobiosis. *Diseases of Aquatic Organisms* 25, 11–16.
- Li, S. and Woo, P.T.K. (1997) Vaccination of rainbow trout, *Oncorhynchus mykiss* (Walbaum) against cryptobiosis: efficacy of the vaccine in fresh and sea water. *Journal of Fish Diseases* 20, 369–374.
- Li, S., Cowey, C.B. and Woo, P.T.K. (1996) The effects of dietary ascorbic acid on *Cryptobia salmositica* infection and on vaccination against cryptobiosis in *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 24, 11–16.
- MacDonald, L.E. (2007) Exploring potential mechanisms mediating *Cryptobia*-induced anorexia in rainbow trout (*Oncorhynchus mykiss*). MSc. thesis, University of Guelph, Guelph, Canada, 104 pp.
- Mehta, M. and Woo, P.T.K. (2002) Acquired cell-mediated protection in rainbow trout, *Oncorhynchus mykiss* against the haemoflagellate, *Cryptobia salmositica*. *Parasitology Research* 88, 956–962.
- Paterson, W.B. and Woo, P.T.K. (1983) Electron microscopic observations of the bloodstream form of *Cryptobia salmositica* Katz, 1951 (Kinetoplastida, Bodonina). *Journal of Protozoology* 39, 431–437.
- 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.
- Sitja-Bobadilla, A. and Woo, P.T.K. (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.
- Tan, C.W., Jesudhasan, R.R.R. and Woo, P.T.K. (2008) Towards a metalloprotease-DNA vaccine against piscine cryptobiosis caused by *Cryptobia salmositica*. *Parasitology Research* 102, 265–275.
- Thomas, P.T. and Woo, P.T.K. (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.
- Thomas, P.T. and Woo, P.T.K. (1989a) An in vitro study on the haemolytic components of *Cryptobia salmositica*. *Journal of Fish Diseases* 12, 89–393.
- Thomas, P.T. and Woo, P.T.K. (1989b) Complement activity in *Salmo gairdneri* Richardson infected with *Cryptobia salmositica* and its relationship to the anaemia in cryptobiosis. *Journal of Fish Diseases* 12, 395–397.
- Thomas, P.T. and Woo, P.T.K. (1990a) In vivo and in vitro cell-mediated immune responses of *Oncorhynchus mykiss* (Walbaum) against *Cryptobia salmositica* Katz, 1951 (Sarcomastigophora, Kinetoplastida). *Journal of Fish Diseases* 13, 423–433.
- Thomas, P.T. and Woo, P.T.K. (1990b) Dietary modulation of humoral immune response and anaemia in *Oncorhynchus mykiss* (Walbaum) infected with *Cryptobia salmositica* Katz, 1951. *Journal of Fish Diseases* 13, 435–446.
- Thomas, P.T. and Woo, P.T.K. (1991) In vitro and in vivo effects of antimicrobial agents on viability of *Cryptobia salmositica* (Sarcomastigophora: Kinetoplastida). *Diseases of Aquatic Organisms* 10, 7–11.
- Thomas, P.T. and Woo, P.T.K. (1992) Anorexia in *Oncorhynchus mykiss* (Walbaum) infected with *Cryptobia salmositica* (Sarcomastigophora, Kinetoplastida), its onset and contribution to the immunodepression. *Journal of Fish Diseases* 15, 443–447.
- Thomas, P.T., Ballantyne, J.S. and Woo, P.T.K. (1992) In vitro oxygen consumption and motility of *Cryptobia salmositica*, *Cryptobia bullocki* and *Cryptobia catostomi*. *Journal of Parasitology* 78, 747–749.
- Verity, C.K. and Woo, P.T.K. (1996) Characterization of a monoclonal antibody against the 47 kDa antigen of *Cryptobia salmositica* Katz and its use in an antigen-capture enzyme-linked immunosorbent assay for detection of parasite antigen in infected rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 19, 91–109.
- Vickerman, K. (1971) Morphological and physiological considerations of extracellular blood protozoa. In: Fallis, A.M. (ed.) *Physiology and Ecology of Parasites*. Toronto University Press, Toronto, pp. 59–91.
- Wales, J.H. and Wolf, H. (1955) Three protozoan diseases of trout in California. *California Fish and Game* 41, 183–187.

- 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. (1970) The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Tropica* 27, 384–386.
- Woo, P.T.K. (1978) The division process of *Cryptobia salmositica* in experimentally infected rainbow trout (*Salmo gairdneri*). *Canadian Journal of Zoology* 56, 1514–1518.
- Woo, P.T.K. (1979) *Trypanoplasma salmositica*: experimental infections in rainbow trout, *Salmo gairdneri*. *Experimental Parasitology* 47, 36–48.
- Woo, P.T.K. (1987a) *Cryptobia* and cryptobiosis in fishes. *Advances in Parasitology* 26, 199–237.
- Woo, P.T.K. (1987b) Immune response of fish to protozoan infections. *Parasitology Today* 3, 186–188.
- Woo, P.T.K. (1990) MISET: an immunological technique for the serodiagnosis of *Cryptobia salmositica* (Sarcocystidophora, Kinetoplastida) infection in *Oncorhynchus mykiss*. *Journal of Parasitology* 76, 389–393.
- Woo, P.T.K. (1992) Immunological responses of fish to parasitic organisms. *Annual Review of Fish Diseases* 2, 339–366.
- Woo, P.T.K. (1994) Flagellate parasites of fishes. In: Kreier, J.P. (ed.) *Parasitic Protozoa*, 2nd edn, Vol. VIII. Academic Press, London, 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. (2006) Diplomonadida (Phylum Parabasalia) and Kinetoplastea (Phylum Euglenozoa). In: Woo, P.T.K. (ed.) *Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections*, 2nd edn. CABI Publishing, Wallingford, Oxon, UK, pp. 116–153.
- Woo, P.T.K. (2007) Protective immunity in fish against protozoan diseases. *Parasitologia* 49, 185–191.
- Woo, P.T.K. and Li, S. (1990) *In vitro* attenuation of *Cryptobia salmositica* and its use as a live vaccine against cryptobiosis in *Oncorhynchus mykiss*. *Journal of Parasitology* 76, 752–755.
- Woo, P.T.K. and Poynton, S.L. (1995) Diplomonadida, Kinetoplastida and Amoebeida (Phylum Sarcocystidophora). In: Woo, P.T.K. (ed.) *Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections*. CABI Publishing, Wallingford, Oxon, UK, pp. 27–96.
- Woo, P.T.K. and Rogers, D.J. (1974) A statistical study of the sensitivity of the haematocrit centrifuge technique in the detection of trypanosomes in blood. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 68, 319–326.
- Woo, P.T.K. and Thomas, P.T. (1992) Comparative *in vitro* studies on virulent and avirulent strains of *Cryptobia salmositica* Katz, 1951 (Sarcocystidophora, Kinetoplastida). *Journal of Fish Diseases* 15, 261–266.
- Woo, P.T.K. and Wehnert, S.D. (1983) Direct transmission of a haemoflagellate, *Cryptobia salmositica* Katz, 1951 (Kinetoplastida, Bodonina) between rainbow trout under laboratory conditions. *Journal of Protozoology* 39, 334–337.
- Woo, P.T.K. and Wehnert, S.D. (1986) *Cryptobia salmositica*, susceptibility of infected trout, *Salmo gairdneri*, to environmental hypoxia. *Journal of Parasitology* 72, 392–396.
- Woo, P.T.K., Wehnert, S.D. and Rodgers, D. (1983) The susceptibility of fishes to haemoflagellates at different ambient temperatures. *Parasitology* 87, 385–392.
- Woo, P.T.K., Leatherland, J.F. and Lee, M.S. (1987) *Cryptobia salmositica*: cortisol increases the susceptibility of *Salmo gairdneri* Richardson to experimental cryptobiosis. *Journal of Fish Diseases* 10, 75–83.
- Wood, J.W. (1979) *Diseases of Pacific Salmon: their Prevention and Treatment*, 3rd edn. State of Washington, Department of Fisheries, Olympia, Washington, DC.
- Zuo, X. and Woo, P.T.K. (1997a) Proteases in pathogenic and nonpathogenic hemoflagellates, *Cryptobia* spp. (Sarcocystidophora: Kinetoplastida) of fishes. *Diseases of Aquatic Organisms* 29, 57–65.
- Zuo, X. and Woo, P.T.K. (1997b) Natural antiproteases in rainbow trout, *Oncorhynchus mykiss*, and brook charr, *Salvelinus fontinalis*, and the *in vitro* neutralization of fish α 2-macroglobulin by the metalloprotease from the pathogenic haemoflagellate, *Cryptobia salmositica*. *Parasitology* 114, 375–382.
- Zuo, X. and Woo, P.T.K. (1997c) The *in vivo* neutralization of proteases from *Cryptobia salmositica* by α 2-macroglobulin in the blood of rainbow trout, *Oncorhynchus mykiss* and brook charr, *Salvelinus fontinalis*. *Diseases of Aquatic Organisms* 29, 67–72.
- Zuo, X. and Woo, P.T.K. (1997d) Purified metalloprotease from the pathogenic haemoflagellate, *Cryptobia salmositica*, and its *in vitro* proteolytic activities. *Diseases of Aquatic Organisms* 30, 177–185.

-
- Zuo, X. and Woo, P.T.K. (1998a) Characterization of purified metallo- and cysteine proteases from the pathogenic haemoflagellate, *Cryptobia salmositica* Katz 1951. *Parasitology Research* 84, 492–498.
- Zuo, X. and Woo, P.T.K. (1998b) *In vitro* secretion of metalloprotease (200 kDa) by the pathogenic piscine haemoflagellate, *Cryptobia salmositica* Katz, and stimulation of protease production by collagen. *Journal of Fish Diseases* 21, 249–255.
- Zuo, X. and Woo, P.T.K. (2000) *In vitro* haemolysis of piscine erythrocytes by purified metalloprotease from the pathogenic haemoflagellate, *Cryptobia salmositica* Katz. *Journal of Fish Diseases* 23, 227–230.
- Zuo, X., Feng, S. and Woo, P.T.K. (1997) The *in vitro* inhibition of proteases from *Cryptobia salmositica* Katz by a monoclonal antibody (MAb-001) against a glycoprotein on the pathogenic haemoflagellate. *Journal of Fish Diseases* 20, 419–426.

4 *Ichthyophthirius multifiliis*

Harry W. Dickerson

College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA

4.1. Introduction

The ciliate, *Ichthyophthirius multifiliis* Fouquet, 1876, is an obligate parasite that infects the epithelia of skin and gills and is one of the most common protozoan pathogens of freshwater fishes. It has significant economic impact and infects a broad spectrum of wild and cultured fish species in most parts of the world. *I. multifiliis* also has been used as a model for elucidating the mechanisms of teleost cutaneous immunity (Dickerson and Clark, 1998; Gonzalez *et al.*, 2007b). This chapter provides a current overview of the biology of the parasite, host pathophysiology and immunity, as well as treatment and prevention of infection.

I. multifiliis is a holotrichous ciliate, class Oligohymenophora, subclass Hymenostomata, order Hymenostomatida, suborder Ophryoglenina, family Ichthyophthiridae (Canella and Rocchi-Canella, 1976; Corliss, 1979; Wright and Lynn, 1995; Van Den Bussche *et al.*, 2000; Lynn, 2008); it and other species in the suborder Ophryoglenina are characterized by the presence of the organelle of Lieberkühn (Canella and Rocchi-Canella, 1976; Lynn *et al.*, 1991). The entire surface of the organism is covered by motile membrane-bound cilia, which are responsible for its progressive motility in the water as well as its movement within the host's epithelium

(Ewing and Kocan, 1992). Like most other ciliate genera in the order Hymenostomatida (e.g. *Tetrahymena*, *Paramecium*) it has a vegetative macronucleus and up to four germ-line micronuclei, which are transcriptionally inactive (Peshkov and Tikhomirova, 1968; Hauser, 1973; Nanney, 1980; Matthews, 1996).

4.2. Life Cycle and Parasite Stages

The direct life cycle of the parasite is comprised of three stages: (i) infective theront; (ii) obligate, fish-associated trophont; and (iii) water-borne reproductive tomont (Fig. 4.1). All stages are ciliated and motile. The theront is oblong (pyriform) in shape, approximately 40 µm in length, with a distinctive caudal cilium (MacIennnon, 1942; Kheisin and Mosevich, 1969; Canella and Rocchi-Canella, 1976; Kozel, 1986; Geisslinger, 1987) (Figs 4.2 and 4.6a). When the pelagic theront encounters a susceptible host it rapidly penetrates into the surface epithelia of the skin and gills through ciliary action and the use of the perforatorium, a specialized membrane-cortical structure on the anterior of the cell (MacIennnon, 1935; Ewing and Kocan, 1992; Buchmann and Nielsen, 1999). The theront is positively phototactic, a function postulated to be attributed to the organelle of Lieberkühn (Wahli and Meier, 1991; Matthews, 2005). Also, the

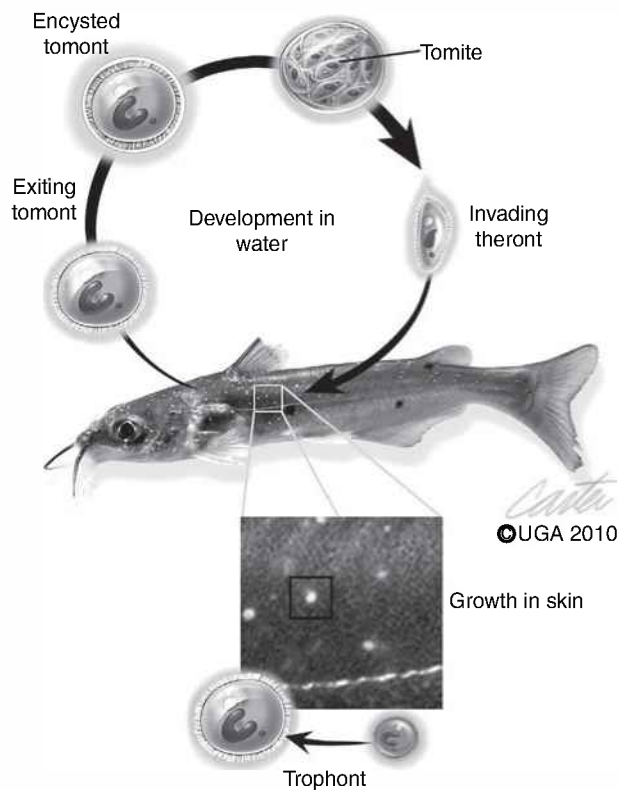


Fig. 4.1. Life cycle of *Ichthyophthirius multifiliis*. All stages of the organism are ciliated. The free-swimming theront penetrates through the mucus and invades into the surface epithelia of the skin and gills. Upon entering the host it transforms into the trophont, which feeds and grows up to 800–1000 μm in size. The trophont actively moves within the epithelium. The parasite exits the fish as the mature tomont, which secretes a protective cyst and divides within it to form 500–1000 daughter cells (tomites). Tomites differentiate into invasive theronts, which bore through the cyst wall and enter into the water.

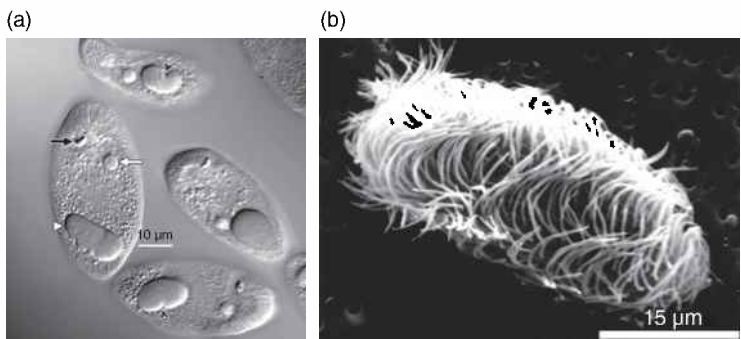


Fig. 4.2. *Ichthyophthirius multifiliis* theronts. (a) The large macronucleus (white arrow head), smaller micronucleus (white arrow) and organelle of Lieberkühn (black arrow) are visible. The typical indented shape of the macronucleus is indicated in several theronts (black arrow heads). Note that surface cilia are not evident in this micrograph (differential interference contrast image). (b) The entire surface of the theront is covered with cilia as seen in this micrograph. The caudal cilium is not evident (scanning electron microscope image).

theront is positively chemotactic to components of fish tissue, including immunoglobulin and mucus (Lom and Cerkasova, 1974; Buchmann and Nielsen, 1999). It has been proposed that the parasite approaches and enters the epithelium of the skin through the goblet cells (Buchmann and Nielsen, 1999). After entry into the host the theront rapidly (within several minutes) differentiates into a feeding trophont, which involves the formation of a functional cytostome and vestibular apparatus (MacIennnon, 1935; Canella and Rocchi-Canella, 1976). *I. multifiliis* is endoparasitic within the epithelium of the host (Matthews, 2005).

The trophont changes from its rigid pyriform theront shape to a polymorphic cellular form propelled by ciliary action between epithelial cells and within tissue spaces created as it feeds in the skin and gill epithelia (Fig. 4.3). The trophont grows rapidly, increasing in size to 200–800 μm , which relates to the duration of feeding (MacIennnon, 1942). The parasite remains on the fish for a variable number of days, depending on the ambient water temperature, and other factors such as the immune status of the fish. Typically, at a temperature of 25°C, it feeds for a period of

5–7 days. The trophont develops and grows within the epithelium, penetrating no deeper than the basal germinal cell layer, which lies immediately adjacent to the underlying dermis (Chapman, 1984). At maturity the parasite leaves the fish, which presumably is triggered by cell volume, size, development and other unknown factors (MacIennnon, 1937, 1942; Ewing *et al.*, 1986; Ewing and Kocan, 1992; Aihua and Buchmann, 2001; Matthews, 2005).

Once it leaves the host and is back in the water the motile organism, now referred to as a tomont, swims for approximately 1 h after which it attaches to any available substrate (e.g. vegetation, inorganic material and other surfaces) by means of a translucent proteinaceous cyst produced by extrusion of the contents of its cortical mucocysts (Ewing *et al.*, 1983). The tomont remains ciliated and rotates within the cyst. It undergoes symmetrical cell divisions and amitotic nuclear divisions at approximately 1 h intervals doubling the number of daughter cells, which are called tomites (Hauser, 1973; Dickerson, 2006). These cells differentiate into infective theronts, which bore through the cyst wall and leave progressively through the perforation(s)

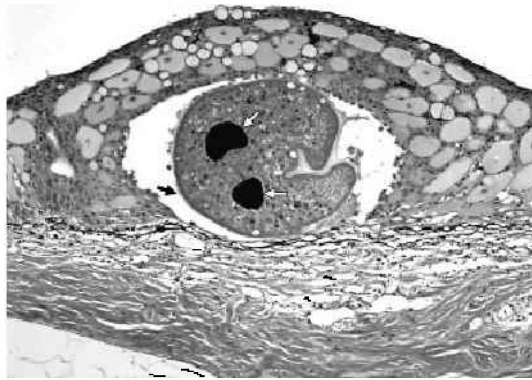


Fig. 4.3. *Ichthyophthirius multifiliis* trophont within the epidermis of a channel catfish. The parasite is actively motile due to action of surface cilia (black arrow) and creates a tissue space within which it feeds on cells and cellular debris that are processed in food vacuoles in the cytoplasm. The polymorphic shape of the trophont with its folded cell membrane is evident, and in this image the single, large 'horse-shoe'-shaped nucleus appears as two parts (white arrows) due to the angle of the section. Numerous alarm cells (larger cells, many have visible nuclei) and goblet cells (smaller cells without visible nuclei and lighter cytoplasm) are visible in the surrounding epithelium that covers the trophont. Inflammatory cells (neutrophils, macrophages and lymphocytes) are present immediately below the parasite in the dermis (haematoxylin-and-eosin-stained paraffin thin section, light microscope image, 100 $\times$ magnification).

created by the first theronts that exit. It takes several minutes for all of the theronts to leave the cyst. The number of theronts produced by each tomont depends on the size of the cell when it leaves the fish and the number of cell divisions within the cyst. Cell division in the tomont is referred to as palintomy because there is no growth between subsequent divisions (Lynn, 2008). Typically, 500–1000 parasites are produced following nine to ten divisions. It has been suggested that adhesion of tomonts to substrate in the immediate environment of a susceptible host population facilitates subsequent infection of the same population, particularly in riparian systems with rapidly flowing water (Matthews, 2005). The life cycle is completed in approximately 16–18 h at 22–25°C. A detailed description of the life cycle and the individual stages with extensive reference to the literature is available (Matthews, 2005; Dickerson, 2006).

The presence of bacteria, which appear to be endosymbiotic, in the cytoplasm of *I. multifiliis* theronts and trophonts was recently described during genomic sequence analysis of the parasite (Sun *et al.*, 2009). Two classes of bacteria were identified, *Rickettsiales* and *Sphingobacteriales*, which were found in laboratory isolates as well as parasites collected from fish naturally infected in the wild. Endosymbiotic bacteria are relatively common in free-living ciliates (Fokin, 2004), and have been previously described at the structural level in *I. multifiliis* as well (Roque *et al.*, 1967; Lobo Da Cunha and Azevedo, 1988; Matthews, 2005). At present, it is not known if these endosymbionts affect virulence or are required for the parasite's survival.

4.3. Transmission and Geographical Distribution

Naturally occurring epizootic outbreaks of ichthyophthiriasis (commonly referred to as 'ich', or 'white spot disease') have occurred on most continents in populations of feral and farm-raised fishes (Paperna, 1972; Nigrelli *et al.*, 1976; Valtonen and Keränen, 1981; Wahli and Meier, 1987; Wurtsbaugh and Tapia, 1988; Bragg, 1991; Buchmann *et al.*,

1995; Rintamaki-Kinnunen and Valtonen, 1997; Traxler *et al.*, 1998; Scholz, 1999; Kim *et al.*, 2002; Thilakaratne *et al.*, 2003; Molnar, 2006; Piazza *et al.*, 2006; Lemos *et al.*, 2007; Jalali *et al.*, 2008; Maceda-Veiga *et al.*, 2009). Low-level infections can occur in natural habitats. The direct life cycle of *I. multifiliis* is conducive to producing explosive outbreaks in dense fish populations, which is often when fish are raised under intense aquaculture. In a 12-month study of pond-reared rainbow trout (*Oncorhynchus mykiss*) in an *I. multifiliis*-endemic region of Turkey, a positive correlation of ambient water temperature and mean intensity of parasite load was clearly demonstrated (Ogut *et al.*, 2005). Outbreaks have been reported to occur in channel catfish (*Ictalurus punctatus*) at ambient temperatures as low as 6–12°C (Bodensteiner *et al.*, 2000) and in rainbow trout at 14–18°C (Ogut *et al.*, 2005), although epizootics are more prevalent at higher temperatures (i.e. 24–28°C). *I. multifiliis* does not usually survive temperatures above 30°C (Dickerson, 2006), however, a South-east Asia isolate apparently can live at 34°C (Bauer and Lunchis, 2001). How the parasite over-winters is not known, but it has been postulated that low numbers of trophonts survive on fish for months in a near-dormant state at low temperatures (Noe and Dickerson, 1995). The parasite is transmitted from fish to fish by infective theronts in the water.

4.4. Diagnosis of Infection and Appearance of Lesions

When exposed to theronts, fish become agitated, hyperactive and rub their gill opercula and flanks against available surfaces. This behaviour is referred to as 'flashing' and is presumably in response to the intense irritation elicited as the parasite bores into the skin and gills and feeds in the tissues. It is a common clinical sign of *I. multifiliis* infection, but it should be noted that any irritant in the water can cause a similar behavioural response.

As the parasite feeds and grows within the skin and gills damage to the epithelia

interferes with normal gaseous exchange, and infected individuals become starved of oxygen and acidotic. As a behavioural response, fish in ponds swim to the surface and rest at the edges in shallow water to gain access to dissolved oxygen and minimize expended energy. Fish in aquaria initially swim near the surface, but eventually sink to the bottom as they weaken due to oxygen depletion in their tissues. Within 3–4 days (at 22–25°C) numerous trophonts appear as vesicular lesions (approximately 0.5–1.0 mm in diameter) disseminated in the skin over the entire surface of the fish (Fig. 4.4). Heavy infections in the gill cause extensive disruption of epithelia and capillary haemorrhage with subsequent loss of physiological function. Severely infected fish die within 5–7 days, which is during the first period of the initial parasite exposure and growth of trophonts. Less severe infections (i.e. those that do not kill fish in the first period of growth) are usually diagnosed by ‘flashing’ behaviour and the presence of white spots in the skin, each of which contains one to four parasites surrounded by hyperplastic epithelial cells (Chapman, 1984; Dickerson, 2006).

Definitive diagnosis of an *I. multifiliis* infection is made by microscopic detection of the parasite in biopsies or tissues taken at necropsy. To prepare specimens for microscopic examination in the field, place skin and mucus scrapings, small pieces of gill lamellae and/or small tail clippings on a glass slide, add several drops of water and place a cover slip over the specimen. Tissues should be taken from either live fish as a

biopsy, or as soon as possible following the death of the fish. It is not necessary to fix or stain the tissue. In unstained preparations the large trophont in skin and gill tissues is easily detected with a low-power objective lens (4×–10× magnification). The large ‘horse-shoe’-shaped nucleus is a pathognomonic diagnostic indicator for *I. multifiliis*. It is usually fairly easy to find the parasite due to its ciliary activity and movement in the tissue. In heavily infected fish the parasite moves freely within the damaged epithelium, presumably due to the naturally loose structure of fish epithelium, which is loosened further by physical and/or enzymatic activity of the feeding parasite.

In haematoxylin-and-eosin-stained histological thin sections of formalin-fixed skin and gills, large parasites are visible within the epithelia under a microscope with 10×–20× objective lenses (see Fig. 4.3). The macronucleus, cytoplasmic food vacuoles and surface cilia are usually visible. The parasite lies within an interstitial tissue space, which contains cellular debris and proteinaceous tissue fluid. The epithelium immediately surrounding the parasite is hyperplastic; the epithelial cells are degenerating, appear hydropic, and necrotic with pyknotic nuclei. The epithelium contains an infiltration of lymphocytes and other inflammatory cells including macrophages and neutrophils (see below). The degree of inflammatory response and tissue damage depends on the number of invading parasites and severity of infection; the response varying from mild to severe. The underlying stratum spongiosum and stratum



Fig. 4.4. Channel catfish fingerling infected with *I. multifiliis*. Trophonts are within vesicular lesions that appear as ‘white spots’ in the skin. Most vesicles contain a single organism, but in heavy infections multiple parasites can occur within the same vesicles due to coalescence of the lesions.

compactum of the dermis appear oedematous, and also contain inflammatory cells. The parasites invade the basal epithelium of the gill lamellae. Gill epithelial cells proliferate in the immediate vicinity of the parasite as well as over the entire gill lamellae (Fig. 4.5). Large trophonts often reside within multiple adjacent lamellae. In severe infections, the entire interlamellar space becomes occluded with hyperplastic epithelium and the tissue takes on a 'clubbed appearance' (Hines and Spira, 1974c). Hyperplasia and excess mucus production in the gills interferes with gaseous exchange (Dickerson, 2006).

4.5. Local and Systemic Pathophysiology

4.5.1. Local response to *I. multifiliis* infection

The pathophysiological effects of chronic and acute *I. multifiliis* infection (ichthyophthiriasis) are attributed to cellular damage and subsequent inflammatory responses in the skin and gills. Expression of the potent inflammatory mediator interleukin-1 beta (IL-1 β) is upregulated in the skin of carp (*Cyprinus carpio*) within 36 h of parasite exposure (Gonzalez *et al.*, 2007a). In rainbow trout both IL-1 β and tumour necrosis factor alpha (TNF- α) are significantly increased at 4 days

(Sigh *et al.*, 2004a). The cells in skin and gills responsible for production of IL-1 β have not been identified, but macrophages, epithelial cells and fibroblasts have been suggested as sources (Sigh *et al.*, 2004a).

The physical integrity of the epithelia is compromised during invasion by theronts and growth of trophonts within the skin and gills, and massive infections with large numbers of parasites can kill the host within 12 h after infection (Ewing *et al.*, 1985; Ventura and Paperna, 1985; Matthews, 1994). Although challenge with fewer theronts (less than 15,000/15–40 g fish) does not usually overwhelm the host at initial infection, subsequent rounds of infection increases the numbers of parasites and susceptible fish are overcome by synchronized waves of theronts (Wang *et al.*, 2002; Swennes *et al.*, 2006; Gonzalez *et al.*, 2007a). Variations in virulence among *I. multifiliis* isolates are determined by differential growth rates on the fish that modulate subsequent infection and immunity (Swennes *et al.*, 2006). The macroscopic and microscopic lesions resulting from parasite invasion and feeding are described in the preceding section.

4.5.2. Systemic response to *I. multifiliis* infection

Severe ichthyophthiriasis leads to significant damage of the skin and gill epithelia, which

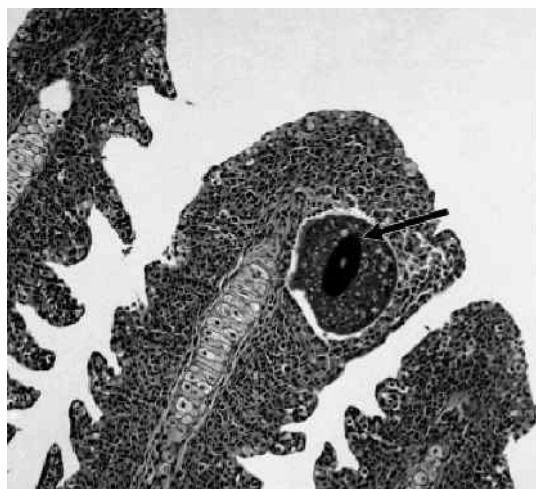


Fig. 4.5. Trophont in gill of a channel catfish. Trophont lies within the epithelium at the tip of a primary lamella of the gill. The macronucleus is indicated by the black arrow. There is extensive hyperplasia of the epithelium and coalescence of the secondary lamellae, which appear 'clubbed'. This biopsy was made 5 days after infection (haemotoxylin-and-eosin-stained paraffin thin section, light microscope image 50 $\times$ magnification).

impedes gaseous exchange resulting in acidosis, oxygen depletion and loss of energy reserves. In carp, a drop in the serum levels of Na^+ , K^+ and Mg^{++} ions, and a rise in blood urea nitrogen occurs (Hines and Spira, 1973b, 1974a, c).

4.6. Protective Control Strategies: Immune Response and Vaccine Strategies

Immunity to *I. multifiliis* was described as early as 1910 (Bushkiel, 1910), and a significant amount of research on the phenomenology of the response and the mechanisms of immune protection has been conducted over the last 40 years. An overview of this is in several comprehensive reviews (Buchmann *et al.*, 2001; Matthews, 2005; Dickerson, 2006). Research has been driven by the need to develop a protective vaccine against this economically important parasite as well as the desire to understand the mechanisms of the long-lasting protection that is elicited following an infection. Further, *I. multifiliis* provides an excellent system to study host-pathogen interactions and mucosal immunity in an early vertebrate model (Dickerson and Clark, 1998).

A basic understanding of immunity to *I. multifiliis* infection has emerged that includes both innate and adaptive immune mechanisms.

4.6.1. Local innate immunity

The mucosal surfaces of the skin and gills serve as a natural barrier to *I. multifiliis* infection. This protection is mediated by physical factors that include the surface mucus barrier, the glycocalyx, and the underlying epithelial cells, as well as constitutively expressed proteins and induced cellular and humoral elements of the inflammatory response (Dickerson, 2009).

In carp the leukocyte response to *I. multifiliis* infection in the skin and gills has been described in detail (Hines and Spira, 1973a; Cross and Matthews, 1993b). The study of Cross and Matthews complements the early

classic research of Hines and Spira, and provides a comprehensive description of the cellular changes in the skin epithelium at both the microscopic and the ultrastructural levels. Tissue lesions and cellular responses in the caudal tail fin were described at sequential time points following challenge in both naïve and immunized fish (Cross and Matthews, 1993b). Within 1 day of exposure of naïve fish to theronts, neutrophils infiltrate the skin and appear within non-vascularized areas of the dermis near the parasite. Increased expression of the chemokines CXCa, CXCR1 and CXCR2 in the skin also occurs at this time, and is probably responsible for the early influx of neutrophils (Gonzalez *et al.*, 2007a). By 2–3 days, these inflammatory cells reach and surround the trophont in the epidermis. At 5–6 days, many leukocytes (eosinophils, neutrophils and basophils) are associated with the parasite in the epidermis and in the dermis directly below it. At this point, the cellular response is predominated by eosinophils, but lymphocytes are also present, primarily in the dermis.

In immune fish, eosinophilic granular cells (EGCs) and macrophages are the predominant leukocytes at days 5–7 following challenge, and they surround the parasite and are in tissue sites from which the parasite has exited (Cross and Matthews, 1993b; Cross, 1994).

The increased expression of CXCa, CXCR1, CXCR2, IL-1 β and TNF- α over the course of *I. multifiliis* infection suggests that these molecules play a role in the recruitment of leukocytes to the skin (Sigh *et al.*, 2004a; Gonzalez *et al.*, 2007a). Necrotic granulocytic cells, cellular membranes and released granules surround the ciliated parasites, which remain motile and show no visible signs of damage (Cross and Matthews, 1993b). The necrotic remains of leukocytes are visible in cytoplasmic food vacuoles of the feeding trophonts (Matthews, 2005), suggesting that inflammatory cells serve as a nutrient source for the feeding organisms. In fact, it has been suggested that their continual ingestion might explain the relatively low inflammatory cell response associated with larger trophonts (Ventura and Paperna, 1985). Enzymes released from degranulated inflammatory

cells probably play a role in the inflammatory response (Matthews, 1994). Tissue breakdown and cellular damage also could be caused by enzymes such as phosphatases and non-specific esterases secreted by the parasite itself (Kozel, 1986; Lobo-Da-Cunha and Azevedo, 1990).

4.6.2. Systemic innate immunity

In carp, a differential shift in circulating leucocytes occurs during the course of parasite infection with fish developing an initial lymphopenia and neutrophilia (Hines and Spira, 1973a). Circulating neutrophils increase in number (up to fivefold) during the acute phase of the infection (Hines and Spira, 1973a). Natural cytotoxic cells (NCCs), first described in channel catfish, have been postulated to play a role in non-specific protection against *I. multifiliis* (Graves *et al.*, 1984, 1985). Following *I. multifiliis* infection in channel catfish, NCCs from the head kidney move into circulation (Graves *et al.*, 1985).

In rainbow trout, *I. multifiliis* infection elicits a decrease in gene expression of complement factor C3 in the head kidney at 24 h (Sigh *et al.*, 2004b). In the spleen, gene expression of complement factor C3 is significantly raised at day 26. In trout injected with live theronts, C3 gene expression in the spleen was upregulated at day 28 (von Gersdorff Jorgensen *et al.*, 2008). In infected trout major histocompatibility complex class II (MHC II) gene expression is depressed at 48 h in the spleen. Extra-hepatic expression of C3 is postulated to be the result of circulating macrophages (Sigh *et al.*, 2004b). The early gene expression (48 h) of IgM, MHC II and complement factor C3 in skin, followed by later systemic expression (4 days) of these genes in the head kidney and spleen suggests that a local immune response to *I. multifiliis* is initiated first, followed later by a systemic response (Sigh *et al.*, 2004b). In rainbow trout, the expression of cytokines IL-1 β and TNF- α in the head kidney and spleen remain elevated at 26 days (Sigh *et al.*, 2004a).

Plasma lysozyme activity increased following the injection of live theronts into the peritoneal cavity of rainbow trout suggesting

that systemic expression of non-specific components of innate immunity may be elicited following exposure to the parasite (Alishahi and Buchmann, 2006). The injection of live theronts also elicited upregulation of genes encoding acute phase proteins in the liver (Alishahi and Buchmann, 2006).

4.6.3. Local adaptive immunity

It is well established that naïve fish surviving infection become resistant to a subsequent challenge and that antibodies are important effectors of immune protection (Hines and Spira, 1974b; Wahli and Meier, 1985; Houghton and Matthews, 1986; Clark *et al.*, 1987; Dickerson and Clark, 1996; Lin *et al.*, 1996). Although both serum and mucus antibodies are elicited against the parasite, specific antibodies in the mucus and epithelia of the skin and gills are believed to be responsible for protection (Lin *et al.*, 1996; Xu *et al.*, 2002; Maki and Dickerson, 2003; Sigh *et al.*, 2004b; Zhao *et al.*, 2008).

For many years it was unclear if teleosts had a mucosal immune system analogous to that in mammals. It is now well established that a mucosal immune system exists in fish, but fundamental gaps in knowledge still remain regarding the sites of induction and differentiation of B and T cells involved in the mucosal antibody response (Zhao *et al.*, 2008; Dickerson, 2009). *I. multifiliis* serves as a useful model to explore the mechanisms of local adaptive immunity because it naturally infects only epithelia of the skin and gills and elicits the production of specific antibodies at these sites (Dickerson and Clark, 1998; Xu *et al.*, 2002; Maki and Dickerson, 2003; Zhao *et al.*, 2008). Acquired protective immunity against *I. multifiliis* is present for at least 2 years in channel catfish held under laboratory conditions, and research suggests that memory B cells and long-lived plasma cells are present in the skin of immune fish (Zhao *et al.*, 2008).

I. multifiliis infection elicits the expression in skin of a number of genes relevant to adaptive immunity, including those encoding IgM and MHC II (Sigh *et al.*, 2004b). IgM is

the primary functional antibody found in fish, and it appears to be the main antibody found in mucus, although at a much lower concentration than in sera (Bradshaw *et al.*, 1971; Zilberg and Klesius, 1997; Maki and Dickerson, 2003). Recent work, however, has suggested that in trout an IgT isotype antibody may function comparably to IgA in mammals (Zhang *et al.*, 2010). In channel catfish and other species of fish in which IgT has not been shown to occur, however, mucosal antibody appears to be structurally and functionally similar to serum antibody, although more research is required to confirm this in light of the recent IgT mucosal antibody discovery.

4.6.4. Systemic adaptive immunity

Serum antibodies are produced in response to infection, but their role as effectors of immunity is questionable because they do not appear to reach surface epithelia under normal physiological conditions (Lobb and Clem, 1981; Lin *et al.*, 1996). For example, although acquired protection is abrogated following treatment of *I. multifiliis*-immune fish with corticosteroids, serum antibody concentrations remain unchanged (Houghton and Matthews, 1986). In passive immunity experiments carried out in channel catfish using protective immobilizing mouse monoclonal antibodies, it was shown that protection was conferred only by IgG antibodies, which can reach parasites located in the skin. In contrast, immobilizing mouse IgM antibodies and *I. multifiliis*-immune catfish serum antibodies, which are much larger molecules, do not reach surface tissues or confer protection following adoptive transfer (Lobb and Clem, 1981; Lin *et al.*, 1996). These findings suggest that serum antibodies are minimally involved (if at all) in protection due to their physiological confinement to the blood. It is possible, however, that serum antibodies reach parasites embedded in the skin and gills when blood enters tissues following inflammation and physical disruption of the epithelia. Inflammatory responses elicited by parasites result in the influx of

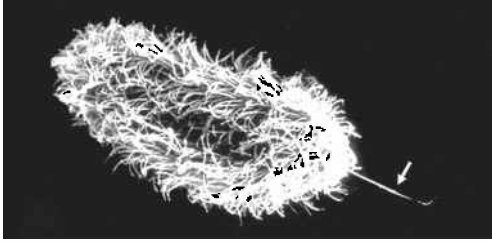
phagocytes into mucosal tissues, and antigens released from the parasite are probably taken up, processed and presented by these cells to B and T cells in the skin and/or lymphoid tissues in the spleen and head kidney. Plasma cells producing *I. multifiliis*-specific antibodies are found in the skin as well as the head kidney (Zhao *et al.*, 2008).

Regardless of whether or not antibodies in the blood play a role in protection, their presence is diagnostic of *I. multifiliis* infection, and they can be used to monitor previous exposure to the parasite. One of the earliest *in vitro* assays used to measure the immune response to the parasite was immobilization, which detects serum antibodies that bind surface antigens on ciliary membranes causing the cilia to stick together with resultant loss of synchronous beating and cessation of swimming (i.e. immobilization; Fig. 4.6) (Hines and Spira, 1974b; Clark *et al.*, 1987, 1988; Cross, 1993; Cross and Matthews, 1993a). Immobilization is serotype-specific depending on the immobilization antigen (i-antigen) displayed on the parasite's surface (Dickerson *et al.*, 1993). To date five serotypes have been identified, but it is likely that more will be characterized as additional isolates are studied (Dickerson, 2006). Several reviews and recent publications are available on i-antigens of *I. multifiliis* (Clark and Forney, 2003; Matthews, 2005; Dickerson, 2006; Xu *et al.*, 2009b). I-antigens play a role in eliciting protective immunity and have been targeted for vaccine production (see below).

4.6.5. Vaccine development

The elicitation of acquired protective immunity following natural infection by *I. multifiliis* suggests that the creation of a vaccine is feasible. Attempts to develop vaccines against the parasite were initiated almost as soon as it was discovered that fish surviving an infection become immune to subsequent challenge (Bushkiel, 1910; Butcher, 1941; Bauer, 1953; Beckert and Allison, 1964; Hines and Spira, 1974b). The use of vaccines is considered an economical and environmentally effective means to protect fish and would be of great

(a)



(b)

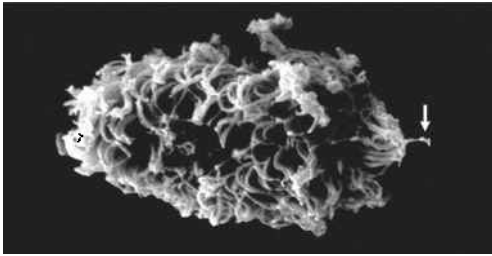


Fig. 4.6. Antibody immobilization. (a) Normal theront. (b) Theront immobilized by mouse monoclonal antibody (IgG). The cilia in the immobilized theront appear thickened and fused. Note the caudal cilium (white arrows), which in the immobilized theront appears to be folded back on itself. Antibodies bind to surface antigens on the cilia resulting in immobilization (scanning electron microscope image, theronts are approximately 40 μm in size).

benefit for the prevention of *I. multifiliis* infections in farm-raised fish.

Extensive research has been conducted in different fish species and this has been previously reviewed (Buchmann *et al.*, 2001; Matthews, 2005; Dickerson, 2006). To date, the most effective means of immunization is to expose fish to controlled surface infections (Parker, 1965; Areerat, 1974; Hines and Spira, 1974b; Burkart *et al.*, 1990) or to inject live parasites (theronts) into the peritoneal region of the coelomic cavity (Dickerson *et al.*, 1985; Burkart *et al.*, 1990; Dickerson and Clark, 1996; Buchmann *et al.*, 2001; Wang and Dickerson, 2002; Xu *et al.*, 2004; Alishahi and Buchmann, 2006; Xu *et al.*, 2008). The fact that the injection of parasites elicits strong mucosal immunity and that surface infection elicits production of systemic antibodies suggests that there is 'cross-talk' between the systemic and mucosal components of the fish immune system (Dickerson, 2009).

Also, vaccines consisting of inactivated parasites (theronts and trophonts) or subunit components (cilia, membrane proteins and purified i-antigens) confer varying degrees of protection under laboratory conditions (Parker, 1965; Areerat, 1974; Beckert, 1975; Burkart *et al.*, 1990; Wang and Dickerson,

2002; Wang *et al.*, 2002; Xu *et al.*, 2008, 2009a). I-antigens injected with Freund's adjuvant into the peritoneal cavity elicit good protection only against challenge by parasites bearing homologous i-antigens on their surface (Wang *et al.*, 2002). In contrast, fishes immunized by injection with or exposure to live theronts are protected against different immobilization serotypes, which suggests that other antigens exist in addition to the i-antigens (Leff *et al.*, 1994; Jarrett, 1997). These cross-reactive antigens have not yet been identified (Swennes *et al.*, 2007).

Since i-antigens elicit protective immunity against homologous *I. multifiliis* immobilization serotypes and limited serotypes exist in nature, it is possible that these antigens could be used as a subunit vaccine. Because *I. multifiliis* is an obligate parasite and cannot be grown in culture, the difficulty to produce large amounts of antigen is a major obstacle to commercial vaccine development. To address this problem, genes encoding i-antigens were transformed and expressed in the free-living and easily cultured ciliate *Tetrahymena pyriformis* with the idea that this organism could be used to produce sufficient amounts of antigen for a vaccine (Gaertig *et al.*, 1999). I-antigen genes have also been expressed in other cell

types including the bacterium *Escherichia coli*, COS-7 mammalian cells and channel catfish (He *et al.*, 1997; Lin *et al.*, 2002). Although work is ongoing, a vaccine using recombinant i-antigens has not yet been developed for field use (He *et al.*, 1997; Wang and Dickerson, 2002).

Methods to present antigens either orally or through the skin and gills by immersion have been investigated but an oral or immersion vaccine against *I. multifiliis* has not yet been developed (Burkart *et al.*, 1990; Wang *et al.*, 2002; Xu *et al.*, 2004).

Studies have indicated that cross-reactive antigens from other organisms, including *Tetrahymena*, elicit a degree of protection against *I. multifiliis* (Goven *et al.*, 1981; Wolf and Markiw, 1982; Dickerson *et al.*, 1984; Ling *et al.*, 1993; Buchmann *et al.*, 1999; Sigh and Buchmann, 2002). Although common antigens on different parasites and ciliate species is possible, it appears that the protection is more likely attributed to an innate immune response against heterologous molecules, which allows sufficient time for the fish to develop an adaptive response against the parasite (Dickerson and Clark, 1994; Matthews, 1994, 2005). Nevertheless, it is becoming evident that elicitation of both innate and adaptive immune responses are required for a vaccine to be effective against *I. multifiliis*.

4.7. Protective Control Strategies: Chemotherapy and Husbandry Practices

The most effective way to eliminate ichthyophthiriasis is to prevent introduction of the parasite (Brown and Gratzek, 1980). This is accomplished through good husbandry practices that include quarantine and prophylactic treatment of new fish before introduction into a pond or aquarium system. Because the periodicity of the life cycle of *I. multifiliis* is inversely related to temperature, fish should be isolated in water of elevated temperature to reduce the time necessary to keep them under observation. At 25–30°C the parasite develops within 3–5 days and trophonts will become easily detectable. It is not always possible to isolate fish before bringing them into

a water system, however, and the introduction of parasites and initiation of devastating epizootic outbreaks is always a threat.

A number of methods have been developed for the treatment of *I. multifiliis*. These include water management strategies as well as chemical treatments. These have been described in detail in a previous review (Matthews, 2005). If logistically possible, daily or semi-daily removal of fish from infected aquaria and transfer to clean water and aquaria is an effective way to eliminate the parasite. Sodium chloride treatments have been used for many years (Cross, 1972), and the addition of NaCl to water at the concentration of 4–5 g/l is effective against theronts (Selosse and Rowland, 1990; Aihua and Buchmann, 2001; Miron *et al.*, 2004). In addition to its detrimental effect on the parasite, salt may also have an ameliorative effect on the osmotic stress elicited by epithelial damage by the parasite (Cross, 1972; Dickerson, 2006).

Treatment with chemicals and drugs is warranted, especially if the majority of fish in the population are not severely infected and do not appear moribund. A number of chemicals are available and many can be used on fish not intended for human consumption (Cross, 1972; Hoffman, 1999). Chemicals that can be used in food fish are limited (Matthews, 2005; Dickerson, 2006). Formalin is the only chemical currently permitted for use in the USA. A recommended treatment is the addition of formaldehyde at a concentration of 25 ppm for 10 days, with water changes every other day. Higher concentrations of 100–250 ppm can be used for up to 1 h (Brown and Gratzek, 1980). It is suggested that formalin is effective as an early treatment to prevent transmission of the parasite from fish to fish during the early stages of an outbreak, but that it is less effective against severe and extensive infections (Matthews, 2005).

Malachite green, which in the past has proven to be an effective drug against the parasite, is now prohibited for use on food fish in the USA and the European Union. The water-soluble, zinc-free oxalate salt is effective against theronts at a concentration of 0.1 ppm for 3–4-day periods with subsequent water changes and re-treatment until the parasite has been eliminated. A non-water-soluble

form has been shown to be effective against trophonts located in the skin and gills when administered orally, and is less toxic to the fish than the water-soluble chemical (Schmahl *et al.*, 1992).

Many chemicals and drugs have been tested or used for the treatment of *I. multifiliis* including copper sulfate (Straus, 1993; Straus *et al.*, 2009), potassium permanganate (Straus and Griffin, 2002), chloramine T (Cross, 1972), sodium percarbonate, garlic (Buchmann *et al.*, 2003) and others (Matthews, 2005). Bronopol has been shown to be efficacious in the control of *I. multifiliis* infection in rainbow trout (Shinn *et al.*, 2003). Efficacy and toxicity varies among fish species and water quality (Straus and Griffin, 2002; Straus *et al.*, 2009).

4.8. Conclusions and Suggestions for Further Study

I. multifiliis has been the subject of applied and basic research for over 100 years (Bushkiel, 1910); a fact attributed to its wide-ranging distribution throughout the world, its ability to infect a broad and diverse spectrum of freshwater fish species, and its economic importance to fish farmers and aquarists.

Also, *I. multifiliis* has emerged as a popular model system for the study of innate and acquired immunity against pathogenic parasites of fish. Although an obligate parasite, it is easily passaged from fish to fish in laboratory aquaria, and because of its relatively large size it is easily observed, collected and manipulated under laboratory conditions. It parasitizes the epithelia of the skin and gills, which facilitates *in vivo* observations of its activity, and quantification of infection. *I. multifiliis* also has disadvantages as an experimental model because it cannot be grown in culture under axenic conditions and viable samples cannot be stored by cryopreservation (Beeler, 1981; Everett *et al.*, 2002). With these disadvantages it is extremely difficult, if not impossible, to carry out basic molecular genetic studies such as the creation of mutants by gene addition or deletion, and the manipulation of genetic crosses. The inability for long-term cryogenic storage makes it difficult

to carry out longitudinal studies on isolates collected from temporally and geographically disparate outbreaks, as well as comparison of virulence. Thus, further attempts to develop an effective method of cryopreservation are critically needed.

A stage-specific transcriptional profile of the expressed genes of *I. multifiliis* has been completed (Cassidy-Hanley *et al.*, 2011). The *I. multifiliis* genome sequencing project has been completed by the J. Craig Venter Institute and is submitted for publication (Robert Coyne, personal communication 8 July 2011). It is likely that this ciliate will be the first protozoan parasite of fish to have its entire genome sequenced, which will bring this organism into the post-genomic era with all the opportunities that this affords. For instance, it should be possible to identify genes encoding potentially important proteins such as enzymes, and diagnostic and protective antigens. Reverse genetic experiments will be easier with a genetic database to mine. The genome sequence will facilitate and stimulate studies that should lead to new discoveries of the parasite's biology. For example, endosymbionts in *I. multifiliis* were identified and characterized as a direct result of the *I. multifiliis* genome sequencing project (Sun *et al.*, 2009). It is expected that the *I. multifiliis* genome database will open up new avenues of research leading to more effective and safer drugs to control parasite infections.

Despite advances in identifying and characterizing protective antigens (such as the i-antigens) and moderate success with protective vaccination in the laboratory using live, inactivated and subunit vaccines (Buchmann *et al.*, 2001; Wang and Dickerson, 2002; Wang *et al.*, 2002; Xu *et al.*, 2008, 2009a), a practical field vaccine against *I. multifiliis* is still not available. Unless a method is developed to easily and inexpensively grow the organism *in vitro*, which is unlikely; the first commercial vaccine probably will come from the development of an inexpensive means to produce recombinant protective antigens. Thus, further research is needed to create new protein expression systems, such as *Tetrahymena thermophila* (Gaertig *et al.*, 1999), or enhance the capabilities of existing systems, such as *E. coli* (Lin *et al.*, 2002).

Successful vaccines will need effective delivery methods, preferably through oral or immersion routes, and adjuvant formulations that stimulate and enhance long-lasting mucosal immunity in the skin and gills. Future research should be focused on eluci-

dating the mechanisms of innate and adaptive immunity including: (i) the sites of antigen presentation and induction; (ii) antibody production and secretion in epithelia; (iii) trafficking of immune cells; and (iv) immune memory.

References

- Aihua, L. and Buchmann, K. (2001) Temperature- and salinity-dependent development of a Nordic strain of *Ichthyophthirius multifiliis* from rainbow trout. *Journal of Applied Ichthyology* 17, 273–276.
- Alishahi, M. and Buchmann, K. (2006) Temperature-dependent protection against *Ichthyophthirius multifiliis* following immunization of rainbow trout using live theronts. *Diseases of Aquatic Organisms*, 72, 269–273.
- Areerat, S. (1974) The immune response of channel catfish, *Ictalurus punctatus* Rafinesque, to *Ichthyophthirius multifiliis*. MSc thesis, Auburn University, Auburn, Alabama, USA.
- Bauer, O.N. (1953) The immune response of channel catfish *Ictalurus punctatus* Rafinesque to *Ichthyophthirius multifiliis*. Fouquet. *Doklady Novaia Servia, SSSR* 93, 377–379.
- Bauer, O.N. and Luchis, O.N. (2001) A new genus of parasitic ciliata from tropical fishes. *Parazitologiya* 35, 142–144.
- Beckert, H. (1975) Observations on the biology of *Ichthyophthirius multifiliis* Fouquet, 1876. Its susceptibility to ethoxyquin, and some immunological responses of channel catfish, *Ictalurus punctatus*, to this parasite. PhD thesis, University of Southwestern Louisiana, Lafayette, Louisiana, USA.
- Beckert, H. and Allison, R. (1964) Some host responses of white catfish to *Ichthyophthirius multifiliis*. *Proceedings Southeastern Association Game Fish Commissioners* 18, 438–441.
- Beeler, C.R. (1981) Controlling cooling rates with a variable vacuum in a Dewar flask. *Cryobiology* 18, 79–81.
- Bodensteiner, L.R., Sheehan, R.J., Wills, P.S., Brandenburg, A.M. and Lewis, W.M. (2000) Flowing water: an effective treatment for ichthyophthiriasis. *Journal of Aquatic Animal Health* 12, 209–219.
- Bradshaw, C.M., Richard, A.S. and Sigel, M.M. (1971) IgM antibodies in fish mucus. *Proceedings of the Society of Experimental and Biological Medicine* 136, 1122–1124.
- Bragg, R.R. (1991) Health status of salmonids in river systems in natal. I. Collection of fish and parasitological examination. *The Onderstepoort Journal of Veterinary Research* 58, 59–62.
- Brown, E.E. and Gratzek, J.B. (1980) *Fish Farming Handbook. Food, Bait, Tropicals and Goldfish*. AVI Publishing, Westport, Connecticut, USA.
- Buchmann, K. and Nielsen, M.E. (1999) Chemoattraction of *Ichthyophthirius multifiliis* (ciliophora) theronts to host molecules. *International Journal of Parasitology* 29, 1415–1423.
- Buchmann, K., Uldal, A. and Lyholt, H.C. (1995) Parasite infections in Danish trout farms. *Acta Veterinaria Scandinavica* 36, 283–298.
- Buchmann, K., Lindénstrom, T. and Sigh, J. (1999) Partial cross protection against *Ichthyophthirius multifiliis* in *Gyrodactylus derjavini* immunized rainbow trout. *Journal of Helminthology* 73, 189–195.
- Buchmann, K., Sigh, J., Nielsen, C.V. and Dalgaard, M. (2001) Host responses against the fish parasitizing ciliate *Ichthyophthirius multifiliis*. *Veterinary Parasitology* 100, 105–116.
- Buchmann, K., Jensen, P.B. and Kruse, K.D. (2003) Effects of sodium percarbonate and garlic extract on *Ichthyophthirius multifiliis* theronts and tomocysts: *in vitro* experiments. *North America Journal of Aquaculture* 65, 21–24.
- Burkart, M.A., Clark, T.G. and Dickerson, H.W. (1990) Immunization of channel catfish, *Ictalurus punctatus* Rafinesque, against *Ichthyophthirius multifiliis* (Fouquet): killed versus live vaccines. *Journal of Fish Diseases* 13, 401–410.
- Bushkiel, A.L. (1910) Beiträge zur kenntnis des *Ichthyophthirius multifiliis*. *Archives für Protistenkunde* 21, 61–102.
- Butcher, A.D. (1941) Outbreaks of white spot or ichthyophthiriasis (*Ichthyophthirius multifiliis* Fouquet, 1876) at the hatcheries of Ballarat fish acclimatization society with notes on laboratory experiments. *Proceedings Royal Society Victoria* 53, 126–144.

- Canella, M.F. and Rocchi-Canella, I. (1976) Biologie des Ophryoglenina (cilies hymenostermes, histophages). *Annals of the University of Ferrara (N.S. Sect. III)* 3 (Suppl. 2), 1–510.
- Cassidy-Hanley, D., Cordonnier-Pratt, M., Pratt, L., Devine, C., Hossain, M., Dickerson, H. and Clark, T. (2011) Transcriptional profiling of stage specific gene expression in the parasitic ciliate *Ichthyophthirius multifiliis*. *Molecular and Biochemical Parasitology* 178, 29–39.
- Chapman, G.B. (1984) Ultrastructural aspects of the host–parasite relationship in ichthyophthiriasis. *Transactions of the American Microscopical Society* 103, 364–375.
- Clark, T. and Forney, J. (2003) Free-living and parasitic ciliates. In: Craig, A. and Sherf, A. (eds) *Antigenic Variation*. Academic Press (Elsevier Science Ltd), London, UK.
- Clark, T.G., Dickerson, H.W., Gratzek, J.B. and Findly, R.C. (1987) *In vitro* response of *Ichthyophthirius multifiliis* to sera from immune channel catfish. *Journal of Fish Biology* 31, 203–208.
- Clark, T.G., Dickerson, H.W. and Findly, R.C. (1988) Immune response of channel catfish to ciliary antigens of *Ichthyophthirius multifiliis*. *Developmental and Comparative Immunology* 12, 581–594.
- Corliss, J. (1979) *The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature*. Pergamon Press, Oxford, UK.
- Cross, D.G. (1972) A review of methods to control ichthyophthiriasis. *The Progressive Fish-Culturist* 34, 165–170.
- Cross, M.L. (1993) Antibody binding following exposure of live *Ichthyophthirius multifiliis* (ciliophora) to serum from immune carp *Cyprinus carpio*. *Diseases of Aquatic Organisms* 17, 159–164.
- Cross, M.L. (1994) Localized cellular-responses to *Ichthyophthirius multifiliis* – protection or pathogenesis. *Parasitology Today* 10, 364–368.
- Cross, M.L. and Matthews, R.A. (1993a) *Ichthyophthirius multifiliis* Fouquet (Ciliophora): the localization of sites immunogenic to the host *Cyprinus carpio* (L.). *Fish and Shellfish Immunology* 3, 13–24.
- Cross, M.L. and Matthews, R.A. (1993b) Localized leukocyte response to *Ichthyophthirius multifiliis* establishment in immune carp *Cyprinus carpio* L. *Veterinary Immunology and Immunopathology* 38, 341–358.
- Dickerson, H.W. (2006) *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (phylum Ciliophora). In: Woo, P.T.K. (ed.) *Fish Diseases and Disorders. Volume 1. Protozoan and Metazoan Infections*, 2nd edn. CABI Publishing, Wallingford, Oxon, UK.
- Dickerson, H.W. (2009) The biology of teleost mucosal immunity. In: Giacomo, Z., Perriere, C., Mathis, A. and Kapoor, B.G. (eds) *Fish Defenses Volume 2: Pathogens, Parasites and Predators*. Science Publishers, Enfield, New Hampshire, USA.
- Dickerson, H.W. and Clark, T.G. (1994) Vaccination against Ich. *Aquaculture* 127, 278–280.
- Dickerson, H. and Clark, T. (1996) Immune response of fishes to ciliates. *Annual Review of Fish Diseases*, 6, 107–120.
- Dickerson, H. and Clark, T. (1998) *Ichthyophthirius multifiliis*: a model of cutaneous infection and immunity in fishes. *Immunological Reviews* 166, 377–384.
- Dickerson, H.W., Brown, J., Dawe, D.L. and Gratzek, J.B. (1984) *Tetrahymena pyriformis* as a protective antigen against *Ichthyophthirius multifiliis* infection – comparisons between isolates and ciliary preparations. *Journal of Fish Biology* 24, 523–528.
- Dickerson, H.W., Lohr, A.L. and Gratzek, J.B. (1985) Experimental intraperitoneal infection of channel catfish, *Ictalurus punctatus* (Rafinesque) with *Ichthyophthirius multifiliis* (Fouquet). *Journal of Fish Diseases* 8, 139–142.
- Dickerson, H.W., Clark, T.G. and Leff, A.A. (1993) Serotypic variation among isolates of *Ichthyophthirius multifiliis* based on immobilization. *Journal of Eukaryotic Microbiology* 40, 816–820.
- Everett, K.D., Knight, J.R. and Dickerson, H.W. (2002) Comparing tolerance of *Ichthyophthirius multifiliis* and *Tetrahymena thermophila* for new cryopreservation methods. *Journal of Parasitology* 88, 41–46.
- Ewing, M.S. and Kocan, K.M. (1992) Invasion and development strategies of *Ichthyophthirius multifiliis*, a parasitic ciliate of fish. *Parasitology Today* 8, 204–208.
- Ewing, M.S., Kocan, K.M. and Ewing, S.A. (1983) *Ichthyophthirius multifiliis*: morphology of the cyst wall. *Transactions of the American Microscopical Society* 102, 122–128.
- Ewing, M.S., Kocan, K.M. and Ewing, S.A. (1985) *Ichthyophthirius multifiliis* (Ciliophora) invasion of gill epithelium. *Journal of Protozoology* 32, 305–310.
- Ewing, M.S., Lynn, M.E. and Ewing, S.A. (1986) Critical periods in development of *Ichthyophthirius multifiliis* (Ciliophora) populations. *Journal of Protozoology* 33, 388–391.
- Fokin, S.I. (2004) Bacterial endocytobionts of Ciliophora and their interactions with the host cell. *International Review of Cytology – a Survey of Cell Biology* 236, 181–249.

- Gaertig, J., Gao, Y., Tishgarten, T., Clark, T. and Dickerson, H. (1999) Surface display of a parasite antigen in the ciliate *Tetrahymena thermophila*. *Nature Biotechnology* 17, 462–465.
- Geisslinger, M. (1987) Observations on the caudal cilium of the tomite of *Ichthyophthirius multifiliis* Fouquet 1876. *Journal of Protozoology* 341, 180–182.
- Gonzalez, S.F., Buchmann, K. and Nielsen, M.E. (2007a) Real-time gene expression analysis in carp (*Cyprinus carpio* L.) skin: inflammatory responses caused by the ectoparasite *Ichthyophthirius multifiliis*. *Fish and Shellfish Immunology* 22, 641–650.
- Gonzalez, S.F., Chatziandreou, N., Nielsen, M.E., Li, W., Rogers, J., Taylor, R., Santos, Y. and Cossins, A. (2007b) Cutaneous immune responses in the common carp detected using transcript analysis. *Molecular Immunology* 44, 1664–1679.
- Goven, B.A., Dawe, D.L. and Gratzek, J.B. (1981) *In vitro* demonstration of serological cross-reactivity between *Ichthyophthirius multifiliis* Fouquet and *Tetrahymena pyriformis* Lwoff. *Developmental and Comparative Immunology* 5, 283–289.
- Graves, S.S., Evans, D.L., Cobb, D. and Dawe, D.L. (1984) Nonspecific cytotoxic cells in fish (*Ictalurus punctatus*) i. Optimum requirements for target cell lysis. *Developmental and Comparative Immunology* 8, 293–302.
- Graves, S.S., Evans, D.L. and Dawe, D.L. (1985) Mobilization and activation of nonspecific cytotoxic cells (ncc) in the channel catfish (*Ictalurus punctatus*) infected with *Ichthyophthirius multifiliis*. *Comparative Immunology Microbiology and Infectious Diseases* 8, 43–51.
- Hauser, M. (1973) Acetomyosin-like filaments in the dividing macronucleus of the ciliated protozoan *Ichthyophthirius multifiliis*. *Chromosoma* 44, 49–71.
- He, J., Yin, Z., Xu, G., Gong, Z., Lam, T.J. and Sin, Y.M. (1997) Protection of goldfish against *Ichthyophthirius multifiliis* by immunization with a recombinant vaccine. *Aquaculture* 158, 1–10.
- Hines, R.S. and Spira, D.T. (1973a) Ichthyophthiriasis in the mirror carp. II. Leukocyte response. *Journal of Fish Biology* 5, 527–534.
- Hines, R.S. and Spira, D.T. (1973b) *Ichthyophthirius multifiliis* (Fouquet) in the mirror carp, *Cyprinus carpio* L. I. Course of infection. *Journal of Fish Biology* 5, 385–392.
- Hines, R.S. and Spira, D.T. (1974a) Ichthyophthiriasis in the mirror carp *Cyprinus carpio* (L.). IV. Physiological dysfunction. *Journal of Fish Biology* 6, 365–371.
- Hines, R.S. and Spira, D.T. (1974b) Ichthyophthiriasis in the mirror carp *Cyprinus carpio* (L.). V. Acquired immunity. *Journal of Fish Biology* 6, 373–378.
- Hines, R.S. and Spira, D.T. (1974c) Ichthyophthiriasis in the mirror carp *Cyprinus carpio* L. III. Pathology. *Journal of Fish Biology* 6, 189–196.
- Hoffman, G. (1999) *Parasites of North American Fishes*. Comstock Publishing Associates, Ithaca, New York, USA.
- Houghton, G. and Matthews, R.A. (1986) Immunosuppression of carp (*Cyprinus carpio* L.) to ichthyophthiriasis using the corticosteroid triamcinolone acetate. *Veterinary Immunology and Immunopathology* 12, 413–419.
- Jalali, B., Barzegar, M. and Nezamabadi, H. (2008) Parasitic fauna of the spiny eel, *Mastacembelus mastacembelus* Banks et Solander (Teleostei: Mastacembelidae) in Iran. *Iranian Journal of Veterinary Research* 9, 158–161.
- Jarrett, L.C. (1997) The immune response of channel catfish against different immobilization serotypes of *Ichthyophthirius multifiliis*. MSc thesis, University of Georgia, Athens, Georgia, USA.
- Kheisin, E.M. and Mosevich, T.N. (1969) Particularities of surface structures in the parasitic ciliate *Ichthyophthirius multifiliis*. *Tsitologiya* 11, 689–694.
- Kim, J.H., Hayward, C.J., Joh, S.J. and Heo, G.J. (2002) Parasitic infections in live freshwater tropical fishes imported to Korea. *Diseases of Aquatic Organisms* 52, 169–173.
- Kozel, T.R. (1986) Scanning electron microscopy of the ontogeny of *Ichthyophthirius multifiliis*: their penetration into host tissue. *Transactions of the American Microscopical Society* 105, 357–364.
- Leff, A.A., Yoshinaga, T. and Dickerson, H.W. (1994) Cross immunity in channel catfish against two immobilization serotypes of *Ichthyophthirius multifiliis*. *Journal of Fish Diseases* 17, 429–432.
- Lemos, J.R.G., Tavares-Dias, M., De Aquino Sales, R.S., Nobre Filho, G.D.R. and Fim, J.D.I. (2007) Parasites in gills of farmed *Brycon amazonicus* (Characidae, bryconinae) in stream channels of Turumirim, Amazonas State, Brazil. *Acta Scientiarum Biological Sciences* 29, 217–222.
- Lin, T.L., Clark, T.G. and Dickerson, H. (1996) Passive immunization of channel catfish (*Ictalurus punctatus*) against the ciliated protozoan parasite *Ichthyophthirius multifiliis* by use of murine monoclonal antibodies. *Infection and Immunity* 64, 4085–4090.

- Lin, Y., Cheng, G., Wang, X. and Clark, T.G. (2002) The use of synthetic genes for the expression of ciliate proteins in heterologous systems. *Gene* 288, 85–94.
- Ling, K.H., Sin, Y.M. and Lam, T.J. (1993) Protection of goldfish against some common ectoparasitic protozoans using *Ichthyophthirius multifiliis* and *Tetrahymena pyriformis* for vaccination. *Aqua-culture* 116, 303–314.
- Lobb, C.J. and Clem, L.W. (1981) The metabolic relationship of the immunoglobulins in fish serum, cutaneous mucus, and bile. *Journal of Immunology* 127, 1525–1529.
- Lobo Da Cunha, A. and Azevedo, C. (1988) Association between xenosomes and glycogen in the cytoplasm of the ciliate *Ichthyophthirius multifiliis*. *Endocytobiosis and Cell Research* 5, 225–231.
- Lobo-Da-Cunha, A. and Azevedo, C. (1990) Enzyme cytochemistry of the alveolar sacs and golgian-like cisternae in the ciliate *Ichthyophthirius multifiliis*. *Journal of Protozoology* 37, 206–211.
- Lom, J. and Cerkasova, A. (1974) Host finding in invasive stages of *Ichthyophthirius multifiliis*. *Journal of Protozoology* 21, 457.
- Lynn, D.H. (2008) *The Ciliated Protozoa Characterization, Classification, and Guide to the Literature*. Springer, Heidelberg, Germany.
- Lynn, D.H., Frombach, S., Ewing, M.S. and Kocan, K.M. (1991) The organelle of Lieberkühn as a synapomorphy for the Ophryoglenina (Ciliophora: Hymenostomatida). *Transactions of the American Microscopical Society* 110, 1–11.
- Maceda-Veiga, A., Salvado, H., Vinyoles, D. and De Sostoa, A. (2009) Outbreaks of *Ichthyophthirius multifiliis* in redtail barbs *Barbus haasi* in a Mediterranean stream during drought. *Journal of Aquatic Animal Health* 21, 189–194.
- MacIennnon, R.F. (1935) Observations on the life cycle of *Ichthyophthirius*, a ciliate parasitic on fish. *North-western Scientist* 9, 12–14.
- MacIennnon, R.F. (1937) Growth in the ciliate *Ichthyophthirius*. I. Maturity and encystment. *Journal of Experimental Zoology* 76, 423–440.
- MacIennnon, R.F. (1942) Growth in the ciliate *Ichthyophthirius*. II. Volume. *Journal of Experimental Zoology* 91, 1–13.
- Maki, J.L. and Dickerson, H.W. (2003) Systemic and cutaneous mucus antibody responses of channel catfish immunized against the protozoan parasite *Ichthyophthirius multifiliis*. *Clinical and Diagnostic Laboratory Immunology* 10, 876–881.
- Matthews, R.A. (1994) *Ichthyophthirius multifiliis* Fouquet 1876: infection and protective responses within the fish host. In: Pike, A.W. and Lewis, J.W. (eds) *Parasitic Diseases of Fish*. Samara Publishing, Cardigan, Dyfed, UK.
- Matthews, R.A. (1996) *Ichthyophthirius*: observations on the life-cycle and indications of a possible sexual phase. *Folia Parasitologica* 43, 203–208.
- Matthews, R.A. (2005) *Ichthyophthirius multifiliis* Fouquet and ichthyophthiriosis in freshwater teleosts. *Advances in Parasitology* 59, 159–241.
- Miron, D.S., Silva, L.V.F., Golombieski, J.I. and Baldisserotto, B. (2004) 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.
- Molnar, K. (2006) Some remarks on parasitic infections of the invasive *Neogobius* spp. (Pisces) in the Hungarian reaches of the Danube River, with a description of *Goussia szekelyi* sp. n. (Apicomplexa: Eimeriidae). *Journal of Applied Ichthyology* 22, 395–400.
- Nanney, D.L. (1980) *Experimental Ciliatology: an Introduction to Genetic and Developmental Analysis in Ciliates*. John Wiley and Sons, New York, USA.
- Nigrelli, R.F., Pokorny, K.S. and Ruggieri, G.D. (1976) Notes on *Ichthyophthirius multifiliis*, a ciliate parasitic on fresh-water fishes, with some remarks on possible physiological races and species. *Transactions of the American Microscopy Society* 95, 607–613.
- Noe, J.G. and Dickerson, H.W. (1995) Sustained growth of *Ichthyophthirius multifiliis* at low temperature in the laboratory. *Journal of Parasitology* 81, 1022–1024.
- Ogut, H., Akyol, A. and Alkahn, M.Z. (2005) Seasonality of *Ichthyophthirius multifiliis* in the trout (*Onchorynchus mykiss*) farms of the eastern Black Sea region of turkey. *Turkish Journal of Fisheries and Aquatic Sciences* 5, 23–27.
- Paperna, I. (1972) Infection by *Ichthyophthirius multifiliis* of fish in Uganda. *The Progressive Fish-Culturist* 34, 162–164.
- Parker, J.C. (1965) Studies on the natural history of *Ichthyophthirius multifiliis* Fouquet 1876 an ectoparasitic ciliate of fish. PhD thesis, University of Maryland, College Park, Maryland, USA.

- Peshkov, M.A. and Tikhomirova, L.A. (1968) Ultra-fine structure of the nuclear apparatus of *Ichthyophthirius multifiliis* at the cystal stage in protomites. *Doklady Novaia Servia* 183, 451–452.
- Piazza, R.S., Martins, M.L., Guiraldelli, L. and Yamashita, M.M. (2006) Parasitic diseases of freshwater ornamental fishes commercialized in Florianopolis, Santa Catarina, Brazil. *Boletim do Instituto de Pesca (Sao Paulo)* 32, 51–57.
- Rintamaki-Kinnunen, P. and Valtonen, E.T. (1997) Epizootiology of protozoans in farmed salmonids at northern latitudes. *International Journal of Parasitology* 27, 89–99.
- Roque, M., De Puytorac, P. and Lom, J. (1967) L'architecture buccale et la stomatogenese d' *Ichthyophthirius multifiliis* fouquet, 1876. *Protistologica* 3, 79–89.
- Schmahl, G., Ruider, S., Mehlhorn, H., Schmidt, H. and Ritter, G. (1992) Treatment of fish parasites. 9. Effects of a medicated food containing malachite green on *Ichthyophthirius multifiliis* Fouquet, 1876 (Hymenostomatida, Ciliophora) in ornamental fish. *Parasitology Research* 78, 183–192.
- Scholz, T. (1999) Parasites in cultured and feral fish. *Veterinary Parasitology* 84, 317–335.
- Selosse, P.M. and Rowland, S.J. (1990) Use of common salt to treat ichthyophthiriasis in Australian warm-water fishes. *Progressive Fish-Culturist* 52, 124–127.
- Shinn, A.P., Wootton, R., Cote, I. and Sommerville, C. (2003) Efficacy of selected oral chemotherapeutants against *Ichthyophthirius multifiliis* (Ciliophora: Ophryoglenidae) infecting rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 55, 17–22.
- Sigh, J. and Buchmann, K. (2002) Comparative analysis of cross-reactivity between *Ichthyophthirius* and *Tetrahymena*. *Bulletin of the European Association of Fish Pathologists* 22, 37–44.
- Sigh, J., Lindenstrom, T. and Buchmann, K. (2004a) Expression of pro-inflammatory cytokines in rainbow trout (*Oncorhynchus mykiss*) during an infection with *Ichthyophthirius multifiliis*. *Fish and Shellfish Immunology* 17, 75–86.
- Sigh, J., Lindenstrom, T. and Buchmann, K. (2004b) The parasitic ciliate *Ichthyophthirius multifiliis* induces expression of immune relevant genes in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 27, 409–417.
- Straus, D.L. (1993) Prevention of *Ichthyophthirius multifiliis* infestation in channel catfish fingerlings by copper sulphate treatment. *Journal of Aquatic Animal Health* 5, 152–154.
- Straus, D.L. and Griffin, B.R. (2002) Efficacy of potassium permanganate in treating ichthyophthiriasis in channel catfish. *Journal of Aquatic Animal Health* 14, 145–148.
- Straus, D.L., Hossain, M.M. and Clark, T.G. (2009) Copper sulfate toxicity to two isolates of *Ichthyophthirius multifiliis* relative to alkalinity. *Diseases of Aquatic Organisms* 83, 31–36.
- Sun, H.Y., Noe, J., Barber, J., Coyne, R.S., Cassidy-Hanley, D., Clark, T.G., Findly, R.C. and Dickerson, H.W. (2009) Endosymbiotic bacteria in the parasitic ciliate *Ichthyophthirius multifiliis*. *Applied and Environmental Microbiology* 75, 7445–7452.
- Swennes, A.G., Noe, J.G., Findly, R.C. and Dickerson, H.W. (2006) Differences in virulence between two serotypes of *Ichthyophthirius multifiliis*. *Diseases of Aquatic Organisms* 69, 227–232.
- Swennes, A.G., Findly, R.C. and Dickerson, H.W. (2007) Cross-immunity and antibody responses to different immobilisation serotypes of *Ichthyophthirius multifiliis*. *Fish and Shellfish Immunology* 22, 589–597.
- Thilakarathne, I., Rajapaksha, G., Hewakopara, A., Rajapakse, R. and Faizal, A.C.M. (2003) Parasitic infections in freshwater ornamental fish in Sri Lanka. *Diseases of Aquatic Organisms* 54, 157–162.
- Traxler, G.S., Richard, J. and McDonald, T.E. (1998) *Ichthyophthirius multifiliis* (Ich) epizootics in spawning sockeye salmon in British Columbia, Canada. *Journal of Aquatic Animal Health* 10, 143–151.
- Valtonen, E.T. and Keränen, A. (1981) Ichthyophthiriasis of atlantic salmon, *Salmo salar* L. At the Montta hatchery in Northern Finland in 1978–1979. *Journal of Fish Diseases* 4, 405–411.
- Van Den Bussche, R.A., Hooper, S.R., Drew, C.P. and Ewing, M.S. (2000) Characterization of histone h3/h4 gene region and phylogenetic affinity of *Ichthyophthirius multifiliis* based on h4 DNA sequence variation. *Molecular and Phylogenetic Evolution* 14, 461–468.
- Ventura, M.T. and Paperna, I. (1985) Histopathology of *Ichthyophthirius multifiliis* infections in fishes. *Journal of Fish Biology* 27, 185–203.
- Von Gersdorff Jorgensen, L., Nemli, E., Heinecke, R.D., Raida, K.M. and Buchmann, K. (2008) Immune-relevant genes expressed in rainbow trout following immunization with live vaccine against *Ichthyophthirius multifiliis*. *Diseases of Aquatic Organisms* 80, 189–197.
- Wahli, T. and Meier, W. (1985) Ichthyophthiriasis in trout: Investigation of natural defence mechanisms. In: Ellis, A.E. (ed.) *Fish and Shellfish Pathology*. Academic Press, London, pp. 347–352.
- Wahli, T. and Meier, W. (1987) Occurrence and significance of *Ichthyophthirius multifiliis* in Switzerland. *Schweiz Arch Tierheilkd* 129, 205–213.

- Wahli, T. and Meier, W. (1991) Affinity of *Ichthyophthirius multifiliis* theronts to light and/or fish. *Journal of Applied Ichthyology* 7, 244–249.
- Wang, X. and Dickerson, H.W. (2002) Surface immobilization antigen of the parasitic ciliate *Ichthyophthirius multifiliis* elicits protective immunity in channel catfish (*Ictalurus punctatus*). *Clinical and Diagnostic Laboratory Immunology* 9, 176–181.
- Wang, X., Clark, T.G., Noe, J. and Dickerson, H.W. (2002) Immunisation of channel catfish, *Ictalurus punctatus*, with *Ichthyophthirius multifiliis* immobilisation antigens elicits serotype-specific protection. *Fish and Shellfish Immunology* 13, 337–350.
- Wolf, K. and Markiw, M.E. (1982) Ichthyophthiriasis: immersion immunization of rainbow trout (*Salmo gairdneri*) using *Tetrahymena thermophila* as a protective immunogen. *Canadian Journal of Fisheries and Aquatic Sciences* 39, 1772–1775.
- Wright, A.D. and Lynn, D.H. (1995) Phylogeny of the fish parasite *Ichthyophthirius* and its relatives *Ophryoglena* and *Tetrahymena* (Ciliophora, Hymenostomatia) inferred from 18s ribosomal RNA sequences. *Molecular and Biological Evolution* 12, 285–290.
- Wurtsbaugh, W.A. and Tapia, R.A. (1988) Mass mortality of fishes in lake Titicaca (Peru-Bolivia) associated with the protozoan parasite *Ichthyophthirius multifiliis*. *Transactions of the American Fisheries Society* 117, 213–217.
- Xu, D.H., Klesius, P.H. and Shelby, R.A. (2002) Cutaneous antibodies in excised skin from channel catfish, *Ictalurus punctatus* Rafinesque, immune to *Ichthyophthirius multifiliis*. *Journal of Fish Diseases* 25, 45–52.
- Xu, D.H., Klesius, P.H. and Shelby, R.A. (2004) Immune responses and host protection of channel catfish, *Ictalurus punctatus* (Rafinesque), against *Ichthyophthirius multifiliis* after immunization with live theronts and sonicated trophonts. *Journal of Fish Diseases* 27, 135–141.
- Xu, D.H., Klesius, P.H. and Shoemaker, C.A. (2008) Protective immunity of Nile tilapia against *Ichthyophthirius multifiliis* post-immunization with live theronts and sonicated trophonts. *Fish and Shellfish Immunology* 25, 124–127.
- Xu, D.H., Klesius, P.H. and Shoemaker, C.A. (2009a) Effect of immunization of channel catfish with inactivated trophonts on serum and cutaneous antibody titers and survival against *Ichthyophthirius multifiliis*. *Fish and Shellfish Immunology* 26, 614–618.
- Xu, D.H., Panangala, V.S., Van Santen, V.L., Dybvig, K., Abernathy, J.W., Klesius, P.H., Liu, Z.J. and Russo, R. (2009b) Molecular characteristics of an immobilization antigen gene of the fish-parasitic protozoan *Ichthyophthirius multifiliis* strain ars-6. *Aquaculture Research* 40, 1884–1892.
- Zhang, Y.A., Salinas, I., Li, J., Parra, D., Bjork, S., Xu, Z., Lapatra, S.E., Bartholomew, J. and Sunyer, J.O. (2010) IgT, a primitive immunoglobulin class specialized in mucosal immunity. *Nature Immunology* 11, 827–835.
- Zhao, X., Findly, R.C. and Dickerson, H.W. (2008) Cutaneous antibody-secreting cells and B cells in a teleost fish. *Developmental and Comparative Immunology* 32, 500–508.
- Zilberg, D. and Klesius, P.H. (1997) Quantification of immunoglobulin in the serum and mucus of channel catfish at different ages and following infection with *Edwardsiella ictaluri*. *Veterinary Immunology and Immunopathology* 58, 171–180.

5 *Miamiensis avidus* and Related Species

Sung-Ju Jung¹ and Patrick T.K. Woo²

¹Chonnam National University, Chonnam, Republic of Korea

²University of Guelph, Guelph, Ontario, Canada

5.1. Introduction

5.1.1. Brief description

Ciliates of the order Scuticociliatida inhabit eutrophic marine coastal waters. Many scuticociliates are facultative parasites of aquatic animals and histophagous members have been problematic to commercial and ornamental fisheries. Several genera, including *Anophryoides*, *Mesanoophrys*, *Miamiensis*, *Philasterides*, *Pseudocohnilembus*, *Tetrahymena* and *Uronema* have been isolated from diseased organisms. Some scuticociliates which cause fish mortality have not been identified to species (Yoshinaga and Nakazoe, 1993; Dyková and Figueras, 1994) because they exhibit very similar morphology and size ranges (Song and Wilbert, 2000). These ciliates are normally considered free-living, but can also be parasites. When they act as parasites, they tend to cause high host mortality.

Scuticociliates are found worldwide in marine aquaculture facilities. They cause high mortality in fishes such as the olive flounder (*Paralichthys olivaceus* = *Paralichthys japonicus*; Yoshinaga and Nakazoe, 1993; Chun, 2000; Jee *et al.* 2001; Kim *et al.*, 2004a), turbot (*Scophthalmus maximus* = *Psetta maxima*; Dyková and Figueras, 1994; Sterud *et al.*, 2000; Iglesias *et al.*, 2001), sea bass

(*Dicentrarchus labrax*; Dragesco *et al.*, 1995), seahorse (*Hippocampus erectus*; Thompson and Moewus, 1964) and southern bluefin tuna (*Thunnus maccoyii*; Munday *et al.*, 1997) (Table 5.1). Mortality is particularly high for flatfishes (e.g. olive flounder and turbot) and, therefore, are of great economic importance. Aetiologic agents of the disease in flatfishes in eastern Asia and Europe are *Miamiensis avidus* and *Philasterides dicentrarchi*, respectively, and there is good evidence that they may be synonymized (Song and Wilbert, 2000; Paramá *et al.*, 2006; Jung *et al.*, 2007). We agree with the proposal and are treating *P. dicentrarchi* as a junior synonym of *M. avidus* in this discussion.

Crustaceans, such as the American lobster (*Homarus americanus*; Cawthorn *et al.*, 1996; Cawthorn, 1997), Norway lobster (*Nephrops norvegicus*; Small *et al.*, 2005a), blue crab (*Callinectes sapidus*; Messick and Small, 1996) and Dungeness crab (*Cancer magister*) are also susceptible to the ciliate (Morado *et al.*, 1999). The ciliate causes systemic invasions which destroy tissues and lead to high host mortality.

This chapter is focused mainly on *M. avidus* (= *P. dicentrarchi*) because its pathogenicity, virulence factor(s), host immunity and prevention are relatively well studied. Diseases caused by other Scuticociliatida are also provided.

Table 5.1. Scuticociliate species, host, distribution and endoparasitic characteristics of scuticociliates worldwide.

Parasite	Host	Endo-parasitic	Region	References
<i>Miamiensis avidus</i> (= <i>P. dicentrarchi</i>)	Seahorse <i>Hippocampus</i> sp.	NA	USA	Thompson and Moewus (1964)
	Olive flounder <i>Paralichthys olivaceus</i> , Righteye flounder <i>Pleuronichthys cornutus</i> , Spotted knifejaw <i>Oplegnathus punctatus</i>	Yes	South Korea, Japan, China	Song and Wilbert (2000), Kim <i>et al.</i> (2004a), Jung <i>et al.</i> (2005), Song <i>et al.</i> (2009b), Moustafa <i>et al.</i> (2010a)
	Groper <i>Polyprion oxygeneios</i> , Kingfish <i>Seriola lalandi</i>	Yes	New Zealand	Smith <i>et al.</i> (2009)
<i>Philasterides dicentrarchi</i> (= <i>M. avidus</i>)	Turbot <i>Scophthalmus maximus</i>	Yes	Spain	Iglesias <i>et al.</i> (2001), Alvarez-Pellitero <i>et al.</i> (2004)
	Sea bass <i>Dicentrarchus labrax</i>	Yes	France	Dragesco <i>et al.</i> (1995)
	Leafy sea dragon <i>Phycodurus eques</i> , Weedy sea dragon <i>Phyllopteryx taeniolatus</i>	Yes	Switzerland (imported from Australia)	Rossteuscher <i>et al.</i> (2008)
<i>Uronema nigricans</i>	Southern bluefin tuna <i>Thunnus maccoyii</i>	Yes	Australia	Munday <i>et al.</i> (1997)
<i>Uronema marinum</i>	Olive flounder <i>P. olivaceus</i>	Yes	South Korea	Jee <i>et al.</i> (2001)
	Indo-Pacific seahorse <i>Hippocampus kuda</i> etc.	Yes/no	USA	Cheung <i>et al.</i> (1980)
<i>Uronema marinum</i>	Groper <i>P. oxygeneios</i>	NA	New Zealand	Smith <i>et al.</i> (2009)
<i>Uronema</i> sp.	Turbot <i>S. maximus</i>	Yes	Norway	Sterud <i>et al.</i> (2000)
	Sand whiting <i>Sillago ciliata</i>	No	Australia	Gill and Callinan (1997)
	Silver pomfret <i>Pampus argenteus</i>	Yes	Kuwait	Azad <i>et al.</i> (2007)
<i>Pseudocohnilembus hargisi</i>	Olive flounder <i>P. olivaceus</i>	No	South Korea	Song <i>et al.</i> (2009a)
<i>Pseudocohnilembus persalinus</i>	Olive flounder <i>P. olivaceus</i>	Yes/No	South Korea	Kim <i>et al.</i> (2004b), Song <i>et al.</i> (2009a)

<i>Pseudocohnilembus persalinus</i>	Rainbow trout <i>Oncorhynchus mykiss</i>	Yes	Canada	Jones <i>et al.</i> (2010)
<i>Tetrahymena corlissi</i>	Guppy <i>Poecilia reticulata</i>	Yes	Japan (imported from Singapore or Sri Lanka)	Imai <i>et al.</i> (2000)
<i>Tetrahymena</i> sp.	Guppy <i>P. reticulata</i>	Yes	Israel, Thailand	Ponpornpisit <i>et al.</i> (2000), Leibowitz <i>et al.</i> (2005)
Unidentified	Turbot <i>S. maximus</i>	Yes	Spain, Portugal	Dyková and Figueras (1994), Alvarez-Pellitero <i>et al.</i> (2004), Puig <i>et al.</i> (2007), Ramos <i>et al.</i> (2007)
Unidentified	Olive flounder <i>P. olivaceus</i>	Yes	Japan	Yoshimizu <i>et al.</i> (1993), Yoshinaga and Nakazoe (1993)
Unidentified	Weedy sea dragon <i>Phyllopteryx taeniolatus</i>	Yes	Japan (imported from Australia)	Umehara <i>et al.</i> (2003)
<i>Anophryoides haemophila</i>	American lobster <i>Homarus americanus</i>	Yes	Canada	Cawthorn <i>et al.</i> (1996, 1997), Athanassopoulou <i>et al.</i> (2004), Greenwood <i>et al.</i> (2005)
<i>Mesanophrys chesapeakeensis</i>	Blue crab <i>Callinectes sapidus</i>	Yes	USA	Messick and Small (1996)
<i>Orchitophrya stellarum</i>	Sea stars <i>Asterina miniata</i> , <i>Pisaster ochraceus</i>	Yes	Canada	Leighton <i>et al.</i> (1991), Claereboudt and Boulard (1994), Bates <i>et al.</i> (2010)
<i>Orchitophrya stellarum</i>	Norway lobster <i>Nephrops norvegicus</i>	Yes	Scotland	Small <i>et al.</i> (2005a)
<i>Mesanophrys pugettensis</i>	Dungeness crab <i>Cancer magister</i>	Yes	USA	Morado and Small (1995), Morado <i>et al.</i> (1999)
<i>Tetrahymena pyriformis</i>	Australian crayfish <i>Cherax quadricarinatus</i>	Yes	Australia	Edgerton <i>et al.</i> (1996)
Unidentified (Orchitophryidae)	Pacific oyster <i>Crassostrea gigas</i> , Kumamoto oysters <i>Crassostrea sikamea</i>	Yes	USA	Elston <i>et al.</i> (1999)

NA, Information not available.

5.1.2. Locations in/on the fish

All parasitic scuticociliates are histophagous and cause lesions on body surfaces. *M. avidus*, *Uronema nigricans*, *Uronema marinum*, *Pseudocohnilembus persalinus* and *Tetrahymena corlissi* cause systemic infections including the brain (Munday *et al.*, 1997; Imai *et al.*, 2000; Iglesias *et al.*, 2001; Jee *et al.*, 2001; Kim *et al.*, 2004a; Jung *et al.*, 2007; Jones *et al.*, 2010). *M. avidus* is highly histophagous in olive flounder and turbot and it causes severe haemorrhages and ulcers on skin muscles, fins and jaws (Fig. 5.1a–c). It is also found in the brain, gills, ascites, spinal cord and digestive tract (Fig. 5.1d) (Iglesias *et al.*, 2001; Jung *et al.*, 2007; Jin *et al.*, 2009; Moustafa *et al.*, 2010a). Masses of ciliates with ingested blood cells and cellular debris are easily detected in wet-mount preparations of organs examined under a light microscope (Fig. 5.1e). *U. nigricans* has been detected in cerebrospinal fluid and has been recovered from the brain cavity and olfactory nerves of infected tuna (Munday *et al.*, 1997). *U. marinum* and *P. persalinus* have been isolated from brains, gills and ulcerated skin of olive flounder in Korea (Jee *et al.*, 2001; Kim *et al.*, 2004b). Heavy *U. marinum* infections have been detected in gills, viscera and body muscles of Atlantic and Pacific marine fishes kept in the

New York Aquarium (Cheung *et al.*, 1980). *T. corlissi* causes obvious scale loss and ulcers in guppies (*Poecilia reticulata*; Imai *et al.*, 2000). It also appears in scale pockets, muscle fibres, abdominal cavities and internal organs such as intestine, liver, eye socket, cranial cavity and spinal cord.

Some infections seem to be restricted only to the body surface and gills. *U. marinum* co-isolated with amoebae exists only in gills of Atlantic salmon (*Salmo salar*) and *Uronema* sp. infesting cultured silver pomfret (*Pampus argentatus*) are only found in skin lesions (Al-Marzouk and Azad, 2007; Dyková *et al.*, 2010). Disease severity is also related to host species and fish size. For example, flatfishes and juvenile fish are more susceptible to scuticociliates. Cultures of *M. avidus* and *Tetrahymena pyriformis* are infective in experimental infections (Ponpornpisit *et al.*, 2000; Paramá *et al.*, 2003; Jung *et al.*, 2007; Moustafa *et al.*, 2010b). However, *U. marinum*, *P. persalinus* and *Pseudocohnilembus hargisi* do not cause mortality in olive flounder which suggests that these species are relatively less pathogenic than *M. avidus* or are only secondary pathogens as proposed by Song *et al.* (2009a).

Anophryoides haemophila which causes 'bumper car disease' in American lobsters is found initially in the gills and connective

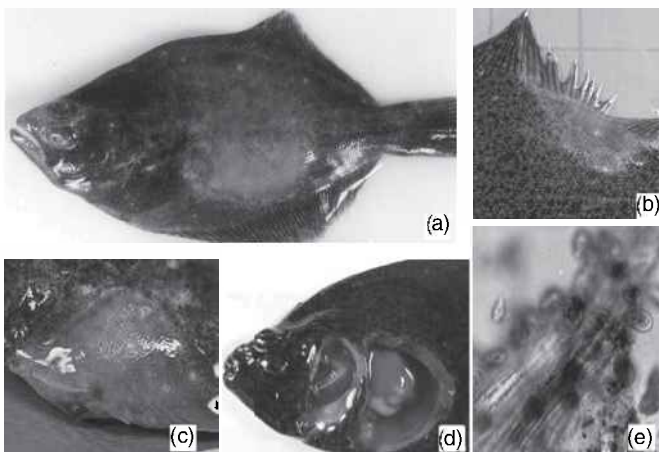


Fig. 5.1. Olive flounder infected with *Miamienis avidus* (a–d). (a) Haemorrhages on large area of the skin with depigmentation of surrounding area; (b) fin erosion and skin ulceration; (c) distended reddish anus accompanying haemorrhages and depigmentation; (d) accumulation of reddish ascites; (e) actively moving ovoid to pyriform ciliates feeding on host tissues and blood cells.

tissue. The ciliate invades the haemolymph at later stages of infection (Athanasopoulou *et al.*, 2004). Other systemic ciliates (e.g. *T. pyriformis*, *Mesanothryx*) have been reported in freshwater crayfish (*Cherax quadricarinatus*) and a variety of crab hosts (Edger-ton *et al.*, 1996; Messick and Small, 1996).

5.1.3. Transmission

The scuticociliates are facultative parasites that can live in the absence of a host. They can be parasitic when the defence capabilities of the fish are compromised which is often due to adverse environmental conditions. Once an outbreak occurs, the disease spreads rapidly to other individuals in the same tank, especially to fry and juvenile fish (Yoshinaga and Nakazoe, 1993). The scuticociliates are difficult to eradicate because they can survive in nutrient-rich water and bottom sediments, and in internal organs of infected fish where the chemical treatment is not very effective (Yoshimizu *et al.*, 1993; Jin *et al.*, 2009).

Experimental *P. dicentrarchi* (= *M. avidus*) infections in farmed turbot using various routes of infection were examined. Intraperitoneal, periorbital and intramuscular inoculations resulted in systemic infections and high fish mortality. Immersion infection was only successful after artificial abrasion of gills and opercula which suggests that lesions in the skin or the gills are entry routes into fish under culture conditions (Paramá *et al.*, 2003). Similarly, experimental infection by immersion was achieved only after abrasion of the gills and muscles in olive flounder as well (Jin *et al.* 2009). However, other studies showed 60–100% mortality via immersion infection without any artificial abrasion or other treatment before infection (Song *et al.*, 2009a; Moustafa *et al.*, 2010b). Although the reason for the different results in immersion infection experiments is not clear, Takagishi *et al.* (2009) suggested low salinity can be a key factor in immersion infection (more details in section 5.4 on protective/control strategies). Unidentified scuticociliate parasites were found in the subepithelial connective tissue of the digestive tract of turbot which may

have entered by an oral route (Dyková and Figueras, 1994). However, Jung *et al.* (2007) proposed it is unlikely that the ciliate invades their host via an oral route because the low pH in the stomach lumen would be a barrier to infection because *M. avidus* can only survive in a pH range of 5–10. As suggested by Iglesias *et al.* (2001), once the ciliate enters the host, it spreads quickly via blood vessels and establishes a systemic infection. Histological examinations demonstrated the presence of ciliates in the blood vessels of gills, the ventricles of the brain, and in blood from the caudal vein. In conclusion, *M. avidus* probably enters the hosts via the body or brachial surfaces, especially if there are lesions on these surfaces. They then spread via blood vessels and lymphatic channels to various internal organs. Similarly, Imai *et al.* (2000) suggested *T. corlissi* infects guppies via the epidermis. Munday *et al.* (1997) proposed that *U. nigricans* infects the southern bluefin tuna through the nasal route because ciliates were consistently found in the axis of olfactory rosettes and nerves in naturally infected fish.

Iglesias *et al.* (2001) suggested that cadavers act as a food source for ciliates in water and are a source of infection for other fish. However, Puig *et al.* (2007) found that scuticociliates in turbot do not survive for long in dead fish so may not be a source of parasite infection.

In crustaceans, moulting increases the probability for ciliate infection (Morado *et al.*, 1999). *Mesanothryx pugettensis* enters the Dungeness crab via lesions associated with adhesions during moulting (Morado and Small, 1995). Once *A. haemophila*, the cause of ‘bumper car disease’ in American lobster, is released from dead lobsters, it can survive in the environment, especially in nutrient-rich water. Immersion infections suggest the gills as the route of infection (Cawthorn, 1997).

5.1.4. Geographical distribution

Scuticociliates have various host species worldwide (Table 5.1). *M. avidus* (= *P. dicentrarchi*) was first reported in 1964 in Miami, Florida from seahorses (Thompson and Moewus, 1964). Dead sea bass in the Mediterranean Sea

(France) were infected with *P. dicentrarchi* (Dragesco *et al.*, 1995). *P. dicentrarchi* was also responsible for outbreaks in turbot farms on the Atlantic coast in Europe (Iglesias *et al.*, 2001; Alvarez-Pellitero *et al.*, 2004). In Asia, *M. avidus* was found in the olive flounder in Korea (Kim *et al.*, 2004a; Jung *et al.*, 2005), Japan (Song *et al.*, 2009b; Takagishi *et al.*, 2009; Moustafa *et al.*, 2010a) and China (Song and Wilbert, 2000). *M. avidus* was found in juvenile gropers (*Polyprion oxygeneios*) and adult kingfish (*Seriola lalandi*) in New Zealand (Smith *et al.*, 2009), and in wild-caught sea dragons (*Phycodurus eques* and *Phyllopteryx taeniolatus*) in southern Australia (Rossteuscher *et al.*, 2008).

U. marinum was the cause of heavy infections in Atlantic and Pacific marine fishes kept in the New York Aquarium (Cheung *et al.*, 1980). *U. nigricans* infects southern bluefin tuna in Australia (Munday *et al.*, 1997). Geographical distributions of other scuticociliate species, including parasites for both fish and crustaceans, are summarized in Table 5.1.

5.1.5. Impact of the disease on production

Scuticociliatosis has been recognized as an emerging problem that causes significant economic loss in aquaculture. Mortality caused by *M. avidus* (= *P. dicentrarchi*) is particularly high for flatfishes such as olive flounder and turbot and results in high economic losses in eastern Asia and Europe, respectively. It is a highly virulent endoparasite which divides rapidly by binary fission. In Korea, the disease has caused mass mortality (30–60%) in many commercial flounder farms since 1995 (Jin *et al.*, 2009). Olive flounder mortality of 12.5–78.9% due to an unidentified scuticociliate occurred in Hokkaido, Japan (Yoshimizu *et al.*, 1993). Olive flounder (12–17 cm long) mortality of 70–80% caused by *M. avidus* occurred in a farm in Japan in July 2005 (Moustafa *et al.*, 2010a). The frequency and severity of scuticociliatosis in turbot cultures in Europe are increasing, with mortality reaching up to 60% in some infected stocks (Sitjà-Bobadilla *et al.*, 2008). Systemic ciliatosis can cause mortality approaching 100% in single units of fry, 30% mortality in the most heavily infected

on-grower units of turbot in Norway (Sterud *et al.*, 2000), and 100% mortality in some tanks in Spain (Iglesias *et al.*, 2001). Higher mortality was observed in younger fish at higher water temperatures (> 20°C) (Iglesias *et al.*, 2001). However, in sea bass, the ciliate has low prevalence and is not associated with ulcers or haemorrhagic lesions (Dragesco *et al.*, 1995). Flatfishes may be more susceptible to the disease because they aggregate and have more skin-to-skin contact which may increase direct transmission because the ciliate occurs in large numbers in skin ulcers and fin lesions. In addition, scuticociliate density is higher at the bottom of the tank than in the water column (Jin *et al.*, 2009). Hence, sedentary, benthic fish are more exposed to infection.

5.2. Diagnosis of the Infection

Infections can be easily detected by microscopic examinations of wet mounts from skin scrapings, gills, brain squash or body cavity fluid. Live ciliates are ovoid to pyriform or elongate in shape (20–50 µm in length and 15–25 µm in width), with variations due to species, fixative condition and feeding status, and actively moving by caudal cilia (Fig. 5.2a, b). They may contain food vacuoles filled with blood cells and/or cellular debris (Iglesias *et al.*, 2001; Azad *et al.*, 2007). Scuticociliatida are morphologically similar. Therefore, various silver impregnation methods (Fig. 5.2c–f) and/or molecular information (e.g. small subunit ribosomal RNA (SSU rRNA) sequence) may aid in species identification (Jung *et al.*, 2005; Smith *et al.*, 2009; Gao *et al.*, 2010). Mitochondrial cytochrome c oxidase subunit 1 (cox1) gene and the internal transcribed spacer genes may help to discriminate between species (Chantangsi and Lynn, 2008; Strüder-Kypke and Lynn, 2010; Jung *et al.*, 2011a, b).

5.3. External/Internal Lesions

5.3.1. Macroscopic lesions

Infected olive flounders have darkened skin, reddening at the base of the fins and around

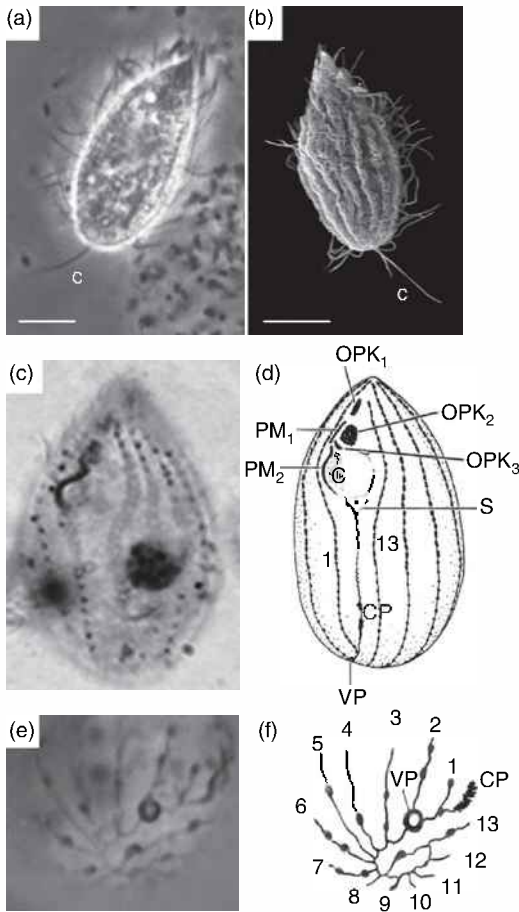


Fig. 5.2. Morphological characteristics of *M. avidus*. (a) Scuticociliate with caudal cilium at the posterior end (labelled 'c') observed under phase contrast microscope; (b) scanning electron microscopy with caudal cilium (labelled 'c'); (c) silver-carbonate-impregnated ciliate; (d) schematic drawing showing somatic and oral infraciliature; (e) wet silver-nitrate-impregnated specimen; and (f) its caudal view illustration. OPK₁, ₂, ₃: Oral polykinetids; PM₁, ₂: paroral membrane; C: cytostome; CP: cypopyge; S: scutico-vestige; VP: contractile vacuole pore. Bar = 10 μ m. (a, b, d: from Iglesias *et al.*, 2001; c, e, f: from Jung *et al.*, 2007; courtesy of *Diseases of Aquatic Organisms*).

the mouth, and skin ulcers with haemorrhages (Fig. 5.1a). Some of these ulcers spread into the muscular tissue, exposing the fin rays (Fig. 5.1b) (Jung *et al.*, 2007; Jin *et al.*, 2009; Moustafa *et al.*, 2010a). These are accompanied by abnormal swimming behaviours, such as convulsions, spinning and spiralling movements. Exophthalmia, protrusion of the eye ball, brain lesions and the distension of the abdominal cavity caused by an accumulation of ascites are seen in naturally infected fish (Moustafa *et al.*, 2010a). In experimental infections, intraperitoneally injected fish at early stages of infection had signs of accumulation of reddish ascetic fluid containing ciliates actively feeding on blood cells (Fig. 5.1c, d) (Puig *et al.*, 2007; Song *et al.*, 2009a; Moustafa *et al.*, 2010b).

Turbot infected by the ciliate have clinical signs similar to those of the olive flounder. Moribund fish have darkened skin and ulcers, temporary alterations in swimming behaviour, exophthalmos, and/or abdominal distension as a result of the accumulation of ascetic fluid in the body cavity (Iglesias *et al.*, 2001; Ramos *et al.*, 2007). Internal organs have little visible alterations except for pale anaemic gills in both turbot and olive flounder. Infected sea dragon has similar clinical signs such as skin ulcerations, whirling and swimming on the water surface, and/or swimming in a lateral position with no obvious changes in the internal organs (Rossteuscher *et al.*, 2008). However, moribund adult sea bass (weighing approximately 250 g) have no skin lesions. Their digestive tract, liver, kidney

and gonads are very congested with ascetic fluids. The prevalence of the disease is low in sea bass (Dragesco *et al.*, 1995).

Southern bluefin tuna infected by *U. nigricans* only exhibit abnormal swimming behaviour 2–8 h before death. The olfactory rosettes are darkened and the brain shows varying degrees of softening/liquefaction (Munday *et al.*, 1997). Silver pomfret infected by *Uronema* sp. have similar clinical signs, with loss of scales, haemorrhages, bleached spots on the skin and dermal necrotic lesions (Al-Marzouk and Azad, 2007). Many of the moribund silver pomfret with brownish skin patches and necrotic lesions also have distended abdomen and the peritoneal fluid.

5.3.2. Histopathology and pathophysiology

Histopathological changes are similar in infected olive flounder (Jung *et al.*, 2007; Jin

et al., 2009; Song *et al.*, 2009a; Moustafa *et al.*, 2010b) and turbot (Iglesias *et al.*, 2001; Paramá *et al.*, 2003; Puig *et al.*, 2007). The ciliate rapidly invades and proliferates in the gills, pharynx, skin, skeletal muscle and fins, with systemic invasion into the brain and digestive tract with accompanying haemorrhages and necrosis of infected areas. Degeneration of gills and necrosis of branchial tissues associated with hyperplasia are commonly seen. Ciliates with red blood cells in their cytoplasm have been seen in the gills and pharynx and in haemorrhagic lesions of the skin, muscle and fins (Fig. 5.3a, b). Ciliates have been found in scale pockets accompanying severe necrosis of the epidermis and dermis with loss of scales (Fig. 5.3c). Skeletal muscles and fins have necrotic degeneration in muscle fibres with severe haemorrhages. Ciliates have been found in the meninges, spinal cord and in the brain in late stages of experimental infections but not consistently in brains of fish showing skin lesions (Moustafa *et al.*, 2010b). In the stomach and intestine, ciliates are located in the lamina

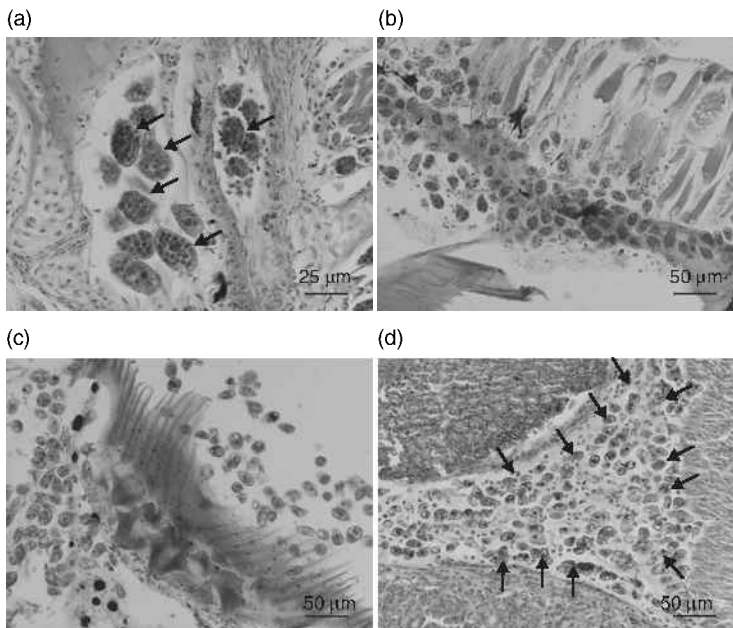


Fig. 5.3. Histopathological changes in experimentally infected olive flounder. (a) Ciliates containing fish erythrocytes (arrows) in the pharynx; (b) ulcerated lesion of fin with necrotized muscle fibre due to heavy ciliate infection; (c) numerous ciliates in scale pocket; (d) ciliates (arrows) in lamina propria of the stomach. Bar = 50 µm (from Jung *et al.*, 2007; courtesy of *Diseases of Aquatic Organisms*).

propria, mainly around blood vessels (Fig. 5.3d). However, the mucosal epithelium of the digestive tract shows different degrees of pathological changes; exhibiting normal appearance in intraperitoneally infected fish (Jung *et al.*, 2007), degenerate with mononuclear cells infiltration (Moustafa *et al.*, 2010a) or complete necrotized and sloughed into the lumen (Jin *et al.*, 2009). Ciliates are rarely seen in the kidney but often seen in the liver and spleen. In Spain, infected turbot showed: (i) muscle fibre degeneration; (ii) hyperplasia of the branchial epithelium; and (iii) severe encephalitis and meningitis associated with different degrees of softening or liquefaction of the brain (Iglesias *et al.*, 2001). In other organs, severe oedema of the intestinal wall, necrosis of the hepatic parenchyma, and oedematous changes in periorbital tissues are associated with the presence of ciliates (Iglesias *et al.*, 2001; Puig *et al.*, 2007). Ciliates are in vascular and perivascular connective tissues and cause vascular and perivascular inflammations. Inflammatory responses were seen in turbot and olive flounder that were naturally infected (Iglesias *et al.*, 2001; Moustafa *et al.*, 2010a). *Uronema* sp. in silver pomfret in Kuwait (Al-Marzouk and Azad, 2007) and in chronically infected sea dragons cause marked inflammatory infiltrate consisting of large numbers of lymphocytes, macrophages and scattered eosinophilic granular cells (Rossteuschler *et al.*, 2008).

Fish mortality is most likely due to a combination of respiratory, excretory and neural dysfunctions. Respiratory failure may be due to the direct damage of respiratory gill lamellae by the ciliates and also by the anaemia caused by haematophagous activity of the ciliates (Cheung *et al.*, 1980; Dyková and Figueras, 1994; Iglesias *et al.*, 2001; Jee *et al.*, 2001; Puig *et al.*, 2007; Song *et al.*, 2009a). Accumulation of ascites in the peritoneum of many fish species infected with scuticociliates suggests excretory dysfunction (Dragesco *et al.*, 1995). Brain and spinal cord damages could affect neurological functions, such as controlling motor, sensory and neurotransmitter systems. Darkened body skin, abnormal behaviours such as convulsions, and difficulty of finding food may be expressions of neural dysfunction.

Information about virulence factors in fish parasites is limited. Among several virulence factors, lytic enzymes, namely proteases, are potential virulence factors for fish and human parasites. Proteases play key roles in the pathogenesis of numerous parasitic diseases, including invasion, immune evasion and nutrient acquisition (Rosenthal, 1999). It has been suggested that cysteine proteinases of *P. dicentrarchi*, *U. marinum*, *Tetrahymena* spp., *Mesanothryx* sp. and *Ichthyophthirius multifiliis* (Ciliophora, Hymenostomatia) participate in the invasion and degradation of host tissue (Kwon *et al.*, 2002; Paramá *et al.*, 2004b; Small *et al.*, 2005b; Jousson *et al.*, 2007; Pimenta Leibowitz *et al.*, 2009). A protease of a scuticociliate that infects turbot induces apoptosis (programmed cell death) of turbot immune cells in head kidney as a means to escape from host immune responses (Paramá *et al.* 2007b). Protease of *U. marinum* is relatively well studied. It has been proposed that the cysteine and metalloproteases excreted by *U. marinum* are involved in the invasion of host tissues and in the pathogenicity of the parasite (Kwon *et al.*, 2002). The cysteine protease gene is homologous to the cathepsin L (ScCtL) genes. The cathepsin B gene (ScCtB) was cloned from a cDNA library of *U. marinum*, and was successfully purified into a functional and enzymatically active form similar to that of the cathepsin L-like cysteine protease (Ahn *et al.*, 2007). Similarly, the recombinant protein produced by cathepsin B gene of *U. marinum* also exhibited typical protease activity (Lim *et al.*, 2005). However, the effect(s) of the recombinant protease on the immune system of olive flounder has not been determined. Cysteine protease of *Tetrahymena* spp. also may contribute to pathogenicity in guppies (Leibowitz *et al.*, 2009). Increasing the knowledge of protease participation in evading host immunity may help provide strategies to control the ciliate.

5.4. Protective/Control Strategies

5.4.1. Environmental control

In experimental infections, cumulative mortality was low at 10°C and increased with temperature

dependently at 15°C and 20°C (Bae *et al.*, 2009). These results agree with field observations of outbreaks of scuticociliatosis in olive flounder which start in late spring when the water temperature is approximately 18–20°C and become epidemic in the summer when water temperatures increase up to 26°C in Korea. Similar results were seen in natural outbreaks in turbot which occur in summer when water temperature is over 20°C (Iglesias *et al.*, 2001; Ramos *et al.*, 2007). Lowering water temperature as a control strategy is impractical in most instances because it is difficult or impossible to reduce water temperature in large-scale farms and because growth rate of flatfish will be reduced. Fish are usually treated with antibiotics or chemotherapeutants (e.g. formalin) promptly in the early stages of infection while the ciliates are on the body surface (see section 5.4.2).

Although *M. avidus* is eurohyaline, it prefers similar osmolality to that in the fish host. Osmolality of the body fluid in teleost is approximately 300 mOsm whereas sea water is approximately 1200 mOsm (35%) (Marshall and Grosell, 2006). Iglesias *et al.* (2003a) tested several *in vitro* culture conditions for the ciliate and concluded that the optimal conditions are 10% osmolality, pH 7.2 and temperature between 18 and 23°C. Experimental immersion infections using full-strength (35%), one-third strength and two-thirds strength of natural sea water also showed higher mortalities under hyposaline conditions (Takagishi *et al.*, 2009). Low salinity is probably a key factor in scuticociliatosis outbreaks and avoiding the use of low salinity sea water may reduce scuticociliatosis mortality in aquaculture farms (Takagishi *et al.*, 2009). This strategy is the exact opposite to using hyposalinity to control diseases caused by several other marine parasites such as *Cryptocaryon irritans* and *Benedenia seriolae*. Tomonts of the *C. irritans* lyse in 10‰ after 3 h and eggs of *B. seriolae* (Monogenea) do not hatch at 10‰ (Colorni, 1985; Ernst *et al.*, 2005).

5.4.2. Chemotherapeutic approaches

Chemotherapeutic trials against scuticociliatosis (including several taxonomically

different parasites) are well reviewed by Harikrishnan *et al.* (2010a). Current strategies depend largely on the use of chemicals such as formalin to kill the parasite. There are several preliminary *in vitro* studies for screening effective drugs (Iglesias *et al.*, 2002; Quintela *et al.*, 2003) and they are summarized in Table 5.2. However, there is no effective chemotherapeutic treatment once the ciliate has invaded the internal organs (Iglesias *et al.*, 2002; Paramá *et al.*, 2003; Fajer-Ávila *et al.*, 2003).

Farmers use formalin, hydrogen peroxide or sodium chloride in combination with antibiotics (such as oxytetracycline, gentamycin and tetracycline) to kill the ciliate and to prevent secondary bacterial infections through skin lesions (Jin *et al.*, 2010). Formalin (37% formaldehyde) is the most effective and widely used chemical to treat scuticociliates. The US Food and Drug Administration (FDA) approves three commercial formaldehyde products of similar formulations (of about 37% formaldehyde) for use in US aquaculture. According to the recommendations on the labels, routine treatment concentrations of formalin ranges from 15 to 250 ppm for control of protozoan and monogenetic trematodes on fish (FDA, 1998; Jung *et al.*, 2001). Treatments of 100–250 ppm for 1–2 h repeated two to five times daily are used for bath treatments against protozoan parasites (Lahnsteiner and Weismann, 2007). For *M. avidus*, 250 ppm for 1 h eliminated all the ciliates. The minimum dose was 25 ppm for 6 h to eliminate 100% of ciliates on the skin and gills (Ruiz de Ocenda *et al.*, 2007). Immersion treatment of olive flounder with hydrogen peroxide (50 ppm/30 min/day for 10 days) or formalin (100–500 ppm/15–20 min/day for 3–5 days) are partially successful (Harikrishnan *et al.*, 2010c). The tolerance to chemicals varies depending on species, fish size and water temperature (Schmahl *et al.*, 1989; Fajer-Ávila *et al.*, 2003). Therefore, the toxicity of the chemical to fish should be determined before applying chemical therapies to control disease epizootics. The anti-inflammatory drug, indomethacin, significantly inhibits ciliate growth under *in vitro* conditions by a mechanism related to the induction of cell death (Paramá *et al.*, 2007c). Resveratrol, a

Table 5.2. Chemotherapeutants (using *in vitro* assay) against scuticociliates.

Chemicals	Lethal dose (time)	Scuticociliate species	References
Formalin	100–400 ppm (1 h)	<i>Philasterides dicentrarchi</i> , ^a <i>Anophryoides haemophila</i> , <i>Uronema nigricans</i> , unidentified	Yoshimizu <i>et al.</i> (1993), Novotny <i>et al.</i> (1996), Crosbie and Munday (1999), Jin <i>et al.</i> (2010)
	62 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Hydrogen peroxide	150–300 ppm (1–1.5 h)	<i>P. dicentrarchi</i> , <i>U. nigricans</i> , unidentified	Choi <i>et al.</i> (1997), Crosbie and Munday (1999), Jin <i>et al.</i> (2010)
Hydrogen peroxide Jenoclean	50 ppm (30 min)	<i>P. dicentrarchi</i>	Jin <i>et al.</i> (2010)
Chloroquine	100 ppm (60% survival, 2 h)	<i>Tetrahymena</i> sp.	Leibowitz <i>et al.</i> (2010)
Monensin	20 min 10 ⁻⁴ M	<i>A. haemophila</i>	Novotny <i>et al.</i> (1996)
Albendazole	100 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
	100 ppm (35% survival, 2 h)	<i>Tetrahymena</i> sp.	Leibowitz <i>et al.</i> (2010)
Niclosamide	100 ppm (23% survival, 2 h)	<i>Tetrahymena</i> sp.	Leibowitz <i>et al.</i> (2010)
	1.5 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Oxyclozanide	1.5 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Bithionol sulfoxide	3.1 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Toltrazuril	6.2 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Furaltadone	25 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Doxycycline hyclate	50 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Carnidazole	100 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Pyrimethamine	100 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Quinacrine hydrochloride	100 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Quinine sulfate	100 ppm (24 h)	<i>P. dicentrarchi</i>	Iglesias <i>et al.</i> (2002)
Pyridothienotriazine (12k)	1.5 ppm	<i>P. dicentrarchi</i>	Quintela <i>et al.</i> (2003), Paramá <i>et al.</i> (2004a)
Resveratrol	50 µM (inhibit growth)	<i>P. dicentrarchi</i>	Leiro <i>et al.</i> (2004), Morais <i>et al.</i> (2009)
(-)-Epigallocatechin-3-gallate	500 µM (inhibit growth)	<i>P. dicentrarchi</i>	Leiro <i>et al.</i> (2004), Lamas <i>et al.</i> (2009)
Indomethacin	100 µM (inhibit growth)	<i>P. dicentrarchi</i>	Paramá <i>et al.</i> (2007a)
Fresh water	100% (10 min), 70% (no effect)	Unidentified	Choi <i>et al.</i> (1997)

^a *Philasterides dicentrarchi* is synonymous with *Miamiensis avidus*.

substance produced by grape vines, exhibits anti-inflammatory and antioxidant properties, induces alterations in mitochondria, generates autophagy, provokes a reduction in the ciliate volume, and also drastically reduces the ciliate endocytic activity. This chemical has therapeutic potential against scuticociliates (Morais *et al.*, 2009); however, there is no information on the efficacy of these candidate drugs in infected fish.

5.4.3. Immunostimulants

The use of immunostimulants can improve the innate immunity of fish against pathogens especially during periods of high stress, such as grading, reproduction, seawater transfer and vaccination (Bricknell and Dalmo, 2005). Immunostimulants are a heterogeneous group of compounds including polymers (e.g. glucan and lipopolysaccharides) and synthetic compounds (e.g. levamisole, hydroxymethyl-butyrate and oligodeoxynucleotides (ODNs) containing CpG motifs). Immunostimulants are very effective in stimulating innate immunity and some may also help with antibody synthesis. Two immunostimulants to control scuticociliatosis are examined to control scuticociliatosis.

Triherbal, a traditional Korean medicine (TKM) is a solvent extract from the leaves of *Punica granatum*, *Chrysanthemum cinerariaefolium* and *Zanthoxylum schinifolium*. In olive flounder, it is effective in increasing innate immunity and disease resistance against *U. marinum* (Harikrishnan *et al.*, 2010b). A 1:1:1 mixture of triherbal at concentrations of 50 and 100 mg/kg body weight clearly enhances the innate immune responses (phagocytosis, respiratory burst, natural haemolytic complement activity and plasma lysozyme activity) and increases disease resistance against *U. marinum* when fed to fish for 30 days.

CpG-containing ODNs can serve as pathogen-associated molecular patterns (PAMPs) and are recognized by pattern recognition receptors (PRRs) in the vertebrate immune system. The immune response induced by CpG is mediated through the Toll-like receptor 9 (TLR 9), PRRs expressed on cells such as B cells, dendritic cells and

macrophages. Recent studies have demonstrated that synthetic ODNs containing CpG motifs (CpG ODNs) can mimic bacterial CpG dinucleotides and produce various immune effects in olive flounder (Liu *et al.*, 2010). Lee and Kim (2009) reported that CpG-ODNs increased resistance against *M. avidis* infection in olive flounder and concluded that CpG-ODNs are potential immunostimulants to reduce fish loss caused by scuticociliates.

5.4.4. Vaccine

Currently, most protozoan infections are controlled using chemotherapy. However, its use is being restricted as there are increasing concerns over food safety and environmental pollution. Vaccination is an attractive alternative to chemotherapy, especially when the ciliate is located in internal organs. Field observations indicate that fish that survived scuticociliate epizootics acquired disease resistance and that they have specific antibodies against the pathogen (Iglesias *et al.*, 2003b). Therefore, vaccination is a viable option.

Formalin-killed scuticociliates alone or in combination with an adjuvant stimulate innate immune factors, enhance the production of specific antibodies, and increase survival in turbot (Iglesias *et al.*, 2003b; Lamas *et al.*, 2008; Sitjà-Bobadilla *et al.*, 2008) and in olive flounder (Jung *et al.*, 2006; Lee and Kim, 2008). In olive flounder, killed vaccine administered in two intraperitoneal injections at 2 week intervals reduced or delayed fish mortality and increased phagocytosis and chemotaxis activity in phagocytes (Jung *et al.*, 2006).

Adjuvants increase the effectiveness of antigen presentation and slow their release, which prolong the period of antigen presentation to the immune system. Combining antigen with adjuvant improved fish survival in scuticociliatosis. Ciliate lysate alone did not induce a detectable antibody response (using ELISA, agglutination tests) and did not protect fish even after they were given a booster injection. However, adding Freund's complete adjuvant (FCA) to the ciliate lysate increased fish survival up to 73.7% (Iglesias *et al.*, 2003b). Because FCA has many undesirable side effects, including production of local

granulomata, autoimmune disease and tuberculin sensitization, it is not permitted in commercial vaccines (Bowden *et al.*, 2003; Afonso *et al.*, 2005). Hence, a new adjuvant based on metabolizable oils currently in use in poultry and fish was tested. Addition of an oil emulsion to a formulation of a metabolizable oil-based non-mineral adjuvant (Seppic MONTANIDE® ISA 763A) has improved the results of formalin-killed vaccines (Sanmartín *et al.*, 2008). It also elevated vaccine efficiency and enhanced the production of specific antibodies in turbot (Sitjà-Bobadilla *et al.*, 2008). Similarly, GERBU adjuvant (lipid microparticles with G-muramyl dipeptide) increases the specific immune responses although there are also some side effects (Palenzuela *et al.*, 2009).

Significant protection from infection was achieved in guppies immunized with *Tetrahymena* sp. using FCA as an adjuvant (Chettri *et al.*, 2009). However, cell lysates or live attenuated parasites alone did not elicit protection against challenge infections suggesting that administration of antigen alone was not sufficient to elicit protective immunity to *Tetrahymena* sp.

The acquired protection of fish against ciliate infections have been reported mainly in *I. multifiliis* (Chapter 4) and the immobilization antigen (i-antigen) has been a target antigen for the development of subunit vaccine against white spot disease (Xu *et al.*, 2006; Swennes *et al.*, 2007). Recent studies indicate the existence of i-antigen variations (different serotypes) in *M. avidus* isolated from olive flounder and turbot (Piazzón *et al.*, 2008; Song *et al.*, 2009b). The main antigenic proteins of *M. avidus* isolated from olive flounder were 30 kDa, 34 kDa and 38 kDa in each of three serotypes (Song *et al.*, 2009b). *M. avidus* serogroups divided by strain i-antigen was well matched with genogroups of mitochondrial *cox1* genes for the strains in Korea and Japan (Jung *et al.*, 2011b). Intraspecific genetic variation of *cox1* was also detected in the ciliate from turbot in Spain (Budiño *et al.*, 2011). The protection induced in turbot by formalin killed vaccine (containing adjuvant MONTANIDE® ISA 763A) protected fish only against the homologous isolate but from different serotypes (Piazzón *et al.*, 2008) indicating i-antigen dependent immunogenicity in

turbot. Conversely, antibody levels (evaluated using agglutination test and ELISA) after vaccination were not correlated with protection (Palenzuela *et al.*, 2009). In addition, Lee and Kim (2008) proposed that *M. avidus* can change its surface i-antigen to evade host antibodies. These studies suggest immune effectors other than i-antigen are involved in immune response and protection and the other candidate proteins are necessary.

Tubulin, cytoskeletal components of microtubules, is another antigen target for protozoans. They are expressed constitutively and are common across related species. It produces full immunoprotection against trypanosomiasis caused by *Trypanosoma brucei* (Lubega *et al.*, 2002). In scuticociliates, antisera against a recombinant beta-tubulin protein from *P. persalinus* showed higher parasitocidal activity than control sera suggesting that beta-tubulin can also be a target antigen (Kim *et al.*, 2006). Although several studies have been conducted to develop a vaccine, there is as yet no commercial vaccine against *M. avidus*.

5.5. Conclusion and Suggestions for Future Studies

Scuticociliates are facultative parasites of aquatic organisms and they have significant economic impacts on marine aquaculture of fishes and crustaceans worldwide. There have been many reports of severe outbreaks of scuticociliatosis since the early 1980s. However, there is confusion on the identification of the parasite to species, especially in *Uronema* and *Miamiensis* (Philasterides), because they are similar in size and have similar oral structures. Recent identifications are based on morphological characteristics as well as SSU rRNA and/or *cox1* gene sequence information. The correct identification of the pathogen will provide a clearer picture of their biological characteristics, pathogenic mechanisms and host-parasite relationships, especially when studies are conducted by different working groups using different host species.

Many scuticociliates can invade internal organs, such as the brain and intestine, making them difficult to kill once established.

There is no effective method to control the parasite once they are in internal organs. Therefore, the best control option may be the development of a vaccine. *M. avidus* can be readily cultured and the results using killed vaccine with adjuvant are encouraging. Recent studies show that different serotypes exist in Asia and Europe and that serotype-specific immunity exists. It is necessary to conduct more extensive surveys to determine the dominant serotype in fish farms in each region and host fish to determine the strain of parasite to be used in vaccine development.

On the other hand, i-antigen-independent adaptive protection is proposed for olive flounder. There is no clear evidence that *M. avidus* can vary its surface antigen to evade the host immune response as seen in other parasites. If confirmed, we need other strategies to develop vaccines that are not based on

surface antigens. Also, their pathogenic mechanisms are not well studied. Recent studies indicate that proteases may be pathogenic factors that help the parasite to survive by consuming host tissue and by reducing immune factors such as immunoglobulin. Recently, a metalloprotease-DNA (MP-DNA) vaccine against the haemoflagellate, *Cryptobia salmositica*, was developed (Chapter 3). The main rationale is that antibodies against proteases (disease-causing factors) will neutralize the metalloprotease secreted by the pathogen on infection. As expected, the vaccine did not protect vaccinated fish from infection; but it lowered parasitaemias, delayed peak parasitaemias, and promoted faster recovery in vaccinated/challenged trout compared to control fish. (Tan *et al.*, 2008; Woo, 2010). Consequently, further research on proteases and common antigens across the scuticociliates and their use in vaccines may be rewarding.

References

- Afonso, A., Gomes, S., da Silva, J., Mariques, F. and Henrique, M. (2005) Side effects in sea bass (*Dicentrarchus labrax* L.) due to intraperitoneal vaccination against vibriosis and pasteurellosis. *Fish and Shellfish Immunology* 19, 1–16.
- Ahn, S.J., Seo, J.S., Kim, M.S., Jeon, S.J., Kim, N.Y., Jang, J.H., Kim, K.H., Hong, Y.K., Chung, J.K. and Lee, H.H. (2007) Cloning, site-directed mutagenesis and expression of cathepsin L-like cysteine protease from *Uronema marinum* (Ciliophora: Scuticociliatida). *Molecular and Biochemical Parasitology* 156, 191–198.
- Al-Marzouk, A. and Azad, I.S. (2007) Growth kinetics, protease activity and histophagous capability of *Uronema* sp. infesting cultured silver pomfret *Pampus argenteus* in Kuwait. *Diseases of Aquatic Organisms* 76, 49–56.
- Alvarez-Pellitero, P., Palenzuela, O., Padrós, F., Sitjà-Bobadilla, A., Riaza, A., Silva, R. and Arán, J. (2004) Histophagous scuticociliatids (Ciliophora) parasitizing turbot *Scophthalmus maximus*: morphology, *in vitro* culture and virulence. *Folia Parasitologica* 51, 177–187.
- Athanassopoulou, F., Speare, D., Cawthorn, R.J., MacMillan, R. and Despres, B. (2004) Pathology of *Anophryoides haemophila* (Scuticociliatida: Orchitophryidae), parasite of American lobster *Homarus americanus* kept under experimental conditions. *Aquaculture* 236, 103–117.
- Azad, I.S., Al-Marzouk, A., James, C.M., Almastar, S. and Al-Gharabally, H. (2007) Scuticociliatosis-associated mortalities and histopathology of natural infection in cultured silver pomfret (*Pampus argenteus* Euphrasen) in Kuwait. *Aquaculture* 28, 202–210.
- Bae, M.J., Im, E.Y., Kim, H.Y. and Jung, S.J. (2009) The effect of temperature to Scuticociliatida *Miamiensis avidus* proliferation, and mortality of infected olive flounder *Paralichthys olivaceus*. *Journal of Fish Pathology* 22, 97–105 (in Korean).
- Bates, A.E., Stickle, W.B. and Harley, C.D.G. (2010) Impact of temperature on an emerging parasitic association between a sperm-feeding scuticociliate and Northeast Pacific sea stars. *Journal of Experimental Marine Biology and Ecology* 384, 44–50.
- Bowden, T.J., Adamson, K., MacLachlan, P., Pert, C.C. and Bricknell, I.R. (2003) Long-term study of antibody response and injection-site effects of oil adjuvants in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Fish and Shellfish Immunology* 14, 363–369.
- Bricknell, I. and Dalmo, R.A. (2005) The use of immunostimulants in fish larval aquaculture. *Fish and Shellfish Immunology* 19, 457–472.

- Budiño, B., Lamas, J., Pata, M.P., Arranz, J.A., Sanmartín, M.L. and Leiro, J. (2011) Intraspecific variability in several isolates of *Philasterides dicentrarchi* (syn. *Miamiensis avidus*), a scuticociliate parasite of farmed turbot. *Veterinary Parasitology* 175, 260–272.
- Cawthorn, R.J. (1997) Overview of 'bumper car disease'-impact on the North American lobster fishery. *International Journal for Parasitology* 27, 162–172.
- Cawthorn, R.J., Lynn, D.H., Despres, B., MacMillan, R., Maloney, R., Loughlin, M. and Bayer, R. (1996) Description of *Anophryoides haemophila* n. sp. (Scuticociliatida: Orchitophryidae), a pathogen of American lobsters *Homarus americanus*. *Diseases of Aquatic Organisms* 24, 143–148.
- Chantangsi, C. and Lynn, D.H. (2008) Phylogenetic relationships within the genus *Tetrahymena* inferred from the cytochrome c oxidase subunit 1 and the small subunit ribosomal RNA genes. *Molecular Phylogenetics and Evolution* 49, 979–987.
- Chettri, J.K., Leibowitz, M.P., Ofirc, R. and Zilberg, D. (2009) Protective immunization against *Tetrahymena* sp. infection in guppies (*Poecilia reticulata*). *Fish and Shellfish Immunology* 27, 302–308.
- Cheung, P.J., Nigrelli, R.F. and Ruggieri, D. (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.
- Choi, S.D., Kim, J.M., Kim, S.Y., Jo, Y.C., Choi, K.K. and Yang, H.C. (1997) Study on distribution and extermination of scuticociliatids parasitizing to Japanese flounder, *Paralichthys olivaceus* in southern Korea. *Journal of Fish Pathology* 10, 21–29 (in Korean).
- Chun, S.K. (2000) Scuticociliatosis. In: *Diseases of Cultures and Fish*. Hanguk Susan Sinbo Press, Seoul, Korea, pp. 89–93 (in Korean).
- Claereboudt, M.R. and Boulard, C. (1994) The effect of parasitic castration by a ciliate on a population of *Asterias vulgaris*. *Journal of Invertebrate Pathology* 63, 172–177.
- Colomi, A. (1985) Aspects of the biology of *Cryptocaryon irritans*, and hyposalinity as a control measure in cultured gilt-head sea bream *Sparus aurata*. *Diseases of Aquatic Organisms* 1, 19–22.
- Crosbie, P.B.B. and Munday, B.L. (1999) Environmental factors and chemical agents affecting the growth of the pathogenic marine ciliate *Uronema nigricans*. *Diseases of Aquatic Organisms* 36, 213–219.
- Dragesco, A., Dragesco, J., Coste, F., Gasc, C., Romestand, B., Raymond, J. and Bouix, G. (1995) *Philasterides dicentrarchi* n. sp. (Ciliophora: Scuticociliatia), a histophagous opportunistic parasite of *Dicentrarchus labrax* (Linnaeus, 1758), a reared marine fish. *European Journal of Protistology* 31, 327–340.
- Dyková, I. and Figueras, A. (1994) Histopathological changes in turbot *Scophthalmus maximus* due to a histophagous ciliate. *Diseases of Aquatic Organisms* 18, 5–9.
- Dyková, I., Tým, T., Kostka, M. and Pecková H. (2010) Strains of *Uronema marinum* (Scuticociliatia) co-isolated with amoebae of the genus *Neoparamoeba*. *Diseases of Aquatic Organisms* 89, 71–77.
- Edgerton, B., O'Donoghue, P., Wingfield, M. and Owens, L. (1996) Systemic infection of freshwater crayfish *Cherax quadricarinatus* by hymenostome ciliates of the *Tetrahymena pyriformis* complex. *Diseases of Aquatic Organisms* 27, 123–129.
- Elston, R.A., Cheney, D., Frelie, P. and Lynn, D. (1999) Invasive orchitophryid ciliate infection in juvenile Pacific and Kumamoto oysters, *Crassostrea gigas* and *Crassostrea sikamea*. *Aquaculture* 174, 1–14.
- Ernst, I., Whittington, I.D., Corneillie, S. and Talbot, C. (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.
- Fajer-Ávila, E.J., Abdo-de la Parra, I., Aguilar-Zarate, G., Contreras-Arce, R., Zaldívar-Ramírez, J. and Betancourt-Lozano, M. (2003) Toxicity of formalin to bukkseye puffer fish (*Sphoeroides annulatus* Jenyns, 1843) and its effectiveness to control ectoparasites. *Aquaculture* 223, 41–50.
- Food and Drug Administration (FDA) (1998) *Certain Other Dosage Form New Animal Drugs; Formalin Solution*. Code of Federal Regulations 21 Part 529.1030, 18 June 1998. US Government Printing Office, Washington, DC.
- Gao, F., Fan, X., Yi, Z., Strüder-Kypke, M. and Song, W. (2010) Phylogenetic consideration of two scuticociliate genera, *Philasterides* and *Boveria* (Protozoa, Ciliophora) based on 18S rRNA gene sequences. *Parasitology International* 59, 549–555.
- Gill, P.A. and Callinan, R.B. (1997) Ulcerative dermatitis associate with *Uronema* sp. infection of farmed sand whiting *Sillago ciliata*. *Australian Veterinary Journal* 75, 5.
- Greenwood, S.J., Després, B.M., Cawthorn, R.J., Lavallée, J., Groman, D.B. and Desbarats, A. (2005) Case report: outbreak of bumper car disease caused by *Anophryoides haemophila* in a lobster holding facility in Nova Scotia, Canada. *Journal of Aquatic Animal Health* 17, 345–352.
- Harikrishnan, R., Balasundaram, C. and Heo, M.S. (2010a) Scuticociliatosis and its recent prophylactic measures in aquaculture with special reference to South Korea. Taxonomy, diversity and diagnosis of

- scuticociliatosis: Part I. Control strategies of scuticociliatosis: Part II. *Fish and Shellfish Immunology* 29, 15–31.
- Harikrishnan, R., Heo, J., Balasundaram, C., Kim, M.C., Kim, J.S., Han, Y.J. and Heo, M.S. (2010b) Effect of traditional Korean medicinal (TKM) triherbal extract on the innate immune system and disease resistance in *Paralichthys olivaceus* against *Uronema marinum*. *Veterinary Parasitology* 170, 1–7.
- Harikrishnan, R., Jin, C.N., Kim, M.C., Kim, J.S., Balasundaram, C. and Heo, M.S. (2010c) Effectiveness and immunomodulation of chemotherapeutants against scuticociliate *Philasterides dicentrarchi* in olive flounder. *Experimental Parasitology* 124, 306–314.
- Iglesias, R., Paramá, A., Alvarez, M.F., Leiro, J., Fernández, J. and Sanmartín, M.L. (2001) *Philasterides dicentrarchi* (Ciliophora, Scuticociliatida) as the causative agent of scuticociliatosis in farmed turbot *Scophthalmus maximus* in Galicia (NW Spain). *Diseases of Aquatic Organisms* 46, 47–55.
- Iglesias, R., Paramá, A., Álvarez, M.F., Leiro, J. and Sanmartín, M.L. (2002) Antiprotozoals effective *in vitro* against the scuticociliate fish pathogen *Philasterides dicentrarchi*. *Diseases of Aquatic Organisms* 49, 191–197.
- Iglesias, R., Paramá, A., Alvarez, M.F., Leiro, J., Aja, C. and Sanmartín, M.L. (2003a) *In vitro* growth requirements for the fish pathogen *Philasterides dicentrarchi* (Ciliophora, Scuticociliatida). *Veterinary Parasitology* 111, 19–30.
- Iglesias, R., Paramá, A., Álvarez, M.F., Leiro, J., Ubeira, F.M. and Sanmartín, M.L. (2003b) *Philasterides dicentrarchi* (Ciliophora: Scuticociliatida) expresses surface immobilization antigens that probably induce protective immune responses in turbot. *Parasitology* 126, 125–134.
- Imai, S., Tsurimaki, S., Goto, E., Wakita, K. and Hatai, K. (2000) *Tetrahymena* infection in guppy, *Poecilia reticulata*. *Fish Pathology* 35, 67–72.
- Jee, B.Y., Kim, Y.C. and Park, M.S. (2001) Morphology and biology of parasite responsible for scuticociliatosis of cultured olive flounder *Paralichthys olivaceus*. *Diseases of Aquatic Organisms* 47, 49–55.
- Jin, C.N., Harikrishnan, R., Moon, Y.G., Kim, M.C., Kim, J.S., Balasundaram, C., Azad, I.S. and Heo, M.S. (2009) Histopathological changes of Korea cultured olive flounder, *Paralichthys olivaceus* due to scuticociliatosis caused by histophagous scuticociliate, *Philasterides dicentrarchi*. *Veterinary Parasitology* 161, 292–301.
- Jin, C.N., Harikrishnan, R., Moon, Y.G., Kim, M.C., Kim, J.S., Balasundaram, C. and Heo, M.S. (2010) Effectiveness of chemotherapeutants against scuticociliate *Philasterides dicentrarchi*, a parasite of olive flounder. *Veterinary Parasitology* 168, 19–24.
- Jones, S.R., Prosperi-Porta, G. and LaPatra, S.E. (2010) First isolation of *Pseudocohnilembus persalinus* (Ciliophora: Scuticociliatida) from freshwater-reared rainbow trout, *Oncorhynchus mykiss*. *Journal of Parasitology* 96, 1014–1016.
- Jousson, O., Di Bello, D., Donadio, E., Felicioli, A., Pretti, C. (2007) Differential expression of cysteine proteases in developmental stages of the parasitic ciliate *Ichthyophthirius multifiliis*. *FEMS Microbiology Letters* 269, 77–78.
- Jung, S.H., Kim, J.W., Jeon, I.G. and Lee, Y.H. (2001) Formaldehyde residues in formalin-treated olive flounder (*Paralichthys olivaceus*), black rockfish (*Sebastes schlegelii*), and seawater. *Aquaculture* 194, 253–262.
- Jung, S.J., Kitamura, S.I., Song, J.Y., Joung, I.Y. and Oh, M.J. (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.
- Jung, S.J., Kitamura, S.I., Aoyama, M., Song, J.Y., Kim, B.K. and Oh, M.J. (2006) Immune response of olive flounder *Paralichthys olivaceus* against *Miamiensis avidus* (Ciliophora: Scuticociliatida). *Journal of Fish Pathology* 19, 173–181 (in Korean).
- Jung, S.J., Kitamura, S., Song, J.Y. and Oh, M.J. (2007) *Miamiensis avidus* (Ciliophora: Scuticociliatida) causes systemic infection of olive flounder *Paralichthys olivaceus* and is a senior synonym of *Philasterides dicentrarchi*. *Diseases of Aquatic Organisms* 73, 227–234.
- Jung, S.J., Bae, M.J., Oh, M.J. and Lee, J. (2011a) Sequence conservation in the internal transcribed spacers and 5.8S ribosomal RNA of parasitic scuticociliates *Miamiensis avidus* (Ciliophora, Scuticociliatida). *Parasitology International* 60, 216–219.
- Jung, S.J., Im, E.Y., Struder-Kypke, M.C., Kitamura, S. and Woo, P.T.K. (2011b) Small subunit ribosomal RNA and mitochondrial cytochrome c oxidase subunit 1 gene sequences of 21 strains of the parasitic scuticociliate *Miamiensis avidus* (Ciliophora: Scuticociliatida). *Parasitology Research* 108, 1153–1161.
- Kim, S.M., Cho, J.B., Kim, S.K., Nam, Y.K. and Kim, K.H. (2004a) Occurrence of scuticociliatosis in olive flounder *Paralichthys olivaceus* by *Philasterides dicentrarchi* (Ciliophora: Scuticociliatida). *Diseases of Aquatic Organisms* 62, 233–238.

- Kim, S.M., Cho, J.B., Lee, E.H., Kwon, S.R., Kim, S.K., Nam, Y.K. and Kim, K.H. (2004b) *Pseudocohnilembus persalinus* (Ciliophora: Scuticociliatida) is an additional species causing scuticociliatosis in olive flounder *Paralichthys olivaceus*. *Diseases of Aquatic Organisms* 62, 239–244.
- Kim, S.M., Lee, E.H., Kwon, S.R., Lee, S.J., Kim, S.K., Nam Y.K. and Kim, K.H. (2006) Preliminary analysis of recombinant β -tubulin of *Pseudocohnilembus persalinus* (Ciliophora: Scuticociliatida) as a vaccine antigen candidate against scuticociliatosis. *Aquaculture* 260, 21–26.
- Kwon, S.R., Kim, C.S., Ahn, K.J., Cho, J.B., Chung, J.K., Lee, H.H. and Kim, K.H. (2002) Protease in cell lysate of *Uronema marinum* (Ciliata: Scuticociliatida), an opportunistic pathogen of cultured olive flounder (*Paralichthys olivaceus*). *Journal of Fisheries Science and Technology* 5, 145–149.
- Lahnsteiner, F. and Weismann, T. (2007) Treatment of ichthyophthiriasis in rainbow trout and common carp with common and alternative therapeutics. *Journal of Aquatic Animal Health* 19, 186–194.
- Lamas, J., Sanmartín M.L., Paramá, A.I., Castro, R., Cabaleiro, S., Ruiz de Ocenda, M.V., Barja, J.L. and Leiro, J. (2008) Optimization of an inactivated vaccine against a scuticociliate parasite of turbot: effect of antigen, formalin and adjuvant concentration on antibody response and protection against the pathogen. *Aquaculture* 278, 22–26.
- Lamas, J., Morais, P., Arranz, J.A., Sanmartín, M.L., Orallo, F. and Leiro, J. (2009) Resveratrol promotes an inhibitory effect on the turbot scuticociliate parasite *Philasterides dicentrarchi* by mechanisms related to cellular detoxification. *Veterinary Parasitology* 161, 307–315.
- Lee, E.H. and Kim, K.H. (2008) Can the surface immobilization antigens of *Philasterides dicentrarchi* (Ciliophora: Scuticociliatida) be used as target antigens to develop vaccines in cultured fish? *Fish and Shellfish Immunology* 24, 142–146.
- Lee, E.H. and Kim, K.H. (2009) CpG-ODN increases resistance of olive flounder (*Paralichthys olivaceus*) against *Philasterides dicentrarchi* (Ciliophora: Scuticociliatida) infection. *Fish and Shellfish Immunology* 26, 29–32.
- Leibowitz, M.P., Ariav, R. and Zilberg, D. (2005) Environmental and physiological conditions affecting *Tetrahymena* sp. infection in guppies, *Poecilia reticulata* Peters. *Journal of Fish Diseases* 28, 539–547.
- Leibowitz, M.P., Ofir, R., Golan-Goldhirsh, A. and Zilberg, D. (2009) Cysteine proteases and acid phosphatases contribute to *Tetrahymena* spp. pathogenicity in guppies, *Poecilia reticulata*. *Veterinary Parasitology* 166, 21–26.
- Leibowitz, M.P., Chettri, J.K., Ofir, R. and Zilberg, D. (2010) Treatment development for systemic *Tetrahymena* sp. infection in guppies, *Poecilia reticulata* Peters. *Journal of Fish Diseases* 33, 473–480.
- Leighton, B.J., Boom, J.D.G., Bouland, C., Hartwick, E.B. and Smith, M.J. (1991) Castration and mortality in *Pisaster ochraceus* parasitized by *Orchitophrya stellarum* (Ciliophore). *Diseases of Aquatic Organisms* 10, 71–73.
- Leiro, J., Arranz, J.A., Iglesias, R., Ubeira, F.M. and Sanmartín, M.L. (2004) Effects of the histiophagous ciliate *Philasterides dicentrarchi* on turbot phagocyte responses. *Fish and Shellfish Immunology* 17, 27–39.
- Lim, S.U., Seo, J.S., Kim, M.S., Ahn, S.J., Jeong, H.D., Kim, K.H., Park, N.G., Kim, J.K., Chung, J.K. and Lee, H.H. (2005) Molecular cloning and characterization of Cathepsin B from a scuticociliate, *Uronema marinum*. *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 142, 283–292.
- Liu, C.S., Sun, Y., Hu, Y.H. and Sun, L. (2010) Identification and analysis of the immune effects of CpG motifs that protect Japanese flounder (*Paralichthys olivaceus*) against bacterial infection. *Fish and Shellfish Immunology* 29, 279–285.
- Lubega, G.W., Byarugaba, D.K. and Prichard, R.K. (2002) Immunization with a tubulin-rich preparation from *Trypanosoma brucei* confers broad protection against African trypanosomiasis. *Experimental Parasitology* 102, 9–22.
- Marshall, W.S. and Grosell, M. (2006) Ion transports, osmoregulation, and acid-base balance. In: Evans, D.H. and Claiborne, J.B. (eds) *The Physiology of Fishes*, 3rd edn. CRC Press, Taylor & Francis Group, Boca Raton, Florida, pp. 177–230.
- Messick, G.A. and Small, E.B. (1996) *Mesanoophrys chesapeakeensis* n. sp., a histophagous ciliate in the blue crab, *Callinectes sapidus*, and associated histopathology. *Invertebrate Biology* 115, 1–12.
- Morado, J.F. and Small, E.B. (1995) Ciliate parasites and related diseases of Crustacea: a review. *Reviews in Fisheries Science* 3, 275–354.
- Morado, J.F., Giesecke, R.H. and Syrjala, S.E. (1999) Molt related mortalities of the Dungeness crab *Cancer magister* caused by a marine facultative ciliate *Mesanoophrys pugettensis*. *Diseases of Aquatic Organisms* 38, 143–150.

- Morais, P., Lamas, J., Sanmartín, M.L., Orallo, F. and Leiro, J. (2009) Resveratrol induces mitochondrial alterations, autophagy and a cryptobiosis-like state in scuticociliates. *Protist* 160, 552–564.
- Moustafa, E.M.M., Naota, M., Morita, T., Tange, N. and Shimada, A. (2010a) Pathological study on the scuticociliatosis affecting farmed Japanese flounder (*Paralichthys olivaceus*) in Japan. *The Journal of Veterinary Medical Science* 72, 1359–1362.
- Moustafa, E.M.M., Tange, N., Shimada, A. and Morita, T. (2010b) Experimental scuticociliatosis in Japanese flounder (*Paralichthys olivaceus*) infected with *Miamiensis avidus*: pathological study on the possible neural routes of invasion and dissemination of the scuticociliate inside the fish body. *The Journal of Veterinary Medical Science* 72, 1557–1563.
- Munday, B.L., O'Donoghue, P.J., Watts, M., Rough, K. and Hawkesford, K. (1997) Fatal encephalitis due to the scuticociliate *Uronema nigricans* in sea-caged, southern bluefin tuna *thunnus maccoyii*. *Diseases of Aquatic Organisms* 30, 17–25.
- Novotny, M.J., Cawthorn, R.J. and Despres, B. (1996) *In vitro* effects of chemotherapeutants on the lobster parasite *Anophryoides haemophila*. *Diseases of Aquatic Organisms* 24, 233–237.
- Palenzuela, O., Sitjà-Bobadilla, A., Ríaza, A., Silva, R., Arán, J. and Alvarez-Pellitero, P. (2009) Antibody responses of turbot *Psetta maxima* against various antigen formulations of scuticociliates Ciliophora. *Diseases of Aquatic Organisms* 86, 123–134.
- Paramá, A., Iglesias, R., Álvarez, M.F., Leiro, J., Aja, C. and Sanmartín, M.L. (2003) *Philasterides dicentrarchi* (Ciliophora: Scuticociliatida): experimental infection and possible routes of entry in farmed turbot (*Scophthalmus maximus*). *Aquaculture* 217, 73–80.
- Paramá, A., Iglesias, R., Álvarez, F., Leiro, J.M., Quintela, J.M., Peinador, C., González, L., Riguera, R. and Sanmartín, M.L. (2004a) *In vitro* efficacy of new antiprotozoals against *Philasterides dicentrarchi* (Ciliophora, Scuticociliatida). *Diseases of Aquatic Organisms* 62, 97–102.
- Paramá, A., Iglesias, R., Álvarez, M.F., Leiro, J., Ubeira, F.M. and Sanmartín, M.L. (2004b) Cysteine proteinase activities in the fish pathogen *Philasterides dicentrarchi* (Ciliophora: Scuticociliatida). *Parasitology* 128, 541–548.
- Paramá, A., Arranz, J.A., Alvarez, M.F., Sanmartín, M.L. and Leiro, J. (2006) Ultrastructure and phylogeny of *Philasterides dicentrarchi* (Ciliophora, Scuticociliatia) from farmed turbot in NW Spain. *Parasitology* 132, 555–564.
- Paramá, A., Castro, R., Arranz, J.A., Sanmartín, M.L., Lamas, J. and Leiro, J. (2007a) Scuticociliate cysteine proteinases modulate turbot leucocyte functions. *Fish and Shellfish Immunology* 23, 945–956.
- Paramá, A., Castro, R., Lamas, J., Sanmartín, M.L., Santamarina, M.T. and Leiro, J. (2007b) Scuticociliate proteinases may modulate turbot immune response by inducing apoptosis in pronephric leucocytes. *International Journal for Parasitology* 37, 87–95.
- Paramá, A., Piazzon, M.C., Lamas, J., Sanmartín, M.L. and Leiro, J. (2007c) *In vitro* activity of the nonsteroidal anti-inflammatory drug indomethacin on a scuticociliate parasite of farmed turbot. *Veterinary Parasitology* 148, 318–324.
- Piazzón, C., Lamas, J., Castro, R., Budiño, B., Cabaleiro, S., Sanmartín, M. and Leiro, J. (2008) Antigenic and cross-protection studies on two turbot scuticociliate isolates. *Fish and Shellfish Immunology* 25, 417–424.
- Pimenta Leibowitz, M., Ofir, R., Golan-Goldhirsh, A. and Zilberg, D. (2009) Cysteine proteases and acid phosphatases contribute to *Tetrahymena* spp. pathogenicity in guppies, *Poecilia reticulata*. *Veterinary Parasitology* 166, 21–26.
- Ponpornpisit, A., Endo, M. and Murata, H. (2000) Experimental infections of a ciliate *Tetrahymena pyriformis* on ornamental fishes. *Fisheries Science* 66, 1026–1031.
- Puig, L., Traveset, R., Palenzuela, O. and Padrós, F. (2007) Histopathology of experimental scuticociliatosis in turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms* 76, 131–140.
- Quintela, J.M., Peinador, C., Gonzalez, L., Iglesias, R., Paramá, A., Álvarez, F., Sanmartín, M.L. and Riguera, R. (2003) Piperazine N-substituted naphthyridines, pyridothienopyrimidines and pyridothienotriazines: new antiprotozoals active against *Philasterides dicentrarchi*. *European Journal of Medicinal Chemistry* 38, 265–275.
- Ramos, M.F., Costa, A.R., Barandela, T., Saraiva, A. and Rodriguez, P.N. (2007) Scuticociliate infection and pathology in cultured turbot *Scophthalmus maximus* from the north of Portugal. *Diseases of Aquatic Organisms* 74, 249–253.
- Rosenthal, P.J. (1999) Proteases of protozoan parasites. *Advances in Parasitology* 43, 105–159.
- Rossteuscher, S., Wenker, C., Jermann, T., Wahli, T., Oldenberg, E. and Schmidt-Posthaus, H. (2008) Severe scuticociliate (*Philasterides dicentrarchi*) infection in a population of sea dragons (*Phycodurus eques* and *Phyllopteryx taeniolatus*). *Veterinary Parasitology* 45, 546–550.

- Ruiz de Ocenda, M.V., Ojea, A., Teijido, A., Couso, N. and Cabaleiro, S. (2007) Efficacy of low-dose formalin treatment against *Philasterides dicentrarchi*. In: *Proceedings of the European Association of Fish Pathologists*. 13th International Conference of Fish and Shellfish Diseases, Grado, Italy, p. 344.
- Sanmartín, M.L., Paramá, A., Castro, R., Cabaleiro, S., Leiro, J., Lamas, J. and Barja, J.L. (2008) Vaccination of turbot, *Psetta maxima* (L.), against the protozoan parasite *Philasterides dicentrarchi*: effects on antibody production and protection. *Journal of Fish Diseases* 31, 135–140.
- Schmahl, G., Taraschewski, H. and Mehlhorn, H. (1989) Chemotherapy of fish parasite. *Parasitology Research* 75, 503–511.
- Sitjà-Bobadilla, A., Palenzuela, O. and Alvarez-Pellitero, P. (2008) Immune response of turbot, *Psetta maxima* (L.) (Pisces: Teleostei), to formalin-killed scuticociliates (Ciliophora) and adjuvanted formulations. *Fish and Shellfish Immunology* 24, 1–10.
- Small, H.J., Neil, D.M., Taylor, A.C., Bateman, K. and Coombs, G.H. (2005a) A parasitic scuticociliate infection in the Norway lobster (*Nephrops norvegicus*). *Journal of Invertebrate Pathology* 90, 108–117.
- Small, H.J., Neil, D.M., Taylor, A.C. and Coombs, G.H. (2005b) Identification and partial characterisation of metalloproteases secreted by a *Mesanoophrys*-like ciliate parasite of the Norway lobster *Nephrops norvegicus*. *Diseases Aquatic Organisms* 67, 225–231.
- Smith, P.J., McVeagh, S.M., Hulston, D., Anderson, S.A. and Gublin, Y. (2009) DNA identification of ciliates associated with disease outbreaks in a New Zealand marine fish hatchery. *Diseases Aquatic Organisms* 86, 163–167.
- Song, J.Y., Kitamura, S.I., Oh, M.J., Kang, H.S., Lee, J.H., Tanaka, S.J. and Jung, S.J. (2009a) Pathogenicity of *Miamiensis avidus* (syn. *Philasterides dicentrarchi*), *Pseudocohnilembus persalinus*, *Pseudocohnilembus hargisi* and *Uronema marinum* (Ciliophora, Scuticociliatida). *Diseases of Aquatic Organisms* 83, 133–143.
- Song, J.Y., Sasaki, K., Okada, T., Sakashita, M., Kawakami, H., Matsuoka, S., Kang, H.S., Nakayama, K., Jung, S.J., Oh, M.J. and Kitamura, S.I. (2009b) Antigenic differences of the scuticociliate *Miamiensis avidus* from Japan. *Journal of Fish Diseases* 32, 1027–1034.
- Song, W. and Wilbert, N. (2000) Redefinition and redescription of some marine scuticociliates from China, with report of a new species, *Metanophrys sinensis* nov. sp. (Ciliophora, Scuticociliatida). *Zoologischer Anzeiger* 239, 45–74.
- Sterud, E., Hansen, M.K. and Mo, T.A. (2000) Systemic infection with *Uronema*-like ciliates in farmed turbot, *Scophthalmus maximus* (L.). *Journal of Fish Diseases* 23, 33–37.
- Strüder-Kypke, M.C. and Lynn, D.H. (2010) Comparative analysis of the mitochondrial cytochrome c oxidase subunit I (COI) gene in ciliates (Alveolata, Ciliophora) and evaluation of its suitability as a biodiversity marker. *Systematics and Biodiversity* 8, 131–148.
- Swennes, A.G., Findly, R.C. and Dickerson, H.W. (2007) Cross immunity and antibody responses to different immobilization serotypes of *Ichthyophthirius multifiliis*. *Fish and Shellfish Immunology* 22, 589–597.
- Takagishi, N., Yoshinaga, T. and Ogawa, K. (2009) Effect of hyposalinity on the infection and pathogenicity of *Miamiensis avidus* causing scuticociliatosis in olive flounder *Paralichthys olivaceus*. *Diseases of Aquatic Organisms* 86, 175–179.
- Tan, C.W., Jesudhasan, P. and Woo, P.T.K. (2008) Towards a metalloprotease-DNA vaccine against piscine cryptobiosis caused by *Cryptobia salmositica*. *Parasitology Research* 102, 265–275.
- Thompson, J.C. and Moewus, L. (1964) *Miamiensis avidus* n. g., n. sp., a marine facultative parasite in the ciliate in the ciliate order Hymenostomatida. *The Journal of Protozoology* 11, 378–381.
- Umehara, A., Kosuga, Y. and Hirose, H. (2003) Scuticociliata infection in the weedy sea dragon *Phyllopteryx taeniolatus*. *Parasitology International* 52, 165–168.
- Woo, P.T.K. (2010) Immunological and therapeutic strategies against salmonid cryptobiosis. *Journal of Biomedicine and Biotechnology* Article ID 341783, 9 pp.
- Xu, D.H., Klesius, P.H. and Pangala, V.S. (2006) Induced cross protection in channel catfish, *Ictalurus punctatus* (Rafinesque), against different immobilization serotypes of *Ichthyophthirius multifiliis*. *Journal of Fish Diseases* 29, 131–138.
- Yoshimizu, M., Hyuuga, S., Oh, M.J., Ikoma, M., Kimura, T.M., Nomura, T. and Ezura, Y. (1993) Scuticociliatida infection of cultured hiramé (*Paralichthys olivaceus*) – characteristics, drug sensitivity, and pathogenicity of clutured scuticociliata. *Fish Pathology* 6, 205–208 (in Japanese).
- Yoshinaga, T. and Nakazoe, J.I. (1993) Isolation and *in vitro* cultivation of an unidentified ciliate causing scuticociliatosis in Japanese flounder (*Paralichthys olivaceus*). *Fish Pathology* 28, 131–134.

6 *Perkinsus marinus* and *Haplosporidium nelsoni*

Ryan B. Carnegie and Eugene M. Bureson

Virginia Institute of Marine Science, College of William & Mary, Virginia, USA

6.1. Introduction

The protistan parasites *Perkinsus marinus* and *Haplosporidium nelsoni* are the two most important pathogens of the eastern oyster (*Crassostrea virginica*) along the Atlantic and Gulf of Mexico coasts of the USA. *H. nelsoni*, a pathogen introduced from Asia, decimated oyster populations in the mid-Atlantic region in the 1950s (Andrews, 1966; Ford and Haskin, 1982); *P. marinus* has probably always been present along the south Atlantic and Gulf coasts, but prolonged drought conditions in the mid-Atlantic region in the late 1980s allowed the pathogen to increase in abundance and expand its range into areas where low salinity had previously prevented its occurrence (Bureson and Ragone Calvo, 1996; Ford, 1996; Ford and Smolowitz, 2007). These two pathogens have devastated ecologically and commercially important oyster populations as far north as New England and Nova Scotia, Canada, but especially in Chesapeake Bay and Delaware Bay. *P. marinus* is the primary pathogen of *C. virginica* in the Gulf of Mexico (Soniati, 1996), where *H. nelsoni* is absent.

6.1.1. *Perkinsus marinus*

P. marinus is classified in the Dinofzoa, family Perkinsidae. It is primarily a parasite of the

eastern oyster *C. virginica*, although it has been reported to experimentally infect the clams *Macoma balthica* and *Mya arenaria* in Chesapeake Bay (Dungan *et al.*, 2007). Geographical distribution and seasonal cycle are controlled by temperature, with the parasite proliferating most rapidly at water temperatures over 25°C (Andrews, 1965); local distribution is mainly controlled by salinity. The parasite occurs throughout the Gulf of Mexico and from Florida to Maine (Bureson *et al.*, 1994; Ford and Smolowitz, 2007). It recently has been introduced to populations of the pleasure oyster (*Crassostrea corteziensis*) on the Pacific coast of Mexico with importations of *C. virginica* from the Gulf of Mexico (Cáceres-Martínez *et al.*, 2008). *P. marinus* is most pathogenic at salinity greater than 12 psu, but it can survive in its host at salinities as low as 1 or 2 psu for extended periods (Ragone Calvo and Bureson, 1993).

P. marinus cells are generally 2–10 µm in diameter, spherical in shape, and characterized by a single nucleus displaced to the cell margin by a large vacuole (Fig. 6.1). Parasites occur in the connective tissue of all organs and in the gut epithelium. *P. marinus* cells are released from the host in faeces or upon death and decomposition of the host. Transmission is direct from oyster to oyster (Chu, 1996) and occurs primarily at the time of maximum host mortality (Ragone Calvo *et al.*, 2003b). Thus,

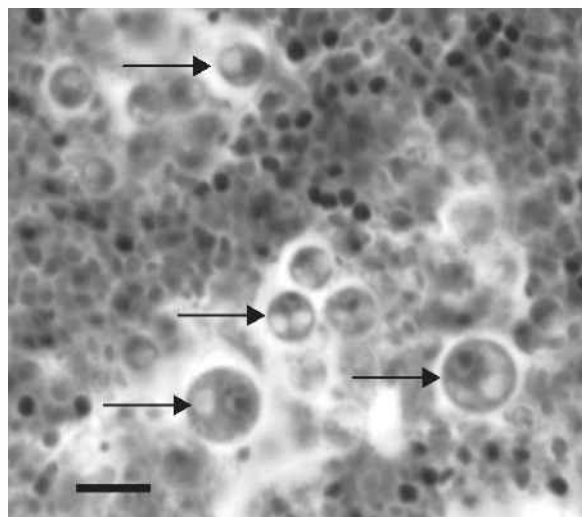


Fig. 6.1. *Perkinsus marinus* cells (arrows) showing vacuole and displaced nucleus with prominent nucleolus. Paraffin histology; bar = 10 μ m.

oysters become infected in the autumn and host mortality peaks in August/September 1 or 2 years later, concurrent with maximum infection intensity. The portal of entry is not well understood, but it may be via the gut with infection facilitated by host haemocytes that phagocytose the parasite in the gut lumen and carry it across the gut epithelium (Alvarez *et al.*, 1992). *P. marinus* cells are often phagocytosed by host haemocytes, but they survive and multiply within haemocytes, eventually causing the host cell to burst, releasing parasites into the tissue, where the phagocytosis/multiplication/cell death cycle is repeated. Within the host, *P. marinus* cells are spread in host haemocytes via the haemolymph.

6.1.2. *Haplosporidium nelsoni*

H. nelsoni is a member of the phylum Haplosporidia, which is characterized by spores with an orifice covered by an external lid or internal flange of wall material. The phylum consists of only four genera and about 30 species, but contains a number of important pathogens of molluscs. *H. nelsoni* infects the Pacific oyster (*Crassostrea gigas*) in Asia, Europe and the west coast of the USA, and *C. virginica* along the east coast of North America from Florida to Nova Scotia, Canada; it is absent from the Gulf of Mexico. Initial

observations of the multinucleate plasmodia in oysters from Delaware Bay in 1957 led to the acronym 'MSX' for Multinucleate Sphere X (unknown). The parasite was not formally described until 9 years after its discovery (Haskin *et al.*, 1966), and the MSX acronym was ingrained in the literature in the interim.

Unlike *P. marinus*, *H. nelsoni* is very sensitive to salinity below 10 psu where it is eliminated from oysters after about 10 days at temperatures above 20°C (Andrews, 1983; Ford, 1985; Ford and Haskin, 1988). It survives at salinities above 10 psu, but is only pathogenic at salinities above 15 psu. Thus, significant oyster mortality occurs primarily in the lower portions of major east coast estuaries and in coastal bays.

H. nelsoni has two life stages: (i) multinucleate plasmodia; and (ii) spores. Plasmodia (Fig. 6.2) occur in the connective tissue or epithelium of all tissues and range from 5 to over 50 μ m in diameter. They may contain 30 nuclei or more. Sporulation occurs only in digestive tubule epithelium (Fig. 6.3a) and primarily in first-year oysters less than 30 mm in shell height (Barber *et al.*, 1991; Burrenson, 1994). Spores are about 7 μ m in length with an external lid covering the spore orifice (Fig. 6.3b). The complete life cycle of *H. nelsoni* is unknown. Direct transmission trials with both plasmodia and spores have been unsuccessful, and the suspected intermediate host has not been identified (Haskin and Andrews, 1988). Oysters

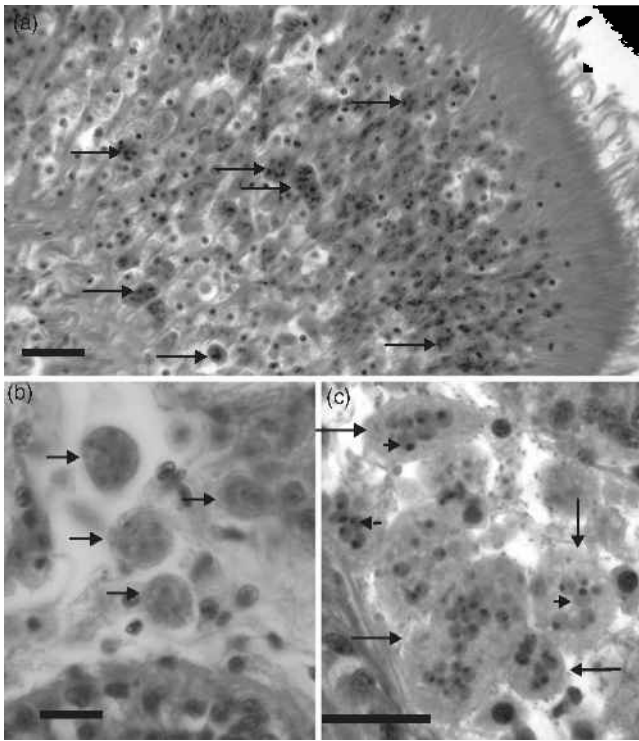


Fig. 6.2. *Haplosporidium nelsoni* multinucleate plasmodia. (a) Plasmodia (arrows) scattered in mantle connective tissue, with moderate haemocytosis host reaction. Paraffin histology; bar = 20 µm. (b) Plasmodia (arrows) in visceral connective tissue; note lack of host reaction. Bar = 10 µm. (c) Plasmodia (arrows) showing nuclei with eccentric nucleolus (arrow heads). Plastic thin section, bar = 10 µm.

become infected in May as water temperature rises above 17°C; the infection period continues through the summer. Initial infections are in the palp or gill epithelium, but the actual mechanism of infection is unknown. Infections develop rapidly with peak oyster mortality in early August only about 3 months after infection (Andrews, 1966). Sporulation can occur at any time of year, but is most prevalent in September. Spores are released in faeces or upon death and decomposition of the host; fate of spores in the environment is unknown.

6.2. Impact of the Diseases on Oyster Production

P. marinus was first observed in oysters in the Gulf of Mexico after reports of significant

oyster mortality that was blamed on the oil industry (Andrews, 1988). However, subsequent reports of *P. marinus*-related oyster mortality in Chesapeake Bay, where there is no oil industry, lead to the conclusion that this parasite and not the oil industry was the cause of mortality in the Gulf of Mexico. Historically, *P. marinus* has caused significant oyster mortality in the Gulf of Mexico and in Chesapeake Bay, especially during drought years. In Chesapeake Bay annual oyster mortality from *P. marinus* averaged about 20% prior to 1985, and this was manageable by industry (Andrews, 1988). Disaster struck in the 1950s with catastrophic oyster mortality from *H. nelsoni* in Delaware Bay beginning in 1957 and in Chesapeake Bay beginning in 1959 (Fig. 6.4). Within a few years 95% of the oyster populations were lost in high salinity areas of both estuaries (Andrews, 1966; Ford and

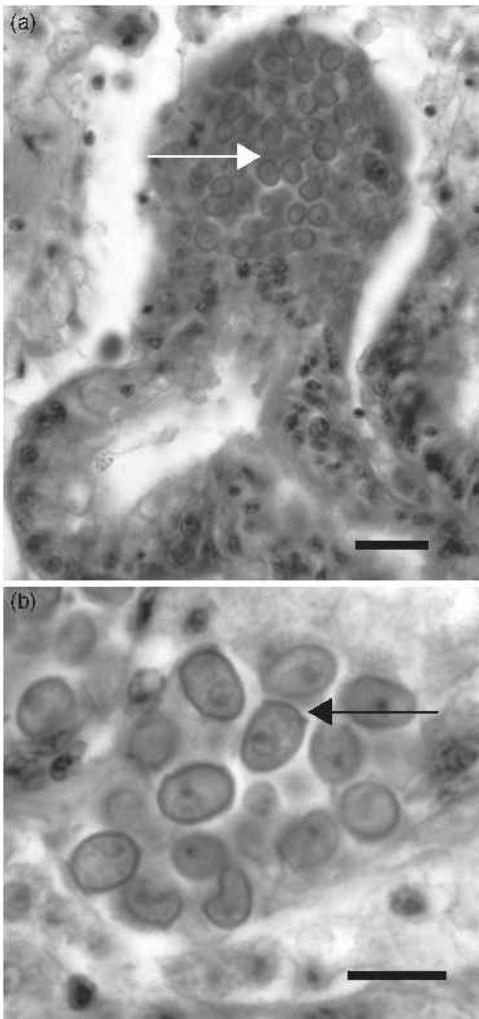


Fig. 6.3. *Haplosporidium nelsoni* spores. (a) Sporocyst with spores (arrow) in epithelium of digestive diverticula. Note hyperplasia of epithelium. Paraffin histology, bar = 20 µm. (b) Spores showing lid (arrow) covering operculum. Paraffin histology, bar = 10 µm.

Haskin, 1982). There is compelling evidence from molecular studies that *H. nelsoni* is an introduced pathogen (Burrenson *et al.*, 2000). *H. nelsoni* is a natural parasite of the Pacific oyster (*C. gigas*) in Asia, where it is not a significant pathogen because prevalence is very low. The parasite was introduced to California with importation of juvenile *C. gigas* from Japan over an 80-year period beginning in 1902 (Friedman, 1996). Exactly how the parasite was introduced to the east coast of the USA is unclear, but there were importations of *C. gigas* from Asia or the west coast of the USA beginning in the 1930s (Burrenson *et al.*, 2000). However, a ballast water introduction cannot

be ruled out as there was much shipping traffic between Asia and the east coast of the USA during and after World War II. *H. nelsoni* appeared in Nova Scotia, Canada in 2000, and there is anecdotal evidence that the parasite was introduced via ballast water from ships originating in Chesapeake Bay. This evidence gives credence to the possibility that the original introduction to the east coast was also via ballast water. After the initial epizootics in the mid-Atlantic region, *H. nelsoni* spread north and south along the east coast causing oyster mortality periodically in Long Island Sound and other areas. In Chesapeake Bay and Delaware Bay mortality varied somewhat after the

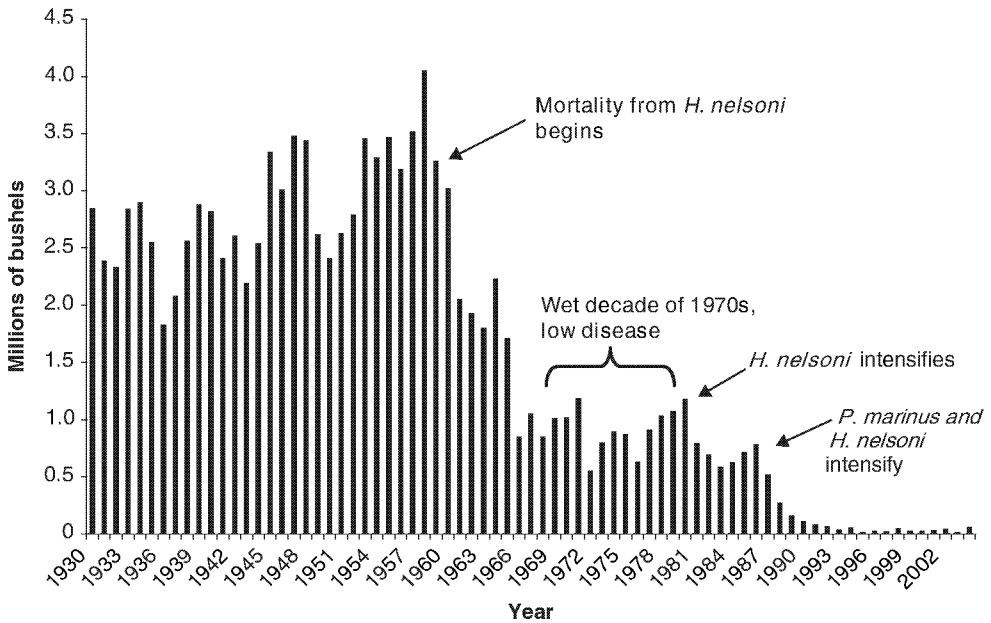


Fig. 6.4. Annual oyster harvest in Virginia, 1931–2005. Data courtesy of Virginia Marine Resources Commission.

1960s due to wet/dry years, but it was always relatively high and oyster stocks never recovered to pre-MSX levels.

In Chesapeake Bay the 1970s were relatively wet years and disease pressure abated somewhat (Fig. 6.4). 1980 and 1981 were very dry and *H. nelsoni* intensified again. Consecutive drought years from 1985–1988 along the US east coast, with concomitant salinity increase, coupled with warm winters, allowed *H. nelsoni* to increase in abundance and to move up estuaries into naïve oyster populations. However, perhaps more important, the drought caused a similar increase in the abundance and distribution of *P. marinus*. Prior to the late 1980s *P. marinus* was not known to occur in the upper Chesapeake Bay or north of the mouth of Chesapeake Bay along the east coast. Drought conditions, with concomitant warm winters, provided favourable conditions for *P. marinus* and the parasite spread throughout Chesapeake Bay and north into Delaware Bay, either naturally or by intentional movement of oysters. Oyster populations in these areas were naïve to

P. marinus and mortality was high (Bureson and Ragone Calvo, 1996) (Fig. 6.4). Because *P. marinus* is tolerant of low salinity the parasite was not affected when climate conditions returned to normal and it continues to cause significant mortality throughout its range during dry years. Along with overharvesting and habitat degradation, the combination of *P. marinus* and *H. nelsoni* has reduced oyster populations to about 1% of what they were in the 1940s in both Delaware Bay and Chesapeake Bay (Fig. 6.4). The pathogens have also greatly slowed the development of oyster aquaculture in Chesapeake Bay.

6.3. Diagnosis

6.3.1. *P. marinus*

There are no clinical signs that are diagnostic for *P. marinus*. The pathogen causes a warm-season general wasting disease resulting in oysters with thin, watery tissue (Fig. 6.5a);

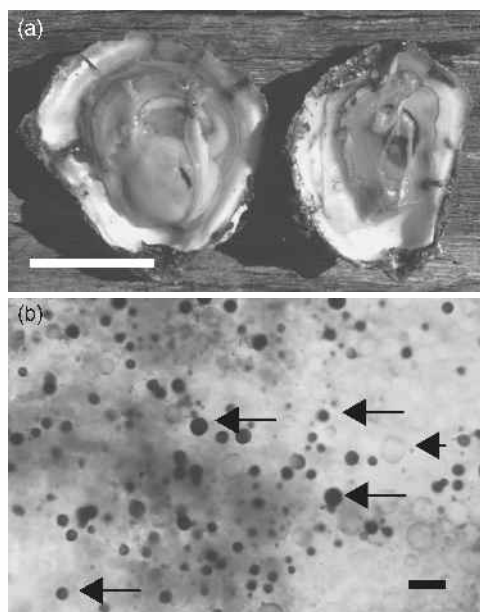


Fig. 6.5. Diagnosis of *P. marinus* infections. (a) Healthy oyster on the left, compared with watery, thin tissue of emaciated oyster on the right, often typical of *P. marinus* infection. Bar = 30 mm. (b) Diagnosis by Ray's fluid thioglycollate medium (RFTM). *P. marinus* cells (arrows) typically appear as black spheres, but some cells deeper in tissue do not stain with Lugol's iodine (arrowhead). Bar = 30 μ m.

however, this syndrome can also be caused by other pathogens or conditions.

The preferred diagnostic technique depends on the purpose of the study. For routine surveillance, where *P. marinus* is the only common species of *Perkinsus* in an area, Ray's fluid thioglycollate medium (RFTM) culture assay is the method of choice (Ray, 1966a). This technique is not species-specific, but it is sensitive, inexpensive and relatively fast. Briefly, infected oyster tissue is cultured for 5 days in the culture medium with antibiotics, macerated on a glass slide, stained with Lugol's iodine and examined using a compound microscope. *P. marinus* cells appear as blue-black spheres (Fig. 6.5b). Histopathological analysis is also acceptable, but it is less sensitive than RFTM diagnosis and may miss low-intensity infections. In histopathology, *P. marinus* cells are spherical with a large vacuole that displaces the nucleus to the margin of the cell (Fig. 6.1). Species-specific molecular diagnostics, PCR primers (Audemard *et al.*, 2004) and DNA probes (Reece *et al.*, 2008), have been developed for *P. marinus* and these are useful for certain research applications, industry certifications for pathogen-free status, or for confirming species identification.

6.3.2. *H. nelsoni*

There are no reliable clinical signs for diagnosis of *H. nelsoni* as mortality is so rapid, usually 2–3 months after infection, that dead oysters can appear otherwise healthy. This is unlike the wasting disease often associated with *P. marinus*.

Histopathological analysis is the preferred method for diagnosis of *H. nelsoni* because it allows determination of life-cycle stages and host reactions. The presence of ~7 μ m-long spores restricted to the epithelium of the digestive diverticula (Fig. 6.3a) is diagnostic for *H. nelsoni*. If spores are absent and only multinucleate plasmodia are present, diagnosis using histology can be problematic because plasmodia of all haplosporidians are similar and there is one other species, *Haplosporidium costale*, which partially overlaps in distribution with *H. nelsoni*. Both species are present in high salinity coastal bays where salinity is greater than about 25 psu and mixed plasmodial infections of the two species have been reported (Stokes and Burreson, 2001). If spores are present the two species are readily distinguishable by location of sporulation: (i) epithelium of the digestive diverticula in *H. nelsoni*; and

(ii) throughout the visceral connective tissue in *H. costale*. In addition, spores of *H. costale* (about 4 µm in length) are much smaller than those of *H. nelsoni*. In major estuaries such as Delaware Bay and Chesapeake Bay, where salinity is less than about 25 psu, *H. costale* is absent, so the presence of plasmodia in these areas can reliably be attributed to *H. nelsoni*. DNA-based diagnostic techniques, both PCR primers and DNA probes, are available for both *H. nelsoni* and *H. costale* (Stokes *et al.*, 1995; Stokes and Bureson, 1995, 2001), so identification can be confirmed using these techniques. PCR is also useful for rapid screening of juvenile oysters for disease-free certifications.

6.4. Internal Lesions

There are no external lesions on the mantle or gill surface of oysters infected with either *P. marinus* or *H. nelsoni*. Internally, *P. marinus* can cause significant disruption of the stomach

or intestine epithelium architecture, which probably interferes with digestion (Fig. 6.6a, b), and also focal lesions in the vesicular connective tissue and/or gonad (Fig. 6.6c, d). Host haemocyte accumulation in the infected region (haemocytosis) is common with both pathogens (Fig. 6.2a), and *P. marinus* cells are readily phagocytosed by host haemocytes, although they are not killed. There is little phagocytosis of *H. nelsoni* plasmodia, probably because they are as large as or larger than the host haemocytes. The large size of *H. nelsoni* plasmodia causes mechanical disruption of tissues and metaplasia of infected epithelium.

6.5. Pathophysiology

6.5.1. *P. marinus*

As noted above, parasitism by *P. marinus* produces a wasting disease in *C. virginica*. Initial infections occur in digestive tract epithelia,

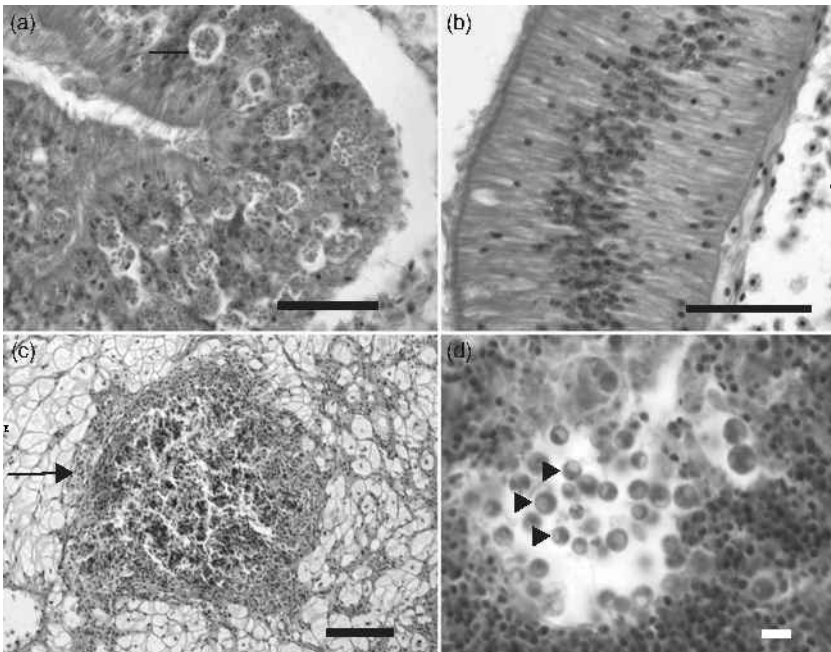


Fig. 6.6. Lesions caused by *P. marinus* in oyster tissue. (a) Stomach epithelium architecture destroyed by *P. marinus* cells. Paraffin histology, bar = 25 µm. (b) Normal stomach epithelium. Paraffin histology, bar = 25 µm. (c) Lesion in visceral connective tissue. Paraffin histology, bar = 100 µm. (d) *P. marinus* cells (arrow heads) in lesion in male gonad. Paraffin histology, bar = 10 µm.

and may occur anywhere from the stomach of an oyster to its rectum. Lytic secretions of *P. marinus* cells disrupt columnar epithelial tissues, an effect that is compounded by infiltrating haemocytes (Mackin, 1951). As an infection progresses the epithelial pathology becomes more extensive, and consequently, epithelial contributions to digestion and nutrition are increasingly compromised. In healthy oysters contributions by the epithelium include: (i) the sorting of food particles in the stomach, to ensure that only the smallest particles enter the ducts of the digestive diverticula; (ii) mixing of particles with digestive enzymes in the stomach; and (iii) enzymatic digestion and nutrient absorption in the mid-gut and rectum (Langdon and Newell, 1996). Parasite sequestration of nutrients, of course, further impacts oyster nutrition (Choi *et al.*, 1989). Even light infections reduce growth (Menzel and Hopkins, 1955), so infections that are primarily epithelial may none the less have energetic costs. Heavier infections bring more serious reductions in growth and in reproductive output. Paynter and Bureson (1991) observed that *P. marinus* infections in waters of moderate salinity (12–15 psu) reduced oyster growth by 60%. In waters of higher salinity (16–20 psu), growth was reduced by 80%. Adverse effects on reproduction are focused primarily on late gametogenesis in the spring and early summer, and include a reduction in fecundity but not egg size or quality. Kennedy *et al.* (1995) found no decrease in oocyte diameters or lipid investments with increasing *P. marinus* infection intensity, but they did observe a decrease in 'reproductive index' (calculated as the average gonad width $\div$ body area $\times$ 100). Even moderate infections reduced the reproductive index by half. Dittman *et al.* (2001) similarly found that gonadal indices (defined as 'the proportion of the cross-sectional visceral mass area occupied by the gonad'), condition index, and the percentage of gametogenic tissue decreased with increasing intensity of *P. marinus* infection. The heaviest infections in an oyster population nearing peak gametogenic development reduced: (i) the mean gonadal index by more than half; (ii) the mean condition index by a third; and (iii) the mean percentage of gametogenic tissue by two-

thirds. Our own observations are that the most intense early-season infections can entirely abolish reproduction.

Interestingly, *P. marinus* infection in one season, provided it is not so intense as to be lethal, does not significantly limit oyster reproduction the next summer. Even when infection reduces the winter glycogen storage that would normally fuel gametogenesis in the spring, such compromised oysters may instead rely on energy assimilated from feeding on the spring phytoplankton bloom to produce gametes (Kennedy *et al.*, 1995; Dittman *et al.*, 2001).

In oysters that are unable to resist *P. marinus*, parasite cells breach the basement membrane of the epithelium and reach the connective tissues and haemolymph spaces, thus allowing infections to become systemic. As *P. marinus* numbers increase in connective tissues, parasite proteases produce widening areas of host tissue liquefaction. Haemocytosis continues, but haemocytes are unable to destroy phagocytosed parasite cells, and rupture from the proliferation of the parasite. Haemocytes, parasite cells and debris can occlude haemolymph vessels and impede circulation (Mackin, 1951). This pathology probably combines with nutrient depletion, which ultimately causes death (Ford and Tripp, 1996).

It is not known if oysters with advanced systemic infections recover. Some do recover from more serious epithelial infections, as indicated by healing epithelium observed in some oysters examined in late winter. Such individuals may have been saved by falling water temperatures in autumn: *P. marinus* is most lethal at water temperatures greater than 25°C, and below 15–20°C the metabolic rate of *P. marinus* is reduced (Chu and La Peyre, 1993; Ford and Tripp, 1996). None the less, it is clear that many oysters, while lightly infected, can resist *P. marinus* proliferation and disease for years. The nature of this resistance remains unclear. Significant lytic activity is a hallmark of *P. marinus* infection (Mackin, 1951), and serine proteases secreted by *P. marinus* are key agents of this lysis (La Peyre *et al.*, 1995). They may suppress oyster haemocyte migration, lysozyme activity and agglutination (Garreis *et al.*, 1996). *C. virginica*

counters parasite proteolytic activity by having protease inhibitors in its plasma (Oliver *et al.*, 1999; Xue *et al.*, 2006, 2009). General protease inhibition (Oliver *et al.*, 2000) and expression and activity of a specific inhibitory protein (La Peyre *et al.*, 2010) are higher in oysters selectively bred for *P. marinus* resistance, which indicates that these proteins may play an important role in suppressing parasite multiplication. *P. marinus* may also be capable of modulating, or interfering with, the production of reactive oxygen species by oyster haemocytes (Anderson, 1999). Resisting superoxide and hydrogen peroxide that have been produced (Schott *et al.*, 2003) may be a key to parasite survival in haemocytes after phagocytosis. Variation among oysters in ability to counter this activity may also contribute to variation in resistance to *P. marinus* but this has not been studied.

6.5.2. *H. nelsoni*

Unlike the more chronic *P. marinus* infections, *H. nelsoni* infections are typically acute. *H. nelsoni*-caused mortality in a susceptible oyster population may exceed 60% within just 3 months of parasite exposure (Ragone Calvo *et al.*, 2003b). The parasite first colonizes gill epithelium, or occasionally gut epithelium, of the oyster (Ford and Haskin, 1982). The host haemocyte response to focal infection of the gills by *H. nelsoni* plasmodia is often intense (Ford and Tripp, 1996), and typically exceeds the response to similar numbers of *P. marinus* infecting gut epithelium. Haemocytes infiltrating gill tissues can be so numerous that the fine plical architecture of a normal oyster gill is obliterated. Systemic *H. nelsoni* infections reduce oyster clearance rates and condition, the latter of which is directly proportional to the amount of glycogen stored in tissues and partly a product of feeding. These reductions have been attributed to impairment of ciliary function in affected gills (Newell, 1985). Focal to multifocal epithelial infections, which were not studied by Newell (1985), presumably cause similar impairment and physiological effects. Barber *et al.* (1988a) found that gill epithelial infection by *H. nelsoni* reduced the oyster condition index by 13% and fecundity

by 35% relative to uninfected oysters. Glycogen in oyster tissues was reduced by 22% (Barber *et al.*, 1988b).

H. nelsoni-resistant oysters, if they become infected at all, can contain the parasite within gill epithelia. In more susceptible oysters *H. nelsoni* penetrates the base of the epithelium and colonizes connective tissues to become systemic. As *H. nelsoni* proliferates in connective tissues and reaches the digestive diverticula, physical damage can include 'mechanical disruption and lysis of tissues ... metaplasia of digestive tubule epithelium, and fibrosis' (Ford and Tripp, 1996). Physiological consequences deepen, with condition index, fecundity and glycogen reduced more sharply (Barber *et al.*, 1988a, b), and with protein levels also depressed in haemolymph (Ford, 1986) and tissues (Barber *et al.*, 1988b). Death is probably caused by the impact of *H. nelsoni* on the metabolic condition of the host. The most susceptible oysters, however, die so quickly that significant pathology and reduction in metabolic condition may not be obvious. This has prompted speculation that a parasite toxin may contribute to mortality (Ford and Tripp, 1996).

6.6. Protective/Control Strategies

Adaptive immunity is not known in oysters or other molluscs, so they cannot be immunized against either *P. marinus* or *H. nelsoni*. The primary means of protection against these pathogens is selective breeding. As early as the 1960s, scientists working with *H. nelsoni* in Delaware Bay began observing increased resistance to *H. nelsoni* parasitism produced from survivors of earlier outbreaks (Haskin and Ford, 1979). These positive results justified investment in breeding programmes for *H. nelsoni* resistance, and aquaculturists in the mid-Atlantic region of the USA continue to cultivate descendants of these early disease-resistant strains. In recent years, resistance to *P. marinus* has also been incorporated into breeding programmes (Ragone Calvo *et al.*, 2003a). Genetic resistance does not confer complete protection: some oysters will still develop *H. nelsoni* infections, and most will become infected by

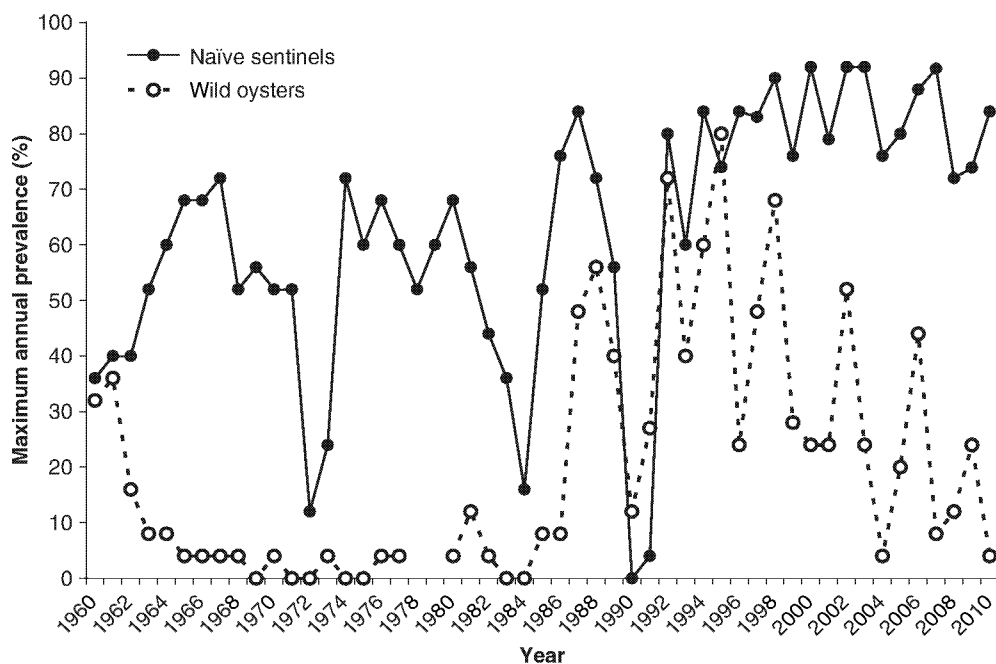


Fig. 6.7. Maximum annual prevalence of *H. nelsoni* in naïve sentinel oysters deployed each spring to the *H. nelsoni*-enzootic York River, Virginia, USA, and in wild oysters from Wreck Shoal, an *H. nelsoni*-enzootic reef in the nearby James River. After colonizing Wreck Shoal during the initial epizootic in the 1960s, *H. nelsoni* was generally absent from Wreck Shoal until the droughts of the 1980s. Oysters at Wreck Shoal have become increasingly resistant to *H. nelsoni* since the mid-1990s. Data for the naïve sentinels indicate that, with the exception of a few wet years, *H. nelsoni* infection pressure has been steadily increasing over time.

P. marinus in parasite-enzootic waters. None the less, fewer infections develop to lethal intensities, and because resistant oysters have also been selected for rapid growth, enough oysters survive to market size (typically 3 inches or 7.6 cm) for oyster aquaculture to be profitable despite disease.

Promotion of genetic resistance to disease is now being applied to the management and restoration of wild oyster populations. While lower salinities in upper parts of estuaries provide some mitigation of parasitism, infection pressure on wild oyster populations in higher salinity waters must be intense. Decades of this pressure have produced more resistant oyster populations. This is especially clear with respect to *H. nelsoni*. In Delaware Bay, major epizootics swept the estuary, purging the most susceptible oysters from the population. Today the parasite is rarely

observed histologically, even though PCR data indicate it remains abundant in the environment (Ford *et al.*, 2009). In Chesapeake Bay, a long-term study (1960 to the present) of naïve sentinel oysters annually deployed to the *H. nelsoni*- and *P. marinus*-enzootic York River reveals *H. nelsoni* levels to be higher than ever, yet levels in wild oysters have been declining (Fig. 6.7), an indication that Chesapeake Bay populations too are increasingly resistant. Resistance to *P. marinus* has been slower to develop, for reasons that are not obvious. Despite continued intense disease caused by *P. marinus* in particular, oyster populations have rebounded in some Chesapeake Bay sub-estuaries where they have been protected from harvest (Schulte *et al.*, 2009). In light of these observations, protection of broodstock oysters in sanctuaries from harvest is being incorporated into management

plans for the Chesapeake Bay oyster population. It is hoped that these sanctuaries will maximize the possibility that resistant oysters will pass on their genes, and thus improve the resistance of the population.

Pathogen control through interference with parasite life cycles or transmission was once reasonably effective for *P. marinus*, which has a simple, direct life cycle. In the 1950s, a distance of 40–50 feet (12–15 m) from sources of *P. marinus* accomplished a reduction in transmission efficiency (Andrews, 1965). Culturists planting small oysters, called seed, extensively on Chesapeake Bay bottom were advised to avoid foci of infection like natural reefs and pilings, and to intensively clean and then fallow beds before planting new seed to slow re-colonization by *P. marinus*. With intensification of *P. marinus* parasitism in the 1980s (Bureson and Andrews, 1988; Bureson and Ragone Calvo, 1996) came a great increase in *P. marinus* abundance, and dispersal distances are now in kilometres (McCollough *et al.*, 2007). Control of *P. marinus* infections by reducing transmission in this manner is no longer practical.

The unresolved life cycle of *H. nelsoni* limits options for its control. The environmental source of this parasite remains a mystery. Manipulating oyster densities and fallowing beds yield no benefit for management. The absence of a relationship between oyster population size and location and *H. nelsoni* infection pressure remains key evidence pointing towards the existence of an intermediate host for this parasite (Haskin and Andrews, 1988).

Neither *P. marinus* nor *H. nelsoni* is fully pathogenic at salinities below 10 psu. The former can withstand long periods of lower salinity, but its disease impact on oyster hosts is reduced. The latter is intolerant of even short periods of salinity below 10 psu, which may purge it from estuarine systems (Ford and Tripp, 1996). Establishing aquaculture farms in waters of relatively lower salinity reduces parasite effects, but lower salinities also reduce the growth rate of *C. virginica* (Shumway, 1996), and very low salinities produced by serious floods that periodically affect these areas can kill oysters (Galtsoff, 1964). For these reasons and also because

oysters are more flavourful when bred in more saline waters, this strategy of disease mitigation is not widely pursued.

Immersion of *C. virginica* for short periods (e.g. 2 weeks) in water of low salinity (below 10 psu) does have potential for treatment of *H. nelsoni* infections (Ford, 1992). While use of disease-resistant seed has largely obviated such strategies in aquaculture, even resistant seed is occasionally found to harbour *H. nelsoni* at 10–20% prevalence. Eventual mortality due to *H. nelsoni* parasitism may be avoided by the immersion method. Such treatment would not be effective for *P. marinus* infections, however, as even longer periods at lower salinities (e.g. 8 weeks at 6 psu; Ragone Calvo and Bureson, 1993) have not been shown to reduce infection.

The nature and scale of oyster aquaculture, with oysters maintained in large arrays of cages or floats in open waters, does not lend itself to the use of chemotherapeutants. Application of chemicals to oysters in wild populations is out of the question. None the less, chemotherapeutants may have utility in small, closed systems, where seed or valuable broodstock are being held. *P. marinus* was originally thought to have fungal affinities, so the earliest study of chemotherapeutant potential focused on the antimycotic cycloheximide. It suppressed *P. marinus* infections and extended the lives of infected oysters, but parasite proliferation resumed when cycloheximide treatment was discontinued (Ray, 1966b). Subsequent research produced similar findings with cycloheximide, but found other anti-coccidial compounds (amprolium, malachite green and sulfadimethoxine) to be ineffective (Calvo and Bureson, 1994). Antibiotics bacitracin (Faisal *et al.*, 1999) and triclosan (Chu *et al.*, 2008) have been shown to slow the proliferation of *P. marinus* in oyster tissues.

6.7. Conclusions and Suggestions for Future Studies

P. marinus and *H. nelsoni* are as relevant today as they ever have been. There are two reasons for this. The first is our continued inability to

effectively control these pathogens, genetic progress notwithstanding. In drought years, even relatively resistant oyster populations may be seriously impacted by *H. nelsoni* parasitism, and annual mortality due to *P. marinus* can exceed 70% (Mann *et al.*, 2009). The second is our renewed interest in oysters. As keystone estuarine species by virtue of their role in benthic-pelagic coupling and the habitat their reefs provide, they are recognized as essential to the ecological restoration of many coastal ecosystems in the eastern and southern USA (Coen *et al.*, 2007). Because of their potential as aquaculture species, which is already being realized in places, we expect them to be central to the economic restoration of many coastal communities. Many of these communities have been in decline since the collapse of oyster and other fisheries decades ago. Diseases caused by *H. nelsoni* and *P. marinus* loom as major impediments to both types of restoration.

Critical gaps in our understanding of these pathogens remain. The most obvious is the *H. nelsoni* life cycle. Resolving the life cycle of *H. nelsoni* may explain the 'irregular' and generally low activity of *H. nelsoni* in polyhaline coastal lagoons (Andrews and Castagna, 1978), and the continued absence of *H. nelsoni* from the Gulf of Mexico coast. It may allow the development of biosecurity guidelines to help ensure that the latter remains the case.

More generally, an understanding of the molecular and cellular bases of pathogenesis is non-existent for *H. nelsoni*, and nearly so in *P. marinus*. Genes and proteins such as the natural resistance-associated macrophage protein (Nramp) and superoxide dismutases that may relate to intrahaemocytic survival of *P. marinus* have begun to be characterized (e.g. Robledo *et al.*, 2004; Fernández-Robledo *et al.*, 2008), but questions remain as to how these molecules relate to observed variability in parasite virulence (Bushek and Allen, Jr, 1996). Our appreciation of the potential interplay between *P. marinus* proteases and the protease inhibitors expressed by *C. virginica* is only marginally more advanced (see references above). Further work to characterize these molecules and others integral to host-parasite interactions and pathogenesis will be

beneficial. This work should extend to a characterization of genetic diversity and to the relative roles of genetics and environmental influences in determining disease outcomes. We may hope that biomarkers for oyster resistance can be identified for use in oyster breeding programmes. Biomarkers for parasite virulence may eventually be used to gauge disease risk.

Finally, we should embrace the view of interactions of *C. virginica* and its parasites as dynamic, not static, and attempt to understand the nature and causes of this dynamism. The great intensification of oyster disease in Chesapeake Bay, especially that caused by *P. marinus*, in 1986 is perhaps the most striking example (Bureson and Andrews, 1988). Others include: (i) the cycling of *P. marinus* in the Gulf of Mexico in connection with El Niño-Southern Oscillation cycles (Soniat *et al.*, 2005); (ii) the progression of *P. marinus* northward from Chesapeake Bay beginning in 1990 (Ford, 1996); and (iii) the decline of *H. nelsoni* impacts in the mid-Atlantic, especially notable in Delaware Bay (Ford *et al.*, 2009). While climate impacts are clearly at work, other influences must be considered. The generally increasing *H. nelsoni* pressure in Chesapeake Bay (Fig. 6.7) suggests an environment increasingly favourable for this parasite. While this may be partly attributable to increasingly milder winter water temperatures (Preston, 2004), it is also the case that a more eutrophic Chesapeake Bay (Kemp *et al.*, 2005) has seen changes in benthic community structure, with smaller, short-lived, opportunistic species of various phyla increasingly favoured (Holland *et al.*, 1987; Long and Seitz, 2009). One of the opportunistic species that has increased in abundance may be the intermediate host for *H. nelsoni*.

In viewing the oyster-parasite system as dynamic, we should also strive to understand the evolutionary forces shaping it. In the Gulf of Mexico, *P. marinus* and *C. virginica* must each act as agents of selection upon the other. In the Atlantic, *H. nelsoni* joins the system, interacting not only with *C. virginica* but, presumably, competing with *P. marinus* as well (Fig. 6.8). How selection operates in this system needs to be studied as it has

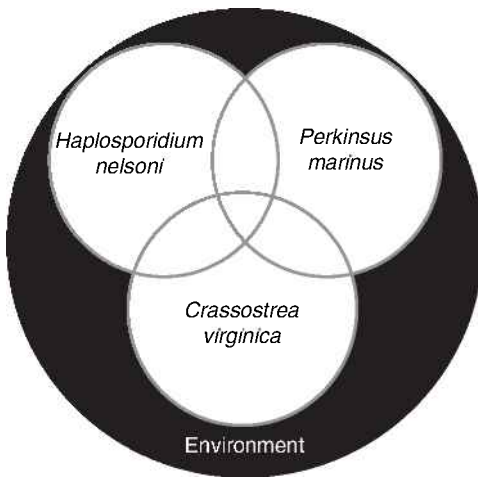


Fig. 6.8. Venn diagram illustrating interactions among oyster host *C. virginica* and parasites *H. nelsoni* and *P. marinus*, with the environment influencing the entire system.

relevance to considerations of host resistance versus susceptibility, and pathogen virulence. We may hypothesize, for example, that decreasing *H. nelsoni* levels in wild oysters (Ford *et al.*, 2009) are a result of parasite-imposed selection for increasing disease resistance. More intriguing is the possibility that *H. nelsoni* may have acted as an agent of selection upon *P. marinus*, selecting for increased virulence in *P. marinus* as a strategy for ensuring transmission from oysters decreased in longevity and density by

introduced *H. nelsoni*. Gene flow (dispersal of host and parasite genotypes) is important, and so is genetic drift: *P. marinus* abundance contracts to very low levels, particularly at more northern latitudes, in the spring, when it becomes nearly undetectable in oysters (Bureson and Ragone Calvo, 1996). Marrying an evolutionary approach to understanding *C. virginica*–*P. marinus*–*H. nelsoni* interactions to the basic molecular and cellular pathobiology would be a most interesting and important direction for future research.

References

- Alvarez, M.R., Friedl, F.E., Hudson, C.M. and O'Neill, R.L. (1992) Uptake and tissue distribution of abiotic particles from the alimentary tract of the American oyster: a simulation of intracellular parasitism. *Journal of Invertebrate Pathology* 59, 290–294.
- Anderson, R.S. (1999) *Perkinsus marinus* secretory products modulate superoxide anion production by oyster (*Crassostrea virginica*) haemocytes. *Fish and Shellfish Immunology* 9, 51–60.
- Andrews, J.D. (1965) Infection experiments in nature with *Dermocystidium marinum* in Chesapeake Bay. *Chesapeake Science* 6, 60–67.
- Andrews, J.D. (1966) Oyster mortality studies in Virginia. V. Epizootiology of MSX, a protistan pathogen of oysters. *Ecology* 47, 19–31.
- Andrews, J.D. (1983) *Minchinia nelsoni* (MSX) infections in the James River seed-oyster area and their expulsion in spring. *Estuarine and Coastal Shelf Science* 16, 255–269.
- Andrews, J.D. (1988) Epizootiology of the disease caused by the oyster pathogen *Perkinsus marinus* and its effects on the oyster industry. *American Fisheries Society Special Publication* 18, 47–63.
- Andrews, J.D. and Castagna, M. (1978) Epizootiology of *Minchinia costalis* in susceptible oysters in seaside bays of Virginia's Eastern Shore, 1959–1976. *Journal of Invertebrate Pathology* 32, 124–138.
- Audemard, C., Reece, K.S. and Bureson, E.M. (2004) Real-time PCR for detection and quantification of the protistan parasite *Perkinsus marinus* in environmental waters. *Applied Environmental Microbiology* 70, 6611–6618.

- Barber, B.J., Ford, S.E. and Haskin, H.H. (1988a) Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. I. Condition index and relative fecundity. *Journal of Shellfish Research* 7, 25–31.
- Barber, B.J., Ford, S.E. and Haskin, H.H. (1988b) Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. II. Tissue biochemical composition. *Comparative Biochemistry and Physiology* 91A, 603–608.
- Barber, R.D., Kanaley, S.A. and Ford, S.E. (1991) Evidence for regular sporulation by *Haplosporidium nelsoni* (MSX) (Ascetosporea Haplosporidiidae) in spat of the American oyster, *Crassostrea virginica*. *Journal of Protozoology* 38, 305–306.
- Burreson, E.M. (1994) Further evidence of regular sporulation by *Haplosporidium nelsoni* in small oysters, *Crassostrea virginica*. *Journal of Parasitology* 80, 1036–1038.
- Burreson, E.M. and Andrews, J.D. (1988) Unusual intensification of Chesapeake Bay oyster diseases during recent drought conditions. In: *Proceedings of the Oceans '88 Conference 1988*, 31 October–2 November 1988, Baltimore, Maryland. Marine Technology Society and the Institute of Electrical and Electronic Engineers, New York, pp. 799–802.
- Burreson, E.M. and Ragone Calvo, L.M. (1996) Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay, with emphasis on data since 1985. *Journal of Shellfish Research* 15, 17–34.
- Burreson, E.M., Alvarez, R.S., Martinez, V.V. and Macedo, L.A. (1994) *Perkinsus marinus* (Apicomplexa) as a potential source of oyster *Crassostrea virginica* mortality in coastal lagoons of Tabasco, Mexico. *Diseases of Aquatic Organisms* 20, 77–82.
- Burreson, E.M., Stokes, N.A. and Friedman, C.S. (2000) Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. *Journal of Aquatic Animal Health* 12, 1–8.
- Bushek, D. and Allen, S.K. Jr (1996) Host–parasite interactions among broadly distributed populations of the eastern oyster *Crassostrea virginica* and the protozoan *Perkinsus marinus*. *Marine Ecology Progress Series* 139, 127–141.
- Cáceres-Martínez, J., Vásquez-Yeomans, R., Padilla-Lardizábal, G. and del Río-Portilla, M.A. (2008) *Perkinsus marinus* in pleasure oyster *Crassostrea corteziensis* from Nayarit, Pacific coast of Mexico. *Journal of Invertebrate Pathology* 99, 66–73.
- Calvo, G.W. and Burreson, E.M. (1994) *In vitro* and *in vivo* effects of eight chemotherapeutants on the oyster parasite *Perkinsus marinus* (Mackin, Owen, and Collier). *Journal of Shellfish Research* 13, 101–107.
- Choi, K.-S., Wilson, E.A., Lewis, D.H., Powell, E.N. and Ray, S.M. (1989) The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of the thioglycollate method. *Journal of Shellfish Research* 8, 125–131.
- Chu, F.-L.E. (1996) Laboratory investigations of susceptibility, infectivity and transmission of *Perkinsus marinus* in oysters. *Journal of Shellfish Research* 15, 57–66.
- Chu, F.-L.E. and La Peyre, J.F. (1993) *Perkinsus marinus* susceptibility and defense-related activities in eastern oysters *Crassostrea virginica*: temperature effects. *Diseases of Aquatic Organisms* 16, 223–234.
- Chu, F.-L.E., Lund, E.D. and Podbesek, J.A. (2008) Effects of triclosan on the oyster parasite, *Perkinsus marinus* and its host, the eastern oyster, *Crassostrea virginica*. *Journal of Shellfish Research* 27, 769–773.
- Coen, L.D., Brumbaugh, R.D., Bushek, D., Grizzle, R., Luckenbach, M.W., Posey, M.H., Powers, S.P. and Tolley, S.G. (2007) Ecosystem services related to oyster restoration. *Marine Ecology Progress Series* 341, 303–307.
- Dittman, D.E., Ford, S.E. and Padilla, D.K. (2001) Effects of *Perkinsus marinus* on reproduction and condition of the eastern oyster, *Crassostrea virginica*, depend on timing. *Journal of Shellfish Research* 20, 1025–1034.
- Dungan, C.F., Reece, K.S., Hamilton, R.M., Stokes, N.A. and Burreson, E.M. (2007) Experimental cross-infections by *Perkinsus marinus* and *P. chesapeaki* in three sympatric species of Chesapeake Bay oysters and clams. *Diseases of Aquatic Organisms* 76, 67–75.
- Faisal, M., La Peyre, J., Elsayed, E. and Wright, D.C. (1999) Bacitracin inhibits the oyster pathogen *Perkinsus marinus* *in vitro* and *in vivo*. *Journal of Aquatic Animal Health* 11, 130–138.
- Fernández-Robledo, J.A., Schott, E.J. and Vasta, G.R. (2008) *Perkinsus marinus* superoxide dismutase 2 (PmSOD2) localizes to single-membrane subcellular compartments. *Biochemical and Biophysical Research Communications* 375, 215–219.

- Ford, S.E. (1985) Effects of salinity on survival of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber and Mackin) in oysters. *Journal of Shellfish Research* 2, 85–90.
- Ford, S.E. (1986) Comparison of hemolymph proteins from resistant and susceptible oysters, *Crassostrea virginica*, exposed to the parasite *Haplosporidium nelsoni* (MSX). *Journal of Invertebrate Pathology* 47, 283–294.
- Ford, S.E. (1992) Avoiding the transmission of disease in commercial culture of molluscs, with special reference to *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX). *Journal of Shellfish Research* 11, 539–546.
- Ford, S.E. (1996) Range extension by the oyster parasite *Perkinsus marinus* into the northeastern United States: response to climate change? *Journal of Shellfish Research* 15, 45–56.
- Ford, S.E. and Haskin, H.H. (1982) History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen, in Delaware Bay, 1957–1980. *Journal of Invertebrate Pathology* 40, 118–141.
- Ford, S.E. and Haskin, H.H. (1988) Comparison of *in vitro* salinity tolerance of the oyster parasite *Haplosporidium nelsoni* (MSX) and hemocytes from the host, *Crassostrea virginica*. *Comparative Biochemistry and Physiology* 90A, 183–187.
- Ford, S.E. and Smolowitz, R. (2007) Infection dynamics of an oyster parasite in its newly expanded range. *Marine Biology* 151, 119–133.
- Ford, S.E. and Tripp, M.R. (1996) Diseases and defense mechanisms. In: Kennedy, V.S., Newell, R.I.E. and Eble, A.F. (eds) *The Eastern Oyster Crassostrea virginica*. Maryland Sea Grant College, College Park, Maryland, pp. 581–660.
- Ford, S.E., Allam, B. and Xu, Z. (2009) Using bivalves as particle collectors with PCR detection to investigate the environmental distribution of *Haplosporidium nelsoni*. *Diseases of Aquatic Organisms* 83, 159–168.
- Friedman, C.S. (1996) Haplosporidan infections of the Pacific oyster, *Crassostrea gigas* (Thunberg), in California and Japan. *Journal of Shellfish Research* 15, 597–600.
- Galtsoff, P.S. (1964) The American oyster *Crassostrea virginica* Gmelin. *Fishery Bulletin* 64, 1–480.
- Garreis, K.A., La Peyre, J.F. and Faisal, M. (1996) The effects of *Perkinsus marinus* extracellular products and purified proteases on oyster defense parameters *in vitro*. *Fish and Shellfish Immunology* 6, 581–597.
- Haskin, H.H. and Andrews, J.D. (1988) Uncertainties and speculations about the life cycle of the eastern oyster pathogen *Haplosporidium nelsoni* (MSX). *American Fisheries Society Special Publication* 18, 5–22.
- Haskin, H.H. and Ford, S.E. (1979) Development of resistance to *Minchinia nelsoni* (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. *Marine Fisheries Review* 41, 54–63.
- Haskin, H.H., Stauber, L.A. and Mackin, J.A. (1966) *Minchinia nelsoni* sp. n. (Haplosporida, Haplosporidiidae): causative agent of the Delaware Bay oyster epizootic. *Science* 153, 1414–1416.
- Holland, A.F., Shaughnessy, A.T. and Hiegel, M.H. (1987) Long-term variation in mesohaline Chesapeake Bay macrobenthos: spatial and temporal patterns. *Estuaries* 10, 227–245.
- Kemp, W.M., Boynton, W.R., Adolf, J.E., Boesch, D.F., Boicourt, W.C., Brush, G., Cornwell, J.C., Fisher, T.R., Glibert, P.M., Hagy, J.D., Harding, L.W., Houde, E.D., Kimmel, D.G., Miller, W.D., Newell, R.I.E., Roman, M.R., Smith, E.M. and Stevenson, J.C. (2005) Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology Progress Series* 303, 1–29.
- Kennedy, V.S., Newell, R.I.E., Krantz, G.E. and Otto, S. (1995) Reproductive capacity of the eastern oyster *Crassostrea virginica* infected with the parasite *Perkinsus marinus*. *Diseases of Aquatic Organisms* 23, 135–144.
- Langdon, C.J. and Newell, R.I.E. (1996) Digestion and nutrition in larvae and adults. In: Kennedy, V.S., Newell, R.I.E. and Eble, A.F. (eds) *The Eastern Oyster Crassostrea virginica*. Maryland Sea Grant College, College Park, Maryland, pp. 231–269.
- La Peyre, J.F., Schafhauser, D.Y., Rizkalla, E.H. and Faisal, M. (1995) Production of serine proteases by the oyster pathogen *Perkinsus marinus* (Apicomplexa) *in vitro*. *Journal of Eukaryotic Microbiology* 42, 544–551.
- La Peyre, J.F., Xue, Q.-G., Itoh, N., Li, Y. and Cooper, R.K. (2010) Serine protease inhibitor cvSI-1 potential role in the eastern oyster host defense against the protozoan parasite *Perkinsus marinus*. *Developmental and Comparative Immunology* 34, 84–92.
- Long, W.C. and Seitz, R.D. (2009) Hypoxia in Chesapeake Bay tributaries: worsening effects on macrobenthic community structure in the York River. *Estuaries and Coasts* 32, 287–297.
- Mackin, J.G. (1951) Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen and Collier. *Bulletin of Marine Science of the Gulf and Caribbean* 1, 72–87.

- Mann, R., Southworth, M., Harding, J.M. and Wesson, J.A. (2009) Population studies of the native eastern oyster, *Crassostrea virginica* (Gmelin, 1791) in the James River, Virginia, USA. *Journal of Shellfish Research* 28, 193–220.
- McCollough, C.B., Albright, B.W., Abbe, G.R., Barker, L.S. and Dungan, C.F. (2007) Acquisition and progression of *Perkinsus marinus* infections by specific-pathogen-free juvenile oysters (*Crassostrea virginica* Gmelin) in a mesohaline Chesapeake Bay tributary. *Journal of Shellfish Research* 26, 465–477.
- Menzel, R.W. and Hopkins, S.H. (1955) The growth of oysters parasitized by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus*. *Journal of Parasitology* 41, 333–342.
- Newell, R.I.E. (1985) Physiological effects of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber & Mackin) on the American oyster *Crassostrea virginica* (Gmelin). *Journal of Shellfish Research* 5, 91–95.
- Oliver, J.L., Lewis, T.D., Faisal, M. and Kaattari, S.L. (1999) Analysis of the effects of *Perkinsus marinus* protease on plasma proteins of the eastern (*Crassostrea virginica*) and Pacific oyster (*Crassostrea gigas*). *Journal of Invertebrate Pathology* 74, 173–183.
- Oliver, J.L., Gaffney, P.M., Allen Jr, S.K., Faisal, M. and Kaattari, S.L. (2000) Protease inhibitory activity in selectively bred families of eastern oysters. *Journal of Aquatic Animal Health* 12, 136–145.
- Paynter, K.T. and Bureson, E.M. (1991) Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*: II. Disease development and impact on growth rate at different salinities. *Journal of Shellfish Research* 10, 425–431.
- Preston, B.L. (2004) Observed winter warming of the Chesapeake Bay estuary (1949–2002): implications for ecosystem management. *Environmental Management* 34, 125–139.
- Ragone Calvo, L.M. and Bureson, E.M. (1993). Effect of salinity on infection progression and pathogenicity of *Perkinsus marinus* in the eastern oyster, *Crassostrea virginica*. *Journal of Shellfish Research* 12, 1–7.
- Ragone Calvo, L.M., Calvo, G.W. and Bureson, E.M. (2003a) Dual disease resistance in a selectively bred eastern oyster, *Crassostrea virginica*, strain tested in Chesapeake Bay. *Aquaculture* 220, 69–87.
- Ragone Calvo, L.M., Dungan, C.F., Roberson, B.S. and Bureson, E.M. (2003b) Systematic evaluation of factors controlling *Perkinsus marinus* transmission dynamics in lower Chesapeake Bay. *Diseases of Aquatic Organisms* 56, 75–86.
- Ray, S.M. (1966a) A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. *Proceedings of the National Shellfisheries Association* 54, 55–69.
- Ray, S.M. (1966b) Cycloheximide: inhibition of *Dermocystidium marinum* in laboratory stocks of oysters. *Proceedings of the National Shellfisheries Association* 56, 31–36.
- Reece, K.S., Dungan, C.F. and Bureson, E.M. (2008) Molecular epizootiology of *Perkinsus marinus* and *P. chesapeakei* infections among wild oysters and clams in Chesapeake Bay, USA. *Diseases of Aquatic Organisms* 82, 237–248.
- Robledo, J.-A.F., Courville, P., Cellier, M.F.M. and Vasta, G.R. (2004) Gene organization and expression of the divalent cation transporter Nramp in the protistan parasite *Perkinsus marinus*. *Journal of Parasitology* 90, 1004–1014.
- Schott, E.J., Pecher, W.T., Okafor, F. and Vasta, G.R. (2003) The protistan parasite *Perkinsus marinus* is resistant to selected reactive oxygen species. *Experimental Parasitology* 105, 232–240.
- Schulte, D., Burke, R.P. and Lipcius, R.N. (2009) Unprecedented restoration of a native oyster metapopulation. *Science* 325, 1124–1128.
- Shumway, S.E. (1996) Natural environmental factors. In: Kennedy, V.S., Newell, R.I.E. and Eble, A.F. (eds) *The Eastern Oyster Crassostrea virginica*. Maryland Sea Grant College, College Park, Maryland, pp. 467–513.
- Soniat, T.M. (1996) Epizootiology of *Perkinsus marinus* disease of eastern oysters in the Gulf of Mexico. *Journal of Shellfish Research* 15, 35–43.
- Soniat, T.M., Klinck, J.M., Powell, E.N. and Hofmann, E.E. (2005) Understanding the success and failure of oyster populations: climatic cycles and *Perkinsus marinus*. *Journal of Shellfish Research* 24, 83–93.
- Stokes, N.A. and Bureson, E.M. (1995) A sensitive and specific DNA probe for the oyster pathogen *Haplosporidium nelsoni*. *Journal of Eukaryotic Microbiology* 42, 350–357.
- Stokes, N.A. and Bureson, E.M. (2001) Differential diagnosis of mixed *Haplosporidium costale* and *Haplosporidium nelsoni* infections in the eastern oyster, *Crassostrea virginica*, using DNA probes. *Journal of Shellfish Research* 20, 207–213.
- Stokes, N.A., Siddall, M.E. and Bureson, E.M. (1995) Detection of *Haplosporidium nelsoni* (Haplosporidia: Haplosporidiidae) in oysters by PCR amplification. *Diseases of Aquatic Organisms* 23, 145–152.

- Xue, Q.-G., Waldrop, G.L., Schey, K.L., Itoh, N., Ogawa, M., Cooper, R.K., Losso, J.N. and La Peyre, J.F. (2006) A novel slow-tight binding serine protease inhibitor from eastern oyster (*Crassostrea virginica*) plasma inhibits perkinsin, the major extracellular protease of the oyster protozoan parasite *Perkinus marinus*. *Comparative Biochemistry and Physiology, Part B* 145, 16–26.
- Xue, Q.-G., Itoh, N., Schey, K.L., Cooper, R.K. and La Peyre, J.F. (2009) Evidence indicating the existence of a novel family of serine protease inhibitors that may be involved in marine invertebrate immunity. *Fish and Shellfish Immunity* 27, 250–259.

7 *Loma salmonae* and Related Species

David J. Speare and Jan Lovy

Atlantic Veterinary College, University of Prince Edward Island, Charlottetown,
Canada

7.1. Introduction

'Microsporidial gill disease of salmon' (MGDS), caused by the xenoma-forming intracellular pathogen *Loma salmonae*, has become increasingly recognized in recent years from many parts of the world as a disease affecting the gills, and to a lesser extent other systemic organs, of farmed and wild salmonids in both freshwater and marine environments. Although, in general, microsporidian infections of animals are difficult to treat, or are refractory to most treatment regimes, recent research into the pathobiology of *L. salmonae* has now begun to yield insight into how this disease might be managed. Although *L. salmonae* appears to have a near global distribution it has its greatest economic effect on the commercial marine cage culture of Pacific salmon, notably chinook salmon (*Oncorhynchus tshawytscha*), in and around coastal British Columbia, Canada (Constantine, 1999); in this region it can also be found in wild salmon (Kent *et al.*, 1998). Here, since the early 1990s MGDS has become an endemic seasonal problem, arising in late summer and early autumn; the disease is recognized by the presence of slow-moving, top-swimming fish, which exhibit laboured respiratory efforts reflecting the underlying severe multifocal chronic inflammatory bronchitis which is the disease's hallmark lesion

(Kent and Speare, 2005). Much of the research introduced in this chapter has its applications aimed at this sector of aquaculture, but the approaches are probably pertinent to other scenarios where *L. salmonae* may be a problem, and, to a degree yet to be determined, other related microsporidial diseases of farmed fish, such as gill and visceral infections of *Loma morhua* in farm-reared Atlantic cod (*Gadus morhua*).

Microsporidians have long been identified as significant pathogens of insects and fish. Their relationship to fungi (Keeling and Fast, 2002), and their abilities to cause disease in mammals, notably in humans suffering from immunosuppression, has sparked interest in: (i) their intracellular biology; (ii) their modes of transmission; (iii) their relationship to other organisms; and (iv) treatment and control measures (Williams, 2009). Unfortunately, despite recent intensive efforts to find clinical management techniques to control microsporidians causing disease in humans, there has been little substantive progress (Bacchi *et al.*, 2002). Treatment regimes tend to be prolonged, prognosis tends to be guarded (Costa and Weiss, 2000) and prevention methods remain elusive. Hampering progress, *in vitro* approaches, which could be used to better understand intracellular biology, are only rarely successful for microsporidia (Bigliardi *et al.*, 2000; Williams, 2009). Many

questions remain and, in this case, a comparative medicine approach has not provided useful starting points.

Microsporidians are well recognized as pathogens of fish and this has provided an extensive background of published reports and reviews describing: (i) infections; (ii) host ranges of different microsporidians; and (iii) ultrastructural descriptions of various stages although often limited to the spore stage (Lom and Nilsen, 2003). Potentially germane to this chapter, there have been several recent reports of microsporidians within the genus *Loma* affecting various non-salmonid aquaculture species; this is succinctly outlined by Casal *et al.* (2009) in their paper on *Loma psittica* in the Amazonian freshwater puffer fish (*Colomesus asellus*). However, until recently there have been only sporadic published reports geared towards examining the clinical and economic consequences of these diseases, pathophysiological consequences of infection, or evidence-based findings in the areas of disease prevention and treatment. Treatment and management programmes devised for *L. salmonae* may provide starting templates or approaches for a myriad of emerging related microsporidial diseases of cultured fish.

The history of MGDS is a relatively recent one, although recognition and description of *L. salmonae* as a salmon parasite and putative pathogen predates this by several years (Morrison and Sprague, 1983; Hauck, 1984; Poynton, 1986). The first report of *L. salmonae* as a cause of disease in salmon cultivated in marine netpens appeared in 1989 (Speare and Ferguson, 1989); it described a 1987 clinical case arising off the coast of British Columbia, which involved a group of 40 g coho salmon (*Oncorhynchus kisutch*), recently transferred from a hatchery setting to a marine netpen grow-out environment. Dying in large numbers, they were found to have a severe gill disease stemming from reaction to the frustule and setae of the diatom *Corethron hystrix*, which had become trapped between gill lamellae, and an interstitial branchitis caused by xenomas of *L. salmonae* located within gill lamellae and deeper tissues of the gill. Since that time, as a disease of notable economic consequence, *L. salmonae* has

established itself as an endemic disease of marine cultured Pacific salmon (notably chinook salmon), becoming known as MGDS and has been found in salmonid culture elsewhere in the world (Bruno *et al.*, 1995; Gandhi *et al.*, 1995). The disease is typically present in late summer and early autumn along coastal British Columbia. Mortality rates can vary from a low of 2.4% to over 70%; in contrast to its initial report MGDS is now most often seen affecting salmon that are nearing market weight, in their second summer of marine culture and thus of high commercial value. Although estimating the costs of disease is a difficult process, a study on one farm site with a 12% mortality rate from MGDS indicated that lost productivity (direct costs) due to mortality specifically from MGDS approached Can\$315,000 during one production cycle (Constantine, 1999); furthermore this study provides an estimate of indirect costs, based on changes to feed conversion efficiency, of Can\$1,470,000.

Currently, MGDS is not reported from chinook salmon hatcheries, although early on it was suspected that hatchery fish carried latent infections that gave rise to MGDS epidemics after the smolt were transferred from freshwater hatcheries to marine sea cages. Stress of transfer was believed to be the trigger for recrudescence. This theory of latency and recrudescence has largely given way to a widely held but as yet unproven alternative theory implicating wild salmon in the marine environment as reservoir sources of the parasite. Assuming this to be true, strategies of screening juveniles prior to transfer to marine netpen sites, previously considered as a key management tool against MGDS, is therefore of less importance than understanding the dynamics of a 'spill-over and amplification' scenario whereby a farmed population acquires its initial infection from transfer from wild reservoirs. As reviewed by Becker and Speare (2007) several studies have addressed the transmission dynamics of *L. salmonae* within carefully controlled laboratory settings to help elucidate factors (host, pathogen or environmental) that affect the horizontal transmission of this pathogen (Mustafa *et al.*, 2000; Becker *et al.*, 2005a, 2006).

To date, experimental transmission models for *L. salmonae* have been developed for rainbow trout (*Oncorhynchus mykiss*), chinook salmon, coho salmon (using the typical strain of *L. salmonae* Rt.) and brook trout (*Salvelinus fontinalis*) (using a variant strain *L. salmonae* Sv. originally isolated from chinook salmon), with successful modes of infection ranging from oral delivery of spores or infectious gill material, intraperitoneal injection of semi-purified spore suspensions, or through cohabitation with infected donor fish. Attempts to establish infection in Atlantic salmon (*Salmo salar*) or Arctic charr (*Salmo alpinus*) have not been successful, although to date the variant strain Sv. has not been tested against these species. Within the permissive host range, there are marked differences between species with respect to susceptibility as demonstrated by Ramsay *et al.* (2002) with chinook and coho salmon developing far greater numbers of gill xenomas following a standard oral challenge as compared with rainbow trout; rainbow trout are more vulnerable than brook trout to the typical strain of *L. salmonae*, but the opposite is true when the Sv. strain is used (Speare and Daley, 2003). To date, *L. salmonae* infections leading to xenomas have not been established in non-salmonid species (Shaw *et al.*, 2000c).

In addition to differences in susceptibility between salmonid species it is interesting to note that, despite the intracellular nature of microsporidians, the organ and tissue distribution of xenomas, and the period of xenoma persistence within host tissues, vary somewhat between susceptible species (Ramsay *et al.*, 2002). In rainbow trout, xenomas develop almost exclusively within the gill (Speare *et al.*, 1998a), where they grow at quantifiable rates within gill pillar cells (Rodriguez-Tovar *et al.*, 2003a, 2004) and persist for 4–5 weeks regardless of water temperature (Becker and Speare, 2004b). In contrast, in chinook salmon, although the gill remains by far the most significant location for xenomas, they can also be present in the heart, spleen and occasionally in other locations (Kent *et al.*, 1995). Although the pillar cell appears to be the preferred/required host cell in rainbow trout, it appears that non-pillar endothelial cells in the gill and endothelial

cells in other organs are also permissive for xenoma development in chinook salmon (Kent *et al.*, 1995; Ramsay *et al.*, 2002). Further, in chinook salmon, although some xenomas rupture in 4–5 weeks as with rainbow trout, others persist for several months (Ramsay *et al.*, 2002). These observations may be critical for future work aimed at developing *in vitro* xenoma expression models by directing researchers towards endothelial cell lines derived from species in which the parasite has a wider tissue and cellular tropism. An interesting contrast is that *L. morhua* in Atlantic cod causes severe systemic infections affecting gills as well as in other organs such as heart and spleen (Rodriguez-Tovar *et al.*, 2003a).

7.2. Diagnosis

It is not difficult to diagnose MGDS: the clinical, gross and subgross signs (Figs. 7.1 and 7.2) when taken together, are distinctive (Constantine, 1999). Fish will swim lethargically at the surface, often close to the net walls, darken in colour, and exhibit laboured respiration and inappetance. The gills show random or coalescing patches of redness from congestion and white patches of hyperplasia; filament fusion may be grossly apparent on larger fish (Figs. 7.1 and 7.2). White- to cream-coloured pin-head-sized xenomas have a random multifocal distribution across all regions of the gill arch; xenomas when fully formed are just large enough to be detected by the naked eye and may be more visible, because of the contrast of white on red, in areas of congestion. In a region where MGDS is endemic, these signs can be used as a definitive non-lethal diagnosis. The presence of xenomas is made more distinct under a dissecting microscope; examining gill whole mounts permits a better assessment of the degree and nature of gill changes (hyperplasia) in the vicinity of xenomas and helps to determine whether other pathogens or foreign bodies (particularly harmful diatoms) are present (Fig. 7.1). Lightly pressing on a gill whole mount can disrupt the xenomas sufficiently to permit visualization and measurement of spores. Histopathology approaches are an effective means to more

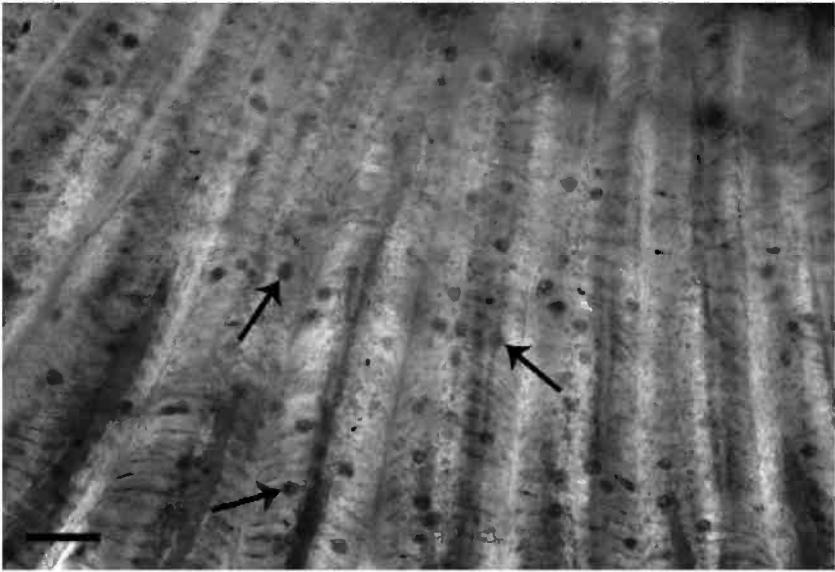


Fig. 7.1. A wet mount of rainbow trout gills 6 weeks after experimental infection with *Loma salmonae*. Xenomas (arrows) are found throughout the tissue. Bar = 200 μ m.



Fig. 7.2. The gills of a farmed chinook salmon clinically affected with microsporidial gill disease caused by *L. salmonae*. The rupture of xenomas causes a multifocal haemorrhagic cystic branchitis.

critically evaluate the host response to xenomas and this diagnostic modality can also be used to stage the infection (e.g. detecting smaller 'pre-xenoma' stages or detection of spores which may be retained in the gills after xenomas have ruptured). Detection of spores is aided by the bi-fringent nature of the microsporidial spore walls (Tiner, 1988), and in our laboratory we find this characteristic for approximately 40% of the spores. Additionally, it is possible to use molecular approaches such as PCR and *in situ* hybridization, or various methods based on the application of monoclonal antibodies (Speare *et al.*, 1998c; Sanchez *et al.*, 1999, 2001b).

7.3. External and Internal Lesions

Although not accompanied by evidence of pathological host response, early stages of *L. salmonae* can be found in the lamina propria of the intestinal tract; the parasite is found subsequently in the cardiac subendocardium, as revealed using *in situ* hybridization studies (Sanchez *et al.*, 2000, 2001a, 2001d) although the mode of the parasite migration is unknown. Lesions visible with routine microscopy and staining are not detected until spore-filled xenomas are formed (and rupture) in the gills and to a much lesser extent in other organs.

The lesions can be divided into two major scenarios, one of which includes the parasite growth and replication phase that occurs within xenomas. In this case the lesion *per se* is the xenoma: an infected host cell that is induced to undergo severe hypertrophy in order to accommodate the developing parasite (Figs. 7.3 and 7.4). The second scenario, a later phase, is when the xenomas rupture, thereby releasing spores into tissue, causing an inflammatory response that leads to tissue damage. The rupture of xenomas causes a multifocal haemorrhagic cystic branchitis.

7.3.1. Early stages and formation of xenomas

The target cell type for *L. salmonae* is either a pillar cell, an endothelial cell or an infected leukocyte that migrates through the basement membrane of a blood vessel and localizes among, or within, pillar and endothelial cells (Rodriguez-Tovar *et al.*, 2002). Several hypotheses (Rodriguez-Tovar *et al.*, 2002) have been put forward to help describe the cellular interactions that permit a circulating leukocyte to cooperatively transfer the immature parasite into another cell which is permissive for the final maturation of the parasite-cell assembly into the characteristic

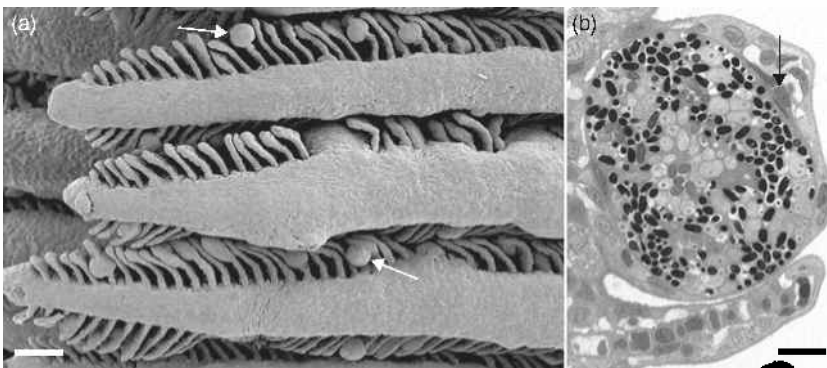


Fig. 7.3. Rainbow trout gills 6 weeks post-infection with *L. salmonae*. (a) A scanning electron micrograph showing the tissue compression caused by multifocal xenomas (arrows) within the lamellae. Bar = 100 μ m. (b) A high resolution light micrograph showing localization of a xenoma within the gill lamellae in close association with a pillar cell (arrow). Staining with toluidine blue. Bar = 10 μ m.

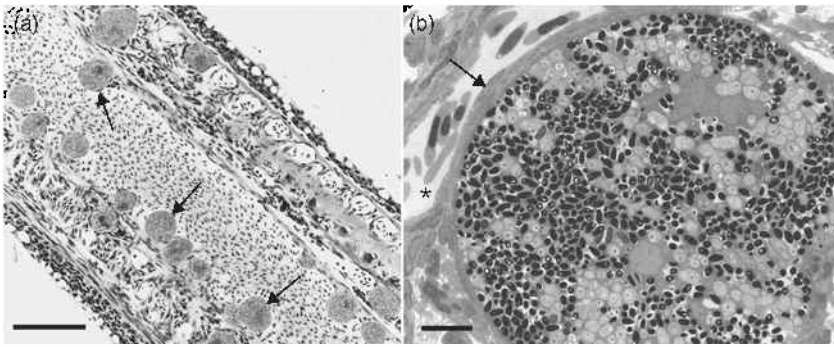


Fig. 7.4. Gills from farmed chinook salmon clinically affected with microsporidial gill disease. (a) A section through a gill filament artery showing numerous xenomas (arrows) attached to the blood vessel wall and protruding into the artery lumen. Staining with haematoxylin and eosin. Bar = 500 µm. (b) A high resolution light micrograph showing a xenoma localized beneath the endothelium (arrow) of the filament artery (the artery lumen is indicated by *). Staining with toluidine blue. Bar = 10 µm.

xenoma. Xenomas can occur within the gill lamellae, where xenomas are found closely associated with pillar cells (Fig. 7.3) or in the gill filament, most frequently within endothelial cells lining arteries and arterioles (Fig. 7.4). Xenomas which rupture within the lamellae often lead to lamellar fusion caused by proliferation of epithelial cells and influx of inflammatory cells. The lamellar lesions tend to be less severe than lesions in the gill filament; in the former location it is likely that the majority of spores will be released into the environment whereas those in the filament will probably become trapped thereby provoking a prolonged host response.

A fascinating, but poorly understood area is the relationship between the parasite and the fish host cell that leads to the development of the xenoma (Williams, 2009); theoretically this may involve inhibition of host-cell apoptosis. Extending the life span of the host cell permits the extended development cycle of the parasite to be completed. Development of the parasite within the xenoma (advancing through merogonial and sporogonial stages), the formation of sporoblasts, and lastly spores is common for *L. salmonae* and many other microsporidia. Through the exploitation of an *in vivo* model and sequential ultrastructural examination of *L. salmonae* development within xenomas, a new hypothesis has emerged on how some

microsporidia exploit host cellular functions, particularly autophagy, to facilitate their development from meronts to sporonts (Lovy *et al.*, 2006b). Autophagy, an intracellular process for eliminating unwanted cytoplasmic elements through degradation, is a common response to the presence of intracellular organisms and injured organelles. Products destined for degradation are enclosed within host endoplasmic reticulum. This forms an autophagosome which subsequently fuses with a lysosome. Degraded proteins are recycled back into the cell. Meronts of *L. salmonae* within xenomas are enclosed by host endoplasmic reticulum membranes as might occur in the first stages of autophagy, however subsequent degradation of the parasite does not occur, but rather the parasite adapts host endoplasmic reticulum membranes for the development of an outer parasite membrane and the limiting membrane of the parasitophorous vacuole. With the parasites enclosed in the former endoplasmic reticulum lumen, containing proteins not freely available in the cytoplasm, development to mature spores occurs (Lovy *et al.*, 2006b). If this process is common among microsporidia it may represent an interesting pathway by which parasites exploit host-cell processes and host-cell structures for their own development. As is the case for highly 'reduced' parasites which offer few targets for chemotherapeutic agents,

further research into how these parasites utilize host-cell machinery, may provide insights into classes of promising chemotherapeutic compounds.

Intracellular localization within the xenoma benefits the developing parasite by helping it to evade host immune responses. *L. salmonae* forms xenomas in the gills of rainbow trout that persist from 4 to 8 weeks, and the xenomas in chinook salmon persist for an even longer period (Ramsay *et al.*, 2002). The intact xenomas elicit, at best, a muted immune response, and the parasite seems to remain well hidden from the host response within the confines of the host cell throughout its development. In *L. salmonae*, intact xenomas are limited by only the xenoma plasma membrane, as this species does not have a thick xenoma wall as in other microsporidian species, such as those of the genus *Glugea*, which has a thick collagenous barrier around the limiting membrane of the xenoma (Lovy *et al.*, 2009a). In *L. salmonae*, although intact xenomas elicit a minimal inflammatory response, there is a fibroblast response which is one or two cell layers thick. This may come about as a result of concentric local tissue damage from compression caused by these massively hypertrophied cells (Fig. 7.5), nevertheless, a unusual feature

of the surrounding fibroblasts is the formation of desmosomes with neighbouring fibroblasts which appears to more efficiently encapsulate the xenomas. The fibroblastic response is not in all xenomas, and often they are only bound by a plasma membrane with minimal response around the periphery. During the late stages of xenoma maturation some phagocytic cells such as neutrophils and macrophages begin to surround the xenoma; this response is thought to be caused by a weak antigenic signal leaking from the xenoma (Rodriguez-Tovar *et al.*, 2003b). Matured xenomas, containing a high proportion of mature spores, also have spores everting their polar filament and piercing host cells around the perimeter of the xenoma. The stimulus to trigger the apparently premature germination of spores within xenomas is unknown. Spore germination within mature xenomas could hypothetically be a method for the parasite to induce or initiate destruction of the xenoma to free other non-germinated pathogenic spores. Small breaches of the xenoma may cause leaking of antigenic signal and subsequent recruitment of inflammatory cells that further enhance xenoma wall destruction; autoinfection is another possibility but this has not been demonstrated in salmonids.

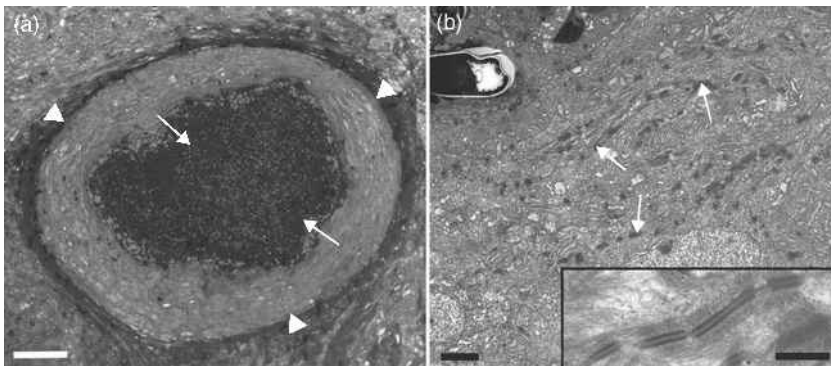


Fig. 7.5. A degraded xenoma of *L. morhua* within the heart of Atlantic cod. (a) A high resolution light micrograph showing the strong 'walling-off' response to a degrading xenoma, with an approximately ten cell-layer thick encapsulation of epithelioid macrophages. Staining with toluidine blue. Bar = 50 μ m. (b) A transmission electron micrograph showing the abundance of desmosomes (arrows) between epithelioid macrophages. Bar = 2 μ m. Inset shows details of the epithelioid macrophage desmosomes. Bar = 500 nm.

7.3.2. Host response subsequent to xenoma rupture

Destruction of the xenoma membrane liberates spores into the surrounding gill tissue and this leads to an inflammatory response that sequentially demonstrates hallmarks of acute reaction (suppurative) transitioning to a chronic reaction (granulomatous). Interestingly, *L. morhua* affecting Atlantic cod causes a typical granulomatous inflammation within the gill, but forms a very different host response within the non-gill organs. *L. morhua* causes severe systemic infections and the degrading, mature xenomas elicit a strong walling-off response, as opposed to the diffuse inflammatory response as seen in salmonids. The response in cod consists of the development of a thick wall of macrophages with an epithelioid-like transformation recognized by large desmosomes (Fig. 7.5). This response is commonly seen in the spleen and heart of cod; a similar response is absent in splenic and cardiac xenomas in salmonids and the significance of the species difference remains to be elucidated.

The host-cell response towards ruptured xenomas begins with neutrophils, but these

are subsequently replaced by macrophages and lymphocytes (Lovy *et al.*, 2007b). Neutrophils are highly phagocytic and within lesions it is typical to see them containing either single or multiple mature spores within a phagosome. However, there is no evidence of spore degradation within neutrophils (Fig. 7.6), which suggests that neutrophils are either unable to degrade the thick exospores/endospores or that the parasite is inhibiting neutrophil functions. It is curious to see such a strong chemotactic and phagocytic response by neutrophils to the spores, especially given the subsequent inability of the neutrophils to degrade them. This raises the possibility that the parasite is using the neutrophil as a method to protect itself and ensure intact spores make it into the environment. In mammals, a strong neutrophil response leads to the development of pustules and the fate of neutrophils is often to be released out of the body through exudates. The neutrophil response in fish with MGDS is heavy enough to cause swelling of the gill filament and epithelial oedema, which can lead to subepithelial blisters full of red blood cells, neutrophils and spores (Fig. 7.7). It is possible that the neutrophils containing

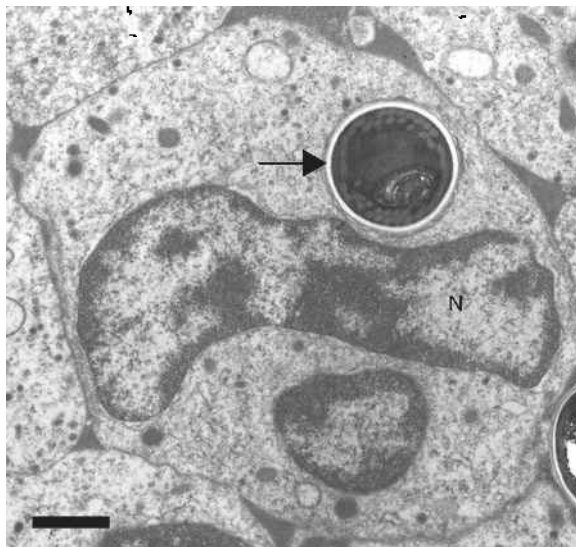


Fig. 7.6. A neutrophil with a segmented nucleus (N) containing a spore of *L. salmonae* (arrow) showing no evidence of lysosomal fusion or degradation. Bar = 1 μ m.

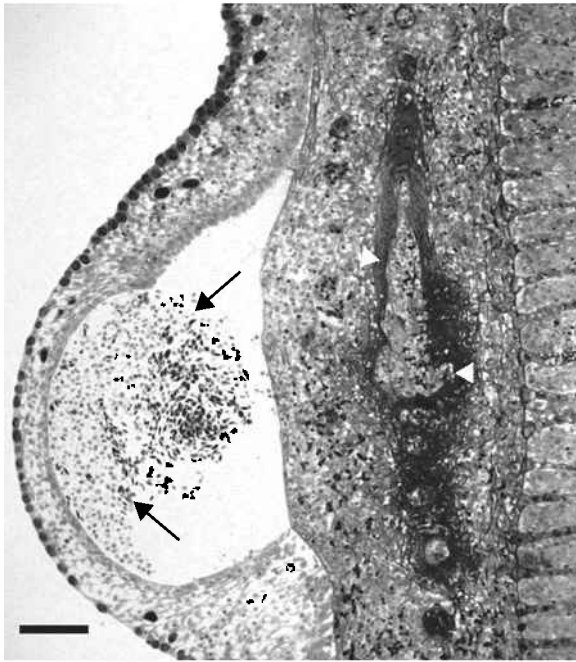


Fig. 7.7. A high resolution light micrograph of a farmed chinook salmon gill with clinical microsporidial gill disease, showing an inflammatory response around the filament artery (arrowheads) causing deposition of fibrin in the adjacent tissue and subepithelial oedema leading to a blister containing red blood cells, neutrophils and spores (arrows). Bar = 100 µm.

intact spores will be released into the environment, making it possible for spores within the gill filament to be released through the exudate. Neutrophils may also be involved in the dissemination of the infection, as it is believed that a leukocyte is responsible for carrying the parasite to the gills during the early stages of infection.

7.3.3. Chronic responses and tissue regeneration

Subsequent to the neutrophil-rich response early after xenoma rupture, macrophages initially arrive in small numbers relative to neutrophils and in chronic inflammatory lesions they become more abundant. In contrast to neutrophils, macrophages are efficiently able to degrade the spores (Fig. 7.8), and chronic inflammatory lesions contain mostly macrophages with degenerated spores.

In addition to phagocytic cells and lymphocytes, cells that resemble Langerhans cells can also be found in areas of acute and chronic inflammation. These cells have peri-centriolar racket-shaped granules containing lattice-structured material structurally similar to Birbeck granules which are characteristic for human Langerhans cells (Lovy *et al.*, 2006a). Although Langerhans cells in mammals are dendritic cells in the epidermis, recent work indicates that these cells in salmonids are typically residents of lymphoid tissues, predominantly found in the spleen and to a lesser extent within the kidney (Lovy *et al.*, 2008b); Langerhans-like cells in salmonids mount systemic responses and can be found within inflammatory lesions. These cells may represent a primitive dendritic cell lineage, forming a key component of the antigen-processing and -presenting system necessary for induction of cell-mediated immunity in salmonids (Lovy *et al.*, 2009b, 2010). Based on their

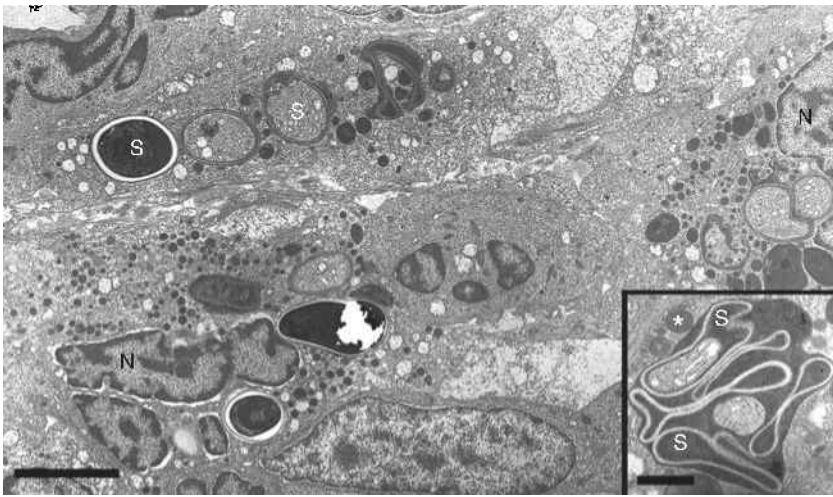


Fig. 7.8. Transmission electron micrograph of a chronic inflammatory lesion within the gill filament of a chinook salmon with clinical MGDS demonstrating three macrophages (N, macrophage nuclei) containing *L. salmonae* spores in various stages of degradation. Bar = 4 μ m. Inset shows degenerated spores (S) and secondary lysosomes (*) within the cytoplasm of the macrophages. Bar = 600 nm.

presence within *L. salmonae*-induced lesions, and given the cell-mediated response that develops in salmonid hosts within 4 weeks following infection (Speare *et al.*, 1998c; Rodriguez-Tovar *et al.*, 2006a), further work into the properties and functions of these cells is clearly warranted.

The endothelial-tropic nature of *L. salmonae* results in vascular pathology being a significant part of the tissue reaction subsequent to xenoma rupture, particularly in farmed chinook salmon clinically affected with MGDS (Lovy *et al.*, 2007b). Arterial damage is evidenced by the deposition of fibrin within blood vessel walls and perivascular connective tissue, and the degree of tissue oedema (Fig. 7.7). *Loma*-induced perivascularitis causes marked alterations to the integrity of the gill vasculature, often accompanied by thrombosis in acute inflammatory lesions and neovascularization in chronic lesions. It is likely that in MGDS-affected chinook salmon, the presence of xenomas, the associated inflammatory response, and the formation of thrombi, when combined, is sufficiently severe so as to alter the course of normal blood flow; vascular remodelling noted late in the disease may be a physiological response to blood flow deficiency (Fig. 7.9). Thrombosis is common

in fish with clinical MGDS sometimes resulting in full obstruction of arteries and arterioles (Fig. 7.10). During xenoma rupture and associated inflammation, the thrombocyte response appears to be a crucial step in early blood vessel repair; neovascularization as a later onset response coincides with other markers of chronic inflammatory lesions such as the presence of macrophages and lymphocytes (Fig. 7.11). A future area of study would be to assess whether the use of treatment drugs aimed at limiting thrombogenesis could reduce mortalities during the pivotal xenoma-rupture stage of MGDS.

Healing of gills following xenoma rupture is an interesting area of study; as inflammation begins to subside, the first evidence of lamellar regeneration appears and this progresses until the gill shows little or no anatomical evidence of prior infection (Fig. 7.12). The regenerative capacity of the gill has not been thoroughly studied to date, but it remains fascinating that fish gills can quickly regenerate. *L. salmonae* in rainbow trout would be an excellent model to better understand the regenerative capacity of gill lamellae after disease and to better understand host and environmental factors that could modify recovery rates.

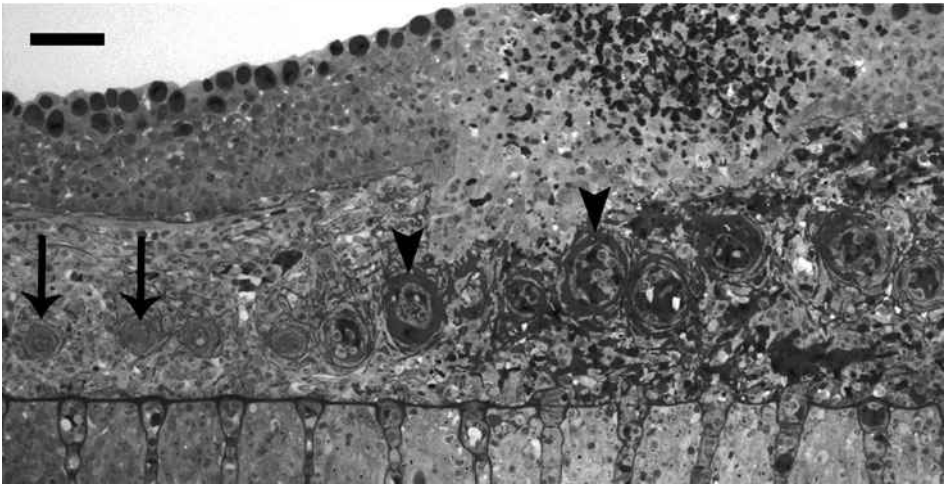


Fig. 7.9. High resolution light micrograph of a farmed chinook salmon gill with clinical MGDS showing vascular remodelling of gill filament arterioles. The affected arterioles (arrowheads) have enlarged lumens and are surrounded with fibrin compared with the adjacent normal arterioles (arrows). Bar = 50 μ m.

7.4. Pathophysiology

7.4.1. Haematology

Although the anatomical pathology of the gill during MGDS has been the subject of intensive study, much work remains to be completed so as to gain a better understanding of the systemic pathophysiological responses of salmonids during MGDS as this may yield vital clues as to treatment geared towards physiological support of fish with MGDS. The remarkably intense inflammatory response noted in the gills is accompanied in the kidney, and to a lesser extent in the spleen, by an increase in myeloid production with a degree of early asynchrony in which immature cells outnumber mature cells. There is a progressive decrease in leukocrit during the course of infection (Powell *et al.*, 2006) suggesting that consumption of white blood cells directed towards gill inflammation exceeds systemic production capacity. No changes were noted in the haematocrit. Assessment of whole-body net ion flux suggests that, despite MGDS causing considerable gill damage, the gill remains able to defend plasma electrolyte concentrations (Powell *et al.*, 2006); however,

it remains to be tested whether, under challenge, infected fish are able to deal with dramatic osmoregulatory issues such as changes in environmental salinity or pH. This may be of interest in the context of the wild salmon fishery, particularly given the recent concerns of high mortality levels of infected wild salmon returning to freshwater rivers and streams to spawn. It could also become of interest in the aquaculture industry if MGDS becomes a hatchery disease as survival of infected smolt subsequent to transport to marine sites could be impacted.

Although the ionoregulatory capacity of MGDS-affected fish remains in place, much work remains to be completed to more fully understand the oxygen exchange disruptions caused by MGDS. In a key study, it has been shown that whereas rainbow trout with MGDS increase their routine metabolic rate, the opposite is true for brook trout (Powell *et al.*, 2005). Furthermore, the maximum post-exercise metabolic rate for infected rainbow trout exceeds that of controls, whereas it remains the same in brook trout (Powell *et al.*, 2005). These may be important factors when we consider the effects incurred by handling/sorting/treating of fish with MGDS, and the

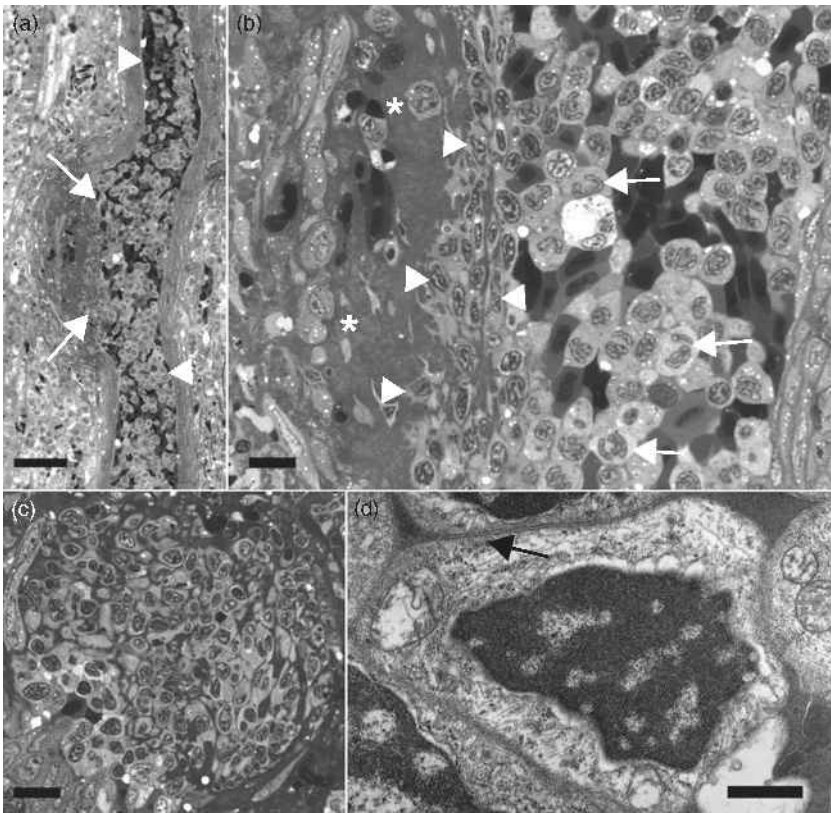


Fig. 7.10. Arterial thrombosis in gills of farmed chinook salmon with clinical MGDS. (a) A major filament artery (arrowheads) with endothelial damage (arrows). Bar = 50 µm. (b) A higher magnification of the damaged endothelial area from (a) showing an aggregation of thrombocytes (arrowheads) and deposition of fibrin (*) in the damaged area. Also notice the abundance of neutrophils (arrows) within the lumen of the artery. Bar = 10 µm. (c) A thrombus fully occluding the luminal space of a filament arteriole. Staining with toluidine blue. Bar = 10 µm. (d) A transmission electron micrograph showing an activated thrombocyte with abundant microtubules and granules (arrow) within the cytoplasm. Bar = 1 µm.

relative cost-effective value of providing supplement oxygen during MGDS outbreaks.

7.4.2. Effects of MGDS on growth indices

Despite many suggestions that diseases of fish limit their growth and/or feed conversion efficiency there are relatively few studies that have identified the extent to which this actually occurs. In studies with rainbow trout, it has been shown that the specific growth rate (SGR) of fish begins to decline coincident with the dissolution of xenomas (Speare *et al.*, 1998c), a temporal pattern

which had been anticipated due to the degree of host response that is elicited by xenoma rupture. Prior to xenoma rupture, growth rates of infected and control fish were identical; the period of SGR reduction persisted for 6 weeks during which all xenomas ruptured from the gills. Significant differences in biomass, stemming from reduced growth rates, were detected by week 9 post-infection. SGR rates of infected fish recovered to match those of control fish by week 10 post-infection (Speare *et al.*, 1998c). A follow-up study demonstrated that SGR reductions were correlated with both a marked reduction in appetite (reduced by 33–46%) and an

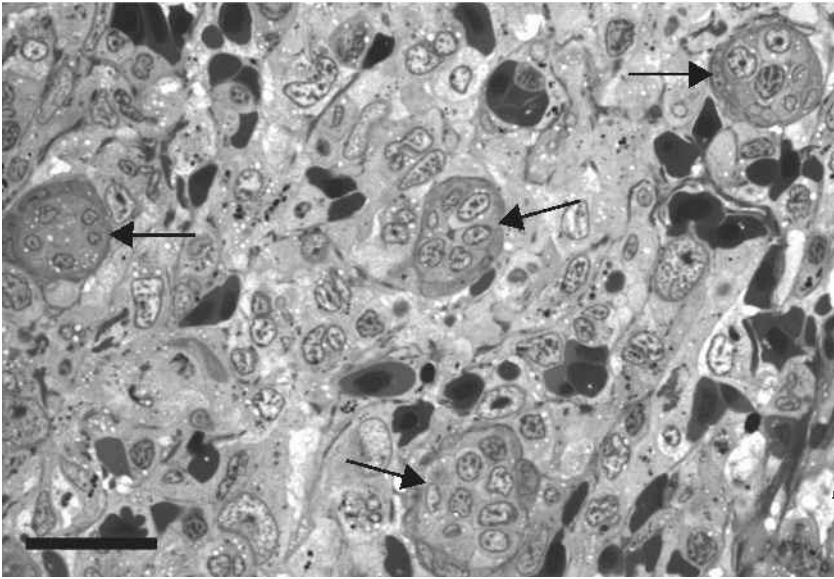


Fig. 7.11. High resolution light micrograph of a chronic inflammatory lesion with neovascularization (arrows) caused by *L. salmonae* in the gill filament of chinook salmon. Staining with toluidine blue. Bar = 20 µm.

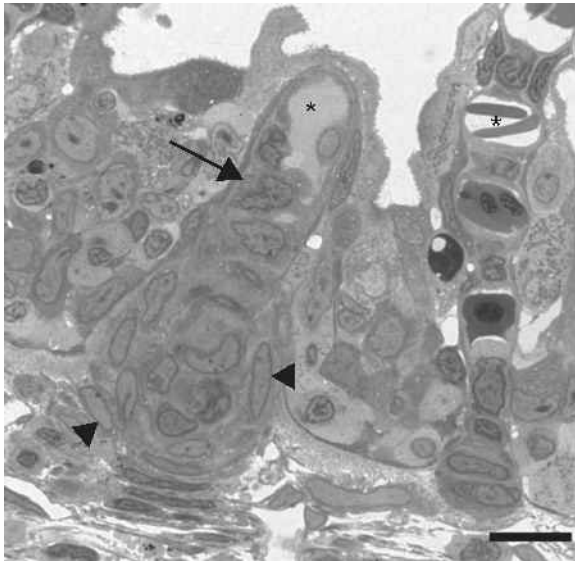


Fig. 7.12. High resolution light micrograph of a regenerating gill lamella from a rainbow trout recovering from infection with *L. salmonae*. An aggregation of non-differentiated cells are observed at the base of the lamella (arrowheads) and a newly formed pillar cell (arrow) is developing its attachments to the basement membrane in the blood vessel lumen (*). Notice the normal structure of the gill lamellae on the right side of the micrograph. Bar = 10 µm.

impairment in feed conversion efficiency (efficiency reduced by 50–95%); compared with appetite reduction, changes in feed conversion efficiency had a later onset and continued for several weeks after appetite had recovered (Ramsay *et al.*, 2004). Based on the timing of changes to appetite, and conversion efficiency relative to changes in SGR, it can be shown that appetite suppression has the greatest negative impact on fish growth, whereas feed conversion impairment, occurring later in the course of MGDS, was more likely linked to diversion of energy into repair of gill tissue rather than somatic growth. A degree of compensatory growth and increased food intake was also noted in the weeks following recovery from MGDS (Ramsay *et al.*, 2004). These results suggest that continuing to feed fish during MGDS outbreaks is likely to be wasteful; however, towards the end of MGDS it would be of benefit to provide increased access to rations, despite the diminished conversion indices, to allow for gill recovery and compensatory growth.

7.4.3. Regulatory effects of water temperature

Given the role of temperature in the metabolism of fish and parasites, it is anticipated that the seasonality and temporal course of MGDS will be heavily influenced by water temperature. However, this relationship has yet to be fully utilized in treatment and control protocols. For example, an understanding of the relationship of disease development with water temperature might suggest allowing harvesting of susceptible populations of fish ahead of forecasted outbreaks. The summer and early autumn pattern of MGDS has been reported for Pacific salmon in marine netpens in Washington State, USA (Kent *et al.*, 1989), in British Columbia, Canada (Speare *et al.*, 1989), and in earlier reports of *L. salmonae* affecting other salmonid species in freshwater (Markey *et al.*, 1994; Bader *et al.*, 1998), although it has also been postulated by some authors that factors other than water temperature, such as seasonal variation in mineral content of water supplies and season per se,

may be critical determinant factors. Perhaps a more important reason to understand the thermal regulatory biology of *L. salmonae* is to help design timing strategies for the use of antimicrosporidial treatments, given that some of these may only be effective on certain stages of the pathogen.

The preferred water temperature for *L. salmonae* is between 13 and 17°C (Beaman and Speare, 1999; Becker *et al.*, 2003; Becker and Speare, 2004b) and within this range, and compared with temperatures slightly outside of this range (11 and 19°C), survival of the parasite through to xenoma formation is optimized. At water temperatures below 11 or above 20°C xenoma formation is sharply inhibited (Beaman and Speare, 1999) and parasite development is abrogated before reaching the gills (Sanchez *et al.*, 2000); the potential for the parasite to continue its life cycle once water temperatures increase to permissive ranges has been demonstrated with a quiescent period of over 4 weeks documented (Beaman and Speare, 1999). Through the preferred temperature range of 13–17°C, the rate of development of xenomas relative to water temperature has been effectively modelled: the rate of development follows temperature through a polynomial relationship [Xenoma onset (days) = $33.4(T) + 0.954(T^2)$], where T is degrees Celsius. This can be further simplified through a thermal unit model that corrects for a 'no development temperature' (NDT) of 7°C below which parasite development halts (Beaman and Speare, 1999). Xenomas can be predicted to form once 260–304 thermal units (TUs) have been logged; working backwards from the time of MGDS outbreaks, one can extrapolate the exposure window and from this construct a treatment protocol (Speare *et al.*, 1999b) for subsequent years. In the Pacific Northwest, based on MGDS outbreaks noted in late August and early September, and further by examining ocean water-temperature data from the sea-cage sites, the TU value of 280 points to a period in June where fish are most likely to be acquiring their initial infections with *L. salmonae*. Accordingly, a treatment window constructed during June well in advance of the first evidence of MGDS (based on

detection of xenomas), is likely to be critical for the success of any chemotherapeutic approach to this disease.

7.5. Protective and Control Strategies

7.5.1. Protecting against exposure to spores

Strict avoidance of exposure to *L. salmonae* can only be achieved with land-based cultivation where control of water sources is possible and where the farm has a closed population and with diligent screening of in-coming stock. Water from a drilled well is unlikely to be contaminated with *Loma* spores, while lake water may also be expected to be *Loma*-free if the lake does not contain a salmonid population. Ultraviolet (UV) treatment of in-coming water can block horizontal transmission of MGDS (Becker and Speare, 2004a) even in situations anticipated to result in high spore loading of in-coming water. The use of recirculation technologies will be of value as it limits the volume of water that may be needed. However, recirculation systems can exacerbate an outbreak by retaining infective spores within the system and particularly so if the recirculation system is used to elevate and maintain water temperature into the range favoured by the parasite (13–17°C) (Beaman and Speare, 1999). Coupling recirculation with the use of UV is therefore suggested.

Avoidance approaches, although suitable to salmon hatcheries and other land-based operations, are not practical for marine netpens. It is expected that salmon in marine netpens will be exposed to spores that are carried in the water column and it is common for juvenile wild salmon to enter netpens, especially when pit lamps are used to alter perceived daylength. MGDS has been transmitted in fresh and salt water and the ease of cohabitation transmission has been demonstrated (Shaw *et al.*, 1998; Becker *et al.*, 2003). Remarkably, the brief cohabitating presence of even one moderately infected fish is sufficient to broadly introduce *L. salmonae* into a naïve population (Becker *et al.*, 2005b).

As discussed by Shaw *et al.* (1999), early suggestions, based on the observations of *L. salmonae* spores in the ovaries of sexually mature chinook salmon, supports the concerns that MGDS may also be transmitted either vertically, or congenitally, from brood-stock to offspring. This has yet to be substantiated. However, for those culture situations where this is suspected to be the reason for persistent multigenerational problems with MGDS, control may prove difficult based on the limited success of iodophor treatments even when iodophor concentrations used are much higher than industry standards for other diseases (Shaw *et al.*, 1999), attesting to the resilience inherent to spore structures.

7.5.2. Marketing ahead of losses

The seasonal nature of MGDS provides an opportunity to develop production and marketing strategies ahead of MGDS outbreaks. A general goal of marketing fish prior to late summer or early autumn is a reasonable strategy, although it is also feasible to use water temperature history, a thermal model (Beaman and Speare, 1999) and sequential screening of fish to more precisely predict MGDS losses.

7.5.3. Use of more resistant strains/related species

Interesting work by Shaw *et al.* (2000b) who assessed three strains of chinook salmon in British Columbia points to the feasibility of selecting strains of fish with enhanced natural resistance to MGDS. Their work showed that although fish strain did not affect prevalence of infection, it did confer a significant effect on the intensity of infection and subsequent mortality. Although the mechanism of this strain-specific resistance was not fully deduced, these authors later investigated species-specific natural resistance and found that macrophages of Atlantic salmon, a species that appears to have natural resistance to *L. salmonae* (Shaw *et al.*, 2000c), have a significantly higher phagocytic response to spores of *L. salmonae* compared with the highly susceptible chinook salmon (Shaw *et al.*, 2001).

Rainbow trout, although susceptible to *L. salmonae*, typically develop far fewer xenomas compared to chinook or coho salmon (Ramsay *et al.*, 2002), and brook trout are far less susceptible than rainbow trout (Shaw *et al.*, 2000c; Sanchez *et al.*, 2001c; Speare and Daley, 2003). The mechanism(s) behind these species variations remains to be fully elucidated although Sanchez *et al.* (2001a) demonstrated that in Atlantic salmon the parasite successfully enters the lamina propria of the intestine, is transported to the heart (potentially reaching the subendocardial macrophages) but fails to subsequently reach the gill. The infection stalls in the heart and the parasite life cycle does not proceed to sporogony. These findings suggest that macrophage-mediated destruction of the parasite within the subendocardial macrophages, or during the translocation from heart to gill, may be key limiting steps.

7.5.4. Site fallowing

It can be estimated that the number of fully formed spores released from a netpen stocked with salmon at commercial densities is in the range of 100 million spores/day, and this rate of spore release is expected to extend over a 4 week period. However, very little is currently known about the physical characteristics of these spores (e.g. settling and dispersion rates, the extracorporeal viability of spores) under a range of environmental conditions. Shaw *et al.* (2000a) demonstrated that spore survival at cool temperatures (4°C), can exceed 3 months, but before fallowing policies can be considered, further work is clearly required to examine variables such as temperature, salinity and microenvironmental conditions in sediment.

The importance of year-class separation is highlighted by Kent *et al.* (1999) and Ramsay *et al.* (2002) who showed that in both chinook and coho salmon, and to a lesser extent in rainbow trout (Ramsay *et al.*, 2001, 2002), xenoma clearance runs for an extended period and fish remain infective for prolonged periods. Furthermore, Ramsay *et al.* (2001) demonstrated that even with rainbow

trout (a species in which branchial xenomas tend to clear relatively quickly) fish will remain potentially infective (have infective spores in their viscera) for over 20 weeks. This 'duration of potential infectivity' is expected to be much longer in chinook and coho salmon compared with rainbow trout.

7.5.5. Antiparasitic treatments

In vitro screening of antimicrosporidial compounds has not yet been possible because there is no cell line that supports parasite growth and multiplication. Consequently, this remains an important future goal. In its absence, the development of refined, standardized *in vivo* models for MGDS (Speare *et al.*, 1998a), with quantifiable outcomes including prevalence and infection intensity (Beaman and Speare, 1999), or survival analysis (Ramsay *et al.*, 2003) have been developed for a range of antiparasitic agents against MGDS. In general, it is unlikely that anti-infective chemotherapeutic strategies will have much effect on *L. salmonae* once it has reached the spore-laden xenoma stage, therefore it will be critical to use the thermal unit model previously described to predict the timing for effective application of promising chemotherapeutic agents.

A high degree of efficacy has been demonstrated with orally administered fumagillin (Kent and Dawe 1994), albendazole (Speare *et al.*, 1999a) and the cationic ionophore monensin (Speare *et al.*, 2000). These treatments resulted in a 70% decrease in the numbers of xenomas forming in treated versus untreated fish. This degree of reduction is likely to translate into a marked reduction in gill pathology and perhaps reduce the disease into a subclinical form. Monensin, a sodium ionophore that is selectively active on post-Golgi endosome and Golgi subcompartments (Dinter and Berger, 1998) which, in microsporidia, may have a key function in the development of the coiled polar tube (Vavra and Larrison, 1999), to date has proven to be the most effective treatment drug, achieving a xenoma reduction rate up to 93% while having no untoward effects on fish growth rates

and feed conversion efficacy (Speare *et al.*, 2000; Becker *et al.*, 2002). Early, rather than later, treatment of MGDS is highlighted by the loss of efficacy of monensin when treatment is not given during the earliest stages of infection (Becker *et al.*, 2002). Some drugs, notably quinine hydrochloride, are effective only in delaying the rate of onset of xenomas (Speare *et al.*, 1998d).

7.5.6. Use of immunomodulators

There is a growing repository of recent papers demonstrating the efficacy of glucans containing β -1,3 and β -1,6 glycosidic linkages (known as beta glucans) against a wide range of pathogens affecting farmed fish. Administration of beta glucan through intraperitoneal injection or with oral delivery in feed can markedly reduce the severity of MGDS in experimental trials (Guselle *et al.*, 2006, 2010), although the efficacy is only noted when used early in the course of infection (Guselle *et al.*, 2007). Given the present-day concerns over the use of drugs in aquaculture, immunostimulation is likely to be the treatment protocol of choice adopted by the aquaculture industry.

7.5.7. Protective host response and vaccine development

The possibility of developing a vaccine for MGDS is based on the observation that recovered fish, even when the initial challenge is at water temperatures that do not permit xenomas to form, demonstrate a remarkably strong resistance to reinfection, even when challenged with massive numbers of infective spores (Speare *et al.*, 1998b; Kent *et al.*, 1999; Beaman *et al.*, 1999). Vaccine prototypes based on killed whole-spore preparations of either the virulent or low-virulence strains of *L. salmonae* have been shown to be effective with rainbow trout developing a strong protective cell-mediated immune response within 3 weeks following vaccination (Sanchez *et al.*, 2001a; Rodriguez-Tovar *et al.*, 2006a, b). Acquired immunity appears to

block the ability of the parasite to transfer from the subendocardial macrophages to the gill pillar cells (Sanchez *et al.*, 2001a), a transfer that is probably mediated by intracellular transport (Rodriguez-Tovar *et al.*, 2002). Protection arising from exposure to these spore-based vaccine prototypes is exceptionally robust and, even without the use of adjuvants, protection exceeds 8 months (Speare *et al.*, 2007). Dexamethasone, known to diminish the innate immune responses to *L. salmonae*, does not diminish the functional protective immunity acquired by *L. salmonae* vaccination (Lovy *et al.*, 2008a). Although rainbow trout and chinook salmon develop effective immune responses against *L. salmonae*, the vaccine in brook trout appears to be less effective (Speare and Daley, 2003) and in the laboratory they can be sequentially reinfected many times. The spore-based prototype vaccine against *L. salmonae* represents the first example of a vaccine developed against a microsporidial disease (Speare *et al.*, 2007). Vaccination should therefore be considered for microsporidial diseases of fish and other vertebrates.

7.5.8. Environmental modulation

Since water temperature affects the host-pathogen interaction the use of cooler water (below 11°C) is expected to markedly constrain MGDS. Also at the higher end of the permissive temperature range (20°C), we can expect salmonids at this temperature to have fewer outbreaks.

Although blood-gas assessments of oxygen and carbon dioxide levels in fish with MGDS have not been completed, the severe degree of gill damage, when combined with the behavioural changes in MGDS fish (see section 7.2), suggest that supplemental oxygenation may prove useful in reducing mortality.

7.5.9. Use of anti-inflammatory agents

Based on the temporal pathobiology of MGDS, in which lesions in the gill arise only after xenomas rupture and host inflammatory

responses are initiated, it appeared useful to examine the potential therapeutic role afforded by the use of anti-inflammatory agents. Previous work by Davis (1994) supports this idea, and showed that catfish with proliferative gill disease (myxosporidial) developed less severe gill lesions when treated with indomethacin, a non-steroidal anti-inflammatory drug that downregulates the manufacture of prostaglandins by inhibiting cyclooxygenase (inducible and constitutive). However, there does not appear to be any benefit of indomethacin administration to rainbow trout with MGDS. Lovy *et al.* (2007a) demonstrated gastric ulceration as a severe side effect of indomethacin treatment, particularly at higher dosages. These results suggest that if anti-inflammatory agents are considered for use in fish, then gastro-protective precautions similar to those used for mammalian treatment regimes must be taken into consideration.

7.6. Conclusions and Suggestions for Future Studies

MGDS is a recurrent problem for the aquaculture production of Pacific salmon, and to a lesser degree in other cultivated species. Through the development of a quantifiable *in vivo* model of MGDS, subsequent research has elucidated much about the life cycle and

transmission of the parasite along with the role of temperature in modulating the infection. Several promising drug treatments including immunostimulants have been evaluated in the laboratory, and a prototype spore-based vaccine has been developed and tested successfully under laboratory conditions. Much remains to be understood with respect to the dynamics of infection, transmission and subsequent amplification of the disease at farm sites. The reason(s) for MGDS striking salmon as they reach market weight is unclear. These important questions could be more fully understood through ongoing surveillance programmes aimed at identifying the MGDS status of salmon during their first and second year at marine sites. With our knowledge of the regulating role of water temperature on infection, and observations of the efficacy of several treatment approaches (administered during the early phases of infection), the stage is set for field trial evaluations. Vaccination against MGDS clearly holds significant promise and developing methods to generate commercial quantities of spores (for killed whole-spore preparations) will be an essential step. Alternatively, work to identify candidate genes for DNA-based vaccines is clearly indicated despite the cautionary note of the historical challenges encountered in developing effective vaccines against parasitic diseases.

References

- Bacchi, C.J., Weiss, L.M., Lane, S. and Wittner, M. (2002) Novel synthetic polyamines are effective in the treatment of experimental microsporidiosis, an opportunistic AIDS-associated infection. *Antimicrobial Agents and Chemotherapy* 46, 55–61.
- Bader, J.A., Shotts, E.B., Steffens, W.L. and Lom, J. (1998) Occurrence of *Loma* cf. *salmonae* in brook, brown and rainbow trout from Buford trout hatchery, Georgia, USA. *Diseases of Aquatic Organisms* 34, 211–216.
- Beaman, H.J., and Speare, D.J. (1999) Regulatory effects of water temperature on *Loma salmonae* (Microspora) development in rainbow trout. *Journal of Aquatic Animal Health*, 11, 237–245.
- Beaman, H.J., Speare, D.J., Brimacombe, M. and Daley, J. (1999) Evaluating protection against *Loma salmonae* generated from primary exposure of rainbow trout, *Oncorhynchus mykiss* (Walbaum) outside of the xenoma-expression temperature boundaries. *Journal of Fish Diseases* 22, 445–450.
- Becker, J.A. and Speare, D.J. (2004a) Ultraviolet light control of horizontal transmission of *Loma salmonae*. *Journal of Fish Diseases* 27, 177–180.
- Becker, J.A. and Speare, D.J. (2004b) Impact of 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 Speare, D.J. (2007) Transmission of the microsporidian gill parasite, *Loma salmonae*. *Animal Health Research Reviews* 8, 59–68.
- Becker, J.A., Speare, D.J., Daley, J. and Dick, P. (2002) Effects of monensin dose and treatment time on xenoma reduction in microsporidian gill disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 25, 673–680.
- Becker, J.A., Speare, D.J. and Dohoo, I.R. (2003) Effect of water temperature and flow rate on the transmission of Microsporidian Gill Disease caused by *Loma salmonae* in rainbow trout *Oncorhynchus mykiss*. *Fish Pathology* 38, 105–112.
- Becker, J.A., Speare, D.J. and Dohoo, I.R. (2005a) Influence of feeding ratio and size on susceptibility to microsporidian gill disease caused by *Loma salmonae* in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 28, 173–180.
- Becker, J.A., Speare, D.J. and Dohoo, I.R. (2005b) Effects 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.
- Becker, J.A., Speare, D.J. and Dohoo, I.R. (2006) Interaction of water temperature and challenge model on xenoma development rates for *Loma salmonae* (Microspora) in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 29, 139–145.
- Bigliardi, E., Bernuzzi, A.M., Corona, S., Gatti, S., Scaglia, M. and Sacchi, L. (2000). *In vitro* efficacy of Nikkomycin Z against the human isolate of the microsporidian species *Encephalitozoon hellem*. *Antimicrobial Agents and Chemotherapy* 44, 3012–3016.
- Bruno, D.W., Collins, R.O. and Morrison, C.M. (1995) The occurrence of *Loma salmonae* (Protozoa: Microspora) in farmed rainbow trout, *Oncorhynchus mykiss* Walbaum, in Scotland. *Aquaculture* 133, 341–344.
- Casal, G., Matos, E., Teles-Grilo, M.L. and Azevedo, C. (2009) Morphological and genetical description of *Loma psittica* sp. n. isolated from the Amazonian fish species *Colomesus psittacus*. *Parasitology Research* 105, 1261–1271.
- Constantine, J. (1999) *Estimating the Cost of Loma salmonae to B.C. Aquaculture*. Animal Health Branch, Ministry of Agriculture and Food, British Columbia.
- Costa, S.F. and Weiss, L.M. (2000) Drug treatment of microsporidiosis. *Drug Resistance Updates* 3, 384–399.
- Davis, S.W. (1994) Effect of indomethacin on the development of proliferative gill disease. *Journal of Aquatic Animal Health* 6, 122–125.
- Dinter, A. and Berger, E.G. (1998) Golgi-disturbing agents. *Histochemistry and Cell Biology* 109, 571–590.
- Gandhi, S., Locatelli, L. and Feist, S.W. (1995) Occurrence of *Loma* sp. (Microsporidia) in farmed rainbow trout (*Oncorhynchus mykiss*) at a farm site in south west England. *Bulletin of the European Association of Fish Pathologists* 15, 58–60.
- Guselle, N.J., Markham, R.J.F. and Speare, D.J. (2006) Intraperitoneal administration of β -1,3/1,6-glucan to rainbow trout, *Oncorhynchus mykiss* (Walbaum), protects against *Loma salmonae*. *Journal of Fish Diseases* 29, 375–381.
- Guselle, N.J., Markham, R.J.F. and Speare, D.J. (2007) Timing of intraperitoneal administration of β -1,3/1,6-glucan to rainbow trout (*Oncorhynchus mykiss*) affects protection against the microsporidian *Loma salmonae*. *Journal of Fish Diseases* 29, 1–8.
- Guselle, N.J., Speare, D.J. and Markham, R.J.F. (2010) Efficacy of intraperitoneally and orally administered ProVale, a yeast beta-(1,3)/(1,6)-D-glucan product, in inhibiting xenoma formation by the Microsporidian *Loma salmonae* on rainbow trout gills. *North American Journal of Aquaculture* 72, 65–72.
- Hauck, A.K. (1984) A mortality and associated tissue reactions of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), caused by the microsporidian *Loma* sp. *Journal of Fish Diseases* 7, 217–229.
- Keeling, P.J. and Fast, N.M. (2002) Microsporidia: biology and evolution of highly reduced intracellular parasites. *Annual Reviews in Microbiology* 56, 93–116.
- Kent, M.L. and Dawe, S.C. (1994) Efficacy of Fumagillin DCH against experimentally induced *Loma salmonae* (Microspora) infections in chinook salmon *Oncorhynchus tshawytscha*. *Diseases of Aquatic Organisms* 20, 231–233.
- Kent, M.L. and Speare, D.J. (2005) Review of the sequential development of *Loma salmonae* (Microsporidia) based on experimental infections with rainbow trout (*Oncorhynchus mykiss*) and chinook salmon (*O. tshawytscha*). *Folia Parasitologia* 52, 1–6.
- Kent, M.L., Elliot D.G., Groff, J.M. and Hedrick R.P. (1989) *Loma salmonae* (Protozoa: Microspora) infections in seawater reared coho salmon *Oncorhynchus kisutch*. *Aquaculture* 60, 211–222.
- Kent, M.L., Dawe, S.C. and Speare, D.J. (1995) Transmission of *Loma salmonae* (Microsporea) to chinook salmon in seawater. *Canadian Veterinary Journal* 36, 98–101.

- Kent, M.L., Traxler, G.S., Kieser, D., Richard, J., Dawe S.C., Shaw, R.W., Prosperi-Porta, G., Ketcheson, J. and Evelyn, T.P.T. (1998) Survey of salmonid pathogens in ocean-caught fishes in British Columbia, Canada. *Journal of Aquatic Animal Health* 10, 211–219.
- Kent, M.L., Dawe, S.C. and Speare, D.J. (1999) Resistance to reinfection in chinook salmon *Oncorhynchus tshawytscha* to *Loma salmonae* (Microsporidia). *Diseases of Aquatic Organisms* 37, 205–208.
- Lom, J. and Nilsen, F. (2003) Fish microsporidia: fine structural diversity and phylogeny. *International Journal for Parasitology* 33, 107–127.
- Lovy, J., Wright, G.M. and Speare, D.J. (2006a) Morphological presentation of a dendritic-like cell within the gills of chinook salmon infected with *Loma salmonae*. *Developmental and Comparative Immunology* 30, 259–263.
- Lovy, J., Wright, G.M., Wadowska, D.W. and Speare, D.J. (2006b) Ultrastructural morphology suggesting a new hypothesis for development of microsporidians seen in *Loma salmonae* infecting the gills of trout. *Journal of Fish Biology* 68, 450–457.
- Lovy, J., Wright, G.M. and Speare, D.J. (2007a) Pathological effects caused by chronic treatment of rainbow trout with indomethacin. *Journal of Aquatic Animal Health* 19, 94–98.
- Lovy, J., Wright, G.M. and Speare, D.J. (2007b) Ultrastructural examination of the host inflammatory response within gills of netpen reared chinook salmon (*Oncorhynchus tshawytscha*) with Microsporidial Gill Disease. *Fish and Shellfish Immunology* 22, 131–149.
- Lovy, J., Speare, D.J., Stryhn, H. and Wright, G.M. (2008a) Effects of dexamethasone on host innate and adaptive immune responses and parasite development in rainbow trout *Oncorhynchus mykiss* infected with *Loma salmonae*. *Fish and Shellfish Immunology* 26, 1–10.
- Lovy, J., Wright, G.M. and Speare, D.J. (2008b) Comparative cellular morphology suggesting the existence of resident dendritic cells within immune organs of salmonids. *The Anatomical Record* 291, 456–462.
- Lovy, J., Kostka, M., Dykova, I., Arsénault, G., Peckova, H., Wright, G.M. and Speare, D.J. (2009a) Phylogeny and morphology of *Glugea hertwigi* from rainbow smelt *Osmerus mordax* found in Prince Edward Island, Canada. *Diseases of Aquatic Organisms*, 86, 235–243.
- Lovy, J., Savidant, G.P., Speare, D.J. and Wright, G.M. (2009b) Langerin CD/207 positive dendritic-like cells in the haemopoietic tissues of salmonids. *Fish and Shellfish Immunology* 27, 365–368.
- Lovy, J., Wright, G.M., Speare, D.J., Tým, T. and Dykova, I. (2010) Comparative ultrastructure of Langerhans-like cells in spleens of ray-finned (Actinopterygii) fishes. *Journal of Morphology* 271, 1229–1239.
- Markey, P.T., Blazer, V.S., Ewing, M.S. and Kocan, K.M. (1994) *Loma* sp. in salmonids from the Eastern United States: associated lesions in rainbow trout. *Journal of Aquatic Animal Health* 6, 318–328.
- Morrison, C.M. and Sprague, V. (1983) *Loma salmonae* (Putz, Hoffman and Dunbar, 1965) in the rainbow trout, *Salmo gairdneri* Richardson, and *L. fontinalis* sp. nov. (Microsporidia) in the brook trout, *Salvelinus fontinalis* (Mitchill). *Journal of Fish Diseases* 6, 345–353.
- Mustafa, A., Speare, D.J., Daley, J., Conboy, G.A. and Burka, J.F. (2000) Enhanced susceptibility of sea water cultured rainbow trout to the microsporidian *Loma salmonae* during a primary infection with the 'sea louse' *Lepeophtheirus salmonis*. *Journal of Fish Diseases* 23, 337–341.
- Powell, M.D., Speare, D.J., Daley, J. and Lovy, J. (2005) Differences in metabolic response to *Loma salmonae* infection in juvenile rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis*. *Diseases of Aquatic Organisms* 67, 233–237.
- Powell, M.D., Speare, D.J. and Becker, J.A. (2006) Whole body net ion fluxes, plasma electrolyte concentrations and haematology during a *Loma salmonae* infection in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 29, 727–735.
- Poynton, S.L. (1986) Distribution of the flagellate *Hexamita salmonis* Moore, 1922 and the microsporidian *Loma salmonae* Putz, Hoffman and Dunbar, 1965 in brown trout, *Salmo trutta* L. and rainbow trout, *Salmo gairdneri* Richardson, in the River Itchen (UK) and three of its fish farms. *Journal of Fish Biology* 29, 417–429.
- Ramsay, J.M., Speare, D.J., Sanchez, J.G. and Daley, J. (2001) The transmission potential of *Loma salmonae* (Microspora) in the rainbow trout, *Oncorhynchus mykiss* (Walbaum), is dependent upon the method and timing of exposure. *Journal of Fish Diseases* 24, 453–460.
- Ramsay, J.M., Speare, D.J., Dawe, S.C. and Kent, M.L. (2002) Xenoma formation during microsporidial gill disease of salmonids caused by *Loma salmonae* is affected by host species (*Oncorhynchus tshawytscha*, *O. kisutch*, *O. mykiss*) but not by salinity. *Diseases of Aquatic Organisms* 48, 125–131.
- Ramsay, J.M., Speare, D.J., Becker, J.A. and Daley, J. (2003) *Loma salmonae*-associated xenoma onset and clearance in rainbow trout, *Oncorhynchus mykiss* (Walbaum): comparisons of *per os* and cohabitation exposure using survival analysis. *Aquaculture Research* 34, 1329–1335.

- Ramsay, J.M., Speare, D.J. and Daley, J. (2004) Timing of changes in growth rate, feed intake and feed conversion in rainbow trout, *Oncorhynchus mykiss* (Walbaum), experimentally infected with *Loma salmonae* (Microsporida). *Journal of Fish Diseases* 27, 425–429.
- Rodriguez-Tovar, L.E., Wright, G.M., Wadowska, D.W., Speare, D.J. and Markham, R.J.F. (2002) Ultrastructural study of the early development and localization of *Loma salmonae* in the gills of experimentally infected rainbow trout. *Journal of Parasitology* 88, 244–254.
- Rodriguez-Tovar, L.E., Wadowska, D.W., Wright, G.M., Groman, D.B., Speare, D.J. and Whelan, D.S. (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.
- Rodriguez-Tovar, L.E., Wright, G.M., Wadowska, D.W., Speare, D.J. and Markham, R.J.F. (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.
- Rodriguez-Tovar, L.E., Speare, D.J., Markham, R.J.F. and Daley, J. (2004) Predictive modelling of post-onset xenoma growth during Microsporidial Gill Disease (*Loma salmonae*) of salmonids. *Journal of Comparative Pathology* 131, 330–333.
- Rodriguez-Tovar, L.E., Becker, J.A., Markham, R.J.F. and Speare, D.J. (2006a) Induction time for resistance to microsporidial gill disease caused by *Loma salmonae* following vaccination of rainbow trout (*Oncorhynchus mykiss*) with a spore-based vaccine. *Fish and Shellfish Immunology* 21, 170–175.
- Rodriguez-Tovar, L.E., Markham, R.J.F., Speare, D.J. and Sheppard, J. (2006b) Cellular immunity in salmonids infected with the microsporidian parasite *Loma salmonae* or exposed to non-viable spores. *Veterinary Immunology and Immunopathology* 114, 72–83.
- Sanchez, J.G., Speare, D.J. and Markham, R.J.F. (1999) Nonisotopic detection of *Loma salmonae* in rainbow trout (*Oncorhynchus mykiss*) gills by *in situ* hybridization. *Veterinary Pathology* 36, 610–612.
- Sanchez, J.G., Speare, D.J. and Markham, R.J.F. (2000) Normal and aberrant tissue distribution of *Loma salmonae* (Microsporida) within rainbow trout (*Oncorhynchus mykiss*) following experimental infection at water temperatures within and outside of the xenoma-expression temperature boundaries. *Journal of Fish Diseases* 23, 235–242.
- Sanchez, J.G., Speare, D.J. and Markham, R.J.F. (2001a) Altered tissue distribution of *Loma salmonae*: effects of natural and acquired resistance. *Journal of Fish Diseases* 24, 33–40.
- Sanchez, J.G., Speare, D.J., Markham, R.J.F. and Jones, S.R.M. (2001b) Experimental vaccination of rainbow trout against *Loma salmonae* using a live low-virulence variant of *L. salmonae*. *Journal of Fish Biology* 59, 427–441.
- Sanchez, J.G., Speare, D.J., Markham, R.J.F. and Jones, S.R.M. (2001c) Isolation of a *Loma salmonae* variant: biological characteristics and host range. *Journal of Fish Biology* 59, 427–441.
- Sanchez, J.G., Speare, D.J., Markham, R.J.F., Wright, G.M. and Kibenge, F.S.B. (2001d) Localization of the initial developmental stages of *Loma salmonae* in rainbow trout (*Oncorhynchus mykiss*). *Veterinary Pathology* 38, 540–546.
- Shaw, R.W., Kent, M.L. and Adamson, M.L. (1998) Modes of transmission of *Loma salmonae* (Microsporida). *Diseases of Aquatic Organisms* 33, 151–156.
- Shaw, R.W., Kent, M.L. and Adamson, M.L. (1999) Iodophor treatment is not completely efficacious in preventing *Loma salmonae* (Microsporida) transmission in experimentally challenged chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). *Journal of Fish Diseases* 22, 311–313.
- Shaw, R.W., Kent, M.L. and Adamson, M.L. (2000a) Viability of *Loma salmonae* (Microsporida) under laboratory conditions. *Parasitology Research* 86, 978–981.
- Shaw, R.W., Kent, M.L. and Adamson, M.L. (2000b) Innate susceptibility differences in chinook salmon *Oncorhynchus tshawytscha* to *Loma salmonae* (Microsporida). *Diseases of Aquatic Organisms* 43, 49–53.
- Shaw, R.W., Kent, M.L., Brown, A.M.V., Whipps, C.M. and Adamson, M.L. (2000c) Experimental and natural host specificity of *Loma salmonae* (Microsporida). *Diseases of Aquatic Organisms* 40, 131–136.
- Shaw, R.W., Kent, M.L. and Adamson, M.L. (2001) Phagocytosis of *Loma salmonae* (Microsporida) spores in Atlantic salmon (*Salmo salar*), a resistant host, and chinook salmon (*Oncorhynchus tshawytscha*), a susceptible host. *Fish and Shellfish Immunology* 11, 91–100.
- Speare, D.J. and Daley, J. (2003) Failure of vaccination in brook trout *Salvelinus fontinalis* against *Loma salmonae* (Microsporida). *Fish Pathology* 38, 27–28.
- Speare, D.J. and Ferguson, H.W. (1989) Clinical and pathological features of common gill diseases of cultured salmonids in Ontario. *Canadian Veterinary Journal* 30, 882–887.

- Speare, D.J., Brackett, J. and Ferguson, H.W. (1989) Sequential pathology of the gills of coho salmon with a combined diatom and microsporidian gill infection. *Canadian Veterinary Journal* 30, 571–576.
- Speare, D.J., Arsenault, G.J. and Buote, M.A. (1998a) Evaluation of rainbow trout as a model species for studying the pathogenesis of the branchial microsporidian *Loma salmonae*. *Contemporary Topics in Laboratory Animal Science* 37, 55–58.
- Speare, D.J., Beaman, H.J., Jones, S.R.M., Markham, R.J.F. and Arsenault, G.J. (1998b) Induced resistance in rainbow trout to gill disease associated with the microsporidian gill parasite *Loma salmonae*. *Journal of Fish Diseases* 21, 93–100.
- Speare, D.J., Daley, J., Markham, R.J.F., Sheppard, J., Beaman, H.J. and Sanchez, G.J. (1998c) *Loma salmonae*-associated growth rate suppression in rainbow trout (*Oncorhynchus mykiss*) occurs during early-onset xenoma dissolution as determined by *in situ* hybridization and immunohistochemistry. *Journal of Fish Diseases* 21, 345–354.
- Speare, D.J., Ritter, G. and Schmidt, H. (1998d) Quinine hydrochloride treatment delays xenoma formation and dissolution in rainbow trout challenged with *Loma salmonae*. *Journal of Comparative Pathology* 119, 459–465.
- Speare, D.J., Athanassopoulou, F., Daley, J. and Sanchez, J.G. (1999a) A preliminary investigation of alternatives to fumagillin for the treatment of *Loma salmonae* infection in rainbow trout. *Journal of Comparative Pathology* 121, 241–248.
- Speare, D.J., Beaman, H.J. and Daley, J. (1999b) Effect of water temperature manipulation on a thermal unit predictive model for *Loma salmonae*. *Journal of Fish Diseases* 22, 277–283.
- Speare, D.J., Daley, J., Dick, P., Novilla, M. and Poe, S. (2000) Ionophore-mediated inhibition of xenoma-expression in trout challenged with *Loma salmonae* (Microspora). *Journal of Fish Diseases* 23, 231–233.
- Speare, D.J., Markham, R.J.F. and Guselle, N.J. (2007) Development of an effective whole-spore vaccine using a low-virulence strain of *Loma salmonae* to protect against Microsporidian Gill Disease in rainbow trout (*Oncorhynchus mykiss*). *Clinical and Vaccine Immunology* 14, 12–18.
- Tiner, J.D. (1988) Birefringent spores differentiate *Encephalitozoon* and other microsporidia from coccidian. *Veterinary Pathology* 25, 227–230.
- Vavra, J. and Larsson, J.I.R. (1999) Structure of the microsporidia. In: Wittner, M. and Weiss, L.M. (eds) *The Microsporidia and Microsporidiosis*. American Society for Microbiology, Washington, DC.
- Williams, B.A.P. (2009) Unique physiology of host–parasite interactions in microsporidia infections. *Cellular Microbiology* 11, 1551–1560.

8 *Myxobolus cerebralis* and *Ceratomyxa shasta*

Sascha L. Hallett and Jerri L. Bartholomew
Oregon State University, Oregon, USA

Myxobolus cerebralis and *Ceratomyxa shasta* are two of over 2000 species of the phylum Myxozoa Grassé, 1970 (Lom and Dyková, 2006) (Fig. 8.1). Both are microscopic, spore-forming parasites that belong to the predominant class, Myxosporidia Bütschli, 1881. They have indirect, freshwater life cycles with two spore stages that develop alternately in fish and worms (Table 8.1 and Fig. 8.2). *M. cerebralis* originated in Europe and has spread across the world through anthropogenic activities, whereas *C. shasta* remains restricted to its native range in North America. Both pathogens were described following disease outbreaks in hatchery rainbow trout (*Oncorhynchus mykiss*) and are problematic in the USA where they have ecological and economic impacts on juvenile salmonids.

8.1. *Myxobolus cerebralis*

8.1.1. Introduction

Description

M. cerebralis has been called one of the most notorious myxosporean species (Lom and Hoffman, 1971). This tiny, metazoan endoparasite was first reported in Germany in 1893. The parasite targets cartilage and infection can manifest in whirling disease (Drehkrankheit).

Cultured rainbow trout and brook trout (*Salvelinus fontinalis*) became infected following importation as specific pathogen-free eggs from the USA (Höfer, 1903). The disease continues to plague fish hatcheries and, more recently, wild salmonid populations in the USA. The organism underwent several name changes (e.g. *Myxosoma cerebralis*) in the years that followed its discovery, but eventually reverted back to the original binomen (Lom and Noble, 1984). *M. cerebralis* is probably the most well-known and certainly the most thoroughly scrutinized member of the Myxozoa.

M. cerebralis spores have two morphologically disparate infective phenotypes: (i) a triactinomyxon-type actinospore; and (ii) a myxobolus-type myxospore (Figs 8.1 and 8.2). The myxospores are broadly oval in frontal view, broadly lenticular in side view with length 8.7 µm, width 8.2 µm and thickness 6.3 µm (Lom and Hoffman, 1971). Two hard valve cells encapsulate a binucleate sporoplasm and two polar capsules which each house a coiled (five to six loops), solid, extrudable polar filament. The actinospore has a triradially symmetric anchor shape. Three valve cells form an axis (~150 µm) with three caudal processes (each ~194 µm) (El-Matbouli and Hoffmann, 1998). Within the apex of the axis are three polar capsules that each contain a coiled polar filament (five loops), and below the capsules is a sporoplasm that contains 64 germ cells.

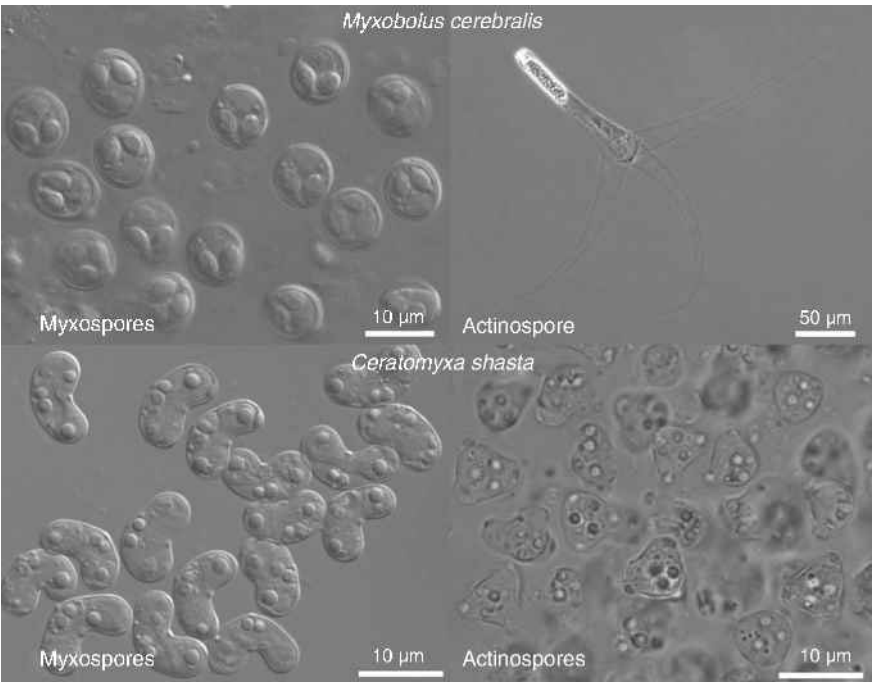


Fig. 8.1. Life cycle counterparts of *Myxobolus cerebralis* (Mc) and *Ceratomyxa shasta* (Cs). Myxospores are from infected rainbow trout and actinospores from an oligochaete (Mc) and a polychaete (Cs). Myxospores, Nomarski phase contrast; Mc actinospore phase contrast; Cs actinospore bright field; all freshly isolated and unstained.

Table 8.1. Characteristics of two ecologically and economically significant freshwater myxozoan parasites.

Parasite species	<i>Myxobolus cerebralis</i>	<i>Ceratomyxa shasta</i>
Disease	Whirling disease	Ceratomyxosis
Vertebrate host (intermediate)	Salmonid fish	Salmonid fish
Invertebrate host (definitive)	Oligochaete worm (<i>Tubifex tubifex</i>)	Polychaete worm (<i>Manayunkia speciosa</i>)
Actinospore morphotype	Triactinomyxon	Tetractinomyxon
Point of entry	Epidermis	Gills
Migration route	Nervous system	Circulatory system
Target organ	Cartilage	Intestine
Development time in fish	Months	Weeks
Origin/current distribution	Europe/worldwide	Pacific Northwest of North America
Sequence data available ^a	rRNA gene array (SSU, ITS1, 5.8S, ITS2, LSU), beta actin mRNA, HSP70 plus eight other protein coding genes	rRNA gene array (SSU, ITS1, 5.8S, ITS2, LSU), beta actin gene (putative), HSP70

^arRNA, ribosomal RNA; SSU, small subunit; ITS1, internal transcribed spacer region 1; ITS2, internal transcribed spacer region 2; LSU, large subunit; HSP70, heat shock protein 70.

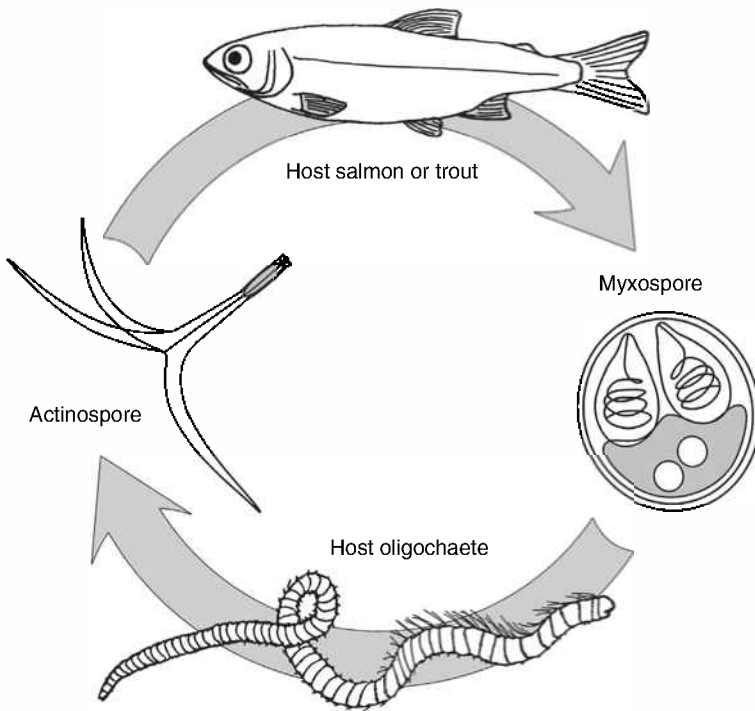


Fig. 8.2. Life cycle of *M. cerebralis*. Triactinomyxon actinospores released into fresh water from infected *Tubifex tubifex* oligochaetes develop into myxobolid myxospores in the cartilage of salmonid fish.

Contemporary descriptions of myxozoans typically include both morphological and molecular (DNA sequence data) details. *M. cerebralis* was one of the first myxozoans to have its small subunit ribosomal RNA gene (ssrRNA) sequenced (Andree *et al.*, 1997). Other genes that have been used to characterize this species are listed in Table 8.1. Genetic information has proven indispensable in separating closely related and morphologically similar (cryptic) species. It has also permitted development of sensitive and specific diagnostic assays, and investigation of myxozoan dispersal and distribution.

Transmission

The life cycle of *M. cerebralis* was the first elucidated for a myxozoan and this momentous discovery revolutionized our understanding not only of a species but of an entire phylum. In 1983, Markiw and Wolf found

that a tubificid oligochaete worm was required for the development of the infective stage for fish, and the following year they (Wolf and Markiw, 1984) described the complete sequence of steps, which involves two immotile water-borne stages and various developmental stages within each obligate host (Fig. 8.2). The two spore phenotypes were later confirmed as the same genotype (Andree *et al.*, 1997). As a consequence, the class Actinosporea was suppressed (Kent *et al.*, 1994). The parasite infects only one invertebrate species (or species assemblage), *Tubifex tubifex* Müller, 1774 (Wolf *et al.*, 1986), and only fishes of the family Salmonidae (Hedrick and El-Matbouli, 2002). However, susceptibility within each host group varies widely (MacConnell and Vincent, 2002; Steinbach *et al.*, 2009).

The triactinomyxon actinospore, released from the worm host, is the infective stage for fish. Chemical signals in the aquatic

environment (fish mucous-derived; primarily nucleosides) and mechanical stimulation by close proximity to a fish trigger the discharge of the polar filaments (Kallert *et al.*, 2005, 2011), which anchor the parasite to a fish. The amoeboid sporoplasm then emerges from between the valve cells and enters the fish epidermis through the secretion opening of a mucous cell (El-Matbouli *et al.*, 1999a). Particular parasite genes appear involved in the invasion process of fish (Eszterbauer *et al.*, 2009). The initial steps of the invasion process – filament attachment and sporoplasm emergence – can occur upon contact with any fish (Kallert *et al.*, 2009), but the sporoplasm can only successfully penetrate and infect salmonids (El-Matbouli *et al.*, 1999a). After penetration, the parasite multiplies mitotically as it migrates through the epidermis, peripheral nerves and central nervous system to reach cartilage (El-Matbouli *et al.*, 1995, 1999a). The host immune response can be effective in eliminating the parasite, but varies widely among salmonids (MacConnell and Vincent, 2002). Early in the infection, parasites that do not reach the nerves are eliminated by host cellular and/or humoral responses (Hedrick *et al.*, 1998). Once within the nerves, developmental stages are sheltered from the host response until they reach the cartilage, where the parasite trophozoites elicit an inflammatory response as they consume host chondrocytes during myxospore formation (Hedrick *et al.*, 1998; MacConnell and Vincent, 2002).

There is some evidence that mature parasite spores are released while the fish host is alive (Taylor and Haber, 1974; Nehring *et al.*, 2002). However, most tissue-trapped myxospores are probably liberated into sediments when the fish dies (Hedrick *et al.*, 1998) then are dispersed passively by water currents (Kerans and Zale, 2002). As *T. tubifex* worms forage, they ingest myxospores which 'hatch' in the gut lumen; polar filaments discharge and attach to the gut epithelium, shell valves open and the sporoplasm penetrates between the cells of the intestinal epithelium. Here ensues an asynchronous developmental series of multiplication, including both mitosis and meiosis (El-Matbouli and Hoffmann, 1998). Ultimately, sporulation creates pansporocysts that contain eight, folded,

triacetinomyxon-type actinospores. Mature actinospores exit their host via the intestinal tract, inflate upon contact with water, then passively float until they contact a fish, or disintegrate. Infected worms can each harbour thousands of spores (Gilbert and Granath, 2001; Hallett *et al.*, 2009), which are released as early as 74 days post-infection at 15°C (Gilbert and Granath, 2001). The water-borne triactinomyxons are viable for 6–15 days at 7–15°C (Markiw, 1992b; El-Matbouli *et al.*, 1999b). Two to ten actinospores are sufficient to infect susceptible fish (Hallett and Bartholomew, 2008) and cause disease (Markiw, 1992a). An infected fish may harbour several million myxospores 52–120 days post-exposure at 7–17°C (Halliday, 1973). The parasite is only transmitted via alternating spore stages; there is no horizontal or vertical transmission within fish or worm populations.

M. cerebralis is dispersed primarily via infected fish (Steinbach *et al.*, 2009), but, as discussed earlier, must metamorphose in a worm to reinfect fish. Intra- and interbasin transfers can occur naturally when infected anadromous fish stray during migration (Engleking, 2002; Zielinski *et al.*, 2010) or via piscivorous fish and birds (Taylor and Lott, 1978; El-Matbouli and Hoffmann, 1991; Arsan and Bartholomew, 2008; Koel *et al.*, 2010). Dissemination may also occur through anthropogenic activities, primarily commercial transfer and stocking of infected fish, but also during recreational pursuits. Fish eggs do not become infected but associated water may contain spores.

Geographic distribution

M. cerebralis originated in Europe but is now exotic in four continents (Asia, Africa, North America and Oceania). Establishment outside its native range was possible because of: (i) the wide distribution of the invertebrate host; (ii) spatial/temporal overlap of these worms with infected fish; and (iii) a combination of environmental factors conducive to parasite persistence. The route of dissemination followed the transfer of live rainbow trout, although imported frozen fish were also implicated as a possible source of entry

to the USA during the 1950s (Hoffman, 1970).

Whirling disease was first described in North American rainbow trout subsequent to their importation into Germany. *M. cerebralis* existed in that region in wild brown trout which have some resistance to the disease, but the introduced trout were naïve to the parasite and were decimated by the infection. In the early 1900s, the pathogen spread throughout trout-rearing facilities in Germany and was detected in Denmark and Finland. Translocated infected rainbow trout may have introduced the parasite, or uninfected fish may have provided a susceptible population in areas where the parasite was enzootic (Hoffman, 1970).

Spread and detection of *M. cerebralis* accelerated post-World War II. Between 1952 and 1975, the parasite was detected in 18 more European countries (France, Italy, Czech Republic, Poland, Bulgaria, Yugoslavia, Sweden, Scotland, Norway, Austria, Belgium, Hungary, England, Ireland, Liechtenstein, Luxemburg, the Netherlands and Spain) and in the USSR, Lebanon, South Africa, Morocco, New Zealand and the USA. *M. cerebralis* has been detected in 25 states in the USA, most recently in Alaska using molecular methods (Arsan *et al.*, 2007a). Its global dissemination has been reviewed earlier (Bartholomew and Reno, 2002).

Dissemination of the parasite to new locations is largely attributable to anthropogenic activities. This is supported by the low level intraspecific variation of *ssrRNA* (<1%) and internal transcribed spacer region 1 (1.7%) DNA sequences between European and North American isolates of *M. cerebralis* (Whipps *et al.*, 2004; Arsan *et al.*, 2007a).

Impact

Whirling disease was initially only a problem for fish culture operations. These facilities raised trout for food, recreation, restoration and conservation. They sustained large economic losses from the reduced quality and quantity of fish and increased costs from the implementation of treatment and control measures. In some cases, destruction of infected fish or closure of the facility was

necessary to curtail contamination of neighbouring waterways (Bartholomew and Reno, 2002; Bartholomew *et al.*, 2007).

In Europe, wild trout populations had presumably evolved resistance to the parasite and were not impacted by the disease (Christensen, 1972). In stark contrast, following its introduction to the USA, the parasite had devastating effects on wild rainbow and cutthroat trout populations, particularly in the Intermountain West (e.g. about 90% reduction of some stocks; Vincent, 1996; Nehring *et al.*, 1998). Whirling disease continues to be a significant fish health issue in the USA (Gilbert and Granath, 2003; Krueger *et al.*, 2006). The malady has changed fish community structure, as vulnerable species are replaced by more resistant species such as brown trout (Baldwin *et al.*, 1998; Granath *et al.*, 2007). Losses have persisted in some areas, while elsewhere populations are recovering (Steinbach *et al.*, 2009). Despite focal points of decimation, there has been no obvious long-term economic harm to recreational fishing in the USA because of a shift to fishing more resistant species (Steinbach *et al.*, 2009).

A web of variables (water flow rates, temperature, sediment, host life histories) governs the impact of *M. cerebralis* on wild fish. Populations may be severely affected in some locations (Vincent, 1996; Nehring *et al.*, 1998), yet persist alongside the parasite elsewhere (see Modin, 1998; Kaeser *et al.*, 2006). Water temperature appears to be the most influential factor, with the critical window being 10–15°C. Temperature affects: (i) parasite development time, (ii) level and duration of spore release from the worm host (El-Matbouli *et al.*, 1999b; Kerans and Zale, 2002; Blazer *et al.*, 2003; Kerans *et al.*, 2005); and (iii) prevalence and severity of infection in the fish host (Baldwin *et al.*, 2000; Schisler *et al.*, 2000; Hiner and Moffitt, 2002; Vincent, 2002; Krueger *et al.*, 2006). Differences in impact have also been associated with the presence of susceptible *T. tubifex* (Beauchamp *et al.*, 2005; Krueger *et al.*, 2006) – a hardy worm with broad distribution from pristine to polluted sediments (Kathman and Brinkhurst, 1998; Granath and Gilbert, 2002).

8.1.2. Diagnosis of the infection and clinical signs of the disease

Clinical signs

Whirling disease was unknown until rainbow trout were introduced to Europe, where native brown trout are disease-resistant hosts of *M. cerebralis*. Clinical signs include whirling behaviour, blacktail, skeletal deformities, stunted growth and death (Fig. 8.3). Abnormal behaviour and a blackened tail may not be evident in fish with chronic infections, and both disease indicators disappear from dead fish.

Parasite development in the fish causes granulomatous inflammation that constricts the spinal cord and compresses the brain stem. This constriction appears responsible for abnormal swimming behaviour in infected fish (Rose *et al.*, 2000). 'Whirling disease' is named for the repeated episodes of tail-chasing – rapid circular swimming – followed by a series of anterior body contractions (Höfer, 1903; Rose *et al.*, 2000). Additionally, fish may adopt an elevated tail posture when not swimming and, less often, brain-stem compression may cause fish to abruptly cease all movement, except opercular movements, and sink (Rose *et al.*, 2000).

Swimming performance generally decreases with increased parasite burden (Ryce *et al.*, 2001, 2005; DuBey *et al.*, 2007).

Blacktail is a consequence of infection of the posterior spinal cartilage (Fig. 8.3); inflammation exerts pressure on root ganglia that control skin melanocytes in the caudal area (Halliday, 1976; Schäperclaus, 1991; El-Matbouli *et al.*, 1995). Skeletal deformities result from disrupted osteogenesis following cartilage damage and associated inflammation, and may include a shortened operculum, indented skull, reduced nose, misaligned jaws and a crooked spine (Andree *et al.*, 2002; MacConnell and Vincent, 2002) (Fig. 8.3). Growth may be reduced during the active phase of infection in severely infected fish, but resume thereafter except in crippled fish (Hedrick *et al.*, 2001; MacConnell and Vincent, 2002). Mortality may occur either directly through physical damage, or indirectly from an inability to feed or evade predators (Hedrick *et al.*, 1998; Steinbach *et al.*, 2009).

Clinical signs may appear 3–8 weeks post-exposure to *M. cerebralis* actinospores (MacConnell and Vincent, 2002). Development and severity of clinical signs depend on: (i) exposure dose and duration; and (ii) the

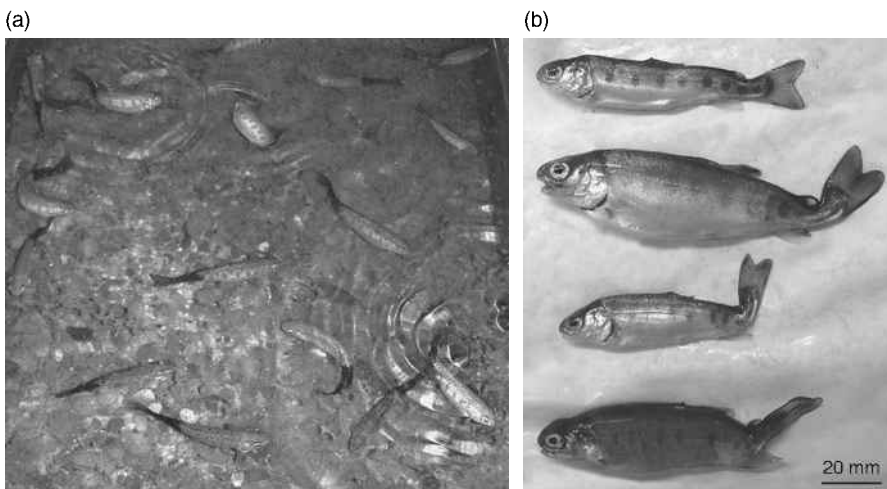


Fig. 8.3. Clinical signs of whirling disease in rainbow trout. Fish were infected with *M. cerebralis* as fry in the laboratory through cohabitation with infected *T. tubifex*. (a) Live fish have distinct black tails and exhibit whirling behaviour when disturbed; (b) skeletal deformities in fish cohabited with infected worms for 6 weeks post-hatch, then held on well water for 5 months and euthanized.

Table 8.2. Susceptibility to whirling disease among species of salmonids following laboratory or natural exposure to *M. cerebralis* at a vulnerable life stage (sources: MacConnell and Vincent, 2002; Sollid *et al.*, 2002, 2004; Vincent, 2002; Wagner *et al.*, 2006; Steinbach *et al.*, 2009; Thompson *et al.*, 2010).

Genus	Species/subspecies	Common name	Susceptibility ^a
<i>Oncorhynchus</i>	<i>mykiss</i>	Rainbow trout	S–hS
	<i>mykiss</i>	Steelhead trout	S–hS
	<i>clarki</i>	Cutthroat trout	S–hS
	<i>c. bouvieri</i>	Yellowstone cutthroat	S–hS
	<i>c. lewisi</i>	Westslope cutthroat	S
	<i>c. pleuriticus</i>	Colorado River cutthroat	S
	<i>c. virginalis</i>	Rio Grande cutthroat	S
	<i>c. stomias</i>	Greenback cutthroat	S
	<i>tshawytscha</i>	Chinook salmon	S
	<i>nerka</i>	Sockeye salmon	hS
	<i>keta</i>	Chum salmon	pR, U
	<i>gorbuscha</i>	Pink salmon	pR, U
	<i>masu</i>	Cherry salmon	pR, U
	<i>kisutch</i>	Coho salmon	pR
	<i>gilae</i>	Gila	hS
<i>Salvelinus</i>	<i>g. apache</i>	Apache	hS
	<i>fontinalis</i>	Brook salmon	S
	<i>malma</i>	Dolly varden	pR, U
	<i>confluentus</i>	Bull trout	pR
<i>Salmo</i>	<i>namaycush</i>	Lake trout	R
	<i>salar</i>	Atlantic salmon	S, U
	<i>trutta</i>	Brown trout	pR
<i>Prosopium</i>	<i>williamsoni</i>	Mountain whitefish	S
<i>Thymallus</i>	<i>thymallus</i>	European grayling	S, U
	<i>arcticus</i>	Arctic grayling	R–pR
<i>Hucho</i>	<i>hucho</i>	Danube salmon	hS

^ahS, Highly susceptible, clinical disease common; pR, partially resistant, clinical disease rare and develops only when exposed to very high parasite doses; R, resistant, no spores develop; S, susceptible, clinical disease common at high parasite doses or when very young, but greater resistance to disease at low doses; U, susceptibility is unclear (conflicting reports or insufficient data).

age, size and strain/species/genus of the salmonid host (Table 8.2; Markiw, 1991, 1992a; MacConnell and Vincent, 2002; Bartholomew *et al.*, 2003; Ryce *et al.*, 2004, 2005). Salmonids can become infected at any age from 2 days post-hatch (Markiw, 1991), but younger fish are most vulnerable to infection and most prone to disease before their cartilage ossifies. Surprisingly, resistance is not associated with the level of skeletal ossification but rather with other age- and size-related factors, such as the stage of development of the central nervous system (Ryce *et al.*, 2005). Myxospore burden is not proportional to disease severity and both decrease with host age. Survivors of long-term infections may only exhibit skeletal deformities (MacConnell and Vincent, 2002).

Diagnosis

Efficient detection is critical, as there is no effective treatment for *M. cerebralis* or whirling disease (Andree *et al.*, 2002). Some infected fish may be carriers of viable stages and do not display clinical signs. Detection methods: (i) permit documentation of parasite distribution; (ii) identify localities with high parasite abundance; (iii) monitor spread; and (iv) determine prevalence and severity of infection in hosts. Accurate detection and diagnosis measures the success of management procedures (Andree *et al.*, 2002).

Myxozoans are identified primarily on the morphological and morphometrical characteristics of the myxospore in the vertebrate host (Lom and Dyková, 2006). Host species

and tissue tropism are also considered, and contemporary novel descriptions are expected to be augmented with molecular (DNA sequence) data.

Diagnosis of whirling disease requires multiple steps. Characteristic gross clinical signs are not unique to the disease and must be observed in combination with the identification of *M. cerebralis* spores in cartilage (Andree *et al.*, 2002). Myxozoan species cannot readily be distinguished based on developmental stages, and formation of mature myxospores takes several months. The genus *Myxobolus* Butschli, 1882 contains over 700 described species (Eiras *et al.*, 2005; Lom and Dyková, 2006). Several species resemble *M. cerebralis* morphologically and six inhabit the cranial tissues of salmonids (Markiw, 1992c; Hogge *et al.*, 2008). *M. cerebralis* is found in cartilage or bone, while *Myxobolus neurobius* (Schuberg and Schröder, 1905), *Myxobolus kisutchi* (Yasutake and Wood, 1957), *Myxobolus arcticus* (Pugachev and Khokhlov, 1979) and *Myxobolus farionis* (Gonzalez-Lanza and Alvarez-Pellitero, 1984) have been described in nerve tissue and *Myxobolus neurotropus* from brain and spinal cord (Hogge *et al.*, 2008). Another commonly encountered myxobolid of salmonids, *Myxobolus squamalis*, is similar in size to *M. cerebralis* but has two distinctive ridges on either side of the suture and is found in scale pockets (Hoffman, 1999). Histopathology can resolve fine tissue tropic differences and discriminate between co-occurring cranial myxobolids whose close proximity would lead to co-purification using other methods. DNA-based methods provide unambiguous identification of *M. cerebralis* (Hogge *et al.*, 2008).

Diagnostic methods for *M. cerebralis* (reviewed by Andree *et al.*, 2002 and Steinbach *et al.*, 2009) range in complexity and vary in sensitivity and specificity. The chosen technique depends on intended purpose (research, monitoring, diagnostics or fish health inspection), and on the source of the sample (fish, worm, water or sediment). There are strict guidelines for inspection purposes in the USA (American Fisheries Society – Fish Health Section, 2010).

Most procedures are lethal and, for non-DNA based methods, the infection must be

sufficiently advanced. Sporogenesis of the parasite is temperature dependant and takes 90 days at 12–13°C or 11 months at 0–7°C (Hedrick and El-Matbouli, 2002). In young fish, spore production peaks 5 months after exposure (>10°C). The general approach is to isolate and/or concentrate spores and then identify these through microscopy (location, spore morphology) or molecular methods. Entire heads or bodies of young fish can be processed, whereas larger fish may need to be sub-sampled by taking a core through the head or halving the head down the midline sagittal plane.

The most common approach for diagnosis is isolation of spores using pepsin-trypsin digest (PTD) and presumptive identification of myxospores, followed by confirmation based on histology (spores of the correct dimensions located in appropriate tissues) or PCR amplification of parasite DNA. PTD uses enzymes and centrifugation to digest and concentrate spores from cartilage, which can then be quantified using a haemocytometer (Markiw and Wolf, 1974; Lorz and Amandi, 1994). Microscopic examination of stained histological sections reveals all stages of development and allows scoring of disease severity (Lorz and Amandi, 1994; Baldwin *et al.*, 2000). The MacConnell-Baldwin numerical scale goes from grade 0 (no abnormalities visible and *M. cerebralis* is not detected) to grade 5 (multifocal to coalescing areas of cartilage necrosis visible with loss of normal architecture).

PCR-based detection methods include: (i) single-round (Schisler *et al.*, 2001; Baldwin and Myklebust, 2002); (ii) nested (Andree *et al.*, 1998); and (iii) qPCR (Kelley *et al.*, 2004; Cavendar *et al.*, 2004). The sensitivity and specificity of PCR allows detection of any life-cycle stage. PCR also permits detection of early or light infections and can distinguish between phenotypically similar species. Nested PCR of non-lethal caudal fin clips appears effective for detection of early parasite stages but becomes less accurate as the infection progresses (Skirpstunas *et al.*, 2006). To ensure meaningful results, appropriate negative and positive controls as well as relevant reference or calibration standards should be included (Hallett and Bartholomew, 2008).

It is important to remember when using sensitive methods (such as PCR) that do not include direct observation of the parasite that detection of pathogen DNA does not imply disease.

Other detection methods include: (i) plankton centrifugation to concentrate spores (O'Grodnick, 1975); (ii) molecular based *in situ* hybridization (Antonio *et al.*, 1998); and (iii) loop-mediated isothermal amplification (LAMP; El-Matbouli and Soliman, 2005). LAMP is a simple, rapid DNA detection method that shows promise for on-site detection of the parasite in fish hatcheries and other non-laboratory situations, but has not been validated for this use.

8.1.3. Lesions

A successful *M. cerebralis* infection culminates in internal, microscopic lesions filled with

myxospores (Fig. 8.4). Lesions can form in the peripheral nerves and epineurium during parasite migration but are predominant in cartilage (Baldwin *et al.*, 2000). Parasite trophozoites digest cartilage as they multiply and mature. Lesions only develop if fish are exposed to a sufficiently high parasite dose (Hedrick *et al.*, 1999a) and only fish that develop lesions have active acquired immunity (MacConnell and Vincent, 2002).

Cartilage lesions are best characterized from rainbow trout, but progress similarly for other species (Hedrick *et al.*, 1999b; Baldwin *et al.*, 2000). Initially, they are small, discrete foci of trophozoites and cartilage degeneration with minimal associated tissue damage and no inflammation (Baldwin *et al.*, 2000; MacConnell and Vincent, 2002). These early lesions progress to extensive cartilage necrosis and degeneration with numerous parasite stages. Older stages are centrally

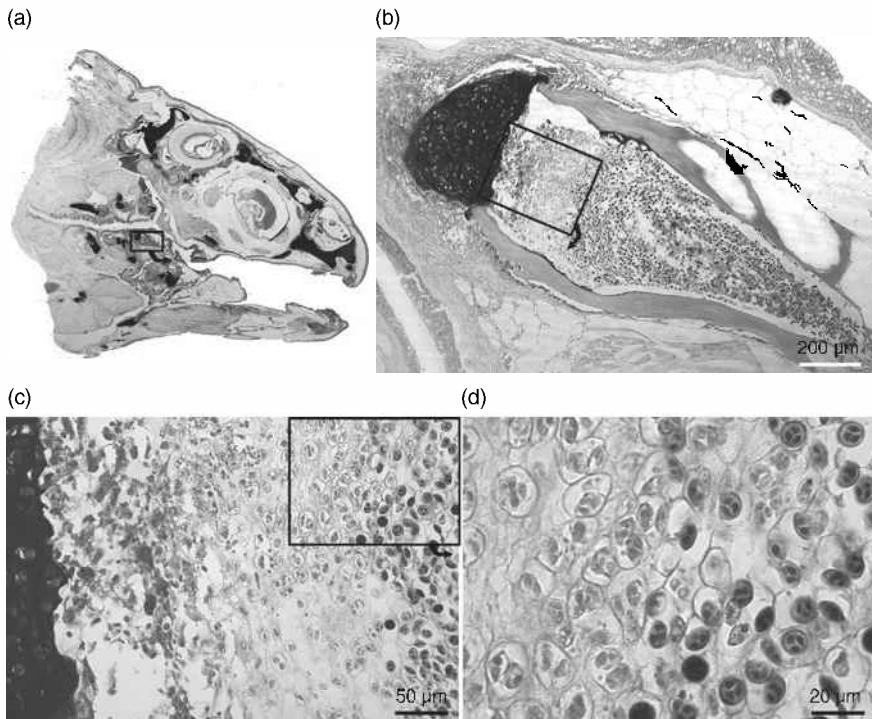


Fig. 8.4. Histological sections of rainbow trout cranial tissues 3 weeks post-exposure to *M. cerebralis* showing cartilage damage. (a) Cross-section through head showing location of subsequent images (box); (b) lesion showing succession of cartilage degradation and progression of parasite front; (c) higher magnification of coalescing regions; (d) developmental stages – mature myxospores with dark staining polar capsules and sporoplasm are conspicuous.

located in necrotic foci with younger stages at the leading edges (Baldwin *et al.*, 2000). Surrounding tissues become involved and granulomatous inflammation is evident (MacConnell and Vincent, 2002). Closely associated with infected cartilage in adjacent soft tissues are mononuclear leukocytes. These and multinuclear leukocytes border and/or infiltrate advanced lesions (Baldwin *et al.*, 2000). As the disease advances, large granulomatous lesions may have necrotic centres that contain spores (Plehn, 1905; Hedrick *et al.*, 1998). Coalescing areas of granulomatous inflammation may become so extensive that the normal structural framework of the cartilage is destroyed (Hedrick *et al.*, 1998; MacConnell and Vincent, 2002). Myxospores can become encased in bone as remaining cartilage ossifies.

Lesions in more resistant brown trout: (i) are smaller than those in highly susceptible rainbow trout; (ii) contain fewer parasite stages; and (iii) have fewer associated leukocytes but more multinucleated giant cells (Baldwin *et al.*, 2000). Any cartilage (cranium, spine, fins, vertebrae, ribs and operculum) can be infected (Antonio *et al.*, 1998) and the principal location of parasite lesions varies among salmonid species. In highly susceptible fish, such as rainbow trout, lesions are found throughout the body but consistently in cranial regions, primarily the ventral calvarium then gill arches (Baldwin *et al.*, 2000). In Yellowstone cutthroat trout, lesions are most prevalent in the lower jaw cartilage (Murcia *et al.*, 2011). In brown trout, lesions are most common in the gill arches and rarely in the calvarial or other cartilages (Hedrick *et al.*, 1999a; Baldwin *et al.*, 2000). In bull trout (*Salvelinus confluentus*) and mountain whitefish (*Prosopium williamsoni*), lesions may be found in the cranium but are often limited to the axial skeleton (MacConnell and Vincent, 2002).

8.1.4. Pathophysiology

In contrast to the profound physical effects *M. cerebralis* has on fish there are only a few described pathophysiological effects. These include chronic inflammation, disrupted osteogenesis and suppressed growth.

Whirling disease is a chronic cartilaginous inflammatory malady of salmonid fish. The early developmental stages of *M. cerebralis* do not cause cellular reaction of the epidermal or nervous tissues, despite the parasite commencing replication soon after entering the fish host and occupying the central nervous system for several weeks. However, passage through the nerves may affect key neurological responses (Hedrick and El-Matbouli, 2002).

Once in the cartilage, maturing developmental stages lyse and digest chondrocytes. As the infection becomes widespread, trophozoites elicit an intense inflammatory response in most susceptible fish species (MacConnell and Vincent, 2002). Following cartilage degeneration, lesions form, which contain granulomatous inflammation. Inflamed regions may coalesce and the normal structure disappears. Granulomatous inflammation can extend into the perineural space and produce ring-like constrictions of the upper spinal cord, sometimes compressing and deforming the lower brain stem (Rose *et al.*, 2000). Pathways that connect the medulla with the spinal cord may also degenerate.

The inflammatory response to the trophozoite stage can disrupt osteogenesis (El-Matbouli *et al.*, 1995; MacConnell and Vincent, 2002). Phagocytosis of chondrocytes destroys the structural framework required for healthy osteocyte formation (Schäperclaus, 1991; MacConnell and Vincent, 2002), which results in irregular bone formation and permanent skeletal deformities.

In severely infected fish, growth rates may be reduced during the active phase of infection, but resume thereafter, except in disabled fish (Hedrick *et al.*, 2001; MacConnell and Vincent, 2002). The physiological parameters associated with these outcomes are unknown. Bioenergetic costs of the disease have not been fully evaluated.

8.1.5. Protective/control strategies

Any management or control programme for *M. cerebralis* necessarily requires a holistic approach that incorporates an understanding of environmental factors of the particular

locality (Murcia *et al.*, 2011), and surveys and monitoring programmes of water, fish and worms (Bartholomew *et al.*, 2007). Numerous control strategies for the parasite have been tested experimentally but few of these have been implemented on a large scale. Current and possible control measures are covered in detail by Wagner (2002). The present discussion is an update on successful and novel approaches.

Evaluations of chemical and physical stressors on spore viability show the actinospore is the more fragile of the two spore stages of *M. cerebralis*. Viability staining indicates that actinospores are killed by: (i) freezing (-20°C); (ii) drying for 1 h; (iii) chlorine concentrations of 130 ppm for 1 min or longer; (iv) hydrogen peroxide concentrations of greater than 10%; and (v) temperatures above 75°C for at least 5 min (Wagner *et al.*, 2003). These approaches are applicable to disinfection of equipment rather than water supplies. The most recent assessment of myxospores measured viability with exposure experiments rather than by vital staining (which tends to overestimate live spores), and revealed that the myxospore stage is less hardy than previously thought (Hedrick *et al.*, 2008). Infectivity is eliminated by: (i) freezing (-20°C) for 7 days; (ii) heating to 20°C for 2 months; (iii) drying; and (iv) treating with alkyl dimethyl benzyl ammonium chloride at 1500 mg/l for 10 min. A dose of ultraviolet (UV) of 40–480 mJ/cm² and chlorine bleach at 500 mg/l for 15 min are largely effective at inactivating myxospores.

Drug efficacy varies widely among myxozoan species and genera (Feist and Longshaw, 2006). No drug or therapeutant treatment exists for *M. cerebralis*. Eleven drugs have been assessed (acetarsone, amprolium, benomyl, clamoxyquin, fumagillin and its analogue TNP-470, furazolidone/furoxone, nicarbazine, oxytetracycline, proguanil and sulfamerazin) but none progressed past the testing phase (Wagner, 2002). Several drugs (e.g. furazolidone, proguanil, benomyl) reduced infection and suppressed disease (inhibited spore formation and/or deformed spores), but none prevented or eliminated infection and some had side effects including toxicity (TNP-470) and reduced growth

(furazolidone) (Hoffman *et al.*, 1962; Taylor *et al.*, 1973; O'Grodnick and Gustafson, 1974; Alderman, 1986; El-Matbouli and Hoffmann, 1991; Staton *et al.*, 2002). Efficacy may depend on the timing of application, relative to parasite development, in particular whether treatment occurred before or after sporulation. Drug development is further impeded by the regulatory environment (at least in the USA) and issues of drug application to wild fish (Wagner, 2002; Steinbach *et al.*, 2009).

Fish culture facilities

Hatcheries and ponds offer greater possibilities for control measures than natural settings. Effective strategies include:

- Conversion of earth-bottom ponds and raceways to concrete, and the regular removal of accumulated organics to eliminate *T. tubifex* habitat.
- Use of a pathogen-free water supply (usually converting from surface-water to ground-water supply) and a strong water flow (Hoffman, 1990; Hallett and Bartholomew, 2008), at least while fish are young and most vulnerable to disease.
- Treatment of incoming water to kill incoming actinospores using ozonation, chlorination and/or UV light (40 mJ/cm²) (Markiw, 1992c; Hedrick *et al.*, 1998, 2000, 2007) or filtration (sand-charcoal rather than membrane; Hoffman, 1962, 1974; Wagner, 2002; Arndt and Wagner, 2003; Arndt, 2005) to remove actinospores.
- Disinfection of ponds with calcium cyanide, calcium cyanamide or chlorine to render both spore stages non-viable and to kill the invertebrate host.
- Regular fish health inspections to detect *M. cerebralis* and careful tracking of fish transfers and stocking.

Natural settings

Once *M. cerebralis* is established, few options exist for its eradication; the goals are to reduce disease severity and mitigate effects on salmonid populations. Risk assessment models

and analyses can identify locations at high risk of parasite introduction and establishment, and highlight the most important variable(s) (Bartholomew *et al.*, 2005; Kaeser *et al.*, 2006; Krueger *et al.*, 2006; Arsan and Bartholomew, 2008, 2009).

In rivers where the fishery is managed for recreational purposes, one of the most simple and effective management strategies is to stock larger fish (Steinbach *et al.*, 2009). Although these fish can still become infected they are less susceptible and produce fewer spores (Ryce *et al.*, 2004). Another effective approach is to selectively stock species or strains of salmonids that are naturally resistant to disease, or whose life histories limit the overlap of fry with seasonal peaks of water-borne actinospores. Two other strategies are being explored: (i) foster the breeding of wild fish populations with high genetic diversity (Miller and Vincent, 2008; Steinbach *et al.*, 2009); and (ii) selective breeding whereby vulnerable native populations are crossed with *M. cerebralis*-resistant fish, such as the domesticated German Höfer strain (Schisler *et al.*, 2006). The aim is to produce progeny with resistance to whirling disease while retaining genetic traits important for survival in the wild.

Comparison of resistant and susceptible fish strains indicates whirling disease severity has a genetic component (Schisler *et al.*, 2006). A major effect quantitative trait locus (WDRES9) region for disease resistance has been identified on chromosome Omy9 of *O. mykiss* (Baerwald *et al.*, 2011). This locus controls a large percentage (50–86%) of phenotypic variation that contributes to whirling disease resistance.

Non-salmonids have been proposed as interceptor fish to lower infection intensity in trout (Kallert *et al.*, 2009). Under laboratory conditions, *M. cerebralis* actinospores attach indiscriminately to fish of any species and more actinospores attach to carp, for example, than to trout (Kallert *et al.*, 2009).

The invertebrate host, *T. tubifex*, is small and often inconspicuous. Significantly, different populations of *T. tubifex* can vary considerably in prevalence of infection and level of actinospore production (Beauchamp *et al.*, 2002; Kerans *et al.*, 2004). This provides one

explanation for the patchy geographic mosaic of whirling disease prevalence (Beauchamp *et al.*, 2005; Hallett *et al.*, 2009). Variations in ability to propagate the parasite have been correlated with host mitochondrial 16S rDNA 'lineage': at the extremes, lineage III is the most susceptible (Beauchamp *et al.*, 2002; Rasmussen *et al.*, 2008; Hallett *et al.*, 2009; Zielinski *et al.*, 2011) whereas lineage IV appears non-susceptible (Arsan *et al.*, 2007b). A survey of *T. tubifex* lineages in a stream offers a tool for risk assessment.

Resistant *T. tubifex* out-competes susceptible strains in exposure experiments conducted under laboratory conditions (Beauchamp *et al.*, 2006) and production per infected worm was reduced in populations dominated by non-susceptible worms (Hallett *et al.*, 2009). These interactions may be exploited to control whirling disease in streams, though they are most applicable to contained water bodies, such as private ponds.

The density of infected *T. tubifex* is positively correlated with whirling disease risk and is associated with fine sediments and lower water temperatures (Krueger *et al.*, 2006). The association between *T. tubifex* populations and point sources of organic enrichment can explain occurrence of the parasite in some systems (Kaeser *et al.*, 2006). Several environmental engineering approaches are being evaluated for their ability to decrease parasite abundance, primarily through reduction of *T. tubifex* populations. Sediment removal reduces favourable *T. tubifex* habitat, and can be achieved through direct excavation or by flushing flows in regulated rivers (Hallett and Bartholomew, 2008). Construction of permeable berms has been used in an attempt to filter and isolate areas of high parasite abundance. Stream restoration efforts include exclusion of grazing livestock along waterways to increase riparian vegetation for shade that lowers stream temperatures. Livestock also contribute significant quantities of nutrients and generate fine sediment (Steinbach *et al.*, 2009).

Public education is also paramount to restricting inadvertent dissemination of *M. cerebralis* through aquatic recreational activities. Pertinent, practical information is

provided to the public via web sites, brochures and signage. Recommended precautions include: (i) no transportation of fish between water bodies; (ii) rinsing all mud and aquatic plants from vehicles, boats, trailers, anchors, axles, waders, boots and fishing equipment with clean water; (iii) draining all water from boats; (iv) allowing boats and gear to dry between trips; and (v) disposing of fish away from waterways, preferably in compost or garbage rather than kitchen disposal (Steinbach *et al.*, 2009). Private fish-pond owners and home aquarists also have a responsibility: individuals should be aware of fish health regulations and appreciate that live invertebrate fish food and associated water can harbour myxozoan infective stages (Lowers and Bartholomew, 2003; Hallett *et al.*, 2005, 2006).

8.2. *Ceratomyxa shasta*

8.2.1. Introduction

Description

C. shasta Noble (1950) was first reported in 1948 as the cause of an epizootic among rainbow trout reared at a hatchery in Shasta County, California, USA. The disease, ceratomyxosis, was described as unusual in the number of tissues and organs affected (Wales and Wolf, 1955); however, the parasite has a tropism for the intestine. *C. shasta* is also atypical for the genus – most *Ceratomyxa* species are coelozoic parasites of marine fishes – though genetic analyses show strong affinity to its marine cousins. It has been labelled ‘a dangerous pathogen of North American salmonids’ and is the most well-known representative of the genus infecting fish in fresh water, although a few non-marine species are known (Lom and Dyková, 2006).

C. shasta has two morphologically distinct spore stages (Fig. 8.1): (i) a ceratomyxa-type myxospore; and (ii) a tetractinomaxon-type actinospore. Myxospores measure 14–17 µm in total length and 6–8 µm wide at the suture line (Yamanoto and Sanders, 1979). Characteristic of the genus, the two spore valves are smooth, elongated and crescent shaped (hence the description as

a kidney bean-shaped spore), and the suture line is distinct. Two subspherical polar capsules, each containing a coiled polar filament, are located mid-spore near the suture line. Mature actinospores are smaller (10 × 8 µm). They have three valve cells that encapsulate three polar capsule cells and one binucleate sporoplasm (Fig. 8.1; Bartholomew *et al.*, 1997).

C. shasta has multiple strains (internal transcribed spacer region 1 (ITS1) genotypes) that differ in their host affinity (Atkinson and Bartholomew, 2010a, b). Generally, the parasite genotypes are host-species-specific. An exception is the species *O. mykiss*, in which the two different forms, steelhead and rainbow trout, are differentially infected.

Transmission

The *C. shasta* life cycle involves two hosts. The actinospore stage develops in a freshwater polychaete worm (*Manayunkia speciosa*), and the myxospore stage develops in a salmonid fish (Fig. 8.5; Bartholomew *et al.*, 1997). The life-cycle counterparts of *C. shasta* were determined through laboratory experiments and, for the first time, supported concurrently by DNA (ssrRNA) sequence data. There is no horizontal or vertical transmission of the parasite between fish or between worms.

Myxospores ingested by the filter-feeding polychaete release their sporoplasms in the gut, which then penetrate between the epithelial cells (Meaders and Hendrickson, 2009). The parasite multiplies and migrates through the nervous system to the epidermal layer of the integument where most development occurs (Bartholomew *et al.*, 1997; Meaders and Hendrickson, 2009) and parallels that described for *M. cerebralis* (El-Matbouli and Hoffman, 1998). Development to mature actinospores occurs in approximately 7 weeks at water temperatures averaging 17°C (Meaders and Hendrickson, 2009). Pansporocysts are released through secretory pores in the polychaete epidermis and rupture, each releasing eight actinospores (Bartholomew *et al.*, 1997; Bjork, 2010), but unlike *M. cerebralis* they do not inflate or change morphologically upon contact with water. Asynchronous development permits prolonged spore release.

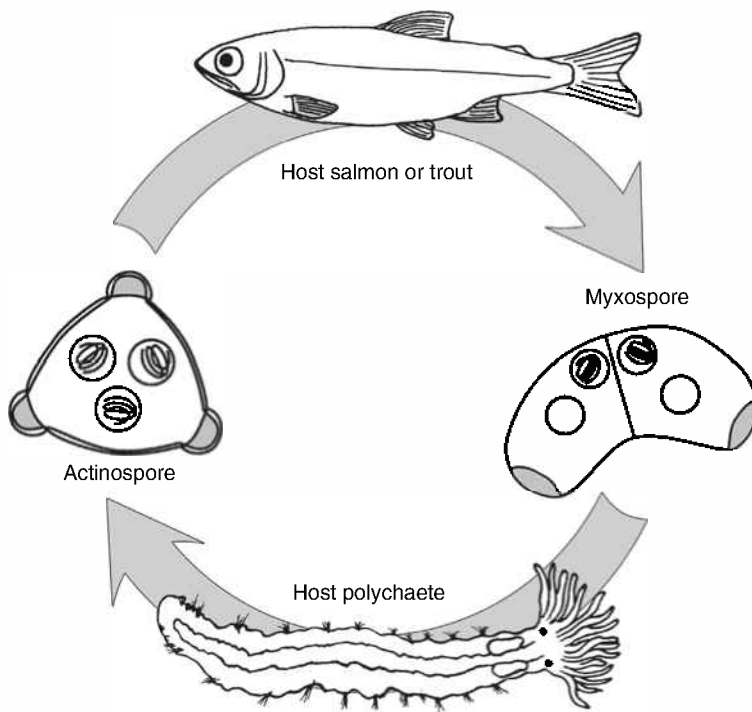


Fig. 8.5. Life cycle of *Ceratomyxa shasta*. Tetractinomyxon actinospores released into fresh water from infected *Manayunkia speciosa* polychaetes develop into ceratomyxid myxospores in the intestine of salmonid fish.

Several hundred actinospores may be released in a single day from an infected polychaete (Bartholomew *et al.*, 2004; Meaders and Hendrickson, 2009). Viability of the actinospores decreases with increasing temperature. Actinospores are viable for up to 13 days at 11°C (Ratliff, 1983), and 3–7 days at 18°C (Foott *et al.*, 2007) under field conditions. In the laboratory actinospores are physically intact for up to 18 days at 4°C and 15 days at 12°C, but for only 6 days at 20°C (Bjork, 2010). Ceratomyxosis occurs seasonally, with release of actinospores in the spring as temperatures rise above 10°C, although infection can occur at temperatures as low as 7°C (Ratliff, 1983). Infection by a single actinospore is sufficient to result in death of highly susceptible strains of salmon and trout (Ratliff, 1983; Bjork and Bartholomew, 2009).

Actinospores attach to the fish gill, and their sporoplasm penetrates the epithelium (Bjork and Bartholomew, 2010). The parasite

migrates to the blood vessels of the gill arch where it replicates in the vessel endothelium, and is delivered to the intestine and other organs via the circulatory system (Bjork and Bartholomew, 2010). Here it develops in small disporic pseudoplasmodia (Yamanoto and Sanders, 1979), which culminate in the myxospore stage at 2 weeks post-exposure at 18°C (R.A. Ray, Oregon State University, personal communication, 2010). Myxospores are released when infected fish die. Adult salmon that die on spawning grounds release millions of spores (Foott *et al.*, 2010), thus returning the parasite to the upper portions of watersheds. Mature parasites are also observed in the intestinal lumen and faecal casts of infected juvenile fish.

Geographical distribution

C. shasta occurs in salmonids in freshwater environments of the Pacific Northwest region

of North America. Unlike the widely dispersed *M. cerebralis*, *C. shasta* remains limited to certain river systems. Although distribution of the parasite requires both salmonid and polychaete hosts, it does not encompass the geographic distribution of either. *C. shasta* is not established in many rivers in the Pacific Northwest where infected salmon migrate. Conversely, the parasite does not occur in the eastern USA where *M. speciosa* is present and where Pacific salmon are introduced. The distribution of *C. shasta* in the Pacific Northwest has been mapped using sentinel fish. Naïve fish held in cages detect the fragile actinospore stage released from polychaetes which indicates parasite establishment. First identified from the Pit River drainage (Schafer, 1968), California, *C. shasta* is now considered endemic in most major Pacific Northwest river drainages, including the San Joaquin, Sacramento, Pit, Klamath, Rogue, Columbia and Fraser Rivers, as well as several smaller water bodies (Nehalem, Alsea and Chehalis Rivers) and Lake Washington (Sanders *et al.*, 1970; Ratliff, 1983; Ching and Munday, 1984a; Hoffmaster *et al.*, 1988; Hendrickson *et al.*, 1989; Stocking *et al.*, 2006, 2007). In Alaska, distribution has been inferred from detection of *C. shasta* in adult salmon, indicating that the parasite is present in several south-central and interior drainages, including the Yukon (Meyers *et al.*, 2008).

Host distribution

The resulting parasite distribution mosaic is reflected in patterns of resistance among salmon and trout, with relative resistance to infection and disease occurring in fish populations that have evolved in waters where the parasite is endemic. Thus, strains of salmonids within the same species may show different susceptibilities to *C. shasta* (Zinn *et al.*, 1977; Buchanan and Sanders, 1983; Ching and Munday 1984b; and reviewed in Bartholomew, 1998). These variations in susceptibility of populations of salmon and trout to infection and disease are one of the best-documented examples of heritable resistance in fishes (Ibarra *et al.*, 1992; Bartholomew, 1998; Bartholomew *et al.*, 2001; Nichols *et al.*, 2003).

C. shasta has been regarded as a single species throughout both its geographic and

its host ranges based on: (i) similarities in the site of infection in fish; (ii) disease manifestations; and (iii) morphology of the myxospore. This conclusion was largely supported by genetic studies, as the ssrRNA sequences of isolates from different geographic locations and from different species were homogeneous (Atkinson and Bartholomew, 2010a). However, recent studies on *C. shasta* in the Klamath River system document the presence of multiple parasite strains based on differences in the ITS1 (Atkinson and Bartholomew, 2010a, b). In rivers with mixed salmonid species, parasite genotypes occur in sympatry yet show marked differences in infection success in different hosts, indicating evolution of host-specific parasite genotypes. Similar to the distribution of their anadromous hosts, some of these parasite strains have been extirpated from portions of rivers with the construction of dams that have blocked fish passage (Atkinson and Bartholomew, 2010a).

Impact

Ceratomyxosis is considered one of the most virulent myxozoan diseases, in part as a result of early epizootics in hatcheries where susceptible strains of salmon and trout were reared on surface waters containing the parasite. Hatcheries where outbreaks occurred were forced to change water sources, treat the water supply or rear more resistant strains of fish. While these practices have decreased epizootics in hatcheries, outbreaks still occur when treatment systems fail, environmental conditions change to favour the parasite, or when susceptible strains of fish are brought on to these facilities. Even when protected from infection in the hatchery, these juvenile fish will be exposed to *C. shasta* following release into rivers where parasite levels are high. Naturally reared fish are similarly at risk of disease, and in some cases this risk may be even higher because of the longer period these fish are exposed to the parasite.

In contrast to whirling disease, size and age of the fish have little effect on the severity of ceratomyxosis (Bjork and Bartholomew, 2009). Estimates of infection and mortality in natural populations of juvenile salmonids

and among hatchery fish following release are difficult to determine and vary widely (Ratliff, 1981; Bartholomew *et al.*, 1992; Margolis *et al.*, 1992; Foott *et al.*, 2004). Information can be deduced from sentinel fish exposures, water sampling and fish trapping (e.g. Foott *et al.*, 2004; Hallett and Bartholomew, 2006; Stocking *et al.*, 2006). It is generally acknowledged that the parasite may significantly affect juvenile survival during years when water flows are low and water temperatures high; however, the consistent high mortality of juvenile salmon that occurs in certain rivers may be indicative of an imbalance in the host–parasite relationship. In the Klamath River (which rises in Oregon and flows through northern California, USA), *C. shasta* infections have caused significant mortality of migrating juvenile salmon (Foott *et al.*, 2004; Stocking *et al.*, 2006), with consequences for commercial fishermen and Native Americans that rely on these fish for their livelihood.

C. shasta is also an important contributor to pre-spawn mortality of infected adult fish (Sanders *et al.*, 1970; Chapman, 1986; Bartholomew *et al.*, 1992).

8.2.2. Diagnosis of the infection and clinical signs of the disease

Clinical signs

Clinical signs of ceratomyxosis vary with level of infection, fish species and fish age. Infected juvenile salmon typically become anorexic, lethargic and darken in colour (especially rainbow trout/steelhead). The anus becomes swollen and haemorrhaged and the abdomen may be distended with ascites (Fig. 8.6a). Exophthalmia is common in fish with ascites. Acutely infected fish may die before clinical signs develop.

Diagnosis

As with other myxozoan infections, visual diagnosis is complicated by the long period required for development of mature myxospores in the fish and by the pleomorphic appearance of the presporogonic stages. However, in contrast to *M. cerebralis*, the location of the parasite in the intestine provides an accessible tissue to sample. Spore maturation is generally simultaneous with the death

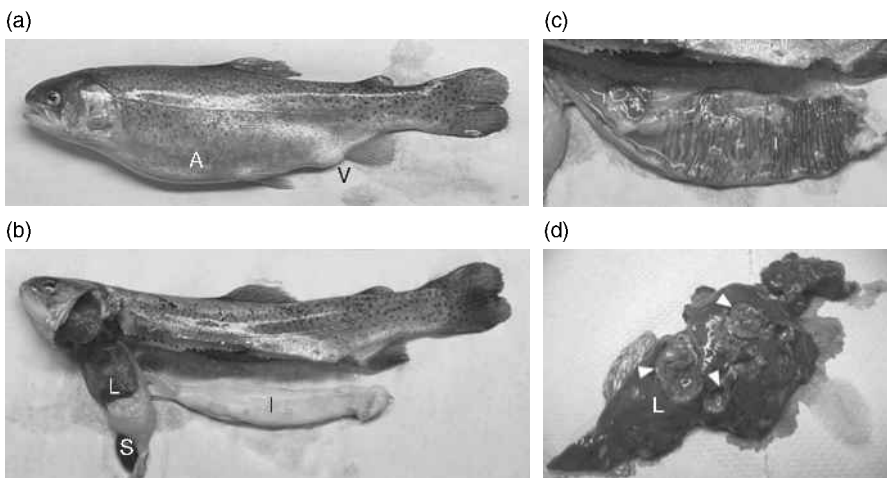


Fig. 8.6. External and internal gross signs of *C. shasta* infection. (a) Clinical ceratomyxosis in an allopatriic rainbow trout showing swollen vent (V) and abdomen (A) distended with ascites; (b) dissected rainbow trout with swollen intestine (I), enlarged spleen (S) and mottled lesions on the liver (L); (c) opened intestine showing haemorrhaging; (d) liver from an adult chinook salmon showing abscessed lesions (images in (a)–(c) provided by Matthew Stinson, Oregon State University; (d) provided by Craig Banner, Oregon Department of Fish and Wildlife).

of the fish host and the process is temperature dependent: for example, the mean time from infection to death for rainbow trout held at 12°C is 55 days; this decreases to 19 days at 20.5°C (Udey *et al.*, 1975). Presumptive diagnosis of *C. shasta* is confirmed by the identification of myxospores with the appropriate morphology or by specific amplification of DNA of presporogonic life stages (Bartholomew, 2003). While other myxozoans such as *Myxidium minterii*, *Chloromyxum* spp. and *Myxobolus* spp. may co-occur with *C. shasta* infections, the ceratomyxid is unique in morphology. Of almost 200 species of *Ceratomyxa* Thélohan, 1892, only five are known from fresh water, and only *C. shasta* infects salmonid fish and intestinal tissue (Lom and Dyková, 2006; Gunter *et al.*, 2009).

PRESUMPTIVE DIAGNOSIS Wet mounts prepared from the wall of the posterior intestine or from ascites are examined for spores.

Lesions in any tissue should also be examined. Wet mounts can be scanned in a systematic manner under phase contrast or brightfield microscopy at 250–400× magnification. Presumptive diagnosis is based on identification of multicellular myxosporean presporogonic stages (trophozoites; Fig. 8.7a) (Bartholomew, 2003). Infected salmonids may not show signs of ceratomyxosis. An alternative to wet mounts are tissue imprints or histological sections of intestinal or other grossly infected tissues. These may be stained with either Giemsa or haematoxylin and eosin stain. In Giemsa-stained sections, multicellular trophozoites appear light blue with the nuclei containing a dark-staining karyosome surrounded by a clear halo (Fig. 8.7b).

CONFIRMATORY DIAGNOSIS Visual confirmation of *C. shasta* infection is by identification of the characteristic kidney bean-shaped myxospores in wet mounts or histological sections.

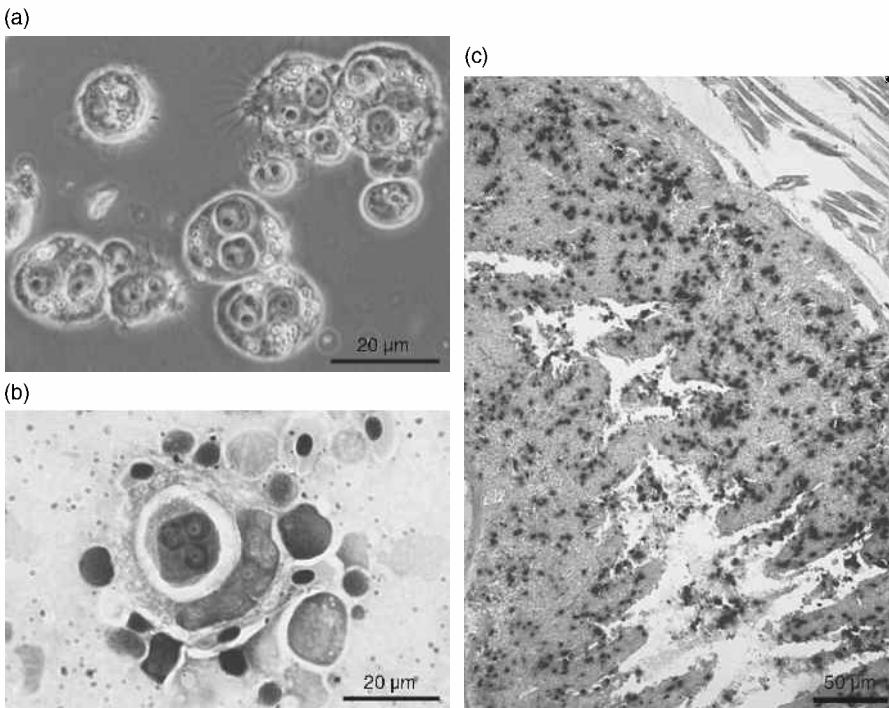


Fig. 8.7. Diagnosis of *C. shasta* infections. (a) Trophozoite, or presporogonic, stages of the parasite in ascites; (b) presporogonic stage in kidney imprint, stained with Giemsa; (c) *in situ* hybridization staining of posterior intestine of a heavily infected rainbow trout, labelled parasites stain dark brown.

Spores are most often detected in the posterior intestine, but may be found in other tissues as well, particularly the kidney, liver, gall bladder and pyloric caeca. Confirmation using molecular diagnosis has become standard, and *C. shasta*-specific PCR primers have been developed (Palenzuela *et al.*, 1999; Palenzuela and Bartholomew, 2002; Bartholomew, 2003).

Non-lethal sampling techniques for adult salmon include intestinal lavage which uses a syringe and flexible tubing to flush the posterior intestine with saline (Coley *et al.*, 1983). Although that study used visual examination to detect spores, molecular methods could also have been employed. Fox *et al.* (2000) modified this technique for juvenile fish and used a swab for collection of intestinal contents combined with PCR for detection. Although this test was not as sensitive as the lethal PCR assay, the parasite could be detected as early as 13 days post-exposure. PCR primers have also been adapted for *in situ* hybridization on histological sections (Fig. 8.7c), although this remains primarily a research tool (Palenzuela and Bartholomew, 2002; Bjork and Bartholomew, 2010). Quantitative PCR (qPCR) allows estimation of parasite density in a sample and, when combined with water filtration, has been used to monitor for water-borne spores in rivers and to predict mortality in migrating juvenile fish (Hallett and Bartholomew, 2006).

8.2.3. External/internal lesions

Macroscopic

The most common external lesion is a haemorrhagic anus that results from severe intestinal lesions. Internally, macroscopic and microscopic lesions are common in *C. shasta*-infected fish and are not restricted to the primary site of infection. In susceptible fish, *C. shasta* invades all intestinal tissue layers and causes necrosis and haemorrhaging, resulting in mortality approaching 100%. Internally, the digestive tract may be grossly swollen, necrotic and haemorrhagic with mucoid contents (Fig. 8.6b, c). The intestine and pyloric caeca may also be lined with caseous material. Additional characteristics may include ascites, lesions in the kidney or liver (fluid

filled blebs/pustules to firm creamy white nodules) and haemorrhaging and (or) necrosis of liver, gall bladder, spleen, gonads, kidney, heart, gills and skeletal musculature.

In adult salmonids, the walls of the intestine and pyloric caeca may be thickened and haemorrhagic. Nodular lesions may develop in the intestinal wall, and perforate the intestine. Gross lesions (which may abscess) can occur in liver, kidney, spleen or musculature (Fig. 8.6d; Conrad and Decew, 1966; Schafer, 1968; Bartholomew *et al.*, 1989).

Microscopic

In the intestine, the parasite triggers an acute inflammatory reaction involving polymorphonuclear leukocytes (PMNL), fibroblasts and macrophages. In severe infections, the epithelial lining necrotizes, fragments and ultimately sloughs, and is replaced by fibrous connective tissue that contains host cells and parasite stages (Bartholomew *et al.*, 2004). The intestinal lumen may contain epithelial cells, epithelial cell fragments, PMNL, fibroblasts and different parasite stages (Bartholomew *et al.*, 1989; Bjork and Bartholomew, 2010). As the trophozoite stages of *C. shasta* proliferate in the intestine and blood vessels, the infection spreads to other organs (Fig. 8.8). Parasites may be detected subsequently in the pyloric caeca, kidney (Fig. 8.8e) and liver, and finally in the capsule of the spleen.

In resistant hosts the parasite can successfully invade the gills and establish in the blood vessels (Bjork, 2010), however, it is cleared from the bloodstream within 2 weeks. Two defence strategies have been observed histologically: (i) parasites are isolated in granulomatous lesions and eliminated; and (ii) parasites migrate through the intestinal layers to the lumen without evidence of host tissue reactions (Bartholomew *et al.*, 1989, 2004; Ibarra *et al.*, 1991; Foott *et al.*, 2007; Bjork, 2010). The latter response may indicate immunological tolerance.

8.2.4. Pathophysiology

Despite our understanding of the pathological effects, the physiological aspects of the disease are largely unknown. Afflicted fish

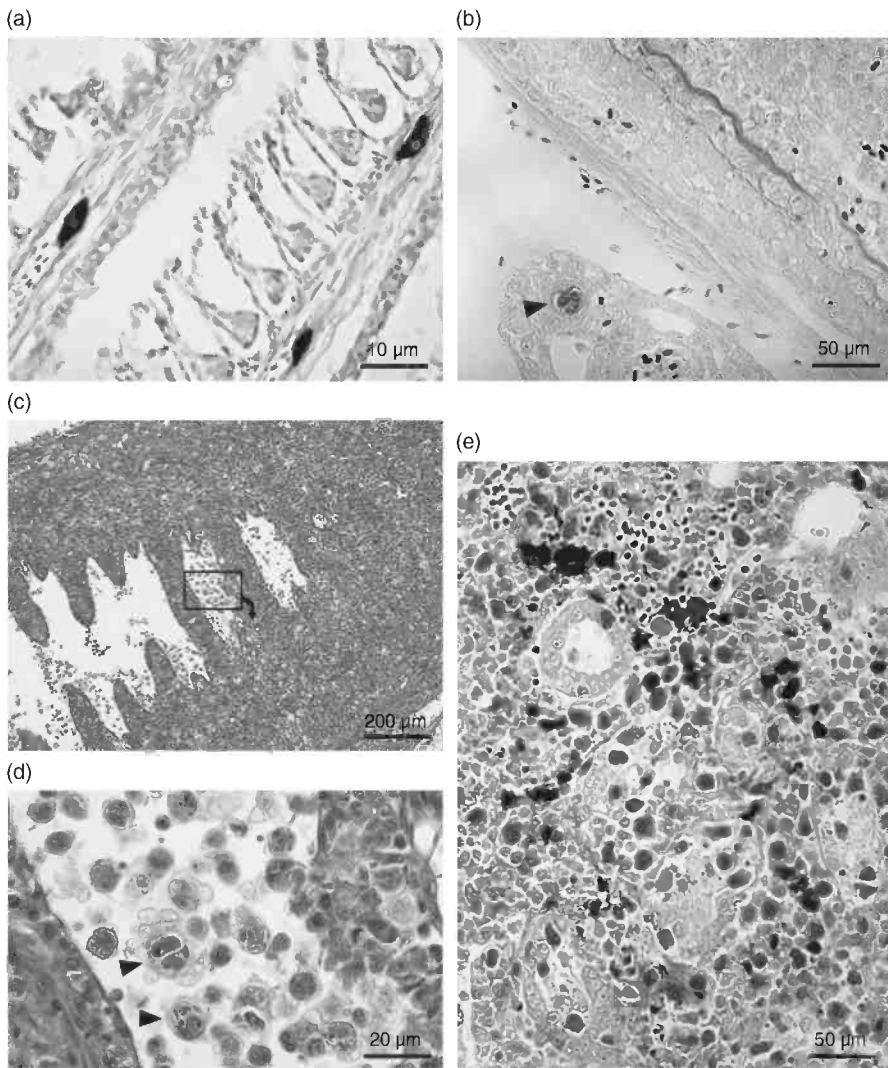


Fig. 8.8. Histological sections of allopatric rainbow trout at different times post-infection. (a) Parasites in gill arch blood vessels; (b) parasite (arrowhead) in blood vessel supplying the intestine; (c) longitudinal section of the posterior intestine late in the infection showing destruction of intestinal epithelium and proliferation of parasites and host immune cells; (d) higher magnification of (c), showing a variety of parasite stages, including disporoblasts (arrowheads); (e) kidney with parasite stages proliferating throughout (image in (a) provided by Sarah Bjork, Oregon State University).

may be immunocompromised and have hampered nutrient uptake and transportation, resulting in reduced growth (Barker *et al.*, 1993). Disease severity is related to: (i) the parasite dose; (ii) the inherent resistance of the fish strain; and (iii) water temperature.

For fish that succumb to infection, death may be a direct result of damage to the intestine or may result from secondary invasion in the damaged tissue by bacterial pathogens. Although empirical observations indicate that parasitized fish are more prone to secondary infection by other pathogens, this has

rarely been experimentally demonstrated. In some cases, co-infections may be a result of a lowered immune capacity as a result of myxozoan infection. However, the coincidence of infections of *C. shasta* with bacterial pathogens of low virulence, such as *Aeromonas hydrophila*, suggests that the lesions that result from *C. shasta* infection allow the environmental bacterium to invade and become a primary pathogen.

The severe granulomatous enteritis that develops in response to *C. shasta* infection also appears to contribute to diminished body condition (Bartholomew *et al.*, 2004), possibly by disturbance of adsorption and transport functions in the intestine (Barker *et al.*, 1993). Protein-losing enteropathy, wasting and ascites are commonly associated with these lesions and negatively impact growth of infected fish. Survivors of infections in hatcheries are undersized (Tipping, 1988) and it has been suggested that *C. shasta* probably affects post-release survival during migration and seawater acclimation either indirectly by decreasing fitness or directly as the disease progresses.

Despite the presence of this parasite in the gills both early (Bjork and Bartholomew, 2010) and late (Bartholomew *et al.*, 1989) in the infection, there is no direct evidence that the parasite interferes with osmoregulatory functions. This should be examined more thoroughly, particularly during the process of smoltification and seawater transition of anadromous fishes.

8.2.5. Protective/control strategies

Hatcheries

Because *C. shasta* infections are not transmitted directly between fish, outbreaks in hatcheries occur only by introduction of the invertebrate host and/or actinospores through the water supply. The invertebrate host for *C. shasta* has different habitat preferences to the host of *M. cerebralis* and is less likely to accumulate in a hatchery environment. Thus the most effective means of disease prevention in a hatchery is by use of uncontaminated water or by rearing *C. shasta*-resistant strains of salmon and

trout. In hatcheries where parasite-free water supplies are unavailable, UV irradiation or chlorination of water supplies can reduce or eliminate the infective actinospore (Bedell, 1971). Sand filtration in combination with either of these methods is more effective in reducing incidence of disease (Sanders *et al.*, 1972; Bower and Margolis, 1985) and ozone treatment of water reduces mortality from ceratomyxosis and also increases fish growth (Tipping, 1988). There has only been limited testing of therapeutants for controlling ceratomyxosis. Two studies investigated the efficacy of fumagillin and its analogue TNP-470 and found no substantial protection when administered either prophylactically or for 53 days post-infection (Ibarra *et al.*, 1990; Whipple *et al.*, 2002). Similarly, glucans fed prophylactically did not provide protection to subsequent parasite exposure (Whipple *et al.*, 2002).

Wild

This parasite has not been as broadly disseminated as other fish pathogens because *C. shasta* is not transmitted horizontally or vertically and the invertebrate host apparently has a restricted range. However, in natural waters where it is present, it continues to cause severe disease as control options are limited. The most widely applied management tool for maintaining sports fisheries in endemic waters has been the stocking of resistant salmonids (Buchanan and Sanders, 1983). Interestingly, a corollary to this practice is based on concerns about hatchery fish interbreeding with native fish. Because of this, highly susceptible strains of fish from non-endemic waters have been used to stock certain watersheds, with the knowledge that these fish would not survive infection to interbreed. While this has been an effective management tool, it elevated parasite levels in some waters to very high levels that could potentially affect natural fish populations (Hurst *et al.*, 2011). Another strategy for minimizing infection of hatchery fish is to time release to occur during periods when parasite abundance is low.

In many rivers construction of dams, diversion of water and destruction of riparian habitat have stabilized water flows, raised

water temperatures and reduced sediment transport. These changes may increase habitat for the polychaete host. In these systems, the need to develop predictive tools for parasite effects has led to development of water sampling methods that both quantify the parasite and distinguish between parasite genotypes (Hallett and Bartholomew, 2006; Atkinson and Bartholomew, 2010a, b). These assays allow predictions of which fish species are likely to become infected and what level of mortality may be expected so that managers can make real-time decisions about water allocation and timing of fish release from hatcheries. These tools also allow better resolution of where focal points for infection occur and in the future it may be possible to reduce disease incidence through implementation of water flows that either reduce the amount of time fish spend in these areas or scour polychaete habitat.

Removal of adult salmon carcasses from spawning grounds was proposed as a management action to reduce myxospore input into the Klamath River system, but the approach was deemed impractical after a pilot study. Although many fish (up to 86%) were infected, myxospores were not always observed. Only a few fish (<10%) contributed high numbers of spores (> one million) back into the system (Foott *et al.*, 2010) and the effect of carcass removal efforts could not easily be measured.

An epizootiological model, a theoretical predictive tool, is being developed to identify parameters important for parasite persistence (Ray *et al.*, 2010). Development of the model will highlight information deficiencies and once values are obtained for all parameters (e.g. transmission efficiency), the model will indicate which link in the cycle should be severed to achieve the best outcome for fish populations.

8.3. Conclusions and Suggestions for Future Studies

Few other pathogens in North America have received as much attention or raised as much public awareness on the importance of healthy fisheries as *M. cerebralis* (Bartholomew

and Wilson, 2002). In response to the impact of the parasite on wild fish populations in the USA, a cooperative research effort was developed between private individuals, federal and state governments and the scientific community (Bartholomew and Wilson, 2002). This was a unique endeavour that endured more than a decade. Research was funded to combat the disease from a number of approaches and resulted in a tremendous increase in our knowledge of this parasite. *M. cerebralis* now serves as a model for studies on other myxosporeans and progress in our understanding this organism has radically altered our view of the entire phylum and impelled myxozoan research.

Identification of the causative agent for whirling disease and its source permitted more extensive and better controlled studies, as evidenced by the explosion of scientific literature in the 1990s (see Hedrick *et al.*, 1998; Bartholomew and Wilson, 2002). Outbreaks of whirling disease in wild trout populations in the US Intermountain West (Nehring and Walker, 1996; Vincent, 1996) further spurred research. Similarly, identification of the infectious stage for ceratomyxosis and its invertebrate host permitted new avenues of experimentation, and continued disease outbreaks in hatcheries and imperilled wild salmonid stocks have funnelled state and federal finances to research.

Despite advances in our knowledge of both *M. cerebralis* and *C. shasta*, we still have numerous unanswered questions because of the impediments and limitations to working with organisms that cannot be cultured, and that require two different hosts to complete their life cycles. In contrast to *M. cerebralis*, *C. shasta* remains a problematic parasite to study under laboratory conditions. A parasite-free stock of polychaetes is difficult to obtain and maintain. This host is sensitive to handling (it dwells in a self-made tube), is almost microscopic and is fastidious. A high actinospore dose is required for transmission to some fish strains, yet worms have a low infection intensity and actinospore development is asynchronous. Improvements in polychaete culture and laboratory challenge models have been made, but working with the parasite remains an unpredictable and challenging venture.

The lack of an adequate test for spore viability has hampered assessments of physical and chemical stressors on spore longevity, particularly for *C. shasta*. Vital stains such as methylene blue (Hoffman and Markiw, 1977), fluorescein diacetate (FDA) and propidium iodide (PI) (Markiw, 1992b; Yokoyama *et al.*, 1997; Wagner *et al.*, 2003) have been used to indicate viability of myxozoans, but have produced variable results and infectivity studies have demonstrated that vital staining underestimates the inactivation of *M. cerebralis* actinospores (Hedrick *et al.*, 2007; Kallert and El-Matbouli, 2008). Thus, the ability to infect the next host remains the best metric for evaluating spore viability (Hedrick *et al.*, 2008) but a simpler, faster method that circumvents such infection experiments would be beneficial.

Less is known about *C. shasta* than *M. cerebralis*. But for both parasites, it is still unclear why they have failed to colonize certain regions, and why disease effects differ among regions where they are established. Preliminary investigations of the polychaete host of *C. shasta* indicate that there are multiple strains (cytochrome oxidase subunit 1 genotypes) of *M. speciosa* in the Pacific Northwest. In contrast to the worm host of *M. cerebralis*, there is no evidence to suggest that susceptibility of polychaetes to *C. shasta* varies with host genotype or that there is a correlation between host genotype and parasite strain.

The discovery that *C. shasta* is comprised of multiple, host-specific genotypes suggests a re-evaluation of previous conclusions regarding susceptibilities of different salmonid species and strains, as testing may have been done using inappropriate parasite genotypes. It also presents opportunities for management, as severe infection in one salmonid species does not necessarily mean all species are at risk. Thus, we need further refinement of genotyping tools to allow better predictive ability for effects and examination of other salmonid species across the parasite range to determine how they are affected by different genotypes.

The development of methods for quantification of parasites in environmental samples leads to opportunities for close to real-time monitoring of parasite levels that could be used to predict disease mortality

and facilitate rapid management responses. Establishment of monitoring programmes on key river systems could provide information for epizootic models and to begin to examine anthropogenic factors and climate change that will affect parasite distribution.

A great deal of progress has been made in understanding host responses to parasite invasion for both species, and this research avenue should be pursued with the aim of determining protective host responses. We are only beginning to investigate genes that are upregulated during parasite invasion (Severin *et al.*, 2007; Baerwald *et al.*, 2008; Bjork, 2010; Zhang *et al.*, 2010), and this should continue with corollary functional studies to determine how these molecules interact with the parasites. We have little understanding of: (i) factors related to parasite virulence; (ii) the mechanisms they use to invade and proliferate in their hosts; and (iii) their migration to very specific tissues. Understanding these mechanisms could provide clues to what treatments might be efficacious in culture, and particularly indicate those that might provide protection after fish are released into endemic waters. However, because the regulatory environment will probably continue to limit chemical therapeutic options in aquaculture, research should proceed down other avenues such as marker-assisted selection for disease resistant traits in captive populations and risk assessments to identify means to minimize disease outbreaks for natural populations.

Acknowledgements

Sam Onjukka (Oregon Department of Fish and Wildlife) kindly provided fish infected with *M. cerebralis*. Harriet Lorz (Oregon State University) isolated the *M. cerebralis* myxospores and prepared the histological sections. Matthew Stinson (Oregon State University) and Craig Banner (Oregon Department of Fish and Wildlife) shared the photographs in Fig. 8.6 parts (a–c) and (d), respectively. Stephen Atkinson (Oregon State University) assisted with photography, figure preparation and reviewed the text.

References

- Alderman, D.J. (1986) Whirling disease chemotherapy. *Bulletin of the European Association of Fish Pathologists* 6, 38–40.
- American Fisheries Society – Fish Health Section (2010) *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens, Blue Book*, 2010 edn. Fish Health Section, American Fisheries Society, Bethesda, Maryland.
- Andree, K.B., Gresoviac, S.J. and Hedrick, R.P. (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.B., MacConnell, E. and Hedrick, R.P. (1998) A nested polymerase 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.B., Hedrick, R.P. and MacConnell, E. (2002) A review of the approaches to detect *Myxobolus cerebralis*, the cause of salmonid whirling disease. In: Bartholomew J.L. and Wilson J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 197–212.
- Antonio, D.B., Andree, K.B., McDowell, T.S. and Hedrick, R.P. (1998) Detection of *Myxobolus cerebralis* in rainbow trout and oligochaete tissues by using a non-radioactive *in situ* hybridization (ISH) protocol. *Journal of Aquatic Animal Health* 10, 338–347.
- Arndt, R. (2005) Pilot project at Midway Hatchery to evaluate several whirling disease filtration techniques. *Utah Division of Wildlife Resources Fisheries Experiment Station Ichthyogram* 16, 1–3.
- Arndt, R.E. and Wagner, E.J. (2003) Filtering *Myxobolus cerebralis* triactinomyxons from contaminated water using rapid sand filtration. *Aquacultural Engineering* 29, 77–91.
- Arsan, E.L. and Bartholomew, J.L. (2008) Potential for dissemination of the non-native salmonid parasite *Myxobolus cerebralis* in Alaska. *Journal of Aquatic Animal Health* 20, 136–149.
- Arsan, E.L. and Bartholomew, J.L. (2009) Potential dispersal of the non-native parasite *Myxobolus cerebralis* in the Willamette River Basin, Oregon: a qualitative analysis of risk. *Reviews in Fisheries Science* 17, 360–372.
- Arsan, E.L., Atkinson, S.D., Hallett, S.L., Meyers, T. and Bartholomew, J.L. (2007a) Expanded geographical distribution of *Myxobolus cerebralis*: first detections from Alaska. *Journal of Fish Diseases* 30, 483–491.
- Arsan, E.L., Hallett, S.L. and Bartholomew, J.L. (2007b) *Tubifex tubifex* from Alaska: distribution and susceptibility to *Myxobolus cerebralis*. *Journal of Parasitology* 93, 1332–1342.
- Atkinson, S.D. and Bartholomew, J.L. (2010a) Disparate infection patterns of *Ceratomyxa shasta* (Myxozoa) in rainbow trout *Oncorhynchus mykiss* and Chinook salmon *Oncorhynchus tshawytscha* correlate with ITS-1 sequence variation in the parasite. *International Journal for Parasitology* 40, 599–604.
- Atkinson, S.D. and Bartholomew, J.L. (2010b) Spatial, temporal and host factors structure the *Ceratomyxa shasta* (Myxozoa) population in the Klamath River basin. *Infection, Genetics and Evolution* 10, 1019–1026.
- Baerwald, M.R., Welsh, A.B., Hedrick, R.P. and May B. (2008) Discovery of genes implicated in whirling disease infection and resistance in rainbow trout using genome-wide expression profiling. *BMC Genomics* 9, 1–11.
- Baerwald, M.R., Petersen, J.P., Hedrick, R.P., Schisler, G.J., and May, B. (2011) A major effect quantitative trait locus for whirling disease resistance identified in rainbow trout (*Oncorhynchus mykiss*). *Heredity* 106, 920–926.
- Baldwin, T.J. and Myklebust, K.A. (2002) Validation of a single-round polymerase chain reaction assay for identification of *Myxobolus cerebralis* myxospores. *Diseases of Aquatic Organisms* 49, 185–190.
- Baldwin, T.J., Peterson, J.E., McGhee, G.C., Staigmiller, K.D., Motteram, E.S., Downs, C.C. and Stanek, D.R. (1998) Distribution of *Myxobolus cerebralis* in salmonid fishes in Montana. *Journal of Aquatic Animal Health* 10, 361–371.
- Baldwin, T.J., Vincent, E.R., Silflow, R.M. and Stanek D. (2000) *Myxobolus cerebralis* infection in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) exposed under natural stream conditions. *Journal of Veterinary Diagnostic Investigation* 12, 312–321.
- Barker, I.K., Van Dreumel, A.A. and Palmer, N. (1993) The alimentary system: chronic inflammatory disease. In: Jubb, K.V.F., Kennedy, P.C. and Palmer, N. (eds) *Pathology of Domestic Animals*, Vol. 2, 4th edn. Academic Press, New York, pp. 120–124.

- 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. (2003) Salmonid ceratomyxosis. In: *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens, Blue Book*, Vol. 2, 5th (2003) edn, Fish Health section. American Fisheries Society, Bethesda, Maryland.
- Bartholomew, J.L. and Reno, P.W. (2002) The history and dissemination of whirling disease. In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 3–24.
- Bartholomew, J.L. and Wilson, J.C. (eds) (2002) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland.
- Bartholomew, J.L., Smith, C.E., Rohovec, J.S. and Fryer, J.L. (1989) Characterization of the 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., Fryer, J.L. and Rohovec, J.S. (1992) Impact of the myxosporean parasite, *Ceratomyxa shasta*, on survival of migrating Columbia River basin salmonids. In: Svrjcek, R.S. (ed.) *Proceedings of the 19th US Japan meeting on Aquaculture*, Ise, Mie Prefecture, Japan, 29–30 October 1990. National Oceanic and Atmospheric Administration (NOAA) Technical Report National Marine Fisheries Service (NMFS) 111. US Department of Commerce, NOAA, NMFS, Springfield, Virginia, pp. 33–41.
- Bartholomew, J.L., Whipple, M.J., Stevens, D.G. and Fryer, J.L. (1997) The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. *American Journal of Parasitology* 83, 859–868.
- Bartholomew, J.L., Whipple, M.J. and Campton, D. (2001) Inheritance of resistance to *Ceratomyxa shasta* in progeny from crosses between high- and low-susceptibility strains of rainbow trout (*Oncorhynchus mykiss*). *Bulletin of the National Research Institute of Aquaculture* Supplement 5, 71–75.
- Bartholomew, J.L., Lorz, H.V., Sollid, S.A. and Stevens, D.G. (2003) Susceptibility of juvenile and yearling bull trout to *Myxobolus cerebralis*, and effects of sustained parasite challenges. *Journal of Aquatic Animal Health* 15, 248–255.
- Bartholomew, J.L., Ray, E., Torell, B., Whipple, M.J. and Heidel, J.R. (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., Kerans, B.L., Hedrick, R.P., MacDiarmid, S.C. and Winton, J.R. (2005) A risk assessment based approach for the management of whirling disease. *Reviews in Fisheries Science* 13, 205–230.
- Bartholomew, J.B., Lorz, H.V., Atkinson, S.D., Hallett, S.L., Stevens, D.G., Holt, R.A., Lujan, K. and Amandi, A. (2007) Evaluation of a management strategy to control the spread of *Myxobolus cerebralis* in a lower Columbia River tributary. *North American Journal of Fisheries Management* 27, 542–550.
- Beauchamp, K.A., Gay, M., Kelley, G.O., El-Matbouli, M., Kathman, R.D., Nehring, R.B. and Hedrick, R.P. (2002) Prevalence and susceptibility of infection to *Myxobolus cerebralis*, and genetic differences among populations of *Tubifex tubifex*. *Diseases of Aquatic Organisms* 51, 113–121.
- Beauchamp, K.A., Kelley, G.O., Nehring, R.B. and Hedrick, R.P. (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.
- Beauchamp, K.A., El-Matbouli, M., Gay, M., Georgiadis, M.P., Nehring, R.B. and Hedrick, R.P. (2006) The effect of cohabitation of *Tubifex tubifex* (Oligochaeta: Tubificidae) populations on infections to *Myxobolus cerebralis* (Myxozoa: Myxobolidae). *Journal of Invertebrate Pathology* 91, 1–8.
- Bedell, G.W. (1971) Eradicating *Ceratomyxa shasta* from infected water by chlorination and ultraviolet irradiation. *Progressive Fish-Culturist* 33, 51–54.
- Bjork, S.J. (2010) Factors affecting the *Ceratomyxa shasta* infectious cycle and transmission between polychaete and salmonid hosts. PhD thesis, Oregon State University, Corvallis, Oregon, 207 pp.
- Bjork, S.J. and Bartholomew, J.L. (2009) Effects of *Ceratomyxa shasta* dose on a susceptible strain of rainbow trout and comparatively resistant Chinook and coho salmon. *Diseases of Aquatic Organisms* 86, 29–37.
- Bjork, S.J. and Bartholomew, J.L. (2010) Invasion of *Ceratomyxa shasta* (Myxozoa) and comparison of migration to the intestine between susceptible and resistant fish hosts. *International Journal for Parasitology* 40, 1087–1095.
- Blazer, V.S., Waldrop, T.B., Schill, W.B., Densmore, C.L. and Smith, D. (2003) Effects of water temperature and substrate type on spore production and release in eastern *Tubifex tubifex* worms infected with *Myxobolus cerebralis*. *Journal of Parasitology* 89, 21–26.

- Bower, S.M. and Margolis, L. (1985) Microfiltration and ultraviolet irradiation to eliminate *Ceratomyxa shasta* (Myxozoa: Myxosporaea), a salmonid pathogen, from Fraser River water, British Columbia. *Canadian Technical Report of Fisheries and Aquatic Sciences* 1364, 11.
- Buchanan, D.V. and Sanders, J.E. (1983) Relative susceptibility of four strains of summer steelhead to infection by *Ceratomyxa shasta*. *Transactions of the American Fisheries Society* 112, 541–543.
- Cavender, W.P., Wood, J.S., Powell, M.S., Overturf, K.E. and Cain, K.D. (2004) Real-time quantitative polymerase chain reaction (QPCR) to identify *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 60, 205–213.
- Chapman, P.F. (1986) Occurrence of the non-infective stage of *Ceratomyxa shasta* in mature summer chinook salmon in the South fork Salmon River, Idaho. *The Progressive Fish Culturist* 48, 304–306.
- Ching, H.L. and Munday, D.R. (1984a) Geographic and seasonal distribution of the infectious stage of *Ceratomyxa shasta* Noble, 1950, a myxozoan salmonid pathogen in the Fraser River system. *Canadian Journal of Zoology* 62, 1075–1080.
- Ching, H.L. and Munday, D.R. (1984b) Susceptibility of six Fraser chinook salmon stocks to *Ceratomyxa shasta* and the effects of salinity on ceratomyxosis. *Canadian Journal of Zoology* 62, 1081–1083.
- Christensen, N.O. (1972) Panel review on myxosomiasis (whirling disease in salmonid fishes). *FI/EIFAC 72/SC II-Symposium* 8, 6.
- Coley, T.C., Chacko, A.J. and Klontz, G.W. (1983) Development of a lavage technique for sampling *Ceratomyxa shasta* in adult salmonids. *Journal of Fish Diseases* 6, 317–319.
- Conrad, J.F. and Decew, M. (1966) First report of *Ceratomyxa* in juvenile salmonids in Oregon. *The Progressive Fish Culturist* 28, 238.
- DuBey, R.J., Caldwell, C.A. and Gould, W.R. (2007) Relative susceptibility and effects on performance of Rio Grande cutthroat trout and rainbow trout challenged with *Myxobolus cerebralis*. *Transactions of the American Fisheries Society* 136, 1406–1414.
- Eiras, J.C., Molnár, K. and Lu, Y.S. (2005) Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporaea: Myxobolidae). *Systematic Parasitology* 61, 1–46.
- El-Matbouli, M. and Hoffmann, R.W. (1991) Effects of freezing, aging, and passage through the alimentary canal of predatory animals on the viability of *Myxobolus cerebralis* spores. *Journal of Aquatic Animal Health* 3, 260–262.
- El-Matbouli, M. and Hoffmann, R.W. (1998) Light and electron microscopic studies on the chronological development of *Myxobolus cerebralis* to the actinosporean stage in *Tubifex tubifex*. *International Journal for Parasitology* 28, 195–217.
- El-Matbouli, M. and Soliman, H. (2005) Development of a rapid assay for the diagnosis of *Myxobolus cerebralis* in fish and oligochaetes using loop-mediated isothermal amplification. *Journal of Fish Diseases* 28, 549–557.
- El-Matbouli, M., Hoffmann, R.W. and Mandok, C. (1995) Light and electron microscopic observations on the route of the triactinomyxon-sporoplasm of *Myxobolus cerebralis* from epidermis into rainbow trout cartilage. *Journal of Fish Biology* 46, 919–935.
- El-Matbouli, M., Hoffmann, R.W., Shoel H., McDowell, T.S. and Hedrick, R.P. (1999a) Whirling disease: host specificity and interaction between the actinosporean stage of *Myxobolus cerebralis* and rainbow trout (*Oncorhynchus mykiss*) cartilage. *Diseases of Aquatic Organisms* 35, 1–12.
- El-Matbouli, M., McDowell, T.S., Antonio, D.B., Andree, K.B. and Hedrick, R.P. (1999b) 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.
- Engelking, H.M. (2002) Potential for introduction of *Myxobolus cerebralis* into the Deschutes River watershed in central Oregon from adult anadromous salmonids. In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 25–31.
- Eszterbauer, E., Kallert, D.M., Grabner, D. and El-Matbouli, M. (2009) Differentially expressed parasite genes involved in host recognition and invasion of the triactinomyxon stage of *Myxobolus cerebralis* (Myxozoa). *Parasitology* 136, 367–377.
- Feist, S.W. and Longshaw, M. (2006) Phylum Myxozoa. In: Woo, P.T.K. (ed.) *Fish Diseases and Disorders*, Volume 1: *Protozoan and Metazoan Infections*, 2nd edn. CABI Publishing, Wallingford, Oxfordshire, UK, pp. 230–296.
- Foott, J.S., Harman, R. and Stone, R. (2004) Effect of water temperature on non-specific immune function and ceratomyxosis in juvenile Chinook salmon and steelhead from the Klamath River. *California Fish and Game* 90, 71–84.

- Foott, J.S., Stone, R., Wiseman, E., True, K. and Nichols, K. (2007) Longevity of *Ceratomyxa shasta* and *Parvicapsula minibicornis* actinospore infectivity in the Klamath River. *Journal of Aquatic Animal Health* 19, 77–83.
- Foott, J.S., Fogerty, R. and Stone, R. (2010) *Ceratomyxa shasta* Myxospore Survey of Fall-run Chinook Salmon Carcasses in Bogus Creek, Shasta River and Klamath River. Component of joint Oregon State University-Yurok Fisheries–California Department of Fish and Game pilot project testing the effect of carcass removal on *C. shasta* myxospore levels in Bogus Creek, 2009–2010. California Nevada Fish Health Center FY2009 Technical Report. California Nevada Fish Health Center, Pacific Southwest Region, United States Department of the Interior, Fish and Wildlife Service, Washington, DC.
- Fox, M.D., Palenzuela, O. and Bartholomew, J.L. (2000) Strategies for diagnosis of *Ceratomyxa shasta* using the PCR: comparison of lethal and non-lethal sampling with microscopic examination. *Journal of Aquatic Animal Health* 12, 100–106.
- Gilbert, M.A. and Granath, Jr, W.O. (2001) Persistent infection of *Myxobolus cerebralis*, the causative agent of salmonid whirling disease, in *Tubifex tubifex*. *Journal of Parasitology* 87, 101–107.
- Gilbert, M.A. and Granath, Jr, W.O. (2003) Whirling disease of salmonid fish: life cycle, biology, and disease. *Journal of Parasitology* 89, 658–667.
- Gonzalez-Lanza, Ma. and Alvarez-Pellitero, Ma.P. (1984) *Myxobolus farionis* n.sp and *M. ibericus* n.sp. of *Salmo trutta* f. *fario* from the Duero basin (NW Spain). Description and population dynamics. *Angewandte Parasitologie* 25, 181–189.
- Granath, Jr, W.O. and Gilbert, M.A. (2002) The role of *Tubifex tubifex* (Annelida: Oligochaeta: Tubificidae) in the transmission of *Myxobolus cerebralis* (Myxozoa: Myxosporidia: Myxobolidae). In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 79–85.
- Granath, Jr, W.O., Gilbert, M.A., Wyatt-Pescador, E.J. and Vincent, E.R. (2007) Epizootiology of *Myxobolus cerebralis*, the causative agent of salmonid whirling disease in the Rock Creek drainage of west-central Montana. *Journal of Parasitology* 93, 104–119.
- Gunter, N.L., Whipps, C.M. and Adlard, R.D. (2009) *Ceratomyxa* (Myxozoa: Bivalvulida): robust taxon or genus of convenience? *International Journal for Parasitology* 39, 1395–1405.
- Hallett, S.L. and Bartholomew, J.L. (2006) Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in water samples. *Diseases of Aquatic Organisms* 71, 109–118.
- Hallett, S.L. and Bartholomew, J.L. (2008) Effects of water flow on the infection dynamics of *Myxobolus cerebralis*. *Parasitology* 135, 371–684.
- Hallett, S.L., Atkinson, S.D., Erséus, C. and El-Matbouli, M. (2005) Dissemination of triactinomyxons (Myxozoa) via oligochaetes used as live food for aquarium fishes. *Diseases of Aquatic Organisms* 65, 137–152.
- Hallett, S.L., Atkinson, S.D., Erséus, C. and El-Matbouli, M. (2006) Myxozoan parasites disseminated via oligochaete worms as live food for aquarium fishes: descriptions of aurantiactinomyxon and raabeia actinospore types. *Diseases of Aquatic Organisms* 69, 213–225.
- Hallett, S.L., Lorz, H.V., Atkinson, S.D., Rasmussen, C., Xue, L. and Bartholomew, J.L. (2009) Propagation of the myxozoan parasite *Myxobolus cerebralis* by different geographic and genetic populations of *Tubifex tubifex*: an Oregon perspective. *Journal of Invertebrate Pathology* 102, 57–68.
- Halliday, M.M. (1973) Studies on *Myxosoma cerebralis*, a parasite of salmonids. II. Development and pathology of *Myxosoma cerebralis* in experimentally infected rainbow trout (*Salmo gairdneri*) fry reared at different water temperatures. *Nordisk Veterinærmedicin* 25, 349–358.
- Halliday, M.M. (1976) The biology of *Myxosoma cerebralis*: the causative organism of whirling disease of salmonids. *Journal of Fish Biology* 9, 339–357.
- Hedrick, R.P. and El-Matbouli, M. (2002) Recent advances with taxonomy, life cycle, and development of *Myxobolus cerebralis* in the fish and oligochaete hosts. In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 45–53.
- Hedrick, R.P., El-Matbouli, M., Adkison, M.A. and MacConnell, E. (1998) Whirling disease: re-emergence among wild trout. *Immunological Reviews* 166, 365–376.
- Hedrick, R.P., McDowell, T.S., Gay, M., Marty, G.D., Georgiadis, M.P. and MacConnell, E. (1999a) Comparative susceptibility of rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* to

- Myxobolus cerebralis*, the cause of salmonid whirling disease. *Diseases of Aquatic Organisms* 37, 173–183.
- Hedrick, R.P., McDowell, T.S., Mukkatira, K., Georgiadis, M.P. and MacConnell, E. (1999b) Susceptibility of selected inland salmonids to experimentally induced infections with *Myxobolus cerebralis*, the causative agent of whirling disease. *Journal of Aquatic Animal Health* 11, 330–339.
- Hedrick, R.P., McDowell, T.S., Marty, G.D., Mukkatira, K., Antonio, K., Andree, K.B., Bukhari, Z. and Clancy, T. (2000) Ultraviolet irradiation inactivates the waterborne infective stages of *Myxobolus cerebralis*: a treatment for hatchery water supplies. *Diseases of Aquatic Organisms* 42, 53–59.
- Hedrick, R.P., McDowell, T.S., Mukkatira, K., Georgiadis, M.P. and MacConnell, E. (2001) Salmonids resistant to *Ceratomyxa shasta* are susceptible to experimentally induced infections with *Myxobolus cerebralis*. *Journal of Aquatic Animal Health* 13, 35–42.
- Hedrick, R.P., Petri, B., McDowell, T.S., Mukkatira, K. and Sealey, L.J. (2007) Evaluation of a range of doses of ultraviolet irradiation to inactivate waterborne actinospore stages of *Myxobolus cerebralis*. *Diseases of Aquatic Organisms* 74, 113–118.
- Hedrick, R.P., McDowell, T.S., Mukkatira, K., MacConnell, E. and Petri, B. (2008) The effects of freezing, drying, ultraviolet irradiation, chlorine and quaternary ammonium treatments in the infectivity of myxospores of *Myxobolus cerebralis* for *Tubifex tubifex*. *Journal of Aquatic Animal Health* 20, 116–125.
- Hendrickson, G.L., Carlton, A. and Manzer, D. (1989) Geographic and seasonal distribution of the infective stage of *Ceratomyxa shasta* (Myxozoa) in northern California. *Diseases of Aquatic Organisms* 7, 165–169.
- Hiner, M. and Moffitt, C.M. (2002) Modeling *Myxobolus cerebralis* infections in trout: associations with habitat variables. In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp 167–179.
- Höfer, B. (1903) Ueber die Drehkrankheit der Regenbogenforelle. *Allgemeine Fischerei Zeitung* 28, 7–8.
- Hoffman, G.L. (1962) *Whirling Disease of Trout*. Fishery Leaflet 508. United States Department of the Interior, Fish and Wildlife Service, Washington, DC.
- Hoffman, G.L. (1970) Intercontinental and transcontinental dissemination and transfaunation of fish parasites with emphasis on whirling disease. In: Snieszko, S.F. (ed.) *Symposium on Diseases of Fishes and Shellfishes*. American Fisheries Society, Bethesda, Maryland, pp. 69–81.
- Hoffman, G.L. (1974) Disinfection of contaminated water by ultraviolet irradiation, with emphasis on whirling disease (*Myxosoma cerebralis*) and its effect on fish. *Transactions of the American Fisheries Society* 103, 541–550.
- Hoffman, G.L. (1990) *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. *Journal of Aquatic Animal Health* 2, 30–37.
- Hoffman, G.L. (1999) *Parasites of North American Freshwater Fishes*, 2nd edn. Cornell University Press, Ithaca, New York, p. 71.
- Hoffman, G.L. and Markiw, M.E. (1977) Control of whirling disease (*Myxosoma cerebralis*). Use of methylene blue staining as a possible indicator of effect of heat on spores. *Journal of Fish Biology* 10, 181–183.
- Hoffman, G.L., Dunbar, C.E. and Bradford, A. (1962) *Whirling Disease of Trouts Caused by Myxosoma cerebralis in the United States*. United States Fish and Wildlife Service, Special Scientific Report Fishery Leaflet 427. United States Department of the Interior, Fish and Wildlife Service, Washington, DC.
- Hoffmaster, J.L., Sanders, J.E., Rohovec, J.S., Fryer, J.L. and Stevens, D.G. (1988) Geographic distribution of the myxosporean parasite, *Ceratomyxa shasta* in the Columbia River basin. *Journal of Fish Diseases* 11, 97–100.
- Hogge, C.I., Campbell, M.R. and Johnson, K.A. (2008) A new species of myxozoan (Myxosporea) from the brain and spinal cord of rainbow trout (*Oncorhynchus mykiss*) from Idaho. *Journal of Parasitology* 94, 218–222.
- Hurst, C.N., Holt, R.A., Tinniswood, W.R. and Bartholomew, J.L. (2011) *Ceratomyxa shasta* in the Williamson River, Oregon: implications for reintroduced anadromous salmon. *Journal of North American Fisheries Management*.
- Ibarra, A.M., Gall, G.A.E. and Hedrick, R.P. (1990) Trials with fumagillin DCH and malachite green to control ceratomyxosis in rainbow trout (*Oncorhynchus mykiss*). *Fish Pathology* 25, 217–223.
- Ibarra, A.M., Gall, G.A.E. and Hedrick, R.P. (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., Hedrick, R.P. and Gall, G.A.E. (1992) Inheritance of susceptibility to *Ceratomyxa shasta* (Myxozoa) in rainbow trout and the effect of length of exposure on the liability to develop ceratomyxosis. *Aquaculture* 104, 219–229.
- Kaesler, A.J., Rasmussen, C. and Sharpe, W.E. (2006) An examination of environmental factors associated with *Myxobolus cerebralis* infection of wild trout in Pennsylvania. *Journal of Aquatic Animal Health* 18, 90–100.
- Kallert, D.M. and El-Matbouli, M. (2008) Differences in viability and reactivity of actinospores of three myxozoan species upon ageing. *Folia Parasitologica* 55, 105–110.
- Kallert, D.M., El-Matbouli, M. and Haas, W. (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., Eszterbauer, E., Grabner, D. and El-Matbouli, M. (2009) *In vivo* exposure of susceptible and non-susceptible fish species to *Myxobolus cerebralis* actinospores reveals nonspecific invasion behaviour. *Diseases of Aquatic Organisms* 84, 123–130.
- Kallert, D.M., Bauer, W., Haas, W. and El-Matbouli, M. (2011) No shot in the dark: Myxozoans chemically detect fresh fish. *International Journal for Parasitology* 41, 271–276.
- Kathman, R.D. and Brinkhurst, R.O. (1998) *Guide to Freshwater Oligochaetes in North America*. Aquatic Resources Center, College Grove, Tennessee.
- Kelley, G.O., Zagmutt-Vergara, F.J., Leutenegger, C.M., Myklebust, K.A., Adkison, M.A., McDowell, T.S., Marty, G.D., Kahler, A.L., Bush, A.L., Gardner, I.A. and Hedrick, R.P. (2004) Evaluation of five diagnostic methods of the detection and quantification of *Myxobolus cerebralis*. *Journal of Veterinary Diagnostic Investigation* 16, 202–211.
- Kent, M.L., Margolis, L. and Corliss, J.O. (1994) The demise of a class of protists: taxonomic and nomenclatural revisions proposed for the protist phylum Myxozoa Grasse, 1970. *Canadian Journal of Zoology* 72, 932–937.
- Kerans, B.L. and Zale, A.V. (2002) The ecology of *Myxobolus cerebralis*. In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 145–166.
- Kerans, B.L., Rasmussen, C., Stevens, R., Colwell, A.E.L. and Winton, J.R. (2004) Differential propagation of the metazoan parasite *Myxobolus cerebralis* by *Limnodrilus hoffmeisteri*, *Ilyodrilus templetoni*, and genetically distinct strains of *Tubifex tubifex*. *Journal of Parasitology* 90, 1366–1373.
- Kerans, B.L., Stevens, R.I. and Lemmon, J.C. (2005) Water temperature affects a host–parasite interaction: *Tubifex tubifex* and *Myxobolus cerebralis*. *Journal of Aquatic Animal Health* 17, 216–221.
- Koel, T.M., Kerans, B.L., Barras, S.C., Hanson, K.C. and Wood, J.S. (2010) Avian piscivores as vectors for *Myxobolus cerebralis* in the greater Yellowstone ecosystem. *Transactions of the American Fisheries Society* 139, 976–988.
- Krueger, R.C., Kerans, B.L., Vincent, E.R. and Rasmussen, C. (2006) Risk of *Myxobolus cerebralis* infection to rainbow trout in the Madison River, Montana, USA. *Ecological Applications* 16, 770–783.
- Lom, J. and Dyková, I. (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitologica* 53, 1–36.
- Lom, J. and Hoffman, G.L. (1971) Morphology of the spores of *Myxosoma cerebralis* (Höfer, 1903) and *M. cartilaginis* (Hoffman, Putz, and Dunbar, 1965). *Journal of Parasitology* 56, 1302–1308.
- Lom, J. and Noble, E.R. (1984) Revised classification of the myxosporea Bütschli, 1881. *Folia Parasitologica* (Prague) 31, 193–205.
- Lorz, H.V. and Amandi, A. (1994) Suggested procedures for the detection and identification of certain fin-fish and shellfish pathogens. In: Thoesen, J.C. (ed.) VI. *Whirling Disease of Salmonids*. Fish Health Section. American Fisheries Society, Bethesda, Maryland, pp 1–7.
- Lowes, J.M. and Bartholomew, J.L. (2003) Detection of myxozoan parasites in oligochaetes imported as food for ornamental fish. *Journal of Parasitology* 89, 84–91.
- MacConnell, E. and Vincent, E.R. (2002) The effects of *Myxobolus cerebralis* on the salmonid host. In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 95–107.
- Margolis, L., McDonald, T.E. and Whitaker, D.J. (1992) Assessment of the impact of the myxosporean parasite *Ceratomyxa shasta* on survival of seaward migrating juvenile chinook salmon, *Oncorhynchus tshawytscha*, from the Fraser River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 49(9), 1883–1889.

- Markiw, M.E. (1991) Whirling disease: earliest susceptible age of rainbow trout to the triactinomyxid of *Myxobolus cerebralis*. *Aquaculture* 92, 1–6.
- Markiw, M.E. (1992a) 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.
- Markiw, M.E. (1992b) Experimentally induced whirling disease. II. Determination of longevity of the infective triactinomyxon stage of *Myxobolus cerebralis* by vital staining. *Journal of Aquatic Animal Health* 4, 44–47.
- Markiw, M.E. (1992c) *Salmonid whirling disease*. Leaflet 17. United States Fish and Wildlife Service, Washington, DC.
- Markiw, M.E. and Wolf, K. (1974) *Myxosoma cerebralis*: isolation and concentration from fish skeletal elements-sequential enzymatic digestions and purification by differential centrifugation. *Journal of the Fisheries Research Board of Canada* 31, 15–20.
- Markiw, M.E. and Wolf, K. (1983) *Myxosoma cerebralis* (Myxozoa: Myxosporea) etiologic agent of salmonid whirling disease requires tubificid worm (Annelida: Oligochaeta) in its life cycle. *Journal of Protozoology* 30, 561–564.
- Meaders, M. and Hendrickson, G. (2009) Chronological development of *Ceratomyxa shasta* in the polychaete host, *Manayunkia speciosa*. *Journal of Parasitology* 95, 1397–1407.
- Meyers, T.R., Burton, T., Bentz, C. and Starkey, N. (2008) *Common Diseases of Wild and Cultured Fishes in Alaska*. Alaska Department of Fish and Game. Commercial Fisheries Division, Juneau, Anchorage, 105 pp.
- Miller, M.P. and Vincent, R.E. (2008) Rapid natural selection for resistance to an introduced parasite of rainbow trout. *Evolutionary Applications* 1, 336–341.
- Modin, J. (1998) Whirling disease in California: a review of its history, distribution, and impacts, 1965–1997. *Journal of Aquatic Animal Health* 10, 132–142.
- Murcia, S., Kerans, B.L., MacConnell, E. and Koel, T.M. (2011) Correlation of environmental attributes with histopathology of native Yellowstone cutthroat trout naturally infected with *Myxobolus cerebralis*. *Diseases of Aquatic Organisms* 93, 225–234.
- Nehring, R.B. and Walker, P.G. (1996) Whirling disease in the wild: the new reality in the intermountain west. *Fisheries* 21, 28–30.
- Nehring, R.B., Thompson, K.G. and Hebein, S. (1998) Impacts of whirling disease on wild trout populations in Colorado. In: Wadsworth, K.G. (ed.) *Transactions of the 63rd North American Wildlife and Natural Resources Conference*. Wildlife Management Institute, Washington, DC, pp. 82–94.
- Nehring, R.B., Thompson, K.G., Taurman, K.A. and Shuler, D.L. (2002) Laboratory studies indicating that living brown trout *Salmo trutta* expel viable *Myxobolus cerebralis* myxospores. In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 125–134.
- Nichols, K.M., Bartholomew, J.L. and Thorgaard, G.H. (2003) Mapping multiple genetic loci associated with *Ceratomyxa shasta* resistance in *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 56, 145–154.
- Noble, E.R. (1950) On a myxosporidian (protozoan) parasite of California trout. *Journal of Parasitology* 36, 457–460.
- O'Gradnick, J.J. (1975) Whirling disease *Myxosoma cerebralis* spore concentration using the continuous plankton centrifuge. *Journal of Wildlife Diseases* 11, 54–57.
- O'Gradnick, J.J. and Gustafson, C.C. (1974) *A Study of the Transmission, Life History, and Control of Whirling Disease of Trout*. Federal Aid to Fish Restoration Progress Report F-35-R-6. United States Fish and Wildlife Service, Washington, DC.
- Palenzuela, O. and Bartholomew, J.L. (2002) Molecular tools for the diagnosis of *Ceratomyxa shasta* (Myxozoa). In: Cunningham, C. (ed.) *Molecular Diagnosis of Fish Diseases*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Palenzuela, O., Trobridge, G. and Bartholomew, J.L. (1999) Development of a polymerase chain reaction diagnostic assay for *Ceratomyxa shasta*, a myxosporean parasite of salmonid fish. *Diseases of Aquatic Organisms* 36, 45–51.
- Plehn, M. (1905) Über die drehkrankheit der salmoniden [(*Lentospora cerebralis*) (Höfer) Plehn]. *Archiv Protistenkunde* 5, 145–166.
- Pugachev, O.N. and Khokhlov, P.P. (1979) Myxosporean parasites of genus *Myxobolus* – parasites of the salmonids head and spinal brain. In: *Sistematika i ekologiya ryb kontinental'nykh vodoemov Dal'nego*

- Vostoka (*Systematics and Ecology of Fish of Continental Water Bodies of the Russian Far East*). Dal'nevost, Vladivostok, Russia, pp. 137–139.
- Rasmussen, C., Zickovich, J., Winton, J.R. and Kerans, B.L. (2008) Variability in triactinomyxon production in *Tubifex tubifex* populations from the same mitochondrial lineage infected with *Myxobolus cerebralis*, the causative agent of whirling disease in salmonids. *Journal of Parasitology* 94, 700–708.
- Ratliff, D.E. (1981) *Ceratomyxa shasta*: epizootiology in chinook salmon of central Oregon. *Transactions of the American Fisheries Society* 110, 507–513.
- Ratliff, 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.
- Ray, R.A., Rossignol, P.A. and Bartholomew, J.L. (2010) Mortality threshold for Chinook salmon (*Oncorhynchus tshawytscha*) in an epidemiological model of *Ceratomyxa shasta*. *Diseases of Aquatic Organisms* 93, 63–70.
- Rose, J.D., Marrs, G.S., Lewis, C. and Schisler, G. (2000) Whirling disease behavior and its relation to pathology of brain stem and spinal cord in rainbow trout. *Journal of Aquatic Animal Health* 12, 107–118.
- Ryce, E.K.N., Zale, A.V. and Nehring, R.B. (2001) Lack of selection for resistance to whirling disease among progeny of Colorado River rainbow trout. *Journal of Aquatic Animal Health* 13, 63–68.
- Ryce, E.K.N., Zale, A.V. and MacConnell, E. (2004) Effects of fish age and parasite dose on the development of whirling disease in rainbow trout. *Diseases of Aquatic Organisms* 59, 225–233.
- Ryce, E.K.N., Zale, A.V., MacConnell, E. and Nelson, M. (2005) Effects of fish age versus size on the development of whirling disease in rainbow trout. *Diseases of Aquatic Organisms* 63, 69–76.
- Sanders, J.E., Fryer, J.L. and Gould, R.W. (1970) Occurrence of the myxosporidian parasite *Ceratomyxa shasta*, in salmonid fish from the Columbia River basin and Oregon coastal streams. In: Snieszko, S.F. (ed.) *A Symposium on Diseases of Fishes and Shellfishes*. American Fisheries Society Special Publication 5. American Fisheries Society, Bethesda, Maryland, pp. 133–144.
- Sanders, J.E., Fryer, J.L., Leith, D.A. and Moore, K.D. (1972) Control of the infectious protozoan *Ceratomyxa shasta* by treating hatchery water supplies. *Progressive Fish-Culturist* 34, 13–17.
- Schafer, W.E. (1968) Studies on the epizootiology of the myxosporidian *Ceratomyxa shasta* Noble. *California Fish and Game* 54, 90–99.
- Schäperclaus, W. (1991) *Fish Diseases*, 5th edn, Volume 2 (translated from German). Akademie-Verlag, Berlin.
- Schisler, G.J., Bergersen, E.P. and Walker, P.G. (2000) Effects of multiple stressors on morbidity and mortality of fingerling rainbow trout infected with *Myxobolus cerebralis*. *Transactions of the American Fisheries Society* 129, 859–865.
- Schisler, G.J., Bergersen, E.P., Walker, P.G., Wood, J. and Epp, J.K. (2001) Comparison of single-round polymerase chain reaction (PCR) and pepsin-trypsin digest (PTD) methods for detection of *Myxobolus cerebralis*. *Diseases of Aquatic Organisms* 45, 109–114.
- Schisler, G.L., Myklebust, K.A. and Hedrick, R.P. (2006) Inheritance of *Myxobolus cerebralis* resistance among F₁-generation crosses of whirling disease resistant and susceptible rainbow trout strains. *Journal of Aquatic Animal Health* 18, 109–115.
- Schuberg, A. and Schröder, O. (1905) Myxosporidien aus dem nervensystem und der haut der bachforelle. *Archiv für Protistenkunde* 6, 46–60.
- Severin, V.I. and El-Matbouli, M. (2007) Relative quantification of immune-regulatory genes in two rainbow trout strains, *Oncorhynchus mykiss*, after exposure to *Myxobolus cerebralis*, the causative agent of whirling disease. *Parasitology Research* 101, 1019–1027.
- Skirpstunas, R.T., Hergert, J.M. and Baldwin, T.J. (2006) Detection of early stages of *Myxobolus cerebralis* in fin clips from rainbow trout (*Oncorhynchus* (sic) *mykiss*). *Journal of Veterinary Diagnostic Investigation* 18, 274–277.
- Sollid, S.A., Lorz, H.V., Stevens, D.G. and Bartholomew, J.L. (2002) Relative susceptibility of selected Deschutes River, Oregon, salmonid species to experimentally induced infection by *Myxobolus cerebralis*. In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 117–124.
- Sollid, S.A., Lorz, H.V., Stevens, D.G., Reno, P.W. and Bartholomew, J.L. (2004) Prevalence of *Myxobolus cerebralis* at juvenile salmonid acclimation sites in northeastern Oregon. *North American Journal of Fisheries Management* 24, 146–153.
- Staton, L., Erdahl, D. and El-Matbouli, M. (2002) Efficacy of Fumagillin and TNP-470 to prevent experimentally induced whirling disease in rainbow trout *Oncorhynchus mykiss*. In: Bartholomew, J.L. and

- Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 239–247.
- Steinbach, E.L.C., Stromberg, K.E., Ryce, E.K.N. and Bartholomew, J.L. (2009) *Whirling Disease in the United States. A Summary of Progress in Research and Management 2009*. Trout Unlimited Whirling Disease Foundation, Bozeman, Montana. 61 pp.
- Stocking, R.W., Holt, R.A., Foott, J.S. and Bartholomew, J.L. (2006) Spatial and temporal occurrence of the salmonid parasite *Ceratomyxa shasta* (Myxozoa) in the Oregon-California Klamath River Basin. *Journal of Aquatic Animal Health* 18, 194–202.
- Stocking, R.W., Lorz, H.L., Holt, R.A. and Bartholomew, J.L. (2007) Surveillance for *Ceratomyxa shasta* in the Puget Sound watershed, WA, USA. *Journal of Aquatic Animal Health* 19, 116–120.
- Taylor, R.E.L. and Haber, M.H. (1974) Opercular cyst formation in trout infected with *Myxosoma cerebralis*. *Journal of Wildlife Diseases* 10, 347–351.
- Taylor, R.L. and Lott, M. (1978) Transmission of salmonid whirling disease by birds fed trout infected with *Myxosoma cerebralis*. *Journal of Protozoology* 25, 105–106.
- Taylor, R.E.L., Coli, S.J. and Junell, D.R. (1973) Attempts to control whirling disease by continuous drug feeding. *Journal of Wildlife Diseases* 9, 302–305.
- Thompson, J.B., Snekvik, K.R. and Vincent, E.R. (2010) The effects of *Myxobolus cerebralis* on Apache and Gila trout in laboratory exposures. *Journal of Aquatic Animal Health* 22, 87–91.
- Tipping, J.M. (1988) Ozone control of ceratomyxosis: survival and growth benefits to steelhead and cutthroat trout. *Progressive Fish-Culturist* 50, 202–210.
- Udey, L.R., Fryer, J.L. and Pilcher, K.S. (1975) Relation of water temperature to ceratomyxosis in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). *Journal of the Fisheries Research Board of Canada* 32, 1545–1551.
- Vincent, E.R. (1996) Whirling disease and wild trout: the Montana experience. *Fisheries* 21, 32–33.
- Vincent, E.R. (2002) Relative susceptibility of various salmonids to whirling disease with emphasis on rainbow and cutthroat trout. In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 109–115.
- Wagner, E. (2002) Whirling disease prevention, control, and management: a review. In: Bartholomew, J.L. and Wilson, J.C. (eds) *Whirling Disease: Reviews and Current Topics*. Symposium 29. American Fisheries Society, Bethesda, Maryland, pp. 217–225.
- Wagner, E.J., Smith, M., Arndt, R. and Roberts, D.W. (2003) Physical and chemical effects on viability of the *Myxobolus cerebralis* triactinomyxon. *Diseases of Aquatic Organisms* 53, 133–142.
- Wagner, E.J., Wilson, C., Arndt, R., Goddard, P., Miller, M., Hodgson, A., Vincent, R. and Mock, K. (2006) Evaluation of disease resistance of the Fish Lake–DeSmet, Wounded Man, and Harrison Lake strains of rainbow trout exposed to *Myxobolus cerebralis*. *Journal of Aquatic Animal Health* 18, 128–135.
- Wales, J.H. and Wolf, H. (1955) Three protozoan diseases of trout in California. *California Fish and Game* 41, 183–187.
- Whipple, M.J., Gannam, A.L. and Bartholomew, J.L. (2002) Lack of a prophylactic effect of orally administered glucan and fumagillin on naturally acquired infection with *Ceratomyxa shasta* in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*). *North American Journal of Aquaculture* 64, 1–9.
- Whipps, C.M., El-Matbouli, M., Hedrick, R.P., Blazer, V. and Kent, M.L. (2004) *Myxobolus cerebralis* internal transcribed spacer 1 (ITS-1) sequences support recent spread of the parasite to North America and within Europe. *Diseases of Aquatic Organisms* 60, 105–108.
- Wolf, K. and Markiw, M.E. (1984) Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. *Science* 225, 1449–1452.
- Wolf, K., Markiw, M.E. and Hiltunen, J.K. (1986) Salmonid whirling disease: *Tubifex tubifex* (Müller) identified as the essential oligochaete in the protozoan life cycle. *Journal of Fish Diseases* 9, 83–85.
- Yamanoto, T. and Sanders, J.E. (1979) Light and electron microscopic observations of sporogenesis in the myxosporidia, *Ceratomyxa shasta* (Noble, 1950). *Journal of Fish Diseases* 2, 411–428.
- Yasutake, W.T. and Wood, E.M. (1957) Some myxosporidia found in Pacific Northwest salmonids. *Journal of Parasitology* 43, 633–642.
- Yokoyama, H., Danjo, T., Ogawa, K. and Wakabayashi, H. (1997) A vital staining technique with fluorescein diacetate (FDA) and propidium iodide (PI) for the determination of viability of myxosporean and actinosporean spores. *Journal of Fish Diseases* 20, 281–286.
- Zhang, Y.A., Salinas, I., Li, J., Parra, D., Bjork, S., LaPatra, S., Bartholomew, J. and Sunyer, J.O. (2010) Convergent evolution of mucosal immunoglobulins in fish and mammals. *Nature Immunology* 11, 827–835.

- Zielinski, C.M., Lorz, H.V. and Bartholomew, J.L. (2010) Detection of *Myxobolus cerebralis* in the lower Deschutes River basin, Oregon, USA. *North American Journal of Fishery Management* 30, 1032–1040.
- Zielinski, C.M., Lorz, H.V., Hallett, S.L., Xue, L. and Bartholomew, J.L. (2011) Comparative susceptibility of Deschutes River, Oregon, *Tubifex tubifex* populations to *Myxobolus cerebralis*. *Journal of Aquatic Animal Health* 23, 1–8.
- Zinn, J.L., Johnson, K.A., Sanders, J.E. and Fryer, J.L. (1977) Susceptibility of salmonid species and hatchery strains of chinook salmon (*Oncorhynchus tshawytscha*) to infections by *Ceratomyxa shasta*. *Journal of the Fisheries Research Board of Canada* 34, 933–936.

9 *Enteromyxum* Species

Ariadna Sitjà-Bobadilla and Oswaldo Palenzuela
Instituto de Acuicultura de Torre de la Sal, CSIC, Castellón, Spain

9.1. Introduction

The myxozoan genus *Enteromyxum* (Palenzuela *et al.*, 2002) consists only of three intestinal species. *Enteromyxum leei*, described as *Myxidium leei* (Diamant *et al.*, 1994), was initially reported in cultured gilthead sea bream (GSB) (*Sparus aurata*) from southern Cyprus. Susceptible hosts include more than 46 marine fishes and the geographical distribution comprises the Canary Islands, the Mediterranean and Red Sea and Western Japan. The parasite has also been transmitted experimentally to freshwater fishes (Diamant *et al.*, 2006). By contrast, *Enteromyxum scophthalmi* (Palenzuela *et al.*, 2002) has only been described in cultured turbot (*Psetta maxima*) and sole (*Solea senegalensis*). The third species, *Enteromyxum fugu* (Yanagida *et al.*, 2004) formerly described as *Myxidium fugu* (Tun *et al.*, 2000), has been reported exclusively from cultured tiger puffer (*Takifugu rubripes*) in Japan.

The impact of these parasites is not limited to direct mortality but also to weight loss, poor conversion rates, delayed growth and reduced marketability of infected fish. *E. leei* is the most devastating parasite in warm-water seawater cultures (Golomazou *et al.*, 2004; Palenzuela, 2006; Rigos and Katharios, 2010). Sharpnose sea bream (*Diplodus puntazzo*) and tiger puffer are the most susceptible, up to a point that the cultivation of this

species is considered doomed in specific enzootic locations (Rigos and Katharios, 2010). Enteromyxosis is subacute in this juvenile fish (< 50 g) a few weeks after introduction into netpens with heavy mortality, while larger fish may remain unaffected. It can also cause 100% losses in aquarium-kept blennids (Padrós *et al.*, 2001). In contrast, enteromyxosis usually cause a subchronic disease in GSB, which can go undetected in netpens, but is conspicuous in land-based facilities, with accumulated mortality below 20%. Although first noticed in the oldest age-class fish, at sustained high temperatures the infection eventually affects all sizes and the severity increases. In other species, such as European sea bass (*Dicentrarchus labrax*), it causes a sub-clinical infection (Sitjà-Bobadilla *et al.*, 2007a). *E. scophthalmi* is very pathogenic to cultured turbot causing serious disease with 100% mortality in some fish stocks (Branson *et al.*, 1999) and stopping of operations in several farms (author's unpublished data). Mortality is often low when it starts in older age classes, but it rapidly increases exponentially, successively affecting younger fish, and typically leading to 100% mortality in a matter of weeks at summer temperatures. However, this parasite seems less virulent for sole with no clinical signs or mortality in experimentally infected fish (Palenzuela *et al.*, 2007). *E. fugu* is the least pathogenic species as the

impact of this disease in tiger puffer cultures is minor and experimentally infected fish do not show a remarkable intestinal pathology nor disease signs (Yanagida *et al.*, 2006).

The spread of enteromyxoses in cultured fish stocks is favoured by the unique mode of transmission of these myxozoans, which can be directly (fish-to-fish) transmitted without the involvement of any invertebrate host. It is believed that pre-sporogonic developmental stages are infectious to fish. Thus far, *E. leei* and *E. scopthalmi* have been experimentally transmitted: (i) by exposure to water from infected tanks (effluent transmission); (ii) by cohabitation with infected fish; (iii) *per os* with intubation of infected intestinal scrapings (Diamant, 1997, 1998; Diamant and Wajsbrodt, 1997; Yasuda *et al.*, 2002, 2005; Redondo *et al.*, 2004; Muñoz *et al.*, 2007; Sitjà-Bobadilla *et al.*, 2007a, Alvarez-Pellitero *et al.*, 2008); and (iv) recently by anal intubation with *E. leei* (Estensoro *et al.*, 2010a). For *E. fugu*, *per os* transmission is also feasible (Yanagida *et al.*, 2006).

Water temperature is a critical risk factor in the transmission and onset of enteromyxosis. A clear relationship between infection and water temperature has been demonstrated for all three species (Redondo *et al.*, 2002; Yanagida *et al.*, 2006; Estensoro *et al.*, 2010a). The onset of the disease is largely delayed or even suppressed at low temperatures. However, the infection can remain latent during the cooler period. This has important epizootological consequences, since false negatives (during winter) are a source of the parasite when water temperature rises. Under

culture conditions, the temperature for developing *E. leei* clinical enteromyxosis in GSB, usually ranges from 18°C (Le Breton and Marques, 1995) to 22°C (Rigos *et al.*, 1999), and outbreaks in French farms have only been observed above 20°C (Fleurance *et al.*, 2008). In turbot, clinical infections by *E. scopthalmi* are seldom noticed below 12°C, but they become devastating above 18°C (Redondo *et al.*, 2002; Quiroga *et al.*, 2006).

9.2. Clinical Signs (Including Microscopic and Macroscopic Lesions)

Common field observations include loss of appetite, poor food conversion rates and difficulties to reach commercial size in the final months of production. Clinical signs of enteromyxosis usually consist of a severe emaciation with epiaxial muscle atrophy (Fig. 9.1). This emaciation can be noticed externally as a knife or razor-like aspect typical of compressiform species (e.g. Sparidae) (Fig. 9.1a, b), or as conspicuous head bony ridges and 'sunken head' in depressiform species (e.g. turbot or Japanese flounder *Paralichthys olivaceus*) (Fig. 9.1c). The emaciation is best noticed in subchronic infections at mild temperatures, with dead fish usually appearing wasted, and it can be imperceptible in very susceptible species and/or at high temperatures (e.g. *D. puntazzo* infections with *E. leei*), because fish die before reaching a

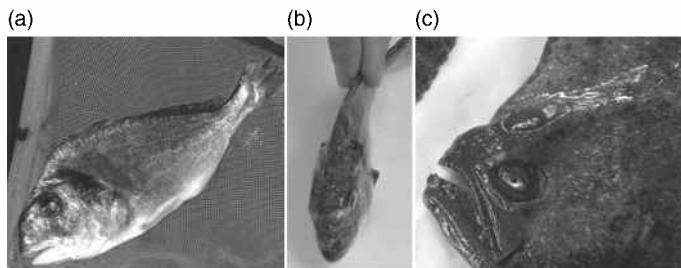


Fig. 9.1. Macroscopic clinical signs of enteromyxosis. (a, b) *Enteromyxum* produces atrophy of epiaxial muscle, with a razor-like aspect typically in *Enteromyxum leei*-infected gilthead sea bream. (c) Conspicuous cranial bony ridges and 'sunken head' are visible in *Enteromyxum scopthalmi*-infected turbot. (b) and (c) are courtesy of Carlos Zarza (Skretting, Spain).

cachectic condition. Distended abdomen and/or rectal prolapse occur in Japanese flounder and tiger puffer infected by *E. leei* or in turbot infected by *E. scopthalmi*. Discoloration and scale loss are less frequent (Athanasopoulou *et al.*, 1999).

At the dissection, macroscopical signs in clinically infected fish are usually restricted to the intestine. Intestine shows focal congestion and haemorrhages, and it can appear fragile and semi-transparent, often filled with mucous liquid. Reduced perivisceral fat deposits, pale internal organs and occasionally green liver are frequent. Enlarged or abnormally coloured gall bladders are common in some hosts (e.g. *D. puntazzo*).

The histopathological study reveals different degrees of catarrhal enteritis and the presence of myxozoan stages located between the enterocytes, or free in the lumen with debris in severe infections (Fig. 9.2). Ribbons of epithelium containing parasite stages are detached, and the submucosae often appear

hypertrophied and infiltrated by immune cells (Fig. 9.2c–d). Oedema is common in *E. scopthalmi*-infected turbot, accompanied with severe lymphoid depletion in lymphohaemopoietic tissues. The nature and degree of the inflammatory response (Fig. 9.2b) varies depending on the host–parasite model. As a general rule, more susceptible species present more marked inflammatory response and detachment of epithelium occurs earlier in the infection. By contrast, more refractory species can harbour large numbers of parasites in the epithelium with little or no inflammation and catarrh. Some degree of re-epithelization can be commonly observed, and the newly built epithelium can eventually be re-colonized by parasites (for more details on the histopathology see Tun *et al.*, 2002; Golomazou *et al.*, 2006a; Fleurance *et al.*, 2008; Alvarez-Pellitero *et al.*, 2008; Bermúdez *et al.*, 2010).

The distribution of the parasites is limited to the digestive system, mainly the intestine,

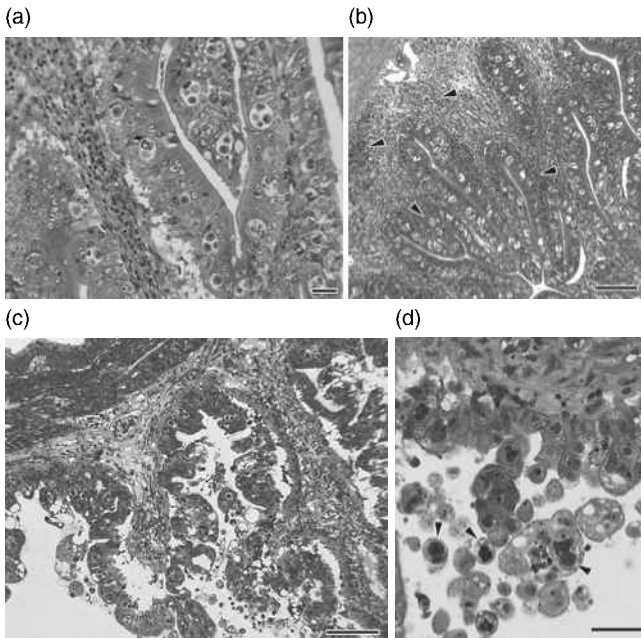


Fig. 9.2. Histopathological effects of *E. leei* (a, b) and *E. scopthalmi* (c, d). Note the detachment of the epithelium from the lamina propria (a, c) and the disintegration of the epithelial layer (b, c). (b) Lymphocyte infiltration is visible at the base of the epithelium and in the lamina propria-submucosae (arrowheads). (d) Parasite stages (arrowheads) and cell debris are released to the intestinal lumen. Stainings: Giemsa (a), haematoxylin and eosin (b), toluidine blue (c, d). Bars = 20 µm (a), 100 µm (b, c), 10 µm (d).

but *E. scopthalmi* stages can also be detected occasionally in the stomach and oesophagus of turbot, and *E. leei* is often reported in the lumen of the gall bladder of some hosts, such as *D. puntazzo* or *Diplodus sargus* (Athanassopoulou *et al.*, 1999; Golomazou *et al.*, 2006a). These locations, however, are neither primary nor consistent. *E. scopthalmi* blood developmental stages have also been detected (Redondo *et al.*, 2004). At early stages of infection, scattered parasite foci are restricted to certain parts of the intestine, spreading to the remaining tissue following a different directional pattern depending on the host-parasite model. In turbot, *E. scopthalmi* stages are initially detected in the pyloric coeca and anterior intestine, whereas *E. leei* stages are first found in the rectum in GSB.

9.3. Diagnosis

Enteromyxosis cannot be diagnosed directly from the clinical signs, since these are non-specific. Field confirmatory diagnosis usually consists of the detection of *Enteromyxum* spores in smears of the intestine, either fresh or stained with diff-quick or May-Grünwald Giemsa (Fig. 9.3c, e, h). However, spores are sometimes scarce or absent, especially in *E. scopthalmi*-clinically diseased fish. Detection of developmental stages in fresh smears is difficult and requires considerable experience (Fig. 9.3a, g). The examination of histological sections of the target tissues is the standard procedure to detect these parasites and the related tissue damage. Stainings with periodic acid-Schiff (PAS) (Fig. 9.3f), or Giemsa or toluidine blue (Fig. 9.3b), or some lectins (Fig. 9.3i) may help in the detection. However, when the parasite is in a latent location, or low numbers of parasites with a patchy distribution are present, the infection may be missed.

More recently, with the availability of gene data on *Enteromyxum* spp. (Palenzuela *et al.*, 2002), oligonucleotide probes have been used for the diagnosis of enteromyxosis using PCR (Palenzuela *et al.*, 2004; Yanagida *et al.*, 2005) and *in situ* hybridization (ISH) (Fig. 9.3j) (Redondo, 2005; Cuadrado *et al.*, 2007).

Non-lethal (NL) sampling procedures have been developed for PCR detection of *E. leei* and *E. scopthalmi* by probing the rectum with a cotton swab (Fig. 9.3k, l). For *E. leei*, this procedure has been validated against a gold standard (histological observation of the whole digestive tract), with a high sensitivity (0.96) and specificity (O. Palenzuela, unpublished data). These molecular methods constitute valuable research tools for the detection and study of parasite entry routes, subclinical infections, putative invertebrate hosts, or concomitant infections by different species, to mention just a few. Moreover, besides their research uses, they constitute powerful monitoring and surveillance tools, and several turbot farms routinely test for *E. scopthalmi* with NL-PCR, because its higher sensitivity allows an early detection of the disease.

9.4. Disease Mechanisms

9.4.1. Pathophysiology

The parasite induces a cascade of events (Fig. 9.4) that end up in a cachectic syndrome, which is featured by decreased haematological values (haematocrit, haemoglobin) and growth performance (lower weight, length, condition factor, specific growth rate). The main cause of cachexia is the reduction of food availability, which is due not only to the damaged intestinal epithelium, whose absorptive function is clearly impaired, but also to anorexia. In GSB, anorexia is progressive and can reach up to 45% of food intake of control fish. However, anorexia only explains about half of the weight reduction (Estensoro *et al.*, 2011). In addition, body weight loss can also be due to an osmoregulatory failure, as suggested by the pathophysiological evidences in *E. leei*-infected tiger puffer (Ishimatsu *et al.*, 2007). *E. leei* disrupts intestinal water uptake, as a significant negative correlation between plasma chloride concentration and condition factor, and significantly higher osmolarity of plasma and major ion concentrations of the intestinal fluid were found in infected fish. Hepatic function was also impaired (Ishimatsu *et al.*, 2007).

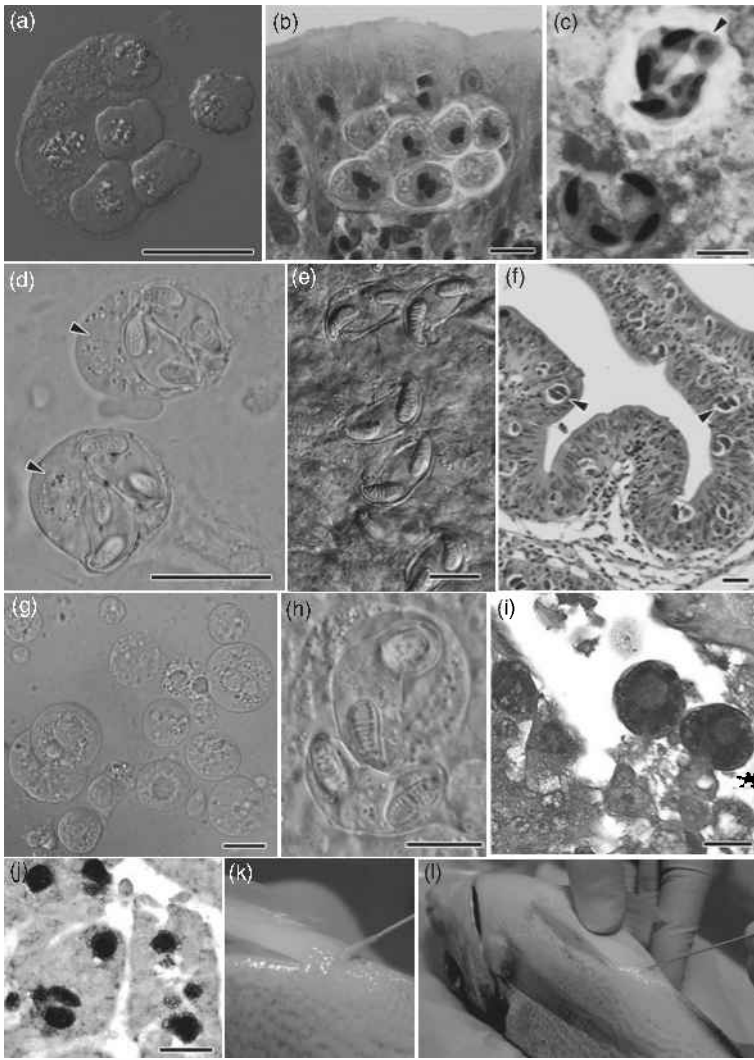


Fig. 9.3. Microscopic detection of *E. leei* (a–f) and *E. scopthalmi* (g–j) stages in fresh intestinal scrapings (a, d, e, g, h), May-Grünwald stained smear (c), Alcian blue-PAS-stained histological section (f), biotinylated SBA (soy bean agglutinin from *Glycine max*) lectin-stained histological section (i) and by *in situ* hybridization (ISH) (j). Note the presence of developmental stages with the cell-in-a-cell pattern (a, b, g), the labelling of the primary cells (i), the disporoblasts with accompanying cells (arrowheads) (c, d, h) and the mature spores with dark stained polar capsules (c). Parasite stages are fuchisine-stained (arrowheads) in (f). Coiled polar filaments are more visible with Nomarski microscopy (e). Rectal probing for non-lethal sampling diagnostic of *E. leei* by PCR (k, l). Bars = 20 μ m (a, d, f, j), 10 μ m (b, c, e, g, h, i). All figures are original from the authors except (j) which is courtesy of Dr M.J. Redondo (IATS, CSIC, Spain); (i) was taken from Redondo *et al.*, 2008, with permission from the publisher.

These pathophysiological effects of *Enteromyxum* are due to the disruption of tight junctions and the electrolyte balance they control, since the intercellular sealing,

the selective diffusion barrier between epithelial cells and the prevention of the free passage of molecules and ions across the paracellular pathway may be altered.

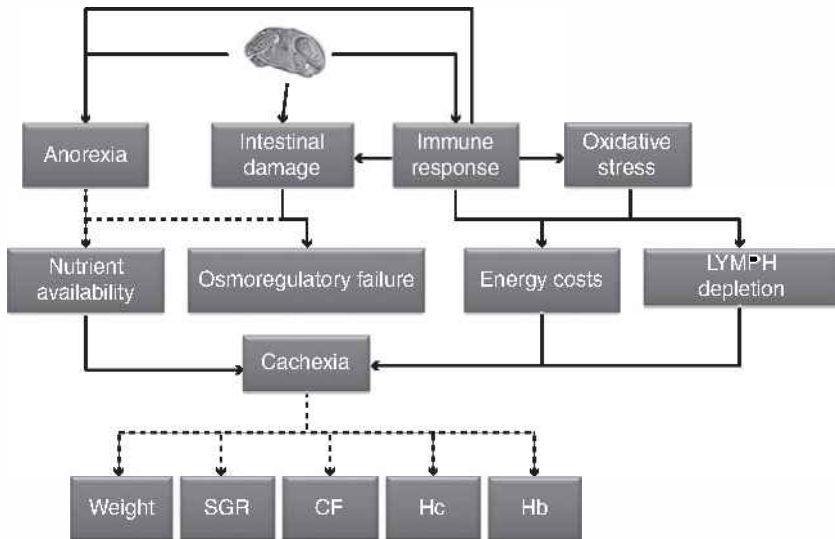


Fig. 9.4. Diagrammatic representation of the disease mechanism of *Enteromyxum* parasites. Dashed arrows stand for negative effects and continuous arrows ones for positive effects on the pointed box. CF, Condition factor; Hb, haemoglobin; Hc, haematocrit; LYMPH, lymphohaemopoietic; SGR, specific growth rate.

Intestinal barrier integrity may also be affected by enterocyte apoptosis and necrosis. It is unclear whether the increased apoptotic rate in infected intestines is a host reaction to prevent parasite spread, or, on the contrary, these apoptotic cells may facilitate parasite survival (Bermúdez *et al.*, 2010), since detached enterocytes which embrace the parasite when released to the lumen may help them to retain their viability in sea water (Redondo *et al.*, 2003a).

The host's immune response (see section 9.5.2.) also has a metabolic cost and adverse effects on growth and feed intake. The immune response is responsible for the production of several cachectic cytokines that induce anorexia. In *E. leei*-infected GSB, transcripts of interleukin-1 beta (IL-1 β) and tumour necrosis factor alpha (TNF- α) were significantly decreased in the intestine at 113 days post-exposure (p.e.) (Sitjà-Bobadilla *et al.*, 2008), whereas IL-1 β expression was increased in head kidney shortly after exposure (Cuesta *et al.*, 2006a). Thus, other anorexigenic factors, such as gastrointestinal neuropeptides or growth factors may be involved. In fact, the number of enteroendocrine cells positive for neuropeptide Y and

substance-P were lower in exposed GSB (Estensoro *et al.*, 2009) and *E. scopthalmi*-infected turbot had significantly increased numbers of epithelial cells positive for cholecystokinin-8 and serotonin. By contrast, the number of both vasoactive intestinal polypeptide (VIP)-immunoreactive endocrine cells and nerve cell bodies and fibres were significantly lower in infected turbot (Bermúdez *et al.*, 2007).

Immune and detoxification systems generate reactive oxygen species (ROS) and reactive nitrogen species (NOS) that, if not counterbalanced, lead to oxidative stress and host tissue damage. The primary enzymatic antioxidant defence system in charge of the removal of these free radicals is the glutathione redox system, which reduces hydrogen peroxide and lipid hydroperoxides by oxidizing reduced glutathione (GSH) to its disulfide form (GSSG), through the intervention of glutathione peroxidases (GPx). In GSB with chronic *E. leei* infections, a reduction in the transcription of GPx-1 was observed (Sitjà-Bobadilla *et al.*, 2008). Plasma total antioxidant capacity and the hepatic GSH:GSSG ratio were also decreased in parasitized GSB bream fed a diet containing vegetable oils as

the major source of lipids (Estensoro *et al.*, 2011). This could render them in a state of oxidative stress with a higher risk of lipid peroxidation and oxidative damage, especially if the production of ROS is maintained high.

9.4.2. Pathogenicity and invasion mechanisms

We are still far from knowing the pathogenic mechanism(s) of *Enteromyxum* species and how the parasite enters the host. Proteases are involved in parasite proliferation in several myxosporeans, but the only information available for *Enteromyxum* is the immunohistochemical detection of a caspase-3-like in some proliferative stages of *E. leei* (Estensoro *et al.*, 2009). This type of cysteine protease has been associated with cytoskeletal remodeling and proliferation in some mammalian cells. In addition, the increased serum total antiproteases and serum alpha-2 macroglobulin (α -2M) in *E. leei*-parasitized sharpshout sea bream (Muñoz *et al.*, 2007) and *E. scophthalmi*-parasitized turbot (Sitjà-Bobadilla *et al.*, 2006), suggest a counteracting role of putative parasite proteases. This is further supported by the significantly increased gene expression of α -2M in the intestine of parasitized GSB (Sitjà-Bobadilla *et al.*, 2008).

We are just starting to decipher the host-parasite interactions occurring at the intestinal epithelium that allow trophozoites to penetrate between enterocytes and dwell in the paracellular space. Receptors present in the intestinal mucin layer can act as binding sites for parasites, and lectin-carbohydrate interactions are frequently involved in the adhesion and penetration of parasites. Carbohydrate residues present on the surface of *E. leei* (Redondo and Alvarez-Pellitero, 2009) and *E. scophthalmi* (Redondo *et al.*, 2008) (Fig. 9.3i) and also in the digestive tract of turbot and GSB (Redondo and Alvarez-Pellitero 2010a) have been detected with lectin histochemistry. Mannose and/or glucose and fucose residues are the most abundant in the membranes of both myxosporeans and at the host-parasite interfaces, and a clear reduction of the number of goblet cells with some carbohydrates in parasitized fish was

observed (Redondo and Alvarez-Pellitero, 2010a).

Once established, the plasmodium interacts with neighbouring enterocytes creating numerous convoluted cytoplasmic projections in direct contact with host-cell membranes, sometimes with bridges similar to gap junctions (Redondo *et al.*, 2003b; Cuadrado *et al.*, 2008). These folds probably play attachment and communication roles with host cells, and also increase the absorptive area and ensure the plasmodium nutrition from the host cells. Somehow the parasite is capable of disguising itself in the epithelium or evading the host reaction, at least at the first steps of the infection, allowing its rapid proliferation. Thus, parasite recognition and antigen presentation by cellular and humoral effectors may be deferred, and therefore the cascade of events leading to the production of specific antibodies is delayed (see section 9.5.2.).

9.5. Protective/Control Strategies

As the life cycle of these parasites is unknown and fish-to-fish transmission favours parasite spread, prevention is the main focus for their management. Once they become established they are generally eradicated only with aggressive actions that include eliminating infected fish, disinfecting tanks, sea cages, drying ponds, etc. Here the possible approaches to control this disease will be described.

9.5.1. Chemotherapeutic approaches

There are no approved antiparasitic preparations for myxosporeans in general, and those tested experimentally, mainly coccidiostats, have had relative success. Oral treatment with toltrazuril did not ameliorate the clinical progress of the disease in *E. scophthalmi*-infected turbot, though the drug induced some negative changes on the parasite (Bermúdez *et al.*, 2006a). The combination of salinomycin and amprolium significantly reduced prevalence, intensity and mortality

in *E. leei*-infected sharpsnout sea bream, without apparent toxic effects (Golomazou *et al.*, 2006c), increased survival rates in *E. scopthalmi*-infected turbot (Palenzuela *et al.*, 2009), and stopped mortality in aquarium-reared yellow tangs (*Zebrasoma flavescens*) with a *Enteromyxum*-like heavy infection (Hyatt, 2009). Other treatments with robenidine plus sulfamides or with diets supplemented with natural extracts improved survival rate of infected turbot. However, none of the treatments stopped the infection (100% final prevalence), but the lower mortality seemed to be due to reduced parasite loads and restricted intestinal invasion. Other drugs, such as fumagillin (Golomazou *et al.*, 2006c) or the combination of narasin and nicarbazine (Palenzuela *et al.*, 2009) had toxic effects on the host or increased the mortality rates.

Recent *in vitro* studies performed with intestinal turbot explants have shown that some parasite carbohydrates are involved in parasite entry, since the addition of the corresponding blocking lectin inhibits *E. scopthalmi* penetration (Redondo and Alvarez-Pellitero, 2010b). This may open a new set of therapeutic targets.

Although the above information suggests some potential for combined therapies in enteromyxosis, the high susceptibility of some hosts, especially under high water temperatures does not allow complete clearance of the parasite. Activity of natural extracts deserves further studies since their use is not restricted by law because they are nutritional supplements and not therapeutics.

9.5.2. Strategies based on the exploitation of the immune system

The characterization of the fish immune response against *Enteromyxum* and how the parasite copes or evades the host defence are crucial for the development of vaccines and other preventive strategies (such as immunomodulation) and selection of disease-resistant strains of fish. Some aspects of the humoral and cellular immune responses against *E. scopthalmi* and *E. leei* have been studied (Sitjà-Bobadilla, 2008) and here the most outstanding ones are outlined.

The invasion of the enteric paracellular space by *Enteromyxum* stages triggers at some point the host cellular response at the site of the infection, with an initial activation of leucopoiesis, followed by leucocyte depletion in lymphohematopoietic organs. Thus, the numbers of granulocytes (Alvarez-Pellitero *et al.*, 2008) and Ig+ cells (Bermúdez *et al.*, 2006b) are increased at the intestine of *Enteromyxum*-infected fish, but its presence is decreased in head kidney and spleen (Cuesta *et al.*, 2006b; Sitjà-Bobadilla *et al.*, 2006; Alvarez-Pellitero *et al.*, 2008; Bermúdez *et al.*, 2010). Enteromyxosis also induces an increase in the respiratory burst of circulating phagocytes (Sitjà-Bobadilla *et al.*, 2006, 2008; Alvarez-Pellitero *et al.*, 2008), serum nitric oxide (NO) levels (Golomazou *et al.*, 2006b) and cell-mediated cytotoxicity (Cuesta *et al.*, 2006b). In spite of all this cellular activation, in susceptible species the parasite keeps on developing and completely invading the intestinal tract.

Some humoral innate factors such as peroxidases, lysozyme (LY) or complement are altered by enteromyxosis, but no single key molecule seems to be involved in parasite clearance. LY was consumed in fighting the parasite, since levels were depleted in exposed turbot and GSB (Sitjà-Bobadilla *et al.*, 2006, 2008). However, in sharpsnout sea bream no LY was detected in either infected or healthy animals (Golomazou *et al.*, 2006b; Sitjà-Bobadilla *et al.*, 2007b), and it was suggested that its absence could contribute to the high pathogenicity in this host (Alvarez-Pellitero *et al.*, 2008). The activity of the complement alternative pathway is initially increased and/or unaltered in response to parasite exposure, but later on it is exhausted for fighting the parasite (Cuesta *et al.*, 2006a; Sitjà-Bobadilla *et al.*, 2006, 2007b). Therefore, it remains to be established if any strategy directed to increase the basal levels of these humoral factors could contribute to cope with the disease.

Both turbot and GSB are also capable of mounting a specific immune response against *Enteromyxum* spp. (Sitjà-Bobadilla *et al.*, 2004; Estensoro *et al.*, 2010b), but the speed of antibody production is relatively slow. When turbot were challenged with *E. scopthalmi*,

specific antibodies against the parasite were detected as soon as 48 days p.e. if fish had been previously exposed (Sitjà-Bobadilla *et al.*, 2007c), whereas naïve animals developed the disease and died without producing antibodies at 40–49 days p.e. (Redondo *et al.*, 2002; Sitjà-Bobadilla *et al.*, 2006). Although some information is available on the parasite structures stained with fish antibodies (Sitjà-Bobadilla *et al.*, 2004; Estensoro *et al.*, 2010b), the specific antigens which trigger this response are unknown. Further knowledge of these antigens is essential to develop vaccines.

Innate resistance of certain fish species and strains against *Enteromyxum* spp. has been reported, but the mechanisms involved in such complex phenomenon have not been elucidated. Inter-specific differences have been reported for *E. leei*, as some marine aquarium-reared (Padrós *et al.*, 2001) and several freshwater fish (Diamant *et al.*, 2006) are refractory to infection, and pathogenic effects even differ among susceptible species (see previous sections). Intra-specific differences were found in turbot, with some stocks having different susceptibility to *E. scophthalmi* (Quiroga *et al.*, 2006; Sitjà-Bobadilla *et al.*, 2006). Similarly, field and experimental data suggest that some GSB individuals or stocks are partially resistant to *E. leei* (Jublanc *et al.*, 2006; Sitjà-Bobadilla *et al.*, 2007a, Fleurance *et al.*, 2008). However, genetic selection, based on the innate resistance has not been exploited. Some turbot companies have started breeding selection programmes as a promising future strategy, but much work remains, as the genetic base is unknown.

The observation of acquired resistance to some enteromyxosis opens a promising door for the future development of vaccines. Thus, *D. puntazzo* that had recovered from *E. leei* infection, when challenged with the parasite were refractory to the disease (Golomazou *et al.*, 2006b). Some turbot surviving *E. scophthalmi* epizootic outbreaks, when experimentally challenged, developed immunity and exhibited also the higher and earlier levels of specific antibodies (Sitjà-Bobadilla *et al.*, 2007c). Lightly or moderately *E. leei*-infected red sea bream (*Pagrus major*) surviving mortalities in Japanese farms do not seem to have recurrent infections (Yanagida *et al.*, 2008).

Finally, different commercial aquafeeds are nowadays formulated to include immunostimulant compounds, some of which are presumed to enhance the fish basal immune system, the mucosal barriers, and the overall potential to fight against pathogens. However, their usefulness in *Enteromyxum* infections has not been fully determined.

9.5.3. Environmental-management measures

The avoidance of *Enteromyxum* infections in marine aquaculture is difficult, and management strategies depend on the type of facility. In GSB land-based facilities, it is essential to avoid the following risk or aggravating factors: (i) year-round elevated water temperatures; (ii) poor water exchange and/or re-intake of contaminated effluent water; (iii) recirculation systems; and (iv) a prolonged culture period necessary for production of large fish (Jublanc *et al.*, 2005; Diamant *et al.*, 2006). Other authors considered enteromyxosis to be associated with overfeeding and the use of diets with a high fat content (Rigos *et al.* 1999); a diet containing vegetable oils as the major source of lipids induced a worse disease outcome in GSB (Estensoro *et al.*, 2011). In land-based facilities and exhibition aquaria, it is also recommended to clean tanks with fresh water, as the viability of pre-sporogonic stages of *E. leei* is reduced with hyposalinity treatment. For euryhaline fish, long-term exposure to hyposalinity may also prevent the invasion of the myxosporean (Yokoyama and Shirakashi, 2007). Cleaning water channels and pipes should also limit the prevalence of the putative intermediate hosts (Jublanc *et al.*, 2005). In turbot farms, 50 µm (nominal) mechanical filtering of the incoming water source was proved effective, since all fish kept in filtered water remained uninfected (Quiroga *et al.*, 2006). However, the use of such filtration in turbot on-growing farms as a routine prophylactic measure is not always affordable due to the large water volumes involved. Some farms located in enzootic waters have managed to overcome the infections with the adoption of combined

income water treatments (ozone, UV and filtration) and effluent water disinfection with ozone, in addition to routine disease surveillance and culling of infected stocks. However, no data on the relative efficacy of each of these measures or recommended dosages has been properly determined. In sea cages the water supply cannot be controlled, and the parasite can enter not only from putative invertebrates present in net fouling and bottoms, but also from neighbouring infected cages or from wild fish. In this situation, daily removal of carcasses with an air pump and a lift hose from a sack device located at the bottom of the cage seemed to reduce the prevalence of infection by *E. leei* in GSB (Dr A. Diamant, National Center for Mariculture, Israel, personal communication, 2010).

Regardless of the type of facility, periodic surveys are suggested to detect infection early. Once detected, culling of affected stocks is often the wisest measure in order to avoid exponential concentration of infective material and dispersion of the disease through contagion or transportation of stocks to disease-free facilities.

9.6. Conclusions and Suggestions for Future Studies

The intense and concerted research conducted on *Enteromyxum* spp. in the last few years has increased our knowledge on the biology and disease mechanisms of these

pathogenic myxosporeans. However, numerous challenges still need to be unveiled. These include the life cycle (invertebrate hosts, undetectable latent stages) and the routes of entry. Efforts still have to be addressed to their structural, genetic and antigenic characterization, which will help to understand their relationship with the host, and to identify possible therapeutic targets for preventive and palliative measures. Future research should also be focused on achieving the *in vitro* culture of these organisms, since this methodological gap thwarts many approaches, such as the production of a constant and reliable source of the parasite for vaccines. More rapid, reliable and easy-to-use diagnostic tools also wait to be developed in the coming years. Finally, much work is still to be done on disclosing the basis of host susceptibility, the molecular mechanisms and the key genes involved in the immune response and resistance to enteromycoses.

Acknowledgements

The authors thank Dr Hiroshi Yokoyama (University of Tokyo, Japan) for updated information on disease status in aquacultured fish. Part of the information gathered in this chapter has been obtained through funding from Spanish research projects (AGL2006-13158-C03-01, AGL2009-13282-C02-01, PROMETEO 2010/006) and the EU research project (MyxFishControl, QLRT-2001-00722).

References

- Alvarez-Pellitero, P., Palenzuela, O. and Sitjà-Bobadilla, A. (2008) Histopathology and cellular response in *Enteromyxum leei* (Myxozoa) infections of *Diplodus puntazzo* (Teleostei). *Parasitology International* 57, 110–120.
- Athanassopoulou, F., Prapas, H. and Rodger, H. (1999) Diseases of *Puntazzo puntazzo* Cuvier in marine aquaculture systems in Greece. *Journal of Fish Diseases* 22, 215–218.
- Bermúdez, R., Alemán, N., Vigliano, F., Vázquez, S., Quiroga, M.I. and Nieto, J.M. (2006a). Effects of symmetric triazinone (toltrazuril) on developmental stages of *Enteromyxum scophthalmi* parasitizing turbot (*Scophthalmus maximus* L.): a light and electron microscopic study. *Aquaculture* 254, 65–71.
- Bermúdez, R., Vigliano, F., Marcaccini, A., Sitjà-Bobadilla, A., Quiroga, M.I. and Nieto, J.M. (2006b) Response of Ig-positive cells to *Enteromyxum scophthalmi* (Myxozoa) experimental infection in turbot, *Scophthalmus maximus* (L.): a histopathological and immunohistochemical study. *Fish and Shellfish Immunology* 21, 501–512.

- Bermúdez, R., Vigliano, F., Quiroga, M.I., Nieto, J.M., Bosi, G. and Domeneghini, C. (2007) Immunohistochemical study on the neuroendocrine system of the digestive tract of turbot, *Scophthalmus maximus* (L.), infected by *Enteromyxum scophthalmi* (Myxozoa). *Fish and Shellfish Immunology* 22, 252–263.
- Bermúdez, R., Losada, A.P., Vázquez, S., Redondo, M.J., Alvarez-Pellitero, P. and Quiroga, M.I. (2010) Light and electron microscopic studies on turbot *Psetta maxima* infected with *Enteromyxum scophthalmi*: histopathology of turbot enteromyxosis. *Diseases of Aquatic Organisms* 89, 209–221.
- Branson, E., Riaza, A. and Alvarez-Pellitero, P. (1999) Myxosporean infection causing intestinal disease in farmed turbot, *Scophthalmus maximus* (L.), (Teleostei: Scophthalmidae). *Journal of Fish Diseases* 22, 395–399.
- Cuadrado, M., Albinyana, G., Padrós, F., Redondo, M.J., Sitjà-Bobadilla, A., Alvarez-Pellitero, P., Palenzuela, O., Diamant, A. and Crespo, S. (2007) An unidentified epi-epithelial myxosporean in the intestine of gilthead seabream (*Sparus aurata* L.). *Parasitology Research* 101, 403–411.
- Cuadrado, M., Marques, A., Diamant, A., Sitjà-Bobadilla, A., Palenzuela, O., Alvarez-Pellitero, P., Padrós, F. and Crespo, S. (2008) Ultrastructure of *Enteromyxum leei* (Diamant, Lom, and Dyková, 1994) (Myxozoa), an enteric parasite infecting gilthead sea bream (*Sparus aurata*) and sharpnosed sea bream (*Diplodus puntazzo*). *The Journal of Eukaryotic Microbiology* 55, 178–184.
- Cuesta, A., Muñoz, P., Rodríguez, A., Salinas, I., Sitjà-Bobadilla, A., Alvarez-Pellitero, P., Esteban, M.A. and Meseguer, J. (2006a) Gilthead seabream (*Sparus aurata* L.) innate defence against the parasite *Enteromyxum leei* (Myxozoa). *Parasitology* 132, 1–10.
- Cuesta, A., Salinas, I., Rodríguez, A., Muñoz, P., Sitjà-Bobadilla, A., Alvarez-Pellitero, P., Meseguer, J. and Esteban, M.A. (2006b) Cell-mediated cytotoxicity is the main innate immune mechanism involved in the cellular defence of gilthead seabream (Teleostei: Sparidae) against *Enteromyxum leei* (Myxozoa). *Parasite Immunology* 28, 657–665.
- 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* 16, 33–39.
- Diamant, A. and Wajsbrodt, N. (1997) Experimental transmission of *Myxidium leei* in gilthead sea bream *Sparus aurata*. *Bulletin of the European Association of Fish Pathologists* 17, 99–103.
- Diamant, A., Lom, J. and Dyková, I. (1994) *Myxidium leei* n. sp., a pathogenic myxosporean of cultured sea bream *Sparus aurata*. *Diseases of Aquatic Organisms* 20, 137–141.
- Diamant, A., Ram, S. and Paperna, I. (2006) Experimental transmission of *Enteromyxum leei* to freshwater fish. *Diseases of Aquatic Organisms* 72, 171–178.
- Diamant, A., Palenzuela, O., Alvarez-Pellitero, P., Athanassopoulou, F., Golomazou, E., Albiñana, G., Padrós, F., Crespo, S., Lipshitz, A., Ghittino, C., Agnetti, F., Marques, A., Le Breton, A. and Raymond, J. (2006) Epizootiology of *Enteromyxum leei* (Myxozoa: Myxosporea) in Mediterranean mariculture systems. In: 5th International Symposium on Aquatic Animal Health, San Francisco, abstract book, p. 94.
- Estensoro, I., Bermúdez, R., Losada, A.P., Quiroga, M.I., Pérez-Sánchez, J., Alvarez-Pellitero, P. and Sitjà-Bobadilla, A. (2009) Effect of *Enteromyxum leei* (Myxozoa) on gastrointestinal neuromodulators and cell apoptosis of gilthead sea bream (*Sparus aurata*). In: *Diseases of Fish and Shellfish*, 14th European Association of Fish Pathologists International Conference, Prague, Czech Republic, abstract book, p. 264.
- Estensoro, I., Redondo, M.J., Alvarez-Pellitero, P. and Sitjà-Bobadilla, A. (2010a) A novel horizontal transmission route for *Enteromyxum leei* (Myxozoa) by anal intubation of gilthead sea bream (*Sparus aurata* L.). *Diseases of Aquatic Organisms* 92, 51–58.
- Estensoro, I., Redondo, M.J., Alvarez-Pellitero, P. and Sitjà-Bobadilla, A. (2010b) Detection of specific antibodies against *Enteromyxum leei* (Myxozoa: Myxosporea) in gilthead sea bream (*Sparus aurata*) by ELISA and immunohistochemistry. In: 1st Symposium of the European Organisation of Fish Immunology, Viterbo (Italy), abstract book p. 44.
- Estensoro, I., Benedito-Palos, L., Palenzuela, O., Kaushik, S., Sitjà-Bobadilla, A. and Pérez-Sánchez, J. (2011) The nutritional background of the host alters the disease course in a fish–myxosporean system. *Veterinary Parasitology* 175, 141–150.
- Fleurance, R., Sauvegrain, C., Marques, A., Le Breton, A., Guereaud, C., Cherel, Y. and Wyers, M. (2008) Histopathological changes caused by *Enteromyxum leei* infection in farmed sea bream *Sparus aurata*. *Diseases of Aquatic Organisms* 79, 219–228.

- Golomazou, E., Karagouni, E. and Athanassopoulou, F. (2004) The most important myxosporean parasite species affecting cultured Mediterranean fish. *Journal of the Hellenic Veterinary Medical Society* 55, 342–352.
- Golomazou, E., Athanassopoulou, F., Vagianou, S., Sabatakou, O., Tsantilas, H., Rigos, G. and Kokkokiris, L. (2006a) Diseases of white sea bream (*Diplodus sargus* L.) reared in experimental and commercial conditions in Greece. *Turkish Journal of Veterinary Animal Science* 30, 389–396.
- Golomazou, E., Athanassopoulou, F., Karagouni, E., Tsagozis, P., Tsantilas, H. and Vagianou, S. (2006b) Experimental transmission of *Enteromyxum leei* Diamant, Lom and Dyková, 1994 in sharpsnout sea bream, *Diplodus puntazzo* C. and the effect on some innate immune parameters. *Aquaculture* 260, 44–53.
- Golomazou, E., Athanassopoulou, F., Karagouni, E., Vagianou, S., Tsantilas, H. and Karamanis, D. (2006c) Efficacy and toxicity of orally administrated anti-coccidial drug treatment on *Enteromyxum leei* infections in sharpsnout seabream (*Diplodus puntazzo* C.). *Israeli Journal of Aquaculture-Bamidgeh* 58, 157–169.
- Hyatt, M. (2009) Successful treatment on enteric myxosporiosis in a collection of yellow tangs *Zebrasoma flavescens* at a public aquarium. *Florida Aqua News* 4, 1–6.
- Ishimatsu, A., Hayashi, M., Nakane, M. and Sameshima, M. (2007) Pathophysiology of cultured tiger puffer *Takifugu rubripes* suffering from the myxosporean emaciation disease. *Fish Pathology* 42, 211–217.
- Jublan, E., Elkirc, N., Toubiana, M., Sri Widada, J., Le Breton, A., Lefebvre, G., Sauvegrain, C. and Marques, A. (2005) Observation on a *Enteromyxum leei* (Myxozoa Myxosporea) parasitosis on farming sea bream *Sparus aurata*. *Journal of Eukaryotic Microbiology* 52, 28S–34S.
- Jublan, E., Toubiana, M., Sri Widada, J., Le Breton, A., Lefebvre, G., Sauvegrain, C. and Marques, A. (2006) Observation of a survival case following infestation by *Enteromyxum leei* (Myxozoa Myxosporea), a pathogenic myxosporidian of the digestive duct of the gilthead sea bream (*Sparus aurata*) in pisciculture. *Journal of Eukaryotic Microbiology* 53, 20S.
- Le Breton, A. and Marques, A. (1995) Occurrence of a histozoic *Myxidium* infection in two marine cultured species: *Puntazzo puntazzo* C. and *Pagrus major*. *Bulletin of the European Association of Fish Pathologists* 15, 210–212.
- Muñoz, P., Cuesta, A., Athanassopoulou, F., Golomazou, E., Crespo, S., Padrós, F., Sitjà-Bobadilla, A., Albiñana, G., Esteban, M.A., Alvarez-Pellitero, P. and Meseguer, J. (2007) Sharpsnout sea bream (*Diplodus puntazzo*) humoral immune response against the parasite *Enteromyxum leei* (Myxozoa). *Fish and Shellfish Immunology* 23, 636–645.
- Padrós, F., Palenzuela, O., Hispano, C., Tosas, O., Zarza, C., Crespo, S. and Alvarez-Pellitero, P. (2001) *Myxidium leei* (Myxozoa) infections in aquarium-reared Mediterranean fish species. *Diseases of Aquatic Organisms* 47, 57–62.
- Palenzuela, O. (2006) Myxozoan infections in Mediterranean mariculture. *Parassitologia* 48, 27–29.
- Palenzuela, O., Redondo, M.J. and Alvarez-Pellitero, P. (2002) Description of *Enteromyxum scopthalmi* gen nov., sp. nov. (Myxozoa), an intestinal parasite of turbot (*Scophthalmus maximus* L.) using morphological and ribosomal RNA sequence data. *Parasitology* 124, 369–379.
- Palenzuela, O., Agnetti, F., Albiñana, G., Alvarez-Pellitero, P., Athanassopoulou, F., Crespo, S., Diamant, A., Ghittino, C., Golomazou, E., Le Breton, A., Lipshitz, A., Marques, A., Padrós, F., Ram, S. and Raymond J. (2004) Applicability of PCR screening for the monitoring of *Enteromyxum leei* (Myxozoa) infection in Mediterranean aquaculture: an epidemiological survey in sparids facilities. In: Adams, S. and Olafsen, J.A. (compilers) *Biotechnologies for Quality*. European Aquaculture Society Special Publication No. 34. European Aquaculture Society, Barcelona, Spain, pp. 639–640.
- Palenzuela, O., Redondo, M.J., López, E. and Alvarez-Pellitero, P. (2007) Cultured sole, *Solea senegalensis* is susceptible to *Enteromyxum scopthalmi*, the myxozoan parasite causing turbot emaciative enteritis. *Parassitologia* 49, 73.
- Palenzuela, O., López-Grandal, E., Zarza, C. and Álvarez-Pellitero, P. (2009) Treatment of turbot enteromyxosis with antiparasitic drugs and bioactive natural extracts-supplemented feeds. Paper presented at the 14th International Conference of the European Association of Fish Pathologists (EAFP) on Diseases of Fish and Shellfish, Prague, Czech Republic, 14–19 September. Book of abstracts, pp. 142–143.
- Quiroga, M.I., Redondo, M.J., Sitjà-Bobadilla, A., Palenzuela, O., Riaza, A., Macías, A., Vázquez, S., Pérez, A., Nieto, J.M. and Alvarez-Pellitero, P. (2006) Risk factors associated with *Enteromyxum scopthalmi* (Myxozoa) infection in cultured turbot (*Scophthalmus maximus* L.). *Parasitology* 133, 433–442.
- Redondo, M.J. (2005) Estudios sobre el ciclo vital y transmisión de *Enteromyxum scopthalmi* (Myxozoa), parásito entérico del rodaballo. PhD thesis, University of Valencia, Valencia, Spain.

- Redondo, M.J. and Alvarez-Pellitero, P. (2009) Lectin histochemical detection of terminal carbohydrate residues in the enteric myxozoan *Enteromyxum leei* parasitizing gilthead seabream *Sparus aurata* (Pisces: Teleostei): a study using light and transmission electron microscopy. *Folia Parasitologica* 56, 259–267.
- Redondo, M.J. and Alvarez-Pellitero, P. (2010a) Carbohydrate patterns in the digestive tract of *Sparus aurata* L. and *Psetta maxima* (L.) (Teleostei) parasitized by *Enteromyxum leei* and *E. scopthalmi* (Myxozoa). *Parasitology International* 59, 445–453.
- Redondo, M.J. and Alvarez-Pellitero, P. (2010b) The effect of lectins on the attachment and invasion of *Enteromyxum scopthalmi* (Myxozoa) in turbot (*Psetta maxima* L.) intestinal epithelium *in vitro*. *Experimental Parasitology* 126, 577–581.
- Redondo, M.J., Palenzuela, O., Riaza, A., Macías, M.A. and Alvarez-Pellitero, P. (2002) Experimental transmission of *Enteromyxum scopthalmi* (Myxozoa), an enteric parasite of turbot *Scophthalmus maximus*. *Journal of Parasitology* 88, 482–488.
- Redondo, M.J., Palenzuela, O. and Alvarez-Pellitero, P. (2003a) *In vitro* studies on viability and proliferation of *Enteromyxum scopthalmi* (Myxozoa), an enteric parasite of cultured turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms* 55, 133–144.
- Redondo, M.J., Quiroga, M.I., Palenzuela, O., Nieto, J.M. and Alvarez-Pellitero, P. (2003b) Ultrastructural studies on the development of *Enteromyxum scopthalmi* (Myxozoa), an enteric parasite of turbot (*Scophthalmus maximus* L.). *Parasitology Research* 90, 192–202.
- Redondo, M.J., Palenzuela, O. and Alvarez-Pellitero, P. (2004) Studies on transmission and life cycle of *Enteromyxum scopthalmi* (Myxozoa), an enteric parasite of turbot *Scophthalmus maximus*. *Folia Parasitologica* 51, 188–198.
- Redondo, M.J., Cortadellas, N., Palenzuela, O. and Alvarez-Pellitero, P. (2008) Detection of carbohydrate terminals in the enteric parasite *Enteromyxum scopthalmi* (Myxozoa) and possible interactions with its fish host *Psetta maxima*. *Parasitology Research* 102, 1257–1267.
- Rigos, G. and Katharios, P. (2010) Pathological obstacles of newly-introduced fish species in Mediterranean mariculture: a review. *Reviews in Fish Biology and Fisheries* 20, 47–70.
- Rigos, G., Christophiligiannis, P., Yiagnisi, M., Andriopoulou, A., Koutsodimou, M., Nengas, I. and Alexis, M. (1999) Myxosporean infection in Greek mariculture. *Aquaculture International* 7, 361–364.
- Sitjà-Bobadilla, A. (2008) Fish immune response to Myxozoan parasites. *Parasite-Journal de la Societe Francaise de Parasitologie* 15, 420–425.
- Sitjà-Bobadilla, A., Redondo, M.J., Macías, M.A., Ferreiro, I., Riaza, A. and Alvarez-Pellitero, P. (2004) Development of immunohistochemistry and enzyme-linked immunosorbent assays for the detection of circulating antibodies against *Enteromyxum scopthalmi* (Myxozoa) in turbot (*Scophthalmus maximus* L.). *Fish and Shellfish Immunology* 17, 335–345.
- Sitjà-Bobadilla, A., Redondo, M.J., Bermúdez, R., Palenzuela, O., Ferreiro, I., Riaza, A., Quiroga, I., Nieto, J.M. and Alvarez-Pellitero, P. (2006) Innate and adaptive immune responses of turbot, *Scophthalmus maximus* (L.), following experimental infection with *Enteromyxum scopthalmi* (Myxosporea: Myxozoa). *Fish and Shellfish Immunology* 21, 485–500.
- Sitjà-Bobadilla, A., Diamant, A., Palenzuela, O. and Alvarez-Pellitero, P. (2007a) Effect of host factors and experimental conditions on the horizontal transmission of *Enteromyxum leei* (Myxozoa) to gilthead sea bream, *Sparus aurata* L., and European sea bass, *Dicentrarchus labrax* (L.). *Journal of Fish Diseases* 30, 243–250.
- Sitjà-Bobadilla, A., Palenzuela, O. and Alvarez-Pellitero, P. (2007b) The innate immune response of sharp-snout sea bream (*Diplodus puntazzo*) in relation to enteromyxosis progression. *Parassitologia* 49, 77.
- Sitjà-Bobadilla, A., Palenzuela, O., Riaza, A., Macías, M.A. and Alvarez-Pellitero, P. (2007c) Protective acquired immunity to *Enteromyxum scopthalmi* (Myxozoa) is related to specific antibodies in *Psetta maxima* (L.) (Teleostei). *Scandinavian Journal of Immunology* 66, 26–34.
- Sitjà-Bobadilla, A., Caldach-Giner, J., Saera-Vila, A., Palenzuela, O., Alvarez-Pellitero, P. and Pérez-Sánchez, J. (2008) Chronic exposure to the parasite *Enteromyxum leei* (Myxozoa: Myxosporea) modulates the immune response and the expression of growth, redox and immune relevant genes in gilthead sea bream, *Sparus aurata* L. *Fish and Shellfish Immunology* 24, 610–619.
- Tun, T., Yokoyama, H., Ogawa, K. and Wakabayashi, H. (2000) Myxosporeans and their hyperparasitic microsporeans in the intestine of emaciated tiger puffer. *Fish Pathology* 35, 145–156.
- Tun, T., Ogawa, K. and Wakabayashi, H. (2002) Pathological changes induce by three myxosporeans in the intestine of cultured tiger puffer, *Takifugu rubripes* (Temminck and Schlegel). *Journal of Fish Diseases* 25, 63–72.

- Yanagida, T., Nomura, Y., Kimura, T., Fukuda, Y., Yokoyama, H. and Ogawa, K. (2004) Molecular and morphological redescrptions 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., Freeman, M.A., Nomura, Y., Takami, I., Sugihara, Y., Yokoyama, H. and Ogawa, K. (2005) Development of a PCR-based method for the detection of enteric myxozoans causing emaciation disease of cultured tiger puffer. *Fish Pathology* 40, 13–29.
- Yanagida, T., Sameshima, M., Nasu, H., Yokoyama, H. and Ogawa, K. (2006) Temperature effects on the development of *Enteromyxum* spp. (Myxozoa) in experimentally infected tiger puffer, *Takifugu rubripes* (Temminck & Schlegel). *Journal of Fish Diseases* 29, 561–567.
- Yanagida, T., Palenzuela, O., Hirae, T., Tanaka, S., Yokoyama, H. and Ogawa, K. (2008) Myxosporean emaciation disease of cultured red sea bream *Pagrus major* and spotted knifejaw *Oplegnathus punctatus*. *Fish Pathology* 43, 45–48.
- Yasuda, H., Ooyama, T., Iwata, K., Tun, T., Yokoyama, H. and Ogawa, K. (2002) Fish-to-fish transmission of *Myxidium* spp. (Myxozoa) in cultured tiger puffer suffering emaciation disease. *Fish Pathology* 37, 29–33.
- Yasuda, H., Ooyama, T., Nakamura, A., Iwata, K., Palenzuela, O. and Yokoyama, H. (2005) Occurrence of the myxosporean emaciation disease caused by *Enteromyxum leei* in cultured Japanese flounder *Paralichthys olivaceus*. *Fish Pathology* 40, 175–180.
- Yokoyama, H. and Shirakashi, S. (2007) Evaluation of hyposalinity treatment on infection with *Enteromyxum leei* (Myxozoa) using anemonefish *Amphiprion* spp. as experimental host. *Bulletin of the European Association of Fish Pathologists* 2, 74–78.

10 *Henneguya ictaluri*

Linda M.W. Pote,¹ Lester Khoo² and Matt Griffin³

¹College of Veterinary Medicine, Mississippi State University, Mississippi, USA

²Thad Cochran National Warmwater Aquaculture Center, College of Veterinary Medicine, Mississippi State University, Mississippi, USA

³Thad Cochran National Warmwater Aquaculture Center, College of Veterinary Medicine; and Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi, USA

10.1. Introduction

The commercial channel catfish industry is the largest warm-water aquaculture industry in the USA. This industry encompasses approximately 99,600 water acres (40,306 ha) with 91.0% of these acres concentrated in Alabama, Arkansas, Mississippi and Texas (USDA NASS, 2011). The inventory of food-size catfish alone in the USA in 2011 was approximately 176 million catfish raised on 909 catfish operations (USDA-NASS, 2011).

In this intensively managed aquaculture system commercial channel catfish (*Ictalurus punctatus*) and blue catfish (*Ictalurus furcatus*) × channel catfish hybrids are raised in open earthen ponds ranging in size from 8 to 20 acres (3.2–8.1 ha) with stocking rates ranging from 1293 to 24,710 fish/ha (USDA, 1997; Avery and Steeby, 2004; Boyd, 2004). The design of the ponds and the management practices used have created an environment conducive for the introduction and perpetuation of many fish parasite life cycles. The following factors contribute to the tremendous challenge in the control and eradication of parasitic diseases in these ponds: (i) multiple-aged fish are raised together in these ponds; (ii) the ponds are seldom drained; and (iii) a wide variety of wild-life feed and live near these ponds year-round.

One parasite that has plagued this industry since its commercialization in the early 1980s is the myxozoan *Henneguya ictaluri*, the causative agent of proliferative gill disease (PGD) or ‘hamburger gill disease’ in channel and hybrid catfish. Outbreaks of this disease have had devastating effects on the industry, with mortality rates often exceeding 50% in affected ponds. While early diagnostic reports and case studies implicated that the *Henneguya* spp. cysts found in catfish gills were associated with this disease (Bowser and Conroy, 1985; Bowser *et al.*, 1985; MacMillan *et al.*, 1989), it was not until 1999 that this disease was linked to the myxozoan *H. ictaluri* (Burtle *et al.*, 1991; Pote *et al.*, 2000).

Currently there are eight *Henneguya* spp. reported in the literature (*Henneguya adiposa*, *Henneguya diversis*, *Henneguya exilis*, *Henneguya longicauda*, *Henneguya limatula*, *Henneguya postexilis*, *Henneguya sutherlandi* and *H. ictaluri*) known to infect *I. punctatus* based on the morphology of the cyst and myxospores and the location of the cysts in the catfish host (Kudo, 1929; Minchew, 1977; Lin *et al.*, 1999; Pote *et al.*, 2000; Griffin *et al.*, 2008b, 2009b). Of these eight species, the 18S small subunit ribosomal RNA (SSU rDNA) has been sequenced for the myxospores of four of these species (*H. adiposa*, *H. exilis*, *H. ictaluri* and

H. sutherlandi). The life cycles have been confirmed using molecular techniques for two of these species, *H. ictaluri* and *H. exilis*, by linking their actinospore stage with their myxospore stage.

Molecular and morphological studies have confirmed that *H. ictaluri* has the typical myxozoan life cycle (Wolf and Markiw, 1984) with the myxospore stage in the catfish host *I. punctatus* (Fig. 10.1), and the actinospore stage (formerly *Aurantiactinomyxon ictaluri*) in the aquatic oligochaete host *Dero digitata* (Fig. 10.2) (Styer *et al.*, 1991; Pote *et al.*, 2000). The genus *Henneguya* has been reported from a wide variety of fishes worldwide with at least eight species reported in channel catfish. However, to date, only the life cycles of *H. ictaluri* and *H. exilis*, both found in the channel catfish, have been confirmed using molecular techniques.

In *H. ictaluri* the only oligochaete identified as the invertebrate host is *D. digitata* which has been confirmed experimentally and molecularly (Styer *et al.*, 1991; Pote *et al.*, 2000). Other species of aquatic oligochaetes and numerous invertebrates found in these catfish ponds cannot be infected with *H. ictaluri* (Bellerud *et al.*, 1995). The *D. digitata* populations in these ponds are ubiquitous and are found year-round in the benthic sediment of commercial catfish ponds with population estimates ranging from 1400 to 20,000

oligochaetes/m² (Bellerud, 1993; Bellerud *et al.*, 1995). Although found year-round, the *D. digitata* populations peak in the spring and there are smaller peaks in the autumn, with pond-water temperatures ranging from 19 to 24°C during this time (Wax *et al.*, 1987), often occurring with seasonal outbreaks of PGD (Bellerud, 1993; Bellerud *et al.*, 1995). Laboratory reared *H. ictaluri*-infected *D. digitata* remain infected for months, and reproduce asexually (Pote *et al.*, 1994) which may contribute to the rapid increase in infected *D. digitata* and the sudden outbreaks of PGD observed in the field. While the prevalence and number of *D. digitata* infected with *H. ictaluri* are higher in ponds experiencing PGD outbreaks, even those ponds considered negative for PGD have *D. digitata* populations infected with *H. ictaluri* maintaining a constant infected reservoir population (Bellerud, 1993; Bellerud *et al.*, 1995).

H. ictaluri actinospores released into the water by the infected *D. digitata* remain infective for at least 24 h, with infectivity decreasing rapidly over time (Wise *et al.*, 2008). Belem and Pote (2001) demonstrated the route of infection for the *H. ictaluri* actinospore into the catfish host occurs through the skin, gills or orally. *In vitro* studies demonstrated that exposure of *H. ictaluri* actinospores to channel catfish blood, mucus and gill tissues resulted

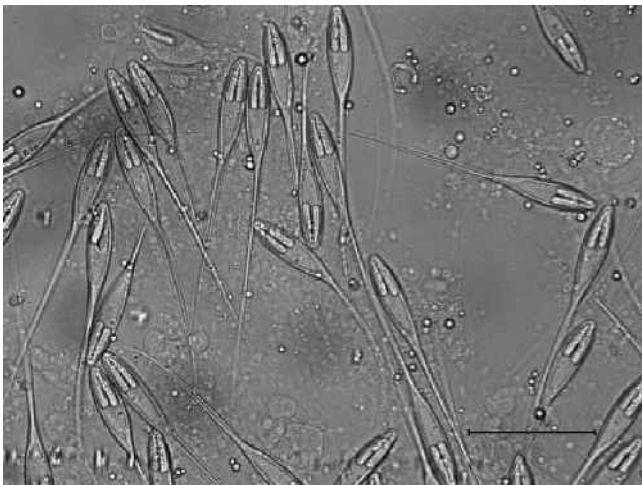


Fig. 10.1. Typical *Henneguya* spp. myxospores isolated from the gills of channel catfish. Bar = ~25 μ m.

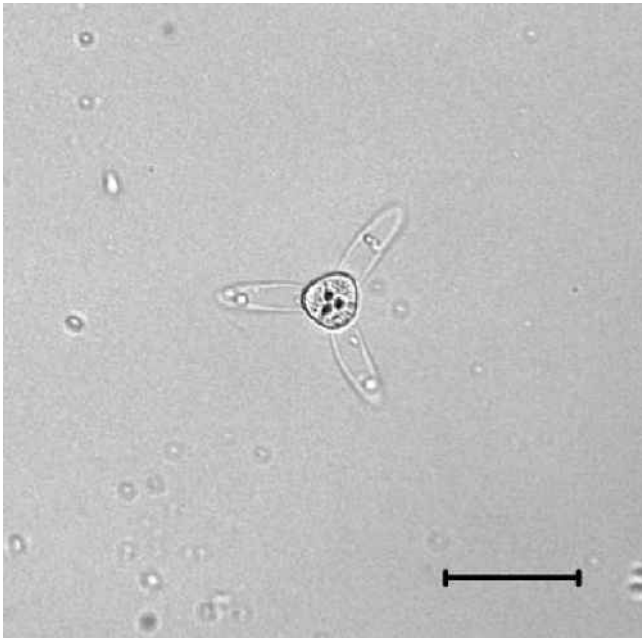


Fig. 10.2. *Henneguya ictaluri* actinospore. Bar = ~50 μ m.

in the discharge of the polar capsule by the actinospore, which indicates this may be one of the cues for host penetration (Pote and Waterstrat, 1993). Studies with other myxozoans have confirmed this observation and have further demonstrated there are several non-specific mechanical and chemical cues at play (Kallert *et al.*, 2005). Factors involved in the interaction of the *H. ictaluri* actinospores and catfish still remain unclear and speculative. Once the actinospore infects the catfish the parasite can be found within 24 h post-infection in the blood (Belem and Pote, 2001; Griffin *et al.*, 2008a). Immature *H. ictaluri* myxospores are in the gills at 3–5 days post-infection and mature cysts at 3 months post-infection (Pote *et al.*, 2000; Griffin *et al.*, 2010).

The channel catfish appears to be the only natural host for *H. ictaluri*. There have been reports of PGD in wild-caught channel catfish in the USA (Thiyagarajah, 1993), rainbow trout (*Oncorhynchus mykiss*) in Germany (Hoffman *et al.*, 1992) and in channel catfish in Italy (Marcer *et al.*, 2004), but in all cases this disease was associated with unknown myxozoan-like parasites in the gills with no molec-

ular data to confirm their species identification. Although blue catfish can become infected with *H. ictaluri*, recent work indicates that *H. ictaluri* does not complete its life cycle in this fish species and there is little pathology associated with the infection (Griffin *et al.*, 2010). Interestingly, hybrid crosses of *I. punctatus* and *I. furcatus* not only become infected, they also exhibit the pathology that is associated with PGD in *I. punctatus*.

10.2. Diagnosis of Infection

Presumptive diagnosis of the disease is based on clinical signs, gross lesions and microscopic examination of wet mounts from gill biopsy. Since acute infections result in respiratory insult, the fish exhibit signs associated with hypoxia and consequently are usually found piping at the surface of the water or swimming listlessly behind the aerator, even when there is sufficient dissolved oxygen. Affected gills have a mottled appearance and are swollen and fragile. This mottled and

swollen appearance resembles that of ground meat, thus this condition is often referred to as 'hamburger gill' by catfish producers. Microscopic examination of gill biopsy wet mounts reveal defects in the filamental cartilage with the associated haemorrhage and swelling of the branchial tissue. The severity of the cartilaginous lesions often correlates well with the severity of infection and clinical signs, especially in fingerling-sized fish. However, in larger fish, especially food-sized fish (0.7–0.9 kg), the damage to the filamental cartilage often does not reflect the severity of infection and there may be only a few fractures or breaks in the cartilage even though fish are succumbing to the disease. Mortality rates can often exceed 50% of a fish population, with the most severe outbreaks in the spring, and to a lesser extent in the autumn, when pond-water temperatures are between 15 and 20°C in the south-eastern USA (Wise *et al.*, 2004). However, there have also been sporadic infections at other temperatures. Also, lesions in the filamental cartilage may take longer to heal during cooler temperatures, therefore gill damage observed in clinical cases submitted during the winter months may reflect delayed healing from an earlier infection rather than an active outbreak. A definitive diagnosis can be made by histological examination of fixed and stained tissues, identifying the characteristic changes together with the presence of the pre-sporegenic vegetative stage. Confirmation can also be made by using species-specific PCR (Whitaker *et al.*, 2001; Pote *et al.*, 2003). Histological identification of the infective organism may require examination of multiple sections of gills as the organisms may not be present in all planes of a section of gill. Also, the infective organism is usually only present during the acute stages of infection and often not readily evident after 14 days post-infection in controlled experimental infections. However, most clinical cases represent more than a one-time exposure and organisms present within gill tissue sections represent several different stages of development. In clinical cases where lesions are suggestive of the disease but organisms are not readily evident, *H. ictaluri* infection can be confirmed using PCR, which is more

sensitive than conventional histological methods (Whitaker *et al.*, 2001; Pote *et al.*, 2003). More recently, a quantitative real-time PCR (qPCR) assay has also been developed, providing a means to not only detect low numbers of these organisms in the tissue but also quantify the amount of parasite DNA within host tissues (Griffin *et al.*, 2008a). Molecular techniques however have the disadvantage that they cannot discriminate between acute infection (when mortalities are often occurring) and more chronic sub-clinical cases. Thus, an accurate diagnosis requires the use of confirmatory tests in conjunction with information from the presumptive diagnosis.

In addition, both molecular assays have been modified to detect the *H. ictaluri* actinospore in pond water (Whitaker *et al.*, 2005; Griffin *et al.*, 2009a); consequently they can be used to identify ponds with PGD, even if the resident fish population does not have clinical signs of the disease. For reasons that are currently unclear, resident fish in a pond have varying degrees of susceptibility to PGD (Wise *et al.*, 2004).

10.3. External/Internal Lesions

10.3.1. Gross

Gills of channel catfish fingerlings with moderate to severe PGD often have a red and white mottled appearance, are swollen, fragile and bleed easily (Fig. 10.3). These gills are often shortened or truncated with portions of the filamental tips missing. In larger food-size fish, the affected gills may only show multiple foci of haemorrhage rather than the 'meaty' appearance seen in fingerlings and the gill filaments are usually not truncated.

10.3.2. Microscopic

Presumptive diagnosis of this disease is based upon microscopic examination of gill biopsies and observing the defects (missing portions that are often semi-circular or fractures) in the cartilage of the gill filaments (Fig. 10.4).



Fig. 10.3. Mottled appearance of gills of a channel catfish fingerling affected by proliferative gill disease (PGD). The operculum has been removed.

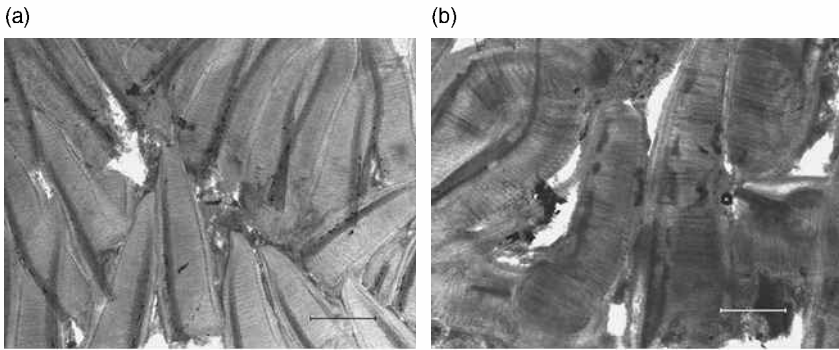


Fig. 10.4. Wet mount of gill clip from a normal (a) and a PGD-affected (b) channel catfish. Note the defects in the cartilage and swelling and haemorrhage of the branchial tissue in the affected fish (b). Bar = ~1000 μ m.

In an acute infection, there is often accompanying multifocal haemorrhage and swelling or expansion of the branchial tissue with loss of detail of the secondary lamellae. In food-size fish, the severity of microscopic lesions often does not reflect the clinical severity of disease. This is perhaps due to just sampling of gill tips for diagnosis because the size of filaments limits the number of tissue sections that can be placed on a glass slide for examination.

Histopathologically, the lesions observed with PGD are dependent on the severity and the chronicity of the disease. At 1 day post-exposure (p.e.) in sub-lethal experimental infections exposing specific pathogen-free

channel catfish fingerlings to pond water of a confirmed PGD infection, the lesions can be relatively non-specific, characterized by: (i) multifocal areas of inflammation with haemorrhage; (ii) epithelial hyperplasia; and (iii) mucus cell hyperplasia resulting in partial filling or obliteration of the lamellar troughs (Fig. 10.5). By 7 days p.e., the inflammatory response is granulomatous and is more intense and expansile. The infiltrate of mononuclear inflammatory cells fills and expands the central portion of the gill filament separating the two ends of the cartilage (Fig. 10.6). Often but not always, one or more cyst-like structures (~20–40 μ m in diameter) containing basophilic (in haematoxylin and eosin

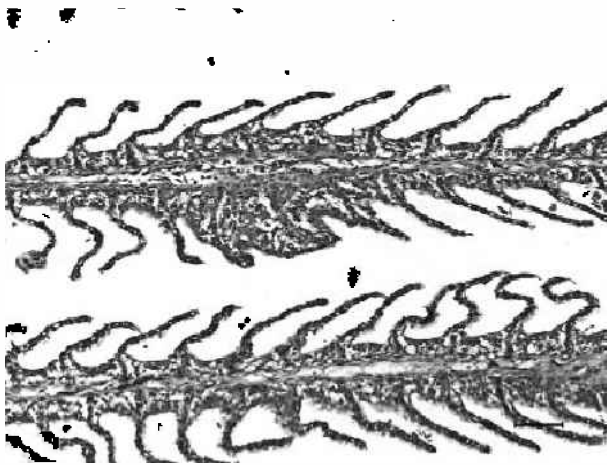


Fig. 10.5. Gills from a channel catfish experimentally infected with PGD, 1 day post-exposure (p.e.). Note the inflammation and epithelial proliferation that partially fills the lamellar troughs and lamellar synechia bridging the troughs. H & E; bar = ~50 μ m.

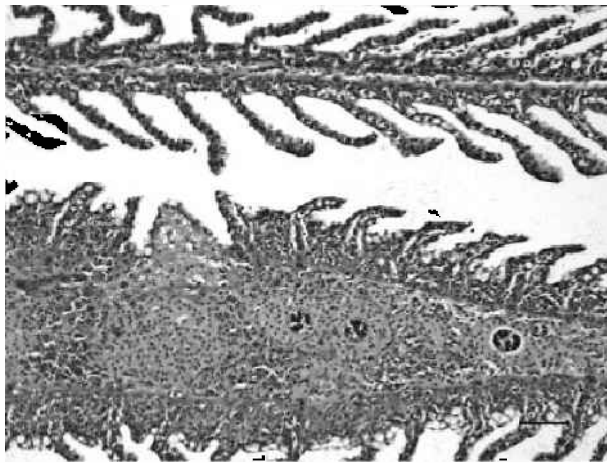


Fig. 10.6. Gills from a PDG-infected channel catfish 7 days p.e. The central portion of the gill is markedly expanded by the influx of inflammatory cells. At least three pre-vegetative spore stages of *H. ictaluri* that are surrounded by macrophages are evident in this section. H & E; bar = ~50 μ m.

(H & E)-stained sections) granular clusters of the developing pre-sporogenic vegetative stage are associated with these foci of intense inflammation. These cyst-like structures are often surrounded by a singular ring of large palisading mononuclear inflammatory cells, presumptively epithelioid macrophages. These changes are consistent with descriptions by Bowser and Conroy (1985) and Duhamel *et al.* (1986) who ascribed the lesions

to *H. exilis* Kudo; prior to the description of *H. ictaluri* as a species by Pote *et al.* (2000). By 14 days p.e., there is some resolution of the inflammatory component and evidence of the healing process is progressing through bridging of cartilaginous defects with callus formation (Fig. 10.7). This is characterized by dyshondroplastic or disorganized irregular, cartilaginous growth consisting of large, pale basophilic chondrocytes. Interestingly at this

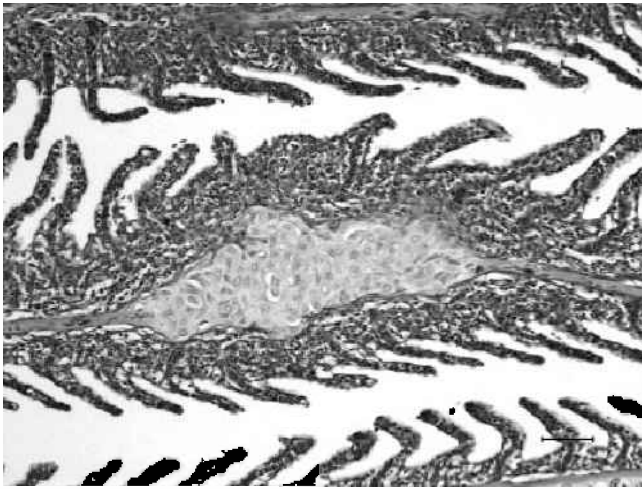


Fig. 10.7. Gills from a PGD-infected channel catfish 14 days p.e. The break in the cartilage has been bridged by cartilaginous hyperplasia. There is still an inflammatory component present, however, the developing sporozoite is no longer readily evident. H & E; bar = ~50 μ m.

time, the infectious agent is no longer readily evident. All that is evident of the infectious agent is a ring of epithelioid macrophages around some faint eosinophilic fibrillar on H & E sections. At 21 days p.e., the inflammatory response is resolving and there is initial remodelling of the callus with partial calcification of the outer or peripheral portions. At this time, the developing sporozoite is often not seen even in areas where the cartilage defect is being remodelled. *H. ictaluri* may be like other myxozoans such as *Sphaerospora* species that have extrasporogenic development where replication takes place in tissues other than those in which sporulation occurs (Kent *et al.*, 2001). If there is indeed non-branchial tissue development sites for *H. ictaluri*, there are no known reports that attribute pathology at these possible sites to the parasites. At 28 days p.e., there is further remodelling of the callus and there is also multifocal mononuclear inflammatory infiltrates that are often not associated with the callus. The developing sporozoite is still not readily evident. There are no significant changes between 28 days and 35 days p.e. Sometime after 35 days p.e. and before 70 days p.e., the developing plasmodia or pseudocyst (presumably of *H. ictaluri* since fish could have

been exposed to more than one species of *Henneguya*) becomes evident. At 70 days p.e., some of these non-epithelium-lined cyst-like structures have characteristic mature *Henneguya* spores with two prominent pyriform polar capsules that are dark blue on Giemsa-stained sections (Fig. 10.8). This is consistent with the findings of Pote *et al.* (2000) where fish were exposed to a challenge of molecularly confirmed *H. ictaluri* actinospores. These plasmodia may not be at or adjacent to the nodular remnant of the callus. The plasmodia of this histozoic myxosporean is often intralamellar (Molnár, 2002) arising just beneath the lamellar epithelium and often balloons out to fill the lamellar trough. The pseudocysts may exceed the dimensions of the lamellar trough and distort the adjacent branchial architecture. The myxozoan spores are separated from the branchial tissue by a thin (~1–2 μ m), pale eosinophilic hyaline (on H & E-stained sections) parasitic wall and there is usually no inflammatory component associated with the intact pseudocyst. With additional time, the cyst-like structures enlarge and through asynchronous development, are filled with mature myxospores. Mature myxospores released from these cyst-like structures when gill biopsies are examined

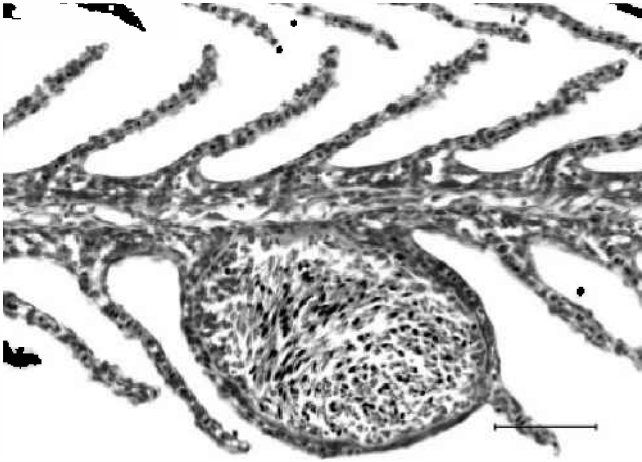


Fig. 10.8. Gill from a PGD-infected channel catfish 70 days p.e. Note the distension due to the pseudocyst containing mature and developing myxozoan spores and the lack of an inflammatory component. Giemsa; bar = ~50 μ m.

have the typical *Henneguya* morphology (Pote *et al.*, 2000) (i.e. spindle-shaped spores with an oval-to-pyriform spore body containing two pyriform polar capsules and bifurcated caudal process or appendage). The caudal process is split the entire length and each is the continuation of the one valve. Both polar capsules are usually the same size and width and are dark blue in Giemsa-stained histological sections.

10.4. Pathophysiology

Belem and Pote (2001) demonstrated that *H. ictaluri* appears to enter channel catfish primarily through the stomach but can also enter through the buccal cavity, gills and skin. After entry the developing sporozoites move or are transported via the blood, appearing in the heart and hepatic vessels and are disseminated to the spleen, kidneys, liver and gills.

Besides respiration, the other major functions of the gills are ion regulation, acid-base balance and excretion (Evans *et al.*, 1999; Speare and Ferguson, 2006) all of which should be adversely affected in fish with PGD. Unfortunately, there is a dearth of literature dealing with the pathophysiology of

PGD. Beecham *et al.* (2010) documented the physiological effects of PGD in sub-lethally infected channel catfish and channel catfish $\times$ blue catfish hybrids at 24, 96 and 168 h p.e. to the parasite. Besides respiratory distress, there was a significant reduction in pO_2 and an increase in pCO_2 at 96 h. There also was a decrease in haematocrit values at 96 h p.e., which corresponds to the haemorrhage seen grossly and microscopically in the gills, which they concluded could also contribute to the changes in pO_2 and pCO_2 . Blue catfish concurrently exposed in the same study did not exhibit pathology or any physiological changes. In their study, Beecham *et al.* (2010) did not see changes in blood-plasma calcium concentration and they concluded that the negative effects of PGD on gaseous exchange were more significant than osmoregulation.

In other infectious diseases involving the gills of fishes, physiological changes other than hypoxemia have also been noted. For example in bacterial gill disease (BGD) caused by *Flavobacterium branchiophilum*, Byrne *et al.* (1991) showed that affected brook trout (*Salvelinus fontinalis*) had hyponatremia, hypochloremia, hypoosmolality, hypoproteinemia and increased packed cell volume or haematocrit; the latter was considered a

compensatory response to the hypoxemia. A later study showed similar changes as well as hypoxemia, hypercapnia and increased blood ammonia in affected BGD rainbow trout that were fed although these changes were less dramatic in unfed fish (Byrne *et al.*, 1995). However, rainbow trout infected with *Loma salmonae* (a microsporidian parasite) had just marginally elevated hypernatremia and hyperchloremia with no changes in plasma K^+ levels (Powell *et al.*, 2006). Perhaps more significantly, in a more closely related disease (i.e. respiratory henneguyosis in *Clarias gariepinus*) Sabri *et al.* (2009) documented decreases in serum proteins, albumin, Na^+K^+ ATPase activity and an increase in globulin levels. Therefore, it would not be surprising if similar changes were seen in PGD-affected fish given the severity of the inflammation and destruction in the gills of affected fish. Unfortunately, this cannot be confirmed as there is a paucity of published studies documenting these changes.

10.5. Protective/Control Strategies

10.5.1. Chemical treatments

Several drugs have been experimented with for the control of myxosporidian infections in fish. Fumagillin (dicyclohexylamine) has been used for the treatment of *Myxobolus cerebralis* infections but with mixed results (Wagner, 2002). This same drug was successful in treating *Thelohanellus hovorkai* in koi carp and *Sphaerospora renicola* in common carp but the drug was unsuccessful for treating *Myxobolus cyprini* and *Thelohanellus nikolskii*. Wagner (2002) concluded that there was different susceptibility for the various myxozoans to the drug. However Buchman *et al.* (1993) found that the time for drug application is very important. If the drug is distributed in the fish tissue before sporogony it will be effective. In contrast, if the drug is administered following sporogony it is not efficacious against spores that are encapsulated and protected in the tissue. Furazolidone, acetarson, amprolium, nicarbazine as well as oxytetracycline and suflamerazine have

also been used for treating *M. cerebralis* infections but with mixed results (Wagner, 2002). The anti-coccidials amprolium and salinomycin have also been demonstrated to be effective against myxozoan infections in other fish species (Athanasopoulou *et al.*, 2004). However, at present none of these drugs have been approved for treating *H. ictaluri* in channel catfish or shown to be efficacious against the organism.

Mischke *et al.* (2001) investigated several potential chemical therapeutics to eradicate the oligochaete host (*D. digitata*) without negatively affecting the pond ecosystem and adversely affecting the resident fish populations. Formalin, chloramines-T, sodium chloride (NaCl), potassium permanganate ($KMnO_4$), copper sulfate ($CuSO_4$), hydrogen peroxide (H_2O_2), Rotenone® ($C_{23}H_{22}O_5$, 5% solution, Prentiss, Inc., Sandersville, Georgia, USA) and Bayluscide® (niclosamide, 70% wettable powder; Bayer Chemical Co., Kansas City, Missouri, USA), were all tested for their ability to eliminate *D. digitata*. Unfortunately, doses required for these agents to be efficacious were cost prohibitive, and required multiple treatments, thus making them an impractical treatment option. Although these chemical agents can be useful tools after an outbreak has occurred and the pond has been drained, they are not successful in eliminating the oligochaetes while fish are present. The benthic substrate and organic matter in these ponds also inhibit the efficacy of chemical treatments requiring higher doses to achieve a LC_{50} (lethal concentration for 50% of the population) for *D. digitata*, resulting in doses that are lethal to catfish (Mischke *et al.*, 2001). Furthermore, Bayluscide® is highly toxic to catfish. As such, management practices specifically designed to reduce the impact of PGD are currently the only feasible solution to ameliorate losses attributed to *H. ictaluri*.

10.5.2. Biological control

Biological control has also been suggested as a potential management strategy. Polyculture with common carp (*Cyprinus carpio*) will

initially reduce the oligochaete populations within the pond, but as the carp increase in size the smaller oligochaetes are no longer a preferred food source. For this strategy to be effective, repeated stocking of appropriately sized carp is required, which is impractical on most commercial operations (Burtle and Styer, 1996). Similarly, the fathead minnow (*Pimephales promelas*) has been proposed as a biological control agent. Fatheads are a small fish which primarily feed on benthic organisms and algae. They can also serve as a dietary supplement (forage fish) for the catfish. Unfortunately, the catfish will decimate the fathead population unless adequate spawning areas are provided. In order for fathead minnows to be an efficient biological control method their numbers need to be above 2000/acre, which has proved to be difficult to maintain (Burtle, 1998). There is also limited evidence that smallmouth buffalo (*Ictiobus bubalus*), which also feed primarily on benthic organisms, can diminish populations of oligochaetes within the pond, indirectly reducing the incidence of PGD. However, reported success is anecdotal and research has yet to establish that polyculture with smallmouth buffalo actually has any noticeable effect on the incidence of PGD.

10.5.3. Supplemental treatments

Palliative therapies for PGD involve restricted feeding to reduce the oxygen demand of the fish, and increased aeration and pond salinity to ameliorate the respiratory insult and help the fish deal with osmoregulatory stress, respectively (Mitchell *et al.*, 1998; Wise *et al.*, 2004). Chloride levels in the pond should also be monitored closely to prevent the onset of nitrite-induced methemoglobinemia, which decreases the oxygen carrying capacity of the blood, potentially exacerbating losses to PGD (Huey *et al.*, 1980; Bowser *et al.*, 1985). Since it is often difficult or almost impossible to remove all of the dead fish, this often leads to an increase in ammonia in the water that is converted to nitrites. Adding salt reduces the deleterious effects of nitrite toxicity because there is competitive uptake of chloride ions and nitrite ion by the chloride cells in the

gills. Supplemental aeration is the most important factor in curbing losses during a PGD outbreak. The actinospore stage is uniformly distributed throughout the pond (Griffin *et al.*, 2009a) even though benthic oligochaetes are not (Bellerud *et al.*, 1995). Although mechanical aerators could potentially increase the dispersal of the actinospore stage throughout the pond, they do not contribute to this distribution any more than natural physical processes. In salmonid aquaculture, the life cycle of myxozoans can be broken by culturing fish in concrete raceways or other culture units that do not provide the earthen substrate required by the oligochaete host (Wagner, 2002). Unfortunately this strategy is not economically feasible in catfish aquaculture.

Another treatment option during a PGD outbreak is to move the fish to another pond where *H. ictaluri* is not present. A reduction in fish mortality and morbidity has been observed in fish moved to a clean, well-oxygenated environment if this was done at the early stage of an outbreak. However, moving the fish is costly in terms of both labour and time and transport-induced stress may result in further loss of clinically and subclinically infected fish. The decision to move should be based on the expected losses if fish are left in the pond and the number of fish that will survive transport (Wise *et al.*, 2004). There is also the potential of introducing the disease into a pond that may be free of *H. ictaluri*. However, research has shown that *H. ictaluri* is endemic on most catfish farms, and the parasite is likely to already be present in a majority of the commercial catfish ponds, although not always at levels sufficient to cause disease (Bellerud *et al.*, 1995; Wise *et al.*, 2004, 2008).

10.5.4. Pond monitoring

The most effective way of reducing losses associated with PGD is an efficient management strategy. In short, naïve fish should not be stocked into ponds with active PGD outbreaks and, if possible, in the incident of a severe outbreak fish should be moved to an environment where *H. ictaluri* actinospores are absent, or at significantly lower levels.

Monitoring and surveillance within a pond using naïve sentinel fish in cages allows producers to determine whether there is an active PGD epizootic in a given pond. This is not only important when identifying ponds for the relocation of fish, but also in determining when a pond is safe to restock following a severe PGD outbreak. Fingerlings stocked into food-fish production ponds are at the greatest risk of developing PGD, especially in the spring or following a severe PGD outbreak. For reasons that are currently unclear, resident populations within a pond have varying degrees of susceptibility to PGD. The multibatch system of stocking used in most commercial catfish ponds means that at any given time there are several populations of fish within a pond. Often, younger fish that have most recently been added to the pond will suffer significant mortalities during an epizootic, while older fish that have been in the pond for a longer period demonstrate little or no clinical signs of the disease. Whether this has to do with an acquired immunity or it simply takes a much higher challenge dose to result in the same level of damage in older, larger fish remains unclear. As such, the resident population of fish does not always provide an accurate assessment of the concentration of *H. ictaluri* actinospores present within the pond. This has necessitated the development of an assessment strategy to determine the risk of losing fish to PGD in newly stocked catfish ponds.

There is a strong correlation between the percentage of damaged or affected gill filaments in sentinel fish and mortalities observed in fish newly introduced into the system (Wise *et al.*, 2008). The Fish Health Management Program at the Thad Cochran National Warmwater Aquaculture Center (Stoneville, Mississippi) developed a lesion scoring system to determine severity of PGD in ponds (Wise *et al.*, 2004). Using parasite-free sentinel fish, the levels of the *H. ictaluri* actinospores within the pond can be estimated and outcome of severity of infection can be predicted. Specific pathogen-free (SPF) fish are held in netpens or cages for 7 days, after which gill biopsy wet mounts (~40–80 filaments) are examined microscopically for the characteristic chondrolytic lesions within the gill filaments.

Quantitative evaluation of the infection is determined by calculating the percentage of primary gill lamellae containing at least one lytic lesion in the cartilage. In the presence of a moderate to severe infection the sampling protocol is repeated. Based on an examination of approximately 40–80 gill filaments a mild infection, described as 1–5% of gill filaments exhibiting chondrolytic lesions, has little to no effect on the health of the fish. Moderate infections, in which no direct mortalities are observed, usually correlate with 6–15% of filaments exhibiting chondrocytic lysis. Severe infections, where mortalities are observed in 1–2 weeks, will have lesions in more than 15% of examined filaments. When the number of filaments demonstrating chondrolysis falls below 5%, the severity of infection decreases from one sampling period to the next and losses do not occur in sentinel fish, the pond can be stocked with little risk of losing the newly introduced fish. Unfortunately in ponds where the levels of infective actinospores are high, severe gill damage and death can occur in caged fish in less than 7 days, calling for a need to repeat this protocol. This results in a delay in determining the state of infection in a given pond (Wise *et al.*, 2004, 2008). Other disadvantages are that the protocol requires a source of SPF fish, transport tanks and equipment and if caged fish die prior to sampling it can be difficult to determine the cause of death. Failure to properly acclimate sentinel fish to ambient pond-water temperatures, especially in early spring, can result in mortalities or predispose fish to other infectious diseases such as saprolegniasis and columnaris, which can be misinterpreted as PGD-related. Additionally, death can occur in these sentinel cages during the summer months when algal blooms cause oxygen depletion or heavy algal growth on netpens restricts water flow to the fish. In order to prevent oxygen depletion, netpens are often placed near mechanical aerators. This can result in swift currents flowing through the cage, which can exhaust fish to the point of death. As a result, sampling bias due to post-mortem autolysis could prevent an accurate evaluation of gill damage in fish that have died prior to sampling and with the mortalities, the number of fish being evaluated would be significantly reduced.

Alternatively, a qPCR assay was developed to directly quantify the number of *H. ictaluri* actinospores within the pond (Griffin *et al.*, 2009a). This has eliminated the need for sentinel fish since the PGD status of a pond can be determined using qPCR analysis of water samples. This has drastically reduced the amount of labour associated with pond monitoring and provided more rapid results. Water samples collected on two separate days, preferably 6–10 days apart, can measure the level of *H. ictaluri* actinospores in the water and determine whether the actinospore level is increasing, decreasing or remaining stable. With levels ranging between 10 and 25 actinospores/l, there is a moderate risk of losing fish, especially if water quality parameters are sub-optimal. At actinospore concentrations ≥ 25 /l, stocking fish would not be recommended. Alternatively, research has shown that with actinospore concentrations ≤ 10 actinospores/l, and a marked decrease from the first to last sampling, producers can stock fish with relatively low risk of losing them to PGD (Griffin *et al.*, 2009a).

10.5.5. Alternative catfish species

Another potential control measure is to culture a catfish species less susceptible to PGD or at least occasionally rotate between channel catfish and a less susceptible catfish species, periodically breaking the life cycle in the ponds. Blue catfish possess several attributes that make them desirable for aquaculture. They have a comparable dressing percentage to channel catfish, are relatively easy to seine, have high individual weight gains in temperate regions and are more resistant to several diseases (such as enteric septicemia and channel catfish virus) that affect channel catfish (Graham, 1999). Also *H. ictaluri* rarely infect blue catfish (Bosworth *et al.*, 2003), and when infection does occur, it may be rapidly cleared by host defences (Griffin *et al.*, 2010). However, blue catfish grow more slowly than channel catfish in the first 2 years of life under culture conditions and are less tolerant to poor water quality. Current processing techniques would also have to be modified due to

slight differences in body conformation. They have however, a better dress-out percentage, are easier to seine and are more uniform size at harvest (Hargreaves and Tucker, 2004).

Similarly, blue catfish $\times$ channel catfish hybrids have increased in popularity in recent years. Their superior growth and relative disease resistance compared to channel catfish also make them desirable to catfish producers. It is currently thought that the hybrid catfish does not suffer PGD-related losses on the same scale as channel catfish, although these claims are speculative and based on anecdotal evidence. In controlled studies, channel catfish and hybrid catfish suffer similar levels of gill damage and mortality when concurrently exposed to ponds with active PGD outbreaks (Griffin *et al.*, 2010). However, the route of infection does not appear to be the same in the two fish species, evident by significantly reduced levels of parasite DNA in hybrid catfish blood compared to channel catfish (Griffin *et al.*, 2008a, 2010). This suggests that *H. ictaluri* may not be able to complete its life cycle in hybrid catfish as efficiently as it does in channel catfish. This may offer an explanation as to why PGD outbreaks do not seem to occur in hybrid catfish ponds as frequently as in channel catfish ponds. If the parasite is unable to complete its life cycle in hybrid catfish, or does so only rarely, ponds used regularly for production of hybrid catfish may not provide the opportunity for the parasite to propagate within the system.

10.5.6. Single batch versus multibatch culture

Rotating production between channel catfish and either hybrid or blue catfish, would require producers to discontinue the use of the multibatch system that is currently used by the majority of the commercial catfish operations. In order to ensure a constant supply of market-ready fish, many producers employ the multibatch system, where fingerlings are continuously understocked into food fish grow-out ponds to replace market-size fish that are continually being harvested. As a consequence of this strategy, ponds on a

given operation have fish at various ages and sizes throughout the year. This provides producers with a constant supply of marketable fish, as more ponds will contain food-size fish than if a single-batch system was used. By maintaining a constant stock of marketable fish, producers can keep up with the demands of the processors. Additionally, in the instance that fish from a single pond are temporarily unmarketable due to off-flavours, having market-size fish in a variety of ponds means having a few ponds with off-flavour problems does not prevent a marketable harvest. However, the multibatch system, coupled with the earthen-bottom ponds commonly used in catfish production, provides an optimal environment for the propagation and proliferation of myxozoan life cycles as most grow-out ponds are in continuous production for several years before the ponds are drained to repair the levees and remove the silt build up in the pond. Consequently, there is never a break in the myxozoan life cycle as there is a continuous supply of potential fish hosts being newly introduced into the system. Also, without draining and drying the pond, the oligochaete populations remain intact.

Alternatively, a single-batch system, where ponds are stocked only once at the beginning of the production cycle, without replacing harvested fish, would provide an opportunity at the end of the production cycle, once all fish have been harvested, to drain and dry the pond prior to the next production cycle. This, in theory, could reduce the number of oligochaete hosts within the system, reducing the level of *H. ictaluri* within the pond, and indirectly preventing the parasite from reaching levels that can result in mortality and lost production. If fingerlings are confirmed to be parasite-free prior to stocking, there is less chance for the parasite to be introduced into the system. A potential drawback to instituting the single-batch culture, at least in terms of PGD, is each time a pond is dried and drained; the pond essentially becomes a new pond, which could theoretically increase the incidence of PGD rather than decrease it. For reasons that are poorly understood, most severe outbreaks are often observed in new or recently re-worked catfish ponds. This is thought to be the consequence

of an ecological shift in the oligochaete host populations, favouring the establishment of *D. digitata*, which are considered an intermediate colonizing organism (Soster and McCall, 1990). In the first several months following new pond construction, or reworking of the pond sediment, *D. digitata* can be one of the predominant oligochaete species within the pond, providing more opportunities for *H. ictaluri* to complete its life cycle.

Admittedly, single-batch culture does not address the potential introduction of this parasite by birds or other vectors, although the role of these vectors in the dissemination of *H. ictaluri* parasites is poorly understood. More research needs to be conducted to determine if a single-batch system could reduce the incidence of PGD enough to make it a favourable management strategy as well as the role of piscivorous birds and other vectors in the spread of *H. ictaluri* throughout the industry.

10.6. Conclusions and Suggestions for Future Studies

While *H. ictaluri* has not been eradicated in the commercial catfish industry there are several promising avenues of research that are going to play a key role in the future control of this parasite. The catfish farmer is not only using the recently developed qPCR for *H. ictaluri* as a tool to determine the safety of restocking after PGD outbreaks, but it is also being used to study these PGD ponds over time to develop models that can be used to predict future PGD outbreaks. Preliminary research demonstrated that blue catfish is less susceptible to *H. ictaluri* infections. This has led to further research to identify factors involved in host susceptibility and host resistance and has given further proof that it may be possible to develop catfish genetic lines that are *H. ictaluri* resistant.

Although the *H. ictaluri* life cycle and its association with PGD have been confirmed, our understanding of this parasite's biology and the host-parasite interactions is far from complete. Currently, the life cycle has not been artificially propagated in the laboratory

which limits the ability to conduct experiments except during natural outbreaks of the disease. The artificial propagation of the *H. ictaluri* life cycle under controlled conditions will significantly increase the avenues of research. Additionally, basic research needs to be done on: (i) the immune response of the catfish to infection; (ii) the pathogenesis of this parasite in the catfish; and (iii) the biochemical host-parasite interactions as *H. ictaluri* enters and invades the catfish and its oligochaete host. Investigations identifying those factors involved in the differences seen

in host susceptibility between the channel and blue catfish could lead to the identification of protective proteins. Further studies are also needed to better understand the population dynamics and ecology of *D. digitata* in catfish ponds and to determine the key factors involved in its infection and susceptibility to *H. ictaluri*. Combined results of this research will be critical in the successful long-term control of this parasite and the eventual development of potential protective vaccines and drugs that target this parasite in its fish and oligochaete host.

References

- Athanassopoulou, F., Karagouni, E., Dotsika, E., Ragias, V., Tavla, J., Christofiloyanis, P. and Vatsos, I. (2004) Efficacy and toxicity of orally administrated anti-coccidial drugs for innovative treatments of *Myxobolus* sp. infection in *Puntazzo puntazzo*. *Diseases of Aquatic Organisms* 62, 217–226.
- Avery, J.L. and Steeby, J.A. (2004) Hatchery management. In: Tucker, C.S. and Hargreaves, J.A. (eds) *Biology and Culture of the Channel Catfish*, 1st edn. Elsevier B.V., Amsterdam, The Netherlands, pp. 145–165.
- Beecham, R.V., Griffin, M.J., LaBarre, S.B., Wise, D., Mauel, M., Pote, L.M. and Minchew, C.D. (2010) The effects of proliferative gill disease on the blood physiology of channel catfish, blue catfish and channel catfish X blue catfish hybrid fingerlings. *North American Journal of Aquaculture* 72, 213–218.
- Belem, A.M. and Pote, L.M. (2001) Portals of entry and systemic localization of proliferative gill disease organisms in channel catfish *Ictalurus punctatus*. *Diseases of Aquatic Organisms* 48, 37–42.
- Bellerud, B.L. (1993) Etiological and epidemiological factors effecting outbreaks of proliferative gill disease on Mississippi channel catfish farms. PhD thesis, College of Veterinary Medicine, Mississippi State University, Stoneville, Mississippi State, USA.
- Bellerud, B.L., Pote, L.M., Lin, T.L., Johnson, M.J. and Boyle, C.R. (1995) Etiological and epizootological factors associated with outbreaks of proliferative gill disease in channel catfish. *Journal of Aquatic Animal Health* 7, 124–131.
- Bosworth, B.G., Wise, D.J., Terhune, J.S. and Wolters, W.R. (2003) Family and genetic group effects for resistance to proliferative gill disease in channel catfish, blue catfish and channel catfish x blue catfish backcross hybrids. *Aquaculture Research* 34, 569–573.
- Bowser, P.R. and Conroy, J.D. (1985) Histopathology of gill lesions in channel catfish associated with *Henneguya*. *Journal of Wildlife Diseases* 21, 177–179.
- Bowser, P.R., Munson, A.D., Jarboe, H.H. and Stiles, F.N. (1985) Transmission trials of proliferative gill disease in channel catfish (*Ictalurus punctatus*). *Mississippi Agricultural and Forestry Experiment Station Research Report* 10(8). Mississippi State University, Stoneville, Mississippi State, USA.
- Boyd, C.E. (2004) Pond hydrology. In: Tucker, C.S. and Hargreaves, J.A. (eds) *Biology and Culture of the Channel Catfish*, 1st edn. Elsevier B.V., Amsterdam, The Netherlands, pp. 196–214.
- Buchmann, K., Slotved, H.-C. and Dana, D. (1993) Epidemiology of gill parasites from carp (*Cyprinus carpio*) in Indonesia and possible control methods. *Aquaculture* 118, 9–21.
- Burtle, G.J. (1998) Control of the oligochaete vector of proliferative gill disease in catfish. In: *1998 Annual Report of the Department of Animal and Dairy Science*. The University of Georgia, College of Agricultural and Environmental Sciences, Athens, Georgia, USA, pp. 22–25.
- Burtle, G.J. and Styer, E.L. (1996) Biological control of proliferative gill disease of channel catfish: fish as predators. In: *1996 Annual Report of the Department of Animal and Dairy Science*. The University of Georgia, College of Agricultural and Environmental Sciences, Athens, Georgia, USA, pp. 1–5.

- Burtle, G.J., Harrison, L.R. and Styer, E.L. (1991) Detection of a triactinomyxid myxozoan in an oligochaete from ponds with proliferative gill disease in channel catfish. *Journal of Aquatic Animal Health* 3, 281–287.
- Byrne, P.J., Ferguson, H.W., Lumsden, J.S. and Ostland, V.E. (1991) Blood chemistry of bacterial disease in brook trout *Salvelinus fontinalis*. *Diseases of Aquatic Organisms* 10, 1–6.
- Byrne, P.J., Ostland, V.E., Lumsden, J.S., MacPhee, D.D. and Ferguson, H.W. (1995) Blood chemistry and acid-base balance in rainbow trout *Oncorhynchus mykiss* with experimentally induced acute bacterial gill disease. *Fish Physiology and Biochemistry* 14(6), 509–518.
- Duhamel, G.E., Kent, M.L., Dybdal, N.O. and Hedrick, R.P. (1986) *Henneguya exilis* Kudo associated with granulomatous branchitis of channel catfish *Ictalurus punctatus*. *Veterinary Pathology* 23, 354–361.
- Evans, D.H., Piermarini, D.R. and Potts, W.T.W. (1999) Ionic transport in fish gill epithelium. *Journal of Experimental Zoology* 282, 641–652.
- Graham, K. (1999) A review of the biology and management of blue catfish. *American Fisheries Society Symposium* 24, 37–49.
- Griffin, M.J., Wise, D.J., Camus, A.C., Mauel, M.J., Greenway, T.E. and Pote, L.M. (2008a) A real-time polymerase chain reaction assay for the detection of the myxozoan parasite *Henneguya ictaluri* in channel catfish. *Journal of Veterinary Diagnostic Investigation* 20(5), 559–566.
- Griffin, M.J., Wise, D.J., Camus, A.C., Mauel, M.J., Greenway, T.E. and Pote, L.M. (2008b) A novel *Henneguya* sp. from channel catfish (*Ictalurus punctatus*) described by morphological, histological and molecular characterization. *Journal of Aquatic Animal Health* 20(3), 127–135.
- Griffin, M.J., Pote, L.M., Camus, A.C., Mauel, M.J., Greenway, T.E. and Wise, D.J. (2009a) Application of a real-time PCR assay for the detection of *Henneguya ictaluri* in channel catfish ponds. *Diseases of Aquatic Organisms* 86, 223–233.
- Griffin, M.J., Wise, D.J. and Pote, L.M. (2009b) Morphology and small-subunit ribosomal DNA sequence of *Henneguya adiposa* (Myxosporidia) from *Ictalurus punctatus* (Siluriformes). *Journal of Parasitology* 95, 1076–1085.
- Griffin, M.J., Wise, D.J., Camus, A.C., Greenway, T.E., Mauel, M.J. and Pote, L.M. (2010) Variation in susceptibility to *Henneguya ictaluri* infection by two species of catfish and their hybrid cross. *Journal of Aquatic Animal Health* 22, 21–35.
- Hargreaves, J.A. and Tucker, C.S. (2004) Industry development. In: Tucker, C.S. and Hargreaves, J.A. (eds) *Biology and Culture of the Channel Catfish*, 1st edn. Elsevier B.V., Amsterdam, The Netherlands, pp. 1–14.
- Hoffman, R. and El-Matbouli, M. and Fischer-Scherl, T. (1992) A proliferative gill disease (PGD) in rainbow trout (*Oncorhynchus mykiss*). *Bulletin of the European Association of Fish Pathologists* 12(4), 139–141.
- Huey, D.W., Simco, B.A. and Criswell, D.W. (1980) Nitrite-induced methemoglobin formation in channel catfish. *Transactions of the American Fisheries Society* 109, 558–562.
- Kallert, D.M., El-Matbouli, M. and Haas, W. (2005) Polar filament discharge of *Myxobolus cerebralis* actinospores is triggered by combined non-specific mechanical and chemical cues. *Parasitology* 131, 1–8.
- Kent, M.L., Andree, K.B., Bartholomew, J.L., El-Matbouli, M., Desser, S.S., Devlin, R.H., Feist, S.W., Hedrick, R.P., Hoffman, R.W., Khattri, J., Hallett, S.L., Lester, R.J.G., Longshaw, M., Palenzeula, O., Siddall, M.E. and Xiao, C. (2001) Recent advances in our knowledge of the myxozoa. *Journal of Eukaryotic Microbiology* 48(4), 395–413.
- Kudo, R.R. (1929) Histoic myxosporidia found in freshwater fishes in Illinois, USA. *Archives Protistenkd* 65, 364–378.
- Lin, D., Hanson, L.A. and Pote, L.M. (1999) Small subunit ribosomal RNA sequence of *Henneguya exilis* (class Myxosporidia) identifies the actinosporean stage from an oligochaete host. *Journal of Eukaryotic Microbiology* 46, 66–68.
- MacMillan, J.R., Wilson, C. and Thiagarajah, A. (1989) Experimental induction of proliferative gill disease in specific-pathogen-free channel catfish. *Journal of Aquatic Animal Health* 1, 245–254.
- Marcer, F., Quaglio, F., Caffara, M., Ferraresi, M. and Floravanti, M. (2004) Proliferative gill disease in channel catfish farmed in Italy. *Ittopatologia* 1, 120–126.
- Minchew, C.D. (1977) Five new species of *Henneguya* (Protozoa: Myxosporidia) from ictalurid fishes. *Journal of Protozoology* 24, 213–220.
- Mischke, C.C., Terhune, J.S. and Wise, D.J. (2001) Acute toxicity of several chemicals to the oligochaete *Dero digitata*. *Journal of the World Aquaculture Society* 32, 184–188.
- Mitchell, A.J., Durborow, R.M. and Crosby, M.D. (1998) *Proliferative Gill Disease*. Southeastern Regional Aquaculture Center (SRAC) publication 475. United States Department of Agriculture Cooperative State Research, Education, and Extension Service, Stoneville, Mississippi, USA.

- Molnár, K. (2002) Site preference of fish myxosporeans in the gill. *Diseases of Aquatic Organisms* 48, 197–207.
- Pote, L.M. and Waterstrat, P. (1993) Motile stage of *Aurantiactinomyxon* sp. (actinosporea: actinomyxina: triactinomyxidae) isolated from *Dero digitata* found in channel catfish ponds. *Journal of Aquatic Animal Health* 5, 213–218.
- Pote, L.M., Bellerud, B.L., Lin, D.L. and Chenney, E.F. (1994) The isolation and propagation of *Dero digitata* infected with *Aurantiactinomyxon* sp. *Journal of World Aquaculture Society* 25, 303–307.
- Pote, L.M., Hanson, L.A. and Shivaji, R. (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.
- Pote, L.M., Hanson, L.A. and Khoo, L. (2003) Proliferative gill disease. In: *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens, Blue Book*, 2010 edn. Fish Health Section, American Fisheries Society, Bethesda, Maryland, USA.
- Powell, M.D., Speare, D.J. and Becker, J.A. (2006) Whole body net ion fluxes, plasma electrolyte concentrations and haematology during a *Loma salmonae* infection of juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 29, 727–735.
- Sabri, D.M., Danasoury, M.A., Eissa, I.A.M. and Khouraiiba, H.M. (2009) Alterations in serum protein fractions and Na⁺K⁺ATPase activity in *Clarias gariepinus* infested with henneguyosis in Ismailia, Egypt. *African Journal of Aquatic Science* 34(1), 103–107.
- Soster, F.M. and McCall, P.L. (1990) Benthos response to disturbance in Western Lake Erie: field experiments. *Canadian Journal of Aquatic Animal Science* 47(10), 1970–1985.
- Speare, D.J. and Ferguson, H.W. (2006) Gills and pseudobranchs. In: Ferguson H.W. (ed.) *Systemic Pathology of Fish*. Scotian Press, London, UK, pp. 24–63.
- Styer, E.L., Harrison, L.R. and Burtle, G.J. (1991) Experimental production of proliferative gill disease in channel catfish exposed to myxozoan-infected oligochaete, *Dero digitata*. *Journal of Aquatic Animal Health* 3, 288–291.
- Thiyagarajah, A. (1993) Proliferative gill disease from the Tennessee-Tombigbee Waterway. *Journal of Aquatic Animal Health* 5, 219–222.
- United States Department of Agriculture (USDA) (1997) *Catfish Epidemiology and Animal Health*. USDA Animal and Plant Health Inspection Service, Fort Collins, Colorado, USA.
- United States Department of Agriculture (USDA) National Agriculture Statistics Service (NASS) (2011) Catfish Production: USDA Counts. ISSN: 1948-271X. Available at: <http://USDA.mannlib.cornell.edu/USDA/current/catfProd> (accessed 24 June 2011).
- Wagner, E.J. (2002) Whirling disease, prevention, control, and management: a review. *American Fisheries Society Symposium* 29, 217–225.
- Wax, C.L., Pote, J.W. and Deliman, N.C. (1987) A climatology of pond temperatures for aquaculture in Mississippi. *Mississippi Agricultural and Forestry Experiment Station Bulletin* 149. Mississippi State University, Stoneville, Mississippi State, USA.
- Whitaker, J.W., Pote, L.M., Khoo, L., Shivaji, R. and Hanson, L.A. (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., Pote, L.M. and Hanson, L.A. (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.
- Wise, D.J., Camus, A., Schwedler, T. and Terhune, J. (2004) Health management. In: Tucker, C.S. and Hargreaves, J.A. (eds) *Biology and Culture of the Channel Catfish*, 1st edn. Elsevier B.V., Amsterdam, The Netherlands, pp. 482–488.
- Wise, D.J., Griffin, M.J., Terhune, J.S., Pote, L.M. and Khoo, L. (2008) Induction and evaluation of proliferative gill disease in channel catfish fingerlings. *Journal of Aquatic Animal Health* 20(4), 236–244.
- Wolf, K. and Markiw, M.E. (1984) Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. *Science* 255, 1449–1452.

11 *Gyrodactylus salaris* and *Gyrodactylus derjavinoïdes*

Kurt Buchmann

Laboratory of Aquatic Pathobiology, University of Copenhagen, Copenhagen, Denmark

11.1. Introduction

Monogenean flatworms of the genus *Gyrodactylus* occur on a wide array of fishes, possess a high degree of host-specificity and it has been estimated the number of species may exceed 20,000 (Bakke *et al.*, 2002) with a few of these parasites infecting salmonids worldwide (Malmberg, 1993). In Europe the Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) are hosts to several important species of which *Gyrodactylus salaris* and *Gyrodactylus derjavinoïdes* are considered the most pathogenic. The biological characteristics of *G. salaris* and *G. derjavinoïdes*, which are both freshwater parasites, have been studied in detail and these species will be discussed here.

G. salaris Malmberg, 1957 (Fig. 11.1) was first described from Baltic salmon sampled at a freshwater hatchery in the Hölle Laboratory where the infection had caused problems in the early 1950s (Malmberg, 2004). The parasite probably originated in rivers draining into the Baltic Sea which is populated by a Baltic strain of the Atlantic salmon. This fish stock comprises numerous subpopulations homing to rivers in Sweden, Finland, Russia, Latvia, Lithuania, Estonia, Poland and Germany draining into the Baltic Sea (Nilsson *et al.*, 2001). The stock has been isolated from

other races of Atlantic salmon for thousands of years following the end of the last glacial period. Norwegian salmon populating rivers draining into the Atlantic had probably always been free of *G. salaris* but anthropogenic transfer of infected salmon from Sweden into Norway occurred in the 1970s on several occasions. This was based on a high demand for salmon for stocking and experimental purposes (Johnsen and Jensen, 1991; Malmberg, 1993; Mo 1994; Bakke *et al.*, 2007). The parasite was new to Norwegian stocks of wild salmon but these fish showed up to be extremely susceptible to the worm which subsequently spread to at least 46 rivers in Norway resulting in severe ecological and economical problems. It is commonly called the 'the Norwegian salmon killer' (Malmberg, 1993; Bakke *et al.*, 2007). Economic losses are related to: (i) diminished fish stocks; (ii) loss of angler tourism; and (iii) parasitological surveys and control measures that have been estimated to cost billions of Euros in the last 30 years. East Atlantic salmon (Scottish, Danish) are also very susceptible to the parasite and show no effective host response whereas Baltic strains activate a clear protective response following a few weeks after infection (Bakke *et al.*, 1990; Bakke and MacKenzie, 1993; Dalgaard *et al.*, 2003; Lindenstrøm *et al.*, 2006; Heinecke *et al.*, 2007; Kania *et al.*, 2007a, 2010). Several different fish species including

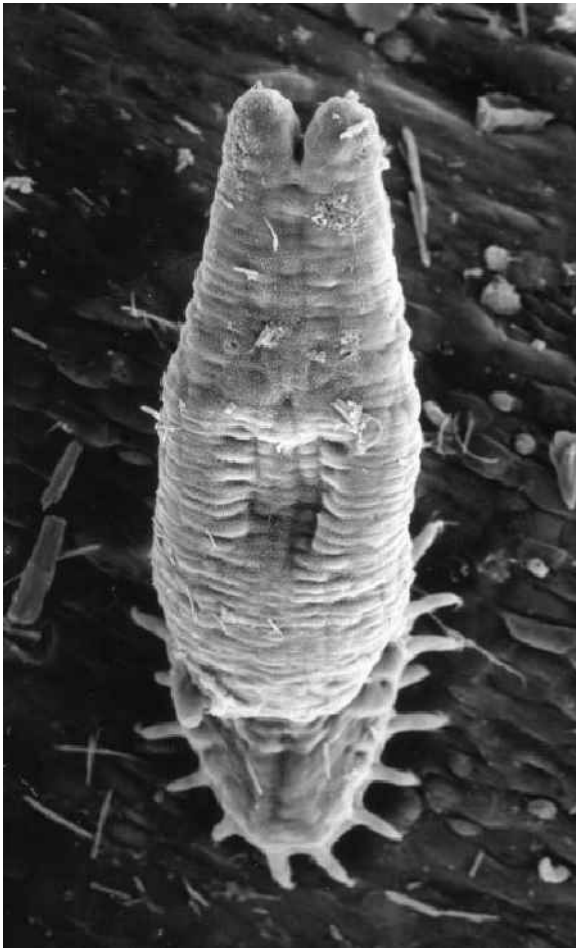


Fig. 11.1. *Gyrodactylus salaris*, *in toto*, on the fin of a Scottish river Conon salmon, dorsal view (scanning electron microscopy (SEM) by K. Buchmann and J. Bresciani).

brown trout, stickleback (*Gasterosteus aculeatus*) and flounder (*Platichthys flesus*) have transient infections (Bakke *et al.*, 2007) whereas others such as Arctic charr (*Salvelinus alpinus*) allow significant persistence and propagation of the parasite (Winger *et al.*, 2008). Recent studies have shown that some strains are pathogenic while others are non-pathogenic to East Atlantic salmon (Lindstrøm *et al.*, 2003b; Jørgensen *et al.*, 2007; Robertsen *et al.*, 2007).

The parasite *G. derjavinoides* (Fig. 11.2) has been studied extensively (under the name *G. derjavini*) in European trout populations (Malmberg and Malmberg, 1993; Mo, 1993, 1997; Buchmann *et al.*, 2000). *G. derjavini* was originally recovered from the Caspian trout

(*Salmo trutta caspius*) and described by Mikailov (1975). However, recent studies of *Gyrodactylus* from Iranian trout (*S. trutta caspius*) have suggested that the European parasite (up until then referred to as *G. derjavini*) differs from the parasite originally described by Mikailov (1975). Consequently the parasite in European trout was renamed *G. derjavinoides* by Malmberg *et al.* (2007). This parasite has probably been endemic in Eurasian populations of brown trout (*S. trutta*) and rarely causes epidemics although heavy parasite burdens in wild fish have been noted (Ergens, 1983; Buchmann *et al.*, 2000). However, the introduced domesticated *O. mykiss* is a susceptible and vulnerable host to this parasite (Buchmann and Bresciani, 1997; Buchmann



Fig. 11.2. *Gyrodactylus derjavinoidea*, in toto, on the fin of a rainbow trout, dorso-lateral view (SEM by K. Buchmann and J. Bresciani).

and Uldal, 1997). Atlantic salmon may host the parasite but rarely allow a significant parasite population increase (Mo, 1993, 1997; Buchmann and Uldal, 1997; Olafsdottir *et al.*, 2003; Jørgensen *et al.*, 2008, 2009).

11.2. Description of the Parasites

Both species are ectoparasitic flatworms with a length of less than 1 mm and a body width of around 0.1 mm. However, the soft body parts of gyrodactylids are affected by compression during slide preparation and their dimensions should not be used for diagnosis. In contrast, hard parts are of taxonomic importance. The ventrally directed opisthaptor is equipped with two large hamuli and 16 marginal hooklets (Figs. 11.3 and 11.4). Shapes and dimensions of these sclerotized parts are used for diagnosis (see Malmberg, 1993; Mo, 1991, 1993). The dimensions of the hard structures are negatively correlated to temperature; consequently if parasites are propagated at low temperatures the anchors increase in size (Mo, 1991, 1993). The anterior part of the body is equipped with cephalic glands and ducts producing secretions. These structures and secretions are for attachment; for example

during migration of the worm from one host to the other or to objects in the aquatic environment (stones, gravel). Using light microscopy the intestinal caeca, pharynx and the cirrus are clearly visible. The most prominent character is the uterus in which the embryo develops. The parasite is viviparous and gives birth to a live young about the same size as the mother, and which may already have its own embryo. This spectacular organization with three generations in one parasite specimen has inspired parasitologists to call the system a Russian doll arrangement (Bakke *et al.*, 2007). Sequencing of genes encoding ribosomal DNA (the internal transcribed spacer (ITS) and intergenic spacer (IGS) regions) and mitochondrial enzymes have proved to be excellent tools for species and strain discrimination (Cunningham *et al.*, 1995, 2003; Cunningham, 1997; Meinilä *et al.*, 2002; Hansen *et al.*, 2003; Huyse *et al.*, 2007, 2008; Collins *et al.*, 2010; Zietara *et al.*, 2010).

11.3. Location on the Host

Both *G. derjavinoidea* and *G. salaris* infect preferentially the fins of the fish but may also be found on the body proper and the head

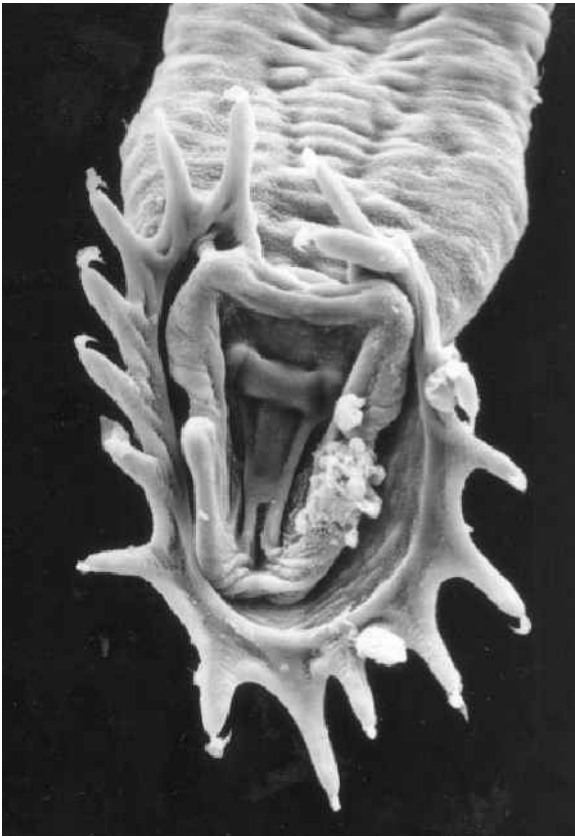


Fig. 11.3. Opisthaptor of *G. salaris*, ventral view showing hamuli and marginal hooklets (SEM by K. Buchmann and J. Bresciani).

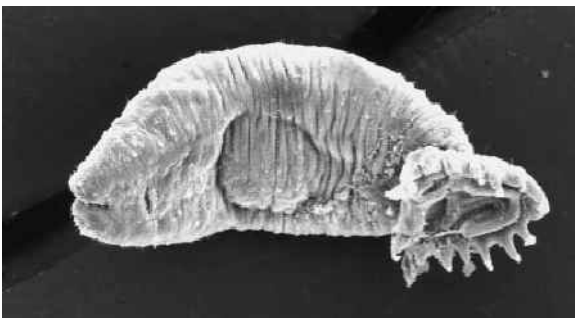


Fig. 11.4. *G. derjavinoiodes*, in toto, latero-ventral view showing ventrally directed hamuli and marginal hooklets (SEM by K. Buchmann and J. Bresciani).

(including nostrils, mouth cavity and cornea). The gills are only rarely infected. However, the location on the host is influenced by: (i) the host strain; (ii) the parasite load; and (iii) the duration of the infection. During its initial colonization *G. salaris* selects preferentially fins and especially the pectoral fins (Fig. 11.5)

of East Atlantic salmon. During an 8-week infection course the relative occurrence on pectoral fins decreases whereas a larger part of the parasite population can be found on the caudal fin. In contrast, the parasites will be found at higher frequency at the caudal fin of Baltic salmon at the initial stage of infection



Fig. 11.5. *G. salaris* colonizing a salmon fin (SEM by K. Buchmann and J. Bresciani).

(Heinecke *et al.*, 2007). Heavily parasitized salmon will harbour parasites all over the body, including skin, fins and corneal surfaces. Moderately infected wild Norwegian salmon harbour the main part of the parasite population on the dorsal, anal and pectoral fins. Heavily infected salmon have parasites at all body sites (Jensen and Johnsen, 1992).

The *G. derjavinoides* population is on the pectoral and pelvic fins on rainbow and brown trout shortly after the initial colonization. During a 6-week infection course the host mounts a response and the relative distribution on the fins and the body changes. The initial colonization sites become partly abandoned whereas previously less colonized sites such as the corneal surface and the tail fin become increasingly populated (Buchmann and Uldal, 1997; Buchmann and Bresciani, 1998). East Atlantic salmon and Baltic salmon obtain merely a weak infection by

G. derjavinoides and then the parasites are found mostly on the tail fin, the pectoral, the pelvic, the anal fins and corneal surface of the host (Buchmann and Uldal, 1997).

11.4. Transmission

Both parasite species are able to spread from one host to another by direct contact between hosts or between hosts and detached parasites occurring on the substrate (stones or gravel or fish-tank walls) or floating in the water column. Both parasite species can detach from the host and move (using leech-like movements) in the fish tank (bottom, walls) or on stones, gravel, plant materials or alternative hosts in their natural aquatic habitat (river, lake). Also direct parasite transmission from dead hosts to live hosts is considered significant (Olstad *et al.*, 2006). The lifespan at

the detached stage is temperature dependent but may for *G. derjavinoidea* last for up to 5 days (Buchmann and Bresciani, 1999) and at least 25 h and probably several days for *G. salaris* (Larsen and Buchmann, 2006). Spreading of the parasite between different rivers and water bodies is mostly observed in connection with translocation of fish (transport and restocking). Fishing tackle which has not been disinfected may allow live parasites to be translocated from one river to another with anglers moving between fishing sites. *G. salaris* is not able to survive in high salinity sea water but may persist for 240 h in 10 ppt and for 42 h in 20 ppt (Soleng and Bakke, 1997). Therefore, migration of infected salmon from infected rivers through low-salinity fjords may explain some cases of parasite introduction to previously uninfected rivers (Soleng and Bakke, 1997). *G. derjavinoidea* may survive in water with 7 ppt sodium chloride and will also be able to spread between rivers in low salinity waters but may not survive on trout migrating in high salinity marine waters (Buchmann, 1997). A series of alternative hosts, especially Arctic charr, may represent an important reservoir for parasites which can infect salmon in previously uninfected sites or following chemical treatment of rivers (Winger *et al.*, 2008).

11.5. Geographical Distribution

The pathogenic form of *G. salaris* is in Norway where a series of subpopulations have been recognized (Hansen *et al.*, 2003). Probably the *G. salaris* in Sweden (Malmberg, 1993; Malmberg and Malmberg, 1993), Finland (Rintamäki-Kinnunen and Valtonen, 1996), Russia (Cunningham *et al.*, 2003; Zietara *et al.*, 2008), Latvia (Hansen *et al.*, 2003) and Poland (Rokicka *et al.*, 2007) may be virulent to East Atlantic salmon. However, this has not yet been confirmed. In Denmark a confirmed non-pathogenic form of *G. salaris* has been isolated from rainbow trout in farms. It shows a predilection towards rainbow trout whereas Atlantic salmon (both East Atlantic and Baltic strains) are not able to sustain the parasite population (Jørgensen *et al.*, 2007). A similar

G. salaris has been reported from Germany (Cunningham *et al.*, 2003) and Italy (Paladini *et al.*, 2009) as well. However, experimental studies on host preference and pathogenicity towards various salmonid species and strains of salmon have not yet been performed for German and Italian isolates. Due to the documented history of frequent rainbow trout transportations from Denmark to Germany and Italy it is likely that at least some of the *G. salaris* from these countries belong to the same non-pathogenic type as reported by Lindenstrøm *et al.* (2003b) and Jørgensen *et al.* (2007). Thus, the *G. salaris* form pathogenic to East Atlantic salmon does not exist in Denmark (Jørgensen *et al.*, 2008) and it can be questioned if the *G. salaris* forms in Italy and Germany are pathogenic to salmon.

11.6. Impact of the Disease on Fish Production

Susceptible Atlantic salmon can have extremely heavy *G. salaris* infections. Several thousand parasites per fish can be on Norwegian, Scottish and Danish salmon if fish are not treated. Farmed and wild fish start dying within 3–5 weeks of infection. At the population level, the impact of infection in wild fish becomes visible after 1–2 years when the fish density has decreased. *G. derjavinoidea* infections elicit morbidity and mortality of rainbow trout and brown trout (Ergens, 1983; Buchmann and Uldal, 1997) and call for frequent use of auxiliary substances in trout farms (Malmberg, 1993; Buchmann and Bresciani, 1997). However, the impact is highly dependent on the intensity of infection. Thus, young fry of brown trout may suffer seriously (30% mortality) already at a parasite load of five to ten parasites per trout. Further, the infection may predispose fish to subsequent bacterial pathogens such as *Flavobacterium psychrophilum* (Busch *et al.*, 2003).

11.7. Diagnosis

The original diagnostic technique is based on morphometric analysis of the opisthaptor hard parts including anchors and marginal

hooklets (Mo, 1991, 1993; Malmberg, 1993; Shinn *et al.*, 1995). Also the location and arrangement of protonephridia can be used for diagnostic purposes (Malmberg, 1970) and the distribution of argentophilic structures on the parasite surface may aid differentiation of species (Shinn *et al.*, 1998). The morphometric method is valuable but the existence of parasite variants possessing different virulence has made it necessary to supplement with alternative methods. Cunningham *et al.* (1995) showed that small subunit (18S) ribosomal RNA (rRNA) gene sequences could be used to differentiate some species (*G. salaris*, *G. derjavinoidea* and *Gyrodactylus truttae*). *In situ* hybridization methods using specific probes binding to immobilized parasite DNA on various membrane types were applied with some success (Cunningham *et al.*, 1995). The ITS gene spanning from 18S over ITS1, 5.8S, ITS2 to 28S was different between *G. salaris*, *G. derjavinoidea* and *G. truttae* and several restriction fragment length polymorphism (RFLP) methods could be used for fast identification (Cunningham, 1997). This method was later shown valid for a congeneric species *Gyrodactylus teuchis* as well (Cunningham *et al.*, 2001). Direct sequencing of DNA following PCR and subsequent comparison is time consuming and therefore RFLP based on PCR, restriction enzyme incubation and finally agarose gel electrophoresis in ethidium bromide is used. Kania *et al.* (2007b) showed that non-pathogenic *G. salaris* could be differentiated from the pathogenic form using a RFLP technique based on a single base substitution in the ITS region. Using a TaqMan probe real-time multiplex PCR assay Collins *et al.* (2010) could reduce the time needed for a valid diagnosis based on ITS sequences even further. The rRNA genes are tandemly repeated and separated by gene regions called the IGS. The sequence variability within the IGS regions is very high and can not easily be used for species identification (Cunningham *et al.*, 2003). Mitochondrial gene sequences encoding various enzymes within both *G. salaris* and *G. derjavinoidea* can be used not only for strain identification but also for species identification (Meinilä *et al.*, 2002; Hansen *et al.*, 2003; Huyse *et al.*, 2007, 2008;

Zietara *et al.*, 2010). Different diagnostic techniques were evaluated by Shinn *et al.* (2010) in a multi-centre test which concluded that combining morphometric and molecular methods provide the most accurate diagnosis.

11.8. Clinical Signs

Heavy mortalities may be the first obvious sign of a *G. salaris* epidemic. Behavioural changes of infected fish may be restricted to lethargy, anorexia and seeking sheltered habitats. External macroscopic lesions and change of appearance may be darkening of the skin and emaciated fins (Fig. 11.5). The microscopic lesions responsible for the disease are visible using scanning electron microscopy (SEM). The worm attaches to the fin or skin surface of the host by inserting 16 marginal hooklets into the epidermis. This action is clearly associated with injuries to the epithelial cells (Fig. 11.6). Also feeding activities of the worm impose epithelium damage. The stimulation of the epidermis elicits an inflammatory reaction which can be seen as a slightly elevated epithelial surface around the parasite's attachment site and feeding area (Fig. 11.7). In heavy infections the injuries are directly correlated to the number of worms in a given surface area and the disturbances from the individual worms may not be discernible in the totally emaciated skin. Also in *G. derjavinoidea* infections the insertion of hooklets (Fig. 11.8) and feeding on epithelium produce openings and lesions (Buchmann and Bresciani, 1997).

11.9. Pathophysiology of the Disease

The extensive emaciation of host epithelia following heavy infections is likely to challenge the osmoregulatory system of the fish. A direct effect of the perforation of epidermal cells may cause problems in coping with the external ion concentrations. The infection may affect the host indirectly as well. Stimulation of trout skin by *G. derjavinoidea* infection elicits a stress response with the release

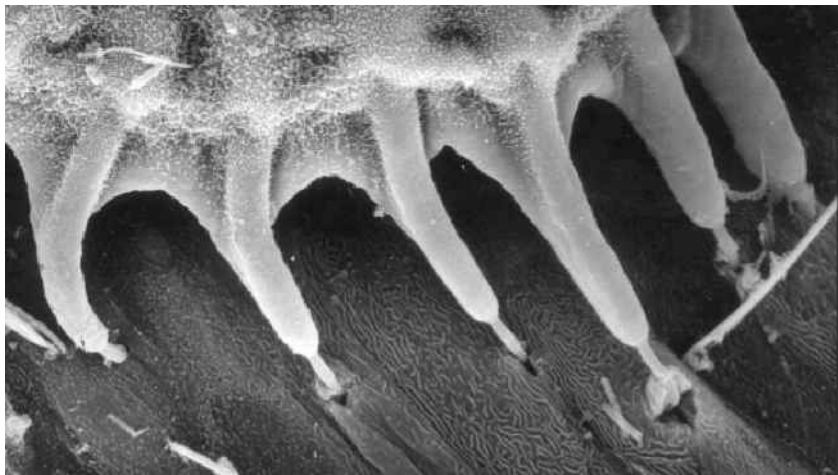


Fig. 11.6. *Gyrodactylus salaris*, marginal hooklets penetrating epithelial cells of the fin of a river Conon salmon (SEM by K. Buchmann and J. Bresciani).

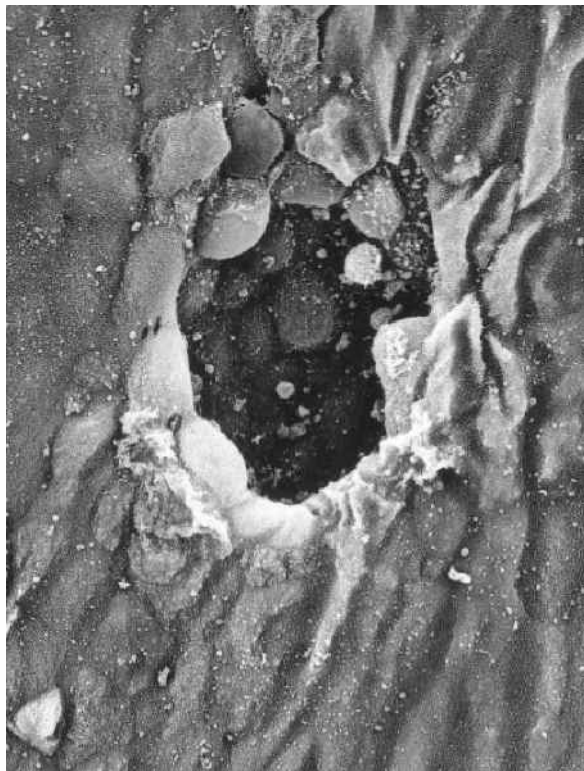


Fig. 11.7. Epithelial damage of salmon fin epidermis following *G. salaris* attachment and feeding. The wound is healing following escape of the worm (SEM by K. Buchmann and J. Bresciani).



Fig. 11.8. *Gyrodactylus derjavinoidea* marginal hooklets (SEM by K. Buchmann and J. Bresciani).

of cortisol in the host. Fish with even low infections have elevated cortisol levels in body fluids (Stoltze and Buchmann, 2001). Due to the immunosuppressive action of cortisol this stress reaction may leave the host more vulnerable to bacterial and fungal pathogens in the environment. Also the physical disturbance of the skin epidermis may give these pathogens an easy access to sub-epidermal compartments in the host and further aggravate the disease.

11.10. Protective/Control Strategies

11.10.1. Immunity

The host immune responses against *G. salaris* and *G. derjavinoidea* reflect intricate interactions between host specificity mechanisms and immunological factors (Buchmann, 1999; Bakke *et al.*, 2002). Host specificity may be influenced by immunological factors but it is possible that other receptor/ligand interactions such as carbohydrate/lectin binding may be involved (Buchmann, 2001; Jørndrup and Buchmann, 2005). However, despite the

presence of a host response against *G. derjavinoidea* (Lindenstrøm and Buchmann, 2000) in rainbow trout and against *G. salaris* in Baltic salmon (Bakke *et al.*, 1990; Dalgaard *et al.*, 2003) no vaccines are available.

G. salaris

The Norwegian, Scottish and Danish strains of the East Atlantic salmon are not able to activate immune factors which confer protection to the host against the Norwegian *G. salaris*. Some subpopulations of the Baltic salmon strain in contrast will with a few exceptions (Bakke *et al.*, 2004) clearly mount a host response following an infection (Bakke *et al.*, 1990; Bakke and MacKenzie, 1993; Dalgaard *et al.*, 2003; Lindenstrøm *et al.*, 2006; Kania *et al.*, 2007a, 2010). The mechanisms behind the response were elucidated by Harris *et al.* (1998), Buchmann *et al.* (2004), Lindenstrøm *et al.* (2006) and Kania *et al.* (2007a, 2010). Complement-like activity in serum and mucus of the host has an adverse effect on *G. salaris in vitro* (Harris *et al.*, 1998). However, this factor from both susceptible and resistant fish did not show differential activity and their involvement *in situ* is still to be

described. The skin mucous cells of salmon are clearly part of the interactions between the host and *G. salaris* (Sterud *et al.*, 1998) and the content of immunologically active substances in these cells may play a role (Buchmann, 1999). Using Western blotting Buchmann *et al.* (2004) demonstrated that plasma antibodies (both from susceptible and resistant salmon exposed for more than 6 weeks) did not bind to *G. salaris* antigens. Gene-expression studies have shown that the susceptible salmon elicits a fast cytokine response (interleukin-1 beta, IL-1 β) and a more extensive epithelial reaction whereas the less susceptible salmon are non-responsive (Lindenstrøm *et al.*, 2006; Kania *et al.*, 2007a, 2010) in the initial phase of the infection. The factor associated with resistance/low susceptibility in the response phase of Baltic salmon is increased expression of: (i) the gene encoding serum amyloid protein A (SAA), which binds to pathogens; and (ii) interferon gamma (IFN- γ), which indicates lymphocyte involvement. Expression of major histocompatibility class II (MHC II) (indicating macrophage activity) and the regulating cytokine IL-10 is also in responding salmon. It was hypothesized that a fast but non-specific epithelial skin reaction may benefit propagation of the parasites due to their feeding preference for epithelial cells and mucus. In contrast, resistant salmon restrict proliferation of epithelial cells and thereby limit nutritional uptake by the parasites which eventually get affected by SAA and other effector molecules and cells in the skin (Kania *et al.*, 2010). This corresponds well with the finding that susceptible salmon exhibit a higher mucous cell density in fins compared to resistant fish and that corticosteroid-treated susceptible salmon has an even higher infection concomitant with an increased mucous cell proliferation (Dalggaard *et al.*, 2003).

G. derjavinoidea

It is well documented that brown trout and rainbow trout develop a temperature-dependent skin response against *G. derjavinoidea* (Buchmann and Uldal, 1997; Buchmann and Bresciani, 1998; Andersen and Buchmann,

1998; Lindenstrøm and Buchmann, 2000). Following infection the parasite propagates on fins and skin but within 4–6 weeks the parasite population decreases markedly. The mechanisms involved in the anti-parasitic response are corticosteroid labile (Lindenstrøm and Buchmann, 1998) which may indicate that the skin factors affecting the parasite adversely are immunological elements. Epidermal mucous cells are affected by *G. derjavinoidea* (Buchmann and Bresciani, 1998; Lindenstrøm and Buchmann, 2000) and a series of mucous factors may affect the worms adversely (Buchmann, 1999). During an infection specific antibodies binding to the parasite are not detected (Buchmann, 1998); this corresponds to the fact that adoptive transfer of serum from immune individuals does not confer protection to naïve fish (Lindenstrøm and Buchmann, 2000). Complement may be involved as judged from immediate killing following exposure to functional complement (Fig. 11.9). At least complement factor C3 has been shown to bind to epitopes of *G. derjavinoidea* at the tegument, the opisthaptor and the cephalic gland ducts (Buchmann, 1998). Also leukocyte products and activity have adverse effects on this parasite (Buchmann and Bresciani, 1999). Molecular studies demonstrated that the reaction is initiated by the production of pro-inflammatory cytokines such IL-1 β and tumour necrosis factor alpha (TNF- α), events which are followed by expression of the gene encoding nitric oxide synthase (iNOS) (Lindenstrøm *et al.*, 2003a, 2004). Atlantic salmon can obtain an infection

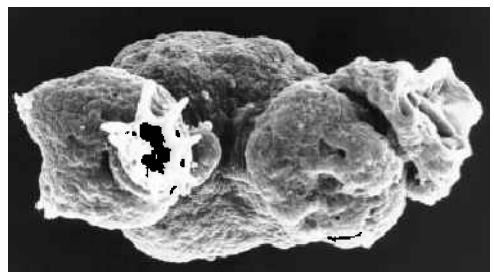


Fig. 11.9. *G. derjavinoidea* after immediate killing due to exposure to complement containing plasma from rainbow trout (SEM by K. Buchmann and J. Bresciani).

with *G. derjavinoidea* but will not experience a build up of infection unless treated with corticosteroids (Olafsdottir *et al.*, 2003). This suggests that host specificity mechanisms, at least partly, are dependent on corticosteroid labile factors, such as immune factors.

11.10.2. Chemotherapy

The treatment strategies are dependent on whether the infection affects wild salmonids in natural rivers and lakes or occur in confined environments (fish tanks). *Gyrodactylus* infections in aquaculture facilities are treated using various anthelmintics and auxiliary substances. Formaldehyde has been applied in traditional fish farming for treatment but the substance must be avoided due to its carcinogenicity, mutagenicity and allergenic properties (Liu *et al.*, 2006; Nielsen and Wolkoff, 2010). Bath treatments (24 h) using mebendazole (1–5 mg/l) and praziquantel (5 mg/l) will eradicate *G. derjavinoidea*. Raising temperature from 11–12 to 18–20°C during exposure augments the efficacy (Lindenstrøm and Buchmann, 1999). Also auxiliary substances such as sodium percarbonate (Buchmann and Kristensson, 2003) and hydrogen peroxide (10 mg/l) eliminate *G. derjavinoidea* from the trout skin (Lindenstrøm and Buchmann, 1999). *G. salaris* may be similarly vulnerable to these treatments which mainly can be applied under fish-farm conditions.

Infections of wild fish in natural habitats pose a special challenge. None the less, chemotherapeutical strategies have been applied against *G. salaris* in wild salmon in Norway. Soleng *et al.* (1999) discovered that low pH and aluminium hydroxide are lethal to this parasite. Based on these observations field trials to eradicate *G. salaris* were performed in a series of Norwegian river systems in which aluminium sulfate was dosed continuously. The infection level in fish fell significantly when using Al in concentrations 100–600 µg/l but success was dependent on temperature and buffer capacity of the water. Many river systems are not suitable for this practice and may need fish fences and rotenone treatment for a satisfactory control of the infection (Poleo *et al.*, 2004).

11.10.3. Zoosanitary measurements and hygiene

Eradication of infections at farm level is most efficiently achieved through stamping out, fallowing, disinfection and drying of ponds and tanks. Subsequently stocking parasite-free fish will secure a healthy fish population. Due to the high pathogenicity of *G. salaris* this approach has been taken with good success in Norwegian fish farms. Norwegian authorities treat entire river systems with the poison rotenone in order to eradicate the infected salmon population whereby the parasite is eliminated as well (Guttvik *et al.*, 2004). Subsequent stocking with non-infected salmon may secure re-population of the river. These non-infected salmon are obtained from disease-free gene-bank hatcheries. This strategy has been used in 28 Norwegian rivers. Sixteen of these have been declared parasite-free after 2 years of monitoring. Additional measures combined with rotenone treatments have been used. Thus, establishing fish fences at river outlets have proved effective in prevention of upward migration of infected fish. At least three rivers have been reinfected following rotenone treatment (Guttvik *et al.*, 2004). As mentioned above, additional trials have involved continuous dosing of aluminium sulfate into rivers. This method could keep the infection level at a satisfactory level but was not able to eradicate the parasite population.

11.10.4. Biotic and abiotic manipulation to interrupt transmission

From a theoretical point of view it would be worth considering the introduction of genes from less susceptible or resistant salmon into salmon with confirmed vulnerability and susceptibility to *G. salaris*. In line with this approach Baltic salmon stocks could be used for stocking in infected areas in order to elevate survival and increase the stock. However, due to conservation issues and a general aim of protecting the original gene pool and diversity of the original Norwegian salmon populations this approach is at present

unacceptable. Selection of parasite-resistant Norwegian salmon following experimental exposure to *G. salaris* and subsequent use of these fish in a breeding programme was suggested by Salte and Bentsen (2004). This approach may be a valid possibility in the future.

11.11. Conclusions and Recommendations

The *G. salaris* story from Norway emphasizes that anthropogenic transfer of fish to new areas with no history of previous occurrence can be catastrophic. The subsequent spread among vulnerable subpopulations has caused serious ecological and economic problems and no solution is at hand. This should serve as a lesson for fish transporters and authorities. At least stringent quarantine precautions should be taken in all cases involving similar transports. *G. derjavinoidea* has not been

shown to elicit similar problems among infected host populations. However, it cannot be excluded that trout populations exist with a similar susceptibility to infection. Accidental infection through similar fish movements and concomitant parasite transfer would prove harmful in such cases. Introduction of genes encoding resistance against parasites would be a theoretical possibility but would also be considered to be genetic interference of a salmon population which conservationists wish to keep intact. Alternatively, establishment of breeding programmes based on Norwegian salmon may be a way forward. Constant dosing of substances into natural waters to kill parasites seems to be problematic from an environmental point of view and the drastic use of rotenone used for killing off infected hosts may be questioned. Alternative measures call for basic research into the physiology of the parasite and especially of host-parasite interactions. Likewise, the use of hyperparasites or predators may be considered viable strategies.

References

- Andersen, P.S. and Buchmann, K. (1998) Temperature dependent population growth of *Gyrodactylus derjavini* on rainbow trout, *Oncorhynchus mykiss*. *Journal of Helminthology* 72, 9–14.
- Bakke, T.A. and MacKenzie, K. (1993) Comparative susceptibility of native Scottish and Norwegian stocks of Atlantic salmon, *Salmo salar*, to *Gyrodactylus salaris* Malmberg: laboratory experiments. *Fisheries Research* 17, 69–85.
- Bakke, T.A., Jansen, P.A. and Hansen, L.P. (1990) Differences in host resistance of Atlantic salmon, *Salmo salar* L., stocks to the monogenean *Gyrodactylus salaris* Malmberg, 1957. *Journal of Fish Biology* 37, 577–587.
- Bakke, T.A., Harris, P.D. and Cable, J. (2002) Host specificity dynamics: observations on gyrodactylid monogeneans. *International Journal for Parasitology* 32, 281–308.
- Bakke, T.A., Harris, P.D., Hansen, H., Cable, J. and Hansen, L.P. (2004) Susceptibility of Baltic and East Atlantic salmon *Salmo salar* stocks to *Gyrodactylus salaris* (Monogenea). *Diseases of Aquatic Organisms* 58, 171–177.
- Bakke, T.A., Cable, J. and Harris, P.D. (2007) The biology of gyrodactylid monogeneans: the 'Russian-doll killers'. *Advances in Parasitology* 64, 161–376.
- Buchmann, K. (1997) Salinity tolerance of *Gyrodactylus derjavini* from rainbow trout *Oncorhynchus mykiss*. *Bulletin of the European Association for Fish Pathologists* 17, 123–125.
- Buchmann, K. (1998) Binding and lethal effect of complement from *Oncorhynchus mykiss* on *Gyrodactylus derjavini* (Platyhelminthes: Monogenea). *Diseases of Aquatic Organisms* 32, 195–200.
- Buchmann, K. (1999) Immune mechanisms in fish skin against monogenean infections – a model. *Folia Parasitologica* 46, 1–9.
- Buchmann, K. (2001) Lectins in fish skin: do they play a role in host–monogenean interactions? *Journal of Helminthology* 75, 227–231.

- Buchmann, K. and Bresciani, J. (1997) Parasitic infections in pond-reared rainbow trout *Oncorhynchus mykiss* in Denmark. *Diseases of Aquatic Organisms* 28, 125–138.
- Buchmann, K. and Bresciani, J. (1998) Microenvironment of *Gyrodactylus derjavini*: association between mucous cell density and microhabitat selection. *Parasitology Research* 84, 17–24.
- Buchmann, K. and Bresciani, J. (1999) Rainbow trout leucocyte activity: influence on the ectoparasitic monogenean *Gyrodactylus derjavini*. *Diseases of Aquatic Organisms* 35, 13–22.
- Buchmann, K. and Kristensson, R.T. (2003) Efficacy of sodium percarbonate and formaldehyde bath treatments against *Gyrodactylus derjavini*. *North American Journal of Aquaculture* 65, 25–27.
- Buchmann, K. and Uldal, A. (1997) *Gyrodactylus derjavini* infections in four salmonids: comparative host susceptibility and site selection of parasites. *Diseases of Aquatic Organisms* 28, 201–209.
- Buchmann, K., Lindenstrøm, T., Nielsen, M.E. and Bresciani, J. (2000) Diagnosis and occurrence of ectoparasite infections (*Gyrodactylus* spp.) in Danish salmonids. *Dansk Veterinærtidsskrift (Danish Veterinary Journal)* 83, 15–19.
- Buchmann, K., Madsen, K.K. and Dalgaard, M.B. (2004) Homing of *Gyrodactylus salaris* and *G. derjavini* (Monogenea) on different hosts and response post-attachment. *Folia Parasitologica* 51, 263–267.
- Busch, S., Dalsgaard, I. and Buchmann, K. (2003) Concomitant exposure of rainbow trout fry to *Gyrodactylus derjavini* and *Flavobacterium psychrophilum*: effects on infection and mortality of host. *Veterinary Parasitology* 117, 117–122.
- Collins, C.M., Kerr, R., McIntosh, R. and Snow, M. (2010) Development of a real-time PCR assay for the identification of *Gyrodactylus* parasites infecting salmonids in northern Europe. *Diseases of Aquatic Organisms* 90, 135–142.
- Cunningham, C.O. (1997) Species variation within the Internal Transcribed Spacer (ITS) region of *Gyrodactylus* (Monogenea: Gyrodactylidae) ribosomal RNA genes. *Journal of Parasitology* 83, 215–219.
- Cunningham, C.O., McGillivray, D.M., MacKenzie, K. and Melvin, W.T. (1995) Discrimination between *Gyrodactylus salaris*, *G. derjavini* and *G. truttae* (Platyhelminthes: Monogenea) using restriction fragment length polymorphisms and an oligonucleotide probe within small subunit ribosomal RNA gene. *Parasitology* 111, 87–94.
- Cunningham, C.O., Mo, T.A., Collins, C.M., Buchmann, K., Thiery, R., Blanc, G. and Lautraite, A. (2001) Redescription of *Gyrodactylus teuchis* Lautraite, Blanc, Thiery, Daniel and Vigneulle, 1999 (Monogenea: Gyrodactylidae), a species identified by ribosomal RNA sequence. *Systematic Parasitology* 48, 141–150.
- Cunningham, C.O., Collins, C.M., Malmberg, G. and Mo, T.A. (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.
- Dalgaard, M.B., Nielsen, C.V. and Buchmann, K. (2003) Comparative susceptibility of two races of *Salmo salar* (Baltic Lule river and Atlantic Conon river strains) to infection with *Gyrodactylus salaris*. *Diseases of Aquatic Organisms* 53, 173–176.
- Ergens, R. (1983) *Gyrodactylus* from Eurasian freshwater salmonidae and thymallidae. *Folia Parasitologica* 30, 15–26.
- Guttvik, K.T., Moen, A. and Skår, K. (2004) Control of the salmon parasite *Gyrodactylus salaris* by the use of the plant-derived poison rotenone. *Norsk Veterinærtidsskrift* 3, 172–174 (in Norwegian).
- Hansen, H., Bachmann, L. and Bakke, T.A. (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.
- Harris, P.D., Soleng, A. and Bakke, T.A. (1998) Killing of *Gyrodactylus salaris* (Platyhelminthes, Monogenea) mediated by host complement. *Parasitology* 117, 137–143.
- Heinecke, R.D., Martinussen, T. and Buchmann, K. (2007) Microhabitat selection of *Gyrodactylus salaris* Malmberg on different salmonids. *Journal of Fish Diseases* 30, 733–743.
- Huysse, T., Plaisance, L., Webster, B.L., Mo, T.A., Bakke, T.A., Bachmann, L. and Littlewood, T. (2007) The mitochondrial genome of *Gyrodactylus salaris* (Platyhelminthes: Monogenea), a pathogen of Atlantic salmon (*Salmo salar*). *Parasitology* 134, 739–747.
- Huysse, T., Buchmann, K. and Littlewood, T. (2008) The mitochondrial genome of *Gyrodactylus derjavinoides* (Platyhelminthes: Monogenea) – a mitogenomic approach for *Gyrodactylus* species and strain identification. *Gene* 417, 27–34.
- Jensen, A.J. and Johnsen, B.O. (1992) Site specificity of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea) on Atlantic salmon (*Salmo salar* L.) in the river Lakselva, Northern Norway. *Canadian Journal of Zoology* 70, 264–267.

- Johnsen, B.O. and Jensen, A.J. (1991) The *Gyrodactylus* story in Norway. *Aquaculture* 98, 289–302.
- Jørgensen, T.R., Larsen, T.B., Jørgensen, L.G., Bresciani, J. and Buchmann, K. (2007) Isolation and characterisation of non-pathogenic form of *Gyrodactylus salaris* from rainbow trout. *Diseases of Aquatic Organisms* 73, 235–244.
- Jørgensen, L.V.G., Heinecke, R.D. Kania, P. and Buchmann, K. (2008) Occurrence of gyrodactylids on wild Atlantic salmon, *Salmo salar* L., in Danish rivers. *Journal of Fish Diseases* 31, 127–134.
- Jørgensen, T.R., Jørgensen, L.G., Heinecke, R.D., Kania, P.W. and Buchmann, K. (2009) Gyrodactylids on Danish salmonids with emphasis on wild Atlantic salmon *Salmo salar*. *Bulletin of the European Association for Fish Pathologists* 29, 123–130.
- Jørndrup, S. and Buchmann, K. (2005) Carbohydrate localization on *Gyrodactylus salaris* Malmberg, 1957 and *G. derjavini* Mikailov, 1975 and corresponding carbohydrate binding capacity of their hosts *Salmo salar* L. and *S. trutta* L. *Journal of Helminthology* 79, 41–46.
- Kania, P.W., Larsen, T.B., Ingerslev, H.C. and Buchmann, K. (2007a) Baltic salmon activates immune relevant genes in fin tissue when responding to *Gyrodactylus salaris* infection. *Diseases of Aquatic Organisms* 76, 81–85.
- Kania, P.W., Jørgensen, T.R. and Buchmann, K. (2007b) Differentiation between a pathogenic and a non-pathogenic form of *Gyrodactylus salaris* using PCR-RFLP. *Journal of Fish Diseases* 30, 123–126.
- Kania, P.W., Evensen, O., Larsen, T.B. and Buchmann, K. (2010) Molecular and immunohistochemical studies on epidermal responses in Atlantic salmon *Salmo salar* L. induced by *Gyrodactylus salaris* Malmberg, 1957. *Journal of Helminthology* 84, 166–172.
- Larsen, T.B. and Buchmann, K. (2006) Host specific *in vitro* colonisation of fish epithelia by gyrodactylids. *Acta Ichthyologica et Piscatoria* 36, 113–118.
- Lindenstrøm, T. and Buchmann, K. (1998) Dexamethasone treatment increases susceptibility of rainbow trout, *Oncorhynchus mykiss* (Walbaum), to infections with *Gyrodactylus derjavini* Mikailov. *Journal of Fish Diseases* 21, 29–38.
- Lindenstrøm, T. and Buchmann, K. (1999) Screening chemotherapeutic compounds against gyrodactylid infections in rainbow trout. Paper presented at the European Association for Fish Pathologist 9th Conference, Diseases of Fish and Shellfish, Rhodes, Greece, 19–24 September.
- Lindenstrøm, T. and Buchmann, K. (2000) Acquired resistance in rainbow trout against *Gyrodactylus derjavini*. *Journal of Helminthology* 74, 155–160.
- Lindenstrøm, T., Buchmann, K. and Secombes, C.J. (2003a) *Gyrodactylus derjavini* infection elicits IL-1 beta expression in rainbow trout skin. *Fish and Shellfish Immunology* 15, 107–115.
- Lindenstrøm, T., Collins, C.M., Bresciani, J., Cunningham, C.O. and Buchmann, K. (2003b) Characterization of a *Gyrodactylus salaris* variant: infection biology, morphology and molecular genetics. *Parasitology* 127, 1–13.
- Lindenstrøm, T., Secombes, C.J. and Buchmann, K. (2004) Expression of immune response genes in rainbow trout skin induced by *Gyrodactylus derjavini* infections. *Veterinary Immunopathology and Immunology* 97, 137–148.
- Lindenstrøm, T., Sigh, J., Dalgaard, M.B. and Buchmann, K. (2006) Skin expression of IL-1beta in East Atlantic salmon, *Salmo salar* L., highly susceptible to *Gyrodactylus salaris* infection is enhanced compared to a low susceptibility Baltic stock. *Journal of Fish Diseases* 29, 123–128.
- Liu, Y.S., Li, C.M., Lu, Z.S., Ding, S.M., Yang, X. and Mo, J.W. (2006) Studies on formation and repair of formaldehyde-damaged DNA by detection of DNA-protein crosslinks and DNA breaks. *Frontiers in Bioscience* 11, 991–997.
- Malmberg, G. (1970) The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Arkiv för Zoologi Serie 2*, 23. Royal Swedish Academy of Science. Stockholm, Sweden.
- Malmberg, G. (1993) Gyrodactylidae and gyrodactylosis of salmonidae. *Bulletin Franchais de Pêche et Pisciculture* 328, 5–46.
- Malmberg, G. (2004) How the ‘salmon killer’ *Gyrodactylus salaris* Malmberg, 1957 was discovered and described in Sweden. Report from the front. In: Buchmann, K. (ed.) *Diagnosis and Control of Fish Diseases*. Research school of Sustainable Control of Fish Diseases in Aquaculture (SCOFDA). Royal Veterinary and Agricultural University, Frederiksberg Bogtrykkeri, Frederiksberg, Denmark, pp. 12–18. Available at: www.dafinet.dk (accessed 15 June 2011).
- Malmberg, G. and Malmberg, M. (1993) Species of *Gyrodactylus* (Platyhelminthes, Monogenea) on salmonids in Sweden. *Fisheries Research* 17, 59–68.

- Malmberg, G., Collins, C.M., Cunningham, C.O. and Jalai, B.J. (2007) *Gyrodactylus derjavinoides* sp. nov. (Monogenea, Platyhelminthes) on *Salmo trutta trutta* L. and *G. derjavini* Mikailov, 1975 on *S. t. caspius* Kessler, two different species of *Gyrodactylus* – combined morphological and molecular investigations. *Acta Parasitologica* 52, 89–103.
- Meinilä, M., Kuusela, J., Zietara, M. and Lumme, J. (2002) Primers for amplifying 820 bp of highly polymorphic mitochondrial COI gene of *Gyrodactylus salaris*. *Hereditas* 137, 72–74.
- Mikailov, T.K. (1975) Fish parasites of the waterbasins of Azerbaijan. *Institute of Zoology, Academy of Sciences, Azerbaijan SSR. ELM, Baku*, 1, 68–69 (in Russian).
- Mo, T.A. (1991) Seasonal variations of opisthaptor hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in laboratory experiments. *Systematic Parasitology* 20, 11–20.
- Mo, T.A. (1993) Seasonal variations of opisthaptor hard parts of *Gyrodactylus derjavini* Mikailov, 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and of Atlantic salmon *Salmo salar* L. parr in the river Sandvikselva, Norway. *Systematic Parasitology* 26, 225–231.
- Mo, T.A. (1994) Status of *Gyrodactylus salaris* problems and research in Norway. In: Pike, A.W. and Lewis, J.W. (eds) *Parasitic Diseases of Fish*. Samara Publishing, Dyfed, Wales, UK, pp. 43–56.
- Mo, T.A. (1997) Seasonal occurrence of *Gyrodactylus derjavini* (Monogenea) on brown trout, *Salmo trutta*, and Atlantic salmon, *S. salar*, in the Sandvikselva river, Norway. *Journal of Parasitology* 83, 1025–1029.
- Nielsen, G.D. and Wolkoff, P. (2010) Cancer effects of formaldehyde: a proposal for an indoor air guideline value. *Archives of Toxicology* 84, 423–446.
- Nilsson, J., Gross, R., Asplund, T., Dove, O., Jansson, H., Kelloniemi, J., Kohlmann, K., Löytynoja, A., Nielsen, E.E., Paaver, T., Primmer, C.R., Titov, S., Vasemagi, A., Veselov, A., Öst, T. and Lumme, J. (2001) Matrilineal phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonisation of the Baltic Sea. *Molecular Ecology* 10, 89–102.
- Olafsdottir, S.H., Lassen, H.P.Ø. and Buchmann, K. (2003) Labile resistance of Atlantic salmon, *Salmo salar* L., to infections with *Gyrodactylus derjavini* Mikailov, 1975: implications for host specificity. *Journal of Fish Diseases* 26, 51–54.
- Olstad, K., Cable, J., Robertsen, G. and Bakke, T.A. (2006) Unpredicted transmission strategy of *Gyrodactylus salaris* (Monogenea: Gyrodactylidae): survival and infectivity of parasites on dead hosts. *Parasitology* 133, 33–41.
- Paladini, G., Gustinelli, A., Fioravante, M.L., Hansen, H. and Shinn, A.P. (2009) The first report of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes, Monogenea) on Italian cultured stocks of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Veterinary Parasitology* 165, 290–297.
- Poleo, A.B.S., Lydersen, E. and Mo, T.A. (2004) Aluminium against the salmon parasite *Gyrodactylus salaris*. *Norsk Veterinærtidsskrift* 3, 176–180 (in Norwegian).
- Rintamäki-Kinnunen, P. and Valtonen, T.E. (1996) Finnish salmon resistant to *Gyrodactylus salaris*: a long-term study at fish farms. *International Journal for Parasitology* 26, 723–732.
- Robertsen, G., Hansen, H., Bachmann, L. and Bakke, T.A. (2007) Arctic charr (*Salvelinus alpinus*) is a suitable host for *Gyrodactylus salaris* (Monogenea, Gyrodactylidae) in Norway. *Parasitology* 134, 1–11.
- Rokicka, M., Lumme, J. and Zietara, M.S. (2007) Identification of *Gyrodactylus* ectoparasites in Polish salmonid farms by PCR-RFLP of the nuclear ITS segment of ribosomal DNA (Monogenea, Gyrodactylidae). *Acta Parasitologica* 52, 185–195.
- Salte, R. and Bentsen, H.B. (2004) Breeding for resistance against *Gyrodactylus salaris*. *Norsk Veterinærtidsskrift* 3, 186–189 (in Norwegian).
- Shinn, A.P., Sommerville, C. and Gibson, D.I. (1995) Distribution and characterization of species of *Gyrodactylus* Nordmann, 1832 (Monogenea) parasitizing salmonids in the UK, and their discrimination from *G. salaris* Malmberg, 1957. *Journal of Natural History* 29, 1383–1402.
- Shinn, A.P., Sommerville, C. and Gibson, D.I. (1998) The application of chaetotaxy in the discrimination of *Gyrodactylus salaris* Malmberg, 1957 (Gyrodactylidae: Monogenea) from species of the genus parasitizing British salmonids. *International Journal of Parasitology* 28, 805–814.
- Shinn, A., Collins, C., Garcia-Vasquez, A., Snow, M., Matejusova, I., Paladini, G., Longshaw, M., Lindstrøm, T., Stone, D.M., Turnbull, J.F., Picon-Camacho, S.M., Rivera, C.V., Duguid, R.A., Mo, T.A., Hansen, H., Olstad, K., Cable, J., Harris, P.D., Kerr, R., Graham, D., Monaghan, S.J., Yoon, G.H., Buchmann, K., Taylor, N.G.H., Bakke, T.A., Raynard, R., Irving, S. and Bron, J. (2010) Multicentre testing and validation of current protocols for the identification of *Gyrodactylus salaris* (Monogenea). *International Journal for Parasitology* 40, 1455–1467.

-
- Soleng, A. and Bakke, T.A. (1997) Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): laboratory studies. *Canadian Journal of Fisheries and Aquatic Sciences* 54, 1837–1845.
- Soleng, A., Poleo, A.B.S., Alstad, N.E.W. and Bakke, T.A. (1999) Aqueous aluminium eliminates *Gyrodactylus salaris* (Platyhelminthes, Monogenea) infections in Atlantic salmon. *Parasitology* 119, 19–25.
- Sterud, E., Harris, P.H. and Bakke, T.A. (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.
- Stoltze, K. and Buchmann, K. (2001) Effect of *Gyrodactylus derjavini* infections on cortisol production in rainbow trout fry. *Journal of Helminthology* 75, 291–294.
- Winger, A.C., Kanck, M., Kristoffersen, R. and Knudsen, R. (2008) Seasonal dynamics and persistence of *Gyrodactylus salaris* in two riverine anadromous Arctic charr populations. *Environmental Biology of Fishes* 83, 117–123.
- Zietara, M.S., Kuusela, J., Veselov, A. and Lumme, J. (2008) Molecular faunistics of accidental infections of *Gyrodactylus* Nordmann, 1832 (Monogenea) parasitic on salmon *Salmo salar* L. and brown trout *Salmo trutta* in NW Russia. *Systematic Parasitology* 69, 123–135.
- Zietara, M.S., Rokicka, M., Stojanovski, S. and Lumme, J. (2010) Introgression of distant mitochondria into the genome of *Gyrodactylus salaris*: nuclear and mitochondrial markers are necessary to identify parasite strains. *Acta Parasitologica* 55, 20–28.

12 *Pseudodactylogyrus anguillae* and *Pseudodactylogyrus bini*

Kurt Buchmann

Laboratory of Aquatic Pathobiology, University of Copenhagen, Copenhagen, Denmark

12.1. Introduction

Wild and farmed eels (genus *Anguilla*) suffer from a series of diseases which include the infections caused by monogenean gill parasites (genus *Pseudodactylogyrus*). The parasites have been recorded in Japan (Kikuchi, 1929) and China (Yin and Sproston, 1948) during the first half of the 20th century but following severe outbreaks of pseudodactylogyrosis in eel farms in the 1970s the disease attracted attention from researchers both in Europe (Molnar, 1983; Lambert *et al.*, 1984; Buchmann *et al.*, 1987a) and in Asia (Ogawa and Egusa, 1976; Egusa, 1979). This was linked to the intensification of pond culture of Japanese and European eel (*Anguilla japonica* and *Anguilla anguilla*) in Japan (Ogawa and Egusa, 1976; Egusa, 1979), China and Taiwan (Chan and Wu, 1984; Chung *et al.*, 1984) and the subsequent development of the water recirculation system in farming of European eel in Europe since the 1980s. *Pseudodactylogyrus* monogeneans are oviparous with a high potential for rapid spread and propagation. The disease is similar to the condition caused by *Dactylogyrus vastator* in common carp (*Cyprinus carpio*) aquaculture (Paperna, 1964) and by *Dactylogyrus lamellatus* in grass carp (*Ctenopharyngodon idella*) farms (Molnar, 1972). *Pseudodactylogyrus* was introduced to European waters due to intercontinental

transfer of live infected eels (Buchmann *et al.*, 1987a; Hayward *et al.*, 2001; Taraschewski, 2006; Kania *et al.*, 2010). However, others had suggested that parasites might have spread with migrations of eel ancestors millions of years ago during continental drift (Cone and Marcogliese, 1995). The European eel is an endangered species (EC, 2007; ICES, 2007) and regardless of their origin these eel parasites cause serious concerns and measures should be taken to control the disease both in farms and, where possible, in wild eels.

12.2. Description of the Parasite

The genus *Pseudodactylogyrus* comprises at least four species, including *Pseudodactylogyrus haze* (Ogawa, 1984) and *Pseudodactylogyrus kamegaiti* (Iwashita *et al.*, 2002), but only *Pseudodactylogyrus bini* and *Pseudodactylogyrus anguillae* are pathogenic to anguillid eels. The former was originally described as *Dactylogyrus bini* by Kikuchi (1929) in Japan and the latter as *Neodactylogyrus anguillae* by Yin and Sproston (1948) in China. The type host was in both cases the Japanese eel (*A. japonica*). The genus *Pseudodactylogyrus* was erected by Gussev (1965) from specimens recovered from Australian eels (*Anguilla reinhardtii*). They are small oviparous monopisthocotylean monogeneans with four eye spots and

have a maximum body length of 1.96 mm (*P. bini*) and 1.66 mm (*P. anguillae*). These are hermaphroditic possessing an ovary, vitellaria (glands producing egg materials), a sclerotized vagina (opening onto the lateral body part), a testis, a prostate gland, a cirrus and an accessory cirrus. The anterior part of the worm is equipped with cephalic openings leading into a series of gland structures producing secretions facilitating attachment during movements (Fig. 12.1). The opisthaptor is located in the posterior part of the body and is equipped with two large ventrally directed hamuli (Fig. 12.2) and 14 marginal hooklets (of larval type) which are used for attachment to the host gills. The parasite uses leech-like movements when translocating on host gills or (when dislodged) on objects in the environment. The oral opening is located at the antero-ventral part of the worm and it feeds on gill epithelia and mucus by grasping the tissue with the mouth and a muscular pharynx (Fig. 12.1). The ingested gill material is digested in the two intestinal caeca which

contain esterases, aminopeptidases and phosphatases (Buchmann *et al.*, 1987b, Buchmann, 1988b). The worm has no anus and undigested material may be regurgitated to the exterior along with enzymes which stimulate the gill epithelium. The excretory system is based on flame cells connected to a system of excretory ducts. The nervous system is composed of a pair of cerebral ganglia connected to ventral and dorsal longitudinal nerves (leading anteriorly and posteriorly) which again are interconnected by transverse commissures (Fig. 12.3). Nervous transmission is based on cholinergic and aminergic elements (Buchmann and Prentø, 1989).

12.3. Location on the Host

Pseudodactylogyus parasites inhabit fish gills (Fig. 12.4). They attach to the host as larvae (oncomiracidia) by using their larval hooklets. Their primary locations are gill filaments or occasionally head/opercula from where



Fig. 12.1. Adult *Pseudodactylogyus bini*. Histological section (3 µm) showing forepart of the worm with cephalic glands and ducts with their openings and a muscular pharynx.



Fig. 12.2. Hamulus tip of *Pseudodactylogyris anguillae* protruding from the covering tegument of the opisthaptor (scanning electron microscopy (SEM) by K. Buchmann and M. Køie). Tip length 25 μ m.

they subsequently migrate to the gill apparatus. Juveniles and adults use their opisthaptors to anchor to the primary gill filaments and their lamellae. The two congeners may be found at all sites in the gill apparatus but studies on smaller eels have shown that these two species have preferred microhabitats. *P. anguillae*, which is more mobile and translocates more easily compared with *P. bini*, selects preferentially the basal and median part of the gill filament on the two posterior gill arches. *P. bini*, in contrast, is more frequently found on the first two gill arches at the median to distal part of the filament (Buchmann, 1989a). Larger eels, possessing a relatively larger gill area, do not show the

same distribution (Buchmann, 1989a; Dzika, 1999; Matejusova *et al.*, 2003; Fang *et al.*, 2008). It has been suggested that congeners, due to a selection force through evolution, select different microhabitats to minimize hybridization (Rohde, 1977). The selective force would be that only intra-specific mating leads to fertile offspring. On the other hand competition could play a role in this segregation. Several competitive mechanisms could be involved. One factor could be based on the fact that *P. bini* elicits a severe hyperplasia and epithelial reaction around its attachment site but remains attached despite the host reaction. In contrast, *P. anguillae* may be less able to cope with the distorted gill tissue and escapes from

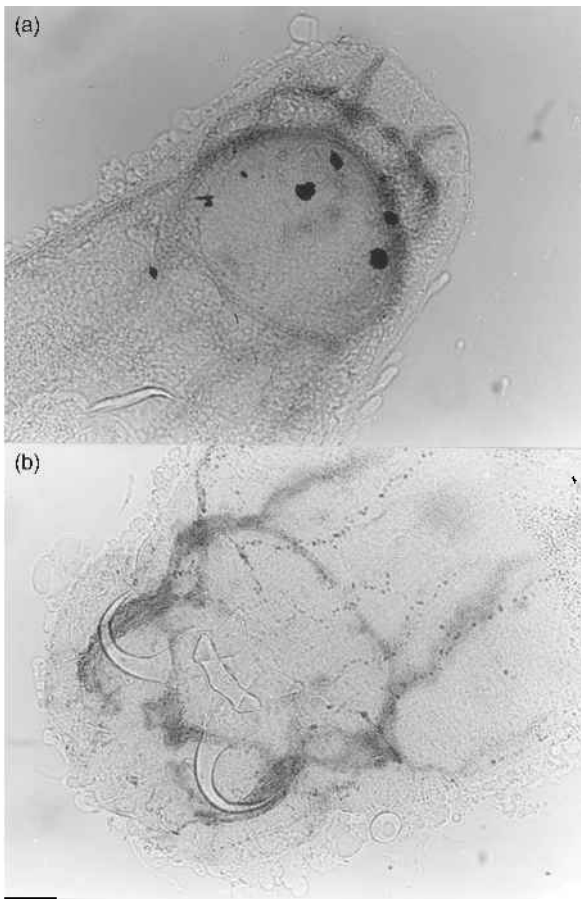


Fig. 12.3. Nervous system of *P. bini* visualized by acetyl-cholinesterase staining. (a) Front part of worm with four eye spots located over the cerebral ganglia and nerve trunks leading anteriorly and posteriorly. (b) Hind part of worm with opisthaptor possessing hamuli and nerve trunks. Length of entire worm 1.5 mm.

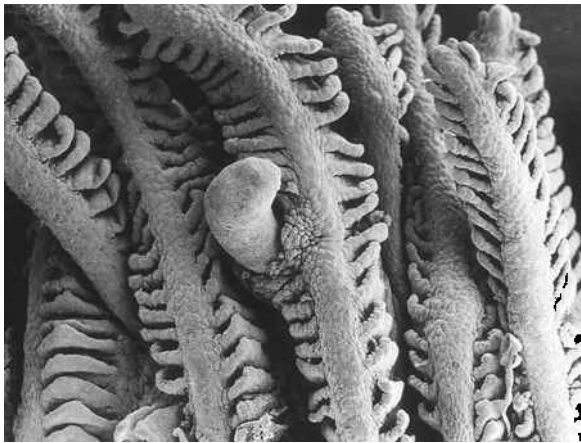


Fig. 12.4. *Pseudodactylogyrus bini* attached to the median part of a primary gill filament of *Anguilla anguilla*. SEM by K. Buchmann and M. Koie. The adult worm 1.5 mm is partly embedded in gill tissue.

sites with inflammatory reactions in the gill apparatus (Buchmann, 1988c). A similar phenomenon, called competitive exclusion, between *Dactylogyrus* congeners on the gills of carp was described by Paperna (1964).

12.4. Transmission

Its life cycle (Fig. 12.5) comprises the adult oviparous worm on the gills, the undeveloped egg released (Fig. 12.6), the oncomiracidium developing inside the egg shell (Fig. 12.7), and the post-larva (Fig. 12.8). Following copulation fertilized eggs are released to the aquatic environment. The oviposition rate is highly temperature dependent. *P. bini* produces no eggs at temperatures below 10°C. At 15°C the parasite produces two or three eggs/day, at 20°C five eggs, at 25°C 12–13 eggs and at 30°C 17 eggs/day. At higher temperatures

(e.g. 32 and 34°C) the egg release rate decreases markedly (Buchmann, 1988d). A similar pattern has been observed for *P. anguillae* although the egg production is lower at all temperatures (Buchmann, 1990b). The time from oviposition to hatching is also highly temperature dependent for both species. Fifty percent of *P. bini* eggs will hatch after 3 days at 30°C, after 4 days at 25°C, after 6 days at 20°C and at 15°C it takes longer than 11 days (Buchmann, 1988d). *P. anguillae* seems to cope better at lower temperatures. Hatching occurs following 3 days at 30°C, after 3.5 days at 25°C, after 4 days at 20°C, after 18 days at 15°C and after 46 days at 10°C (Buchmann, 1990b).

The oncomiracidium escaping the egg shell is ciliated and equipped with four eye spots, 14 marginal hooklets and two undeveloped hamuli. The oncomiracidium moves in elegant spirals before attaching to the host. This free-living stage is relatively short lived

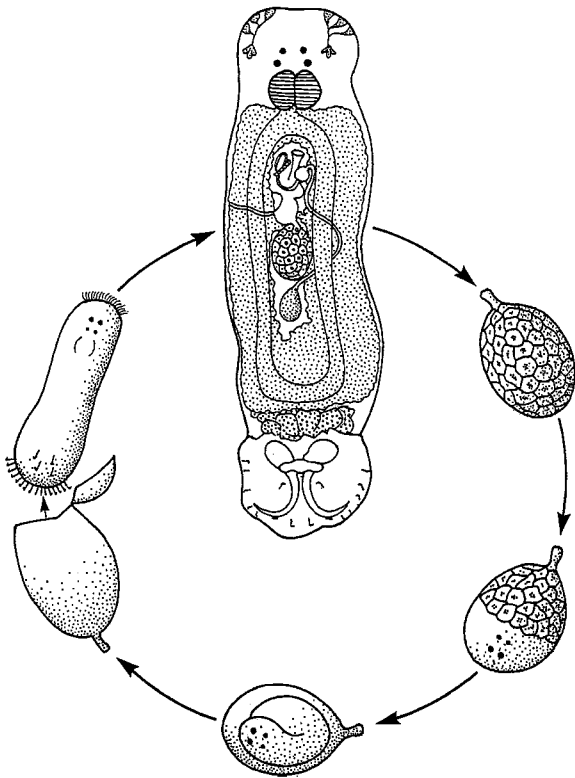


Fig. 12.5. Life cycle of *Pseudodactylogyrus* parasites. The drawing is showing a mature hermaphroditic and oviparous worm delivering an egg which embryonates and hatches whereby a free-swimming ciliated larva (oncomiracidium) is liberated. This larval stage attaches to the host gill, sheds its ciliated cells and develops through the post-larval stage to the adult egg-producing parasite.

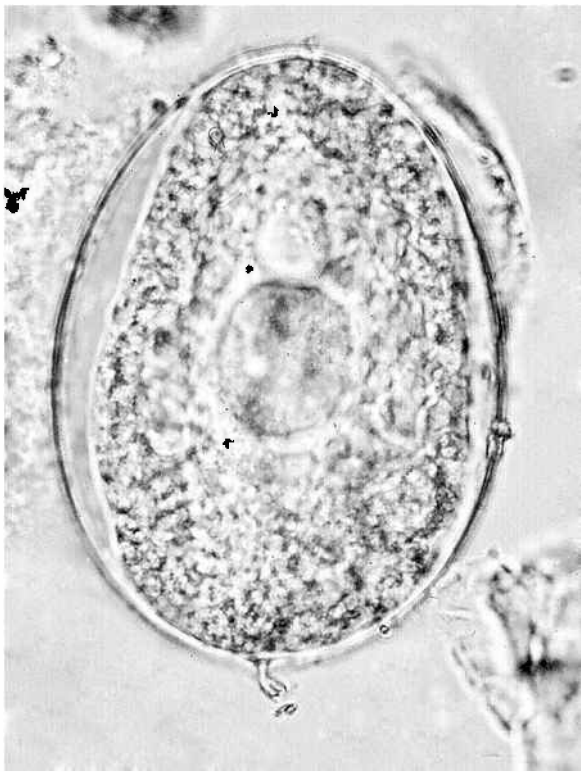


Fig. 12.6. Newly produced and undeveloped egg of *P. anguillae*. Length of egg 52 μm .

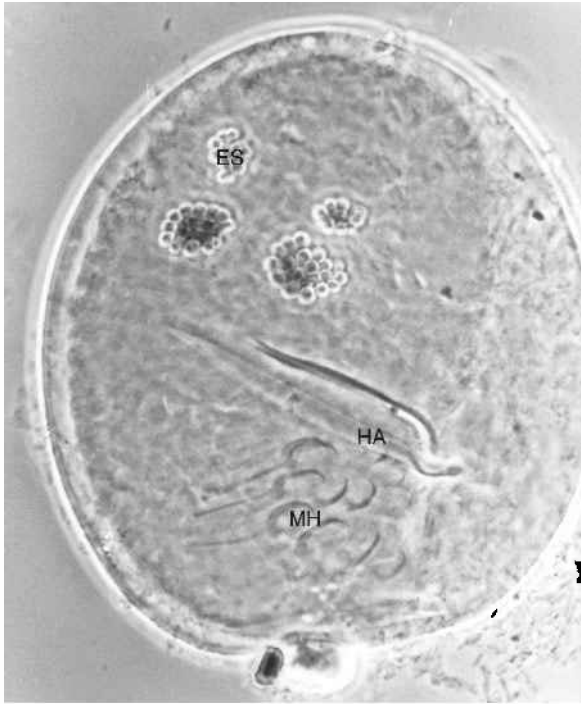


Fig. 12.7. Fully embryonated egg containing an oncomiracidium of *P. anguillae*. The eye spots (ES), hamuli anlagen (HA) and marginal hooklets (MH) are visible in the larva inside the egg shell.

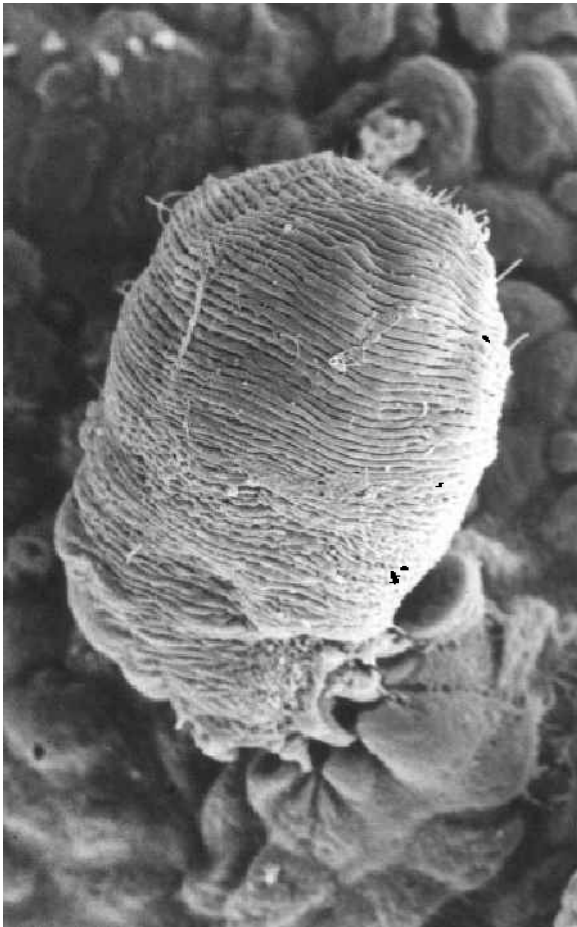


Fig. 12.8. Post-larva of *P. anguillae* attached to a gill filament of *A. anguilla*. SEM by K. Buchmann and M. Køie. Length of post-larva 200 μm .

(up to 6 h) (Golovin, 1977; Imada and Muroga, 1978).

Following attachment to the host the oncomiracidium sheds its ciliated cells and starts moving in a leech-like manner to its preferred microhabitat. The time to reach the adult stage is also highly dependent on temperature. At 25–30°C *P. anguillae* produces its first eggs 6–7 days post-infection (Imada and Muroga, 1978; Buchmann, 1990b). This maturation period is 8–9 days for *P. bini* (Buchmann, 1988d).

The lifespan of *P. anguillae* is more than 210 days at 10°C. This is considerably shortened at 20°C (62 days), at 25°C (47 days), at 30°C (30 days) and at 34°C (14 days). The generation time (time from egg deposition to the adult reproducing worm stage) is around 10

days for *P. anguillae* at 25–30°C and around 11–12 days for *P. bini* (Buchmann, 1988d, 1990b).

12.5. Geographical Distribution

The parasites have been recorded in Japan (Kikuchi, 1929; Ogawa and Egusa, 1976; Fang *et al.*, 2008), China (Yin and Sproston, 1948; Chan and Wu, 1984), Taiwan (Chung *et al.*, 1984), Indian Ocean (Sasal *et al.*, 2008), Russia (Golovin, 1977), Australia (Gusse, 1965; Hayward *et al.*, 2001), Europe (Molnar, 1983; Lambert *et al.*, 1984; Buchmann *et al.*, 1987a; Køie, 1988; Nie and Kennedy, 1991; Dzika *et al.*, 1995; Saraiva, 1995; Gelnar *et al.*, 1996;

Skorikova *et al.*, 1996; Sures *et al.*, 1999; Matejusova *et al.*, 2003; Aguilar *et al.*, 2005; Kania *et al.*, 2010), Africa (Christison and Baker, 2006), North America (Hayward *et al.*, 2001) and Canada (Cone and Marcogliese, 1995; Kania *et al.*, 2010). Due to the initial isolation of the parasites in Japan (Kikuchi, 1929) and China (Yin and Sproston, 1948) it has generally been accepted that their original distribution was the Pacific region. Hence it has been considered an introduced species in Europe, Africa and North America. This is further supported by the finding that the European eel is significantly more susceptible to the parasites when compared with the Japanese eel (Fang *et al.*, 2008). This view has been challenged by Cone and Marcogliese (1995) who suggested the parasites have been associated both with the American eel (*Anguilla rostrata*) and the European eel (*A. anguilla*) since its original ancient spread from the Pacific.

12.6. Disease Impact on Wild and Farmed Fish

Commercial production of European eel (*A. anguilla*) has been severely hampered due to pseudodactylogyrosis both in Europe and in Japan (Ogawa and Egusa, 1976; Egusa, 1979). Also farming of Japanese eels is affected (Chan and Wu, 1984; Chung *et al.*, 1984) but generally Japanese eels are less susceptible to the parasite (Fang *et al.*, 2008). In European eel decrease of feed intake and lethargy are some of the first indications of infection. If the parasitosis is left untreated marked disease signs and high mortality (up to 90%) may subsequently occur. Following introduction of the water recirculation system of eel farming in Europe around 1980 it soon became evident that pseudodactylogyrosis was associated with high mortality and that strict monitoring of the parasite occurrence and regular control measures in farms should be implemented to secure a stable production (Buchmann *et al.*, 1987a). The impact on wild eels has not been elucidated; however, significant infections of wild European eels have been recorded in some freshwater lakes (Køie, 1988; Nie and Kennedy, 1991; Dzika *et al.*, 1995; Saraiva, 1995; Gelnar *et al.*, 1996; Skorikova *et al.*, 1996;

Sures *et al.*, 1999; Dzika, 1999; Matejusova *et al.*, 2003; Aguilar *et al.*, 2005) and it can be assumed that the wild population at certain locations may be affected by this parasitosis. Since Japanese eels appear to be less susceptible to both parasite species (Fang *et al.*, 2008) it may be hypothesized that the wild populations of *A. japonica* are also less affected by these parasites.

12.7. Diagnosis

Correct diagnosis of the infection requires: (i) euthanasia of the host; (ii) dissection of the gill apparatus; (iii) recovery of the gill-monogeneans under the dissection microscope; and (iv) subsequent mounting of worms on microscope slides whereby they can be examined at 100–400× magnification for morphometric analysis. The hard parts (sclerotized structures) should be used for diagnosis only. Soft parts may be stained but due to the distorting effect of compression under slide preparation these parts should not be used for diagnostic purposes. In order to differentiate between *P. anguillae* and *P. bini* the morphology and size of the hamuli (anchors) and marginal hooklets must be recorded. *P. anguillae* possess long and slender hamuli (Fig. 12.9) whereas *P. bini* anchors are short and stout (Fig. 12.10). The distance between the base of the hamulus curvature and the highest point of the shaft (where it is joining the flexible upper part) is suitable for diagnosis. Thus, this parameter is 53–61 µm in *P. bini* and 95–114 µm in *P. anguillae*.

Molecular techniques may supplement the classical morphometric analysis. The DNA sequence of the genes encoding ribosomal RNA (18S, 28S and the internal transcribed spacer (ITS) region including 5.8S) can be used to differentiate the two species. The methods are dependent on lysis of the parasite (by incubation with proteinase K), and subsequent PCR using specific primers and finally sequencing (Hayward *et al.*, 2001; Kania *et al.*, 2010).

Anon-lethal and fast diagnostic procedure for clinical use was presented by Buchmann (1990a). This method includes anaesthesia of the host and subsequent insertion of a micro-



Fig. 12.9. Hamuli from *P. anguillae* showing the long and slender shape to be used for species discrimination (light microscopy). The distance between the hamulus curvature and the upper shaft at the junction with the flexible part is 100 μm .



Fig. 12.10. Hamuli from *P. bini* showing the short and stout shape of the anchor (light microscopy). The distance between the hamulus curvature and the upper shaft at the junction with the flexible part is 60 μm .

endoscope through the opercular opening into the gill chamber of the eel immersed in water. Live parasites present on the gill filaments can

be detected and enumerated without killing the host. The parasites will, however, merely be diagnosed to genus level by this technique.

12.8. Clinical Signs and Behavioural Effect on Eels of Infection

Slight infections do not seem to affect the eel seriously. However, if left uncontrolled even weak infections will, due to the short generation time of the parasite, in a few weeks develop into severe infections eliciting serious morbidity and mortality.

The impact of the parasite burden is dependent not on the intensity (number of parasites per fish) but rather on the number of parasites in relation to the size of the eel. The gill surface of fish increases markedly with body length (Hughes, 1966) and the space available for parasite attachment therefore increases with host size (Buchmann, 1989b). Therefore glass eels and young fingerlings will suffer from even a small number of parasites which will cause no problems in larger eels. Heavy infections make the eels lethargic and anorectic. The first sign is a decrease of feeding activity and a second clear sign of gill-disease is the fish seeking the water surface (with higher oxygen saturation) due to decreased uptake of oxygen by the affected gills. When reaching a threshold level eels turn upside down and eventually die. In recirculation fish-farm systems (applying a continuous flow of water through tanks and biofilters) the weakened eels are not able to remain at their positions in the fish tank and flow with water currents. This leads eventually to trapping of diseased eels in grids at the outlet.

12.9. Macroscopic and Microscopic Lesions

Severe pathological reaction may be induced by the infection (Egusa, 1979; Buchmann, 1988b, c). The parasite inserts its hamuli and marginal hooklets into gill tissue which initiates an inflammatory reaction and a cellular host response (Fig. 12.11). Hyperplasia of mucous cells is associated with excess production of mucus. Hyperplasia of gill epithelial cells leads to clubbing of primary gill filaments, fusion of gill lamellae and even adjacent primary filaments (Fig. 12.12). These reactions reduce the respiratory gill surface

area leading to an impaired gas exchange (release of carbon dioxide from the host and uptake of oxygen). The extensive tissue reaction may lead to partial embedding of *P. bini* (Buchmann, 1988b, c). This parasite species has relatively small hamuli and is less mobile. When the parasite is removed severe malformations of dysfunctional gill tissue can be observed. Haemorrhages due to parasite feeding and insertion of hooks may be evident and telangiectasis are found in heavily infected eels. Further, the injuries produced by the action of attachment hooks and parasite feeding may allow facultative pathogens (bacteria and fungi) to colonize and gain access to the host tissues.

12.10. Pathophysiology of the Disease

The effect of the parasites on the host is dependent on the intensity in relation to the host size. Smaller eels (glass eels and fingerlings) suffer from even low infections which cause no or merely a slight problem to larger eels. This is based on the anatomical relation between host length and gill surface area which make the available colonization area of the gills much higher in large eels (Hughes, 1966). Therefore clinical signs in larger eels do normally first occur at relatively high infection intensities. In all cases the surface area of the gill apparatus is markedly reduced due to the severe pathological reactions caused by the infection. This will evidently affect gas exchange and ammonia excretion via gill epithelia. No information from controlled experiments on the effect of *Pseudodactylogyrus* parasites on oxygen uptake and swimming performance of eels are available. However, Molnar (1994), working with a comparable system (fry of common carp infected with *D. vastator*) demonstrated intensity-dependent morbidity of hosts induced by hypoxia. The parasites feed primarily and directly on the gill epithelia and mucus but in severe cases even blood leaking from haemorrhages may be ingested as well. Since this monopisthocotylean parasite is a surface browser, it normally does not ingest blood, consequently

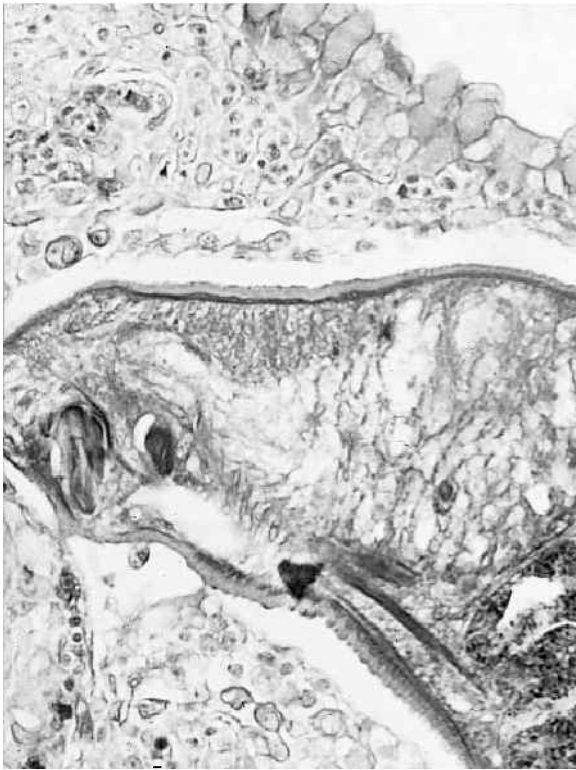


Fig. 12.11. Histological section showing extensive hyperplasia around the opisthaptor of *P. bini* attached to and partly embedded in gill filaments of European eel.

anaemia is not directly linked to pseudodactylogyrosis but may occur as a secondary effect (due to decreased food intake of eels).

12.11. Control Strategies

12.11.1. Immunity

The host reacts strongly to infections by epithelial and mucous cell hyperplasia. The reaction is spectacular in *P. bini* infections where epithelial outgrowth may partly encapsulate and embed the parasite. Also, *P. anguillae* infections are associated with general gill epithelial hyperplasia. An acquired and partly protecting host reaction has been demonstrated in the European eel. Fingerlings of *A. anguilla* were immunized by an experimental primary infection (mixed infection of *P. anguillae* and *P. bini*) which subsequently

was eliminated (by bath treatment using the anthelmintic mebendazole). Challenge infections by exposure of eels to infective oncomiracidia at 14 and 33 days post-treatment showed that previously infected eels had a reduced worm burden compared to naïve eels (Slotved and Buchmann, 1993). However, this protective effect is on its own not sufficient for control of the disease under farming conditions but together with additional control measures host immune responses may contribute to acceptable infection levels in farms. The immune reactions mounted in eels comprise both innate and adaptive responses. The cellular elements of the reactions are evident from the histopathological picture discussed earlier. The humoral response is represented by a weak, but specific, antibody reactivity in the blood against a few parasite antigens as demonstrated using Western blotting by Buchmann (1993), Mazzanti *et al.* (1999) and Monni and Cognetti-Varriale (2002).



Fig. 12.12. Extensive gill tissue reaction of eel gills with clubbing and fusion of gill filaments due to *P. bini* infection. SEM by K. Buchmann and M. K  ie. The adult partly embedded worm is 1.5 mm in length.

12.11.2. Chemotherapy

Fish farmers have traditionally applied various auxiliary substances such as potassium permanganate, ammonia, formaldehyde and sodium chloride in order to control this parasitosis (Chan and Wu, 1984; Buchmann *et al.*, 1987a). Formaldehyde has been used frequently (regular bath treatments at 30–90 ppm) with some efficacy. However, the well-defined stress effect of formaldehyde on fish (J  rgensen and Buchmann, 2007) and documented carcinogenic and allergenic properties of this chemical call for alternative measures. Sodium chloride is generally regarded as a relatively environmentally friendly substance for treating ectoparasites. However, it has no effect on *Pseudodactylogyrus*. *P. bini* may be affected by 20 ppt sodium chloride but *P. anguillae* seems to be more salt tolerant and survive this treatment (Buchmann

et al., 1992). The organophosphate metrifonate (trichlorphon, Neguvon) tested by Chinese researchers (Chan and Wu, 1984) showed efficacy but due to its high toxicity to eels its use is not recommended. A series of commercially available anthelmintics have been tested. Some anthelmintics were shown to have toxic effects on *A. anguilla* (e.g. niclosamide, ivermectin) and should therefore be avoided (Buchmann *et al.*, 1990a). However, a number of drugs showed high efficacy against the parasites and low toxicity to the host and could be recommended for eel culture. Especially benzimidazoles such as mebendazole, luxabendazole and flubendazole (1 mg/l) were found to have a high parasitocidal effect and low toxicity to the host (Szekely and Molnar, 1987; Buchmann and Bjerregaard, 1990a, b). Also praziquantel (10 mg/l) showed an excellent effect (Buchmann, 1987; Buchmann *et al.*, 1990b).

12.11.3. Zoosanitation

Widespread use of mechanical filters in modern fish farming to remove excess organic material from the water has been shown to prevent gill parasite infections as well. Mechanical filters with mesh sizes of 40 µm or less (commonly used in modern fish farms) are able to remove a considerable number of eggs and larvae from the water. The free-living larva may be vulnerable to ultraviolet (UV) irradiation. UV light has been proved to kill infective ciliates with high efficacy (Gratzek *et al.*, 1983) and monogenean larvae are likely to be vulnerable to this energy-rich irradiation. Studies have shown that parasite eggs can be trapped and ingested by elements of the free-living microfauna in recirculation fish-tank systems. Thus, newly produced eggs were ingested and eliminated by turbellarians (*Stenostomum* sp.) and copepods eliminated parasite larvae (Buchmann, 1988a). These organisms may contribute to a reduction of the infection pressure in farms but due to difficulties in controlling the population size of the microfauna this biocontrol method needs further development before implementation in farms.

12.12. Conclusions and Recommendations

The European eel is an endangered species (EC, 2007; ICES, 2007) probably due to deterioration of habitats for juvenile eels in brackish and freshwater locations but also other species within the genus *Anguilla* are under pressure. Diseases caused by bacterial

(Haenen and Davidse, 2001), viral (Haenen *et al.*, 2002) and parasitic organisms (Tarschewski, 2006) may have contributed to the crisis which may be further aggravated by increasing temperatures in the aquatic environment. These may provide excellent opportunities for the oviparous monopisthocotyleans of the genus *Pseudodactylogyrus* with a proven predilection for higher temperatures. Pseudodactylogyrosis may be controlled in aquaculture enterprises by mechanical and medical measures but wild eel stocks may be exposed to a less well-defined and uncontrolled infection pressure. Therefore, the disease should be monitored regularly in natural eel populations as part of conservation and management programmes (EC, 2007). The diagnostic methods available are already useful for general purposes but further in-depth research on the genome of the parasites may assist in future high-resolution detection of various strains of parasites. Additional molecular markers, apart from the ITS region (e.g. intergenic spacer (IGS) and mitochondrial genes), may prove to be useful tools in the future for tracing anthropogenic transfer of the two parasites between continents. Sustainable control methods in farms should be further developed. Mechanical filtering and medical methods of control have shown their strength but alternative methods based on biological control and immune responses of the host may assist this purpose. Immunization may confer a relative protection against reinfection and additional research efforts should be initiated to explore the possibilities for immuno-prophylaxis.

References

- Aguilar, A., Alvarez, M.F., Leiro, J.M. and Sanmartin, M.L. (2005) Parasite populations of the European eel (*Anguilla anguilla* L.) in the rivers Ulla and Tea (Galicia, northwest Spain). *Aquaculture* 249, 85–94.
- Buchmann, K. (1987) The effects of praziquantel on the monogenean gill parasite *Pseudodactylogyrus bini*. *Acta Veterinaria Scandinavica* 28, 447–450.
- Buchmann, K. (1988a) Epidemiology of pseudodactylogyrosis in an intensive eel-culture system. *Diseases of Aquatic Organisms* 5, 81–85.
- Buchmann, K. (1988b) Feeding of *Pseudodactylogyrus bini* (Monogenea) from *Anguilla anguilla*. *Bulletin of the European Association for Fish Pathologists* 8, 79–81.

- Buchmann, K. (1988c) Interactions between the gill-parasitic monogeneans *Pseudodactylogyrus anguillae* and *P. bini* and the fish host *Anguilla anguilla*. *Bulletin of the European Association for Fish Pathologists* 8, 98–100.
- Buchmann, K. (1988d) Temperature-dependent reproduction and survival of *Pseudodactylogyrus bini* (Monogenea) on the European eel (*Anguilla anguilla*). *Parasitology Research* 75, 162–164.
- Buchmann, K. (1989a) Microhabitats of monogenean gill parasites on European eel (*Anguilla anguilla*). *Folia Parasitologica* 36, 321–329.
- Buchmann, K. (1989b) Relations between host-size of *Anguilla anguilla* and the infection level of the monogeneans *Pseudodactylogyrus* spp. *Journal of Fish Biology* 36, 599–601.
- Buchmann, K. (1990a) Endoscope-technology for detection of monogenean gill-parasites from eels. *Bulletin of the European Association for Fish Pathologists* 10, 60–61.
- Buchmann, K. (1990b) Influence of temperature on reproduction and survival of *Pseudodactylogyrus anguillae* (Monogenea) from the European eel. *Folia Parasitologica* 37, 59–62.
- Buchmann, K. (1993) A note on the humoral immune response of infected *Anguilla anguilla* against the gill monogeneans *Pseudodactylogyrus bini*. *Fish and Shellfish Immunology* 3/5, 397–399.
- Buchmann, K. and Bjerregaard, J. (1990a) Comparative efficacies of commercially available benzimidazoles against *Pseudodactylogyrus* infestations in eels. *Diseases of Aquatic Organisms* 9, 117–120.
- Buchmann, K. and Bjerregaard, J. (1990b) Mebendazole treatment of pseudodactylogyrosis in intensive eel-culture systems. *Aquaculture* 86, 139–153.
- Buchmann, K. and Prentø, P. (1989) Cholinergic and aminergic elements in the nervous system of *Pseudodactylogyrus bini* (Monogenea). *Diseases of Aquatic Organisms* 6, 89–92.
- Buchmann, K., Møllergaard, S. and Køie, M. (1987a) *Pseudodactylogyrus* infections in eel: a review. *Diseases of Aquatic Organisms* 3, 51–57.
- Buchmann, K., Køie, M. and Prentø, P. (1987b) The nutrition of the gill parasitic monogenean *Pseudodactylogyrus anguillae*. *Parasitology Research* 73, 532–537.
- Buchmann, K., Székely, Cs. and Bjerregaard, J. (1990a) Treatment of *Pseudodactylogyrus* infestations of *Anguilla anguilla* I. Trials with niclosamide, toltrazuril, phenolsulfonphthalein and rafoxanide. *Bulletin of the European Association for Fish Pathologists* 10, 14–17.
- Buchmann, K., Székely, Cs. and Bjerregaard, J. (1990b) Treatment of *Pseudodactylogyrus* infestations of *Anguilla anguilla* II. Trials with bunamidine, praziquantel and levamisole. *Bulletin of the European Association for Fish Pathologists* 10, 18–20.
- Buchmann, K., Felsing, A. and Slotved, H.C. (1992) Effects of metrifonate, sodium chloride and bithionol on an European population of *Pseudodactylogyrus* spp. and the host *Anguilla anguilla*. *Bulletin of the European Association for Fish Pathologists* 12, 57–60.
- Chan, B. and Wu, B. (1984) Studies on the pathogenicity, biology, and treatment of *Pseudodactylogyrus* for the eels in fish farms. *Acta Zoologica Sinica* 30, 173–180.
- Christison, K.W. and Baker, G.C. (2006) First record of *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) (Monogenea) from South Africa. *African Zoology* 42, 279–285.
- Chung, H.-Y., Lin, I.H. and Kou, G.-H. (1984) Study of the parasites on the gill of cultured eel in Taiwan. *Council of Agriculture Fisheries Series, No. 10, Fish Diseases Research* 4, 24–33.
- Cone, D.K. and Marcogliese, D.J. (1995) *Pseudodactylogyrus anguillae* on *Anguilla rostrata* in Nova Scotia – an endemic or an introduction? *Journal of Fish Biology* 47, 177–178.
- Dzika, E. (1999) Microhabitats of *Pseudodactylogyrus anguillae* and *P. bini* (Monogenea: Dactylogyridae) on the gills of large-size European eel *Anguilla anguilla* from Lake Gaj, Poland. *Folia Parasitologica* 46, 33–36.
- Dzika, E., Wlasow, T. and Gomulka, P. (1995) The first recorded case of the occurrence of two species of the genus *Pseudodactylogyrus* on the eel *Anguilla anguilla* (L.) in Poland. *Acta Parasitologica* 40, 165–167.
- Egusa, S. (1979) Notes on culture of the European eel (*Anguilla anguilla* L.) in Japanese eel-farming ponds. *Rapports et procès-Verbaux des Reunions, Conseil International pour l'Exploration de la Mer* 174, 51–58.
- European Community (EC) (2007) Council Regulation no. 1100/2007 establishing measures for the recovery of the stock of European eel. September 18, 2007. *Official Journal of the European Union* L248/17–L248/23.
- Fang, J., Shirakashi, S. and Ogawa, K. (2008) Comparative susceptibility of Japanese and European eels to infections with *Pseudodactylogyrus* spp. (Monogenea). *Fish Pathology* 43, 144–151.

- Gelnar, M., Scholz, T., Matejusova, I. and Konecny, R. (1996) Occurrence of *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) and *P. bini* (Kikuchi, 1929), parasites of eel, *Anguilla anguilla* L., in Austria. *Annalen Naturhistorischen Museum Wien* 98B, 1–4.
- Golovin, P.P. (1977) Monogeneans of eel during its culture using heated water. *Investigations of Monogeneoidea in USSR* (Zoological Institute, USSR, Academy of Sciences, Leningrad) 1, 144–150.
- Gratzek, J.B., Gilbert, J.P., Lohr, A.L., Shotts, E.B. and Brown, J. (1983) Ultraviolet light control of *Ichthyophthirius multifiliis* Fouquet in a closed fish culture recirculation system. *Journal of Fish Diseases* 6(2), 145–153.
- Gusse, A.V. (1965) A new genus of monogenetic trematodes from the eel, genus *Anguilla*. *Trudy Zoologiya* (Zoological Institute, USSR, Academy of Sciences, Leningrad) 35, 119–125.
- Haenen, O.L.M. and Davidse, A. (2001) First isolation and pathogenicity studies with *Pseudomonas anguilliseptica* from diseases European eel *Anguilla anguilla* (L.) in the Netherlands. *Aquaculture* 196 (1/2), 27–36.
- Haenen, O.L.M., Dijkstra, S.G., van Tulden, P.W., Davidse, A., van Nieuwstadt, A.P., Wagenaar, F. and Wellenberg, G.J. (2002) *Herpesvirus anguillae* (HVA) isolations from disease outbreaks in cultured European eel, *Anguilla anguilla*, in the Netherlands since 1996. *Bulletin of the European Association of Fish Pathologists* 22(4), 247–257.
- Hayward, C.J., Iwashita, M., Crane, J.S. and Ogawa, K. (2001) First report of the invasive eel pest *Pseudodactylogyrus bini* in North America and in wild American eels. *Diseases of Aquatic Organisms* 44, 53–60.
- Hughes, G.M. (1966) The dimensions of fish gills in relation to their function. *Journal of Experimental Biology* 45, 179–195.
- Imada, R. and Muroga, K. (1978) *Pseudodactylogyrus microrchis* (Monogenea) on the gills of cultured eels. II. Oviposition, hatching and development on the host. *Bulletin of the Japanese Society for Scientific Fisheries* 44, 571–576.
- International Council for Exploration of the Sea (ICES) (2007) European eel. Report of the ICES Advisory Committee on Fishery Management, Advisory Committee on the Marine Environment and Advisory Committee on Ecosystems, 2007. *ICES Advice* 9, 86–92.
- Iwashita, M., Hirata, J. and Ogawa, K. (2002) *Pseudodactylogyrus kamegaki* sp. n. (Monogenea: Pseudodactylogyridae) from wild Japanese eel, *Anguilla japonica*. *Parasitology International* 51, 337–342.
- Jørgensen, T.R. and Buchmann, K. (2007) Stress response in rainbow trout during infection with *Ichthyophthirius multifiliis* and formalin bath treatment. *Acta Ichthyologica et Piscatoria* 37, 25–28.
- Kania, P.W., Taraschewski, H., Han, Y.-S., Cone, D.K. and Buchmann, K. (2010) Divergence between Asian, European and Canadian populations of the monogenean *Pseudodactylogyrus bini* indicated by ribosomal DNA patterns. *Journal of Helminthology* 84, 404–409.
- Kikuchi, H. (1929) Two new species of Japanese trematodes belonging to Gyrodactylidae. *Annotationes Zoologia Japon* 12, 175–186.
- Køie, M. (1988) Parasites in European eel *Anguilla anguilla* (L.) from Danish freshwater, brackish and marine localities. *Ophelia* 29, 93–118.
- Lambert, A., Le Brun, N. and Pariselle, A. (1984) Presence en France de *Pseudodactylogyrus anguillae* (Yin et Sproston, 1948) Gussev, 1965 (Monogenea, Monopisthocotyles) parasite branchial de l'anguille européenne, *Anguilla anguilla*, en eau douce. *Annales Parasitologie Humaine et Compare* 60, 91–92.
- Matejusova, I., Simkova, A., Sasal, P. and Gelnar, M. (2003) Microhabitat distribution of *Pseudodactylogyrus anguillae* and *P. bini* among and within gill arches of the European eel (*Anguilla anguilla* L.). *Parasitology Research* 89, 290–296.
- Mazzanti, C., Monni, G. and Varriale, A.M.C. (1999) Observations on antigenic activity of *Pseudodactylogyrus anguillae* (Monogenea) on the European eel (*Anguilla anguilla*). *Bulletin of the European Association for Fish Pathologists* 19, 57–59.
- Molnar, K. (1972) Studies on gill parasitosis of the grass carp (*Ctenopharyngodon idella*) caused by *Dactylogyrus lamellatus* Achmerow, 1952. IV. Histopathological changes. *Acta Veterinaria Academiae Scientiarum Hungariae* 22, 9–24.
- Molnar, K. (1983) Das Vorkommen von parasitären Hakensaugwürmern bei der Aalaufsicht in Ungarn. *Zeitschrift für Binnenfischerei der DDR* 30, 341–345.
- Molnar, K. (1994) Effects of decreased water oxygen content on common carp fry with *Dactylogyrus vastator* (Monogenea) infection of varying severity. *Diseases of Aquatic Organisms* 20, 153–157.

- Monni, G. and Cognetti-Varriale, A.M. (2002) Antigenicity of *Pseudodactylogyrus anguillae* and *P. bini* (Monogenea) in the European eel (*Anguilla anguilla*, L.) under different oxygenation conditions. *Fish and Shellfish Immunology* 13, 125–131.
- Nie, P. and Kennedy, C.R. (1991) Occurrence and seasonal dynamics of *Pseudodactylogyrus anguillae* (Yin & Sproston) (Monogenea) in eel, *Anguilla anguilla* (L.) in England. *Journal of Fish Biology* 39, 879–900.
- Ogawa, K. (1984) *Pseudodactylogyrus haze* sp. n. a gill monogenean from the Japanese goby, *Acanthogobius flavimanus*. *Japanese Journal of Parasitology* 33, 403–405.
- Ogawa, K. and Egusa, S. (1976) Studies on eel pseudodactylogyrosis. I. Morphology and classification of three eel dactylogyrids with a proposal of a new species, *Pseudodactylogyrus microorchis*. *Bulletin of the Japanese Society for Scientific Fisheries* 42, 395–404.
- Paperna, I. (1964) Competitive exclusion of *Dactylogyrus extensus* by *D. vastator* (Trematoda, Monogenea) on the gills of reared carp. *Journal for Parasitology* 50, 94–98.
- Rohde, K. (1977) A non-competitive mechanism responsible for restricting niches. *Zoologische Anzeiger* 199, 164–172.
- Saraiva, A. (1995) *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) Gussev, 1965 and *P. bini* (Kikuchi, 1929) Gussev, 1965 (Monogenea: Monopisthocotylea) in Portugal. *Bulletin of the European Association for Fish Pathologists* 15, 81–83.
- Sasal, P., Taraschewski, H., Valade, P., Grondin, H., Wielgoss, S. and Moravec, F. (2008) Parasite communities in eels of the Island of Reunion (Indian Ocean): a lesson in parasite introduction. *Parasitology Research* 102, 1343–1350.
- Skorikova, B., Scholz, T. and Moravec, F. (1996) Spreading of introduced monogeneans *Pseudodactylogyrus anguillae* and *P. bini* among eel populations in the Czech Republic. *Folia Parasitologica* 43, 155–156.
- Slotved, H.-C. and Buchmann, K. (1993) Acquired resistance of *Anguilla anguilla* L. against challenge infections with gill monogeneans. *Journal of Fish Diseases* 16, 585–591.
- Sures, B., Knopf, K., Würtz, J. and Hirt, J. (1999) Richness and diversity of parasite communities in European eels *Anguilla anguilla* of the River Rhine, Germany, with special reference to helminth parasites. *Parasitology* 111, 323–330.
- Szekely, Cs. and Molnar, K. (1987) Mebendazole is an efficacious drug against pseudodactylogyrosis in the European eel (*Anguilla anguilla*). *Journal of Applied Ichthyology* 3, 183–186.
- Taraschewski, H. (2006) Hosts and parasites as aliens. *Journal of Helminthology* 80, 99–128.
- Yin, W.-Y. and Sproston, N.G. (1948) Studies on the monogenetic trematodes of China, Parts 1–5. *Sinensia* 19, 57–85.

13 *Benedenia seriolae* and *Neobenedenia* Species

Ian D. Whittington

Monogenean Research Laboratory, Parasitology Section, The South Australian Museum; Marine Parasitology Laboratory and Australian Centre for Evolutionary Biology and Biodiversity at The University of Adelaide, Adelaide, Australia

13.1. Introduction

Capsalidae are epithelium-feeding Monogenea (Monopisthocotylea) comprising ~180 species (Perkins *et al.*, 2009). These ectoparasitic flatworms infect diverse sites on marine teleosts and elasmobranchs (Whittington, 2004). *Benedenia seriolae* (Figs. 13.1a, 13.2a, b) and *Neobenedenia* species (Figs. 13.1b, 13.2c) are capsalids that cause disease, production losses and mortality to teleosts in aquaculture threatening profitability and viability (Ogawa, 2005; Whittington, 2005; Whittington and Chisholm, 2008). For a comprehensive background on monogeneans, consult Kearn (1998), Hayward (2005), Whittington (2005) and Whittington and Chisholm (2008). The life cycle is direct (Fig. 13.1). Unlike gyrodactylids (Chapter 11), capsalids are oviparous and lay tetrahedral eggs singly (Fig. 13.1c, d). Eggs from parasites on wild hosts drift in sea water; their long filamentous appendage may tangle on substrates. After embryonation, an infective larva, the oncomiracidium, hatches (Fig. 13.1f, g). Eggs from parasites on caged stock may catch on nets (Fig. 13.1e; Ogawa and Yokoyama, 1998) so oncomiracidia hatch close to fish. At high water temperatures eggs embryonate and parasites mature rapidly (Lackenby *et al.*, 2007 for *B. seriolae*; Bondad-Reantaso *et al.*, 1995a for *Neobenedenia*). Efficient transmission causes parasite numbers to increase if captive fish

densities are high and host immunity is compromised by stress, suboptimal nutrition, water quality and/or other pathogens.

Described as *Epibdella seriolae* from wild carangids in Japan (Yamaguti, 1934), *B. seriolae* became a major pathogen when Japanese *Seriola* culture intensified in the 1950s (Egusa, 1983; Whittington *et al.*, 2001a). *Seriola* species are globally distributed in warm waters and *B. seriolae* is reported from several wild species (Japan: Yamaguti, 1934; New Zealand: Sharp *et al.*, 2003; Australia: Hutson *et al.*, 2007a). On farms outside Japan, infections occur in South and Central America (Chile, Ecuador, Mexico) and Australasia (Australia, New Zealand) (Whittington and Chisholm, 2008) but not on *Seriola dumerili* farmed off the Balearic Islands, western Mediterranean Sea (Grau *et al.*, 1999).

Impacts by *B. seriolae* on cultivated *Seriola* production globally are reported in Japan (e.g. Hoshina, 1968; Egusa, 1983; Ogawa and Yokoyama, 1998; Ogawa, 2005) and Australasia (Whittington *et al.*, 2001b; Ernst *et al.* 2002; Chambers and Ernst, 2005; Diggles and Hutson, 2005; Hutson *et al.*, 2007b). If unmanaged, infections may kill captive *Seriola* (see Ernst *et al.*, 2002). Production costs in Japan are especially high, reportedly twice those for Atlantic salmon in Norway, and *B. seriolae* management contributes 20% to total production expenses (Ernst *et al.*, 2005).

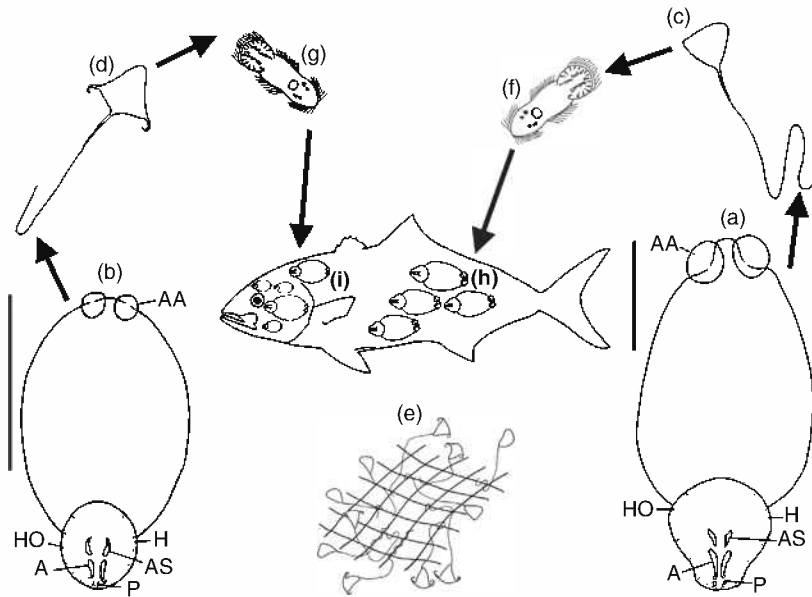


Fig. 13.1. Life cycle of (a) *Benedenia seriolae* and (b) *Neobenedenia* species (Monogenea: Capsalidae) here co-infecting skin of *Seriola dumerili* (Carangidae). Eggs (c, d) drift in seawater but may tangle on sea cages (e). Ciliated larvae (f, g) hatch, infect a host and worm populations (h, i) can grow rapidly on captive fish. A, Anterior hamulus; AA, anterior attachment organ; AS, accessory sclerite; H, haptor; HO, hooklet; P, posterior hamulus. Not to scale, but for comparison, scale bars for (a) and (b) = 2 mm.

Impacts on fish health by *Neobenedenia* species are less clear because of uncertainty that shrouds the specific identity or identities of the pathogen(s). *Neobenedenia melleni*, described as *Epibdella melleni* (reported as *B. melleni* by some) from numerous fish species in the New York Aquarium (NYA) by MacCallum (1927), is now allegedly known from more than 100 species in more than 30 families and five orders from wild, aquarium and farmed teleosts worldwide (Whittington and Horton, 1996). Among the most host specific of all metazoan parasites, commonly a monogenean species may parasitize only one host species (Whittington *et al.*, 2000). *B. seriolae* is specific to host genus so the peculiar ubiquity of *N. melleni* from copious unrelated fish species is extraordinary! Whittington and Horton (1996) tentatively suggested and Whittington (2004, 2005) has hypothesized that *N. melleni* and *Neobenedenia girellae* may each represent a complex of several species currently impossible to differentiate morphologically. This is the reason 'Neobenedenia

species' is in the chapter title and pathogenic *Neobenedenia* are considered a collection of potentially many undifferentiated species. Identities from publications are maintained but occur in quotation marks (e.g. '*N. melleni*').

Published information about *Neobenedenia* on wild fish is scarce. Brooks and Mayes (1975) reported more than 100 '*N. girellae*' on the skin of *Pimelometopon pulchrum* (Labridae) and Gaida and Frost (1991) reported up to 42 '*N. girellae*' on the skin of *Medialuna californiensis* (Kyphosidae) off California. For '*N. melleni*', there are these reports: (i) low mean abundance on skin of *Sebastes capensis* (Sebastidae) off northern Chile (González and Acuña, 1998); (ii) small numbers from the skin of *Trachinotus carolinus* (Carangidae) in the Gulf of Mexico (Bullard *et al.*, 2003); (iii) low mean abundance on skin of *Sphoeroides annulatus* (Tetraodontidae) off Sinaloa, Mexico (Fajer-Ávila *et al.*, 2004); (iv) different infection intensities on skin of three sympatric Caribbean surgeonfish species (Acanthuridae) (see Sikkell *et al.*, 2009); and (v) low

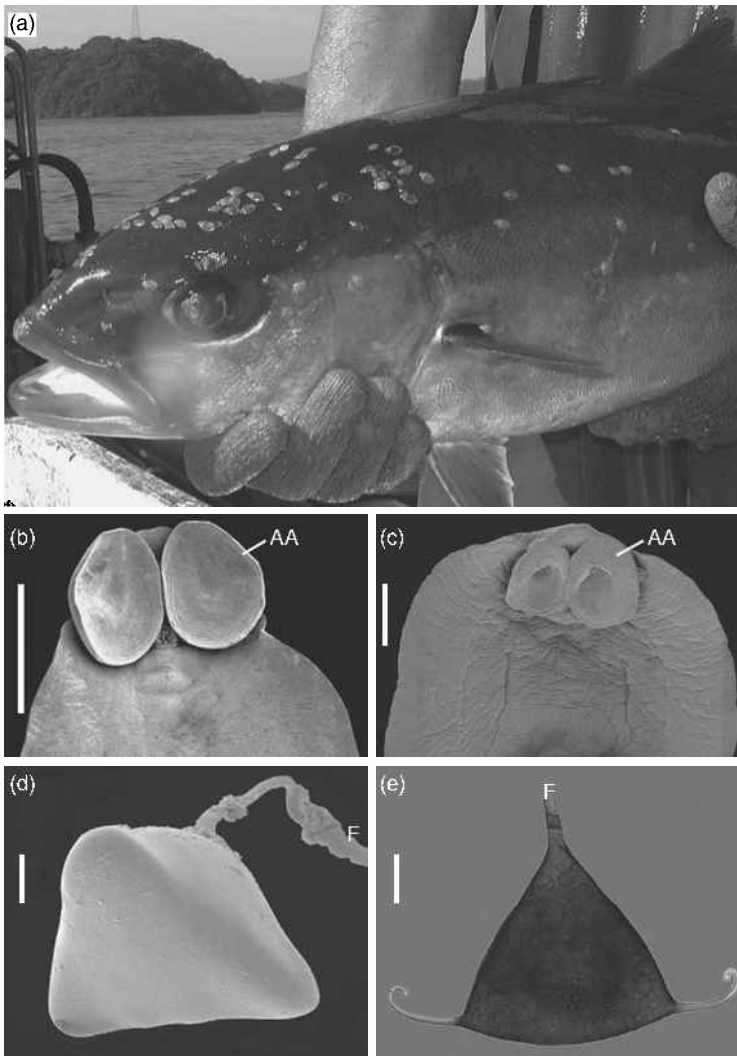


Fig. 13.2. (a) *Seriola quinqueradiata* (Carangidae) from Japanese sea-cage culture after brief treatment in freshwater showing *B. seriolae* (Monogenea: Capsalidae) as prominent, white, blister-like ovals. Anterior end of (b) *B. seriolae* and (c) a *Neobenedenia* species by scanning electron microscopy (SEM). Scale bars: (b) 1 mm; (c) 200 μ m. Tetrahedral eggs of (d) *B. seriolae* viewed by SEM and (e) a *Neobenedenia* species by light microscopy. Scale bars = 20 μ m. AA, Anterior attachment organ; F, filamentous egg appendage (full lengths not shown; see Fig. 13.1c, d).

infection intensity from skin of *Trichiurus lepturus* (Trichiuridae) off Brazil (Carvalho and Luque, 2009). In view of massive infections reported from captive fish, relatively low infection intensities on wild hosts may seem surprising. These, however, probably reflect normal parasitaemia of presumably healthy,

wide-ranging hosts at natural population densities in their regular environment.

In contrast, *Neobenedenia* populations on captive fish can be enormous. Jahn and Kuhn (1932) reported more than 2000 adult '*N. melleni*' from a 'Galapagos labroid' in the NYA and Ogawa *et al.* (1995) observed 2000

'*N. girellae*' on Japanese flounder (*Paralichthyes olivaceus*, Paralichthyidae), in Japanese sea-cage culture! Pathogenic reports on skin of many teleost species abound globally in public aquaria (Alaska, Australia, Bermuda, Chicago, Las Vegas, Mexico, New York, Philadelphia and Taiwan; Whittington and Chisholm, 2008). In especially heavy aquarium infections, '*N. melleni*' was reported from 'gill and nasal cavities' (Jahn and Kuhn, 1932). In marine aquaculture worldwide, *Neobenedenia* species have caused disease and death to many fish species (Table 13.1).

In aquaria, costs to monitor and manage infections and replace fishes killed in outbreaks are incalculable. Impacts in marine aquaculture are huge as demonstrated by outbreaks reported in Table 13.1, some of which killed stock (Kaneko *et al.*, 1988; Ogawa *et al.*, 1995; Ogawa and Yokoyama, 1998; Deveney *et al.*, 2001; Ogawa *et al.*, 2006). Fifty tonnes of *Lates calcarifer* worth AUS\$500,000 died during a 3-week outbreak of '*N. melleni*' in Australia (Deveney *et al.*, 2001). There are two characteristics of *Neobenedenia* epizootics in aquaculture: (i) the infection source is often unknown (Kaneko *et al.*, 1988; Deveney *et al.*, 2001; Ogawa *et al.*, 2006); and (ii) fish susceptibility to *Neobenedenia*, and associated inherent difficulties to manage infections, may limit development, progress and expansion of sea-cage culture (e.g. cobia in Taiwan, Liao *et al.* 2004; spotted halibut and Japanese flounder in Japan, Hirazawa *et al.*, 2004).

13.2. Diagnosis of the Infection

Both species are dorsoventrally flattened, generally oval (Figs. 13.1a, b and 13.2a) and infect body surfaces including flanks, head, fins and eyes. Live worms can be virtually transparent but are sometimes pigmented (Whittington, 1996). Capsalids feed on epidermis. If skin pigment is ingested, appearance in the branched intestine and worm transparency conceals mild and moderate infections by live parasites. A clinical manifestation of infection may be due to feeding which may 'irritate' fish so they rub their body (= 'flashing') against nearby substrates presumably trying to dislodge attached, feeding capsalids.

13.2.1. *Benedenia seriolae*

Adults infect skin, sometimes eyes, of *Seriola* species (Fig. 13.2a). Adults range from 4 to 12 mm in length, and from 1 to 6 mm in width (Whittington *et al.*, 2001c). Live specimens on hosts may be difficult to detect but a brief dip in dechlorinated tap water highlights infection turning worms opaque as prominent white, raised, blister-like ovals (Fig. 13.2a). This technique rarely detaches parasites from fish. They attach by strong suction maintained even when dead on fish and smooth inert surfaces like glass and plastic. Attachment is supplemented by proteinaceous sclerites (accessory sclerites, two pairs of hamuli and 14 hooklets at the edge of the posterior attachment organ, the haptor; Fig. 13.1a). Tetrahedral eggs (Figs. 13.1c, 13.2d; side length: 130–150 µm; Hoshina, 1968) may be detected if infected fish are isolated and tank water is screened (Whittington and Chisholm, 2008). All *Benedenia* species, however, and many monopisthocotyleans including *Neobenedenia* species, lay tetrahedral eggs so their presence does not confirm *B. seriolae* infection.

13.2.2. *Neobenedenia* species

Adults infect flanks, head, fins and eyes of numerous fish species (e.g. Table 13.1) so host taxon provides no diagnostic help. The total length range of adults is 2–7 mm (Whittington and Horton, 1996; Whittington and Chisholm, 2008). Transparency and pigment may hide live worms but bathing in dechlorinated tap water turns specimens *in situ* milky white. In Japan, farmed *S. dumerili* and *Seriola quinqueradiata* can be co-infected by *B. seriolae* and '*N. girellae*'. Kinami *et al.* (2005) treated infected fish with fresh water and determined distinct shape differences (compare Figs. 13.1a and b) and plotting length of anterior attachment organs against total parasite length differentiated them. The body of *Neobenedenia* species is broad anteriorly at the level of the anterior attachment organs (Figs. 13.1b, 13.2c) and does not taper like *B. seriolae* (Figs. 13.1a, 13.2b). Anterior attachment organs and the

Table 13.1. Outbreaks of *Neobenedenia* 'species' in marine sea-cage aquaculture arranged chronologically emphasizing host and geographic ranges.

<i>Neobenedenia</i> 'species'	Host species (host family)	Locality	Source
<i>Neobenedenia</i> sp. (as <i>Benedenia</i> sp.)	<i>Oreochromis aureus</i> (Cichlidae)	Cuba	Prieto <i>et al.</i> (1986)
' <i>N. melleni</i> '	<i>Oreochromis mossambicus</i>	Hawaii	Kaneko <i>et al.</i> (1988)
' <i>N. melleni</i> '	<i>Oreochromis urolepis hornorum</i> × <i>O. mossambicus</i>	Bahamas	Mueller <i>et al.</i> (1992), Ellis and Watanabe (1993)
' <i>N. melleni</i> '	<i>O. mossambicus</i> , <i>Coryphaena hippurus</i> (Coryphaenidae), <i>Sparus aurata</i> (Sparidae)	Israel	Colorni (1994)
' <i>N. girellae</i> '	<i>Epinephelus akaara</i> , <i>Epinephelus</i> <i>cyanopodus</i> , <i>Epinephelus</i> <i>malabaricus</i> , <i>Epinephelus</i> <i>suillus</i> (Serranidae), <i>Lateolabrax japonicus</i> (Lateolabracidae), <i>Paralichthys</i> <i>olivaceus</i> (Paralichthyidae), <i>Plectropomus leopardus</i> (Serranidae), <i>Pseudocaranx dentex</i> , <i>Seriola dumerili</i> , <i>Seriola lalandi</i> , <i>Seriola quinqueradiata</i> , <i>Seriola</i> <i>rivoliana</i> (Carangidae), <i>Takifugu</i> <i>rubripes</i> (Tetraodontidae), <i>Tilapia</i> <i>nilotica</i> (Cichlidae)	Japan	Ogawa <i>et al.</i> (1995)
<i>Neobenedenia</i> sp. II of Leong (1997)	<i>Epinephelus coioides</i> (Serranidae), <i>Lates calcarifer</i> (Latidae), <i>Lutjanus</i> <i>argentimaculatus</i> , <i>Lutjanus johnii</i> , <i>Pinjalo pinjalo</i> (Lutjanidae)	South-east Asia (including Malaysia, Philippines, Singapore, Thailand)	Leong (1997)

(Continued)

Table 13.1. *Continued*

<i>Neobenedenia</i> 'species'	Host species (host family)	Locality	Source
' <i>N.girellae</i> '	<i>Pagrus major</i> (Sparidae), <i>Paralichthys olivaceus</i> , <i>S. dumerili</i> , <i>S. quinquerradiata</i> , <i>T. rubripes</i>	Japan	Ogawa and Yokoyama (1998)
' <i>N. girellae</i> '	<i>Cromileptes altivelis</i> (Serranidae)	Indonesia	Koesharyani <i>et al.</i> (1999)
' <i>N. melleni</i> '	<i>Lates calcarifer</i>	Australia	Deveney <i>et al.</i> (2001)
<i>Neobenedenia</i> sp.	<i>Rachycentron canadum</i> (Rachycentridae)	Taiwan	Lopez <i>et al.</i> (2002), Liao <i>et al.</i> (2004)
' <i>N. melleni</i> '	<i>Pseudosciaena crocea</i> (Sciaenidae), <i>S. dumerili</i>	China	Wang <i>et al.</i> (2004)
' <i>N. girellae</i> '	<i>S. dumerili</i>	China	Wang <i>et al.</i> (2004)
' <i>N. girellae</i> '	<i>Verasper variegatus</i> (Pleuronectidae)	Japan	Hirazawa <i>et al.</i> (2004), Ogawa (2005)
' <i>N. girellae</i> '	<i>S. dumerili</i>	Japan	Kinami <i>et al.</i> (2005)
' <i>N. melleni</i> '	<i>Epinephelus awoara</i> (Serranidae)	China	Li <i>et al.</i> (2005)
' <i>N. girellae</i> '	<i>P. crocea</i>	China	Li <i>et al.</i> (2005)
' <i>N. girellae</i> '	<i>R. canadum</i>	Taiwan	Ogawa <i>et al.</i> (2006)
' <i>N. melleni</i> '	<i>Lutjanus sanguineus</i> (Lutjanidae)	China	Rao and Yang (2007)
' <i>N. melleni</i> '	<i>Lates calcarifer</i>	Indonesia	Rückert <i>et al.</i> (2008)
' <i>N. melleni</i> '	<i>Epinephelus marginatus</i> (Serranidae)	Brazil	Sanches (2008)

haptor in *Neobenedenia* species are usually small relative to total parasite size (Fig. 13.1b). A significant, taxonomically important difference is the presence of a vagina in *Benedenia* species and its absence in *Neobenedenia* species. This however is rarely obvious. It is challenging to detect the narrow vagina in many *Benedenia* species. Eggs of some *Neobenedenia* species reportedly bear short, hooked appendages on two of four poles of the tetrahedron plus a long filament (Fig. 13.1d, 13.2e; e.g. MacCallum, 1927; Jahn and Kuhn, 1932) which may promote entrapment on substrate (Fig. 13.1e). It is not clear whether hooked appendages are characteristic for all *Neobenedenia* species or only for some species.

13.3. External/Internal Lesions

There are no studies on feeding and attachment by *B. seriolae* or *Neobenedenia* species. Injuries are inferred from how the best-researched capsalid, *Entobdella soleae*, feeds (Kearn, 1963) and attaches (Kearn, 1964). The pharynx uses proteolytic secretions to disassociate epithelial cells. Haptoral sclerites (Fig. 13.1a, b) may penetrate host epidermis and may injure fish skin. Proliferating parasite numbers can cause lesions. Whittington *et al.* (2001b) observed large capsalid populations grazing on captive fish injured and eroded epithelium faster than it could be replaced. In contrast, wild fish support smaller natural capsalid populations and parasite mobility may spread injuries (Whittington, 2005). In farmed fish, lesions may worsen from: (i) epithelial aggravation from flashing; (ii) host health deterioration affecting the immune system; and (iii) secondary infection (bacteria, viruses, fungi) of capsalid-inflicted wounds.

13.3.1. *B. seriolae*

Lesions in heavy infections are common (Hoshina, 1968). Feeding erodes epidermis causing attrition and skin haemorrhage (Hoshina, 1968), sometimes producing wounds that deeply penetrate the epidermis

(Williams *et al.*, 2007). Inflammatory cells and/or secondary infection may also stimulate flashing behaviour. No studies have specifically investigated attachment but it is thought to cause insignificant damage (Williams *et al.*, 2007). Whittington and Chisholm (2008) provided the following observations on infected *Seriola lalandi* in sea cages in Spencer Gulf, South Australia: (i) flashing behaviour, presumably stimulated by feeding, led to dark epithelial patches; and (ii) lesions worsened by skin damage from flashing. Damage to *Seriola* eyes is not reported.

13.3.2. *Neobenedenia* species

MacCallum (1927) noted pierced and destroyed corneas of several host species in the NYA within 3 weeks of infection. Jahn and Kuhn (1932) confirmed corneal destruction even in mild infections followed by entire damaged eyes, assisted by secondary infections, if parasites were uncontrolled. In heavy outbreaks, body epidermis was severely injured with scale disruption and loss, large areas of connective and muscle tissue exposed and eventual death. Thoney and Hargis (1991) described open lesions penetrating to the bone in *Chaetodipterus faber* (Ephippidae) with secondary infection by motile, rod-shaped bacteria.

Llewellyn (1957) commented that fish eyes are effectively immunologically privileged due to vascular absence and therefore lack blood-borne antibodies. Corneas lack mucous cells (Kearn, 1999) and fish mucus has high immunological activity (Buchmann, 1999). Therefore, *Neobenedenia* epizootics on captive fish may flourish on eyes. In captivity another factor may relate to the breakdown in host specificity permitting exploitation of abnormal host species (Thoney and Hargis, 1991).

In marine mariculture, '*N. melleni*' infection foci on tilapia (*Oreochromis mossambicus*) in sea cages off Hawaii were anterodorsal head regions and corneas (Kaneko *et al.*, 1988). Heavily infected fish (>400 '*N. melleni*' per host) had significant mucus secretion, discoloured skin, epithelium and scale loss and haemorrhagic lesions. Eyes suffered intense

pathology with the following chronology: (i) opaque cornea; (ii) corneal ulceration; (iii) eye enlarges; (iv) eye bursts; (v) disintegration of internal eye structure; (vi) scarring; and (vii) blindness (Kaneko *et al.*, 1988). *Cromileptes altivelis* infected by '*N. girellae*' in Indonesia had eye opacity and excess mucus production and haemorrhagic and abrasive body lesions (Koesharynari *et al.*, 1999). *Epinephelus marginatus* infected by '*N. melleni*' off Brazil showed darkened skin, eye opacity, eye lesions and body haemorrhages (Sanches, 2008).

Ogawa *et al.* (2006) observed '*N. girellae*' concentrated on the dorsal head region, especially eyes, in cobia (*Rachycentron canadum*) from sea cages in Taiwan. The cornea in uninfected fish comprised several layers of squamous epithelial cells of uniform shape and size but infected eyes were opaque, corneal squamous epithelial cells lost uniformity, became irregularly thickened and were sometimes lost. Below the cornea, upper layers of the collagenous stroma became thickened, oedematous and infiltrated by inflammatory cells; however no co-infection with other pathogens was apparent. Histological sections of '*N. girellae*' attached to epithelium surrounding the eye indicated: (i) mucus in the attachment region suggesting a 'strong irritating effect'; (ii) the haptor was applied firmly and closely to epithelium but was lined with cellular debris and mucus; and (iii) the distal tips of the accessory sclerites (Fig. 13.1b) had penetrated and disrupted epithelial tissue.

Epidermis of *S. dumerili* experimentally infected by '*N. girellae*' was thin compared with uninfected fish (Sato *et al.*, 2008; Hirayama *et al.*, 2009) suggesting that epithelial cells do comprise the parasites' diet (Sato *et al.*, 2008) or that thinning is a response to infection. Sato *et al.* (2008) also suggested epidermal thinning may lead to increased bruising from flashing behaviour. Mucous cells were seldom observed in epidermis of infected fish compared to uninfected fish, indicating that mucus production at infection sites may be low. Hirayama *et al.* (2009) noted a worm migration as infection progressed with most adults recovered from the fish belly where haemorrhage was observed at infections of $>0.735 \pm 0.096$ worms/cm² but no dermal penetration occurred.

The relative contributions to fish lesions from capsalid infection versus possible secondary pathogen infection is usually unquantified, but Ogawa *et al.* (2006) specifically noted no co-infection by other pathogens in *R. canadum* parasitized by *N. girellae*. Lopez *et al.* (2002), however, reported a disease outbreak in caged cobia off Taiwan where vibriosis and photobacteriosis were associated with severe head and eye ulcers and suggested bacteria may gain entry via skin damage from a *Neobenedenia* sp.

13.4. Pathophysiology

At natural population levels, monogeneans typically cause minimal damage with no notable pathogenic response (Whittington, 2005). Epizootics, often due to imbalance(s) in parasite–host interactions, are promoted by unnatural and/or unfavourable conditions. Farmed fish are maintained at one location where parasite eggs, larvae and adults intensify (Fig. 13.1). At high stocking densities, captive fish may become stressed affecting their ability to control infections. Also, these 'immobile' fish are a perfect environment for capsalids to reproduce, invade and establish large populations rapidly. Capsalid pathology is inferred but rarely *definitively* credited to a single aetiology and co-infection is seldom discounted. Pathophysiology of monogenean infections (i.e. broad manifestations of parasites and their effects on host organ systems, physiology and metabolism) is totally neglected. It seems obvious that epidermal loss, mucus hypersecretion, lesions, appetite loss and emaciation lead to poor nutrition, stress, impaired osmoregulation, growth and immunity and high incidences of secondary infection that ultimately ends in fish disease and/or death.

13.4.1. *B. seriola*

Hoshina (1968) reported anorexia and growth retardation in infected *S. quinqueradiata*. For infected *S. lalandi*, Whittington and Chisholm (2008) included a time course after appearance

of skin lesions: (i) reduced growth and food conversion ratios; (ii) aggravated epithelial lesions; (iii) onset of secondary infections; (iv) appetite loss; and (v) high likelihood for mass stock mortality if parasite and secondary infections are untreated. Most research has focused on methods to control infections (see section 13.5) and not on pathophysiological changes.

13.4.2. *Neobenedenia* species

Nigrelli (1932) drew attention to eyes as a preferred site for '*N. melleni*'. In heavy infections, the eye was destroyed and the fish eventually starved to death. Blindness (see section 13.3.2) probably occurs at the corneal opacity stage, well before further eye damage. Ogawa *et al.* (2006) speculated that parasitized cobia may be able to suppress '*N. girellae*' infection via active immune substances in skin mucus (perhaps complement) which may cause parasites to retreat to the eyes.

Heavy parasitaemia is associated with severe body epidermal injuries leading to scale loss, exposure of connective and muscle tissues and secondary infection by bacteria followed by death within days (Kaneko *et al.*, 1988; Thoney and Hargis, 1991). Robinson *et al.* (2008) reported no significant differences in lymphocytes, plasma cells, neutrophils, monocytes and macrophage counts between uninfected hybrid tilapia (*Oreochromis aureus* × *O. mossambicus*) and those infected by '*N. melleni*' in Jamaica and no evidence of a humoral response. Sato *et al.* (2008) used ^{13}C -labelled fatty acids in supplemented feeding experiments to *S. dumerili* in Japan. No ^{13}C -labelled fatty acids were detected in epidermal mucus suggesting that cell metabolism was fast. Hirayama *et al.* (2009) used the same model system to explore and quantify the effect of different '*N. girellae*' infection levels on *S. dumerili* growth. At populations $>0.285 \pm 0.042$ worms/cm², host growth significantly slowed and the feed conversion ratio was positively correlated with infection size. Lower haematocrit levels when infected by $>0.735 \pm 0.096$ worms/cm² were attributed to epidermal haemorrhage. Rare occurrence

of epidermal mucous cells was suggested to decrease resistance to bacterial invasion. Ten days after exposure to oncomiracidia, host appetite declined and death occurred after 12 days when infection was 1.393 ± 0.276 worms/cm². This study noted that longer infection duration and greater '*N. girellae*' numbers led to thinner host epidermis. Infected host epidermis was thinner in fish reared at 25°C and 30°C but not at 20°C (Hirazawa *et al.*, 2010).

13.5. Protective/Control Strategies

There are no methods to prevent *B. seriolae* and *Neobenedenia* infections, most allow only temporary respite by removing parasites (e.g. fresh water or chemical baths) and none provides any protection against immediate reinfection and are therefore best termed 'treatments'. Control methods are presented as mechanical, chemical, biological and new technologies.

13.5.1. *B. seriolae*

Protection

Leef and Lee (2009) investigated *B. seriolae* survival when exposed for 8 h at 17°C to diluted serum and mucus of naïve or infected *S. lalandi* from New Zealand but observed little to no difference. However *B. seriolae* was susceptible to serum exposure with 50% mortality within 1 h at dilutions $>1:20$ at 17°C and this effect was removed by heat treatment of serum. Living on skin, *B. seriolae* rarely encounters host blood and Leef and Lee (2009) considered the serum killing activity had little relevance but noted that addition of 5 mM ethylene-diamine-tetraacetic acid inhibited killing ability, suggesting antiparasitic activity was probably mediated by the alternative, rather than the classical, complement pathway. Leef and Lee (2009) showed that cutaneous *S. lalandi* mucus had no effect on *B. seriolae* which is not surprising since it lives in and on this host secretion.

Control

Broodstock of *S. lalandi* from the wild in South Australia are maintained at low density in recirculation tanks. They are usually given fresh water, hydrogen peroxide or formalin baths before introduction to tanks and mechanical filtration generally controls *B. seriolae*. Treatment before introduction is required because monogenean eggs are resistant to chemicals due to their proteinaceous shell (Whittington and Chisholm, 2008). Sharp *et al.* (2004) found most *B. seriolae* eggs from New Zealand kingfish exposed to 250 and 400 ppm formalin baths for 1 h remained viable. Ernst *et al.* (2005) studied effects of temperature, salinity, desiccation and chemical treatment on embryonation and hatching success of *B. seriolae* from *S. quinquerediata* in Japan. Temperature influenced embryonation with hatching 5 days after laying at 28°C but 16 days at 14°C and >70% hatching success at each temperature but no hatching at 30°C. The embryonation period increased at low and high salinities: (i) >70% hatched at salinities ranging from 25 to 45‰ but few or no eggs hatched at 10 and 15‰; and (ii) eggs, however, do not hatch if desiccated for 3 min, immersed in water at 50°C for 30 s or treated with 25% ethanol for 3 min. These results are relevant for parasite management in closed or semi-closed systems such as aquaria, nurseries and flow-through hatcheries.

The Japanese *Seriola* industry grows wild caught fingerlings in sea cages, and freshwater bathing (for 3–5 min, Egusa, 1983; up to 10 min, Ogawa, 2005; 5 min, Chambers and Ernst, 2005) is widely used (Ogawa and Yokoyama, 1998). In South Australia, freshwater treatment is impractical because cages are some distance offshore and fresh water is uncommon. Bathing in 300 ppm hydrogen peroxide is the treatment of choice (Chambers and Ernst, 2005) as it has no food-safety concerns (Mansell *et al.*, 2005; APVMA, 2010); it can, however, be toxic to some fish but it is related to water temperature (Treves-Brown, 2000). Hydrogen peroxide is also an approved treatment in Japan (Ogawa, 2005). Caprylic acid, a natural medium-chain fatty acid in coconut and other edible oils, tested *in vitro* against larvae and adults stopped larval movement immediately, caused

lysis within 25 min and death after 2 h, whereas adults contracted in 20 min but remained alive after a 2 h treatment (Hirazawa *et al.*, 2001). There are no published studies using caprylic acid in feed.

An anthelmintic, praziquantel, synthesized to treat endoparasitic flatworms of mammals, has been tested against a range of blood- and epidermal-feeding Monogenea from fish. Praziquantel is the active ingredient of Hadaclean® registered to treat *B. seriolae* in Japan. Williams *et al.* (2007) tested oral praziquantel efficacy against *B. seriolae* on *S. lalandi* in South Australia and determined fish fed a lower daily dose (50 and 75 mg/kg body weight (BW)/day for 6 days) had fewer parasites than fish fed a higher daily dose (100 and 150 mg/kg BW/day for 3 days) but noted highly medicated feed was unpalatable to fish. Assessing bioavailability and pharmacokinetics in *S. lalandi*, Tubbs and Tingle (2006) studied maximum praziquantel concentrations in skin and plasma when administered in solution and in feed. Results suggested oral treatment every 24 h may expose parasites to highly variable praziquantel concentrations. They recommended a dose interval of less than 24 h to potentially alleviate variable, sub-therapeutic praziquantel levels in host tissues and ensure it reaches feeding monogeneans.

Using skin epithelial extracts from *S. quinquerediata*, *Pagrus major* and *Paralichthys olivaceus*, Yoshinaga *et al.* (2002) developed an assay to assess larval attachment. No clear differences in the ability of the three extracts to induce larval attachment were found indicating that either the attachment-inducing capacity is not host specific or that the assay was insufficiently sensitive. Addition of the lectins wheat germ and concanavalin A to skin epithelial extracts from *S. quinquerediata* and *P. olivaceus* suppressed larval attachment suggesting that sugar-related chemicals are responsible.

Farm husbandry

Environmental parameters (water temperature, salinity) influence: (i) egg embryonation; (ii) hatching success; (iii) parasite growth; and (iv) development and fecundity (Japan: Hoshina, 1968; Ernst *et al.*, 2005; Mooney *et al.*, 2008; Australia: Ernst *et al.*, 2002; Lackenby

et al., 2007; New Zealand: Tubbs *et al.*, 2005). *In vitro* studies (e.g. Tubbs *et al.*, 2005) are less meaningful than those *in vivo* (e.g. Lackenby *et al.*, 2007; Mooney *et al.*, 2008) because parasite behaviour when attached to hosts is more representative than detached worms in dishes of sea water. Under *in vivo* conditions, Mooney *et al.* (2008) determined that *B. seriolae* on *S. quinqueriata* at ~24°C laid eggs continuously throughout the 24 h period with a mean egg production of ~58 eggs/worm/h. On farms, eggs tangle on net mesh (Fig. 13.1e; Ogawa and Yokoyama, 1998) but regular cleaning or net changes to reduce egg load may have limited efficacy at high summer temperatures when eggs hatch rapidly. Large cages and steel enclosures in Japan cannot be changed easily (Ogawa, 2005). Ernst *et al.* (2002) correlated egg retention on cage material with fouling organisms and noted up to 64,000 eggs/m² on nets in Japan which, if distributed evenly over one, 30 m diameter cage, was 165 million eggs!

Chambers and Ernst (2005) hypothesized that the largest contribution to reinfection of treated stock was from parasites on fish in nearby cages. They assessed infection pressure within and between neighbouring sea-cage leases in South Australia using fish sentinels free of infection. On the same farm, eggs in plankton samples were only found at sites in line with tidal current. Fish sentinels had higher infections when in line with, but not across, tidal current. Infection pressure between farm leases reduced with increased distance from infected stock. For effective parasite management in Spencer Gulf, South Australia, independent management units (IMUs; i.e. different farm leases) need to be more than 8 km apart due to dispersal of *B. seriolae* eggs. Farms arrange sea cages in line with currents to help maintain cage shape, for functional effectiveness and mooring efficacy. These perceived operational efficiencies may contribute to more efficient monogenean transmission (Chambers and Ernst, 2005). Intensity of sea cages and farms in South Australia is low and IMUs are possible.

Administration of bath or in-feed treatments requires strategically timed dual delivery for optimal results to kill adult parasite populations on fish (first treatment) followed

by another delivery (second treatment) to kill immature, growing parasites that invaded treated fish as larvae from eggs and oncomiracidia resident in and around the farm (Fig. 13.1e). Timing of the second treatment is important because it must kill all new recruits before they become egg layers. Successful treatment timing must use local water temperature and salinity data to predict parasite growth rates. Lackenby *et al.* (2007) assessed growth rates and age at sexual maturity for *B. seriolae* on farmed *S. lalandi* simulating annual seawater temperatures in Spencer Gulf. For maximum benefit, every cage on each farm or IMU must be treated within a short time frame.

13.5.2. *Neobenedenia* species

Protection

Nigrelli (1932) reported that black triggerfish (*Melichthys bispinosus*, now *Melichthys niger* (Balistidae)) heavily infected by '*N. melleni*' shed worms and were not reinfected and that some *Epinephelus* species demonstrated natural immunity throughout epizootics and were not parasitized. Bondad-Reantaso *et al.* (1995b) showed acquired protection by *P. olivaceus* against larval infection demonstrated by a reduction in number and size of worms on previously infected fish. No significant difference, however, was found in serum antibody levels between primed and control fish. Experimental inoculation of parasite homogenate indicated that protection from previous infections was not associated with a humoral antibody. In tilapia infected by '*N. melleni*' Robinson *et al.* (2008) showed that mucus of infected fish exhibited maximum parasite-killing activity 9 weeks after infection and continued until 15 weeks which corresponded with a decline in mean infestation intensity, but immunoassays failed to show evidence of a humoral response. Hatanaka *et al.* (2005) identified an antigen expressed on the ciliary surface of larval '*N. girellae*' from spotted halibut (*Verasper variegatus*) which under *in vitro* conditions caused agglutination/immobilization of oncomiracidia. Intraperitoneal injection of either sonicated or intact ciliary proteins with adjuvant induced

immunoglobulin production that, when injected into *P. olivaceus*, immobilized parasites *in vitro*. While this discovery may be useful for vaccination, it is unclear whether fish antibodies against this antigen prevent '*N. girellae*' infection (Hatanaka *et al.*, 2005). Studies have also characterized highly concentrated serum lectins in *V. variegatus* which bind to the ciliary surface glycoprotein and agglutinate '*N. girellae*' larvae *in vitro* (Hatanaka *et al.*, 2008). Experiments by Ohno *et al.* (2008) on susceptibility of different farmed fish species in Japan indicate that *S. dumerili* is more susceptible to '*N. girellae*' larvae than *S. quinquerediata* and *P. olivaceus*. Parasites grow fastest on *S. dumerili*, slowest on *P. olivaceus* and both species acquired partial protection against reinfection by '*N. girellae*'. According to Ogawa (2005), *P. olivaceus* is 'very susceptible' to '*N. girellae*'. *V. variegatus* is thought to be less susceptible to '*N. girellae*' than other cultured Japanese species and much must be determined about the biological functions of fish lectins including their potential role in pathogen immunity (Hatanaka *et al.*, 2008).

Control

In the NYA '*N. melleni*' has been relentless since the 1920s (personal communication: Dennis Thoney, Vancouver Aquarium, British Columbia, Canada, 1995; Alistair Dove, Georgia Aquarium, Atlanta, USA, 2001) and in aquaria globally (section 13.1). An initial step to control infections in aquaria is to quarantine fish *before* introduction into exhibition tanks. Nigrelli (1932) indicated that removal of fish species susceptible to '*N. melleni*' to a tank with circulation separate from the main NYA display 'has become one of the most effective means of controlling the parasites'.

Chemical control has been widely studied (Thoney and Hargis, 1991; Whittington and Chisholm, 2008). Nigrelli (1932) reported sodium chloride treatments in the NYA caused parasites to fall from hosts within 1 h after raising the relative water density to 1.035. In sea-cage aquaculture, freshwater baths are effective. Kaneko *et al.* (1988) dipped tilapia infected by '*N. melleni*' and recorded death of all parasites and 100% host survival after freshwater treatment for 120 s and 150 s.

By applying 2 min freshwater baths every 2–4 weeks across infected cages on the Hawaiian farm, the '*N. melleni*' population on tilapia declined. Freshwater bathing is used routinely to control *Neobenedenia* on several farmed fish species in South-east Asia (Leong, 1997), '*N. girellae*' in Japan (Ogawa and Yokoyama, 1998) and '*N. melleni*' off Brazil (Sanches, 2008). In laboratory experiments, Mueller *et al.* (1992) determined that '*N. melleni*' egg hatching failed from Florida red tilapia when exposed to fresh water for 72 h and for 96 h. Treatment for 5 days with hyposaline water (15 g/l) prohibited egg hatching and eliminated juveniles and adults from fish (Ellis and Watanabe, 1993). Similar studies at 25°C on '*N. girellae*' in Japanese experimental culture demonstrated that hyposalinities at 8, 17 and 24 ppt for 5 h reduced egg laying *in vitro*, lowered hatching rates when incubated for 15 days and numbers of non-swimming oncomiracidia were higher at 8 and 17 ppt over 5 h (Umeda and Hirazawa, 2004). In tanks in Mexico, a 60 min exposure to fresh water removed 99% of immature and adult *Neobenedenia* sp. from *Sphoeroides annulatus* (see Fajer-Ávila *et al.*, 2008). Failure to remove all parasites with prolonged freshwater treatment highlights broad variability that is probably dependent on the physiological tolerances of parasites and hosts. A 2 min freshwater bath, however, significantly increased susceptibility to reinfection (Ohno *et al.*, 2009). After treatment, a white mucoid material presumed to be host skin mucus was observed in the bath water and it was suggested loss of this layer probably reduced the resistance of treated *S. dumerili* and *S. quinquerediata* which led to increased reinfection by '*N. girellae*'. Other chemical baths for '*N. melleni*' include: (i) a 14 day treatment using 0.15–0.18 ppm copper sulfate; (ii) a 1 h bath in 250 ppm formalin; (iii) two to three treatments every 2–3 days using 0.5 ppm trichlorfon (Thoney and Hargis, 1991); and (iv) 1:2000 formalin for 10 min (Sanches, 2008).

As for *B. seriolae*, oral administration of chemical therapeutants in feed is also a major advance to treat *Neobenedenia* on cultured fish. Okabe (2000) recommended an oral praziquantel dose against '*N. girellae*' infecting *S. quinquerediata* of 150 mg/kg BW/day for

3 days. Hirazawa *et al.* (2004) investigated praziquantel against '*N.girellae*' on *V.variegatus* and 40 mg/kg BW/day for 11 days was strongly antiparasitic. Trials using a higher praziquantel dose for shorter durations (150 mg/kg BW/day for 3 days) caused appetite problems and strongly medicated feed was regurgitated (Hirazawa *et al.*, 2004) contrary to the study of Okabe (2000; see above). Antibiotics (oxytetracycline, florfenicol, ampicillin, erythromycin or sulfamonomethoxine) were not effective against '*N.girellae*' (see Ohno *et al.*, 2009).

Expense of chemical treatments (initial development, then field trials), possible toxicity to fish, barriers to approved use on food fish, deployment and regulation in industry and environmental concerns have stimulated studies seeking alternative control methods for '*N.girellae*'. In Japan, this pathogen causes heavy losses to six fish species (Table 13.1; Ogawa and Yokoyama, 1998; Hirazawa *et al.*, 2004; Ogawa, 2005). Buffers containing different metallic ions (Ca^{2+} , Mg^{2+}) were assessed *in vitro* and *in vivo* against '*N.girellae*' on *V.variegatus* and a significant effect against percentage parasite survival was found using Ca^{2+} / Mg^{2+} -free buffer: it disrupted worm intercellular junctions but did not affect hosts (Ohashi *et al.*, 2007a). Other approaches have investigated larval behavioural responses to potentially interfere with and reduce infection. Attachment-inducing capacities of various fish extracts for '*N.girellae*' larvae determined that fish skin epithelium but not gill, muscle and intestine were effective but no significant differences in attachment induction were detected between skin epithelia of *Oncorhynchus mykiss* (Salmonidae), *Pagrus major*, *Paralichthys olivaceus* and *S.quinqueradiata* (see Yoshinaga *et al.*, 2000). They showed that '*N.girellae*' larvae are phototactic. Infection trials by Ishida *et al.* (2007) using *P.olivaceus* and *V.variegatus* exposed to '*N.girellae*' larvae showed that black-and-white contrast was important for finding the host.

In the well-studied Caribbean '*N.melleni*'-Florida red tilapia sea-cage system, Cowell *et al.* (1993) compared the capacity of three tropical cleaner fish species to control parasites and determined that final infections on tilapia maintained without cleaner fish were

significantly greater in two of three experiments. They detected Monogenea in cleaner fish gut contents, found gobies were more effective than a labrid and suggested cleaning symbionts could provide biological control for '*N.melleni*' in sea-cage tilapia culture. Another Caribbean field experiment investigated the ability of cleaner shrimps to remove '*N.melleni*' from acanthurids for extended durations open to a constant, natural supply of infective larvae in large enclosures under semi-natural conditions (McCammon *et al.*, 2010). The study allowed shrimps access to natural habitat including alternative food sources but fish regularly visited shrimps. Pederson shrimp (*Periclimenes pedersoni*, Palaemonidae) significantly reduced the number and size of '*N.melleni*' from *Acanthurus coeruleus* (Acanthuridae), the primary host at their Virgin Islands' study site (Sikkel *et al.*, 2009).

Hirazawa *et al.* (2006) determined that '*N.girellae*' from *V.variegatus* in Japan has four serine proteases in adults and two in oncomiracidia. Proteinase inhibitors, pH and temperature inhibited swimming ability of larvae and suppressed egg laying under *in vitro* conditions and they concluded that serine proteases are important for parasite survival, but had no evidence of their functional significance. To clarify host specificity in '*N.girellae*', Ohashi *et al.* (2007b) purified a glycoprotein that induces larval attachment to skin of *Takifugu rubripes* and using N-terminal amino acid sequencing, identified it as Wap 65-2 but also found other, unidentified glycoproteins that influenced larval attachment. Interference with gametogenesis, a technique to sterilize pests that is used successfully to control crop-eating insects, was studied by Ohashi *et al.* (2007c) to isolate *vas*-related genes, a gene family with germ-cell-specific expression in many organisms. They isolated three *vas*-related cDNAs expressed in germ cells of '*N.girellae*' from *V.variegatus*, used RNA interference (RNAi) to achieve partial or complete germ cell loss and also noted significantly decreased egg hatching from parasites showing partial germ cell loss. By demonstrating that sterilized '*N.girellae*' can be generated by RNAi, Ohashi *et al.* (2007c) claimed it could pave the way for new control

methods by interfering with parasite reproduction. Delivery of this technique in marine aquaculture, however, will be problematic. In China, Rao and Yang (2007) focused on cysteine proteases which probably have many roles in parasites including feeding and digestion, host invasion and immune evasion. Using '*N. melleni*' from *Lutjanus sanguineus*, they investigated cathepsin L, isolated the full-length cDNA for a cathepsin L-like cysteine protease, determined its expression in swimming larvae, juveniles and adults but not in fresh eggs or newly hatched oncomiracidia. This was interpreted as evidence that cathepsin L is important for growth and to maintain the parasite–host association.

13.6. Conclusions and Suggestions for Future Studies

13.6.1. Farm husbandry, Integrated Parasite Management (IPM) and mathematical models

Detailed knowledge of monogenean biology, transmission, life cycle, potential biological control and chemical intervention combined into a well-conceived, strategic plan using best practice husbandry is needed to establish IPM. But if '*N. melleni*' control in aquaria has been difficult, is there hope for capsalid control in sea cages where segregation of fish from pathogens is impractical? Chambers and Ernst (2005) recognized the value of IMUs for IPM for *B. seriolae* on *S. lalandi* in South Australia. Methods used to control sea lice on salmon farms (e.g. site fallowing, strict separation of fish year classes in separate IMUs and regular cage relocation to new sites) will probably contribute positively to IPM where sea-cage and farm-lease density is low. In South-east Asia, many small independent farms operate in a finite area precluding IMUs and IPM unless cage and farm density and their arrangement and management are addressed. This requires a significant culture change. Without this, however, 'control' in intense culture is improbable. In South Australia, knowledge of local factors that influence the *B. seriolae* life cycle (Chambers and

Ernst, 2005; Lackenby *et al.*, 2007) is applied to minimize infections. Three-dimensional numerical models have predicted dispersal of sea lice between wild and farmed salmon (Amundrud and Murray, 2009; Murray, 2009). Capsalid management could be achieved using mathematical models to integrate all available parasite data. Monitoring to establish population size, fecundity, egg viability, dispersion and transmission of eggs and larvae, background infection levels and stage survival and mortality between infection sources (cages, leases, farms) and throughout bays and gulfs should be integrated with local oceanographic information. These data would improve timing of strategic control measures (e.g. cage cleaning, cage changes and chemical intervention) but may only benefit South-east Asian farms if spatial and temporal coordination of husbandry was viable.

13.6.2. Biological control

Cleaner organisms (fish, shrimp) probably exist even in temperate waters. Observations by diving clubs on cleaning symbioses in fish-farming regions could provide beneficial data about potential local biological controls but risks of co-culture need thorough assessment (e.g. Treasurer and Cox, 1991). Grazing herbivorous fish could reduce algal fouling on sea cages but investigations must ensure they are not infection reservoirs for capsalids or other pathogens.

13.6.3. Chemical treatments versus vaccines

Chemicals, applied as baths or in feed, if delivered against recommended guidelines (e.g. lower concentrations to cut costs), can lead to sub-therapeutic doses raising the likelihood of the emergence of resistance. Thoney and Hargis (1991) reported acquired resistance to trichlorfon in '*N. melleni*'. Highly variable praziquantel concentrations in *S. lalandi* serum and skin (Tubbs and Tingle, 2006) suggest its wide use in feed may be ineffective and could lead to resistance. If

geographically widespread resistance to hydrogen peroxide and/or praziquantel developed, no effective alternative products are currently available to treat capsalids. Social change, however, has turned against chemical use in food production. Multidisciplinary approaches incorporating parasitologists, veterinarians, statisticians, chemists, nutritionists, physiologists, ecologists and economists are needed to develop well-designed trials to ensure that environmentally responsible antiparasitic compounds reach parasites at appropriate dose and cost. In feed, immunostimulants to boost the immune system of captive fish is another viable therapy. Future research, however, is likely to explore vaccines. Innate and acquired immunity against Monogenea is implied and mucus is important (Buchmann, 1999). Host responses are probably not uncommon (Buchmann and Bresciani, 2006) but are poorly understood. Initial vaccines for Monogenea are likely in the *Gyrodactylus salaris*–salmonid association (Chapter 11). Immunoprophylaxis against capsalids requires detailed studies on protection mechanisms to select optimum candidate antigens, adjuvants and formulations for field trials. Benefits of vaccines versus chemicals include specific and sustained action within fish and no environmental impact, withdrawal period or flesh residues. Host responses against many monogeneans are only partially expressed suggesting the parasites may secrete immune evasion or immunosuppressive substances (Buchmann and Bresciani, 2006), a valuable focus using new technologies.

13.6.4. New technologies

Advanced sequencing enables huge volumes of genetic data to be generated cheaply. Whole genomes are therefore a reality for fish and their parasites. Parasite genomics will provide data which, with appropriate bioinformatics, may help predict and identify new drug targets against reproduction, feeding, metabolism, neurotransmitters and immune evasion. Isolation, characterization and expression of genes and their products will help us to interfere with a parasite's ability to infect, establish,

feed and reproduce. RNAi can produce mutant, deficient and knockdown parasites and hosts to expand knowledge of the parasite–host association (Sitjà-Bobadilla, 2008). Characterization of fish immune mechanisms may help control '*N. girellae*' infections of *P. olivaceus* and *T. rubripes* because continuous cell lines for these fish are developed (Alvarez-Pellitero, 2008) enabling studies of their immune systems and *in vitro* parasite cultivation. Advanced genetic techniques on resistant versus susceptible hosts may also shed light on parasite-resistant fish strains (Sitjà-Bobadilla, 2008) to breed for culture. What induces capsalid larvae to attach to hosts is inconclusive but glycoproteins, proteoglycans and polysaccharides are implicated (Yoshinaga *et al.*, 2000, 2002). Knowledge of oncomiracidial attraction to hosts and host specificity could help develop 'traps' to guide parasite larvae away from fish stocks. This information could also be used to selectively breed or genetically modify hosts devoid of larval attractant and/or settlement cues. Gene technology to investigate and synthesize natural marine antifoulants could reduce sea-cage fouling and so reduce entanglement of capsalid eggs.

13.6.5. Capsalid biology, ecology and identity

New technologies, however, should not replace fundamental studies of parasite biology, ecology and identity where multidisciplinary approaches are necessary. Detailed studies on feeding and attachment have value. A quantitative assessment of the volume of epidermis ingested per unit time by adult *B. seriolae* and *Neobenedenia* species could inform farm managers about total parasite population trigger levels to alert when stock *must* be treated to prevent disease and death. Specificity by *B. seriolae* for several *Seriola* species is known, but lack of specificity in '*Neobenedenia* species' is mysterious. My view that '*N. melleni*' and '*N. girellae*' represent complexes of morphologically indistinguishable species (Whittington *et al.*, 2004; Whittington, 2004, 2005) is not demonstrated.

Partial 28S sequence data showed two geographically widespread samples identified morphologically as '*N. melleni*' differed genetically (Whittington *et al.*, 2004). Wang *et al.* (2004) also used partial 28S sequence data to compare '*N. melleni*' and '*N. girellae*' from Chinese farms but found little genetic diversity. Li *et al.* (2005) used internal transcribed spacer region 1 (ITS1) and partial 28S sequence data and PCR-based single strand conformation polymorphism (SSCP) to compare several capsalids including '*N. melleni*' and '*N. girellae*' in Chinese aquaculture but found identical SSCP bands and sequence data. These studies indicate that genes used to assess differences between '*Neobenedenia* species' are not ideal. Appropriate spatial and temporal sampling strategies are needed for *Neobenedenia* populations throughout their distribution from wild hosts to compare with samples from cultured stock. To resolve identity, mounted vouchers for morphological study and vouchers in undenatured ethanol for future DNA analyses using improved

markers, must be deposited in museums (Whittington, 2004). A multi-locus approach including nuclear coding genes and mitochondrial markers is likely to help clarify the biology, ecology and identity of *Neobenedenia* species.

Acknowledgements

I thank T. Benson and L. Chisholm (South Australian Museum, Adelaide), M. Deveney (Marine Biosecurity, South Australian Research and Development Institute, Aquatic Sciences, Adelaide) and E. Perkins (Heron Island Research Station) for valuable comments on a previous draft. D. Vaughan (Aquatic Animal Health Research, Two Oceans Aquarium, Cape Town, South Africa) provided helpful advice on aquarium husbandry. I. Ernst (Aquatic Animal Health Program, Australian Government Department of Agriculture, Fisheries and Forestry, Canberra) gave permission to use the image in Fig. 13.2a.

References

- Alvarez-Pellitero, P. (2008) Fish immunity and parasite infections: from innate immunity to immunophy-lactic prospects. *Veterinary Immunology and Immunopathology* 126, 171–198.
- Amundrud, T.L. and Murray, A.G. (2009) Modelling sea lice dispersion under varying environmental forcing in a Scottish sea loch. *Journal of Fish Diseases* 32, 27–44.
- Australian Pesticides and Veterinary Medicines Authority (APVMA) (2010) APVMA. Australian Govern-ment. Available at: <http://permits.apvma.gov.au/PER12169.PDF> (accessed 5 August 2010).
- Bondad-Reantaso, M.G., Ogawa, K., Fukudome, M. and Wakabayashi, H. (1995a) Reproduction and growth of *Neobenedenia girellae* (Monogenea: Capsalidae), a skin parasite of cultured marine fishes of Japan. *Fish Pathology* 30, 227–231.
- Bondad-Reantaso, M.G., Ogawa, K., Yoshinaga, T. and Wakabayashi, H. (1995b) Acquired protection against *Neobenedenia girellae* in Japanese flounder. *Fish Pathology* 30, 233–238.
- Brooks, D.R. and Mayes, M.A. (1975) *Phyllodistomum scrippsi* sp. n. (Digenea: Gorgoderidae) and *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963 (Monogenea: Capsalidae) from the California sheephead, *Pimelometopon pulchrum* (Ayres) (Pisces: Labridae). *Journal of Parasitology* 61, 407–408.
- Buchmann, K. (1999) Immune mechanisms in fish skin against monogeneans – a model. *Folia Parasitologica* 46, 1–9.
- Buchmann, K. and Bresciani, J. (2006) *Monogenea (Phylum Platyhelminthes)*. In: Woo, P.T.K. (ed.) *Fish Diseases and Disorders – Volume 1. Protozoan and Metazoan Infections*, 2nd edn. CABI Publishing, Wallingford, Oxon, UK.
- Bullard, S.A., Goldstein, R.J., Hocking, R. and Jewell, J. (2003) A new geographic locality and three new host records for *Neobenedenia melleni* (MacCallum) (Monogenea: Capsalidae). *Gulf and Caribbean Research* 15, 1–4.
- Carvalho, A.R. and Luque, J.L. (2009) Ocorrência de *Neobenedenia melleni* (Monogenea; Capsalidae) em *Trichiurus lepturus* (Perciformes; Trichiuridae), naturalmente infestados, no litoral do Rio de Janeiro, Brasil. *Revista Brasileira de Parasitologia Veterinária, Jaboticabal* 18, 74–76.

- Chambers, C.B. and Ernst, I. (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.
- Colomni, A. (1994) Hyperparasitism of *Amyloodinium ocellatum* (Dinoflagellidae: Oodiniidae) on *Neobenedenia melleni* (Monogenea: Capsalidae). *Diseases of Aquatic Organisms* 19, 157–159.
- Cowell, L.E., Watanabe, W.O., Head, W.D., Grover, J.J. and Shenker, J.M. (1993) Use of tropical cleaner fish to control the ectoparasite *Neobenedenia melleni* (Monogenea: Capsalidae) on seawater-cultured Florida red tilapia. *Aquaculture* 113, 189–200.
- Deveney, M.R., Chisholm, L.A. and Whittington, I.D. (2001) First published record of the pathogenic monogenean parasite *Neobenedenia melleni* (Capsalidae) from Australia. *Diseases of Aquatic Organisms* 46, 79–82.
- Diggles, B. and Hutson, K.S. (2005) Diseases of kingfish (*Seriola lalandi*) in Australasia. *Aquaculture Health International* 3, 12–14.
- Egusa, S. (1983) Disease problems in Japanese yellowtail, *Seriola quinqueradiata*, culture: a review. *Rapports et Proces-verbaux des Reunion Conseil International pour Exploration de la Mer* 182, 10–18.
- Ellis, E.P. and Watanabe, W.O. (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., Whittington, I., Corneillie, S. and Talbot, C. (2002) Monogenean parasites in sea-cage aquaculture. *Austasia Aquaculture* February/March, 46–48.
- Ernst, I., Whittington, I.D., Corneillie, S. and Talbot, C. (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.
- Fajer-Ávila, E.J., Roque, A., Aguilar, G. and Duncan, N. (2004) Patterns of occurrence of the platyhelminth parasites of the wild bullseye puffer (*Sphoeroides annulatus*) off Sinaloa, Mexico. *Journal of Parasitology* 90, 415–418.
- Fajer-Ávila, E.J., Martínez-Rodríguez, I., Abdo de la Parra, M.I., Álvarez-Lajonchere, L. and Betancourt-Lozano, M. (2008) Effectiveness of freshwater treatment against *Lepeophtheirus simplex* (Copepoda: Caligidae) and *Neobenedenia* sp. (Monogenea: Capsalidae), skin parasites of bullseye puffer fish, *Sphoeroides annulatus* reared in tanks. *Aquaculture* 284, 277–280.
- Gaida, I.H. and Frost, P. (1991) Intensity of *Neobenedenia girellae* (Monogenea: Capsalidae) on the half-moon, *Medialuna californiensis* (Perciformes: Kyphosidae), examined using a new method for detection. *Journal of the Helminthological Society of Washington* 58, 129–130.
- González, M.T. and Acuña, E. (1998) Metazoan parasites of the red rockfish *Sebastes capensis* off northern Chile. *Journal of Parasitology* 84, 783–788.
- Grau, A., Riera, F. and Carbonell, E. (1999) Some protozoan and metazoan parasites of the amberjack from the Balearic Sea (western Mediterranean). *Aquaculture International* 7, 307–317.
- Hatanaka, A., Umeda, N., Yamashita, S. and Hirazawa, N. (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.
- Hatanaka, A., Umeda, N. and Hirazawa, N. (2008) Characterization of highly concentrated serum lectins in spotted halibut *Verasper variegatus*. *Parasitology* 135, 359–369.
- Hayward, C.J. (2005) Monogenea Polyopisthocotylea (ectoparasitic flukes). In: Rohde, K. (ed.) *Marine Parasitology*. CSIRO Publishing, Melbourne, Australia, pp. 55–63.
- Hirayama, T., Kawano, F. and Hirazawa, N. (2009) Effect of *Neobenedenia girellae* (Monogenea) infection on host amberjack *Seriola dumerili* (Carangidae). *Aquaculture* 288, 159–165.
- Hirazawa, N., Oshima, S. and Hata, K. (2001) *In vitro* assessment of the antiparasitic effect of caprylic acid against several fish parasites. *Aquaculture* 200, 251–258.
- Hirazawa, N., Mitsuboshi, T., Hirata, T. and Shirasu, K. (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., Umeda, N., Hatanaka, A. and Kuroda, A. (2006) Characterization of serine proteases in the monogenean *Neobenedenia girellae*. *Aquaculture* 255, 188–195.
- Hirazawa, N., Takano, R., Hagiwara, H., Noguchi, M. and Narita, M. (2010) The influence of different water temperatures on *Neobenedenia girellae* (Monogenea) infection, parasite growth, egg production and emerging second generation on amberjack *Seriola dumerili* (Carangidae) and the histopathological effect of this parasite on fish skin. *Aquaculture* 299, 2–7.

- Hoshina, T. (1968) On the monogenetic trematode, *Benedenia seriola*, parasitic on yellow-tail, *Seriola quinqueradiata*. *Bulletin de l'Office International des Epizooties* 69, 1179–1191.
- Hutson, K.S., Ernst, I., Mooney, A.J. and Whittington, I.D. (2007a) Metazoan parasite assemblages of wild *Seriola lalandi* (Carangidae) from eastern and southern Australia. *Parasitology International* 56, 95–105.
- Hutson, K.S., Ernst, I. and Whittington, I.D. (2007b) Risk assessment for metazoan parasites of yellowtail kingfish *Seriola lalandi* (Perciformes: Carangidae) in South Australian sea-cage aquaculture. *Aquaculture* 271, 85–99.
- Ishida, M., Kawano, F., Umeda, N. and Hirazawa, N. (2007) Response of *Neobenedenia girellae* (Monogenea) oncomiracidia to brightness and black-and-white contrast. *Parasitology* 134, 1821–1830.
- Jahn, T.L. and Kuhn, L.R. (1932) The life history of *Epibdella melleni* MacCallum, 1927, a monogenetic trematode parasitic on marine fishes. *Biological Bulletin (Woods Hole)* 62, 89–111.
- Kaneko, J.J., Yamada, R., Brock, J.A. and Nakamura, R.M. (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.
- Kearn, G.C. (1963) Feeding in some monogenean skin parasites: *Entobdella soleae* on *Solea solea* and *Acanthocotyle* sp. on *Raia clavata*. *Journal of the Marine Biological Association of the United Kingdom* 43, 749–766.
- 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. (1998) *Parasitism and the Platyhelminths*. Chapman and Hall, London, UK.
- Kearn, G.C. (1999) The survival of monogenean (platyhelminth) parasites on fish skin. *Parasitology* 119, S57–S88.
- Kinami, R., Miyamoto, J., Yoshinaga, T., Ogawa, K. and Nagakura, Y. (2005) A practical method to distinguish between *Neobenedenia girellae* and *Benedenia seriola*. *Fish Pathology* 40, 63–66.
- Koesharyani, I., Zafran, Yuasa, K. and Hatai, K. (1999) Two species of capsalid monogeneans infecting cultured humpback grouper *Cromileptes altivelis* in Indonesia. *Fish Pathology* 34, 165–166.
- Lackenby, J.A., Chambers, C.B., Ernst, I. and Whittington, I.D. (2007) Effect of water temperature on reproductive development of *Benedenia seriola* (Monogenea: Capsalidae) from *Seriola lalandi* in Australia. *Diseases of Aquatic Organisms* 74, 235–242.
- Leef, M.J. and Lee, P.S. (2009) Preliminary investigation into the killing effect of kingfish (*Seriola lalandi*) serum and mucus against the monogenean parasites *Benedenia seriola* and *Zeuxapta seriola*. *Aquaculture International* 17, 607–614.
- Leong, T.S. (1997) Control of parasites in cultured marine finfishes in Southeast Asia – an overview. *International Journal for Parasitology* 27, 1177–1184.
- Li, A.-X., Wu, X.-Y., Ding, X.-J., Lin, R.-Q., Xie, M.-Q., Lun, Z.-R. and Zhu, X.-Q. (2005) PCR-SSCP as a molecular tool for the identification of Benedeniinae (Monogenea: Capsalidae) from marine fish. *Molecular and Cellular Probes* 19, 35–39.
- Liao, I.C., Huang, T.S., Tsai, W.S., Hsueh, C.M., Chang, S.L. and Leaño, E.M. (2004) Cobia culture in Taiwan: current status and problems. *Aquaculture* 237, 155–165.
- Llewellyn, J. (1957) Host-specificity in monogenetic trematodes. In: Baer, J.G. (ed.) *First Symposium on Host-Specificity Among Parasites of Invertebrates*. P. Attinger, Neuchâtel, Switzerland, pp. 199–212.
- Lopez, C., Rajan, P.R., Lin, J.H.-Y., Kuo, T.-Y. and Yang, H.-L. (2002) Disease outbreak in seafarmed cobia (*Rachycentron canadum*) associated with *Vibrio* spp., *Photobacterium damsela* ssp. *piscicida*, monogenean and myxosporean parasites. *Bulletin of the European Association of Fish Pathologists* 22, 206–211.
- MacCallum, G.A. (1927) A new ectoparasitic trematode, *Epibdella melleni*, sp. nov. *Zoopathologica* 1, 291–300.
- McCammon, A., Sikkil, P.C. and Nemeth, D. (2010) Effects of three Caribbean cleaner shrimps on ectoparasitic monogeneans in a semi-natural environment. *Coral Reefs* 29, 419–426.
- Mansell, B., Powell, M.D., Ernst, I. and Nowak, B.F. (2005) Effects of the gill monogenean *Zeuxapta seriola* (Meserve, 1938) and treatment with hydrogen peroxide on pathophysiology of kingfish, *Seriola lalandi* Valenciennes, 1833. *Journal of Fish Diseases* 28, 253–262.
- Mooney, A.J., Ernst, I. and Whittington, I.D. (2008) Egg-laying patterns and *in vivo* egg production in the monogenean parasites *Heteraxine heterocerca* and *Benedenia seriola* from Japanese yellowtail *Seriola quinqueradiata*. *Parasitology* 135, 1295–1302.
- Murray, A.J. (2009) Using simple models to review the application and implications of different approaches used to simulate transmission of pathogens among aquatic animals. *Preventative Veterinary Medicine* 88, 167–177.

- Mueller, K.W., Watanabe, W.O. and Head, W.D. (1992) Effect of salinity on hatching in *Neobenedenia melleni*, a monogenean ectoparasite of seawater-cultured tilapia. *Journal of the World Aquacultural Society* 23, 199–204.
- Nigrelli, R.F. (1932) The life history and control of a destructive fish parasite at the New York Aquarium. *Bulletin of the New York Zoological Society* 34, 123–129.
- Ogawa, K. (2005) Effects in finfish culture. In: Rohde, K. (ed.) *Marine Parasitology*. CSIRO Publishing, Melbourne, Australia, pp. 378–391.
- Ogawa, K. and Yokoyama, H. (1998) Parasitic diseases of cultured marine fish in Japan. *Fish Pathology* 33, 303–309.
- Ogawa, K., Bondad-Reantaso, M.G., Fukudome, M. and Wakabayashi, H. (1995) *Neobenedeniagirellae* (Hargis, 1955) Yamaguti, 1963 (Monogenea: Capsalidae) from cultured marine fishes of Japan. *Journal of Parasitology* 81, 223–227.
- Ogawa, K., Miyamoto, J., Wang, H.-C., Lo, C.-F. and Kou, G.-H. (2006) *Neobenedeniagirellae* (Monogenea) infection of cultured cobia *Rachycentron canadum* in Taiwan. *Fish Pathology* 41, 51–56.
- Ohashi, H., Umeda, N., Hirazawa, N., Ozaki, Y., Miura, C. and Miura, T. (2007a) Antiparasitic effect of calcium and magnesium ion-free buffer treatments against a common monogenean *Neobenedeniagirellae*. *Parasitology* 134, 229–236.
- Ohashi, H., Umeda, N., Hirazawa, N., Ozaki, Y., Miura, C. and Miura, T. (2007b) Purification and identification of a glycoprotein that induces the attachment of oncomiracidia of *Neobenedeniagirellae* (Monogenea, Capsalidae). *International Journal for Parasitology* 37, 1483–1490.
- Ohashi, H., Umeda, N., Hirazawa, N., Ozaki, Y., Miura, C. and Miura, T. (2007c) Expression of *vasa* (*vas*)-related genes in germ cells and specific interference with gene functions by double-stranded RNA in the monogenean, *Neobenedeniagirellae*. *International Journal for Parasitology* 37, 515–523.
- Ohno, Y., Kawano, F. and Hirazawa, N. (2008) Susceptibility by amberjack (*Seriola dumerili*), yellowtail (*S. quinqueradiata*) and Japanese flounder (*Paralichthys olivaceus*) to *Neobenedeniagirellae* (Monogenea) infection and their acquired protection. *Aquaculture* 274, 30–35.
- Ohno, Y., Kawano, F. and Hirazawa, N. (2009) The effect of oral antibiotic treatment and freshwater bath treatment on susceptibility to *Neobenedeniagirellae* (Monogenea) infection of amberjack (*Seriola dumerili*) and yellowtail (*S. quinqueradiata*) hosts. *Aquaculture* 292, 248–251.
- Okabe, K. (2000) Chemotherapeutic drug (Hada-clean) of oral administering type to control fish parasites. *Doyaku Kenkyu* 60, 1–12 (in Japanese).
- Perkins, E.M., Donnellan, S.C., Bertozzi, T., Chisholm, L.A. and Whittington, I.D. (2009) Looks can deceive: molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification. *Molecular Phylogenetics and Evolution* 52, 705–714.
- Prieto, A., Fajer, E., Cartaya, R. and Vinjoy, M. (1986) *Oreochromis aureus* cultivada en ambiente marino. *Benedenia* sp. (Monogenea: Capsalidae) en tilapia. Primera comunicación. *Revista de Salud Animal* 8, 141–145.
- Rao, Y.Z. and Yang, T.B. (2007) cDNA cloning, mRNA expression and recombinant expression of a cathepsin L-like cysteine protease from *Neobenedenia melleni* (Monogenea: Capsalidae). *Aquaculture* 269, 41–51.
- Robinson, R.D., O'Connor, N.P.G. and Steele, R.D. (2008) Interactions between cage-cultured hybrid tilapia and a marine monogenean, *Neobenedenia melleni*, in Jamaica. *North American Journal of Aquaculture* 70, 68–73.
- Rückert, S., Palm, H.W. and Klimpel, S. (2008) Parasite fauna of seabass (*Lates calcarifer*) under mariculture conditions in Lampung Bay, Indonesia. *Journal of Applied Ichthyology* 24, 321–327.
- Sanches, E.G. (2008) Controle de *Neobenedenia melleni* (MacCallum, 1927) (Monogenea: Capsalidae) em garoupa-verdadeira, *Epinephelus marginatus* (Lowe, 1834), cultivada em tanques-rede. *Revista Brasileira de Parasitologia Veterinária* 17, 145–149.
- Sato, S., Hirayama, T. and Hirazawa, N. (2008) Lipid content and fatty acid composition of the monogenean *Neobenedeniagirellae* and comparison between the parasite and host fish species. *Parasitology* 135, 967–975.
- Sharp, N., Poortenaar, C.W., Diggles, B.K. and Willis, T.J. (2003) Metazoan parasites of yellowtail kingfish, *Seriola lalandi*, in New Zealand: prevalence, intensity, and site preference. *New Zealand Journal of Marine and Freshwater Research* 37, 273–282.
- Sharp, N.J., Diggles, B.K., Poortenaar, C.W. and Willis, T.J. (2004) Efficacy of Aqu-i-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.

- Sikkel, P.C., Nemeth, D., McCammon, A. and Williams, E.H. Jr (2009) Habitat and species differences in prevalence and intensity of *Neobenedenia melleni* (Monogenea: Capsalidae) on sympatric Caribbean surgeonfishes (Acanthuridae). *Journal of Parasitology* 95, 63–68.
- Sitjà-Bobadilla, A. (2008) Living off a fish: a trade-off between parasites and the immune system. *Fish and Shellfish Immunology* 25, 358–372.
- Thoney, D.A. and Hargis, W.J. Jr (1991) Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annual Review of Fish Diseases* 1, 133–151.
- Treasurer, J. and Cox, D. (1991) The occurrence of *Aeromonas salmonicida* in wrasse (Labridae) and implications for Atlantic salmon farming. *Bulletin of the European Association of Fish Pathologists* 11, 208–210.
- Treves-Brown, K.M. (2000) *Applied Fish Pharmacology*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Tubbs, L.A. and Tingle, M.D. (2006) Bioavailability and pharmacokinetics of a praziquantel bolus in kingfish *Seriola lalandi*. *Diseases of Aquatic Organisms* 69, 233–238.
- Tubbs, L.A., Poortenaar, C.W., Sewell, M.A. and Diggles, B.K. (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.
- Umeda, N. and Hirazawa, N. (2004) Response of the monogenean *Neobenedenia girellae* to low salinities. *Fish Pathology* 39, 105–107.
- Wang, J., Zhang, W., Su, Y. and Ding, S. (2004) Genetic relationship between *Neobenedenia girellae* and *N. melleni* inferred from 28S rRNA sequences. *Acta Oceanologica Sinica* 23, 709–716.
- 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. (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: Rohde, K. (ed.) *Marine Parasitology*. CSIRO Publishing, Melbourne, Australia, pp. 63–72.
- Whittington, I.D. and Chisholm, L.A. (2008) Diseases caused by Monogenea. In: Eiras, J.C., Segner, H., Wahli, T. and Kapoor, B.G. (eds) *Fish Diseases Volume 2*. Science Publishers Inc., Enfield, New Hampshire, USA, pp. 683–816.
- Whittington, I.D. and Horton, M.A. (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., Cribb, B.W., Hamwood, T.E. and Halliday, J.A. (2000) Host-specificity of monogenean (platyhelminth) parasites: a role for anterior adhesive areas? *International Journal for Parasitology* 30, 305–320.
- Whittington, I.D., Corneillie, S., Talbot, C., Morgan, J.A.T. and Adlard, R.D. (2001a) 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., Ernst, I., Corneillie, S. and Talbot, C. (2001b) Sushi, fish and parasites. *Australasian Science* 22(3), 33–36.
- Whittington, I.D., Deveney, M.R. and Wyborn, S.J. (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.
- Whittington, I.D., Deveney, M.R., Morgan, J.A.T., Chisholm, L.A. and Adlard, R.D. (2004) A preliminary phylogenetic analysis of the Capsalidae (Platyhelminthes: Monogenea: Monopisthocotylea) inferred from large subunit rDNA sequences. *Parasitology* 128, 511–519.
- Williams, R.E., Ernst, I., Chambers, C.B. and Whittington, I.D. (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.
- Yamaguti, S. (1934) Studies on the helminth fauna of Japan. Part II. Trematodes of fishes I. *Japanese Journal of Zoology* 5, 249–541.
- Yoshinaga, T., Nagakura, T., Ogawa, K. and Wakabayashi, H. (2000) Attachment-inducing capacities of fish tissue extracts on oncomiracidia of *Neobenedenia girellae* (Monogenea, Capsalidae). *Journal of Parasitology* 86, 214–219.
- Yoshinaga, T., Nagakura, T., Ogawa, K., Fukuda, Y. and Wakabayashi, H. (2002) Attachment-inducing capacities of fish skin epithelial extracts on oncomiracidia of *Benedenia seriolae* (Monogenea: Capsalidae). *International Journal for Parasitology* 32, 381–384.

14 *Heterobothrium okamotoi* and *Neoheterobothrium hirame*

Kazuo Ogawa

Department of Aquatic Bioscience, The University of Tokyo, Tokyo, Japan

Heterobothrium okamotoi Ogawa, 1991 and *Neoheterobothrium hirame* Ogawa, 1999 belong to the family Diclidophoridae (Monogenea: Polyopisthocotylea). Infection with the two parasites causes serious disease in their respective host, tiger puffer (*Takifugu rubripes*; Tetraodontidae) and olive flounder or Japanese flounder (*Paralichthys olivaceus*; Paralichthyidae). They share many features concerning biology and pathological effects on their hosts. However, they differ from each other in their origin: *H. okamotoi* is a parasite indigenous to Japan, whereas *N. hirame* is an introduced parasite. Besides, *H. okamotoi* infection is a problem in aquaculture, whereas *N. hirame* infection is primarily a problem with wild fish populations.

14.1. *Heterobothrium okamotoi*

14.1.1. Introduction

Monogeneans of the genus *Heterobothrium* infect tetraodontid fishes. Four species have been described in Japan, all hosts being members of the genus *Takifugu* (Tetraodontidae) (Ogawa, 1991). The parasites are species specific, and *H. okamotoi* is known only from the tiger puffer (*T. rubripes*).

H. okamotoi infection was first reported from tiger puffer cultured in the Inland Sea in western Japan (Okamoto, 1963). Because of

its high market value, puffer was cultured in the 1950s–1960s by maintaining fish, caught in the spring and summer, in enclosures until marketed in the winter. Without knowledge of effective control measures, this parasitic disease was a major limiting factor in puffer culture at that time (Okamoto, 1963). Since the 1980s, when artificially produced seedlings were introduced, tiger puffer has been cultured in more locations and on a larger scale in floating net cages. Most typically juvenile puffers are introduced into net cages in the summer and cultured for 1.5 years until the winter of the following year. *H. okamotoi* propagates readily in this culture system, and its infection has since been a recurrent disease problem. This is mainly because of its high fecundity and production of long egg filaments which entangle with the culture nets.

H. okamotoi is a large monogenean, up to 23 mm long, with the body proper, attenuated posteriorly in the form of isthmus and haptor bearing four pairs of clamps of typical diclidophorid-type at its posterior end (Fig. 14.1; Ogawa, 1991). Adult worms infect the branchial cavity wall of the host (Okamoto, 1963; Ogawa and Inouye, 1997a), which is different from typical diclidophorids that infect the gills. In most cases, the site of attachment is on the ventral side of the branchial cavity wall close to the gills. A few to dozens of worms are clustered in heavily infected fish (Fig. 14.2).

Its life cycle is relatively straightforward (Fig. 14.3). Eggs are connected, at both ends, with previous and successive ones through a continuous filament, forming a long egg string (Fig. 14.4; Ogawa, 1997). Eggs hatch and oncomiracidia settle on the gill filaments.

Post-larvae are first found on the basal part of the gill filaments, then with the development of clamps, they gradually move towards the distal part, and migrate to the branchial cavity wall after they grow on the gills for 1–1.5 months (Ogawa and Inouye, 1997a, b).

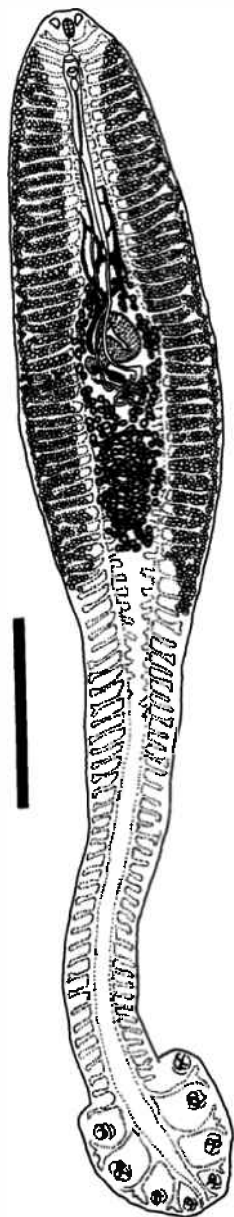


Fig. 14.1. Line drawing of *Heterobothrium okamotoi* Ogawa, 1991. Bar = 3 mm (from Ogawa, 1991).



Fig. 14.2. Adults of *H. okamotoi* on the branchial cavity wall of tiger puffer (*Takifugu rubripes*). The posterior part of the body is embedded in the host tissue. Note some of them group together to form a cluster. Photo by M. Nakane.

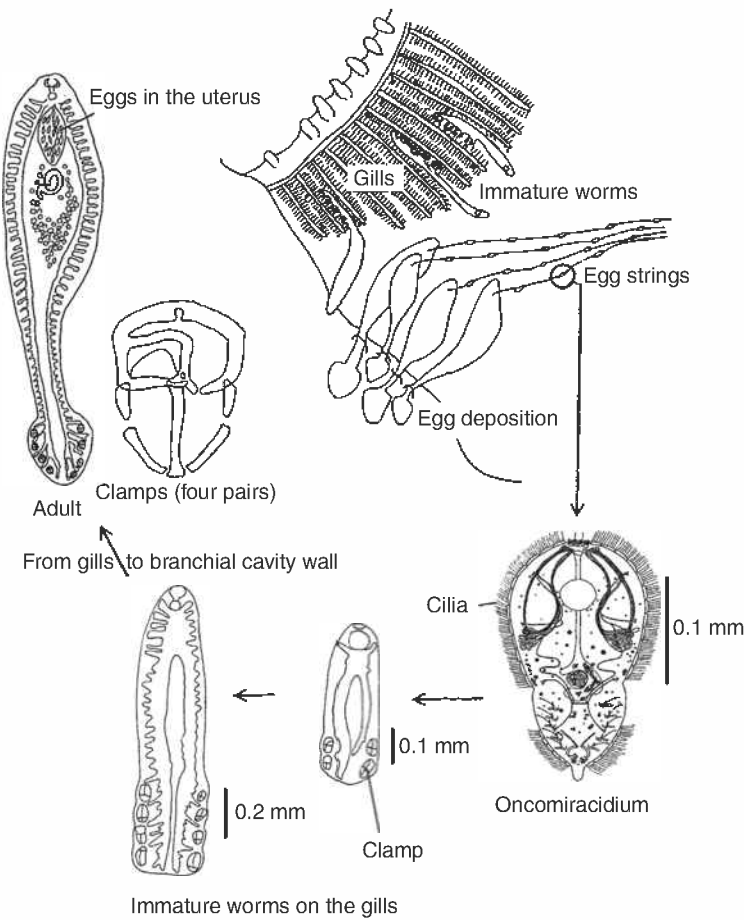


Fig. 14.3. Life cycle of *H. okamotoi*.

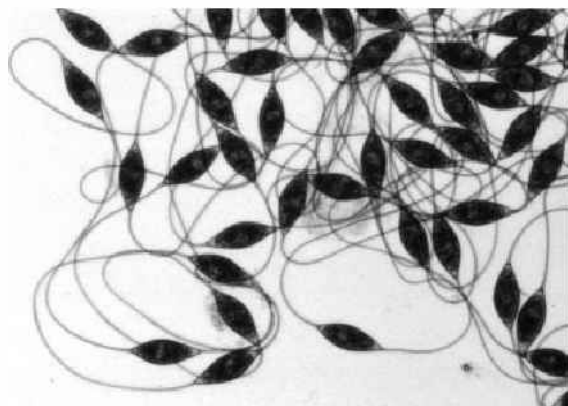


Fig. 14.4. Egg string of *H. okamotoi* (from Ogawa, 2002).

There is only one report of *Heterobothrium* infection in wild tiger puffers caught in the Inland Sea (Okamoto and Ogasawara, 1965); only older fish (2+ years) were infected. However, infection among cultured tiger puffer is common. It was detected in all cultured areas in western and southern Japan surrounded by the Pacific, the East China Sea and the Sea of Japan. Tiger puffer cultured in China was also infected with this monogenean (K. Ogawa, unpublished observation). *H. okamotoi* is highly host specific as well as highly site specific (Ogawa, 1991; Ogawa and Inouye, 1997a; Ohhashi *et al.*, 2007). No similar monogeneans have been recorded from tiger puffer (Ogawa and Yokoyama, 1998).

14.1.2. Diagnosis of the infection

The posterior body part (isthmus and haptor) of *H. okamotoi* is embedded within the host tissue, and only the body proper appears outside, which is readily observable by the naked eye, when the operculum is cut open. Dead worms are sometimes found encapsulated in the host hyperplastic tissue. Worms on the gill filaments are always immature and are up to 6 mm long (Ogawa and Inouye, 1997a).

No signs of external disease are noticed in lightly infected fish. Heavily infected fish are anaemic and lethargic. They tend to swim

inactively and leave the school of puffers in the same net cage. Prolonged infection often leads to emaciation and death of the host.

Propagation of *H. okamotoi* is highly temperature dependent. The optimal temperature is approximately 25°C, with the highest mean production rate of 453 eggs per parasite/day (Yamabata *et al.*, 2004). Eggs produced above 26°C are often morphologically abnormal. Eggs laid and kept at 10°C did not hatch, but when transferred to 15°C, they hatch within several days. *Heterobothrium* infections in cultured puffers tend to be milder in the summer than in other seasons (M. Sameshima, Kumamoto Prefectural Fisheries Research Center, personal observation, 2010). Frequency distribution of body length of the parasite collected from a single puffer population indicates that the winter-spring generation mostly disappeared in the summer, and it was replaced by an autumn generation (Ogawa and Inouye, 1997a).

The uterus contains a maximum of 1580 eggs, which, when deposited, forms an egg string of 2.83 m (Ogawa, 1997). These egg strings entangle with the culture nets, which results in egg accumulation within the culture system. Eggs are easily collected with lines or small pieces of nets hung down from the water surface, and this can be used for monitoring infection.

The oncomiracidium (200–300 µm long; Fig. 14.3), has a life span of about 9.1, 7.3 and 4.7 days at 15, 20 and 25°C, respectively

(Ogawa, 1998), compared with less than 24 h for oncomiracidia of most monogenean species (Llewellyn, 1963; Buchmann and Bresciani, 2006). Infectivity decreases as the larvae age, but some of the 4-day-old larvae may still be infective (Chigasaki *et al.*, 2000). The oncomiracidium has two types of movements: (i) a swimming phase with strong ciliary beatings; and (ii) a stationary phase with ciliary beatings too weak to generate any directional motion (Shirakashi *et al.*, 2010). It lacks eye spots and hence does not have phototactic reactions. These behavioural characteristics may contribute to its long life at the larval stage.

14.1.3. External/internal lesions

Infection of immature worms on the gill lamellae induces no apparent responses in the host, whereas adults induce marked inflammation by the action of clamps at the attachment site. Upon migration from the gills to the branchial cavity wall, the clamps take hold of the wall. Prolonged action of the clamps induces disruption of the skin, and the haptor reaches the underlining muscle tissue (Fig. 14.5a). The action of clamps also induces host inflammatory responses. Host tissue surrounds the posterior part of the parasite, but as the host encapsulation is incomplete, the surrounding tissue becomes necrotic (Fig. 14.5b) due to invasion of sea water through the eroded tissue (Ogawa and Inouye, 1997a).

14.1.4. Pathophysiology

H. okamotoi is a blood feeder, and heavily infected tiger puffer are anaemic. In an infection experiment, where puffers (205–345 g in body weight) were exposed to an oncomiracidial suspension, blood parameters deteriorated as the parasite grew. On 81 days post-exposure (p.e.) with between two and 38 adults on the branchial cavity, the haemoglobin content was reduced from 6.5 g/100 ml of blood to lower than 4.0 g, and the mean haematocrit dropped from 25.1 to 12.8% (Ogawa and Inouye, 1997b).

The number of haematin cells in the gut of the oncomiracidia ranged from 14 in worms at day 7 p.e. to 114 at day 13 p.e. and up to 665 at day 19 p.e., reflecting a sharp increase in the amount of blood taken by the worms as they grew (Yasuzaki *et al.*, 2004). Ogawa *et al.* (2005) injected fluorescent microspheres (1 µm in diameter) into tiger puffer to estimate the blood taken by a single parasite. In an experimental period of 12 h the volume of blood ingested by a single adult was estimated to be 1.38 µl/day.

14.1.5. Protective/control strategies

Host reaction

Tsutsui *et al.* (2003) identified a novel man-nose-specific lectin in the skin mucus of tiger puffer. This lectin was detected in epithelial cells in the skin and gills (Tsutsui *et al.*, 2005) and it binds to *H. okamotoi* under *in vitro* conditions (Tsutsui *et al.*, 2003). This suggests that the lectin may bind to *H. okamotoi* both on the gills and on the branchial cavity wall; however, it has not yet been demonstrated that it plays a role in the immuno-protection against *H. okamotoi*.

Nakane *et al.* (2005) showed that persistently infected fish established immunity against *H. okamotoi* infection, though the fish did not completely clear the parasite. When infected fish were cohoused with naïve fish in an aquarium for 70 days, the latter fish became much more heavily infected on the gills than the former, which showed no change in the infection level. The persistently infected fish had much fewer worms with zero to one pair of clamps on the gills and no new infection on the branchial cavity wall, suggesting that immunity takes effect first when the oncomiracidium settles on the gills, secondly when the parasite develops to one with a pair of clamps, and thirdly when it migrates to the branchial cavity wall (Nakane *et al.*, 2005). These observations suggest that immune-prophylactic measures may have effect in the future control programme.

Naturally infected puffer produced antibody against adult *H. okamotoi* (Wang

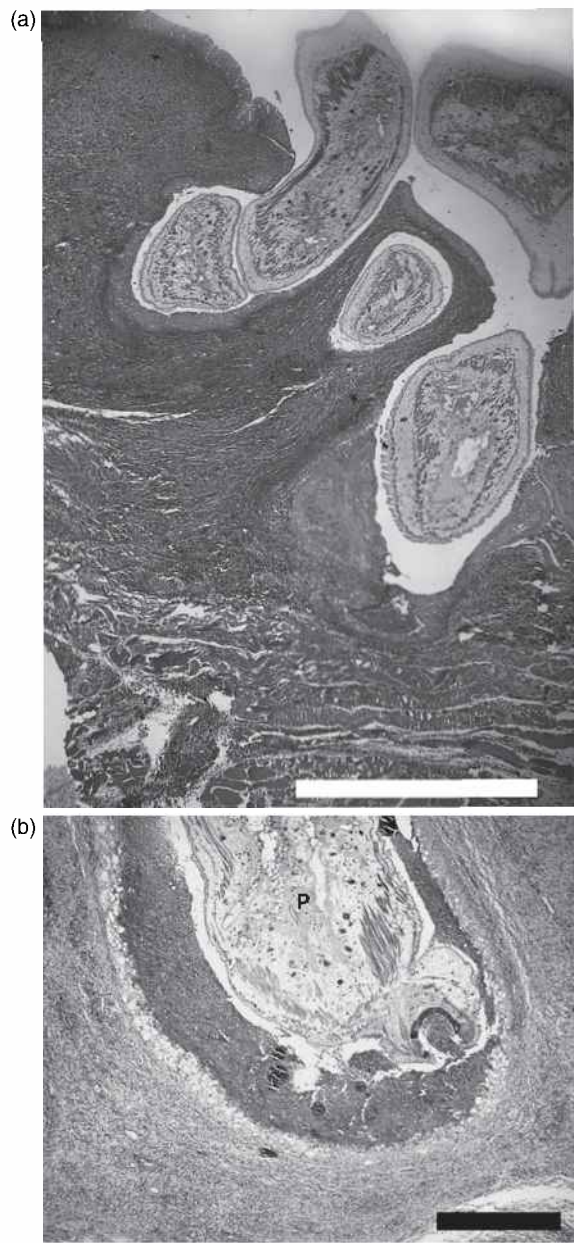


Fig. 14.5. Histological section of an adult worm on the branchial cavity wall of tiger puffer. (a) Haptor reaching the underlining muscle tissue of the host. Bar = 2 mm. (b) Host inflammatory responses to the parasite. Note that the host tissue around the parasite (P) is necrotic due to invasion of sea water through the eroded tissue. Bar = 0.5 mm (from Ogawa, 2002).

et al., 1997; Nakane *et al.*, 2005). In contrast, Umeda *et al.* (2007) demonstrated antibody against oncomiracidium and its cilia, but not against immature worms or adults in fish persistently infected for 89 days. Umeda *et al.* (2007) also stated that specific

antibodies against adult worms were detected from tiger puffer persistently infected for 2 years, suggesting that tiger puffer would take a considerable period to produce specific antibodies. Puffer intraperitoneally injected with oncomiracidium or its cilia showed no effect on prevention of infection. It is still inconclusive whether antibodies against adult worms play a role in preventing infection.

Control measures

In the 1980s–1990s, farmers routinely treated infected fish with diluted formalin, which was subsequently discarded into the sea. For fear of formalin residues in treated fish and pollution of the coastal environment, the use of formalin in aquaculture was banned in 2003. It was replaced with hydrogen peroxide (bath treatment in 0.6 g/l solution for 20–30 min), which is effective to remove immature worms on the gills, but not for adults on the branchial cavity wall (Ogawa and Yokoyama, 1998). In 2004, oral administration of febantel (25 mg/kg fish body weight for 5 consecutive days), a prodrug of fenbendazole, was approved for commercial use and is now widely used, which is effective both against immature parasites and against adults (Kimura *et al.*, 2006, 2009). Also oral administration of praziquantel (4 g/kg diet) or caprylic acid (2.5 g/kg diet) to tiger puffer was effective to control *Heterobothrium* infection (Hirazawa *et al.*, 2000), but a long-term administration was required (e.g. for 30 consecutive days). These chemicals were used only in experimental studies.

Although anthelmintics may show high efficacy, the total eradication of the parasite is not expected using chemotherapy.

Mechanical control and management: deposited eggs form long continuous filaments, which easily entangle with the culture nets, and constitute a source of reinfection. Thus, at the time of chemical treatment, farmers change the culture nets to remove eggs on the nets (Ogawa and Yokoyama, 1998).

Hatching was completely suppressed when eggs were treated in 40°C sea water or air-dried for 1 h, while freshwater treatment of eggs for 24 h was not effective (Hirazawa

et al., 2003). Heat or air-drying treatment can be used to kill eggs in an aquarium or tanks when they are emptied.

14.1.6. Conclusions and suggestions

H. okamotoi has been one of the most serious pathogens of cultured tiger puffer, causing severe anaemia (Ogawa and Yokoyama, 1998; Ogawa, 2002). Eradication of the parasite from the culture environments is practically impossible since the infection is maintained between 0-year and 1-year fish at the culture sites. Chemotherapy using hydrogen peroxide and fenbendazole is now widely used for the control of infection. This parasitic disease is now not as serious as it was before chemicals were approved for commercial use. Although no resistance against these anthelmintics has so far been noticed, it should carefully be monitored in puffer farms. Removal of parasite eggs entangled on the culture net is effective to reduce the chances of new infection, but no promising method of egg removal has been developed. It is recommended to use the host immune responses for more effective control, but it remains to be studied in detail. Persistently infected fish produced antibodies against the worm, but it is also not clear how and to what extent the antibodies contribute to protection against infection. Host innate immunity may also be involved, but it needs further careful studies. Tiger puffer is one of the fish with a completely sequenced genome and the sequences are available, which has opened a way to elucidate how the puffer's defence mechanism works on *H. okamotoi* infection.

The disease problem aside, tiger puffer and *H. okamotoi* provide an ideal model for studies on monogenean infections. Tiger puffer is commercially available and quite easy to maintain in a recirculating water system in a laboratory and *H. okamotoi* is also easily available from puffer culture sites. Tens of thousands of *Heterobothrium* eggs can be collected daily from this laboratory system. Its oncomiracidium has a long lifespan and is easier to handle because it has no phototactic response. For these reasons, experiments

using this host–parasite system will contribute to better understanding of monogeneans in general.

14.2. *Neoheterobothrium hirame*

14.2.1. Introduction

A disease of wild and, less frequently, cultured olive flounder or Japanese flounder (*P. olivaceus*) with severe anaemia was first confirmed in the 1990s (Michine, 1999; Miwa and Inouye, 1999; Ogawa, 1999; Yoshinaga *et al.*, 2000b). A large-scale epizootiological study conducted of wild flounders showed 31% (130/416) were anaemic, and 90% of the anaemic fish were infected with a monogenean and/or had vestiges of the parasite (Mushiake *et al.*, 2001). Ogawa (1999) described the monogenean as a new diclidophorid species, and named it *Neoheterobothrium hirame*.

N. hirame is a slender and large (14–33 mm long) monogenean, with the body proper attenuated posteriorly in form of isthmus and haptor bearing four pairs of pedunculate clamps (Fig. 14.6). As a member of Diclidophoridae, *N. hirame* has a similar life cycle to that of *H. okamotoi*. Adults attach to the buccal cavity wall. Very young worms attach to the gill filaments with marginal hooks and hamuli and later with clamps. As they grow, they move to the gill arches or rakers, and then to the buccal cavity wall where they mature (Anshary and Ogawa, 2001).

Based on histological observations a viral aetiology was first suspected as the cause of anaemia in olive flounders (Miwa and Inouye, 1999). However, flounders challenged with *N. hirame* and those subjected to repeated bleedings both reproduced the same anaemic condition as found in wild flounder (Yoshinaga *et al.*, 2001b; Nakayasu *et al.*, 2002). Besides, infected flounder recovered from anaemia after the parasite was removed from infected hosts (Yoshinaga *et al.*, 2001c). All these data suggest that the severe anaemia in wild and cultured olive flounders is caused by *N. hirame*.

N. hirame collected from flounders in different localities from Hokkaido to Kyushu confirmed its existence in the northern Sea of Japan (Anshary *et al.*, 2001), and expanded its distribution to coastal areas of the western Sea of Japan and to the Pacific (Fig. 14.7). Sudden appearance and rapid expansion in the geographical distribution suggest that this monogenean is an introduced parasite.

Hayward (2005), on the other hand, speculated that *N. hirame* naturally spread from the USA through the Bering Sea to Japan; he assumed that *N. hirame* is a synonym of *Neoheterobothrium affine*, a parasite of summer flounders (*Paralichthys dentatus*) in the USA. Recently, Yoshinaga *et al.* (2009) morphologically and molecularly compared *N. hirame* from olive flounders with diclidophorids collected from summer flounders and southern flounders (*Paralichthys lethostigma*) from the USA, and demonstrated that *N. hirame* is originally a parasite of southern flounders and different from *N. affine* of summer flounders. Also experimental infection demonstrates that southern flounders can serve as the host of *N. hirame* (Yoshinaga *et al.*, 2001a). These findings strongly suggest that *N. hirame* was introduced into Japanese waters with infected southern flounders. Infection was also confirmed on wild olive flounders caught in Korean waters (Hayward *et al.*, 2001).

Recently, the commercial catch of olive flounders has declined considerably in south-western Japan. In this region, 0-year flounder newly recruited in the spring became infected with *N. hirame* in the summer. Fish density was extremely reduced from late summer to autumn, which was probably caused by the death of heavily infected fish (Anshary *et al.*, 2002). The commercial catch declined by more than 80% which has remained low (Shirakashi *et al.*, 2008). In contrast, no apparent decrease in the commercial catch has been noticed in northern regions of Japan, in spite of high prevalence of infection (Shirakashi *et al.*, 2006; Tomiyama *et al.*, 2009). In the northern Pacific region, where water temperature was below 10°C in the winter, the intensity of infection was about one-third of that in the temperate Sea of Japan area, where the

infection level was likely to have no apparent effect on the size of the local host population (Shirakashi *et al.*, 2006).

14.2.2. Diagnosis of the infection

Adult worms can be seen with the naked eye except the posterior part of body (isthmus and haptor) which is embedded within the host tissue. Sometimes worms are clustered at the attachment site. In wild flounders, not only live worms but also vestiges of the posterior part of

the parasite are often noticed in hyperplastic tissues of the buccal cavity wall (Mushiake *et al.*, 2001). Worms on the gills are 1.3 ± 0.8 mm long with zero to four pairs of clamps while those on the gill arches or rakers are 5.8 ± 1.9 mm long with four pairs of clamps (Anshary and Ogawa, 2001). Unlike *H. okamotoi*, eggs of *N. hirame* are not connected, and the uterus is narrow and contains only a few eggs (Ogawa, 1999). *N. hirame* has high fecundity, producing 781 eggs daily at 20°C (Tsutsumi *et al.*, 2002). The oncomiracidium are 250–320 μ m long (Ogawa, 2000), but their biological characteristics remain to be studied.

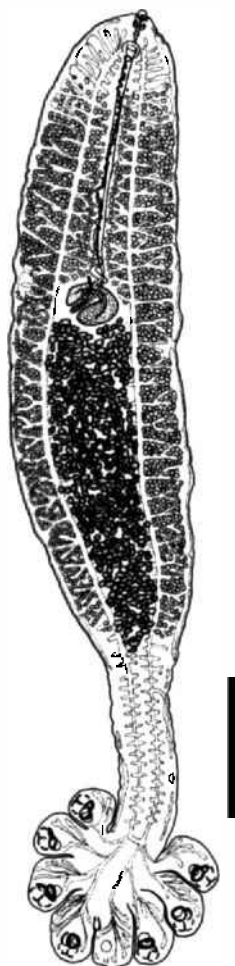


Fig. 14.6. Line drawing of *Neoheterobothrium hirame* Ogawa, 1999. Bar = 3 mm (from Ogawa, 1999).

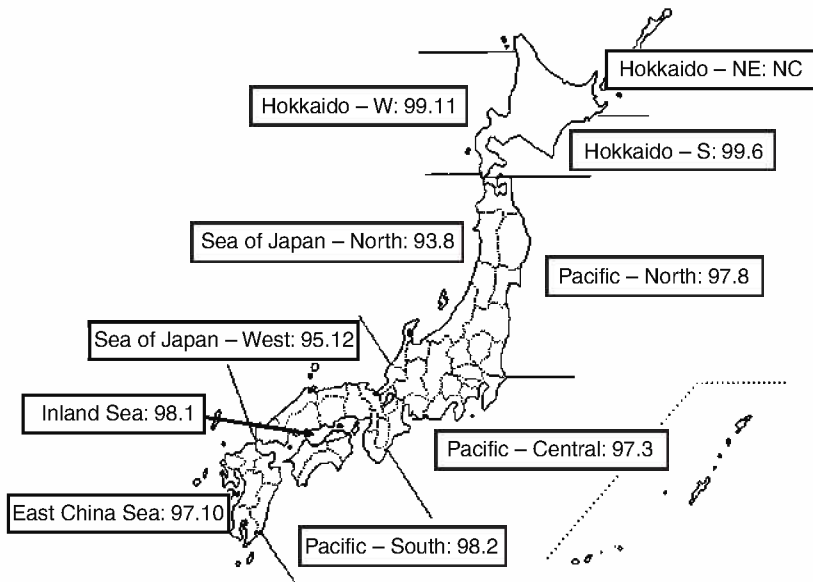


Fig. 14.7. Geographical distribution of *N. hirame*, with the first record of its occurrence (indicated by the year in 1900s and month) on olive flounder (*Paralichthys olivaceus*) within the ten separated Japanese waters. Earliest specimens were collected from olive flounder caught in the northern Sea of Japan in August, 1993, which is surrounded by the box in bold.

Incidence of wild anaemic flounders tends to be low in June–October and high in December–February, and it decreases as fish age: 0-year fish (52.9%), 1-year fish (39.1%) and 2-year fish (28.3%) (Mushiaki *et al.*, 2001).

Shirakashi *et al.* (2005) experimentally demonstrated that at 8°C, oncomiracidial attachment and its subsequent development on flounders were negatively affected. A considerable number of worms disappeared from the host before reaching maturation. This suggests that the low temperature is not optimal for the propagation of this parasite.

Infected flounders altered their behaviour in that there is: (i) increased activity level (Fig. 14.8a); (ii) altered diel activity; (iii) poor burrowing performance (Fig. 14.8b); and (iv) lowered swimming endurance (Shirakashi *et al.*, 2008). There is experimental evidence that such infected fish are more susceptible to predation by larger fish. Infected fish also have lowered feeding efficiency, which makes them more vulnerable to predation during feeding (Shirakashi *et al.*, 2009).

14.2.3. External/internal lesions

Infected wild flounder are emaciated, have pale gills (white to pink in colour) and the unpigmented side of the body appears pale blue (Miwa and Inouye, 1999; Mushiaki *et al.*, 2001). The heart is enlarged and so is the pale liver (Mushiaki *et al.*, 2001).

14.2.4. Pathophysiology

Wild olive flounders had a negative correlation between the number of adult parasites and haemoglobin levels (Mushiaki *et al.*, 2001). Haematocrit values of wild anaemic flounders ranged from 1.0 to 12.6% (Miwa and Inouye, 1999). The anaemia is characterized by the appearance of many immature erythrocytes and abnormal staining in the cytoplasm of erythrocytes (Yoshinaga *et al.* 2000b). As the haemoglobin content lowers, more immature erythrocytes tend to appear in

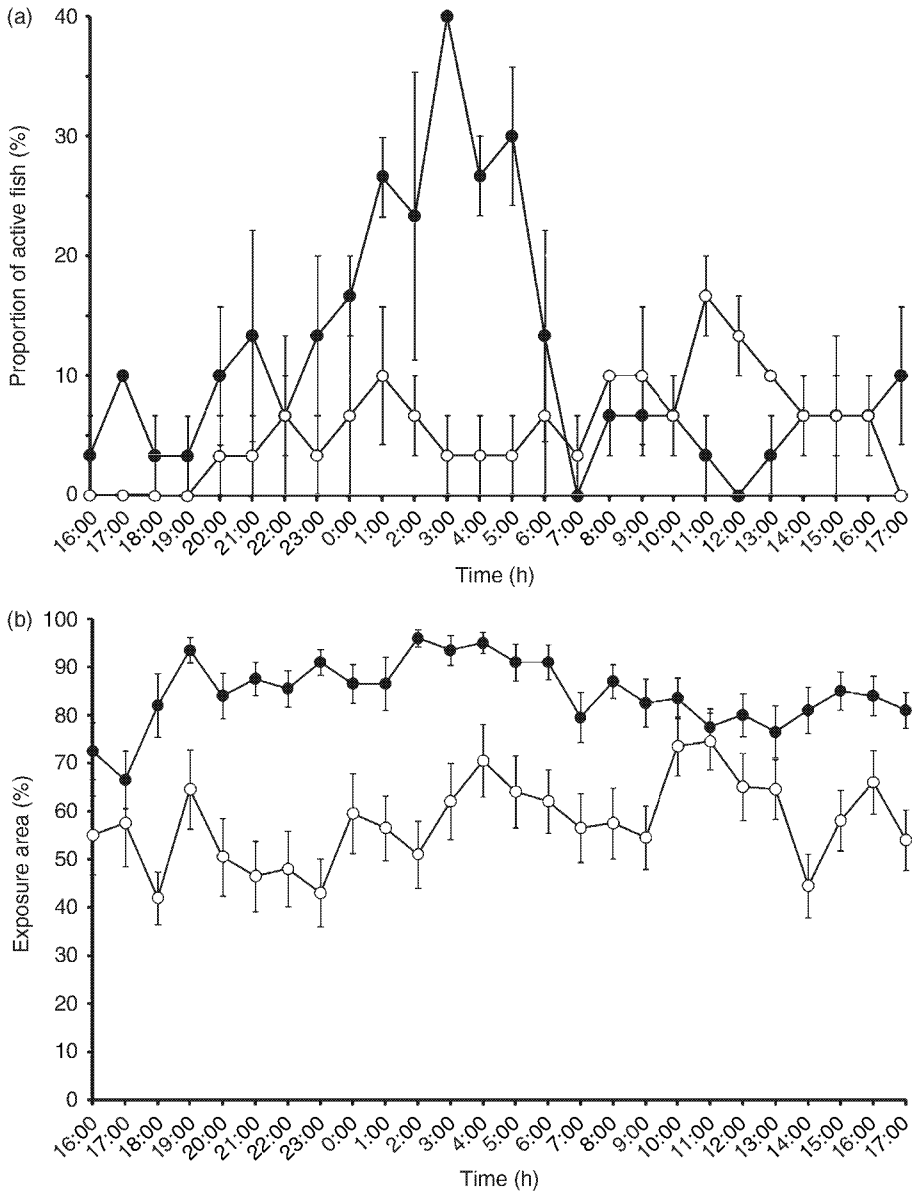


Fig. 14.8. Behavioural changes of olive flounder infected with *N. hirame*. (a) Temporal change in the proportion of active fish (mean $\pm$ SEM) during 25 h of monitoring; (b) temporal change in the exposed body area (mean $\pm$ SEM) during 25 h of monitoring. Light hours were 06:00–18:00. Open circles represent uninfected controls; closed circles, infected fish (from Shirakashi *et al.*, 2008).

the peripheral blood (Mushiake *et al.*, 2001). In fish with no anaemia (haemoglobin content more than 4 g/100 ml blood), erythrocytes constitute an average of 97.2% mature and

1.7% immature ones, whereas fish with severe anaemia have a haemoglobin content of less than 1 g/100 ml blood and an average of 1.2% mature and 87.9% immature erythrocytes.

14.2.5. Protective/control strategies

Host reaction

The host reaction induced by *N. hirame* is similar to that in *H. okamotoi*. The inflammatory response is minimal at the attachment sites on the gill filaments and in the epithelium of the gill arches and rakers, whereas a strong host response including inflammation and hyperplasia and necrosis are associated with the prolonged attachment of adult parasites to the buccal cavity wall (Anshary and Ogawa, 2001). Leucocytes constituting monocytes/macrophages, granulocytes and dense granular cells infiltrate and adhere to adult parasites with the monocytes/macrophages most often observed. Vacuolation of the parasite tegument (Fig. 14.9) adjacent to the adherent site of the leucocytes is common (Nakayasu *et al.*, 2003). The tegument disrupts partially and is phagocytosed by the infiltrated host cells which leads to the death and elimination of the parasite (Nakayasu *et al.*, 2005). This host reaction was not observed in infected fish under starvation.

cDNA microarray gene expression patterns of peripheral blood leucocytes were

analysed to search for specific molecular biomarkers in response to *N. hirame* (Matsuyama *et al.*, 2007). Some candidate genes were selected, but further analysis is needed to target specific subsets of leucocytes.

In experimentally infected flounders, antibody was detected after the parasite had moved to the buccal cavity wall. Antibody production was enhanced after the death of the parasite induced a host reaction (Tsumi *et al.*, 2003).

No investigation has been made as to whether the host inflammatory response and antibody production against *N. hirame* induce immunity to reinfection.

Control measures

No chemical has been approved for commercial use to treat *N. hirame*-infected olive flounders. No data is available on the efficacy against *N. hirame* of hydrogen peroxide, febantel or praziquantel, chemicals that are effective to treat *H. okamotoi*-infected tiger puffers. Water temperature, salinity or chlorine treatment of eggs in culture facilities is impractical to prevent infection from *N. hirame* (Yoshinaga *et al.*, 2000a). In culture facilities using running water, eggs released into the water can be

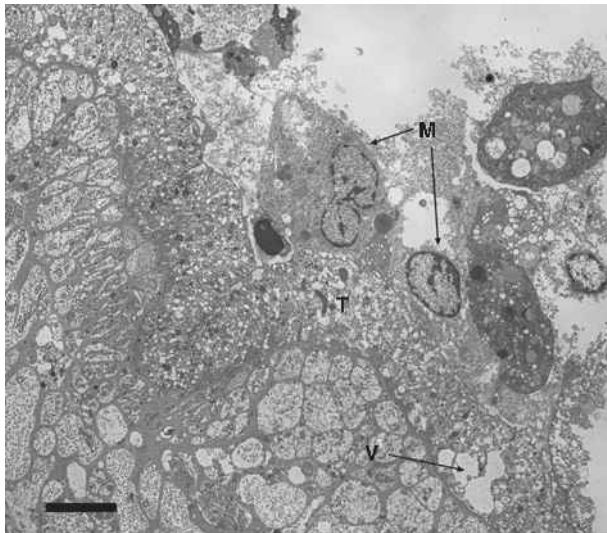


Fig. 14.9. Transmission electron micrograph of olive flounder infected with *N. hirame*, showing that host leucocytes adhere to the parasite tegument, inducing vacuolation. M, macrophage; T, parasite tegument; V, vacuolation of the tegument. Bar = 4 µm (modified from Nakayasu *et al.*, 2003).

flushed out before they hatch (Y. Fukuda, Oita, personal communication, 2003). A treatment of NaCl-supplemented sea water (3% w/v) for 60 min is effective against immature worms on the gills (Yoshinaga *et al.*, 2000c), and 8% NaCl-supplemented sea water for 5 min is effective against adult worms on the buccal cavity wall (Isshiki *et al.*, 2003). The latter treatment can also be used to eliminate parasites from spawner flounders in hatcheries.

14.2.6. Conclusions and suggestions

N. hirame infection can induce severe anaemia in wild olive flounders and decrease its

population considerably in Japan. Severity of infection is dependent on water temperature; in northern Japan, infection is common, but it appears to have no serious effects on flounder populations, whereas in south-western Japan, the intensity of infection is several times higher which results in considerable decline in the flounder catch.

It is still not known how long the high infection levels will continue in the wild flounder populations. *N. hirame* may provide a good example to show how an introduced pathogen can cause serious damage to wild stocks, and such a study requires long-term observations which have been initiated.

References

- Anshary, H. and Ogawa, K. (2001) Microhabitats and mode of attachment of *Neoheterobothrium hirame*, a monogenean parasite of Japanese flounder. *Fish Pathology* 36, 21–26.
- Anshary, H., Ogawa, K., Higuchi, M. and Fujii, T. (2001) A study of long-term change 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.
- Anshary, H., Yamamoto, E., Miyahara, T. and Ogawa, K. (2002) Infection dynamics of the monogenean *Neoheterobothrium hirame* infecting Japanese flounder in the western Sea of Japan. *Fish Pathology* 37, 131–140.
- Buchmann, K. and Bresciani, J. (2006) Monogenea (Phylum Platyhelminthes). In: Woo, P.T.K. (ed.) *Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections*, 2nd edn. CABI Publishing, Wallingford, Oxon, UK, pp. 297–344.
- Chigasaki, M., Ogawa, K. and Wakabayashi, H. (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.
- Hayward, C. (2005) Monogenea Polyopisthocotylea (ectoparasitic flukes). In: Rohde, K. (ed.) *Marine Parasitology*. CSIRO Publishing, Collingwood, Victoria, Australia, pp. 55–63.
- Hayward, C.J., Kim, J.H. and Heo, G.J. (2001) Spread of *Neoheterobothrium hirame* (Monogenea), a serious pest of olive flounder *Paralichthys olivaceus*, to Korea. *Diseases of Aquatic Organisms* 45, 209–213.
- Hirazawa, N., Ohtaka, T. and Hata, K. (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., Goto, T. and Shirasu, K. (2003) Killing effect of various treatments on the monogenean *Heterobothrium okamotoi* eggs and oncomiracidia and the ciliate *Cryptocaryon irritans* cysts and theronts. *Aquaculture* 223, 1–13.
- Isshiki, T., Tochino, M. and Nagano, T. (2003) Treatments of *Neoheterobothrium* infection in Japanese flounder by 8% NaCl-supplemented seawater bathing. *Suisanzoshoku* 51, 363–364.
- Kimura, T., Sameshima, M., Nomura, Y., Morita, J., Mizoguchi, H. and Ishihara, M. (2006) Efficacy of orally administered febantel against monogenean *Heterobothrium okamotoi* infection of cultured tiger puffer *Takifugu rubripes*. *Fish Pathology* 41, 147–151.
- Kimura, T., Nomura, Y., Kawakami, H., Itano, T., Iwasaki, M., Morita, J. and Enomoto, J. (2009) Field trials of febantel against gill fluke disease caused by the monogenean *Heterobothrium okamotoi* in cultured tiger puffer *Takifugu rubripes*. *Fish Pathology* 44, 67–71.

- Llewellyn, J. (1963) Larvae and larval development of monogeneans. *Advances in Parasitology* 1, 287–326.
- Matsuyama, T., Fujiwara, A., Nakayasu, C., Kamaishi, T., Oseko, N., Tsutsumi, N., Hirono, I. and Aoki, T. (2007) Microarray analyses of gene expression in Japanese flounder *Paralichthys olivaceus* leucocytes during monogenean parasite *Neoheterobothrium hirame* infection. *Diseases of Aquatic Organisms* 75, 79–83.
- Michine, A. (1999) *Neoheterobothrium* sp. found on Japanese flounder cultured commercially or maintained as spawners. *Researches of the Shimane Prefectural Center of Cultural Fisheries* 2, 15–23 (in Japanese).
- Miwa, S. and Inouye, K. (1999) Histopathological study of the flounder with anemia found in various places in Japanese coastal waters. *Fish Pathology* 34, 113–119 (in Japanese with English abstract).
- Mushiaki, K., Mori, K. and Arimoto, M. (2001) Epizootiology of anemia in wild Japanese flounder. *Fish Pathology* 36, 125–132 (in Japanese with English abstract).
- Nakane, M., Ogawa, K., Fujita, T., Sameshima, M. and Wakabayashi, H. (2005) Acquired protection of tiger puffer *Takifugu rubripes* against infection with *Heterobothrium okamotoi* (Monogenea: Diclidophoridae). *Fish Pathology* 40, 95–101.
- Nakayasu, C., Yoshinaga, T. and Kumagai, A. (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., Tsutsumi, N., Yoshitomi, T., Yoshinaga, T. and Kumagai, A. (2003) Identification of Japanese flounder leucocytes involved in the host response to *Neoheterobothrium hirame*. *Fish Pathology* 38, 9–14.
- Nakayasu, C., Tsutsumi, N., Oseko, N. and Hasegawa, S. (2005) Role of cellular response in elimination of the monogenean *Neoheterobothrium hirame* in Japanese flounder *Paralichthys olivaceus*. *Diseases of Aquatic Organisms* 64, 127–134.
- Ogawa, K. (1991) Redescription of *Heterobothrium tetrodonis* (Monogenea: Diclidophoridae) and other related new species from puffers of the genus *Takifugu* (Teleostei: Tetraodontidae). *Japanese Journal of Parasitology* 40, 388–396.
- Ogawa, K. (1997) Copulation and egg production of the monogenean *Heterobothrium okamotoi*, a gill parasite of cultured tiger puffer (*Takifugu rubripes*). *Fish Pathology* 32, 219–223.
- Ogawa, K. (1998) Egg hatching of the monogenean *Heterobothrium okamotoi*, a gill parasite of cultured tiger puffer (*Takifugu rubripes*), with a description of its oncomiracidium. *Fish Pathology* 33, 25–30.
- 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. (2000) The oncomiracidium of *Neoheterobothrium hirame*, a monogenean parasite of Japanese flounder *Paralichthys olivaceus*. *Fish Pathology* 35, 229–230.
- Ogawa, K. (2002) Impacts of diclidophorid monogenean infections on fisheries in Japan. *International Journal for Parasitology* 32, 373–380.
- Ogawa, K. and Inouye, K. (1997a) *Heterobothrium* infection of cultured tiger puffer, *Takifugu rubripes* (Teleostei: Tetraodontidae) – a field study. *Fish Pathology* 32, 15–20.
- Ogawa, K. and Inouye, K. (1997b) *Heterobothrium* infection of cultured tiger puffer, *Takifugu rubripes* – experimental infection. *Fish Pathology* 32, 21–27.
- Ogawa, K. and Yokoyama, H. (1998) Parasitic diseases of cultured marine fish in Japan. *Fish Pathology* 33, 303–309.
- Ogawa, K., Yasuzaki, M. and Yoshinaga, T. (2005) Experiments on the evaluation of the blood feeding of *Heterobothrium okamotoi* (Monogenea: Diclidophoridae). *Fish Pathology* 40, 169–174.
- Ohhashi, Y., Yoshinaga, T. and Ogawa, K. (2007) Involvement of host recognition and survivability in the host specificity of the monogenean parasite *Heterobothrium okamotoi*. *International Journal for Parasitology* 37, 53–60.
- Okamoto, R. (1963) On the problems of a monogenetic trematode infection of tiger puffers from the Inland Sea of Japan. *Suisanzoshoku (Special Issue)* 3, 17–29 (in Japanese).
- Okamoto, R. and Ogasawara, Y. (1965) Parasite occurrences of tiger puffer in natural waters. *Annual Report of Naikai Regional Fisheries Laboratory, Series A* 2, 42–43 (in Japanese).
- Shirakashi, S., Yoshinaga, T., Oka, M. and Ogawa, K. (2005) Larval attachment and development of the monogenean *Neoheterobothrium hirame* under low temperature. *Fish Pathology* 40, 33–35.
- Shirakashi, S., Yamada, T., Yamada, T. and Ogawa, K. (2006) Infection dynamics of *Neoheterobothrium hirame* (Monogenea) on juvenile olive flounder in coastal Japan. *Journal of Fish Diseases* 29, 319–329.
- Shirakashi, S., Teruya, K. and Ogawa, K. (2008) Altered behaviour and reduced survival of juvenile olive flounder infected by an invasive monogenean parasite *Neoheterobothrium hirame*. *International Journal for Parasitology* 38, 1513–1522.

- Shirakashi, S., Nishioka, N. and Ogawa, K. (2009) *Neoheterobothrium hirame* (Monogenea) alters the feeding behaviour of juvenile olive flounder, *Paralichthys olivaceus*. *Fisheries Science* 75, 121–128.
- Shirakashi, S., Nakatsuka, S., Udagawa, A. and Ogawa, K. (2010) Oncomiracidial behavior of *Heterobothrium okamotoi* (Monogenea: Diclidophoridae). *Fish Pathology* 45, 51–57.
- Tomiya, T., Watanabe, M. and Kurita, Y. (2009) Rapid fluctuation in infection levels of *Neoheterobothrium hirame* (Monogenea) in Japanese flounder *Paralichthys olivaceus* in the Joban area, Japan. *Journal of Fish Biology* 75, 172–185.
- Tsutsui, S., Tasumi, S., Suetake, H. and Suzuki, Y. (2003) Lectins homologous to those of monocotyledonous plants in the skin mucus and intestine of pufferfish, *Fugu rubripes*. *Journal of Biological Chemistry* 278, 20882–22089.
- Tsutsui, S., Tasumi, S., Suetake, H., Kikuchi, K. and Suzuki, Y. (2005) Demonstration of the mucosal lectins in the epithelial cells of internal and external body surface tissues in pufferfish (*Fugu rubripes*). *Developmental and Comparative Immunology* 29, 243–253.
- Tsutsumi, N., Mushiaki, K., Mori, K., Yoshinaga, T. and Ogawa, K. (2002) Effects of temperature on the egg-laying of the monogenean *Neoheterobothrium hirame*. *Fish Pathology* 37, 41–43.
- Tsutsumi, N., Yoshinaga, T., Kamaishi, T., Nakayasu, C. and Ogawa, K. (2003) Effects of temperature on the development and longevity of the monogenean *Neoheterobothrium hirame* on Japanese flounder *Paralichthys olivaceus*. *Fish Pathology* 38, 41–47.
- Umeda, N., Hatanaka, A. and Hirazawa, N. (2007) Immobilization antibodies of tiger puffer *Takifugu rubripes* induced by i.p. injection against monogenean *Heterobothrium okamotoi* oncomiracidia do not prevent the infection. *Parasitology* 134, 853–863.
- Wang, G., Kim, J.-H., Sameshima, M. and Ogawa, K. (1997) Detection of antibodies against the monogenean *Heterobothrium okamotoi* in tiger puffer by ELISA. *Fish Pathology* 32, 179–180.
- Yamabata, N., Yoshinaga, T. and Ogawa, K. (2004) Effects of water temperature on egg production and egg viability of the monogenean *Heterobothrium okamotoi* infecting tiger puffer *Takifugu rubripes*. *Fish Pathology* 39, 215–217.
- Yasuzaki, M., Ogawa, K. and Yoshinaga, T. (2004) Early development of the monogenean *Heterobothrium okamotoi* on the gills of tiger puffer *Takifugu rubripes*. *Fish Pathology* 39, 153–158.
- Yoshinaga, T., Segawa, I., Kamaishi, T. and Sorimachi, M. (2000a) Effect of temperature, salinity and chlorine treatment on egg hatching of the monogenean *Neoheterobothrium hirame* infecting Japanese flounder. *Fish Pathology* 35, 85–88.
- Yoshinaga, T., Kamaishi, T., Segawa, I., Kumagai, A., Nakayasu, C., Yamano, K., Takeuchi, T. and Sorimachi, M. (2000b) Hematology, histopathology and the monogenean *Neoheterobothrium hirame* infection in anemic flounder. *Fish Pathology* 35, 131–136.
- Yoshinaga, T., Kamaishi, T., Segawa, I. and Yamamoto, E. (2000c) Effects of NaCl-supplemented seawater on the monogenean, *Neoheterobothrium hirame*, infecting the Japanese flounder. *Fish Pathology* 35, 97–98.
- Yoshinaga, T., Tsutsumi, N., Shima, T., Kamaishi, T. and Ogawa, K. (2001a) Experimental infection of the southern flounder *Paralichthys lethostigma* with *Neoheterobothrium hirame* (Monogenea: Diclidophoridae). *Fish Pathology* 36, 237–239.
- Yoshinaga, T., Kamaishi, T., Segawa, I., Yamano, K., Ikeda, H. and Sorimachi, M. (2001b) Anemia caused by challenges with the monogenean *Neoheterobothrium hirame* in the Japanese flounder. *Fish Pathology* 36, 13–20.
- Yoshinaga, T., Kamaishi, T., Ikeda, H. and Sorimachi, M. (2001c) Experimental recovery from anemia in Japanese flounder challenged with the monogenean *Neoheterobothrium hirame*. *Fish Pathology* 36, 179–182.
- Yoshinaga, T., Tsutsumi, N., Hall, K.A. and Ogawa, K. (2009) Origin of the diclidophorid monogenean *Neoheterobothrium hirame* Ogawa, 1999, the causative agent of anemia in olive flounder, *Paralichthys olivaceus*. *Fisheries Science* 75, 1167–1176.

15 *Diplostomum spathaceum* and Related Species

Anssi Karvonen

Department of Biological and Environmental Science, University of Jyväskylä,
Jyväskylä, Finland

15.1. Introduction

Trematodes of the genus *Diplostomum* are ubiquitous parasites of freshwater fishes; they infect the eyes of over 100 fish species worldwide (Chappell, 1995). Some of the species are found also in other parts of the fish body. *Diplostomum* flukes may also be very abundant with tens or even hundreds of parasites in an individual fish (Chappell, 1969; Wootton, 1974; Valtonen and Gibson, 1997; Valtonen *et al.*, 1997). Infections are also found in aquaculture (e.g. Stables and Chappell, 1986a; Field and Irwin, 1994; Buchmann and Bresciani, 1997; Karvonen *et al.*, 2006a) causing significant problems by impairing the fish's vision. This chapter describes the loss of vision in fish (development of parasitic cataracts), explains the effects of the infection (physiology, growth, appearance, behaviour) and discusses control strategies to prevent *Diplostomum* in aquaculture.

Most of the research has been on one species, *Diplostomum spathaceum* s.l., mainly because the infection results in notable deleterious effects in fish with significant economical importance. The taxonomy of *Diplostomum* parasites, however, is very complex and still not completely resolved. For example, recent molecular studies have identified a range of new species in wild fishes

(Locke *et al.*, 2010a, b), suggesting that the currently known taxonomic species composition is not complete. Thus, in the earlier literature, the species name *D. spathaceum* has been commonly used collectively to describe species found in the lens. The same approach is taken in the present discussion except when referring to studies where the species has been properly verified as other than *D. spathaceum*.

15.1.1. Parasite life cycle

The life cycle of *D. spathaceum* is typical for trematodes and includes three host species (Fig. 15.1). Sexual reproduction takes place in the definitive host, which is a fish-eating bird, such as a gull. Adult hermaphroditic worms mate in the intestine and start producing eggs 3–4 days after establishment. An individual bird is typically infected with several hundreds of worms, each of which can release several hundreds of eggs/day (Karvonen *et al.*, 2006b). Eggs are released to the aquatic environment with host faeces, where they hatch to miracidia larvae in 2 weeks in summer temperatures. Miracidia are short-lived and actively seek out the first intermediate host, a freshwater snail. Several snail species can act as hosts for *Diplostomum* parasites, but those of the genus *Lymnaea* are the most common.

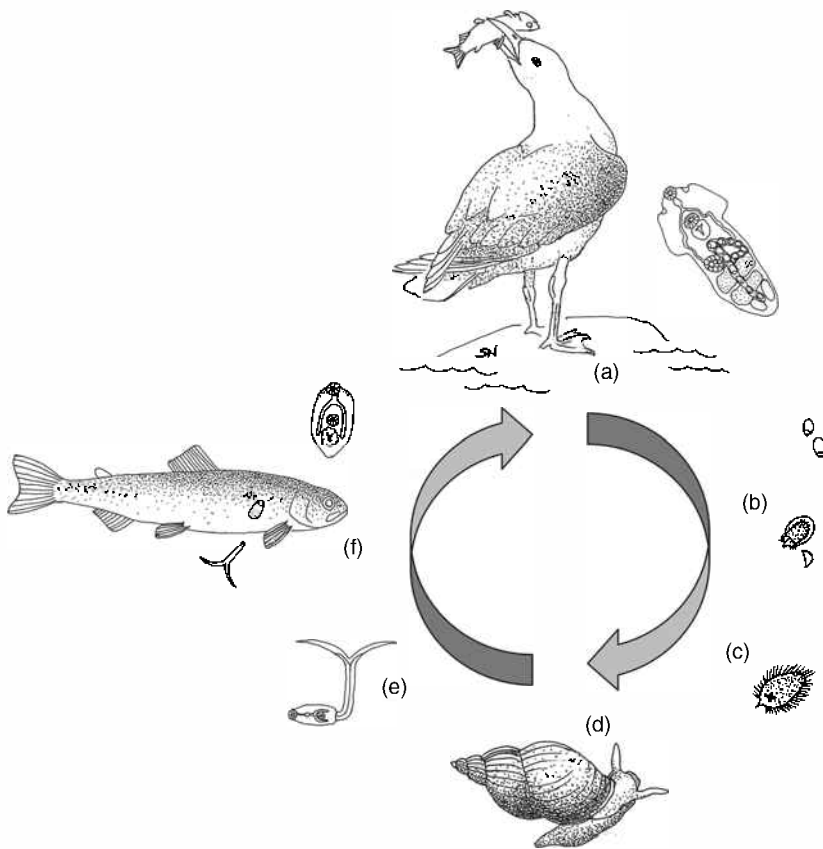


Fig. 15.1. Life cycle of *Diplostomum spathaceum*. Adult worms reproduce in an avian definitive host (a) and produce eggs (b). Eggs hatch in water to miracidia (c), which infect the first intermediate host, an aquatic snail (d). Asexual reproduction in the snail gives rise to thousands of cercariae (e) that penetrate the epithelium of fish (f), the second intermediate host, and settle in the eye lenses as metacercariae (f). The life cycle is completed when an infected fish is eaten by a bird.

Within the snail, the parasite reproduces asexually by forming sporocysts, which occupy the reproductive system of the snail leading to castration. Cercariae are formed within the sporocysts and are released to the water in very high numbers. For example, one individual of *Lymnaea stagnalis* can release tens of thousands of cercariae/day for several weeks (Lyholt and Buchmann, 1996; Karvonen *et al.*, 2004a). In northern latitudes, this mainly takes place during the summer months when the water temperature exceeds 10°C (Stables and Chappell, 1986a; Karvonen *et al.*, 2004b). Cercariae are equipped with a bifurcated tail. After a contact with a fish, cercariae penetrate the epithelium of gills and skin, drop their tail and enter the

host body. It is still poorly understood which routes the cercariae (at this stage called diplostomulae) use in their migration towards the eye (Ratanarat-Brockelman, 1974; Whyte *et al.*, 1991). The tissue migration from penetration to establishment in the lens is typically completed within 24 h (Whyte *et al.*, 1991), but can take longer in low temperatures (Lyholt and Buchmann, 1996). Subsequently, diplostomulae exhaust their limited energy reserves and are killed by the host immune system.

After reaching the eye lens, diplostomulae develop to metacercariae and cause the disease diplostomiasis. They grow considerably in size, first becoming elongated before taking their typical oval or cylindrical shape

(Sweeting, 1974). This process is also controlled by the water temperature; development of the metacercariae is completed within a few weeks in 15–20°C, but may be halted completely at low temperatures. For example, parasites establishing in fish in late autumn may overwinter as undeveloped metacercariae and continue their development in the following spring (Karvonen *et al.*, unpublished). It is generally believed that the metacercariae can survive in fish for years. As such, infections accumulate in fish with time (Marcogliese *et al.*, 2001). The life cycle of the parasite is completed when an infected fish is eaten by a bird definitive host.

15.2. Signs of the Infection

15.2.1. Parasitic cataracts

The most notable sign of *D. spathaceum* infection in fish is cataract formation, when the eye lens becomes opaque and grey, and the vision of the fish is impaired. Quantification of the

cataracts is best done with an ophthalmological microscope (Karvonen *et al.*, 2004c). In low-level infections, individual parasites are typically surrounded by small clouds of granules or thread-like formations (Shariff *et al.*, 1980; Karvonen *et al.*, 2004c). In more severe infections, damage caused by individual parasites overlap resulting in opacity of the lens. Eventually the eye lens may begin to appear whitish, which is visible just by looking at the fish. This is the chronic stage of the infection. Parasitic cataracts have recently been described quantitatively both from farmed and from wild fish species (Marcogliese *et al.*, 2001; Seppänen *et al.*, 2008; Seppälä *et al.*, 2011).

The main factor influencing the severity of cataracts is the number of parasites in the lens (Fig. 15.2). The relationship, however, may be influenced by several factors. For example, cataract coverage typically varies greatly among individual fish so that the same infection intensity does not necessarily cause similar cataracts in all individuals (Fig. 15.2). This can at least partly be explained by metacercarial distribution in the lens; parasites

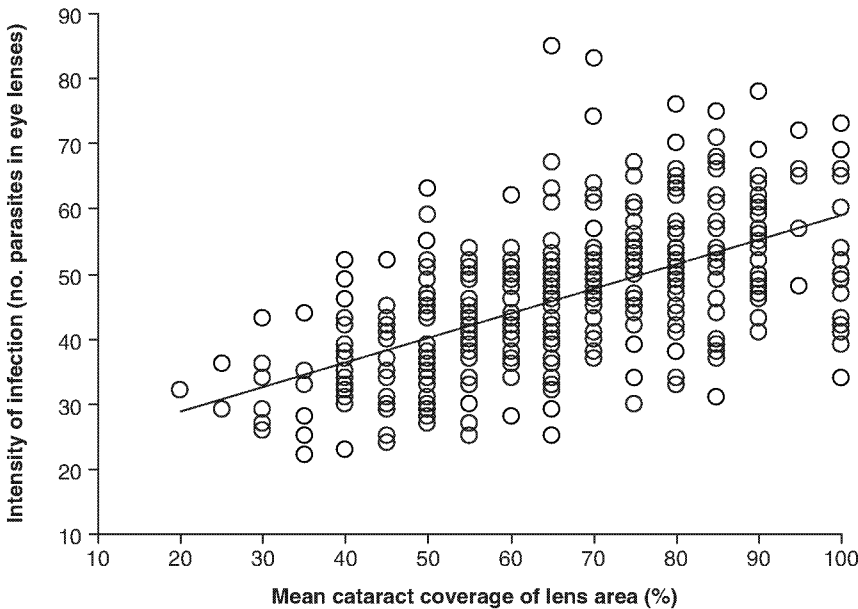


Fig. 15.2. Relationship between intensity of *D. spathaceum* infection (i.e. the total number of parasites in the lenses of the right and left eye) and mean cataract coverage in the eye lenses of whitefish (*Coregonus lavaretus*) experimentally exposed to the parasite. The fitted line represents linear regression (data from Karvonen and Seppälä, 2008b).

may be aggregated resulting in cataracts only in certain parts of the lens. However, in higher infection intensities parasites typically occupy the whole lens resulting in cataracts covering the whole lens area. In an individual fish, parasites may also be unevenly distributed between the right and left eye. Thus, it is possible that cataracts mainly develop in one eye, while the other remains less affected.

Recently established parasites do not usually cause strong cataracts. Instead, cataracts mainly develop when the metacercariae are reaching full development (Seppälä *et al.*, 2005a). The rate of metacercarial development is strictly controlled by water temperature. Consequently, temperature also influences cataract formation as parasites become less active in cold water. For example, if fish become infected in late summer or autumn, followed by a decrease in water temperature, development of cataracts may take place in the following spring, several months after the infection (Karvonen *et al.*, unpublished). In such cases, it may be sometimes difficult to link the timing of infection and emergence of negative effects in fish.

Variation in cataracts may also be host induced as individual fish may show differences in susceptibility to parasite-inflicted damage. For example, smaller fish may be more susceptible to cataracts as smaller eye lenses may become covered with cataracts even in low infection intensities. Young fish are also often more susceptible to infection before the development of specific immune responses (see below), which may further intensify cataract development. Cataracts may also show differences among fish species, or among populations of one species, as a consequence of physiological or genetic predisposition to infection and cataracts (Betterton, 1974; Sweeting, 1974; Rintamäki *et al.*, 2004; Kuukka-Anttila *et al.*, 2010). In general, factors that contribute to cataract formation are not completely understood.

15.2.2. Other pathological effects in the eye

A chronic *D. spathaceum* infection may also cause other severe pathological effects on the

lens. A fish eye lens is composed of fibrous cells, which are variably compressed in different parts of the lens. This gives the lens its typical structure with softer outer parts and a very hard nucleus. *Diplostomum* metacercariae commonly occupy the outermost layers of the lens, which may lead to destruction of the crystalline structure of the cells (Shariff *et al.*, 1980). In the most severe cases, the lens capsule may rupture releasing materials into the eye and resulting in a significant inflammatory reaction (Shariff *et al.*, 1980). This is often accompanied by retinal detachment and dislocation of the lens to the anterior chamber. At this stage, the fish becomes completely blind. Such signs, however, are rare and have been reported mainly from aquaculture units in association with very high infection intensities. Their occurrence in wild fish is unknown.

Leaking of the lens material can also lead to a reduction in the size of the lens (Shariff *et al.*, 1980; Karvonen and Seppälä, 2008a). This is known in several wild and farmed fish species so that the more heavily infected lens of an individual fish is usually smaller (Karvonen and Seppälä, 2008a). Also, at a population level, more heavily infected fish have smaller eye lenses compared to less infected conspecifics (Karvonen and Seppälä, 2008a). Reduction in lens size induced by *D. spathaceum* infection may lead to increased susceptibility to cataracts, which develop as a function of infection intensity and are likely to depend on the lens volume. Also, the ability of fish to focus its vision depends on the lens radius and may therefore be sensitive to changes in lens size. It is important to note that the lens size reduction can take place even at low infection intensities (Karvonen and Seppälä, 2008a), and before the development of major pathological changes in the eye. This suggests that focusing and vision ability of the eye could be affected even at low infection intensities.

15.3. Effects of Infection on Fish

The effects of infection can be divided into two types: (i) acute effects, caused directly by

the cercarial invasion; and (ii) chronic effects, induced by metacercariae and cataracts. Several studies have investigated these effects, focusing, for example, on mortality, physiology, feeding, growth and behaviour of infected fish. Most of the research has been conducted using economically important farmed salmonid species, but studies using naturally infected wild fish species also exist.

15.3.1. Acute mortality

Penetration of the parasite cercariae and migration of the diplostomulae can be detrimental especially to young fish. In addition to damage to the epidermis, histological studies have shown that diplostomulae penetrate blood vessels causing internal haemorrhages and inflammatory responses (e.g. Ratanarat-Brockelman, 1974). In a high-level exposure, this can result in acute mortality of fish. For example, mortality of small rainbow trout (*Oncorhynchus mykiss*) with a body length of 5–6 cm begins when the fish are exposed to 300–600 cercariae and reaches 100% in doses of 1000 cercariae per fish (Larsen *et al.*, 2005). However, larger fish are typically more resistant and rarely experience mortality from the acute infection.

15.3.2. Physiology

Acute *Diplostomum* infection typically is a stressor for fish and thus initiates a range of physiological responses. Laitinen *et al.* (1996) showed that an acute exposure to *Diplostomum pseudospathaceum* cercariae results in increased heart and ventilation rates in fish. They also observed an increase in swimming activity of fish, which may be related to attempt to escape the exposure (Karvonen *et al.*, 2004b). Using an elegant design, Laitinen *et al.* (1996) also demonstrated that fish did not respond to the presence of cercariae of a different parasite species, *Plagiorchis elegans*, which does not infect fish. In other words, the mere presence of cercariae in the water did not elicit the physiological responses; it required penetration of the

parasites to host tissues. Some of these responses may last for up to 3 days and peak at different stages of parasite establishment (Laitinen *et al.*, 1996), which shows the complexity of these responses.

Chronic *Diplostomum* infection can also have physiological effects in fish. For example, studies have reported increased oxygen consumption among infected Arctic charr (*Salvelinus alpinus*) (Voutilainen *et al.*, 2008), but also decreased standard metabolic rate of the fish (Seppänen *et al.*, 2009). Such effects can be related to increased food intake, as suggested by Voutilainen *et al.* (2008), or reduced efficiency of energy metabolism, as suggested by Seppänen *et al.* (2009). Overall, it seems clear that chronic *Diplostomum* infection has metabolic consequences, which again can affect other traits such as growth and fecundity.

15.3.3. Feeding and growth

Impaired vision caused by the parasite may also interfere with fish growth. For example, it has been shown that the infection decreases the weight and condition of rainbow trout in farming conditions (Buchmann and Uldal, 1994), which may be related to reduction in feeding efficiency of fish (Crowden and Broom, 1980; Owen *et al.*, 1993). More recently, studies have linked fish feeding and growth to cataracts. Cataracts have a central role in impairment of vision and typically show high variance among infected individuals. For example, severity of cataracts is known to correlate negatively with feeding efficiency of Arctic charr in terms of reaction time to prey and number of prey items caught (Voutilainen *et al.*, 2008). It has also been shown experimentally that growth of whitefish (*C. lavaretus*) is impaired with increasing cataract coverage in aquaculture conditions (Karvonen and Seppälä, 2008b). However, this negative effect is evident only when cataracts cover the whole lens in both eyes (Fig. 15.3). In other words, individuals with cataracts covering up to 80–95% of the lens area are still growing at the same rate as individuals with significantly fewer cataracts. One explanation for this surprising result is that fish with poor

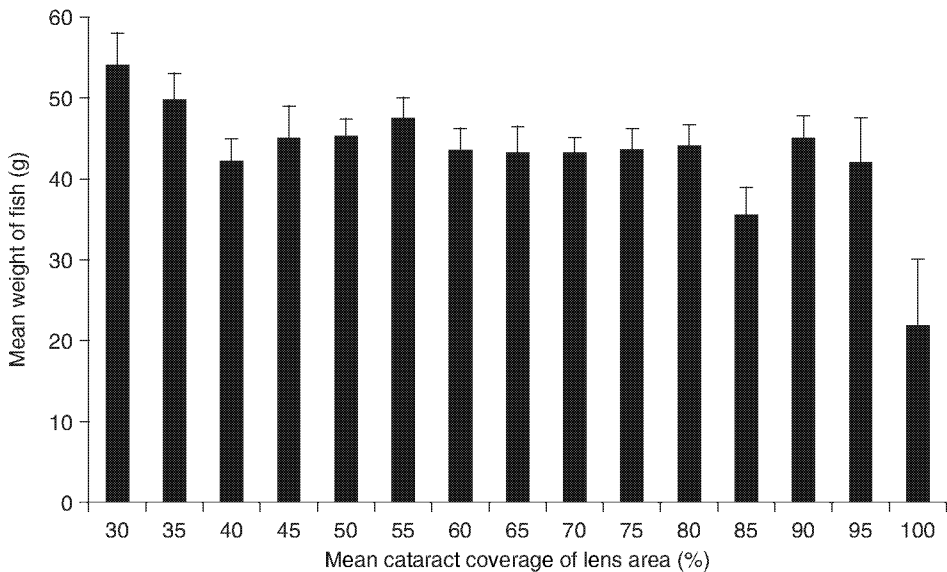


Fig. 15.3. Mean weight ($\pm$ SE) of whitefish in relation to mean cataract coverage in the eye lenses. Fish were experimentally exposed to *D. spathaceum* and reared thereafter in tanks for 8 weeks (data from Karvonen and Seppälä, 2008b).

vision may be able to follow feeding cues (movement to the surface when food is introduced) from less infected conspecifics in a tank using other sensory mechanisms such as the lateral line. This suggests that effects of the infection on fish growth in farming conditions may not become apparent until the fish is practically blind.

15.3.4. Predator avoidance

Cryptic coloration is an important mechanism for fish to hide from predators. Coloration of fish is regulated by the amount of light entering the eye; when light intensity increases fish respond by becoming lighter. However, when the eye lens is infected with *D. spathaceum* metacercariae, observation of light intensity is impaired and fish remain darker. This is primarily caused by the incapability of infected fish to match their coloration with a lighter background (Seppälä *et al.*, 2005b). In farming conditions, heavily infected fish are often notably dark and can be separated from less infected individuals.

The lack of cryptic coloration may also be one of the reasons *D. spathaceum*-infected fish are more vulnerable to predation. Darker fish that cannot properly adjust their colour, or seek shelter from a matching background, are more easily seen by predators (Seppälä *et al.*, 2005b). This is accompanied by their reduced ability to detect approaching avian predators, which is also positively linked to coverage of cataracts (Seppälä *et al.*, 2005a). Ultimately, this serves as a benefit for the parasite by increasing its likelihood to reach the final bird host and completing its life cycle.

15.4. Control Strategies and Prevention

Because of the deleterious effects of *D. spathaceum* in fish, a range of control strategies have been put forward to limit and control the infections in aquaculture. One of the problems in designing such protocols, however, is that epidemics of *D. spathaceum* infection are usually very unpredictable with high variance in prevalence and intensity of the infection among

aquaculture units and years. Moreover, problems (e.g. fish beginning to show cataracts and stopping feeding) often appear with a long time lag when control of the infection is no longer possible. It is to some extent possible to medicate infected fish by chemotherapy (Bylund and Sumari, 1981), but this has not been widely used. Furthermore, the cercariae are released from snails over a period of several months, which makes short-term actions against the cercariae (such as chemical treatments) unfeasible. However, several strategies against the parasite have been applied, and these can roughly be divided into: (i) those limiting the parasite establishment in fish (immunization); and (ii) those interrupting the parasite life cycle (control strategies against the snail intermediate hosts).

15.4.1. Immunization

The first invasion of *D. spathaceum* cercariae into an individual fish elicits an immune response, which involves both innate and specific branches of the immune system (Chappell *et al.*, 1994). Specific responses develop within a few weeks from the exposure and significantly reduce the number of parasites establishing in subsequent exposures (Stables and Chappell, 1986b; Höglund and Thuvander, 1990; Whyte *et al.*, 1990; Karvonen *et al.*, 2005). Thus, several researchers have explored the possibility of developing a vaccine against the parasite, for example by using attenuated infective stages (Bortz *et al.*, 1984; Speed and Pauley, 1985; Whyte *et al.*, 1990). Although such protocols decrease subsequent parasite establishment, an effective vaccine against *Diplostomum* has not yet been developed. One reason for this is that interactions among the immune responses mobilized by the infection (antibodies, leucocytes, cytokines, complement) are still not completely understood (Chappell, 1995).

Moreover, the effect of the immunization against *D. spathaceum* is not complete, meaning that some parasites still establish in the lens regardless of acquired immune responses (Karvonen *et al.*, 2005). Considering the efficiency of immunization, it is important to determine the degree of damage these

parasites are causing. It has been shown that previously immunized rainbow trout become heavily infected and develop intensive cataracts when re-exposed to the parasite in cages under natural conditions (Karvonen *et al.*, 2004b, 2010). This suggests that immunization alone is inefficient to prevent the infection. However, fish also use behavioural means in their defence against the parasite and escape the infection (Karvonen *et al.*, 2004b), a trait which is effectively eliminated in limited space such as a tank or cage of a fish farm. The absence of such a defence could very well provide an explanation for seemingly high parasite infection success under those conditions. Overall, in the light of the current knowledge, it seems unlikely that immunization of fish against *D. spathaceum* alone could provide a feasible or economically sustainable method to prevent the parasite in aquaculture.

15.4.2. Interruption of the parasite life cycle

Perhaps the most efficient strategy to control diplostomiasis in aquaculture is to interrupt the parasite life cycle. In practice, this means eradication of the snail intermediate host since control of the avian definitive host flying over a fish farm is much more challenging. Snails thrive especially in ponds with vegetation and high primary production. Typically these snails are responsible for most of the infections within a farm, but parasite cercariae can also be brought into a farm by incoming water (Karvonen *et al.*, 2005). Snails can be removed physically or chemically, and this can be ideally done in connection with draining and cleaning of the ponds. Further establishment of snails can be reduced by removing the vegetation or constructing the ponds in a way which inhibits vegetation growth.

Changes in the infrastructure of a farm can also help in controlling the infections. For example, increasing the water flow rate and turbulence decreases infection intensities in fish (Field and Irwin, 1994). Furthermore, as snails typically inhabit littoral zones of lakes, taking the incoming water from a basin of a

lake can decrease the number of cercariae entering the farm. Cercariae can also be filtered from the water (Larsen *et al.*, 2005) although this is applicable only with very small volumes of water. It should be stressed, however, that the above actions should be considered complementary to the most important measure of prevention, which is the eradication of snails inside the farm.

15.5. Future Prospects

Despite considerable research efforts, *D. spathaceum* s.l. remains a problem in aquaculture. For example, fish in facilities using surface water sources often harbour infections from *Diplostomum* parasites. Although these infections do not necessarily affect the fish in any way in farming conditions, and may even be beneficial to some extent as they evoke immunity towards future infections, they may have an effect after the fish have been stocked into more challenging conditions in the wild. Stocking protocols are common in many countries to maintain endangered salmonid fish populations. Thus, there is still a need for more detailed studies on the effects of these parasites on fish feeding, growth and survival in wild conditions, particularly in

relation to different intensities of infection and severity of cataracts. Such information would also have a direct applied value when assessing the quality of young fish intended for stocking, as well as evaluating the success of fish stocking protocols in general.

Future studies should also explore the genetic predisposition of different fish species and populations to infection and cataracts. This work has begun only recently. For example, information on less susceptible fish populations could be helpful when designing fish breeding protocols which aim to develop fish stocks with better parasite resistance. Similarly, information on genetic differences in infectivity and virulence among *Diplostomum* species and strains of one species is important as they determine the ability of parasites to cause damage in their hosts. Overall, untangling these interactions requires investigations both from the host's and the parasite's perspective.

Acknowledgements

I thank Christian Rellstab, Otto Seppälä and Tellervo Valtonen for discussions and comments. Special thanks to Sven Nikander for producing the life cycle figure.

References

- Betterton, C. (1974) Studies on the host specificity of the eyefluke, *Diplostomum spathaceum*, in brown and rainbow trout. *Parasitology* 69, 11–29.
- Bortz, B.M., Kenny, G.E., Pauley, G.B., Garcia-Ortigoza, E. and Anderson, D.P. (1984) The immune response in immunized and naturally infected rainbow trout (*Salmo gairdneri*) to *Diplostomum spathaceum* as detected by enzyme-linked immunosorbent assay (ELISA). *Developmental and Comparative Immunology* 8, 813–822.
- Buchmann, K. and Bresciani, J. (1997) Parasitic infections in pond-reared rainbow trout *Oncorhynchus mykiss* in Denmark. *Diseases of Aquatic Organisms* 28, 125–138.
- Buchmann, K. and Uldal, A. (1994) Effects of eyefluke infections on the growth of rainbow trout (*Oncorhynchus mykiss*) in a mariculture system. *Bulletin of the European Association of Fish Pathologists* 14, 104–107.
- Bylund, G. and Sumari, O. (1981) Laboratory tests with Droncit against diplostomiasis in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 4, 259–264.
- Chappell, L.H. (1969) The parasites of the three-spined stickleback *Gasterosteus aculeatus* L. from a Yorkshire pond. II. Variation of the parasite fauna with sex and size of fish. *Journal of Fish Biology* 1, 339–347.
- Chappell, L.H. (1995) The biology of diplostomatid eyeflukes of fishes. *Journal of Helminthology* 69, 97–101.

- Chappell, L.H., Hardie, L.J. and Secombes, C.J. (1994) Diplostomiasis: the disease and host-parasite interactions. In: Pike, A.W. and Lewis, J.W. (eds) *Parasitic Diseases of Fish*. Samara Publishing Limited, Dyfed, Wales, UK, pp. 59–86.
- Crowden, A.E. and Broom, D.M. (1980) Effects of the eyefluke, *Diplostomum spathaceum*, on the behaviour of dace (*Leuciscus leuciscus*). *Animal Behaviour* 28, 287–294.
- Field, J.S. and Irwin, S.W.B. (1994) The epidemiology, treatment and control of diplostomiasis on a fish farm in Northern Ireland. In: Pike, A.W. and Lewis, J.W. (eds) *Parasitic Diseases of Fish*. Samara Publishing Limited, Dyfed, Wales, UK, pp. 87–100.
- Höglund, J. and Thuvander, A. (1990) Indications of non-specific protective mechanisms in rainbow trout *Oncorhynchus mykiss* with diplostomosis. *Diseases of Aquatic Organisms* 8, 91–97.
- Karvonen, A. and Seppälä, O. (2008a) Eye fluke infection and lens size reduction in fish: a quantitative analysis. *Diseases of Aquatic Organisms* 80, 21–26.
- Karvonen, A. and Seppälä, O. (2008b) Effect of eye fluke infection on the growth of whitefish (*Coregonus lavaretus*) – an experimental approach. *Aquaculture* 279, 6–10.
- Karvonen, A., Kirsi, S., Hudson, P.J. and Valtonen, E.T. (2004a) Patterns of cercarial production from *Diplostomum spathaceum*: terminal investment or bet hedging? *Parasitology* 129, 87–92.
- Karvonen, A., Seppälä, O. and Valtonen, E.T. (2004b) Parasite resistance and avoidance behaviour in preventing eye fluke infections in fish. *Parasitology* 129, 159–164.
- Karvonen, A., Seppälä, O. and Valtonen, E.T. (2004c) Eye fluke-induced cataract formation in fish: quantitative analysis using an ophthalmological microscope. *Parasitology* 129, 473–478.
- Karvonen, A., Pauku, S., Seppälä, O. and Valtonen, E.T. (2005) Resistance against eye flukes: naïve versus previously infected fish. *Parasitology Research* 95, 55–59.
- Karvonen, A., Savolainen, M., Seppälä, O. and Valtonen, E.T. (2006a) Dynamics of *Diplostomum spathaceum* infection in snail hosts at a fish farm. *Parasitology Research* 99, 341–345.
- Karvonen, A., Cheng, G.-H., Seppälä, O. and Valtonen, E.T. (2006b) Intestinal distribution and fecundity of two species of *Diplostomum* parasites in definitive hosts. *Parasitology* 132, 357–362.
- Karvonen, A., Halonen, H. and Seppälä, O. (2010) Priming of host resistance to protect cultured rainbow trout against eye flukes and parasite-induced cataracts. *Journal of Fish Biology* 76, 1508–1515.
- Kuikka-Anttila, H., Peuhkuri, N., Kolari, I., Paananen, T. and Kause, A. (2010) Quantitative genetic architecture of parasite-induced cataract in rainbow trout, *Oncorhynchus mykiss*. *Heredity* 104, 20–27.
- Laitinen, M., Siddall, R. and Valtonen, E.T. (1996) Bioelectronic monitoring of parasite-induced stress in brown trout and roach. *Journal of Fish Biology* 48, 228–241.
- Larsen, A.H., Bresciani, J. and Buchmann, K. (2005) Pathogenicity of *Diplostomum* cercariae in rainbow trout, and alternative measures to prevent diplostomosis in fish farms. *Bulletin of the European Association of Fish Pathologists* 25, 20–27.
- Locke, S.A., McLaughlin, J.D., Dayanandan, S. and Marcogliese, D.J. (2010a) Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome c oxidase I and internal transcribed spacer sequences. *International Journal for Parasitology* 40, 333–343.
- Locke, S.A., McLaughlin, J.D. and Marcogliese, D.J. (2010b) DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among Diplostomoidea (Platyhelminthes: Digenea) parasitizing freshwater fishes in the St. Lawrence River, Canada. *Molecular Ecology* 19, 2813–2827.
- Lyholt, H.C.K. and Buchmann, K. (1996) *Diplostomum spathaceum*: effects of temperature and light on cercarial shedding and infection of rainbow trout. *Diseases of Aquatic Organisms* 25, 169–173.
- Marcogliese, D.J., Dumont, P., Gendron, A.D., Mailhot, Y., Bergeron, E. and McLaughlin J.D. (2001) Spatial and temporal variation in abundance of *Diplostomum* spp. in walleye (*Stizostedion vitreum*) and white suckers (*Catostomus commersoni*) from the St. Lawrence River. *Canadian Journal of Zoology* 79, 355–369.
- Owen, S.F., Barber, I. and Hart, P.J.B. (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.
- Ratanarat-Brockelman, C. (1974) Migration of *Diplostomum spathaceum* (Trematoda) in the fish intermediate host. *Zeitschrift für Parasitenkunde* 43, 123–134.
- Rintamäki-Kinnunen, P., Karvonen, A., Anttila, P. and Valtonen, E.T. (2004) *Diplostomum spathaceum* metacercarial infection and colour change in salmonid fish. *Parasitology Research* 93, 577–581.
- Seppälä, O., Karvonen, A. and Valtonen, E.T. (2005a) Manipulation of fish hosts by eye flukes in relation to cataract formation and parasite infectivity. *Animal Behaviour* 70, 889–894.
- Seppälä, O., Karvonen, A. and Valtonen, E.T. (2005b) Impaired crypsis of fish infected with a trophically transmitted parasite. *Animal Behaviour* 70, 895–900.

- Seppälä, O., Karvonen, A. and Valtonen, E.T. (2011) Eye fluke-induced cataracts in natural fish populations: is there potential for host manipulation? *Parasitology* 138, 209–214.
- Seppänen, E., Kuukka, H., Huuskonen, H. and Piirainen, J. (2008) Relationship between standard metabolic rate and parasite-induced cataract of juveniles in three Atlantic salmon stocks. *Journal of Fish Biology* 72, 1659–1674.
- Seppänen, E., Kuukka, H., Voutilainen, A., Huuskonen, H. and Peuhkuri, N. (2009) Metabolic depression and spleen and liver enlargement in juvenile Arctic charr *Salvelinus alpinus* exposed to chronic parasite infection. *Journal of Fish Biology* 74, 553–561.
- Shariff, M., Richards, R.H. and Sommerville, C. (1980) The histopathology of acute and chronic infections of rainbow trout *Salmo gairdneri* Richardson with eye flukes, *Diplostomum* spp. *Journal of Fish Diseases* 3, 455–465.
- Speed, P. and Pauley, G.B. (1985) Feasibility of protecting rainbow trout, *Salmo gairdneri* Richardson, by immunizing against eye fluke, *Diplostomum spathaceum*. *Journal of Fish Biology* 26, 739–744.
- Stables, J.N. and Chappell, L.H. (1986a) The epidemiology of diplostomiasis in farmed rainbow trout in north-east Scotland. *Parasitology* 92, 699–710.
- Stables, J.N. and Chappell, L.H. (1986b) Putative immune response of rainbow trout, *Salmo gairdneri*, to *Diplostomum spathaceum* infections. *Journal of Fish Biology* 29, 115–122.
- 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.
- Valtonen, E.T. and Gibson, D.I. (1997) Aspects of the biology of diplostomid metacercarial (Digenea) populations occurring in fishes in different localities in northern Finland. *Annales Zoologici Fennici* 34, 47–59.
- Valtonen, E.T., Holmes, J.C. and Koskivaara, M. (1997) Eutrophication, pollution, and fragmentation: effects on parasite communities in roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in four lakes in central Finland. *Canadian Journal of Fisheries and Aquatic Sciences* 54, 572–585.
- Voutilainen, A., Figueiredo, K. and Huuskonen, H. (2008) Effects of the eye fluke *Diplostomum spathaceum* on the energetics and feeding of Arctic charr *Salvelinus alpinus*. *Journal of Fish Biology* 73, 2228–2237.
- Whyte, S.K., Chappell, L.H. and Secombes, C.J. (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., Secombes, C.J. and Chappell, L.H. (1991) Studies on the infectivity of *Diplostomum spathaceum* in rainbow trout (*Oncorhynchus mykiss*). *Journal of Helminthology* 65, 169–178.
- Wootton, R. (1974) Observations on strigeid metacercariae in the eyes of fish from Hanningfield Reservoir, Essex, England. *Journal of Helminthology* 48, 73–83.

16 *Sanguinicola inermis* and Related Species

Ruth S. Kirk

School of Life Sciences, Kingston University, Kingston upon Thames, UK

16.1. Introduction

16.1.1. The parasite

Sanguinicola inermis Plehn, 1905 (Strigeida, Schistosomatoidea) is a digenean trematode that inhabits the blood vascular system of freshwater cyprinid fish in Europe and Asia. These small, hermaphrodite blood flukes (mean length: 550 µm) have caused considerable taxonomic confusion due to their morphological and developmental features that are atypical of the Digenea. The adults and cercariae lack an obvious oral or ventral sucker. They possess a pre-oesophageal muscular organ resembling a modified sucker for attachment rather than a pharynx for feeding (McMichael-Phillips *et al.*, 1994). The adults have a reduced, lobed intestine and a short, uncoiled uterus reduced to a metraterm (Fig. 16.1). Eggs produced and released by mature worms accumulate in blood vessels associated with the gills and they hatch *in situ* to release miracidia (Kirk and Lewis, 1993). It is not surprising, therefore, that *S. inermis* was initially described as a turbellarian endoparasite in carp (*Cyprinus carpio* L.) by Plehn (1905) and then as a member of the monozoic Cestodaria (Plehn, 1908), before it was correctly identified as a trematode by Odhner (1911).

The family-group name for sanguinicolid fish blood flukes has also been a matter of uncertainty. Sanguinicolidae von Graff, 1907 and Aporocotylidae Odhner, 1912 have both been used for the single family name and for separate families. Examination of the early German literature by Bullard *et al.* (2009) established that the correct family name is Aporocotylidae Odhner, 1912 and that Sanguinicolidae Poche, 1926 is the junior subjective synonym. The flukes of the Aporocotylidae collectively show an extensive geographical distribution in a wide range of freshwater and marine hosts. The Aporocotylidae is the most diverse family of blood flukes in comparison to the Schistosomatidae of birds and mammals and the Spirorchidae of turtles and is currently thought to comprise over 100 species (Smith, 1997a, b; Bullard *et al.*, 2008 and subsequent new species descriptions), but there are probably many more species that are, as yet, unreported and unidentified (Bullard *et al.*, 2008).

16.1.2. Life cycle

S. inermis has an indirect life cycle transmitted between fish and aquatic snails. All varieties of carp (scaled, mirror, leather and koi) act as the major definitive hosts (Kirk and Lewis, 1994a), but the parasite has also been reported

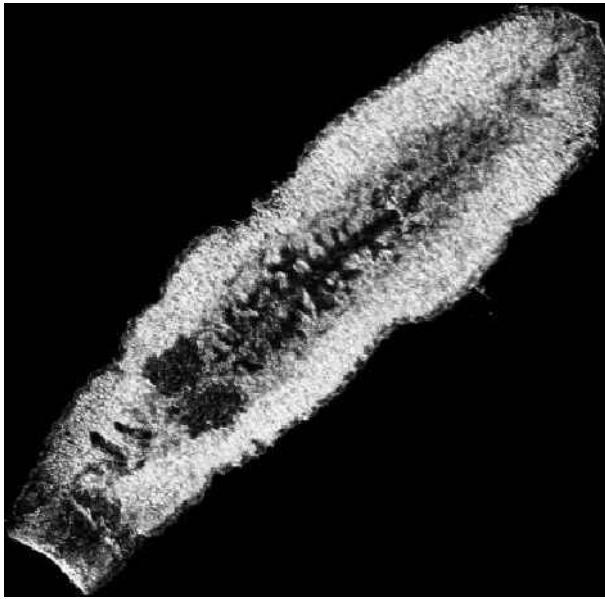


Fig. 16.1. Adult *Sanguinicola inermis*, 4 weeks p.i. (×250).

from bream (*Abramis brama*), crucian carp (*Carassius carassius*), goldfish (*Carassius auratus*), nase (*Chondrostoma nasus*), roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*), silver bream (*Blicca bjoerkna*) and tench (*Tinca tinca*) (Smith, 1997b¹). It is an autogenic parasite that has been disseminated between the carp farms and fisheries of Eurasia by anthropochore movements of carp and was widespread in mainland Europe by the 1960s (Bauer, 1962). Establishment of *S. inermis* in new foci has been facilitated by the widespread distribution of the intermediate snail hosts, most commonly *Radix auricularia* and *Radix peregra*, and also *Lymnaea stagnalis* in cyprinid fisheries (Kirk and Lewis, 1994a).

Cyprinid fish are infected by the direct penetration of cercariae through the epidermis of the skin, fins, opercular cavity and gill lamellae. Juvenile flukes migrate through the dermis, connective tissue and muscle to enter the blood vascular system (Kirk and Lewis, 1996). The majority of flukes that survive host defences mature in the ventral aorta, afferent branchial arteries, bulbus arteriosus, ventricle and atrium, although flukes can also establish in the dorsal aorta, cephalic, renal and hepatic

vessels. Migration to the blood system is completed within 1 month at 20°C (Kirk and Lewis, 1996). Adults cross-fertilize and commence egg development. The maximum lifespan of adults is between 56 and 70 days at 20°C (Kirk and Lewis, 1996).

The eggs are released from adults while immature and develop further in the blood and tissue of the fish host. Most eggs accumulate in the gills because the majority of adults are present in the ventral blood system (Kirk and Lewis, 1993). They are carried by the ventral blood flow until they lodge in the gill and visceral capillaries. The accumulation of eggs causes the delicate blood vessels to rupture and discharge eggs into adjacent tissue. Miracidia develop inside thin, pliable, non-operculate egg capsules and hatch *in situ* within approximately 7 days (McMichael-Phillips *et al.*, 1992a; Kirk and Lewis, 1996). Those miracidia present in the distal regions of the gills and some miracidia in the proximal regions, escape into the water using the stylet and rodlet complex of the apical papilla, possibly aided by enzyme secretions from lateral glands (McMichael-Phillips *et al.*, 1992b; Kirk and Lewis, 1996). Ciliated miracidia locate and penetrate snail

intermediate hosts. Eggs and miracidia that do not escape from the gills, and those that are sequestered in visceral and connective tissue, become trapped within granulomatous tissue and degrade (Kirk and Lewis, 1996). Within the snail host the miracidium transforms into a mother sporocyst in which daughter sporocysts develop and migrate into the interlobular spaces of the digestive gland. Furcocercous cercariae develop inside daughter sporocysts within 4–5 weeks at 20°C. There is no metacercarial stage. The cercariae emerge from snails in late afternoon to early evening to locate the fish definitive hosts (Kirk and Lewis, 1993).

S. inermis exhibits a well-defined seasonal cycle of development in carp fisheries in temperate regions. Sporocyst infections in snails and miracidial infections in carp overwinter until increasing temperatures in spring facilitate development (Bobiatynska-Ksok, 1964; Lee, 1990). Cercariae emerge from snails in spring/early summer to infect carp, and miracidia migrate from the gill tissue to infect newly hatched snails. Summer adult flukes in carp produce eggs and die by the autumn. Further snails are infected by miracidia. Naïve carp are infected during the second peak of cercarial emergence in the late summer. A second generation of adult flukes develop and produce eggs until declining autumn temperatures inhibit further development. Adults overwintering in the heart of carp hosts reproduce in the spring and then die by early summer (Naumova, 1961; Lee, 1990).

16.1.3. Impact on fish production

Most fish mortalities attributed to *S. inermis* have occurred in 0–1+ year old carp in intensively farmed fish populations. The parasite was responsible for serious economic problems on carp farms in mainland Europe and Asia from the 1950s to the 1980s having caused parasite-induced mortalities and impaired growth of fry and fingerlings (Bauer, 1962; Lucky, 1964; Moravec, 1984). Disease problems were exacerbated by old fish-farming practices of rearing fry with older fish in silted ponds containing high populations of snails (Bobiatynska-Ksok,

1964; Hlond *et al.*, 1977), but improvements in husbandry and use of molluscicides have reduced the prevalence of sanguinicoliasis (Sapozhnikov, 1988). The blood fluke was not detected in the UK until 1977 when it was reported to be the cause of 90% mortality of the year's fry on a carp farm in western England (Sweeting, 1979). *S. inermis* was subsequently reported from a number of sites in southern, eastern and central England and was associated with the mortality of young carp in farms and management problems in extensive leisure fisheries as sanguinicoliasis prevented carp movements from infected sites to restock other fisheries (Kirk and Lewis, 1994a). Destruction of the gills, kidney, liver and brain tissue was reported in 0–1+ UK farmed carp with long-term infections (12–16 months post-infection (p.i.)), poor growth, osmoregulatory and respiratory failure (Iqbal and Sommerville, 1986). Mortalities associated with infections of *S. inermis* in extensive fisheries occurred in combination with secondary factors such as post-spawning stress and low dissolved oxygen (Kirk, unpublished observations). Recent surveys of the literature by Kirk (unpublished) in the UK indicate that *S. inermis* is no longer reported as a significant pathogen in carp farms or in extensive fisheries, despite mandatory and discretionary monitoring of parasitic fauna prior to fish movements. Routine use of anthelmintics, such as praziquantel, against tapeworm infections may have reduced incidence. In addition, carp in densely stocked leisure fisheries, common in the UK, may decrease or eliminate infections due to the consumption of intermediate snail hosts (B. Brewster, Kingston, personal communication, 2010). Reports of the parasite have also decreased in mainland Europe, but this may be partly due to reduced veterinary screening in some countries.

In China, *Sanguinicola* species have been associated with epizootics of cyprinid fingerlings in pond farms. *Sanguinicola lungensis* Tang and Ling, 1975 caused extensive losses of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) in South Fukien (Tang and Ling, 1975). *Sanguinicola megalobramae* Li, 1980 killed blunt-snout bream (*Megalobrama amblycephala*) in Hupei

Province (Li, 1980 cited by Smith 1997a). *Sanguinicola* species in North America caused mortalities of young salmonids in hatcheries until husbandry was improved to eliminate intermediate hosts from water circulation systems. *Sanguinicola davis* Wales, 1958 was responsible for heavy losses of young rainbow and steelhead trout (*Oncorhynchus mykiss*) and cutthroat trout (*Oncorhynchus clarkii clarkii*) in hatcheries in Oregon and California (Wales, 1958; Davis *et al.*, 1961). *Sanguinicola fontinalis* Hoffman, Fried and Harvey, 1985 caused severe disease in 400,000 brook trout (*Salvelinus fontinalis*) in a Pennsylvania state hatchery, necessitating a cull of approximately 100,000 of the most severely infected fish (Hoffman *et al.*, 1985). *Sanguinicola idahoensis* Schell, 1974 caused significant disease problems and losses in steelhead trout in a hatchery in Idaho (Schell, 1974). Wales (1958) also reported mass mortalities of cutthroat trout in a California hatchery attributed to *Sanguinicola klamathensis* Wales, 1958.

16.2. Diagnosis and Clinical Signs

Diagnosis of sanguinicoliasis caused by *S. inermis* is currently determined by morphological identification of adult flukes during post-mortem of fish. Serological or molecular techniques for differential diagnosis are not available. Adult flukes can be detected by examination of the ventral and dorsal vascular system of fish using low power microscopy and then differentially identified using phase contrast microscopy. Adults of *S. inermis* can be distinguished from sympatric congeners *Sanguinicola armata* Plehn, 1905, *Sanguinicola intermedia* Ejsmont, 1926 and *Sanguinicola volgensis* (Rasin, 1929) McIntosh, 1934 by a lack of marginal spines. Immature and mature triangular-shaped eggs with a single dorsal spine on the convex edge and miracidia can be observed in gill tissue and tissue squashes from the kidney, liver and spleen examined at high magnification (400–1000 $\times$) using a high power microscope (Fig. 16.2), but they have a similar structure and size to those of *S. armata* and *S. intermedia*.

Carp fingerlings (~3.5 cm in length) can develop epithelial haemorrhage and oedema

and die within 5 h when exposed to 2500 cercariae per fish (Kirk and Lewis, 1992). After infection has established, carp fingerlings may become darkened in colour, emaciated, lethargic and anorexic with distended opercula and exophthalmia (Fig. 16.3). They often exhibit loss of balance by spiral swimming, aggregation at aeration sources and gulping at the water surface due to respiratory distress (Sapoznikov, 1988; Kirk and Lewis, 1998). Hlond *et al.* (1977) also reported septicaemia in infected carp fry. However, not all infected fish show signs of clinical disease. Young carp with low infection intensities and older carp with chronic infections may present with no or few external clinical signs except for reduced growth rate, raised scales, oedema and/or exophthalmia (Sapoznikov, 1988; Kirk and Lewis, 1994b).

16.3. Pathology (Internal Lesions)

Histopathological changes are most significant in fry and fingerling fish and the extent of damage is dependent upon parasite intensity. The spines of invading cercariae tear epithelial cells. Migrating juveniles mechanically damage dermal, connective and muscle tissue. Adult flukes puncture the pericardium and blood vessels on entry and then brace against the walls, utilizing club-shaped setae and a lobed tegument to resist the blood flow. Their presence elicits hyperplasia of the endothelial lining and high numbers of aggregated flukes impede blood flow from the heart to the gills (Kirk and Lewis, 1998) (Fig. 16.4).

Most of the pathology, however, is associated with the presence of eggs in host tissue and emigration of miracidia from the gills. The accumulation of eggs in branchial capillaries ruptures pillar cells and vessel walls, releasing eggs into adjacent tissues (Kirk and Lewis, 1998). The eggs induce hyperplasia of primary and secondary lamellae, reducing functional respiratory surface. Vascular obstruction and vessel destruction results in ischaemia in the gills, which leads to necrosis of gill tissue. In addition, miracidia emigrating from the gills cause mechanical damage and haemorrhage (Fig. 16.5) (Hlond *et al.*,

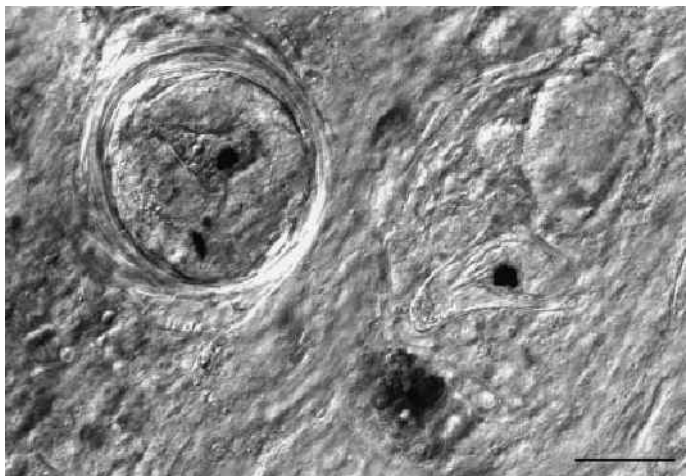


Fig. 16.2. Eggs of *S. inermis* in a kidney squash. Bar = 25 μ m.



Fig. 16.3. Uninfected 3-month carp fingerling (above) and *S. inermis* infected carp fingerling (below) ($\times 1$).



Fig. 16.4. Adult *S. inermis* occluding the bulbus arteriosus of a carp fingerling. H&E section. Bar = 50 μ m.

1977; Iqbal and Sommerville, 1986; Kirk and Lewis, 1998). Destruction of gill tissue ultimately results in establishment of secondary infections (Kirk and Lewis, 1998). Iqbal and Sommerville (1986) also reported hyperplasia, necrosis and haemorrhage of skin tissue resulting in osmoregulatory and respiratory failure. Some eggs and miracidia in the proximal part of the gills (Fig. 16.6), and eggs

lodged in other visceral sites, are sequestered in the branchial, hepatic, splenic and pancreatic tissue and then are eventually degraded within periovular granulomas (Kirk and Lewis, 1998).

Similar pathological changes occur in both young and older carp, but the effects of disease are more acute in younger carp due to their smaller size and laboratory experiments

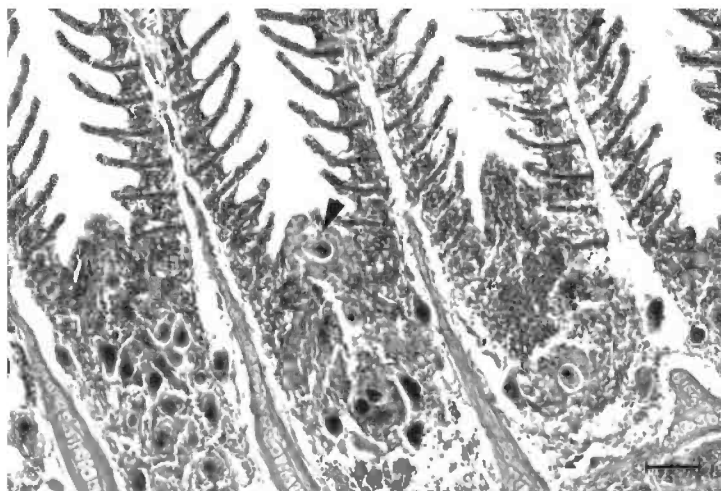


Fig. 16.5. *S. inermis* eggs in the gills of a carp fingerling, note emigrating miracidium (arrowed). H&E section. Bar = 50 μ m.

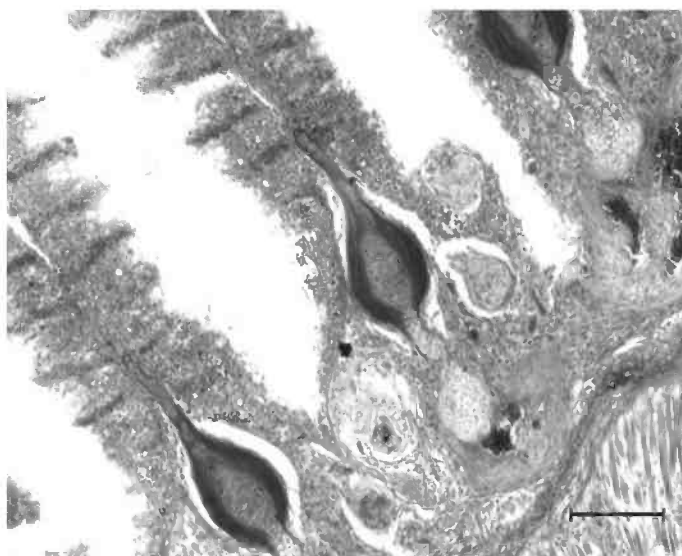


Fig. 16.6. Periovular granulomas surrounding *S. inermis* eggs in the gills of a carp fingerling. Masson's trichrome section. Bar = 100 μ m.

indicate that they are more susceptible to infection (Lee, 1990). The chronic effects of *S. inermis* in older carp may become significant when the carp are exposed to secondary biological and environment stressors (Kirk and Lewis, 1994b). Comparable damage to the respiratory and renal tissue has been noted in fish infected with salmonid *Sanguinicola* species (Davis *et al.*, 1961; Evans, 1974a; Schell, 1974; Hoffman *et al.*, 1985). In addition, *S. idahoensis* has been reported to cause significant pathology to the choroid coat and iris stroma of steelhead trout (Schell, 1974).

16.4. Pathophysiology and Immune Responses

16.4.1. Pathophysiology

Pathology caused by *S. inermis* is thought to result in impairment of respiratory, osmoregulatory and haemopoietic functions (Lucky, 1964; Iqbal and Sommerville, 1986; Kirk and Lewis, 1994b), but few studies have been carried out to elucidate and quantify pathophysiological changes. Ivasik and Svirepo (1971) reported on blood parameters of overwintering carp in the Ukraine. In comparison to uninfected carp, the haemoglobin levels of carp lightly and heavily infected with *S. inermis* were 20% and 61% lower, respectively. Serum protein levels of infected fish were lower by a mean of 69.9% in infected carp. Similarly, significantly reduced packed cell volumes were observed from brook trout with high intensities of *S. fontinalis* (Hoffman *et al.*, 1985; Holliday and Fried, 1986), and cutthroat trout experimentally infected with *S. klamathensis* (Evans, 1974b). When 1–3-year-old red roach (*Achondrostoma arcasii* reported as *Rutilus arcasii*) were experimentally infected with *Sanguinicola* sp. (later identified as *Sanguinicola rutili* Simon-Martin, Rojo-Vázquez and Simon-Vicente, 1988), the haemoglobin concentration of infected fish decreased to a low level in the youngest fish after 3 weeks p.i. (Gomez-Bautista and Simon-Martin, 1987).

Iqbal and Sommerville (1986) measured the growth performance of *S. inermis*-infected fingerling carp (11–12 months p.i.) that were

moved from farm tanks and then maintained in optimum conditions over a 16-week period at 20°C. Heavily infected carp (100% prevalence) showed a consistently poor growth performance with a specific growth rate of 1.41%/day compared with logarithmic growth in lightly infected fish (20% prevalence) at 1.62%/day. Food conversion and protein efficiency ratios were reported as below standard in both groups. Daily food intake and daily protein intake were particularly low in the heavily infected group (109.17 and 53.49 mg, respectively) compared with the lightly infected group (240.83 and 118.01 mg, respectively) due to systemic impairment (Iqbal and Sommerville, 1986).

16.4.2. Immune responses

Eggs, adults and cercariae of *S. inermis* induce a cell-mediated response in carp. Ultrastructural studies by Richards *et al.* (1994a) have shown that granuloma formation around clusters of eggs in the mesonephric interstitial tissue commences with the aggregation of eosinophils around 0–1-week-old eggs (5 weeks p.i.). The eosinophils degranulate into an amorphous layer of cell debris around the eggs, not directly on to the egg shell surface. The eggs, therefore, are undamaged at this stage, but eosinophil degranulation may promote infiltration by neutrophils and macrophages which surround the eggs 1–2 weeks later (6 weeks p.i.). Eggs become surrounded by layers of macrophages at 2–3 weeks of age, and show evidence of degradation by macrophages within a granulomatous lesion at 5–6 weeks after release (9 weeks p.i.). The macrophages probably stimulate the deposition of collagenous and fibrotic connective tissue around the eggs as in schistosomiasis (Richards *et al.*, 1994a). Early events in the cell-mediated reaction to sequestered eggs in tissues and pathogenesis in the gills are reflected by changes in the cellular composition of lymphoid organs. Further ultrastructural studies by Richards *et al.* (1994b) have shown that levels of pronephric and splenic erythrocytes were reduced in infected carp between 5 and 9 weeks p.i., compared with controls, probably due to haemorrhage in the gill tissue.

Increasing numbers of splenic thrombocytes in infected carp were associated with subsequent coagulation processes. Decreases in the numbers of pronephric and splenic eosinophils over time reflected their early involvement in the response to egg antigens and migration into associated sites of inflammation. Conversely, increases in splenic and pronephric macrophages and pronephric neutrophils occurred when these cells predominated in the encapsulating granulomatous tissue around eggs (Richards *et al.*, 1994b).

Extracts of cercariae and adults have been shown to elicit proliferation of carp pronephric and splenic lymphocytes under *in vitro* conditions in a dose-dependent manner. This response is comparable with levels produced by recognized T-cell and B-cell mitogens (Richards *et al.*, 1996a). Blastogenic response is dependent on temperature, host lymphoid organ and parasite stage. The highest levels of pronephric lymphocyte proliferation occur at 20°C, whereas the highest levels of splenic lymphocyte proliferation are elicited at 10°C. Adult extracts are more mitogenic than those of cercariae at both temperatures. Humoral immune responses, probably mediated by pronephric lymphocytes, may therefore be important in reducing fluke numbers in carp during the summer, but may not operate during lower temperatures. A reduced cell-mediated immune capacity, possibly mediated by T cells in the spleen, may enable penetration of cercariae and survival of juveniles during spring and autumn and permit adult flukes to overwinter in carp (Richards *et al.*, 1996a). This hypothesis is supported by longevity data of the parasite from experimental infections in which adults survived up to 10 weeks p.i. at 20°C (Kirk and Lewis, 1996) and up to ~210 days at 15–18°C (Sommerville and Iqbal, 1991). Live adults and cercariae induce polarization of pronephric neutrophils and eosinophils (Richards *et al.*, 1996b). Leucocytes readily attach to cercariae within 12 h and cause tegumental damage, but fewer leucocytes attach to post-penetration juveniles and adults. This suggests that transformation from cercariae to juveniles and adult stages may involve changes in the tegument that enable the adult flukes to evade the cellular immune response (Richards *et al.*, 1996c). There is also evidence

that *S. inermis* elicits a humoral response which involves specific antibody production and non-specific complement activity. An ELISA developed by Roberts *et al.* (2005) showed that parasite-specific antibodies were detected in the serum of carp intra-peritoneally injected with 150 live cercariae of *S. inermis* and maintained at 20 or 25°C, but not when exposed to 500 cercariae (Roberts *et al.*, 2005). Serum antibody levels peaked after 7 days at both temperatures and then remained at a constantly elevated titre for 63 days at 25°C, but declined at 20°C to below control levels, again emphasizing the influence of temperature on immune reactions to the parasite. The lack of an antibody response in carp naturally exposed to cercariae may be associated with the capability of *S. inermis* to bind host-like antibodies to its surface tegument or modulate antibody levels as part of an immune evasion/suppression strategy (Roberts *et al.*, 2005). Similar evasion strategies are demonstrated by mammalian adult schistosomes (reviewed in Schroeder *et al.*, 2009). Although immune evasion strategies may operate in *S. inermis*, they appear to offer limited protection since less than 7% of the original exposure dose survived to produce eggs in the vascular system of carp in experimental infections at 20°C (Richards *et al.*, 1994b; Kirk and Lewis, 1996). Deficiency in defence mechanisms may, however, reduce pathogenicity in the host and therefore be advantageous for the parasite. Evidence for a partial acquired resistance in carp is indicated in studies by Roberts (1997) which show that when carp receive a challenge infection at 8 months post-primary infection (p.p.i.), significantly fewer adult flukes established compared with the number present at 35 days p.i. in the primary infection. Numbers were not significantly decreased in a challenge infection administered at 13 months p.p.i.

Complement activity is induced by infection with cercariae of *S. inermis*. Fish intra-peritoneally injected with 150 live cercariae and kept at 20 or 25°C displayed a peak in haemolytic complement activity at 3 weeks p.i. while those fish exposed to 500 cercariae and maintained at 20°C showed a peak in complement activity at 5 weeks p.i., coincident with egg production, and then a decline to control levels (Roberts *et al.*, 2005). Elevated

levels of complement activity probably result from increases in macrophages in the spleen and pronephros and other immune cells as complement in carp is associated with pronephric granulocytes and macrophages (Nakao *et al.*, 2003) and peripheral lymphocytes (Nakao *et al.*, 2004). The cercariae and eggs of *S. inermis* are potent inducers of complement activity as is also demonstrated in the mammalian immune response to schistosome eggs (Van Egmond *et al.*, 1981; Schroeder *et al.*, 2009).

16.5. Control Measures

In a pond farm environment, an integrated approach involving parasite and snail control has been found to be effective in eliminating sanguinicoliasis from carp ponds within 1–2 years (Sapozhnikov, 1988; Lee, 1990). Snails and aquatic weed may be excluded from filled ponds and supply systems by the use of wire-mesh traps. It is recommended that snails are eliminated by the annual or biannual draining, liming and drying of ponds and application of molluscicides such as copper sulfate (5–10 g/m³) (Sapozhnikov, 1988). Environmental management of the pond farm should include removal of snail biotopes like ditches and pits (Sapozhnikov, 1988). It has also been suggested that the biological control of cercariae, miracidia and snail hosts can be implemented by the use of crustaceans and fish (Sapozhnikov and Petrov, 1980; Sapozhnikov, 1988), but this intervention must be risk assessed as these organisms would then act as reservoirs of infection for a range of parasites.

A range of anthelmintics administered in food have been tested against the parasite. A number of trials have shown efficacy of anthelmintics to be variable and without total cure (reviewed in Smith, 1997a). Treatment failure may be due to pellet rejection, particularly as sanguinicoliasis will cause reluctance to feed in heavily infected young fish. Praziquantel (Coriban, Droncit®) is one of the most effective broad spectrum anthelmintics used against *S. inermis* and will also reduce or eliminate infections of other fish helminths

such as *Khawia sinensis* and *Bothriocephalus acheilognathii*. When Didenko *et al.* (1979) administered Coriban to 1+ carp at a dose of 50 ml/kg feed at 6% total fish body weight daily for 10 days, intensities of adult flukes were reduced by 82.6% and 78.3%. Praziquantel can now be administered as a soluble formulation (Fluke-Solve™) to ponds and tanks, so eliminates the problem of pellet rejection. Trials have shown that there are no toxic effects of Fluke-Solve™ to non-target organisms in a pond (Fish Treatment Ltd, 2010). If an infection does occur, despite these interventions, the stock must be destroyed and the farm disinfected. In large extensive fisheries, sanguinicoliasis will be more difficult to control due to the economic costs and logistics of snail elimination and carp treatment. There are no commercial incentives to develop a vaccine as sanguinicoliasis is no longer reported as a widespread problem in carp fisheries.

16.6. Conclusions and Future Studies

Sanguinicola species are pathogenic to their fish hosts due to the intense cellular response elicited by sequestered eggs leading to periovular granuloma formation, and mechanical damage caused by migrating juveniles, adults and miracidia. Pathophysiological impairment of organ systems due to sanguinicoliasis has been inadequately studied, but there is little incentive to fund studies now that effective interventions have been developed against the disease in cultured fish. However, the *S. inermis*–carp model shows great potential for the investigation of blood fluke immune evasion/suppression strategies and many aspects of the fish immune system including complement and Th2 (helper T cell) type responses. The immunopathological response of carp hosts to *S. inermis* eggs shows a similarity to granuloma formation in schistosome-infected mice in which interleukin (IL)-4 has a major role in driving the Th2 response to the eggs, including the inducement of alternative activation of macrophages (Sandor *et al.*, 2003). The *S. inermis*–carp model may, therefore, be used in the investigation of

alternatively activated macrophages in fish immune systems (Joerink *et al.*, 2006).

Most of the recent research on aporocotylids has been focused on the discovery of new species. While this is important, Bullard *et al.* (2008) have highlighted that more information is required on the phylogenetic relationships of the Aporocotylidae as no clade-based phylogenetic analysis of morphological or molecular data for the majority of the genera has been reported. This is partly

due to the lack of availability of suitable specimens. Among the European *Sanguinicola* congeners, *S. inermis* is the only species for which sequence data is known (Olson *et al.*, 2003) and this is limited to one population of cercariae from *L. stagnalis* in Poland. In particular, an integrated approach incorporating morphological study coupled with the use of multiple molecular markers would facilitate a timely revision of the genus *Sanguinicola*.

Note

¹ Note that the record for *Esox lucius* is incorrect in Smith (1997b) according to the original reference Bykhovskaya-Pavlovskaya *et al.* (1964).

References

- Bauer, O.N. (1962) The ecology of parasites of freshwater fish. Parasites of freshwater fish and the biological basis for their control. *Bulletin of the State Scientific Research Institute of Lake and River Fisheries* 49, 3–215.
- Bobiatynska-Ksok, E. (1964) Circulation cycle of *Sanguinicola* Plehn in the Dojlidy fish pond farm near Bialystok. *Wiadomosci Parazytologiczne* 10, 516–517 (in Polish).
- Bullard, S.A., Snyder, S.D., Jensen, K. and Overstreet, R.M. (2008) New genus and species of Aporocotylidae (Digenea) from a basal Actinopterygian, the American paddlefish, *Polyodon spathula*, (Acipenseriformes: Polyodontidae) from the Mississippi Delta. *Journal of Parasitology* 94, 487–495.
- Bullard, S.A., Jensen, K. and Overstreet, R.M. (2009) Historical account of the two family-group names in use for the single accepted family comprising the 'fish blood flukes'. *Acta Parasitologica* 54, 78–84.
- Bykhovskaya-Pavlovskaya, I.E., Gusev, A.V., Dubinina, M.N., Izyumov, N.A., Smirnova, T.S., Sokolovskaya, I.L., Shtein, G.A., Shulman, S.S. and Epshtein, V.M. (1964) *Key to Parasites of Freshwater Fish of the USSR*. Israel Programme for Scientific Translation, Jerusalem, Israel.
- Davis, H.S., Hoffman, G.L. and Surber, E.W. (1961) Notes on *Sanguinicola davis* (Trematoda: Sanguinicolidae) in the gills of trout. *Journal of Parasitology* 47, 512–514.
- Didenko, P.P., Sapozhnikov, G.I., Babii, Yu. A., Verbovyi, G.N. and Parokhonyak, P.I. (1979) Coriban in *Sanguinicola* infection. *Veterinariya (Moscow, USSR)* 5, 49–50 (in Russian).
- 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.
- Fish Treatment Ltd (2010) Available at: www.fish-treatment.co.uk (accessed 1 December 2010).
- Gomez-Bautista, M. and Simon-Martin, F. (1987) Haematological alterations in *Rutilus arcasi* (Cyprinidae) experimentally infected with *Sanguinicola* sp. (Digenea: Sanguinicolidae). *Revista Ibérica de Parasitología* (Vol. Extraordinario), 189–193 (in Spanish).
- Hlond, S., Kozłowski, F. and Szaryk, A. (1977) Gill necrosis in carp fry on the basis of trematode infection with *Sanguinicola inermis* Plehn. *Roczniki Nauk Rolniczych, Poland, Seria H* 98, 65–76 (in Polish).
- Hoffman, G.L., Fried, B. and Harvey, J.E. (1985) *Sanguinicola fontinalis* sp. nov. (Digenea: Sanguinicolidae): a blood parasite of brooke trout, *Salvelinus fontinalis* (Mitchill), and longnose dace, *Rhinichthys cataractae* (Valenciennes). *Journal of Fish Diseases* 8, 529–538.
- Holliday, C.W. and Fried, B. (1986) Some physiological effects of *Sanguinicola fontinalis* (Trematoda: Sanguinicolidae) infection in the brook trout *Salvelinus fontinalis*. *Journal of Parasitology* 72, 189–190.

- Iqbal, N.A.M. and Sommerville, C. (1986) Effects of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae) infection on growth performance and mortality in carp, *Cyprinus carpio* L. *Aquaculture and Fisheries Management* 17, 117–122.
- Ivasik, V.M. and Svirepo, B.G. (1971) *Sanguinicola inermis* in carp in the winter (Ukrainian SSR). *Helminthologia Year 1969* 10, 103–105 (in Russian).
- Joerink, M., Forlenza, M., Ribeiro, C.M.S., De Vries, B.J., Savelkoul, H.F.J. and Wiegertjes, G.R. (2006) Differential macrophage polarisation during parasitic infections in common carp (*Cyprinus carpio* L.). *Fish and Shellfish Immunology* 21, 561–571.
- Kirk, R.S. and Lewis, J.W. (1992) The laboratory maintenance of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae). *Parasitology* 104, 121–127.
- Kirk, R.S. and Lewis, J.W. (1993) The life-cycle and morphology of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae). *Systematic Parasitology* 25, 125–133.
- Kirk, R.S. and Lewis, J.W. (1994a) The distribution and host range of species of the blood fluke *Sanguinicola* in British freshwater fish. *Journal of Helminthology* 68, 315–318.
- Kirk, R.S. and Lewis, J.W. (1994b) Sanguinicoliasis in cyprinid fish in the UK. In: Pike, A. and Lewis, J.W. (eds) *Parasitic Diseases of Fish*. Samara Publishing Ltd for the British Society for Parasitology and the Linnean Society of London, Dyfed, Wales, UK, pp. 101–117.
- Kirk, R.S. and Lewis, J.W. (1996) Migration and development of the blood fluke *Sanguinicola inermis* Plehn, 1905 (Trematoda: Sanguinicolidae) in carp, *Cyprinus carpio* L. *Parasitology* 113, 279–285.
- Kirk, R.S. and Lewis, J.W. (1998) Histopathology of *Sanguinicola inermis* infection in carp, *Cyprinus carpio*. *Journal of Helminthology* 72, 33–38.
- Lee (Kirk), R.S. (1990) The development of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae) in the common carp (*Cyprinus carpio* L.). PhD thesis, University of London, London, UK.
- Lucky, Z. (1964) Contribution to the biology and pathogenicity of *Sanguinicola inermis* in juvenile carp. *Proceedings of the Symposium on Parasitic Worms and Aquatic Conditions*. Czechoslovak Academy of Sciences Prague, Czech Republic, pp. 153–157.
- McMichael-Phillips, D.F., Lewis, J.W. and Thorndyke, M.C. (1992a) Ultrastructure of the egg of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae). *Journal of Natural History* 26, 895–904.
- McMichael-Phillips, D.F., Lewis, J.W. and Thorndyke, M.C. (1992b) Ultrastructural studies on the miracidium of *Sanguinicola inermis* (Digenea: Sanguinicolidae). *Parasitology* 105, 435–443.
- McMichael-Phillips, D.F., Lewis, J.W. and Thorndyke, M.C. (1994) Ultrastructure of the digestive system of adult *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae). *Journal of Helminthology* 68, 149–154.
- Moravec, F. (1984) Occurrence of endoparasitic helminths in carp (*Cyprinus carpio* L.) from the Macha lake fishpond system. *Vestník Československé Společnosti Zoologické* 48, 261–278.
- Nakao, M., Fujiki, K., Konodo, M. and Yano, T. (2003) Detection of complement receptors on head kidney phagocytes of common carp *Cyprinus carpio*. *Fisheries Science* 65, 929–935.
- Nakao, M., Miura, C., Itoh, S., Nakahara, M., Okumura, K., Mutsuro, J. and Yano, T. (2004) A complement C3 fragment equivalent to mammalian C3d from the common carp (*Cyprinus carpio*): generation in serum after activation of the alternative pathway and detection of its receptor on the lymphocyte surface. *Fish and Shellfish Immunology* 16, 139–149.
- Naumova, A.M. (1961) Seasonal dynamics of *Sanguinicola inermis* infections in carp. *Doklady Moskovskoi Sel'sko-Khozyaistvennoi Akademii imenika K.A. Timiryazeva* 69, 211–216 (in Russian).
- Odhner, T. (1911) *Sanguinicola* M. Plehn—a digenean Trematode! With an appendix on previous observations of Prof. A. Looss, Kairo. *Zoologischer Anzeiger* 38, 33–45 (in German).
- Olson, P.D., Cribb, T.H., Tkach, V.V., Bray, R.A. and Littlewood, D.T. (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal of Parasitology* 33, 733–755.
- Plehn, M. (1905) *Sanguinicola armata* and *inermis* (n. gen. n. sp.) n. fam. Rhynchostomida. Endoparasitic Turbellaria in the blood of cyprinids. *Zoologischer Anzeiger* 29, 244–252 (in German).
- Plehn, M. (1908) Monozoic cestodes as blood parasites (*Sanguinicola armata* und *inermis* Plehn). *Zoologischer Anzeiger* 33, 427–440 (in German).
- Richards, D.T., Hoole, D., Lewis, J.W., Ewens, E. and Arme, C. (1994a) 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., Hoole, D., Lewis, J.W., Ewens, E. and Arme, C. (1994b) 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., Hoole, D., Lewis, J.W., Ewens, E. and Arme, C. (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., Hoole, D., Lewis, J.W., Ewens, E. and Arme, C. (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., Hoole, D., Lewis, J.W., Ewens, E. and Arme, C. (1996c) Adherence of carp leucocytes to adults and cercariae of the blood fluke *Sanguinicola inermis*. *Journal of Helminthology* 70, 63–67.
- Roberts, M.L. (1997) The immune response of carp (*Cyprinus carpio* L.) to the blood fluke *Sanguinicola inermis* Plehn, 1905 (Trematoda: Sanguinicolidae). PhD thesis, Keele University, Keele, Staffordshire, UK.
- Roberts, M.L., Lewis, J.W., Wiegertjes, G.F. and Hoole, D. (2005) Interaction between the blood fluke, *Sanguinicola inermis* and humoral components of the immune response of carp, *Cyprinus carpio*. *Parasitology* 131, 261–271.
- Sandor, M., Weinstock, J.V. and Wynn, T.A. (2003) Granulomas in schistosome and mycobacterial infections: a model of local immune responses. *Trends in Immunology* 24, 44–52.
- Sapozhnikov, G.L. (1988) *Sanguinicola* infestation in carp and its control. *Veterinariya (Moscow, USSR)* 8, 36–38 (in Russian).
- Sapozhnikov, G.L. and Petrov, Yu.F. (1980) The role of the living components of pond biocoenoses in the eradication of *Sanguinicola*. *Veterinariya (Moscow, USSR)* 9, 45–47 (in Russian).
- 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.
- Schroeder, H., Skelly, P.J., Zipfel, P.F., Losson, B. and Vanderplasschen, A. (2009) Subversion of complement by hematophagous parasites. *Developmental and Comparative Immunology* 33, 5–13.
- 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.
- Sommerville, C. and Iqbal, N.A.M. (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.
- Sweeting, R.A. (1979) *Sanguinicola*—a case study. *Proceedings of the Institute of Fisheries Management 10th Annual Study Course* 10, 217–220.
- Tang, C.C. and Ling, S.M. (1975) *Sanguinicola lungensis* sp. nov. and the outbreaks of sanguinicolosis in Lien-yü nursery ponds in South Fukien. *Journal of Xiamen University (Natural Science)* 2, 139–160 (in Chinese).
- Van Egmond, J.G., Deelder, A.M. and Daha, M.R. (1981) *Schistosoma mansoni*: complement activity by antigenic preparations. *Experimental Parasitology* 51, 188–194.
- Wales, J.H. (1958) Two new blood fluke parasites of trout. *California Fish and Game* 44, 125–136.

17 *Bothriocephalus acheilognathi*

Tomáš Scholz,¹ Roman Kuchta¹ and Chris Williams²

¹*Institute of Parasitology, Biology Centre of the Academy of Sciences
of the Czech Republic, České Budějovice, Czech Republic*

²*Environment Agency, Cambridgeshire, UK*

17.1. Introduction

The Asian tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda: Bothriocephalidea), is the most important pathogenic cestode of cyprinid fish, which causes bothriocephalosis and one of the most dangerous helminth parasites of cultured carp (Bauer *et al.*, 1977; Nie and Hoole, 2000). The parasite has also been recorded in a range of other freshwater teleost fishes, prompting concern of the disease in wild fish populations (Clarkson *et al.*, 1997; Heckmann, 2000). It is listed as a 'Pathogen of Regional Concern' by the US Fish and Wildlife Service (2010).

B. acheilognathi has been reported under more than 20 different specific names and the most frequently used are *Bothriocephalus gotokongensis* Yeh, 1955 and *Bothriocephalus opsariichthydis* Yamaguti, 1934 (for a list of synonyms – see Kuchta and Scholz, 2007). According to Pool and Chubb (1985) and Pool (1987), all descriptions of *Bothriocephalus* tapeworms from cyprinid hosts represent the same parasite, differing only in length and the shape of the scolex because different methods were used to fix the worm.

B. acheilognathi is indigenous to East Asia, but has spread rapidly throughout the world with the trade of fish. The parasite has now been recorded from every continent excluding Antarctica. The ability to success-

fully colonize new geographical regions has been facilitated by its simple, two-host life cycle (involving common copepod species as an intermediate host) and euryxenous host specificity (very wide range of suitable fish hosts). This has led to the transmission and establishment of *B. acheilognathi* to many new host species in areas where it has been introduced (Scholz, 1999; Salgado-Maldonado and Pineda-López, 2003). Once established it may endanger native fish populations, including ecologically sensitive species and fishes that are phylogenetically unrelated to those in which it was introduced (Font and Tate, 1994; Dove and Fletcher, 2000).

B. acheilognathi can have pronounced detrimental effects on fish. These include severe damage to the intestinal tract, physiological disturbance, reduced growth, condition loss and death. Records of 100% mortality in hatchery reared common carp (*Cyprinus carpio*) highlight the pathogenic potential of this parasite.

17.1.1. Description

B. acheilognathi typically measures between 3.5 and 8 cm in length and up to 4 mm in width (Yeh, 1955), although specimens of 60 cm and even 1 m have been observed (Baer and Fain, 1958; Granath and Esch, 1983a).

Size is a highly variable morphological parameter as it depends on: (i) ecological conditions (Nevada and Mutafova, 1988); (ii) host size (Davydov, 1978); (iii) host species (Molnár and Murai, 1973; Granath and Esch, 1983a); (iv) host age; and (v) intensity of infection (Davydov, 1978). Upon relaxation, parasites can also increase in length by a factor of 1.5–2 (Pool, 1987). Consequently, parasite size is not a valid feature on which to base identification, despite earlier beliefs (Molnár and Murai, 1973).

An important morphological characteristic of *B. acheilognathi* is its heart-shaped scolex, with a weakly developed apical disc and a pair of deep, slit-like grooves (bothria) positioned dorsoventrally along the scolex (Figs. 17.1a and 17.2). The scolex is much wider than the first body segments (proglottides). The strobila (body) of the tapeworm consists of numerous proglottides (Fig. 17.1b), each containing one set of reproductive organs

(Fig. 17.1c). The shape of these segments differs with maturity. Immature segments lacking fully developed genital organs are always wider than they are long, whereas more developed gravid segments bearing eggs are rectangular and longer than they are wide. However, contraction and relaxation of the segments also causes extreme variation in the length and width ratio of the strobila (Brandt *et al.*, 1981).

The male reproductive system is formed by numerous spherical testes situated in the medulla (the region internal to the inner longitudinal musculature). A muscular cirrus-sac localized anterior to the ovary opens on the dorsal side of segments into a common genital atrium, which is situated alongside the median line of the body.

The female reproductive system is composed by a bi-lobed ovary situated near the posterior margin of each segment. The vagina, which is short and slightly sinuous, opens

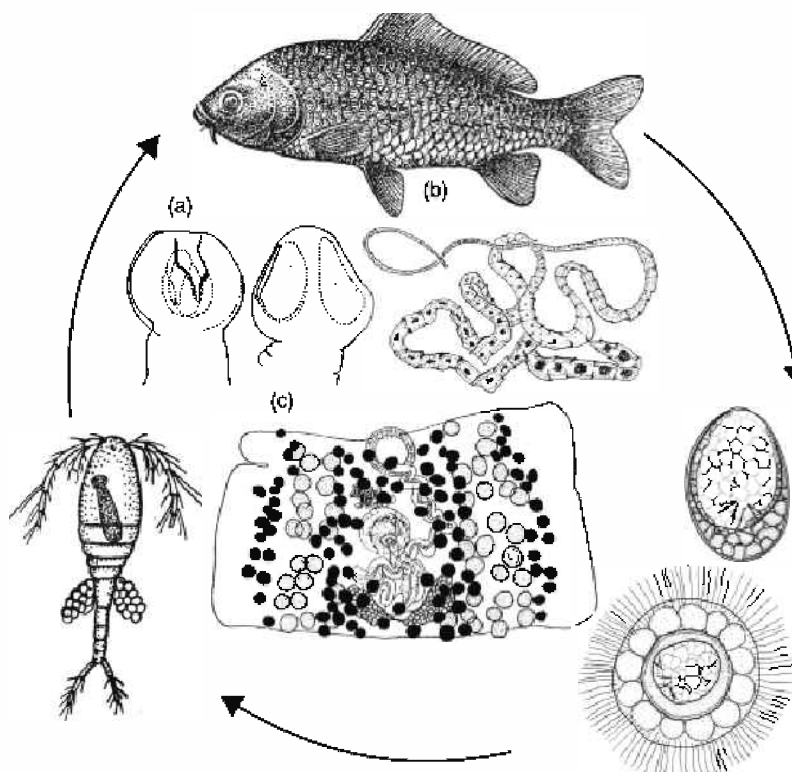


Fig. 17.1. Life cycle and morphology of *Bothriocephalus acheilognathi*. a, scolex; b, total view (segmented body); c, mature segment (proglottis).

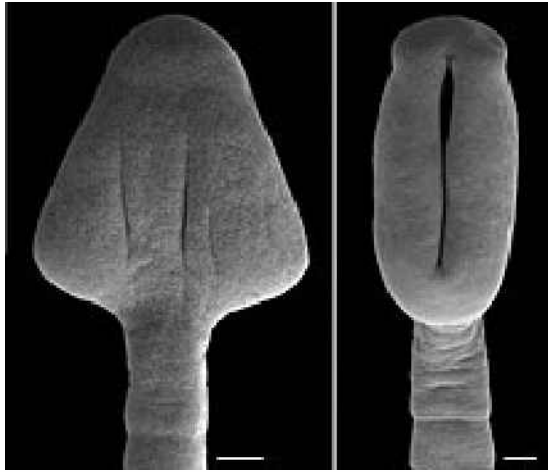


Fig. 17.2. Scanning electron micrographs of the scolex of *B. acheilognathi*. Bar = 100 μ m.

into the common genital atrium posterior to the male genital pore. Vitelline follicles are very numerous, circumcortical and confluent between segments. The uterus is saccular, spherical to oval, and opens on the ventral side in the anterior third of the segment. The eggs are thick-walled, operculate (i.e. with a cap – the operculum) on a narrower pole, and usually unembryonated (without a formed embryo) when released into the water.

17.1.2. Life cycle and transmission

B. acheilognathi has a simple two-host life cycle, involving a planktonic copepod (Copepoda: Cyclopidae) as an intermediate host (Fig. 17.1). In favourable conditions, the life cycle may be completed in about 1 month. Eggs shed by adult parasites into the gut lumen are released into the water with faeces. Depending on water temperature, an embryo (six-hooked oncosphere or hexacanth) is formed within the egg in a few days. The larva (coracidium) is surrounded by ciliated cells which enable its active movement in the water after hatching from the egg.

A number of copepods are suitable intermediate hosts in both experimental and natural conditions. These include species of *Acanthocyclops*, *Cyclops*, *Macrocylops*, *Megacyclops*, *Mesocyclops*, *Thermocyclops* and

Tropocyclops (Marcogliese and Esch, 1989) which are considered common in most fresh waters throughout the world (Pool, 1984). Other planktonic crustaceans, such as diaptomids and cladocerans, are not suitable intermediate hosts (Molnár, 1977).

After ingestion, the coracidium loses its ciliature and penetrates the gut into the body cavity where it develops from an oncosphere into a plerocercoid (previously also called pro-cercoid – see Chervy, 2002 for terminology of cestode larvae). Larval development is completed in a few weeks depending on the water temperature: within 21–23 days at 28–29°C (Liao and Shih, 1956), but 1.5–2 months at 15–22°C (Davydov, 1978). The life cycle is completed when fish ingest infected copepods. Once established within the intestine of a suitable fish, egg production may begin in as little as 20 days (Liao and Shih, 1956).

It has been shown that transmission of adult parasites can occur from fish to fish via predation by piscivorous fish on infected prey, a phenomenon known as postcyclic transmission (Odening, 1976; Hansen *et al.*, 2007). Local spread caused by aquatic birds, such as *Anas platyrhynchos* and *Chlidonias niger*, was assumed to take place based on experiments conducted by Prigli (1975) and field observations (finding of *B. acheilognathi* in *Ixobrychus minutus*) by Borgarenko (1981), but this mode of parasite dissemination needs verification. There are also records of the

tapeworm in an amphibian (axolotl *Ambystoma dumerilii* – García-Prieto and Osorio-Sarabia, 1991) and a snake *Thamnophis melanogaster* (Pérez-Ponce de León *et al.*, 2001) although these records may represent only accidental infection.

17.1.3. Definitive (fish) hosts

The most suitable hosts of *B. acheilognathi* are cyprinids, especially the common carp (*C. carpio*) and grass carp (*Ctenopharyngodon idella*). However, the parasite has been reported from approximately 200 species of freshwater fishes, representing ten orders and 19 families (Salgado-Maldonado and Pineda-López, 2003; R. Kuchta – unpublished data). Nevertheless, maturity of the worm may be reached in only a proportion of these fish species (Dove and Fletcher, 2000).

Holmes (1979) identified three classes of host, in terms of their ability to allow the maturation of parasites: (i) 'required hosts'; (ii) 'suitable hosts'; and (iii) 'unsuitable hosts'. In 'required hosts' parasites usually obtain full maturity. In 'suitable hosts' parasites may gain sexual maturity, but are only found in small numbers, while in 'unsuitable hosts' parasites may establish but do not reach maturity. Consequently, although a parasite may infect many fish species, the maintenance of the parasite population may rely on a much narrower range in which reproduction takes place (Riggs *et al.*, 1987).

Small fish are more commonly and intensively infected with *B. acheilognathi* than large hosts (Leong, 1986). Brouder (1999) detailed a strong negative correlation between size of host and infection intensity of *B. acheilognathi*.

17.1.4. Geographical distribution

B. acheilognathi was originally described from a small cyprinid fish, *Acheilognathus rhombeus* (Temminck & Schlegel) (Cypriniformes: Cyprinidae), from Lake Ogura, Kyoto Prefecture, Honshu, in Japan (Yamaguti, 1934). The parasite is endemic in China, Japan and the Amur River, Asia (Yamaguti, 1934; Yeh, 1955;

Dubinina, 1971). Initial movements of *B. acheilognathi* were linked closely with the spread of carp westwards from Japan and China to Europe during the 1960s and 1970s (Minervini *et al.*, 1985).

The spread of *B. acheilognathi* throughout most parts of the world has been well documented (Bauer and Hoffman, 1976) and represents one of the best examples of parasite translocation through man-assisted activities (Bauer and Hoffman, 1976; Dove and Fletcher, 2000). The rapid spread of this parasite has been assisted by the trade in many cyprinid species for: (i) aquaculture (Minervini *et al.*, 1985); (ii) the ornamental fish industry (Edwards and Hine, 1974; Evans and Lester, 2001); (iii) aquatic weed control (Maitland and Campbell, 1992); (iv) mosquito control (Dove and Fletcher, 2000); and (v) the fishing bait industry (Heckmann, 2009).

The tapeworm was introduced between 1954 and 1962 into the European part of the former USSR as a result of uncontrolled imports of grass carp and common carp from the River Amur and China. Infections of *B. acheilognathi* became widespread in farmed carp as well as in a variety of wild fishes (Malévitskaya, 1958; Radulescu and Georgescu, 1962; Bauer and Hoffman, 1976). The increased importance of carp for food and weed control led to the rapid spread of *B. acheilognathi* throughout Europe.

By 1970–1975, the tapeworm had colonized several countries of Central and Eastern Europe (Austria, Bulgaria, former Czechoslovakia, Germany, Hungary, Poland and former Yugoslavia – Buza *et al.*, 1970; Petkov, 1972; Bauer and Hoffman, 1976; Minervini *et al.*, 1985). By the 1980s, the parasite became established in France (Denis *et al.*, 1983) and the UK (Andrews *et al.*, 1981).

The origin of African populations of *B. acheilognathi*, first described as *Bothriocephalus (Clesthobothrium) kivuensis* from barbels (*Barbus* spp.) by Baer and Fain (1958) in Zaire (now the Democratic Republic of the Congo), is not clear. The tapeworm has since been reported from Egypt and South Africa (Rysavy and Moravec, 1975; Brandt *et al.*, 1981).

The tapeworm was introduced to the New World in 1965 with grass carp imported

from China to Mexico (López-Jiménez, 1981), then to the USA (Texas) in 1975 and Canada (British Columbia) in 1983 (Hoffman, 1999; Choudhury *et al.*, 2006). It was suspected that bait minnows (*Plagopterus argentissimus*) represented the main source of the tapeworm infecting fish populations in the western USA (Heckmann, 2000, 2009). To date, *B. acheilognathi* has been reported in 13 states of the USA (Hoffman, 1999; Choudhury *et al.*, 2006), most recently from the Great Lakes area (Marcogliese, 2008). There is only one published record of *B. acheilognathi* from South America (Rego *et al.*, 1999), most probably as a result of the import of carp from Europe to Brazil (Cornelio Procópio, Paraná).

Records of *B. acheilognathi* from India, Iraq, Israel, Korea, Malaysia, the Philippines, Sri Lanka and Turkey confirm its wide distribution in Asia (Paperna, 1996; Hoffman, 1999).

In Australia, *B. acheilognathi* was detected in goldfish (*Carrasius auratus* (L.)) and koi carp (*Cyprinus carpio* (L.)) (Langdon, 1992). The tapeworm is now widely distributed in many native finfish species in eastern Australia (Dove and Fletcher, 2000). The parasite was imported to New Zealand with grass carp but was then eradicated during quarantine (Edwards and Hine, 1974). The ability of *B. acheilognathi* to colonize isolated geographical localities has been confirmed by records of the parasite in Puerto Rico, Hawaii and Mauritius and remote subterranean sinkholes (cenotes) in Yucatan (Bunkley-Williams and Williams, 1994; Font and Tate, 1994; Scholz *et al.*, 1996).

B. acheilognathi has so far been recorded in six continents as a result of the introduction of cyprinids (grass carp, carp), and guppies (mosquito-fish – *Gambusia* and *Poecilia*) by man to control mosquito larvae (Hoffman and Schubert, 1984; Salgado-Maldonado and Pineda-López, 2003). However, the distribution area is limited between 60°N and 40°S. The spread of *B. acheilognathi* further north or further south in the southern hemisphere is unlikely, because the cestode is thermophilic with an optimum temperature between 22 and 25°C (Bauer *et al.*, 1981; Granath and Esch, 1983a; Hanzelová and Žitňan, 1986).

17.1.5. Importance of the disease

B. acheilognathi is an important pathogen in aquaculture in Asia and Europe (Bauer *et al.*, 1981; Heckmann, 2009). Losses of juvenile fish, with up to 100% mortality, occur in hatchery ponds. In commercial carp farms, fry (length 38–42 mm) can be infected 28–29 days after hatching (Hanzelová and Žitňan, 1986). The susceptibility of fry is probably because copepods make up a large proportion of the diet of these fish, and the limited space within the intestinal tract to accommodate these large parasites. Heavy tapeworm burdens cause blockage of the intestine and severe pathological changes, leading to reduced growth, condition and survival (Scott and Grizzle, 1979; Granath and Esch, 1983b; Hoole and Nissan, 1994; Hansen *et al.*, 2006). The tapeworm has also been the cause of disease problems in ornamental fish farms in Australia and Central Europe, involving *Poecilia reticulata* and *Xiphophorus maculatus* (Evans and Lester, 2001; R. Kuchta, unpublished data) and mortality of koi carp (Han *et al.*, 2010).

Far less information exists on the impact of *B. acheilognathi* in wild fish populations. The introduction of this alien tapeworm to new localities can endanger native fish species (Dove and Fletcher, 2000; Heckmann, 2000, 2009; Salgado-Maldonado and Pineda-López, 2003). This may be particularly serious in fish that attain only a small size at maturity, with potential for reduced recruitment, growth, fitness and survival. However, equilibrium between host and parasite can develop in a relatively short period, limiting disease impacts (Hoffman, 1999). It is recognized that identification and evaluation of the effects of parasites in wild fish populations is problematic, as sick fish are rapidly removed by predators, water flow and necrophages (Blanc, 1997).

17.2. Diagnosis of Infection and Clinical Signs

Infections can be readily detected at autopsy, with the recovery of entire tapeworms

followed by microscopic examination of the scolex (Figs. 17.2, 17.3. and 17.4). The examination of faecal material may reveal detached segments from adult tapeworms or eggs, which possess an operculum at the apex. The presence of *B. acheilognathi* may also be achieved by a squash plate method. Glass slides or plates are used to flatten the intestinal tract and the worms are detected by reflected light and low-power microscopy. Although mature parasites may be conspicuous within the intestine of infected fish (Figs. 17.3 and 17.4), the detection of light infections or presence of juvenile parasites and plerocercoids requires microscopic examination. While these infections may hold little importance to the disease status of individuals, detection may be critical for effective disease control and limiting the spread of infected fish.

Infected fish may become sluggish and swim close to the water surface (Hoole, 1994). This may be accompanied by inappetence, slow growth, condition loss, emaciation and signs of anaemia (Liao and Shih, 1956; Edwards and Hine, 1974; Scott and Grizzle, 1979; Hoole and Nisan, 1994; Sopinska and Guz, 1997). Infected fish may be more susceptible to secondary bacterial infections due to the debilitating effects of the parasite (Clarkson *et al.*, 1997) and destruction of the epithelial layer of the intestine (Bauer *et al.*, 1981). Heavy tapeworm burdens can cause the body of infected carp fry to become noticeably distended and swollen (Scott and Grizzle, 1979; Brandt *et al.*, 1981). In very small fish, the movement of tapeworms may be seen through the body wall prior to dissection.

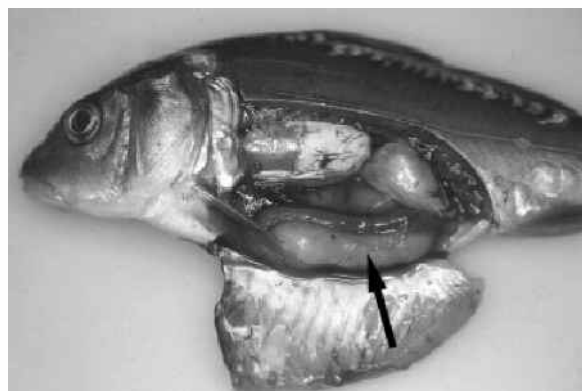


Fig. 17.3. Juvenile common carp (*Cyprinus carpio*) with infection of *B. acheilognathi* (arrow).

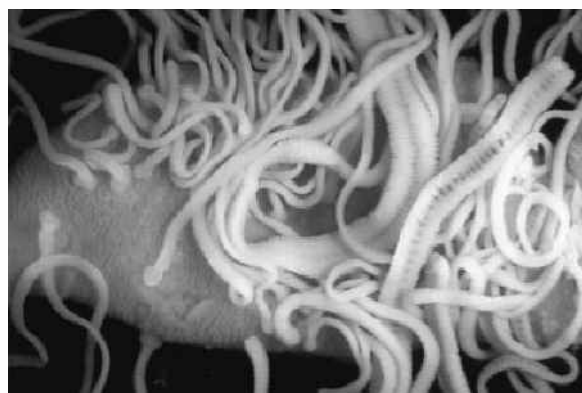


Fig. 17.4. Opened intestine of common carp (*C. carpio*) infected with *B. acheilognathi*.

The intestinal tract of infected fish is often grossly enlarged, very thin-walled and obviously occluded. In such cases the gut wall can be stretched to the point of transparency, revealing the white-coloured worms within (Fig. 17.3) and even allowing individual body segments of parasites to be distinguished. Internal organs of infected hosts may become enlarged and the gall bladder swollen and turgid. Parasites usually accumulate at the posterior part of the first loop of the intestine, posterior to the common bile duct opening. Small focal haemorrhages may be detected at the point of scolex attachment, extending in severity to full haemorrhagic enteritis (Hoole and Nisan, 1994). In extreme cases, intestinal perforation may result with rupture of the intestinal tract (Scott and Grizzle, 1979; Heckmann, 2000).

Intensity of infection may range from 30 to 156 mature, gravid worms per pond-reared carp (90–160 mm in length), and up to 20, although usually less than ten, in fully-grown grass carp (Scott and Grizzle, 1979). The highest number of parasites recorded in a single fish was 467 worms (Liao and Shih, 1956), but no data on their size were provided. However, small numbers of very large tapeworms can also cause pronounced pathological changes. These records suggest that disease results from the overall mass of parasites, rather than a defined intensity of infection (Davydov, 1977). The pathogenicity of *B. acheilognathi* may also vary at different times of year due to changes in the number of parasites present, water temperature, metabolic rates, nutritional status of the host and duration of infection (Scott and Grizzle, 1979).

17.3. Macroscopic and Microscopic Lesions

Histopathological studies have been conducted on a wide range of fish species including the common carp (Sekrearyuk, 1983; Hoole and Nisan, 1994), grass carp (*C. idella* – Liao and Shih, 1956; Scott and Grizzle, 1979), spottail shiner (*Notropis hudsonius*), fat-head minnow (*Pimephales promelas*), woundfin (*Plagopterus argenteus*) and roundtail

chub (*Gila robusta* – Heckmann, 2000). The pathology may be divided generally into: (i) damage caused by scolex attachment; and (ii) damage caused by the presence of strobila within the intestine lumen (Scott and Grizzle, 1979; Hoole and Nisan, 1994). Other organs may exhibit signs of pathological change. For example, infected fish can show signs of nutritional deficiency, with atrophy of hepatocytes within the liver. In severe cases, these changes are consistent with starvation (C. Williams, unpublished data).

17.3.1. Pathological changes caused by attachment of *B. acheilognathi*

B. acheilognathi attaches to the gut wall by its bothria, which engulf the intestinal folds. This causes compression of the mucosal epithelium, focal pressure necrosis and haemorrhage (Fig. 17.5). Scolex attachment may also be associated with increased mucus production (Scott and Grizzle, 1979). Tapeworm attachment also provokes a localized inflammatory response, consisting mainly of lymphocytes. In heavy infections, increased numbers of lymphocytes may occur throughout the *lamina propria*. Lesions associated with the scolex depend upon the force exerted by the bothria to maintain attachment. The scolex is often pushed firmly against the gut wall causing compression and the formation of localized pits, extending as far as the muscularis (Figs 17.5 and 17.6). These attachment sites lead to pronounced thinning of the intestine at these points. Scolex attachment can also result in a loss of brush border and an overall reduction in thickness of the terminal web. In advanced cases of infection, scolex attachment can cause localized ulceration. Desquamative catarrhal enteritis and proliferation of connective tissue around the point of scolex attachment have also been recorded (Bauer *et al.*, 1973).

Heckmann (2000) provided unique accounts of advanced scolex penetration. Studies on woundfin and roundtail chub revealed severe pathology associated with penetration of the gut wall up to the muscularis, resulting in a prominent inflammatory response, extensive haemorrhaging and

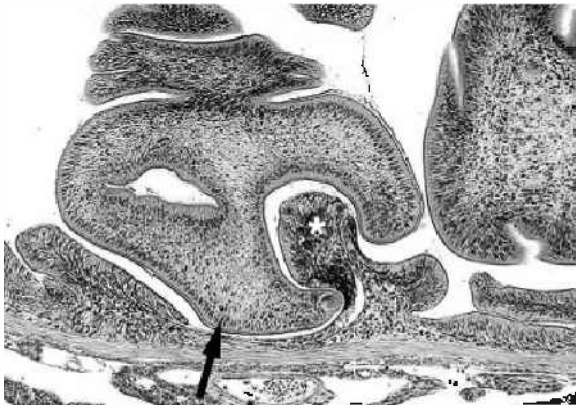


Fig. 17.5. Scolex of *B. acheilognathi* engulfing the intestine of common carp causing compression of the mucosa (arrow) and localized haemorrhage (star).

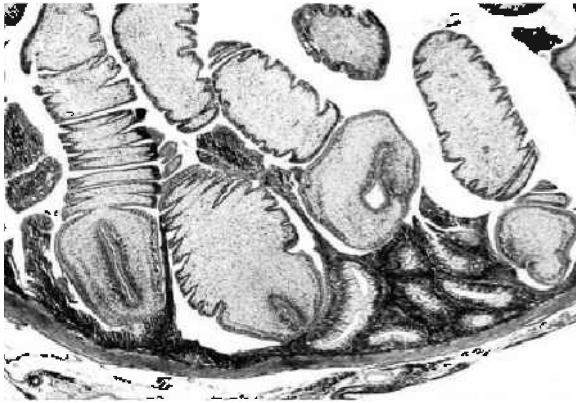


Fig. 17.6. Marked thinning of the intestine, with formation of pits in the intestinal wall caused by the attachment of numerous tapeworms.



Fig. 17.7. Transverse section of common carp intestine showing attenuation of the gut and partial occlusion from tapeworms within.

necrosis. The scolex of some parasites even continued to penetrate the intestine wall into the body cavity, extending as far as the liver and gonads (Heckmann, 2000). This represents an unusual and rarely reported consequence of *B. acheilognathi* infection.

Scolex attachment causes considerable disruption to the intestine, including: (i) destruction of the desmosomal junctions; (ii) loss of the gut microvillous border; (iii) separation and loss of enterocytes; (iv) release of host-cell debris into the gut lumen; and (v) infiltration of leukocytes into the infected area. In hosts less than 4 cm in length, damage through attachment can be extensive and is consistent with disruption of gut enzymes (Hoole and Nissan, 1994). In many places, the plasmalemma between the microtriches and that of the epithelial cells of the host intestine are lacking, so that the matrix of the tegument is in direct contact with the cytoplasm of the host cells. Lysosomes have been demonstrated surrounding the microtriches embedded in the host cytoplasm (Smyth and McManus, 1989).

17.3.2. Pathological changes caused by the strobila of *B. acheilognathi*

The pathological changes caused by the strobila of *B. acheilognathi* generally exceed those

associated with scolex attachment. The extent and severity of this damage can vary depending on host size, parasite size and intensity of infection (Davydov, 1978; Hoole and Nissan, 1994). In small cyprinid fish, pathological changes are characterized by distension of the intestine, compression of the intestinal folds and pronounced thinning of the gut wall (Fig. 17.7). In very heavy infections severe distension may be accompanied by a complete loss of normal gut architecture, with occlusion of the intestine, congestion, compression, pressure necrosis, thinning and atrophy of the mucosa (e.g., Nakajima and Egusa, 1974a) (Fig. 17.8). Separation and degeneration of the epithelium, with regions of complete epithelial loss can occur. Lysis of large areas of the mucosa with necrotic changes has also been observed in heavily infected carp fry (Davydov, 1977; Sekretaryuk, 1983).

The presence of parasite eggs caught between the parasites and gut wall can lead to epithelial abrasion, exfoliation of host cells and indentation of the mucosa. This damage, combined with an already compressed gut wall, can lead to ulceration. Inflammatory changes may be pronounced during heavy tapeworm burdens, with increased numbers of lymphocytes and eosinophilic granular cells occurring throughout infected regions.

Paradoxically, heavy parasite infections and marked pathological changes have been

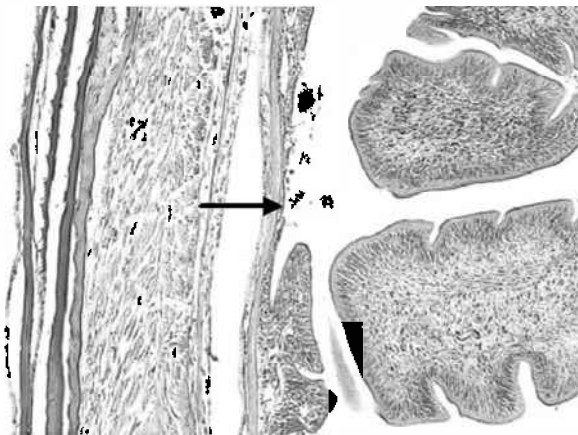


Fig. 17.8. Severe intestinal compression, with necrosis and complete loss of epithelium (arrowhead). The damage within this region is approaching intestinal rupture.

observed in apparently healthy fish (C. Williams, unpublished data). Nakajima and Egusa (1974a) described no signs of mortality, despite serious histopathological changes in common carp fry. What is seldom clear in these cases are the metabolic and physiological costs of these infections and the energetic or behavioural demands upon infected fish to maintain condition.

17.4. Disease Mechanism

B. acheilognathi causes a number of physiological changes in juvenile fish. These include: (i) protein depletion; (ii) altered digestive enzyme activity; (iii) elevated muscle fatigue in heavily infected hosts; and (iv) mortality of young fishes (Liao and Shih, 1956; Davydov, 1978; Scott and Grizzle, 1979; Granath and Esch, 1983b; Brouder, 1999; Hansen *et al.*, 2006). Bothriocephalosis also reduces fat content and causes a decrease in kidney, liver and spleen weight (Balakhnin, 1979; Žitňan and Hanzelová, 1982).

According to Clarkson *et al.* (1997), *B. acheilognathi* infection causes a reduction in reproductive capacity, depressed swimming ability and elevated muscle fatigue in heavily infected hosts. Granath and Esch (1983b) also showed reduced ability of mosquito-fish to adapt to changing water temperatures, resulting in the mortality of infected fish.

Relatively little is known about the relationship between the fish immune response and *B. acheilognathi* infections, although inflammation occurs in intestines of infected fish and leukocytes were noted on the surface of the parasite (Hoole and Nisan, 1994; Nie and Hoole, 2000). The interaction between the parasite and pronephric lymphocytes of carp was studied by examining proliferation of lymphocytes isolated from both naïve fish and fish injected intraperitoneally with cestode extract (Nie *et al.*, 1996). Parasite extracts increased antibody production and pronephric antibody-producing cells in injected fish (Nie and Hoole, 1999), and stimulated proliferation of pronephric lymphocytes *in vitro* after 5 and 10 days post-injection (Nie *et al.*, 1996).

Reduced haemoglobin and total blood volume were reported in infected carp (Kudryashova, 1970; Pár, 1978; Svobodová,

1978), but opinions vary as to the intensity of infection necessary to induce these pathogenic effects. Kudryashova (1970) found these effects in fish with more than five worms while Svobodová (1978) did not find any significant effect on various physiological indices in fish harbouring between one and 21 worms and attributed the elevated leukocyte count to inflammation of the gut. According to Pár (1978), fish with between one and 29 worms had an elevated leukocyte count and those with more than 15 specimens showed marked damage to the gut.

Biochemical studies showed reduction in activities of enzymes, such as alanine and aspartate aminotransferase, intestinal trypsin and chymotrypsin, amylase and acid phosphatase (Kudryashova, 1970; Matskási, 1984). Reduction in total serum proteins, disrupted carbohydrate and protein metabolism, and elevated oxygen consumption was observed in infected grass carp (Davydov, 1978). Morbidity and mortality in winter were attributed to changes in alanine and aspartate aminotransferase activities, which interfered with protein synthesis (Lozinska-Gabska, 1981).

Inflammation of the gut, severe catarrhal-haemorrhagic enteritis at the parasite attachment point, with proliferation of the peripheral connective tissue, and thinning of the intestinal wall have been attributed to increased acid phosphatase activity during infections (Pár, 1978; Svobodová, 1978; Scott and Grizzle, 1979; Sekretaryuk, 1983).

There is some evidence to suggest *B. acheilognathi* secretes toxic materials (their composition is not known) leading to intoxication of the host (Degger and Avenant-Oldewage, 2009) and damage to the epithelial lining (Hoole and Nisan, 1994). According to Bauer *et al.* (1981) intoxication of the entire host can produce degenerative processes in organs. Hoole and Nisan (1994) revealed through ultrastructural studies that electron-dense secretions are released from the surface of *B. acheilognathi* which may have a protective function for the parasite. Worms adhering to the host's gut wall injure the lining epithelium, produce and secrete toxic materials, and obstruct the passage of the intestinal contents (Bauer *et al.*, 1977).

17.5. Protective/Control Strategies

Due to the economic importance of *B. acheilognathi*, its global distribution and expanding host range, considerable efforts have been made to limit disease impacts. These include: (i) the intervention of stringent legislative controls; (ii) extensive prophylaxis; (iii) veterinary examination of fish stocks; (iv) reduction of intensive stock management; and (v) active therapy (Weirowski, 1984).

Control of the parasite can be directed at either the copepod intermediate hosts (drainage of the ponds in the spring to eliminate planktonic invertebrates) or the fish stage of the life cycle, although these measures are governed by economic and practical considerations. Fish ponds can be allowed to dry and disinfected with unslaked lime (Shcherban, 1965).

European fish farmers control bothrioccephalosis by drying the ponds annually or treating drained wet ponds with calcium chloride (about 70 kg/ha) or calcium hydroxide (about 2 t/acre) or calcium hypochlorite (HTH) to kill the copepod intermediate host, and treating the fish with anthelmintics. Insecticides employed as ectoparasiticides include Neguvon (Masoten or Dipterex – at 25 ppm, i.e. at 25 g of Masote per ton) or similar compounds (Bromex; Naled), can be used to reduce populations of copepods in ponds (Hoffman, 1983). However, these are now banned in many countries as a result of environmental and health concerns.

A wide range of chemotherapeutic agents have been employed with varied success including natural products such as: (i) tobacco dust (Avdosyev, 1973); (ii) lupin seeds (Balatskii *et al.*, 1976); (iii) conifer needles (Klenov, 1969a); and (iv) horseradish leaves (Klenov, 1969b). Also a number of compounds and insecticides have been used (Molnár, 1970; Edwards and Hine, 1974; Fijan *et al.*, 1976; Pár *et al.*, 1977; Brandt *et al.*, 1981).

A comprehensive review of chemotherapeutic treatments used for the control and eradication of *B. acheilognathi* is in Bauer *et al.* (1981) and Williams and Jones (1994). Drugs are usually administered orally. Such preparations are often mixed in oil (corn, soy and

fish) and sprayed on to pellets or mixed with feeds. Recent efforts have focused on waterborne chemotherapeutics, which alleviate some of the problems associated with inappetence and dosage. It is important to distinguish the use of treatment to reduce parasite burden and treatment to achieve complete eradication of the infection.

Tapeworms may shed segments during adverse conditions or periods of stress, regenerating when conditions become more favourable. Another important consideration is whether anthelmintics are ovicidal (i.e. kill parasite eggs). This is necessary to avoid the discharge of large numbers of infective eggs to the environment when the worm is evacuated from the fish.

The eggs of *B. acheilognathi* can be killed rapidly by drying, freezing and ultraviolet rays. Among 11 chemicals tested for ovicidal effect, two chlorine-based compounds were found to be effective: (i) 3.1 ppm of sodium dichloroisocyanurate; and (ii) 9 ppm of bleaching-powder (Nakajima and Egusa, 1974b). However, there are very few chemical treatments currently licensed for use for tapeworm infections. Furthermore, the use of chemicals for the control of parasites in open water bodies can be very difficult, ineffective, harmful, expensive and illegal.

17.6. Conclusions and Suggestions for Future Studies

The Asian tapeworm is pathogenic to freshwater fishes, especially young carp fry, and may cause great economic loss in hatcheries and fish farms. It has the ability to colonize new regions, and adapt to a wide spectrum of fish hosts. It represents one of the most impressive and deplorable examples of a parasite widely disseminated by man-assisted movements of fish. The rate of dissemination and success of colonization has been aided by the cosmopolitan distribution of both intermediate and definitive hosts. However, the spread of *B. acheilognathi* to many parts of the world has also been the result of inadequate legislative controls, poor preventative measures and lack

of appropriate health-checking procedures prior to fish introductions (Scholz and Di Cave, 1993; Hoffman, 1999; Heckmann, 2000).

Recent data indicate that the impact of the tapeworm in Europe may have decreased during the last decade. However, surveillance should be maintained to prevent its further expansion to new areas. Efforts are underway to identify the resistance of different strains of common carp used in European aquaculture. Hoole (1994) proposed the development of a vaccine against *B. acheilognathi*, although practical and economic constraints continue to limit this approach. Exported fish, especially cyprinids and ornamental species (like guppies), should be inspected by veterinarians before their translocation to prevent further dissemination of the tapeworm into new regions. Control measures are generally effective, including treatment of infected fish, but the use of some anthelmintics are no longer allowed because of their negative effect on human health or the environment. Future work must therefore seek to accommodate novel and effective treatments to minimize economic loss.

Many aspects of the biology, ecology and pathology of *B. acheilognathi* are well understood and comprehensively documented. However, many of these observations are restricted to cultured fish populations. Due to the expanding host and geographical ranges of *B. acheilognathi*, the importance of the parasite to wild fish populations requires further assessment and documentation. This is an important consideration in view of declining global biodiversity and the growing conservation efforts to protect aquatic environments. Comparative studies are needed to understand differences in species susceptibility and disease potential in newly infected hosts and the consequences of the parasite in new environments.

Sublethal effects of the parasite on fish growth, fitness, fecundity, behaviour or tolerance to environmental changes may also hold important ecological implications. The physiological and bioenergetic costs of the parasite under natural conditions also requires clarification. This information is necessary to provide better understanding of future disease risks and to evaluate the role of this introduced parasite on the health and stability of fish populations.

References

- Andrews, C., Coles, T. and Dearsley, A. (1981) *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda: Pseudophyllidea) in the British Isles. *Journal of Fish Diseases* 4, 89–93.
- Avdosyev, B.S. (1973) The use of tobacco dust in the control of *Bothriocephalus gowkongensis* infection in carp. *Rybnoe Khozyaistvo* 1973, 109–118 (in Russian).
- Baer, J.C. and Fain, A. (1958) *Bothriocephalus* (*Cleistobothrium*) *kivuensis* n. sp., cestode parasite d'un barbel du Lac Kivu. *Annales de la Société Royale Zoologique de Belgique* 88, 287–302.
- Balakhnin, I.A. (1979) Changes in the correlations between the intestinal organs of carp with bothriocephaliasis. *Gidrobiologicheskii Zhurnal* 15, 45–49 (in Russian).
- Balatskii, K.P., Ivasik, V.P., Boyarchuk, V.M. and Novosad, N.L. (1976) *Bothriocephalus* infection in carp. *Veterinariya* 1976, 55 (in Russian).
- Bauer, O.N. and Hoffman, G.L. (1976) Helminth range extension by translocation of fish. In: Page, L.A. (ed.) *Wildlife Diseases*. Proceedings of the 3rd International Wildlife Disease Conference, Munich, 1975. Plenum Press, New York, USA, pp. 163–172.
- Bauer, O.N., Musselius, V.A. and Strelkov, Yu.A. (1973) *Diseases of Pond Fishes*. Program for Scientific Translations, Jerusalem, Israel.
- Bauer, O.N., Musselius, V.A., Nikolaeva, V.M. and Strelkov, Yu.A. (1977) *Ichthyopatologiya*. Izdatel'svo Pishchevaya Promyshlennost, Moscow, USSR. 431 pp.
- Bauer, O.N., Musselius, V.A. and Strelkov, Yu.A. (1981) *Diseases of Freshwater Fish*. Legkaya i Pischevaya Promyshlennost, Moscow, USSR (in Russian).
- Blanc, (1997) Introduced pathogens in European aquatic ecosystems: Theoretical aspects and realities. *Bulletin Français de la Pêche et de la Pisciculture* 344–345, 489–513.

- Borgarenko, L.F. (1981) Cestodes of the order Pseudophyllidea in birds in Tadzhikistan. *Izvestiya Akademii Nauk Tadzhikskoi SSR Otdelenie Biologicheskikh Nauk* 1979, 99–100.
- Brandt, F.W., Van As, J.G. and Hamilton-Attwell, V.L. (1981) The occurrence and treatment of bothriocephalosis in the common carp, *Cyprinus carpio* in fish ponds with notes on its presence in the large-mouth yellowfish *Barbus kimberleyensis* from the Vaal Dam, Transvaal. *Water SA* 7, 34–42.
- Brouder, M.J. (1999) Relationship between length of roundtail chub and infection intensity of Asian fish tapeworm *Bothriocephalus acheilognathi*. *Journal of Aquatic Animal Health* 11, 302–304.
- Bunkley-Williams, L. and Williams, E.H., Jr (1994) *Parasites of Puerto Rican Freshwater Sport Fishes*. Puerto Rico Department of Natural and Environmental Resources, San Juan, Puerto Rico, and Department of Marine Sciences, University of Puerto Rico, Mayaguez, Puerto Rico, 168 p.
- Buza, L., Molnár, K. and Szakolczai, J. (1970) *Bothriocephalus gowkongensis* elofor dulasa magyarorszagon. *Holaszat* 16, 42–43.
- Chervy, L. (2002) The terminology of larval cestodes or metacestodes. *Systematic Parasitology* 52, 1–33.
- Choudhury, A., Charipar, E., Nelson, P., Hodgson, J.R., Bonar, S. and Cole, R.A. (2006) Update on the distribution of the invasive Asian fish tapeworm, *Bothriocephalus acheilognathi*, in the U.S. and Canada. *Comparative Parasitology* 73, 269–273.
- Clarkson, R.W., Robinson, A.T. and Hoffnagle, T.L. (1997) Asian tapeworm (*Bothriocephalus acheilognathi*) in native fishes from the Little Colorado River, Grand Canyon, Arizona. *Great Basin Naturalist* 57, 66–69.
- Davydov, O.N. (1978) Growth, development and fecundity of *Bothriocephalus gowkongensis* (Yeh, 1955), a parasite of cyprinid fish. *Gidrobiologicheskii Zhurnal* 14, 70–77.
- Davydov, V.G. (1977) Host tissue reaction to different types of cestode attachment. *Biologiya Vnutrennykh Vod, Informatsionnyi Byulletin* 33, 45–48 (in Russian).
- Degger, N. and Avenant-Oldewage, A. (2009) Metal accumulation analysis within tissue of *Bothriocephalus acheilognathi*. *Journal of the South African Veterinary Association* 80, 127–128.
- Denis, A., Gabrion, C. and Lambert, A. (1983) The presence in France of two parasites of East Asian origin: *Diplozoon nipponicum* (Monogenea) and *Bothriocephalus acheilognathi* (Cestoda) in *Cyprinus carpio*. *Bulletin Français de Pisciculture* 289, 128–134.
- Dove, A.D.M. and Fletcher, A.S. (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. *Journal of Helminthology* 74, 121–127.
- Dubinina, M.N. (1971) Cestodes from fishes of the River Amur's basin. *Parazitologicheskii Sbornik* 25, 77–119 (in Russian).
- Edwards, D.J. and Hine, P.M. (1974) Introduction, preliminary handling and diseases of grass carp in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 8, 441–454.
- Evans, B.B. and Lester, R.J.G. (2001) Parasites of ornamental fish imported into Australia. *Bulletin of the European Association of Fish Pathologists* 21, 51–55.
- Fijan, N., Kezic, N., Teskeredzic, E. and Kajgana, L. (1976) Treatment of carp bothriocephaliasis. *Veterinarski Arhiv* 46, 245–252.
- Font, W.F. and Tate, D.C. (1994) Helminth parasites of native Hawaiian freshwater fishes: an example of extreme ecological isolation. *Journal of Parasitology* 80, 682–688.
- García-Prieto, L. and Osorio-Sarabia, D. (1991) Distribución actual de *Bothriocephalus acheilognathi* en México. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoología* 62, 523–526.
- Granath, W.O. and Esch, G.W. (1983a) 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.
- Granath, W.O. and Esch, G.W. (1983b) Survivorship and parasite-induced host mortality among mosquitofish in a predator-free, North Carolina cooling reservoir. *American Midland Naturalist* 110, 314–323.
- Han, J.E., Shin, S.P., Kim, J.H., Choresca, C.H., Jr, Jun, J.W., Gomez, D.K. and Park, S.C. (2010) Mortality of cultured koi *Cyprinus carpio* in Korea caused by *Bothriocephalus acheilognathi*. *African Journal of Microbiology Research* 4, 543–546.
- Hansen, S.P., Choudhury, A., Heisey, D.M., Ahumada, J.A., Hoffnagle, T.L. and Cole, R.A. (2006) Experimental infection of the endangered bonytail chub (*Gila elegans*) with the Asian fish tapeworm (*Bothriocephalus acheilognathi*): impacts on survival, growth, and condition. *Canadian Journal of Zoology* 84, 1383–1394.
- Hansen, S.P., Choudhury, A. and Cole, R.A. (2007) Evidence of experimental postcyclic transmission of *Bothriocephalus acheilognathi* in bonytail chub (*Gila elegans*). *Journal of Parasitology* 93, 202–204.

1



2



3

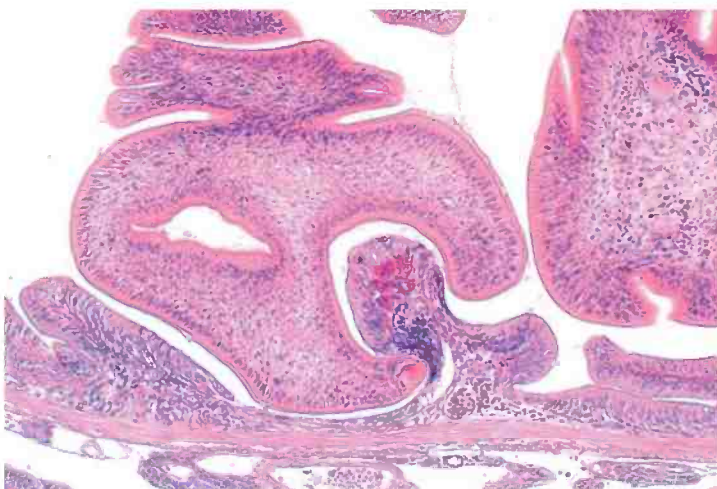


Plate 1. Carp (*Cyprinus carpio*) with infection of *B. acheilognathi*.

Plate 2. Intestine of carp (*C. carpio*) infected with *B. acheilognathi*.

Plate 3. Scolex of *B. acheilognathi* engulfing the intestine of common carp causing compression of the mucosa and localized haemorrhage.

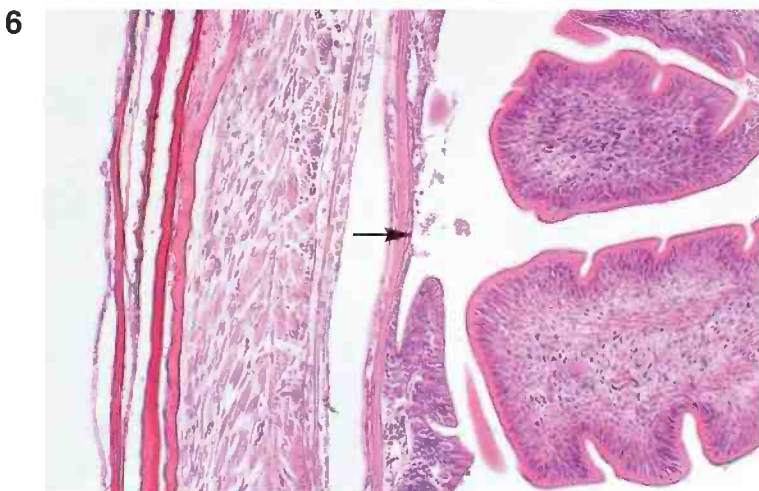
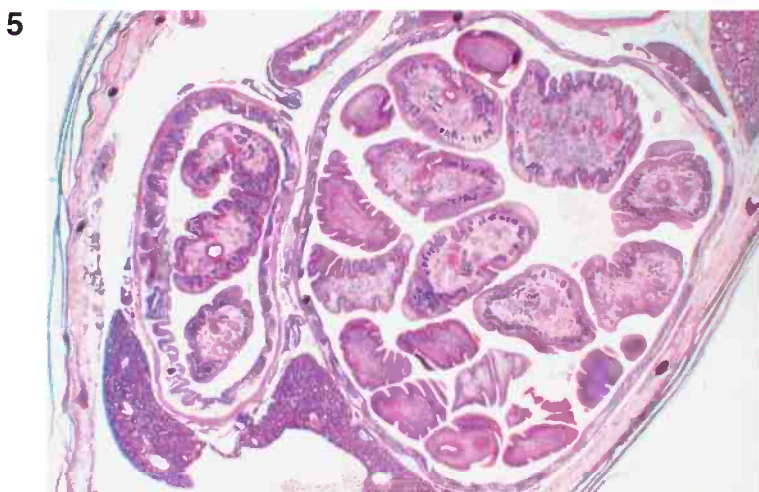


Plate 4. Marked thinning of the intestine wall caused by the attachment of numerous tapeworms.

Plate 5. Transverse section of common carp intestine showing attenuation of the gut and partial occlusion from tapeworms within.

Plate 6. Severe intestinal compression, with necrosis and complete loss of epithelium (arrowhead). The damage within this region is approaching intestinal rupture.

- Hanzelová, V. and Žitňan, R. (1986) Embryogenesis and development of *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda) in the intermediate host under experimental conditions. *Helminthologia* 23, 145–155.
- Heckmann, R.A. (2000) Asian tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934, a recent cestode introduction into the western United States of America: control methods and effect on endangered fish populations. *Proceedings of Parasitology* 29, 1–24.
- Heckmann, R.A. (2009) The fate of an endangered fish species (*Plagopterus argentissimus*) due to an invasive fish introduction (*Cyprinella lutrensis*) infected with Asian tapeworm (*Bothriocephalus argentissimus*); recovery methods. *Proceedings of Parasitology* 47, 43–52.
- Hoffman, G.L. (1983) *Asian Fish Tapeworm*, *Bothriocephalus opasariichthydis*, *Prevention and Control*, revised edn. US Department of the Interior Fish and Wildlife Service.
- Hoffman, G.L. (1999) *Parasites of North American Freshwater Fishes*. University of California Press, Berkeley, California, USA.
- Hoffman, G.L. and Schubert, G. (1984) Some parasites of exotic fishes. In: Courtenay, W.R. and Stauffer, J.R. (eds) *Distribution, Biology, and Management of Exotic Fishes*. John Hopkins University Press, Baltimore, Maryland, USA, pp. 233–261.
- Holmes, J.C. (1979) Parasite populations and host community structure. In: Nickol, B.B. (ed.) *Host–Parasite Interfaces*. Proceedings of the Symposium at the University of Nebraska-Lincoln, 5–7 October. Academic Press, London, UK, pp. 27–46.
- Hoole, D. (1994) Tapeworm infections in fish: past and future problems. In: Pike, A.W. and Lewis, J.W. (eds) *Parasitic Diseases of Fish*. Samara Publishing Limited, Tresaith, Wales, UK, pp. 119–140.
- Hoole, D. and Nisan, H. (1994) Ultrastructural studies on the intestinal response of carp, *Cyprinus carpio* L. to the pseudophyllidean tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934. *Journal of Fish Diseases* 17, 623–629.
- Klenov, A.P. (1969a) Coniferous needles tested against *Bothriocephalus* in grass carp. *Veterinariya* 46, 65–67 (in Russian).
- Klenov, A.P. (1969b) Testing ‘phytoncides’ (onion and leaves of horse-radish) against *Bothriocephalus* infection in grass carp. *Veterinariya* 46, 59 (in Russian).
- Kuchta, R. and Scholz, T. (2007) Diversity and distribution of fish tapeworms of the ‘Bothriocephalidea’ (Eucestoda). *Parassitologia* 49, 21–38.
- Kudryashova, Yu.V. (1970) The effect of *Bothriocephalus gowkongensis* on the hematological indicators of 2-year-old carp. *Doklady Moskovskoi Sel’skokhozyaistvennoi Akademii im. K.A. Timiryazeva* 164, 345–349.
- Langdon, J.S. (1992) Major protozoan and metazoan parasitic diseases of Australian finfish. In: Munday, B. (ed.) *Fin Fish Workshop Refresher Course for Veterinarians*, Proceedings 182. Post Graduate Committee in Veterinary Science, University of Sydney, Sydney, Australia (1992), pp. 1–27.
- Leong, T.S. (1986) Seasonal occurrence of metazoan parasites of *Puntius binotatus* in an irrigation canal, Pulau Pinang, Malaysia. *Journal of Fish Biology* 28, 9–16.
- Liao, H.-H. and Shih, L.-C. (1956) Contribution to the biology and control of *Bothriocephalus gowkongensis* Yeh, a tapeworm parasitic in young grass carp (*Ctenopharyngodon idellus* C. a. V.). *Acta Hydrobiologica Sinica* 2, 129–185 (in Chinese).
- López-Jiménez, S. (1981) Céstodos de peces I. *Bothriocephalus* (*Clestopbothrium*) *acheilognathi* (Cestoda: Bothriocephalidae). *Anales del Instituto de Biología Universidad Nacional Autónoma de México, Serie Zoológica* 51, 69–84.
- Lozinska-Gabska, M. (1981) Activity of aspartate and alanine aminotransferase in the alimentary canal of carp (*Cyprinus carpio* L.) infected with tapeworms *Bothriocephalus gowkongensis* Yeh, 1955 or *Khawia sinensis* Hsu, 1935. *Wiadomosci Parazytologiczne* 27, 717–743.
- Maitland, P.S. and Campbell, R.N. (1992) *Freshwater Fishes of the British Isles*. Harper Collins Publishers, London, UK.
- Malevitskaya, M.A. (1958) O zavoze parazita so slozhnym ciklom razvitiya *Bothriocephalus gowkongensis* pri akklimatizacii amurskich ryb. Dokl. Doklady Akademii Nauk USSR 123, 572–575 (in Russian).
- Marcogliese, D.J. (2008) First report of the Asian fish tapeworm in the Great Lakes. *Journal of Great Lakes Research* 34, 566–569.
- Marcogliese, D.J. and Esch, G.W. (1989) Experimental and natural infection of planktonic and benthic copepods by the Asian tapeworm, *Bothriocephalus acheilognathi*. *Proceedings of the Helminthological Society of Washington* 56, 151–155.

- Matskási, I. (1984) The effect of *Bothriocephalus acheilognathi* infection on the protease and α -amylase activity in the gut of carp fry. In: Olaha, J. (ed.) *Fish, Pathogens and Environment in European Polyculture* (Proceedings of an International Seminar, 23–27 June 1981, Szarvas). *Symposia Biologica Hungarica* 23, 119–125.
- Minervini, R., Lombardi, F. and Cave, D. (1985) L'introduzione di *Bothriocephalus acheilognathi* Yamaguti, 1934, in Italia: osservazioni su popolazioni naturali e di allevamento di carpa (*Cyprinus carpio*). *Rivista Italiana di Piscicoltura e Ittiopatologia* 20, 27–32.
- Molnár, K. (1970) An attempt to treat fish bothriocephalosis with devermin. Toxicity for the host and anti-parasitic effect. *Acta Veterinaria Academiae Scientiarum Hungaricae* 20, 325–331.
- Molnár, K. (1977) On the synonyms of *Bothriocephalus acheilognathi* Yamaguti, 1934. *Parasitologia Hungarica* 10, 61–62.
- Molnár, K. and Murai, E. (1973) Morphological studies on *Bothriocephalus gowkongensis* Yeh, 1955 and *B. phoxini* Molnár, 1968 (Cestoda, Pseudophyllidea). *Parasitologia Hungarica* 6, 99–108.
- Nakajima, K. and Egusa, N. (1974a) *Bothriocephalus opsariichthydis* Yamaguti (Cestoda: Pseudophyllidea) found in the gut of cultured carp, *Cyprinus carpio* (Linne) – I. Morphology and taxonomy. *Fish Pathology* 9, 31–39 (in Japanese).
- Nakajima, K. and Egusa, N. (1974b) *Bothriocephalus opsariichthydis* Yamaguti (Cestoda: Pseudophyllidea) found in the gut of cultured carp, *Cyprinus carpio* (Linne) – III. Anthelmintic effects of some chemicals. *Fish Pathology* 9, 46–49 (in Japanese).
- Nedeva, I. and Mutafova, T. (1988) On the morphology of *Bothriocephalus acheilognathi* Yamaguti, 1934 (Bothriocephalidae). *Khel'mintologiya* 26, 39–46 (in Bulgarian).
- Nie, P. and Hoole, D. (1999) Antibody response of carp, *Cyprinus carpio* to the cestode, *Bothriocephalus acheilognathi*. *Parasitology* 118, 635–639.
- Nie, P. and Hoole, D. (2000) Effects of *Bothriocephalus acheilognathi* on the polarization response of pronephric leucocytes of carp, *Cyprinus carpio*. *Journal of Helminthology* 74, 253–257.
- Nie, P., Hoole, D. and Arme, C. (1996) Proliferation of pronephric lymphocytes of carp, *Cyprinus carpio* induced by extracts of *Bothriocephalus acheilognathi*. *Journal of Helminthology* 70, 127–131.
- Odening, K. (1976) Conception and terminology of hosts in parasitology. *Advances in Parasitology* 14, 1–93.
- Paperna, I. (1996) *Parasites, Infections and Diseases of Fishes in Africa: an Update*. CIFA Technical Paper 31, Food and Agriculture Organization of the United Nations, Rome, Italy.
- Pár, O. (1978) Low-intensity invasion by tapeworm *Bothriocephalus gowkongensis*, as acting on the physiological and condition parameters of the health state of the carp. *Bulletin VÚRH Vodňany* 14, 26–33.
- Pár, O., Párová, J. and Prouza, A. (1977) Mansonil – an effective anthelmintic for the treatment of botriocephalosis in the carp. *Bulletin VÚRH Vodňany* 1, 17–25.
- Pérez-Ponce de León, G., Jiménez-Ruiz, F.A., Mendoza-Garfias, B. and García-Prieto, L. (2001) Helminth parasites of garter snakes and mud turtles from several localities of the Mesa Central of Mexico. *Comparative Parasitology* 68, 9–20.
- Petkov, P. (1972) Occurrence of *Bothriocephalus gowkongensis* in carp bred in artificial water reservoirs in the Pleven district. *Veterinarnomeditsinski Nauki* 9, 75–78.
- Pool, D. (1984) A scanning electron microscope study of the life cycle of *Bothriocephalus acheilognathi* Yamaguti, 1934. *Journal of Fish Biology* 25, 361–364.
- Pool, D.W. (1987) A note on the synonymy of *Bothriocephalus acheilognathi* Yamaguti, 1934, *B. aegyptiacus* Ryšavý and Moravec, 1975 and *B. kivuensis* Baer and Fain, 1958. *Parasitology Research* 73, 146–150.
- Pool, D.W. and Chubb, J.C. (1985) A critical scanning electron microscope study of the scolex of *Bothriocephalus acheilognathi* Yamaguti, 1934, with a review of the taxonomic history of the genus *Bothriocephalus* parasitizing cyprinid fishes. *Systematic Parasitology* 7, 199–211.
- Prigly, M. (1975) The role of aquatic birds in spreading *Bothriocephalus gowkongensis* Yeh, 1955 (Cestoda). *Parasitologia Hungarica* 8, 61–62.
- Radulescu, I. and Georgescu, R. (1962) Contributii la cunoasterea parazitofaunei speciei *Centropharyngodon idella* in primul an de acclimatizare in R.P. Romina. *Buletinul Institutului de Cercetari si Proiectari Piscicole* 21, 85–91.
- Rego, A.A., Chubb, J.C. and Pavanelli, G.C. (1999) Cestodes in South American freshwater teleost fishes: keys to genera and brief description of species. *Revista Brasileira de Zoologia* 16, 299–367.
- Riggs, M.R., Lemly, A.D. and Esch, G.W. (1987) The growth, biomass, and fecundity of *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir. *Journal of Parasitology* 73, 893–900.

- Ryšavý, B. and Moravec, F. (1975) *Bothriocephalus aegyptiacus* sp. n. (Cestoda: Pseudophyllidea) from *Barbus bynni*, and its life cycle. *Věstník Československé Společnosti Zoologické* 39, 68–72.
- Salgado-Maldonado, G. and Pineda-López, R.F. (2003) The Asian fish tapeworm *Bothriocephalus acheilognathi*: a potential threat to native freshwater fish species in Mexico. *Biological Invasions* 5, 261–268.
- Scholz, T. (1999) Parasites in cultured and feral fish. *Veterinary Parasitology* 84, 317–335.
- Scholz, T. and Di Cave, D. (1993) *Bothriocephalus acheilognathi* (Cestoda: Pseudophyllidea) parasite of freshwater fish in Italy. *Parassitologia* 34, 155–158.
- Scholz, T., Vargas-Vázquez, J., Moravec, F., Vivas-Rodríguez, C. and Mendoza-Franco, E. (1996) Cestoda and Acanthocephala of fish from cenotes (sinkholes) of the Peninsula of Yucatan, Mexico. *Folia Parasitologica* 43, 141–152.
- Scott, A.L. and Grizzle, J.M. (1979) Pathology of cyprinid fishes caused by *Bothriocephalus gowkongensis* Yeh, 1955 (Cestoda: Pseudophyllidea). *Journal of Fish Diseases* 2, 69–73.
- Sekretaryuk, K.V. (1983) Morphogistokhimicheskie issledobaniya kishechnika karpa pri botriocephaleze. *Parazitologiya* 17, 203–208 (in Russian).
- Shcherban, M.P. (1965) *Cestode Infections of Carp*. Izdatelstvo Urozhai, Kiev, USSR.
- Smyth, J.D. and McManus, D.P. (1989) *The Physiology and Biochemistry of Cestodes*. Cambridge University Press, Cambridge, UK.
- Sopinska, A. and Guz, L. (1997) Fenbendazole treatment against *Bothriocephalus acheilognathi* in carp, *Cyprinus carpio*. *Bulletin of the European Association of Fish Pathologists* 17, 86–87.
- Svobodová, Z. (1978) Values of some conformation, condition and physiological parameters of two-year-old carp invaded by the tapeworm *Bothriocephalus gowkongensis*. *Bulletin VÚRH Vodňany* 3, 21–25.
- Weirowski, F. (1984) Occurrence, spread and control of *Bothriocephalus acheilognathi* in the carp ponds of the German Democratic Republic. In: Olaha, J. (ed.) *Fish, pathogens and environment in European polyculture*. Proceedings of an International Seminar, June 23–27, 1981, Szarvas. *Symposia Biologica Hungarica* 23, 149–155.
- Williams, H. and Jones, A. (1994) *Parasitic Worms of Fish*. Taylor & Francis, London, UK.
- Yamaguti, S. (1934) Studies on the helminth fauna of Japan. Part 4. Cestodes of fishes. *Japanese Journal of Zoology* 6, 1–112.
- Yeh, I.S. (1955) On a new tapeworm *Bothriocephalus gowkongensis* n. sp. (Cestoda: Bothriocephalidae) from freshwater fish in China. *Acta Zoologica* 7, 69–74 (in Chinese).
- Žitňan, R. and Hanzelová, V. (1982) Negative effects of bothriocephalosis on weight gains in carp. *Folia Veterinaria* 26, 173–181.

18 *Anisakis* Species

Arne Levsen¹ and Bjørn Berland²

¹National Institute of Nutrition and Seafood Research, Bergen, Norway

²University of Bergen, Bergen, Norway

18.1. Introduction

The members of the nematode genus *Anisakis* (Order Ascaridida, Family Anisakidae), commonly known as the herring or whale worm, occur at their third larval stage in numerous marine teleost fish species around the globe, except apparently from strictly Antarctic waters. Several of the commonly infected fish host species are among the most valuable fisheries resources. Historically, only very few species were recognized within the genus *Anisakis* (Davey, 1971), with *Anisakis simplex* as the most widespread and consequently the most intensively studied. However, based on biochemical and molecular techniques, there are now nine nominal *Anisakis* species within two main phylogenetic clades. The first clade currently contains six species including the *A. simplex* complex whose members share the larval morphology known as *Anisakis* Type I (*sensu* Berland, 1961). *A. simplex* (*sensu stricto*) seems to occur circumpolarly in subarctic, temperate and subtropical waters of the northern hemisphere. It has been recorded in both the western and eastern Atlantic and Pacific Oceans, and appears to have its southern limit in North-east Atlantic waters near Gibraltar. A comprehensive review of the molecular systematics of anisakid nematodes including the genus *Anisakis* is provided by Mattiucci and Nascetti (2008).

Anisakis species have indirect, complex life cycles which involve various whale species as definitive hosts while planktonic or pelagic crustaceans act as first intermediate or transport hosts, and fish and squid (Cephalopoda, Decapodiformes) as second intermediate or transport hosts. The adult worms live in the stomach of various cetaceans such as dolphins and porpoises (Odontoceti) or baleen whales (Mysticeti). After the final two moults, maturation and copulation, the female worms shed the eggs which with the definitive host's faeces are voided into the sea. There they embryonate producing tiny third-stage larvae (Køie *et al.*, 1995), which are ingested by crustaceans such as copepods or euphausiids (krill) in which they grow within the haemocoel. Fish or squid become infected by eating crustaceans containing third-stage larvae which bore through the wall of the digestive tract into the viscera and body cavity, followed by host-induced encapsulation. When an infected fish is eaten by another fish the encapsulated larvae become free thus repeating the larval fish host cycle. This is important from an epidemiological perspective since the repeated transmission of larvae between fishes may result in extensive accumulation. Some large and older piscivorous fish, sometimes harbour hundreds or thousands of encapsulated larvae. The definitive hosts become infected by eating fish or squid containing the larvae.

In a fish, the majority of *A. simplex* larvae are typically encapsulated as flat, tight spirals (measuring 4–5 mm across) in and on the visceral organs. However, a smaller number of larvae may migrate from the abdominal cavity into the flesh. This behaviour eventually results in worms in fillets, which again may draw the attention of consumers and food-safety authorities. Most of the flesh-invading larvae reside in the belly flaps but some may penetrate deeply into the dorsal musculature of the fish host. However, due to their small size and transparency, most *Anisakis* larvae in the fish flesh may remain undetected during industrial processing, and are hence still present when the final product reaches the market. When liberated from the capsule, the worm, 20–30 mm long, moves vigorously. The third-stage larvae of *Anisakis* spp. and the other frequently occurring anisakid species in fish (e.g. *Hysterothylacium aduncum*, *Pseudoterranova decipiens* and *Contracaecum* spp.) have a projecting boring tooth on their head which may act as a piercing device during their migration across the gut wall. However, enzymes secreted from gland cells in the oesophagus are probably important in facilitating the larvae's migration from the gut into the body cavity. As the encapsulated larvae may live for years in the fish hosts, it is possible that the boring tooth lacerates the inner capsule wall permitting body growth and access to host cells (see Berland, 2006). *Anisakis* sp. third-stage larvae are clearly distinguished from those of the other anisakid species by a comparatively broad and elongate oesophageal ventricle which in live worms is clearly visible at low magnification due to its somewhat opaque appearance (Fig. 18.1).

During the past two or three decades there has been an almost explosive increase in research activities on anisakid nematodes and *A. simplex* in particular, consequently resulting in a vast body of literature. Numerous studies focus on the quality-reducing or actual consumer pathogenic properties of the parasite. These efforts have been accompanied or supported by the extensive research and development in methodology and insight regarding the taxonomy, molecular systematics and co-evolutionary host–parasite relationships of this group of nematodes. Partly due to the growing popularity of Asian-inspired seafood based on semi-processed or raw fish meat, increasing numbers of 'anisakiasis' cases in humans have been reported worldwide in the past few years. Moreover, various studies have demonstrated that *A. simplex* larvae, both dead and alive, may cause allergic reactions after consumption of infected seafood (see Audicana *et al.*, 2002; Chai *et al.*, 2005; Valls *et al.*, 2005; Audicana and Kennedy, 2008).

In contrast to the many studies on the consumer health implications, systematics and ecology of *Anisakis* species, comparatively few works deal with the detrimental effects of these worms on the health, condition or fitness of the fish hosts. Thus, this chapter discusses some key pathobiological features or effects of *Anisakis* species, with emphasis on *A. simplex* (*sensu lato*), on various economically important fish species including Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), saithe (*Pollachius virens*) and Atlantic mackerel (*Scomber scombrus*).

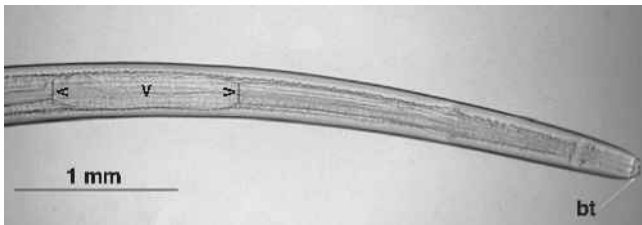


Fig. 18.1. Anterior body of *Anisakis simplex* third-stage larva. Note boring tooth (bt) and oesophageal ventricle (v).

18.2. Diagnosis and Clinical Signs of the Infection

18.2.1. Macroscopic appearance

At first glance, any fish that is more or less heavily infected with *Anisakis* sp. larvae usually appears healthy. The intensity or any macroscopic signs of the infection become obvious on visual examination of the visceral organs, mesenteries and peritoneal linings. Depending on various factors such as fish host species, host size and infection intensity, the larvae may occur scattered singly or in clusters with sometimes hundreds of worms, on the organs and mesenteries of the visceral cavity (Fig. 18.2). The larvae are typically surrounded by a fibrous connective tissue capsule generated by the host. Especially in heavy infections, host connective tissue capsules may also be formed around clusters of larvae (Fig. 18.3). Each capsule consists of at least three layers. The inner layer mostly consists of host-cell debris with traces of pycnosis, and surrounds each coil of the larva. The middle layer has a denser fibrous appearance due to the presence of fibroblastic elements, while the outermost is dominated by blood vessels, large fibroblasts and extravascular erythrocytes and their remnants (Margolis, 1970). The capsule thickness seems to depend on the infection site (abundance of connective tissue) and the age of infection. Older capsules may gradually decrease in size, the

connective tissue layers become more compact, sometimes accompanied by deposition of calciferous granules. Another frequently observed feature of infections with *Anisakis* sp. is the presence of melanomacrophage aggregates around larval infection sites on the liver (Fig. 18.4). The nature of these aggregates and their role in fish pathology was reviewed by Agius and Roberts (2003) who suggested that the melanomacrophage centres develop focally in association with late-stage chronic inflammations due to various pathogens including parasites. However, the presence of macrophage aggregates in the vicinity of encapsulated *A. simplex* larvae on the surface or in the parenchyma of the liver of flounder (*Platichthys flesus*) (Dezfuli *et al.*, 2007), Atlantic salmon (Murphy *et al.*, 2010) and blue whiting (*Micromesistius poutassou*) (A. Levsen, personal observation) is apparently not associated with any significant tissue damage.

While *Anisakis* sp. seems to be by far the most prevalent anisakid in pelagic or semi-pelagic fish species such as blue whiting, herring (*Clupea harengus*), mackerel or saithe, mixed infections with the larvae of two or more anisakid species (e.g. *Anisakis* sp., *P. decipiens* and *Contracaecum* sp.) are commonly observed in demersal fish such as cod or monkfish (*Lophius* spp.) from offshore or coastal waters. Thus, the primary cause of lesions or other detrimental effects in mixed infections may not always be obvious since the actual anisakid species may interact.



Fig. 18.2. Massive infection of *A. simplex* third-stage larvae on the liver and stomach of Atlantic cod (*Gadus morhua*).

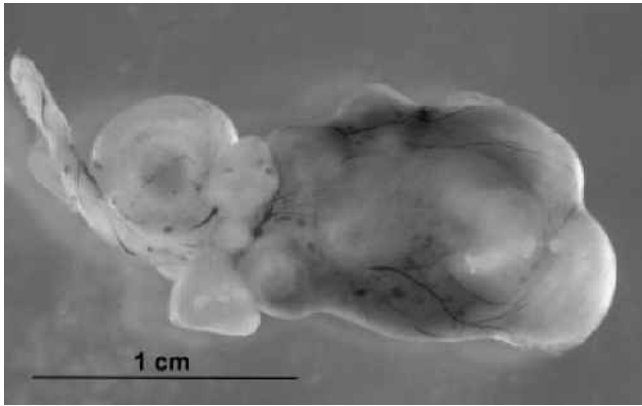


Fig. 18.3. Host-induced connective tissue capsule around a cluster of *A. simplex* third-stage larvae in the visceral cavity of saithe (*Pollachius virens*) from western Norway. Note blood vessels in the outer tissue layer of the capsule.

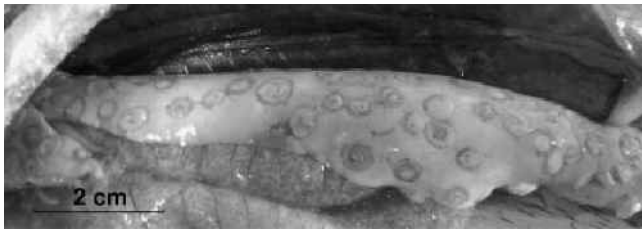


Fig. 18.4. *Anisakis simplex* third-stage larvae on the liver of blue whiting (*Micromesistius poutassou*). Numerous melanomacrophage aggregates appear as tiny black spots on or around the encapsulated larvae.

18.3. Gross Pathology and Host Tissue Damage

As with many other parasitic infections, the ability and extent to which *Anisakis* sp. larvae may pathologically affect the fish host appears to depend largely upon the intensity of infection and the infection site (e.g. lots of larvae in or on vital organs such as the liver are more likely to induce more tissue damage than would clusters of worms on the mesenteries). Thus, heavy infections of *Anisakis* sp. larvae have been reported to cause severe damage to the liver in several species of fish. For example, heavily infected livers of cod and hake (*Merluccius merluccius*) may be flaccid and reddish-brown with local haemorrhages, or even appear green due to the destruction of bile ducts (Margolis, 1970, and

references therein). The latter may occur in cases where larvae have penetrated deeply into the liver parenchyma (Kahl, 1938; A. Levsen, personal observation).

18.3.1. The 'stomach crater syndrome' of cod

The 'stomach crater syndrome' was frequently observed in larger cod (> 5 kg) caught at the spawning grounds off the Lofoten Islands, northern Norway (Berland, 1981). The stomach wall of heavily infected fish was very thick, up to 1 cm, and had in its mucosa several 'craters' or pits from which the tails of numerous *A. simplex* larvae were protruding, their heads penetrating deeply into the pits (Fig. 18.5). Apparently these fish had repeatedly been

infected by new larvae over the years which gradually resulted in extensive accumulation. Thus, sections of the stomach wall revealed the presence of densely packed larvae (Fig. 18.6), however, without inducing any significant tissue damage apart from the sheer presence of lots of larvae. Presumably, the physical thickness of the stomach wall impeded the larvae's migration through the mucosa. Rapid onset of the host-induced encapsulation process subsequently stopped the larvae halfway in their

tracks. The syndrome appeared to reach a peak in 1974–1975 when more than 50% of the cod stomachs investigated ($n = 300$ –500 annually over a 12-year period) showed this condition. Any possible long-term pathobiological implications of the disease on affected cod or the local cod population were not investigated. The underlying reasons for the apparent sudden rise in prevalence of the syndrome in 1969, and its marked decrease 12 years later, also remain unclear.

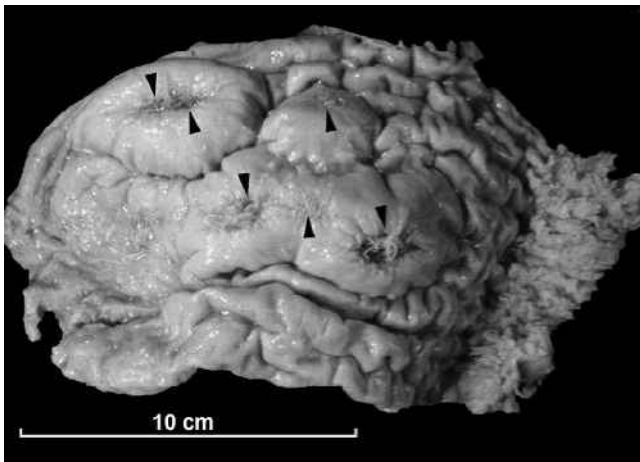


Fig. 18.5. Gross appearance of the 'stomach crater syndrome' in Atlantic cod (*G. morhua*).

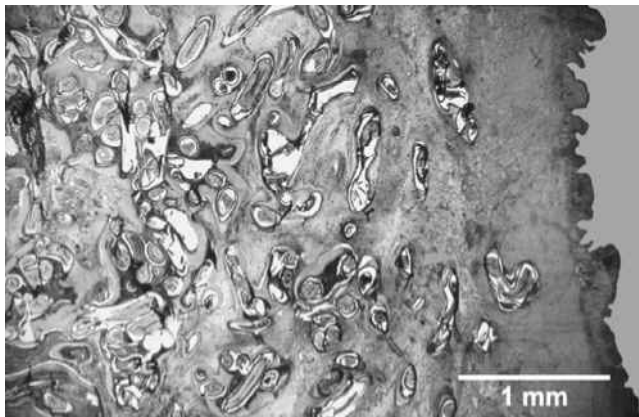


Fig. 18.6. *Anisakis simplex* third-stage larvae in the stomach wall ('stomach crater syndrome') of Atlantic cod (*G. morhua*).

18.3.2. The 'red vent syndrome' (RVS) of wild Atlantic salmon

During early summers of 2006 and 2007, many wild Atlantic salmon returning to rivers in Scotland, England and Wales had bleeding, swollen and haemorrhagic vents. This condition, popularly named 'red vent syndrome' or RVS, was subsequently attributed to large numbers of *A. simplex* (*sensu stricto*) third-stage larvae in the tissues surrounding the vent and urogenital papilla (Beck *et al.*, 2008; Noguera *et al.*, 2009). RVS occurred mainly in adult one sea-winter salmon of both sexes, although it was also recorded from some two sea-winter salmon and sea trout (*Salmo trutta*). By the end of 2007, most major salmon rivers in mainland England, Scotland and Wales had confirmed records of affected fish. Moreover, the condition was also reported from Atlantic salmon returning to rivers in Iceland in 2007 and Norway and Quebec, Canada, in 2008.

External clinical signs include a swollen, protruding and haemorrhagic vent, sometimes accompanied by erosion of the skin, scale loss and moderate to severe bleedings in affected tissue areas. Histologically, affected vent regions showed gross lesions around lots of unencapsulated *A. simplex* larvae, with haemorrhages and moderate to severe inflammation dominated by eosinophilic granular cells and melanomacrophages. There was apparently no correlation between larval intensity in the organs and mesenteries of the body cavity, and the numbers of larvae found in the discrete area of the vent and urogenital papilla. It was noted, however, that independent of the severity of the lesions, affected fish were generally in good overall conditions. Moreover, there was no evidence of RVS-induced wild salmon mortality or any other pathogenic viral or primary bacterial infections (Beck *et al.*, 2008; Noguera *et al.*, 2009). It also turned out that the extent of the lesions differed depending on how long fish had been in fresh water since salmon captured after they had spent some time upstream in the rivers, showed signs of recovery.

The causative reason for RVS has as yet not been fully elucidated. Noguera *et al.* (2009) hypothesized that climate-driven spa-

tial or temporal changes in the North-east Atlantic pelagic ecosystem may have influenced the physiological stage of the fish. This was supported by the findings of strong eosinophilic inflammatory responses predominantly in early summer fish still in the pre-spawning phase.

18.4. Pathophysiological Effects

No information seems to exist regarding any direct pathophysiological effect of *Anisakis* sp. infections in fish. There are indications, however, that heavy larval infections may – at least in some pelagic or semi-pelagic fish species – indirectly impede body growth and/or sexual maturity and hence, adversely affect the fecundity of the actual hosts. Although *Anisakis* sp. larvae appear to be generalists at the fish host level, different fish species represent different microhabitats, each characterized by specific physiological properties which may also be expressed as different immune responses to the parasite. Several pelagic and demersal fish species including herring and Atlantic cod show an increase in prevalence and abundance of *Anisakis* sp. larvae with age and size (Petrie and Wootten, 2009; Levsen and Lunestad, 2010). In Atlantic mackerel, however, an opposite infection pattern seems to occur (Levsen and Midthun, 2007). Thus, preliminary results from an ongoing investigation of the occurrence and spatial distribution of *A. simplex* third-stage larvae in mackerel from the North-east Atlantic indicate that both prevalence and intensity of the larvae are significantly higher in smaller fish (< 500 g) compared with larger mackerel (> 500 g) (A. Levsen, personal observation). The relationship between larval intensity and fish host body weight for mackerel examined in 2008 ($n = 237$) is illustrated in Fig. 18.7. The infection pattern suggests that at least smaller and younger mackerel are capable of reducing the infection immunologically. This is supported by frequent findings of dead larvae and, what appeared to be disintegrated capsules, on the visceral organs and the fillets, especially in mackerel weighing < 500 g (Fig. 18.8).

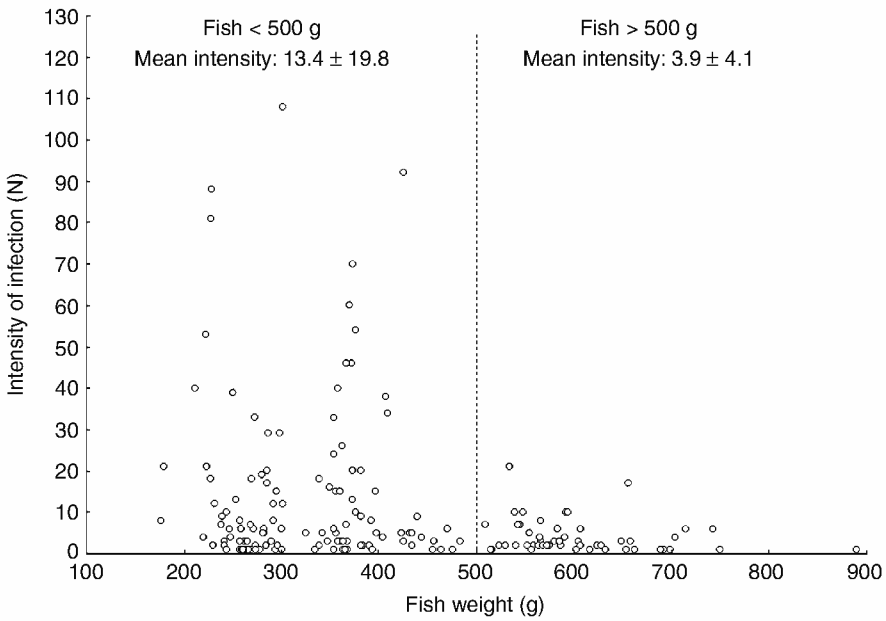


Fig. 18.7. Relationship between fish weight and infection intensity of *A. simplex* third-stage larvae in Atlantic mackerel (*Scomber scombrus*) from the northern North Sea in 2008.

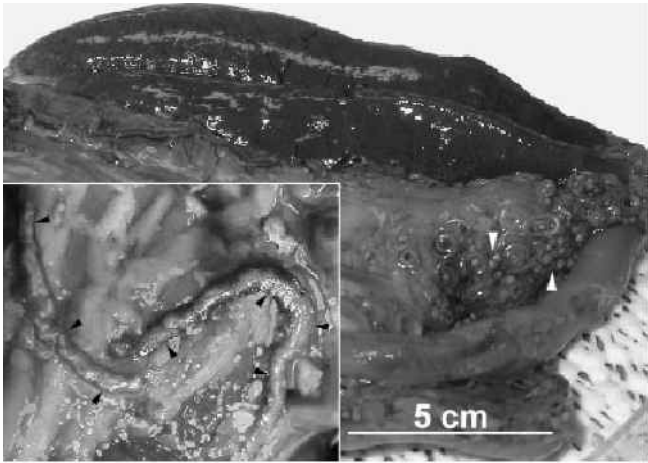


Fig. 18.8. Dead *A. simplex* third-stage larva (inserted image) and disintegrated capsules (arrowheads) on the viscera of Atlantic mackerel (*S. scombrus*) from the northern North Sea.

Basically the same trend in *A. simplex* infection pattern has been found in saithe from the North-east Atlantic (Priebe *et al.*, 1991). In this species, smaller and younger fish (3–4 years old) show a significantly higher intensity of *A. simplex* larvae in the

body musculature (fillets and belly flaps) compared with saithe older than 5 years. However, and somewhat in contrast to mackerel, up to 10% of the larvae in the muscle of older saithe were dead while only living larvae were found in the flesh of younger fish.

Subsequent indirect ELISA analysis of various serum samples of the different age groups revealed a moderate correlation between antibody titre height and the age of under-feeding saithe (post-spawning), and a close correlation in fish caught during feeding periods (pre-spawning). The authors concluded that the migration ability and lifetime of the muscle-lodging larvae in saithe is influenced by a specific immune response which increases with age of the fish.

Differences in immune response to the same parasite species are known from several more-or-less closely related fish host species. For example, the plerocercoids of the pseudophyllidean cestode *Ligula intestinalis* provoke a pronounced cellular response in roach (*Rutilus rutilus*) including massive infiltration of various leucocytes into the body cavity, often accompanied by extensive deposition of connective tissue fibres. In gudgeon (*Gobio gobio*), another frequently occurring cyprinid species in Europe, no cellular response to *L. intestinalis* is ever found (Arme, 1997). Although *Ligula* from European roach and gudgeon may be different strains, various host factors including immunological responses may account for the different response patterns in these two cyprinid fish hosts as well (Arme, 1997; Stefka *et al.*, 2009). Basically the same phenomenon is known from infections of different salmonid fish species with the ectoparasitic copepod *Lepeophtheirus salmonis*. Thus, while there is little evidence of host-tissue responses in Atlantic salmon at the parasite's feeding or attachment sites, coho salmon (*Oncorhynchus kisutch*) show strong tissue responses to *L. salmonis*, characterized by pronounced epithelial hyperplasia and inflammation (Wagner *et al.*, 2008).

These observations support the hypothesis that the *A. simplex* infection pattern, at least in some pelagic or semi-pelagic fish species, is not only related to specific life-history traits (e.g. the feeding habits and size/age of the actual fish host), but may also be influenced by host- and/or age-specific immunological characteristics. Consequently, in heavily infected smaller mackerel there may be a trade-off in metabolic energy use between the necessity to cope with the infection and

the need to grow and to optimize fecundity. However, further investigations are needed in order to elucidate the possible impact of heavy *A. simplex* infections on body growth, fecundity and, consequently, the fitness of individual mackerel, or even on the robustness and recruitment of the North-east Atlantic mackerel stock.

18.4.1. Effect on the condition of fish

Several workers have studied the possible effect of *Anisakis* sp. larvae on the condition factor of various fish host species including sculpin (*Myoxocephalus scorpius*) and Baltic herring. Thus, Petrushevsky and Kogteva (1954, cited by Margolis, 1970) found a decrease in Fulton's condition coefficient with increasing intensity of *Anisakis* sp. larvae in the liver of sculpin from the White Sea. However, Podolska and Horbowy (2003) reported a significantly positive relationship between Fulton's condition factor and the prevalence of *A. simplex* larvae in Baltic herring (i.e. for a condition factor ranging from 0.59 to 0.73, the larval prevalence approximately doubled). The latter authors concluded that a better condition at high larval prevalence probably reflects good feeding conditions and therefore a higher chance of infection. This was supported by the fact that infection intensity had no significant effect on the condition.

18.4.2. *Anisakis* larvae and farmed fish

The larvae of *Anisakis* sp. have no significance as disease-causing parasites in cultured fish. A number of studies from different countries or areas have shown that several sea-caged salmonid fish species including Atlantic salmon, coho salmon and rainbow trout (*Oncorhynchus mykiss*), do not carry *Anisakis* larvae (Pacific North America – Deardorff and Kent, 1989; France – Angot and Brasseur, 1993; Japan – Inoue *et al.*, 2000; Norway – Lunestad, 2003; Chile – Sepúlveda *et al.*, 2004; Denmark – Skov *et al.*, 2009). Marty (2008) recorded a single anisakid larva penetrating

the intestinal caecum of one Atlantic salmon from British Columbia, Canada, but a species identification was not performed. Additionally, Penalver *et al.* (2010) have recently demonstrated the absence of anisakid larvae in farmed European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) in south-east Spain.

The apparent absence of *Anisakis* sp. larvae in artificially hatched and net-pen reared fish may be explained by the widespread application of pelleted compound feed. During the extrusion process, the feed is treated at high temperature and pressure which destroys all nematode larvae that might have been present in the raw material. Although fish are often reared in open floating cages in coastal areas where anisakid nematodes are abundant, the probability that farmed fish come into contact with infected benthic or pelagic invertebrates is very low. Additionally, the use of artificial diets seems to reduce the risk of opportunist feeding on wild small fish that may occasionally enter the pens. Indeed, Skov *et al.* (2009) found that only two of 166 sea-caged rainbow trout had the remains of small fish in their stomach. Although this represents a potential infection route for farmed fish, the lack of infection indicates that the risk is very low.

There are, however, two farming practices which both may pose an increased risk of infection with larval anisakid nematodes. These practices are: (i) the feeding of caged fish with unprocessed marine fish offal; and (ii) the capture of juvenile wild fish for subsequent on-growing in net-pens. Thus, the presence of *A. simplex* third-stage larvae in the stomach lumen or abdominal cavity of sea-caged cobia (*Rachycentron canadum*) in Taiwan was linked to the occasional feeding of the cobias with chopped unfrozen raw fish or residuals thereof (Shih *et al.*, 2010). Moreover, at comparing the parasite fauna of farmed and stationary wild cod in Norway, Heuch *et al.* (2009) found 100% prevalence of *A. simplex* larvae in the viscera of 35 wild caught and subsequently sea-caged cod in Finnmark county, northern Norway. No additional infection descriptors were provided by the authors. At the time of capture, the body weight of the actual cod was approximately 400 g, indicating that the fish acquired the worms while

still free-living. It is important to note, however, that none of these infections were associated with disease in the infected fish.

18.5. Protective or Control Strategies

The effect of eight different anthelmintics (mebendazole, flubendazole, parbendazole, triclabendazole, piperazine dihydrochloride, netobimin, trichlorfon and nitroscanate) on the survival and post-treatment development of *Anisakis simplex* third-stage larvae in experimentally infected rainbow trout was investigated by Tojo *et al.* (1994). They found that none of the drugs showed any larvicidal activity nor did they affect the ability of the larvae to undergo ecdysis to the fourth larval stage. Dziekonska-Rynko *et al.* (2002) and Arias-Diaz *et al.* (2006) investigated the *in vitro* survival of *A. simplex* third-stage larvae upon treatment with ivermectin and albendazole. Both drugs reduce the survival of the larvae but their effect appears to depend on the duration and dosage of the treatment, as well as the pH condition of the aqueous culture solution. For example, all *A. simplex* larvae were killed after a 48 h exposure to 1 µg/ml ivermectin or albendazole at pH 7.0. At pH 2.0, however, 100% lethality was observed only at concentrations ≥ 50 µg/ml and ≥ 200 µg/ml of ivermectin and albendazole, respectively (Dziekonska-Rynko *et al.*, 2002). In another study, the *in vitro* effect of various monoterpenic derivatives from different essential oils on *A. simplex* third-stage larvae was investigated by Navarro *et al.* (2008) who found that α -pinene, ocimene and cineole had high larvicidal activity at a concentration of 125 µg/ml for 48 h while only α -pinene and ocimene were active at 62.5 µg/ml. It must be emphasized, however, that all the above *in vitro* trials were conducted at high temperatures (36–37°C) since they were primarily aimed at exploring the applicability of the actual drugs or compounds against human anisakiasis. A recommended drug against intestinal nematodes such as *Oxyuris* spp., *Capillaria* spp. and *Camallanus cotti* parasitizing ornamental freshwater fishes is levamisole hydrochloride (Sandford/Loaches online, 2007). However,

the possible effect of the drug on encapsulated nematode larvae in the visceral cavity of fish has yet to be investigated.

18.6. Conclusions

The third-stage larvae of *Anisakis* spp. have to be considered as parasites of generally low pathogenicity and virulence in fishes. Some more-or-less detrimental *Anisakis*-related disease outbreaks (e.g. the RVS in Atlantic salmon) appear to be geographically or temporarily isolated events, presumably induced by a chain of concurrent environmental changes at a particular time or locality. In general, however, the effects of heavy infections of *Anisakis* sp. larvae in fish seem to be governed by factors that are linked to the actual host individual or

species rather than to the parasite itself. For example, considerable differences seem to exist between actual fish species with respect to their ability to respond immunologically against the larvae. Thus, at least some stages or age groups of Atlantic mackerel appear to be capable of reducing the *A. simplex* infection by immunological means. Since the latter fish species is among the most valuable fish stocks in the North Atlantic, further studies on the significance of heavy *A. simplex* infections on the growth and fecundity of Atlantic mackerel should be carried out. Considering the predicted rise in average water temperature in the northern hemisphere, *Anisakis* sp. in Atlantic mackerel may provide a useful model for studying the dynamics or adaptability of a discrete pelagic fish host-parasite system during changing environmental conditions.

References

- Agius, C. and Roberts, R.J. (2003) Melano-macrophage centres and their role in fish pathology. *Journal of Fish Diseases* 26, 499–509.
- Angot, V. and Brasseur, P. (1993) European farmed Atlantic salmon (*Salmo salar* L.) are safe from anisakid larvae. *Aquaculture* 118, 339–344.
- Arias-Diaz, J., Zuloaga, J., Vara, E., Balibrea, J. and Balibrea, J.L. (2006) Efficacy of albendazole against *Anisakis simplex* larvae *in vitro*. *Digestive and Liver Disease* 38, 24–26.
- Arme, C. (1997) Ligulosis in two cyprinid hosts: *Rutilus rutilus* and *Gobio gobio*. *Helminthologia* 34, 191–196.
- Audicana, M.T. and Kennedy, M.W. (2008) *Anisakis simplex*: from obscure infectious worms to inducer of immune hypersensitivity. *Clinical Microbiological Reviews* 21, 20–25.
- Audicana, M.T., Ansotegui, I.J., Corres, D.E. and Kennedy, M.W. (2002) *Anisakis simplex*: dangerous – dead and alive? *Trends in Parasitology* 18, 20–25.
- Beck, M., Evans, R., Feist, S.W., Stebbing, P., Longshaw, M. and Harris, E. (2008) *Anisakis simplex sensu lato* associated with red vent syndrome in wild adult Atlantic salmon *Salmo salar* in England and Wales. *Diseases of Aquatic Organisms* 82, 61–65.
- Berland, B. (1961) Nematodes from some Norwegian marine fishes. *Sarsia* 2, 1–50.
- Berland, B. (1981) Massenbefall von *Anisakis simplex*-Larven am Magen des Kabeljaus (*Gadus morhua* L.). In: *IV. Wissenschaftliche Konferenz zu Fragen der Physiologie, Biologie und Parasitologie von Nutzfischen*. Wilhelm-Pieck-Universität, Rostock, Germany, pp. 125–128.
- Berland, B. (2006) Musings on nematode parasites. *Fisken og Havet* 11, 1–26.
- Chai, J.Y., Murrel, K.D. and Lymbery, A.J. (2005) Fish-borne parasitic zoonoses: status and issues. *International Journal of Parasitology* 35, 1233–1254.
- Davey, J.T. (1971) A revision of the genus *Anisakis* Dujardin, 1845 (Nematoda: Ascaridida). *Journal of Helminthology* 45, 51–72.
- Deardorff, T.L. and Kent, M.L. (1989) Prevalence of larval *Anisakis simplex* in pen-reared and wild-caught salmon (Salmonidae) from Puget Sound, Washington. *Journal of Wildlife Diseases* 25, 416–419.
- Dezfuli, B.S., Pironi, F., Shinn, A.P., Manera, M. and Giari, L. (2007) Histopathology and ultrastructure of *Platichthys flesus* naturally infected with *Anisakis simplex* s.l. larvae (Nematoda: Anisakidae). *Journal of Parasitology* 93, 1416–1423.
- Dziekonska-Rynko, J., Rokicki, J. and Jablonowski, Z. (2002) Effects of ivermectin and albendazole against *Anisakis simplex* *in vitro* and in guinea pigs. *Journal of Parasitology* 88, 395–398.

- Heuch, P.A., MacKenzie, K., Haugen, P., Hansen, H., Sterud, E., Jansen, P.A. and Hemmingsen, W. (2009) The parasite fauna of farmed cod and adjacent wild local cod in Norway. CODPAR project report, National Veterinary Institute, Oslo, Norway, 21 pp. (in Norwegian with English summary).
- Inoue, K., Oshima, S.-I., Hirata, T. and Kimura I. (2000) Possibility of anisakid larvae infection in farmed salmon. *Fisheries Science* 66, 1049–1052.
- Kahl, W. (1938) Nematoden in Seefischen. II. Erhebungen über den Befall von Seefischen mit Larven von *Anacanthocheilus rotundatus* (Rudolphi) und die durch diese Larven hervorgerufenen Reaktionen des Wirtsgewebes. *Zeitschrift für Parasitenkunde* 10, 513–534.
- Køie, M., Berland, B. and Burt, M.D.B. (1995) Development to third-stage larvae occurs in the eggs of *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae). *Canadian Journal of Fisheries and Aquatic Sciences* 52(Suppl. 1), 134–139.
- Levsen, A. and Lunestad, B.T. (2010) *Anisakis simplex* third stage larvae in Norwegian spring spawning herring (*Clupea harengus* L.), with emphasis on larval distribution in the flesh. *Veterinary Parasitology* 171, 247–253.
- Levsen, A. and Midtthun, E. (2007) Occurrence and spatial distribution of *Anisakis* sp. in three commercially important pelagic fish stocks from the NE Atlantic, with comments on the significance to consumer safety. *Parassitologia* 49(Suppl. 2), 402–403.
- Lunestad, B.T. (2003) Absence of nematodes in farmed Atlantic salmon (*Salmo salar* L.) in Norway. *Journal of Food Protection* 66, 122–124.
- Margolis, L. (1970) Nematode diseases of marine fishes. In: Snieszko, S.F. (ed.) *A Symposium on Diseases of Fishes and Shellfishes*. American Fisheries Society, Washington, DC, pp. 190–208.
- Marty, G.D. (2008) Anisakid larva in the viscera of a farmed Atlantic salmon (*Salmo salar*). *Aquaculture* 279, 209–210.
- Mattiucci, S. and Nascetti, G. (2008) Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host–parasite co-evolutionary processes. *Advances in Parasitology* 66, 47–148.
- Murphy, T.M., Berzano, M., O’Keeffe, S.M., Cotter, D.M., McEvoy, S.E., Thomas, K.A., Maoileidigh, N.P.O. and Whelan, K.F. (2010) Anisakid larvae in Atlantic salmon (*Salmo salar* L.) grilse and post-smolts: molecular identification and histopathology. *Journal of Parasitology* 96, 77–82.
- Navarro, M.C., Noguera, M.A., Romero, M.C., Montilla, M.P., González de Selgas, J.M. and Valero, A. (2008) *Anisakis simplex* s.l.: larvicidal activity of various monoterpenic derivatives of natural origin against L3 larvae *in vitro* and *in vivo*. *Experimental Parasitology* 120, 295–299.
- Noguera, P., Collins, C., Bruno, D., Pert, C., Turnbull, A., McIntosh, A., Lester, K., Bricknell, I., Wallace, S. and Cook, P. (2009) Red vent syndrome in wild Atlantic salmon *Salmo salar* in Scotland is associated with *Anisakis simplex sensu stricto* (Nematoda: Anisakidae). *Diseases of Aquatic Organisms* 87, 199–215.
- Penalver, J., Dolores, E.M. and Munoz, P. (2010) Absence of anisakid larvae in farmed European sea bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.) in Southeast Spain. *Journal of Food Protection* 73, 1332–1334.
- Petrie, A.B. and Wootten, R. (2009) A survey of *Anisakis* and *Pseudoterranova* in Scottish fisheries and the efficacy of current detection methods. Report of the Food Standards Agency – Project S14008. Food Standards Agency Scotland, Aberdeen, UK.
- Podolska, M. and Horbowy, J. (2003) Infection of Baltic herring (*Clupea harengus membras*) with *Anisakis simplex* larvae, 1992–1999: a statistical analysis using generalized linear models. *International Council for Exploration of the Sea (ICES) Journal of Marine Science* 60, 85–93.
- Priebe, K., Huber, C., Märtlbauer, E. and Terplan, G. (1991) Nachweis von Antikörpern gegen Larven von *Anisakis simplex* beim Seelachs *Pollachius virens* mittels ELISA. *Journal of Veterinary Medicine B* 38, 209–214.
- Sanford, S. /Loaches online (2007) Levamisole Hydrochloride – its Application and Usage in Freshwater Aquariums. Available at: <http://loaches.com/Members/shari2/levamisole-hydrochloride-1> (accessed 22 June 2011).
- Sepúlveda, F., Marín, S.L. and Carvajal, J. (2004) Metazoan parasites in wild fish and farmed salmon from aquaculture sites in southern Chile. *Aquaculture* 235, 89–100.
- Shih, H.-H., Ku, C.-C. and Wang, C.-S. (2010) *Anisakis simplex* (Nematoda: Anisakidae) third-stage larval infections of marine cage cultured cobia, *Rachycentron canadum* L., in Taiwan. *Veterinary Parasitology* 171, 277–285.
- Skov, J., Kania, P.W., Olsen, M.M., Lauridsen, J.H. and Buchmann, K. (2009) Nematode infections of maricultured and wild fishes in Danish waters: a comparative study. *Aquaculture* 298, 24–28.

-
- Stefka, J., Hypsa, V. and Scholz, T. (2009) Interplay of host specificity and biogeography in the population structure of a cosmopolitan endoparasite: microsatellite study of *Ligula intestinalis* (Cestoda). *Molecular Ecology* 18, 1187–1206.
- Tojo, J.L., Santamarina, M.T., Leiro, J.L., Ubeira, F.M. and Sanmartin, M.L. (1994) Failure of antihelminthic treatment to control *Anisakis simplex* in trout (*Oncorhynchus mykiss*). *Japanese Journal of Parasitology* 43, 301–304.
- Valls, A., Pascual, C.Y. and Martín Esteban, M. (2005) *Anisakis* allergy: an update. *Revue Française d'Allergologie et d'Immunologie Clinique* 45, 108–113.
- Wagner, G.N., Fast, M.D. and Johnson, S.C. (2008) Physiology and immunology of *Lepeophtheirus salmonis* infections of salmonids. *Trends in Parasitology* 24, 176–183.

19 *Anguillicoloides crassus*

François Lefebvre,¹ Géraldine Fazio² and Alain J. Crivelli³

¹*Independent researcher, scientific associate at the Natural History Museum, London, UK and the Station Biologique de la Tour du Valat, Arles, France*

²*Institute of Integrative and Comparative Biology, University of Leeds, Leeds, UK*

³*Station Biologique de la Tour du Valat, Arles, France*

The nematode *Anguillicoloides crassus* has spread across four continents in just a few decades, and now infects at least six eel species. Anguillicolosis causes severe pathology in the hosts' swimbladder, including lesions, inflammation, haemorrhaging and fibrosis. Losses have been reported in both wild and farmed eels. Concerns have also arisen over the capability of affected silver eels (i.e. mature individuals) to complete their deep-sea reproductive migration upon which all restocking and cultivation activities exclusively rely. Anguillicolosis is now listed among the main potential threats to the eel fishing industry and to the survival of both the European and the American eel species. These concerns are reflected in the number of published research articles since the first record of this invasive parasite outside of Asia (≈470 between 1982 and 2010).

crassus (sub-genus *Anguillicoloides* Moravec and Taraschewski, 1988) until a recent systematic revision transferred it to the genus *Anguillicoloides* (Moravec, 2006). It belongs to the taxonomic family Anguillicolidae, comprising four other species divided into two genera (*Anguillicoloides australiensis*, *Anguillicoloides novaezelandiae*, *Anguillicoloides papernai* and *Anguillicola globiceps*). Adult anguillicolids are all strictly parasitic to the eel genus *Anguilla*. *A. crassus* is known to infect six of the 15–20 eel species currently described worldwide (see Table 19.1 and Fig. 19.1; for further information on the host systematics and distributions, see Tesch, 2003; Froese and Pauly, 2010). Key characters for *A. crassus* identification are described in Moravec (2006) and briefly illustrated in Fig. 19.2.

19.1.2. Life cycle

19.1. General Biology and Distribution

19.1.1. Systematics

The nematode was first described from cultured eels in Japan as *Anguillicola crassa* Kuwahara, Niimi and Itagaki, 1974. It has been commonly referred to as *Anguillicola*

A. crassus is a trophically transmitted parasite whereby the completion of its life cycle depends upon predator–prey interactions. Typically, it involves one intermediate host in addition to the eel definitive host where reproduction takes place (Fig. 19.2). Eggs leave the swimbladder *via* the pneumatic duct, pass down the intestine and hatch in

Table 19.1. Hosts of *Anguillicoloides crassus*. For intermediate and paratenic hosts, only the most representative families (percentage of the total number of species), and most commonly recorded species per family, are given. Data extracted from both experimental and natural infection studies.

	Family	Species
Definitive hosts (one family/six species)	Anguillidae (100%)	<i>Anguilla anguilla</i> (European eel) <i>Anguilla japonica</i> (Japanese eel) <i>Anguilla rostrata</i> (American eel) <i>Anguilla bicolor</i> (Indonesian shortfin eel) <i>Anguilla marmorata</i> (giant mottled eel) <i>Anguilla mossambica</i> (African longfin eel)
Intermediate hosts (seven families/23 species)	Cyclopidae (65%) Candonidae (9%) Temoridae (4%)	<i>Paracyclops fimbriatus</i> <i>Cypria ophtalmica</i> <i>Eurytemora affinis</i>
Paratenic hosts (20 families/50 species)	Cyprinidae (46%) Gobiidae (10%) Percidae (6%)	<i>Alburnus alburnus</i> (bleak) <i>Neogobius fluviatilis</i> (monkey goby) <i>Gymnocephalus cernuus</i> (ruffe)

water, although some may hatch inside the swimbladder (De Charleroy *et al.*, 1990). Newly hatched second-stage larvae (L2) attach to the substratum by their caudal extremity and wriggle intensively (Kim *et al.*, 1989), probably to stimulate predation by aquatic invertebrates (Thomas and Ollevier, 1993a). Free-living larvae can survive and remain infective for days, especially at low water temperature and salinity (Kennedy and Fitch, 1990). Once ingested, L2 pierce the intestinal wall and invade the haemocoel where they start to grow immediately (Thomas and Ollevier, 1993a). The second moult (from L2 to third-stage larvae (L3)) takes place within 4–12 days, after which larvae can remain infective for weeks (Kim *et al.*, 1989; Petter *et al.*, 1989). Eels may become infected by feeding on a range of aquatic organisms. We compiled from the literature a total of 23 crustacean species that may serve as intermediate hosts (see Table 19.1), of which most were found in Europe. In Asia, the ostracod *Physocypria nipponica* and the copepods *Thermocyclops hyalinus*, *Eucyclops serrulatus* and *Eucyclops euacanthus* are known to harbour L3 (e.g. Ooi *et al.*, 1997), but there is yet no identified intermediate host in America and Africa. Most of the crustacean host species have natural preference for the epibenthic zone (Kirk, 2003), where young eels predominantly forage (Tesch, 2003). Eels of larger size can also get infected by preying

on a range of other aquatic organisms containing L3 (paratenic hosts; see Table 19.1). From the literature, we compiled a total of 50 species that may serve as paratenic hosts (all from Europe except the shortfin silverside, *Chirostoma humboldtianum*, recently recorded from Mexican aquaculture; G. Salgado-Maldonado, Mexico, personal communication, 2010). The list contains taxonomically diverse species from aquatic insect larvae to amphibian tadpoles (Moravec and Škoríková, 1998). Also, the possibility of eel infection via cannibalism was experimentally verified (De Charleroy *et al.*, 1990; Kennedy and Fitch, 1990). Once ingested by an eel, L3 pass through the intestinal wall, migrate inside the peritoneal cavity and reach the swimbladder wall within 1 week (Haenen *et al.*, 1989). In the swimbladder wall, L3 presumably feed on the host tissues (Polzer and Taraschewski, 1993). The time to metamorphosis into fourth-stage larvae (L4) is temperature dependent, and may vary from 2–3 weeks (De Charleroy *et al.*, 1990) up to 3 months post-infection (Haenen *et al.*, 1989). The diet of L4 is still uncertain with some authors arguing for histotrophy (Würtz and Taraschewski, 2000) and others for strict haematophagy (De Charleroy *et al.*, 1990). At high adult populations, *A. crassus* larvae in the swimbladder wall arrest their development in a density-dependent manner (Ashworth and Kennedy, 1999; Fazio *et al.*, 2008a). After a final moult

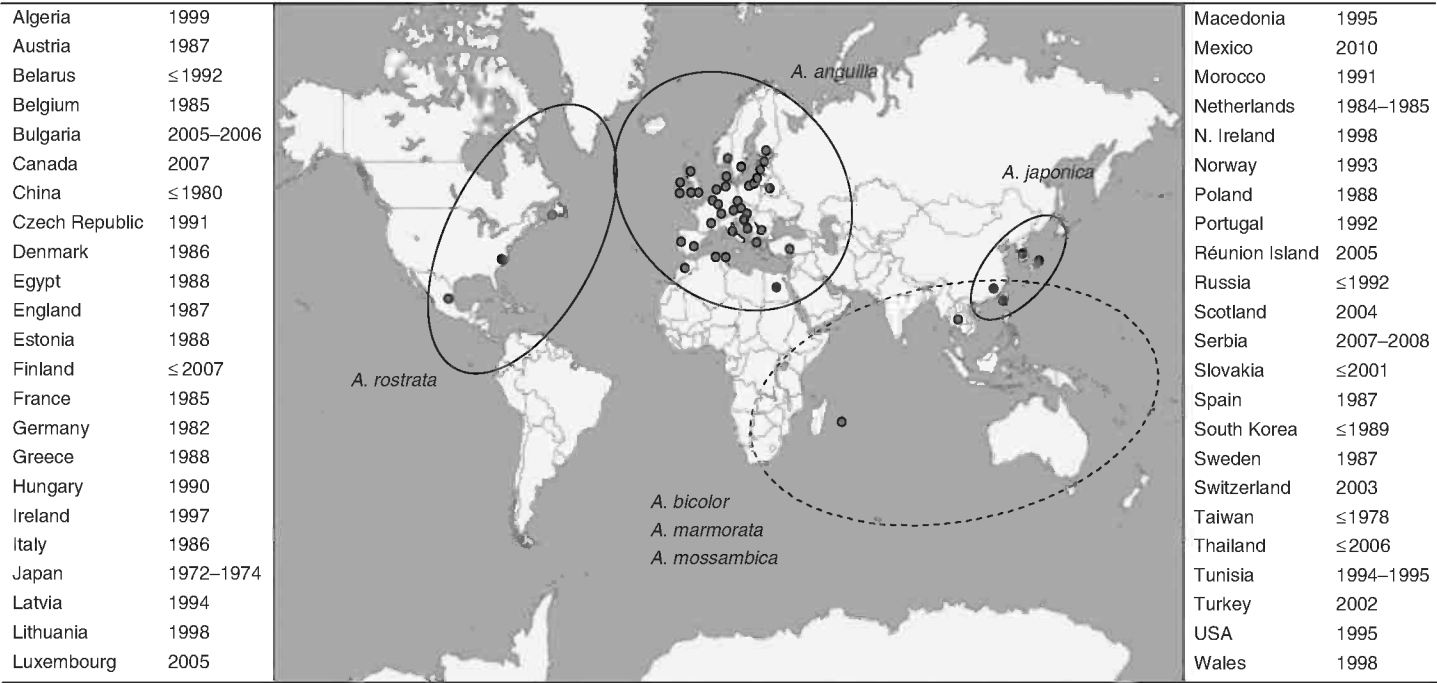


Fig. 19.1. Geographical distribution of the six eel (*Anguilla*) host species according to FishBase (Froese and Pauly, 2010) and first records of *Anguillicoloides crassus* by geopolitical countries (whether in coastal or inland waters, in an eel farm or in the wild). Data extracted from the literature (see Kirk, 2003; Moravec, 2006; Jakob *et al.*, 2009a), except for Mexico (G. Salgado-Maldonado, Mexico, personal communication, 2010) and Serbia (A. Hegedis and M. Lenhardt, Beograd, Serbia, personal communication, 2010).

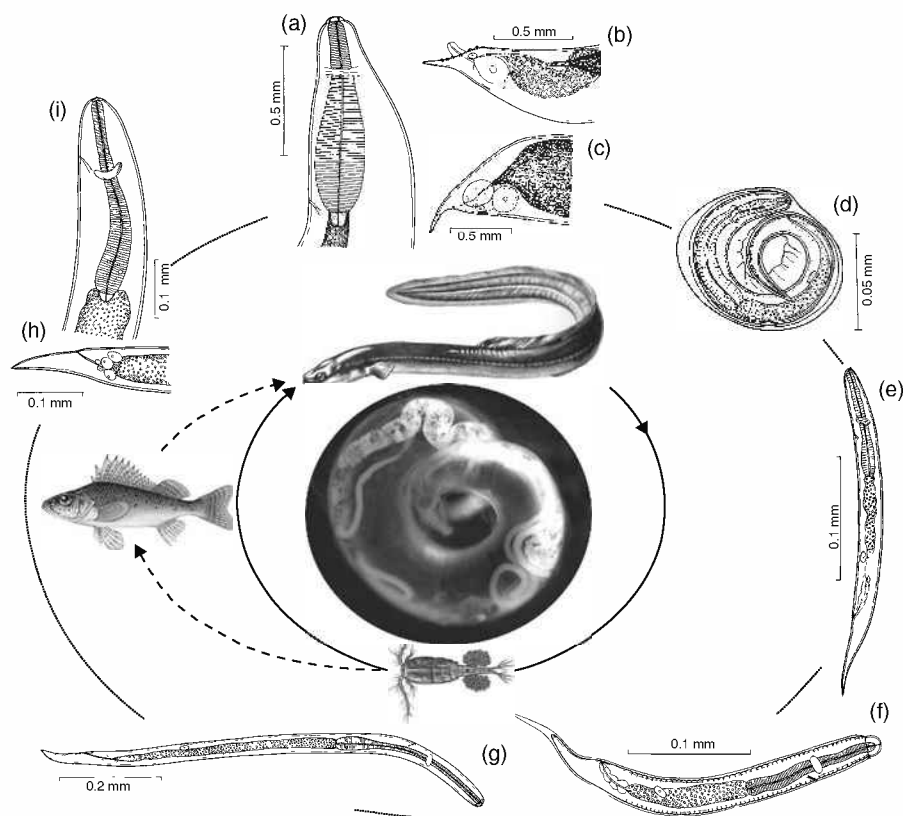


Fig. 19.2. Life cycle of *A. crassus* in European continental waters, showing the definitive host, the European eel *Anguilla anguilla*, and typical intermediate and paratenic hosts, the copepod *Cyclops strenuus* and the ruffe *Gymnocephalus cernuus*, respectively. Photo (courtesy of J. Lecomte): alive adult female. Drawings (courtesy of F. Moravec, after Moravec, 2006): (a) anterior end of gravid female; (b) caudal end of male; (c) caudal end of female; (d) second-stage larva (L2) inside egg shell; (e, f) L2 from copepods, 1 and 10 days post-infection (p.i.), respectively; (g) third-stage infective larva (L3) from a copepod, 20 days p.i.; (h, i) caudal end and anterior part, respectively, of a young fourth-stage larva (L4) from an eel, 23 days p.i.

(from L4 to pre-adult stage), the parasites feed on the capillary systems from the inside of the swimbladder. Adult males and females copulate in the lumen of the swimbladder, and females lay eggs containing a fully developed embryo (the first moult from L1 to L2 occurring *in utero*). Estimation of fecundity of a single female varies from 100,000–150,000 to 500,000 eggs (see, respectively, Thomas and Ollevier, 1993b, and Kennedy and Fitch, 1990). First eggs have been observed 6–8 weeks post-infection (Moravec *et al.*, 1994). The life cycle (from egg to eggs, without the intercalation of paratenic hosts) can be

completed in 2–3 months in optimal conditions (in 2 months at 20°C according to De Charleroy *et al.*, 1990), but it would take longer in the field (presumably over 4–6 months; Haenen *et al.*, 1989).

19.1.3. Epizootiology

The first unambiguous records of *A. crassus* occurred in Japan (Kuwahara *et al.*, 1974; for considerations on the possible origin of *A. crassus* see Moravec, 2006). It is now also

present in China, Korea and Taiwan, infecting both wild and farmed Japanese eels, and a number of other eel species imported there for cultivation purposes (notably the European and the American eels). In the Japanese eel, *A. crassus* seems to have out-competed the supposedly native *A. globiceps*, so that the latter almost disappeared from East Asia (Münderle *et al.*, 2006; F. Moravec, České Budějovice, Czech Republic, and H. Taraschewski, Karlsruhe, Germany, personal communications, 2010). In Europe, it was first detected in 1982 from wild European eels in northern Germany (Weser-Ems region; Neumann, 1985). Population genetics data recently suggested that 'Europe was invaded only once from Taiwan' (Wielgoss *et al.*, 2008), giving support to early statements based on eel import records (Koops and Hartmann, 1989).

In less than two decades it had invaded most of the geographical range of its new host (Jakob *et al.*, 2009a), including North Africa, with the exception of the very northernmost European countries (e.g. Iceland; A. Kristmundsson, Reykjavik, Iceland, personal communication, 2010). In America, *A. crassus* was first documented in 1995 in Texas aquaculture (Johnson *et al.*, 1995) and the same year from wild American eels in South Carolina (Fries *et al.*, 1996). Subsequent investigations tended to support an east coast origin for the introduction of the parasite, and possibly via the importation of Japanese eels from Japan (Wielgoss *et al.*, 2008). Recently, the parasite has been recorded in Cape Breton Island (Nova Scotia, Canada), the northernmost infected site yet for the American continent (Rockwell *et al.*, 2009). Southwards, the parasite has reached Mexico, both in wild and farmed American eels (G. Salgado-Maldonado, Mexico, personal communication, 2010). The nematode has also been documented in the Réunion Island (Indian Ocean) in three additional hosts, namely the Indonesian shortfin eel, the giant mottled eel and the African longfin eel (Sasal *et al.*, 2008; see Table 19.1 and Fig. 19.1). Here again, human-mediated transfers for cultivation purposes are suspected, and the authors suggested a Baltic Sea origin for the imported parasite population(s). So far, *A. crassus* has never been recorded in the Australasian eels, probably in relation to the strict

import limitations that are applied in these countries (C. Kennedy, Exeter, UK, personal communication, 2010), and/or competitive exclusion by local anguillicolid species.

Overall, we listed *A. crassus* in 46 countries worldwide. To our knowledge, there is no instance of any large-scale infected area later recorded as being cleared of the parasite. According to Kennedy (2007), once introduced, 'it is here to stay'. Following introduction, prevalences generally soon reach high values, up to 100% on occasion (Kennedy and Fitch, 1990; Taraschewski, 2006). Typically however, after a few years of high infection pressure, mean intensities and abundances start to decrease or level off (Audenaert *et al.*, 2003; Lefebvre and Crivelli, 2004). Although this may resemble a phase of stabilization due to the host immune response, it rather seems to reflect the fact that the infected organs are getting so damaged by repetitive infection events that they become unsuitable for further parasite establishment.

19.2. Diagnosis of Infection

19.2.1. Detection by non-invasive methods

Altered eel behaviours have been frequently reported in association with *A. crassus* infection, for example: (i) 'moribund behaviour' (Molnár *et al.*, 1991); (ii) reduced swimming performance (Sprengel and Luchtenberg, 1991; Palstra *et al.*, 2007); or (iii) abnormal hanging near the surface (Van Banning and Haenen, 1990). Morphological changes have also been observed, such as body emaciation (Egusa, 1979) or swollen abdomen (Ooi *et al.*, 1996). Anal redness has been specifically investigated as a simple means to reveal *A. crassus* infection (Van Banning and Haenen, 1990; Crean *et al.*, 2003). However, phenotypic changes are generally observed only in a small number of the infected hosts, and multiple confounding factors may explain these clinical signs (e.g. viral and bacterial infections, ingestion of solid matters such as crayfishes).

Serodiagnostic methods (immunoblotting and ELISA) have been applied for the

detection of specific antigens or antibodies (Buchmann *et al.*, 1991; Inui *et al.*, 1999). However, for economic and logistic reasons, their applicability to epizootiological studies appear somehow limited (Knopf *et al.*, 2000). Copro-analyses by identification of eggs and/or L2 in the faeces of eels have also been attempted for diagnosing *A. crassus* infections (e.g. Shin and Chen, 2000). The specificity and predictability of this method proved to be satisfactory, thus offering a cheap and convenient alternative for detection of anguillicolosis.

Among other non-invasive methods, radiography has been employed to visualize *A. crassus* worms in the swimbladder lumen. Data from X-ray imageries proved to be consistent with the findings of later dissection (Beregi *et al.*, 1998). The method also works for recording the severity of the pathology in the swimbladder, thus constituting a tool of primary importance in assessing and monitoring the infection status of live eels (e.g. Székely *et al.*, 2005; Palstra *et al.*, 2007).

19.2.2. Detection at autopsy

Upon excision of the swimbladder, adult and pre-adult worms are so conspicuous (several millimetres long for the smallest and typical dark brown or black coloration, see Fig. 19.2) that they are unmistakable to the naked eye. Also, *A. crassus* is almost definitely the sole metazoan parasite encountered in the swimbladder of the European, American and Japanese eels, both in the wild and in aquaculture. Only the larval stages of the nematode *Daniconema anguillae* can occasionally be found in the swimbladder of eels (for morphological identification, see Moravec and Køie, 1987). A binocular microscope ($\times 10$ magnification) is needed to look for the larval stages of *A. crassus* in the swimbladder wall. Swimbladder material can be observed as such or flattened between two glass slides and eventually fixed in 10% buffered formalin (e.g. Nimeth *et al.*, 2000). Recently, a simple PCR-based method was developed for detecting the presence of L3/L4 stages within the swimbladder wall.

Furthermore, subsequent sequencing of the amplified marker gene yields a reliable method for the identification of the parasite at the species level (Heitlinger *et al.*, 2009). Also, since damages to the swimbladder have been observed in the absence of any concomitant infections by other pathogens (e.g. Csaba *et al.*, 1993; Würtz and Taraschewski, 2000), observations of gross pathology in the swimbladder organ may constitute another line of investigation to infer the presence of larvae and/or the occurrence of past infections.

19.3. Macroscopic and Microscopic Lesions

19.3.1. Histopathologies

In the original description of *A. crassus*, the authors reported a young worm 'sucking blood with the mouth attached to the capillaries distributed in the wall of the air bladder' (Kuwahara *et al.*, 1974), and Egusa (1979) reported that 'heavy infections produce various pathological changes in the swimbladder'. Bloodsucking marks of about 30 μm in length had then been observed, which approximately corresponds to the size of the mouth of adult *A. crassus* (Würtz and Taraschewski, 2000). Mechanical injuries of repeated blood sucking by adult and pre-adult worms cause epithelial lesions, and dilatation of the blood vessels (Molnár *et al.*, 1993). Migrations and feeding activities of larvae also cause microscopic lesions in the width of the swimbladder wall (including the *rete mirabile*) and in the pneumatic duct. Tunnel formations were observed as a result of migrating L3 and L4 through the swimbladder wall (Van Banning and Haenen, 1990). However, it is assumed that the observed macroscopic changes do not directly correspond to mechanical injuries resulting from parasite activities, but mostly to the initial – cellular – phase of the host immune response (Molnár *et al.*, 1993; Nielsen and Esteve-Gassent, 2006). Evidence of a cellular immune response was found on the observation of macrophages and

granulocytes around infection sites of L3 and L4, which form parasitic nodules in the walls of both the intestine and the swimbladder (Molnár *et al.*, 1993). Under high infection pressure, the wall of the swimbladder showed degenerative, inflammatory and proliferative changes (Molnár *et al.*, 1993; Haenen *et al.*, 1996). All layers of the swimbladder wall are affected (Fig. 19.3) but perhaps the most characteristic change is the anarchic – ‘cauliflower-like’ – proliferation of the epithelial cells (facing the lumen) that form hyperplastic tissues (Würtz and Taraschewski, 2000). The swimbladder wall may thus exhibit considerable thickening from 0.2–0.5 mm (normal state) up to 5 mm, occasionally leading to total collapse with no lumen remaining (Molnár *et al.*, 1993; Würtz and Taraschewski, 2000) (Fig. 19.4). However, the most harmful phase of anguillicolosis seems to result from the rupture of the swimbladder wall and the subsequent hatching and aberrant migration of L2 (Molnár *et al.*, 1995; Sokolowski and Dove, 2006). In these situations, L2 have been found in the swimbladder and intestine walls, in the muscular tissues, and also in vital organs such as the liver and kidneys, which may occasionally develop fibrosis (Van Banning and Haenen, 1990).

19.3.2. Dynamics of the degradations

Experimental investigations have demonstrated that no histopathological damage in the swimbladder can be detectable after a single dose with up to 20–40 larvae (Haenen *et al.*, 1996; Würtz and Taraschewski, 2000). The severe pathology observed in wild eels is probably as a result of recurrent *A. crassus* infections. Indeed, infection is possible almost anytime during the eel continental phase, from glass eel to silver eel stages (e.g. Nimeth *et al.*, 2000). The recovery rate of damaged swimbladders, if any, seems to be rather slow. By means of X-ray radiographs, Székely *et al.* (2005) demonstrated that after 3 months (in the absence of reinfection), the health status of the swimbladders had deteriorated in 55% of the initial eel sample, while the tendency to improve merely reached 1%.

Csaba *et al.* (1993) schematically identified three pathogenic stages in the swimbladder following a massive infection: (i) lumen worms and transparent wall; (ii) inflamed wall and lumen fluids; and (iii) thickened wall and no lumen worms. Actually, the normal development of *A. crassus* appears impeded in severely damaged swimbladders (Dekker and Van Willigen, 1988), and fibrosis probably constitutes a poor basis for

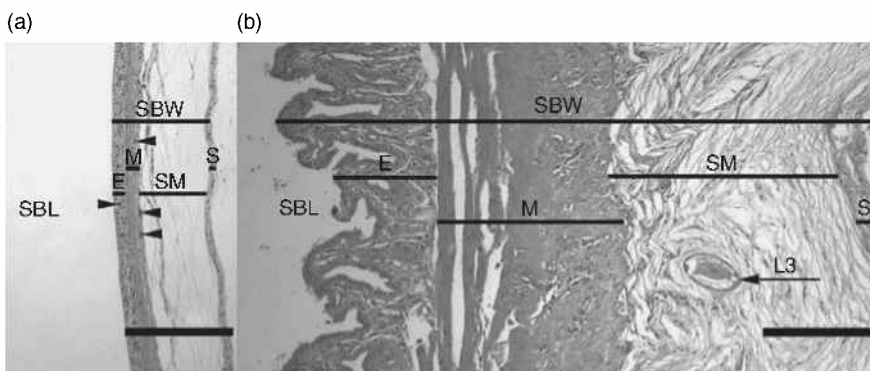


Fig. 19.3. Microphotographic sections of eel swimbladders (courtesy of M. Sokolowski). (a) Swimbladder of an uninfected eel, showing the swimbladder lumen (SBL) and the structure of normal swimbladder wall (SBW), with its four layers: the innermost mucosa or epithelium (E), the muscularis mucosa (M), the submucosa (SM), the serosa (S, the outermost layer), and blood vessels (arrowheads). (b) Section of a damaged swimbladder, showing a migrating third-stage larva (L3 indicated by arrow), and the considerable thickening and structural changes in all four layers. Haematoxylin and eosin stain was used. Bars = 200 µm.

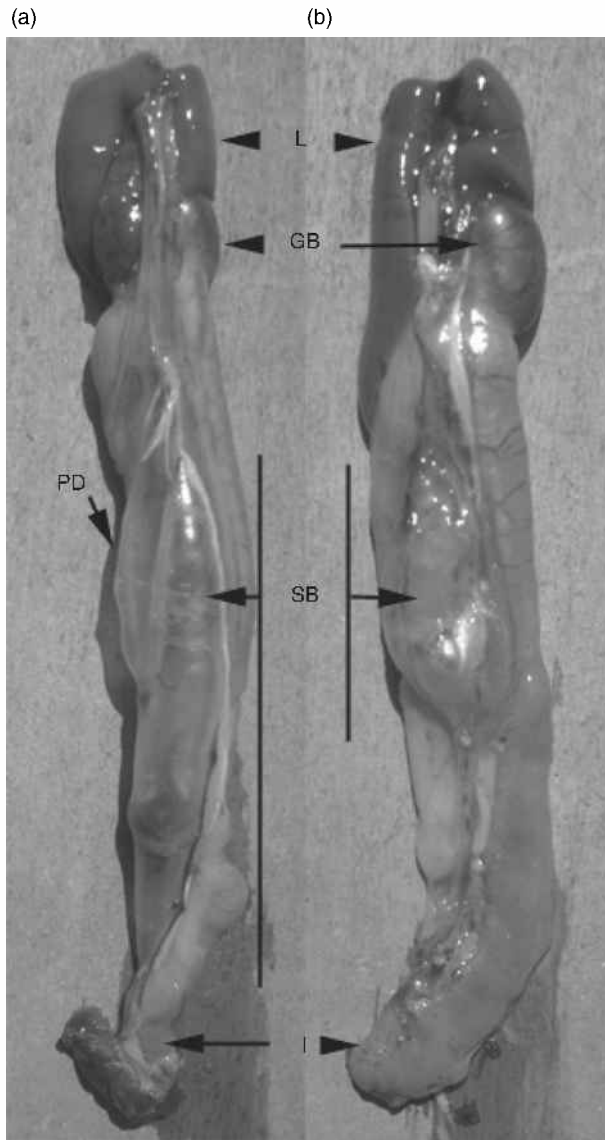


Fig. 19.4. Photos of *in situ* swimbladders (SB) along with the liver (L), gallbladder (GB) and intestine (I) (modified from Lefebvre *et al.*, 2011). (a) Healthy swimbladder showing adult worms inside, and visible pneumatic duct (PD); (b) degraded swimbladder showing overall shrinkage, and no worms inside the few lumen remaining.

reinfection (Van Banning and Haenen, 1990). This found support in field datasets showing a marked decrease in live worm abundance among eels with fibrotic swimbladders (Lefebvre *et al.*, 2002; Audenaert *et al.*, 2003). In the continuation of previous workers, Lefebvre *et al.* (2002) proposed a codified metric, the

Swimbladder Degenerative Index, based on the severity of gross pathology observed in three defined criteria: (i) opacity; (ii) abundance in pigmentation/exudates; and (iii) thickness (see Fig. 19.4). More recently an easy alternative metric was introduced (EEL-REP, 2005; also see Palstra *et al.*, 2007), based

on the observation that swimbladders shorten following infections, so that the severity of the damage can be expressed as a linear measure of the infected organ in relation to body size (see Fig. 19.4). With the development of swimbladder metrics, we now have the tools to record the past infection history which, in conjunction with classical epidemiologic counts of living worms, allow estimation of the proportion of eels really affected by the disease. For instance, in a study of silver eels in four habitats of southern France, Lefebvre *et al.* (2003) showed that when considering individuals with worms at the autopsy (52–77%) plus those showing signs of past infections in the swimbladder (40–78%), the proportion of potential spawners really affected by anguillicolosis ranged from 71% up to 95%.

19.4. Pathobiology of the Infection

Egusa (1979) first reported that ‘infected eels lose their appetite and vitality and become emaciated’. Since then, numerous investigations have looked at the potential impact of *A. crassus* on the life history traits of the eel hosts (Table 19.2). Most studies (87%) were done on the European eel ($n = 59$ for *A. anguilla*; $n = 5$ for *A. rostrata*; $n = 4$ for *A. japonica*). It seems that the Japanese eel suffers a comparatively lower pathogenicity than the two Atlantic eel species, with no reported cases of mortality or reduced growth/condition. In the European eel, proxy indicators such as spleen size, plasma glucose and cortisol reveal a physiological response to *A. crassus* infection (Sures *et al.*, 2001; Gollock *et al.*, 2005). Moreover, the swimbladder is involved in both gas exchange and buoyancy control, and so the partial or total reduction of its functional volume is likely to have an impact at the phenotypic level (Würtz and Taraschewski, 2000).

19.4.1. Mortality

Two mass mortalities of eels had been reported under field conditions. The first occurred in the Lake Balaton in Hungary

(Molnár *et al.*, 1991), and the second in the Morava River system in the Czech Republic (Baruš *et al.*, 1999). These events present striking similarities: (i) high density of large eels and intermediate/paratenic hosts; and (ii) incidences concentrated during the hot summer months. Examination of dead or dying eels revealed exceptionally high prevalence and intensity values, with severe damage to the swimbladder. However, subsequent investigations have cast doubts on the primary aetiological role of *A. crassus*. For instance, Nemcsóck *et al.* (1999) suggested a possible role of insecticides in these mass eel devastations. In aquaculture, mortality has been reported several times, although some authors invoked other aetiological causes (e.g. Liewes and Schaminee-Main, 1987; Kamstra, 1990). Ooi *et al.* (1996), however, noted that anthelmintic drugs administered during an outbreak period almost stopped mortality in the days afterwards. Both in the wild and in captivity, *A. crassus*-infected eels were suspected to be less resistant to viral, bacterial or fungal infections (Van Banning and Haenen, 1990). Also, authors have reported mortality among infected eels following transportation and handling (Koops and Hartmann, 1989). In semi-experimental conditions, parasite burden and/or pathological damage positively correlated with the mortality rate under hypoxia (Molnár, 1993). There is as yet no experimental evidence for a direct effect of the infection on host survival, and it seems that a combined action of *A. crassus* and environmental stressors might occur to cause high mortalities.

19.4.2. Condition and swimming performance

Studies on the impacts on body condition, growth and swimming behaviour have produced a similarly ambiguous picture (Table 19.2), partly because data are often very hard to evaluate and validate. A commonly advanced hypothesis to explain some counter-intuitive results is that active individuals in good condition were more likely to get infected simply because they eat more host preys (Koops

Table 19.2. Number of published works (non-exhaustive dataset) with evidence for negative (–), positive (+) and no effect (=) of *A. crassus* infection (past and/or current) on four life-history traits of eels *Anguilla* spp.

	Survival			Condition/growth			Swimming			Reproduction ^a		
	–	=	+	–	=	+	–	=	+	–	=	+
Experimental infections ^b	0	2	0	0	3	0	0	1	1	0	0	0
Natural infections ^c												
Farm	4	1	0	3	4	0	1	0	0	0	0	0
Wild	10	5	0	6	20	3	5	2	0	6	5	2

^a The trait reproduction only concerns silver eels and may include swimming performance, gonad mass or the degree of achieved maturity.

^b Experimental investigations have been conducted in the laboratory and involve comparisons between uninfected and artificially infected individuals, all things being equal.

^c Natural infection data (for which the infection pressure is assessed afterwards) in aquaculture conditions (i.e. similar density, *ad libitum* food, etc.) and in field conditions (i.e. in the wild where there are many confounding factors).

and Hartmann, 1989). In support of this, some field data indicated that infected eels contained more ingested prey in the gut than non-infected eels (Moser *et al.*, 2001). Another explanation may come from possible methodological artefacts. Indeed, in most studies, the effect of *A. crassus* was investigated by studying body mass in relation to body length, assuming that the growth in length is not affected by the infection. Also, comparisons of body dimensions were generally performed according to the infection status of the eels at the time of the autopsy (presence/absence or number of living worms within the swimbladder), with no consideration for the infection history of each individual. However, it now seems obvious that damage in the swimbladder wall has a much higher pathogenic impact on the host than the mere number of worms in the swimbladder lumen (Molnár, 1993). In the report by EELREP (2005), the authors clearly showed that the condition factor increases with the parasite biomass in the swimbladder lumen, but decreases with the severity of the swimbladder damage.

19.4.3. Reproduction

Reproductive physiology does not seem to be altered to a great extent, as two separate teams have succeeded in artificially bringing female eels to sexual maturity despite being infected with *A. crassus* (Müller *et al.*, 2003; EELREP, 2005). There are data to suggest that infection may even accelerate the silvering

process (Durif *et al.*, 2006; Fazio *et al.*, 2008b). Fazio and co-workers found that eels infected by *A. crassus* had a higher level of gene expression in deep-sea rod opsin, a retinal protein that permits a better vision in deep-sea oceanic waters, than uninfected eels. As suggested by the authors, silver-phase eels in stressful conditions could anticipate their pre-migratory metamorphosis, investing more in reproduction than in somatic growth, so as to limit the potential impact of the infection on their reproductive fitness. A negative correlation between the number of parasites in the swimbladder and the relative gonad mass was detected among wild caught silver eels (unpublished data of Palstra, 2005, cited in Székely *et al.*, 2009).

A greater level of agreement is achieved concerning the potential impact of *A. crassus* infection on the reproductive migration. Workers have long feared that the induced damage to the swimbladder organ might interfere with the capacity of Atlantic eels to reach their spawning grounds in the Sargasso Sea (e.g. Dekker and Van Willigen, 1988; Sprengel and Luchtenberg, 1991). To reproduce indeed, European silver eels need to swim over 5500 km and perform diel vertical migrations between depths of 200 and 1000 m (Tesch, 2003; Aarestrup *et al.*, 2009). Also, it was shown that the parasite can survive for long periods in eels kept in sea water (Kirk *et al.*, 2000), and so is likely to affect the use of the swimbladder as a hydrostatic organ in the ocean and to impose substantial metabolic

costs. The conclusion of the last international collaborative investigation into the reproductive capacities of the European eel (EELREP, 2005) was explicit enough: 'In the case of heavy swimbladder infection and/or damage ... eels ... will in fact never reach the spawning grounds' (also see Palstra *et al.*, 2007).

If such is the case, *A. crassus* would be quite similar in effect to closely related philometrid nematodes that establish in the gonads and castrate their fish hosts (Moravec, 2006). And this imposes an overlooked cost at the population level: not only are such 'sterilized' individuals excluded from the reproductive pool, but they keep competing with unaffected potential breeders during their growing phase.

19.5. Protective/Control Strategies

19.5.1. Chemotherapeutic treatments

Of the 19 anthelmintics that have been tested against *A. crassus*, Levamisole proved to be the drug of choice. The compound induced paralysis, death and expulsion of pre-adult and adult worms, and had no detectable toxicity for the fish (Taraschewski *et al.*, 1988; Hartmann, 1989). Hartmann (1989) investigated three methods of administration and found that 24 h water bath with Levamisole at 2 mg/l was the most effective procedure. However, larval stages L3 and L4 in the wall of the swimbladder are not affected by any medication (Taraschewski *et al.*, 1988; Kamstra, 1990), and it is recommended to repeat the treatment over an extended period until all adults maturing from larvae are killed. Furthermore, precautions must be taken since the L2 larval stage (whether inside the egg shell or free in the water) remain infective to intermediate hosts, and so are a latent risk of eel reinfection (Kamstra, 1990). In aquaculture, anthelmintic drugs do not seem widely applied nowadays, and there has been no published research on the subject for the last 15 years.

Other authors suggested disrupting the life cycle by adding chemicals to the water to eliminate intermediate crustacean hosts (Egusa and Hirose, 1983). Out of the several drugs that have been tested, Trichlorfon and

Diflubenzuron proved to be effective in single-dose application at concentrations (e.g. 0.01–0.02 mg/l) which are not dangerous to eels (Kamstra, 1990; Kim *et al.*, 1989). For an efficient control, however, the treatment has to be renewed about every week (Kamstra, 1990). Using chemicals to kill crustacean hosts is not considered to be very practical and seems virtually impossible to do routinely. This solution is moreover not environmentally acceptable as the effluent from the ponds would in turn contaminate natural water bodies (Kennedy, 2007). It seems that keeping intermediate host populations at low levels is a more sustainable and cost-effective alternative in eel farms. This may be accomplished by avoiding the accumulation of organic matter and/or filtering water in recycle systems (Kamstra, 1990). Moreover, eel farmers nowadays immediately take out and euthanize 'ill-looking' individuals (with pigmentation, haemorrhages, etc.) from newly arrived elvers to reduce the risks of introducing *A. crassus* and other pathogens at their farms. This has proved to be a simple but effective method (O. Haenen, Lelystad, The Netherlands, personal communication, 2010).

19.5.2. Immunology and vaccination

Both the European and Japanese eels are capable of mounting a cellular and humoral immune response against *A. crassus* (reviewed in Knopf, 2006; Nielsen and Esteve-Gassent, 2006). Also, antibacterial drugs (Flumequine and Oxytetracyclin) have been shown to enhance the natural immune system of the European eel (Van der Heijden *et al.*, 1996). However, reinfection experiments failed to demonstrate any indication for acquired immunity resulting from primary infections in the European eel (Haenen *et al.*, 1996; Knopf, 2006). There is no available vaccine against *A. crassus* on the market. Recent investigations using weakened (irradiated) L3 demonstrated a significant reduction of adult worms (both in size and number) in the case of later reinfections with normal L3, but only for the Japanese eel (Knopf and Lucius, 2008). The authors concluded that adaptive

immunity plays a role in protection in the Japanese eel but that the level of antibody response was too weak to be protective in the European eel. Possibly, Atlantic eels will develop the same level of defence efficiency as achieved by the Japanese eel after a long enough co-evolution period with *A. crassus* and/or *A. globiceps* (Taraschewski, 2006).

19.5.3. Environmental approach

It has been repeatedly noted that eel farms on sea coasts were most often parasite free (Kamstra, 1990), so the use of salt water may be worth further consideration in aquaculture. In the wild, multiple studies have revealed negative correlations between salinity values and infection parameters (e.g. Sauvaget *et al.*, 2003; Jakob *et al.*, 2009b). We re-investigated this relationship on a larger scale by compiling published data of 64 local studies (all eel species included). Clearly, parasite prevalence is at its lowest level at high salinities ($R_s = -0.35$, $P < 0.001$). Laboratory investigations demonstrated that egg hatching, L2 survival and infectivity, all decline with increasing salinity (Kennedy and Fitch, 1990). Even for adult worms, high salinities impose an ionic stress with some worms unable to osmoconform to the plasma of their hosts, thus presenting severe tissue damage (Kirk *et al.*, 2000). In addition, brackish and marine waters seem to provide a narrower range of suitable intermediate/paratenic hosts, and hence a lower prospect of transmission efficiency (Kirk *et al.*, 2000). Eels staying in a saline environment are thus at lower risk of becoming infected with *A. crassus*. For the welfare of natural eel populations and for the quality of future spawners, a sensible proposal would thus be to protect coastal waters and lagoons as areas free of eel-fishing pressure (Sauvaget *et al.*, 2003).

19.6. Conclusions and Suggestions for Future Studies

Based on historical records (fishery catchments and scientific monitoring), Atlantic eel

populations may have dropped as much as 90% since the 1960s (Dekker, 2008). Clearly, *A. crassus* is not the primary cause of the decline, as statistics on eel stocks started to decrease long before the parasite was introduced on the European and American continents. It is more likely that the downward trend in Atlantic eel populations results from the combination of multiple factors (e.g. habitat loss, over-fishing, oceanic changes) now including anguillicolosis (Tesch, 2003; Dekker, 2008). It is therefore essential to quantify the net losses due to *A. crassus* infection, in order to integrate this new threat into the development of appropriate management measures for the eel resource. In particular, we need to: (i) assess the quality of future spawners; and (ii) estimate the proportion of affected silver eels not being able to reach their spawning grounds. Tools are now available to assess non-invasively the health status of silver eels (e.g. radio imagery and swimbladder indices, for a review see Lefebvre *et al.*, 2011), and to follow their oceanic migrations individually in the long term (e.g. satellite tracking; see the ongoing EELIAD project (2008–2011). International collaborative efforts, which have already proved to be very fruitful (e.g. the EELREP project), are needed to combine the two techniques in such an ambitious research objective.

In aquaculture, anguillicolosis may no longer be an economic threat if basic sanitary measures can be applied (Taraschewski, 2006; Han *et al.*, 2008). Eel farms in Europe are usually free of the parasite or harbour a sustainable level compatible with high quality production, and hence for many years there has been no report in the literature of high mortality events. In natural water bodies that support eel fisheries through stocking, the fish densities can be maintained at levels compatible with commercial benefits and low risk of infections, as was done in the Balaton Lake, Hungary, since the massive outbreaks in the 1990s (Székely *et al.*, 2009). There is not much to do in the field apart from limiting intercontinental trade and transfers, and applying stringent controls of imported eels. It is worth mentioning that the European eel has been recently listed in the Annex II of the Convention on International Trade in

Endangered Species (CITES, 2007). It is crucial to keep reinforcing such drastic policies on a worldwide scale because many countries in Asia, Oceania and Africa are currently running pilot projects on establishing eel farms with local and/or imported eel species (see Münderle *et al.*, 2006; Sasal *et al.*, 2008). In parallel, efforts have to be pursued to master artificial eel reproduction (e.g. Abe *et al.*, 2010), so as not to deplete further the wild stocks for cultivation purposes.

Acknowledgements

This review could not have been done without the participation of dozens of colleagues worldwide. We are grateful to all those who sent us their own work and/or kindly

helped to locate some of the most obscure literature on the subject, and particularly Csaba Székely. Thanks to multiple individual contributions we almost reach 'exhaustivity' in terms of literature coverage. We would also like to thank all ichthyo-parasitologists who helped us to track down the spread of *A. crassus*. We are in great debt to František Moravec for kindly providing us with the original drawings of the species description, and for his insightful discussions on the possible origin of *A. crassus*. Finally, many thanks to our two editors, Patrick T.K. Woo and Kurt Buchmann, and to the many people who commented at some stages on the manuscript, namely: Willem Dekker, Kirsten Foley, Olga Haenen, Clive Kennedy, Klaus Knopf, Kalman Molnár and Csaba Székely.

References

- Aarestrup, K., Okland, F., Hansen, M.M., Righton, D., Gargan, P., Castonguay, M., Bernatchez, L., Howey, P., Sparholt, H., Pedersen, M.I. and McKinley, R.S. (2009) Oceanic spawning migration of the European eel (*Anguilla anguilla*). *Science* 325, 1660.
- Abe, T., Ijiri, S., Adachi, S. and Yamauchi, K. (2010) Development of an *in vitro* culture system for producing eel larvae from immature ovarian follicles in Japanese eel *Anguilla japonica*. *Fisheries Science* 76, 257–265.
- Ashworth, S.T. and Kennedy, C.R. (1999) Density-dependent effects on *Anguillicola crassus* (Nematoda) within its European eel definitive host. *Parasitology* 118, 289–296.
- Audenaert, V., Huyse, T., Goemans, G., Belpaire, C. and Volckaert, F.A.M. (2003) Spatio-temporal dynamics of the parasitic nematode *Anguillicola crassus* in Flanders, Belgium. *Diseases of Aquatic Organisms* 56, 223–233.
- Baruš, V., Moravec, F. and Prokeš, M. (1999) Anguillicolosis of the European eel (*Anguilla anguilla*) in the Czech Republic. *Czech Journal of Animal Science* 44, 423–431.
- Beregi, A., Molnár, K., Békési, L. and Székely, C. (1998) Radiodiagnostic method for studying swimbladder inflammation caused by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Diseases of Aquatic Organisms* 34, 155–160.
- Buchmann, K., Pedersen, L.Ø. and Glamann, J. (1991) Humoral immune response of European eel *Anguilla anguilla* to a major antigen in *Anguillicola crassus* (Nematoda). *Diseases of Aquatic Organisms* 12, 55–57.
- Convention on International Trade in Endangered Species (CITES) (2007) Inclusion of *Anguilla anguilla* (L.) in Appendix II. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CoP14), The Hague, The Netherlands.
- Crean, S.R., Dick, J.T.A., Evans, D.W., Elwood, R.W. and Rosell, R.S. (2003) Anal redness in European eels as an indicator of infection by the swimbladder nematode, *Anguillicola crassus*. *Journal of Fish Biology* 62, 482–485.
- Csaba, G., Láng, M., Sályi, G., Ramotsa, J., Glávits, R. and Rátz, F. (1993) Az *Anguillicola crassus* (Nematoda, Anguillicolidae) fonálféreg és szerepe az 1991. évi balatoni angolnapusztulásban. *Magyar Állatorvosok Lapja* 48, 11–21.
- De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C. and Ollevier, F. (1990) The life cycle of *Anguillicola crassus*. *Diseases of Aquatic Organisms* 8, 77–84.

- Dekker, W. (2008) Coming to grips with the eel stock slip-sliding away. *American Fisheries Society Symposium* 62, 335–355.
- Dekker, W. and Van Willigen, J.A. (1988) Abundance of *Anguillicola crassa* in Dutch outdoor waters and the reaction of its host *Anguilla anguilla*. *International Council for the Exploration of the Sea/Committee Meeting M* 13, 1–6.
- Durif, C.M.F., Dufour, S. and Elie, P. (2006) Impact of silvering stage, age, body size and condition on reproductive potential of the European eel. *Marine Ecology Progress Series* 327, 171–181.
- EELIAD (2011) European Eels in the Atlantic: Assessment of their Decline. Available at: <http://www.eeliad.com> (accessed 27 June 2011).
- EELREP (2005) Estimation of the reproduction capacity of European eel. Final report of the EU project Q5RS-2001-01836. Available at: http://www.fishbiology.net/EELREP_final_report.pdf (accessed 27 June 2011).
- Egusa, S. (1979) Notes on the culture of the European eel (*Anguilla anguilla* L.) in Japanese eel-farming ponds. *Rapports et Procès-Verbaux des Réunions. Conseil International pour l'Exploration de la Mer* 174, 51–58.
- Egusa, S. and Hirose, H. (1983) Anguillicolosis of eels. In: Egusa, S. (ed.) *Fish Pathology (Infectious and Parasitic Diseases)*. Koseisha Koseikaku, Tokyo, Japan, pp. 308–310.
- Fazio, G., Sasal, P., Da Silva, C., Fumet, B., Boissier, J., Lecomte-Finiger, R. and Moné, H. (2008a) Regulation of *Anguillicola crassus* (Nematoda) infrapopulations in their definitive host, the European eel, *Anguilla anguilla*. *Parasitology* 135, 1707–1716.
- Fazio, G., Moné, H., Lecomte-Finiger, R. and Sasal, P. (2008b) Differential gene expression analysis in European eels (*Anguilla anguilla*, L. 1758) naturally infected by macroparasites. *Journal of Parasitology* 94, 571–577.
- Fries, L.T., Williams, D.J. and Johnson, S.K. (1996) Occurrence of *Anguillicola crassus*, an exotic parasitic swim bladder nematode of eels, in the Southeastern United States. *Transactions of the American Fisheries Society* 125, 794–797.
- Froese, R. and Pauly, D. (2010) FishBase. World Wide Web electronic publication. Available at <http://www.fishbase.org> (accessed 27 June 2011).
- Gollock, M.J., Kennedy, C.R. and Brown, J.A. (2005) 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.
- Haenen, O.L.M., Grisez, L., De Charleroy, D., Belpaire, C. and Ollevier, F. (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., van Wijngaarden, T.A.M., van der Heijden, M.H.T., Höglund, J., Cornelissen, J.B.J.W., van Leengoed, L.A.M.G., Borgsteede, F.H.M. and van Muiswinkel, W.B. (1996) Effects of experimental infections with different doses of *Anguillicola crassus* (Nematoda, Dracunculoidea) on European eel (*Anguilla anguilla*). *Aquaculture* 141, 41–57.
- Han, Y.-S., Chang, Y.-T., Taraschewski, H., Chang, S.-L., Chen, C.-C. and Tzeng, W.-N. (2008) The swim-bladder parasite *Anguillicola crassus* in native Japanese eels and exotic American eels in Taiwan. *Zoological Studies* 47, 667–675.
- Hartmann, F. (1989) Investigations on the effectiveness of Levamisole as a medication against the eel parasite *Anguillicola crassus* (Nematoda). *Diseases of Aquatic Organisms* 7, 185–190.
- Heitlinger, E.G., Laetsch, D.R., Weclawski, U., Han, Y.-S. and Taraschewski, H. (2009) Massive encapsulation of larval *Anguillicoloides crassus* in the intestinal wall of Japanese eels. *Parasites and Vectors* 2, 48.
- Inui, T., Ushikoshi, R., Nogami, S. and Hirose, H. (1999) A competitive-ELISA for the serodiagnosis of anguillicolosis in Japanese eel, *Anguilla japonica*. *Fish Pathology* 34, 25–31.
- Jakob, E., Walter, T. and Hanel, R. (2009a) A checklist of the protozoan and metazoan parasites of European eel (*Anguilla anguilla*): checklist of *Anguilla anguilla* parasites. *Journal of Applied Ichthyology*, 1–49.
- Jakob, E., Hanel, R., Klimpel, S. and Zumholz, K. (2009b) Salinity dependence of parasite infestation in the European eel *Anguilla anguilla* in northern Germany. *International Council for the Exploration of the Sea (ICES) Journal of Marine Science* 66, 358–366.
- Johnson, S.K., Fries, L.T., Williams, J. and Huffman, D.G. (1995) Presence of the parasitic swim bladder nematode, *Anguillicola crassus*, in Texas aquaculture. *World Aquaculture* 26, 35–36.
- Kamstra, A. (1990) *Anguillicola* in Dutch eelfarms: current state. *Internationale Revue Der Gesamten Hydrobiologie* 75, 867–874.
- Kennedy, C.R. (2007) The pathogenic helminth parasites of eels. *Journal of Fish Diseases* 30, 319–334.

- Kennedy, C.R. and Fitch, D.J. (1990) Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in the eel, *Anguilla anguilla*, in Britain. *Journal of Fish Biology* 36, 117–131.
- Kim, Y.-G., Kim, E.-B., Kim, J.-Y. and Chun, S.-K. (1989) Studies on a nematode, *Anguillicola crassa* parasitic in the air bladder of the eel. *Journal of Fish Pathology* 2, 1–18.
- Kirk, R.S. (2003) The impact of *Anguillicola crassus* on European eels. *Fisheries Management and Ecology* 10, 385–394.
- Kirk, R.S., Lewis, J.W. and Kennedy, C.R. (2000) Survival and transmission of *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 (Nematoda) in seawater eels. *Parasitology* 120, 289–295.
- Knopf, K. (2006) The swimbladder nematode *Anguillicola crassus* in the European eel *Anguilla anguilla* and the Japanese eel *Anguilla japonica*: differences in susceptibility and immunity between a recently colonized host and the original host. *Journal of Helminthology* 80, 129–136.
- Knopf, K. and Lucius, R. (2008) Vaccination of eels (*Anguilla japonica* and *Anguilla anguilla*) against *Anguillicola crassus* with irradiated L₃. *Parasitology* 135, 633–640.
- Knopf, K., Naser, K., van der Heijden, M.H.T. and Taraschewski, H. (2000) 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.
- Koops, H. and Hartmann, F. (1989) *Anguillicola*-infestations in Germany and in German eel imports. *Journal of Applied Ichthyology* 5, 41–45.
- Kuwahara, A., Niimi, A. and Itagaki, H. (1974) Studies of a nematode parasitic in the air bladder of the eel. I. Description of *Anguillicola crassa* n. sp. (Philometridea, Anguillicolidae). *Japanese Journal of Parasitology* 23, 275–279.
- Lefebvre, F.S. and Crivelli, A.J. (2004) Anguillicolosis: dynamics of the infection over two decades. *Diseases of Aquatic Organisms* 62, 227–232.
- Lefebvre, F., Contournet, P. and Crivelli, A.J. (2002) The health state of the eel swimbladder as a measure of parasite pressure by *Anguillicola crassus*. *Parasitology* 124, 457–463.
- Lefebvre, F., Acou, A., Poizat, G. and Crivelli, A.J. (2003) Anguillicolosis among silver eels: a 2-year survey in four habitats from Camargue (Rhône delta, South of France). *Bulletin Français de la Pêche et de la Pisciculture* 368, 97–108.
- Lefebvre, F., Fazio, G., Palstra, A.P., Székely, C. and Crivelli, A.J. (2011) An evaluation of indices of gross pathology associated with the invasive nematode *Anguillicoloides crassus* in eels. *Journal of Fish Diseases* 34, 31–45.
- Liewes, E.W. and Schaminee-Main, S. (1987) Onderzoek aal parasiet vordet. *Aquacultuur* 2, 5–17.
- Molnár, K. (1993) Effect of decreased oxygen content on eels (*Anguilla anguilla*) infected by *Anguillicola crassus* (Nematoda: Dracunculoidea). *Acta Veterinaria Hungarica* 41, 349–360.
- Molnár, K., Székely, C. and Baska, F. (1991) Mass mortality of eel in Lake Balaton due to *Anguillicola crassus* infection. *Bulletin of the European Association of Fish Pathologists* 11, 211–212.
- Molnár, K., Baska, F., Csaba, G., Glávits, R. and Székely, C. (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., Szakolczai, J. and Vetési, F. (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. (2006) *Dracunculoid and Anguillicoloid Nematodes Parasitic in Vertebrates*. Academia, Prague, Czech Republic.
- Moravec, F. and Kóie, M. (1987) *Daniconema anguillae* gen. et sp. n., a new nematode of a new family Daniconematidae fam. n. parasitic in European eels. *Folia Parasitologica* 34, 335–340.
- Moravec, F. and Škoríková, B. (1998) Amphibians and larvae of aquatic insects as new paratenic hosts of *Anguillicola crassus* (Nematoda: Dracunculoidea), a swimbladder parasite of eels. *Diseases of Aquatic Organisms* 34, 217–222.
- Moravec, F. and Taraschewski, H. (1988) Revision of the genus *Anguillicola* Yamaguti, 1935 (Nematoda: Anguillicolidae) of the swimbladder of eels, including descriptions of two new species, *A. novaezealandiae* sp. n. and *A. papernai* sp. n. *Folia Parasitologica* 35, 125–146.
- Moravec, F., Di Cave, D., Orecchia, P. and Paggi, L. (1994) Experimental observations on the development of *Anguillicola crassus* (Nematoda: Dracunculoidea) in its definitive host, *Anguilla anguilla* (Pisces). *Folia Parasitologica* 41, 138–148.
- Moser, M.L., Patrick, W.S. and Crutchfield, J.U.J. (2001) Infection of American eels, *Anguilla rostrata*, by an introduced nematode parasite, *Anguillicola crassus*, in North Carolina. *Copeia* 3, 848–853.

- Müller, T., Váradi, B., Horn, P. and Bercsényi, M. (2003) Effects of various hormones on the sexual maturity of European eel (*Anguilla anguilla* L.) females from farm and lakes. *Acta Biologica Hungarica* 54, 313–322.
- Münderle, M., Taraschewski, H., Klar, B., Chang, C.W., Shiao, J.C., Shen, K.N., He, J.T., Lin, S.H. and Tzeng, W.N. (2006) Occurrence of *Anguillicola crassus* (Nematoda: Dracunculoidea) in Japanese eels *Anguilla japonica* from a river and an aquaculture unit in SW Taiwan. *Diseases of Aquatic Organisms* 71, 101–108.
- Nemcsók, J., Bálint, T., Fazakas, J., Kátai, F., Kiss, I., Hieu, L.H., Kufcsák, O., Láng, G., Polyhos, C., Szabó, I. and Szegletes, T. (1999) The contribution of a pyrethroid insecticide to the massive eel (*Anguilla anguilla*) devastation, in Lake Balaton, in 1995. *Acta Biologica Hungarica* 50, 161–173.
- Neumann, W. (1985) Schwimmblasenparasit *Anguillicola* bei Aalen. *Fischer und Teichwirt* 36, 322.
- Nielsen, M.E. and Esteve-Gassent, M.D. (2006) The eel immune system: present knowledge and the need for research. *Journal of Fish Diseases* 29, 65–78.
- Nimeth, K., Zwerger, P., Würtz, J., Salvenmoser, W. and Pelster, B. (2000) Infection of the glass-eel swim-bladder with the nematode *Anguillicola crassus*. *Parasitology* 121, 75–83.
- Ooi, H.-K., Wang, W.-S., Chang, H.-Y., Wu, C.-H., Lin, C.-C. and Hsieh, M.-T. (1996) An epizootic of anguillicolosis in cultured American eels in Taiwan. *Journal of Aquatic Animal Health* 8, 163–166.
- Ooi, H.-K., Lin, C.-C., Chen, M.-C. and Wang, W.-S. (1997) *Eucyclops euacanthus* (Copepoda: Cyclopidae) is an intermediate host of *Anguillicola crassus* (Nematoda: Dracunculoidea) in Taiwan. *Taiwan Journal of Veterinary Medicine and Animal Husbandry* 67, 135–138.
- Palstra, A.P., Heppener, D.F.M., van Ginneken, V.J.T., Székely, C. and van den Thillart, G.E.E.J.M. (2007) Swimming performance of silver eels is severely impaired by the swim-bladder parasite *Anguillicola crassus*. *Journal of Experimental Marine Biology and Ecology* 352, 244–256.
- Petter, A.J., Fontaine, Y.A. and Le Belle, N. (1989) Étude du développement larvaire de *Anguillicola crassus* (Dracunculoidea, Nematoda) chez un Cyclopidae de la région parisienne. *Annales de Parasitologie Humaine et Comparée* 64, 347–355.
- Polzer, M. and Taraschewski, H. (1993) Identification and characterization of the proteolytic enzymes in the developmental stages of the eel-pathogenic nematode *Anguillicola crassus*. *Parasitology Research* 79, 24–27.
- Rockwell, L.S., Jones, K.M.M. and Cone, D.K. (2009) First record of *Anguillicoloides crassus* (Nematoda) in American eels (*Anguilla rostrata*) in Canadian estuaries, Cape Breton, Nova Scotia. *Journal of Parasitology* 95, 483–486.
- Sasal, P., Taraschewski, H., Valade, P., Grondin, H., Wielgoss, S. and Moravec, F. (2008) Parasite communities in eels of the Island of Reunion (Indian Ocean): a lesson in parasite introduction. *Parasitology Research* 102, 1343–1350.
- Sauvaget, B., Fatin, D. and Briand, C. (2003) Contamination par *Anguillicola crassus* de cinq populations d'anguilles (*Anguilla anguilla*) du littoral de Bretagne sud (France). *Bulletin Français de la Pêche et de la Pisciculture* 368, 21–26.
- Shin, J.-W. and Chen, C.-C. (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.
- Sokolowski, M.S. and Dove, A.D.M. (2006) Histopathological examination of wild American eels infected with *Anguillicola crassus*. *Journal of Aquatic Animal Health* 18, 257–262.
- Sprengel, G. and Lüchtenberg, H. (1991) Infection by endoparasites reduces maximum swimming speed of European smelt *Osmerus eperlanus* and European eel *Anguilla anguilla*. *Diseases of Aquatic Organisms* 11, 31–35.
- Sures, B., Knopf, K. and Kloas, W. (2001) Induction of stress by the swimbladder nematode *Anguillicola crassus* in European eels, *Anguilla anguilla*, after repeated experimental infection. *Parasitology* 123, 179–184.
- Székely, C., Molnár, K. and Rácz, O.Z. (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.
- Székely, C., Palstra, A., Molnár, K. and van den Thillart, G. (2009) Impact of the swim-bladder parasite on the health and performance of European eels. *Fish and Fisheries Series* 30, 201–226.
- Taraschewski, H. (2006) Hosts and parasites as aliens. *Journal of Helminthology* 80, 99–128.
- Taraschewski, H., Renner, C. and Mehlhorn, H. (1988) Treatment of fish parasites. 3. Effects of levamisole HCl, metrifonate, fenbendazole, mebendazole, and ivermectin on *Anguillicola crassus* (nematodes) pathogenic in the air bladder of eels. *Parasitology Research* 74, 281–289.

- Tesch, F.-W. (2003) *The Eel*, 5th edn. Blackwell Science, Oxford, UK.
- Thomas, K. and Ollevier, F. (1993a) Hatching, survival, activity and penetration efficiency of second-stage larvae of *Anguillicola crassus* (Nematoda). *Parasitology* 107, 211–217.
- Thomas, K. and Ollevier, F. (1993b) First estimate of the egg production of *Anguillicola crassus* (Nematoda: Dracunculoidea). *Folia Parasitologica* 40, 104.
- Van Banning, P. and Haenen, O.L.M. (1990) Effects of the swimbladder nematode *Anguillicola crassus* in wild and farmed eel, *Anguilla anguilla*. In: Perkins, F.O. and Cheng, T.C. (eds) *Pathology in Marine Science*. Academic Press, New York, USA, pp. 317–330.
- Van der Heijden, M.H.T., Helders, G.M., Booms, G.H.R., Huisman, E.A., Rombout, J.H.W.M. and Boon, J.H. (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.
- Wielgoss, S., Taraschewski, H., Meyer, A. and Wirth, T. (2008) Population structure of the parasitic nematode *Anguillicola crassus*, an invader of declining North Atlantic eel stocks. *Molecular Ecology* 17, 3478–3495.
- Würtz, J. and Taraschewski, H. (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.

20 *Argulus foliaceus*

Ole Sten Møller

Institute of Biosciences, University of Rostock, Rostock, Germany

20.1. Introduction

The Branchiura is a group of crustaceans parasitizing primarily freshwater fishes (Piascecki and Avenant-Oldewage, 2008; Møller, 2009). Termed ‘carp lice’ colloquially, the Branchiura is often mixed up with the unrelated caligid copepods ‘salmon lice’ or ‘sea lice’ (normally referring to members of the genera *Lepeophtheirus* or *Caligus*), while they in fact are more closely related to the holoparasitic Pentastomida (Wingstrand, 1972; Zrzavý, 2001). The Branchiura move about on the fish (Avenant-Oldewage and van As, 1990; Avenant-Oldewage, 1994), readily leave the host, and they are generally excellent swimmers (Wilson, 1902; Thiele, 1904; Fryer, 1968; 1969). The morphology of members of the genus *Argulus* is well studied, especially the widespread species which include *Argulus foliaceus*, *Argulus japonicus* and *Argulus coregoni* (Leydig, 1889; Wilson, 1902; Thiele, 1904; Monod, 1928; Tokioka, 1936; Meehan, 1940; Rushton-Mellor, 1992; Avenant-Oldewage and Swanepoel, 1993; Møller *et al.*, 2007; Kaji *et al.*, 2011).

A. foliaceus are 3–15 mm in length (Fig. 20.1a, c) and like all branchiurans their dorsoventrally flattened body comprises: (i) a head with five cephalic appendages; (ii) a

thorax with four biramous thoracopods; and (iii) a short unsegmented bilobed abdomen with short furcal rami (Fig. 20.1a). Anteriolaterally in the carapace is a pair of large compound eyes (Fig. 20.1c), and slightly posterior to them, a small median nauplius eye with three pigment cups. The carapace extends posteriorly as a large lobate shield, covering the legs on either side of the body (Figs. 20.1a and 20.2a). In some *Argulus* species, the carapace also extends to cover the abdomen (Yamaguti, 1963; Cressey, 1972; Thatcher, 1991) and the shape is sometimes used for identification purposes. The carapace contains the highly branched gut caeca as well as two specialized areas for osmoregulation (Haase, 1975), which normally are referred to (wrongly) as ‘respiratory areas’, and are often used for species determination (Grobben, 1908; Rushton-Mellor, 1994; Boxshall, 2005). *In vivo*, *A. foliaceus* has a greenish hue and is relatively transparent, except gravid females where the yellow/whitish egg mass in the ovaries is prominent (Fig. 20.1c). The cephalic appendages are almost exclusively adapted for attachment to the host. The small first antenna has a large hook on the first podomere (Figs 20.1a and 20.2a, b), and the first maxillae are equipped distally with strong suction-disc structures (Fig. 20.1a, b).

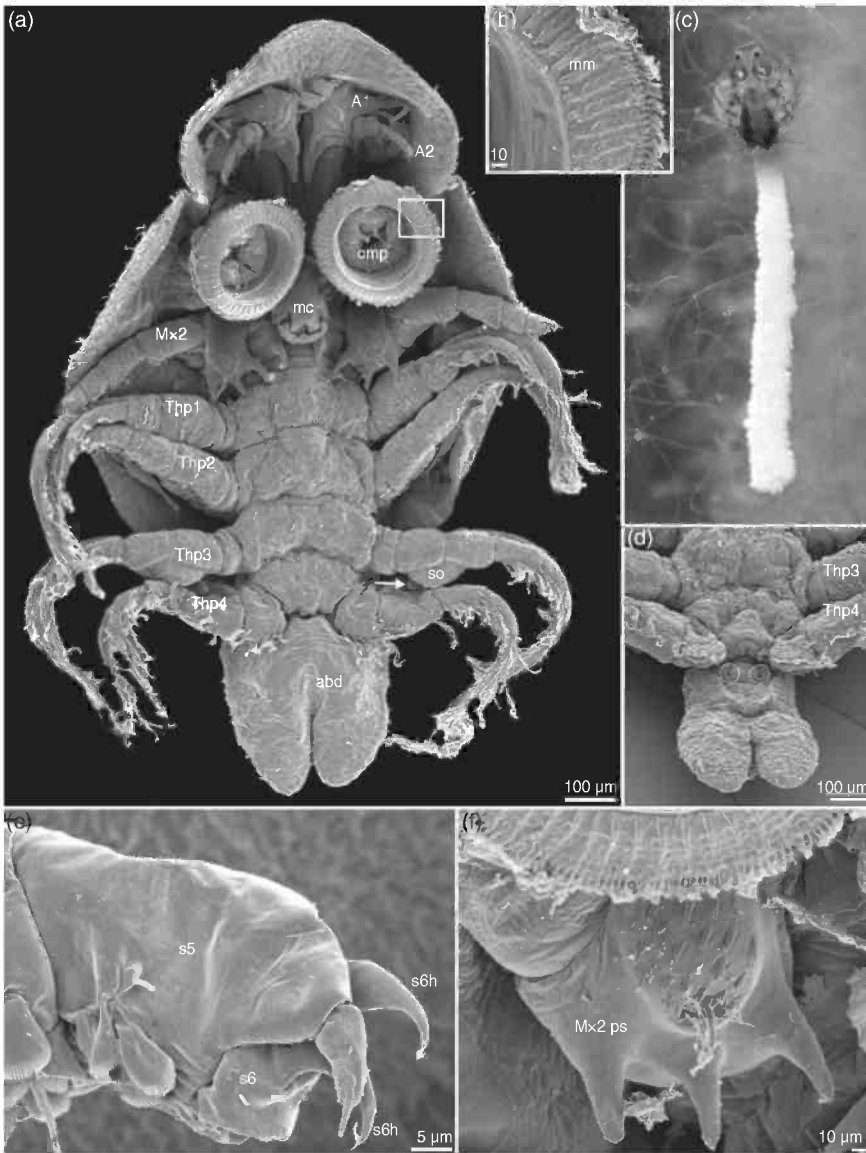


Fig. 20.1. *Argulus foliaceus*, light photography and scanning electron microscopy (SEM). (a) Adult male, ventral view. Note the peg (arrow) and socket (so) system of the male external genital system, of the third and fourth thoracopod, respectively. (b) Detail of boxed area in (a), showing the first maxilla suction disc marginal membrane with the sclerotized support structures. (c) Adult female depositing eggs on the front glass pane of an aquarium. Note the whitish colour of the egg string; it later turns yellowish-brown. (d) The posterior thorax region and abdomen of an adult female; tilted ventral view from posterior. Note the openings of the spermatheca (dotted circles). (e) Detail of the distal segments of the second maxilla, showing the two hooks. (f) Detail of the second maxilla proximal segment with the characteristic teeth directed posteriorly. Abbreviations: A1, first antenna; A2, second antenna; abd, abdomen; cmp, central movable part; mc, mouth cone; mm, marginal membrane; Mx2, second maxilla; Mx2 ps, second maxilla proximal segment; s5–6, segments five to six; so, 'socket' of the male external genital system, third thoracopod. s6h, sixth segment hook; Thp1–4, thoracopods: one to four.

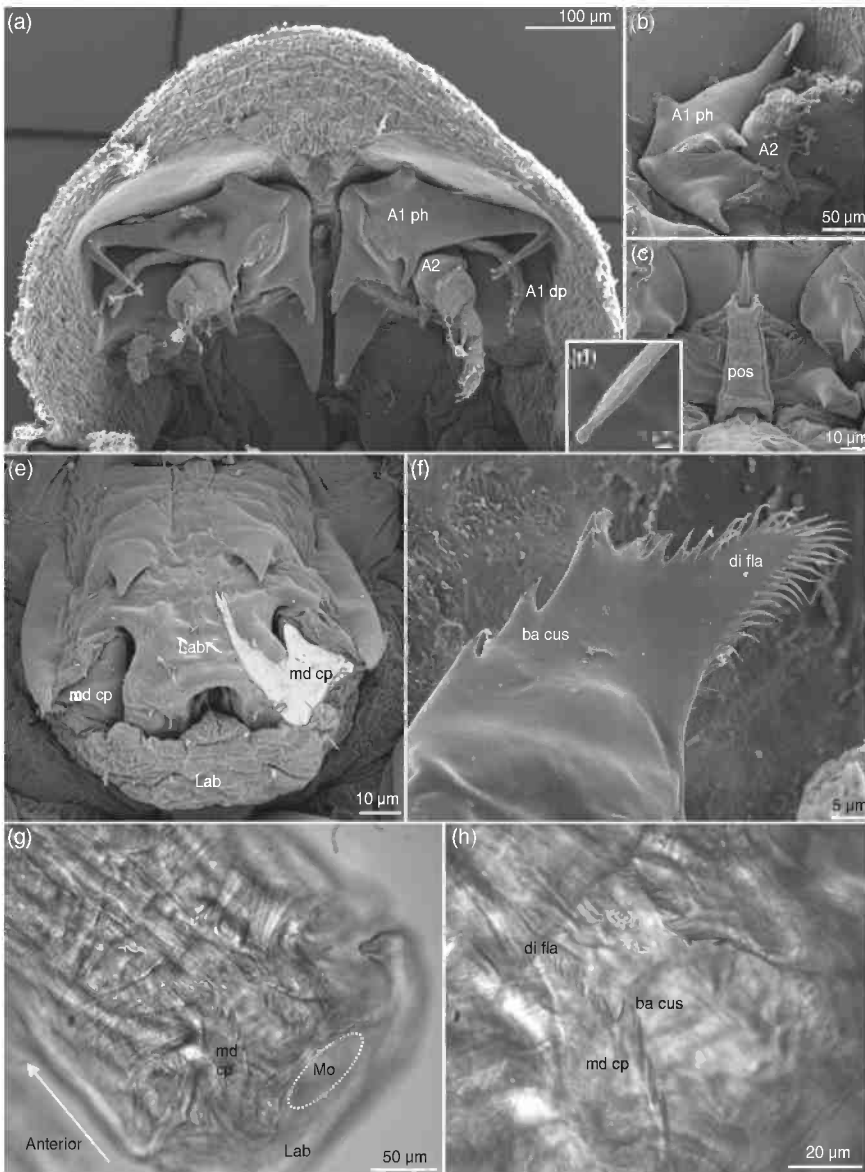


Fig. 20.2. *Argulus foliaceus*, cephalic details, Differential Interference Contrast Light Microscopy (DIC-LM) and SEM. (a) Anterior cephalic region, ventral view. (b) Detail of first and second antennae, lateral view. (c) Pre-oral spine, view from posterior. (d) Tip of pre-oral spine showing duct opening. (e) Mouth cone, juvenile. The mandibular coxal process from a dissected specimen (in white) is superimposed onto the specimen, showing its approximate position within the cone. (f) Detail of a dissected mandibular coxal process from an adult specimen. (g) Tip of the mouth cone, adult specimen, cleared with lactophenol. The 'upright' position of the coxal processes within the oral cavity is evident. (h) Detail from (g). Abbreviations: A1 dp, first antenna distal part; A1 ph, first antenna proximal hook; A2, second antenna; ba cus, basal cusps of the coxal process; di fla, distal 'flange' of the coxal process; Lab, labium; Labr, labrum; md cp, mandibular coxal process; Mo, mouth opening; pos, pre-oral spine.

The second maxilla is also used for attachment and has three posteriorly directed stout 'teeth' on the first podomere (Fig. 20.1f) and two small hooks apically (Fig. 20.1e) (Møller *et al.*, 2008). The mouth opening is situated at the tip of the mouth cone, the latter being of varying length within the genus, but is always completely fused into a tube in all Branchiura (Martin, 1932; Gresty *et al.*, 1993) (Figs. 20.1a and 20.2e, g). The sickle-shaped mandibular coxal processes are situated within the oral cavity (Fig. 20.2e–h), but can be rotated so as to rip and bite into the host tissue. Anterior to the mouth cone, but continuous with its base, is the pre-oral spine or stylet (Fig. 20.2c, d). This thin cuticular structure is very movable and can be completely retracted. The apical pore (Fig. 20.2d) is connected with glands at the base of the spine, but the precise function is debatable and the detailed innervation is still unknown (Swanepoel and Avenant-Oldewage, 1992; Gresty *et al.*, 1993).

A spermatophore in *Argulus* was not unequivocally confirmed until recently (Avenant-Oldewage and Everts, 2010). Copulation in *Argulus* can take place on, as well as off, the host, but probably not while swimming (Clark, 1902; Wilson, 1902; Avenant-Oldewage and Everts, 2010). The eggs fertilized by sperm stored in the spermatophore are deposited on stones, leaves and roots, from shallow brinks down to as deep as 8.5 m (Walker *et al.*, 2004; Harrison *et al.*, 2006) (Fig. 20.1d). Each egg mass contains from 50 to several hundred eggs (Fig. 20.1c). In extreme cases multiple layers of eggs on the same stone have been reported for *A. coregoni* in fish ponds (Mikheev *et al.*, 2001), and hatching rates are consistently very high (> 90%) (Hakalahti and Valtonen, 2003; Hakalahti *et al.*, 2003).

A. foliaceus and *A. japonicus* are not host specific and are found on many freshwater fishes including small stickleback (*Gasterosteus aculeatus* L.), rudd (*Scardinius erythrophthalmus* L.), perch (*Perca fluviatilis* L.), carp (*Cyprinus carpio* L.), carp bream (*Abramis brama* L.), tench (*Tinca tinca* L.), eel (*Anguilla anguilla* L.), large pike (*Esox lucius* L.), trout (*Salmo trutta* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) (Kollatsch, 1959; Menezes *et al.*, 1990; Paperna, 1991, 1996; Buchmann and Bresciani, 1997; Evans and

Matthews, 2000; Taylor *et al.*, 2009). *A. coregoni* is seemingly more host specific and prefers salmonids (Bandilla *et al.*, 2004; Pasternak *et al.*, 2004; Mikheev *et al.*, 2007).

A. foliaceus is found all over Europe and the UK, and in southern Scandinavia extending into Finland where it co-occurs with *A. coregoni* (Hakalahti *et al.*, 2006; Mikheev *et al.*, 2007; Bandilla *et al.*, 2008). The eastern distribution limit is not known, but other species (*A. indicus* and *A. japonicus*) take over gradually towards India and the Far East. In higher latitudes, seemingly *A. coregoni* gradually replaces *A. foliaceus*, (Schram *et al.*, 2005), although this has yet to be confirmed. Both *A. foliaceus* and *A. japonicus* have spread widely with the transport of live fish especially with the expansion of aquaculture fish production and the increasing popularity of recreational carp fisheries, for example koi carp as well as ornamental carp breeding (Menezes *et al.*, 1990; Rushton-Mellor, 1992; Paperna, 1996; Northcott *et al.*, 1997; Bandilla *et al.*, 2004; Hakalahti *et al.*, 2004; Catalano and Hutson, 2010).

20.2. Diagnosis of Infection and Clinical Signs of the Disease

A. foliaceus is easily spotted on fish; the best visual cues are the two compound eyes. Typically the attachment site is at the base of fins (Kollatsch, 1959; Schlüter, 1978; Mikheev *et al.*, 1998). In some host fish, *A. foliaceus* is also commonly found in the mouth cavity and under the gill covers (e.g. in pike; personal observation). In extreme infections more than 250 adults and more than 1500 juvenile *Argulus* have been reported from a single fish (Kruger *et al.*, 1983; Northcott *et al.*, 1997). Such heavy infections result in severe damage to the integument of the host which leads to high mortality (Walker *et al.*, 2004), but even small numbers of parasites can cause mortality in fish larvae (Poulin, 1999). Infected fish are lethargic, show erratic swimming behaviour and changes in shoal size, and under laboratory conditions an active avoidance of parasitized conspecifics was shown in sticklebacks (Poulin and FitzGerald, 1989; Dugatkin *et al.*, 1994; Poulin, 1999; Barber *et al.*, 2000).

20.3. Macroscopic and Microscopic Lesions

Argulus feed by penetrating/damaging the integument of the host and feeding on the haemorrhaging fluids (Gresty *et al.*, 1993; Paperna, 1996; Tam and Avenant-Oldewage, 2006). The eversible mandibular coxal processes are effective biting and ripping tools (Fig. 20.2f, h), which are present already in the first larval stage (Møller *et al.*, 2007). The wound made during feeding is effectively sealed by the labrum and labium (Fig. 20.2e), while the musculature in the proboscis sucks the blood into the oral cavity (Gresty *et al.*, 1993; Rushton-Mellor and Boxshall, 1994; Tam and Avenant-Oldewage, 2006). In addition, the pre-oral spine (Fig. 20.2c, d) is used as an 'ice-pick-like tool' to further increase the flow from the wound (personal observation), possibly by injecting lytic substances; no direct toxic effect of the injected fluid has been proven (Shimura, 1983; Shimura and Inoue, 1984). It is important to emphasize that no direct feeding can take place through the spine as it is not directly connected to the digestive system (Swanepoel and Avenant-Oldewage, 1992; Gresty *et al.*, 1993). The feeding causes severe local damage to the host integument, and as the parasites move around on the host, the damaged epithelium is highly prone to secondary infections by bacteria, fungi, etc. (Walker *et al.*, 2004; Boxshall, 2005; Piasecki and Avenant-Oldewage, 2008). The presence of trypsin or peroxidase-secreting glands as they are known from *Lepeophtheirus salmonis* (Tully and Nolan, 2002), has not been confirmed in *Argulus*. A serious effect of an infection with *A. foliaceus* is the spreading of the spring viraemia of carp virus, which is a highly lethal disease causing massive fish death among cyprinids (Ahne, 1985; Walker *et al.*, 2004).

20.4. Pathophysiology

Infected fish are generally weakened and clinical signs include suppression of appetite, anorexia and ultimately growth cessation (Kabata, 1985; Piasecki and Avenant-Oldewage,

2008). Specific changes to the haematological parameters of infected fish include: (i) increased monocyte and granulocyte counts indicating an immune system response; and (ii) after a longer exposure a general decrease in the levels of several other parameters like haemoglobin and haematocrit values, and erythrocyte and leucocyte counts (Tavares-Dias *et al.*, 1999; Piasecki and Avenant-Oldewage, 2008). A specific immune response to *A. foliaceus* antigens was reported in rainbow trout by Ruane *et al.* (1995), and Walker *et al.* (2004) summarized data from other investigations showing increased expression of the interleukin-1 and tumour necrosis factor alpha genes in response to *Argulus* infections. In general, the immune response in the investigated hosts is not as strong as could be expected, hinting at the presence of immunorepressive secretions as described from caligid copepods (Tully and Nolan, 2002). Marshall *et al.* (2008) showed that osmoregulation is directly affected in infected killifish (*Fundulus heteroclitus*) and that the effect is directly related to the amount of tissue damage to the osmoregulatory active tissues. Typical histopathological indications are epithelial hyperplasia/hypertrophy of the wound margins, and damage to the stratum compactum have been reported (Walker *et al.*, 2004). The damage is aggravated by the active moving around on the host by the parasite, creating multiple wounds. Bandilla *et al.* (2006) cross-infected rainbow trout with a bacterium (*Flavobacterium columnare*) and *A. coregoni*, and demonstrated a significantly higher mortality in trout infected with both pathogens than in trout infected with either alone. In general, one of the greatest risks for the host is from secondary infections or pre-existing infections becoming systemic. The role of Argulids as stress inducers was reviewed by Walker *et al.* (2004) and they concluded that only high infection rates induce any detectable stress responses in the hosts.

20.5. Treatment and Control

Many methods to control and treat infections with *Argulus* have been suggested. Methods

to intercept egg laying are probably the most effective and environmentally tenable, and some progress has already been made, for example by placing boards of various colours and at various depths to attract *Argulus* to deposit their eggs. Frequent removal of the boards almost completely eliminated the parasites from ponds, thus stopping the infection (Gault *et al.*, 2002; Harrison *et al.*, 2006). A complete drying out of the pond/basins to kill off deposited eggs is in most cases untenable. The presence of just a handful of gravid females in a large fish pond represents enough reproductive power to restart the parasite infection in the system, and the 'bet-hedging' strategies of the parasites ensures an extended infection period (Mikheev *et al.*, 2001; Fenton and Hudson, 2002; Hakalahti and Valtonen, 2003; Hakalahti *et al.*, 2003, 2004, 2005; Bandilla *et al.*, 2007; Mikheev *et al.*, 2007). Relying only on physical removal and prevention of reinfection is not sufficient, and a combined physical and chemical approach is called for, of course with careful attention to the environmental impact.

Organochlorine and organophosphate pesticides have proved to be effective against *Argulus* infections, and there is a rich literature on this subject (Walker *et al.*, 2004; Piasecki and Avenant-Oldewage, 2008). As an example Tavares-Dias *et al.* (1999) used the chlorinated organophosphate Triclorphon at 0.4 mg/500 l water, while similar chemicals have been used at concentrations of 2.5 mg/l and 0.25 ppm in other cases (Walker *et al.*, 2004; Piasecki and Avenant-Oldewage, 2008). Both groups of chemicals affect the nervous system of the parasite: (i) organochlorines via Na⁺-ion channel activation and subsequent synaptic hyperactivity; and (ii) organophosphates are acetylcholinesterase (AChE) inhibitors causing AChE build up in the synaptic cleft (Niesink *et al.*, 1996) but are highly toxic to humans and some of the commercially available products have been banned in the European Union. Thus their use is generally discouraged (Paperna, 1991, 1996; Piasecki and Avenant-Oldewage, 2008). Plant-derived pyrethroid compounds (Na⁺-ion channel activators) are less toxic to humans (the LD₅₀ is estimated at ca 1 g/kg) but more toxic to aquatic invertebrates and have also been used

with relative success against branchiuran infections at 20–200 ppm (Piasecki and Avenant-Oldewage, 2008). Several other substances with less acute human toxicity have also been applied in branchiuran infection control, for example in-feed treatments with emamectin benzoate (a GABA-receptor binding Cl-channel activator, derived from an actinomycete secondary metabolite) were tested and found to be successful in controlling an infection by *A. coregoni* at a concentration of 50 mg/kg fish by Hakalahti *et al.* (2004). Finally, compounds from the so-called invertebrate developmental inhibitors (IDIs) have proved to be efficient, for example commercially available flea-treatments like Lufenuron and Diflubenzuron. These compounds (benzoyl-phenylureas) are chitin-production/polymerization inhibitors, and have been used in feed (10 mg/kg body weight) or in the water at 15 mg/l to successfully control an *Argulus* infection (Wolfe *et al.*, 2001; personal observation).

20.6. Conclusions and Future Studies

In conclusion, *Argulus* infections rarely cause serious impacts to natural populations of fish. However, they can be severe in farmed fish populations, especially the secondary infections, and the risk of spring viraemia infections are to be taken seriously. It remains questionable to what extent *Argulus* actually cause stress in the fish, but the feeding activity and the damage it causes can be serious. In comparison with other teleost host-parasite systems, the specific host reactions (e.g. of the immune and endocrine systems) as a response to *Argulus* infections, let alone other branchiurans like *Dolops ranarum*, are poorly known. Studies on both hosts and parasites are necessary to unravel the precise cause/effect systems of the interaction, and not just at the individual level, but also at the population level.

Further studies should include a large-scale investigation of the 'natural range' of the three most widely spread *Argulus* species: *A. foliaceus*, *A. japonicus* and *A. coregoni*, for example using haplotype techniques and/or DNA-barcoding to try to determine the

geographic origin and subsequent dispersal of the parasites. A better understanding of the natural range of the parasites is a prerequisite for the prevention of parasitic infections spreading from natural to farmed fish stocks, and vice versa. The need to prevent infection and explore ways to treat infected fish clearly still exists, even if some progress has been made with regards to physical measures to counter infections. Environmentally safe and

sustainable therapies combining both chemical and physical approaches must be investigated further, in order to increase their efficiency. The fact remains that even if *Argulus* are not among the most virulent or economically important parasites, the branchiurans are highly specialized fish parasites with a tremendous reproductive and ecological potential for dispersal and deleterious host impact.

References

- Ahne, W. (1985) *Argulus foliaceus* (L.) and *Piscicola geometra* L. as mechanical vectors of spring viraemia of carp virus (SVCV). *Journal of Fish Diseases* 8, 241–242.
- 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. and Everts, L. (2010) *Argulus japonicus*: sperm transfer by means of a spermatophore on *Carassius auratus* (L.). *Experimental Parasitology* 126, 232–238.
- Avenant-Oldewage, A. and Swanepoel, J.H. (1993) The male reproductive system and mechanism of sperm transfer in *Argulus japonicus* (Crustacea: Branchiura). *Journal of Morphology* 215, 51–63.
- Avenant-Oldewage, A. and van As, J.G. (1990) The digestive system of the fish ectoparasite *Dolops ranarum* (Crustacea: Branchiura). *Journal of Morphology* 204, 103–112.
- Bandilla, M., Hakalahti, T., Hudson, P.J. and Valtonen, E.T. (2004) Aggregation of *Argulus coregoni* (Crustacea: Branchiura) on rainbow trout (*Oncorhynchus mykiss*): a consequence of host susceptibility or exposure? *Parasitology* 130, 169–176.
- Bandilla, M., Valtonen, E.T., Suomalainen, L.-R., Aphalo, P.J. and Hakalahti, T. (2006) A link between ectoparasite infection and susceptibility to bacterial disease in rainbow trout. *International Journal for Parasitology* 36, 987–991.
- Bandilla, M., Hakalahti-Sirén, T. and Valtonen, E.T. (2007) Experimental evidence for a hierarchy of mate- and host-induced cues in a fish ectoparasite, *Argulus coregoni* (Crustacea, Branchiura). *International Journal for Parasitology* 37, 1343–1349.
- Bandilla, M., Hakalahti-Sirén, T. and Valtonen, E.T. (2008) Patterns of host switching in the fish ectoparasite *Argulus coregoni*. *Behavioral Ecology and Sociobiology* 62, 975–982.
- Barber, I., Hoare, D. and Krause, J. (2000) Effects of parasites on fish behaviour: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries* 10, 131–165.
- Boxshall, G.A. (2005) Crustacean parasites: Branchiura. In: Rohde, K. (ed.) *Marine Parasitology*. CSIRO Publishing, Collingwood, Victoria, Australia, pp. 145–147.
- Buchmann, K. and Bresciani, J. (1997) Parasitic infection in pond-reared rainbow trout *Oncorhynchus mykiss* in Denmark. *Diseases of Aquatic Organisms* 28, 125–138.
- Catalano, S.R. and Hutson, K.S. (2010) Harmful parasitic crustaceans infecting wild arripids: a potential threat to southern Australian finfish aquaculture. *Aquaculture* 303, 101–104.
- Clark, F.N. (1902) *Argulus foliaceus*. A contribution to the life history. *Proceedings of the South London Entomological and Natural History Society* 12–21.
- Cressey, R.F. (1972) The genus *Argulus* (Crustacea: Branchiura) of the United States. In: *Biota of Freshwater Ecosystems Identification Manual*, No. 2. 18050 ELD. The Environmental Protection Agency, Washington, DC, pp. 1–14.
- Dugatkin, L.A., FitzGerald, G.J. and Lavoie, J. (1994) Juvenile three-spined sticklebacks avoid parasitized conspecifics. *Environmental Biology of Fishes* 39, 215–218.
- Evans, D.W. and Matthews, M.A. (2000) First record of *Argulus foliaceus* on the European eel in the British Isles. *Journal of Fish Biology* 57, 529–530.
- Fenton, A. and Hudson, P.J. (2002) Optimal infection strategies: should macroparasites hedge their bets? *Oikos* 96, 92–101.

- Fryer, G. (1968) The parasitic Crustacea of African freshwater fishes: their biology and distribution. *Journal of Zoology, London* 156, 45–95.
- Fryer, G. (1969) A new freshwater species of the genus *Dolops* (Crustacea: Branchiura) parasitic on a galaxiid fish of Tasmania – with comments on disjunct distribution patterns in the southern hemisphere. *Australian Journal of Zoology* 17, 49–64.
- Gault, N.F.S., Kilpatrick, D.J. and Stewart, M.T. (2002) Biological control of the fish louse in a rainbow trout fishery. *Journal of Fish Biology* 60, 226–237.
- Gresty, K.A., Boxshall, G.A. and Nagasawa, K. (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* 339, 119–135.
- Grobbe, K. (1908) Beiträge zur Kenntnis des Baues und der systematischen Stellung der Arguliden. *Sitzungsberichte der kaiserlichen Akademie der Wissenschaften in Wien* 117, 191–233.
- Haase, W. (1975) Ultrastruktur und Funktion der Carapaxfelder von *Argulus foliaceus* (L.) (Crustacea, Branchiura). *Zeitschrift für Morphologie der Tiere* 81, 161–189.
- Hakalahti, T. and Valtonen, E.T. (2003) Population structure and recruitment of the ectoparasite *Argulus coregoni* Thorell (Crustacea: Branchiura) on a fish farm. *Parasitology* 127, 79–85.
- Hakalahti, T., Pasternak, A.F. and Valtonen, E.T. (2003) Seasonal dynamics of egg laying and egg-laying strategy of the ectoparasite *Argulus coregoni* (Crustacea: Branchiura). *Parasitology* 128, 655–660.
- Hakalahti, T., Lankinen, Y. and Valtonen, E.T. (2004) 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., Bandilla, M. and Valtonen, E.T. (2005) Delayed transmission of a parasite is compensated by accelerated growth. *Parasitology* 131, 647–656.
- Hakalahti, T., Karvonen, A. and Valtonen, E.T. (2006) Climate warming and disease risks in temperate regions *Argulus coregoni* and *Diplostomum spathaceum* as case studies. *Journal of Helminthology* 80, 93–98.
- Harrison, A.J., Gault, N.F.S. and Dick, J.T.A. (2006) Seasonal and vertical patterns of egg-laying by the freshwater fish louse *Argulus foliaceus* (Crustacea: Branchiura). *Diseases of Aquatic Organisms* 68, 167–173.
- Kabata, Z. (1985) Branchiura. In: *Parasites and Diseases of Fish Cultured in the Tropics*. Taylor and Francis, London, pp. 255–265.
- Kaji, T., Møller, O. S. and Tsukagoshi, A. (2011) A bridge between original and novel states: ontogeny and function of ‘suction discs’ in the Branchiura (Crustacea). *Evolution & Development*, 13, 119–126.
- Kollatsch, D. (1959) Untersuchungen über die Biologie und Ökologie der Karpfenlaus (*Argulus foliaceus* L.). *Zoologische Beiträge* 5, 1–36.
- Kruger, I., van As, J.G. and Saayman, J.E. (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.
- Leydig, F. (1889) Ueber *Argulus foliaceus*. Neue Mittheilung. *Archiv für mikroskopische Anatomie* 33, 1–51.
- Marshall, W.S., Cozzi, R.R.F. and Strapps, C. (2008) Fish louse *Argulus funduli* (Crustacea: Branchiura) ectoparasites of the euryhaline teleost host, *Fundulus heteroclitus*, damage the ion-transport capacity of the opercular epithelium. *Canadian Journal of Zoology* 86, 1252–1258.
- Martin, M.F. (1932) On the morphology and classification of *Argulus* (Crustacea). *Proceedings of the Zoological Society of London* 771–806.
- Meehan, O.L. (1940) A review of the parasitic Crustacea of the genus *Argulus* in the collections of the United States National Museum. *Proceedings of the United States National Museum* 88, 459–522.
- Menezes, J., Ramos, M.A., Pereira, T.G. and da Silva, A.M. (1990) Rainbow trout culture failure in a small lake as a result of massive parasitosis related to careless fish introduction. *Aquaculture* 89, 123–126.
- Mikheev, V.N., Valtonen, E.T. and Rintamäki-Kinnunen, P. (1998) Host searching in *Argulus foliaceus* (L.) (Crustacea: Branchiura): the role of vision and selectivity. *Parasitology* 116, 425–430.
- Mikheev, V.N., Pasternak, A.F., Valtonen, E.T. and Lankinen, Y. (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., Pasternak, A.F. and Valtonen, E.T. (2007) Host specificity of *Argulus coregoni* (Crustacea: Branchiura) increases at maturation. *Parasitology* 134, 1767–1774.
- Møller, O.S. (2009) Branchiura (Crustacea) – Survey of historical literature and taxonomy. *Arthropod Systematics and Phylogeny* 67, 41–55.
- Møller, O.S., Olesen, J. and Waloszek, D. (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.

- Møller, O.S., Olesen, J., Avenant-Oldewage, A., Thomsen, P.F. and Glenner, H. (2008) First maxillae suction discs in Branchiura (Crustacea): development and evolution in light of the first molecular phylogeny of Branchiura, Pentastomida, and other 'Maxillopoda'. *Arthropod Structure and Development* 37, 333–346.
- Monod, T. (1928) Les Argulidés du Musée du Congo. *Revue de Zoologie et de Botanique Africaines* 16, 242–274.
- Niesink, R.J.M., de Vries, J. and Hollinger, M.A. (1996) *Toxicology: Principles and Applications*. CRC Press, Boca Raton, Florida, USA.
- Northcott, S.J., Lyndon, A.R. and Campbell, A.D. (1997) An outbreak of freshwater fish lice, *Argulus foliaceus* (L.), seriously affecting a Scottish stillwater fishery. *Fisheries Management and Ecology* 4, 73–75.
- Paperna, I. (1991) Diseases caused by parasites in the aquaculture of warm water fish. *Annual Review of Fish Diseases* 155–194.
- Paperna, I. (1996) Parasitic crustacea. In: *Parasites, Infections and Diseases of Fishes in Africa. An Update*. CIFA Technical Paper No. 31. Food and Agriculture Organization of the United Nations (FAO), Rome, 220 pp. Available at: <http://www.fao.org/docrep/008/v9551e/V9551E00.htm#TOC> (accessed 28 June 2011).
- Pasternak, A.F., Mikheev, V.N. and Valtonen, E.T. (2004) Growth and development of *Argulus coregoni* (Crustacea: Branchiura) on salmonid and cyprinid hosts. *Diseases of Aquatic Organisms* 58, 203–207.
- Piasecki, W. and Avenant-Oldewage, A. (2008) Diseases caused by crustacea. In: Eiras, J.C., Segner, H., Wahli, T. and Kapoor, B.G. (eds) *Fish Diseases*. Science Publishers, New Hampshire, USA, pp. 1115–1200.
- Poulin, R. (1999) Parasitism and shoal size in juvenile sticklebacks: conflicting selection pressures from different ectoparasites? *Ethology* 105, 959–968.
- Poulin, R. and FitzGerald, G.J. (1989) A possible explanation for the aggregated distribution of *Argulus canadensis* Wilson, 1916 (Crustacea: Branchiura) on juvenile sticklebacks (Gasterosteidae). *Journal of Parasitology* 75, 58–60.
- Ruane, N.M., McCarthy, T.K. and Reilly, P. (1995) Antibody response to crustacean ectoparasites in rainbow trout, *Oncorhynchus mykiss* (Walbaum), immunized with *Argulus foliaceus* L. antigen extract. *Journal of Fish Diseases* 18, 529–537.
- 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. (1994) The genus *Argulus* (Crustacea: Branchiura) in Africa: identification keys. *Systematic Parasitology* 28, 51–63.
- Rushton-Mellor, S.K. and Boxshall, G.A. (1994) The developmental sequence of *Argulus foliaceus* (Crustacea: Branchiura). *Journal of Natural History* 28, 763–785.
- Schlüter, U. (1978) Observations about host attacking by the common fish louse *Argulus foliaceus* L. (Crustacea, Branchiura). *Zoologischer Anzeiger* 200, 85–91.
- Schram, T.A., Iversen, L., Heuch, P.A. and Sterud, E. (2005) *Argulus* sp. (Crustacea: Branchiura) on cod, *Gadus morhua* from Finmark, northern Norway. *Journal of the Marine Biological Association of the United Kingdom* 85, 81–86.
- Shimura, S. (1983) SEM observations on the mouth tube and preoral sting of *Argulus coregoni* Thorell and *Argulus japonicus* Thiele (Crustacea: Branchiura). *Fish Pathology* 18, 151–156.
- Shimura, S. and Inoue, K. (1984) Toxic effects of extract from the mouth-parts of *Argulus coregoni* Thorell (Crustacea: Branchiura). *Bulletin of the Japanese Society of Scientific Fisheries* 50, 729.
- Swanepoel, J.H. and Avenant-Oldewage, A. (1992) Comments on the morphology of the pre-oral spine in *Argulus* (Crustacea: Branchiura). *Journal of Morphology* 212, 155–162.
- Tam, Q. and Avenant-Oldewage, A. (2006) The digestive system of larval *Argulus japonicus* (Branchiura). *Journal of Crustacean Biology* 26, 447–454.
- Tavares-Dias, M., Martins, M.L. and Kronka, S.N. (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., Wootten, R. and Sommerville, C. (2009) The influence of risk factors on the abundance, egg laying habits and impact of *Argulus foliaceus* in stillwater trout fisheries. *Journal of Fish Diseases* 32, 509–519.
- Thatcher, V.E. (1991) Amazon Fish Parasites. *Amazoniana* 11, 263–572.
- Thiele, J. (1904) Beiträge zur Morphologie der Arguliden. *Mitteilungen aus der Zoologischen Sammlung des Museums für Naturkunde Berlin* 2, 5–51.

-
- Tokioka, T. (1936) Larval development and metamorphosis of *Argulus japonicus*. *Memoirs of the College of Science, Kyoto Imperial University, Series B* 12, 93–114.
- Tully, O. and Nolan, D.T. (2002) A review of the population biology and host–parasite interactions of the sea-louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Parasitology* 124, S165–S182.
- Walker, P.D., Flik, G. and Bonga, S.E.W. (2004) The biology of parasites from the genus *Argulus* and a review of the interactions with its host. In: Wiegertjes, G.F. and Flik, G. (eds) *Host–Parasite Interactions*. Garland Science/BIOS Scientific Publishers (Taylor and Francis), Abingdon, Oxon, UK, pp. 107–129.
- Wilson, C.B. (1902) North American parasitic copepods of the family Argulidae, with a bibliography of the group and a systematic review of all known species. *Proceedings of the United States National Museum* 25, 635–742.
- 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, 1–72.
- Wolfe, B.A., Harms, C.A., Groves, J.D. and Loomis, M.R. (2001) Treatment of *Argulus* sp. infestations of river frogs. *Contemporary Topics in Laboratory Animal Science* 40, 35–36.
- Yamaguti, S. (1963) *Parasitic Copepoda and Branchiura of Fishes*. Interscience Publishers, New York.
- Zrzavý, J. (2001) The interrelationships of metazoan parasites: a review of phylum- and higher-level hypotheses from recent morphological and molecular phylogenetic analyses. *Folia Parasitologica* 48, 81–103.

21 *Lernaea cyprinacea* and Related Species

Annemarië Avenant-Oldewage
University of Johannesburg, Johannesburg, South Africa

21.1. Introduction

The lernaeids are commonly known as 'anchor worms', a misleading term for these mesoparasitic crustaceans. The vernacular name is derived from the body shape of the vermiform adult female with its highly metamorphosed thorax which enlarges disproportionately after attachment. The thorax contains the ovaries and bears two conspicuous egg-filled sacs terminally. A minute abdomen and head completes the body arrangement (Figs. 21.1 and 21.2). Adult females reach a length of 12–16 mm without the egg sacs which may add 6 mm to the length.

Larval lernaea occur on the gills but adult females are mostly lodged in the musculature where the epizootics cause unsightly red sores on the host (Fig. 21.3) and, in severe cases or in small fish or fry, cause death of the hosts. Barson *et al.* (2008) reported 100% prevalence (mean intensity of up to 149 parasites per fish) in two *Oreochromis* species in impoundments in the south-eastern lowveld of Zimbabwe.

21.1.1. Host range

Lernaeids occur in freshwater fishes both in natural water systems (Kularatne *et al.*, 1994a;

Barson *et al.*, 2008) and in aquaculture environments. They are notorious killers specifically of small fishes (Woo and Shariff, 1990), and are the cause of great economic loss (Kabata, 1985; Shariff and Roberts, 1989; Hoffman, 1999; Piasecki *et al.*, 2004; Hemaprasanth *et al.*, 2008). They are suspected of transmitting viruses and/or bacteria which result in secondary infections (Noga, 1986; Woo and Shariff, 1990).

Currently 43 valid *Lernaea* species are listed in the World of Copepods database (Walter and Boxshall, 2008). They occur on all continents but the majority of species occur in Africa (Piasecki *et al.*, 2004; Piasecki and Avenant-Oldewage, 2008). *Lernaea cyprinacea* L. has a cosmopolitan distribution, frequently as an introduced parasite, and can infect a variety of hosts (Kabata, 1979; Shariff *et al.*, 1986; Paperna, 1996). For the other species restricted host ranges are reported (Shariff *et al.*, 1986; Paperna, 1996) and they are parasites of freshwater teleosts, specifically cyprinids, but occur also on salmonids and other fishes such as tilapia (Kabata, 1979; Shariff *et al.*, 1986; Paperna, 1996; Robinson and Avenant-Oldewage, 1996; Barson *et al.*, 2008).

Lernaeids have also been recorded on: (i) frogs (*Rana boylei*; Kupferberg *et al.*, 2009); (ii) tadpoles in North America

(Baldauf, 1961; Tidd and Shields, 1963; Kupperberg *et al.*, 2009), South America (Martins and Souza, 1996; Alcalde and Batistoni, 2005) and Asia (Ming, 2001); and (iii) axolotl (Carnavia and Speranza, 2003; Melidone *et al.*, 2004). Furthermore, their copepodids occur on the gills of many freshwater fish species (Shields and Tidd, 1974) and on the gills of *Rana* frogs (Fryer, 1966; Shields and Tidd, 1974).

21.1.2. Life cycle

Lernaea has a direct life cycle, commonly involving a single host. However, Wilson (1917) reported *Lernaea variabilis* copepodids from short-nosed gar (*Lepistomus platostomus*), whereas their adult females occurred on the bluegill (*Lepomis palidus*). Similarly, Fryer (1966) and Thurston (1969) reported *Lernaea barnimiana* and *L. cyprinacea*, respectively, on

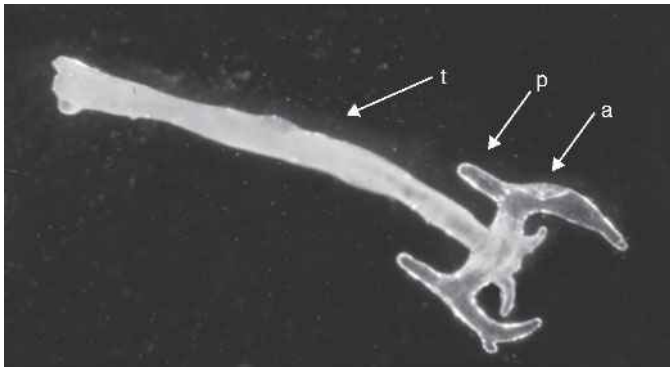


Fig. 21.1. *Lernaea cyprinacea* female after detachment from the host and removal of the host capsule. a, Anterior process of the anchor; t, thorax; p, posterior process of the anchor (outgrowth).

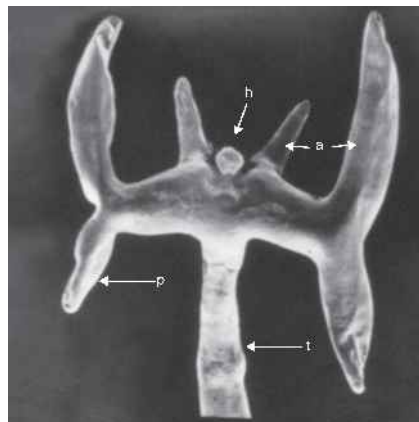


Fig. 21.2. Scanning electron micrograph of *L. cyprinacea* female, anterior part of the body showing the head and anchors. a, Anterior process of the anchor; h, head; t, thorax; p, posterior process of the anchor (outgrowth).



Fig. 21.3. *L. cyprinacea* in situ on *Labeo rosae*, ventral view.

Bagrus, but the adult females on tilapia species.

The life cycle consists of three nauplius stages and five copepodid stages of which the last stage gives rise to male and female cyclopoids (Fig. 21.4). After copulation the males die and females attach permanently to a host (Piasecki and Avenant-Oldewage, 2008). The naupliar stages are free-swimming and non-feeding (Shields and Tidd, 1974). The third stage moults into the first copepodid stage.

Copepodids of both sexes are frequently encountered on the host's gills and apparently feed on epidermal and dermal tissues (Shields and Tidd, 1974; Goodwin, 1999). They are not permanently attached and periods of attachment are interspersed with bouts of energetic swimming in the vicinity of the gill filaments. After insemination, females attach permanently to the host by burrowing with the aid of the mouthparts into the host tissue. This process is further enhanced by the secretion of what appears to be digestive or histolytic enzymes (Shields and Goode, 1978; Shariff and Roberts, 1989). Metamorphosed

females feed on erythrocytes and host tissue debris resulting from the damage they cause while burrowing for attachment (Shariff and Roberts, 1989). They then undergo metamorphosis of the cephalic region to form lateral processes, the anchors (Fig. 21.2), which embed the parasite in soft host tissue, usually in the superficial layer of the skin, although they have also been reported from the gills and the buccal cavity (McNeil, 1961; Fryer, 1966; Ghittino, 1987). The shape of the anchors differs from species to species, and is also affected by the consistency of the surrounding tissue (Fryer, 1968). After attachment the thorax expands disproportionately to form the main part of the parasite body.

In adult females, the anterior end is embedded in host tissue while the thorax and abdomen remain on the surface of the host allowing the parasite access to feeding on host tissue while the eggs are released directly in the environment. Eggs sacks are produced within 4 days after attachment.

In small fishes the parasite frequently penetrates into the internal organs, and this is probably the cause of many deaths.

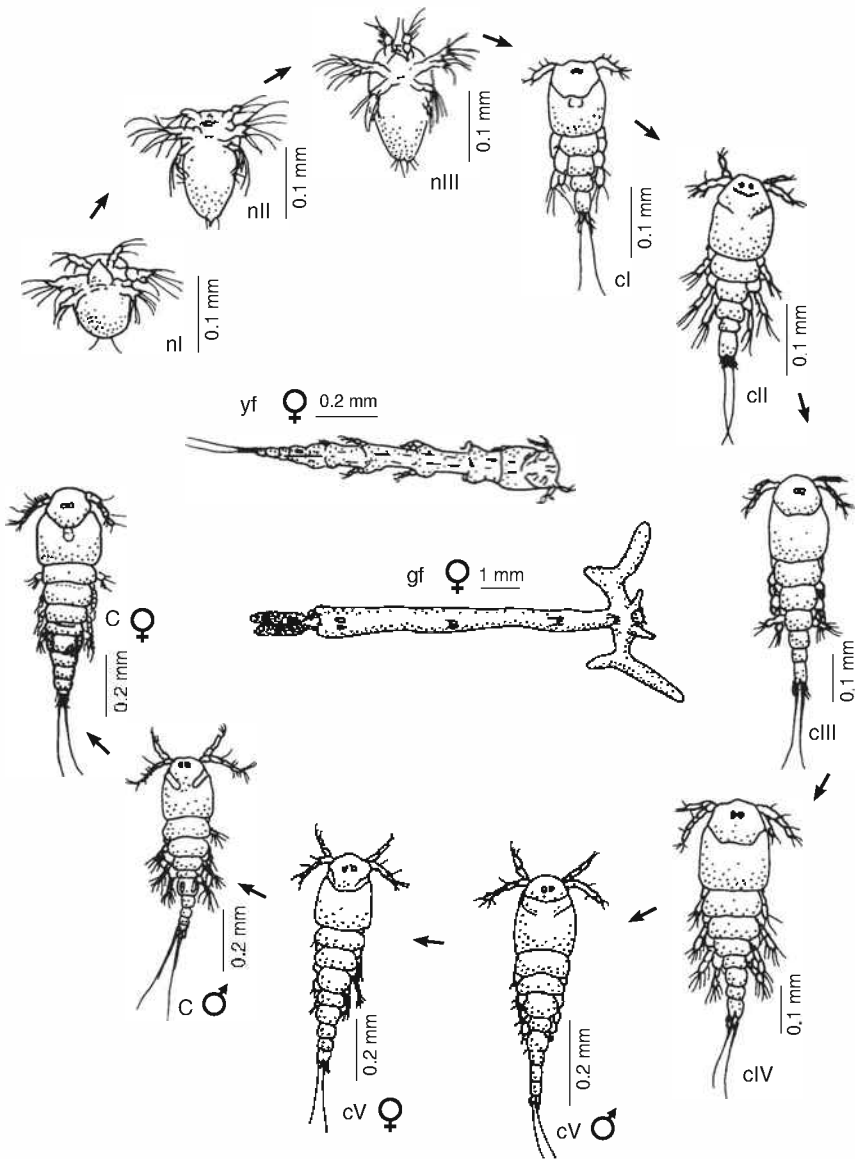


Fig. 21.4. Line drawing of life cycle of *L. cyprinacea*. nl, nauplius I; nII, nauplius II; nIII, nauplius III; cl, copepodite I; cII, copepodite II; cIII, copepodite III; cIV, copepodite IV; cV, copepodite V; C, cyclopoid; yf, young female; gf, gravid female (nl-yf; redrawn from Grabda, 1963; yf, redrawn from Kasahara, 1962).

The development rate of larval *Lernaea* stages depends on temperature, and in temperate regions it has been reported that metamorphosed females overwintered on the hosts (Shields and Tidd, 1968).

21.1.3. Distribution

On the host

Parasites attach to all exterior parts of the host body and also inside the mouth, in the gill

chambers (Noga, 1986; Barson *et al.*, 2008), occasionally on the gill filaments or even in the eye of fishes (Woo and Shariff, 1990) in stagnant or slow-flowing water. In fast-flowing water they are found on protected areas such as behind the fins. Parasite intensity increases in dry seasons due to the reduced volume of the water (Robinson and Avenant-Oldewage, 1996; Manna *et al.*, 1999; Medeiros and Maltchik, 1999) and consequently infection increases as a result of immunosuppression caused by environmental stress (Plaul *et al.*, 2010).

Geographical

Lernaea cyprinacea has a cosmopolitan distribution. However, according to Piasecki *et al.* (2004) and Figueira and Ceccarelli (1991) it was introduced into North and South America and Australia (Lymbery *et al.*, 2010) along with imported cyprinids. In a *Lernaea* outbreak in Arkansas, USA most of the channel catfish (*Ictalurus punctatus*) on a farm where *Hypophthalmichthys nobilis* was present died (Goodwin, 1999). It has spread to many states in the USA. In Bulgaria it became widespread, presumably after human introduction (Daskalov and Georgiev, 2001). Similarly, in Egypt it was reported to infect native Nile tilapia and common carp after the introduction of *Carassius auratus* (Mahmoud *et al.*, 2009), and it was introduced into central and southern Africa (Fryer, 1968; Paperna, 1996; Robinson and Avenant-Oldewage 1996; Boane *et al.*, 2008; Barson *et al.*, 2008). It was also introduced into Brazil (Silva-Souza *et al.*, 2000; Gallo *et al.*, 2007), and Argentina (Vanotti and Tanzola, 2005) where most of the imported cyprinid species became infected.

The occurrence of the parasite is regulated by temperature; in temperate regions it occurs mostly during late summer, the optimal temperature being in the 25–30°C range (Shields and Tidd, 1968; Noga, 1986; Marcogliese, 1991; Hoffman, 1998). It is prevalent in slow-flowing water and therefore intensive culture conditions or manmade lakes are preferred environments (Pérez-Bote, 2010). Temperature affects the rate of development of the larval stages (Shields and Tidd, 1968).

Noga (1986), Tumuli and Shanbhogue (1996a), Gutiérrez-Galindo and Lacasa-Millán

(2005) and Pérez-Bote (2010) found that larger fish were more prone to infection (higher prevalence) and had higher numbers of parasites. Contrary to these reports Tasawar *et al.* (2009), found that *Lernaea* was significantly more prevalent on *Ctenopharyngodon idella* smaller than 15 cm with a mixed infection containing four *Lernaea* species.

21.1.4. Impact on production

Infected fishes had a significantly lower condition factor than non-parasitized fishes and the haematocrit value was also lower (Kabata, 1985; Pérez-Bote, 2010). As few as six parasites can cause the death of a fingerling (Daskalov *et al.*, 1999).

21.2. Diagnosis of the Infection

21.2.1. Host behaviour

Only 4 days post-infection with *L. polymorpha*, naïve fish displayed swift, agitated movements, interspersed with periods of resting. Soon thereafter they rubbed their bodies against the gravel substrate or even against other fish in the tank (Shields and Goode, 1978; Woo and Shariff, 1990). In fish with severe parasitaemia movement became sluggish and mortality occurred (Shariff and Roberts, 1989; Tumuli and Shanbhogue, 1996a). Similar behaviour was reported in *Helostoma temminckii* infected by *L. cyprinacea* (Woo and Shariff, 1990).

21.2.2. Clinical signs

Adult female parasites can be observed macroscopically and are surrounded by a haemorrhagic area on the skin (Fig. 21.3). The parasite extends out from the wound and it is not unusual to observe two egg sacs attached to the posterior end of the parasite (Fig. 21.4gf). An area of up to 1 cm in diameter surrounding the parasite is red and inflamed. Lesions without parasites are also common (Berry *et al.*, 1991) (and see Fig. 21.3).

Larval (copepodid) infections occur on the gills and skin. The larvae are small (0.6 mm) and can be observed only with a dissection microscope and may therefore go unnoticed. Infected fish may display respiratory difficulty (Kabata, 1985).

21.3. External/Internal Lesions (Macroscopic and Microscopic)

21.3.1. Larvae

Larvae (copepodids) do not permanently attach to the gills, but cause disruption and necrosis, and even the death of the host (Khalifa and Post, 1976). Copepodids in high intensities on the gills of *I. punctatus* resulted in epithelial hyperplasia, telangiectasis, haemorrhage and death (Goodwin, 1999).

21.3.2. Adult

In naïve fish adult females penetrate the host at an angle by sliding between overlapping scales (Shariff and Roberts, 1989). They penetrate via the epidermis to the dermis, causing necrosis and punctuate haemorrhages measuring up to 5 mm in diameter (Khalifa and Post, 1976). These lesions are detectable by the naked eye (Fig. 21.3) and, in *L. polymorpha*, they are visible 8–24 h after metamorphosis of the cyclopoid stage (Shariff and Roberts, 1989). Haemorrhage occurs when the female's head penetrates the host tissue, which is followed by an acute inflammatory response in the immediate surrounding area (Joy and Jones, 1973). Haemorrhaging also occurs along the path of entry, under the scales, between muscle bands and below the scales, resulting in pockets of subepithelial erythrocytes and large aggregations of melanin within the dermal layer. In *L. polymorpha* granulosomes (mellanosomes) are released to the surface (Shariff and Roberts, 1989).

Necrosis of the host's muscles occurs at the anterior end of the parasite which is surrounded by infiltrating leucocytes and giant cells (Daskalov *et al.*, 1999). Blood from sev-

ered blood vessels may ooze into the water behind the parasite. Behind the head, epidermal cells form an irregular cumulus in an apparent attempt to seal the lesion off from the environment (Shariff and Roberts, 1989).

Acute inflammation sets in, blood vessels become congested with leukocytes and oedematous swelling of the surrounding tissue occurs. Myofibres adjacent to the parasite anchors show necrosis of the sarcoplasm.

Approximately 3 days after infection, leucocytes and monocytes, interspersed with exudates, are present at the sites of penetration and the point of entry becomes blocked by a nodule resulting from inflammatory exudates. An increase in vascularization of the area occurs. At 5 days post-infection, degeneration of the inflammatory cells occurs, damaged muscle fibres start to degenerate, the fragmented dermis thickens, and a mesh of collagen forms adjacent to the inserted parasite head and anchors. Ten days after infection mononuclear and club cells are abundant and spongiosis is present. At 3 weeks after attachment eosinophilic granule cells (ECGs) and cells resembling lymphocytes are reported in *Micropterus salmoides* infected with *L. polymorpha* (Noga, 1986; Shariff and Roberts, 1989).

Chronic inflammation results in a layer of vascular chronic granulomatous fibrosis that encapsulates the part of the parasite embedded in the fish and even extends out from the fish to form a collar (Khalifa and Post, 1976; Shields and Goode, 1978; Berry *et al.*, 1991). The capsule is more prominent towards the anterior horns of the anchor (Shariff and Roberts, 1989). Blindness resulted when the eyes were infected (Uzman and Rayner, 1958; Shariff, 1981).

In immune fish lesions differ markedly: the epidermal breach is relatively small, but extensive haemorrhaging occurs below the epidermis and around the scale beds. The epidermis around the edges of the lesion is thickened and spongiotic with many ECGs and lymphocytes. The dermis is oedematous with distended blood vessels with ECGs with lymphocytes around them (Noga, 1986; Shariff and Roberts, 1989). Noga (1986) observed remnants of recently

metamorphosed *Lernaea cruciata* females in the lesions and the wounds were secondarily infected with *Aeromonas* bacteria and fungi.

In small fish the anchor of the parasite frequently extends into the internal organs and the traumatic damage to vital organs results in death (Otte, 1965; Khalifa and Post, 1976; Shariff and Roberts, 1989).

Manual removal of the parasite is complicated by the collar and frequently the parasite breaks when an attempt is made to pull it from the host. Removal is more successful when the scale anterior to the parasite is lifted or removed and the parasite is then pulled by the neck, dislodging both parasite and collar. The collar should be removed, preferably prior to fixation, because the shape of the anchors is an important taxonomic feature. Remove the collar by inserting two Dumont tweezers into the opening of the collar; pull in opposite directions to tear the collar and thereby release the parasite undamaged.

21.4. Pathophysiology

Kurovskaya (1984) reported that the weight and size of infected carp fry was not affected by lernaeosis, although alkaline phosphatase activity was reduced and the activities of amylase and protease increased, indicating that parasites affect the fish's nutritional status. Various other researchers reported weight loss. Infected fishes had a significantly lower condition factor than non-parasitized fishes (Kabata, 1985; Faisal *et al.*, 1988; Pérez-Bote, 2010) and Shariff and Sommerville (1986) noted that infested carp were up to 35% lighter. In infected fish the haematocrit count is lower and fish may display respiratory difficulty (Kabata, 1985). Furthermore, Silva-Souza *et al.* (2000) indicated that the haematocrit displayed intense lymphocytopenia and neutrophilia as well as a very high number of immature leucocytes.

Parasites cause open wounds, allowing opportunistic microbial infections (Noga, 1986). They also cause fluid, protein and ion losses, due to disruption of the host integument and the difference in osmotic pressure

between the fish and the surrounding water. Even though the epidermal cells form a collar, a complete cover is not achieved due to constant movement of the distal parts of the parasite's body and the inflammatory exudate is therefore constantly exposed to the environment.

21.4.1. Host immune response

Silva-Souza *et al.* (2000) reported lymphocytopenia and a significant increase in neutrophils in *Schizodon intermedium* both with lesions and infected by *Lernaea*.

Lesions on immune fish were very different from those on naïve fish. In naïve *L. polymorpha* infection in *Aristichthys nobilis* the epidermis had a relatively small opening, but the underlying tissue exhibited very extensive haemorrhaging. The edges of the ulcer were greatly thickened and spongiotic, with an infiltration of EGCs and lymphocytes, distended blood vessels and oedematous dermis (Shariff and Roberts, 1989). In the later stages of infection a reduction in the number of parasites occurred, probably due to a cellular response (Shields and Goode, 1978; Noga, 1986; Shariff and Roberts, 1989; Woo and Shariff, 1990). In recovered fish the host rejects the copepods indicating a protective immunity due to an anamnestic response elicited from memory cells as observed in recovered *Helostoma temminckii* (Woo and Shariff, 1990). The protection was complete in some recovered fish if the challenge dose was low. However, if the dose was high the fish were still susceptible to infection. Furthermore, the fecundity of the parasites was suppressed presumably due to immunological starvation of parasites and those on recovered fish lost more egg sacks and the eggs did not hatch or were non-infective even to naïve fish (Woo and Shariff, 1990). Lesions contained remnants of recently metamorphosed females (Noga, 1986).

Protective immunity was not observed in *Puntius gonionotus* infected by *Lernaea minuta*, this being attributed to the fact that the pathology in this species is less severe (Kularatne *et al.*, 1994b).

21.5. Protective/Control Strategies

Inorganic chemicals and/or toxic organophosphates are still used to treat lernaeciosis, but these have severe effects on the environment as they are non-specific, kill non-target organisms, and cause residues that potentially affect human health – Ghittino (1987) discontinued treatment at least 1 month before eels treated with organophosphates were prepared for marketing. The primary mechanism of action of organophosphate pesticides is inhibition of carboxyl ester hydrolases, particularly acetylcholinesterase. Effective elimination of the embedded lernaed females (from a pond) usually requires treatment over a period of time to disrupt the life cycle since embedded parasites are mostly not susceptible to treatment.

However, it is possible to eradicate copepodite stages prior to attachment. Treatment of ponds with organophosphate insecticides are successful particularly trichlorphon such as Dipterex, Nevugon and Masoten at 0.25 ppm. Treatments should be repeated to coincide with the duration of larval metamorphosis, which is temperature dependent. Recommended intervals for the treatment of *L. cyprinacea* copepodites are: 12 days at 20°C, 9 days at 25°C, 7 days at 30°C and 5 days at 35°C. Below 20°C, monthly treatment suffices (Sarig, 1971; Paperna, 1996) and should be repeated until all females have died. Trichlorphon at 0.25 ppm kills the copepod stages but not the nauplii or adults (Kabata, 1985) whereas Bromex (dimethyl-1,2-bromo-2,2-dichloroethylphosphate) at 0.12–0.15 ppm kills nauplii and copepodids (Sarig, 1971). Malathion at 0.01–0.02% repeated three times with 10 days intervals successfully killed lernaecids on a farm (Manal *et al.*, 1995).

To eradicate adult females Shariff *et al.* (1986) recommend the use of the organophosphate insecticides Dipterex (trichlorphon) (0.16 ppm) and Unden (2-isopropoxyphenyl-N-methylcarbamate) at a dosage of 0.16 ppm with weekly intervals for 5 weeks, because both are biodegradable. However, fish treated with Dipterex tend to fast for the full period of treatment, with a resultant effect on their condition. Furthermore, after the fourth treatment copepodids also became resistant to Unden.

The insecticide Dimilin® (Philips-Dupar, Netherlands; UniRoyal Chemical, USA), an insect growth regulator, is effective against adult females at concentrations of 0.03–0.05 ppm (Hoffman and Lester, 1987). This insecticide has not been approved for use with food fish. Also, its degradation in the environment is slow, and contaminated water should not be released until at least 30 days after treatment.

The organochlorine chloroquine Lindane, another insecticide, also known as gamma-hexachlorocyclohexane (HCH) and benzene hexachloride (BHC), has been used at 10 ppm for 72–90 h every 2 weeks to eradicate *Lernaea* with varying success (McNeil, 1961). This insecticide is not registered for use in fisheries in many countries.

Dipping of fish in a powerful oxidizer, potassium permanganate (KMnO₄) at 20–25 ppm for 2–3 h, or the application of an 8 ppm concentration to ponds, effectively kills attached female lernaecids (Sarig, 1971; Kabata, 1985; Faisal *et al.*, 1988; Vulpe *et al.*, 2000) but the fish become severely distressed and the eggs and free-living stages remain viable (Tamuli and Shanbhogue, 1996a). Great caution should be exercised because the effective concentrations are very close to toxic levels (safety index 1.7–2.0). The treatment is suitable only for fish of over 25 g, and tolerance will vary with species. Increased aeration of the ponds is suggested as KMnO₄ reduces the oxygen-binding potential of water. Tamuli and Shanbhogue (1996b) found that brushing concentrated KMnO₄ onto each individual was less stressful for the fish but killed female *Lernaea* effectively. Alternatively, clipping the female parasites off the fish is very effective.

Sodium percarbonate, at 100 mg/l, is effective against *L. cyprinacea* (Pavlov and Nikolov, 2007).

Doramectin (Dectomax; Pfizer) a chloride channel activator affecting the nervous system and a fermentation-derived endectocidal agent of the avermectin class, in pelleted feed at 1 mg/kg body weight cured young *Labeo fimbriatus* fish and fingerlings of *L. cyprinacea* within 18 days, as opposed to 42 days for untreated fish. A decrease in number of eggs per egg sac was observed. The treatment had the additional benefit that wound

healing was augmented (Hemaprasanth *et al.*, 2008). However, the safety testing of this drug in aquatic organisms has not been completed and it was previously reported to cause the death of fish (Palmer *et al.*, 1997; Katoch *et al.*, 2003) and other sediment-dwelling organisms (Davies *et al.*, 1997, 1998).

Sodium chlorite is a non-residual alternative (Dempster *et al.*, 1988). When applied at a concentration of 20–40 mg/l at a pH above 6 the chlorite killed *L. cyprinacea* from a commercial aquarium, but at the same time killed the bacteria in the biological filter. Therefore, the water needs to be exchanged for at least 2 weeks after treatments to reduce the ammonia and nitrite levels until the chlorite-resistant bacteria in the filters recuperate to become biologically active again. Herbal remedies are discussed by Kabata (1985). Furthermore, Toro *et al.* (2003) recently found steamed *Pinus* resin fractions were effective treatment of lernaeciosis in *Leptorinus piau*.

21.5.1. Biological control

The piscine immune system is well developed, plays a vital role in controlling diseases and can be exploited against pathogens. Woo and Shariff (1990) reported that only 50% of the eggs produced by *Lernaea* from recovered hosts were viable, whereas 100% of the eggs from naïve hosts hatched, indicating a reduction in parasite fecundity, probably due to immunological lesion starvation, which would also affect parasite longevity. Noga (1986) reported that only 2% of lesions contained visible females while the remainder of lesions contained remnants of dead *L. cruciata* parasites. If no naïve fish are introduced into a pond, there will, after a period of time, be no infective larvae and the system will be safe for restocking. In this regard, Shields (1978) recommended increasing the frequency of water changes, while Shariff and Sommerville (1986) suggested that at 25–29°C all fish should be removed from a pond for a minimum of 7–9 days as this would cause all nauplii and copepodids to die.

Kabata (1985) found that the copepod *Mesocyclops* feeds on free-swimming larvae

and suggested predation as an alternative treatment. It was also observed that goldfish removed maturing parasites from each other (Shields, 1978) and tilapia (*Oreochromis mossambicus*) effectively reduce the number of parasites in tanks where *Catla catla* with *Lernaea* occurred (Tamuli and Shanbhogue, 1995). Ashraf *et al.* (2008) reported that an increase in vitamin C in the diet of the fish reduced the parasite numbers.

21.6. Conclusions and Suggestions for Future Studies

It is well documented that the immune response effectively reduces the number of eggs produced as well as the viability of the eggs, therefore the possibility of vaccination should be addressed in future studies. Crude parasite products have been used against other crustacean parasites with a fair amount of success and this should be tested against *Lernaea* too.

Protection is, however, not complete and that aspect should receive attention too. In this regard rotational farming practices should be considered where pond utilization is rotated between three to four ponds to include a period where each pond will be devoid of fishes. The effect will be that eggs will hatch in fish-free ponds and starvation of larvae will occur. Fish should be returned to the pond before all parasites have died so that fish will receive an immunological challenge, which will provide immunological protection against disastrous parasite outbreaks.

Environmental stressors appear to have an effect on parasitaemia (Avenant-Oldewage, 2003; Almeida *et al.*, 2008) and it seems as if some pollutants increase the intensity of parasites, probably due to the stress they induce on the hosts' immune response. Therefore, the effect of pollutants should be evaluated when studying immunity. Furthermore, the effect of global warming, which would affect the rate of completion of the life cycle, should be considered. Preliminary results have shown that global warming may be responsible for an increase in *Lernaea* parasitaemia (Kupferberg *et al.*, 2009).

Mechanical removal of parasites appears to be effective and the application of this technique on large-scale operations should be evaluated. It may be sufficient to harm the parasite in a treatment plant just enough to elicit the effect that was obtained by Tamuli and Shanbhogue (1996b) who clipped the parasites – a practice

which would have serious manpower implications.

Kabata's (1985) suggestion of using *Mesocyclops* for biological control could also be investigated further. Biological control seldom represents complete eradication and so would allow resistance to develop while preventing disastrous outbreaks.

References

- Alcalde, L. and Batistoni, P. (2005) *Hyla pulchella cordobae* (Cordoba treefrog). *Herpetological Review* 36, 302.
- Almeida, D., Almodóvar, A., Nicola, G.C. and Elvira, B. (2008) Fluctuating asymmetry, abnormalities and parasitism as indicators of environmental stress in cultured stocks of goldfish and carp. *Aquaculture* 279, 120–125.
- Ashraf, M., Ayub, M. and Rauf, A. (2008) Effect of vitamin C on growth, survival and resistance to *Lernaea* infection in mrigal (*Cirrhinus mrigala*) fingerlings. *Pakistan Journal of Zoology* 40, 165–170.
- Avenant-Oldewage, A. (2003) *Lamproglana* and *Lernaea* as possible indicators of environmental deterioration in a river in South Africa. *Journal of the South African Veterinary Association* 74, 96.
- Baldauf, R.J. (1961) Another case of parasitic copepods in amphibians. *Journal of Parasitology* 47, 195.
- Barson, M., Mulonga, A. and Nhiwatiwa, T. (2008) Investigation of a parasitic outbreak of *Lernaea cyprinacea* Linnaeus (Crustacea: Copepoda) in fish from Zimbabwe. *African Zoology* 43, 175–183.
- Berry, C.R., Babey, G.J. and Shrader, T. (1991) Effect of *Lernaea cyprinacea* (Crustacea: Copepoda) on stocked rainbow trout (*Oncorhynchus mykiss*). *Journal of Wildlife Diseases* 27, 206–213.
- Boane, C., Cruz, C. and Saraiva, A. (2008) Metazoan parasites of *Cyprinus carpio* L. (Cyprinidae) from Mozambique. *Aquaculture* 284, 59–61.
- Carnevia, D. and Speranza, G. (2003) First report of *Lernaea cyprinacea* L., 1758 in Uruguay, introduced by goldfish, *Carassius auratus* (L., 1758) and affecting axolotl *Ambystoma mexicanum* (Shaw, 1798). *Bulletin of the European Association of Fish Pathologists* 23, 255–256.
- Daskalov, C. and Georgiev, L. (2001) Research on diagnosis, therapy and prophylaxis of lernaecosis on carp. *Zhivotnov dni Nauki* 38, 50–52.
- Daskalov, H., Stoikov, D. and Grozeva, N. (1999) A preliminary hygienic view in case of lernaecosis in the common carp (*Cyprinus carpio* L.) based on clinical and pathomorphological observations. *Bulgarian Journal of Veterinary Medicine* 2, 59–64.
- Davies, I.M., McHenery, J.G. and Rae, G.H. (1997) Environmental risk from dissolved ivermectin to marine organisms. *Aquaculture* 158, 263–275.
- Davies, I.M., Gillibrand, P.A., McHenery, J.G. and Rae, G.H. (1998) Environmental risk of ivermectin to sediment dwelling organisms. *Aquaculture* 163, 29–46.
- Dempster, R.P., Morales, P. and Glennon, F.X. (1988) Use of sodium chlorite to combat anchor worm infestation of fish. *Progressive Fish-Culturist* 50, 51–55.
- Faisal, M., Easa, M.el-S., Shalaby, S.I. and Ibrahim, M.M. (1988) Epizootics of *Lernaea cyprinacea* (Copepoda: Lernaeidae) in imported cyprinids to Egypt. *Tropenlandwirt*, 89, 131–141.
- Figueira, L.B. and Ceccarelli, P.S. (1991) Observações sobre a presença de ectoparasitas em pisciculturas de interior (CEPTA e Região). *Boletim Técnico do CEPTA* 4, 57–65.
- Fryer, G. (1966) Habitat selection and gregarious behavior in parasitic crustaceans. *Crustaceana* 10, 199–209.
- Fryer, G. (1968) The parasitic Crustacea of African freshwater fishes, their biology and distribution. *Journal of Zoology (London)* 156, 45–95.
- Gallio, M., da Silva, A.S. and Moteiro, S.G. (2007) Parasitismo por *Lernaea cyprinacea* em *Astyanax bimaculatus* provenientes de um acude no município de Antonio Prado, Rio Grande do Sul. *Acta Scientiae Veterinariae* 35, 209–212.

- Ghittino, C. (1987) Positive control of buccal lernaeosis in eel farming. *Revista Italiana di Piscicoltura alttiopatologia* 22, 26–29.
- Goodwin, A.E. (1999) Massive *Lernaea cyprinacea* infestation damaging the gills of channel catfish polyculture with bighead carp. *Journal of Aquatic Animal Health* 11, 406–408.
- Grabda, J. (1963) Life cycle and morphogenesis of *Lernaea cyprinacea* L. *Acta Parasitologica Polonica* 11, 169–198.
- Gutiérrez-Galindo, J.F. and Lacasa-Millán, M.I. (2005) Population dynamics of *Lernaea cyprinacea* (Crustacea: Copepoda) on four cyprinid species. *Diseases of Aquatic Organisms* 67, 111–114.
- Hemaprasanth, K.P., Raghavendra, A., Sing, R., Sridhar, N. and Raghunath, M.R. (2008) Efficacy of doramectin against natural and experimental infections of *Lernaea cyprinacea* in carps. *Veterinary Parasitology* 156, 261–269.
- Hoffman, G.L. (1998) *Parasites of North American Freshwater Fishes*. Cornell University Press, Ithaca, New York, USA.
- Hoffman, G. (1999) *Parasites of North American Freshwater Fishes*, 2nd edn. University of California Press, Berkeley, California, USA.
- Hoffman, G.L. and Lester, R.J.G. (1987) Workshop 4F: crustacean parasites of fish. *International Journal for Parasitology* 17, 1030–1031.
- Joy, J.E. and Jones, L.P. (1973) Observations on the inflammatory response within the dermis of a white bass, *Morone chrysops* (Rafinesque), infected with *Lernaea cruciata* (Copepoda: Caligidea). *Journal of Fish Biology* 5, 21–23.
- Kabata, Z. (1979) *Parasitic Copepoda of British Fishes*. The Ray Society, London, UK.
- Kabata, Z. (1985) *Parasites and Diseases of Fish Cultured in Tropics*. Taylor and Francis, London, 302 pp.
- Khalifa, K.A. and Post, G. (1976) Histopathological effect of *Lernaea cyprinacea* (a copepod parasite) on fish. *The Progressive Fish Culturist* 38, 110–113.
- 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.
- Katoch, R., Khurana, S.R., Chatterjee, S., Telang, R.S. and Agnihotri, R.K. (2003) Ivermectin toxicity in fish: a controlled trial. *Journal of Veterinary Parasitology* 17, 79–80.
- Kularatne, M., Shariff, M. and Subasinghe, R.P. (1994a) Comparison of larval morphometrics of *Lernaea minuta*, a copepod parasite of *Puntius gonionotus* from Malaysia, with those of *L. cyprinacea* and *L. polymorpha*. *Crustaceana* 67, 288–295.
- Kularatne, M., Subasinghe, R.P. and Shariff, M. (1994b) Investigations on the lack of acquired immunity by the Javanese carp, *Puntius gonionotus* (Bleeker), against the crustacean parasite, *Lernaea minuta* (Kuang). *Fish and Shellfish Immunology* 4, 107–114.
- Kuperberg, S.J., Catenazzi, A., Lunde, K., Lind, A.J. and Palen, W.J. (2009) Parasitic Copepod (*Lernaea cyprinacea*) outbreaks in foothill yellow-legged frogs (*Rana boylei*) linked to unusually warm summers and amphibian malformations in northern California. *Copeia* 3, 529–537.
- Kurovskaya, L.Y. (1984) The influence of parasite infection of carp fry on the activity of their intestinal enzymes. *Sbornik Nauchnykh Trudov Vsesoyuznogo Nauchno-Issledovatel'skogo Instituta Prudovogo Rybnogo Khozyaistva* 40, 68–73.
- Lymbery, A.J., Hassan, M., Morgan, D.L., Beatty, S.L. and Doupé, R.G. (2010) Parasites of native and exotic freshwater fishes in south-western Australia. *Journal of Fish Biology* 76, 1770–1785.
- Mahmoud, M.A., Aly, S.M., Diab, A.S. and John, G. (2009) The role of ornamental goldfish *Carassius auratus* in transfer of some viruses and ectoparasites to cultured fish in Egypt: comparative ultrapathological studies. *African Journal of Aquatic Science* 34, 111–121.
- Manal, E.M.A.A., Oifatt, M.A. and El, S.E.M. (1995) Lernaeosis outbreak in cultured freshwater fish fingerlings at Kafr El Sheikh governorate, Egypt. *Egyptian Journal of Comparative Pathology and Clinical Pathology* 8, 109–121.
- Manna, A.K., Paria, T. and Ghosh, S. (1999) Incidence and intensity of anchor worm *Lernaea* infection on the Asiatic rice fish *Oryzias metastigma*. *Environment and Ecology* 17, 73–75.
- Marcogliese, D.J. (1991) Occurrence of *Lernaea cyprinacea* in fishes in Belews Lake, North Carolina. *The Journal of Parasitology* 77, 326–327.
- Martins, M.L. and Souza, F.L.D. (1996) Experimental infestations of *Rana catesbeiana* Shaw tadpoles by copepodids *Lernaea cyprinacea* Linnaeus (Copepoda, Lernaeidae). *Revista Brasileira de Zoologia* 12, 619–625.
- McNeil, P.L., Jr (1961) The use of benzene hexachloride as a copepodicide and some observations on lernaeal parasites in trout rearing units. *Progressive Fish-Culturist* 23, 127–133.

- Medeiros, S.F. and Maltchik, L. (1999) The effects of hydrological disturbance on the intensity of infestation of *Lernaea cyprinacea* in an intermittent stream fish community. *Journal of Arid Environments* 43, 351–356.
- Melidone, R., Chow, J.A. and Principato, M. (2004) *Lernaea cyprinacea* infestation in an axolotl. *Exotic DVM* 6, 24–25.
- Ming, L.T. (2001) Parasitic copepods responsible for limb abnormalities? *Froglog* 46, 3.
- Noga, E.J. (1986) The importance of *Lernaea cruciata* (Le Sueur) in the initiation of skin lesions in large-mouth bass, *Micropterus salmoides* (Lacépède), in Chowan River, North Carolina, USA. *Journal of Fish Diseases* 9, 295–302.
- Otte, E. (1965) An observed heavy infection of rainbow trout (Osteichthyes) with a previously unknown *Lernaea* species, new species *Lernaea minima* (Copepoda). *Wien Tierärztliche Monatschrift* 5, 21–25.
- Palmer, R., Coyne, R., Davey, S. and Smith, P. (1997) Case notes on adverse reaction associated with ivermectin therapy of Atlantic salmon. *Bulletin of the European Association of Fish Pathologists* 17, 62–67.
- Paperna, I. (1996) *Parasites, Infections and Diseases of Fishes in Africa. An Update*. Committee for Inland Fisheries of Africa (CIFA) Technical Paper No. 31. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
- Pavlov, A. and Nikolov, G. (2007) Investigation of sodium percarbonate as a means to fight with *Lernaea cyprinacea* in some freshwater fish. *Journal of Agriculture and Forest Science* 6, 21–23.
- Pérez-Bote, J.L. (2010) *Barbus comizo* infestation by *Lernaea cyprinacea* in the Guadiana River, south-western Spain. *Journal of Applied Ichthyology* 26, 592–595.
- Piasecki, W. and Avenant-Oldewage, A. (2008) Diseases caused by Crustacea. In: Eiras, J. Segner, H., Wahli, T. and Kapoor, B.G. (eds) *Fish Diseases*. Science Publishers, Enfield, New Hampshire, USA, pp. 1115–1200.
- Piasecki, W., Goodwin, A.E., Eiras, J.C. and Nowak, B.F. (2004) Importance of Copepoda in freshwater aquaculture. *Zoological Studies* 43, 193–205.
- Plaul, S.E., Garcia Romero, N. and Barbeito, C.G. (2010) Distribution of the exotic parasite, *Lernaea cyprinacea* (Copepoda, Lernaeidae) in Argentina. *Bulletin of the European Association of Fish Pathologists* 30, 65–73.
- Robinson, J. and Avenant-Oldewage, A. (1996) Aspects of the morphology of the parasitic copepod *Lernaea cyprinacea* Linnaeus, 1758 and notes on its distribution in Africa. *Crustaceana* 69, 610–626.
- Sarig, S., (1971) The prevention and treatment of diseases of warmwater fish under subtropical conditions, with special emphasis on intensive fish farming. T.F.H. Publications Inc., Jersey City, New Jersey, USA.
- Shariff, M. (1981) The histopathology of the eye of big head carp *Aristichthys nobilis* (Richardson) infested with *Lernaea piscinae* Harding, 1950. *Journal of Fish Diseases* 4, 161–168.
- Shariff, M. and Roberts, R.J. (1989) The experimental histopathology of *Lernaea polymorpha* Yu, 1938, infection in naive *Aristichthys nobilis* (Richardson) and a comparison with the lesion in naturally infected clinically resistant fish. *Journal of Fish Diseases* 12, 405–414.
- Shariff, M. and Sommerville, C. (1986) Effects of *Lernaea polymorpha* on the growth of big head carp, *Aristichthys nobilis*. In: International Congress of Parasitology (ICOPA) VI Handbook. Australian Academy of Sciences, Canberra, Abstract no. 598, p. 227.
- Shariff, M., Kabata, Z. and Sommerville, C. (1986) Host susceptibility to *Lernaea cyprinacea* L. and its treatment in a large aquarium system. *Journal of Fish Diseases* 9, 393–401.
- Shields, R.J. (1978) Procedures for the laboratory rearing of *Lernaea cyprinacea* L. (Copepoda). *Crustaceana* 35, 259–264.
- Shields, R.J. and Goode, R.P. (1978) Host rejection of *Lernaea cyprinacea* L. (Copepoda). *Crustaceana* 35, 301–307.
- Shields, R.J. and Tidd, W.M. (1968) Effect of temperature on the development of larval and transformed females of *Lernaea cyprinacea* L. (Lernaeidae). *Crustaceana* 1, 87–95.
- Shields, R.J. and Tidd, W.M. (1974) Site selection on hosts by copepods of *Lernaea cyprinacea* L. (Copepoda). *Crustaceana* 27, 225–230.
- Silva-Souza, A.A., Almeida, S.C. and Machado, P.M. (2000) Effect of the infestation by *Lernaea cyprinacea* Linnaeus, 1758 (Copepoda, Lernaeidae) on the leucocytes of *Schizodon intermedius* Garavello & Britski, 1990 (Osteichthyes, Anostomidae). *Revista Brasileira de Biologia* 60, 217–220.
- Tamuli, K.K. and Shanbhogue, S.L. (1995) Biological control of *Lernaea* L. infection employing *Oreochromis mossambica*, Peters. *Journal of the Assam Science Society* 37, 123–128.

- Tamuli, K.K. and Shanbhogue, S.L. (1996a) Incidence and intensity of anchor worm *Lernaea bhadraensis* infection on cultivated carps. *Environment and Ecology* 14, 282–288.
- Tamuli, K.K. and Shanbhogue, S.L. (1996b) Acquired immunity of Indian major carp *Catla catla* to infection of the anchor worm *Lernaea bhadraensis*. *Environment and Ecology* 14, 518–523.
- Tasawar, Z., Zafar, S., Lashari, M.H. and Hayat, C.S. (2009) The prevalence of lernaeid ectoparasites in grass carp (*Ctenopharyngodon idella*). *Pakistan Veterinary Journal* 29, 95–96.
- Thurston, J.P. (1969) The biology of *Lernaea barnimiana* (Crustacea: Copepoda) from Lake George, Uganda. *Revue de Zoologie et de Botanique Africaines* 60, 15–33.
- Tidd, W.M. and Shields, R.J. (1963) Tissue damage inflicted by *Lernaea cyprinacea* Linnaeus, a copepod parasitic on tadpoles. *Journal of Parasitology* 49, 693–696.
- Toro, R.M., Gessner, A.A.F., Furtado, N.A.J.C., Ceccarelli, P.S., de Albuquerque, S. and Bastos, J.K. (2003) Activity of the *Pinus elliottii* resin compounds against *Lernaea cyprinacea* in vitro. *Veterinary Parasitology* 118, 143–149.
- Uzman, J.R. and Rayner, H.J. (1958) Record of the parasitic copepod *Lernaea cyprinacea* L. in Oregon and Washington fishes. *Journal of Parasitology* 44, 452–453.
- Vanotti, M.D. and Tanzola, R.D. (2005) Relación entre la carga parasitaria total y algunos parámetros hematológicos de *Rhamdia* sap oval. (Pisces) en condiciones naturales. *Biología Acuática* 22, 247–256.
- Vulpe, V., Nastasa, V. and Cura, P. (2000) Studies about the therapeutic modalities in parasites of the fish culture. *Lucrai Stiintifice Medicina Veterinara Universitatea de Stiinte* 43, 376–379.
- Walter, T.C. and Boxshall, G.A. (2008) World of Copepods database. Available at: <http://www.marinespecies.org/copepoda>. Consulted on 2010-08-17 (accessed 17 August 2010).
- Wilson, C.B. (1917) North American parasitic copepods belonging to the lernaeidae with a revision of the entire family. *Proceedings of the US National Museum* 53, 1–150.
- Woo, P.T.K. and Shariff, M. (1990) *Lernaea cyprinacea* L. (Copepoda: Caligidae) in *Helistoma temminckii* Cuvier and Valenciennes: the dynamics of resistance in recovered and naïve fish. *Journal of Fish Diseases* 13, 485–494.

22 *Lepeophtheirus salmonis* and *Caligus rogercresseyi*

John F. Burka, Mark D. Fast and Crawford W. Revie
*Atlantic Veterinary College, University of Prince Edward Island, Charlottetown,
Canada*

22.1. Introduction

Sea lice are parasitic copepods in the order Siphonostomatoida, family Caligidae. There are 36 genera within this family which include approximately 42 *Lepeophtheirus* and 300 *Caligus* species (Walter and Boxshall, 2010). *Lepeophtheirus salmonis* and various *Caligus* species are adapted to salt water and are major ectoparasites of farmed and wild Atlantic salmon (*Salmo salar*), feeding on the mucus, epidermal tissue and blood of host fish. *L. salmonis* is the primary sea louse of concern in the northern hemisphere and much is known about its biology and interactions with its salmon host. *Caligus rogercresseyi* has recently become a significant parasite of concern on salmon farms in Chile (Bravo, 2003) and studies are underway to gain a better understanding of the parasite and the host-parasite interactions. This review will focus on these two species. Recent evidence is also emerging that *L. salmonis* in the Atlantic has sufficient genetic differences from *L. salmonis* from the Pacific, suggesting that Atlantic and Pacific *L. salmonis* may have independently co-evolved with Atlantic and Pacific salmonids, respectively (Yazawa *et al.*, 2008).

22.2. Diversity and Hosts: Sea Lice on Wild Fish

Most of our understanding of the biology of sea lice, other than the early morphological studies, is based on laboratory studies designed to understand issues associated with the parasite infecting fish on salmon farms. Knowledge of sea louse biology and interactions with wild fish is unfortunately sparse and further research is required in these areas.

Many sea lice species are specific to host genera; for example *L. salmonis* has high specificity for salmonids, including the widely farmed Atlantic salmon. *L. salmonis* can parasitize other salmonids to varying degrees, including brown trout (sea trout: *Salmo trutta*), Arctic char (*Salvelinus alpinus*) and all species of Pacific salmon (*Oncorhynchus* spp.). Coho and pink salmon (*Oncorhynchus kisutch* and *Oncorhynchus gorbuscha*, respectively) mount strong tissue responses to attaching *L. salmonis*, which lead to rejection of the parasite within the first week of infection (Wagner *et al.*, 2008). Pacific *L. salmonis* can also develop, but does not appear to complete its life cycle on the three-spined stickleback (*Gasterosteus aculeatus*) (Jones *et al.*, 2006).

While Atlantic *L. salmonis* have also been observed on non-salmonid hosts (Bruno and Stone, 1990; Pert *et al.*, 2006), these interactions do not appear to be as prevalent or as lengthy as those between Pacific *L. salmonis* and the three-spined stickleback.

C. rogercresseyi was originally identified as *Caligus flexispina*, but detailed characterization indicated it was a different species (Boxshall and Bravo, 2000). *C. rogercresseyi* infests a number of native South American marine fishes, including the Patagonian blennie (*Eleginops maclovinus*), the Peruvian silverside smelt (*Odontesthes regia*), the small-eye flounder (*Paralichthys microps*) and the introduced brown trout (*S. trutta*) (Carvajal *et al.*, 1998; Boxshall and Bravo, 2000; Bravo *et al.*, 2006). Farmed Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*), which are now infested with *C. rogercresseyi*, are not indigenous to Chile and originated as parasite-free eggs from North America or Europe. It is apparent that the *C. rogercresseyi* on the introduced salmonids originates from native fish species, particularly those noted above (Carvajal *et al.*, 1998) and confirms that the parasite has a broad host range. Interestingly, introduced coho salmon is not as susceptible to *C. rogercresseyi* as Atlantic salmon (Bravo, 2003).

Temperature, light and currents are major factors that affect the dispersal of the planktonic stages of both *L. salmonis* and *C. rogercresseyi*, and their survival depends on salinity above 25‰ (Costelloe *et al.*, 1998; Genna *et al.*, 2005; Brooks, 2005, 2009; Costello, 2006; Bravo *et al.*, 2008a). It has been hypothesized that *L. salmonis* copepodids migrate upwards towards light and salmon smolt moving downwards at daybreak facilitate host finding (Heuch *et al.*, 1995). Several field and modelling studies have examined copepodid populations in intertidal zones and have shown that the planktonic stages can be transported tens of kilometres from their source by tides and currents (McKibben and Hay, 2004; Costello, 2006). Some adaptation to altered lower salinity can occur: (i) *C. rogercresseyi* from sites where there is a continual inflow of fresh water show better adaptation to low salinity than sea lice from sites with constantly high salinity; and (ii) females have

greater tolerance to lower salinity (20‰) than males (Bravo *et al.*, 2008a).

It has always been a mystery where and how sea lice reside between the time when they fall off the adult salmon and when they attach to the juveniles of the next generation. It is possible sea lice survive on fish that remain in the estuaries or that they transfer to an as yet unknown alternate host to spend the winter. Nonetheless, smolts get infected with sea lice larvae, or even possibly adults, when they enter the estuaries in the spring. As noted above, the anadromous three-spined stickleback can serve as a host for the Pacific *L. salmonis* (Jones *et al.*, 2006) while other hosts, especially in the Atlantic, have not yet been defined. It is also not known how sea lice get from one fish to another in the wild. Adult stages of *Lepeophtheirus* spp. can transfer under laboratory conditions, but the frequency is low (Ritchie, 1997). *Caligus* spp. transfer quite readily and between different species of fish (Oines *et al.*, 2006) as noted above for *C. rogercresseyi* (Carvajal *et al.*, 1998).

22.3. Morphology and Development: Possible Targets for Integrated Pest Management

Sea lice have both free swimming (planktonic) and parasitic life stages. All stages are separated by moults and development is dependent on temperature (Johnson and Albright, 1991a, b; Schram, 1993; González and Carvajal, 2003). The development rate from egg to adult varies with temperature from 19 days (at 17°C), 43 days (at 10°C), to 93 days (at 5°C) for *L. salmonis* (Wadsworth *et al.*, 1998) and 26 days (at 15°C) to 45 days (at 10.3°C) for *C. rogercresseyi* (González and Carvajal, 2003). The life cycle of *L. salmonis* is shown in Fig. 22.1 and anatomical descriptions of the developmental stages, based on Johnson and Albright (1991a) and Schram (1993), are extensively reviewed by Pike and Wadsworth (1999). In contrast to *Lepeophtheirus* species, *C. rogercresseyi* has only eight developmental stages and there is no pre-adult stage with the chalimus going directly to mobile adults (Boxshall and Bravo, 2000).

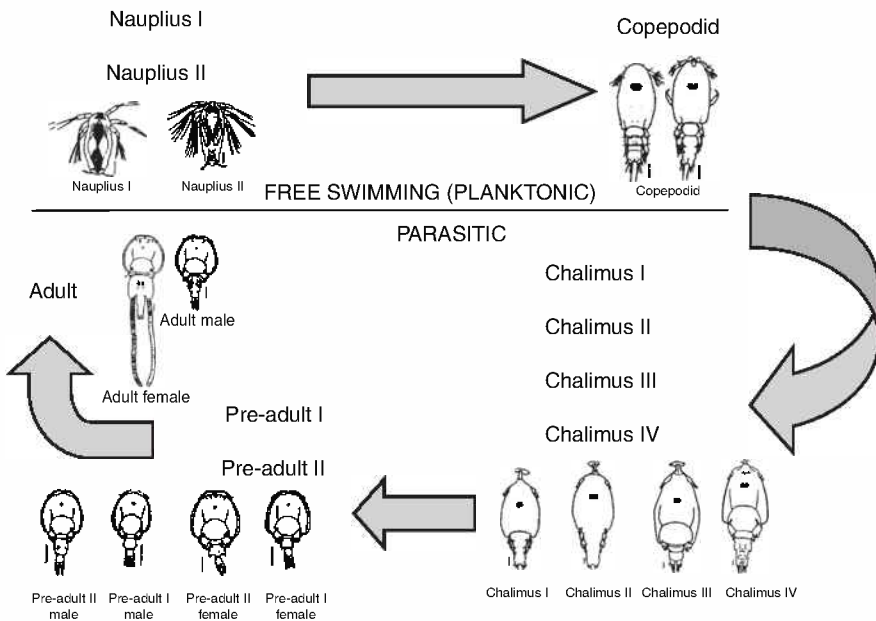


Fig. 22.1. *Lepeophtheirus salmonis* life cycle (adapted from Schram, 1993).

Eggs hatch into nauplius I which moults to a second naupliar stage; both naupliar stages are non-feeding. They depend on yolk reserves for energy, and are adapted for swimming. The copepodid stage is the infectious stage which searches for an appropriate host using chemo- and mechanosensory clues. Receptors on the antennules have been associated with chemoreception (Gresty *et al.*, 1993) and ablation of the distal tips of the antennules reduces host finding as well as mating behaviour (Hull *et al.*, 1998). Semiochemicals, or kairomones, play an integral role for sea lice to identify an appropriate host and avoid non-hosts (Bailey *et al.*, 2006). Two semiochemicals from Atlantic salmon, isophorone and 6-methyl-5-hepten-2-one, attract *L. salmonis* copepodids whereas semiochemicals from a non-host turbot (*Scophthalmus maximus*) does not. Similarly, water conditioned from rainbow trout and Atlantic salmon is attractive to male *C. rogercesseyi*, whereas water conditioned from a non-host blennid (*Hypsoblennius sordidus*) is repulsive (Pino-Marambio *et al.*, 2007). Pheromones released by female sea lice have attractive properties for conspecific males, suggesting

that semiochemical traps could be used in integrated pest management for sea louse control (Ingvarsdottir *et al.*, 2002; Pino-Marambio *et al.*, 2007). Alternative strategies preventing copepodid attachment could also include confounding chemicals (i.e. masking compounds) that block kairomones and pheromones or repellents which could be administered in feed and redistributed to the skin and mucus to deter copepodids from attaching to the host (Mordue and Birkett, 2009).

Water currents, salinity, light and other factors also will assist copepodids in finding a host (Genna *et al.*, 2005). Salinity below 30‰ results in decreased development of *L. salmonis* eggs to the copepodid stage (Johnson and Albright, 1991b). Preferred settlement of copepodids on the fish occurs in areas with the least hydrodynamic disturbance, particularly the fins and other protected areas, and under medium to low light conditions (10–300 lx) (Bron *et al.*, 1991; Genna *et al.*, 2005). Copepodids on a suitable host feed for a period of time prior to moulting to the chalimus I stage. Their development continues through three additional chalimus stages,

each separated by a moult. A characteristic feature of all four chalimus stages of *L. salmonis* and *C. rogercresseyi* is that they are physically attached to the host by their frontal filaments with unique adhesive components (Bron *et al.*, 1991; Johnson and Albright, 1991a; Gonzalez-Alanis *et al.*, 2001; González and Carvajal, 2003). Interference with frontal filament development and/or attachment could be an intervention for sea louse control. Chitin synthesis inhibitors which interfere with moulting are already actively used and are discussed below.

L. salmonis tends to be approximately twice the size of *Caligus* spp. The body lengths of adult male and female *C. rogercresseyi* are approximately 5 mm long (Boxshall and Bravo, 2000) whereas *L. salmonis* adult females are approximately 10 mm long and males 5 mm long (Johnson and Albright, 1991a). Considerable variations have been reported for *L. salmonis* depending on their origin (i.e. wild versus farmed, location and season) (Pike and Wadsworth, 1999). The body consists of the cephalothorax, fourth leg-bearing segment, genital complex and abdomen. The cephalothorax forms a broad shield that includes all of the body segments up to the third leg-bearing segment. It acts like a suction cup in holding the louse on the fish. All species have mouth parts shaped as a siphon or oral cone (characteristic of the Siphonostomatoida). The second antennae and oral appendages are modified to assist in holding the parasite on the fish and are also used by males to grasp the female during copulation (Anstenrud, 1990). The adult females develop a very large genital complex which makes up the majority of the body mass. With the exception of a short period during the moult, the pre-adult and adult stages are mobile on the fish and, in some cases, can move between host fish. Adult females occupy relatively flat body surfaces on the posterior ventral and dorsal midlines and may actually out-compete pre-adults and males at these sites (Todd *et al.*, 2000). Patterns of pair formation and mating have been described for *L. salmonis* (Hull *et al.*, 1998). Newly moulted adult males preferentially mate with virgin adult females > pre-adult II females >> pre-adult I females. Adult

males are more mobile than adult females and display more inter-host transfer.

Two egg strings are produced averaging about 285 eggs per egg string for *L. salmonis* (Heuch *et al.*, 2000) and 29 eggs per egg string for *C. rogercresseyi* (Bravo, 2010) that darken with maturation and are approximately the same length as the female's body. The first egg strings a female produces are always shorter than subsequent strings. One female can produce between six and 11 pairs of egg strings in a lifetime of approximately 7 months (Heuch *et al.*, 2000; Mustafa *et al.*, 2001; Bravo, 2010). Egg strings are longer and contain more eggs in sea lice from areas of lower salinity as well as in the winter, although eggs at colder temperatures are smaller and less viable (Heuch *et al.*, 2000; Bravo *et al.*, 2009). Development of egg strings also takes four times longer at 7°C than at 12°C and the time between extrusion of egg strings doubles in the colder temperature (Heuch *et al.*, 2000). Thus temperature has a direct influence on egg development in both sea lice.

Egg production in *L. salmonis* has become a novel potential therapeutic target in vaccine development (Dalvin *et al.*, 2009). As the adult female matures egg production begins to occur, as indicated by transcription of genes encoding major yolk proteins following post-moulting growth of the abdomen and genital segment (Eichner *et al.*, 2008). Egg development occurs in both inseminated and virgin females. Yolk proteins are essential for embryogenesis and early larval development since the yolk provides the nutrients through to the copepodid stage. A novel yolk-associated protein, LsYAP, which appears to be involved in vitellin formation and utilization, and two major vitellogenins, LsVT1 and LsVT2, have since been characterized (Dalvin *et al.*, 2009, 2011). LsYAP and vitellogenin production takes place in the subcuticular tissue where the proteins are produced and stored before being taken up into the eggs. LsYAP appears to have a critical role in embryogenesis resulting in normal development and survival of nauplii since deformed phenotypes occur in LsYAP RNA interference (RNAi) experiments (Dalvin *et al.*, 2009).

Germ cell and embryonic development is also controlled by a nuclear steroid receptor,

LsRXR1, which is involved in steroidogenesis and fatty acid metabolism (Eichner *et al.*, 2010). This receptor is highly expressed in the ovary, oocytes and oviduct and knockdown experiments indicate that functional LsRXR1 receptors are necessary for egg-string development and successful hatching, moulting and growth, thus affecting larval development. This same research group has also described a unique coagulation factor LsCP1 which resembles vitellogenins (Skern-Mauritzen *et al.*, 2007). LsCP1 is also critical in embryonic patterning and RNAi-induced LsCP1 deficiency reduces larval fitness (Skern-Mauritzen *et al.*, 2010). However, LsCP1 deficiency was not lethal to adult females since it is presumed, as with other organisms, there is considerable redundancy in clotting mechanisms.

Thus, these proteins and pathways could be specific targets for either potential vaccines or drugs. In particular, the egg proteins and vitellogenin-like compounds have so far been exploited in anti-sea lice vaccine development (Ross *et al.*, 2006; Frost *et al.*, 2007).

22.4. Pathophysiology

22.4.1. Feeding habits

Pre-adult and adult sea lice, especially gravid females, are aggressive feeders and, in some cases, feed on blood in addition to tissue and mucus (Fig. 22.2). *L. salmonis* is known to secrete large amounts of trypsin into the

host's mucus which may assist in feeding and digestion (Firth *et al.*, 2000; Wagner *et al.*, 2008). Other compounds, such as prostaglandin E_2 (PGE_2), have also been identified in *L. salmonis* secretions and may assist in feeding and/or serve the parasite in avoiding the immune response of the host by regulating it at the feeding site (Fast *et al.*, 2005; Wagner *et al.*, 2008).

22.4.2. Sea louse–host interactions

Sea lice cause physical and enzymatic damage at their sites of attachment and feeding which results in abrasion-like lesions that vary in their nature and severity depending upon a number of factors. These include: (i) host species; (ii) age; and (iii) general health of the fish. It is not clear whether stressed fish are particularly prone to infestation. Sea lice infection itself causes a generalized chronic stress response in fish since feeding and attachment cause changes in the mucus consistency and damage to the epithelium resulting in loss of blood and fluids, electrolyte changes and cortisol release. This can decrease salmon immune responses and make them susceptible to other diseases and reduces growth and performance (Johnson and Albright, 1992a, b; Ross *et al.*, 2000).

Successful host responses to *L. salmonis* infection have been characterized by hyperplastic and inflammatory responses involving rich neutrophil infiltration at the site of attachment within 24 h followed by significant

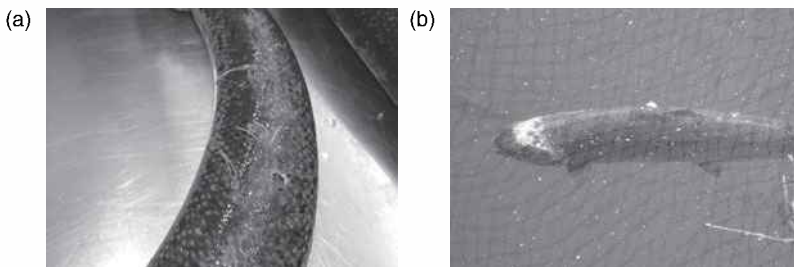


Fig. 22.2. Gravid female *L. salmonis* on Atlantic salmon (*Salmo salar*). (a) Mild infection causing minor abrasion and fluid loss. (b) Severe infection where the lice have eaten through skin and flesh to expose the skull.

decreases in parasite abundance within 72 h (Johnson and Albright, 1992a, b; Fast *et al.*, 2002; Johnson and Fast, 2004). Both within the epidermis/underlying dermis and systemically (i.e. head kidney), strong pro-inflammatory gene stimulation to attached life stages is also observed (Jones *et al.*, 2007). This response has been observed in *Oncorhynchus* spp., such as coho and pink salmon; however, *Salmo* spp. infections are characterized by: (i) little to no hyperplastic response; (ii) delayed neutrophil involvement; (iii) restricted epidermal and systemic pro-inflammatory gene stimulation; and (iv) maintenance of high numbers of parasites (Johnson and Albright, 1992a, b; Fast *et al.*, 2006a, b; Skugor *et al.*, 2008). While localized/systemic pro-inflammatory gene responses in *Oncorhynchus* spp. appear to be maintained throughout infection and to some degree even after rejection, a downregulation of these responses occurs in Atlantic salmon throughout the attached chalimi stages, only to be stimulated again following moulting of the parasite to pre-adults (Fast *et al.*, 2006a, b; Skugor *et al.*, 2008). At this latter point, the parasite has entered a mobile life stage and, despite the significant host response, may exemplify the ineffective nature of immune mechanisms against a moving, external target. Similarly, *Oncorhynchus* spp. maintain high abundances of *L. salmonis* mobile life stages in the wild and have exhibited higher parasite burdens when cohabited with *Salmo* spp. carrying mobile life stages as compared with those with early/attached parasite life stages (Nagasawa *et al.*, 1993; Fast *et al.*, 2002; Beamish *et al.*, 2005). This highlights the importance of the rapidity of the host response to infection and the need to eliminate *L. salmonis* either prior to or shortly after attachment. Within the *Oncorhynchus* spp. greater susceptibility can be induced through injection of cortisol, leading to a delayed/reduced inflammatory response and higher *L. salmonis* burdens in coho salmon and extremely small size upon seawater entry (<0.5 g) in pink salmon (Johnson and Albright, 1992b; Pacific Salmon Forum, 2009).

L. salmonis is known to secrete bioactive compounds, such as trypsin and PGE₂, which may contribute to reducing host inflammation at the site of attachment (Wagner *et al.*,

2008). These secretions change based on the *L. salmonis* host (Fast *et al.*, 2003). This may help explain the ability of less susceptible species to mount rapid inflammatory responses in the absence/reduced presence of *L. salmonis* immunomodulatory compounds. However, while host immunosuppression may be counterproductive to the parasite from the stand point of increasing rates of host mortality and potentially reducing parasite transmission, high density infections can result in osmoregulatory stress to the fish which indirectly leads to opportunistic infection and chronic or acute mortality.

Heavy infections of farmed Atlantic salmon and wild sockeye salmon (*Oncorhynchus nerka*) by *L. salmonis* can lead to deep lesions, particularly on the head region, even exposing the skull. Disease of this magnitude has been absent in farmed fish due to the efficacy of anti-sea lice therapeutants, namely emamectin benzoate used in the salmon culture industry from the mid-1990s until recently (2009). However, from 2009 to 2010 significant pathology has returned to the salmon culture industry in Eastern Canada where lice exhibiting resistance to current control methods are creating morbidly high infection levels on Atlantic salmon, discussed in greater detail below.

22.4.3. Sea lice as vectors of diseases

Sea lice are carriers of bacteria and viruses that are probably obtained from their attachment to and feeding on tissues of contaminated fish (Nylund *et al.*, 1993). Sea lice intestine will contain infectious salmon anaemia virus (ISAv) after lice feed on infected fish. However, it is not known how long the virus remains viable in the lice nor whether lice can actively transmit ISAv when feeding (Nylund *et al.*, 1993). Epizootiological studies have shown that the presence of sea lice in salmon cages is a risk factor for ISAv infection in Atlantic salmon (McClure *et al.*, 2005) and that ISAv infection frequency is reduced when salmon are more frequently deloused (Hammell and Dohoo, 2005). Recent studies have shown that *L. salmonis* can also harbour *Aeromonas salmonicida*, *Pseudomonas*

fluorescens, *Tenacibaculum maritimum*, *Vibrio* spp. and infectious haematopoietic necrosis virus (IHNV) both externally and internally (Barker *et al.*, 2009; Lewis *et al.*, 2010; Stull *et al.*, 2010). However, active transmission of these bacteria and virus has not yet been proven using Koch's postulates.

22.5. Protective/Control Strategies

22.5.1. Control on salmon farms

Integrated pest management programmes for sea lice are instituted or recommended in a number of countries including: (i) Canada (Health Canada, 2003; British Columbia Ministry of Agriculture and Lands, 2008); (ii) Norway (Heuch *et al.*, 2005); (iii) Scotland (Rosie and Singleton, 2002); and (iv) Ireland (Grist, 2002). Identification of epizootiological factors as potential risk factors for sea louse abundance (Revie *et al.*, 2003) with effective sea louse monitoring programmes have been shown to effectively reduce sea louse levels on salmon farms (Saksida *et al.*, 2007a).

Natural predators

Cleaner fish, including five species of wrasse (Labridae), are used on fish farms in Norway and to a lesser extent in Scotland, Shetland and Ireland in integrated pest management programmes (Treasurer, 2002). Wrasse, mostly sourced from the wild, are stocked with farmed salmon to reduce lice burdens. Wrasse have little, if any, effect on larval stages, but snatch adult lice from fish surfaces. Good farming practices must be ensured so that the wrasse or the netting are of adequate size to prevent escape and that fouling is reduced so that wrasse are not diverted from eating lice. Concerns have been raised that wrasse could be vectors of salmon diseases, such as infectious salmon anaemia or infectious pancreas necrosis; however, evidence to date indicates this is not the case (Treasurer, 2002). Trade literature describes ballan wrasse (*Labrus bergylta*) being used on organic salmon farms in Norway, virtually reducing the requirement of drugs to control

sea lice (FishNewsEU.com, 2009). The potential of cleaner fish has not been realized in other fish-farming regions, such as Pacific and Atlantic Canada or Chile since there are no indigenous cleaner fish in these regions. It is inadvisable to introduce foreign species which could become invasive. However, studies continue to determine if any local fish may act as cleaner fish (New Brunswick Salmon Growers Association, 2010).

Husbandry

Good husbandry techniques include: (i) fallowing; (ii) removal of dead and sick fish; and (iii) prevention of net fouling, etc. Bay management plans are in place in most fish-farming regions to keep sea lice below a level that could lead to health concerns on the farm or affect wild fish in surrounding waters. These include: (i) separation of year classes; (ii) counting and recording of sea lice on a prescribed basis; (iii) use of parasiticides when sea louse counts increase; and (iv) monitoring for resistance to parasiticides (Revie *et al.*, 2009).

Salmon breeding

Early findings suggested genetic variation in the susceptibility of Atlantic salmon to *Caligus elongatus* (Mustafa and MacKinnon, 1999). Research then began to identify trait markers (Jones *et al.*, 2002); recent studies have shown that susceptibility of Atlantic salmon to *L. salmonis* can be identified to specific families and that there is a link between major histocompatibility complex (MHC) Class II and susceptibility to lice (Glover *et al.*, 2007; Gharbi *et al.*, 2009). Studies continue to discern salmon families with minimal sea louse settlement while maintaining optimal growth and quality.

Immunostimulation

The role of the immune system appears to be integral to attachment, settlement and development of sea lice on their host. Thus, by enhancing systemic and, subsequently, localized inflammatory mechanisms through immunostimulation prior to *L. salmonis*

exposure, it may be possible to both accelerate and boost Atlantic salmon responses to *L. salmonis* leading to greater protection against infection. Currently there are several products on the salmon feed market sold as immunostimulant additives that have reported enhanced protection in Atlantic salmon to sea lice infection, but still have yet to be used in and show protection in large-scale production. Bio-Mos® (Alltech Inc.), which includes yeast extracts such as mannan oligosaccharides (MOS), provides 22–48% protection against multiple stages of *Lepeophtheirus* and *Caligus* spp. in a Norwegian sea-cage system (Sweetman *et al.*, 2010). EWOS also produces a feed supplement (BOOST®) containing microbial-based nucleotides and, in conjunction with pyrethroid baths, reports significant protection against *C. rogercresseyi* (Gonzalez and Troncoso, 2009). Similar studies with nucleotide-enriched yeast extracts (Nupro®, etc.), used as feed supplements for enhanced growth, are also currently being extended to sea lice (M.D. Fast, personal observation). Other potential immunostimulants include specific forms of β -glucan, which in rainbow trout have been shown to provide protection against the gill microsporidian *Loma salmonae* (Guselle *et al.*, 2010). Stimulation of non-specific mucosal immunity directly at the site of the host-parasite interface should provide interesting areas of future research. The positive 'side effects' of immunostimulant supplements, including increased growth, reduced handling stress and potentially reduced gut pathogenesis, make oral immunostimulation an attractive component within a multi-faceted approach to sea lice control. Used in conjunction with other therapeutants, enhanced protection windows may be achieved in an integrated management system.

22.5.2. Drugs

The range of therapeutants for farmed fish has been limited, particularly in some jurisdictions due to regulatory processing limitations. All drugs used have been assessed for environmental impact and risks (Burridge, 2003; Haya *et al.*, 2005). The parasiticides are

classified into bath and in-feed treatments as follows.

Bath treatments

There are both advantages and disadvantages to using bath treatments. Bath treatments are more difficult than in-feed treatments and need more manpower to administer, requiring skirts or tarpaulins to be placed around the cages to contain the drug. Since the volume of water is imprecise, the required drug concentration is not guaranteed. Crowding of fish to reduce the volume of drug can also stress the fish. Recent use of wellboats containing the drugs has reduced both the concentration and the environmental concerns, although transferring fish to the wellboat and back to the cage is stressful for fish. Recent studies in New Brunswick, Canada, indicated that therapeutic doses of Alpha Max® (deltamethrin) and Salmosan® (azamethiphos) could not be attained or maintained, even with tarpaulins (Beattie and Brewer-Dalton, 2010a). It is not yet clear what causes drug concentrations to fall; high organic content in the waters of the Bay of Fundy is one possibility.

The major advantage to bath treatments is that all the fish will be treated equally, in contrast to in-feed treatments where the amount of drug ingested can vary for a number of reasons. Prevention of reinfection is a challenge since it is practically impossible to treat an entire area in a short time and the drifting of the drug to adjacent cages provides sub-therapeutic doses which may promote drug resistance.

ORGANOPHOSPHATES Organophosphates are acetylcholinesterase inhibitors and cause excitatory paralysis leading to death of sea lice when given as a bath treatment. Dichlorvos was used for many years in Europe and later replaced by azamethiphos, the active ingredient in Salmosan®, which is more potent and safer for operators to handle (Burka *et al.*, 1997). It is effective in killing the mobile stages of sea lice, but apparently less effective in targeting the larval chalimus stages (Roth *et al.*, 1996). Treatment methods recommend fully enclosing the net pens and administering azamethiphos (0.2 ppm when

using a tarpaulin and 0.3 ppm when using a skirt) for 30–60 min, depending on temperature, accompanied by vigorous oxygenation (Findlay, 2009; Fish Vet Group, 2008). Laboratory studies have shown that azamethiphos is toxic to other crustaceans, such as lobsters and shrimp, but field studies indicated no mortalities of lobsters in sentinel cages, no decrease in juvenile lobsters, and no drug in water samples in the vicinity of treated cages because azamethiphos is water soluble and is broken down relatively quickly in the environment (Burridge *et al.*, 1999; Burridge, 2003; Beattie and Brewer-Dalton, 2010b).

Resistance to organophosphates began to develop in Norway in the mid-1990s, apparently due to acetylcholinesterases being altered as a result of mutation of sea lice (Fallang *et al.*, 2004). Its use also declined considerably with the introduction of SLICE®, emamectin benzoate. However, it has recently been reintroduced for bath treatments, particularly in Canada, for emergency-use only, where therapeutic failure of emamectin benzoate has occurred (Fish Vet Group, 2008).

PYRETHROIDS Pyrethroids are direct stimulators of sodium channels in neuronal cells where they induce rapid depolarization and spastic paralysis leading to death. The effect is specific to the parasite since the drugs are only slowly absorbed by the host and rapidly metabolized once absorbed. Cypermethrin (Excis®, Betamax®) and deltamethrin (Alpha Max®) are two pyrethroids commonly used to control both juvenile and adult stages of sea lice (Grant, 2002). Treatments typically use skirts, but tarpaulin use is recommended to provide more accurate dosing (Alexandersen, 2009). Low water temperatures increase the toxic effects of deltamethrin to fish and, as with azamethiphos treatment, oxygenation is required. Resistance to pyrethroids has been reported in Norway (Sevatdal and Horsberg, 2003) and appears to be due to a mutation leading to a structural change in the sodium channel which prevents pyrethroids from activating the channel (Fallang *et al.*, 2005). Use of deltamethrin has been increasing as an alternate treatment with the rise in resistance observed with emamectin benzoate. Alpha Max® (3 ppb for 40 min, using a tarpaulin) was

introduced under emergency registration in Canada in 2009 (New Brunswick Agriculture and Aquaculture, 2009) and is undergoing environmental trials. Sentinel organisms are not affected by Alpha Max® nor is the drug detectable in the water column at the farm site or downcurrent 10 min after the release of the skirts (Beattie and Brewer-Dalton, 2010b).

HYDROGEN PEROXIDE Bathing fish with hydrogen peroxide (1500 mg/l for 20 min) will remove mobile *L. salmonis* from salmon (Grant, 2002). Hydrogen peroxide also appears to show efficacy against both chalimus (56% reduction) and mobile stages (98% reduction) of *C. rogercresseyi* (Bravo *et al.*, 2010). It is environmentally friendly since hydrogen peroxide (H_2O_2) dissociates to water and oxygen, but can be toxic to operators and fish. Its toxicity depends on water temperature and time of exposure (Grant, 2002). Toxicity to fish increases with increasing temperatures, especially above 14°C. The mechanism of toxicity of hydrogen peroxide to sea lice has not been clearly elucidated, but may be related to its free-radical properties, as well as liberation of oxygen in the gut and haemolymph. It dislodges sea lice from the fish, leaving them capable of reattaching to other fish and reinitiating an infection. However, there is also a degree of toxicity to the sea lice. Egg development is suppressed by about half and, of those that survive, none of the nauplii moult to the copepodid stage (Johnson *et al.*, 1993).

Hydrogen peroxide may be a suitable therapeutant to include in an integrated pest management strategy. However, its use can be limited by inaccurate dosing, resistance development and potential toxicity to the host fish (Treasurer *et al.*, 2000a, b; Bravo *et al.*, 2010). The use of wellboats is being investigated to allow controlled dosing conditions which provide increased efficacy and reduced toxicity (Brugge and Armstrong, 2010).

In-feed treatments

In-feed treatments are easier to administer and pose less environmental risk than bath treatments. Feed is usually coated with the drug and drug distribution to the parasite is dependent on the pharmacokinetics of the

drug reaching the parasite in sufficient quantity. The drugs have high selective toxicity for the parasite, are quite lipid soluble so that there is sufficient drug to act for approximately 2 months, and any unmetabolized drug is excreted so slowly that there are few environmental concerns. A disadvantage of in-feed treatments is that diseased or stressed fish may not feed and, thus, underdosing in these fish may lead to resistance development.

AVERMECTINS Avermectins belong to the family of macrocyclic lactones and have been the major drugs used as in-feed treatments to kill sea lice. These drugs selectively open glutamate-gated chloride channels in arthropod neuromuscular tissues (Rohrer *et al.*, 1992) to cause hyperpolarization and flaccid paralysis leading to death. The first avermectin used was ivermectin at doses close to the therapeutic level, but was never submitted by its manufacturer for legal approval for use on fish. Ivermectin is toxic to some fish, causing sedation and central nervous system depression as the drug crosses the blood–brain barrier and stimulates GABA-gated channels in the central nervous system (Høy *et al.*, 1990). Emamectin benzoate, which is the active agent in the formulation SLICE® (Intervet Schering-Plough Animal Health, 2009), has been used since 1999 and has a greater safety margin on fish as it does not accumulate in the brain (Sevatdal *et al.*, 2005). It is administered at 50 µg/kg/day for 7 days and is effective for 2 months, killing both chalimus and mobile stages. Withdrawal times vary with jurisdiction, from zero in Canada to 175 degree days in Norway. Emamectin benzoate has relatively low environmental concerns and is less toxic than ivermectin in all fish taxa tested (Haya *et al.*, 2005; Telfer *et al.*, 2006). Decreased efficacy and sensitivity to emamectin benzoate has been noted for *C. rogercresseyi* and *L. salmonis* on Chilean (Bravo *et al.*, 2008b) and North Atlantic (Lees *et al.*, 2008b, c; Horsberg, 2010; Westcott *et al.*, 2010) fish farms, respectively. The resistance is probably due to prolonged use of the drug leading to upregulation of P-glycoprotein in the parasite which results in decreased drug at the target site (Tribble *et al.*, 2008); this is similar to that seen in nematode resistance to macrocyclic lactones (Lespine *et al.*, 2008). Resistance

concerns with emamectin benzoate have prompted: (i) the use of other agents; (ii) modifications in management strategies; and (iii) increased research efforts in finding alternative treatments (Horsberg, 2010).

GROWTH REGULATORS Teflubenzuron, the active agent in the formulation Calicide® (European Agency for the Evaluation of Medicinal Products, 1999; Ritchie *et al.*, 2002), is a chitin-synthesis inhibitor which prevents moulting. It is administered in feed at 10 mg/kg/day for 7 consecutive days and blocks further development of larval stages of sea lice, but has no effect on adults. It has been used only sparingly in sea louse control, largely due to concerns that it may affect the moult cycle of non-target crustaceans, although this has not been shown at the recommended concentrations (Burridge, 2003). A similar molecule, diflubenzuron, formulated as Lepsidon®, is not being sold in 2010. No resistance concerns have been noted to date for any of the growth regulator agents (Horsberg, 2010).

22.5.3. Vaccines

A number of studies are underway to examine various antigens, particularly from the gastrointestinal tract and reproductive endocrine pathways, as vaccine targets. The first targets sought were proteins from the gastrointestinal tract of *L. salmonis*, particularly trypsin-like proteases. These proteases are produced and secreted from cells in the midgut (Johnson *et al.*, 2002; Kvamme *et al.*, 2004) and have also been isolated from *L. salmonis* secretions and found in host mucus during infections (Firth *et al.*, 2000; Fast *et al.*, 2007). A vaccine against recombinant *L. salmonis* trypsin has been shown to decrease sea lice counts on Atlantic salmon (administered intraperitoneally 6 weeks prior to infectious copepodid challenge) by approximately 20% in a cohabitation trial with unvaccinated fish (Ross *et al.*, 2006). This protection was observed up to 20 days post-infection, prior to development of the mobile stage. Following pre-adult development and potential re-assortment on hosts, no differences were observed between treatments.

As noted earlier, vitellin and vitellogenin proteins, LsYAP, LsVT1 and LsVT2, are unique sea lice targets for vaccine development (Dalvin *et al.*, 2009; Dalvin *et al.*, 2011). A recombinant vaccine has been developed against specific sea lice egg proteins, including vitellogenin, which induce high levels of specific antibodies in both rabbits and Atlantic salmon and reduce prevalence and abundance of female *L. salmonis* on Atlantic salmon hosts (Frost *et al.*, 2007). A recombinant vaccine has been developed against specific egg proteins, including vitellogenin, which when administered intraperitoneally with 200 µg protein reduces prevalence and abundance of female *L. salmonis* on Atlantic salmon in both cohabitation and individual trials (Frost *et al.*, 2007). Sea lice were monitored from the time of infection with copepodids to 3 weeks after the first egg string was observed on adult female lice. Males are not significantly reduced, and about 80% of the vaccinated fish had no skin pathology. The egg proteins used to make the vaccine are common to both *L. salmonis* and *Caligus spp.*, suggesting the vaccine may also be effective against *C. rogercesseyi*.

A novel akirin homologue, expressed in eggs and the gastrointestinal tract of all development stages of *C. rogercesseyi*, has also recently been characterized and proposed as a vaccine target (Carpio, 2010). Akirin is a nuclear factor involved in innate immunity.

22.5.4. Implementation of integrated control strategies

As the salmon aquaculture sector has grown over the past three decades much knowledge has been gained regarding the management of diseases. This is amply illustrated in the case of sea lice. However, moving from anecdotal to evidence-based approaches remains a challenge. For example:

- How can key risk factors best be identified?
- What empirical evidence exists for the benefit of a particular intervention?
- How best can a rational integrated strategy be devised?

In order to adequately respond to these and similar questions two key elements must be in place: (i) large-scale epizootiological data together with appropriate analysis; and (ii) mathematical models to capture a system's complexity and allow decision makers to explore alternatives. Over the past decade these two elements have been increasingly apparent in the sea lice research literature and have begun to influence the practice of integrated sea lice management.

As far as data sets are concerned the situation at the end of the 1990s was summed up in what remains one of the most comprehensive reviews to date (Pike and Wadsworth, 1999). Despite running to over 100 pages, this review referenced virtually no empirical data regarding sea lice control because, as the authors note, 'published information on prevalence and intensity of infection with sea lice is surprisingly sparse for cage-cultured salmon, considering the frequency with which the parasites occur' (Pike and Wadsworth, 1999). Most studies in the literature prior to 1999 were laboratory based, while those farm-based studies which were available related to only two to three sites in a single year (Grant and Treasurer, 1993) or to a single site over a few years (Bron *et al.*, 1993). Given the inherent ecological variability relating to sea lice infestations on farms it is not surprising that these were inadequate to generate strong associations or to adequately assess risk factors. However, in the past decade many industry operators have been collecting data which, together with research-focused material, has been used to explore relationships and risks. The first large-scale study using farm-based data (with lice counts from 1996 to 2000 on over 88,000 fish from around 40 Scottish farms) was published by Revie *et al.* (2002a). It quantified previous anecdotal reports that *L. salmonis* infestation in the second year of production was significantly higher than the first year, with levels of mobile lice being three to ten times higher in the latter year of the production cycle. This contrasted with the abundance of mobile *C. elongatus*, which were seen to be consistently higher in the first year of production (Revie *et al.*, 2002b). The pattern of seasonable infestation on Scottish farms with *C. elongatus* was

also highly regular and thus amenable to modelling using time series methods (McKenzie *et al.*, 2004), something not possible for *L. salmonis* (Revie, 2006). The clear differences in infection dynamics may indicate some form of competitive pressure between species (Revie *et al.*, 2005a, Revie 2006) and highlights the importance of clearly distinguishing between parasite (and host) species rather than talking in broad, and potentially confusing, terms of 'sea lice infestation'.

The first papers to formally explore risk factors for sea lice infestation on salmon farms were also based on this data set from Scotland. An initial study looked at: (i) stocking type; (ii) geographical region; (iii) level of coastal exposure; and (iv) mean sea water temperature (Revie *et al.*, 2002c). None of these factors appeared to be associated with significant differences in *L. salmonis* infestation. However, treatment intervention did have a major impact, emphasizing the importance of adjusting for such interventions as a potential confounding variable in any epizootiological study of risk factors for sea lice infestation. In a subsequent and more extensive analysis, 15 risk factors were incorporated into a linear regression model (Revie *et al.*, 2003). This analysis indicated that not only was sea water temperature variation across sites not a risk factor, but neither were differences in total biomass, stocking density or number of weeks of fallow. In addition to treatment frequency and type, mean current speed at a site, overall flushing time of the loch and cage volume were found to be risk factors.

The collection of large data sets and creation of descriptive epizootiological summaries was adopted by other researchers and resulted in a range of studies from: (i) Norway (Heuch *et al.*, 2003, 2009); (ii) Chile (Bravo *et al.*, 2010); (iii) Ireland (O'Donohoe *et al.*, 2008); and (iv) Canada (Saksida *et al.*, 2007a). This approach was also applied to update the situation in Scotland (Lees *et al.*, 2008a). The use of formal risk factor analysis has been less widely reported, the exceptions being a study in Chile (Zagmutt-Vergara *et al.*, 2005) and one in British Columbia (Saksida *et al.*, 2007a). This latter study highlighted the value of such data sets in making an assessment on

treatment efficacy. Not only can overall levels be estimated, as in the case for SLICE[®] use in British Columbia (Saksida *et al.*, 2007b), Maine (Gustafson *et al.*, 2006), Norway (Ramstad *et al.*, 2002) and Scotland (Treasurer *et al.*, 2002), but an investigation of changes in efficacy can indicate potential development of tolerance within a population. This approach was successfully used in Scotland (Lees *et al.*, 2008b, c) to formalize anecdotal reports of tolerance to SLICE[®], 2 years prior to the publication of *in vitro* evidence (Tildesley *et al.*, 2010). It has recently been applied in British Columbia to demonstrate that this region does not appear to share the reductions in efficacy seen elsewhere (Saksida *et al.*, 2010).

With the increasing pace of growth of information systems and calls for greater transparency in access to data relating to fish farming, it is likely these types of data sets will continue to increase both in scale and in scope. This will bring its own challenges: for example, steps must be taken to ensure that the natural clustering of parasites which occurs in net pens (Revie *et al.*, 2005b) does not introduce undue bias into the sampling process (Revie *et al.*, 2007). Policy makers are becoming attuned to these issues and efforts are underway to standardize surveillance practices around the globe (Revie *et al.*, 2009, 2010). In addition new technologies, such as passive monitoring, may lead to prevalence becoming a standard infestation metric (Baillie *et al.*, 2009). The integration of field- and lab-based data sets from molecular to population scales should provide novel scientific insight that will ultimately improve the management of this host-parasite relationship (Westcott *et al.*, 2010).

However, in addition to the collection and analysis of large data sets, it has become increasingly important to build models that aid our understanding of key interactions and help predict the likely impact of intervention strategies. As has been the case for diseases affecting human and terrestrial animals, the past decade has seen a growth in the application of mathematical modelling to the transmission dynamics of aquatic pathogens (Reno, 1998; McCallum *et al.*, 2004; Murray, 2009; Green, 2010). This has included the use

of hydrodynamic models to explore interactions between sea lice from farmed and wild sources (Murray and Gillibrand, 2006; Krkošek *et al.*, 2006; Foreman *et al.*, 2009; Brooks, 2009). There is insufficient space here to review this sometimes controversial area; an excellent summary is provided by Krkošek (2009). A limited number of models have specifically addressed the biological development of lice populations in either the laboratory (Tucker *et al.*, 2002; Stien *et al.*, 2005) or the field (Revie *et al.*, 2005c; Krkošek *et al.*, 2009). The *SLiDES*im (Sea Lice Difference Equation Simulation) model uses delay differential equations to predict sea lice infestation dynamics on Scottish (Revie *et al.*, 2005c) and Norwegian (Gettinby *et al.*, 2010)

farms. This model has also been used to explore the impact of varying the frequency, timing and efficacy of topical treatments on sea lice infestation dynamics (Robbins *et al.*, 2010).

While comprehensive data sets and mathematical modelling research have yet to be developed for *C. rogercresseyi*, there is no reason why the approaches described above should not be equally applicable to salmon farms in Chile. It seems likely that the confluence of large data sets and more robust models will provide an environment not only to better understand host-parasite interactions but also to give decision makers appropriate tools to implement and evaluate integrated intervention strategies.

References

- Alexandersen, S. (2009) Practical experience with Alpha Max[®] against sea lice: experience from 12 years of use in Norway. Available at: http://0101.nccdn.net/1_5/038/248/08d/Integrated-Pest-Management-Workshop-Dec-10-2009.pdf (accessed 27 June 2011).
- Anstensrud, M. (1990) Molting and mating in *Lepeophtheirus pectoralis* (Copepoda: Caligidae). *Journal of the Marine Biological Association of the United Kingdom* 70, 269–281.
- Bailey, R.J.E., Birkett, M.A., Ingvarsdóttir, A., Mordue (Luntz), A.J., Mordue, W., O'Shea, B., Pickett, J.A. and Wadhams, J.J. (2006) The role of semiochemicals in host location and non-host avoidance by salmon louse (*Lepeophtheirus salmonis*) copepodids. *Canadian Journal of Fisheries and Aquatic Sciences* 63, 448–456.
- Baillie, M., Lees, F., Gettinby, G. and Revie, C.W. (2009) The use of prevalence as a measure of lice burden: a case study of *Lepeophtheirus salmonis* on Scottish Atlantic salmon (*Salmo salar* L) farms. *Journal of Fish Diseases* 32, 15–25.
- Barker, D.E., Braden, L.M., Coombs, M.P. and Boyce, B. (2009) Preliminary studies on the isolation of bacteria from sea lice, *Lepeophtheirus salmonis*, infecting farmed salmon in British Columbia, Canada. *Parasitology Research* 105, 1173–1177.
- Beamish, R.J., Neville, C.M., Sweeting, R.M. and Ambers, N. (2005) Sea lice on adult Pacific salmon in the coastal waters of central British Columbia, Canada. *Fisheries Research* 76, 198–208.
- Beattie, M.J. and Brewer-Dalton, K.E. (2010a) Industry update on pesticide use. Available at: http://0101.nccdn.net/1_5/1a8/3d4/3b7/Sea-Lice-Research-Workshop-Report-Jan-2010.pdf (accessed 27 June 2011).
- Beattie, M.J. and Brewer-Dalton, K.E. (2010b) AlphaMax[®] and Salmosan[®]: sea lice environmental control trials in New Brunswick. In: *Sea Lice 2010 Proceedings*, 14. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- Boxshall, G.A. and Bravo, S. (2000) On the identity of the common *Caligus* (Copepoda: Siphonostomatoida: Caligidae) from salmonid netpen systems in southern Chile. *Contributions to Zoology* 69, 137–146.
- Bravo, S. (2003) Sea lice in Chilean salmon farms. *Bulletin of the European Association of Fish Pathologists* 23, 197–200.
- Bravo, S. (2010) The reproductive output of sea lice *Caligus rogercresseyi* under controlled conditions. *Experimental Parasitology* 125, 51–54.
- Bravo, S., Perroni, M., Torres, E. and Silva, M.T. (2006) Report of *Caligus rogercresseyi* in the anadromous brown trout (*Salmo trutta*) in the Río Gallegos Estuary, Argentina. *Bulletin of the European Association of Fish Pathologists* 26, 186–193.

- Bravo, S., Pozo, V. and Silva, M.T. (2008a) The tolerance of *Caligus rogercresseyi* to salinity reduced in southern Chile. *Bulletin of the European Association of Fish Pathologists* 28, 198–206.
- Bravo, S., Sevattal, S. and Horsberg, T.E. (2008b) Sensitivity assessment of *Caligus rogercresseyi* to emamectin benzoate in Chile. *Aquaculture* 282, 7–12.
- Bravo, S., Erranz, F. and Lagos, C. (2009) A comparison of sea lice, *Caligus rogercresseyi*, fecundity in four areas in southern Chile. *Journal of Fish Diseases* 32, 107–113.
- Bravo, S., Treasurer, J., Sepulveda, M. and Lagos, C. (2010) Effectiveness of hydrogen peroxide in the control of *Caligus rogercresseyi* in Chile and implications for sea louse management. *Aquaculture* 303, 22–27.
- British Columbia Ministry of Agriculture and Lands (2008) Sea Lice Management Strategy 2007/2008. Available at: http://www.al.gov.bc.ca/ahc/fish_health/SL%20Mgmt%20Strat%202007%202008%20Final.pdf (June 27 2011).
- Bron, J.E., Sommerville, C., Jones, M. and Rae, G.H. (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., Sommerville, C., Wootten, R. and Rae, G.H. (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.
- Brooks, K.M. (2005) The effects of water temperature, salinity, and currents on the survival and distribution of the infective copepodid stage of sea lice (*Lepeophtheirus salmonis*) originating on Atlantic salmon farms in the Broughton Archipelago of British Columbia, Canada. *Reviews in Fisheries Science* 13, 177–204.
- Brooks, K.M. (2009) Considerations in developing an integrated pest management programme for control of sea lice on farmed salmon in Pacific Canada. *Journal of Fish Diseases* 32, 59–73.
- Brugge, E. and Armstrong, I. (2010) Hydrogen peroxide treatments – significant efficacy improvements achieved utilizing modern wellboat technology. In: *Sea Lice 2010 Proceedings*, 20. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- Bruno, D.W. and Stone, J. (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.
- Burka, J.F., Hammell, K.L., Horsberg, T.E., Johnson, G.R., Rennie, D.J. and Speare, D.J. (1997) Drugs in salmonid aquaculture – a review. *Journal of Veterinary Pharmacology and Therapeutics* 20, 333–349.
- Burridge, L.E. (2003) Chemical use in marine finfish aquaculture in Canada: a review of current practices and possible environmental effects. *Canadian Technical Report of Fisheries and Aquatic Sciences* 2450, 97–131.
- Burridge, L.E., Haya, K., Waddy, S.L. and Wade, J. (1999) The lethality of anti-sea lice formulations Salmo-sar® (Azamethiphos) and Excis® (Cypermethrin) to stage IV and adult lobsters (*Homarus americanus*) during repeated short-term exposures. *Aquaculture* 182, 27–35.
- Carpio, Y. (2010) A novel akirin homolog exists in salmon louse *Caligus rogercresseyi*. In: *Sea Lice 2010 Proceedings*, 21. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (27 June 2011).
- Carvajal, J., González, L. and George-Nascimento, M. (1998) Native sea lice (Copepods: Caligidae) infestation of salmonids reared in netpen systems in southern Chile. *Aquaculture* 166, 241–266.
- Costello, M.J. (2006) Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology* 22, 475–483.
- Costelloe, M., Costelloe, J., O'Donohue, G., Coghlan, N.J., Oonk, M. and van der Heijden, Y. (1998) Planktonic distribution of sea lice larvae *Lepeophtheirus salmonis*, in Killary harbour, west coast of Ireland. *Journal of the Marine Biological Association of the United Kingdom* 78, 853–874.
- Dalvin, S., Frost, P., Biering, E., Hamre, L.A., Eichner, C., Krossøy, B. and Nilsen, F. (2009) Functional characterization of the maternal yolk-associated protein (LsYAP) utilising systemic RNA interference in the salmon louse (*Lepeophtheirus salmonis*) (Crustacea: Copepoda). *International Journal for Parasitology* 39, 1407–1415.
- Dalvin, S., Frost, P., Loeffen, P., Skern-Mauritzen, R., Baban, J., Rønnestad, I. and Nilsen, F. (2011) Characterisation of two vitellogenins in the salmon louse *Lepeophtheirus salmonis*: molecular, functional and evolutionary analysis. *Diseases of Aquatic Organisms* 94, 211–224.
- Dalvin, S., Skern-Mauritzen, R., Eichner, C., Frost, P. and Nilsen, F. (2010) Reproduction and yolk proteins in salmon louse (*Lepeophtheirus salmonis* Krøyer 1837). In: *Sea Lice 2010 Proceedings*, 23. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).

- Eichner, C., Frost, P., Dysvik, B., Jonassen, I., Kristiansen, B. and Nilsen, F. (2008) Salmon louse (*Lepeophtheirus salmonis*) transcriptomes during post molting maturation and egg production, revealed using EST-sequencing and microarray analysis. *BMC Genomics* 9, 126–140.
- Eichner, C., Malde, K., Dalvin, S., Skern-Mauritzen, R. and Nilsen, F. (2010) Knockdown of the nuclear receptor LsRXR1 in salmon louse (*Lepeophtheirus salmonis* Krøyer 1837). In: *Sea Lice 2010 Proceedings*, 24. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- European Agency for the Evaluation of Medicinal Products (1999) Committee for Veterinary Medicinal Products: Teflubenzuron, Summary Report. Available at: <http://www.ema.europa.eu/pdfs/vet/mrls/054799en.pdf> (27 June 2011).
- Fallang, A., Ramsay, J.M., Sevatdal, S., Burka, J.F., Jewess, P., Hammell, K.L. and Horsberg, T.E. (2004) Evidence for occurrence of an organophosphate-resistant type of acetylcholinesterase in strains of sea lice (*Lepeophtheirus salmonis* Krøyer). *Pest Management Science* 60, 1163–1170.
- Fallang, A., Denholm, I., Horsberg, T.E. and Williamson, M.S. (2005) Novel point mutation in the sodium channel gene of pyrethroid-resistant sea lice *Lepeophtheirus salmonis* (Crustacea: Copepoda). *Diseases of Aquatic Organisms* 65, 129–136.
- Fast, M.D., Ross, N.W., Mustafa, A., Sims, D.E., Johnson, S.C., Conboy, G.A., Speare, D.J., Johnson, G. and Burka, J.F. (2002) Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. *Diseases of Aquatic Organisms* 52, 57–68.
- Fast, M.D., Burka, J.F., Johnson, S.C. and Ross, N.W. (2003) Enzymes released from *Lepeophtheirus salmonis* in response to mucus from different salmonids. *Journal of Parasitology* 89, 7–13.
- Fast, M.D., Johnson, S.C., Eddy, T.D., Pinto, D. and Ross, N.W. (2007) *Lepeophtheirus salmonis* secretory/excretory products and their effects on salmonid immune gene regulation. *Parasite Immunology* 29, 179–189.
- Fast, M.D., Ross, N.W. and Johnson, S.C. (2005) Prostaglandin E₂ modulation of gene expression in an Atlantic salmon (*Salmo salar*) macrophage-like cell line (SHK-1). *Developmental and Comparative Immunology* 29, 951–963.
- Fast, M.D., Muise, D.M., Easy, R.E., Ross, N.W. and Johnson, S.C. (2006a) The effects of successive *Lepeophtheirus salmonis* infections on the immunological status of Atlantic salmon (*Salmo salar*). *Fish and Shellfish Immunology* 21, 228–241.
- Fast, M.D., Ross, N.W., Muise, D.M. and Johnson, S.C. (2006b) Differential gene expression in Atlantic salmon, *Salmo salar*, infected with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Journal of Aquatic Animal Health* 18, 116–127.
- Findlay, C. (2009) Salmosan® sea lice treatment. Available at: http://0101.nccdn.net/1_5/038/248/08d/Integrated-Pest-Management-Workshop-Dec-10-2009.pdf (accessed 27 June 2011).
- Firth, K.J., Johnson, S.C. and Ross, N.W. (2000) Characterization of proteases in the skin mucus of Atlantic salmon (*Salmo salar*) infected with the salmon louse (*Lepeophtheirus salmonis*) and in whole-body louse homogenates. *Journal of Parasitology* 86, 1199–1205.
- FishNewsEU.com (2009) Shetland researchers investigate Ballan wrasse solution for salmon sea lice control. Available at: <http://www.fishnews.eu.com/latest-news/scottish/1885-shetland-researchers-investigate-ballan-wrasse-solution-for-salmon-sea-lice-control.html> (accessed 27 June 2011).
- Fish Vet Group (2008) Salmosan® – sea lice treatment. Available at: <http://www.salmosan.net> (accessed 6 September 2011).
- Foreman, M.G.G., Czajko, P., Stucchi, D.J. and Guo, M. (2009) A finite volume model simulation for the Broughton Archipelago, Canada. *Ocean Modelling* 30, 29–47.
- Frost, P., Nilsen, F. and Hamre, L.A. (2007) Novel sea lice vaccine. International Publication Number WO/2007/039599 A1. World Intellectual Property Organization, Geneva, Switzerland.
- Genna, R.L., Mordue, W., Pike, A.W. and Mordue (Luntz), A.J. (2005) Light intensity, salinity, and host velocity influence presettlement intensity and distribution on hosts by copepodids of sea lice, *Lepeophtheirus salmonis*. *Canadian Journal of Fisheries and Aquatic Sciences* 62, 2675–2682.
- Gettinby, G., Robbins, C., Lees, F., Heuch, P.A., Finstad, B. and Revie, C.W. (2010) Modelling *L. salmonis* sea lice populations on farms in the Hardangerfjord. In: *Sea Lice 2010 Proceedings*, 29. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- Gharbi, K., Glover, K.A., Stone, L.C., MacDonald, E.S., Matthews, L., Grimholt, U. and Stear, M.J. (2009) Genetic dissection of MHC-associated susceptibility to *Lepeophtheirus salmonis* in Atlantic salmon.

- BMC Genetics 10. Available at: <http://www.biomedcentral.com/content/pdf/1471-2156-10-20.pdf> (accessed 27 June 2011).
- Glover, K.A., Grimholt, U., Bakke, H.G., Nilsen, F., Storset, A. and Skaala, Ø. (2007) Major histocompatibility complex (MHC) variation and susceptibility to the sea louse *Lepeophtheirus salmonis* in Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 76, 57–66.
- González, J. and Troncoso, J. (2009) Reducing *Caligus rogercresseyi* infestation in *Salmo salar* fed a diet supplemented with microbial based nucleotides and immunostimulants. Abstract from the World Aquaculture Conference, Veracruz, Mexico, 25–29 September.
- González, L. and Carvajal, J. (2003) Life cycle of *Caligus rogercresseyi*, (Copepoda: Caligidae) parasite of Chilean reared salmonids. *Aquaculture* 220, 101–117.
- Gonzalez-Alanis, P., Wright, G.M., Johnson, S.C. and Burka, J.F. (2001) Frontal filament morphogenesis in the salmon louse *Lepeophtheirus salmonis*. *Journal of Parasitology* 87, 561–574.
- Grant, A.N. (2002) Medicines for sea lice. *Pest Management Science* 58, 521–527.
- Grant, A.N. and Treasurer, J.W. (1993) The effects of following on caligid infestations on farmed Atlantic salmon (*Salmo salar* L.) in Scotland. In: Boxshall, G.A. and Defaye, D. (eds) *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood, Chichester, West Sussex, UK, pp. 255–260.
- Green, D.M. (2010) A strategic model for epidemic control in aquaculture. *Preventive Veterinary Medicine* 94, 119–127.
- Gresty, K.A., Boxshall, G.A. and Nagasawa, K. (1993) Antennular sensors of the infective copepodid larva of the salmon louse, *Lepeophtheirus salmonis* (Copepods: Caligidae). In: Boxshall, G.A. and Defaye, D. (eds) *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood, Chichester, West Sussex, UK, pp. 83–98.
- Grist, B. (2002) The regulatory system for aquaculture in the Republic of Ireland. *Pest Management Science* 58, 609–615.
- Guselle, N.J., Speare, D.J. and Markham, R.J.F. (2010) Efficacy of intraperitoneally and orally administered ProVale, a yeast β -(1,3)/(1,6)-D-glucan product, in inhibiting xenoma formation by the microsporidian *Loma salmonae* on rainbow trout gills. *North American Journal of Aquaculture* 72, 65–72.
- Gustafson, L., Ellis, S., Robinson, T., Marengi, F. and Endris, R. (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.
- Hammell, K.L. and Dohoo, I.R. (2005) Risk factors associated with mortalities attributed to infectious salmon anaemia virus in New Brunswick, Canada. *Journal of Fish Diseases* 28, 651–661.
- Haya, K., Burrridge, L.E., Davies, I.M. and Ervik, E. (2005) A review and assessment of environmental risk of chemicals used for the treatment of sea lice infestations of cultured salmon. *Handbook of Environmental Chemistry* 5(M). Springer-Verlag, Berlin, Germany, pp. 305–340.
- Health Canada (2003) Integrated Pest Management of Sea Lice in Salmon Aquaculture. ISBN 0-662-34002-7. Available at: http://www.hc-sc.gc.ca/cps-spc/alt_formats/pacrb-dgapcr/pdf/pubs/pest/fact-fiche/lice-pou-eng.pdf (accessed 27 June 2011).
- Heuch, P.A., Parsons, A. and Boxaspen, K. (1995) Diel vertical migration: a possible host finding mechanism in salmon lice (*Lepeophtheirus salmonis*) copepodids? *Canadian Journal of Fisheries and Aquatic Sciences* 52, 681–689.
- Heuch, P.A., Nordhagen, J.R. and Schram, T.A. (2000) Egg production in the salmon louse [*Lepeophtheirus salmonis* (Krøyer)] in relation to origin and water temperature. *Aquaculture Research* 31, 805–814.
- Heuch, P.A., Revie, C.W. and Gettinby, G. (2003) A comparison of epidemiological patterns of salmon lice (*Lepeophtheirus salmonis*) infections in Norway and Scotland. *Journal of Fish Diseases* 26, 539–551.
- Heuch, P.A., Bjørn, P.A., Finstad, B., Holst, J.C., Asplin, L. and Nilsen, F. (2005) A review of the Norwegian action plan against salmon lice on salmonids: the effect on wild salmonids. *Aquaculture* 246, 79–92.
- Heuch, P.A., Stigum, O., Malkenes, R., Revie, C.W., Gettinby, G., Baillie, M., Lees, F. and Finstad, B. (2009) The spatial and temporal variations in *Lepeophtheirus salmonis* infection on salmon farms in the Hardanger fjord 2004–2006. *Journal of Fish Diseases* 32, 89–100.
- Horsberg, T.E. (2010) Sea lice treatments: effects, side effects and resistance development. In: *Sea Lice 2010 Proceedings*, 37. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- Høy, T., Horsberg, T.E. and Nafstad, I. (1990) The disposition of ivermectin in Atlantic salmon (*Salmo salar*). *Pharmacology and Toxicology* 67, 307–312.

- Hull, M.Q., Pike, A.W., Mordue (Luntz), A.J. and Rae, G.H. (1998) Patterns of pair formation and mating in an ectoparasitic caligid copepod *Lepeophtheirus salmonis* (Krøyer, 1837): implications for its sensory and mating biology. *Philosophical Transactions of the Royal Society of London B* 353, 753–764.
- Ingvarsdottir, A., Birkett, M.A., Duce, L., Genna, R.L., Mordue, W., Pickett, J.A., Waldhams, L.J. and Mordue (Luntz), A.J. (2002) Role of semiochemicals in mate location by parasitic sea louse, *Lepeophtheirus salmonis*. *Journal of Chemical Ecology* 28, 2107–2117.
- Intervet Schering-Plough Animal Health (2009) SLICE Premix. Available at: <http://www.spaquaculture.com/default.aspx?pageid=545>.
- Johnson, S.C. and Albright, L.J. (1991a) The developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). *Canadian Journal of Zoology* 69, 929–950.
- Johnson, S.C. and Albright, L.J. (1991b) Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. *Journal of the Marine Biological Association of the United Kingdom* 71, 425–436.
- Johnson, S.C. and Albright, L.J. (1992a) Comparative susceptibility and histopathology of the host response of naïve Atlantic, Chinook, and coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Diseases of Aquatic Organisms* 14, 179–193.
- Johnson, S.C. and Albright, L.J. (1992b) Effects of cortisol implants on the susceptibility and the histopathology of the responses of naïve coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Diseases of Aquatic Organisms* 14, 195–205.
- Johnson, S.C., Constible, J.M. and Richard, J. (1993) Laboratory investigations on the efficacy of hydrogen peroxide against the salmon louse *Lepeophtheirus salmonis* and its toxicological and histopathological effects on Atlantic salmon *Salmo salar* and chinook salmon *Oncorhynchus tshawytscha*. *Diseases of Aquatic Organisms* 17, 197–204.
- Johnson, S.C., Ewart, K.V., Osborne, J.A., Delage, D., Ross, N.W. and Murray, H.M. (2002) Molecular cloning of trypsin cDNAs and trypsin gene expression in the salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Parasitology Research* 88, 789–796.
- Johnson, S.C. and Fast, M.D. (2004) Interactions between sea lice and their hosts. In: Wiegertjes, G.F. and Flik, G. (eds). *Host-parasite interactions*. BIOS Scientific Publishers, Oxford, UK, pp. 131–161.
- Jones, C.S., Lockyer, A.E., Verspoor, E., Secombes, C.J. and Noble, L.R. (2002) Towards selective breeding of Atlantic salmon for sea louse resistance: approaches to identify trait markers. *Pest Management Science* 58, 559–568.
- Jones, S.R.M., Prosperi-Porta, G., Kim, E., Callow, P. and Hargreaves, N.B. (2006) The occurrence of *Lepeophtheirus salmonis* and *Caligus clemensi* (Copepoda: Caligidae) on threespine stickleback *Gasterosteus aculeatus* in coastal British Columbia. *Journal of Parasitology* 92, 473–480.
- Jones, S.R.M., Fast, M.D., Johnson, S.C. and Groman, D.B. (2007) Differential rejection of salmon lice by pink and chum salmon: disease consequences and expression of proinflammatory genes. *Diseases of Aquatic Organisms* 75, 229–238.
- Krkošek, M. (2009) Sea lice and salmon in Pacific Canada: ecology and policy. *Frontiers in Ecology and the Environment* 8, 201–209.
- Krkošek, M., Lewis, M.A., Morton, A., Frazer, L.N. and Volpe, J.P. (2006) Epizootics of wild fish induced by fish farms. *Proceedings of the National Academy of Sciences USA* 103, 15506–15510.
- Krkošek, M., Morton, A., Volpe, J.P. and Lewis, M.A. (2009) Sea lice and salmon population dynamics: effects of exposure time for migratory fish. *Proceedings of the Royal Society B* 276, 2819–2828.
- Kvamme, B.O., Skern, R., Frost, P. and Nilsen, F. (2004) Molecular characterisation of five trypsin-like peptidase transcripts from the salmon louse (*Lepeophtheirus salmonis*) intestine. *International Journal of Parasitology* 34, 823–832.
- Lees, F., Gettinby, G. and Revie, C.W. (2008a) Changes in epidemiological patterns of sea lice infestation on farmed Atlantic salmon (*Salmo salar* L) in Scotland between 1996 and 2006. *Journal of Fish Diseases* 31, 251–262.
- Lees, F., Baillie, M., Gettinby, G. and Revie, C.W. (2008b) The efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon (*Salmo salar* L) in Scotland between 2002 and 2006. *Public Library of Science One* 3, e1549.F
- Lees, F., Baillie, M., Gettinby, G. and Revie, C.W. (2008c) Factors associated with changing efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on Scottish salmon farms. *Journal of Fish Diseases* 31, 947–951.
- Lespine, A., Alvinerie, M., Vercruysse, J., Prichard, R.K. and Geldhof, P. (2008) ABC transporter modulation: a strategy to enhance the activity of macrocyclic lactone anthelmintics. *Trends in Parasitology* 24, 293–298.

- Lewis, D.L., Barker, D., Stull, A., Sandeman-Allen, M., Martin, R., Novak, C. and Jakob, E. (2010) The ectoparasitic copepod (*Lepeophtheirus salmonis*) as a carrier of *Aeromonas salmonicida* infecting Atlantic salmon, *Salmo salar*. In: *Sea Lice 2010 Proceedings*, 48. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- McCallum, H., Kuris, A., Harvell, C.D., Lafferty, K.D., Smith, G.W. and Porter, J. (2004) Does terrestrial epidemiology apply in marine systems? *Trends in Ecological Evolution* 19, 585–591.
- McClure, C.A., Hammell, K.L. and Dohoo, I.R. (2005) Risk factors for outbreaks of infectious salmon anaemia in farmed Atlantic salmon, *Salmo salar*. *Preventive Veterinary Medicine* 72, 263–280.
- McKenzie, E., Gettinby, G., McCart, K. and Revie, C.W. (2004) Time series models of sea lice *Caligus elongatus* (Nordmann) abundance on Atlantic salmon *Salmo salar* L. in Loch Sunart, Scotland. *Aquaculture Research* 35, 764–772.
- McKibben, M.A. and Hay, D.W. (2004) Distributions of planktonic sea lice larvae *Lepeophtheirus salmonis* in the inter-tidal zone in Loch Torrindon, western Scotland in relation to salmon farm production cycles. *Aquaculture Research* 35, 742–750.
- Mordue (Luntz), A.J. and Birkett, M.A. (2009) A review of host finding behaviour in the parasitic sea louse, *Lepeophtheirus salmonis* (Caligidae: Copepoda). *Journal of Fish Diseases* 32, 3–13.
- Murray, A.G. (2009) Using simple models to review the application and implications of different approaches used to simulate transmission of pathogens among aquatic animals. *Preventive Veterinary Medicine* 88, 167–177.
- Murray, A.G. and Gillibrand, P.A. (2006) Modeling salmon lice dispersal in Loch Torridon, Scotland. *Marine Pollution Bulletin* 53, 128–135.
- Mustafa, A. and MacKinnon, B.M. (1999) Genetic variation in susceptibility of Atlantic salmon to the sea louse *Caligus elongatus* Nordmann 1882. *Canadian Journal of Zoology* 77, 1332–1335.
- Mustafa, A., Conboy, G.A. and Burka, J.F. (2001) Life-span and reproductive capacity of sea lice, *Lepeophtheirus salmonis*, under laboratory conditions. *Aquaculture Association of Canada Special Publication* 4, 113–114.
- Nagasawa, K., Ishida, Y., Ogura, M., Tadokoro, K. and Hiramatsu, K. (1993) The abundance and distribution of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on six species of Pacific salmon in offshore waters of the north Pacific Ocean and Bering Sea. In: Boxshall, G.A. and Defaye, D. (eds) *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood, Chichester, West Sussex, UK, pp. 166–178.
- New Brunswick Agriculture and Aquaculture (2009) Alpha Max® trials for sea lice control on salmon farms. Available at: <http://www.nbsga.com/wharftalk/Alphamax%20Information%20Sheet%20June%202012%202009%20Final-2.pages.pdf> (accessed 6 September 2011).
- New Brunswick Salmon Growers Association (2010) Sea Lice Research Development Workshop Report. Available at: http://0101.nccdn.net/1_5/1a8/3d4/3b7/Sea-Lice-Research-Workshop-Report-Jan-2010.pdf (accessed 27 June 2011).
- Nordhagen, J.R., Heuch, P.A. and Schram, T.A. (2000) Size as indicator of origin of salmon lice *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Contributions to Zoology* 69, 99–108.
- Nylund, A., Wallace, C. and Hovland, T. (1993) The possible role of *Lepeophtheirus salmonis* (Krøyer) in the transmission of infectious salmon anaemia. In: Boxshall, G.A. and Defaye, D. (eds) *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood, Chichester West Sussex, UK, pp. 367–373.
- O'Donohoe, P., Kane, F., Kelly, S., McDermott, T., Drumm, A. and Jackson, D. (2011) National Survey of Sea Lice (*Lepeophtheirus salmonis* Krøyer and *Caligus elongatus* Nordmann) on Fish Farms on Ireland – 2010. Available at: <http://www.marine.ie/NR/rdonlyres/88D0A537-5AF3-41F7-8AE4-CF-753BEBDDC7/0/IFBno34.pdf> (accessed 27 June 2011).
- Oines, O., Simonsen, J.H., Knutsen, J.A. and Heuch, P.A. (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.
- Pacific Salmon Forum (2009) Final report and recommendations to the government of British Columbia. Available at: <http://www.farmedanddangerous.org/wp-content/uploads/2011/01/BCPSFFinRptqSm.pdf> (accessed 27 June 2011).
- Pert, C.C., Urquhart, K. and Bricknell, I.R. (2006) The sea bass (*Dicentrarchus labrax* L.): a peripatetic host of *Lepeophtheirus salmonis* (Copepoda: Caligidae)? *Bulletin of the European Association of Fish Pathologists* 26, 162–165.
- Pike, A.W. and Wadsworth, S.L. (1999) Sealice on salmonids: their biology and control. *Advances in Parasitology* 44, 233–337.

- Pino-Marambio, J., Mordue (Luntz), A.J., Birkett, M., Carvajal, J., Asencio, G., Mellado, A. and Quiroz, A. (2007) Behavioural studies of host, non-host and mate location by the sea louse, *Caligus rogercresseyi* Boxshall & Bravo, 2000 (Copepoda: Caligidae). *Aquaculture* 271, 70–76.
- Ramstad, A., Colquhoun, D.J., Nordmo, R., Sutherland, I.H. and Simmons, R. (2002) Field trials in Norway with SLICE (0.2% emamectin benzoate) for the oral treatment of sea lice infestation in farmed Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 50, 29–33.
- Reno, P.W. (1998) Factors involved in the dissemination of disease in fish populations. *Journal of Aquatic Animal Health* 10, 160–171.
- Revie, C.W. (2006) The development of an epi-informatics approach to increase understanding of the relationships between farmed fish and their parasites. PhD thesis, University of Strathclyde, Glasgow, Scotland, UK.
- Revie, C.W., Gettinby, G., Treasurer, J.W., Grant, A.N. and Reid, S.W.J. (2002a) Sea lice infestation on Atlantic salmon and the use of ectoparasitic treatments. *The Veterinary Record* 151, 753–757.
- Revie, C.W., Gettinby, G., Treasurer, J.W. and Rae, G.H. (2002b) 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., Gettinby, G., Treasurer, J.W., Rae, G.H. and Clark, N. (2002c) Temporal, environmental and management factors influencing the epidemiological patterns of sea lice (*Lepeophtheirus salmonis*) infestations on farmed Atlantic salmon (*Salmo salar* L.) in Scotland. *Pest Management Science* 58, 576–584.
- Revie, C.W., Gettinby, G., Treasurer, J.W. and Wallace, C. (2003) Identifying epidemiological factors affecting sea lice (*Lepeophtheirus salmonis*) abundance on Scottish salmon farms using general linear models. *Diseases of Aquatic Organisms* 57, 85–95.
- Revie, C.W., Gettinby, G., McKenzie, E., Kelly, L., Wallace, C. and Treasurer, J.W. (2005a) Evidence of inter-species interaction between sea lice in Scottish salmon farms? In: *Society for Veterinary Epidemiology and Preventive Medicine: Meeting Proceedings*, Nairn, Scotland, UK, 30 March–1 April 2005, pp. 124–134.
- Revie, C.W., Gettinby, G., Treasurer, J.W. and Wallace, C. (2005b) Evaluating the effect of clustering when monitoring the abundance of sea lice populations on farmed Atlantic salmon. *Journal of Fish Biology* 66, 773–783.
- Revie, C.W., Robbins, C., Gettinby, G., Kelly, L. and Treasurer, J.W. (2005c) 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.
- Revie, C.W., Hollinger, E., Gettinby, G., Lees, F. and Heuch, P.A. (2007) Clustering of parasites within cages on Scottish and Norwegian salmon farms: alternative sampling strategies illustrated using simulation. *Preventive Veterinary Medicine* 81, 135–147.
- Revie, C., Dill, L., Finstad, B. and Todd, C.D. (2009) Salmon Aquaculture Dialogue Working Group Report on Sea Lice, commissioned by the Salmon Aquaculture Dialogue. Available at: <http://www.worldwildlife.org/what/globalmarkets/aquaculture/WWFBinaryitem11790.pdf> (accessed 27 June 2011).
- Revie, C., Heuch, P.A. and Gettinby, G. (2010) A short history of sea lice sampling strategies on Atlantic salmon farms and the use of empirical evidence to determine best practice. In: *Sea Lice 2010 Proceedings*, 68. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- Ritchie, G. (1997) The host transfer ability of *Lepeophtheirus salmonis* (Copepods: Caligidae) from farmed Atlantic salmon. *Journal of Fish Diseases* 20, 153–157.
- Ritchie, G., Rønsberg, S.S., Hoff, K.A. and Branson, E.J. (2002) Clinical efficacy of teflubenzuron (Calicide®) for the treatment of *Lepeophtheirus salmonis* infestations of farmed Atlantic salmon *Salmo salar* at low water temperatures. *Diseases of Aquatic Organisms* 51, 101–106.
- Robbins, C., Hollinger, E., Gettinby, G., Lees, F. and Revie, C.W. (2010) Assessing topical treatment interventions on Scottish salmon farms using a sea lice (*Lepeophtheirus salmonis*) population model. *Aquaculture* 306, 191–197.
- Rohrer, S.P., Meinke, P.T., Hayes, E.C., Mrozik, H. and Schaeffer, J.M. (1992) Photoaffinity labeling of avermectin binding sites from *Caenorhabditis elegans* and *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences USA* 89, 4168–4172.
- Rosie, A.J. and Singleton, P.T.R. (2002) Discharge consents in Scotland. *Pest Management Science* 58, 616–621.

- Ross, N.W., Firth, K.J., Wang, A., Burka, J.F. and Johnson, S.C. (2000) Changes in hydrolytic enzyme activities of naive Atlantic salmon (*Salmo salar*) skin mucus due to infection with the salmon louse (*Lepeophtheirus salmonis*) and cortisol implantation. *Diseases of Aquatic Organisms* 41, 43–51.
- Ross, N.W., Johnson, S.C., Fast, M.D. and Ewart, K.V. (2006) Recombinant vaccines against caligid copepods (sea lice) and antigen sequences thereof. International Publication Number WO/2006/010265. World Intellectual Property Organization, Geneva, Switzerland.
- Roth, M., Richards, R.H., Dobson, D.P. and Rae, G.H. (1996) Field trials on the efficacy of the organophosphorus compound azamethiphos for the control of sea lice (Copepoda: Caligidae) infestation of farmed Atlantic salmon (*Salmo salar*). *Aquaculture* 140, 217–239.
- Saksida, S., Karreman, G.A., Constantine, J. and Donald, A. (2007a) Differences in *Lepeophtheirus salmonis* abundance levels on Atlantic salmon farms in the Broughton Archipelago, British Columbia, Canada. *Journal of Fish Diseases* 30, 357–366.
- Saksida, S., Constantine, J., Karreman, G.A. and McDonald, A. (2007b) Evaluation of sea lice abundance levels on farmed Atlantic salmon (*Salmo salar* L.) located in the Broughton Archipelago of British Columbia from 2003 to 2005. *Aquaculture Research* 38, 219–231.
- Saksida, S.M., Morrison, D. and Revie, C.W. (2010) The efficacy of emamectin benzoate against infestations of sea lice, *Lepeophtheirus salmonis*, on farmed Atlantic salmon, *Salmo salar* L., in British Columbia. *Journal of Fish Diseases* 33, 913–917.
- Schram, T.A. (1993) Supplementary descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). In: Boxshall, G.A. and Defaye, D. (eds) *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood, Chichester, West Sussex, UK, pp. 30–47.
- Sevatdal, S. and Horsberg, T.E. (2003) Determination of reduced sensitivity in sea lice (*Lepeophtheirus salmonis* Krøyer) against the pyrethroid deltamethrin using bioassays and probit modelling. *Aquaculture* 218, 21–31.
- Sevatdal, S., Magnusson, Å., Ingebrigtsen, K., Haldorsen, R. and Horsberg, T.E. (2005) Distribution of emamectin benzoate in Atlantic salmon (*Salmo salar* L.). *Journal of Veterinary Pharmacology and Therapeutics* 28, 101–107.
- Skern-Mauritzen, R., Frost, P., Hamre, L.A., Kongshaug, H. and Nilsen, F. (2007) Molecular characterization and classification of a clip domain containing peptidase from the ectoparasite *Lepeophtheirus salmonis* (Copepoda, Crustacea). *Comparative Biochemistry and Physiology, Part B* 146, 289–298.
- Skern-Mauritzen, R., Dalvin, S., Eichner, C., Frost, P. and Nilsen, F. (2010) LsCP1 – a putative development and hemostatic protein in *Lepeophtheirus salmonis* (Krøyer 1837). In: *Sea Lice 2010 Proceedings*, 73. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- Skugor, S., Glover, K.A., Nilsen, F. and Krasnov, A. (2008) Local and systemic gene expression responses of Atlantic salmon (*Salmo salar* L.) to infection with the salmon louse (*Lepeophtheirus salmonis*). *BMC Genomics* 9, 498–516.
- Stien, A., Bjørn, P.A., Heuch, P.A. and Elston, D. (2005) Population dynamics of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon and sea trout. *Marine Ecology Progress Series* 290, 263–275.
- Stull, A., Barker, D., Garver, K., Lewis, D., Sandeman-Allen, M., Martin, R. and Jakob, E. (2010) Potential role of *Lepeophtheirus salmonis* as a carrier for infectious haematopoietic necrosis virus. In: *Sea Lice 2010 Proceedings*, 76. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- Sweetman, J.W., Torrecillas, S., Dimitroglou, A., Rider, S., Davies, S.J. and Izquierdo, M.S. (2010) Enhancing the natural defenses and barrier protection of aquaculture species. *Aquaculture Research* 41, 345–355.
- Telfer, T.C., Baird, D.J., McHenery, J.G., Stone, J., Sutherland, I. and Wislocki, P. (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.
- Tildesley, A.S., McHenery, J.G., Roy, W.J. and Endris, R.G. (2010) Reduced sensitivity to emamectin benzoate in a farm population of sea lice (*Lepeophtheirus salmonis*) demonstrated by *in vivo* and *in vitro* testing of efficacy of Slice. In: *Sea Lice 2010 Proceedings*, 81. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- Todd, C.D., Walker, A.M., Hoyle, J.E., Northcott, S.J., Walker, A.F. and Ritchie, M.G. (2000) Infestations of wild adult Atlantic salmon (*Salmo salar* L.) by the ectoparasitic copepod sea louse *Lepeophtheirus salmonis* (Krøyer): prevalence, intensity and the spatial distribution of males and females on the host fish. *Hydrobiologia* 429, 181–196.

- Treasurer, J.W. (2002) A review of potential pathogens of sea lice and the application of cleaner fish in biological control. *Pest Management Science* 58, 546–558.
- Treasurer, J.W., Wadsworth, S. and Grant, A. (2000a) Resistance of sea lice, *Lepeophtheirus salmonis* (Krøyer), to hydrogen peroxide on farmed Atlantic salmon, *Salmo salar*. *Aquaculture Research* 31, 855–860.
- Treasurer, J.W., Grant, A. and Davis, P.J. (2000b) Physical constraints of bath treatments of Atlantic salmon (*Salmo salar*) with a sea lice burden (Copepoda: Caligidae). *Contributions to Zoology* 69, 129–136.
- Treasurer, J.W., Wallace, C. and Dear, G. (2002) Control of sea lice on farmed Atlantic salmon *S. salar* L. with the oral treatment emamectin benzoate (SLICE). *Bulletin of the European Association of Fish Pathologists* 22, 375–380.
- Tribble, N.D., Burka, J.F., Kibenge, F.S.B. and Wright, G.M. (2008) Identification and localization of a putative ATP-binding cassette transporter in sea lice (*Lepeophtheirus salmonis*) and host Atlantic salmon (*Salmo salar*). *Parasitology* 135, 243–255.
- Tucker, C.S., Norman, R., Shinn, A.P., Bron, J.E., Sommerville, C. and Wootten, R. (2002) A single cohort time delay model of the life-cycle of the salmon louse *Lepeophtheirus salmonis* on Atlantic salmon *Salmo salar*. *Gyobyo Kenkyu (Fish Pathology)* 37, 107–118.
- Wadsworth, S., Grant, A. and Treasurer, J. (1998) Strategic approach to lice control. *Fish Farmer* 4, 52.
- Wagner, G.N., Fast, M.D. and Johnson, S.C. (2008) Physiology and immunology of *Lepeophtheirus salmonis* infections of salmonids. *Trends in Parasitology* 24, 176–183.
- Walter, T.C. and Boxshall, G. (2010) Caligidae. In: *World Copepoda database. World Register of Marine Species*. Available at: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=135513>.
- Westcott, J.D., Revie, C.W., Giffin, B.L. and Hammell, K.L. (2010) Evidence of sea lice *Lepeophtheirus salmonis* tolerance to emamectin benzoate in New Brunswick, Canada. In: *Sea Lice 2010 Proceedings*, 85. Available at: http://sealice2010.com/resources/SeaLice2010_abstract_booklet.pdf (accessed 27 June 2011).
- Yazawa, R., Yasuike, M., Leong, J., von Schalburg, K.R., Cooper, G.A., Beetz-Sargent, M., Robb, A., Davidson, W.S., Jones, S.R. and Koop, B.F. (2008) EST and mitochondrial DNA sequences support a distinct Pacific form of salmon louse, *Lepeophtheirus salmonis*. *Marine Biotechnology (New York)* 10, 741–749.
- Zagmutt-Vergara, F.J., Carpenter, T.E., Farver, T.B. and Hedrick, R.P. (2005) Spatial and temporal variations in sea lice (Copepoda: Caligidae) infestations of three salmonid species farmed in net pens in southern Chile. *Diseases of Aquatic Organisms* 64, 163–173.

Index

AGD *see* Amoebic gill disease

Amoebic gill disease (AGD)

Atlantic salmon

dorsal aorta cannulation 6

experimental exposure, *N. perurans* 2

freshwater bathing 8–9

gene expression changes 7

haemoglobin 7

isolated amoebae 2

lower cardiac output 6

Tasmania 2

white gross lesions 3, 4

coho salmon 2

co-infections 12

mortalities 3

N. branchiphila 2

Amyloodiniosis

chloroquine diphosphate 24

clinical signs 22

copper levels 24

piscidins 25

recovery 25

treatment 22, 23

Amyloodinium ocellatum

acquired resistance 26

aquarium fish 19

damsel fish 20

description 19

diagnosis, infection

freshwater bath 20

histopathology 20, 21

indirect illumination 19

oligonucleotide primers, PCR assay
21

serum antibody 21–22

tricaine anesthetic 20

trophonts/tomonts, identification 20

environmental treatments

dinospores 25

lowering, temperature 24

repeated water changes 25

salinity 25

external/internal lesions

clinical signs 22

gill hyperplasia 21, 22

innate resistance

diet 26

HLPs 25

host factors 25

serum, anti-*Amyloodinium* activity 25

life cycle 19

medical treatments

chloroquine 24

copper 24

flush treatment, formalin 24

hydrogen peroxide 24

prophylaxis 22, 23

outbreaks 19

pathophysiology 22

Anguillicoloides crassus

Atlantic eel populations 321

chemotherapeutic treatments

chemicals 320

eel farmers 320

Levamisole and administration 320

condition and swimming performance

damage, swimbladder wall 319

growth and swimming behaviour 318,
319

infected eels 319

Anguillicoloides crassus continued

- diagnosis, infection
 - adult and pre-adult worms 313, 315
 - eel behaviour 314
 - larval stages 315
 - radiography 315
 - serodiagnostic methods 315
- drastic policies 322
- dynamics, degradations
 - development, swimbladder metrics 318
 - experimental investigations 316
 - gross pathology 317
 - pathogenic stages 316
- environmental approach
 - brackish and marine waters 321
 - laboratory investigations 321
 - salt water 321
- epizootiology 314
- histopathologies
 - bloodsucking 315
 - cellular immune response 315–316
 - eel swimbladders 316
 - in situ* swimbladders 316, 317
 - tunnel formations 315
- immunology and vaccination
 - antibacterial drugs 320
 - protection, adaptive immunity 320–321
 - reinfection experiment 320
- life cycle *see* Life cycle, *A. crassus*
- mortality
 - aquaculture 318
 - dying eels 318
 - parasite burden 318
- proxy indicators 318
- reproduction
 - gene expression 319
 - population level 320
 - swimbladder infection 319–320
- sanitary measures 321
- stocking 321
- systematics
 - eel species 310–312
 - taxonomic family 310

Anisakis sp.

- anterior body, *A. simplex* third-stage larva 299
- Asian-inspired seafood 299
- gross pathology and host tissue damage
 - infections 301
 - RVS, wild Atlantic salmon 303
 - 'stomach crater syndrome' cod 301–302
- herring/whale worm 298
- larvae's migration 299
- larval fish host cycle 298
- low pathogenicity and virulence, fishes 307
- macroscopic appearance
 - A. simplex* third-stage larvae, blue whiting liver 300, 301

host-induced connective tissue capsule
300, 301

infections feature 300
massive infection, *A. simplex* third-stage
larvae 300

pathophysiological effects
Anisakis larvae and farmed fish 305–306
dead larvae and disintegrated capsules
303, 304

fish condition 305
gudgeon 305
infection pattern 303, 304
larval intensity and fish host body
weight, mackerel 303, 304

phylogenetic clades 298
protective/control strategies 306–307
systematics and ecology 299

Antibodies, *I. multifiliis*

IgM and MHC II 62–63
immobilization 63, 64
mucosal immune system 62
phagocytes 63
protection 63
treatment 63

Antimicrobial polypeptides (AMPPs) 25–26

Argulus foliaceus

apical pore 329, 330
clinical signs and diagnosis
mouth cavity 330
swimming behaviour 330
description, Branchiura 327
fish production 330
flattened body 327, 328
freshwater fishes 330
haplotype techniques 332–333
host reactions 332
macroscopic and microscopic lesions
damaged epithelium 331
feeding 329, 331
pre-oral spine 329, 331
mouth cone 329, 330
osmoregulation 327
pathophysiology
cross-infected rainbow trout 331
immune response 331
infected fish 331
spermatophore 330
treatment and control
branchiuran infection 332
IDIs 332
nervous system 332
organochlorine and organophosphate
332
parasite infection 332
yellow/whitish egg 327, 328

Atlantic salmon
cages 11

- co-infection 12
- mixed-sex diploid 11
- stocking density 11
- triploid 11
- see also* Amoebic gill disease (AGD)
- Bacterial gill disease (BGD) 184–185
- Bath treatments
 - advantages and disadvantages 357
 - hydrogen peroxide 358
 - organophosphates 357–358
 - pyrethroids 358
- Benedenia serialae*
 - biological control 238
 - capsalidae 225
 - capsalid biology, ecology and identity 239–240
 - chemical treatments versus vaccines 238–239
 - control strategy 234
 - diagnosis, infection
 - adults 228
 - life cycle, and *Neobenedenia* species 226, 228
 - S. quinquerradiata* 227, 228
 - external/internal lesions 231
 - farm husbandry 234–235
 - impacts 225, 226
 - IPM and mathematical models, farm husbandry 238
 - N. melleni* 226
 - pathophysiology
 - monogenean infections 232
 - time course, skin lesions 232–233
 - protection strategy 233
 - technologies 239
- BGD *see* Bacterial gill disease
- Bothriocephalus acheilognathi*
 - Asian tapeworm 292
 - Bothriocephalidea 282
 - definitive fish hosts 285
 - detrimental effects, fish 282
 - disease mechanism
 - causes, juvenile fish 290–291
 - enzymes activities, reduction 291
 - reduced haemoglobin and total blood volume 291
 - disease significance 286
 - electron micrographs scanning, scolex 283, 284
 - fish populations 293
 - geographical distribution
 - African populations 285
 - Australia 286
 - China and Japan 285
 - cyprinid species 285
 - tapeworm 285–286
 - infection diagnosis and clinical signs
 - carp 286–287
 - intensity 288
 - squash plate method 287
 - life cycle and transmission
 - copepods 284
 - postcyclic 284
 - male and female reproductive system 283–284
 - morphological characteristics 283
 - morphology and life cycle 283
 - pathological changes, attachment
 - intestine wall causes, numerous tapeworms 288, 289
 - scolex 288, 289
 - protective/control strategies
 - chemotherapeutic agents, natural products 292
 - chlorine-based compounds 292
 - European fish farmers 292
 - impacts 291
 - size 283
 - strobila, pathological changes
 - carp intestine, gut attenuation and partial occlusion 289–290
 - intestinal rupture 290
- Caligus rogercresseyi*
 - body lengths 353
 - chalmus stages 352–353
 - developmental stages 351
 - diversity and hosts
 - adaptation 351
 - characterization 351
 - salmonids 351
 - temperature, light and currents 351
 - host–parasite interactions 350
 - maturation 353
 - protective/control strategies
 - challenges 360
 - collection, large data sets 361
 - drugs 357–359
 - growth, information systems 361
 - husbandry 356
 - immunostimulation 356–357
 - models and interactions 361–362
 - natural predators 356
 - risk factors 361
 - salmon breeding 356
 - sea lice infestations 360
 - vaccines 359–360
 - rainbow trout 352
 - salt water 350
- Ceratomyxa shasta*
 - adequate test 152
 - ceratomyxosis 143

Ceratomyxa shasta continued

- clinical signs 146
 - diagnosis
 - infection 147–148
 - non-lethal sampling techniques 148
 - presumptive 147
 - spore maturation 146–147
 - external/internal lesions 148
 - genotyping tools 152
 - geographical distribution
 - freshwater 144–145
 - polychaetes 145
 - host distribution
 - parasite strains 145
 - salmon and trout 145
 - impact
 - estimation and mortality 145–146
 - hatcheries 145
 - investigations 152
 - monitoring programmes 152
 - multiple strains 143
 - parasite invasion 152
 - pathophysiology
 - afflicted fish 148–149
 - damage 149
 - granulomatous enteritis 150
 - infections 150
 - protective/control strategies
 - adult salmon carcasses 151
 - disease prevention 150
 - epizootiological model 151
 - stocking 150
 - water sampling methods 151
 - water supplies, hatcheries 150
 - spore stages 143, 144
 - transmission
 - actinospores 144
 - myxospores 143–144
- Cryptobia*-resistant fish 41–42
- Cryptobia* (*Trypanoplasma*) *salmositica*
- adaptive (acquired) immunity
 - live vaccine 42–43
 - metalloprotease-DNA vaccine 43–45
 - body measurements 31
 - chemotherapy
 - Amphotericin B 45
 - isometamidium chloride 45, 46
 - contractile vacuoles 31
 - cryptobiosis
 - chinook salmon 34
 - Fraser River drainage 33
 - in vitro* multiplication 35
 - mortality 34, 35
 - post-spawning 34
 - description 31
 - diagnosis, infection
 - immunological techniques 37

- parasitological techniques 36–37
 - environmental modification and vector control
 - leeches 48
 - water temperature 48
 - immunochemotherapy 48
 - innate (natural) immunity
 - Cryptobia*-resistant fish 41–42
 - Cryptobia*-tolerant fish 42
 - forms 40
 - pathology
 - anaemia 37
 - endovasculitis and mononuclear infiltration 38
 - haemolysis 37–38
 - 200 kDa metalloprotease 38
 - necrosis 38
 - pathophysiology
 - anorexia 38, 39
 - attenuated vaccine strain 39
 - immunodepression 38
 - red blood cell 30, 31
 - salmonid cryptobiosis 35–36
 - serological protection
 - Cs-gp200 40
 - intraperitoneal implantation, cortisol 39
 - mAb-001 antibody 40
 - transmission
 - direct 32–33
 - indirect 32
- Cryptobia*-tolerant fish 42
- Cryptobiosis, *C. salmositica*
- 17 β -estradiol 35
 - chinook salmon 34
 - females 35
 - Fraser River drainage 33
 - mortality 34

Delayed-type hypersensitivity (DTH) 37

Diplostomiasis

- control strategy 266
- diplostomulae 261

Diplostomum spathaceum

- control strategies and prevention
 - epidemics 265–266
 - immunization 266
 - interruption, parasite life cycle 266–267
- fish populations 267
- infection effects, fish
 - acute mortality 264
 - feeding and growth 264–265
 - physiology 264
 - predator avoidance 265
 - types 263–264
- parasite life cycle
 - diplostomiasis 261

- eggs 260
 - host species 260, 261
 - snail 261
 - parasitic cataracts
 - chronic stage, infection 262
 - metacercariae 263
 - parasite-inflicted damage 263
 - relationship, intensity 262
 - pathological effects, eye 263
 - problem, aquaculture 267
 - taxonomy 260
 - trematodes 260
- Enteromyxum* sp.
- clinical signs
 - catarrhal enteritis and myxozoan stages 165
 - distribution 165–166
 - emaciation 164–165
 - epiaxial muscle 164
 - inflammatory response 165
 - intestine 165
 - described, enteromyxosis 163
 - diagnosis
 - detection, spores 166, 167
 - oligonucleotide probes 166
 - tissue damage 166, 167
 - efforts 172
 - in vitro* culture 172
 - intestinal species 163
 - mortality 163–164
 - pathogenicity and invasion mechanisms
 - host–parasite interactions 169
 - plasmodium 169
 - proliferation 169
 - pathophysiology
 - cachectic syndrome 166, 168
 - cytokines 168
 - disruption 167
 - enteroendocrine cells 168
 - immune and detoxification systems 168–169
 - intestinal barrier integrity 168
 - weight reduction 166
 - protective/control strategies
 - characterization, fish immune response 170
 - enzootic waters 171–172
 - fumagillin 170
 - host cellular response 170
 - innate resistance 171
 - land-based facilities 171
 - marine aquaculture 171
 - periodic surveys 172
 - peroxidases and lysozyme (LY) 170
 - salinomycin and amprolium 169–170
 - turbot and antibodies 170–171
 - vaccines, development 171
 - transmission 164
 - water temperature 164
- Epizootiology, *A. crassus*
- cultivation purposes 314
 - investigations 314
 - population genetics data 314
 - prevalence 314
- External/internal lesions
- A. ocellatum*
 - clinical signs 22
 - gill hyperplasia 21, 22
 - B. seriolae* 231
 - C. shasta*
 - adult salmonids 148
 - gills and blood vessels 148
 - parasite triggers 148
 - tissue layers 148
 - H. ictaluri*
 - branchial tissue 181
 - caudal process 184
 - cyst-like structures 182
 - healing process 182, 183
 - infectious agent 183
 - inflammatory cells 181, 182
 - mottled appearance, gills 180, 181
 - myxozoan spores 183, 184
 - PGD infection 181, 182
 - plasmodia development 183
 - remodelling, callus 183
 - wet mount, gill clip 180, 181
 - H. okamotoi* 249, 250
 - L. cyprinacea*
 - chronic inflammation 342
 - collar 343
 - epidermis 342–343
 - haemorrhage 342
 - infection 342
 - larvae 342
 - metamorphosis 342
 - necrosis 342
- Neobenedenia* sp.
- epidermis, *S. dumerili* 232
 - eyes suffered intense pathology, chronology 232
 - farmed fish, lesions 231
 - N.girellae* attachment, epithelium surrounding 232
- N. hirame* 254
- N. perurans*
- chloride and mucous cells 5–6
 - eosinophils 6
 - gills 5
 - inflammatory cells 6
 - interlamellar vesicles formation 5
 - squamation-stratification, epithelium 5

- Gyrodactylus salaris* and *G. derjavinioides*
 anthropogenic transfer, fish 204
 Baltic salmon sampled, freshwater hatchery 193, 194
 biotic and abiotic manipulation, interrupt transmission 203–204
 chemotherapy 203
 clinical signs
 epithelial damage, salmon fin epidermis 199, 200
 infections, hooklets insertion and feeding on epithelium 199, 201
 marginal hooklets penetrating epithelial cells 199, 200
 diagnosis 198–199
 disease impact, fish production 198
 European trout populations 194, 195
 geographical distribution 198
 host location
 colonization, salmon fin 196, 197
 infection 196–197
 immunity
 complement-like activity, host serum and mucus 201
 complement, rainbow trout 202
 host specificity 201
 infection 202–203
 resistance/low susceptibility factor, Baltic salmon 202
 skin mucous cells, salmon 201–202
 parasites
 opisthaptor 195, 196
 ventrally directed hamuli and marginal hooklets 195, 196
 worm migration 195
 pathophysiology, disease 199, 201
 ‘the Norwegian salmon killer’ 193
 transmission 197–198
 zoosanitary measurements and hygiene 203
- Haematocrit centrifuge technique (HCT) 36
- Haplosporidium nelsoni*
 description 92
 diagnosis
 epithelium 97
 sporulation 97–98
 diseases, oyster production
 ballast water 95
 data, Virginia 94, 96
 drought conditions 96
 mortality 94
 populations 96
 genes and proteins 103
 intensification, oyster disease 103
 interactions, *Crassostrea virginica* 103–104
 internal lesions 98
 life stages 93, 94
 maximum annual prevalence 101
 molluscs 93
 pathophysiology
 connective tissues 100
 gill epithelium 100
 infections and mortality 100
 protective/control strategies
 breeding programmes 100–101
 chemotherapeutants 102
 disease-resistant seed 102
 lower salinities 102
 restoration 101
 transmission 102
 wild oyster populations 101
 resolving, life cycle 103
 salinities 93
 spores 94, 95
- HCT *see* Haematocrit centrifuge technique
- Henneguya icaluri*
 actinospores 178–179
 artificial propagation 190
 biological control
 fathead minnows 186
 oligochaete populations 185–186
 smallmouth buffalo 186
 blue and channel catfish hybrids 188
Dero digitata populations and PGD 178
 diagnosis
 affected gills 179–180
 filamental cartilage 180
 infective organism 180
 mortality rates 180
 PCR and PGD 180
 eradication, parasitic diseases 177
 external/internal lesions *see* External/internal lesions
 interaction 179
 investigations 190
 myxozoan life cycle 178
 pathophysiology
 BGD 184–185
 physiological effects, PGD 184
 rainbow trout 185
 respiration 184
 polar capsule 178–179
 pond monitoring
 disadvantages 187
 qPCR assay 188
 quantitative evaluation 187
 sentinel fish and mortalities 187
 stocking 187
 safety, restocking 180
 single batch versus multibatch culture
 dissemination 189
 pond construction 189

- rotating production 188–189
 - stocking 189
- species identification 179
- treatments
 - chemical 185
 - supplemental 186
- Heterobothrium okamotoi*
 - control measures 251
 - description 245
 - diagnosis, infection
 - oncomiracidium 248–249
 - propagation 248
 - egg string 246, 248
 - external/internal lesions 249, 250
 - gill filaments 246
 - host reaction
 - infected fish 249
 - infected puffer 249–250
 - lectin 249
 - infection 245
 - life cycle 246, 247
 - line drawing, *H. okamotoi* 245, 246
 - tiger puffer 251–252
 - worms clustered, infected fish 245, 247
- Ichthyophthirius multifiliis*
 - description 55
 - diagnosis, infection
 - epithelium 59
 - flashing behaviour 58–59
 - gill epithelial cells 60
 - microscopic detection 59
 - trophonts 59
 - disadvantages 66
 - genome sequencing project 66
 - life cycle 55–58
 - pathophysiology
 - cellular damage 60–61
 - inflammatory mediator 60
 - theronts and trophonts 60
 - protective control strategies
 - antibodies 62–63
 - cellular changes 61
 - chemicals and drugs, treatment 65
 - chemokines 61
 - circulating leucocytes 62
 - enzymes 61–62
 - feeding 61
 - gene expression 62
 - immune protection 62
 - plasma lysozyme activity 62
 - temperature 65
 - theronts and trophonts 65–66
 - vaccine development 63–65
 - water management 65
 - protein expression systems 66
 - transmission and geographical distribution
 - epizootic outbreaks 58
 - low-level infections 58
 - temperatures 58
- IDIs *see* Invertebrate developmental inhibitors
- IGS *see* Intergenic spacer
- Immunostimulants, *Miamiensis avidus*
 - CpG motifs 84
 - pathogens, high stress 84
 - triherbal 84
- In-feed treatments
 - advantages and disadvantages 357
 - avermectins 359
 - growth regulators 359
- Integrated Parasite Management (IPM) 238
- Intergenic spacer (IGS)
 - defined 199
 - sequencing, genes encoding ribosomal DNA 195
- Internal transcribed spacer (ITS)
 - gene spanning 199
 - region 216, 221
 - sequencing, genes encoding ribosomal DNA 195
- Invertebrate developmental inhibitors (IDIs) 332
- IPM *see* Integrated Parasite Management
- ITS *see* Internal transcribed spacer
- Lepeophtheirus salmonis*
 - bacteria and viruses 355–356
 - diversity and hosts
 - adaptation 351
 - adult stages 351
 - salmonids 350
 - temperature, light and currents 351
 - three-spined stickleback 350–351
 - feeding habits 354
 - host–parasite interactions 350
 - life cycle
 - body lengths 353
 - cephalothorax 353
 - chalmus stages 352–353
 - egg production 353
 - maturation 353
 - naupliar and copepodid stages 352
 - nuclear steroid receptor 353–354
 - pair formation and mating 353
 - pheromones 352
 - semiochemicals 352
 - temperature 351
 - protective/control strategies
 - challenges 360
 - collection, large data sets 361
 - drugs 357–359
 - growth, information systems 361
 - husbandry 356

Lepeophtheirus salmonis continued

protective/control strategies continued

- immunostimulation 356–357
- models and interactions 361–362
- natural predators 356
- risk factors 361
- salmon breeding 356
- sea lice infestations 360
- vaccines 359–360

salt water 350

sea louse–host interactions *see* Sea louse–host interactions, *L. salmonis**Lernaea cyprinacea*

'anchor worms' 337

anterior process 337, 338

diagnosis, infection

- clinical signs 341–342
- host behaviour 341

distribution

- cyprinids and carp 341
- gill filaments 341
- infection 341
- temperature 341

environmental stressors 345–346

external/internal lesions 342–343

host range

- copepodids 337–338
- cosmopolitan distribution 337
- frogs, tadpoles and axolotl 337–338
- notorious killers 337

larval lernaea 337, 339

life cycle

- development rate 340
- feeding 339–340
- insemination 339
- metamorphosis 338, 339
- nauplius and copepodid stages 339, 340

pathophysiology

- epidermal cells 343
- haematocrit 343
- protective immunity 343–344
- ulcer 343
- weight loss 343

production 341

protection 345

protective/control strategies

- adult females 344
- Doramectin 344–345
- feeds 345
- inorganic chemicals 344
- insecticides 344
- piscine immune system 345
- potassium permanganate (KMnO₄) 344
- sodium chlorite 345
- treatments 344
- water changes 345

red sores 337, 339

vaccination 345

Life cycle

A. crassus

- crustacean species 311
- eel infection 311
- fecundity, estimation 313
- metamorphosis 311
- paratenic hosts 311
- preadult stage 313
- predator–prey interactions 310

I. multifiliis

- cell division 58
- endosymbiotic bacteria 58
- stages 55, 56
- theront 55, 57
- tomont 57–58
- trophont 57

L. cyprinacea

- development rate 340
- feeding 339–340
- insemination 339
- metamorphosis 338, 339
- nauplius and copepodid stages 339, 340

L. salmonis

- body lengths 353
- cephalothorax 353
- chalmus stages 352–353
- egg production 353
- maturation 353
- naupliar and copepodid stages 352
- nuclear steroid receptor 353–354
- pair formation and mating 353
- pheromones 352
- semiochemicals 352
- temperature 351

Loma salmonae

chronic responses and tissue regeneration

- arterial damage 117, 118
- healing, gills 118
- Langerhans cells 117–118
- macrophages and lymphocytes 118, 121
- thrombosis 118, 120

description, MGDS 109

diagnosis

- detection, spores 113
- gills, farmed chinook salmon 111, 112
- histopathology approaches 111, 113

disease, marine netpens 110

early stages and formation

- cellular interactions 113
- chemotherapeutic agents 114–115
- degradation 114
- development, parasite 114
- fibroblasts 115
- host immune response 115
- pillar cells 113, 114
- spore germination 115

- effects, MGDS
 - outbreaks 122
 - rainbow trout 120
 - SGR reductions 120, 122
 - haematology
 - gill damage 119
 - ionoregulatory capacity, MGDS 119–120
 - salmonids 119
 - hatcheries 110
 - host-cell response
 - desmosomes 115, 116
 - neutrophils 116
 - spore degradation 116
 - swelling 116–117
 - infected host cell 113, 114
 - lamina propria 113
 - microsporidians
 - hampering progress and *in vitro* approaches 109–110
 - immunosuppression 109
 - infections 109
 - published reports 110
 - treatment and management programmes 110
 - mortality rates 110
 - pillar cell 111
 - protective and control strategies
 - anti-inflammatory agents 125–126
 - avoidance approaches 123
 - dexamethasone 125
 - environmental modulation 125
 - in vivo* models 124
 - immunomodulators 125
 - marketing ahead, losses 123
 - monensin 124–125
 - rainbow trout 124
 - site fallowing 124
 - spores 123
 - strains 123
 - ultraviolet (UV) treatment 123
 - vaccine prototypes 125
 - rainbow trout 111
 - regulatory effects, water temperature
 - disease development 122
 - factors 122
 - MGDS outbreaks 122
 - thermal unit model 122
 - xenoma formation 122
 - transmission models 111
- Metalloprotease-DNA vaccine, *C. salmositica*
- agglutinating antibodies 45
 - neutralization 44
 - plasmid vaccine 44
- MGDS *see* Microsporidial gill disease of salmon
- Miamiensis avidus*
- 'bumper car disease' 76–77
 - chemotherapeutic approaches
 - chemotherapeutants 82, 83
 - formalin and treatments 82
 - resveratrol 82, 84
 - crustaceans 73
 - cultures 76
 - diagnosis, infection
 - caudal cilia 78, 79
 - silver impregnation 78, 79
 - disease impact, production
 - economic losses 78
 - olive flounder mortality 78
 - skin-to-skin contact 78
 - environmental control
 - antibiotics 82
 - osmolarity 82
 - water temperature 82
 - geographical distribution
 - olive flounder and turbot 78
 - Uronema marinum* 78
 - Uronema nigricans* 78
 - haemorrhages and ulcers, olive flounder 76
 - histopathology and pathophysiology
 - blood vessels 80–81
 - cysteine protease gene 81
 - fish mortality 81
 - inflammatory responses 81
 - red blood cells 80
 - scale pockets 80
 - virulence factors and proteases 81
 - identification and morphological characteristics 85
 - immersion infection
 - artificial abrasion 77
 - cadavers act 77
 - gills and muscles, olive flounder 77
 - moulting, crustaceans 77
 - pH range and blood vessels 77
 - immunostimulants 84
 - internal organs 85–86
 - macroscopic lesions
 - abnormal swimming behaviours 79
 - fin erosion and skin ulceration 76, 79
 - moribund fish and internal organs 79–80
 - silver pomfret 80
 - scuticociliate
 - description 73
 - species 73–75
 - Uronema marinum* infections 76
 - vaccine 84–86
- Microsporidial gill disease of salmon (MGDS)
- cause 109
 - L. salmonae*
 - anti-inflammatory agents 125–126
 - cohabitation transmission 123

Microsporidial gill disease of salmon *continued**L. salmonae continued*

- description 109
- diagnosis 111
- drug treatments 126
- hatcheries 110
- in vivo* models 124
- ionoregulatory capacity 119–120
- mortality rates 110
- neutrophil 116
- outbreaks 122
- SGR reductions 120, 122
- strains 123
- thrombosis 118, 120
- ultraviolet (UV) treatment 123
- vaccination 125, 126
- water temperature 122

Myxobolus cerebralis

- adequate test 152
- characteristics 131, 132
- clinical signs
 - blacktail 136
 - development and severity 136–137
 - granulomatous inflammation 136
 - growth 136
 - whirling disease 136

diagnosis

- detection methods 137, 139
- isolation, spores 138
- PCR 138–139
- purpose 138
- temperature 138
- whirling disease 138

genes 132, 133

geographic distribution

- brown trout 135
- dissemination 135
- spread and detection 135
- whirling disease 134–135

identification, causative agent 151

impact

- economic losses 135
- water temperature 135
- wild trout populations 135

infective phenotypes 131

investigations 152

lesions

- brown trout 140
- cartilage 139–140
- myxospores 139

monitoring programmes 152

parasite invasion 152

pathophysiology

- cartilage 140
- growth rates 140
- osteogenesis 140
- whirling disease 140

polychaetes 151

protective/control strategies

- comparison, fish strains 142
- drug efficacy 141
- environmental factors 140–141
- evaluations 141
- fish culture facilities 141
- interactions 142
- non-salmonids 142
- precautions 143
- recreational purposes, rivers 142
- risk assessment models 141–142
- Tubifex tubifex* populations 142
- whirling disease prevalence 142

transmission

- developmental stages 134
- dissemination 134
- host immune response 134
- intestinal epithelium and sporulation 134
- triacinomyxon actinospore 133–134
- tubificid oligochaete worm 133

Neobenedenia sp.

biological control 238

capsalid biology, ecology and identity 239–240

chemical treatments versus vaccines 238–239

control strategy

N. melleni 236

NYA 236

sea-cage aquaculture, freshwater baths 236

serine and cysteine proteases 237–238

diagnosis, infection

B. seriolae 227, 228

marine sea-cage aquaculture 228–230

external/internal lesions

epidermis, *S. dumerili* 232

eyes suffered intense pathology, chronology 232

farmed fish, lesions 231

N. girellae attachment, epithelium surrounding 232

farm husbandry, IPM and mathematical models 238

pathophysiology

Capsalid 232

eyes, *N. melleni* 233

heavy parasitaemia 233

strategy, protection 235–236

technologies 239

'treatments' 233

Neoheterobothrium hirame

diagnosis, infection

adult worms 253

- behavioural changes, olive flounder 254, 255
- external/internal lesions 254
- geographical distribution 252, 254
- infection, anaemia 257
- olive flounders 252
- pathophysiology 254–255
- pedunculate clamps 252, 253
- protective/control strategies
 - control measures 256–257
 - host reaction 256
- Neoparamoeba perurans*
 - AGD infections 1
 - clinical signs
 - endosymbionts 3–4
 - PCR, gill swabs 3
 - white gross lesions 3, 4
 - coho salmon 2
 - description 1
 - eukaryotic endosymbiont 12
 - external/internal lesions 5–6
 - geographic distribution 3
 - in vitro* culture 2
 - isolated amoebae 2
 - mortalities 3
 - Paramoeba pemaquidensis* 1
 - pathophysiology
 - chloride cells reduction 6
 - epithelial hyperplasia 6–7
 - gene expression changes 7
 - haemoglobin subunit beta 7
 - heart morphology 6
 - respiration 6
 - protective/control strategies
 - cage netting and fouling 11
 - copper sulfate concentrations 11
 - disinfectants 9
 - freshwater bathing 8–9
 - immunostimulants 9
 - levamisole 9
 - oral treatments 9
 - resistance, exposure 9, 10
 - selective breeding 9
 - stocking density 11
 - vaccination 9
 - salinity 3
 - salmon farms 1
- New York Aquarium (NYA)
 - destroyed corneas, host species 231
 - N. melleni* 226, 227, 236
 - sodium chloride treatments 236
- Olive flounder *see* *Miamiensis aoidus*
- PAIC *see* Polyclonal antibodies-conjugated drug
- PCR *see* Polymerase chain reaction
- Perkinsela amoebae*-like organisms (PLOs) 3–4
- Perkinsus marinus*
 - cells 92–93
 - diagnosis
 - cells 97
 - RFTM 97
 - watery tissue 96–97
 - diseases, oyster production
 - ballast water 95
 - data, Virginia 94, 96
 - drought conditions 96
 - mortality 94
 - populations 96
 - ecological restoration 103
 - genes and proteins 103
 - intensification, oyster disease 103
 - interactions, *Crassostrea virginica* 103–104
 - internal lesions 98
 - oyster–parasite system 103–104
 - pathophysiology
 - connective tissues 99
 - infections and epithelium 99
 - proteins 100
 - reproduction 99
 - water temperatures 99
 - protective/control strategies
 - breeding programmes 100–101
 - chemotherapeutants 102
 - disease-resistant seed 102
 - lower salinities 102
 - restoration 101
 - transmission 102
 - wild oyster populations 101
 - temperature 92
- Polyclonal antibodies-conjugated drug (PAIC) 48
- Polymerase chain reaction (PCR)
 - detection methods 138–139
 - Hennequya ictaluri* infection 180
 - primers 147, 148
- Proliferative gill disease (PGD), *H. ictaluri*
 - description 177
 - infected channel catfish, gills 183, 184
 - outbreaks 178, 186–188
 - smallmouth buffalo 186
 - stocking, fingerlings 187
- Pseudodactylogyrus anguillae* and *P. bini*
 - aquaculture enterprise 221
 - clinical signs and behavioural effect, infection
 - eels 218
 - control strategies
 - chemotherapy 220
 - immunity 219
 - zoosanitation 221
 - diagnosis
 - hamuli 216, 217
 - infection 216

- Pseudodactylogyrus anguillae* and *P. bini* continued
 disease impact, wild and farmed fish 216
 geographical distribution 215–216
 host location
 attachment, primary gill filament
 median part 210, 212
 congeners 211
 gill filaments 211, 212
 macroscopic and microscopic lesions
 extensive gill tissue reaction 218, 220
 extensive hyperplasia 218, 219
 monogenean gill parasites 209
 parasite
 adult 210
 hamulus tip 210, 211
 nervous system 210, 212
 species 209
 pathophysiology, disease 218–219
 transmission
 fully embryonated egg, oncomiracidium
 P. anguillae 213, 214
 life cycle, *Pseudodactylogyrus*
 parasites 213
 newly produced and undeveloped egg
 213, 214
 post-larva, *P. anguillae* 213, 215
- Ray's fluid thioglycollate medium (RFTM) 97
 Red vent syndrome (RVS) 303
 Reproduction, *A. crassus*
 gene expression 319
 population level 320
 swimbladder infection 319–320
 Restriction fragment length polymorphism (RFLP)
 199
 RFLP *see* Restriction fragment length
 polymorphism
 RFTM *see* Ray's fluid thioglycollate medium
- Salmonid cryptobiosis
 clinical signs 35–36
 diagnosis, infection
 immunological techniques 37
 parasitological techniques 36–37
- Sanguinicola inermis*
 aporocotylids 279
 control measures 278
 diagnosis and clinical signs
 carp fingerlings 273, 274
 eggs, kidney smear 273, 274
 sanguinicoliasis 273
 immune responses
 cercariae and adults 277
 complement activity 277–278
 eosinophils 276
 humoral 277
 T-cell and B-cell mitogens 277
 impact, fish production
 disease problems 272
 mortalities 272, 273
 internal lesions pathology
 adult, carp fingerling bulbus arteriosus
 273, 274
 chronic effects 276
 eggs, carp fingerling gills 273, 275
 hyperplasia 273
 periovarian granulomas 275
 life cycle
 carp 270–271
 cyprinid fish 271
 eggs 271–272
 snails 272
 parasite
 adult 270, 271
 blood vascular system, freshwater
 cyprinid fish 270
 pathophysiology 276
 S. inermis–carp model 278–279
- Sanguinicoliasis
 diagnosis 273
 elimination, carp ponds 278
 organ systems pathophysiological
 impairment 278
 prevalence 272
 treatment failure 278
- Sea louse–host interactions, *L. salmonis*
 attachment and feeding 354
 emamectin benzoate 355
 mobile life stages 355
 neutrophil infiltration 354–355
Salmo spp. infections 355
 trypsin and PGE₂ 355
- Specific growth rate (SGR) reduction 120, 122
 Squash plate method 287
 Stomach crater syndrome, cod
 gross appearance 301, 302
 simplex third-stage larvae, stomach wall 302
- Treatments
 A. foliaceus
 branchiuran infection 332
 IDLs 332
 nervous system 332
 organochlorine and
 organophosphate 332
 parasite infection 332
 H. ictaluri
 actinospore stage 186
 agents 185
 chloride levels 186
 drug application 185

-
- fish mortality and morbidity 186
 - fumagillin 185
 - life cycle, myxozoans 186
 - oligochaete host 185
 - palliative therapies, PGD 186
 - Turbot *see Miamiensis avidus*
 - Vaccines
 - I. multifiliis*
 - fish protection 63–64
 - heterologous molecules 65
 - i-antigens 64–65
 - immunization 64
 - theronts and trophonts 64
 - L. salmonis*
 - proteases 359
 - sea lice egg proteins 360
 - M. avidus*
 - antigen presentation 84–85
 - cell lysates 85
 - i-antigen variations 85
 - intraperitoneal injections 84
 - metabolizable oils 85
 - metalloprotease-DNA 86
 - tubulin 85
 - 'Velvet disease' 22
 - Whirling disease
 - clinical signs 136
 - described 135
 - diagnosis 138
 - impact 135
 - susceptibility 137
 - T. tubifex* 142
 - Zoosanitary measurements and hygiene 203