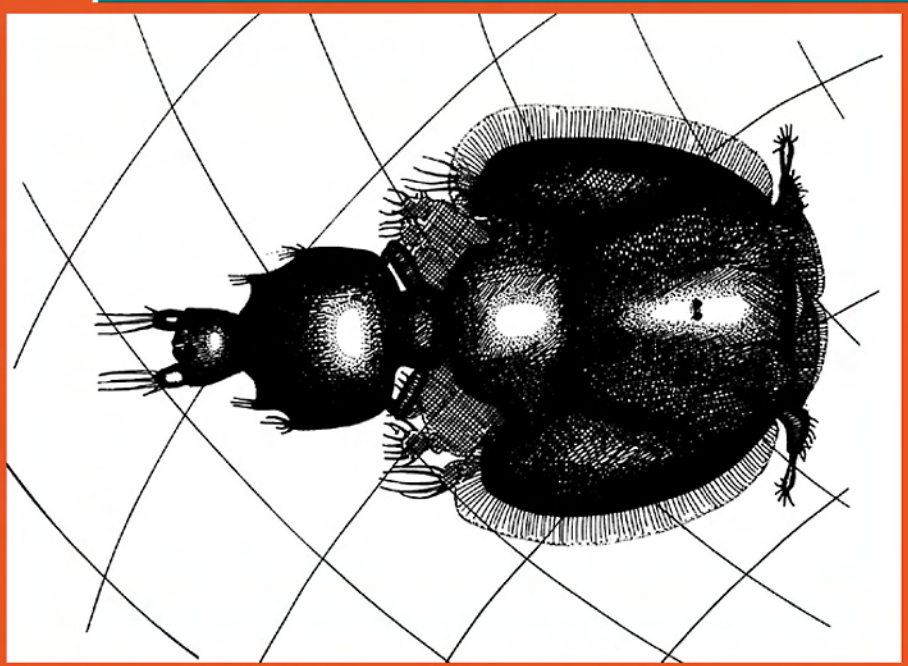


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AQUACULTURE AND FISHERIES SUPPORT

PATHOGENS OF WILD AND FARMED FISH: sea lice

editors

Geoffrey A. Boxshall and Danielle Defaye



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Sea Lice

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PATHOGENS OF WILD AND FARMED FISH

Sea Lice

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Programme AIR, DGXIV
Commission of the European Communities



ELLIS HORWOOD

NEW YORK LONDON TORONTO SYDNEY TOKYO SINGAPORE



First published 1993 by
Ellis Horwood Limited
Market Cross House, Cooper Street
Chichester
West Sussex, PO19 1EB
A division of
Simon & Schuster International Group

This edition published in the Taylor & Francis e-Library, 2006.

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Library of Congress Cataloging-in-Publication Data

Available from the publisher

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN 0-203-01132-5 Master e-book ISBN

ISBN 0-203-19133-1 (Adobe eReader Format)
ISBN 0-13-015504-7 (hbk)

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Preface

Sea lice are a serious problem for commercial salmon farming in the Northern Hemisphere and for the smaller-scale coastal fish farms around the Mediterranean Sea and in South-East Asia. Sea lice are parasitic copepods which typically infest the external surfaces of marine and brackish-water fish. The family Caligidae, to which the sea lice belong, comprises over 400 species but only a handful of these species have been reported as pests in fish-farming facilities. Although common on wild fish, sea lice rarely occur in epizootic proportions in nature. On farms, however, they may account for losses in excess of 10% of total production.

Since the rapid growth of the salmon-farming industry during the 1970s, there has been considerable interest in the development of methods for the control of sea lice. Initially research was concentrated on chemotherapeutic methods of control but in the last decade research activity has also been directed towards providing basic information on the biology of caligid sea lice as well as towards the development of novel methods for their control. The international nature of the sea lice problem led to the establishment of numerous independent research programmes worldwide and it became apparent that the time was right for an international workshop, focused on the biology and control of sea lice, that would provide a medium for the exchange of the latest results and ideas between research groups.

The workshop was organized by G.A.Boxshall (London) and A.Raibaut (Montpellier) and was held in Paris on the 3 and 4 September 1992 during the First European Crustacean Conference. It was attended by over 80 researchers and fish health experts interested in caligid sea lice or their relatives within the Crustacea. This volume comprises the majority of the oral and poster papers on sea lice presented during the workshop, plus additional contributions from some researchers who were unable to come to Paris.

We are grateful to the main organizing committee of the host conference in Paris for their work and wholehearted support of the sea lice workshop. We would like to thank the following organizations for their generous support of the host conference: the Université Pierre et Marie Curie, the Muséum National d'Histoire Naturelle, the

École Normale Supérieure, the Centre National de la Recherche Scientifique, the Institut Français de Recherche pour l'Exploitation de la Mer, the Ministère des Affaires Étrangères, the Ministère de l'Éducation Nationale et de la Culture, the Ministère de la Francophonie, the Commission of the European Communities (DG XII), the United Nations Educational Scientific and Cultural Organization, the Crustacean Society, the International Association of Astacology, the Parc National des Cévennes and the Aquariums Coutant.

We are especially grateful to the Commission of the European Communities (DG XIV) Program AIR, for a generous grant which enabled us to provide financial support for some participants and which supported the publication of this volume. We would like to acknowledge additional financial support for publication, received for the organizing committee of the conference.

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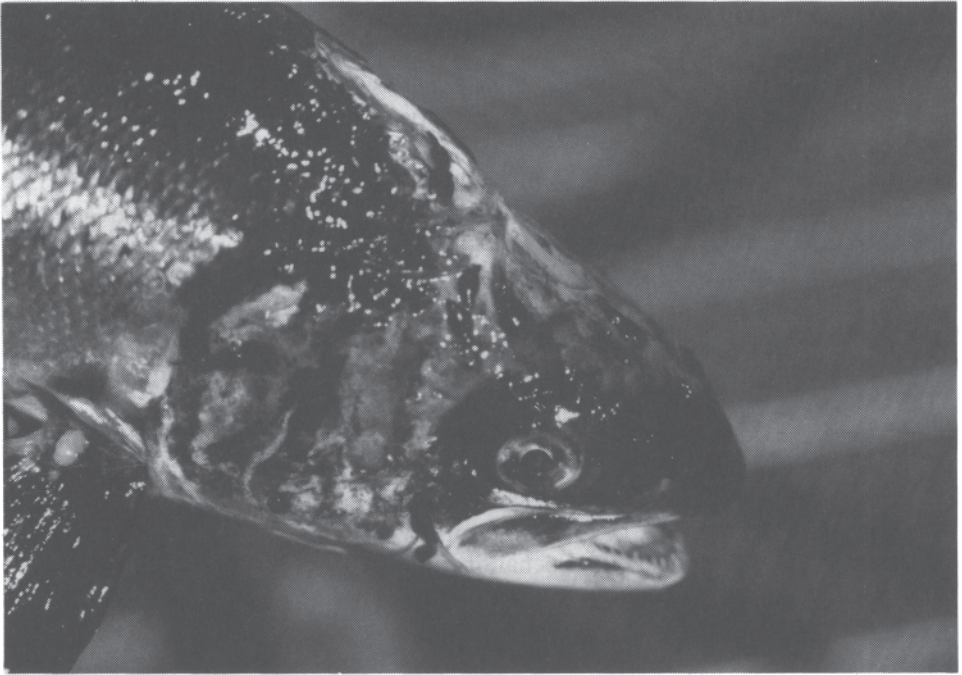
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Frontispiece. A farmed Atlantic salmon, showing the lesions caused by sea lice, *Lepeophtheirus salmonis*. The brain of the salmon has been exposed by the feeding activity of the sea lice. Photograph by James Bron.

Part I Biology of sea lice

Part Ia Life cycle stages

1

Life history of *Caligus epidemicus* Hewitt parasitic on tilapia (*Oreochromis mossambicus*) cultured in brackish water

Ching-Long Lin and Ju-shey Ho

ABSTRACT

The life history of *Caligus epidemicus* was studied by infesting fingerling tilapia with copepodids reared in the laboratory at $24.5 \pm 0.5^\circ\text{C}$ in 20 ppt water. Eleven stages were found, including two nauplii, one copepodid, six chalimus stages, one preadult stage and the adult. First nauplii moulted into second nauplii in about 6 h and the latter in turn moulted into copepodids in 14 h. The period between attaching to the host and production of egg sacs is about 15 days. Sexual dimorphism appeared first in chalimus IV. Mate guarding was observed as early as female chalimus V. Nauplii hatched approximately 28 h after the appearance of eggs in the newly formed egg sac. The process of hatching involved two steps: breaking of the chamber (egg sac wall) and rupture of the egg membrane. The ontogeny of *C. epidemicus* comprises the largest number of instars in the caligid copepods studied so far. The addition of two chalimus stages is chiefly due to the delay in the formation of the adult armature of leg 4.

INTRODUCTION

Caligus epidemicus Hewitt is a parasite of low-salinity water in the coastal and estuarine zones of the western Pacific. It has been reported from both wild (Hewitt 1971, Roubal 1981) and cultured (Natividad *et al.* 1986) fishes. Ruangpan and Kabata's (1984) discovery of *C. epidemicus* on the cultured tiger shrimp (*Penaeus monodon* Fabricius) from Chantaburi Province, Thailand, is most unusual, not only for this species but for the entire family of sea lice (Caligidae).

The occurrence of *C. epidemicus* in Taiwan was first noticed in March 1990 on the Mozambique tilapia (*Oreochromis mossambicus*) being reared in the salt-water

ponds at the Tainan branch of the Fisheries Research Institute of Taiwan. The infestation was so severe that many fish were killed. Since our knowledge of the life history of *C. epidemicus* is still incomplete—only a few larval stages were reported by Hewitt (1971)—a study on its ontogeny was undertaken using ovigerous parasites taken from moribund tilapia. An overview of the life history of *C. epidemicus* is given here but a detailed account of the morphology of each larval stage will be reported separately.

MATERIALS AND METHODS

Ovigerous *C. epidemicus* taken from the moribund tilapia were kept one each in a Petri dish (35 mm in diameter) filled with 20 ppt sterile brackish water. After removing the host debris (tissues, scales, mucus, etc.) and replacing with clean water, the dishes (each containing an ovigerous parasite and 10 ml of 20 ppt sterile water) were placed in a shaker bath maintained at $24.5 \pm 0.5^\circ\text{C}$. Water was changed every 6 h.

Nauplii hatched in the dish were transferred with a wide-mouth pipette to a new dish. Then, every 2 h, two nauplii were removed and fixed in 10% formalin for examination. At each water change exuviae and dead nauplii were also removed.

All copepodids that moulted from the nauplii within an hour of one another were collected in one dish. Twenty of them were then randomly selected and transferred to a 250 ml beaker containing 100 ml of 20 ppt brackish water and a fingerling Mozambique tilapia (24–30 mm in length). Twenty such replicates were prepared. Additional replicates were made to keep all copepodids from the same clutch of eggs in a dish for study of their survival in the absence of the host. After removing all nauplii, the female parasite left in the dish was kept in 21–29 ppt brackish water at $10\text{--}23^\circ\text{C}$ in order to study new egg sac production.

To ensure good water quality in the beaker, fish were not fed throughout the experiment and faeces were removed as soon as they appeared, particularly during the first 6 h. Following introduction of copepodids, one fish was sacrificed and fixed in 10% formalin every 4 h to ascertain whether the copepodids had attached. When the parasite reached the chalimus stage, removal of host fish for fixation and examination was carried out every 12 h during the earlier stages of ontogeny and every 24 h during the later stages. Water changes were made every day.

Preserved larvae and adults were cleared in lactic acid and examined both entire and dissected. The copepodid and chalimus larvae that attached to the scales or fins were removed from their hosts together with the attached tissue. Dissection of larvae was done under a dissecting microscope with a pair of fine needles. Between four and six specimens of each stage were dissected for examination of the changes in the appendages.

RESULTS

Ovigerous *C. epidemicus* obtained from the pond-cultured tilapia may carry as many as 21 eggs in each of its egg sacs. The egg number can differ slightly between the two sacs of the same individual. As shown in Fig. 1, our rearing experiments indicate that the life cycle of *C. epidemicus* is rather long, comprising two nauplii, one copepodid, six chalimus stages, one preadult stage and the adult. Each stage is separated by a moult.

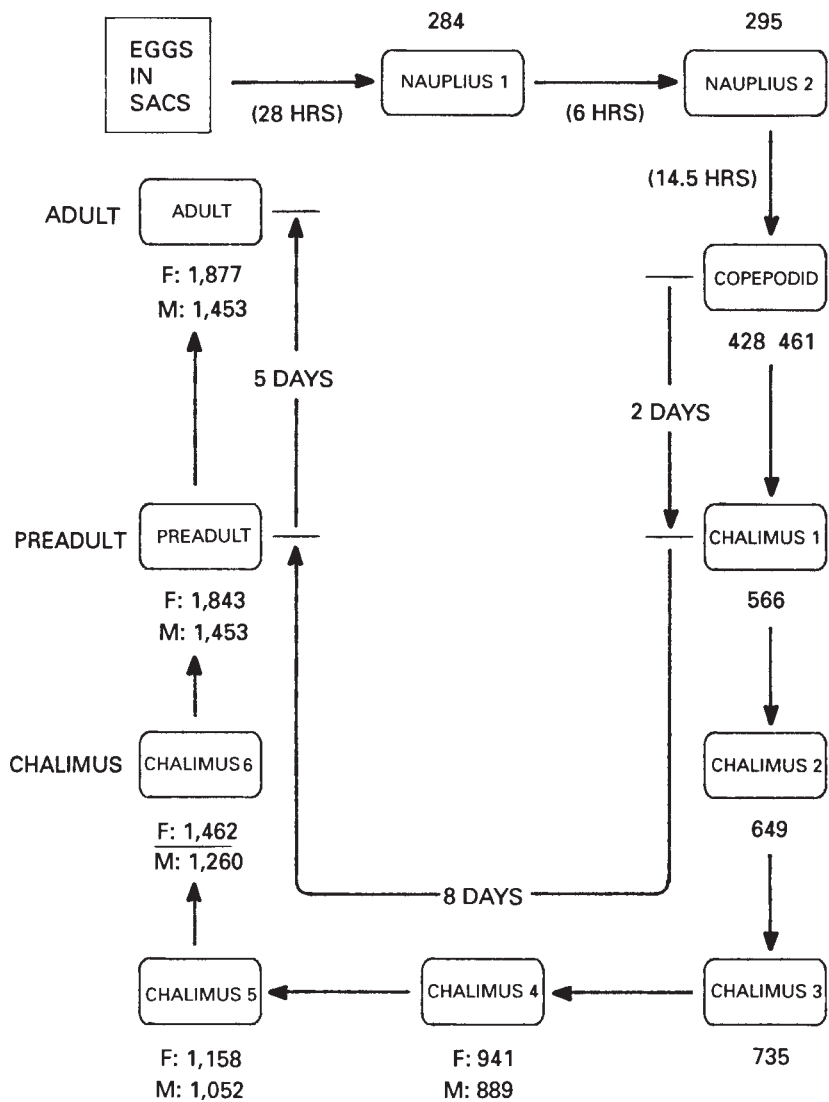


Fig. 1. Flow chart depicting the life cycle of *C. epidemicus*. Numbers next to the stage are average body lengths in micrometres. The larger of the two numbers under COPEPODID is for the one with the frontal organ, and the smaller, the one without.

Hatching

The cytoplasm of newly formed eggs in the egg sac is uniformly distributed. But, a few minutes later, it concentrates on the medial side (towards the central axis of the parasite body) and the egg colour changes from colourless to green. Approximately 6 h later,

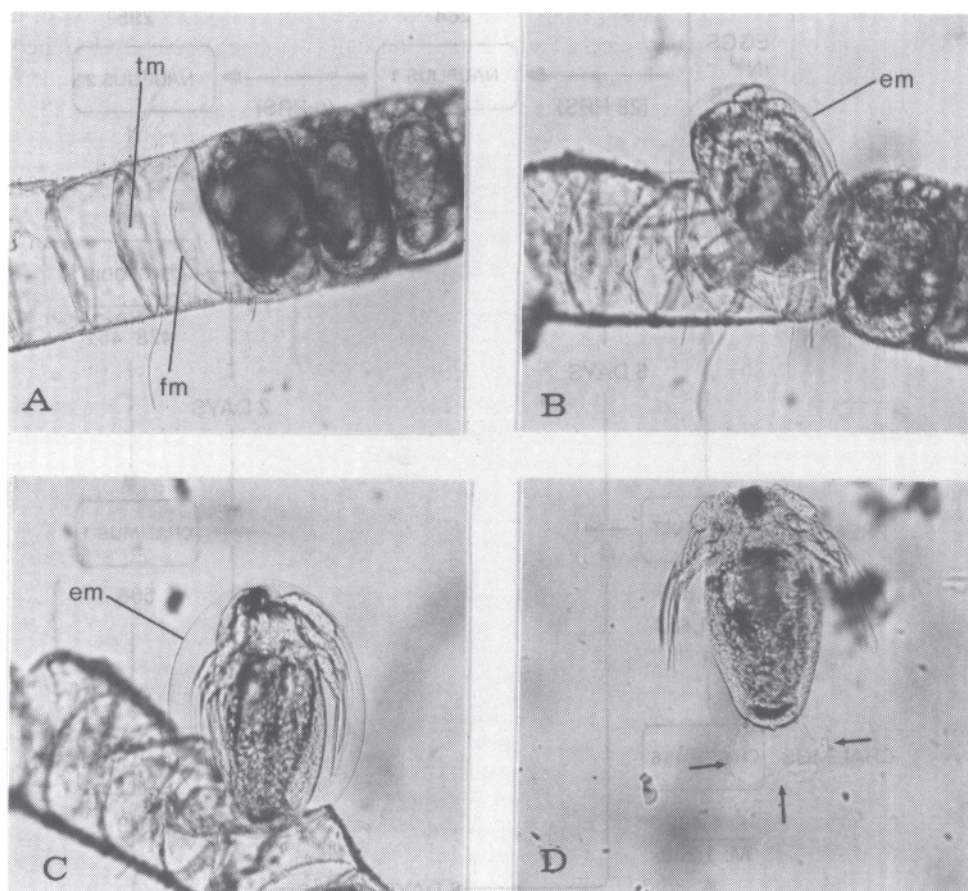


Fig. 2. Hatching of *C. epidemicus*. (A) Swelling of the egg membrane, (B) rupture of the egg sac chamber and (C) emergence of the hatching egg. Note the egg membrane wrapping around the nauplius in C. (D) Newly hatched nauplius I with ruptured egg membrane (indicated by arrows) caught on the balancers. Abbreviations: em=egg membrane, fm = closely attached egg and transverse membranes, tm=transverse membrane.

the dark pigment on both medial and outer sides appears in the distal egg in the sac. In about 4 h, all eggs in the sac appear with such peripheral, dark pigment. At this time the movement of the embryo within the egg membrane is noticeable. The ovigerous parasite frequently raises its egg sacs and vibrates them horizontally. Hatching of nauplii commences from the distal end of the sac and proceeds proximally in sequence.

All embryos in the egg sac invariably have their head ends facing outward, so as to facilitate the releasing of nauplii at hatching. Shortly before hatching, the egg swells (Fig. 2A) (by absorption of water) until it bursts the egg sac (Fig. 2B). After a few minutes, while the egg is still sitting in its own compartment in the egg sac, the nauplius within the egg membrane spreads out its appendages (Fig. 2C) and, with a vigorous movement, rapidly ejects itself out of the egg membrane. Newly hatched nauplii are motionless and carry the broken egg membrane on their balancers (arrowed

in Fig. 2D). At the beginning, the nauplius holds its appendages out, trailing from the head end, but a few seconds later it starts to swim with the typical jerky, naupliar movement.

New egg sac formation

After all the eggs in the two sacs have hatched, the empty sacs may remain attached to the parasite for some time. However, if there are mature eggs in the oviducts, the empty sacs become detached about 5 min after completion of hatching. In some instances, the egg sac was discarded even before completion of hatching all the eggs. About 20 min after detaching the old egg sac, a new sac bud appears, and almost immediately the first egg is extruded into the sac bud. Thenceforth, the eggs are extruded into the new sac one after the other. It takes 2–4 min to fill a sac with 19 or 20 eggs. If there are no mature eggs in the oviduct, the sac bud may extend to a certain length and remain without eggs for some time.

Usually, the number of eggs in the new sacs is less than in the previous pair. Hatching of those newly produced eggs does not start until about 2 days later. Following ovulation, the genital complex appears empty, but in about 2 h eggs reappear in the oviduct and the genital complex turns greyish-green. In our experiments, two new sets of egg sacs were produced by a female parasite kept alone in a culture dish without host.

Nauplius

The first nauplius is elliptical and weakly phototactic. About 6 h following hatching, it moults into the second nauplius. The moulting begins with a break of the cuticle at the anterior end of the larva. First, the cuticle splits horizontally between the bases of the antennules and then the nauplius II moves forward to escape from the old cuticle. The second nauplius is only 11 μm longer than the first (Fig. 1) and, morphologically, it is distinguishable by the presence of a bifurcate ventral sclerite on the posterior third of the body and a slight protrusion of the posterior end between the balancers.

The second nauplius lasts for about 14.5 h. With the approach of moulting, it becomes less active and sinks to the bottom of the dish. It moults in the same manner as nauplius I. After the commencement of the second moult, it takes about 3 h for an entire clutch of 42 nauplius II stages to moult into copepodids.

Copepodid

The copepodid is initially much more active than the nauplius, moving swiftly in the water with the two pairs of one-segmented, biramous legs. Later, however, the copepodid spends more and more time resting on the bottom of the dish. The survival time of this larva without food (host) was about three to four days. However, when a fish is introduced, the larvae suddenly become active and attach to the host by means of their strong, prehensile antennae. Some time after securing a place on the host, the copepodid shows through its cuticle a large frontal organ anterior to the eyes and between the bases of the antennae. As indicated in Fig. 1, the copepodid with the frontal organ (containing the frontal filament) is slightly larger than the one without. In about two days, the copepodid develops into the first chalimus, which attaches to the fish by means of a frontal filament.

Table 1. Developmental changes of armature of legs 1–4 of *C. epidemicus*. Roman numerals indicate spines and Arabic numerals denote setae. Semicolon indicates segmentation of ramus. Numbers in parentheses indicate armature without limb bud. Abbreviations: ADT=adult, CH1–6 =chalimus I–VI, COP=copepodid, enp=endopod, exp=exopod, PRA=preadult

Stage	Leg 1		Leg 2		Leg 3		Leg 4
	exp	enp	exp	enp	exp	enp	exp
COP	I,II,I,3	6	I,II,I,3	6	(2)	—	—
CH1	1,7	2	1,7	1,6	5	4	(2)
CH2	1,3,1,3	2	1–0;7	0–1;6	1,6	1,3	(2)
CH3	1–0;3,4	2	1–0;7	0–1;6	1,6	1,3	2
CH4	I–0;III,1,3	2	I–1;II,I,5	0–1;7	I–0;III,4	0–1;4	1,3
CH5	I–0;III,1,3	2	I–1;III,I,5	0–1;8	I–0;IV,5	0–1;5	1,3
CH6	I–0;III,1,3	2	I–1,I–1;II,I,5	0–1;0–2;6	I–0;I–1;III,4	0–1;6	1,3
PRA	I–0;III,1,3	2	I–1;I–1;II,I,5	0–1;0–2;6	I–0;I–1;III,4	0–1;6	I,1,II
ADT	I–0;III,1,3	2	I–1;I–1;II,I,5	0–1;0–2;6	I–0;I–1;III,4	0–1;6	I,1,II

Chalimus stages

The antennae, which functioned as the major attachment organ in the copepodid, are markedly degenerated in chalimus I. In about 12 h the larva moults into chalimus II, which lasts about one day, and then moults into chalimus III. These first three chalimus stages have a similar body form, comprising a large, elliptical, non-segmented prosome and a cylindrical, poorly segmented metasome. Apart from size, the other differences shown between them are the segmentation and armature of their legs (Table 1).

The third chalimus lives for about a day and moults into chalimus IV. The body of this new chalimus stage is quite different from the previous three stages. It assumes a typical caligid form with a broad, saucer-shaped cephalothorax comprising the cephalosome plus the fused first three pedigerous somites. The frontal filament of chalimus IV is characteristic in having a node midway along its length. Interestingly, the section of this filament distal to the node is about as long as the entire filament of the preceding chalimus III. Sexes are distinguishable for the first time in this stage by the shape of the posterolateral corners of the genital complex: rounded in the female but protruded in the male. The lunules, hyaline marginal membranes around the cephalothoracic shield, sternal furca, and limb buds of leg 4 are also formed in this stage.

Passing three more moults, approximately 1 day apart, the larvae develop through two more chalimus stages (fifth and sixth) to become preadults. However, unlike chalimus IV, the frontal filaments of chalimus V and chalimus VI are shorter and lack the node at midlength. The reproductive organs can be seen in the genital complex of chalimus IV but not in chalimus V.

Preadult

Frequently, the larvae of this stage are found attached with a frontal filament to the host. However, they are easily detached. Both sexes attain the adult complement of armature on leg 4 in this stage (Table 1). However, fine structures of the armature still differ from those of the adult.

When a male preadult attaches in the vicinity of a female larva, it attempts to grasp the female. We observed this attempted grasping behaviour several times as male preadults ‘swing’ on their frontal filaments, trying to reach nearby female larvae.

Mating

Approximately eight days are required for the male to develop from the first chalimus to a mature adult. Mating pairs can be seen in one of the three following situations: both members attached (to fish with the frontal filament); a free male holding an attached female; or both members free. In the last situation, the mating pair may move together on the fish body, on the bottom of the beaker or in the water. Analysis of 13 mating pairs revealed that the male is invariably in the adult stage but the female is not; it can be in chalimus VI (30%) or chalimus V (8%) in addition to the preadult (62%).

In amplexus, the male holds the female from behind with its antennae clasping the partner’s ‘waist’ (between the fourth pediger and the genital complex) in a precopula position as described for *Lepeophtheirus pectoralis* (Müller) by Anstensrud (1990). When they separate after copulation, the female carries a pair of spermatophores on the ventral surface of the genital complex towards the midposterior margin. Five days after separation, egg sacs begin to appear and about 28 h later hatching of these new eggs begins.

DISCUSSION

Complete developmental cycles have so far been studied for 12 species of Caligidae (Table 2). It is interesting that while the larval stages of the four species of *Lepeophtheirus* are consistent in comprising two nauplii, one copepodid, four chalimus stages and two preadult stages, the numbers of chalimus and preadult stages in the eight species of *Caligus* are variable. However, aside from *Caligus centrodoni* Baird, the rest of the *Caligus* species have the same total number of developmental stages (see references in Table 2). Thus the documented information indicates that the life cycle of *Caligus* comprises nine stages and that of the *Lepeophtheirus* ten stages.

The developmental cycle of *C. epidemicus* on tilapia clearly differs from this norm. It has two additional stages in the chalimus phase—11 stages in total. Examination of developmental changes in the leg armature (Table 1) revealed that the addition of chalimus stages is essentially due to the delay in the formation of leg 4. The limb

bud of this leg does not appear until chalimus III (see Table 1), while in the other species of *Caligus* it appears in chalimus II. With the addition of two larval stages, the time required for completion of the life cycle is consequently longer in *C. epidemicus*.

Table 2. Number of stages in the five developmental phases of caligid copepods. Abbreviations: N=nauplius; C=copepodid; Ch=chalmus, Pa=preadult, A=adult

	N	C	Ch	Pa	A	Total	Reference
<i>Caligus</i>							
<i>centrodonti</i>	2	1	4	?	1	(8)	Gurney (1934)
<i>curtus</i>	2	1	4	1	1	9	Heegaard (1947) ^a
<i>orientalis</i>	2	1	4	1	1	9	Hwa (1965) ^a
<i>spinosus</i>	2	1	4	1	1	9	Izawa (1969) ^a
<i>clemensi</i>	2	1	4	1	1	9	Kabata (1972)
<i>minimus</i>	2	1	5	—	1	9	Caillet (1979)
<i>pageti</i>	2	1	4	1	1	9	Ben Hassine (1983) ^b
<i>elongatus</i>	2	1	4	1	1	9	Hogans and Trudeau (1989)
<i>epidemicus</i>	2	1	6	1	1	11	(present work)
<i>Lepeophtheirus</i>							
<i>dissimulatus</i>	2	1	4	2	1	10	Lewis (1963) ^a
<i>hospitalis</i>	2	1	4	2	1	10	Voth (1972) ^a
<i>pectoralis</i>	2	1	4	2	1	10	Boxshall (1974b)
<i>salmonis</i>	2	1	4	2	1	10	Johnson and Albright (1991)

^aStages based on Kabata’s (1972) interpretation

^bTwo stages of preadult in male.

According to Ben Hassine (1983), at temperatures between 24 and 26°C the developmental cycle of *Caligus pageti* Russell was completed in 10–11 days, depending on the water salinity. In our study of *C. epidemicus* at about the same temperature (24.5±0.5°C), it took 17 days to develop from hatching to the ovigerous female. Regrettably, published accounts of the development of other species of *Caligus* do not contain sufficient information for further comparison on the duration of the developmental cycle.

Heegaard (1947) reported that the egg sac of *Caligus curtus* Müller ‘is divided by partitions into a number of chambers each enclosing an egg’. However, such partitioning of the egg sacs into chambers was not confirmed by Boxshall (1974a) in his study of the development of *L. pectoralis*. Nevertheless, our study of *C. epidemicus* shows that there is a transverse membrane located in the egg sac at the distal end of each egg. This membrane is discernible only after the egg is hatched (see tm in Fig. 2A). When the egg swells prior to hatching, the egg membrane is pushed closely against the transverse membrane and appears as one thick membrane (fm in Fig. 2A).

Information on the hatching in caligids is still scanty. Wilson (1905), Lewis (1963), Hwa (1965), Izawa (1969) and Johannessen (1978) briefly described the hatching of caligid nauplii. They all allude to hatching as a one-step process involving only a break of the egg sac wall by the nauplius. However, Boxshall (1974a) reported that hatching in *L. pectoralis* involved two steps: a transverse splitting of the egg sac

membrane, followed by expansion and rupture of the egg membrane to release the nauplius. As shown in Fig. 2, the hatching in *C. epidemicus* also involves two stages: (1) swelling of the egg (Fig. 2A) to break down the wall of the chamber (Fig. 2B,C); and (2) rupture of the egg membrane to project away the nauplius (Fig. 2D). Similar hatching, however, has been reported by Schram (1979) for *Lernaeenicus sprattae* (Sowerby) (Pennellidae) and by Piasecki (1989) for *Tracheliastes maculatus* Kollar (Lernaeopodidae).

Our experiments on the production of new egg sacs in female *C. epidemicus* do not support Heegaard's (1947:33–34, 1959:232) contention on the formation of tubular-shaped egg sacs. He hypothesized that the shaping of the egg sac into a tubular form was an external mechanism, due to the resistance of the water acting 'as a backward-directed pull or drag on the newly laid egg masses' as the fish leaped forward. In our experiments, all egg sacs produced by the female *C. epidemicus* kept alone in dishes assumed the same tubular form as those produced by females attached to the fish. We did not see any egg sacs produced in the dish bearing the same irregular forms as shown by Heegaard (1947: fig. 3, 1959: fig. 2) in his experiments with *C. curtus*.

Heegaard's (1947) account of the formation of the first frontal filament by the copepodid was confirmed by Lewis (1963). However, they are not in agreement with each other on the fate of this first frontal filament. Heegaard (1947:93–94) claimed that a new filament was secreted at each subsequent moulting throughout development, but Lewis's (1963:240) observations showed that the original filament remained attached to the developing larvae. Our observations on the development of *C. epidemicus* show a third possibility concerning the formation of the frontal filament. We shall discuss this matter in detail in our later work in connection with the 'node' on the frontal filament of chalimus IV.

Mate guarding was not mentioned in all previous works listed in Table 2. Anstensrud (1992) studied in detail mate guarding in *L. pectoralis*. According to him the male, contrary to Boxshall's (1990) observations, never establishes precopula (mate guarding) with chalimus larvae. In *C. epidemicus* the youngest female being guarded by an adult male is chalimus V, whereas in *L. pectoralis*, as reported by Boxshall (1990), the guarding can occur as early as when the female is in her chalimus IV.

ACKNOWLEDGEMENTS

The senior author (C.L.L.) would like to express his particular thanks to Shiu-Nan Chien and Guang-Hsiung Kuo (National Taiwan University) for their financial support and the use of their laboratory facilities during the present studies, and to I-Chiu Liao (Director General, Taiwan Fisheries Research Institute) and J.C. Lee (Head of Fisheries, Council of Agriculture) for their constant support and encouragement. Chin-Lii Wu (Tainan Branch of Taiwan Fisheries Research Institute) is acknowledged for her skilful assistance in various facets of the experiments. Completion of the work was supported in part by California State University, Long Beach, to the junior author (J.S.H.)

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2

Developmental stages of *Caligus punctatus* Shiino, 1955 (Copepoda: Caligidae)

Il-Hoi Kim

ABSTRACT

Hundreds of juvenile *Caligus punctatus* removed from the goby, *Chaenogobius castaneus* (O'Shaughnessy), caught in a brackish lagoon on the eastern coast of Korea (Sea of Japan side), were found to comprise four distinct size groups. The three smaller size groups were identified as chalimus I, chalimus II and chalimus III. The largest size group was composed of a mixture of chalimus IV and young adults. These five stages could easily be distinguished by the characteristic appearance of the base of their frontal filaments. The identification of developmental stages was confirmed by rearing experiments. In total, eight developmental stages (two nauplius stages, one copepodid, four chalimus, and adult) were described and compared with the ontogeny of other caligid species.

INTRODUCTION

Caligus punctatus Shiino, 1955 is known from Japanese waters and occurs on five species of fish (Shiino 1955, 1959): *Tribolodon hakonensis* (Günther), *Triakis scyllium* Müller and Henle, *Takifugu vermicularis* (Temminck and Schlegel), *Acanthogobius flavimanus* (Temminck and Schlegel), and *Liza menada* Tanaka. These hosts represent five families belonging to four orders in two classes.

In September 1991, a small benthic goby, *Chaenogobius castaneus* (O'Shaughnessy) living in Lake Hwajinpo, was found to harbour the larval stages and adults of *C. punctatus*. Since *C. punctatus* parasitizes various phylogenetically remote fish and can complete its life cycle in a brackish lagoon where the environmental conditions are extremely variable, it is regarded as a pest with potential threat to the development of sea farming. Thus its developmental stages are described in this chapter for future reference.

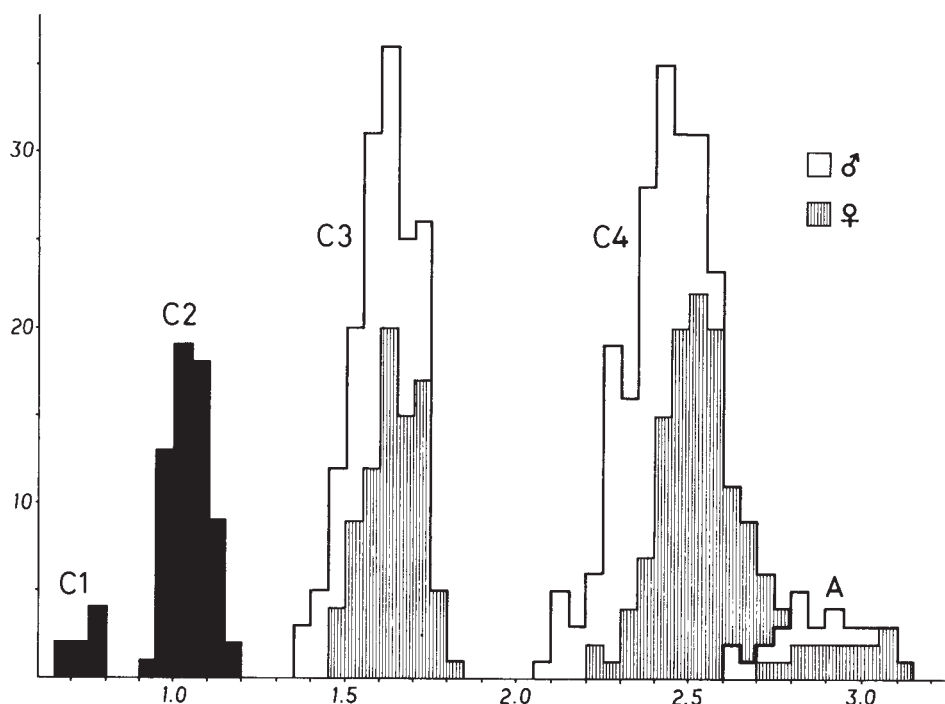


Fig. 1. Frequency of body lengths of attached larval stages and young adults of *Caligus punctatus* (n=485) removed from 500 host fish *Chaenogobius castaneus*. Cl-4: chalimus I-IV; A: young adults with frontal filament.

MATERIALS AND METHODS

Approximately 1300 *Chaenogobius castaneus* (about 5 cm long) were caught on 7 December 1991 in a set net from Lake Hwajinpo, a brackish lagoon located on the eastern coast of Korea. The lake has an average depth of 2 m and an area of about 3 km² and is occasionally connected to the Sea of Japan. At the time of collection (in winter) the salinity was 17 ppt and water temperature was 7°C. Five hundred gobies were randomly selected and fixed immediately in 5% formalin. The preserved gobies were rinsed carefully with tap water and examined under the dissecting microscope for copepods. In total 485 larvae were removed from their fins, preserved in 70% alcohol, measured and grouped according to their lengths (Fig. 1).

The rearing experiments were carried out from May to July 1992 with artificial brackish water (salinity approximately 17 ppt; mixture of the same amounts of sea water with fresh water) in 11 beakers kept at 15–20°C. Egg sacs of the copepod were removed and placed in the beakers. About 24 h after hatching, all nauplii moulted into copepodids. Further developmental stages were obtained by infesting the gobies with copepodids.

Measurements and dissections were carried out after soaking the specimens in lactic acid for at least 5 h. In the following description for each stage, only the differences

from the preceding stage are given. In case of males, only sexually dimorphic structures are mentioned.

DESCRIPTION

First nauplius (Fig. 2a–d)

Body (Fig. 2a) oval, 385 μm (356–401 μm) long and 173 μm (165–176 μm) wide, based on ten specimens, with three pairs of appendages on anteroventral surface. Balancers on posterior end of body 110 μm long, slender and arched.

Antennule (Fig. 2b) indistinctly two-segmented. Proximal segment with two unequal, naked setae, minute spinules, and two indistinct lines. Distal segment armed apically with two long setae with serrate margins, one slender aesthete and three denticles. Aesthete less than half length of setae. Antenna (Fig. 2c) biramous, with basal segments of both rami fused to sympod. Exopod five-segmented. Second to fifth segments each armed with one large inner distal seta; all setae plumose on inner margin and serrate on outer margin. Fifth segment with one spiniform process in addition to seta fused basally to segment. Endopod shorter than exopod and two-segmented. Distal segment with two large setae, one small inner seta and one spiniform process 6.3 μm long (slightly shorter than inner seta). Serrate margin of two large setae facing each other.

Mandible (Fig. 2d) biramous. Exopod four-segmented. First segment fused with sympod, each remaining segment armed with one large seta. Terminal seta fused to distal segment. All setae serrate on outer margin and plumose on inner margin. Endopod one-segmented, indistinctly divided from sympod, with one small inner aesthete-like element and two large terminal setae. Serrate margins of both setae facing each other.

Second nauplius (Fig. 2e–g)

Body (Fig. 2e) 416 μm (405–423 μm) long and 156 μm (154–161 μm) wide, based on ten specimens, longer and slender than nauplius I, with a bifurcate structure (?maxillule) on posterior third of ventral surface (Fig. 2e). Balancer 100 μm long, straight, with broad distal half.

Antennule as in first nauplius, except for addition of one spine and two spinules on terminal segment (Fig. 2f). Antenna with longer spiniform process (length 23 μm) on terminal segment of endopod (Fig. 2g).

Copepodid (Figs. 2h–k, 3a–h)

Body (Fig. 2h) divided into two tagmata: cephalothorax and posterior part. Body length 565 μm (547–576 μm) and maximum width 224 μm (221–228 μm), based on ten specimens. Cephalothorax (Fig. 2i) oval, partly divided laterally by transverse constriction between antenna and postantennary process, and with a distinct ventral bulge in front of leg 1. Both anterior and posterior margins of cephalothorax truncate. First pedigerous somite incorporated into cephalothorax.

Posterior part of body comprising four distinct somites. First somite (second pediger) 53×104 μm in size. Second somite 51×85 μm , with rudimentary third legs on posterolateral corners and a pair of setules on dorsal surface. Third somite unarmed,

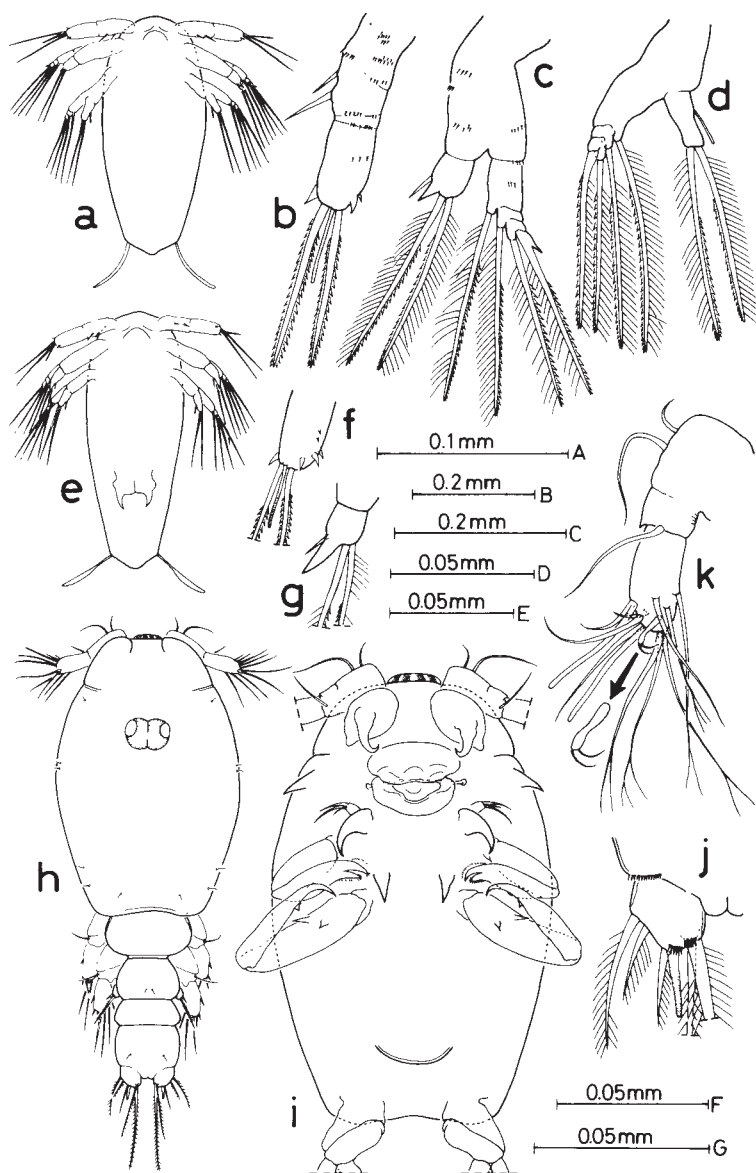


Fig. 2. *Caligus punctatus*. First nauplius: (a) habitus, ventral; (b) antennule; (c) antenna; (d) mandible. Second nauplius: (e) habitus, ventral; (f) distal part of antennule; (g) endopod of antenna. Copepodid: (h) habitus, dorsal; (i) cephalothorax, ventral; (j) caudal ramus, ventral; (k) antennule. Scales: a, e=B; b-d, f, g=A; h=C; i=D; j=E; k=F.

32×77 μm in size. Anal somite 48×80 μm , with a pair of dorsal setules. Caudal ramus (Fig. 2j) 26 μm long and 25 μm wide, with five setae, one aesthete, and two rows of spinules on distal margin of ventral side.

Antennule (Fig. 2k) two-segmented. Basal segment partly subdivided by transverse line on anterodorsal surface of distal third, with pointed anterodistal corner and three naked setae. Terminal segment slightly shorter than basal one, armed with 11 naked setae and two aesthetes; five posterior setae longer, each with branched tip, one seta specialized as rod tipped with two setules. Antenna (Fig. 3a) three-segmented. Basal segment short, but broad, and produced into spiniform process. Middle segment bearing, in basal region, a large, pointed inner process, covered with hyaline material on anterior side. Terminal segment a strongly curved claw, with one basal seta. Postantennary process simple, appearing as sharply pointed process near lateral margin of cephalothorax (Fig. 2i).

Mandible (Fig. 3b) consisting of four indistinctly defined parts; terminal part flat, armed with 11 teeth. Maxillule (Fig. 3c) consisting of a claw-like process and a papilla tipped with three unequal setae. Maxilla (Fig. 3d) two-segmented; brachium slightly longer than lacertus, with a relatively large flagellum on anterodistal margin; calamus with two strips of serrated membranes on anterior margin, and one posterior row of setules; canna with one row of setules on posterior margin. Maxilliped (Fig. 3e) two-segmented; corpus armed with two spiniform processes on medial surface; shaft slender, with subterminal process on inner margin; terminal claw bearing a basal denticle. A pair of sharp, postoral processes situated between bases of maxillae and maxillipeds (Fig. 2i).

Leg 1 (Fig. 3f) and leg 2 (Fig. 3g) biramous, with one-segmented rami and two-segmented sympod. Both legs with outer seta on basis of sympod. Inner corner of coxa of leg 2 acutely projected. Endopods of both legs with pointed process at outer distal corner and a short fissure near middle of outer margin. Second inner proximal seta on leg 1 endopod naked and distinctly smaller than neighbouring setae. Setation formula of these legs as follows:

P1: Sympod 0–0; 1–0; Exp IV, I, 3; Enp 7

P2: Sympod 0–0; 1–0; Exp III, I, 3; Enp 6

Leg 3 represented by small process bearing two unequal setae (Fig. 3h).

First chalimus (Figs 3i–o, 4a–c)

Body (Fig. 3i) 734 μm long (685–776 μm) and 310 μm (271–339 μm) wide, based on eight specimens, without distinct segmentation. Cephalothorax spindle-shaped and posterior tagma nearly cylindrical, with two indistinct divisions respectively located just posterior to leg 3 and near distal area of body. Base of frontal filament (Fig. 3j) clearly delimited ventrally from anterior region of cephalothorax with slightly notched posterior border. Later in this stage, a pair of circular bodies appear internally in frontal area of cephalothorax (Fig. 3i,j). Caudal ramus incompletely demarcated from posterior part of body, with six naked setae.

Antennule (Fig. 3k) armed with three setae on basal segment, and 11 setae+two aesthetes on terminal segment. All setae simple and naked. Antenna (Fig. 3l) one-segmented, terminated in an irregularly shaped process carrying three spiniform elements. Postoral processes lost.

Mandible basically as in adult bearing blade with 12 teeth on one side and hyaline membrane on other (Fig. 3m). Maxillule unchanged from preceding stage, except for

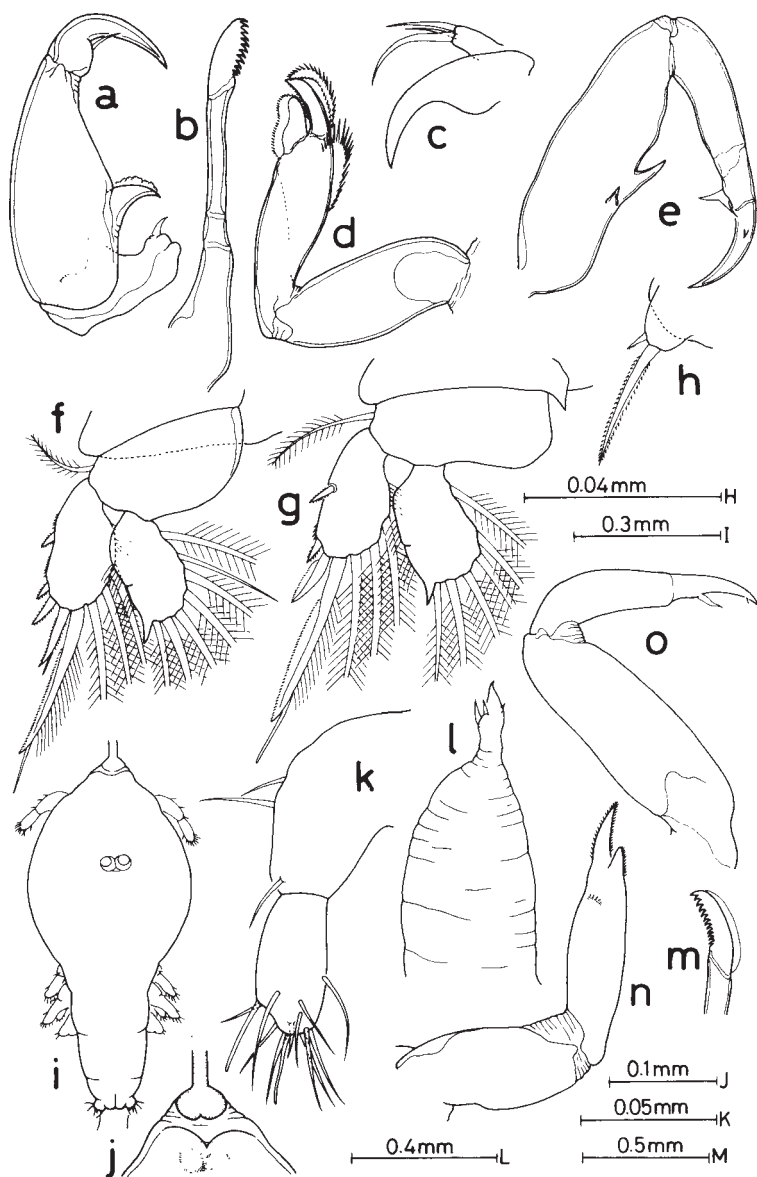


Fig. 3. *Caligus punctatus*. Copepodid: (a) antenna; (b) mandible; (c) maxillule; (d) maxilla; (e) maxilliped; (f) leg 1; (g) leg 2; (h) leg 3, ventral. First chalum: (i) habitus, dorsal; (j) frontal region of cephalothorax, ventral; (k) antennule; (l) antenna; (m) distal part of mandible; (n) maxilla; (o) maxilliped. Scales: a, c, d, l=G; b, m=H; e–g=D; h=F; i=I; j=J; k, o=K, n=E.

sharper posterior process. Maxilla (Fig. 3n) reduced, brachium as long as lacertus and carrying simplified flagellum, calamus and canna simplified. Maxilliped (Fig. 3o) also reduced, with unarmed corpus and shaft; terminal claw, with minute subterminal spinule and basal seta on inner margin.

Legs 1–3 (Fig. 4a–c) with unsegmented sympod and two rami, both fused to sympod. All setae naked and inner setae on both rami usually with blunt tip. Leg 1 with prominent medial protuberance bearing coxal seta in proximal region of sympod; exopod armed with eight setae and endopod reduced to a smaller ramus tipped with two long setae. Leg 2 with medial protuberance on inner coxal region of sympod, as in leg 1 but less prominent; exopod armed with eight setae (inner proximal one obscure), endopod with six setae. Leg 3 consisting of two small rami vaguely delimited from sympod; exopod armed with four weak setae, endopod unarmed.

Second chalmus (Fig. 4d–m)

Body (Fig. 4d) has regained its tagmata, 1.05 mm (0.91–1.17 mm) long and 0.53 mm (0.50–0.59 mm) wide, based on 62 specimens. Cephalothorax well defined posteriorly by posterolateral corners. Frontal plate weakly defined, without suture line. Frontal filament with two bases (Fig. 4e), original, distal base and newly formed, proximal, bilobate base. First three pedigers fused to cephalosome. Fourth pediger delimited incompletely both anteriorly and posteriorly. Genital complex delimited from anal somite by shallow groove. Caudal ramus much wider than long, bearing six setae.

Antennule (Fig. 4f) with seven setae on basal segment and 12 setae+two aesthetes on terminal segment; all setae naked. Antenna (Fig. 4g) with small basal protuberance, one apical process and three subterminal, spiniform processes. Postantennary process a small hemisphere accompanied by two setules (Fig. 4h).

Process of maxillule conical and tipped with spinule; papilla carrying distally one large and two small setae (Fig. 4i).

Legs 1–3 with unsegmented sympod. Leg 1 (Fig. 4j) with one-segmented rami fused to sympod, which bears a small protuberance on basal inner margin, coxal seta near inner and outer basal seta near outer corners; proximal outer seta on exopod isolated farther from remaining seven setae; endopod more reduced, less than one-third of exopod length and tipped with two small setae. Rami of leg 2 (Fig. 4k) weakly demarcated from sympod, which bears two protuberances on basal inner margin, and outer basal seta near distal corner; exopod armed with nine setae; endopod shorter than exopod, incompletely segmented, and armed with eight setae, one of which is isolated from the rest and located near base of inner margin. Leg 3 (Fig. 4l) with one-segmented rami incompletely separated from sympod, which carries one seta on outer distal corner; exopod armed with eight setae, with incomplete segmentation; endopod hemispherical, armed with five small, tubercle-like setae. Leg 4 (Fig. 4m) one-segmented, about 1.7 times as long as wide, armed with one lateral and two distal, tubercle-like setae.

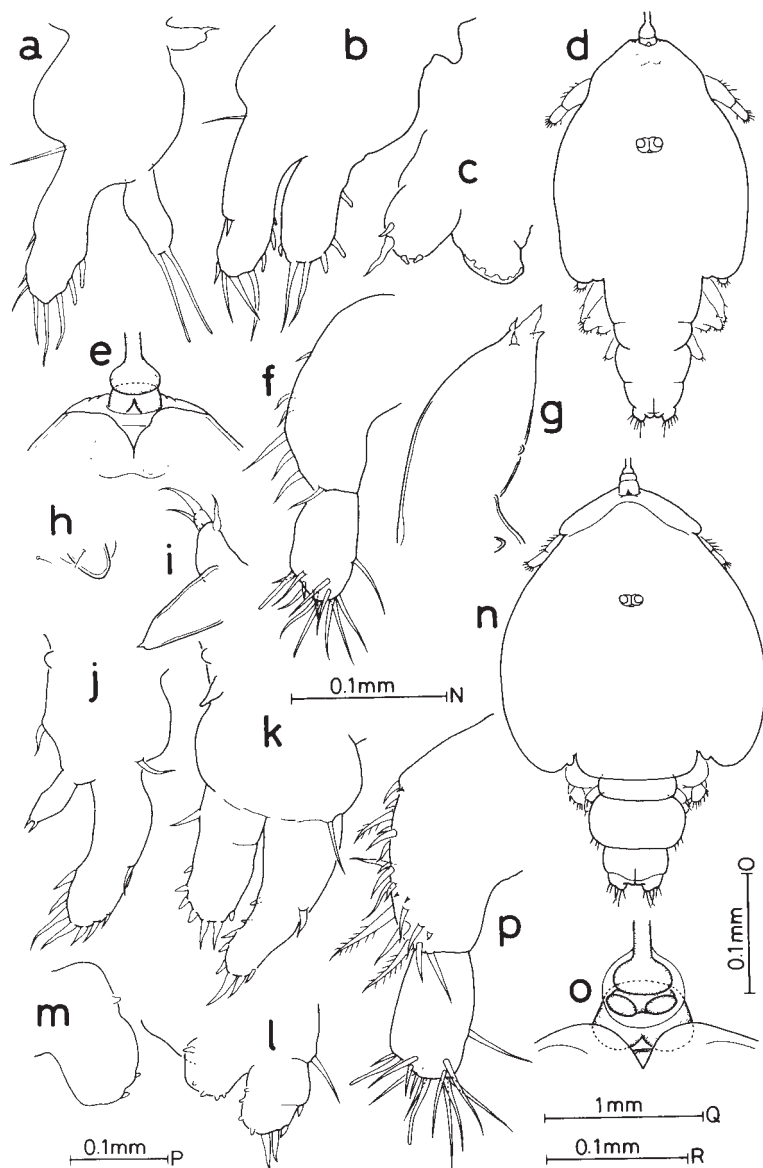


Fig. 4. *Caligus punctatus*. First chalmus: (a) leg 1; (b) leg 2; (c) leg 3. Second chalmus: (d) habitus, dorsal; (e) frontal region of cephalothorax, ventral; (f) antennule; (g) antenna; (h) postantennary process; (i) maxillule; (j) leg 1; (k) leg 2; (l) leg 3; (m) leg 4. Third chalmus: (n) habitus, dorsal; (o) frontal region of cephalothorax, ventral; (p) antennule. Scales: a–c=E; d=L; e, o=J; j–l=A; g–i, m=K; n=M; p=N.

Third chalimus (Figs 4n–p, 5a–h)*Female*

Body (Fig. 4n) 1.64 mm (1.48–1.81 mm) long, based on 83 specimens. Cephalothorax approaching adult form, with marked frontal plate and shallow posterior sinuses. Posterior tagma, including fourth pedigerous somite, distinctly three-segmented as in adult. Genital complex better developed, broader than other somites of posterior tagma. Frontal filament with three bases (Fig. 4o); distal and median bases enclosed in a common cuticle; median base consisting of a pair of ovoid bodies; newly formed, proximal base incised posteriorly.

Antennule (Fig. 4p) with 22 setae on basal segment, including four tiny and several larger plumose setae, and 12 setae+two aesthetes on terminal segment. Antenna (Fig. 5a) longer, with stronger distal process, accompanied subterminally by two setae and two tiny, ventral setules. Postantennary process (Fig. 5b) better developed, longer than wide, but not claw-like, with two small papillae bearing branched setules.

Maxillule (Fig. 5c) with stronger posterior process.

Leg 1 (Fig. 5d) endopod further reduced and tipped with small knob. Sympod weakly segmented; distal segment with inner and outer setae; rami not demarcated from sympod; exopod two-segmented, segmentation incomplete, with seta on outer distal corner of first segment and seven setae on second segment; all setae naked. Leg 2 (Fig. 5e) with unsegmented sympod carrying inner coxal and outer distal setae; both rami weakly demarcated from sympod; exopod armed with 11 marginal setae; endopod showing incipient segmentation with shallow groove dividing ramus into basal part carrying one inner seta and terminal part carrying eight setae. Leg 3 (Fig. 5f) with sympod bearing one outer and one inner setae; rami demarcated from sympod; exopod one-segmented, armed with one outer proximal spine and nine setae; endopod two-segmented; basal segment with one inner seta, and distal segment five setae. Leg 4 (Fig. 5g) uniramous; sympod armed with distal seta, exopod with one lateral and three distal setae. Leg 5 represented by three setae on posterolateral margin of genital complex.

Male

Body 1.62 mm (1.39–1.74 mm) long, based on 81 specimens. Body form, including shape of genital complex, not distinguishable from female. Sexual dimorphism chiefly seen in antenna (Fig. 5h), which is stouter and abruptly tapered at tip.

Fourth chalimus (Fig. 5i–t)*Female*

Body (Fig. 5i) 2.51 mm (2.20–2.78 mm) long, based on 110 specimens. Cephalothorax with distinct frontal plate and weakly curved, H-shaped suture lines delimiting cephalic, thoracic and lateral zones. Frontal filament with four bases (Fig. 5j). Later in this stage rudimentary lunules visible through cuticle of frontal plate (Fig. 5i). Genital complex larger. All setae on caudal rami plumose.

Antennule (Fig. 5k) carrying adult armature with 29 setae on basal segment and 12 setae+two aesthetes on terminal segment. Some larger setae on basal segment plumose. Aesthetes of terminal segment barely distinguishable from setae. Antenna (Fig. 5l) indistinctly two-segmented. Proximal segment with medial nodular process at base.

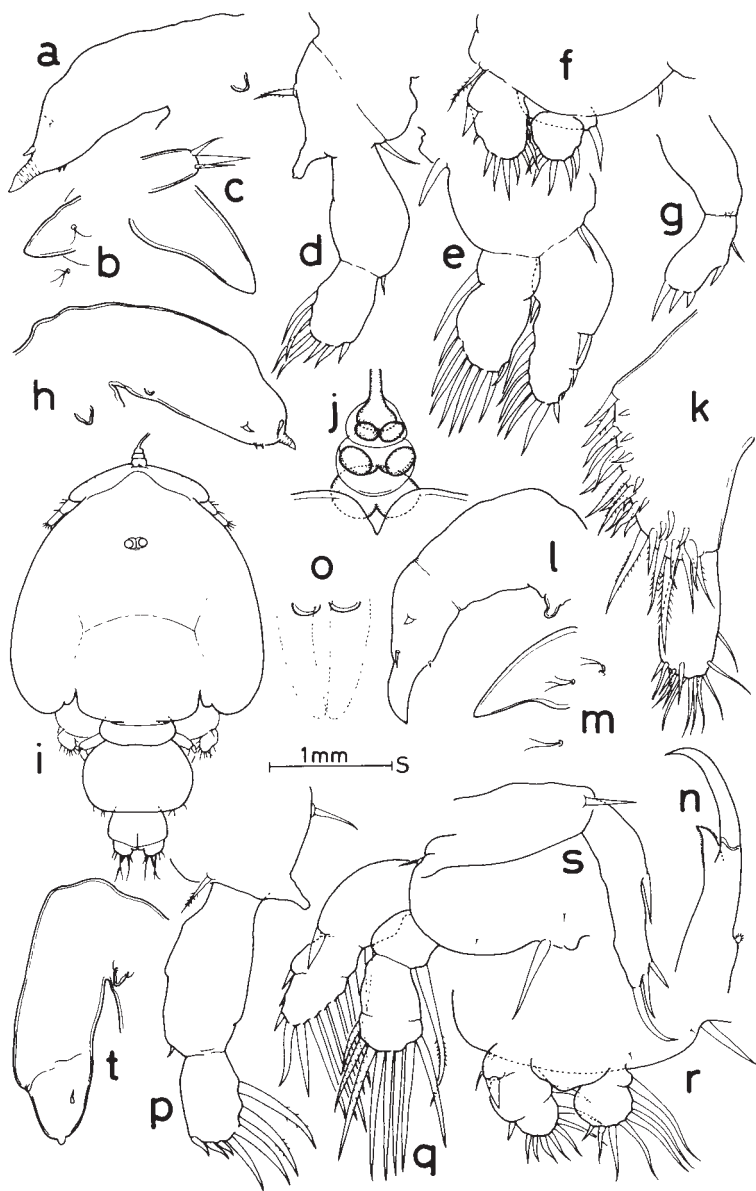


Fig. 5. *Caligus punctatus*. Third chalimus (a) female antenna; (b) postantennary process; (c) maxillule; (d) leg 1; (e) leg 2; (f) leg 3; (g) leg 4; (h) male antenna. Fourth chalimus: (i) habitus (female/male), dorsal; (j) base of frontal filament, ventral; (k) antennule; (l) female antenna; (m) postantennary process; (n) distal part of maxilla; (o) sternal furca; (p) leg 1; (q) leg 2; (r) leg 3; (s) leg 4; (t) male antenna. Scales: a, g, h, m=N; b=E; c=A; d, o=O; e, l, t=P; f, k=J; i=Q; j, p, s=C; n=R; q, r=B.

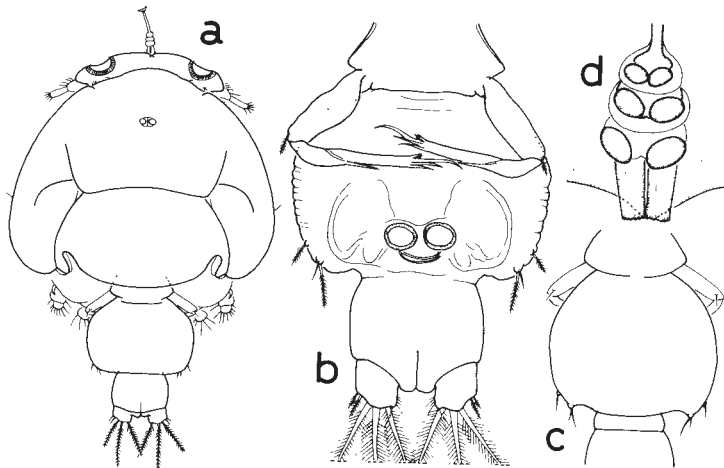


Fig. 6. *Caligus punctatus*, young adult with frontal filament: (a) habitus (female/male), dorsal; (b) female urosome, ventral; (c) genital complex of male, dorsal; (d) base of frontal filament, ventral. Scales: a=S; b=L; c=M; d=C.

Distal segment with claw-like apical process and three setae. Postantennary process (Fig. 5m) consisting of claw-like process and three minute papillae bearing two or three setules.

Flagellum on maxilla appearing as a small pectinate papilla (Fig. 5n). Calamus unarmed but canna bipectinate. Sternal furca (Fig. 5o) appearing as a pair of sclerotized lobes, but later in this stage a pair of long cuticular tines visible underneath cuticle.

Leg 1 (Fig. 5p) with distinct suture between sympod and exopod; both rami unchanged from preceding stage, but inner four setae on distal segment of exopod better developed. Leg 2 (Fig. 5q) with one more seta on indistinctly three-segmented exopod; first and second segments each with one outer and one inner setae; third segment armed with eight setae; endopod three-segmented, but segmentation between distal two segments incomplete; first segment with one weakly plumose seta on inner distal corner; other setae on this leg naked; second segment unarmed; third segment armed with eight setae. Leg 3 (Fig. 5r) with velum between bases of rami; both three-segmented exopod and two-segmented endopod with incomplete segmentation; armature on these rami as in adult; all setae naked. Leg 4 (Fig. 5s) two-segmented; both segments nearly equal in length; exopod with better developed distal seta.

Male

Body closely resembling that of female. Body length 2.38 mm (2.05–2.60 mm), based on 110 specimens. Genital complex slightly narrower than that of female. Antenna (Fig. 5t) stout and bearing distally a small protuberance and one subterminal setule.

Young adult (Fig. 6)

After moulting from chalimus IV, young adult (Fig. 6a) remains attached to host by means of frontal filament, but can be readily detached, with poorly developed genital complex (Fig. 6b,c). Length of female 2.96 mm (2.71–3.15 mm), based on 18 specimens, and that of male 2.81 mm (2.61–3.07 mm), based on 13 specimens. Frontal filament consisting of five sets of bases with three middle ones made up of a pair of ovoid bodies (Fig. 6d). The proximal set bilobate initially, but later transformed into a pair of long rods (Fig. 6d). About 25% of attached female young adults carried a pair of spermatophores. Young adults of both sexes matured sexually without further moulting.

DISCUSSION

A complete developmental cycle is known of 11 species of caligid copepods, five of them belonging to the genus *Caligus* Müller and the rest to the genus *Lepeophtheirus* Nordmann. Kabata (1972), after comparing his work on the development of *Caligus clemensi* Parker & Margolis with Gurney's (1934) *Caligus centrodoni* Baird, Heegaard's (1947) *Caligus curtus* Müller, Lewis's (1963) *Lepeophtheirus dissimulatus* Wilson, Hwa's (1965) *Caligus orientalis* Gussev, Izawa's (1969) *Caligus spinosus* Yamaguti, and Voth's (1972) *Lepeophtheirus hospitalis* Fraser, concluded that the life cycle of Caligidae comprised five phases and ten stages. The phases are nauplius (two stages), copepodid (one stage), chalimus (four stages), preadult (two stages) and adult. Although this conclusion was supported by Boxshall (1974a) on his work on *Lepeophtheirus pectoralis* (Müller) and Johnson and Albright (1991) in their study of *Lepeophtheirus salmonis* (Krøyer), Caillet (1979) found nine stages in *Caligus minimus* Otto, and Ben Hassine (1983) reported for *Caligus pageti* Russell nine stages in the female and ten stages in the male. Furthermore, with the present discovery of eight stages in *C. punctatus*, it seems that there is no uniform number of stages for caligid development.

The second nauplius of *C. punctatus* is characterized by having a bifurcate sclerite (precursor of maxillule?) on the posteroventral surface of the body. This structure has previously been reported for only one other caligid, *C. pageti*, by Ben Hassine (1983). Another characteristic feature of *C. punctatus* is the absence of a rostrum in the copepodid. At the time of attachment to the host, the frontal filament is distinctly visible inside the anterior region of the copepodid. When anchored to the host's fin, by anterior stretching of the antennae, the larvae continuously jabbed the fin ray of the host with its frontal filament.

As described above, the chalimus larvae can be easily identified to stage by the basal structure of the frontal filament. With one base being added at each moult, chalimus I has only one base, and attached young adults carry five such bases. Such 'stage indicator' is known of four other species of *Caligus*: *centrodoni*, *curtus*, *orientalis* and *pageti*. In *C. punctatus*, the identification of chalimus stages can be further confirmed by the discrete size ranges of each stages (Fig. 1). Although the size frequencies of chalimus stages are known for other caligids (Lewis 1963, Izawa 1969, Boxshall 1974b, Urawa *et al.* 1979), they are not as discrete as in the present species.

Antennules and the oral appendages, including the maxillipeds, show fewest changes in development throughout the chalimus stages. For example, the mandible

and maxillule of the copepodid stage are virtually in the adult form and change very little throughout the succeeding stages. The postantennary process and sternal furca change more markedly. The antenna, which is functional in the copepodid, is reduced in the early chalimus stages. This appendage in *C. punctatus* shows sexual dimorphism as early as chalimus III. The development of postantennary process in caligids varies with species. In *C. punctatus*, the process appears first at the copepodid stage, as in *C. clemensi* and *C. orientalis*; however, only in *C. orientalis* is it retained throughout the remaining stages. In the other two species it disappears at the following stage and reappears in chalimus II in *C. punctatus* and in chalimus III in *C. clemensi*. For the other caligids, this process appears first in chalimus I in *L. pectoralis*, in chalimus II in *C. curtus*, *C. centrodoni*, *C. pageti* and *C. spinosus*, and in chalimus III in *C. minimus*, *L. dissimulatus* and *L. hospitalis*. In these eight species, the process is present continuously after its first appearance.

A pair of enigmatic processes has been reported for the copepodid of *C. punctatus*, *C. centrodoni* and *L. pectoralis*. They are located between the bases of maxillae and maxillipeds and are called 'sternal furca' by Boxshall (1974a). Kabata (1972) claimed that the copepodid of *C. clemensi* has a sternal furca located 'in the midventral line behind the bases of the maxillipeds'. However, these structures disappear and a true sternal furca does not appear until chalimus IV.

Legs 1 and 2 of copepodid in all species have single-segmented rami. The exopod of leg 1 is universal in having eight elements, whereas the endopod is armed with six or seven elements. Leg 3 appears in the copepodid stage as one (in *L. dissimulatus*) or two (in all other species) setae. Leg 4 appears first either in chalimus I (in *C. centrodoni*, *C. curtus*, *C. minimus*, *C. pageti* and all *Lepeophtheirus* species) or in chalimus II (in the other four species of *Caligus*) as a small lobe. *C. clemensi* is unusual in having its leg 5 appearing in the preadult stage. In other species this leg appears as early as in chalimus II (in *C. centrodoni*, *C. curtus*, *C. minimus* and *C. pageti*) as a single seta or in chalimus III (in other species) as two or three setae.

ACKNOWLEDGEMENT

I am grateful to Dr Ju-Shey Ho, Department of Biology, California State University, Long Beach, who kindly read the drafts of this manuscript.

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3

Supplementary descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae)

Thomas A.Schram

ABSTRACT

Johnson and Albright (1991) recently described the nine developmental stages and adult of *Lepeophtheirus salmonis* from the Pacific. This chapter provides additional information, based on Atlantic material, on the morphology, size, pigmentation and other relevant characteristics of each stage. This is presented to facilitate practical identification of the larval stages. Throughout development, Atlantic larvae were all smaller than corresponding stages from the Pacific. Before the preadult stage, the maxillule is not bifid and the sternal furca is not a separate appendage.

INTRODUCTION

The salmon louse, *Lepeophtheirus salmonis*, had been known for at least 50 years before Krøyer published his description in 1837 (Berland and Margolis 1983). During the past decade, this parasite has become an increasingly serious problem in the pen rearing of salmonids, especially Atlantic salmon (Brandal and Egidius 1979). Nevertheless, more than 200 years elapsed before Johnson and Albright (1991) published a complete description of the morphology of its developmental stages. Their excellent paper also includes detailed descriptions of all appendages of the nine larval stages.

The life cycle of caligid copepods typically comprises five phases and ten stages (Fig. 1). These are two free-swimming nauplius stages, one free-swimming infective copepodid stage, four attached chalimus stages, two preadult stages, and the adult (Kabata 1972).

The objective of the present chapter is to give *additional* information on morphology, size, pigmentation and other characteristics of each stage. I hope this will facilitate the practical identification of the different stages of salmon lice. Throughout the chapter comparison is made with the results of Johnson and Albright (1991).

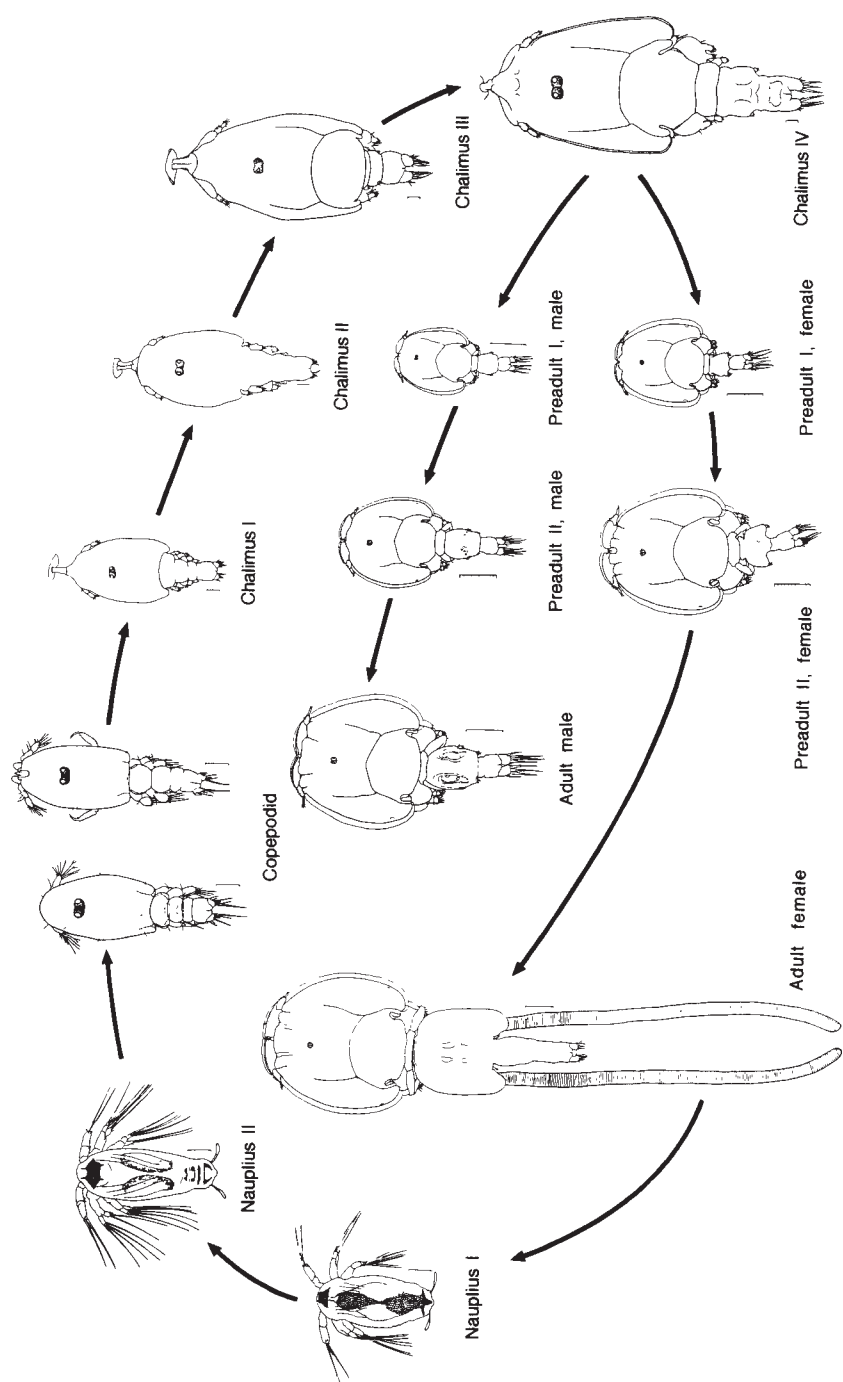


Fig. 1. Life cycle of *Lepeophtheirus salmonis*. Scale bars: nauplius–chalimus=0.1 mm, preadult–adult=1 mm.

MATERIALS AND METHODS

Developmental stages were obtained by rearing eggs in the laboratory (10–12°C and salinity 30–31‰) using the technique described by Schram and Anstensrud (1985) with Atlantic salmon (*Salmo salar* L.) as host. Additional specimens were collected from Atlantic salmon in various farms in western Norway. Pelagic stages were also collected with a plankton net (mesh size 100 μm) at a fish farm. Parasites were studied alive, after preservation in 4% formaldehyde in sea water or cleared in lactic acid. Specimens for scanning electron microscopy were preserved in 4% formaldehyde, post-fixed in 2.5% glutaraldehyde, and thereafter treated as described in Schram (1991).

RESULTS

First nauplius

The newly hatched larva has a mean length of c. 0.5 mm and is on average 0.2 mm broad (greatest width in the middle of the body) with wavy lateral margins. Wildcaught, free-swimming larvae are similar in length but somewhat slimmer (Table 1, Fig. 2a).

Live larvae are almost translucent: the yolk and bands of longitudinal muscle are visible, as are two types of pigment. Black pigment may be seen anteriorly (dorsally and around the eyes) and at the posterior end of the body. Brown pigment is found approximately in the middle of the body, distributed symmetrically on both sides of the intestine. Eyespots are under development anteriorly, but are difficult to see due to black pigment situated more dorsally in the larva.

The appendages of the first nauplius larva (Fig. 3a) are all unpigmented except the proximal segment of the antennule, where some individuals may show black spots ventrally, close to the body. The posterior balancers are unpigmented.

Second nauplius

The free-swimming nauplius is translucent, smooth, oval and slender and has black and dark brown pigment. The average length is 0.6 mm and width 0.205 mm (Fig. 2b, Table 1). In older nauplii the pigmentation of the copepodid is visible through the naupliar cuticle.

Appendages are similar to those of the first nauplius except for an additional blunt spine near the apex of the distal segment of the antennule (=cuticular ridge seen by Johnson and Albright (1991) in nauplius I). Moreover, the length of the broad terminal spine of the endopod of the antenna increases from c. 10 μm in nauplius I to 30 μm in nauplius II, and the weaker spine also grows from 10 μm to 20–23 μm .

In live larvae, black pigment may be seen anteriorly in three areas: dorsally, between and around the eyes, and on the ventral bump (Fig. 2c,d). Posteriorly, black pigment is found as two patches, and an additional band is seen ventrally across the body (Fig. 2d). In older larvae the pigment of the copepodid urosome is visible through the naupliar cuticle as three to four bands across the larva, which become progressively broader posteriorly (Fig. 2b,e). Dark brown pigment is seen dorsally as two C-shaped figures, on each side of the intestine, and laterally as a more tripartite figure. Brown pigment is most widely distributed ventrally (Fig. 2b,d,e). Black pigment is also present here in the middle of the larva. The appendages of nauplius II are all unpigmented.

Table 1. Dimensions in millimetres of the developmental stages of *Lepeophtheirus salmonis* (N=number of specimens, SD=standard deviation, L=mean length, W=mean width)

Stage	N	L	SD	Range	N	W	SD	Range
Nauplius I								
Cultivated	56	0.504	0.014	0.450-0.530	32	0.210	0.009	0.20-0.23
Wild	30	0.511	0.024	0.470-0.575	30	0.188	0.008	0.165-0.200
Nauplius II								
Free sw.	22	0.606	0.01	0.590-0.620	17	0.205	0.01	0.195-0.230
Copepodid								
Free sw.	15	0.684	0.016	0.658-0.709	15	0.229	0.007	0.223-0.252
Attached	24	0.793	0.026	0.720-0.830	24	0.295	0.018	0.260-0.330
Chalimus I	21	1.063	0.024	1.025-1.139	21	0.483	0.018	0.456-0.506
Chalimus II	15	1.284	0.084	1.15-1.44	15	0.516	0.043	0.46-0.60
Chalimus III								
Young	13	1.78	0.16	1.41-1.98	13	0.782	0.072	0.73-0.90
Old	44	2.064	0.135	1.840-2.310	44	0.951	0.073	0.820-1.04
Chalimus IV	39	2.291	0.140	2.040-2.720	39	1.048	0.072	0.950-1.27
Preadult I								
Male	33	3.352	0.162	2.7-3.6	33	1.583	0.05	1.4-1.7
Female	75	3.585	0.12	3.3-3.8	75	1.885	0.05	1.8-2.0
Preadult II								
Male	35	4.314	0.137	4.1-4.5	34	2.168	0.07	2.0-2.3
Female	26	5.18	0.55	4.48-5.97	26	2.91	0.31	2.48-3.50
Adult male	32	5.06	0.24	4.5-5.6	32	2.72	0.12	2.5-3.1
	13	6.22	0.29	5.7-6.6	13	3.02	0.12	2.8-3.3
	68	6.03	0.21	5.60-6.65	—	—	—	—
Adult female	18	8.39	1.18	7.0-10.5	18	4.02	0.27	3.6-4.5
	11	11.52	0.07	11.45-11.59	—	—	—	—

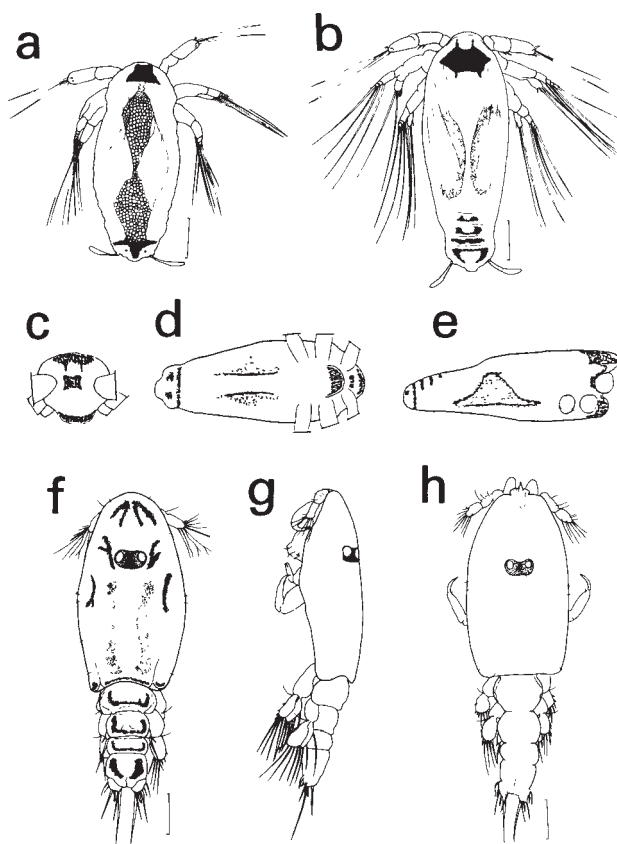


Fig. 2. (a) Newly hatched first nauplius, dorsal; (b–e) free-swimming second nauplius; (b) dorsal, (c) frontal, (d) ventral, (e) lateral; (f,g) free-swimming copepodid; (f) dorsal, (g) lateral; (h) attached copepodid, dorsal. Scale lines 0.1 mm.

Copepodid

The free-swimming copepodid is on average 0.7 mm long and 0.2 mm wide (Table 1). The cephalothorax is slender and oval in outline. Anteriorly it bears a well developed rostrum c. 20–25 μm long, which is deflected ventrally. Two dark red, heavily pigmented, contiguous eyes are present dorsally, each with a clear spherical lens. The antennae are flexed underneath the cephalothorax (Fig. 2f,g).

The general body colour of the live copepodid is black and dark brown. Brown pigment is present beneath the cephalothorax on both sides of the intestine. Dorsally it is concentrated in two dense C-shaped patches. Black pigment is present dorsally, anterior and posterior to the eyes and also in the ventral part of the body. Dense patches of black pigment are also seen on the trunk segments, increasing in intensity towards the posterior end (Fig. 2f). There is some individual variation in the black

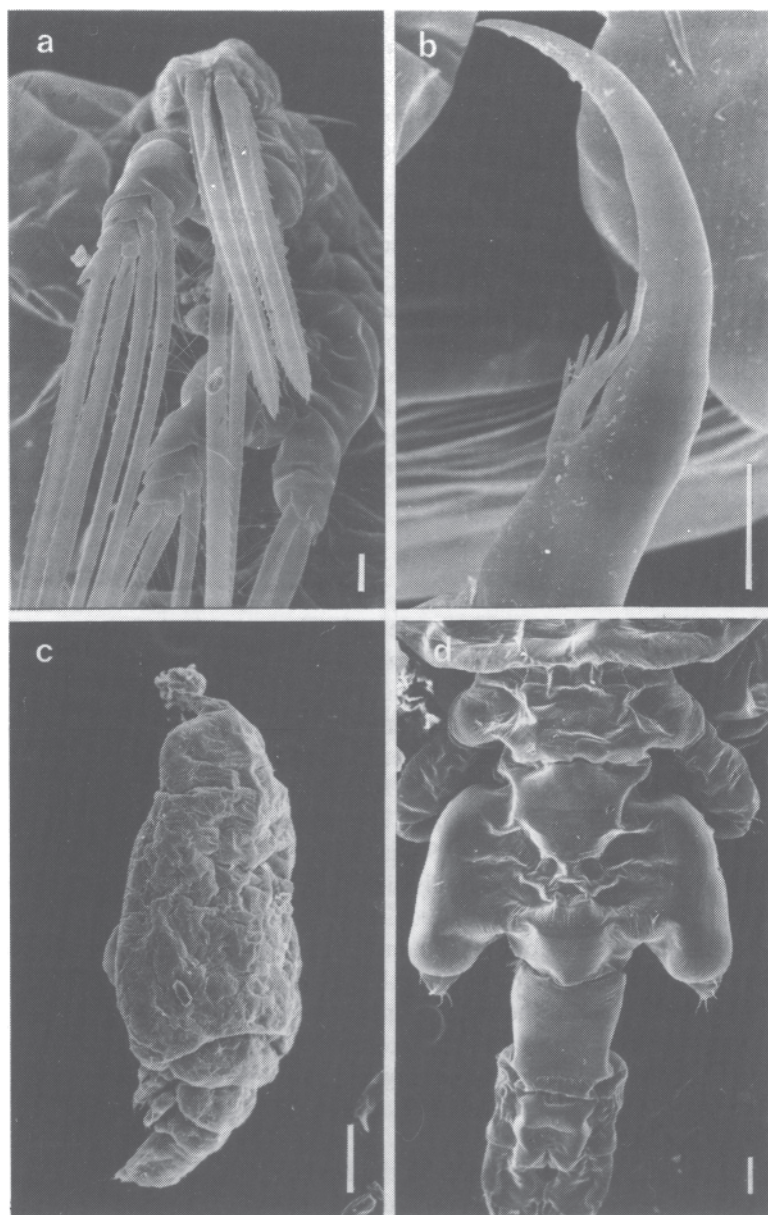


Fig. 3. (a) Appendages of first nauplius, lateral; scale 10 μm ; (b) tip of maxilliped of copepodid, lateral, scale 10 μm ; (c) first chalimus, lateral; scale 100 μm ; (d) urosome of second preadult female, dorsal; scale 100 μm .

pigmentation. Some specimens have additional patches lateral to the eyes and a pair of patches more posteriorly. Finally, the posterior border of the cephalothorax may be black.

The copepodid attaches itself to the host by the antennae which point forward and can be seen in dorsal view. Similarly, the maxillipeds which grip the fish surface are directed outwards and forwards (Fig. 2h). The maxilliped consists of a robust basal segment articulating with a distal subchela. The subchela is indistinctly subdivided and has a slender branching barb close to the suture line at the inner surface. The barb (Fig. 3b) has five to six 'fingers' (cf. Johnson and Albright 1991: three fingers).

The cephalothorax is more rectangular than in free-swimming larvae and the rostrum is anteriorly directed. The older attached copepodid is on average c. 0.8 mm long and 0.3 mm wide (Table 1), i.e. somewhat longer and wider than the free-swimming larva.

First chalimus

The larva has a frontal filament, average length 1.1 mm and width 0.5 mm (Table 1). The armature of all appendages has generally been reduced in length and setules are absent. The margin between anterior and posterior tagmata is present, whereas boundaries between segments in the urosome are indistinct or absent (Figs 3c, 4a,b). The caudal rami are clearly delimited from the abdomen.

The cephalothorax of *younger* larvae is broader and more pear-shaped than that of *older* narrower specimens (Fig. 4a,b). The shape of the first chalimus larva as described by Johnson and Albright (1991) is similar to that of the younger specimens of the present material, but the body divisions are different. Thus the third thoracic segment of the Pacific larva is included in the cephalothorax, and the boundary between cephalothorax and body segments is indistinct. Moreover, the urosome segments are all delimited (Johnson and Albright 1991).

From this stage onwards, the black pigment found in the free-swimming stages disappears, but brown pigment is distributed widely throughout the cephalothorax. An area around the eyes is without pigment in this and all following stages. Furthermore, brown pigment is concentrated in bands posteriorly in the cephalothorax and in the urosome.

A new accessory seta has developed on the medial surface of the distal segment of the antenna, close to the base of the terminal claw (Fig. 4d). This seta is not mentioned by Johnson and Albright (1991), but the antenna of chalimus II of *Lepeophtheirus pectoralis* (Müller) carries two small setae and a small conical process on its apex (Boxshall 1974).

Rudiments of a postantennal process are present ventrally near the lateral margin of the cephalothorax. The postantennal process described by Johnson and Albright (1991) is not the same as the blunt-tipped bulb in Atlantic specimens. The latter is more similar to the process described in chalimus II of *L. pectoralis* by Boxshall (1974).

Second chalimus

The most characteristic feature is the absence of distinct segmentation (Fig. 1). The boundaries between anterior and posterior tagmata and the urosome segments have disappeared. Chalimus II is 1.3 mm long and 0.5 mm broad (Fig. 4c, Table 1). The morphology of this stage is as described by Johnson and Albright (1991).

The live larva has brown pigment distributed over the dorsal surface similarly to

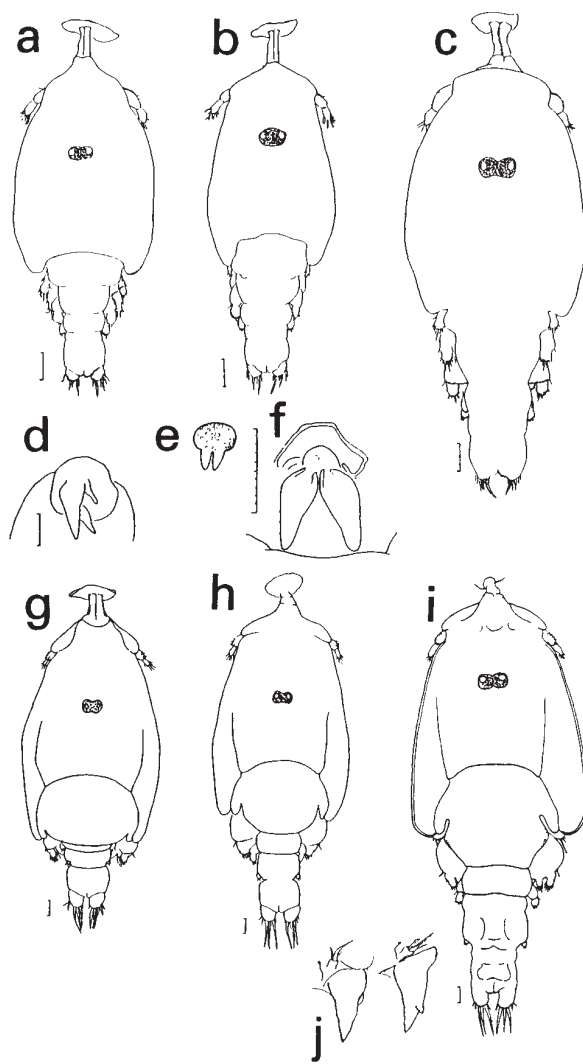


Fig. 4. (a) Young first chalimus, dorsal; (b) older first chalimus, dorsal; (c) second chalimus, dorsal; (d) tip of antenna of first chalimus, ventral; (e) developing sternal furca of third chalimus, ventral; (f) sternal furca of fourth chalimus, below cuticula, ventral; (g) young third chalimus, dorsal; (h) older third chalimus, dorsal; (i) fourth chalimus, dorsal; (j) maxillules of chalimus IV, ventral. Scale lines 0.1 mm; (d) scale line 0.01 mm.

the first chalimus, i.e. pigment is lacking around the eyes and there are denser patches on the urosomal segments. Ventrally, the pigmentation is more scanty, but brown spots are found all over the surface and somewhat more concentrated along the sides. The symphys of the third legs are also sparsely pigmented.

A postantennal process is present. The third leg has developed greatly to a broad plate-like structure, easily seen both in dorsal and ventral views.

Third chalimus

The *young* chalimus larva is relatively thin, hyaline or light grey with brown pigment. Total length is c. 1.8 mm and width 0.8 mm (Fig. 4g, Table 1). The cephalothorax has become more pointed anteriorly and its greatest width is approximately in the middle. Some external segmentation in the urosome has been regained, and the cephalothoracic grooves have developed into an H. The posterior sinuses are distinct, small and V-shaped. In the youngest chalimus III larvae the posterior extension of the thoracic area is small, extending only slightly beyond the lateral lobes, and the posterior margin is almost straight. The cephalothorax now includes the first three pedigerous segments, as in the adult. The fourth pedigerous segment is the first free segment in the urosome and, in dorsal view, is partly overlapped by the posterior portion of the median cephalothoracic area. The telescoping of the urosome is characteristic of the youngest chalimus III, but the length of the free part, and thus also the exposed surface of the fourth leg, varies individually.

The *older* chalimus III larva is 2.1 mm long and c. 1 mm broad, and differs from younger specimens in that the lateral areas of the cephalothorax are broader and the posterior sinuses larger, and have a more open U shape (Fig. 4h, Table 1). The median thoracic area extends posteriorly as a tongue of mean length 80 μm and with a broad curved margin, and all three segments in the urosome are seen in dorsal view.

In older chalimus III larvae the developing sternal furca may be present beneath the exoskeleton. In larvae shorter than 2 mm it is only visible as a simple protuberance, whereas it is divided in specimens measuring 2.2–2.3 mm, with small tines (c. 20 μm) arising from a common basis (Fig. 4e). The rudimentary sternal furca is difficult to detect in larvae preserved in formaldehyde, but clearing in lactic acid reveals it.

The live larva has red eyes and brown pigment distributed over the body as in chalimus II. The anterior part of the cephalothorax is unpigmented. The brown pigment is more concentrated close to the suture lines on the cephalothorax. Posteriorly, the third legs are pigmented, as are the first and second segments (genital complex) in the urosome. However, the abdominal segment is sparsely pigmented and the caudal rami are colourless.

The chalimus III larva from the Pacific (Johnson and Albright 1991) is very similar to older Atlantic specimens in that the border between genital complex and abdomen is indistinct, whereas the caudal rami are clearly delimited.

Fourth chalimus

Chalimus IV is c. 2.3 mm long and 1.1 mm broad. It is thick, firm, opaque and more dorsoventrally flattened than chalimus III (Fig. 4i, Table 1). It has definite shape, which is easily restored if the larva has been subjected to pressure. The cephalothorax is widest posteriorly and the lateral margins converge towards the pointed anterior end. Inside and parallel with these margins, lines describing the lateral rim of the ventral body surface are visible in dorsal view. Posterior sinuses are further developed and U-shaped, but may be difficult to detect due to underlying lateral extensions of the median thoracic area. The median thoracic area is large and extends a long way

posteriorly. From the sinuses, its lateral margins taper posteriorly, angled first outwards and then inwards before they connect to the posterior margin, which is almost straight. In some of the oldest chalimus IV larvae, lobe-like structures are present posterolaterally beneath the cuticle in the first urosomal segment. These are precursors of the cuticular folds seen in the female preadult I (and II). The segment boundaries in the urosome are indistinct, especially that between genital complex and abdomen and the sutures between abdomen and caudal rami.

The pigmentation of the last chalimus stage is similar to that of chalimus III, with little or no pigment anteriorly, and the lateral areas of the cephalothorax somewhat lighter brown and not as densely pigmented as the rest of the shield. Pigment is concentrated close to the suture lines of the cephalothorax. The protopodites of the third legs and the urosome segments are pigmented, but the greater part of the abdomen and the caudal rami is colourless.

The sternal furca has developed further but is still covered by the cuticle. The tines (c. 0.1 mm) are angled slightly outwards and bluntly rounded distally (Fig. 4f, length 0.104 ± 0.007 mm, $N=36$).

The posterior process of the maxillule is broad at the base and bears a single heavy spine directed ventrolaterally. In some older individuals, a small dentiferous process arises from the medial margin. This is a precursor of the tine seen in the preadult larvae (Fig. 4j).

Appendages and legs are generally larger than in chalimus III. Thus the mean width of the third leg increases from 552 μm in young chalimus III to 650 μm in late chalimus III, and to 760 μm in chalimus IV. The cephalothorax is 2.1 times longer than the urosome and 1.5 times longer than its maximum width.

The third and fourth chalimus larvae are similar in appearance, and closer study is therefore necessary to identify these stages. Johnson and Albright (1991) mention that the posterior sinuses of the fourth chalimus are more developed, that rudimentary thoracic valves are present and that the genital complex and abdomen are indistinctly separated. These differences are also apparent in Atlantic specimens as are the six minor differences they mention in the structure of the appendages in chalimus III and IV. However, these latter differences are so small that they are of no practical help in identifying the stage.

Johnson and Albright (1991) indicated two major differences between these stages. In chalimus IV, unlike chalimus III, the maxillule bears a posterior process consisting of small medial and large lateral tines, and a sternal furca is present. According to Boxshall (1974) the same is true of the chalimus larvae of *L. pectoralis*.

The posterior process of the maxillule of most fourth chalimus larvae in my Atlantic material consists of a single spine only. Further development with a precursor of a medial tine is seen only in rare cases. Moreover, the sternal furca is not functional, as it is developing below the cuticle of the fourth chalimus larva. It is therefore not visible on SEM photographs, where the structure is covered by the exoskeleton (Fig. 5a,b). Preserved larvae must be cleared in lactic acid to reveal the shape of the tines.

There are sexual differences in shape and size at the fourth chalimus stage. The cephalothorax, third leg and urosome are wider in females than in the relatively slim males. The cuticular folds are a positive character for females, which are therefore easier to pick out among chalimus IV larvae.

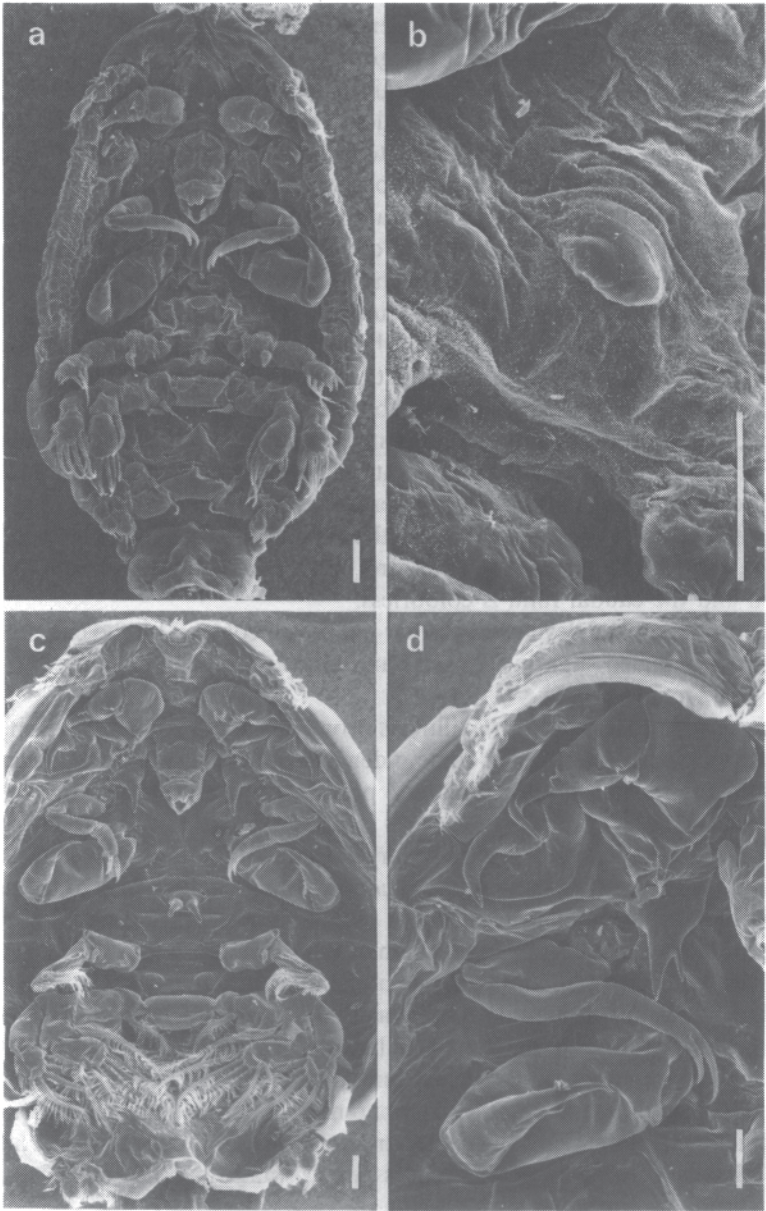


Fig. 5. (a) Cephalothorax of chalcus IV, ventral; (b) sternal furca covered by cuticle centrally in chalcus IV larva, ventral; (c) cephalothorax of first preadult male, ventral; (d) appendages of second preadult male, ventral. Scale lines 100 μ m.

First preadult, female

The larva has a mean length of 3.6 mm and width of 1.9 mm (Table 1). It is freemoving

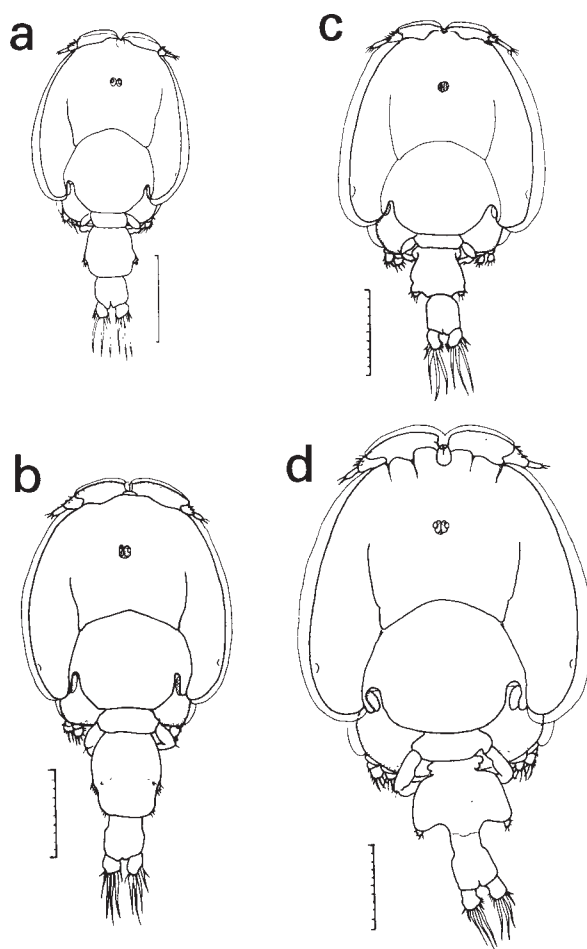


Fig. 6. (a) First preadult male, dorsal; (b) second preadult male, dorsal; (c) first preadult female; (d) second preadult female. Scale lines 1 mm.

on the host, or temporarily attached by the frontal filament during moulting. The filament emerges from the frontal organ (*sensu* Anstensrud 1990) situated ventrally close to the anterior margin of the cephalothorax. The larva has a shape similar to the adult except for the urosome segments (Fig. 6c). The urosome has distinct segments. The genital complex has ovoid lateral margins which diverge posteriorly. There is a posterolateral and ventral extension of the complex representing the fifth leg with four setae, and cuticular folds are present on the anterolateral margins.

Brown pigment is scattered all over the larva, except on the posterior part of the abdomen and the caudal rami. The pigment may be concentrated along suture lines in the cephalothorax and laterally in the genital complex.

The maxillule is broad at the base, and divided posteriorly. The sternal furca has now developed into a furcal box, which is robust and carries slender blunt tines of uniform width.

First preadult, male

The average length is 3.4 mm and width 1.6 mm. This stage is generally similar to the first preadult female except for the genital segment (Table 1, Figs 5c, 6a). The genital segment of a young preadult I male is barrel-shaped, and in dorsal view three of the four setae of the fifth leg may be seen laterally. In ventral view all four setae are seen on the bulbous outgrowths of the fifth leg. Rudiments of the sixth leg are located more posteriorly, each with two setae.

In larvae longer than c. 3 mm the genital segment is more ovoid in shape and the venterolateral bulbous outgrowths are visible in dorsal view. The fifth legs are situated more anteriorly on the genital segment of the male than in the corresponding female. Male larvae have no cuticular folds on the genital segment.

The distribution of brown pigment is similar to that in the female, but there is no lateral concentration on the genital segment.

Second preadult, female

The average length is c. 5.2 mm and width 3 mm (Table 1). The general morphology is similar to that of the adult female, except for differences in size and shape of genital complex and abdomen. The genital complex is larger and it has cuticular folds anterolaterally and diverging lateral margins which form large lobes on the posterolateral corners (Figs 3d, 6d). The bulbous fifth leg is located ventrally at these corners. The suture between the genital complex and abdomen may be indistinct. The abdomen narrows close to the genital complex. Pigment is distributed as in preadult I female.

Second preadult, male

The average length is c. 4.3 mm and width 2.2 mm (Table 1). The shape of cephalothorax is similar to that of other preadult stages, but narrower than in the second preadult female, maximum width only 0.3 mm greater than the cephalothorax of the first preadult female (Figs 5d, 6b). The genital complex is longer and more ovoid than in the preceding stage. Rudiments of reproductive organs are seen. The fifth and sixth legs are represented by bulbous outgrowths from the genital complex and bear four and three setae, respectively. The anterior part of the abdomen has a lateral constriction. Pigment is more or less evenly distributed but, as before, a triangular area on the abdomen and the caudal rami are unpigmented.

Adult male

The total length is 5–6 mm and maximum average width c. 3 mm. The size varies with season and locality (Table 1, Fig. 7c). The cephalothorax is similar to that of the second preadult stage. Adhesion pads are present on the antenna and on the body wall medial to the base of the maxillules. There is an additional small tine on the posterior process of the maxillule (Fig. 8a). The genital complex is ovoid, larger and more

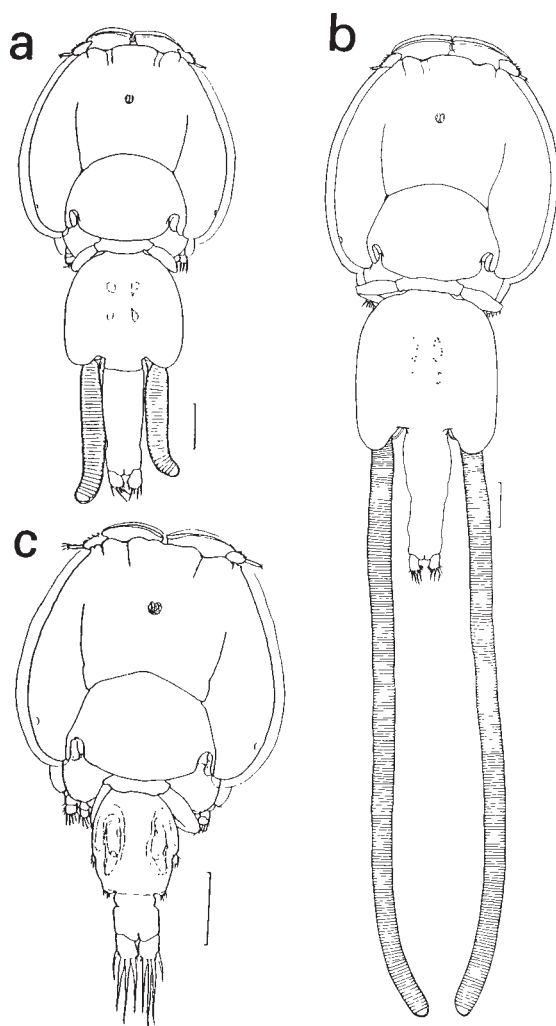


Fig. 7. (a) Young adult female, dorsal; (b) older adult female, dorsal; (c) adult male. Scale lines 1 mm.

developed, but very similar to that of the second preadult stage. Fifth legs bear four setae and are located close to lateral margins nearly in the middle of the segment. The sixth legs on the posterolateral corners of the genital complex are each armed with three setae. The abdomen has a lateral constriction anteriorly.

The pigmentation of the adult specimens, females as well as males, varies from light brown through various shades of brown to terracotta, copper or Venetian red. Both vividly coloured and paler specimens were found within the same batch of parasites, living under similar conditions. In pale specimens, the chromatophores are contracted and the pigment is seen as widely spaced dots. There seem to be no other differences between pale and brightly coloured specimens, and pairs of different colour

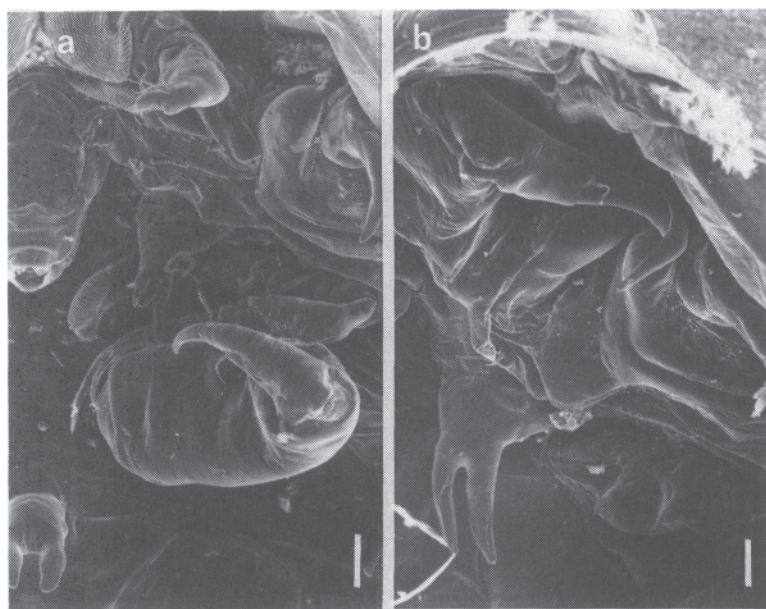


Fig. 8. (a) Appendages of adult male, ventral; (b) appendages of adult female, ventral. Scale lines 100 μm .

are often seen in precopula. Bleeding of pigment from the chromatocytes, which results in a characteristic carmine colouring, is a good criterion of death in the salmon louse.

The lateral zones of the cephalothorax of the male are more evenly and lightly pigmented than the cephalic and median thoracic areas. The first segment in the urosome and the genital segment stand out as the most heavily pigmented tagmata of the male. In the former, both pigment bands across the segment and dark pigment on the legs are present. Both the anterior and posterior parts of the genital segment have darker pigment than the median area. In transmitted light, the paired spermatophores are seen as light yellow bean-shaped structures. The abdomen is still sparsely pigmented, and the caudal rami are unpigmented.

Adult female

The total length is between 8 and 11 mm and width c. 4 mm (Table 1). The cephalothorax is round or more or less oval, and the fourth leg-bearing segment is short. The genital complex of younger females is about as long as wide, with narrow sloping anterolateral margins (Fig. 7a). In older females it is longer, with rounded anterolateral corners, parallel lateral margins and prominent rounded posterolateral corners. The abdomen is cylindrical, and about as long as the genital complex. The caudal rami are small and subquadrate (Fig. 7b).

There is often darker pigmentation at some distance from the rim of the genital complex. Another characteristic is that four small oval areas in the central area of the genital complex (Fig. 7a,b) stand out in transmitted light, as unpigmented, with no structures lying between the dorsal and ventral exoskeleton.

The medial and lateral tines of the maxillule are almost equal in length (Fig. 8b).

The egg strings are c. 0.5 mm in diameter and contain 15–17 eggs per millimetre. Young females have short strings, about as long as the abdomen (c. 3 mm). In older females the strings may be as long as the parasite (c. 12 mm) or at least up to twice this length (c. 20 mm). Thus each string may carry between 180 and 300 eggs.

DISCUSSION

Pelagic stages of parasitic copepods are morphologically very similar and information on pigmentation is therefore of great supplementary value to enable positive identification of living or newly preserved larvae. Johnson and Albright (1991) gave no information on the pigmentation of the developmental stages of *L. salmonis*. Johannessen (1978), stated that living nauplii of the salmon louse are almost transparent, with deep bluish pigment spots mainly concentrated in four distinct areas. This corresponds roughly to the present description although, in my view, the pigment he describes as bluish is in fact black. Johannessen (1978) did not mention any brown pigment.

White (1942) illustrated the attached copepodid of *L. salmonis*, showing pigment scattered all over the larva, but tending to be found in large dense patches. The colour of the pigment is not stated. White (1942) did, however, recognize that the pigment of the chalimus stages (II–IV) is arranged in smaller patches than in the copepodid. The distribution of pigment in these larval stages, as shown in his illustrations, is very similar to my observations on Atlantic *L. salmonis*.

The larva of *L. pectoralis* differs markedly from *L. salmonis* in that its antennule is highly pigmented and has pigment spots arranged serially along its length (Boxshall 1974). This stage is approximately the same size as nauplii of the salmon louse, and has a characteristic pigmentation pattern with black pigment on the antennule, around the eyes and across the posterior end, in addition to two or three pairs of lateral spots in nauplius I and II, respectively (own studies).

Four patches of pigment in the naupliar stages have also been observed in other species, e.g. *Caligus clemensi* Parker & Margolis (Kabata 1972), *Caligus curtus* Müller (Heegaard 1947), *Caligus elongatus* Nordmann (present author) and *Lernaeenicus sprattae* (Sowerby) (Schram 1979). Red pigment seems to be relatively common in nauplii, and has been found in *Lepeophtheirus kareii* Yamaguti (Lopez 1976), *Lepeophtheirus dissimulatus* Wilson (red and blue, Lewis 1963), *C. curtus* (Heegaard 1947), *C. elongatus* (present author), and *Caligus spinosus* Yamaguti (Izawa 1969). Although pelagic stages of parasitic copepods are rare in plankton samples, they may be more numerous in studies made in special areas, e.g. close to fish farms. Pigment data will aid in the identification of larvae from such plankton samples.

Johnson and Albright (1991) measured only small numbers of most developmental stages, with the exception of pelagic larvae, and took a total of about 190 length measurements. In the present study, approximately 400 larvae were measured: the number measured at each stage was always larger than in Johnson and Albright's study, and sometimes considerably larger.

The mean length of most stages of salmon lice from the Pacific was greater than the corresponding stages in the present study. The greatest differences between Atlantic and Pacific larvae were found in chalimus IV and the first preadult males. Chalimus IV from the Pacific ($N=12$) were on average 0.48 mm longer than those from the Atlantic ($N=39$), whereas first preadult males from the Pacific ($N=10$) were on average 0.45 mm *shorter* than the Atlantic sample ($N=33$). Similarly, the width of all Pacific developmental stages was greater than that of the Atlantic specimens. Thus larvae from the Pacific were generally larger than corresponding stages from the Atlantic.

According to Johnson and Albright (1991), the sexes of *L. salmonis* and other *Lepeophtheirus* species can first be distinguished morphologically in the preadult stage. I agree with this but, as mentioned above, the males are generally slimmer, and some chalimus IV larvae may be sexed using this and other criteria.

The pattern of development observed by Johnson and Albright (1991) for the maxillule of *L. salmonis* (divided in chalimus IV stage) is identical to that of *L. pectoralis* (Boxshall 1974). In my material the posterior process of the maxillule is not bifid until the first preadult stage, although a precursor of a medial tine is occasionally seen in chalimus IV. The somewhat delayed development of the Atlantic larvae may be related to their smaller size (average length of Atlantic chalimus IV larvae 2.3 mm, corresponding Pacific larvae 2.8 mm). The development of the maxillule of *L. salmonis* from the Atlantic is similar to that of *L. hospitalis*, which is also not bifid before the first preadult stage (Voth 1972).

Rudiments of the sterna furca may first be seen developing beneath the exoskeleton of the third chalimus larva (Lewis 1963, Boxshall 1974, Johnson and Albright 1991, present study) but the structure first becomes readily visible in the fourth chalimus stage (Johnson and Albright 1991). The morphology of the developing sternal furca in *L. pectoralis*, *L. salmonis* from the Pacific (Boxshall 1974, Johnson and Albright 1991) and their Atlantic relatives is broadly similar. However, the tines of chalimus IV specimens in my material are not functional, being situated beneath the larval exoskeleton. The sternal furca appears externally as a functional structure in the first preadult stage as in *C. spinosus* (Izawa 1969).

Except for the points discussed here and some minor differences, my observations of the appendages of different stages generally agree with the excellent descriptions provided by Johnson and Albright (1991).

ACKNOWLEDGEMENT

I thank Alison J. Coulthard, who has given editorial assistance and corrected the English.

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Part Ib Developmental factors

4

The development of *Caligus elongatus* Nordmann from hatching to copepodid in relation to temperature

A.W.Pike, A.J.Mordue (Luntz) and G.Ritchie

ABSTRACT

The development of the free-living stages of *Caligus elongatus* has been investigated at 5, 10 and 15°C in a 12 h light: 12 h dark regime using egg strings obtained from gravid adult lice and maintained in 32 ppt sea water. Duration of stages was temperature dependent. For the nauplius I the duration at the three temperatures was 36.9 h, 27.6 h and 16.6 h, respectively. The times for the nauplius II were 159.1 h, 68.1 h and 41.1 h, respectively. Development to the copepodid stage was more successful at temperatures of 10–15°C. The morphology of each stage is described and figured. Successful development of the early free-swimming stages of *C. elongatus* is very dependent upon high water quality.

INTRODUCTION

Caligus elongatus Nordmann, belonging to the family Caligidae, is one of the most important ectoparasites of farmed salmonids, and is a non-specific parasite of many fish species (Kabata 1979). Like most caligid copepods the life cycle involves two naupliar, one copepodid (infective), four larval and two preadult stages before reaching the final adult stage. The first phase of the life cycle, the nauplius, is divided into two stages, nauplius I and II, which are separated by a single moult. The nauplii are planktonic, non-feeding stages, propelling themselves through the water by means of three pairs of appendages.

The ontogeny of caligid copepods has been described for several species including *C. elongatus* (Gurney 1934, Hewitt 1971, Hogans and Trudeau 1989, Kabata 1979, Parker 1969, Tully 1989, Wootten *et al.* 1982), but no data exist on the effect of temperature on the generation times or development rates of *C. elongatus* nauplii.

Both Johannessen (1978) and Johnson and Albright (1991) have described the duration of the naupliar stages of *Lepeophtheirus salmonis* (Krøyer) in the temperature range 5–16°C and found a correlation.

The aim of the present work was to examine how temperature influences both development rate and survival of *C. elongatus* nauplii. This together with detailed descriptions of both morphology and behaviour of the free-living larval stages will provide a sound basis for the establishment of laboratory cultures of *C. elongatus*.

MATERIALS AND METHODS

Gravid adult female *C. elongatus* were collected from benzocaine-anaesthetized, seawater cultured, rainbow trout (*Oncorhynchus mykiss* Walbaum) at a fish farm in north-east Scotland. The lice were transported back to the laboratory in cooled sea water.

Culturing techniques

The parasites were held in the laboratory in 25-litre tanks of well-aerated sea water at 10°C, salinity 32 ppt. No hosts were present. Mature eggs were recognizable by their characteristic purple pigmented embryos. In contrast, immature eggs were white or pale yellow (Wilson 1905, Johannessen 1978). Females with mature egg strings were removed from the 25-litre tank and placed individually in 1-litre glass beakers with 900 ml of gently aerated, twice-filtered, fresh sea water. Beakers were placed in temperatures of 5, 10 and 15°C with a photoperiod of 12 h light: 12 h dark. Females with immature egg strings were held until the eggs matured (no longer than three days). The water was changed and dead specimens were removed daily. Fresh sea water was collected every third day, during high tide, at Cove Bay, Aberdeen. This was stored well aerated at 10°C. High-quality sea water, without any trace of nitrogenous waste, was essential for successful culturing.

Development rates

Females with mature egg strings, at each temperature, were observed every hour to record when the first nauplius I stage was released. Observations were made every half hour once hatching began. The hatching period was measured from the release of the first nauplius I larva to the last from each egg string. As nauplii emerged from the egg string a sample of 10–36 individuals was gently pipetted from the beaker and placed in a 100 ml glass beaker with 90 ml of twice filtered sea water (no aeration) which was changed daily. Samples of nauplii were taken from the hatched strings of several different females and maintained at the temperature at which hatching took place. Regular readings were taken to record the development rates and duration of the naupliar stages. Moribund specimens were removed, the stage at which mortality occurred noted and from this the survival of nauplii at the three temperatures was assessed. Changes in morphology during development were examined by light microscopy.

RESULTS

Studies of the exuvia and living nauplii showed that, after hatching, *C. elongatus* passed through two, free-swimming, naupliar stages before moulting to the copepodid.

Morphology and behaviour

Nauplius I stage

Shortly before hatching, twitching movements of the developing nauplii were seen within the egg membrane. The nauplii were released sequentially from the distal end of the ovisac and were characteristically pigmented (Fig 1A–C).

Several morphological changes were observed as the nauplius I developed, resulting in the differentiation of three different stages (early, mid and late). Newly hatched nauplii are ovoid and unsegmented (Fig. 1A). The dorsal surface is more convex than the ventral. The mean body length and width of the early nauplius I stages are given in Table 1. Anteriorly, on the ventral surface, are three pairs of appendages, similar to those of other caligid nauplii (Kabata 1972, Johannessen 1978), lying perpendicularly to the body, with the setae pointing posteriorly. The antennules are uniramous and terminate in two setae. The antennae and mandibles are biramous, with each of the four exopodal segments bearing a long seta. The one-segmented endopod terminates in two similar setae. Two ‘balancer organs’ are present at the posterior end (Wilson 1905, Kabata 1972, Johannessen 1978). Some internal structures are visible. Anterior patches of red pigment are associated with the naupliar eye. The large area of yolk is situated posteriorly. These small globules are held within the midgut, which at this stage is closed at either end. A small transverse slit, situated anteriorly, on the ventral surface represents the mouth. No proctodaeum was observed. Immediately posterior to the eye is a transparent yellowish structure, possibly the brain. Movements at this stage were restricted to twitching of the appendages.

Gradually, activity increased with more frequent twitching of the appendages. Slight photokinetic responses were observed. The mid nauplius I (active) (Fig. 1B) has a more elongate body which is oval in shape and slightly attenuated posteriorly. The mean body length had increased while the width had decreased slightly (Table 1). The appendages are now directed anteriorly. In addition to the pigmentation of the naupliar eye and the yolk within the midgut, there are concentrations of red pigment which extend along the sides of the body. The nauplius I remained at this particular morphological stage for the longest duration during development.

The late (pre-ecdysial) nauplius shows diminished activity. The antennae and mandibles are directed posteriorly and the antennules are held perpendicular to the body. The body is still unsegmented but has a fuller shape and smoother outline (Fig. 1C). The nauplius I is now slightly longer but narrower posteriorly (Table 1). The increase in length was associated with apolysis of the cuticle, with the nauplius II being clearly visible beneath the old cuticle. The more developed midgut is visible. As the nauplius I entered ecdysis the cuticle split immediately anterior to the antennules. Twitching movements further split the exuvia until the nauplius II was free. Moulting took approximately 1 h at 10°C.

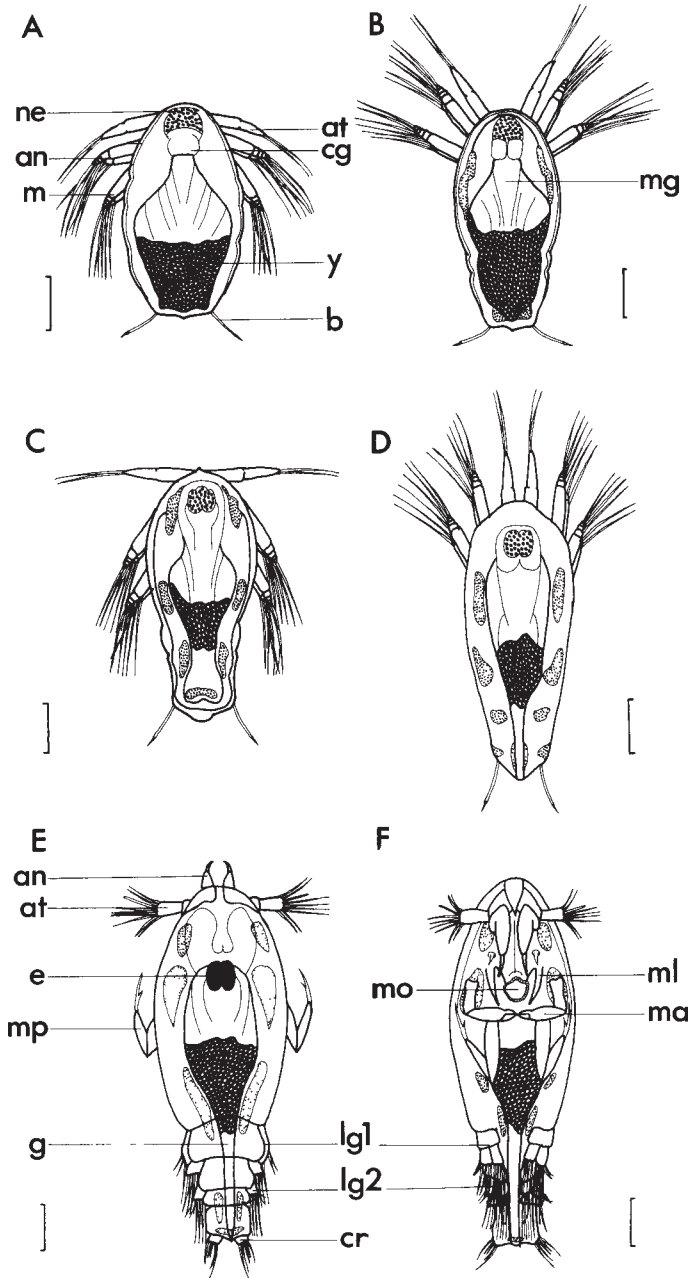


Fig. 1. *C. elongatus* cultured at 10°C. (Stippled areas represent pigment patches. Bar represents 100 μm .) (A) Nauplius I, dorsal view. (B) Nauplius I mid stage (active), dorsal view. (C) Nauplius I late stage (pre-ecdysis), dorsal view. (D) Nauplius II early-mid stage (active), dorsal view. (E) Copepodid stage, dorsal view. (F) Copepodid stage, ventral view. Abbreviations: an, antenna; at, antennule; b, balancer; cg, cerebral ganglion; cr, caudal rami; e, copepodid eye; g, gut; lg1, leg 1; lg2, leg 2; m, mandible; ma, maxilla; mg, midgut; ml, maxillule; mo, mouth; mp, maxilliped; ne, nauplius eye; y, yolk.

Table 1. Variation in length and width ($\pm$ SE) of the naupliar and copepodid stages of *C. elongatus*, cultured at 10°C with a 12 h light/dark cycle ($n=20-24$). All parameters are significantly different from each other at $p\leq 0.05$

Stage	Age	Mean length $\pm$ SE (μ m)	Mean width $\pm$ SE (μ m)
Nauplius I	Early	404 $\pm$ 3	226 $\pm$ 3
	Mid	444 $\pm$ 3	208 $\pm$ 3
	Late	481 $\pm$ 1	194 $\pm$ 2
Nauplius II	Early to mid	504 $\pm$ 3	182 $\pm$ 1
	Late	550 $\pm$ 3	226 $\pm$ 2
Copepodid	Early	756 $\pm$ 5	272 $\pm$ 2

Nauplius II stage

After moulting, the early to mid nauplii were more active than previously and showed positive photokinesis (active nauplius II stage, Fig. 1D). The nauplius II has a distinctive contoured and elongate body. The appendages are directed forward and show wide sweeping movements which are responsible for the rapid swimming movements. The gut has undergone further development, but is still non-functional; no proctodaeum was observed. The concentration of yolk within the midgut had decreased. The number of pigmented lateral areas had increased and a pigmented region was now visible at the posterior end (Fig 1D).

As the nauplius II developed, activity decreased, apolysis occurred and the copepodid could be seen within the old cuticle (late nauplius II). The length and width had increased (Table 1). As the nauplius approached pre-ecdysis, activity decreased further and the appendages, mouth, gut and pigmented eyes of the copepodid became apparent. The gut was now fully formed within the copepodid. The second ecdysis occurred in a similar fashion to the first but was more rapid (<1 h) at 10°C.

Copepodid stage

Kabata (1972) gave a detailed description of the copepodid stage of caligid copepods. The copepodid was larger (Table 1) than both naupliar stages. It consists of a cephalothorax and a segmented ‘pseudoabdomen’ (Kabata, 1972) (Fig. 1E,F). Mouthparts and gut were fully developed but not yet in use (Richmond and Pike, unpublished observations). The swimming legs rendered the copepodid faster and more mobile than the naupliar stages. The copepodid is positively phototactic.

Development

Hatching

The disc-shaped eggs were contained within elongate cylindrical strings. The number of eggs per string produced per female was variable. Hatching occurred sequentially from the distal end of the egg string. There was no correlation between the time of hatching and the time of day. Over 85% of the nauplii hatched from egg strings that matured 0–3 days after collection at each of the three temperatures. This material was

Table 2. Hatching period (time between hatching of first and last egg in egg string) of *C. elongatus* egg strings at 5, 10 and 15°C; *n*=the number of paired egg strings

Temperature (°C)	Mean hatching period (h)
5	4.25 ± 0.38 (<i>n</i> = 3)
10	3.25 ± 0.22 (<i>n</i> = 6)
15	2.28 ± 0.48 (<i>n</i> = 5)

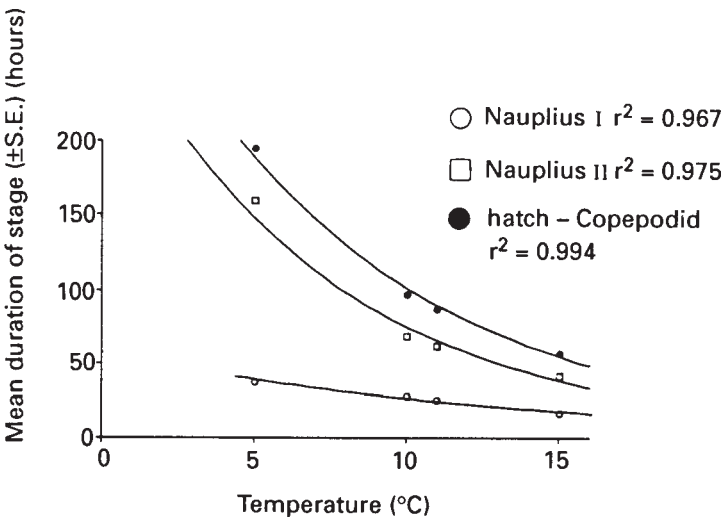


Fig. 2. The duration (hours) of the naupliar stages of *C. elongatus* at different temperatures: O, nauplius I (hatching to post first ecdysis); □, nauplius II (post first ecdysis to post second ecdysis); ●, total naupliar stages (hatching to copepodid); *n*=2–10 samples (no. of nauplii per sample varied from 9 to 36).

used to study the development of the nauplii. Egg strings that matured later showed poor hatching.

Temperature directly affected the hatching period of *C. elongatus* (Table 2). As temperature increased the mean hatching period decreased and the decrease in hatching period between 5 and 15°C was significant (*p*<0.05).

Development of the naupliar stages

The duration of the naupliar stages and the total duration from hatching to the copepodid stage at 5, 10, 11 and 15°C are shown in Fig. 2. Mean duration of the nauplius I stage showed a negative correlation with temperature. At 5°C the mean duration was 36.9 h, decreasing to 27.6 h at 10°C, 25.2 h at 11°C and 16.6 h at 15°C. The durations at 5 and 15°C were significantly different (*p*<0.001).

At each temperature the duration of the nauplius II stage was longer than that of the

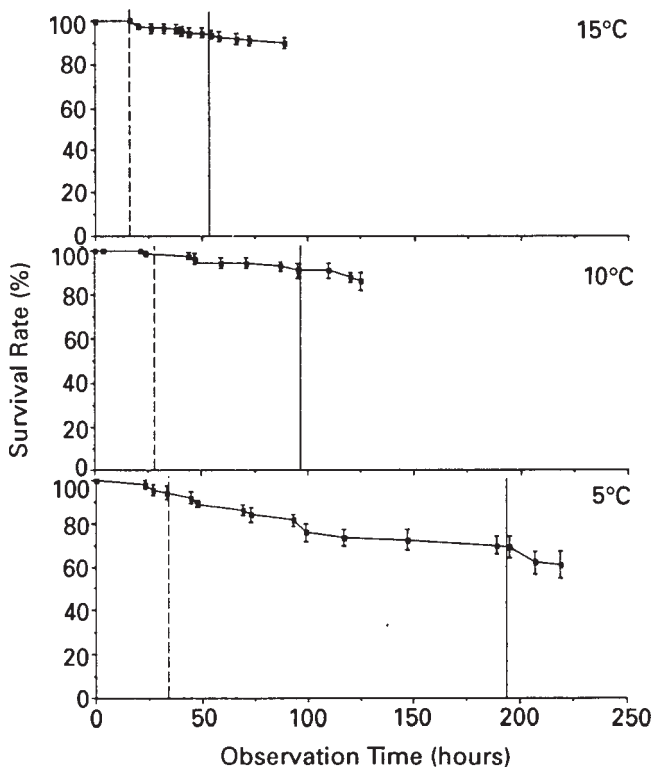


Fig. 3. The survival rate of *C. elongatus* nauplii at 5, 10 and 15°C $\pm$ SE; $n=10$ (except at 10°C, where $n=5$), where n =number of samples (one sample=10–36 nauplii). The vertical lines show the time at which 50% of the population has moulted to nauplius II (---) and copepodid (—) stages.

nauplius I stage. Again a negative correlation existed, with the duration decreasing from 159.1 h at 5°C to 68.1 h at 10°C, 61.4 h at 11°C and 41.1 h at 15°C. These differences were significant ($p<0.001$ in all cases).

The total durations of the naupliar stages, at each temperature, were again significantly different ($p<0.001$). Specimens with longer durations than indicated generally did not develop into the next stage.

Nauplius survival

The survival rate (%) of *C. elongatus* nauplii at 5, 10 and 15°C is shown in Fig. 3. Observations were discontinued when all the nauplii had either reached the copepodid stage or had died. At 15°C the mean survival rate of nauplii to the copepodid stage was 90%. The number of nauplius II mortalities was slightly higher than those of the nauplius I. Several nauplii did not develop to copepodid but survived for over 70 h. Only two deaths occurred during moulting. A similar result was obtained at 10°C with 86% of the nauplii reaching the copepodid stage. The numbers of nauplius I and II mortalities were equal.

Mortalities were considerably higher at 5°C. The percentage of nauplii surviving to the copepodid stage was approximately 60%. Mortalities were higher at the nauplius I stage, particularly during first ecdysis. After 50 h many nauplii were still attempting to complete the first moult. Most nauplii which delayed this moult were unsuccessful in reaching the nauplius II stage. They often reached the late naupliar I stage but failed to ecdyse and became infected with ciliated protozoans.

DISCUSSION

C. elongatus possesses two naupliar stages divided by a single moult. Both stages are non-feeding and free living. Their morphology is similar to that described for other species of caligid (Wilson 1905, Hewitt 1971, Kabata 1972, Johannessen 1978, Hogans and Trudeau 1989). Distinct variation occurs between the stages, and internal differences were observed within a single stage as development proceeds. The most notable difference overall is the increase in length of the naupliar stages to the copepodid. Behaviour patterns were similar to those described by Johannessen (1978) for *L. salmonis* nauplii, with both naupliar stages being responsive to tactile and phototactic stimuli, particularly the nauplius II stage.

Alterations in water temperature directly affected hatching rate, development rate and survival of nauplii. The mean hatching period in this study with egg strings of approximately 80 eggs was considerably lower than that found previously for *L. salmonis*. Johannessen (1978) reported that the hatching period of egg strings with 100–500 eggs was more than 30 h, with the majority hatching within 5–10 h. Johnson and Albright (1991) found the hatching period to range from 18 to 65 h at 10°C. Egg strings of *Caligus spinosus* Yamaguti (Izawa 1969) hatched within 3–8 h but only carried between 10 and 20 eggs. In both these studies hatching took place in static water. From our observations, the hatching period of *C. elongatus* was considerably longer in static rather than aerated water. Oxygenated and moving water appear to be important conditions in maximizing hatching. The activity of nauplii emerging from the egg string was considerably reduced at 5°C. This partly accounts for the longer hatching period at that temperature, since the larvae rupture the egg case very slowly.

Positive correlations between durations of individual stages and temperature were observed. Nauplii which develop faster or slower than the average show the same differential in the next stage. At each of the four temperatures the duration of the nauplius I stage was shorter than that of nauplius II. Hogans and Trudeau (1989) found the duration of both stages to be the same at 10°C. The variation in instar length with temperature in this study corresponded well with studies on *L. salmonis* by Johannessen (1978), Wootten *et al.* (1982) and Johnson and Albright (1991). With the exception of the results by Hogans and Trudeau (1989) the duration of the nauplius I stage is shorter than that of nauplius II in all caligid species studied to date.

Alterations in temperature affected the survival of nauplii. Increases in temperature seemed to have little effect on the survival of nauplii compared to survival at an ambient temperature of 10°C. However, reduction in temperature led to high mortality. Comparable data for the survival of caligid nauplii at various temperatures do not exist, but Schram (1979) reported high mortalities of *Lernaeenicus sprattae* (Sowerby)

nauplii at 5°C. It has been suggested that, in several species of Crustacea, shedding of the cuticle is blocked at low temperature although the life processes continue (Johannessen 1978). In many cases, internal development was observed although no moult occurred. The survival rates of nauplii at lower temperatures may be different for females with egg strings collected at these lower temperatures.

Overall, the experiments have demonstrated that *C. elongatus* larvae hatched, grew and survived well at a temperature of 10°C (12 h light: 12 h dark) in clean, aerated and moving sea water and that any reduction in water quality usually caused development to fail.

ACKNOWLEDGEMENTS

The support of Brian Shaw and the Cromarty Salmon Co. is gratefully acknowledged. G.R. was supported by an Aberdeen University Research Committee Grant awarded to A.J.M. and A.W.P.

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5

Comparative life history of two species of sea lice

T.DeMeeüs, A.Raibaut and F.Renaud

ABSTRACT

In the Mediterranean, the parasitic copepod *Lepeophtheirus thompsoni* specifically parasitizes turbot, a marine scophthalmid, on which female copepods are haematophagous. The congeneric *Lepeophtheirus europaensis* infects brill (a marine scophthalmid) and flounder (a pleuronectid inhabiting lagoons) on which females are mucophagous like the other parasitic stages of both caligid species. The study of some life history traits such as female fertility, life span, developmental success and duration of early development reveals the probable influence of the diet and of the host species utilized. The importance of such studies in the understanding of the epidemiology of sea lice in general is discussed.

INTRODUCTION

In the Gulf of Lions (Mediterranean, France), *Lepeophtheirus thompsoni* Baird, 1850 and *Lepeophtheirus europaensis* Zeddam, Berrebi, Renaud, Raibaut & Gabrion, 1988 parasitize three different species of flatfish (Heterosomata). *L. thompsoni* is specific to one host, the turbot (*Psetta maxima* L. 1758), a marine scophthalmid, and *L. europaensis* is found on both brill (*Scophthalmus rhombus* L. 1758), a marine scophthalmid, and flounder (*Platichthys flesus* L. 1758), a pleuronectid inhabiting lagoons. For these parasites, egg laying takes place within the gill chamber of their host. There, *L. thompsoni* females attach to the gill filaments and become haematophagous, whereas *L. europaensis* females are mucophagous like the other stages (Zeddam *et al.* 1988). De Meeüs *et al.* (Chapter 11) describe other differences in specialization between the two copepod species studied (experimental host specificity and salinity tolerance) and reveal the existence of some heterogeneities within the Mediterranean populations of *L. europaensis* with respect to the host species parasitized (brill versus flounder).

Little is known about sea lice life history and, in particular, even basic information

about life span and fecundity of adults and survival characteristics of free-living stages is unavailable (Pike 1989). This chapter is intended to emphasize the life history traits of *L. thompsoni* and *L. europaensis* that can be correlated with the observed differences in specialization, host species parasitized and diet. For this purpose the numbers of eggs per egg sac are analysed for females sampled on each of the three host species. Experimental infections provide complementary data on the number of clutches (i.e. two egg sacs) produced, on development times and on life span of attached stages. The developmental success of eggs and of free stages, and the survivalship of infective stages (copepodids) in the absence of a host are also studied.

The results reveal that haematophagy appears to be correlated with higher female fertility. The exploitation of two different hosts (brill and flounder) by *L. europaensis* is associated with heterogeneities in female fertility and in development time of free stages. These observations are discussed and compared with what can be found on similar organisms. This work provides new data on the poorly known life history of sea lice in general.

MATERIAL AND METHODS

Ovigerous female copepods and natural clutches

Ovigerous females were collected in fishing ports (Sète and Grau-du-Roi, France) from the gill cavities of their hosts. Flounder copepods were collected on hosts caught in lagoons (Etang d'Ingril, Etang de Mauguio, Etang du Ponant) by craft fishermen. *L. thompsoni* females are found attached to gill filaments, while *L. europaensis* females are found on the wall of the gill chamber and on the inner surface of the operculum (Zeddiam *et al.* 1988). Eggs contained in one egg sac per pair and per female were counted using a binocular microscope.

Experimental hosts

Some parasite-free turbot were bought from fish farms. The different flatfish species used for experimental infections were caught along the Languedoc coast (Mediterranean, France) and in lagoons (flounders). Turbot and brill were caught at sea by craft fishermen, or inshore, and flounder were caught in lagoons by craft fishermen. In the laboratory and under a binocular microscope, these fish, anaesthetized with 3-aminobenzoic acid ethyl ester (Sigma A 5040), were observed and only the parasite-free hosts were kept for experiments.

Experimental clutches

Sampled eggs were incubated at 15°C in filtered sea water. After hatching and development through the nauplius phase, the infective stages (copepodids) were isolated, counted and placed in a 50-litre tank containing fish to be infected. After development and mating, all parasites except females were removed. Observations of the anaesthetized infected fish took place every 3 days. Ovigerous females were regularly removed from the host, relieved of their egg sacs and reintroduced into the gill chamber of the host until the next egg laying. Eggs contained in these egg sacs were then

counted. The experiment stopped at the disappearance of the last female from the experimental host.

Two flounder and two turbot were infected by flounder and turbot copepods, respectively. Because brill does not survive well in experimental conditions, *L. europaensis* from brill were studied on two flounder and on only one brill.

Development of eggs and survival of free stages

Clutches were incubated at 15°C in filtered sea water (35‰ salinity). After hatching and development through the nauplius phase, the infective stages (copepodids) were counted daily. The mean survival (in days) was then calculated for each clutch. These observations provided data on the developmental success of clutches, eggs and free stages, and also on the development times from egg laying to infective stage.

RESULTS

Size of natural clutches

The number of eggs per egg sac is 66 ($s^2=398$) for turbot copepods ($n=247$ egg sacs examined), 52 ($s^2=287$) for brill copepods ($n=251$) and 61 ($s^2=407$) for flounder copepods ($n=335$). Variances are not homogeneous (F -tests for homogeneity of variances, $p<0.01$). A non-parametric test (one-tailed Kolmogorov–Smirnov) is thus used for paired comparisons. This reveals that *L. thompsoni* lays more eggs per egg sac than *L. europaensis* from brill ($p<0.001$) and flounder ($p<0.02$). Moreover, flounder parasites produce larger clutches than brill parasites ($p<0.001$).

Experimental clutches

Experimental clutches contain fewer eggs than natural ones (Fig. 1). This point is only tested for flounder copepods raised on flounder because more data are available for this experiment. The difference between these data and natural clutches is very significant ($p<0.001$, one-tailed Kolmogorov–Smirnov). The experimental results must therefore be interpreted with care. Females lay a number of clutches which can be relatively large (e.g. up to 16 clutches for one flounder copepod) compared with what has been reported in the literature (Lewis 1963). First and last clutches contain fewer eggs than intermediate clutches. This may explain the large variances observed in natural clutches.

During these experiments, we obtained some data on the development times of attached stages (from copepodid to the first egg laying) and on the delay between the production of consecutive egg sacs (in 15°C sea water). For reasons given above, these results, presented in Table 1, must be considered with caution and are only presented here as a basis for discussion. In all experiments, development times of the attached phases appear homogeneous (about 36 days) as does the delay between successive clutches (6–8 days).

Developmental success and survival of free stages

The observation of clutch development in sea water revealed that 10%, 4% and 6% of clutches do not develop at all (absence of pigmentation) for turbot, brill and flounder copepods, respectively (χ^2 not significant, 1 d.f., $p>0.1$). Furthermore, the developmental

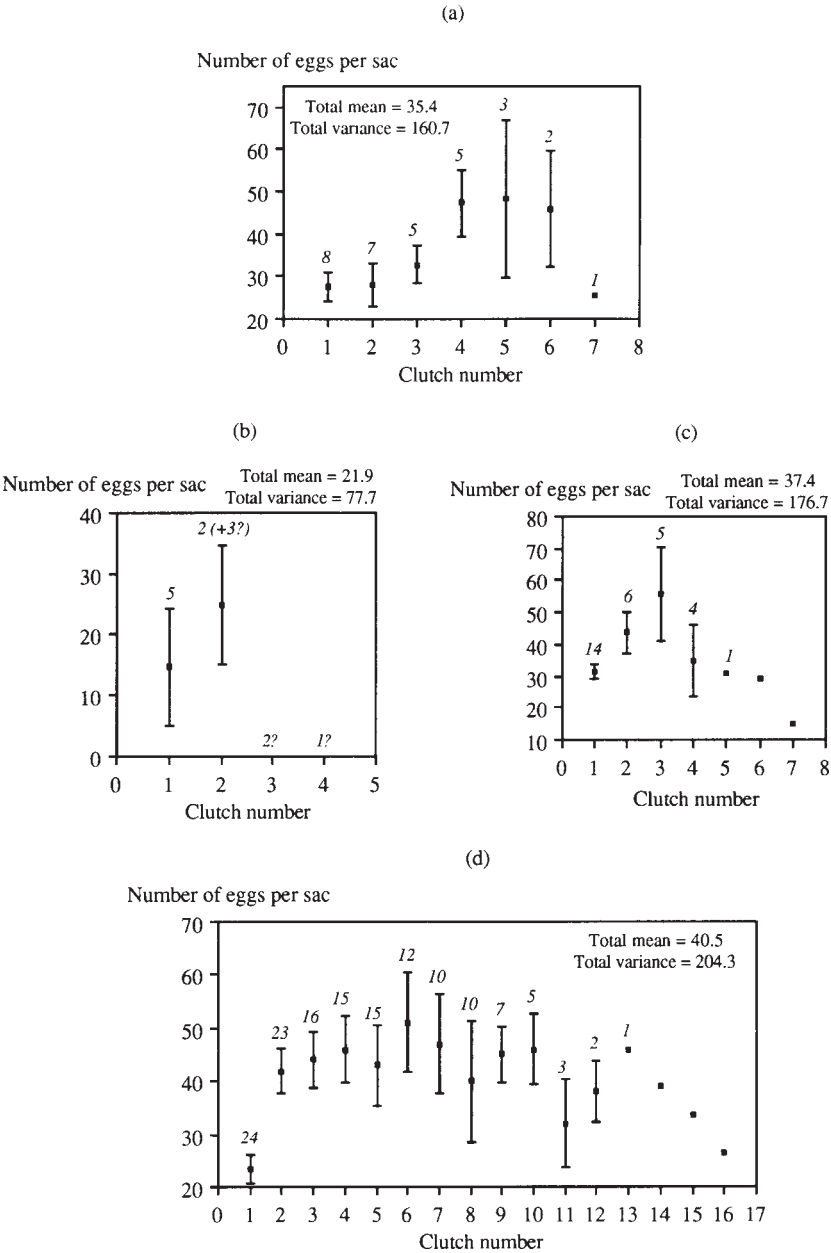


Fig. 1. Experimental clutch production observed for turbot copepods on turbot (CT/T) (a), for brill copepods on brill (CB/B) (b) or on flounder (CB/F) (c) and for flounder copepods on flounder (CF/F) (d). Successive numbers of gravid females are given within the graphs (italics).

Table 1. Development times (in days) of the attached phase (from the fixed copepodid to the first egg laying) and time between successive clutches, observed for different copepods raised on different experimental hosts

		CT/T	CB/B	CB/F	CF/F
W1	<i>N</i>	3	1	2	2
	Days	42,37,38	47	35,31	37,35
	<i>n</i>	10	3	9	16
$W_i - W_{i+1}$	Mean (days)	5.7	7.7	7.6	6.1
	s_{mean}	0.7	0.9	1.0	0.3

N: number of experiments; *n*: number of successive clutches observed; W1: first egg laying; $W_i - W_{i+1}$: time between laying of successive clutches. CT/T: turbot copepods raised on turbot; CB/B: brill copepods raised on brill; CB/F: brill copepods raised on flounder; CF/F: flounder copepods raised on flounder.

Table 2. Development times (in days) from fertilized eggs to copepodids for turbot (CT), brill (CB) and flounder (CF) copepods

	CT	CB	CF
<i>n</i>	42	51	58
Mean	8.2	6.3	7.8
s^2	6.3	6.8	4.5
s_{mean}	0.36	0.36	0.28

n: number of clutches; s^2 : variance; s_{mean} : standard error of the mean.

success from fertilized eggs to copepodids at 35‰ is higher for flounder copepods (86.4% of copepodids) than for those of brill and turbot, which do not differ significantly from each other (73.9% and 75.1% of copepodids, respectively) (Chapter 11).

The survival (in days) of the copepodids left in sea water without a host corresponds to the mean survival obtained for each clutch (considered thus as single individuals). Copepodids from turbot survive 6.8 days ($s^2=16.9$, $n=27$ clutches), those from brill survive 7.5 days ($s^2=12.4$, $n=23$) and those from flounder survive 5.5 days ($s^2=12$, $n=20$). Variances are homogeneous (*F*-test, $p>0.1$). A single classification ANOVA does not discriminate between the results obtained for turbot, brill and flounder ($p>0.1$).

Development times of eggs and free stages

The different development times, from fertilized eggs to copepodids, are presented in Table 2. Variances are homogeneous (*F*-test, $p>0.05$). A single-classification ANOVA indicates that the three samples are heterogeneous ($p<0.001$). Paired comparisons (*t*-tests) reveal that this heterogeneity comes from brill parasites, which develop faster (6.3 days) than turbot parasites (8.2 days) and flounder parasites (7.8 days) ($p<0.001$). The differences between turbot and flounder parasites are not significant ($p>0.5$).

DISCUSSION

Despite the fact that the present study is restricted to certain conditions (35‰ sea water at 15°C, experimental constraints), it provides detailed quantitative information on the life history of two sea lice species on their respective hosts. These data concern essential parameters of the fitness of those organisms: fecundity, developmental success and survival and duration of the different life cycle phases. Such data are not frequent in the literature (Pike 1989). It is nevertheless known that the concept of the ‘highest possible number of eggs’, postulated for parasites in general, has many exceptions in parasitic copepods (Kabata 1981). Indeed, on a basis of ten clutches laid by one *L. thompsoni* female (the most fertile), we would only have obtained 1320 eggs, a number comparable to that found in non-parasitic copepods (e.g. Uye 1981).

High survival must compensate for low fecundity (Hirschfield and Tinkle 1975). In accordance with this, the developmental success appears high for *Lepeophtheirus salmonis* (Johannessen 1978) as it does for the copepods studied in this chapter. Moreover, if we use the experimental fixation rates of copepodids on their original hosts (Chapter 11) as the ratio of successfully infective copepodids, the proportions of eggs leading to infective copepodids become 68% for *L. thompsoni* and 71% and 81% for *L. europaensis* from brill and flounder, respectively. Furthermore, copepodid survival without a host is also high. With a general mean of 7 days, some copepodids survived more than 20 days without a host, a result close to the maximum of 1 month observed for *L. salmonis* by Johannessen (1978), although this period may be exceptional. Such results can have important epidemiological and evolutionary consequences. The free-swimming phase indeed represents a critical step in parasitic life cycles. A good dispersal ability appears to be a basic necessity for organisms displaying habitat selection (De Meeüs *et al.* 1993), which in the present case may be equivalent to host specificity.

The compilation of all the results obtained on the duration of the different life cycle phases provides an approximate estimate of the life cycle duration. The period from fertilized eggs to the first egg laying is about 44 days for all the copepods studied in this chapter. The maximum life span of a single female, from fertilized egg to death, is 135 days (observed for one *L. europaensis* from flounder).

Our study also reveals important heterogeneities between the different copepods studied. Possibly as a correlation with its haematophagous diet, *L. thompsoni* appears more fertile than *L. europaensis* (mucophagous). Moreover, *L. europaensis* from flounder lay more eggs than those from brill, the free phase of which (fertilized eggs to copepodid) appears shorter, perhaps as a compensation. In the North Sea, flounder are parasitized by two different species of ectoparasitic copepods: *Lepeophtheirus pectoralis* (Müller, 1776), the adult females of which are found on the inner surface of pectoral and pelvic fins; and *Acanthochondria depressa* (Scott, 1901), which occupies the gill chamber (Boxshall 1974). In this area, Anstensrud (1990) found a maximum of 70 eggs per clutch. Such a value is just above half of the mean observed for *L. europaensis* (122 eggs per clutch) and is far from the maximum observed for this species (218). It is possible that the gill chamber represents a more favourable environment (more oxygen, more mucus available) than the pectoral and pelvic fins.

To conclude, these data obtained on the life history of *L. thompsoni* and *L. europaensis* may lead to a better understanding of sea lice epidemiology. Differences in diets (mucophagy, haematophagy), in location on the host (gill chamber, fins), in

the host species parasitized (brill versus flounder for *L. europaensis*) and in external environment (sea and lagoons) appear to be correlated with important life history traits. Changes might occur within the populations of *L. salmonis*, for example the adoption of haematophagy, when faced with farmed rather than wild salmon. Such possibilities of consequential changes should be taken into account in studies dealing with such a major pathogen of farmed salmon (Pike 1989).

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6

A comparison of development and growth rates of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on naive Atlantic (*Salmo salar*) and chinook (*Oncorhynchus tshawytscha*) salmon

S.C. Johnson

ABSTRACT

The development and growth rate of the economically important marine ectoparasitic copepod *Lepeophtheirus salmonis* on naive Atlantic salmon *Salmo salar* and naive chinook salmon *Oncorhynchus tshawytscha* were compared under laboratory conditions. The abundance of *L. salmonis* declined significantly on both host species over time. Copepods occurred and developed on the gills, fins and other body surfaces up to the first preadult moult, after which they moved to the other body surfaces. *L. salmonis* developed faster on Atlantic salmon, with adult males and females first recorded at 25 and 30 days post-infection, respectively. Adult males and females were first recorded at 40 and 45 days post-infection, respectively, on chinook salmon. Nutritional and/or non-specific host defence mechanisms are most likely responsible for the difference in development rate, but their exact roles remain to be determined. Although it has been previously suggested that *L. salmonis* develops at different rates on different regions of the host body, no differences in development rate were observed in this study.

INTRODUCTION

Lepeophtheirus salmonis is a common marine ectoparasitic copepod of wild and penreared salmonids throughout the Northern Hemisphere (Kabata 1979, 1988, Wootten *et al.* 1982). This species has a broad range of salmonid hosts, including *Oncorhynchus clarki* Richardson (= *Salmo clarki*) (coastal cutthroat trout), *Oncorhynchus gorbuscha* (Walbaum) (pink salmon), *Oncorhynchus keta* (Walbaum)

(chum salmon), *Oncorhynchus kisutch* (Walbaum) (coho salmon), *Oncorhynchus masou* (Brevort) (cherry or masu salmon), *Oncorhynchus mykiss* (Walbaum) (= *Salmo gairdneri*) (rainbow or steelhead trout), *Oncorhynchus nerka* (Walbaum) (sockeye salmon), *Oncorhynchus tshawytscha* (Walbaum) (chinook salmon), *Salmo salar* L. (Atlantic salmon) and *Salvelinus fontinalis* (Mitchill) (brook trout) (Kabata 1979, 1988, Wootten *et al.* 1982, Nagasawa 1987). On the Pacific coast of Canada (British Columbia), *L. salmonis* is prevalent on pen-reared Atlantic salmon as well as on both wild and penreared chinook salmon (unpublished data).

Host effects on the biology of ectoparasitic copepods have been previously documented. These include changes in their distribution on the host, interruption of egg sac production, loss of egg sacs, failure of eggs to develop, and reduced infectivity of the copepodid stage (Shariff 1981, Paperna and Zwerner 1982, Woo and Shariff 1990). Johnson and Albright (1992a) demonstrated that naive chinook salmon are more resistant than naive Atlantic salmon to experimental infection with *L. salmonis*. Furthermore, they reported that the developmental rate of *L. salmonis* appeared to be higher on Atlantic than chinook salmon.

The objective of the present study was to investigate further the differences in the development and growth rates of *L. salmonis* on naive chinook and naive Atlantic salmon under controlled laboratory conditions. This information furthers our understanding of the population dynamics of *L. salmonis*.

MATERIALS AND METHODS

Ovigerous *L. salmonis* were collected from wild chinook and sea-farmed Atlantic salmon from the Strait of Georgia, British Columbia, Canada. Eggs and developing nauplii were cultured in 45-litre tanks supplied with flowing sand-filtered sea water (1 gal min⁻¹) with a salinity of 29–31‰ and a temperature of 9.1–10.3°C. Circulation in the tanks was maintained by gentle aeration. Large surface area screens (100 µm Nitex mesh) were attached to the tank drains to prevent copepod loss.

Sixty-five naive Atlantic and 65 naive chinook salmon were introduced into separate 500 litre tanks, acclimated for 17 days, and then exposed for 24 h to approximately 3000 newly moulted copepodid larvae. The infections were carried out under conditions of darkness, low water flow, and aeration. Large surface area screens (180 µm Nitex mesh) were attached to the tank drains to prevent copepodid loss. Both species of salmon had been smolted and maintained in sand-filtered sea water to ensure no previous exposure to *L. salmonis*.

Fish were maintained in flowing sand-filtered sea water with ambient salinity (29–31‰). Temperatures of the tanks were recorded hourly and daily averages calculated from these readings. Fish were fed a commercial dry pellet feed at 1 % body weight per day. Samples were collected every 5 days post-infection. Fish were rapidly killed by a blow to their head, and their fork lengths and wet weights determined. The body surfaces including the gills were examined for copepods and the distribution of the copepods on the fish was recorded. An average copepod stage was calculated for each fish as follows: (1×COP+2×CH1+3×CH2+4×CH3+5×CH4+6×PRE1 +7×PRE2+8×adult)/total number, where COP=copepodid; CH1=first chalimus; CH2=second chalimus; CH3=third chalimus; CH4=fourth chalimus; PRE1=first preadult; PRE2=second preadult.

Intensity data were corrected to a standard wet body weight to compensate for differences in size among hosts.

For each copepod reared in the laboratory, measurements were made of total length (anterior margin to base of caudal rami), and maximum cephalothorax width (excluding marginal membrane). The total length, cephalothorax length, cephalothorax width, genital complex length, genital complex width, abdomen length, and the number of eggs carried in the egg sacs were determined for adult female copepods collected from both mature chinook and mature Atlantic salmon reared at the farm from which the eggs were collected.

STATISTICAL METHODS

Intensity data were log ($x+1$) transformed and differences in copepod intensity on the different body regions and among the different body regions were examined by analysis of variance (ANOVA). For each salmon species comparisons of copepod intensity for each body region over time as well as between body regions at each sampling period were made using Scheffé's tests (Zar 1984).

Stage distribution data were compared between host species and among different body regions of individual host species using a χ^2 statistic. Differences in the proportion of each stage on the different host species were investigated by t -tests of arcsin transformed proportion data.

Size data were log ($x+1$) transformed and differences in sizes of the different copepod stages on the different body regions investigated by ANOVA. For each stage comparisons of copepod size between host body regions at each sampling period were made using t -tests or Scheffé's tests.

RESULTS

The intensity of *L. salmonis* on the gills, fins and other body surfaces of Atlantic and chinook salmon over time is presented in Fig. 1. The intensity of infection for each body region was significantly different over time for both host species (one-way ANOVA; Atlantic salmon (gills, fins and other body surfaces, $p<0.001$); chinook salmon (gills, fins and other body surfaces, $p<0.001$)). The results of Scheffé's multiple range tests of copepod intensity for each body region of Atlantic and chinook salmon over time are summarized in Table 1.

Copepods were collected from the gills of Atlantic salmon between 5 and 20 days post-infection, with no significant difference in the intensity of infection over this time (Scheffé's test; $p<0.05$; Table 1). The fins of Atlantic salmon bore copepods up to 25 days post-infection, with significantly fewer copepods present at 20 and 25 days post-infection than earlier in the experiment (Scheffé's test; $p<0.05$; Table 1). Copepods were present on the other body surfaces of Atlantic salmon throughout the experiment.

Copepods were present on the gills and fins of chinook salmon up to 30 days post-infection. There was no significant difference in copepod intensity on the gills between 5–15, 15–25 and 20–30 days post-infection, or on the fins between 5–20 or 25–30 days post-infection (Scheffé's test; $p<0.05$; Table 1). Copepods were present on the other body surfaces throughout the experiment.

At 5, 10 and 15 days post-infection there was no significant difference in the intensity

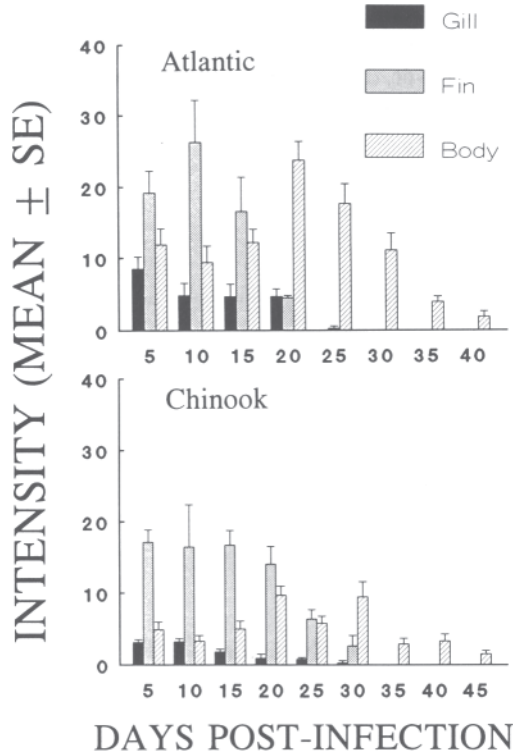


Fig. 1. Mean ($\pm$ SE) intensity of *L. salmonis* on different body regions of naive Atlantic and naive chinook salmon at various times post-infection. Fish were maintained at 9.0–11.3°C and ambient salinity (29–31‰).

of copepods between the fins and other body surfaces of Atlantic salmon, and between the other body surfaces and the gills (Scheffés test; $p < 0.05$). At 20 and 25 days post-infection there were significantly fewer copepods present on the fins and gills than on the other body surfaces, and no significant difference in copepod intensity between the fins and gills (Scheffés test; $p < 0.05$).

There were significantly more copepods present on the fins of chinook salmon when compared to both the other body surfaces and the gills, and no significant difference in copepod intensity between the other body surfaces and the gills (Scheffés test; $p < 0.05$) at 5 and 10 days post-infection. At 15 days post-infection there were significantly more copepods present on the fins when compared to both the other body surfaces and the gills, and significantly more copepods present on the body than on the gills (Scheffés test; $p < 0.05$). There was no significant difference in copepod intensity between the fins and the body, and significantly fewer copepods on the gills when compared to both the fins and the other body surfaces (Scheffés test; $p < 0.05$) at 20 and 25 days post-infection. At 30 days post-infection there were significantly more copepods present on the other body surfaces than on the fins or the gills, and no significant difference in copepod intensity between the fins and the gills (Scheffés test; $p < 0.05$).

Table 1. Results of multiple range tests (Scheffés test; $p<0.05$) of intensity of *L. salmonis* on various body regions of Atlantic and chinook salmon over time. Values within rows are the days post-infection between which there is no significant difference in *L. salmonis* intensity

Host species	Body region	Scheffés test results
Atlantic	Gills	5, 10, 15, 20 25, 30, 35, 40
Atlantic	Fins	5, 10, 15 20 25, 30, 35, 40
Atlantic	Other body regions	5, 10, 15, 20, 25 5, 10, 15, 30 35, 40
Chinook	Gills	5, 10, 15 15, 20, 25 20, 25, 30, 35, 40, 45
Chinook	Fins	5, 10, 15, 20 25, 30, 35, 40, 45
Chinook	Other body regions	5, 10, 15, 20, 25, 35, 40 5, 10, 15, 25, 35, 40, 45

The stage composition and the average copepod stage of *L. salmonis* on both host species over time are presented in Fig. 2. Significant differences in the stage distributions of *L. salmonis* on the two host species occurred on each day sampled (Pearsons χ^2 ; $p<0.001$ for all samples). *L. salmonis* developed faster on Atlantic salmon with adult males and females first recorded at 25 and 30 days post-infection, respectively. Adult males and females were first recorded at 40 and 45 days post-infection, respectively, on chinook salmon.

The stage composition of *L. salmonis* by body region on Atlantic and chinook salmon at various times post-infection are given in Figs 3 and 4. Significant differences in the stage distributions on the different body regions of Atlantic salmon occurred at 5 and 20 days post-infection (Pearsons χ^2 ; $p<0.01$ for both samples). Higher proportions of first chalimus larvae occurred on the fins and other body surfaces when compared to the gills of Atlantic salmon at 5 days post-infection. At 20 days post-infection higher proportions of first preadult males and females occurred on the other body surfaces when compared to the fins and gills.

Significant differences in the stage distributions on the different body regions of chinook salmon occurred at 20 and 25 days post-infection (Pearsons χ^2 ; $p<0.001$ for both samples). At 20 days post-infection 100% of the copepods present on the gills were third chalimus larvae. At 25 days post-infection there were higher proportions of preadult males and females on the other body surfaces when compared to the fins and gills.

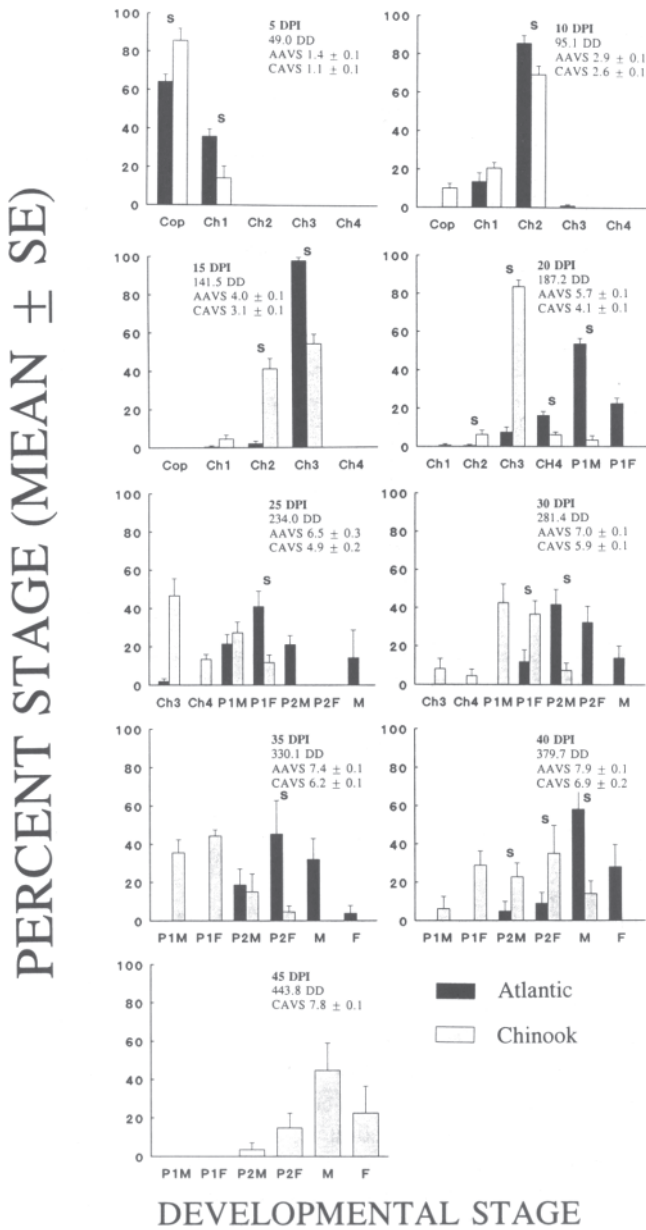


Fig. 2. *L. salmonis* stage distributions on naive Atlantic and naive chinook salmon at various times post-infection. Values are the mean percent stage $\pm$ SE for total copepods collected. An ‘S’ above the bars indicate stages which have statistically significant differences in their proportions between host species (*t*-test; *p*<0.05). Also shown are the degree days and the mean $\pm$ SE copepod stage. Fish were maintained at 9.0–11.3°C and ambient salinity (29–31‰). (AAVS, Atlantic mean copepod stage; CAVS, chinook mean copepod stage; DD, degree days; DPI, days post-infection; Cop, copepodid; Ch1, first chalimus; Ch2, second chalimus; Ch3, third chalimus; Ch4, fourth chalimus; P1M, first preadult male; P1F, first preadult female; P2M, second preadult male; P2F, second preadult female; M, adult male; F, adult female.)

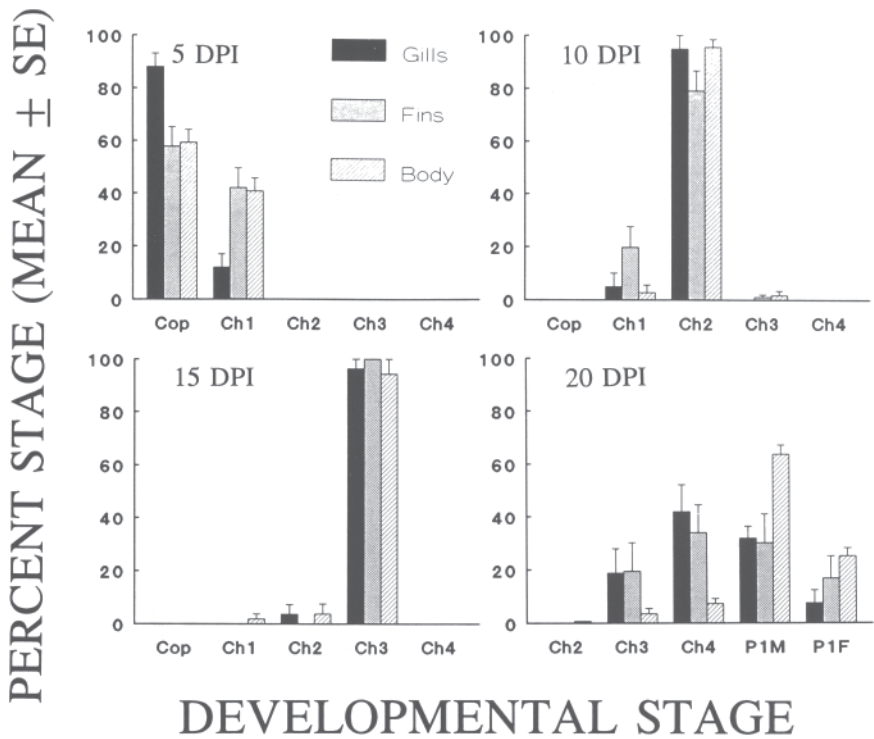


Fig. 3. *L. salmonis* stage distributions by body region on naive Atlantic salmon at various times post-infection. Values are the mean percent stage±SE for total copepods collected from that region. Fish were maintained at 9.0–11.3°C and ambient salinity (29–31‰). (Abbreviations as Fig. 2.)

In general, there were no significant differences in the total length, cephalothorax length or cephalothorax width of individual developmental stages growing on different regions of the body of Atlantic salmon over the period of 5–20 days post-infection, or of chinook salmon over the period of 5–25 days post-infection (Tables 2 and 3).

With exception of the size of the genital complex there were no significant differences in the dimensions of the bodies of adult female *L. salmonis* collected from mature Atlantic (5 years old) and mature chinook salmon (4 years old) reared at the same farm site (Table 4). There were highly significant differences in the numbers of eggs carried by the copepods collected from these two host species. Copepods collected from Atlantic salmon carried on average twice as many eggs as those collected from chinook salmon (Table 4).

DISCUSSION

Understanding the population dynamics of sea lice is an important step in the development of effective control and/or management strategies to reduce their impact on salmon stocks. Important to our understanding of the population dynamics is a

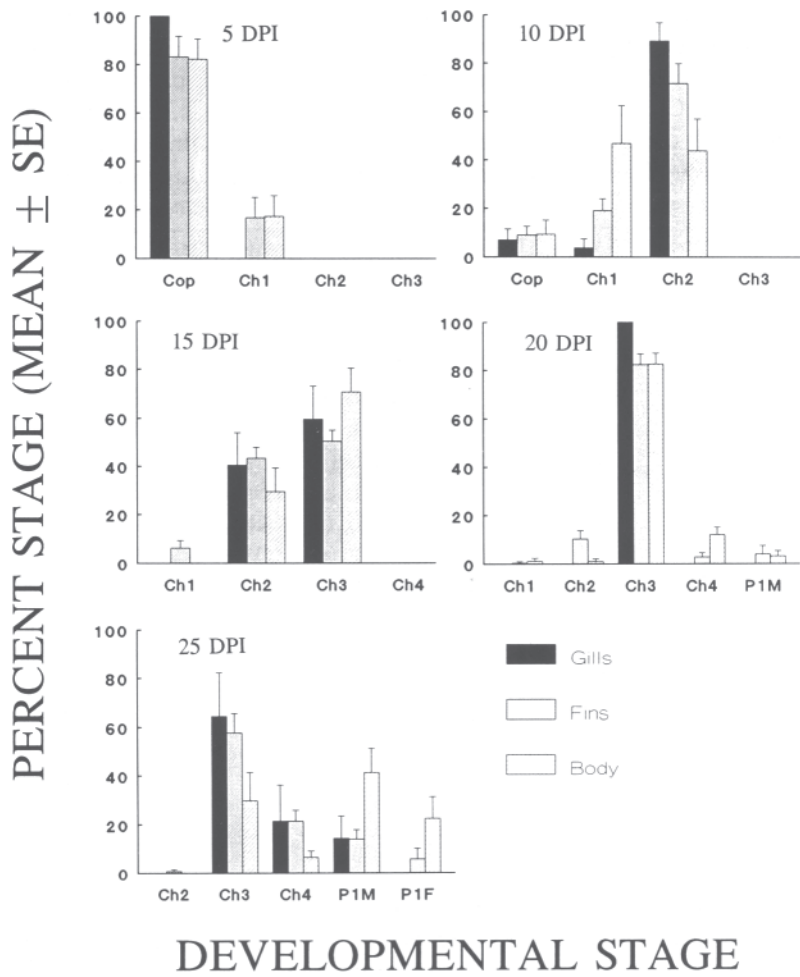


Fig. 4. *L. salmonis* stage distributions by body region on naive chinook salmon at various times post-infection. Values are the mean percent stage±SE for total copepods collected from that region. Fish were maintained at 9.0–11.3°C and ambient salinity (29–31‰). (Abbreviations as Fig. 2.)

knowledge of interactions between sea lice and their hosts. Due to their economic importance, the effects of sea lice on their salmonid hosts have been well documented (Brandal and Egidius 1979, Wootten *et al.* 1982). However, only recently have the effects of the host on the biology of sea lice come under investigation, and as yet few of the mechanisms behind these effects are known.

Several factors acting independently or in conjunction with each other may be responsible for the significant reductions in the intensity of *L. salmonis* which occurred on both host species over time. These factors include active host rejection, natural mortality of the copepods independent of any host response, and the effects of water

Table 2. Results of ANOVA of body dimensions of *L. salmonis* developmental stages growing on different body regions of Atlantic salmon. Values are the dimensions measured and its corresponding probability (Cop, copepodid; Ch1, first chalimus; Ch2, second chalimus; Ch3, third chalimus; Ch4, fourth chalimus; P1F, first preadult female; P1M, first preadult male; TL, total length; CL, cephalothorax length; CW, cephalothorax width; G, gill; F, fin; B, body; ns, no significant difference)

Host species	DPI	Stage	Dimensions (probability)	Scheffés test
Atlantic	5	Cop	TL (ns); CL (ns); CW (< 0.05)	CW G > (F = B)
	5	Ch1	TL (<0.001); CL (ns); CW (ns)	TL (ns)
	10	Cop	TL (ns); CL (ns); CW (ns)	
	10	Ch1	TL (ns); CL (ns); CW (ns)	
	10	Ch2	TL (ns); CL (<0.05); CW (ns)	
	15	Ch1	TL (ns); CL (ns); CW (<0.05)	CW B > F
	15	Ch2	TL (<0.05); CL (ns); CW (ns)	TL (ns)
	15	Ch3	TL (ns); CL (ns); CW (ns)	
	20	Ch2	TL (ns); CL (ns); CW (ns)	
	20	Ch3	TL (ns); CL (ns); CW (<0.05)	CW (ns)
	20	Ch4	TL (ns); CL (ns); CW (ns)	
	20	P1M	TL (ns); CL (ns); CW (ns)	
	20	P1F	TL (ns); CL (<0.05); CW (<0.05)	CL (F = B) > G CW (F = B) > G

flow in the tanks. Active host rejection of the cyclopoid copepods *Lernaea cyprinacea* L. and *Lernaea polymorpha* Yü has been demonstrated for both naive and previously exposed hosts to be due, at least in part, to cellular responses (Shields and Goode 1978, Shariff and Roberts 1989, Woo and Shariff 1990). Non-specific host defence mechanisms have been demonstrated to be important in reducing the intensity of *L. salmonis* on naive coho salmon (Johnson and Albright 1992b). Different water velocities have been demonstrated to affect the rate of decrease in *L. salmonis* intensity on naturally infected Atlantic salmon after their transfer to tanks (Jaworski and Holm 1992). The lowest rate of copepod loss occurred in the tank with the lowest current velocity, and the more motile preadult stages and adult male stages were more readily lost than adult females. Further studies are required to investigate non-naive hosts' resistance to infection with sea lice and their rate of parasite loss. As infections of the host species were carried out in separate tanks no comparisons of infection intensity between host species have been made.

Loss of copepods from the gills and fins after 25 days post-infection in Atlantic salmon and 30 days post-infection in chinook salmon was due to moulting to the first preadult stage and movement to the other body surfaces. The presence of copepods on

Table 3. Results of ANOVA of body dimensions of *L. salmonis* developmental stages growing on different body regions of chinook salmon. Values are the dimension measured and its corresponding probability (Abbreviations as Table 2)

Host species	DPI	Stage	Dimensions (probability)	Scheffé's test
Chinook	5	Cop	TL (<0.001); CL (<0.001); CW (ns)	TL G > F > B CL G > (F = B)
	5	Ch1	TL (<0.001); CL (n < 0.001); CW (<0.05)	TL F > B CL F > B CW F > B
	10	Cop	TL (ns); CL (ns); CW (ns)	
	10	Ch1	TL (ns); CL (ns); CW (ns)	
	10	Ch2	TL (<0.05); CL (<0.001); CW (<0.001)	TL (ns) CL G > (F = B) CW G > (F = B)
	15	Ch2	TL (ns); CL (ns); CW (ns)	
	15	Ch3	TL (<0.05); CL (<0.01); CW (ns)	TL (F = B) > G CL (F = B) > G
	20	Ch2	TL (ns); CL (ns); CW (ns)	
	20	Ch3	TL (ns); CL (ns); CW (<0.05)	CW (ns)
	20	Ch4	TL (ns); CL (ns); CW (ns)	
	20	P1M	TL (ns); CL (ns); CW (ns)	
	25	Ch3	TL (ns); CL (ns); CW (ns)	
	25	Ch4	TL (ns); CL (ns); CW (ns)	
	25	P1M	TL (ns); CL (ns); CW (ns)	
	25	P1F	TL (ns); CL (ns); CW (ns)	

Table 4. Results of ANOVA of body dimensions and total number of eggs of female *L. salmonis* living on mature Atlantic and chinook salmon. Body dimensions are reported in millimetres as the mean $\pm$ SD (ns, not significantly different)

	Atlantic	Chinook	F value (probability)
Total length	12.4 $\pm$ 0.4	12.2 $\pm$ 0.5	1.48 (ns)
Cephalothorax length	5.3 $\pm$ 0.3	5.3 $\pm$ 0.2	0.00 (ns)
Cephalothorax width	4.7 $\pm$ 0.1	4.6 $\pm$ 0.2	3.34 (ns)
Genital complex length	4.2 $\pm$ 0.2	3.9 $\pm$ 0.3	5.34 (0.05)
Genital complex width	3.8 $\pm$ 0.3	3.1 $\pm$ 0.2	56.74 (0.001)
Abdomen length	3.2 $\pm$ 0.3	3.2 $\pm$ 0.4	1.16 (ns)
Total eggs	879.2 $\pm$ 112.5	430.0 $\pm$ 100.0	70.30 (0.001)

the gills of experimentally infected Atlantic and chinook salmon and wild sockeye salmon has been previously reported (Johnson and Albright 1992a). *L. salmonis* is able to develop to the preadult stage on the gills as evidenced by attached preadults on the gills of both host species. The importance of the gills as a site of attachment in naturally infected fish needs to be assessed.

Differences in the stage distribution of *L. salmonis* between chinook and Atlantic salmon were evident in the first sample taken at 5 days post-infection (49 degree days) and in all subsequent samples. These results confirm the observation of Johnson and Albright (1992a), who reported that *L. salmonis* appeared to develop at a slower rate on naive chinook salmon than on naive Atlantic salmon. The mechanism behind this difference in development rate remains to be determined. As these fish were naive with respect to *L. salmonis* infection, either nutritional and/or non-specific host defence mechanisms are most likely explanations. Numerous non-specific humoral factors have been identified in the body fluids of fish. These include substances such as lectins, lysozyme, complement, C-reactive protein, transferrin, properdin, interferon and other naturally occurring agglutinins, lysins and precipitins (Sindermann 1990). These factors have been demonstrated to have both antiviral and antibacterial activities, but their possible effects on sea lice and other ectoparasites remain to be determined. It is possible that one or a combination of these factors present in chinook salmon may interfere with *L. salmonis* feeding activities, thereby reducing the development rate. Further studies are needed to determine if the development rate of *L. salmonis* differs on immunosuppressed and non-naive hosts. Such experiments would help to determine the role of nutritional factors in controlling the development rate on these species.

Significant differences in the stage distribution of *L. salmonis* on different regions of the host body occurred only at 5 and 20 days post infection in Atlantic salmon and at 20 and 25 days post-infection in chinook salmon. Significant differences in the developmental stages present on the other body surfaces when compared to the fins and gills in the samples from 20 and 25 days post-infection are related to maturation to the preadult stage and movement onto the other body surfaces. The data presented here do not support the observation of Johnson and Albright (1992a), who suggested that *L. salmonis* developed at a slower rate on the gills when compared to the fins and other body surfaces.

Current investigations of seasonal differences in the reproductive output of sea lice most commonly relate egg production to environmental conditions such as sea-water temperature and photoperiod. In the present study approximately twice as many eggs were carried by *L. salmonis* growing on mature Atlantic salmon than on those growing on mature chinook salmon reared at the same site. Higher numbers of eggs have also been reported from *L. salmonis* growing on mature versus immature coho salmon reared at the same site (personal observation). These differences suggest that host nutritional and/or immunological factors may play an important role in controlling the reproduction of sea lice. Future research needs to consider host factors when investigating seasonal cycles of sea lice reproduction.

ACKNOWLEDGEMENTS

I thank Drs T.P.T.Evelyn and L.Margolis for critically reviewing the manuscript. This research was funded by the Department of Fisheries and Oceans Biological Sciences Branch, Pacific Region, and by a Natural Sciences and Engineering Research Council of Canada Post-doctoral Fellowship.

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Part Ic Anatomy

7

Antennular sensors of the infective copepodid larva of the salmon louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae)

Karen A.Gresty, Geoffrey A.Boxshall and Kazuya Nagasawa

ABSTRACT

The infective copepodid stage of *Lepeophtheirus salmonis* possesses 13 setation elements on the distal segment of the antennule. There are five long branched setae, three long unarmed setae, three short unarmed setae and two aesthetascs. The ultrastructure of the long unarmed setae is very similar to that of the long branched setae, both types having paired dendrites, each densely packed with microtubules. The short setae possess a different arrangement of microtubules in their dendrites. The terminal dendritic segments arise from either a double or a single ciliary basal body surrounded by a scolopale, and all are considered to be mechanoreceptors. Two ultrastructurally very similar aesthetascs are reported from this copepodid for the first time. These elements are characterized by their thin cuticle and the large number of small dendrites, and are considered to be chemoreceptors.

INTRODUCTION

In recent years the salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837), has been the subject of intense study because it is a major pathogen of farmed Atlantic salmon (*Salmo salar* L.) in sea-cage sites (Brandal *et al.* 1976, Brandal and Egidius 1979, Wootten *et al.* 1982, Hahnenkamp and Fyhn 1985, Nagasawa 1987, Tully 1989, Pike 1989, Bron *et al.* 1991, Johnson and Albright 1991a,b, Spencer 1992).

Most research on control methods, excluding fallowing, has been concentrated on removing lice attached to the fish rather than on preventing infection. However, a potential weak point in the life cycle of the parasite is the infective copepodid stage. If it were possible to disrupt normal host location behaviour, then this could be developed as a means of control. Very little work has been published on the sensory biology of

sea lice and the mechanisms by which the infective copepodid locates its host. In *Lepeophtheirus pectoralis* (Müller, 1776), a parasite of flatfish, Boxshall (1976) reported that initial contact with the host resulted from the copepodid's response to water flow generated by the respiratory and fin movements of the host.

The antennule is the main site of chemical perception in the Crustacea, via setation elements referred to as aesthetascs (Ache 1982, Heimann 1984, Laverack and Barrientos 1985, Gill 1986, Spencer and Linberg 1986, Grünert and Ache 1988). These chemoreceptors, combined with the array of setal mechanoreceptors, make the antennule an extremely important sensory appendage. In caligids the antennules presumably provide most of the sensory data used by the parasite in its host location and recognition. *L. salmonis* copepodids, as all caligids, possess a two-segmented antennule; the proximal segment carrying three setae, the distal segment 13 elements (Johnson and Albright 1991a). The external structure of the antennular elements of *Lepeophtheirus* copepodids has been documented (White 1942, Boxshall 1974, Johnson and Albright, 1991a) but their fine structure and functional morphology have yet to be investigated.

The aim of this study is to characterize the individual elements on the distal segment of the copepodid antennule, by means of light and electron microscopy. A tentative, functional interpretation of the distal antennular elements is proposed.

MATERIALS AND METHODS

Egg sacs from adult female *L. salmonis* were collected from farmed salmon on the west coast of Scotland and maintained in sea water below 10°C for a few days. After hatching they were left until the infective copepodid stage appeared. Copepodids were then processed in one of three ways, as follows.

Light microscopy

Fixed in 80% industrial methylated spirit (IMS), then cleared and stained in chlorazol black in lactophenol. Drawings were made using a camera lucida on a Leitz Diaplan microscope with Nomarski interference contrast.

Scanning electron microscopy (SEM)

Fixed in 3% glutaraldehyde/phosphate and sucrose buffer for 1–1.5 h. Washed in phosphate buffer, then fixed and stained in 1% osmium tetroxide for 1 h. Washed in phosphate buffer again, then placed in a weak detergent solution (one drop of RBS in 100 ml of distilled water) for 15 min before sonication in the detergent solution for 10s only. Dehydrated in a standard acetone series, then, after critical-point drying, mounted on stubs and sputter coated with gold/palladium. Observations were made on a Hitachi FE S-800.

Transmission electron microscopy (TEM)

Fixed and stained with glutaraldehyde and osmium tetroxide as for SEM. Stained with 2% aqueous uranyl acetate for 1 h in the dark. Dehydrated through a standard acetone series and then gradually infused with Spurr resin (medium hardness), by increasing the concentration of the resin until in pure Spurr. Cured at 60°C for 9 h. The specimens were orientated on the ultramicrotome to allow for sectioning of the antennule

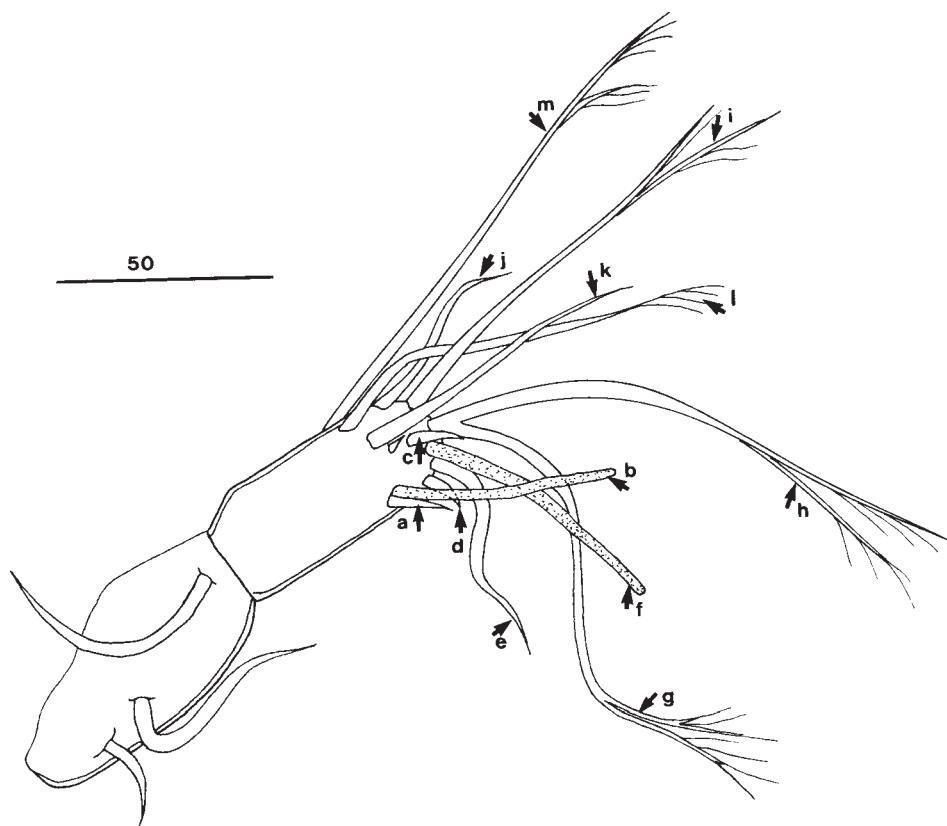


Fig. 1. Antennule of *L. salmonis* copepodid, showing 13 setation elements (*a-m*) on distal segment and three setae on proximal segment.

only. Sections were stained with lead citrate on formvar-coated grids and observed on a Siemens Elmiskop 101. All scale bars on figures are given in μm .

RESULTS

Light microscopy

The number and position of the antennular setae are constant and a lettering system has been applied (*a-m*) to the elements on the distal segment. The proximal segment (Fig. 1) possesses three unarmed setae anteroventrally: two near the anterior margin, the other distally. The terminal segment has 11 setae and two aesthetascs distally and subdistally; one aesthetasc (*b*) located ventrally, associated with a short seta (*a*) and the other (*f*) on the anterior margin. There are five long setae (*g* and *h*, which share a common base, *i*, *l* and *m*) with distally branching tips and six unarmed, sharply tapered setae: three long (*e*, *k* and *j*) and three short (*a*, *d* and *c*). At the base of seta *c* is a structure which resembles a very small seta (Figs 1 and 2B,D). It appears to be a type of cuticular pore and can also be seen in the TEM sections (Fig. 3B,C).

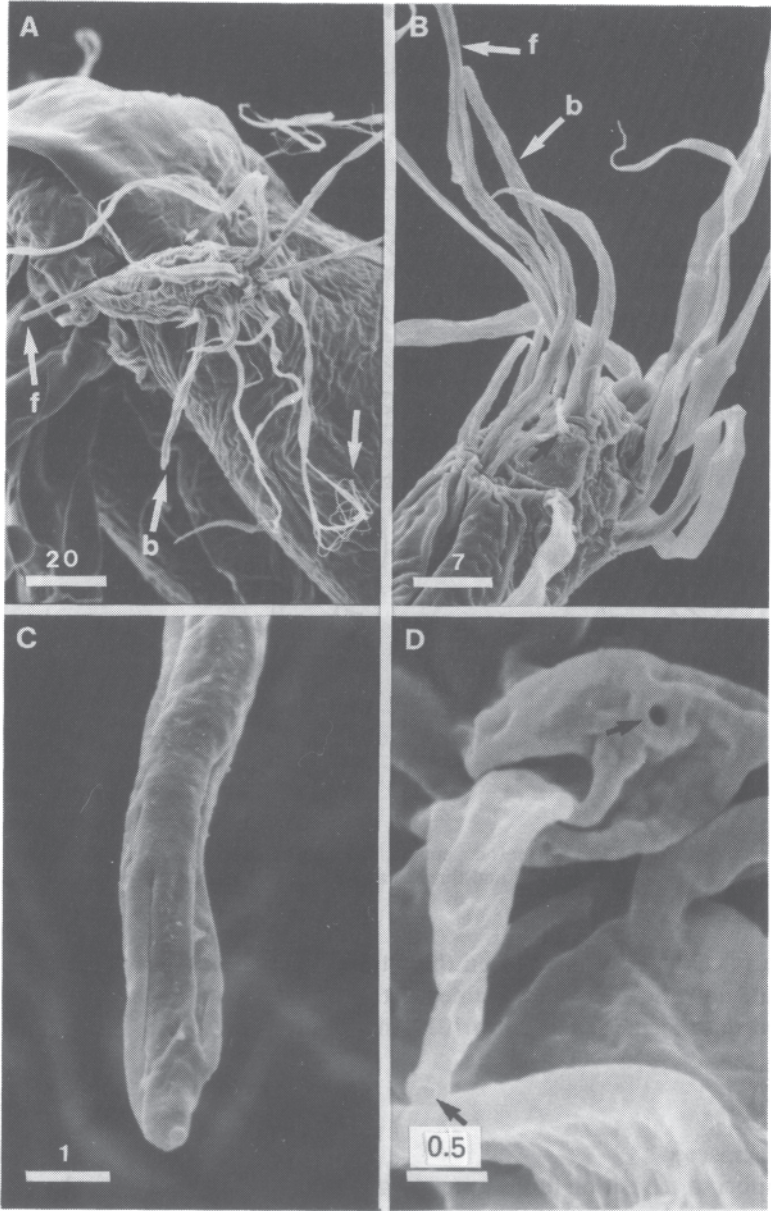


Fig. 2. Scanning electron micrographs of *L. salmonis* antennule. (A) Antennule with 13 distal elements displayed; aesthetascs *b* and *f* are labelled and a branched seta arrowed. (B) Distal setation elements, with aesthetascs labelled and tube pore at base of seta *c* arrowed. (C) Tip of aesthetasc. (D) Tube pore at base of seta *c* (pores arrowed).

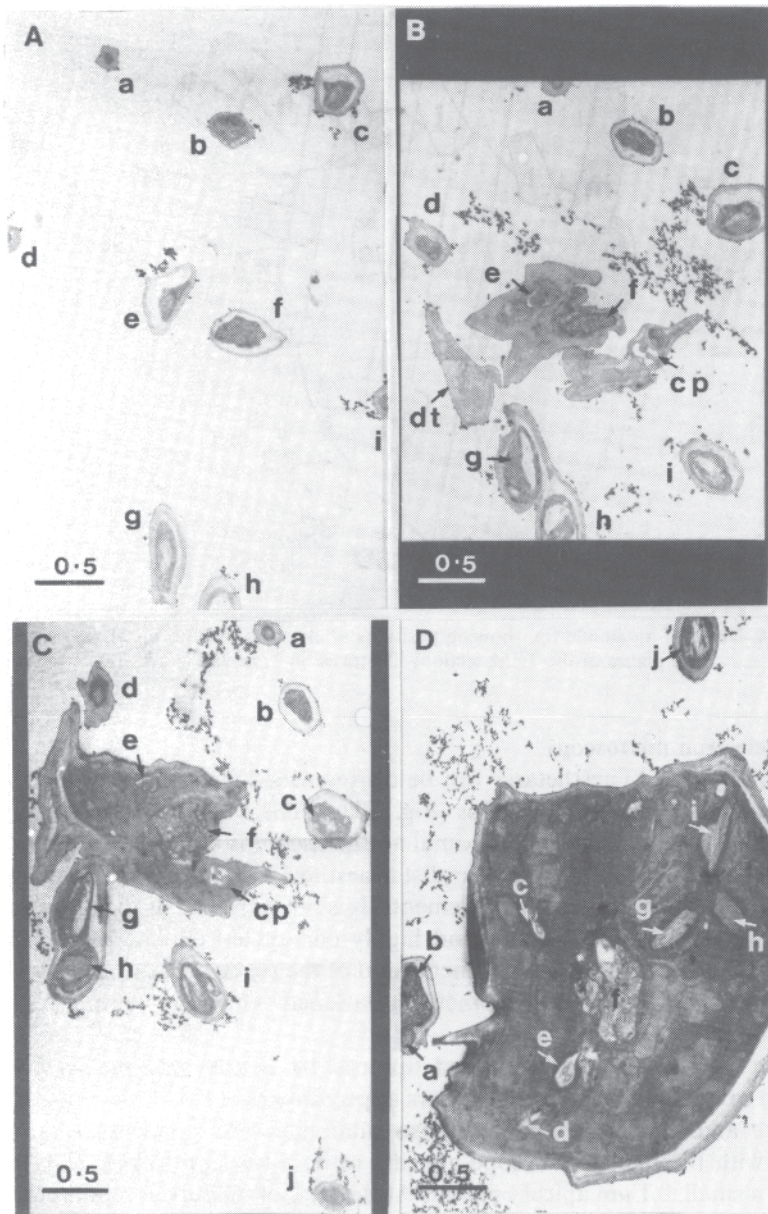


Fig. 3. Transmission electron micrographs of *L. salmonis* antennule. (A) Section through tips of the apical setation elements on distal segment. (B) Same, in more proximal plane, showing nine distal elements (a-i) close to or at their origins on the segment (dt=distal tip of antennule, cp=cuticular pore). (C) Same, in more proximal plane, showing ten distal elements (a-j) at or near their origins on the antennule (cp=cuticular pore). (D) Section through distal segment showing individually identified nerves from setation elements c-i.

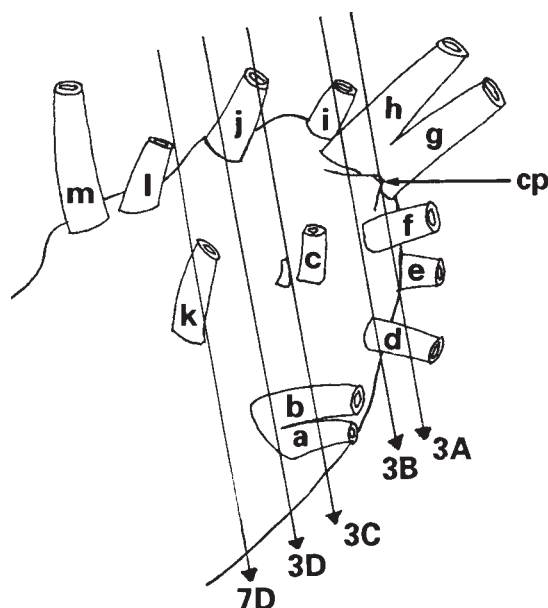


Fig. 4. Detail of antennule tip, showing positions of elements *a–m* on distal segment, and the planes of the TEM sections illustrated in Figs. 3A–D and 7D.

Scanning electron microscopy

Under SEM (Fig. 1A) aesthetascs can be distinguished from setae by their rounded appearance and relatively blunt tips (Fig. 2B,C). There is no opening at the tip of either aesthetasc (Fig. 2C). The proximal aesthetasc (*b*) is 40 μm long and associated with a short, unarmed seta (*a*). The distal aesthetasc (*f*) is 50 μm long and often harder to distinguish from the setal elements. It is not as robust as the other aesthetasc but has a characteristic blunt tip and highly corrugated cuticle. In the specimens observed the cuticle of the distal elements and of the segment itself appears collapsed and wrinkled, and the setae are somewhat flattened. This may be an artefact of the SEM processing.

The five long setae with bifurcated tips can be clearly seen by SEM (Fig. 2A, unlabelled arrow). The bifurcation occurs approximately two-thirds along the length of the seta and then these branches also subdivide. The small, seta-like structure observed with the light microscope appears to be a tube pore (Fig. 2D). It is 2 μm long with a small, 0.1 μm apical opening. At the base of the tube is a bulbous swelling, which also carries a small 0.1 μm pore. It is not known if these two pores are associated.

Transmission Electron Microscopy

The antennule was sectioned in the planes indicated in Fig. 4. At low magnifications, the internal differences between the distal elements are very obvious (Fig. 3A,B). The setae can be placed in a number of different categories. Setae *h*, *g*, *l*, *i* and *m* (not

shown) are all branched and exhibit a similar structure. Seta *h* is described as typical for this group. Setae *a*, *d* and *c* are all short, unarmed and similar in structure. Seta *a* is described as typical for this group. Setae *e*, *k* and *j* are all long and unarmed. Seta *j* is described as typical of this group. The two aesthetascs (*b* and *f*) have very similar internal structure, so only aesthetasc *b* is described in detail.

The section in Fig. 3B shows the distal elements just at the level of their origin on the distal antennular segment. Seta *e* and aesthetasc *f* are already fused to the segment, and setae *g* and *h* originate from a common base. The pore-like structure (Fig. 3B, cp), adjacent to seta *f*, has a hollow centre and an irregular shape. It contains some tissue that could possibly be nervous in origin. This raised pore is located at the extreme tip of the antennule and can be seen with the light microscope, near the base of the double setae *g* and *h*. The distal tip of the antennule is highly folded in the specimens sectioned. Fig. 3C shows a section proximal to that in Fig. 3B. The setation elements are much closer together. The mass of dendrites of aesthetasc *f* is readily identifiable inside the antennular segment, as is the nerve and scolopale of seta *e*. In the plane of section of Fig. 3D three free elements (*a* and *b* fused, and *j*), distal to the origins on the segment, are visible and the nerves of the other elements, already fused with the antennule, can be individually traced within the distal segment.

Towards its tip aesthetasc *b* (Fig. 5A) contains approximately 65 dendrites, of a similar size (approx. $0.15\ \mu\text{m}$ in diameter), within the receptor lymph fluid of the lumen. These dendrites are ovaly compressed and each contains between one and eight internal microtubules. The cuticle of the aesthetasc is thin ($0.25\ \mu\text{m}$), with an underlying granular layer that terminates without a distinct cellular boundary. Surrounding the outside of the aesthetasc cuticle is a layer of osmophilic debris, fixed when the copepodid was transferred from sea water into the initial fixative.

The 65 outer dendritic segments persist proximally in the aesthetasc (Fig. 5B). They become tightly packed and fill the lumen. The number of microtubules in each dendrite increases up to ten or more. The indistinct cuticular layer is adjacent to the dendrites but there is no definite cellular boundary. Receptor lymph fluid does not surround the dendrites to the same extent as in the distal part of the aesthetasc.

Near the base of the aesthetasc the dendrites become very tightly packed in the centre of the lumen. They are enclosed by a membranous structure and are separated from the cuticle by receptor lymph fluid (Fig. 5C). The number of dendrites appears to be similar along the aesthetasc.

Aesthetasc *b* joins seta *a* before its origin on the antennule proper (Fig. 5D). These two elements have a common base with a thickened outer cuticle but their nerves remain separate. The microtubules present in the dendrite of seta *a* are enveloped by a scolopale, which incompletely encircles the nervous tissue. No such structure was found in the aesthetasc. The area of nervous tissue inside the aesthetasc is almost three times that of seta *a*, and, at this level, small vesicles are also present inside some of the dendrites.

Seta *h* is long and branched. It contains two large dendrites, each with a number of small vesicles (Fig. 6A). The central area is surrounded by a unit membrane that separates the nervous tissue from the cuticle. The cuticle ($0.5\ \mu\text{m}$) is thicker than that of the aesthetasc. Proximally, seta *h* merges with seta *g* to form a double seta set on a large common base (Fig. 6B). The nerves to the two setae remain separate. At this level, the two dendrites of seta *h* are filled with tightly packed,

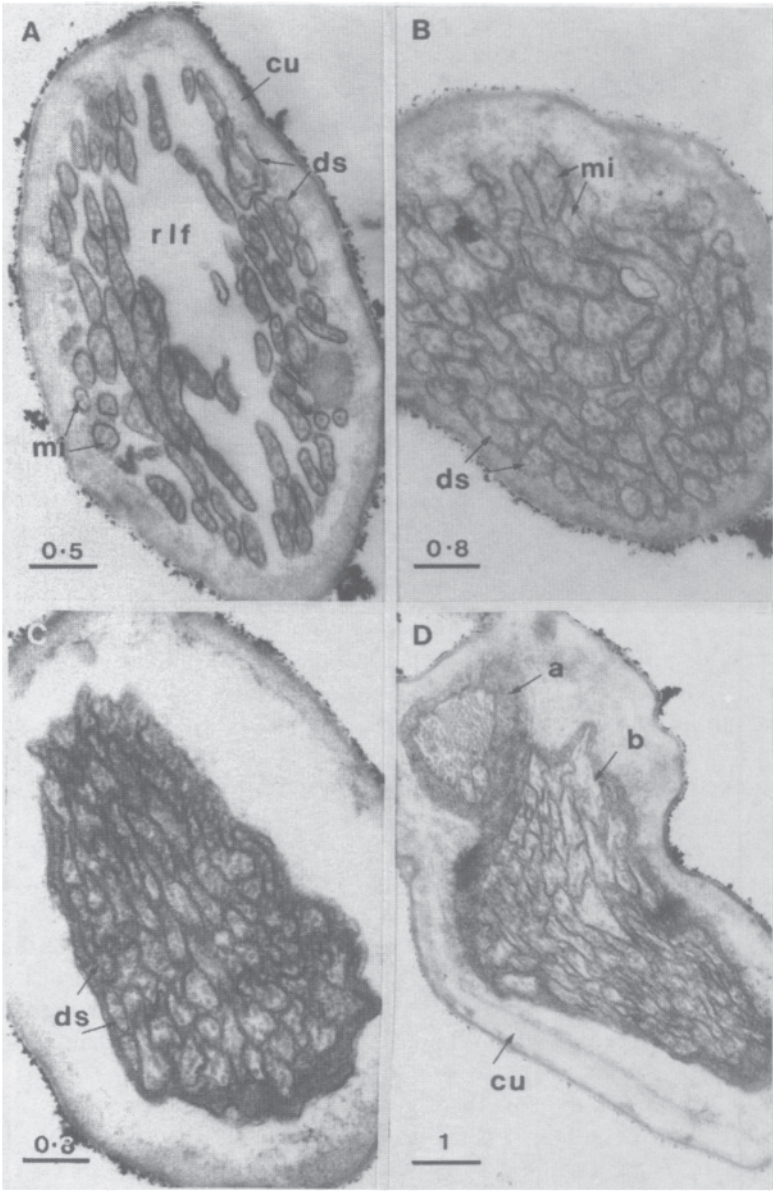


Fig. 5. Transmission electron micrographs of *L. salmonis* antennular aesthetascs. (A) Tip of aesthetasc *b* (cu=cuticle, rlf=receptor lymph fluid, ds=dendritic segment, mi=microtubule). (B) Same in more proximal plane, showing dendritic segments (ds) tightly packed together with many internal microtubules (mi). (C) Aesthetasc *f*, before it merges with seta *a*, showing dendritic segments (ds) concentrated in central lumen. (D) Section through common base of aesthetasc *b* and seta *a* (cu=cuticle of common base).

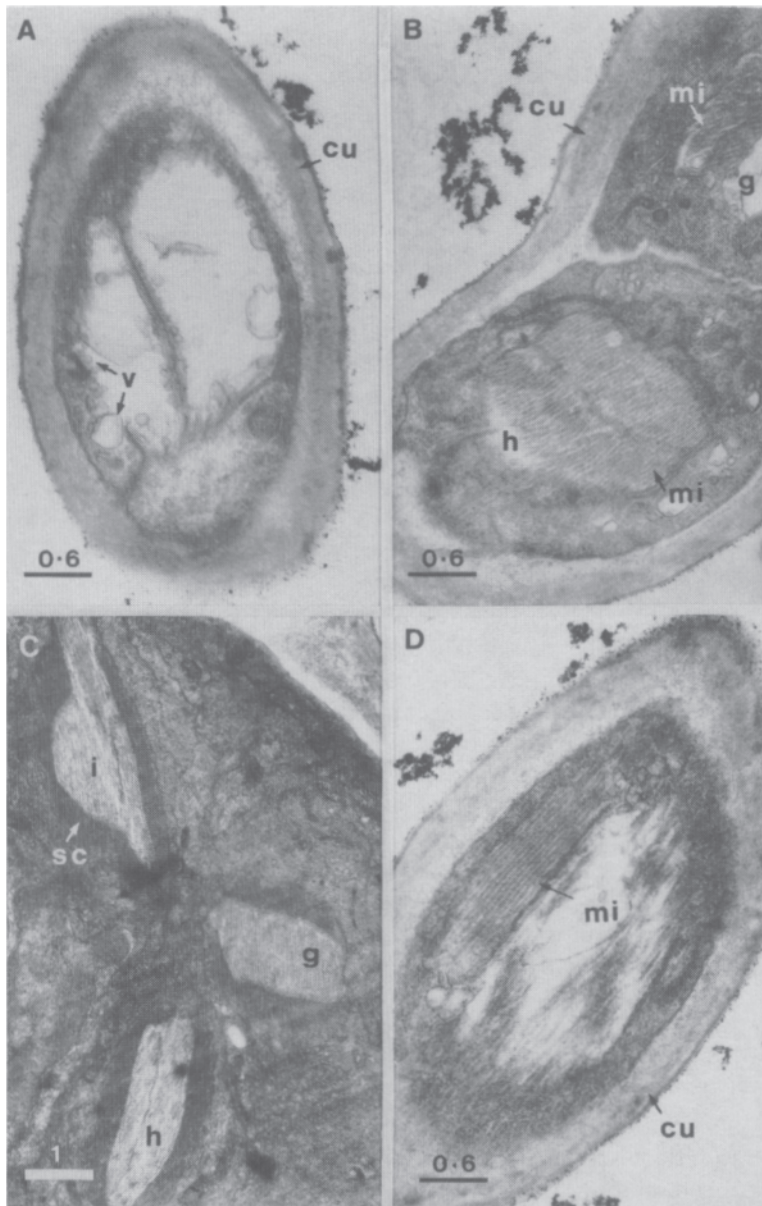


Fig. 6. Transmission electron micrographs of *L. salmonis* antennular setae. (A) Seta *h* (long, branched setal type) containing two dendrites containing vesicles (v), setal cuticle (cu) thick. (B) Section through common base of seta *h* and seta *g*, showing paired dendrites of each seta packed with microtubules (mi) which are orientated at right angles to each other in the two setae. (C) Nerves of setae *g*, *h* and *i* inside antennule segment, showing a tripartite configuration. Each pair of dendrites containing closely packed microtubules (mi) is surrounded by a scolopale (sc). (D) Seta *j* (long, unarmed setal type) with thick cuticle (cu) and paired dendrites containing microtubules (mi).

parallel microtubules. The dendrites of seta *g* are also packed with microtubules but they are orientated at right angles to those of seta *h*. Surrounding the central area of microtubules is a dense cellular layer consisting of vesicles and granular cytoplasm.

Setae *g* and *h* form a tripartite structure together with seta *i* (Fig. 6C). The microtubules packing the two dendrites in each of these setae are orientated in parallel to the other. Inside the segment the dendrites are surrounded by a dark-staining scolopale. The cytoplasm in this region is granular and numerous mitochondria are present.

Seta *j* is long, unarmed and, in transverse section, is similar to the long branched setae (Fig. 6D). There is a thick, robust cuticle ($0.7\ \mu\text{m}$) and a central area of nervous tissue separated from the cuticle by a unit membrane. In the middle of this tissue are the two dendrites, each packed with parallel microtubules and some vesicles. The scolopale and paired ciliary bodies of seta *j* can also be seen in Fig. 7C.

Seta *a* is short, robust and, internally, very different from the long setae. In its distal part (Fig. 7A) seta *a* appears square in cross-section, with its thick cuticle merging into a homogeneous, granular core. In the centre, a darkly staining structure, possibly a distal extension of the scolopale, is just apparent, alongside the three dendrites. Two of these dendrites are the same size: one has five microtubules in it and the other has four. There is also a thin, membranous structure bearing numerous septate desmosomes, looking like a zip in section, around these dendrites that may hold the dendrites together. The third and largest dendrite has an obscured centre and no microtubules are visible in it at this level.

Proximally, seta *a* becomes larger. The scolopale now incompletely surrounds the central dendrites in a horse-shoe shape, and appears to possess microtubules (Fig. 7B). The three dendrites all contain microtubules now, the smaller dendrites having the same numbers as before and the largest dendrite having 20. In Fig. 5D, seta *a* has joined with the aesthetasc but its nerve remains distinct. The two small dendrites persist with the same number of microtubules but the largest dendrite now has over 100. The scolopale surrounds the setal nervous tissue. In Fig. 7D, the scolopale and single ciliary body of seta *a* can be seen. Unlike the long and branched setae, which possess double ciliary basal bodies, the shorter seta *a* has a single ciliary body.

In the most proximal section shown (Fig. 7D) seta *l* is almost at its origin on the main antennule, leaving only the long, branched seta *m* still separate. The basal bodies of the distal elements are visible inside the segment, as are the dendrites of the aesthetascs. Each scolopale now encloses two ciliary basal bodies (except for seta *a* type). At this level in the antennule the central nerve appears to be dividing into a dorsal and a ventral root.

The double ciliary basal body of a long seta, enclosed by a scolopale, is shown in Fig. 7C. The basal bodies are equal in size ($0.5\ \mu\text{m}$) and have an internal configuration of $9 + 0$ microtubules. The scolopale is a dense, highly granular structure in close contact with the surrounding cytoplasm and rough endoplasmic reticulum. The two ends of the scolopale overlap, incompletely sealing off the two ciliary bodies from a concentric membranous structure of unknown function.

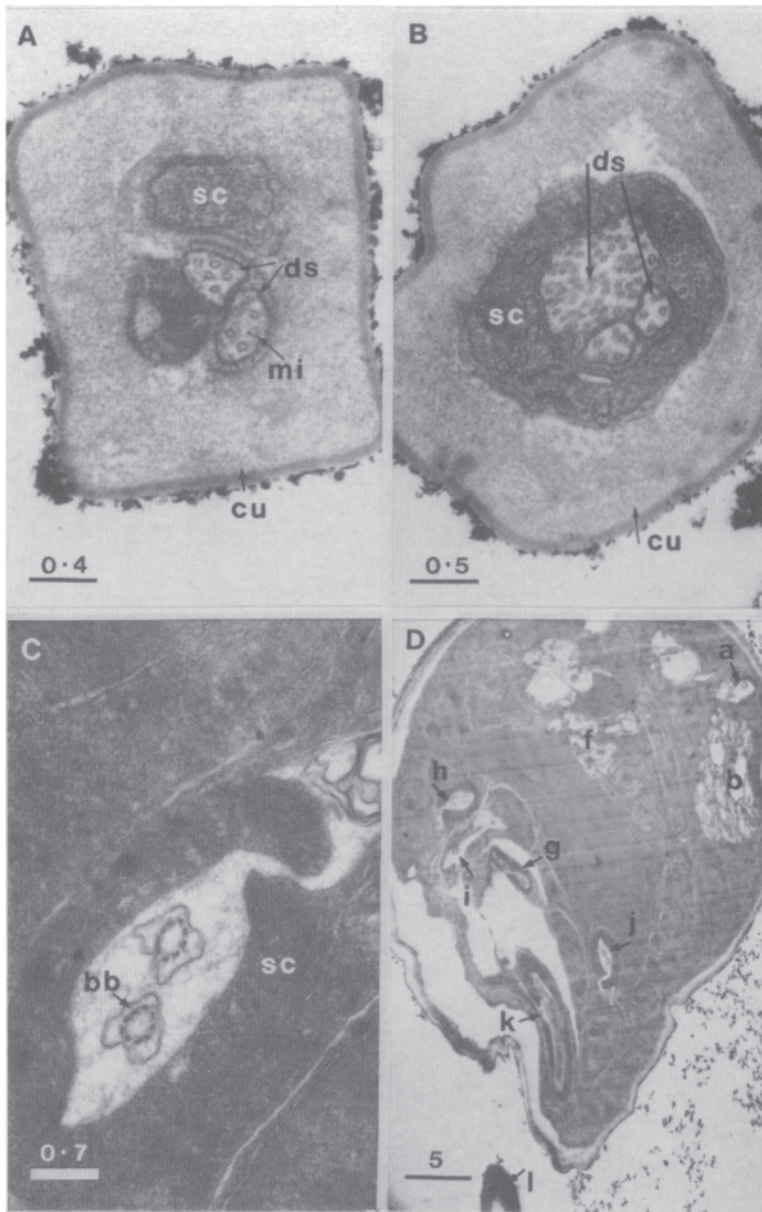


Fig. 7. Transmission electron micrographs of *L. salmonis* antennule. (A) Seta *a* (short, unarmed setal type) with thick cuticle (cu), scolopale (sc) just starting to form around central dendritic segments which contain microtubules (mi). (B) Same, in more proximal plane, showing well-formed scolopale (sc) enclosing three dendritic segments (ds). (C) Double ciliary basal body (bb) from a long, branched seta, with 9+0 microtubules enclosed by a dark-stained scolopale (sc). (D) Section through distal segment of antennule at about the level of origin of seta *l*, showing double ciliary basal bodies of long and branched setae *g*, *h* and *j*, and single ciliary basal body of short unarmed seta *a*. The mass of dendrites of aesthetascs *b* and *f* is also apparent.

DISCUSSION

There has been a great deal of research on crustacean sensory biology but the majority of this work centres upon the large decapods (Laverack and Ardill, 1965, Mill and Lowe 1973, Ball and Cowan 1977, Altner and Prillinger 1980, Laverack and Barrientos 1985). In copepods research has been concentrated upon the adult, free-living forms (Strickler and Bal 1973, Gill 1986, Légier-Visser *et al.* 1986, Yen *et al.* 1992); the exception being Fraile (1989), who studied both adult and copepodid stages in the caligid parasite *Caligus minimus* Otto 1848.

The antennules, particularly in copepods, are important sensory structures with mechanoreceptive capabilities (Strickler and Bal 1973, Gill 1986, Yen *et al.* 1992). Setae appear to be modified from ciliary structures and it has been suggested that they function as mechanoreceptors by detecting gravitational and inertial forces, transmitted by fluid mechanical means (Strickler and Bal 1973, Yen *et al.* 1992).

The characteristic feature of arthropod cuticular mechanoreceptors is an accumulation of microtubules in the distal region of the dendrite that is in contact with the base of the seta (McIver 1975). All known, presumed crustacean mechanoreceptors (Ball and Cowan 1977) and proprioceptors are characterized by a well-developed scolopale, which is lacking in identified chemoreceptors (Grünert and Ache 1988). Eleven of the 13 distal elements on the antennules of *L. salmonis* copepodids display the typical microtubule-packed dendrites and scolopale. In each of the long and branched setae, there are two dendrites packed with microtubules extending from inside the antennule into the seta. This double dendrite structure has also been documented by Strickler and Bal (1973), Gill (1986), Fraile (1989) and Yen *et al.* (1992).

The three short setae lack this double dendrite structure, having fewer microtubules distributed in two or more small, circular dendrites. The small setae also possess only a single ciliary basal body inside the scolopale. Fraile (1989) found both single and double ciliary basal bodies in the copepodid but did not correlate them with the different types of setae present. Nevertheless, the setal mechanoreceptors he described in *C. minimus* are very similar to those observed in this study of *L. salmonis*. Gill (1986) suggested that the presence of two or more dendrites ending at the same seta may indicate directional sensitivity.

Both the long/branched and short setae possess the 9+0 configuration of microtubules inside the ciliary basal body, as is common in Crustacea (Strickler and Bal 1973, Ball and Cowan 1977, Gill 1986, Yen *et al.* 1992). Fraile (1989) demonstrated that these 9+0 microtubules of the ciliary basal bodies are not continuous with the microtubules found in the distal part of the dendrite. The transition zone between the basal bodies and packed microtubules in the distal dendritic segment is very abrupt and it is not known how individual microtubules are joined to the ciliary structures.

Sensory systems are not perfect instruments for the measurement of physical quantities but are shaped by natural selection to serve the needs of the animal (Tautz 1990). Sensitivity of setae may be correlated with the surface area presented to the environment: the larger the surface, the greater the coupling with the external medium and the more sensitive the unit (Laverack and Barrientos 1985, Breithaupt and Tautz 1990). The short setae lack elaborate, 'feathered' tips and consequently may be less sensitive to

slight disturbances in the local water currents than the longer setae. However, they may be more sensitive to oscillating signals. Sensory cells react to the displacement of the setal shaft and the velocity of the water movement (Breithaupt and Tautz 1990). However, the magnitude of the effective movement at the dendritic ending depends on the structural and material properties of the cuticular apparatus of the sensory structure (Steinbrecht 1992). Tautz *et al.* (1981) determined that simple, conical hairs on the antennule of *Astacus leptodactylus* Eschscholtz were stimulated directly by low-amplitude vibration caused by water movement. It is possible that the short setae on the antennules of *L. salmonis* copepodids are important in detecting repetitive disturbances in the local environment and are able to fine-tune stimuli already detected by the long setae.

Yen *et al.* (1992) found that bending the distal setae of a copepod elicited neural responses and that some of them were probably directionally sensitive. In *L. salmonis* it is possible that the array of three setae with different orientations (*g*, *h* and *i*) could provide directionality in detecting signals. Yen *et al.* (1992) also found that copepods were capable of very quick reactions to sudden displacement of setae. In free-living forms, this would probably cause an escape reaction; however, in the parasitic *L. salmonis* it could allow the copepodid to respond to the presence of a fish host nearby.

There are five long plumose setae on the distal antennular segment of *L. salmonis* copepodids (Johnson and Albright 1991a, and present account). However, White (1942) described six long plumose setae in the copepodid, which he termed metanauplius. The presence of two branched setae joined at the base has been noted in other caligid copepodids (Boxshall 1974). Johnson and Albright (1991a) also found three short setae on the distal segment, but noted four long unarmed setae (one of which is presumably an aesthetasc as they found only one).

Two aesthetascs are present in *L. salmonis*. Johnson and Albright (1991a) noted only one aesthetasc—the more anterior (*f*) of the two—and drew the other as a long unarmed seta. Kabata (1972) also observed only one aesthetasc, in his developmental study of *Caligus clemensi* Parker and Margolis, 1964. Only Boxshall (1974), in his study of *L. pectoralis*, described both aesthetascs in the caligid copepodid. It seems that the posterior aesthetasc is the more difficult to identify by light microscopy. No morphological differences were noted between the two aesthetascs in *L. salmonis*. There may be functional differences since they are probably sensitive to different chemical stimuli.

Laverack and Ardill (1965), Heimann (1984), Gill (1986) and Spencer and Linberg (1986) found that some chemosensory organs have modified ciliary structures. The nerves from the aesthetascs have not been completely traced back to the antennular nerve but no ciliary basal bodies have been found associated with aesthetascs in the copepodids of *L. salmonis*.

The aesthetascs of planktonic copepods studied by Gill (1986) lack a terminal pore, are innervated by a large number of dendrites containing numerous small microtubules, and have a thin cuticle. The lack of a terminal pore in an assumed chemosensor has been noted by Laverack and Ardill (1965), Heimann (1984) and Spencer and Linberg (1986), and was also commented on by Fraile (1989) in his study of *Caligus minimus*.

Heimann (1984) was able to demonstrate that dissolved substances penetrate the poreless cuticle of these structures instantaneously.

The *L. salmonis* copepodid is equipped with an array of antennular setal elements, which enable it to detect and locate a potential salmonid host. The mechanosensory elements appear to be orientated in specific planes, which may confer some directional sensitivity onto particular sensors. The full array of mechanosensors may therefore provide information on the direction, strength and even frequency of mechanical signals generated by potential hosts.

ACKNOWLEDGEMENTS

We would like to thank James Bron (Stirling University) and Jim Treasurer (Marine Harvest International) for valuable help, especially concerning the collection of sea lice and eggs. We are also grateful to Sue Barnes and the staff of the photographic unit (Natural History Museum, London) for help and advice with electron microscopy, and Rony Huys for assistance with light microscopy. This research was funded by a generous grant from the Great Britain–Sasakawa Foundation.

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8

Ultrastructure of the frontal filament in chalimus larvae of *Caligus elongatus* and *Lepeophtheirus salmonis* from Atlantic salmon, *Salmo salar*

A.W.Pike, K.Mackenzie and A.Rowand

ABSTRACT

The frontal filament of *Caligus elongatus* is a complex, discrete structure arising from the frontal organ of the chalimus. It is composed of a wider cylindrical proximal region containing fibres arranged as helicoids within an outer sheath. The filament tapers rapidly to a very slender, thread-like portion which contains straight fibres inside a prominent sheath. The distal region of the filament expands into a broad basal plate applied directly to the fish scale surface. The frontal filament is pre-formed inside the body of the copepodid larva. In contrast, the frontal filament of *Lepeophtheirus salmonis* is simpler and forms an integral part of the anterior body of the chalimus. It is attached beneath the fish epidermis but above the scale by an adhesive secretion. Internally the filament is composed of straight fibres inside a thinner sheath. The basal plate is an expanded portion of the filament and its surface is extensively branched. The differences between the two species are highlighted and the possibility of disrupting the filament briefly discussed.

INTRODUCTION

The frontal filament is a feature of most, if not all, siphonostomatoid Copepoda (Kabata 1981); its function is to attach the chalimus stages to the host's skin. Heegaard (1947) described frontal filament production in *Caligus curtus* Müller copepodids, and Lewis (1963) and Bron *et al.* (1991) have done so for *Lepeophtheirus dissimulatus* Wilson 1905 and *Lepeophtheirus salmonis* (Krøyer), respectively. However, Johnson and Albright (1991) state that some preadults of *Caligus clemensi* Parker & Margolis, *Caligus spinosus* Yamaguti and most species of *Lepeophtheirus* are reported to be

attached during this stage. Anstensrud (1990) similarly reported that a frontal filament is also produced as a temporary holdfast, just before moulting, of the preadult stages of *Lepeophtheirus pectoralis* (Müller). The frontal filament therefore may fulfil a role in temporary attachment of the preadult stages as well as its primary role in chalimus larvae attachment.

Heegaard (1947) stated that the filament is formed *de novo* at each moult of the chalimus and rejected Gurney's (1934) view that the filament is formed just once during the progression of fixed chalimus stages. Evidence from the more recent work of Anstensrud (1990) and Johnson and Albright (1991) suggests that the filament may indeed be formed more than once.

Anstensrud (1990) seems also to have resolved the debate about the function of a widely reported frontal organ on the anterior margin of the cephalothorax of caligid copepods. In a set of excellent scanning electron micrographs, Anstensrud (1990) shows that the organ in *L. pectoralis*, which has the same structure as similar organs described by Kabata (1981) and Oldewage and Van As (1989), is the origin of the frontal filament in preadults.

The structure and chemical composition of the frontal filament is poorly known even though the filament represents a potential weak link in the developmental cycle. The copepodid would be effectively prevented from establishing itself on the host if it was possible to interfere with the formation or attachment of the frontal filament. With this possibility in mind a study was carried out mainly to elucidate the structure of the filaments of *Caligus elongatus* Nordmann and *L. salmonis*, two common species of sea lice on salmon.

MATERIALS AND METHODS

Copepodids were obtained by incubating eggs from gravid female lice obtained from farmed salmonids. *C. elongatus* were removed from sea-water rainbow trout on the north-east coast of Scotland and *L. salmonis* from salmon at a west coast of Scotland site. Developing egg strings were removed and maintained in cleaned, aerated sea water until the nauplii hatched. Copepodids were harvested from the holding container at various times and prepared for electron microscopy as follows. Chalimus larvae were collected directly from infected fish on the sites used for collecting adult lice.

Transmission electron microscopy (TEM)

Live copepodid, chalimus I and chalimus II larvae of both species of sea lice were fixed in 4% glutaraldehyde in 0.2 M sodium cacodylate buffer containing 0.1 M sodium chloride and 0.35 M sucrose at pH 7.2 for 24 h at 4°C. The specimens were then washed in 0.2 M sodium cacodylate buffer containing 0.3 M sodium chloride at pH 7.2, post-fixed in 1% osmium tetroxide in 0.2 M sodium cacodylate buffer containing 0.35 M sodium chloride at pH 7.2 for 2 h, then dehydrated with ethanol and embedded in epon resin. Thick sections (0.5 µm) were cut for light microscopy and stained with 1% toluidine blue. Ultrathin sections for TEM were stained with uranyl acetate and lead citrate, and then examined with a Philips 301 transmission electron microscope at 80 kV.

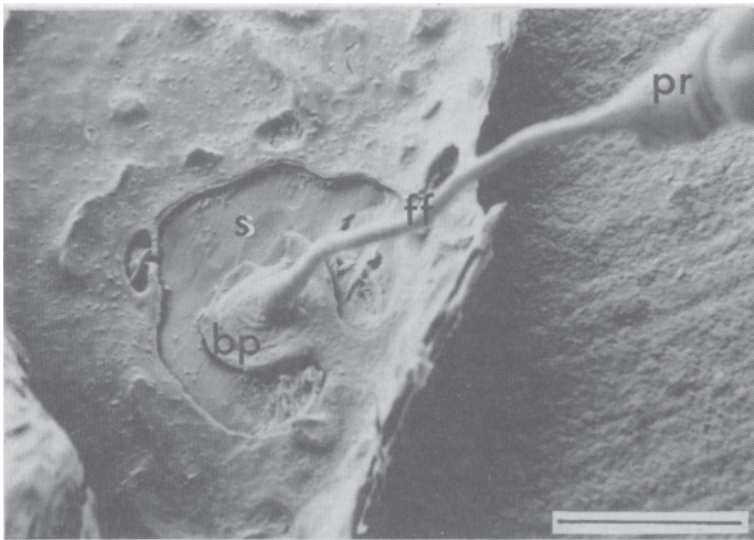


Fig. 1. SEM of the frontal filament of chalimus I of *C. elongatus*. Scale bar=100 μm . Abbreviations: adh, adhesive; b, bacteria; bp, basal plate; bs, branching surface; ct, cephalothorax; e, epiphytes; f, fibres; ff, frontal filament; fr, frontal region; fo, frontal organ; h, helicoid fibres; kf, kinking in fibres; m, microvillar surface; pr, proximal region; s, fish scale; sh, filament sheath.

Scanning electron microscopy (SEM)

Specimens were fixed in the same way as for TEM, dehydrated through a graded series of ethanols and critical point dried with liquid carbon dioxide. Dried specimens were then mounted onto stubs, sputter coated with gold and examined in a Cambridge Instruments S600 scanning electron microscope at 7.5 kV.

RESULTS

Morphology of the frontal filaments

The appearance of the filaments of *C. elongatus* and *L. salmonis* is distinctly different and reflects a seemingly different mode of production and attachment to the host. The filament of *C. elongatus* is long and slender (Fig. 1), rather like an umbilical cord, and is fixed directly to the fish scale by a large basal plate (Fig. 2). The filament surface is smooth and straight for most of its length but proximally its diameter increases and it forms an almost S-shaped bend before inserting into the anteroventral surface of the cephalothorax (Fig. 3). The filament of *L. salmonis* is by comparison short and stumpy (Fig. 4) and is inserted into the epidermis, covering the scale, where it produces an adhesive. Its surface is also usually smooth but is sometimes contoured (Fig. 4). Whereas the filament in *C. elongatus* is a discrete structure and clearly not part of the chalimus body, in *L. salmonis* the surface of the filament is continuous with that of the chalimus body.

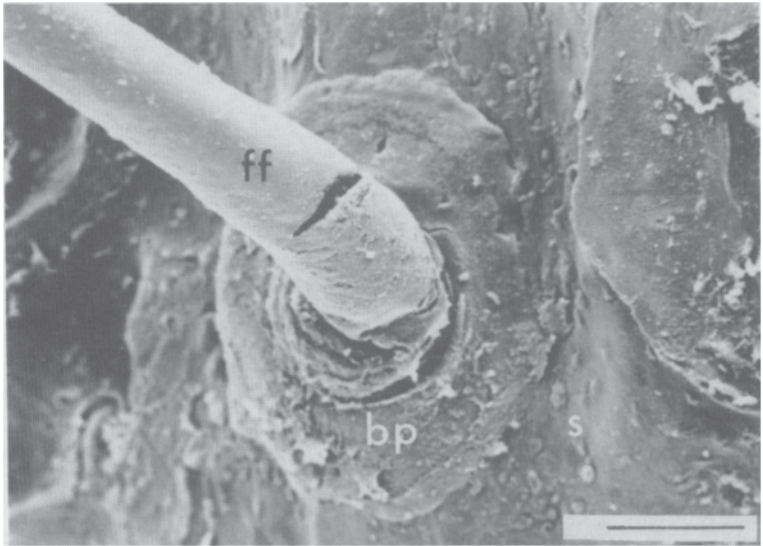


Fig. 2. SEM of chalimus I of *C. elongatus* showing the frontal filament basal plate on the fish scale. Scale bar=20 μm . Abbreviations: see legend to Fig. 1.

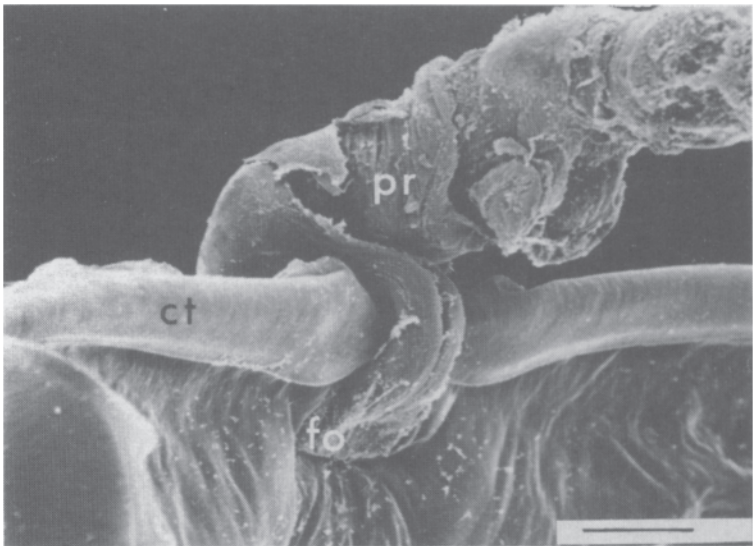


Fig. 3. SEM of the proximal end of *C. elongatus* chalimus I, frontal filament at its origin on the anteroventral surface of the cephalothorax. Scale bar=40 μm . Abbreviations: see legend to Fig. 1.

Internal structure of the frontal filaments

The filaments of both species are enclosed in a non-cellular sheath. In *C. elongatus* this sheath is substantial and composed of fine fibres orientated perpendicular to the

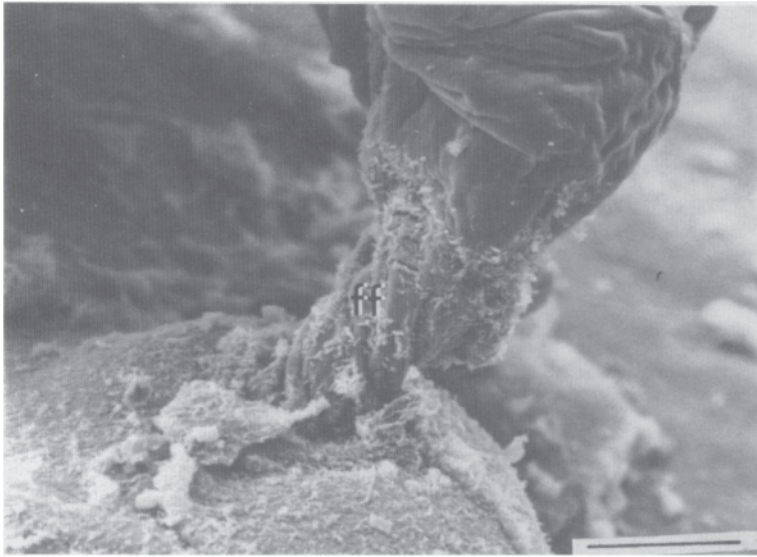


Fig. 4. SEM of the frontal filament of *L. salmonis* chalimus I. Scale bar=40 μm . Abbreviations: see legend to Fig. 1.

long axis of the filament (Fig. 5). Within the sheath there are densely packed bundles of fibres running longitudinally through the slender part of the filament. These fibres appear to be divided into segments at approximately 5 μm intervals (Figs 5 and 6). The fibres are constrained by the sheath as shown by Fig. 6, where the sheath has been disrupted by fracture of the filament. In the proximal region of the filament (Fig. 3) the internal structure is more complex, with the fibres arranged in the form of helicoids (Fig. 7). The fibres are approximately 160 nm in diameter and are composed of bundles of extremely fine fibrils. The appearance and arrangement of these fibres is revealed in Fig. 8, where the sheath has been removed during specimen processing. Near to the intersection between the slender distal portion and the wider proximal portion of the filament (Fig. 3) there is a transitional region (Fig. 9) in which the helicoid arrangement changes to the parallel arrangement of fibres (Fig. 10).

In this region of the filament dense colonies of bacteria were identified lying inside the sheath (Figs 7 and 9). Colonies of another species of bacterium (Professor G. Gooday, personal communication) and protozoans were attached to the surface of the sheath.

The filament in *L. salmonis* is a considerably simpler, more homogeneous structure. Within the thinner sheath there are parallel rows of fine fibres which do not adopt the helicoid arrangement (Fig. 11). A duct is visible in some sections running through the filament. There are no apparent regional differences of structure except at the distal end of the filament. Here the filament expands into a basal plate (Fig. 12) in which the surface is highly pitted and branched (Fig. 13). Within this part of the filament there are numerous cellular inclusions (Fig. 12).

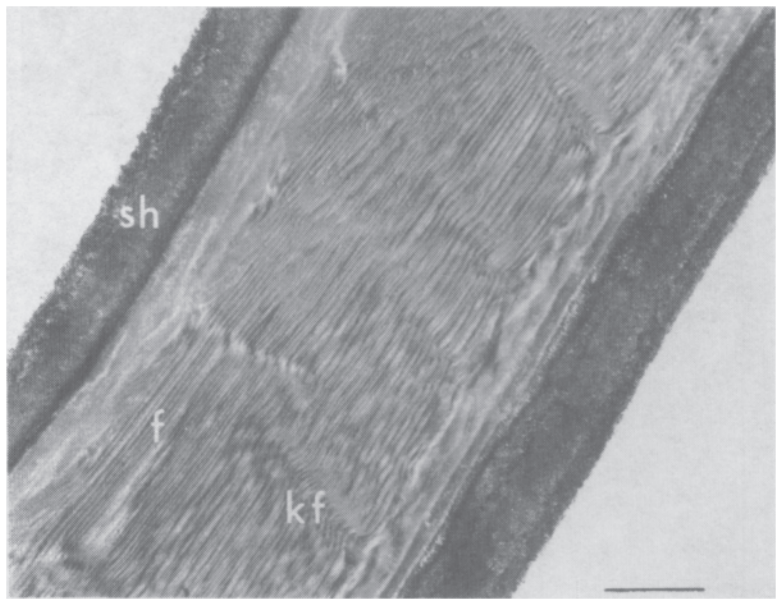


Fig. 5. Longitudinal TEM section of *C. elongatus* chalimus I, mid-region of frontal filament showing sheath and internal fibres. Note the kinking of the internal fibres. Scale bar=2.5 μ m. Abbreviations: see legend to Fig. 1.

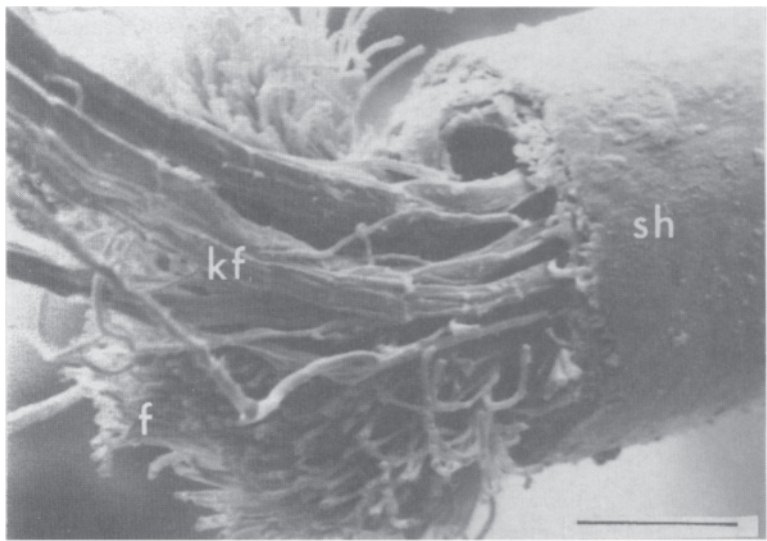


Fig. 6. SEM of *C. elongatus* chalimus I, mid-region of fractured frontal filament revealing filamentous structure inside sheath. The kinking of the fibres seen in Fig. 5 is also visible here. Scale bar=4 μ m. Abbreviations: see legend to Fig. 1.

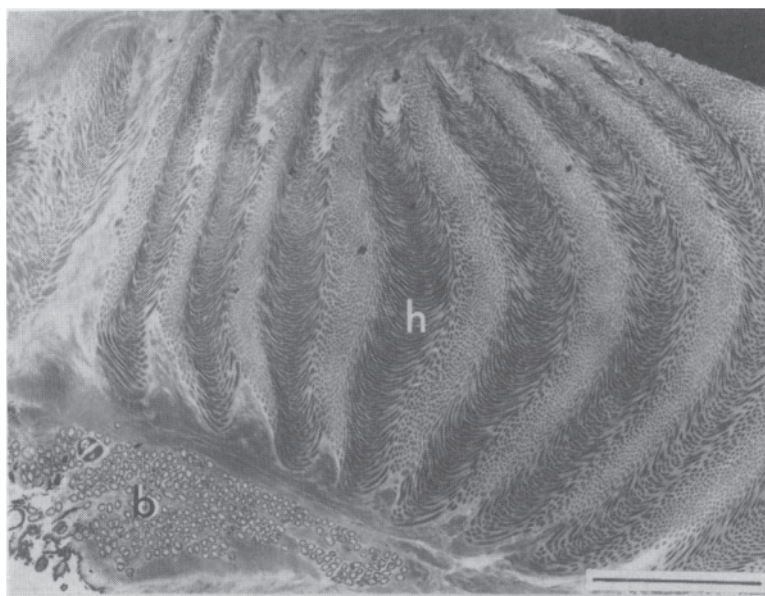


Fig. 7. Transverse TEM section of *C. elongatus* chalimus I, frontal filament proximal region showing helicoid arrangement of fibres. Note the bacterial colony beneath the sheath. Scale bar=10 μm . Abbreviations: see legend to Fig. 1.

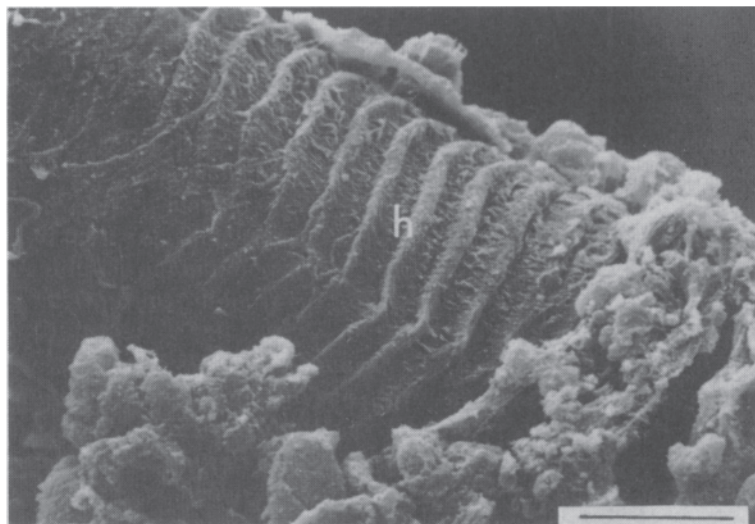


Fig. 8. SEM of *C. elongatus* chalimus I, frontal filament proximal region. The sheath has been damaged in preparation to reveal the surface appearance of the helicoids. Scale bar=20 μm . Abbreviations: see legend to Fig. 1.

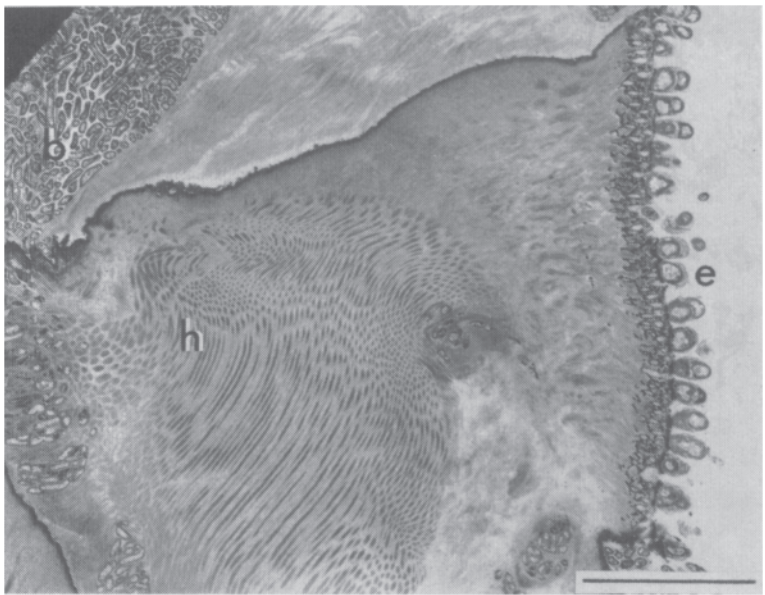


Fig. 9. Transverse TEM section of chalimus I of *C. elongatus* at the junction between the proximal and mid-regions of the filament. Note bacterial colonies and epiphytes. Scale bar=10 μ m. Abbreviations: see legend to Fig. 1.

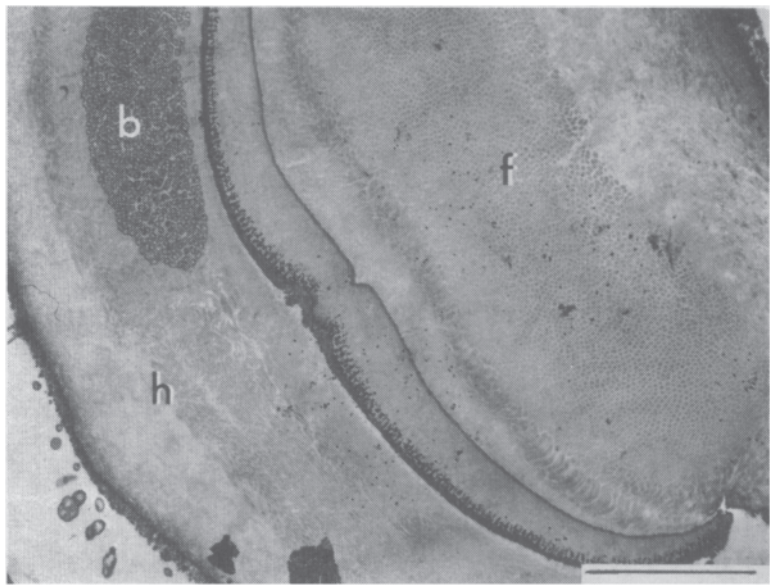


Fig. 10. Transverse TEM section of chalimus I of *C. elongatus* at the junction between the proximal and mid-regions of the filament. Scale bar=10 μ m. Abbreviations: see legend to Fig. 1.

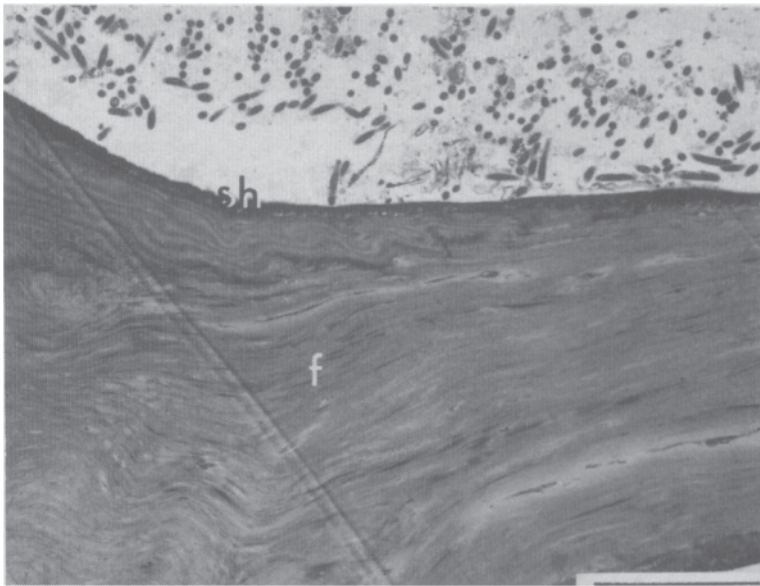


Fig. 11. Longitudinal TEM section of chalimus I of *L. salmonis* tapering portion of frontal filament showing the sheath and internal fibres. Note also the folding of some proximal fibres similar to the helicoid seen in Fig. 7. Scale bar=10 μ m. Abbreviations: see legend to

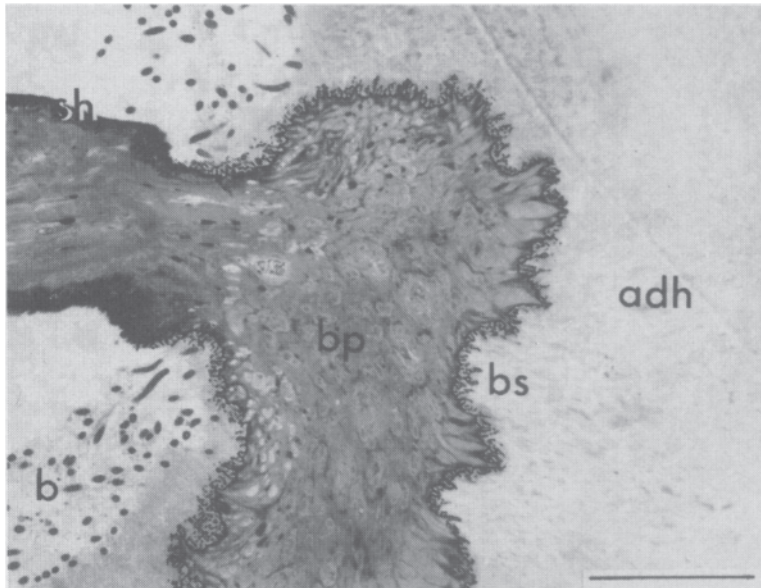


Fig. 12. Longitudinal TEM section of chalimus I of *L. salmonis* frontal filament basal plate showing its highly branched surface, internal cellular inclusions and adhesive. Scale bar=10 μ m. Abbreviations: see legend to Fig. 1.

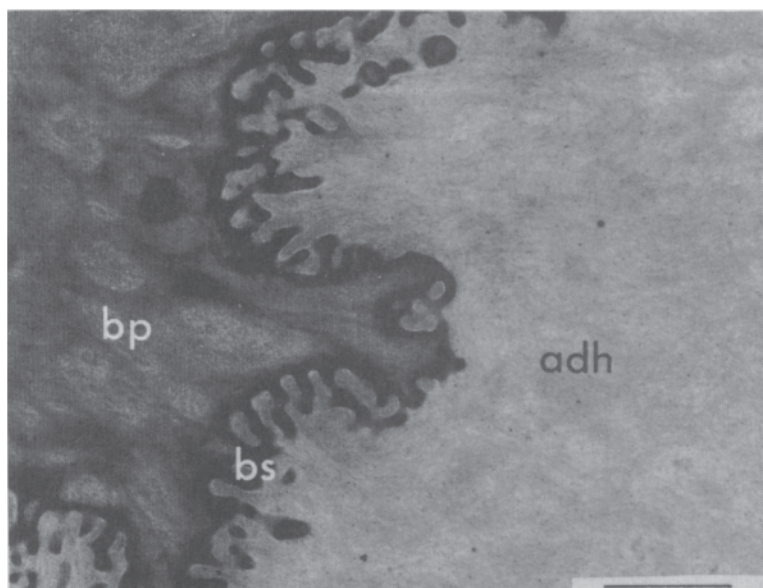


Fig. 13. Longitudinal TEM section of chalimus I of *L. salmonis* surface of the frontal filament basal plate. Scale bar=1 μm . Abbreviations: see legend to Fig. 1.

Origin of the frontal filament

The frontal filament of *C. elongatus* emerges from a discrete location on the extreme anteroventral surface of the chalimus body (Fig. 4) which corresponds in adult lice to the frontal organ (Fig. 14). Serial sectioning through this organ in the chalimus stages reveals a shallow, circular depression (Fig. 15) lined with a microvillar surface (Fig. 16). Fibres, continuous with the frontal filament, emerge from between the microvilli but it is unclear where their source is located.

A very similar structure is present in the chalimus of *L. salmonis*, in which the same type of microvillar surface seems to be the site of origin of bundles of fibres (Fig. 17). However, it is less clear how this relates to the frontal filament of this species because there is no direct connection between the two. In fact the microvillar surface appears to be inside the cuticle of the chalimus some distance from the filament.

Examination of copepodids of *C. elongatus* has revealed a very obvious structure believed to be the precursor of the frontal filament inside the body of 100 h old free-swimming specimens (Fig. 19). Examination of younger copepodids revealed developing stages of this precursor in 38 h copepodids but not in nauplius II larvae. It appears from these observations that the frontal filament is ready to be deployed before the copepodid has settled on a host fish. No evidence has been obtained that a similar structure exists in the free-swimming copepodid of *L. salmonis*, where sections of the anterior end of copepodids of various ages have failed to reveal any structure resembling a frontal filament.

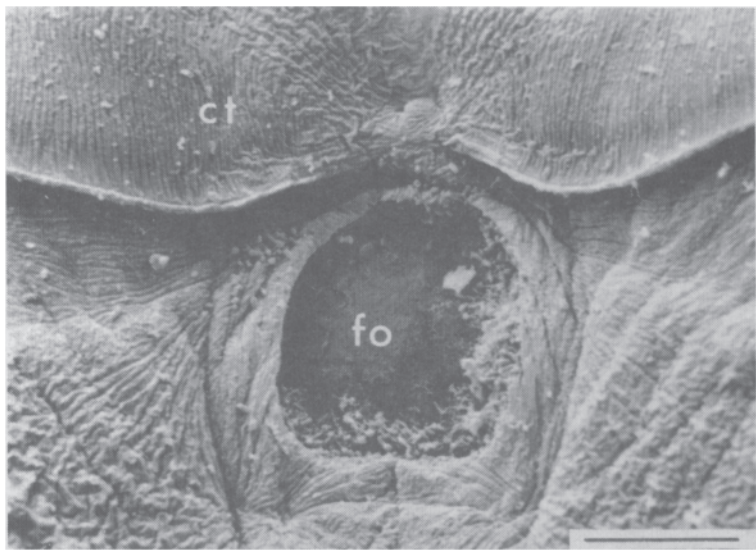


Fig. 14. SEM of adult *C. elongatus* frontal organ. Scale bar=20 μ m. Abbreviations: see legend to Fig. 1.

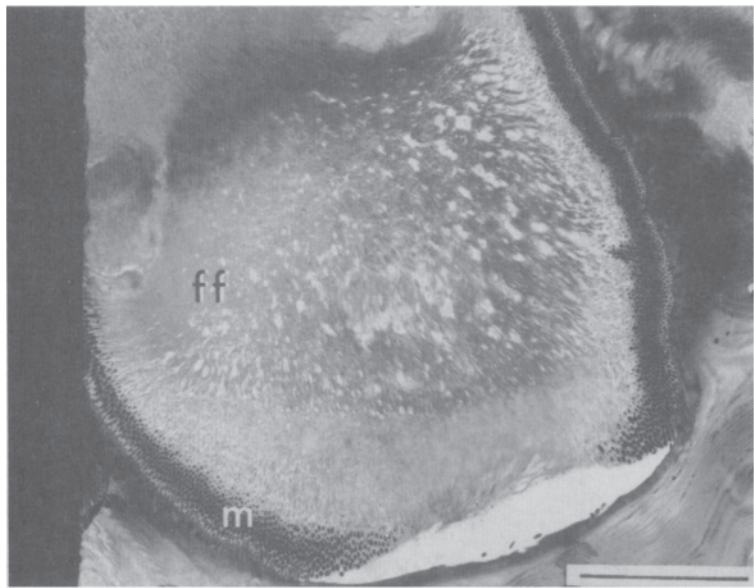


Fig. 15. Longitudinal TEM section of chalimus I of *C. elongatus* frontal organ with emerging frontal filament. Scale bar=10 μ m. Abbreviations: see legend to Fig. 1.

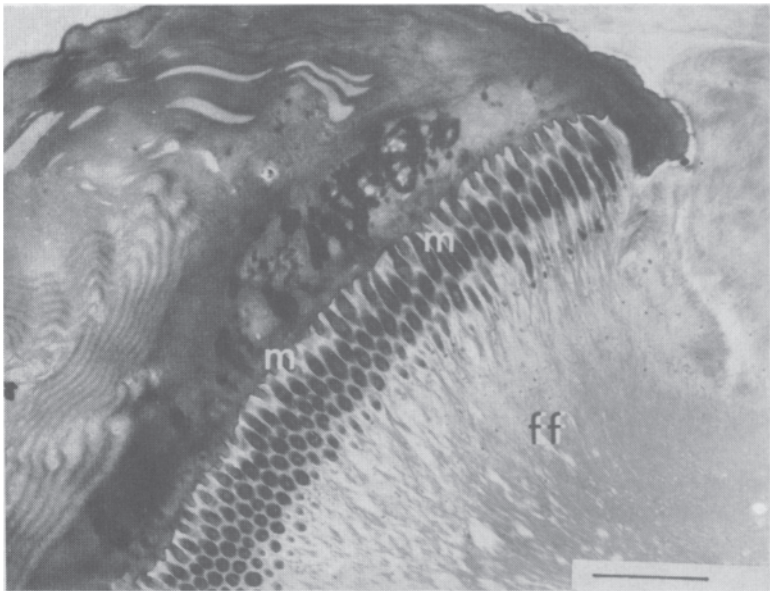


Fig. 16. Longitudinal TEM section of chalimus I of *C. elongatus* microvillar surface of frontal organ and frontal filament. Scale bar=2 μ m. Abbreviations: see legend to Fig. 1.

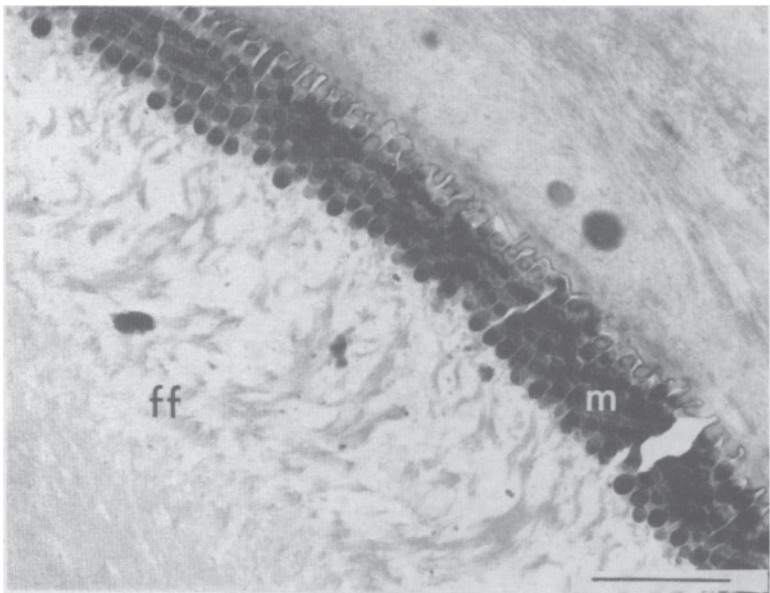


Fig. 17. Longitudinal TEM section of chalimus I of *L. salmonis* microvillar surface of presumed frontal organ. Scale bar=2 μ m. Abbreviations: see legend to Fig. 1.

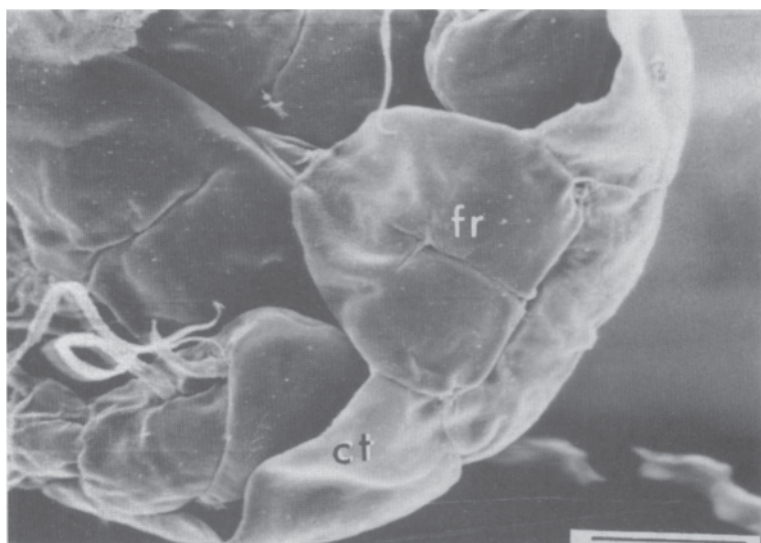


Fig. 18. SEM of copepodid of *C. elongatus* frontal region. Scale bar=20 μ m. Abbreviations: see legend to Fig. 1.

DISCUSSION

This study has shown that there is very little similarity between the frontal filaments of *C. elongatus* and *L. salmonis* with respect to either morphology or internal structure. Whereas the former possesses a filament which is discrete and formed as a separate appendage to the chalimus body, the filament of *L. salmonis* is an integral part of the body of the chalimus.

The filament of *C. elongatus* is attached by the copepodid directly to the fish scale after the juvenile has excavated the epidermal layer covering the scale. The means of attachment is not clear but the basal plate contains fibres similar to those in the filament. In contrast, the filament of *L. salmonis* apparently releases an adhesive which spreads subepidermally over the basement membrane (Bron *et al.* 1991) to form the basal plate. The filament of *L. salmonis* contains a duct leading from a glandular apparatus (Bron *et al.* 1991), which is described as conducting the adhesive to the tip of the filament. The duct has been observed in our TEM sections but has not been traced. However, the distal end of the filament contains cellular inclusions which could perhaps be associated with this duct, although we have no firm evidence of this. The enlarged surface area of the filament tip may improve adhesion between the adhesive and the filament. The anterior end of the copepodid of *C. elongatus* (Fig. 18) has an identical structure to that described for *L. salmonis* (Bron *et al.* 1991), including the presence of a filament duct, but it is unlikely that it performs the function ascribed to it in *L. salmonis*.

With regard to the debate concerning the replacement of the filament during moulting it seems that there could be marked differences again between the two species. Whereas the filament of *C. elongatus* is attached firmly to the scale, and is unlikely to be

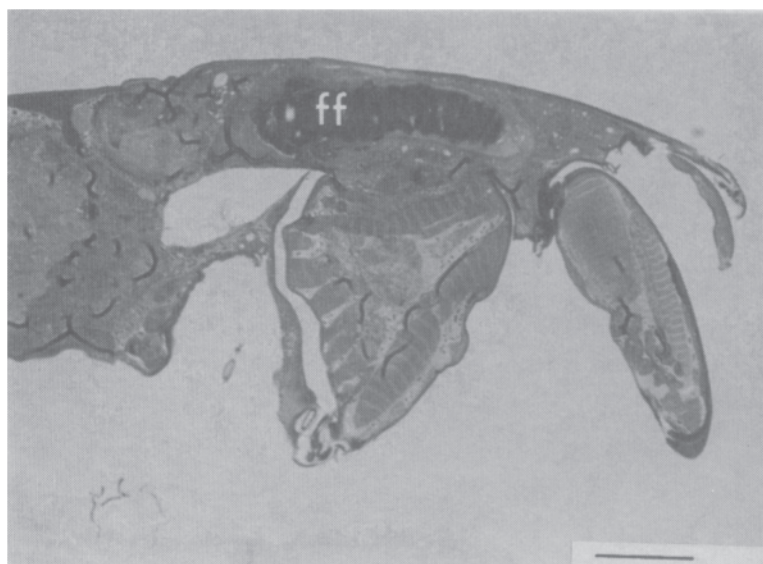


Fig. 19. Longitudinal light microscopy resin section of a 100 h copepodid of *C. elongatus*. Scale bar=100 μ m. Abbreviations: see legend to Fig. 1.

dislodged, the attachment of *L. salmonis* within the epidermis (Bron *et al.* 1991) seems more tenuous given the lability of this tissue. Perhaps there is more reason to believe that replacement of the filament during moulting would be more likely for *L. salmonis* than for *C. elongatus*. The fact that *L. pectoralis* preadults can produce a temporary filament during moulting (Anstensrud 1990) adds weight to this argument. Furthermore, if attachment is through the secretion of an adhesive substance there seems no good reason to assume that such an event would be impossible. By contrast the more complex filament of *C. elongatus* could be more permanent; perhaps even less readily replaced. A careful examination of the chalimus stages of *C. elongatus* has failed to reveal any evidence of an internally developing filament such as exists in the copepodid (Fig. 19), suggesting that the filament may not be reformed at moulting.

The presence of what has been described as the filament precursor but which could be a fully-formed filament within the copepodid is not new. Previous descriptions exist in the literature for several species including *C. clemensi* by Kabata (1972), *Salmincola edwardsii* by Fasten (1919) and *Salmincola californiensis* by Kabata and Cousens (1973). The early stages of formation have been observed in copepodids of *C. elongatus* but details of the developmental process and source of the chemical components are still lacking. We also have no information on the means by which the filament is attached to the fish scale. The deployment of the frontal filament from within the body of the copepodid and the release of a cement from the frontal gland has been described for *S. californiensis* by Kabata and Cousens (1973). We have not observed settlement in *C. elongatus* so cannot confirm that a similar process occurs. What is evident, however, is that the prominent basal plate described by Bron *et al.* (1991) and seen in our TEM sections as a homogeneous substance (Fig. 12) does not exist in *C. elongatus*.

A further point of interest concerning the deployment of the frontal filament relates to its passage through the microvillar layer in the frontal organ. Perhaps it is in a liquid crystal form such as that referred to by Neville (1988) for other skeletal helicoids. The origin of the sheath is also unknown but must be produced at the same time as the internal elements of the filament.

The components of the filament bear some morphological resemblance to the byssus threads of mussels, the chemistry of which has been studied extensively by Waite (1991). It would be interesting to pursue the comparison by studying the chemistry of the frontal filament and its precursor in the body of the copepodid. Whilst the possibility of finding a way to disrupt filament production may be remote, a clearer understanding of its chemistry and mode of production could yield new clues to ways of making this possible.

ACKNOWLEDGEMENT

This work was supported by a grant from the Scottish Salmon Growers Association.

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9

Sensory innervation of the antennule of the preadult male *Caligus elongatus*

M.S.Laverack and M.Q.Hull

ABSTRACT

The tip of the antennule of preadult male *Caligus* carries 13 setae forming an apical or terminal tuft. Three of these setae lie at the posterior border of the distal article and are long (about 130 μm), thin (c. 4 μm at the insertion) and gradually tapering, one being bifid. Anteriorly nine setae are rather shorter, broader, rectilinear and sharply tapered. The final seta lies between these two groups and is similar to the first group, though shorter. A numerical convention has been adopted, with I–V referring to the longer setae, and VI–XIII to the shorter ones.

Transmission electron microscopic studies show that setae I–IV carry two large-diameter dendrites; seta VI has two large-, two small-diameter long and 13 small-diameter shorter nerve fibres; setae VIII and IX have one large and two short small fibres; XII has one large and one short dendrites; XIII has one large and two short fibres, while V and X have many small long fibres. Internal differences exist amongst the individual dendritic endings in terms of neurotubules and ciliary figures at the bases.

The sensitivities of these varied fibres is conjectural though it is likely that multiple long, thin dendrites may be chemoreceptors as in aesthetasc setae in Decapoda (Grünert and Ache 1988) and others mechanically sensitive (possession of scolopale). Seta number VI may be a contact bifunctional mechano-chemoreceptor. Functional and physiological investigations are required for verification of such suggestions.

INTRODUCTION

The location of distant prey by parasites must depend on the detection of sensory clues emanating from the host organism(s). These may be of several different modalities, such as chemical (metabolites, pheromones, mucus) and mechanical (e.g. vibration, water eddies from swimming). Combined physicochemical events may occur when the parasite makes contact with the host. For most parasites these are also specific and identifiable from particular host species.

Sensors involved in detecting such information are probably carried at many sites in invertebrates, and amongst Crustacea are known over the whole body. They may be found on large surfaces (e.g. carapace, abdomen) or concentrated in certain areas on specific limbs. Amongst the best known of these are the aesthetasc setae of the antennules of decapods (Laverack and Ardill 1965, Grünert and Ache 1988), where large numbers of chemically sensitive units are situated.

Caligus Müller takes up its external parasitic habit on a fish host which it locates by sensory means. The major sources of sensory input are probably carried anteriorly and may reside in the limbs of the head, notably the antennules. The present investigation was carried out to determine whether or not the setae of the antennule of the preadult are innervated and what numbers and types of sensory neurones are present. This should be compared with the findings of Gresty *et al.* (Chapter 7) on the copepodid of *Lepeophtheirus salmonis* (Krøyer).

MATERIALS AND METHODS

Preadult male *Caligus elongatus* Nordmann were taken by plankton net sampling in St Andrews Bay, Scotland. They were fixed in crustacean fixative (Barrientos and Laverack 1985) and external features examined using a JEOL JSM 35CF scanning electron microscope. Internal details were obtained after processing for transmission electron microscopy (TEM), araldite embedding, and sectioning with a Diatome diamond knife. Sections were stained with uranyl acetate/lead citrate and observed in a Philips 301 transmission electron microscope.

RESULTS

Scanning electron microscopy

The anterior portion of the preadult *C. elongatus* is shown in Fig. 1(a,b) and the antennule in Fig. 1(c). The animal is provided with numerous presumptive sensory setae and the distal portion of the antennule carries 13 such structures (Fig. 1d). These have been numbered as shown in Fig. 2, which gives three views of the antennulatory tip, from the dorsal and ventral sides and distally from end on. Hairs numbered I–V are long (c. 130 μm); VI–XIII are short (30–55 μm). The long setae are about 4 μm in diameter, while the shorter setae may be rather stouter, up to 8 μm at the base. Seta IX is short (c. 5 μm) and fine (c. 0.8 μm). Seta IV appears as two separate structures but TEM studies show that the seta is actually bifid, the two branches being fused about 10 μm from the base. All of the setae are smooth, though they show varying diameters at the base and distally.

Transmission electron microscopy

Information regarding the innervation of these setae is restricted to the shaft portion, and does not include the details of the tip or deep into the cuticle surface. The internal structures vary and are not consistent from seta to seta.

Posterior long setae: I–IV

The setae are covered with a thin epicuticle (0.15 μm thick) continuous with the general body epicuticle. The endocuticle is thick and lamellar in structure, with a

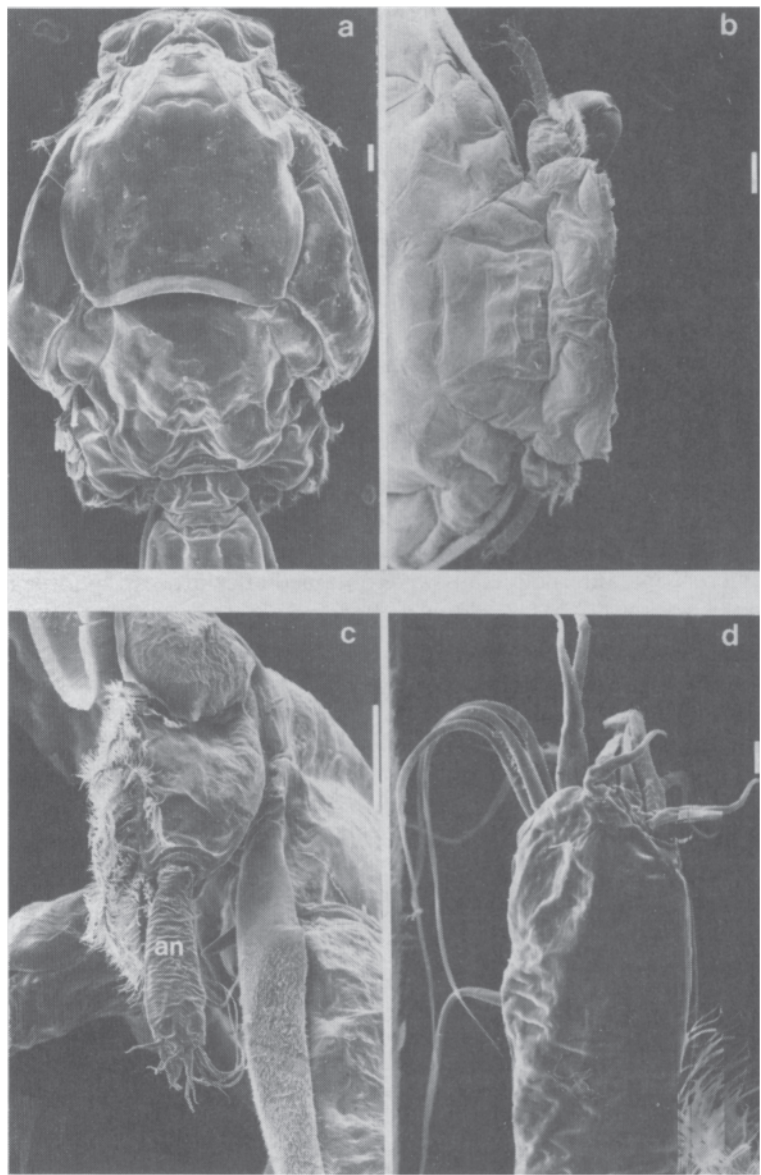


Fig. 1. (a) EM of the anterior portion of preadult male *Caligus*. Anterior towards the top. Scale bar=100 μ m. (b) Head of *Caligus* with right and left antennules extended laterally. Scale bar=100 μ m. (c) Left antennule (an) of *Caligus*. Scale bar=100 μ m. (d) Tip of left antennule to show cluster of distal setae. Ventral view. Scale bar=10 μ m.

substantial lumen at the base that is occluded by a flocculent ‘honeycombed’ material distally, the seta ending in solid cuticular tip. Seta I is bifid above the base (Fig. 3a) and two sensory dendrites are observed in each branch, though it is not known if they

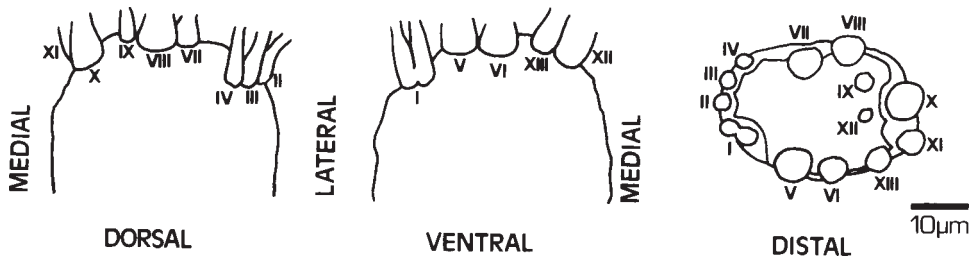


Fig. 2. This diagram represents the tip of the antennule of preadult male *C. elongatus* as seen from the dorsal, ventral and distal aspects. The numbering convention adopted for the 13 distal setae is indicated.

join at a level below this. Within the lumen of these setae a sheath invests two large-diameter dendrites that extend about 40% of the length of the shaft, where the lumen is pinched off.

Mid/anterior short setae: V–XIII

Two setae have been studied in detail: VI and XIII. The remainder have been observed in varying degrees of completeness. Seta VI has a lumen that runs almost to the tip of the hair, and within lies a sheath enclosing the nervous elements.

Innervation

Various modifications of internal arrangements occur, and details of numbers of identified dendrites are indicated in Table 1. Setae numbered I–IV each contain two large-diameter neurones (Fig. 3d), VI, VIII and IX also have two such dendrites, but additionally two smaller-diameter and other endings (Fig. 3b,c). Seta I is bifid above the base and two dendrites are observed in each branch, though it is not known if they join at a level below this. The total number of nerve endings in setae VIII and IX is uncertain. Setae VII and XI have one large- and two smaller-diameter plus an additional group, while XII has one large and one small, XIII has one large and two small, while the remaining setae, V and X, each contain up to 200 small-diameter dendrites. These dendrites are the distal endings of bipolar nerve cells. The larger-diameter dendrites are around 75 µm in length in all cases and in the case of long, thin setae the tip of the neurone reaches the limit of the luminal space, the tip being occluded. In the shorter setae the dendrites end before the sheath and lumen become limiting, though in seta VI there is some evidence of a continuing thread that attaches the bundle to the end of the sheath. These large-diameter units have a substantial scolopale structure wrapped around them in the proximal region (Fig. 3c). At the base there is a distinct ciliary basal body (Fig. 3e) and distal to this in sequence are regions demonstrating clear ciliary structures in a clear cytoplasm (Fig. 3f,g). In the outer segment numerous microtubules are located, extending as far as the tip.

The shorter setae (except V and X) carry small-diameter, long dendrites in pairs and have distal segments with a ciliary basal body and peripheral arrays of microtubules. Number VI uniquely possesses a bunch of 13 short distal segments (Fig. 4a,b), though the number of individual sensory neurones underlying these is not known. There may be peripheral division of a smaller number of originating cells.

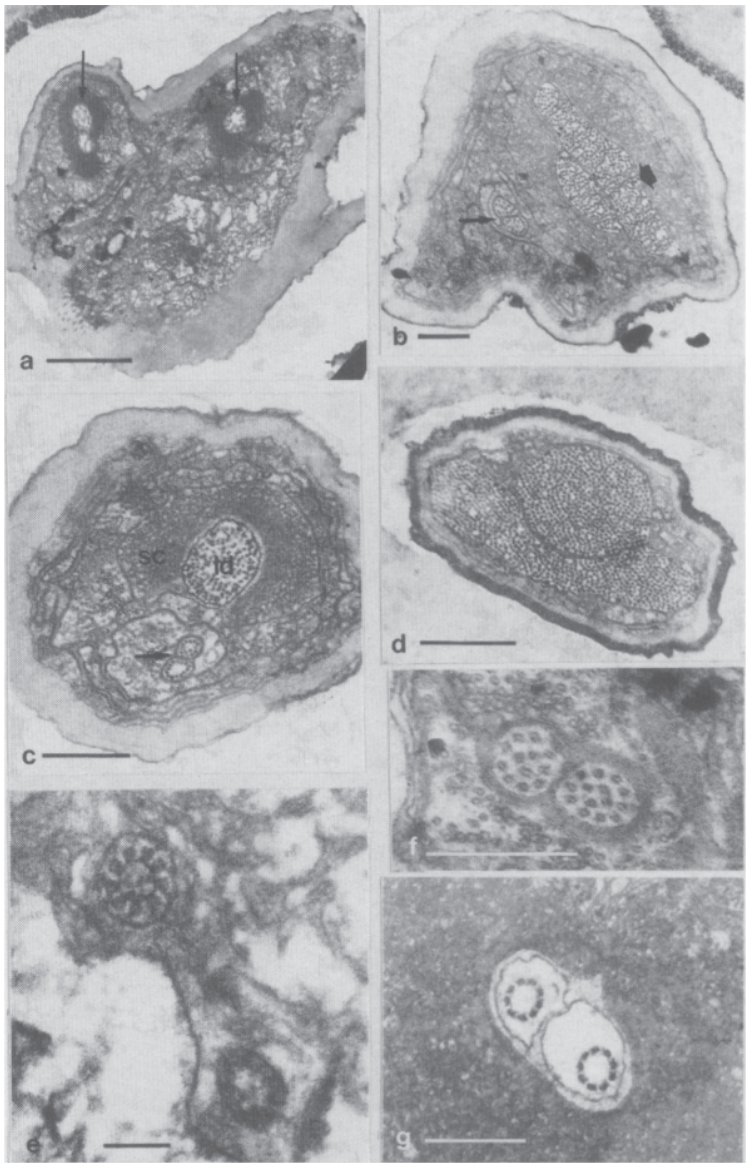


Fig. 3. (a) Seta I is bifid, appearing as two separate setae distally but fused at the base. Dendrites (arrowed) appear within each shaft. Scale bar=1 μ m. (b) Setae VIII and IX each carry two small dendritic profiles (arrowed) and two larger elements (large arrows). Scale bar=0.5 μ m. (c) Seta XIII has two small sensory neurones (arrow) and one larger (ld) which is invested with scolopale (sc) material. Scale bar=0.5 μ m. (d) Setae II, III and IV possess two large units, with one always partly surrounded by the second. Scale bar=0.5 μ m. (e) Seta VI; typical ciliary basal bodies and other ciliated figures appear in a variety of setae at slightly different levels, but usually at the setal bases. These are from the two major dendrites in this seta. Scale bar=0.25 μ m. (f) Seta XIII; the tips of the dendrites show a number of microtubules, but arranged in specific numbers or patterns related to ciliary tubules, (g) Basal region of the large diameter dendrites, as found in setae I to IV. Scale bar=0.25 μ m.

Table 1.

Seta no.	Large diameter	Small, long	Small, short	Very small, long
Ia	2	—	—	—
Ib	2	—	—	—
II	2	—	—	—
III	2	—	—	—
IV	2	—	—	—
V	—	—	—	100–200
VI	2	2	13	—
VII	1	2	?	—
VIII	2	2	?	—
IX	2	2	?	—
X	—	—	—	c. 200
XI	1	2	?	—
XII	1	1	—	—
XIII	1	2	—	—

Some ciliary bases have been identified in the proximal reaches of the outer segments; the internal microtubules are in the form of doublets, while peripherally they are reduced to single entities.

Setae V and X contain up to 200 dendrites each of 0.1–0.2 μm diameter and with up to 30 microtubules in the cytoplasm (Fig. 4c–f).

DISCUSSION

Gresty *et al.* (1993) describe the innervation of the setae of the infective copepodid of *Lepeophtheirus salmonis*. In essence their findings reflect those described for *Caligus elongatus* though there appear to be some small differences. The total number of setae present differs in that the larval stage has 12 distal setae, while the preadult *Caligus* has 13. Of these one (seta I) is bifid and each shaft possesses two dendrites of probable mechanoreceptor function. Gresty *et al.* (1993) consider this to be two elements fused into a single base rather than one divided seta; in view of the double innervation this may be the correct interpretation. Laverack (1988) has remarked upon the continual addition of sensory neurons during the many moulting stages of Crustacea, and the early larvae of many organisms are known to increase the numbers of setae, and hence presumably the sensory inclusions, throughout all stages up to the terminal moult (e.g. Letourneau 1976). In view of the fact that the observations for *Lepeophtheirus* (Chapter 7) and *Caligus* (this chapter) were conducted on different stages this may account for alterations in numbers of setae. It is also conceivable that there are species-specific differences.

The setal tuft of the tip of the antennule of *Caligus* shows considerable variability in structure, both in the nature of the type of setal shaft, the cuticular structure and

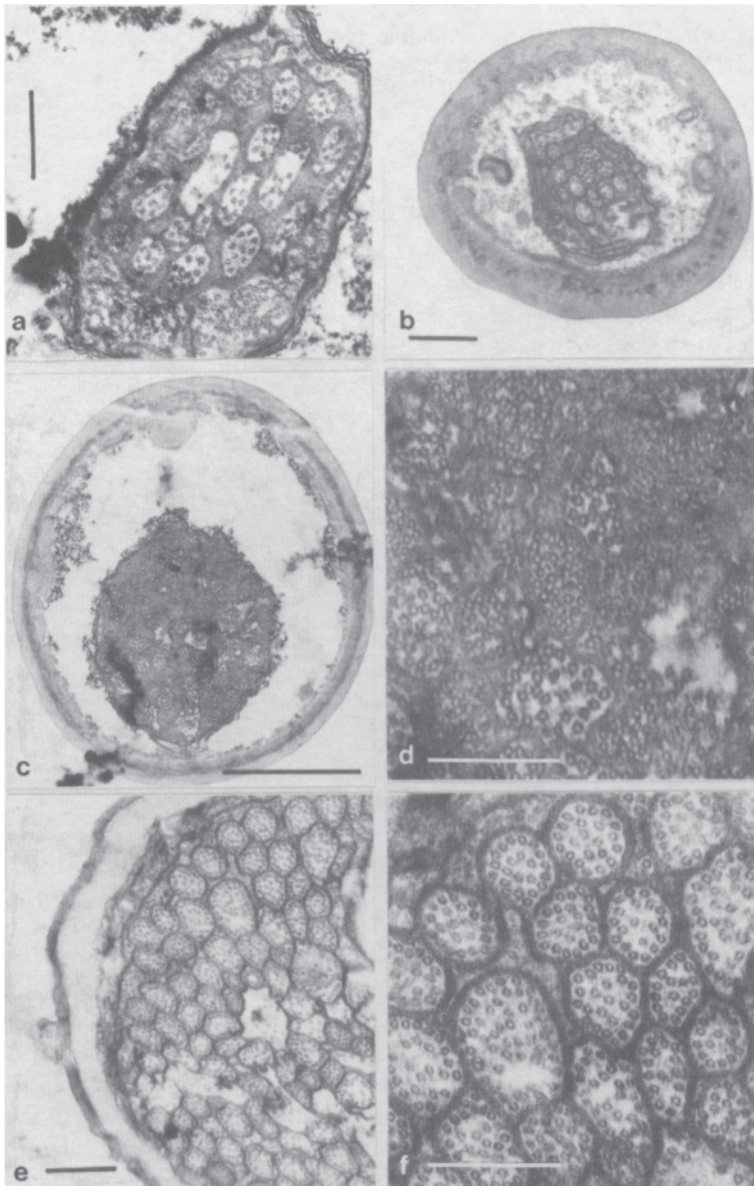


Fig. 4. (a,b) Seta VI possesses not only two distinct larger units, and two rather smaller, but a bunch of 13 small-diameter long dendrites that extend a considerable distance along the seta. This section is from a distal position. Scale bar= $0.5\ \mu\text{m}$ (a) and $1.0\ \mu\text{m}$ (b). (c) Seta V; a cluster of about 100 closely packed dendrites is found in this seta. Scale bar= $1.0\ \mu\text{m}$. (d) Seta V; each dendrite is densely provided with microtubules in no particular arrangement. Scale bar= $0.25\ \mu\text{m}$. (e) Seta X; up to 200 dendrites are found in this seta tightly adjacent to one another, and of slightly varying diameters. Scale bar= $1\ \mu\text{m}$. (f) Seta X; the individual dendrites lie close together with some interstitial extracellular material among them, and internally they show a ring of 10–20 peripheral microtubules and some (three to eight) randomly arranged microtubules in the centre. Scale bar= $0.5\ \mu\text{m}$.

internal contents. The lengths of setae vary; the cuticle may provide a long internal lumen, or be occluded and fill the lumen with an amorphous material that precludes extension of the setal contents. The centres of the setae contain variable numbers of dendritic endings. Large-diameter endings, often in pairs, with an attached scolopale seem clearly to be mechanoreceptors, having sufficient similarities with other described such sensors in Crustacea to be taken as being involved in mechanical detection (see Bush and Laverack 1982, for a review). The size and position of the setae suggest that they are probably concerned with determining contact with a solid substrate (e.g. the host fish). A probing function during settlement would be appropriate, rather than water movement, which demands a lightly pivoted hydrodynamic sensor (e.g. Laverack 1963, Vedel and Clarac 1976), though the setae with two pairs of dendrites (VIII and IX) *may* perform two distinct types of mechanoreception. Setae V and X, on the other hand, contain many dendrites of small diameter, more reminiscent of aesthetasc chemoreceptors in decapod Crustacea (Grünert and Ache 1988). The possibility exists that these represent numerous branches from a small number of neurones, as shown by Heimann (1984) for the isopod *Asellus aquaticus*. The large number of neurones located on anterior setae argues for chemoreception for these two setae. Seta VI is more problematical since it contains not only two large mechanoreceptor type units, and two smaller dendrites extending the length of the seta, but additionally 13 small-diameter endings like those of V and X. We suggest that comparison with receptor organs such as the stout subdivided setae of the decapod periopod chelae (Altner *et al.* 1983) indicates that the limited number of small-diameter neurones coupled with the presence of two rather larger units may be indicative of a dual function. Since the decapod organ is used in contact rather than distance chemoreception it is possible that this seta is involved in chemoreception on the host to assist selection and attachment. Gresty *et al.* (1993) do not comment on a seta with this kind of innervation and it may represent a late addition to the sensory armament of the host-seeking parasite.

In summary, there may be three distinct populations of sensors in the apical tuft of the antennule, the first composed of two setae (aesthetascs) filled with many dendrites and probably concerned in chemoreception in behaviour such as host detection. The second group are mechanoreceptors, and there may be some distinction between long and shorter hairs. It should be remembered that all setae will be buoyed up when the animal is in the normal watery habitat, and the longer setae may well be extended as far as the outer border of the antennule in water. Appearances by scanning electron microscopy (SEM) may be misleading in this respect. Lightly built hairs pivoted at the base may well be stimulated by water movements (Vedel and Clarac, 1976), while stouter, more substantial setae are probably displaced by contact with a solid body (e.g. the host). The third group is composed of only a single seta (VI) with two distinct types of ending and this may be crucial in final settlement on a host, since it represents a contact chemoreceptive organ.

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Part Id Behaviour

10

Aspects of the behaviour of copepodid larvae of the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837)

J.E.Bron, C.Sommerville and G.H.Rae

ABSTRACT

A study has been carried out to investigate the sensory organs and behaviour of copepodid larvae of *Lepeophtheirus salmonis* (Krøyer, 1837). A description is given of the antennule, nauplius eye and integumental organs of the larva and a pattern of behaviour is proposed from experimental studies which might allow the copepodid to locate and infect host fish.

Larvae are positively phototactic and move upwards in response to increased pressure. This may serve to enhance the probability of host contact. No chemotactic response was observed and it is proposed that the principal method of host contact is through a burst-swimming response to water flow or mechanical vibration generated by the host. Settlement occurs by 'grappling' using the clawed antennae. Copepodid larvae did not infect non-salmonid hosts and it is suggested that host identification is carried out via high-threshold contact chemoreceptors on the antennules. The site of settlement is suggested to be mediated by local current flow and epidermal characteristics. The results of this study are discussed with reference to previous studies on parasitic and free-living copepods.

INTRODUCTION

In order to maximize the chances of survival, successful host interception and settlement, copepodid larvae must be able to respond to cues present in the external environment. Despite the recent attention given to the general biology and taxonomy of caligids, little work has been carried out on behaviour, particularly that of the infective copepodid stage. Many of the observations in the literature are largely anecdotal and relate principally to the response of the larvae to light.

A strong positive phototactic response has been reported for copepodids of *Lepeophtheirus salmonis* (Krøyer, 1837) by Johannessen (1975) and Wootten *et al.*

(1982). Such a response has also been reported for copepodids of *Lepeophtheirus dissimulatus* Wilson by Lewis (1963) and of *Lepeophtheirus pectoralis* (Müller) by Boxshall (1976). This positive response decreased with age (Lewis 1963, Boxshall 1976). Negative phototactic responses have been noted for some caligids, notably *Caligus curtus* Müller, *Caligus bonito* Wilson and *Caligus elongatus* Nordmann (Wilson 1905, Heegaard 1947).

Copepodids of *L. pectoralis* displayed increased activity when exposed to water turbulence or changes in light intensity, and exhibited a positive rheotactic response when subjected to a directional water current (Boxshall 1976). Experiments by Cabral and Fraile (cited by Raibaut 1985) suggest that copepodids of *Caligus minimus* (Otto) are attracted to currents caused by respiratory and body movements of *Dicentrarchus labrax* (L.) and furthermore are attracted to scales and fresh/freeze-dried mucus taken from the host. Despite the observation of host-seeking behaviour in some caligids, it has been noted that some species, e.g. *Lepeophtheirus kareii* Yamaguti, show no evidence of directed host-seeking behaviour (Lopez 1976).

The approach of the present study has been to hypothesize, by reference to other free-living and parasitic copepods, the methods by which a copepodid might be expected to seek out a host and then to examine specific aspects of morphology and behaviour that may support or refute such hypotheses.

In order to locate a host effectively, a copepodid larva must first be able to detect stimuli present in the external medium and, second, must be able to react to them in an appropriate manner. Because the copepodid stage is, as are the preceding two nauplius stages, lecithotrophic, it must furthermore locate the host with a minimum possible expenditure of its limited energy resources.

Using the behaviour of other parasitic and free-living copepods for reference, one might expect the behavioural repertoire of the copepodid larva to cater for one or more of the following activities:

1. Predator avoidance.
2. Avoidance of adverse environmental conditions.
3. Movement into, or maintenance within, host-rich environments.
4. Host detection.
5. Host contact/settlement.
6. Confirmation of host suitability.

The literature concerning behaviour of parasitic and free-living copepods indicates that the following cues might play a role in determining the directed movement and behaviour of the copepodid larva of *L. salmonis*.

1. Light (as described for *L. salmonis* by Johannessen 1975, Wootten *et al.* 1982).
2. Chemical (as suggested for *C. minimus* by Raibaut 1985).
3. Pressure (as suggested for *L. salmonis* (Johannessen 1975), *Calanus finmarchicus* (Gunnerus) (Hardy and Paton 1947, Rice 1962) and numerous other marine invertebrates (Morgan 1984)).
4. Water flow/vibration (as suggested for *L. pectoralis* (Boxshall 1976) and *C. minimus* (Raibaut 1985)).

SENSORY MORPHOLOGY

The copepodid is equipped with a range of sensory structures which have been studied using a combination of light and electron microscopy (see Bron in preparation for detailed methodology). The following organ types are briefly described: antennules, nauplius eye, sensillae and pores. This does not represent an exhaustive list of the sensory structures possessed by the copepodid; Dudley (1972), for example, showed that the infective copepodid stage of the notodelphyid *Doropygus seclusus* Illg possesses a pair of large chemosensors in the frontal region of the head, now identified as the organ of Bellonci (Boxshall 1992) and these have also been observed in copepodids of *L. salmonis* in the present study.

Antennules

The antennules are the primary sensory interface of copepods. The large, well-innervated antennules of the copepodid of *L. salmonis* comprise a large basal segment which carries three anteriorly directed setae and a distal segment equipped with 13 elements: two aesthetascs, five branched setae, three short unarmed and three long unarmed setae. In addition, there is a single tube pore (see Chapter 7). This concentration of mechanosensory and chemosensory elements suggests that the antennules are important for the detection of water flow/vibration stimuli and host-derived or other chemical stimuli in the environment.

Eyes

The nauplius eye consists of a pair of dorsal ocelli and a single ventral ocellus. The structure of the nauplius eye agrees broadly with that described by Elofsson (1966) for the adult male of *Caligus acutus* Kirtisinghe and with the more general description of the eye of *L. pectoralis* given by Scott (1901). Each dorsal ocellus possesses a large lens which projects anterodorsally through a window in the pigmented central cells (Fig. 1A). The dorsal ocelli each possess nine retinular cells arranged in a basket surrounding the lens. The ventral eye has no lens and is equipped with ten retinular cells. Within the retinular cells are the rhabdoms, which are composed of multilayers of regularly folded membranes (Figs 1B and 2A). The array of membranes appears in a different configuration according to the plane of the section (compare O and M in Fig. 2A).

Dorsal and ventral ocelli each possess a pronounced tapetum comprising two cells containing stacked crystalline inclusions set in membrane pockets (Fig. 2A). The tapetal layer acts as a mirror reflecting light back into the retinular cells and enhancing the light-gathering properties of the eye (Land 1984).

The combination of lens and mirror optics, plus the presence of effective pigment shielding with a small distal aperture in the dorsal ocelli, suggests that they have good light-gathering properties and may have image-forming capacity allowing for precise location of a light or shadow source. While the dorsal ocelli cover the anterodorsal field of view, the ventral ocellus probably provides information on the level of illumination from behind and below the animal. Innervation for the ocelli enters the cerebrum anterodorsally, though major fibres can be traced into the interior of the cerebrum.

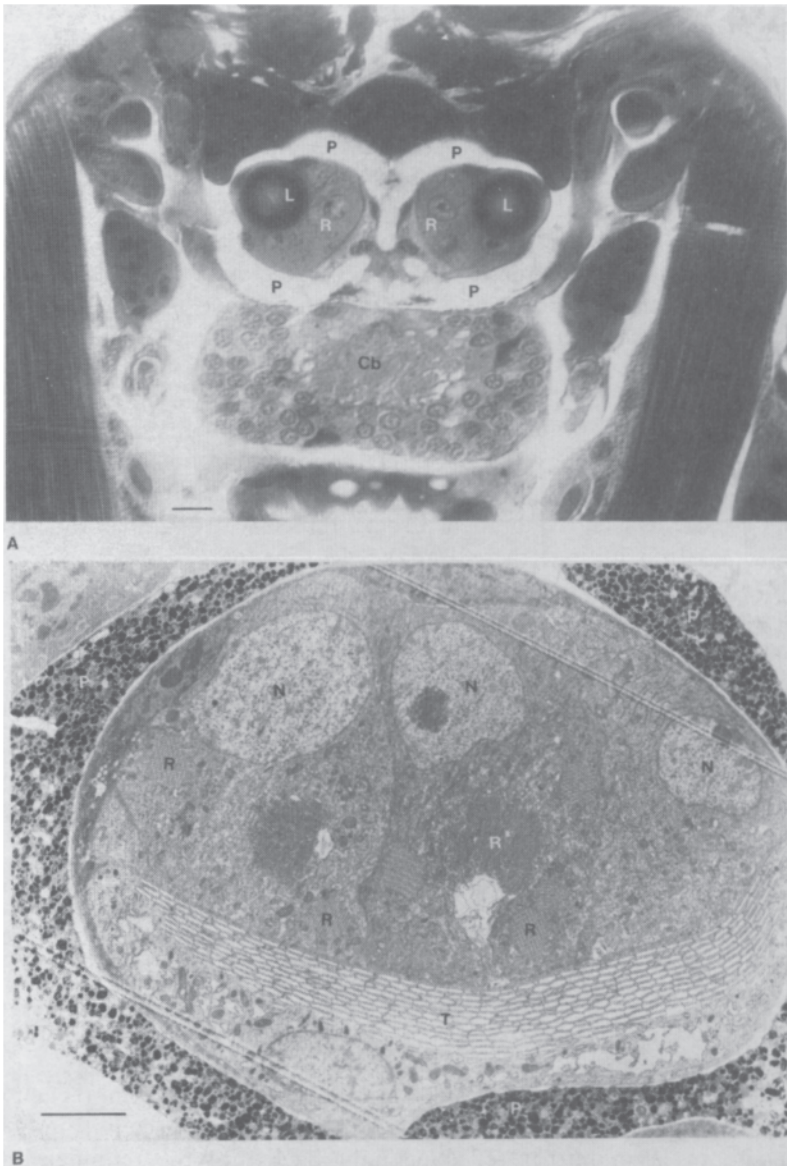


Fig. 1. (A) Light micrograph showing paired dorsal ocelli (stained PAS, haematoxylin). L, lens; R, retinular cells; P, pigment cells; Cb, cerebrum. Scale bar=10 μm . (B) Transmission electron micrograph (TEM) of dorsal ocellus showing general structural components. N, nucleus of retinular cell; P, pigment cell; R, rhabdom of retinular cell; T, tapetum. Scale bar=3 μm .

Integumental sensory organs

A number of integumental organs are arranged bilaterally over the dorsal surface of the cephalothorax and free pedigerous somites (Fig. 3). These organs, many of which

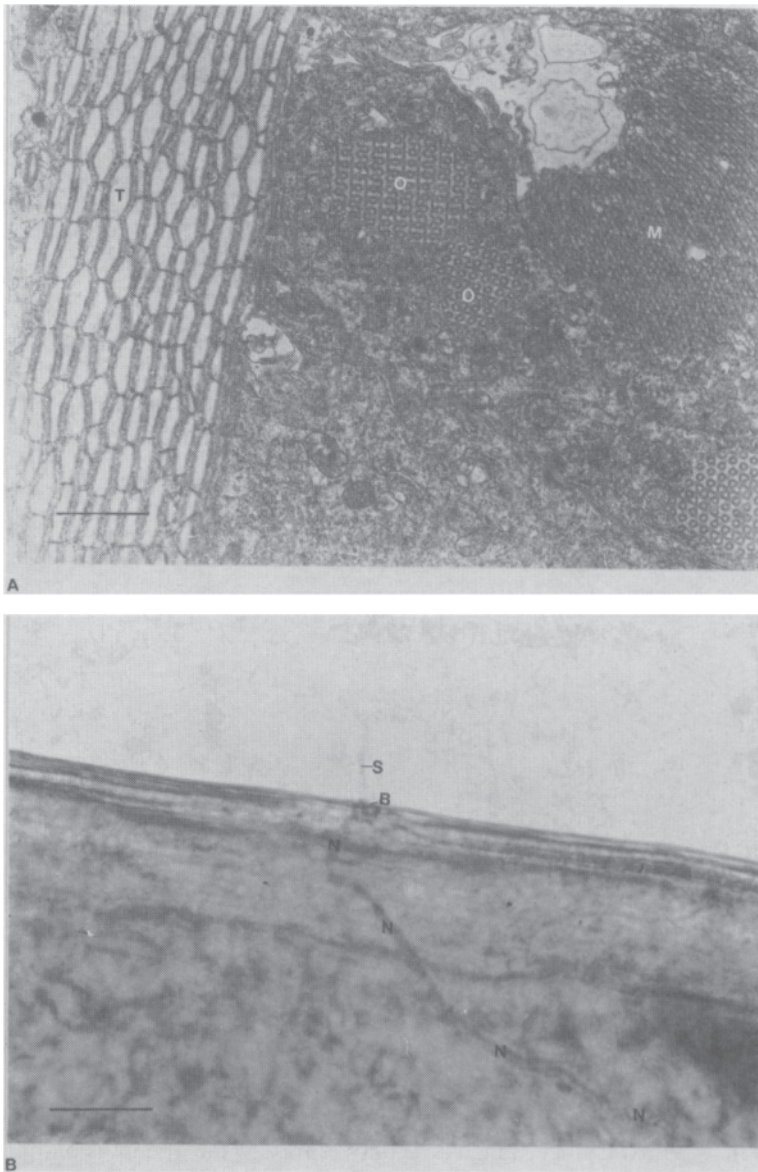


Fig. 2. (A) TEM of dorsal ocellus showing tapetum and rhabdoms of reticular cells cut in two different planes (O and M). T, tapetum. Scale bar=1 μm . (B) Light micrograph showing a single hair sensillum and its nerve (stained for cholinesterase). S, sensillum; B, base of sensillum; N, nerve stained for cholinesterase. Scale bar=20 μm .

are innervated by cholinergic nerves (Fig. 2B), appear to be principally mechanoreceptors, corresponding to those described previously in Crustacea as hair and peg sensillae (Fig. 4A). The absence of these organs from the ventral body surface

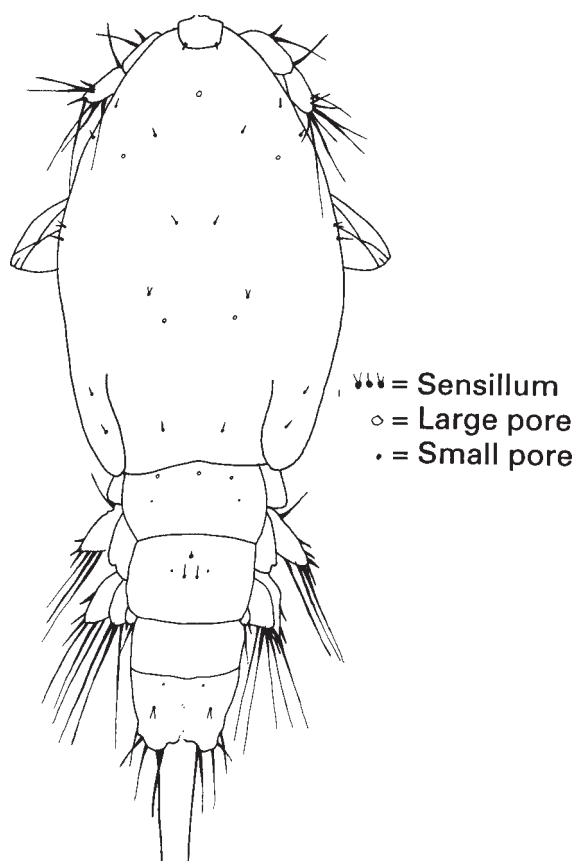


Fig. 3. The distribution of sensillae and integumental pores over the body of the copepodid larva.

may be due to the effect that water turbulence associated with swimming activity would have on the sensitivity of such mechanoreceptors. The distribution of these organs in *L. salmonis* agrees with that shown by Johnson and Albright (1991a) for the same species and with that of *L. pectoralis* (Boxshall 1974). In addition to these sensillae, there are a number of pores penetrating the dorsal and ventral integument, the function of which is uncertain. These may represent the openings of integumental glands, larger internal glands or even chemosensory pits, but their function has yet to be established. Such pores are commonly found in free-living copepods (see Von Vaupel Klein 1982).

It is clear from the number and variety of sensory organs that the copepodid larva of *L. salmonis* is equipped to detect a range of stimuli in the external environment. The ability to detect a given stimulus does not, however, imply that the stimulus is important to the copepodid or that it will elicit a behavioural response. This study also therefore investigated the reaction of the copepodid larva to stimuli presented under controlled conditions. The principal reaction to such stimuli was a measurable directional response although some stimuli elicited a non-directional behavioural response.

Copepodids for use in the following experiments were obtained by hatching egg

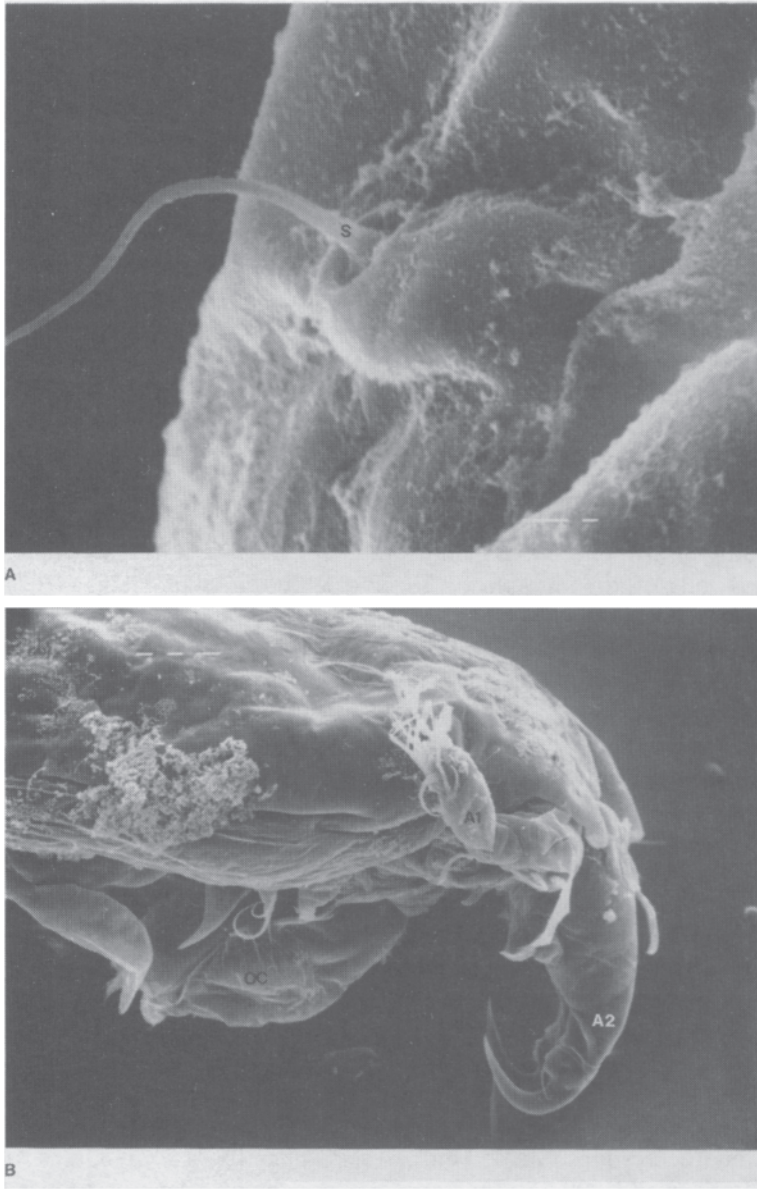


Fig. 4. (A) SEM showing single hair sensillum on body surface of copepodid. S, sensillum. Scale bar= $1\text{ }\mu\text{m}$. (B) SEM showing anterior of copepodid with prominent clawed antennae. A1, antennule; A2, antenna; OC, oral cone. Scale bar= $10\text{ }\mu\text{m}$.

strings from female *L. salmonis* and incubating them in glass dishes containing sea water at a salinity of c. 30‰, at 10–11°C. Copepodids were used between 24 and 72 h after moulting from the second nauplius stage.

BEHAVIOURAL RESPONSES

Light

Copepodid responses to light were tested in a modification of the test chamber described by Forward (1986). This consisted of a multi-chambered trough along which a horizontal beam of light was directed. The position of copepodids in this trough following exposure to different intensities and wavelengths of light was recorded after replacement of moveable partitions. Replicates of 25 copepodids were tested after exposure to different light intensities ranging from 240 to 2.4 lux for a period of 180 s. Similar tests were carried out at wavelengths between 700 nm and 400 nm.

During both the light intensity and wavelength sensitivity experiments, only five out of 1750 copepodids used were found in the compartment of the trough lying furthest away from the light source (see Bron in preparation). For this reason, positive phototaxis was chosen over negative phototaxis. This response was significantly greater in tested intensities and wavelengths than in dark controls (Mann-Whitney U -test, $p < 0.005$).

The strong positive phototaxis demonstrated for *L. salmonis* copepodids agrees with the observations of Johannessen (1978) and Wootten *et al.* (1982). Although this behaviour is also seen in aquaria with diffuse lighting, it has been suggested (Forward 1988 *inter alia*) that the demonstration of positive phototaxis in zooplankton is an artefact in many species, resulting from the experimental stimulus being a narrow, directed beam. Nevertheless, such directed movement does indicate sensitivity of the copepodid larva to the tested intensity or wavelength.

At the highest light intensities, the copepodids swim almost directly (i.e. in the test case, horizontally) towards the light source with little or no lateral component to the direction of swimming (in contrast to the more normal sink and swim behaviour seen in diffuse non-directional light). At lower intensities, swimming was more meandering, with less direct progress towards the light source during each swimming burst and a more pronounced lateral component. The regression in Fig. 5 shows that the level of response was highly positively correlated with light intensity ($p < 0.001$). A positive correlation of phototactic response with intensity has also been observed in free-living copepods such as *Acartia tonsa* Dana (Stearns and Forward 1984a) and other parasitic copepods such as *Salmincola edwardsii* Olsson (Fasten 1913).

Fig. 6 illustrates the response of copepodids to different wavelengths. The response to light at 400 nm was significantly less (STP test, $p < 0.05$) than to 550, 600 or 650 nm, and the response at 450 nm was significantly less than that at 550 nm. Between 500 nm and 700 nm, the response to light wavelengths tested was fairly uniform. Overall, the peak response was to light of 550 nm and the lowest to light at 400 nm.

The wide spectral sensitivity displayed by *L. salmonis* mirrors that of *A. tonsa* at high light intensities (Stearns and Forward 1984b). Sensitivity in the 500 nm band has been suggested to be useful for vertical migration in free-living plankton since this is the region in which maximum quanta are transmitted at twilight (Forward and Douglass 1989) and it may be that the maximal response of *L. salmonis* close to this region derives from this same migrating mechanism.

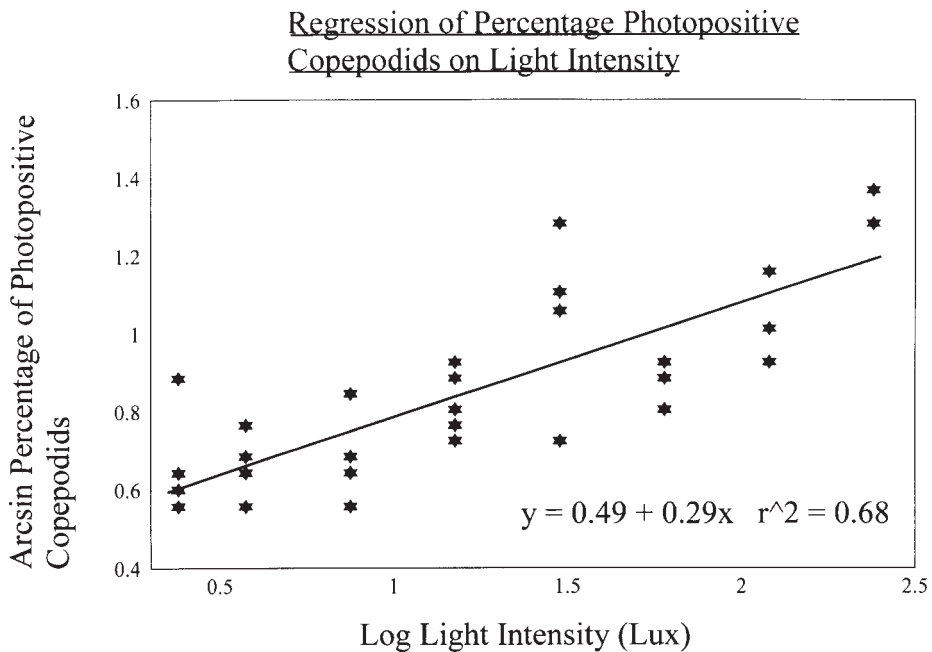


Fig. 5. Regression showing correlation between photopositive copepodid response (as arcsin percentage) and light intensity (as log).

In addition to light intensity and wavelength responses, the response of copepodids to shadows was tested using simulated static and moving hosts in a controlled aquarium environment (see Bron in preparation for details of experimental design). Potential responses to surface albedo were also tested using simulated black, grey, white and silvered surfaces.

No behavioural changes were observed in copepodids exposed to either mobile or static shadows. There was also no observed response to the various tested surfaces. This appears to indicate a lack of shadow or albedo-mediated response.

Forward (1986, 1988) and Forward and Cronin (1977) have suggested that in many free-living crustacean and fish larvae, shadow responses function in predator avoidance. It is perhaps for this reason that the copepodids of *L. salmonis* show no such response, although the authors' unpublished observations indicate that nauplii and mobile stages of this species show a pronounced response to shadow/decreasing light intensity. Such a response may be suppressed or absent in the copepodid stage as it might interfere with successful host contact. This contrasts with copepodids of *S. edwardsii*, which were reported as displaying a strong host-finding shadow response by Poulin *et al.* (1990), as were those of *Salmincola californiensis* Dana (Kabata and Cousens 1977).

Chemical

Various experimental designs were used to test the response of copepodids to chemical cues from the host (see Bron in preparation for details of experimental design). A

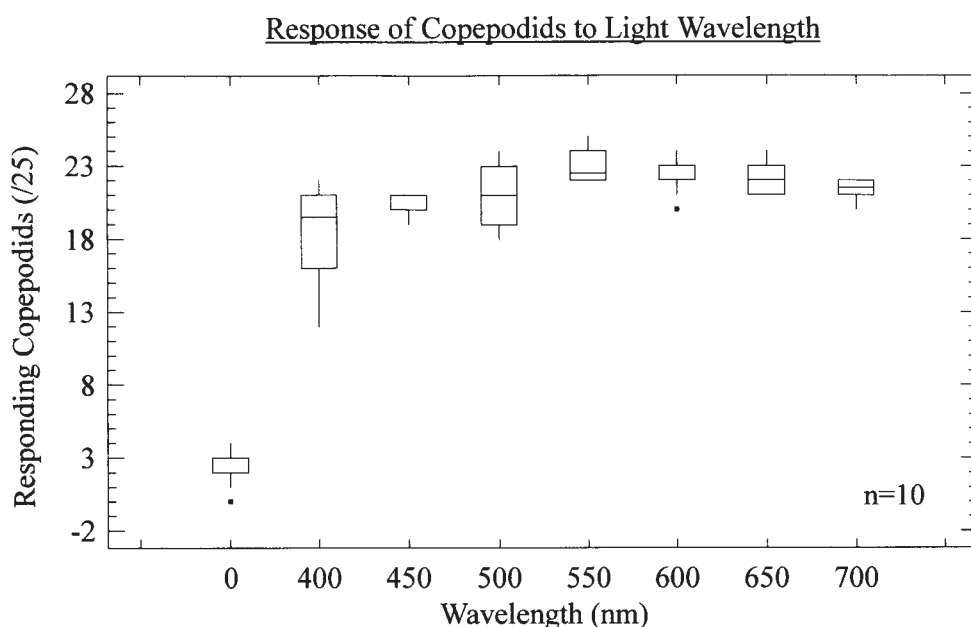


Fig. 6. Box and whisker plot of copepodid response to differing light wavelengths (0=dark control). Graph shows median, upper/lower quartiles, range and outliers.

range of host tissues and products was tested: blood, bile, faeces/urine, mucus, skin and whole host specimens.

The experiments were carried out using a variety of test chambers and systems. These fall into two basic categories: 'static' and 'flow-through'. The static system was, at its simplest, a partitioned circular chamber. The system suggested by Bartel and Davenport (1965) was also modified and used. This system required an active 'choice' to be made by the copepodids. The flow-through systems were similar in design to the static systems but utilized a flexible flow rate control which channelled water from reservoirs containing the test chemical. A system modified from Raibaut (1985) was also used to test responses to chemical stimuli presented in conjunction with different flow regimes.

No significant differences were found between the response of copepodids to the tested host components and to sea-water controls (STP test, $p < 0.05$). In the static system where the copepodids were in closer contact with the chemical components, there were significant differences between fish mucus and fish blood/faeces, though not between any of these components and the sea-water controls (Fig. 7). During infection experiments where copepodids were exposed to relatively immobile hosts, parasites were observed to approach to within c. 1 mm of hosts before swimming away. This supports the suggestion that copepodids are unable to detect hosts chemotactically even over short distances.

Crisp and Meadows (1963) have suggested that it may be unrealistic to expect small organisms to home in on a chemical source when simultaneously exposed to the vagaries of the aquatic environment. Such a problem is accentuated when the source

Response of Copepodids to Host Components (Static System 1)

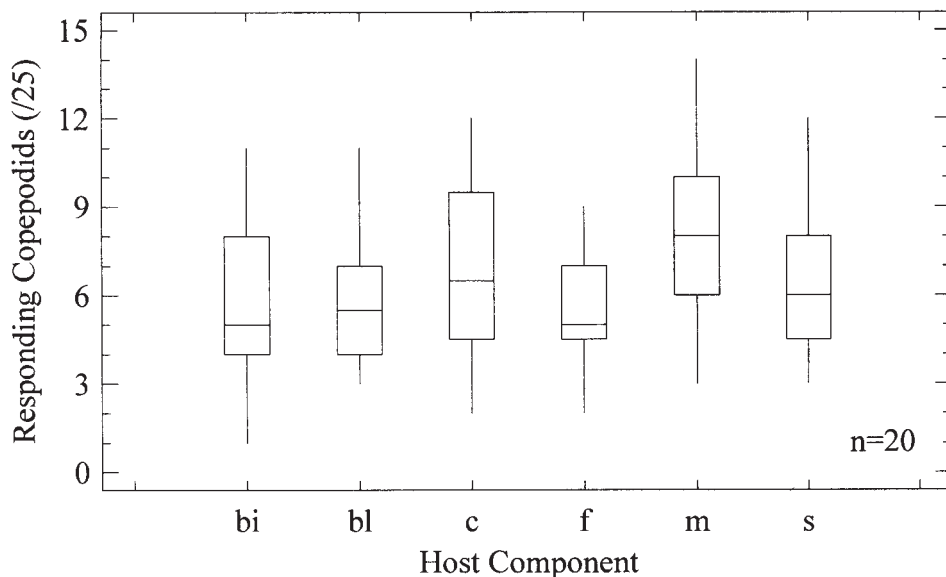


Fig. 7. Box and whisker plot of copepodid response to various host components in static test system 1. bi, bile; bl, blood; c, control (sea water); f, faeces/urine; m, mucus; s, skin. Graph shows median, upper/lower quartiles, range and outliers.

is on the move, as is the case with the salmonid host of *L. salmonis*. This may explain the apparent lack of response to host-derived chemical cues by *L. salmonis* copepodids. There is, however, evidence that some copepods parasitic on fish respond to chemical cues. Copepodids of *S. edwardsii* respond to the gills of different trout species (Fasten 1913), and *C. minimus* responds to scales and fresh/freeze-dried mucus from its host *Dicentrarchus labrax* but not *Mugil cephalus* L. (Fraile cited in Raibaut 1985). Hogans and Trudeau (1989) have also reported pronounced chemotactic behaviour in response to *Salmo salar* L. by copepodids of *C. elongatus*.

Water flow

Using a flow system similar to that noted above for measuring chemical stimuli, responses of copepodids to a laminar flow regime were tested at water input rates of 10, 20, 50, 100 and 200 ml min⁻¹ (see Bron in preparation for experimental details). No positive rheotactic responses were gained when copepodids were exposed to these various flow rates; all moved or were taken 'downstream' in the current.

In addition, copepodids were placed in a glass dish and their response to a highly directional flow resulting from the rapid expulsion of water from a pipette was observed. Copepodids demonstrated fast 'looping' or 'spiralling' behaviour composed of what appeared to be a mixture of short bursts of swimming and tight turns in the vicinity of the stimulus. Some copepodids entered the pipette during the course of such manoeuvres. Similar behaviour was also observed when copepodids were exposed to fish or other

objects passing near them. On removal of the flow stimulus, behaviour returned immediately to normal.

C. minimus tested with similar equipment showed a pronounced tendency to swim into the current flow (Raibaut 1985) but no such tendency was observed in *L. salmonis*. Despite this, *L. salmonis* mirrors *L. pectoralis* (as observed by Boxshall 1976) in its apparent response to strongly directional water currents. The 'looping' behaviour executed by the copepodid of *L. salmonis* when subjected to the jet of a pipette or the passage of a fish may be interpreted as a host contact mechanism and, indeed, appears to be the principal observable mechanism of this kind.

Pressure response

The response of copepodids to increasing and decreasing pressure was tested using a manually raised water manometer filled with sea water to simulate tidal/depth-related pressure increase (details of experimental design given in Bron in preparation).

An increase of head of water between 0 and 3 m generated little instant response in the copepodids. Copepodids showed what appeared to be a holding pattern of sinking passively and then swimming upwards in short 1–2 cm bursts. Over time (5 min+) a gradual upward vertical movement was apparent.

At 3–4 m head of water, an upward swimming trend was clearly apparent, with copepodids swimming upward in 3 cm bursts with short refractory periods between. Their response to a fall in head of water was manifested by a return to normal sink/swim behaviour with a sinking trend. A 5 m head of water gave an even more pronounced upward swimming pattern, with individual copepodids making vertical swimming bursts of up to 15 cm with short refractory periods between.

Increased and decreased pressure therefore resulted in active upward swimming and passive sinking responses respectively. Larger increases in pressure resulted in more active upward (geonegative) responses.

No evidence has been presented for a pressure response in copepodids of parasitic copepods, though it has been little studied. Johannessen (1975) suggested that a pressure response was possible in *L. salmonis* and this study appears to support such a suggestion. Such a response is common in free-swimming invertebrates (Morgan 1984) and should not be unexpected in the larvae of parasitic copepods.

Experimental infection of non-salmonid species

L. salmonis is highly specific to salmonids in the marine environment, at least in the adult/preadult stages (Kabata 1979). For the infective copepodid stage, the present study has also demonstrated that this specificity extends to the fresh-water parr and first-feeder phases of Atlantic salmon, indicating that the host recognition factor is not limited to smoltified fish. The following experiments were designed to investigate whether this host specificity is generated by the infection behaviour of the copepodid stage or whether it is a factor of differential post-settlement survival or migration.

Juvenile fish of various species were captured from the wild by a variety of methods and were exposed to a standard infection trial. The trial infection consisted of individual test fish being placed in 1 litre of water (salinity c. 30‰) held in a plastic tank and

cooled to 10°C using an external water bath. In each experiment, infection was initiated by the addition of an infective dose of 100 copepodids which were left for 1 h. After 1 h, the fish were narcotized with Benzocaine and examined for the presence of settled copepodids using a low-power (10×) dissection microscope. The infection experiments were observed throughout and notes taken on copepodid behaviour. In the course of these experiments, the following fish species were tested:

Cod, *Gadus morhua* L.

Saithe, *Pollachius virens* (L.)

Three-spined stickleback, *Gasterosteus aculeatus (armatus)* L.

Butterfish, *Pholis gunnellus* (L.)

Lumpsucker, *Cyclopterus lumpus* L.

Flounder, *Platichthys flesus* L.

Eel, *Anguilla anguilla* (L.) (elvers)

Copepodids placed with the above species displayed ‘burst-swimming’ and ‘looping’ behaviour when stimulated by the fish swimming nearby but did not attach to or settle on them despite occasional contact. In addition to observations made *in vitro*, saithe (*P. virens*) and various wrasse species (see Bron and Treasurer 1992) captured from cages containing infected salmon showed no settled larval *L. salmonis*.

Despite reports that *P. virens* may act as a host for *L. salmonis* (Bruno and Stone 1990), the present work suggests that copepodid larvae do not infect this or any of the non-salmonid species examined. This is supported by studies of parasitic infections of *P. virens* taken from Scottish (Harvey 1988) and Norwegian salmon farms (Nylund personal communication). Host specificity of *L. salmonis* appears, therefore, to be maintained by active selective behaviour of the copepodid larvae.

The basis of the active selection of hosts by *L. salmonis* is likely to stem from close-range or contact surface chemical recognition mediated via high-threshold chemoreceptors. Such specificity is common in parasitic copepods and has been noted in particular for copepodids of *L. pectoralis* exposed to different hosts (Boxshall 1976) and for copepodids of *S. edwardsii* exposed to gills excised from a number of trout species (Fasten 1913). Boxshall (1976) expressed the opinion that copepodid attachment was dependent on the nature of host chemical factors adsorbed on or diffusing from the host surface.

It has been suggested (Chapter 9) that one of the setation elements on the antennule of adult *C. elongatus* may function as a high-threshold contact chemoreceptor. The two chemosensory aesthetascs on the antennules of *L. salmonis* copepodids may well be a site of contact chemoreception and hence, potentially, host recognition.

Settlement responses

Initial attachment to the host is obtained by ‘grappling’ with the hooked antennae (Figs 4B and 8A). Once attached, the maxillae were used to help lever the antennae deeper into the epidermis, this being responsible for the ‘prodding’ activity that often accompanies attachment. Eventually, the leading edge of the cephalic shield was also driven into the epidermis (Fig. 8B). During this activity, the antennules are in close proximity to or in contact with the host surface and are thus well positioned for high-threshold contact chemoreception (Fig. 8A,B).

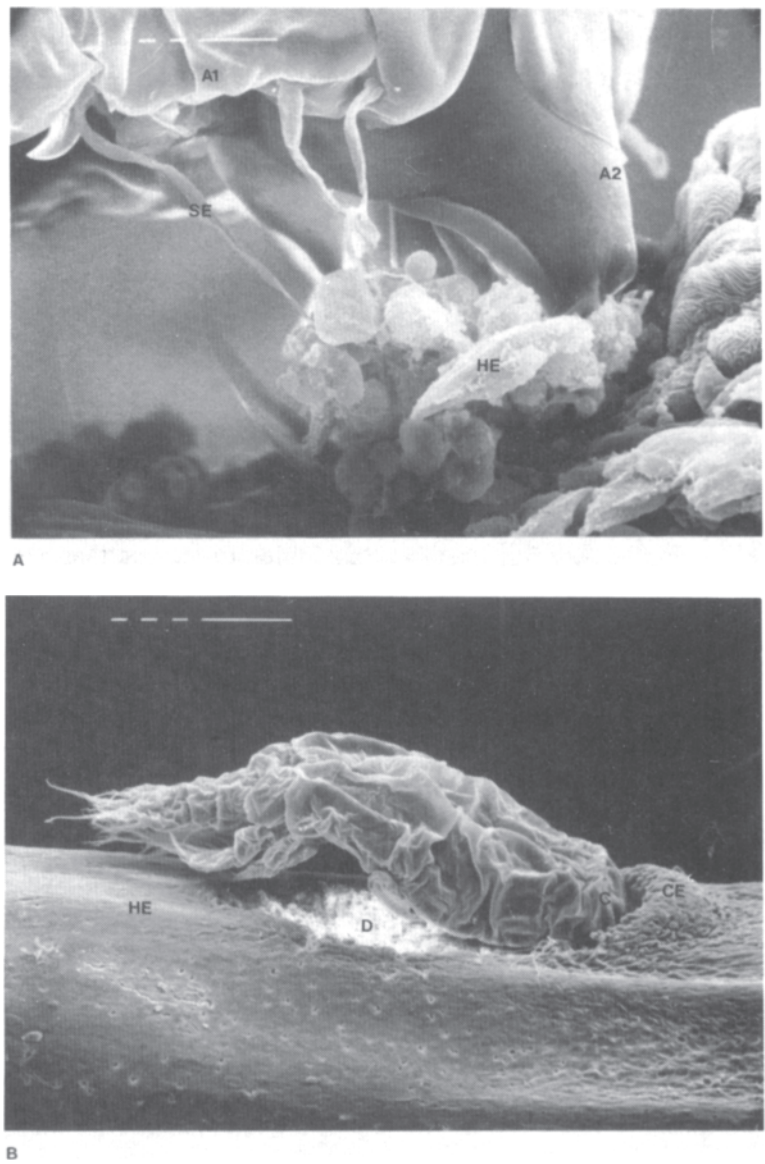


Fig. 8. (A) SEM showing antennae embedded in host epidermis and proximity of the antennules to the host. A1, antennule; SE, sensory element of antennule; A2, antennae; HE, host epidermis. Scale bar=10 μ m. (B) SEM of copepodid embedded in host epidermis illustrating compression of host epidermis anterior to the cephalic shield and intimate contact between the antennules and the host surface. C, cephalic shield; D, damage to host epidermis; CE, compressed host epidermis in front of copepodid; HE, host epidermis. Scale bar=100 μ m.

Kabata (1979, 1981) also observed that the antennae are often the principal means of initial attachment for infective copepodid stages of parasitic copepods. The prodding behaviour of *L. salmonis* has also been noted in *L. dissimulatus* (Lewis 1963) and *Lernaeocera branchialis* (L.) (Kabata 1981).

In naturally and experimentally infected fish, significantly more copepodids were found on the fins than on the body surface, although experimentally infected fish also showed large numbers of copepodids on the gills (Bron *et al.* 1991, Johnson and Albright 1991b).

The predominance of settlement on host fins seen in *L. salmonis* has also been noted in *Salmincola salmoneus* L., which was found to settle disproportionately on the pectoral fins and around the opercula (Kabata and Cousens 1977). A similar distribution is found in natural infections of *L. pectoralis* (Boxshall 1976). In both these latter instances, the distribution was explained in terms of movement of copepodids towards pronounced sources of water movement. Anstensrud and Schram (1988), observing a similar predominance of settled copepodids of *Lernaeenicus sprattae* (Sowerby) on host fins, attributed the distribution to migration to the fins post-settlement by copepodids homing on water currents. We suggest that the distribution pattern observed in *L. salmonis* is determined more by the ability of copepodids to attach initially and subsequently remain attached in a given area than by selection of particular areas. The suitability of a given area for settlement may therefore be dependent upon the nature of the epidermis (which varies over the host body surface) and the exposure to water currents at different sites. It is suggested that the large numbers of copepodids found on the gills of experimentally infected fish are attributable to an enhancement of settling opportunity brought about by reduced swimming speeds of fish kept in tanks.

CONCLUSIONS

The copepodid is well equipped with an array of sensors which allow it to detect a range of stimuli. Experiments in the laboratory indicate the nature of the behavioural responses to perceived stimuli but may (particularly in the case of unidirectional light experiments) give artefactual results which make extrapolation from laboratory to nature problematic. A tentative scheme of events may, however, be drawn from the reactions and may be used as a testable working hypothesis.

Copepodid larvae move towards a light source and into areas of low pressure. If this is true in the natural environment, this would tend to bring them into the surface waters—a location which might allow them better chances of host contact (at least in coastal and estuarine waters). The lack of a response to host-derived chemical components or to whole immobile fish suggests that copepodids cannot detect hosts at a distance by tracing a chemical plume back to its source. The principal method of host detection appears to be through water flow or mechanical vibration generated by the host. The detection of a moving object in its vicinity elicits a combination of 'burst-swimming' and 'looping' behaviour from the copepodid which we have interpreted as an attempt to 'grapple' the host with the antennae (this response being non host-specific). On successful grappling of the putative host the identification of the host is probably via high-threshold chemoreceptors on the antennules. This chemical determination will prompt settlement on suitable hosts, or alternatively, if the substrate is unsuitable, will cause the copepodid to re-enter the water column.

The site of settlement is probably mediated by the speed of local current flow and the nature of the epidermis in a given area, such that copepodids in more exposed positions become detached.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge funding from Marine Harvest International which enabled this study to be carried out. Thanks are also due to Jim Treasurer, David Morley, Alison Arbuthnott, Marianne Pearson, Amanda Maclean, Alison Dawson and all staff working at Lochailort. Thanks also to the managers and staff of the farms from which we collected lice and without whose cooperation this study could not have been carried out. Many thanks to the Zoological Society of London for allowing the reproduction of Fig. 8B.

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11

Speciation and specificity in parasitic copepods: caligids of the genus *Lepeophtheirus*, parasites of flatfish in the Mediterranean

T.De Meeüs, A.Raibaut and F.Renaud

ABSTRACT

Two species of *Lepeophtheirus* are studied: *L. thompsoni*, a specific parasite of turbot (a marine scophthalmid); and *L. europaensis*, which exploits both Mediterranean brill (a marine scophthalmid) and flounder (a pleuronectid inhabiting lagoons). Studies of survival when exposed to low and high salinities, dispersion patterns and female fecundity confirm the different degree of specialization between the two species. The exploitation of two different hosts (brill and flounder) by *L. europaensis* seems to have led the parasite to develop some heterogeneity within the Mediterranean population. This may represent the starting point of a speciation event. The phenotypic plasticity and genetic variability observed in *L. europaensis*, coupled with the dispersion patterns observed on its new Mediterranean host (flounder), suggest that such characteristics should be taken into account when studying fish-farming pests such as *L. salmonis*.

INTRODUCTION

Host specificity is the product of a more or less high degree of specialization on the part of the parasite with regard to its host. The different ecological, ethological, physiological and genetic components of the host collectively represent the main ecological factors defining the parasite's niche. Hosts thus represent the 'habitat/resource system' of the parasite (Renaud 1992). This is why host-parasite systems offer useful biological models in the study of evolutionary biology.

Sea lice, with their simple life cycle, permanent ectoparasitism and economic importance, are ideal subjects for experimental studies of their population biology.

In the Gulf of Lions, *Lepeophtheirus thompsoni* Baird, 1850 and *Lepeophtheirus europaensis* Zeddam, Berrebi, Renaud, Raibaut and Gabrion, 1988 utilize three different host species of flatfish (Heterosomata). *L. thompsoni* is specific to turbot (*Psetta maxima*

L. 1758), whereas *L. europaensis* parasitizes brill (*Scophthalmus rhombus* L. 1758) and flounder (*Platichthys flesus* L. 1758). Turbot and brill are marine scophthalmids, while flounder is a pleuronectid inhabiting lagoons (brackish waters). *L. europaensis* exploits two fairly different hosts (brill and flounder) but appears to be unable to infest turbot in the wild (Zeddiam *et al.* 1988). It has, nevertheless, been shown experimentally that these two parasite species can produce viable and fertile hybrids on the same host (turbot) (De Meeüs *et al.* 1990). In order to understand the observations made by De Meeüs *et al.* (1990), we propose in this chapter to investigate the different infective capabilities of those sea lice species with different host species and different salinities. The dispersion patterns found in the wild and female fecundities are also studied. Finally, the evolutionary biology of such organisms is discussed in the light of the different patterns of host use encountered in the geographical distribution of these parasites.

MATERIALS AND METHODS

Experimental hosts

Parasite-free turbot (10 cm long) were bought from fish farms. The different flatfish species used for experimental infestations were caught along the Languedoc coast (Mediterranean, France) and in lagoons (flounders). Brill (20–25 cm long) were caught at sea by craft fishermen, while smaller brill (5.6–7 cm long) and turbot (3.5–17 cm long) were caught inshore. Flounder (7–20 cm long) were caught in lagoons by craft fishermen. In the laboratory, these fish, anaesthetized with 3-aminobenzoic acid ethyl ester (Sigma A 5040), were examined under a binocular microscope and naturally infested fish (rare for the size range used) were rejected.

Ovigerous female copepods, infective stages and experimental infestations

Ovigerous female copepods were obtained from fishing ports (Sète and Grau-du-Roi, France). Flounder copepods were collected on hosts caught in lagoons (Etang d'Ingril, Etang de Mauguio, Etang du Ponant) by craft fishermen. *L. thompsoni* females are haemophagous and found attached to the gills, while *L. europaensis* females are mucophagous and found on the wall of the gill chamber and on the inner surface of the operculum (Zeddiam *et al.* 1988). Collected eggs were incubated at 15°C in filtered sea water. After hatching and development of the larvae, the infective stages (copepodids), were isolated, counted and placed in a 50-litre tank containing fish to be infested. An average of 100 copepodids per infestation was used.

Observation of the anaesthetized fish commenced 3 days after infestation. Attached parasites were counted using a binocular microscope.

Development and survival at different salinities

Eggs and free stages of copepods were subjected to different salinities, ranging from 20‰ to 45‰, which was measured with a manual refractometer (Atago, S/Mill). Only the youngest (without pigment) and complete (two egg sacs) clutches were tested. Each egg sac was placed in a sterile Petri dish filled with water of a given salinity at increments of 5‰ within the range 20–45‰. For each clutch, one of the

Table 1. Successful fixation (percentages) obtained during single fish experiments

		CT	CB	CF
Turbot	<i>n</i>	15	8	8
	Mean	41.3	20.5	11.87
	s^2	534	99.7	97.3
	s_{mean}	6	3.5	3.5
Brill	<i>n</i>	9	5	3
	Mean	1.8	36.5	35.6
	s^2	1.1	338.9	338.3
	s_{mean}	0.35	8.2	10.6
Flounder	<i>n</i>	4	5	5
	Mean	0	44	52.4
	s^2	—	721.8	412
	s_{mean}	—	12	9.1

CT, turbot copepods; CB, brill copepods; CF, flounder copepods;
 s^2 , variance; s_{mean} , standard error of the mean.

pair of egg sacs was placed in standard sea water (35‰). This control allowed rejection of the unfertile clutches from the final results. Petri dishes were then placed in a temperature-controlled incubator (15°C), with a 12 h light/dark photoperiod. For each Petri dish, the proportion of copepodids (infective stage) obtained was calculated with respect to the initial number of eggs. Results concerning turbot copepods represent unpublished data. Other results come from De Meeüs *et al.* (1992). For this study, flounder copepods were obtained from fish caught in a single lagoon (Etang d'Ingril).

RESULTS

Fixation of different copepods on the three hosts

Only one fish was exposed to infestation throughout these experiments. All possible combinations (infestation of turbot, brill or flounder with parasites from turbot, brill or flounder) were made and the fixation rates (percentages) compared by non-parametric statistics (Wilcoxon, Mann-Whitney *U*-test). The influence of host size on fixation rate was tested using Spearman's coefficient of rank correlation *S*. The use of such statistics is justified by the heterogeneity of variances in these experiments. Mean fixation rates, variances and standard errors are presented in Table 1.

Fig. 1 shows the relationship between host size and fixation rates of parasites for each type of experiment where a sufficiently wide range of host sizes was available. As far as the host range used in this study is concerned, fixation rate seems to be independent of host size, according to Spearman's coefficient computed for those experiments (Fig. 1). Significance levels obtained for paired comparisons are given in Table 2.

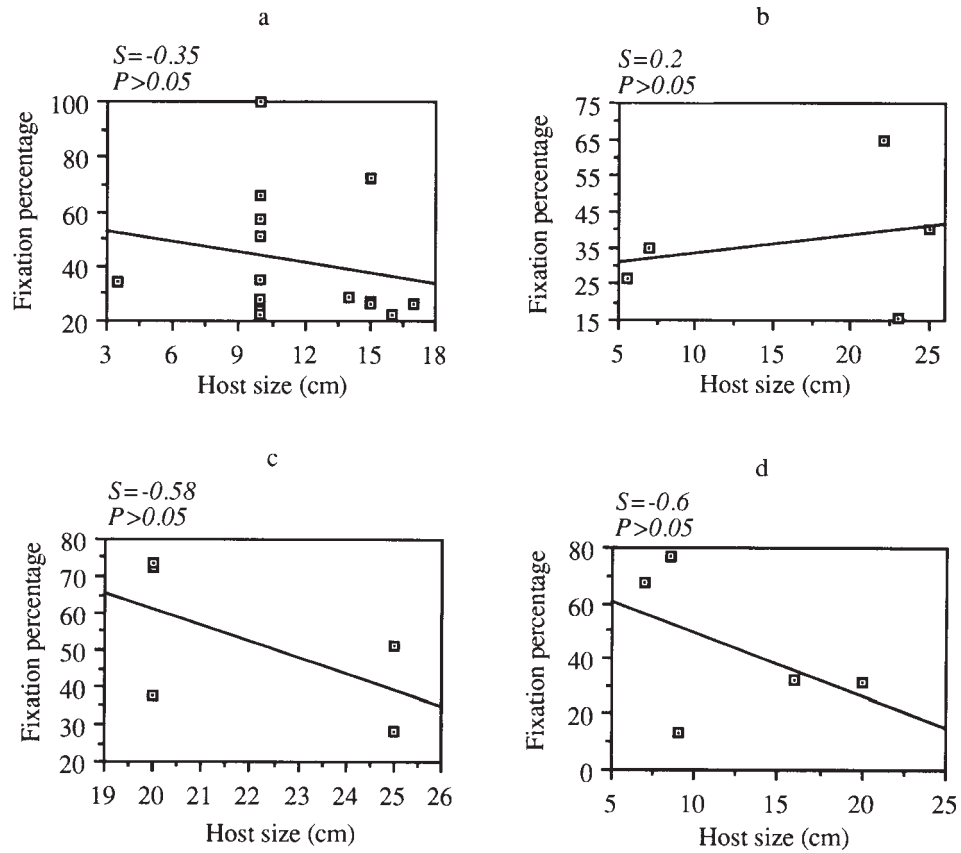


Fig. 1. Relationships between host size and fixation rates of different copepods. (a) Infestation of turbot by *L. thompsoni*. (b) Infestation of brill by *L. europaensis* from brill. (c) Infestation of flounder by *L. europaensis* from flounder. (d) Infestation of flounder by *L. europaensis* from brill. S, Spearman coefficient (probabilities are given for one-tailed tests).

Tables 1 and 2 reveal that *L. thompsoni* (turbot parasite) is a specialist which colonizes brill and flounder with much lower rates than its natural host. In comparison, *L. europaensis* settles on a non-natural host at a higher rate than *L. thompsoni*. However, *L. europaensis* infested turbot with lower rates than *L. thompsoni*. Fixation rates appear identical for both parasite species when infesting their natural hosts (*L. thompsoni* on turbot, *L. europaensis* on brill or flounder). Heterogeneity within the species *L. europaensis* was found. Flounder copepods showed distinctly lower fixation rates on turbot compared to brill and flounder, whereas brill parasites settle equally well on each of the three host species.

Development of eggs and survival of free-living stages in different salinities

The results of this study are given in Table 3. The results of using χ^2 tests (1 d.f.) can be summarized as follows:

Table 2. Results of Wilcoxon Mann–Whitney *U*-tests for single fish experiment comparisons

(a)

	CT/T	CT/B	CB/B
CT/B	$p < 0.002$	—	—
CB/B	$p > 0.2$	$p < 0.002$	—
CB/T	$p < 0.002$	$p < 0.002$	$p > 0.1$

(b)

	CT/T	CT/F	CF/F
CT/F	$p < 0.002$	—	—
CF/F	$p > 0.1$	$p = 0.02$	—
CF/T	$p < 0.002$	$p < 0.001$	$p = 0.01$

(c)

	CF/F	CF/B	CB/B
CF/B	$p > 0.2$	—	—
CB/B	$p > 0.2$	$p > 0.2$	—
CB/F	$p > 0.2$	$p > 0.2$	$p > 0.2$

Example: CT/B indicates infestation of brill (B) with turbot copepod (CT).

- At 20%, only those copepods from flounder can reach the infective stage (copepodid).
- At 25%, *L. europaensis* from flounder develop better than those from brill ($p < 0.001$), which in turn develop better than turbot copepods ($p < 0.001$).
- At 30%, the difference is significant ($p < 0.05$) for brill copepods, which develop better than those from flounder and than those from turbot ($p < 0.001$).
- At 35%, the flounder copepods develop better than either brill or turbot copepods ($p < 0.001$), which do not significantly differ from each other ($p > 0.05$).
- At 45%, flounder copepods develop much better than brill copepods ($p < 0.001$), which in turn develop much better than turbot copepods ($p < 0.001$).

Population dynamics

In the Mediterranean, the flatfish copepods do not display a seasonal cycle of abundance like that described for *L. pectoralis* (Müller, 1776) in the North Sea by Boxshall (1974a).

Since males are not regularly sampled, this section deals only with gravid females. The results obtained fall within the range found for *L. pectoralis* on North Sea plaice (*Pleuronectes platessa* L. 1758) (Boxshall 1974b). *L. thompsoni* (turbot copepod) is more aggregated on its host population (mean abundance=5.5, prevalence=0.84, $k=0.98$, $n=221$ turbot examined) than *L. europaensis* on brill (mean abundance=3.6,

Table 3. Development from egg to infective (copepodid) stage in different salinities

Salinities (‰)		Number of clutches	Initial number of eggs	Final number of copepodids	Percentage copepodids obtained
20	CT	16	1037	0	0
	CB	21	1147	0	0
	CF	15	928	43	4.6
25	CT	20	1487	713	47.9
	CB	22	1196	777	65
	CF	22	1421	1028	72.3
30	CT	20	1260	902	71.6
	CB	29	1438	1213	84.4
	CF	15	1022	775	75.8
35	CT	65	5581	4190	75.1
	CB	76	4417	3265	73.9
	CF	45	2957	2555	86.4
45	CT	17	901	9	1
	CB	25	1413	209	14.8
	CF	7	404	148	36.6

CT, turbot copepods; CB, brill copepods; CF, flounder copepods.

prevalence=0.89, $k=2.46$, $n=261$ brill examined). However, *L. europaensis* on flounder are the most aggregated (mean abundance=12.5, prevalence=0.72, $k=0.43$, $n=75$ flounder examined). It appears that *L. europaensis* females encounter much higher densities within the gill chambers of flounder than within brill gill chambers.

A detailed study of clutch sizes is presented in Chapter 5. *L. thompsoni* females lay larger clutches (66 eggs per sac, variance=398, $n=247$ clutches) than *L. europaensis* from flounder (61 eggs per sac, variance=407, $n=335$ clutches). On brill, despite lower female densities in the gill chambers, clutches appear smaller (52 eggs per sac, variance=287, $n=251$ clutches). All differences proved to be significant (one-tailed Kolmogorov–Smirnov test, 2 d.f., $p<0.02$).

DISCUSSION

L. thompsoni displays a strong preference for turbot as its host even in experimental conditions. In the wild, the haematophagous *L. thompsoni* females are found in relatively larger numbers on their hosts and have larger clutches than the mucophagous *L. europaensis* females on brill. Furthermore, the eggs and free larvae of *L. thompsoni* appear less tolerant to salinity variations than those of *L. europaensis*. *L. europaensis*

is thus a generalist which displays a broader host specificity in the wild where both brill (marine scophthalmid) and flounder (pleuronectid inhabiting lagoons) are exploited in the Mediterranean. Moreover, this species is also capable of infesting all three host species under the experimental conditions.

Turbot, brill and flounder can be found outside the Mediterranean (i.e. in the Atlantic and North Sea). In those areas turbot and brill are inhabited by the same sea lice fauna as in the Mediterranean (Zeddarn *et al.* 1988). In contrast, flounders are exploited by two other copepod species: *L. pectoralis*, the adult female of which is found on the inner surfaces of the pectoral and pelvic fins; and *Acanthochondria depressa* (Scott, 1929) (a member of the family Chondracanthidae), inhabiting the gill chamber (Boxshall 1974c). These parasites are absent from Mediterranean flounder. The three host species studied, like most of the flatfish, are of northern origin (Quignard 1972) and colonized the Mediterranean during the last glaciations. During this migration into the Mediterranean, flounders must have lost their original ectoparasitic fauna and, after becoming established, must have acquired *L. europaensis* (a generalist species) from brill. The plasticity of *L. europaensis* allows it to exploit different environments, and has resulted in the heterogeneity exhibited within populations of this species. As revealed in the present study, *L. europaensis* females are found more often, at higher densities, and with larger clutch sizes, on flounder than on brill. Eggs and free-living stages tolerate a wider range of salinities if they are from flounder. This last point clearly indicates an adaptive heterogeneity within *L. europaensis* populations. Moreover, flounder and brill occupy different environments and only encounter each other during the winter when the flounder undertakes its spawning migrations (Zeddarn *et al.* 1988). Such a phenomenon must considerably reduce the chance of exchange of copepods between the two fish species. A reduced gene flow can contribute considerably to the stability of adaptive polymorphisms (Moran 1959, Eyland 1971) and may even lead the population further towards genetic isolation (Balkau and Feldman 1973). We may consider that, in the Mediterranean, *L. europaensis* populations exploiting brill and flounder may be in such a dynamic situation and that speciation is currently in progress within this parasite taxon.

The utilization of flounder by *L. europaensis* must be a recent phenomenon and has rapidly resulted in the development of heterogeneities within this parasite species. These heterogeneities are observed in aspects of the population dynamics (dispersion pattern, fecundity) and population genetics (adaptive traits). Sea lice are major pathogens for farmed salmon (Pike 1989). Within fish farms, such parasites must be faced with conditions fairly different from the wild. The results presented in this chapter suggest that long-term control programmes should profitably take into account the plasticity (*sensu lato*) that such parasites display.

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Part Ie Epidemiology

12

The reproductive output of *Lepeophtheirus salmonis* adult females in relation to seasonal variability of temperature and photoperiod

G. Ritchie, A.J.Mordue (Luntz), A.W.Pike and G.H.Rae

ABSTRACT

The reproductive output of *Lepeophtheirus salmonis* adult females, collected from two salmon farms on the west coast of Scotland, has been investigated over a period of 18 months. Distinct seasonal variation exists between winter and summer generations at the sites examined. Adult females from winter generations are significantly larger, produce longer egg strings and a greater number of smaller eggs than females from summer generations. Temperature has a greater effect on female size, egg string length and number of eggs than photoperiod. Egg size is affected more significantly by photoperiod. Work is currently being undertaken to investigate the level of reproductive investment throughout the year in terms of both fertility and fecundity.

INTRODUCTION

Continual infestation of *Lepeophtheirus salmonis* Krøyer, 1838) on Atlantic salmon (*Salmo salar* L. 1758), within a fish farm, is dependent on a precise and successful mating strategy involving mate location, copulation, insemination and brood production.

Very few studies on sea lice have included information on reproductive biology and strategies (Wilson 1905, Scott and Scott 1913, Anstensrud 1990a,b). Anstensrud (1990a), in a most comprehensive and meticulous study on *Lepeophtheirus pectoralis* (Müller, 1776), made observations of mating behaviour and reproductive strategies within the Caligidae. Adult male *L. pectoralis* take up precopulatory positions with preadult females. Copulation proceeds after the female's final moult when the male attaches paired spermatophores to the female's genital complex (Anstensrud 1990a,b, Kabata 1981). The reproductive behaviour and strategies described for *L. pectoralis* also

occur in *L. salmonis* (Ritchie, unpublished observations). After insemination the female releases eggs from her genital complex in paired egg strings which may reach over 20 mm in length.

Currently there is a paucity of information on the reproductive output of sea lice. The number of eggs produced within an egg string has been shown to vary considerably from less than 100 up to 700 (Wootten *et al.* 1982), confirming that there are wide variations in reproductive output. Variations in the quality of eggs have never been examined. Figures for the number of broods produced by a single female have not been recorded but there are believed to be at least six (Ritchie, unpublished observations).

Many studies, on numerous crustacean groups, have shown that exogenous factors (temperature, photoperiod, salinity and food availability) interact with endogenous factors, singly or collectively, to generate alterations in the fecundity of populations (Nelson 1980, Sastry 1983, Williams 1985, Johnston and Dykeman 1987). Variations in the levels of lice infection between seasons have been well documented (Wootten *et al.* 1982, Tully 1989, Hogans and Trudeau 1989), and these differences suggest alterations in the reproductive strategy and output as a consequence of fluctuating environmental parameters which interact with each other and with the population. Tully (1989) observed the mean fecundity (number of eggs per string) of *L. salmonis* to be significantly higher in January than August. Alterations in fecundity could possibly result in changes in the level of infestation within a fish farm.

The objective of this study was to examine the reproductive output of *L. salmonis* at two salmon farms in terms of quantity and quality of eggs related particularly to seasonal variation of temperature and photoperiod.

MATERIALS AND METHODS

Monthly samples of *L. salmonis* were collected at two salmon farms on the west coast of Scotland: farm 1 (April to August 1991) and farm 2 (October 1991 to August 1992). Adult females were carefully removed from salmon (3–6 kg) prior to their harvesting and immediately placed in 10% buffered formalin. The mean monthly water temperature was recorded at the farm site and the photoperiod was obtained from published sources.

Lice were sorted into the various categories shown in Fig. 1. Virgin females were not inseminated and had a reduced genital complex. Non-gravid females lacked egg strings. Aged females were also non-gravid and larger than females from other groups. Ovigerous females were subdivided into those with viable and non-viable egg strings. Non-viable strings had no definitely formed eggs and the few eggs present were completely disorganized, while eggs from viable strings were highly organized in sequence. Using a calibrated microscope, at 40 $\times$, each female with viable egg strings was measured for egg string length (right only), number of viable eggs per string (right only), egg length ($\times 100$) and cephalothorax length. Egg strings were measured from the point of their attachment to the genital complex to the posterior tip of the string. Brood size is defined here as the number of viable eggs from one string per female. Eggs which appeared discoloured or disorganized were not counted. Egg length was determined by measuring the distance between the divisions of eight to ten eggs from

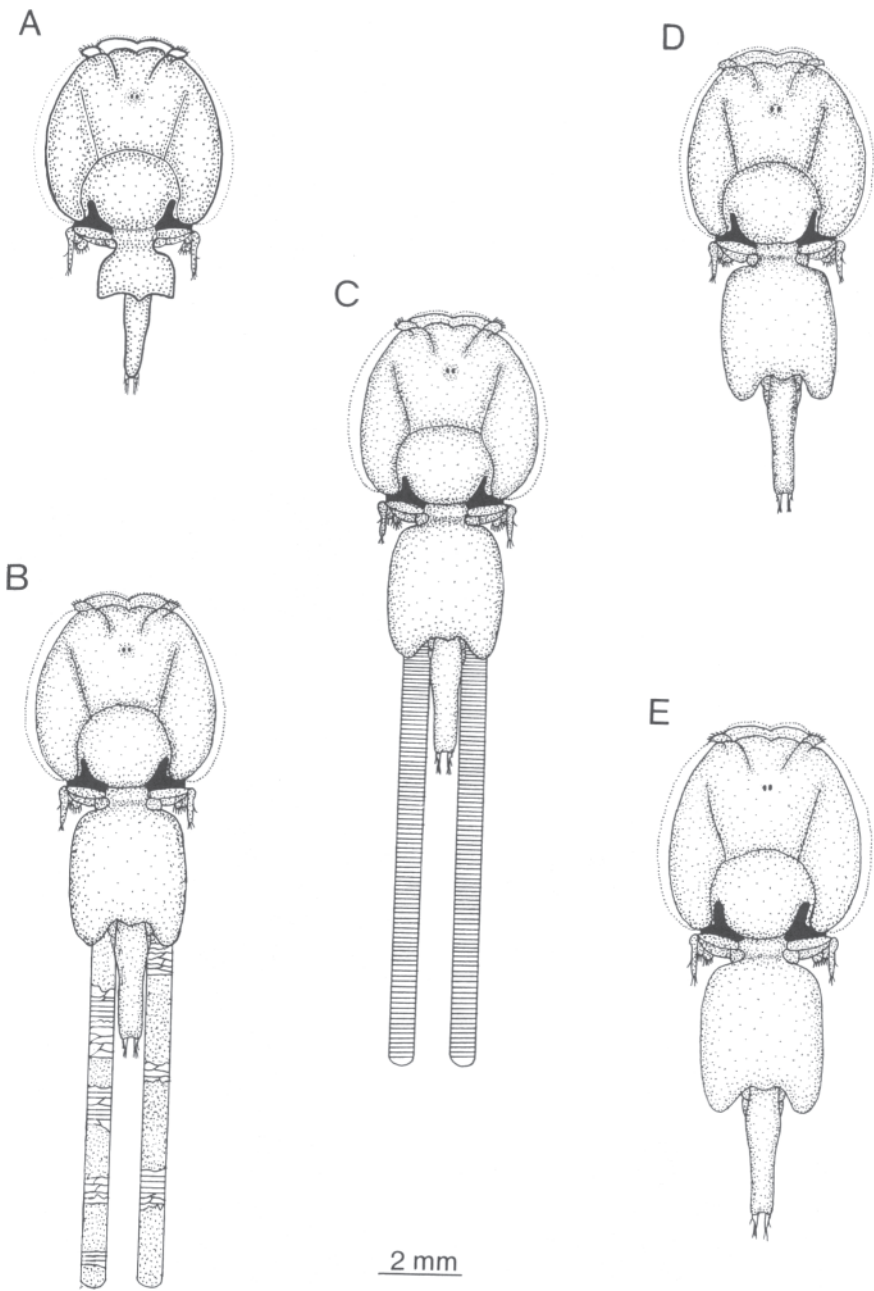


Fig. 1. Reproductive categories of adult female *L. salmonis*: (A) virgin; (B) non-viable egg strings; (C) viable egg strings; (D) non-gravid; (E) aged.

various locations in the egg string. The cephalothorax length was measured from the joint of the cephalothorax to the anterior tip of the frontal organ.

Any alteration of these reproductive parameters was compared with fluctuations in mean monthly water temperature and photoperiod over the sample period. Significant differences between mean monthly measurements were investigated by analysis of variance (ANOVA) followed by Tukey's multiple comparison test to show whether any significant seasonal variations occur. Stepwise regression analysis was used to determine which environmental parameter has the greatest influence on each of the reproductive parameters. Changes in environmental parameters will affect reproductive output several weeks later and not immediately. These exogenous factors are believed to combine with endogenous factors to alter reproductive investment before changing reproductive output. As a consequence, monthly measurements were tested against the temperature and photoperiod from the previous month.

RESULTS

Each monthly sample was collected approximately 4 weeks after fish had been treated with Aquagard (Ciba Geigy). In June and July (1991) treatments were less than 2 weeks prior to sampling. It was not possible to collect a sample in December 1991 at farm 2. Over 40 adult females with viable egg strings were examined each month, with the exception of June and July 1991 ($n=23$ and 22 , respectively). Since the samples for April and May (1992), at farm 2, were only 14 days apart, and louse treatment was carried out 7 days prior to sampling in May, the reproductive parameters for these two sample months were averaged.

Egg string length (Fig. 2)

Egg strings were examined at different stages of maturity and it was found that length did not increase progressively with the advancement of egg development in the sacs. Therefore, egg strings containing eggs at all stages of maturity were used for measurement.

Fig. 2 shows that the mean egg string length (MESL), in both populations, appears to be seasonally variable (ANOVA, $p<0.001$ for farm 2, $p<0.05$ for farm 1). Neither temperature nor photoperiod was associated with the changes observed in the farm 1 population.

MESL at farm 2 increased progressively from October to March and then gradually decreased to August. The MESL in March (18.29 mm) was significantly larger compared to other sample months, except February (Tukey's test, $p<0.01$). The MESL in February was significantly larger than in October and November ($p<0.05$). As in the farm 1 population, there was only slight variation in length between April and August. Over the total sample period temperature was negatively correlated with egg string length, accounting for 73.4% of the variability ($r^2=0.73$, $p<0.01$), while photoperiod had no significant effect (Table 1).

Brood size (Fig. 3)

Both populations produced similar numbers of eggs per string from April to August. Brood size in both populations changed significantly over the sample period,

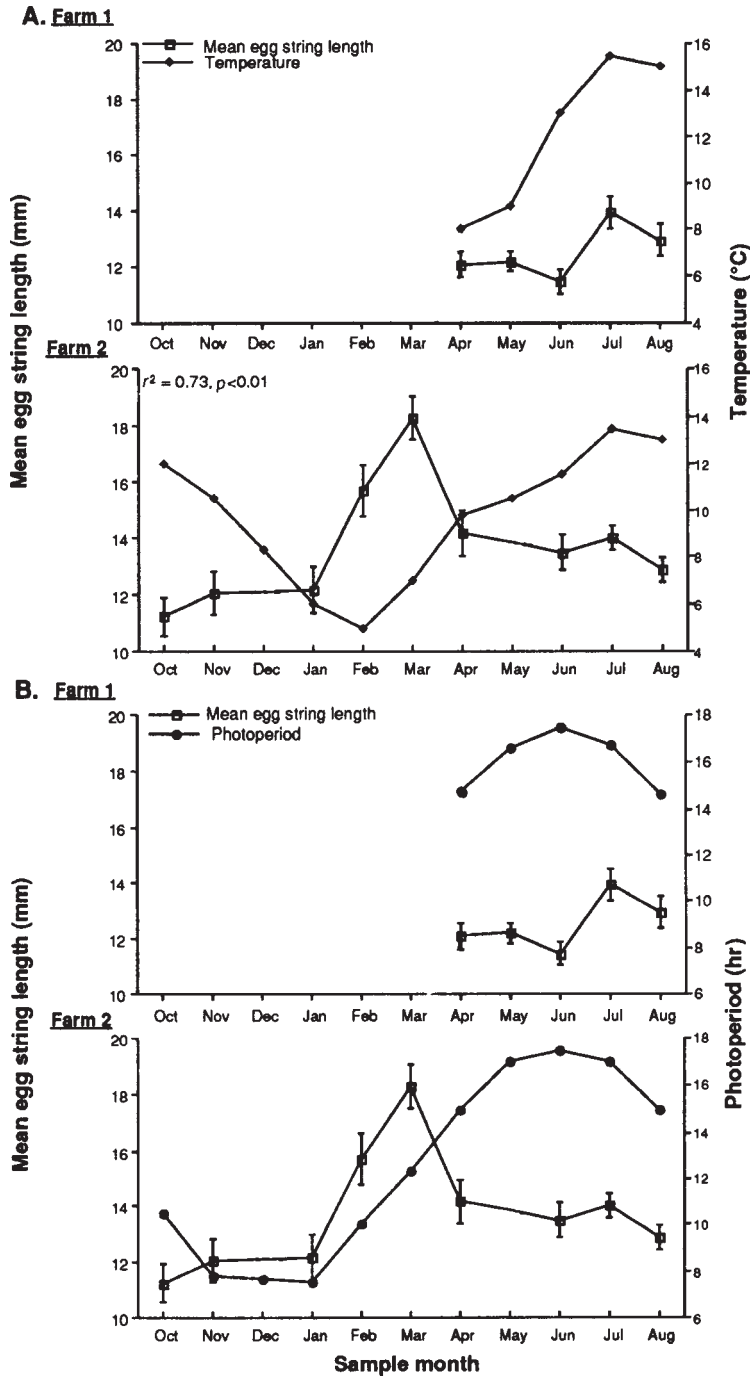


Fig. 2. Changes in the mean viable egg string length (mm) of all adult females per month ($n > 22$) ($\pm$ SE) from two populations of *L. salmonis* (farm 1 and farm 2) with (A) temperature and (B) photoperiod.

Table 1. Values of r^2 and significance from step wise regression of environmental parameters on the reproductive parameters of *L. salmonis* at both farms. MNE, mean number egg; MCL, mean cephalothorax length; MESL, mean egg string length; MEL, mean egg length (n.s., not significant)

Environmental parameter	Farm 1		Farm 2			
	MNE	MCL	MESL	MNE	MEL	MCL
Temperature	0.59	0.81	0.73	0.77	0.22	0.45
	n.s.	$p < 0.05$	$p < 0.01$	$p < 0.001$	n.s.	$p < 0.05$
Photoperiod	0.79	0.84	0.035	0.036	0.68	0.001
	$p < 0.05$	$p < 0.05$	n.s.	n.s.	$p < 0.05$	n.s.

suggesting that the number of eggs produced is seasonally variable (ANOVA, $p < 0.001$ for both populations). The mean number of eggs per string (MNE), in the farm 1 population, fell significantly from 183 to 142 (April to August, $p < 0.01$). Stepwise regression analysis showed that photoperiod was negatively correlated with MNE, accounting for 79% of the variability ($r^2 = 0.79$, $p < 0.05$), temperature having no significant effect on brood size at farm 1 (Table 1). Although MESL increased significantly from June to July (Fig. 2) at this farm there was no significant increase in the number of eggs (Fig. 3). More disorganized eggs and empty egg cases were observed in strings of viable females collected in July. By August, egg strings appeared normal again. This unusual finding could be a result of the recent Aquagard treatment prior to sampling.

At farm 2, variation in MNE was positively correlated with the change in MESL ($r^2 = 0.96$, $p < 0.001$). MNE increased significantly from 147 to 246 (October to March, $p < 0.001$) before gradually declining to 175 (August). MNE was significantly greater in March than any other sample month, except February ($p < 0.05$). There was no significant decrease in egg number from April to August, in contrast to the farm 1 population. As with string length, egg number was negatively correlated with temperature, which accounted for 77% of the variability ($r^2 = 0.77$, $p < 0.01$). Photoperiod had no significant effect on egg number (Table 1).

From both data sets it appears that distinct seasonal variation exists in the number of eggs produced by adult females. Significantly more eggs are produced in winter and early spring than in summer and autumn.

Egg length (Fig. 4)

Mean egg lengths (MEL) were only measured for April and August at farm 1, increasing significantly from 73 to 82 μm ($p < 0.01$). Fig. 4 shows the variation in MEL with temperature and photoperiod at farm 2. ANOVA showed that distinct seasonal variation in egg length exists, decreasing from October to February (73.65–62.70 μm) before increasing again and remaining relatively constant over the summer months ($p < 0.001$). MEL in January and February were significantly smaller than in other sample months ($p < 0.01$). Changes in length are positively correlated with photoperiod ($r^2 = 0.68$, $p < 0.05$).

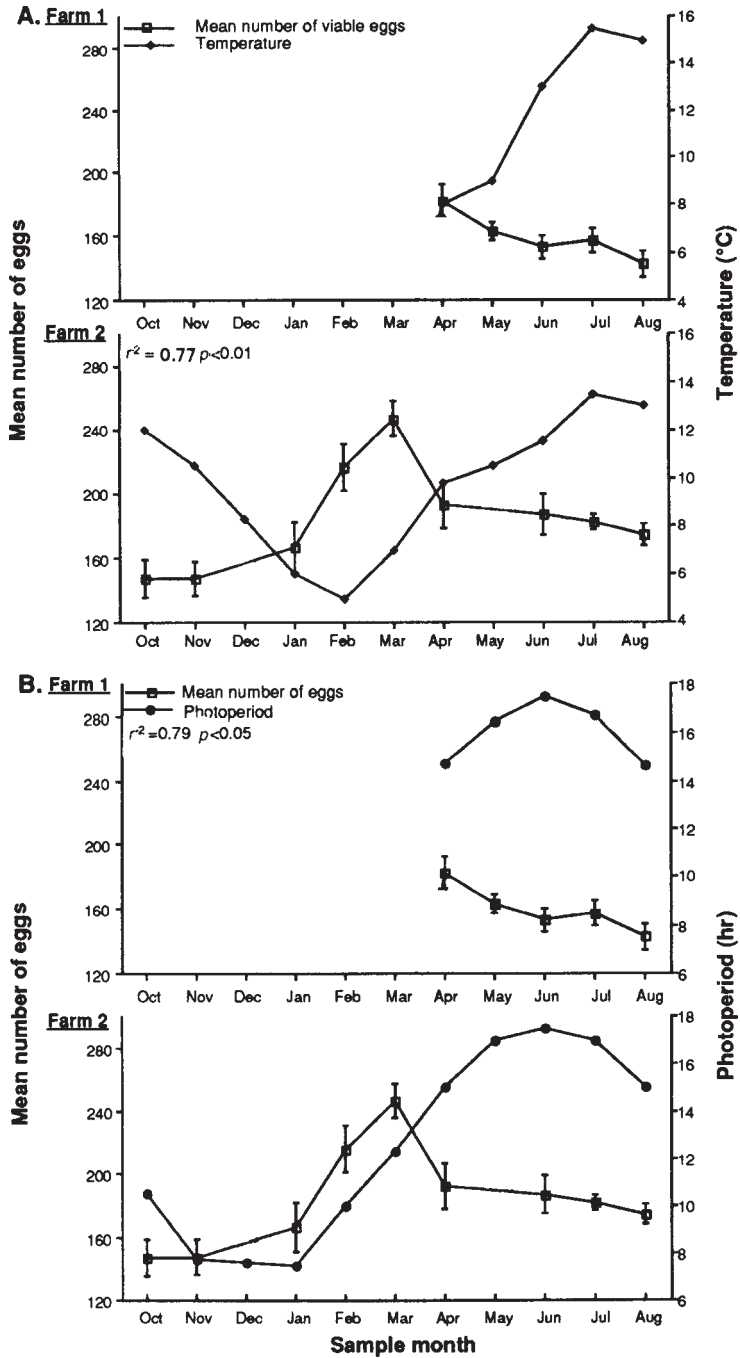


Fig. 3. Changes in the mean number of viable eggs produced per string from all adult females per month ($n > 22$) ($\pm$ SE) from two populations of *L. salmonis* (farm 1 and farm 2) with (A) temperature and (B) photoperiod.

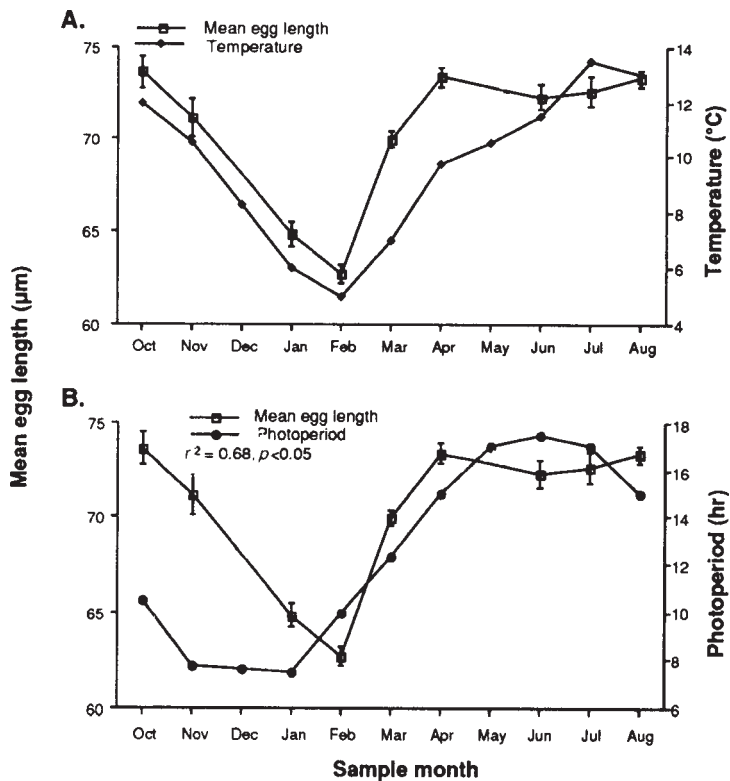


Fig. 4. Changes in mean viable egg size (in μm) from all adult females of *L. salmonis* per month ($n>22$) ($\pm\text{SE}$) from farm 2 with (A) temperature and (B) photoperiod.

Temperature had no influence on egg length (Table 1). It appears that the variation in environmental conditions is expressed more rapidly in MEL than in string length or brood size and as a result a poor relationship is obtained between these reproductive parameters.

Size of adult females with viable egg strings (Fig. 5)

Variations in mean cephalothorax length (MCL) were observed from both sites. ANOVA showed that significant variation occurred in MCL over the sample period in both populations, suggesting a seasonal change in size ($p<0.001$). At farm 1 there was a steady, significant increase in size from 4.55 to 4.92 mm (April to August, $p<0.01$). At farm 2 MCL increased from 4.30 to 4.98 mm (October 1991 to March 1992, $p<0.001$) and, in contrast to the farm 1 values, fluctuated over the following months (March to August) with no net change. MCL in March was significantly larger than in other sample months, except February and June ($p<0.001$).

At farm 2, MCL was negatively correlated with water temperature from October to March ($r^2=0.79, p<0.05$). Using stepwise regression, the variation in MCL over the total sample period was also negatively correlated with water temperature

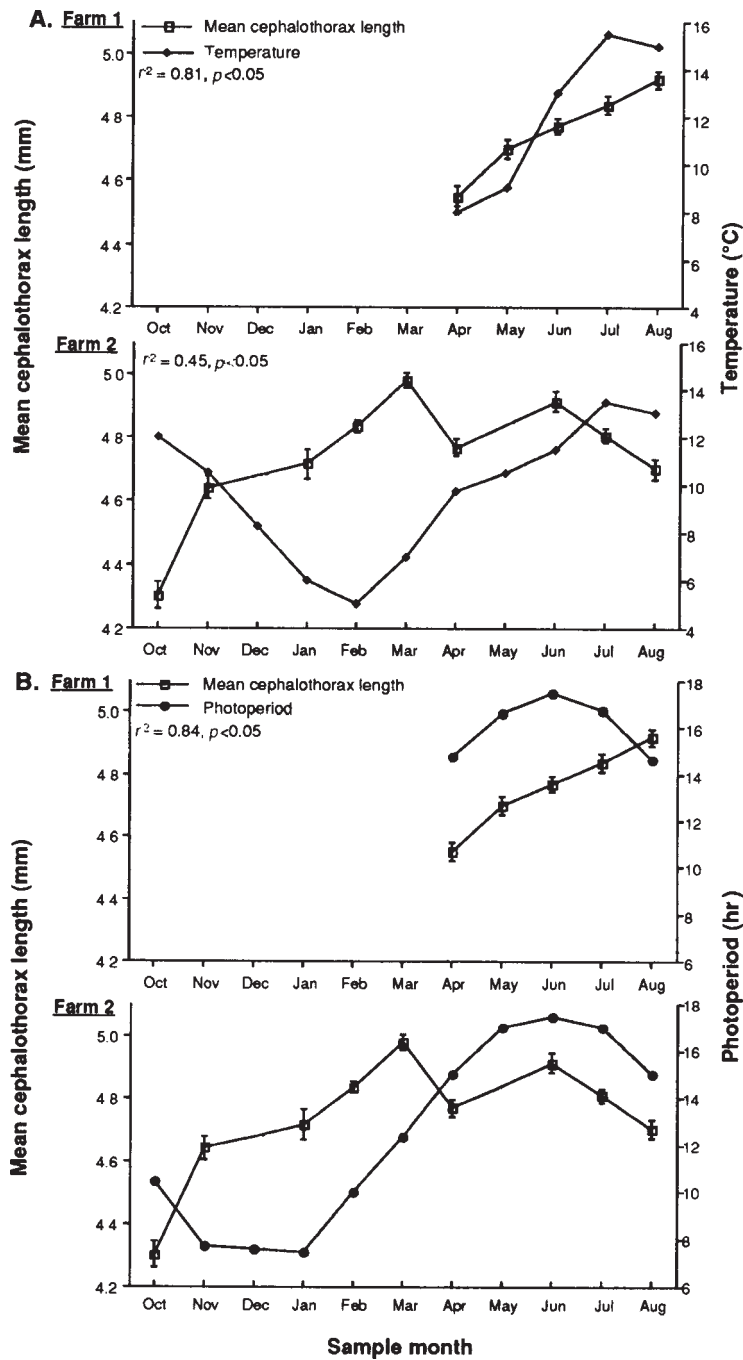


Fig. 5. Changes in the mean cephalothorax length (mm) of all adult females per month ($n > 22$) ($\pm$ SE) from two populations of *L. salmonis* (farm 1 and farm 2) with (A) temperature and (B) photoperiod.

($r^2=0.45$, $p<0.05$). There was no significant effect of photoperiod on the variability in MCL (Table 1). This suggests that the seasonal variation in size observed is a result of changes in water temperature. Neither temperature nor photoperiod was correlated with size between April and August.

MCL was positively correlated with temperature ($r^2=0.81$, $p<0.05$) and photo-period ($r^2=0.84$, $p<0.05$) in the farm 1 population (Table 1). While only temperature correlates with size in the farm 2 population, both environmental parameters correlate with viable adult female size in the farm 1 population.

The recent Aquagard treatments before sampling in June and July 1991 may have affected the measurements obtained since fewer adult females with viable egg strings were collected. Stepwise regression showed that changes in the size (MCL) at farm 2 had no influence on egg string length or egg number, although correlations do exist ($r^2=0.50$, $p<0.05$ for egg string length and $r^2=0.62$, $p<0.05$ for egg number). There was no correlation between the size of viable adult females and brood size within any sample month.

DISCUSSION

Although no information exists on the life span of adult female *L. salmonis*, Anstensrud (1990b) mentioned that adult males of *L. pectoralis* could live up to 101 days. Assuming that adults of *L. salmonis* survive a similar time to *L. pectoralis*, we would expect different generations of egg-bearing adult females to be present over the two sample periods examined (April to August 1991 and October 1991 to August 1992).

It is evident, from the two farms examined, that these generations have marked differences in reproductive output and strategies. Within these populations of *L. salmonis* seasonal variations in egg string length, egg number, egg length (size) and adult female size are evident. It has been demonstrated that winter generations of *L. salmonis* produce significantly longer strings than those of summer generations. Anstensrud (1990b) showed that egg string length was significantly greater in adult females that mated immediately after moulting, compared with those that had remained virgins for longer in the adult stage. Low numbers of males compared to females would result in more virgin adult females in a population. Since sex ratios in the populations from farms 1 and 2 were near unity throughout the sample period, it is assumed that smaller egg strings collected in summer were not a result of egg-bearing females that were virgin for a longer period.

Associated with seasonal alterations in egg string length is the change in the number of viable eggs produced. Unfortunately no winter data were collected from farm 1; however, farm 2 shows that winter generations have significantly greater numbers of eggs than those in summer. The larger range in temperature at farm 1 from 8 to 15.5°C (April to August) may be responsible for the significant reduction in egg number over this time. The low temperature in April means winter generations of adult females, producing more eggs, were present. The dramatic increase in temperature after April may have stimulated the rapid emergence of summer generations with fewer eggs. At farm 2 the temperature range between April and August was lower and changes were more gradual, producing a lower variation in viable egg number. The larger standard error bars are associated with the gradual alteration in generations with temperature. More eggs and regions of viable egg

strings appeared discoloured and disorganized in winter generations (Ritchie, unpublished observation).

Other authors have commented on variations in egg number (Wootten *et al.* 1982, Hogans and Trudeau 1989). Tully (1989) observed a significant reduction in the number of eggs produced per string by winter and summer generations (315 ± 35 in January and 107 ± 19 in August). The large variation observed by Tully (1989) in January may be a consequence of mixing generations, depending on previous temperature values. Nelson (1980), studying various amphipod species, showed that brood size was larger in winter than summer populations, and that it was similarly seasonally variable in species of Decapoda, Isopoda, Mysidacea and Cumacea.

In contrast to greater egg string length and egg number, egg size was reduced in winter. In laboratory trials Johnston and Dykeman (1987) showed that warmer temperatures significantly increase egg size in *Salmincola salmoneus* (L.).

In both farm populations, mean cephalothorax length appeared to be seasonally controlled. The changes in size observed in farm 1 may be attributable to the larger range in temperature. As in farm 2, Tully (1989) showed that the mean length of adult male *L. salmonis* was negatively correlated, from August (1987) to January (1988), with water temperature ($r^2=0.96$, $p<0.001$). Negative correlation between size and temperature, as observed in farm 2, has also been reported for other crustacean species (Sheader 1983, Murtaugh 1989). Nelson (1980) showed that females from winter populations were larger than summer populations. Johnston and Dykeman (1987), however, showed the opposite, whereby size increased at higher temperatures. It has been shown for other crustacean groups that female size has a direct influence on the number of eggs per brood (Sheader 1983, Williams 1985) and that the number produced is dependent on brood pouch size. Unlike these groups, the number of eggs produced by *L. salmonis* and other caligid species is not controlled by a brood pouch. It was shown (by stepwise regression) that changes in size did not directly account for any variability in reproductive output; however, the positive correlations observed between size, egg string length and egg number mean we cannot discount the influence of size on changes in reproductive output.

The observed variations in the reproductive parameters suggest that environmental factors are, perhaps, generating alterations in reproductive output of the two populations observed. At farm 2, changes in mean egg string length, mean egg number and mean cephalothorax length are correlated with, and therefore influenced by, changes in temperature rather than photoperiod. Temperature has been shown to have an effect on reproductive output in other crustacean groups (Sheader 1983, Johnston and Dykeman 1987). In this study, the variation observed in egg size between generations was correlated with photoperiod, suggesting egg size is influenced by photoperiod. Williams (1985) demonstrated that reproductive activity in *Talitrus saltator* (Montagu) is strongly influenced by photoperiod. It has been suggested that changes in reproductive output could be based on a combination of temperature and photoperiod and conditions which reduce reproductive output may be different from those that increase output (Moore and Francis 1986). Since temperature and photoperiod are closely associated with each other we cannot discount the effect of their combination on the reproductive parameters examined.

Since the number and size of eggs differ between generations in this study it appears that temperature may be interacting with endogenous factors to cause alterations in the expenditure of energy for reproductive output. If this is the case, greater reproductive investment per egg in summer will result in fewer though larger eggs, while in winter a greater number of smaller eggs will be produced. Assuming both greater reproductive investment per egg in summer and higher survival rates of nauplii (along with shorter generation times), as a consequence, the prevalence of infection at a fish farm may increase at this time. The expected outcome would thus be higher prevalence of infection during summer and autumn and lower prevalence in winter and spring.

Since changes in egg size appear before changes in string length and egg number, photoperiod may be triggering, directly or indirectly, the alteration in reproductive investment. The variation in female size suggests that winter generations are perhaps directing more energy into growth and maintenance than summer generations.

It has been demonstrated that distinct seasonal variation exists in the reproductive output of *L. salmonis* on the farms examined. Winter generations of adult female *L. salmonis* were found to be larger, producing significantly longer egg strings and a greater number of smaller eggs compared with summer generations. These differences in reproductive output are influenced by the environmental parameters of temperature and photoperiod. Investigations are now under way to support the idea that reproductive parameters are directly influenced by environmental factors and that summer and winter generations of adult females have different levels of reproductive investment which directly affect egg viability.

ACKNOWLEDGEMENTS

Financial support for G.R. was provided by a SERC CASE award in association with the Scottish Salmon Growers Association. The help and assistance provided by Marine Harvest International in collecting samples is gratefully acknowledged.

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13

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

Kazuya Nagasawa, Yukimasa Ishida, Miki Ogura, Kazuaki Tadokoro and Kazuhiko Hiramatsu

ABSTRACT

Examination of six species of Pacific salmon (genus *Oncorhynchus*) captured with long-lines from offshore waters of the North Pacific Ocean and Bering Sea revealed a marked difference in the prevalence, mean intensity and abundance of *Lepeophtheirus salmonis* infection between host species. Pink salmon (*O. gorbuscha*) had highest prevalence, mean intensity and abundance, followed by chinook salmon (*O. tshawytscha*) and steelhead trout (*O. mykiss*). Relatively high levels of infection were found on coho (*O. kisutch*) and chum (*O. keta*) salmon. Sockeye salmon (*O. nerka*) were very rarely infected. About 78% and 15% of the *L. salmonis* found occurred on pink and chum salmon, respectively; the remaining four species of salmonids carried only about 6% of copepods. The infection level increased with host ocean age and size. The frequency distributions of *L. salmonis* within salmonid populations and within ocean-age groups of chum salmon is described by the negative binomial. Pink and chum salmon, especially pink salmon, are considered the most important hosts of *L. salmonis*.

INTRODUCTION

With increased importance of salmonid mariculture, the caligid copepod *Lepeophtheirus salmonis* (Krøyer), salmon louse or sea louse, has emerged as a serious pathogen which causes damage and fish mortality (Wootten *et al.* 1982, Pike 1989). In recent years, various aspects of the biology, pathogenicity and control of this parasite have

been studied extensively (see this volume). Compared with such abundant information on *L. salmonis* from cultured salmonids, very little work has been done on the biology of the parasite on wild, especially offshore, salmonids, although it is generally believed to be common on sea-migrating salmonids.

Six species of Pacific salmon (genus *Oncorhynchus* Suckley) are distributed in offshore waters of the North Pacific Ocean and Bering Sea: pink salmon, *Oncorhynchus gorbuscha* (Walbaum); sockeye salmon, *Oncorhynchus nerka* (Walbaum); chum salmon, *Oncorhynchus keta* (Walbaum); coho salmon, *Oncorhynchus kisutch* (Walbaum); chinook salmon, *Oncorhynchus tshawytscha* (Walbaum); and steelhead trout, *Oncorhynchus mykiss* (Walbaum). While these species are relatively closely related to each other and largely overlap in ocean distribution, their ecologies are fairly different at sea (Groot and Margolis 1991). Pacific salmon thus provide a good opportunity to study host species utilization by *L. salmonis* in the ocean.

Nagasawa (1987) and Nagasawa and Takami (1993) examined the occurrence of adult female *L. salmonis* on Pacific salmon from the central North Pacific Ocean and Sea of Japan, respectively, and found differences in infection level between salmonid species. However, the infection levels of *L. salmonis* they reported were probably underestimated, because they used surface gill-nets to capture salmonids and, according to Nagasawa (1985), this type of gear removes *L. salmonis* from the body surface of salmonids during fishing operation and entanglement. The long-line is preferred gear, rather than surface gill-nets, for more precise assessment of the level of *L. salmonis* infection (Nagasawa 1985).

The aims of the present study, based on catch by long-lines, are to evaluate the relative importance of six species of Pacific salmon as hosts of *L. salmonis* and to determine the relationships of host age and size to infection levels.

MATERIALS AND METHODS

Sampling

Pacific salmon (*Oncorhynchus* species) were collected with long-lines by the RV *Wakatake maru* and *Shin-Riasu maru* at 42 locations in the central North Pacific Ocean and Bering Sea from 14 June to 12 July 1991 (Fig. 1). During the cruise, the *Wakatake maru* conducted both south–north and west–east transect surveys from 40°30' N to 58°30' N along 179°30' W and from 177°30' W to 176°30' E along 56° 30' N. Long-lines were usually set approximately 30 min before sunset (rarely before sunrise) and allowed to fish for 60 min. Thirty hachi (unit of long-line; one hachi contains 120 m long-line and 49 hooks) were used for each operation. Bait was salted Japanese anchovy (*Engraulis japonicus*). Some gill-netted fish were also examined, but the data were not used in this chapter.

Fish examination

When the long-line was hauled aboard, fish were removed from hooks, identified and examined for adult female *L. salmonis*. The fork length and body weight of each fish were measured and sex and gonad weight also recorded. One or two scales were removed from the INPFC (International North Pacific Fisheries Commission) preferred area on the lateral side of each fish, for age determination (Major *et al.* 1972).

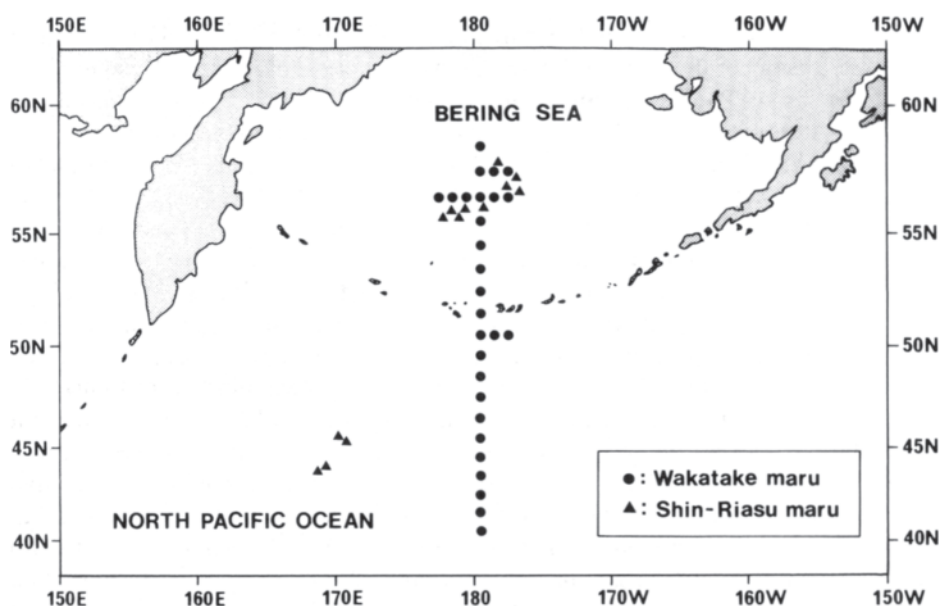


Fig. 1. Map of the North Pacific Ocean and Bering Sea, indicating locations where Pacific salmon were collected during the cruises of the RV *Wakatake maru* and *Shin-Riasu maru* in June–July 1991.

The ocean ages of Pacific salmon were determined by the number of annuli on scales according to the criteria of Koo (1962); ocean age 1 and ocean age 2 fish, for example, are those that have spent one and two winters at sea, respectively.

Data analysis

The terms prevalence, mean intensity and abundance follow the definitions of Margolis *et al.* (1982): prevalence is the percentage of infected fish, mean intensity the mean number of parasites per infected fish, and abundance the mean number of parasites per fish examined. The term infection level is also used to embrace the concepts of prevalence, mean intensity and abundance of infection.

Observed frequencies of *L. salmonis* within the host populations were tested for goodness-of-fit to the Poisson and negative binomial distributions using the χ^2 statistic. Sockeye salmon and steelhead trout were not tested because insufficient numbers of infected copepods were collected for sockeye salmon and because of the small sample size for steelhead trout. The frequency distributions of *L. salmonis* within ocean age groups 1–4 of chum salmon were also fitted to the negative binomial model. The variance to mean ratio ($s^2/\bar{x}$) and the parameter k of the negative binomial model were also calculated.

Table 1. Occurrence of *L. salmonis* on six species of Pacific salmon from offshore waters of the North Pacific Ocean and Bering Sea in June–July 1991

Salmonid species	Percent prevalence (infected/examined)	No. of copepods found (%)	Intensity Mean (range)	Abundance
Pink salmon	91.8 (975/1062)	5686 (78.4)	5.83 (1–33)	5.35
Sockeye salmon	5.9 (6/102)	8 (0.1)	1.33 (1–3)	0.08
Chum salmon	44.6 (505/1133)	1151 (15.9)	2.28 (1–26)	1.02
Coho salmon	60.2 (118/196)	270 (3.7)	2.29 (1–7)	1.38
Chinook salmon	75.7 (28/37)	85 (1.2)	3.04 (1–7)	2.30
Steelhead trout	86.7 (13/15)	54 (0.7)	4.15 (1–10)	3.60

RESULTS

Occurrence on Different Host Species

Pink salmon had highest prevalence, mean intensity and abundance of infection (Table 1). Relatively high levels of infection also occurred on steelhead trout and chinook salmon, which were followed by coho and chum salmon. Sockeye salmon were very rarely infected.

A total of 7254 *L. salmonis* was found on Pacific salmon examined (Table 1). The number of *L. salmonis* found on each host species represents the parasite's relative population size on each species of Pacific salmon because the long-line catch based on constant fishing effort at all locations could be used as a measure of abundance of each salmonid species. Pink salmon harboured *L. salmonis* most abundantly, accounting for about 78% of copepods found. Chum salmon followed pink salmon, with the number of *L. salmonis* exceeding 15%. Other salmonids carried only a small number of *L. salmonis*: about 4% for coho salmon and 2% for chinook and sockeye salmon and steelhead trout.

Effect of host age

There were no pink and coho salmon of ocean age ≥ 2 , and all specimens of these two salmonid species sampled were ocean age 1 fish. Despite their short residence at sea, the infection level on pink salmon was the highest of all six salmonid species, and that on coho salmon was relatively high (Table 2).

In the other four salmonids, fish of ocean age ≥ 2 were included. In chum salmon, there were distinct host age-related changes in infection level (Table 2). Prevalence increased steadily from ocean age 1 to 5. Mean intensity and abundance changed similarly but declined at ocean age 5, although this may be an effect of the small sample size. Likewise, chinook salmon and steelhead trout showed increasing trends in infection level with ocean age. Despite the low level of infection, the prevalence, mean intensity and abundance on sockeye salmon all slightly increased from ocean age 2 to 3 where many samples were available.

Table 2. Occurrence of *L. salmonis* on six species of Pacific salmon of different ocean age groups from offshore waters of the North Pacific Ocean and Bering Sea in June–July 1991. *n*, sample size; P, prevalence (%); M, mean intensity; A, abundance

Ocean age	Pink salmon				Sockeye salmon				Chum salmon			
	<i>n</i>	P	M	A	<i>n</i>	P	M	A	<i>n</i>	P	M	A
1	1062	91.8	5.83	5.35	1	0	—	0.00	115	15.7	1.28	0.20
2					58	5.2	1.00	0.05	588	39.6	1.84	0.73
3					36	8.3	1.67	0.14	270	57.8	2.67	1.54
4					2	0	—	0.00	72	70.8	3.52	2.50
5									6	100	2.00	2.00

Ocean age	Coho salmon				Chinook salmon				Steelhead trout			
	<i>n</i>	P	M	A	<i>n</i>	P	M	A	<i>n</i>	P	M	A
1	196	60.2	2.29	1.38	6	66.7	1.00	0.67	9	77.8	4.86	3.78
2					22	83.4	3.16	2.73	2	100	3.50	3.50
3					3	66.7	4.50	3.00				

Effect of Host Size

Prevalence, mean intensity and abundance generally increased with host size, while size classes with small samples (*n*<10) did not show a consistent trend (Table 3). Such host size-related changes were distinct both between 30 and 59 cm size classes for pink salmon and between 30 and 69 cm size classes for chum salmon.

Infection levels were also affected by local variations in host size: *L. salmonis* was more common on chum salmon from the northern area along 179° 30'W, and from the western area along 56° 30' N (Fig. 2). This correlates with longitudinal and latitudinal changes in size of chum salmon.

Copepod frequency distributions

The frequency distributions of *L. salmonis* within the populations of six salmonid species are shown in Fig. 3. In the four species, excluding sockeye salmon and steelhead trout, *L. salmonis* was overdispersed with the variance (*s*²) of the parasite, counts being greater than their mean ($\bar{x}$) (Table 4). The negative binomial model provided a better fit to the observed frequencies than did the Poisson (Table 4). The fit to the negative binomial was good for coho and chinook salmon (*p*>0.05), but not for pink and chum salmon (0.01<*p*<0.05 and *p*<0.01); this is because in pink salmon observed frequencies at some intensity levels (14 and 16 copepods per fish) differed markedly from expected values and because five ocean groups with different means were combined in the chum salmon data.

The frequency distribution of *L. salmonis* within each ocean age group of chum salmon is shown in Fig. 4. The parasite was overdispersed in ocean age groups 1–4

Table 3. Occurrence of *L. salmonis* on six species of Pacific salmon of different size classes from offshore waters of the North Pacific Ocean and Bering Sea in June–July 1991. *n*, sample size; P, prevalence (%); M, mean intensity; A, abundance

Size class	Pink salmon				Sockeye salmon				Chum salmon			
	<i>n</i>	P	M	A	<i>n</i>	P	M	A	<i>n</i>	P	M	A
20–29									2	50.0	6.00	3.00
30–39	55	85.5	4.19	3.58	1	0	—	0.00	179	29.6	2.06	0.61
40–49	942	91.9	5.88	5.40	49	4.1	1.00	0.04	603	38.6	1.66	0.64
50–59	65	95.4	6.47	6.17	38	7.9	1.00	0.08	257	58.0	2.96	1.71
60–69					13	7.7	3.00	0.23	90	75.6	3.04	2.30
70–79					1	0	—	0.00	2	50.0	2.00	1.00

Size class	Coho salmon				Chinook salmon				Steelhead trout			
	<i>n</i>	P	M	A	<i>n</i>	P	M	A	<i>n</i>	P	M	A
30–39					7	71.4	1.00	0.71				
40–49	44	61.4	2.11	1.30	9	77.8	3.43	2.67	1	0	—	0.00
50–59	148	60.1	2.34	1.41	12	83.3	3.30	2.75	9	88.9	4.38	3.89
60–69	4	50.0	2.50	1.25	6	66.7	3.25	2.17	1	100	4.00	4.00
70–79					3	66.7	5.00	1.67	4	100	3.75	3.75

(variance to mean ratio=1.25–4.49), and the distributions of *L. salmonis* within these ocean age groups were described by the negative binomial model (Fig. 4). The fit to this model was good for fish of ocean ages 3 and 4 ($p>0.05$), but not for those of ocean age 2 ($0.01<p<0.05$). Values of the mean $\bar{x}$ (=abundance of infection) increased with host ocean age, whereas there were no distinct age-related trends in the value of the parameter k of the negative binomial model ($k=0.611, 0.638, 0.691$ and 0.967 for ocean age groups 1–4).

DISCUSSION

The present study showed various levels of infection by *L. salmonis* on different species of salmonids. These results agree with but differ somewhat from those of Nagasawa (1987), who also examined the presence of *L. salmonis* on six species of Pacific salmon from the central North Pacific Ocean in spring. In the present study, pink salmon were found to be most frequently and heavily infected, but Nagasawa (1987) reported that the highest levels of infection were found on chinook salmon and steelhead trout, followed by pink salmon. This difference is likely due to sampling methods, because Nagasawa used surface gill-nets as fishing gear, and it is possible that the degree of loss of *L. salmonis* from the body surface of gill-netted salmonids is different between the host species. As evidenced by Nagasawa (1985), the long-line gear which was used

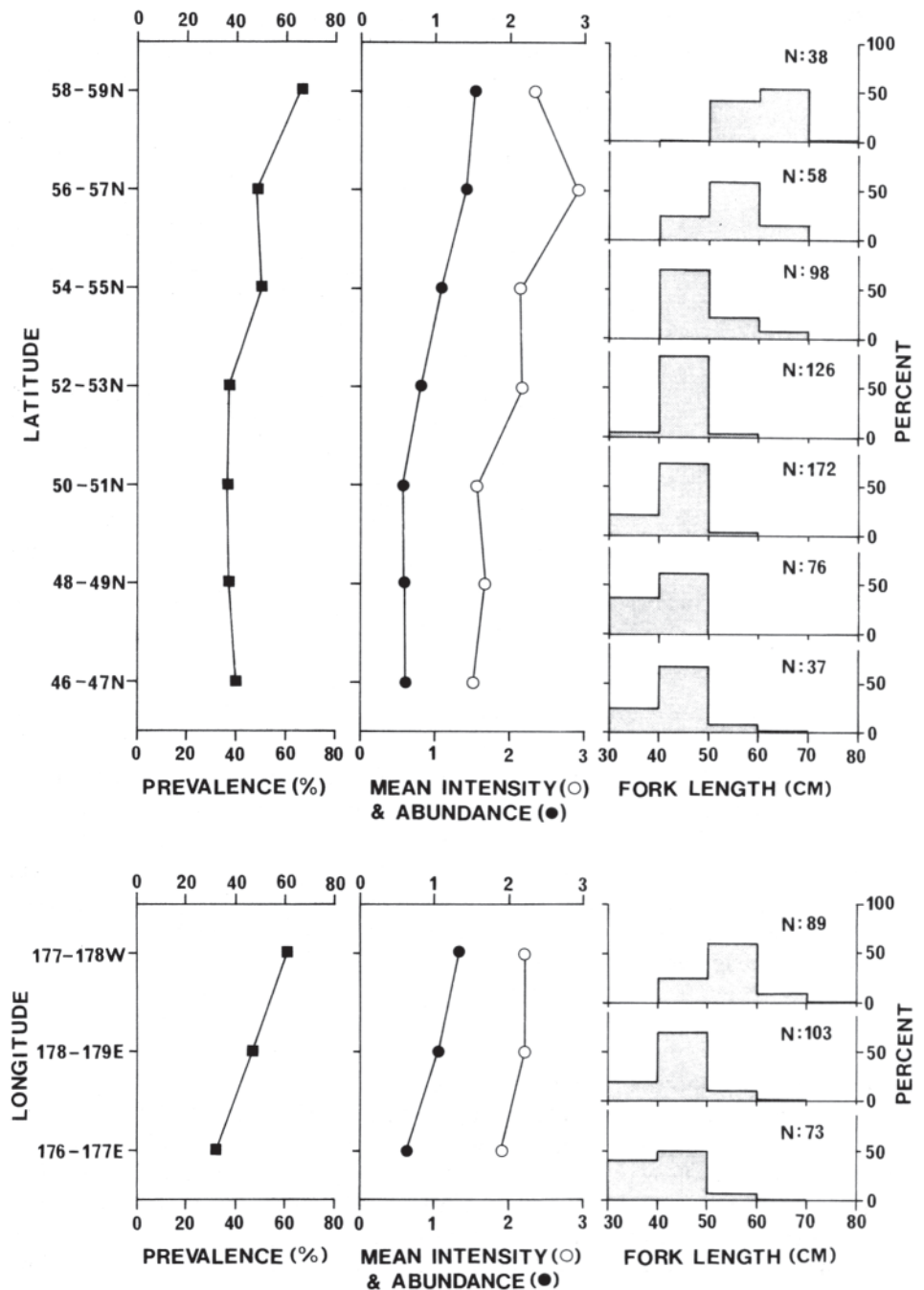


Fig. 2. Latitudinal (top) and longitudinal (bottom) changes in infection level of *L. salmonis* on chum salmon and size frequency distribution of chum salmon along 179°30' W and 56° 30' N, respectively.

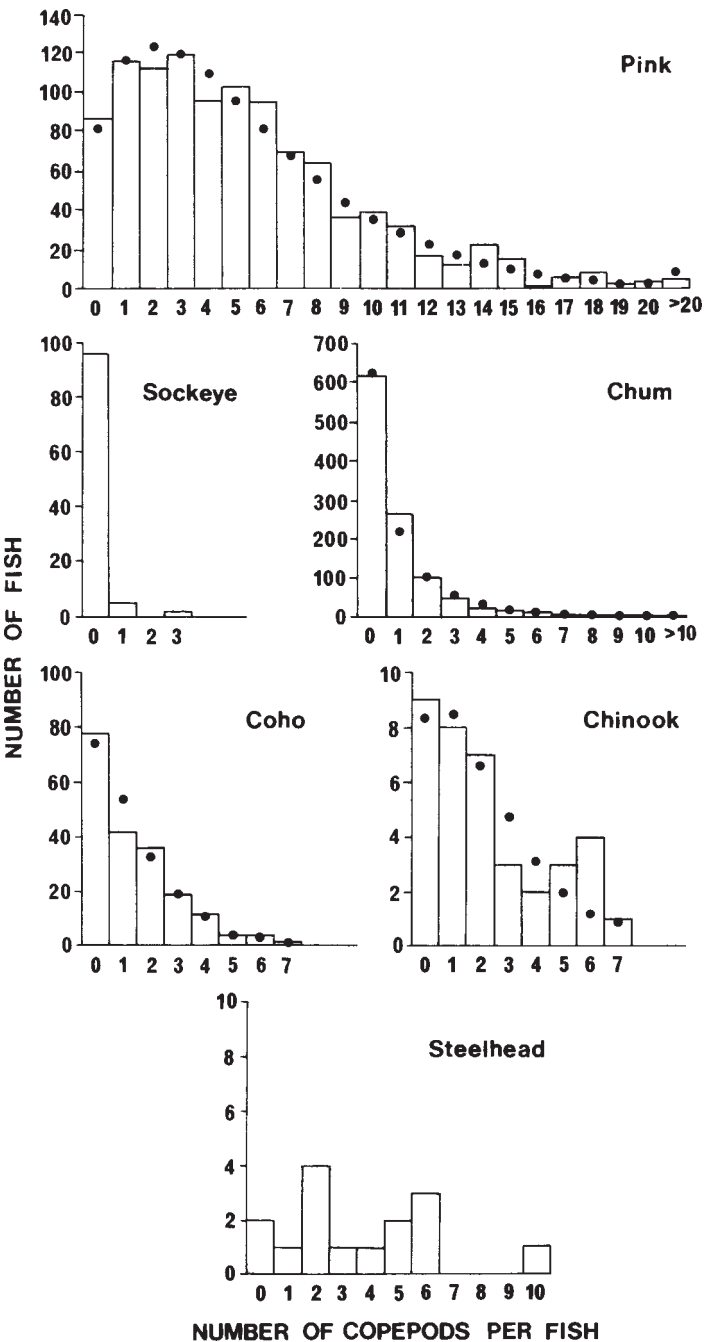


Fig. 3. The frequency distributions of *L. salmonis* within six populations of Pacific salmon. The histograms represent the observed distributions and the dots show the fitted negative binomial distributions.

Table 4. Calculated values for Poisson and negative binomial distributions fitted to frequency distributions of *L. salmonis* on four species of Pacific salmon from offshore waters of the North Pacific Ocean and Bering Sea in June–July 1991. Relative goodness-of-fit to each model is judged by values of χ^2 . d.f., degrees of freedom

Salmonid species	No. of fish	Mean ($\bar{x}$)	Variance to mean ratio	Poisson		Negative binomial	
				χ^2	d.f.	k	d.f.
Pink salmon	1062	5.35	3.66	2576.1	10	1.965	17
Chum salmon	1133	1.02	3.64	391.0	3	0.533	6
Coho salmon	196	1.38	1.75	41.3	3	1.502	4
Chinook salmon	37	2.30	2.03	6.3	2	1.809	1

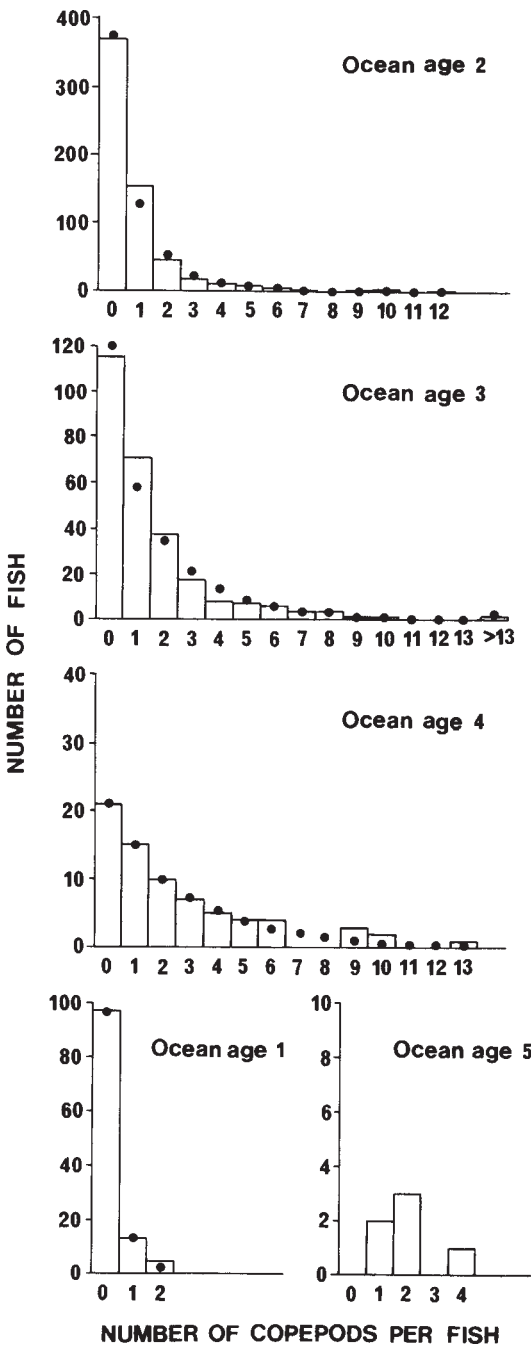


Fig. 4. The frequency distribution of *L. salmonis* within ocean age groups of chum salmon. The histograms represent the observed distributions and the dots show the fitted negative binomial distributions.

in this study is more reliable than surface gill-nets when precise data on the infection level of *L. salmonis* are needed.

About 78% and 15% of the number of *L. salmonis* found occurred on pink and chum salmon. This is consistent with the findings of Nagasawa (1987), who calculated that about 67% and 24% of the estimated population of *L. salmonis* occur on these salmonids. From these results, it is evident that pink salmon and, to a lesser extent, chum salmon are the most important as hosts of *L. salmonis* in offshore waters of the North Pacific Ocean and Bering Sea.

One of the most interesting results in this study is a marked difference in infection level between host species; pink salmon with 1-year ocean residence had the highest infection level, while sockeye salmon spending usually 2–3 years at sea were very rarely infected. Nagasawa (1987) attributed such a difference to both diversity in ocean distribution and duration of ocean residence of hosts and to their differential susceptibility to infection. In particular, he suggested that pink and sockeye salmon are the highest and least susceptible to infection, respectively. However, most mature sockeye salmon returning to Canadian coastal waters are, sometimes very heavily, infected (L. Margolis, Pacific Biological Station, Nanaimo, BC, Canada, personal communication), indicating that sockeye salmon are susceptible to *L. salmonis*. Thus some further explanations are needed for the observed difference in infection level. For example, as the success in settlement and attachment of *L. salmonis* infective larvae is considered to be largely dependent upon the host's behaviour, the swimming speed of salmonids may be related to the infection of *L. salmonis*. According to Brett (1982), pink salmon can only perform at about 80% of the level of prolonged speed achieved by sockeye salmon of comparable size. It is possible that *L. salmonis* copepodids can settle on and attach to the slower-swimming pink salmon more easily than to sockeye salmon. One of the reasons why pen-cultured salmonids become heavily infected with *L. salmonis* might be that the movement of caged salmonids is suppressed in the confined space.

While it is not yet known where the infective larvae of *L. salmonis* are distributed in the ocean, they probably occur in the upper water layer, since these larvae actively swim upwards and are photosensitive (Johannessen 1978, Wootten *et al.* 1982). Thus, although all species of Pacific salmon are epipelagic in principle, the precise swimming depth of each species would influence the encounter rate with infective larvae.

Further, if there are differences in structure and function of epidermis between salmonid species, differential suitability of epidermis as a site for the settlement and attachment of *L. salmonis* may also be a factor contributing to the differences in level of infection on different salmonids. In addition, since salmonid maturation is accompanied by structural and functional changes in the epidermis (Pickering and Richards 1980), such changes may also affect the occurrence of *L. salmonis*. Pink salmon examined in this study were all maturing, and there are trends that maturing salmonids are frequently and heavily infected with *L. salmonis* (Nagasawa 1985). The relationships of *L. salmonis* infection to epidermal changes associated with fish maturation should be taken into account in future studies.

The overdispersed distribution of *L. salmonis* within salmonid populations could result from a non-random distribution of infective larvae. Boxshall (1974) made a similar suggestion by studying the population of *Lepeophtheirus pectoralis* (Müller)

on plaice, *Pleuronectes platessa* L., from Sandsend Bay, Yorkshire. Some work on free-living copepods may confirm the relevance of such a hypothesis (e.g. Heip and Engels 1977). As another possible explanation, non-random characteristics of host biology could be responsible for the overdispersion of *L. salmonis*.

The observed frequencies of *L. salmonis* within each ocean age group of chum salmon were well described by the negative binomial distribution, but when all the ocean age groups were combined the fit to this model was poor. This is probably because several different negative binomial distributions were compounded in the latter case.

In conclusion, *L. salmonis* parasitizes the six species of Pacific salmon at various levels of infection, but pink and chum salmon, especially pink salmon, are the most important hosts of this parasite. Since very little information is as yet available on the detailed biology of *L. salmonis* in wild, especially offshore, salmonid populations, more information is needed to understand the host–parasite dynamics of this parasite. Future work should aim to identify factors resulting in the differences in infection level between salmonid species.

ACKNOWLEDGEMENTS

We thank the captains, officers and crew of the RV *Wakatake maru* and *Shin-Riasu maru*, and Nancy D. Davis, Fisheries Research Institute, University of Washington, for their assistance during the study. Thanks are also due to Soto-o Ito of the National Research Institute of Far Seas Fisheries for age determinations of Pacific salmon.

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14

Salmon lice on wild salmon (*Salmo salar* L.) in western Norway

Bjørn Berland

ABSTRACT

Salmon lice occur on wild marine and farmed salmon, and have become a serious problem in marine salmon aquaculture in Norway. In heavy infections the lice may erode the skin to produce sores, exposing underlying tissue and even the skull roof.

In 1973 the lice infection in 35 wild Atlantic salmon caught in gill-nets near Bergen, Norway, was studied by Johannessen; for *Lepeophtheirus salmonis* he found a prevalence of 100%, mean intensity 11.7, range 1–37, abundance 11.7. For *Caligus elongatus* the prevalence was 48.6%, mean intensity 2.3, range 1–8, abundance 1.1.

The infection in 157 wild salmon caught in the same area in June 1988 was for *L. salmonis*: prevalence 93%, mean intensity 7.44, range 1–40, abundance 6.92; all salmon above 70 cm body length were infected. The infection with *C. elongatus* was much lower; prevalence 17%, mean intensity 1.42, range 1–34, abundance 0.24. In addition seven salmon judged by the fishermen as escaped farmed salmon were infected with *L. salmonis* only; for these the infection was: prevalence of 85.7%, mean intensity 25.2 and abundance 21.6. These values must be regarded as minima, as lice may have been scraped off during netting and handling.

The infection in 45 wild salmon caught in weirs on the outer coast in June–July 1992 was: *L. salmonis* 97.7%, 20.18, 1–66, 19.9. For *C. elongatus* the corresponding values were: 97.7%, 23.9, 2–171, 23.4.

The 1973 study was carried out when salmon aquaculture in Norway was in its infancy, while by 1988 and 1992 it had grown immensely. One would expect the high louse infection in farmed fish to have greatly influenced the infection in wild salmon. In 1988 infection did not differ markedly from that in 1973, while in 1992 the infection with both species of lice was much higher.

INTRODUCTION

It is well known that the salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837), is parasitic on marine salmon, *Salmo salar* L. and sea trout, *Salmo trutta* L. It

was common knowledge in the countries of northern Europe that the lice left the fish in fresh water and will survive only a few days in brackish or fresh water (Hahnenkamp and Fyhn 1985, McLean *et al.* 1990). This conspicuous parasite is one of a few that was given a vernacular name—laxlus and lakselus—in Scandinavian countries.

The salmon is also host to another caligid, *Caligus elongatus* Nordmann, 1832, which it shares with many other teleosts (see Kabata 1979).

From a modest start at the end of the 1960s, marine salmon aquaculture has grown immensely in several countries. The major producer country is Norway, but Scotland, Iceland and the Faroes have many salmon farms. Salmon is also farmed in the Baltic, but as this sea is very brackish the marine *L. salmonis* does not thrive there.

L. salmonis appeared in due course also on cultured marine salmon, but it was not possible to predict *a priori* that this parasite should become a serious pest in marine aquaculture. Feeding on the fish's skin, mucus and blood, the lice causes small haemorrhages and sores, and may erode the skin and expose the underlying tissue, in severe cases even revealing the bones in the skull roof. Injuries to, and death of, wild salmon were reported by White (1940, 1942).

In spite of its known presence on farmed salmon, solid data on the infection seem to be lacking. Johannessen (1990) states that 400–500 lice on a single cultured salmon is not exceptional, and further that close to 2000 lice have been recorded from a single salmon; these numbers have more anecdotal than statistical value. Lice on wild salmon are the source of infection in farmed marine salmon. What is the infection in wild salmon returning from the open sea on the homeward spawn run?

Johannessen (1975) studied 35 gill-netted salmon on the outer coast near Bergen in June–July 1973, and landed at Sekkingstad, Sotra. His data are presented in Table 1. The summer of 1988 was the last season in which drift gill-netting was permitted in Norway; the opportunity was taken to study the parasites of wild salmon, also at Sekkingstad, Sotra. A paper, in Norwegian, on the salmon lice of wild salmon has been published (Berland 1991), and one on the metazoan parasites by Bristow and Berland (1991). The present chapter aims at conveying facts relating to lice on wild salmon to an international audience.

MATERIALS AND METHODS

In June 1988 the author spent 7 working days at Sekkingstad, Sotra, waiting for commercial drift-netters to land their catches of wild salmon. A total of 157 wild salmon were, after recording body length, searched for external parasites and the species and numbers recorded from each fish. In addition, one sea trout (*S. trutta*) and seven salmon, which on morphological criteria were judged by the fishermen to be escaped farmed salmon (escapees), were also examined.

In June–July 1992 a total of 45 wild marine salmon were caught in wedge-shaped weirs (Norwegian: kilenot) on the outer coast at Taelavåg, Sotra, near Bergen. Upon being taken out of the trap, and after taking blood and mucus smears, each fish was placed in an individual plastic bag within minutes, kept on ice for a few hours until a thorough inspection for lice could be made (by me), and viscera

Table 1. Infections with *L. salmonis* and *C. elongatus* on wild salmon, Sekkingstad, Sotra, June–July 1973, based on Johannessen's (1975) data

Salmon		<i>L. salmonis</i> infection					<i>C. elongatus</i> infection						
Size group (cm)	Number of fish	Infected	Sum <i>L. s.</i>	Prev. (%)	Range/ max.	Mean	Abundance	Infected	Sum <i>C. e.</i>	Prev. (%)	Range/ max.	Mean	Abundance
60–69	5	5	47	100	1–17	9.4	9.4	2	2	40.0	1	1.0	0.4
70–79	14	14	151	100	1–37	9.6	9.6	4	14	28.6	1–8	3.5	1.0
80–89	7	7	111	100	3–28	15.8	15.8	4	6	40.0	1–3	1.5	0.6
90–99	7	7	105	100	3–32	15.0	15.0	6	15	85.7	1–6	2.5	2.1
100–109	2	2	12	100	3–9	6.0	6.0	1	2	50.0	1	2.0	1.0
Sum	35	35	426	100	1–37	11.7	11.7	17	39	48.6	1–8	2.3	1.1

sampled for tissue parasites (by G.A.Bristow); the fish and their viscera were frozen for later study. On simple sketches of salmon for each fish the position of each attached louse was plotted, by species; loose ones in the plastic bag were also noted. The generic identity of small and medium specimens was checked in a stereo microscope at the time of collection. All lice from each fish were preserved in 70% ethanol.

The terms used for infection are those proposed by Margolis *et al.* (1982).

RESULTS

The infection with the two louse species—*L. salmonis* and *C. elongatus*—on salmon samples studied in 1988 and 1992 are presented in Tables 2 and 3, respectively.

The seven ‘escapees’ referred to above, size range 57–75 cm, carried a total of 151 *L. salmonis*, no *C. elongatus*, and one fish was clean. This gives a prevalence of 85.7%, mean intensity 25.2 and abundance 21.6. The single sea trout, 57 cm long, carried two *L. salmonis*.

On 16 June 1988 a 70 cm long salmon was found to be carrying five specimens of the lernaepodid copepod *Salmincola salmoneus* (L.) on its gills.

DISCUSSION

When fish become tangled in, and when removed from, the fishing nets, and during handling and transport, lice may be scraped or drop off; the numbers recorded must always be regarded as minima.

For *L. salmonis*, Johannessen (1975) found the prevalence to be 100%, intensity range 1–37 and mean intensity (= abundance) 11.7, while Berland’s (1991) corresponding values are 93%, 1–40 and 7.44 (6.92) (see Tables 1 and 2). The corresponding values for 1992 (Table 3) are higher; 97.7%, range 1–66, mean intensity 20.18 and abundance 19.7. Tables 1–3 show that for *L. salmonis* the mean intensity of infection increases with fish length—the largest fish have most lice. The high recorded infection in 1992 may be real; note that each fish was placed in an individual plastic bag minutes after being lifted from the sea, and a heavy loss of lice may have been prevented. If that is the case, the recorded values for 1973 and 1988 may be underreporting the infection, but they are the best we have.

Johannessen’s infection parameters for *C. elongatus* in 1973 (Table 1) are 48.6%, 1–8, 2.3 and 1.1, while those found by Berland in 1988 (Table 2) are 17%, 1–3, 1.42 and 0.24. The parameters recorded in 1992 are exceptional (Table 3): 97.7%, range 2–171, mean intensity 23.9 and abundance 23.4. These values are much higher than those recorded earlier.

Johannessen’s (1975) infection data were obtained when Norwegian marine aquaculture was in its infancy, whereas salmon farming had grown to a huge industry when Berland (1991) recorded the infection in 1988 and 1992 (present chapter). Although Johannessen’s 1973 sample is rather small, the infection parameters in the years 1973 and 1988 are comparable; there are no marked differences in infection, measured as prevalence, mean intensity and abundance, for the two species, particularly for *L. salmonis*.

Table 2. Infection with the salmon lice *L. salmonis* and *C. elongatus* on wild salmon, Sekkingstad, Sotra, June 1988

Salmon	<i>L. salmonis</i> infection							<i>C. elongatus</i> infection						
	Size group (cm)	Number of fish	Infected	Sum <i>L. s.</i>	Prev. (%)	Range/ max.	Mean	Abundance	Infected	Sum <i>C. e.</i>	Prev. (%)	Range/ max.	Mean	Abundance
	50-59	40	34	133	85	1-21	3.91	3.33	4	7	10	1-3	1.75	0.18
	60-69	67	62	433	92	1-40	6.98	6.46	12	14	17.9	1-2	1.17	0.21
	70-79	23	23	189	100	1-19	8.22	8.22	5	6	22	1-2	1.2	0.26
	80-89	24	24	276	100	1-30	11.5	11.5	4	8	16.7	1-3	2.0	0.33
	90-99	2	2	37	100	15-22	18.5	18.5	0	0	0	—	0	0
	100-109	1	1	18	100	18	18	18	1	2	100	2	2.0	2.0
Sum		157	146	1086	93	1-40	7.44	6.92	26	37	17	1-3	1.42	0.24

Table 3. Infection with salmon lice *L. salmonis* and *C. elongatus* from wild salmon, Taelavåg, Sotra, Sotra, 30 June to 8 July 1992

Salmon	<i>L. salmonis</i> infection						<i>C. elongatus</i> infection							
	Size group (cm)	Number of fish	Infected	Sum <i>L. s.</i>	Prev. (%)	Range/ max.	Mean	Abundance	Infected	Sum <i>C. e.</i>	Prev. (%)	Range/ max.	Mean	Abundance
	50-59	19	19	298	100	1-43	15.7	15.7	19	355	100	2-79	18.7	18.7
	60-69	6	6	80	100	3-30	13.3	13.3	5	72	83.3	2-79	14.4	12.0
	70-79	8	7	126	87.5	11-22	18.0	15.8	8	206	100	2-62	25.8	25.8
	80-89	10	10	284	100	2-94	28.4	28.4	10	396	100	3-171	39.6	39.6
	90-99	2	2	100	100	34-66	50.0	50.0	2	22	100	3-19	11.0	11.0
Sum		45	44	888	97.7	1-94	20.18	19.7	44	1051	97.7	2-171	23.9	23.4

The many *L. salmonis* on cultured salmon reproduce during almost the entire year, and must produce enormous quantities of nauplii, which in due course become infective copepodids. One would expect the coastal waters in 1988 to be full of infective copepodids that would infect wild salmon to give an elevated infection in wild salmon. That was obviously not the case.

Adult females of *L. salmonis* on wild salmon are mainly attached at the sides of, and behind, the dorsal and adipose fins where, being dark brown, they do not show well against the dark colour of the fish. A major site is either side of and just behind the anal fin, where they are very conspicuous. Single adult females may attach anywhere on the fish's body, but few are found on top of the head, where they seem to aggregate in farmed salmon. The smaller specimens—males, young females and preadults—are of lighter brownish colour, and may be found all over the body and head, and some even inside the mouth and under the opercula. Many small ones wedge themselves under scales.

The large brown females, with and without egg strings, were specifically recorded in 1992; but egg strings may have been torn off in the nets or during handling. At least 50% of the *L. salmonis* found were mature females; a more detailed study of the preserved material may yield more exact numbers.

The presence of chalimus larvae was noted on 14 of the salmon inspected in 1992; they were mainly attached at the sides and base of the dorsal, anal and tail fins, but a few were also attached to scales. This shows that the wild salmon had become infected recently, and this infection probably took place in the coastal current.

We know that salmon caught in the fjords and on the coast are infected with lice, i.e. the conspicuous *L. salmonis*. But what is the infection in the open ocean, far from any coast? For the Atlantic nothing seems to be known, but for the Pacific infection data are available. Examining more than 6000 specimens, of the six Pacific *Oncorhynchus* Suckley species, Nagasawa (1987) found the infection to vary between species; chinook had the highest infection (prevalence 46%, abundance 1.67), while sockeye had the lightest infection (0.6% and 0.01, respectively). He also found that the parasite burden increased with fish size. Nagasawa (1985) found that by drift-netting lice are lost; the infection on fish caught by long-lining was higher than on those caught by gill-nets. Nagasawa's results indicate that, in the Pacific, salmon caught near the continental coasts have a higher louse infection than those caught in the open ocean.

The presence of *C. elongatus* on farmed salmon is well known, and it is a very common louse in the Ireland Scotland–Shetland–Faroe area. It is also known from farms in Norway; Berland (1991) referred to information from a veterinarian who recorded 332 specimens, and a single *L. salmonis*, from salmon on a farm on the west coast of Norway.

Infection by *C. elongatus* was exceptional in the summer of 1992. Some of the salmon searched were teeming with this louse when caught; the agile copepods could be seen to swarm jerkily over the fish's body. Reports from fish farmers of infection with this small louse in the summer of 1992 have been received. One report, with a fixed sample of *C. elongatus*, from a veterinarian stated that the mass infection in several farms on the outer coast near Bergen appeared quite suddenly in July; the fish were bristling with this louse, making them 'look like

hedgehogs'. Is the Norwegian west coast becoming 'Scottish' with respect to salmon lice?

Why did *C. elongatus* 'explode' in 1992? If this is a southern species, the very warm and sunny spring and early summer in northern Europe in 1992 may have been favourable to this species, boosting the population enormously. But as this louse is known from many teleosts, would not the build-up of the population on salmon have been noted earlier, or were they parasites on other fishes? There may be an interchange of ectoparasites between the salmon inside the holding pens and gadoids on the outside; Bruno and Stone (1990) reported that near the salmon pens saithe, *Pollachius virens* L., became infected with *L. salmonis* preadults, and in tests found that *L. salmonis* regularly transferred between salmon and saithe.

Several reports, namely: (a) Sea Trout Action Group Report: an investigation into the 1989/90 collapse of sea trout stocks in Galway and South Mayo, *Sea Trout News* no. 2, 30 January 1991, 24 pp.; (b) Annual report no. XXXV (report for the year ended 31 December 1990), *The Salmon Research Agency of Ireland Inc.*, 24 pp.; (c) Sea Trout Action Group, 1991 Report, *Sea Trout News* no. 3, February 1992, 24 pp.; (d) Report on the working group on pathology and diseases of marine organisms, Ostend, 19–22 February 1991, *ICES*, C.M. 1991/F: 42, 44 ss.; tell of a recent disastrous decline in sea trout catches in the west of Ireland; the smolts 'disappear' at sea and never return. A similar situation may appertain in Scotland. The reports link this decline to the many salmon farms on the Irish coast; the sea trout are believed to become so heavily infected by salmon lice that they succumb.

We do know that both wild and farmed salmon become infected with infective copepodids of *L. salmonis*; so also do sea trout, and we must assume that smolts of both species will be attacked. A large, grown salmon can carry a burden of several dozen lice, but what about the tender smolts? On their seaward migration, they have to pass waters literally teeming with infective copepodids. If infected, the larvae will in a matter of weeks become large lice. If exceeding critical numbers, these lice may cause the smolt to become distressed or perish. It is conceivable that the vast numbers of lice larvae 'waiting in the wings' in the plankton may kill off a substantial part of the smolt recruits, leading to a decline in the salmon population at sea and poor home runs in a few years' time. The smolts leaving the rivers near the head of a fjord have to travel further, running so to speak 'a longer gauntlet', than smolts coming from rivers near the coast.

The salmon catches in Norway have been declining for years. The Norwegian drift-netting for salmon was closed permanently in 1989, and the pelagic salmon fishery near the Faroes has been phased out. With natural spawning and artificial cultivation, one would expect the Norwegian salmon catches in the sea and in the rivers to increase. This does not seem to be the case. If they continue to decline, in spite of the countermeasures, we may have a more serious problem to cope with. Until we know more, these views are admittedly only qualified guesses, but Berland and Vasshaug (1992) alerted the Norwegian anglers to the problem.

ACKNOWLEDGEMENTS

The following persons and institutions are thanked for their help and support: the firm Konrad Sekkingstad a/s, Fjell; local fishermen at Taelavåg, Sund, in particular Ivar M. Øvretveit, who caught most of the salmon, and let us use his boathouse as living quarters and a makeshift lab; NFFR (project no. V 110.006) and 'Directorate for Nature Management', Trondheim for financial support.

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15

Lice infestation of farmed salmon in Ireland

D. Jackson and D. Minchin

ABSTRACT

Two caligid species, *Lepeophtheirus salmonis* and *Caligus elongatus*, were present on cultivated salmon in Irish waters sampled during April to September 1991 and in May 1992. *L. salmonis* was more common on west and north-west coasts and *C. elongatus* on the south-west, except in September when *L. salmonis* predominated. Fewer lice were found on south-west coast farms; however, there was no clear separation of the three coastal areas studied on the basis of infestation parameters. Smolts had the lowest numbers of lice but could be infested soon after introduction to the sea. Two-sea-winter fish normally had larger lice loads.

INTRODUCTION

Salmonid cultivation began on the west coast of Ireland in 1972. Since this time cultivation has expanded to the south-west and north coasts. Production of salmon can be modified by the presence of the salmon louse, *Lepeophtheirus salmonis* (Krøyer), which feeds on mucus, skin (Kabata 1974) and blood of salmonids (Brandal *et al.* 1976). *L. salmonis* is known to occur on salmonids and has seldom been found on other species (Kabata 1973). Recently Bruno and Stone (1990) have found specimens on saithe, *Pollachius virens* L., associated with Scottish salmon farms. A further species, *Caligus elongatus* Nordmann, appears in Irish waters and is known from a wide range of teleost and elasmobranch species (Kabata 1979). Sea lice cause erosion of dermal tissue principally in the head region (Egidius 1985); this can lead to problems of osmotic regulation and may lead to secondary infections (Wootten *et al.* 1982) and in severe cases death. Infestations on Irish marine farms result in serious stress for salmon and it has been found necessary to treat for this condition.

Sea lice infestations are normally treated using preparations of dichlorvos dissolved in various solvents and added to sea water (Brandal and Egidius 1979, Jackson and Costello 1991). This treatment is only effective on the later free-moving preadult and adult stages, and because of this serial treatments are necessary. Ivermectin, which is

incorporated as an additive in food pellets, is currently being studied as an alternative control method.

Other techniques used in Irish waters include the use of wrasse that behave as cleaner fishes (Costello and Bjordal 1990). This treatment is not currently widespread in Ireland. The isolation of furunculosis in stocked wrasse has led to the suspension of some studies while these results are evaluated.

In Europe the most frequent louse on salmon is *L. salmonis* (Jackson and Minchin 1992). On the Atlantic coast of Canada it is present in low numbers during the summer and is not present on fish during the winter. The dominant species present throughout the year is *C. elongatus* (Hogans and Trudeau 1989a,b). In Canada two other crustacean parasites present are *Caligus curtus* (Hogans and Trudeau 1989a) and the branchiuran *Argulus alosae* (Stuart 1990). These latter two species were not found on Irish farmed salmon (Jackson and Minchin 1992) but there have been isolated records of the praniza larvae of isopods of the genus *Gnathia* (Drinan and Rodger 1990).

While qualitative comments are made on the effects of treatments on lice numbers it was not intended that this work would investigate the effects of specific treatments on lice numbers or population structure. This study was carried out to assess the prevalence and intensity of sea lice infestations on farmed salmon in Ireland.

METHODS

Sampling at 20 sites took place from Bantry Bay along the western coast to Lough Swilly during April–May 1991 (Fig. 1). Thereafter sampling took place at monthly intervals until October at five sites from the south-west, west and north coasts.

A sample of 30 salmon from a single cage was taken for each year class in cultivation, except broodstock. The same cage was sampled on subsequent visits. Cages were not sampled unless a minimum of 7 days had elapsed since the last dichlorvos treatment. Fish were encouraged to feed, then selected using a hoop net. A total of 2048 salmon were examined in 1991.

Following capture smolts were either placed directly in separate bags or held in a bin before being placed in bags. Water from bins was sieved for detached lice, which were preserved separately. Fish were examined within hours of capture for all juvenile and adult louse stages. Lice from each fish were preserved in 70% ethanol.

Salmon from other year classes were anaesthetized after capture using ethyl-4-aminobenzoate (benzocaine) in acetone. Up to five fish were held within a bin at a time prior to examination. Each fish removed from the bin was examined for free louse stages, which were removed using forceps. Twenty-five fish were examined in this way. Contents of bins were sieved and preserved for each age class sampled. In addition five of the one- and two-sea-winter fish were killed after removal, placed directly in separate bags and all lice stages were later removed.

Preserved specimens of free-moving stages were identified to species and numbers 1 of attached specimens (chalimus and copepodid stages) recorded. Data on total numbers of lice of each species and numbers of ovigerous females of each species were treated

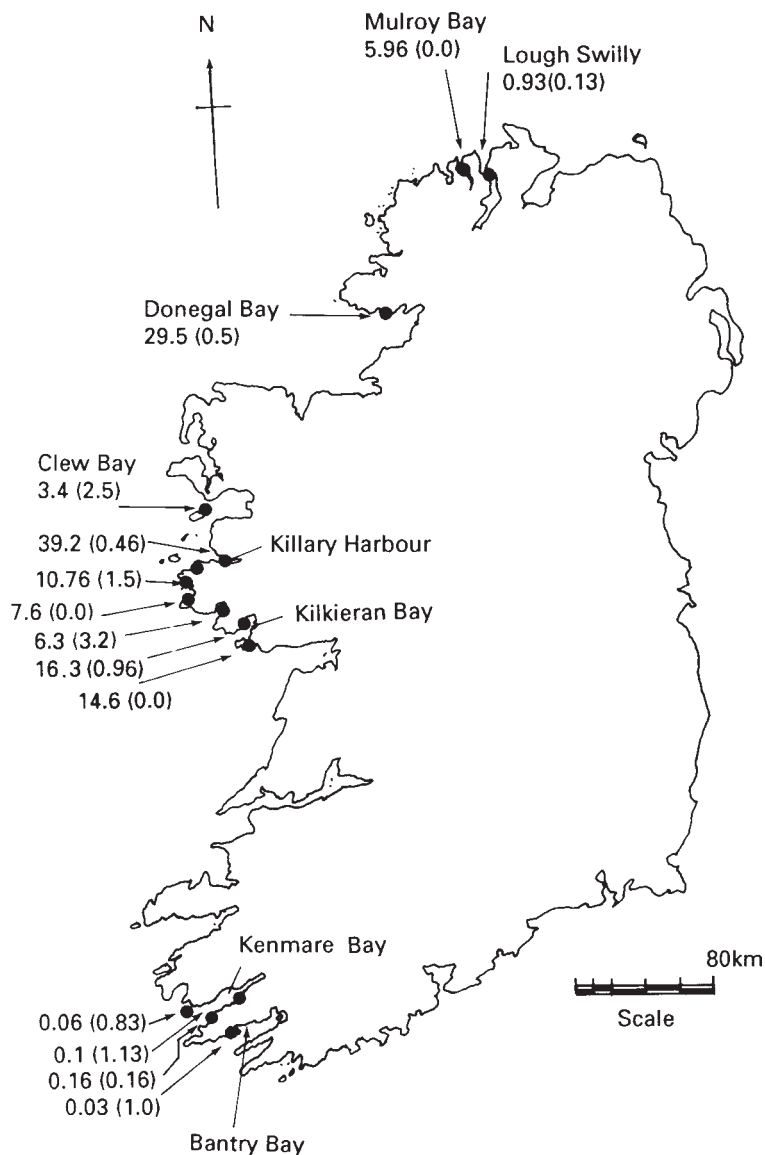


Fig. 1. Distribution of sampling sites for April–May 1991 survey. Values given are gross abundance of *L. salmonis* and *C. elongatus* (in parentheses) on one-sea-winter fish.

using cluster analysis. The clustering method used was median clustering and the distance measure was squared Euclidean distances.

In May 1992 a second survey of the 20 sites from Bantry Bay to Lough Swilly was carried out. On this occasion only one-sea-winter fish were sampled and all 30 fish from each sample were anaesthetized and examined for free-moving lice on site.

RESULTS

Two species of lice were found on Irish farmed salmon: *L. salmonis* and *C. elongatus*. Copepodid and chalimus stages of both species were present. They were frequently found on fins, particularly the dorsal fin. A total of 23 004 preadult and adult *L. salmonis* were obtained. This was the most abundant species at most sites surveyed (Fig. 1). *C. elongatus* was present on all coasts, although in low numbers. It was the most abundant species at certain sites off the south-west coast and 3981 were collected.

Smolts acquired lice with a low prevalence soon after being introduced to the sea (Table 1). Numbers on smolts during May were low, with an intensity of less than one per fish. No egg-bearing lice were found on smolts in May 1991. Although the intensity and prevalence of lice slowly increased on smolts, only one adult was found on a smolt later in the year.

Mean sampling efficiency for preadult and adult *L. salmonis* and *C. elongatus* calculated from lice numbers sieved from bins and related to the total numbers obtained was 89.4% and 75.3%, respectively. One- and two-sea-winter fish placed in bags had lower lice numbers than those recorded from anaesthetized fish. The mean numbers from killed and anaesthetized fish did not vary significantly, however. All data from each cage sampled were therefore combined.

The greatest number of lice was found within bays on the west coast. *L. salmonis* was present on west and north-west farms on all fish age groups. The mean abundance of this species in these two areas varied according to site (Table 1). This ranged from 0.84 to 28.6 in the north-west and from 3.37 to 82.38 in the west for one-sea-winter fish. Oldest fish normally had the most lice and smolts the least, at any one site where all age groups were cultivated. Two-sea-winter fish on the west coast farms normally had higher lice loads but also demonstrated greater variability between sites (mean abundance 1.9–202.8). Sites where there was a high intensity of infestation on one-sea-winter fish had more than twice this intensity on two-sea-winter fish. A relationship between these was found ($r=0.81$, $p=0.05$).

C. elongatus was present on all coasts in low numbers and had a mean incidence ranging from 0.0 to 3.1 (Table 2). Although present in low frequencies *C. elongatus* was dominant during the late spring and summer on the south-west coast but replaced by *L. salmonis* in the early autumn (Fig. 5). *C. elongatus* was present on all age groups in all coastal areas but was not recorded on all farm sites (Fig. 1).

All samples from the 20 sites sampled in April-May 1991 were treated using cluster analysis. Thirty-three samples formed a cluster (cluster 1) with strong linkage between samples. This cluster contained samples from all areas and all year classes sampled. A second cluster (cluster 2) of two samples consisted of a sample of two-sea-winter fish and a sample of one-sea-winter fish and was weakly linked to cluster 1. A third cluster of two samples of two-sea-winter fish with heavy lice loads was quite distinct from the other two clusters (Fig. 2). All four samples in clusters 2 and 3 were from the western region. When all samples for 1991 were clustered (Fig. 3) the four samples comprising clusters 2 and 3 in Fig. 2 again separated from the main body of the samples which formed a single strongly linked cluster (cluster 1).

Table 1. Abundance of infestation by preadult and adult *L. salmonis* on cultivated salmon in April–May 1991. Standard deviations are provided in parentheses

Locality	Free <i>L. salmonis</i>		
	Smolts	One-sea-winter	Two-sea-winter
<i>North-west coast</i>			
Lough Swilly	0.1 (0.2)	0.84 (1.05)	4.70 (2.82)
Mulroy Bay	0.2 (0.4)	5.77 (3.03)	12.77 (6.26)
McSwyne Bay	0.0	28.6 (6.61)	
Mean	0.1	11.74	8.74
<i>West coast</i>			
Clare Island	0.1 (0.3)	3.37 (2.46)	1.90 (1.90)
Killary Harbour		26.83 (11.75)	92.10 (38.86)
Ballinakill Bay	0.0 (0.2)	7.33 (5.37)	148.0 (44)
Ardbear Bay	0.9 (1.2)	10.26 (4.14)	
Mannin Bay		82.38 (27.15)	202.8 (45.73)
Bertraghboy Bay		6.27 (4.31)	
Kilkieran Bay		15.13 (7.96)	
Lettermullen	0.1 (0.2)		23.4 (12.37)
Ardnamackan Rk	0.3 (0.7)		
Mean	0.3	21.65	93.64
<i>South-west coast</i>			
Dinish Island	0.0	0.07 (0.36)	
Kilmakilloge	0.0	0.16 (0.45)	0.2 (0.5)
Coulagh Bay	0.0	3.00 (0.30)	
Bere Haven	0.0 (0.2)	0.03 (1.18)	
Mean	0.0	0.82	0.2

Comparison of the gross mean (i.e. adjusted for sampling efficiency) louse numbers for April–May 1991 and May 1992 (Fig. 4) shows no great changes in infestation levels. However, there is an increase in lice numbers in the north-west and south-west, the increase in the south-west being the more marked. Increased infestation levels were also recorded from some sites in the west.

DISCUSSION

Prevalence and abundance of both louse species varied between farmed sites in Ireland but lay within the range of observations made elsewhere (Brandal and Egidius 1977, Tully 1989, Hogans and Trudeau 1989a). The south-west Irish coast had a low louse count. Initially in 1991 *C. elongatus* was present in low numbers, and by the end of the

Table 2. Abundance of infestation by preadult and adult *C. elongatus* on cultivated salmon in April–May 1991. Standard deviations are provided in parentheses

Locality	Free <i>C. elongatus</i>		
	Smolts	One-sea-winter	Two-sea-winter
<i>North-west coast</i>			
Lough Swilly	0.0	0.1 (0.3)	0.4 (0.5)
Mulroy Bay	0.0	0.0	0.2 (0.5)
McSwyne Bay	0.0	0.5 (0.6)	0.5 (0.6)
Mean	0.0	0.2	0.37
<i>West coast</i>			
Clare Island	1.3 (1.7)	2.8 (2.9)	0.7 (0.9)
Killary Harbour		0.1 (0.2)	0.1 (0.2)
Ballinakill Bay	0.0	0.0	0.0
Ardbear Bay	0.0	0.2 (0.2)	
Mannin Bay		1.7 (1.3)	1.3 (1.5)
Bertraghboy Bay		3.1 (2.6)	
Kilkieran Bay		0.4 (0.7)	
Lettermullen	0.0		0.2 (0.4)
Ardnamackan Rk	0.1 (0.3)		
Mean	0.28	1.18	0.46
<i>South-west coast</i>			
Dinish Island	0.0	0.8 (1.0)	
Kilmakilloge	0.0	0.0 (0.2)	0.2 (0.5)
Coulagh Bay	0.0 (0.2)	1.1 (1.2)	
Bere Haven	0.0 (0.2)	0.1 (0.4)	
Mean	0.0	0.5	0.2

summer *L. salmonis* had become more frequent. Louse abundance is probably influenced by general and local hydrographic conditions.

Numbers of *L. salmonis* present on farms varied according to treatment. Declines in numbers were found following treatments of dichlorvos and ivermectin. In one case a treatment of ivermectin resulted in a decline to 1% of the maximum observed abundance.

Copepodid and chalimus stages cause localized damage on salmon and were often difficult to locate. The majority attached to fins, particularly the base of the dorsal fin. These areas correspond to those found by Jones *et al.* (1990) and Wootten *et al.* (1982). Johnson and Albright (1991b) in a laboratory study found most copepodid and chalimus stages attached to gills. In this study none was found on the gills of killed fish. Attached stages are unlikely to result in significant osmotic stress, because the area of damage

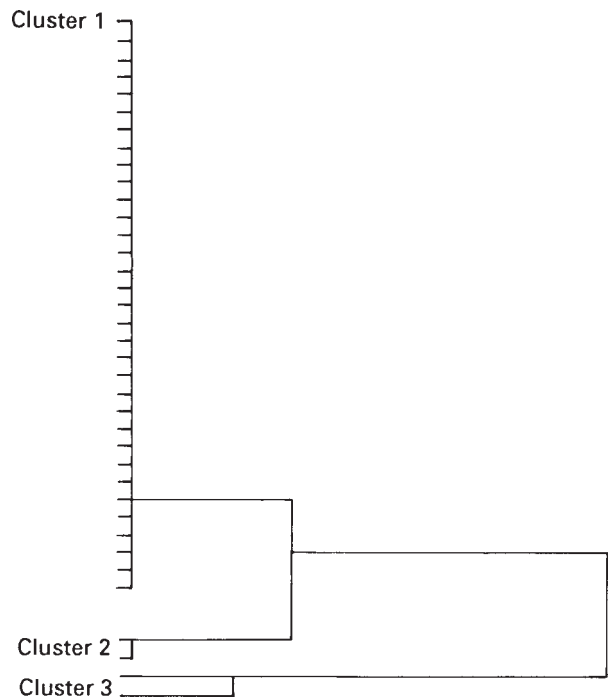


Fig. 2. May samples (squared Euclidean distance/median).

is small and dermal erosion becomes apparent only in later chalimus stages (Jones *et al.* 1990).

Free-moving louse stages were found predominantly on the dorsal surface. Egg-bearing females predominated in groups behind the adipose and anal fins. Dermal erosion or oedema was noticed in heavily infested fish, and adult lice were often found within a discoloured depression.

Killed fish had a lower count of preadult and adult stages than those that had been anaesthetized. They were more active before being dispatched and may have shed lice. This could account for the small differences obtained between the two sampling methods.

Numbers of attached lice are probably underestimated on account of their small size. Those on the darker dorsal surface were difficult to find and small discolorations were important in locating them. Fish colour changed rapidly following death. In 15 cases where fish were frozen before examination skin discoloration and build-up of mucus obscured attached lice stages and resulted in lower counts.

At a given site the infestation level with *L. salmonis* varied between age classes. Two-sea-winter fish had greater lice loads. These would be expected to have higher numbers because they were being prepared for market and were withdrawn from dichlorvos treatments. Only one farm visited had mean lice levels exceeding 200 lice per fish (maximum level recorded 202.8); this is considerably lower than that reported by Brandal and Egidius (1977). Progressively older year classes of chinook and chum

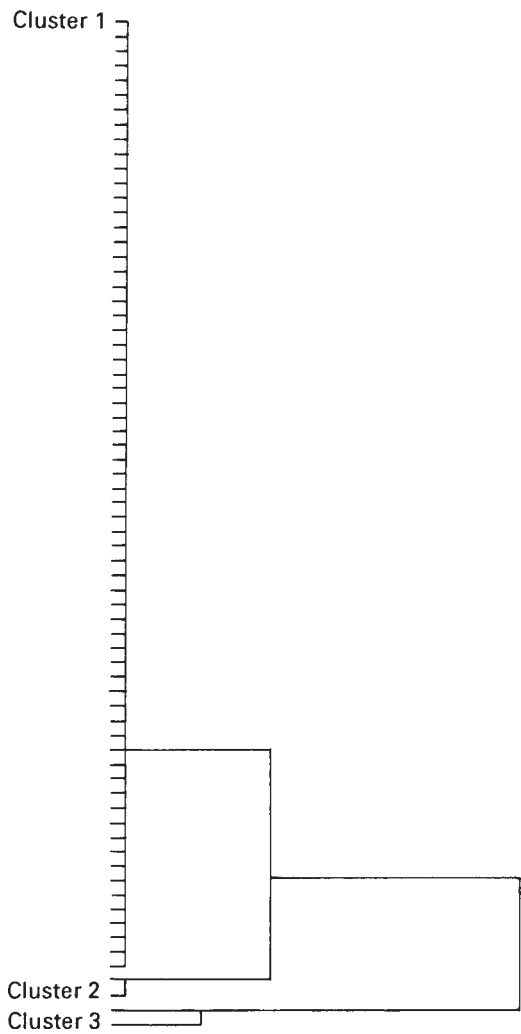


Fig. 3. All samples (squared Euclidean distance/median).

salmon from the Pacific Ocean were found to have greater numbers of lice (Nagasawa 1987). This pattern may relate to the greater surface area available for attachment and a greater duration of time for their establishment. Hogans and Trudeau (1989b), however, found no difference in infestations of *C. elongatus* between smolts and market-sized salmon. This they explain as being due to the effect of low sea temperatures on the life cycle of this louse.

C. elongatus occurred on all Irish coasts and most sites visited. *C. elongatus* can be found on many other native fish species (Kabata 1979). Lice on wild fish may become displaced and attach to salmon in nearby cages, thus causing sudden increases in infestation. Transfers of *C. elongatus* in the opposite direction are also possible but Tully (1989) considers transfers between salmon and native species to be negligible. Bruno and Stone (1990) demonstrated vertical transfers of *Lepeophtheirus* from older

age groups to smolts. The relationship between infestation levels on one- and two-sea-winter fish at the same site found in this study would also suggest that vertical transfers of infestation occur. The position of *L. salmonis* as the most abundant/dominant species on farmed salmon in Ireland may be due to the relative fecundity of *L. salmonis* and *C. elongatus* on farmed fish (Jackson and Minchin 1992).

Wootten *et al.* (1982) commonly found *C. elongatus* at Scottish fish farm sites, where it also appeared on smolts soon after transfer to sea. They refer to a peak of

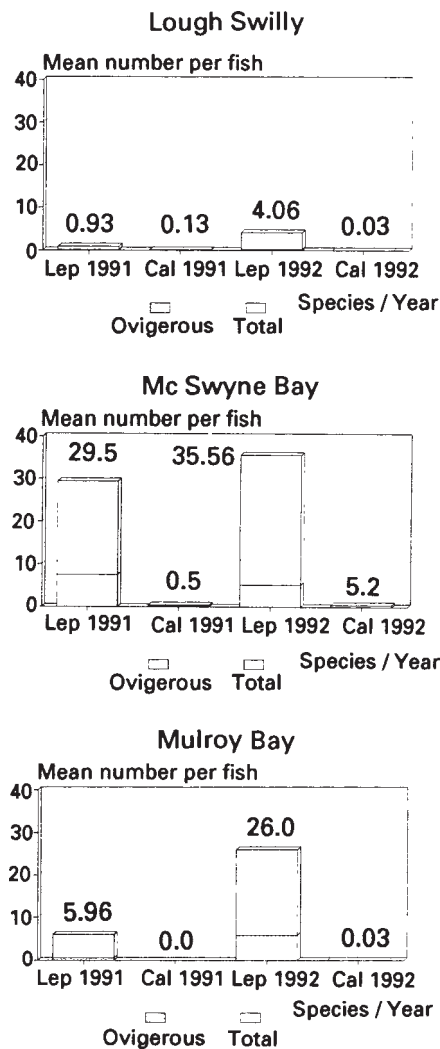


Fig. 4(a). Sea lice in Ireland, May 1991 and 1992 (adjusted for sampling efficiency: (a) north-west; (b) west; (c) south-west.

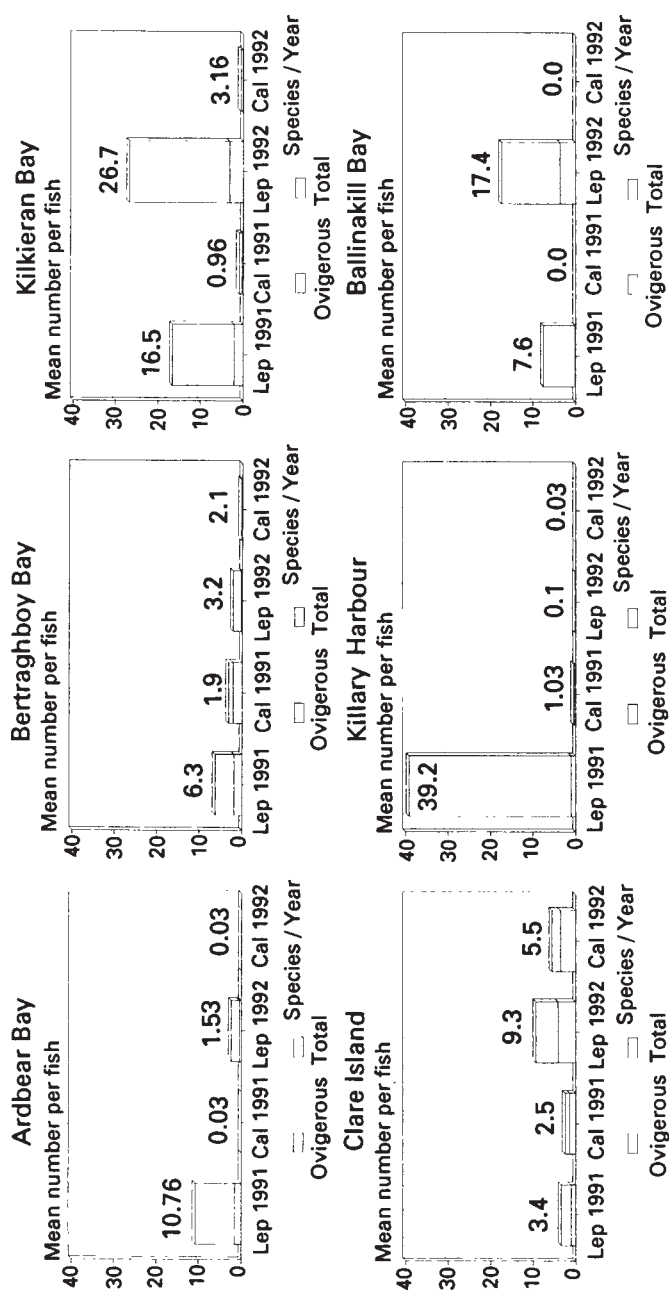


Fig. 4. (b)

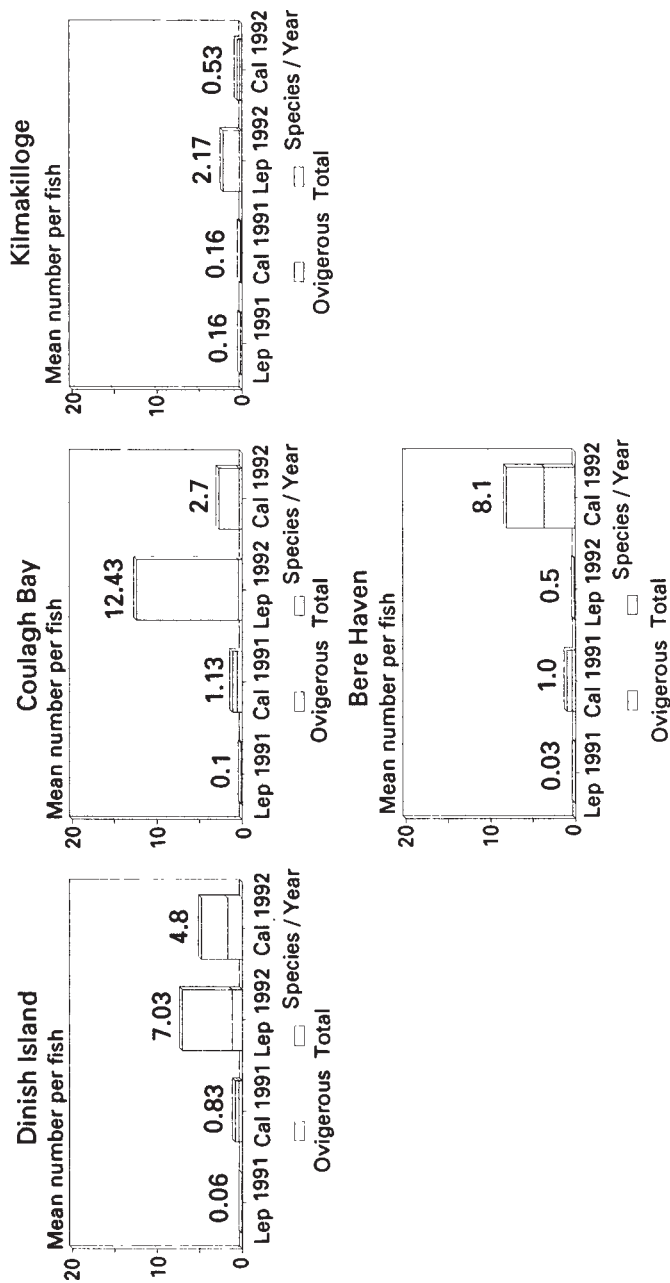


Fig. 4. (c)

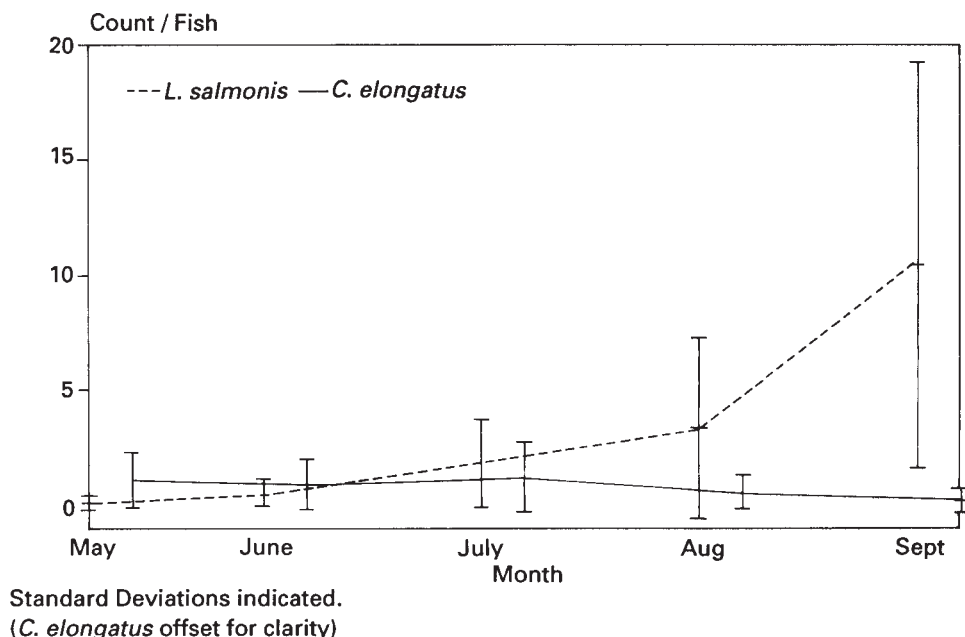


Fig. 5. *L. salmonis* and *C. elongatus* mean numbers on south-west coast (one-sea-winter fish).

abundance during late summer and autumn, but do not provide figures of lice prevalence or abundance. In Ireland greatest numbers were found during the spring and early summer. Hogans and Trudeau (1989b) found that the abundance and distribution of three caligid species increased with higher sea temperatures. Numbers of lice in Irish waters during the winter and early spring are not known but numbers of *L. salmonis* increased during the late summer and autumn. Tully (1989) examined the abundance of both louse species on smolts over a 7-month period in the west of Ireland from July 1987 to January 1988. Numbers of both species declined during September and then rose again during November. In the autumn of 1986 Taylor (1987), working on smolts in a neighbouring bay, found a greater proportion of *C. elongatus* present on the fish throughout the sampling period (April–September).

Cluster analysis showed that the infestation levels at sites could not be grouped according to geographical area, year class of fish or type of site, with all but four samples, with relatively high infestation levels, forming a single cluster.

The slight rise in infestation levels in May 1992 relative to 1991 may be due to difficulties with achieving effective treatments just prior to the sampling period. Many salmon growers reported ineffective treatments, which they attributed to low water temperatures at this time.

At one site, Killary Harbour, which was fallowed for 2 1/2 months, the lice numbers on smolts stocked after the fallowing were dramatically reduced (Fig. 4b). It would appear that the fallow period prevented vertical transmission of lice infestation.

CONCLUSIONS

Two species of sea lice are present on Irish cultivated salmon. The most frequent species, *L. salmonis*, is present throughout the year but the incidence of infestation varies during the year according to age class and site farmed. Smolts have lowest loads and two-sea-winter fish the highest. Variability between farmed sites is greater than between the three main geographical areas where salmon are farmed. The highest incidence was from bays on the west coast.

ACKNOWLEDGEMENTS

We would like to thank those who cooperated by allowing us access to their farms.

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Parameters and possible causes of epizootics of *Lepeophtheirus salmonis* (Krøyer) infesting sea trout (*Salmo trutta* L.) off the west coast of Ireland

O.Tully, W.R.Poole, K.F.Whelan and S.Merigoux

ABSTRACT

Infestation parameters were determined for *Lepeophtheirus salmonis* (Krøyer) infesting sea trout post smolts (*Salmo trutta* L.) during May 1992 at 14 locations off the west coast of Ireland. Prevalence ranged from 14.3% to 100%, mean intensity from 7 to 124.7 and the maximum number of lice recorded on an individual fish was 325. Maximum infestations of over 100 lice per fish were recorded at ten of the 14 locations sampled. Cluster analysis of the infestation parameters isolated three groups of sites which had different levels of infestation and within which infestations were statistically homogeneous.

Infesting lice were predominantly chalimi which were, invariably, responsible for the heaviest infestations. A higher proportion of adults occurred at sites where infestations were light. The chalami were attached mainly to the fins of the fish, particularly the dorsal fin, but also on the general body surface and the gills. Lice damage to the dorsal fin was severe and extensive grazing marks and skin erosion were evident on the dorsal body surface.

Epizootics of *L. salmonis* on sea trout occurred annually between 1989 and 1992 in the west of Ireland. Increased production of nauplii from infested farmed Atlantic salmon (*Salmo salar* L.), which accounts for the major proportion of total larval production of *L. salmonis* in the areas concerned, has probably increased exposure of sea trout to the infective copepodid in recent years. In addition, higher sea-water temperatures decreased generation times and increased the rates of development, maturation and potential production of the parasite between 1989 and 1992 relative to the previous 8 years. However, although no disease was detected in sea trout during a 3-year sampling period, the possibility that compromised fish health reduces host reaction or resistance to infestation needs to be established before a firm conclusion on the cause(s) of the epizootics can be reached.

INTRODUCTION

Heavy infestations of *Lepeophtheirus salmonis* (Krøyer) occurred on wild migratory

brown trout or sea trout (*Salmo trutta* L.) at a number of locations in the west of Ireland, annually, between 1989 and 1991 (Tully *et al.* 1993). The infestations were associated with significant host pathology, and moribund and dead fish were observed (Whelan 1991, Tully *et al.* 1993). However, the significance of these epizootics for sea trout populations was not established and the proportion of fish that contracted lethal infestations is unknown. The spatial and temporal occurrence of the epizootics did, however, coincide with the collapse of a number of populations (Whelan 1993).

An important feature associated with both the collapse in sea trout populations and the occurrence of epizootics of *L. salmonis* was the premature return of sea trout post smolts to estuarine and fresh water within 2–3 weeks of migrating to sea (Tully *et al.* 1993). Usually, smolts migrate to sea during April and May and return to fresh water in the autumn of the same year, with a smaller proportion overwintering at sea or in estuaries. A high proportion of prematurely returning fish had heavy sea lice infestations. Sampling concentrated on these fish, and the infestation parameters given by Tully *et al.* (1993) for 1990 and 1991 and those given in this chapter for 1992 refer only to the proportion of the populations that returned prematurely to fresh water. These data cannot be extrapolated to the entire population as it is not known if fish that remained at sea suffered similar levels of lice infestation or if fish returned prematurely because of the infestation.

This chapter presents data on the infestation of wild sea trout by *L. salmonis* at 14 locations off the west coast of Ireland during May 1992. Similar data for 1990 and 1991 can be found in Tully *et al.* (1993). We also discuss the possible causes of epizootics of *L. salmonis* that occurred on sea trout in the west of Ireland annually between 1989 and 1992. These include a correlation between local production of nauplius I of the parasite from farmed salmon and subsequent levels of infestation that develop on sea trout, an increase in the rate of production of the parasite because of higher sea-water temperatures and increased susceptibility to infestation.

METHODS

Sea trout were captured at 14 locations (Fig. 1) by gill-netting in the upper reaches of estuaries. The majority of these fish were prematurely returning smolts but newly migrating fish may also have been caught by this procedure. Infestation parameters may therefore be underestimates.

Lice were removed under dissecting microscope and counted and staged using descriptions of Johnson and Albright (1991a). Chalimus 1 and 2 or 3 and 4 were not distinguished from each other. Parasitological terms follow Margolis *et al.* (1982).

RESULTS

Some 260 sea trout were sampled from 14 locations during May 1992 (Fig. 1). Lice infestation parameters are given in Table 1. The infestation levels were similar in intensity to those recorded in 1990 and 1991 (Tully *et al.* 1993) and the heaviest infestations also occurred in generally the same locations as in previous years; i.e. between Gal way Bay and Clew Bay (Fig. 1). There were indications, however, that in 1992 infestations on the south-west coast were higher than in previous years.

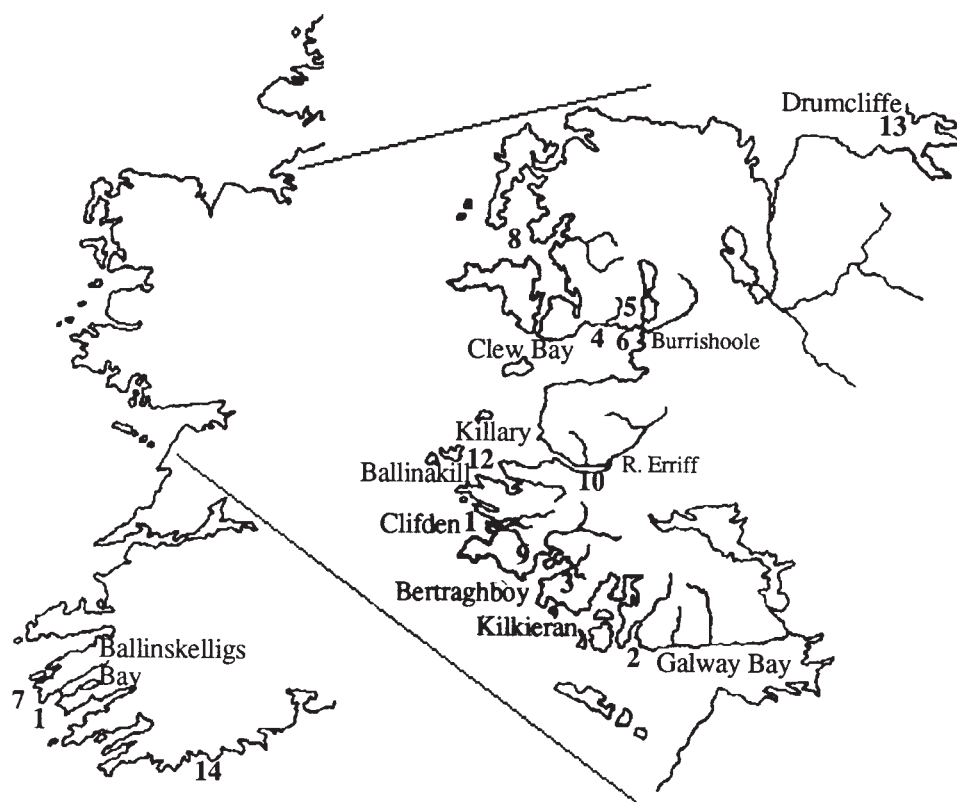


Fig. 1. Locations of 14 estuarine sites in the west of Ireland where sea trout were sampled during May 1992. Numbers represent the ranking of the site in median parasitic intensity as in Table 1.

Maximum recorded levels at two sites in this area were 47 and 36 in 1991 and 109 and 325 in 1992, indicating that a proportion of the population may have contracted heavy infestations in 1992.

In 1992 prevalence varied from 14.3% to 100%, mean intensity from 7 to 124.7, and the maximum number of lice recorded on an individual fish was 325. High maxima (>100 lice per fish) were recorded at ten of the 14 locations sampled. The abundance of lice varied significantly among sites (ANOVA of log-transformed data: $F=9.57$, d.f.=12, $p<0.0001$). Inter-site similarity in infestation parameters was defined, using data in Table 1, by a cluster analysis (Fig. 2). Three clusters of sites were recognized. Clusters formed statistically homogeneous groupings within which no significant differences in mean abundance of lice existed among constituent sites (cluster 1: $F=1.4$, $p>0.05$; cluster 2: $F=0.43$, $p>0.05$; cluster 3: $F=1.94$, $p>0.05$). Significant differences in both mean abundance and mean intensity were apparent among clusters (log abundance: $F=9.57$, d.f.=2, $p<0.0001$; log intensity: $F=8.08$, d.f.=2, $p<0.001$). Infestation parameters for each cluster of sites are given in Table 2. The level of infestation at sites in cluster 1 was lowest in prevalence, abundance, intensity and maximum numbers of lice per

Table 1. Infestation parameters for *L. salmonis* parasitic on sea trout post smolts at 14 locations in the west of Ireland during May 1992. N, number of fish; IQR, interquartile range; Max., maximum number of lice per fish. Locations are arranged in order of decreasing median intensity

Location	N	Prev. (%)	Abundance		Intensity			Max.	Mean fish length (cm)
			Mean	Variance	Mean	Median	IQR		
(1) Clifden	6	100.0	77.5	3896	77.5	79.5	116.0	154	21.3
(2) Costello	7	71.4	74.9	6887	104.8	69.0	134.5	235	23.3
(3) Gowla	14	100.0	75.4	3842	75.4	60.5	87.7	198	20.8
(4) Owengarve	45	95.5	70.5	4155	73.8	57.0	97.0	269	22.4
(5) Burrishoole	7	100.0	51.1	1128	51.1	56.0	48.0	107	21.9
(6) Newport	7	100.0	55.0	569	55.0	52.0	45.0	94	25.4
(7) Inny River	73	89.0	45.2	2748	50.8	39.0	61.5	325	21.5
(8) Owenduff	6	33.3	8.2	168	24.5	24.5	9.0	29	16.4
(9) Ballynahinch	5	80.0	14.2	162	17.5	21.5	26.0	27	27.3
(10) Killary	28	85.7	33.8	2379	39.4	19.5	39.0	166	24.2
(11) Currane	21	33.3	13.0	937	39.1	17.0	88.0	109	22.1
(12) Dowras	15	100.0	34.7	1386	34.7	14.0	55.0	123	21.7
(13) Drumcliffe	7	14.3	1.0		7.0	7.0	0.0	7	26.6
(14) Argideen	19	26.3	8.4	1097	31.8	3.0	74.0	145	21.9

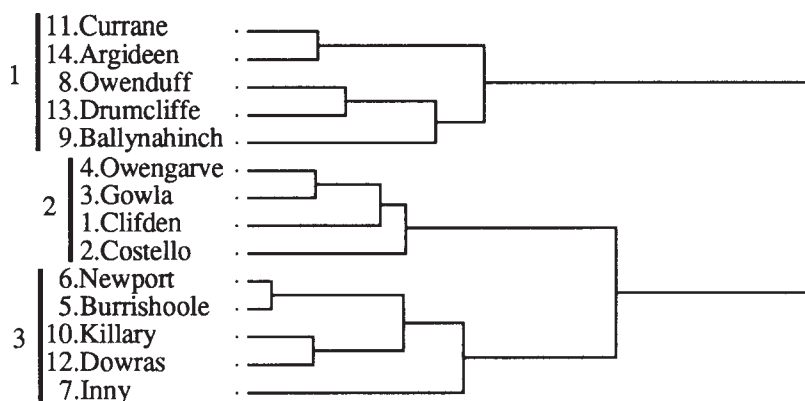


Fig. 2. Cluster analysis (Euclidean distance and complete linkage algorithms) of 14 locations using infestation parameters given in Table 1. Three clusters are recognized. Numbers represent the ranking of the site in median parasitic intensity as in Table 1.

fish. Fish from sites in cluster 2 had highest infestations (average intensity=76.7 lice per fish). The frequency distribution of lice among host fish is shown for each site cluster in Fig. 3. Cluster 2 had a greater proportion of fish in higher infestation classes than clusters 1 or 3.

Sites within individual embayments usually had similar levels of infestation. Three estuaries in Clew Bay—Owengarve, Burrishoole and Newport—ranked 4, 5 and 6 in

Table 2. Infestation parameters for *L. salmonis* parasitic on sea trout at three groups of locations in the west of Ireland during May 1992. N, number of fish; IQR, interquartile range. Locations were grouped by a cluster analysis (see Fig. 2) of the infestation parameters in Table 1

Cluster	N	Prevalence (%)	Abundance		Intensity			Maximum
			Mean	Variance	Mean	Median	IQR	
1	58	32.7	9.6	718	29.5	17.0	27.0	145
2	72	94.4	71.4	4160	75.5	60.0	99.0	269
3	130	90.7	42.4	2301	46.7	34.5	59.2	325

terms of infestation intensity. The Inny and Currane estuaries in Ballinskelligs Bay (Fig. 1) had mean infestation levels of 50.8 and 39.1 and ranked 7 and 11, respectively. Ballynahinch and Gowla in Bertraghboy Bay did not rank closely, although only five fish were sampled in Ballynahinch. In 1991 close ranking of infestation levels in estuaries within embayments was also apparent (Tully *et al.* 1993).

POPULATION STRUCTURE OF *L. SALMONIS*

As in 1990 and 1991 (Tully *et al.* 1993) the louse population was immature and dominated by chalimus stages (Table 3). No copepodids were recorded, indicating that infestations had not occurred for a number of days prior to the fish being captured. Most adult and preadult lice were recorded at sites where infestations were comparatively light, e.g. Owenduff, Drumcliffe, Argideen (Table 3). As in 1990 and 1991 heaviest infestations were invariably due to early developmental stages.

DISTRIBUTION OF LICE ON HOST FISH AND IMPACTS TO THE HOST

The highest percentage of lice, which, as indicated above were mainly chalimi, was attached to the fins, particularly the dorsal fin (Table 4). Significant numbers also occurred on the distal ends of the gill filaments of the first branchial arch. The gills of a number of fish were not examined as they had been taken for other analyses.

The morphological impact of lice on host sea trout was significant. The rays of the dorsal fin were exposed and extensive grazing marks and skin erosion were evident on the dorsal side of the fish. This morphological damage is correlated with physiological changes, including anaemia, hypoproteinaemia and increases in plasma sodium and chloride (unpublished data). Mortality of infested fish was directly observed each year. Mortality of fish as a result of lice infestation was also suggested in 1990 and 1991 by the persistent immaturity of the louse population over time. This could have occurred if fish died as the lice developed to preadult and adult stages although, alternatively, it could also have arisen because of mortality of the parasite during development (Tully *et al.* 1993). The epizootics have also coincided, geographically and temporally, with a drop in marine survival of post smolt and adult sea trout in the areas concerned (Whelan 1993).

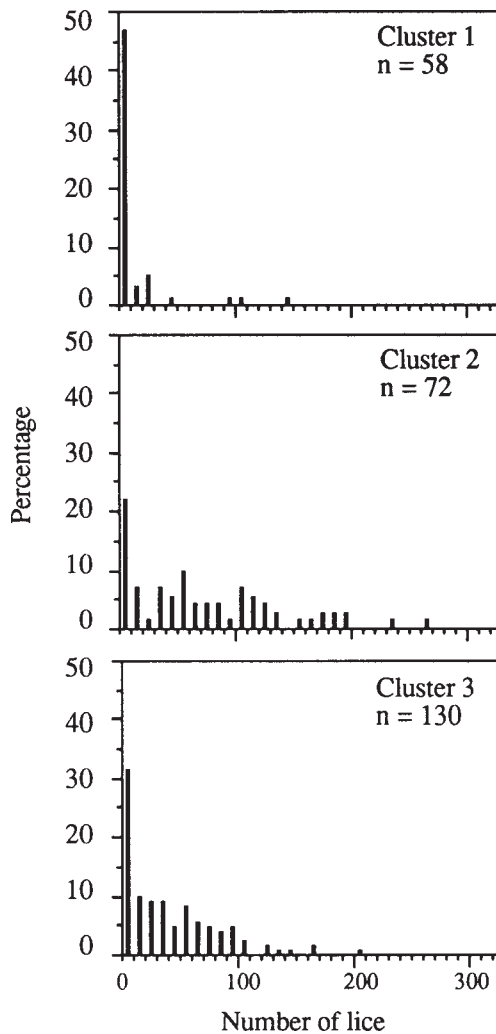


Fig. 3. Frequency distribution (in classes of ten) of *L. salmonis* among sea trout in three clusters of sites identified in Fig. 2. *n*, number of fish.

DISCUSSION

Sea lice epizootics in wild fish populations appear to be rare. A previous estimate of *L. salmonis* infestation of wild sea trout by Boxshall (1974) in the North Sea indicated a mean intensity of only four lice per fish (range 0–12). White (1940) documented an epizootic of *L. salmonis* on *Salmo salar* in the Moser River estuary of Nova Scotia which caused substantial mortality. Parker and Margolis (1964) reported fin loss and mortality in wild pink salmon smolts (*Oncorhynchus gorbuscha* (Walbaum 1792)) due to infestation by *Caligus clemensi* Parker and Margolis. Sockeye salmon (*Oncorhynchus nerka* (Walbaum 1792)) in the Alberni Inlet, British Columbia, suffered high mortality in the autumn of 1990, apparently

Table 3. Population structure of *L. salmonis* parasitic on sea trout at 14 locations in the west of Ireland during May 1992. C1,2, chalimus 1 and 2; C3,4, chalimus 3 and 4; PAM1 and 2 and PAF1 and 2, preadult male and female 1 and 2; AM, adult male; AF, adult female. Location codes are the same as in Table 1

Location	Life cycle stage							
	C1,2	C3,4	PAM1	PAF1	PAM2	PAF2	AM	AF
(1) Gowla	72.5	27.3	0	0.3	0	0	0	0
(2) Clifden	52.7	28.2	3.2	9.0	6.2	0.2	0.4	0
(3) Owengarve	23.8	42.7	11.8	7.0	6.4	2.4	3.8	1.6
(4) Burrishoole	46.6	42.2	5.9	2.8	1.1	0.8	0.6	0
(5) Costello	48.8	35.7	4.9	4.5	2.7	2.3	1.0	0
(6) Newport	32.5	45.5	10.1	4.2	4.4	1.3	2.1	0
(7) Inny	28.7	53.1	5.6	4.7	4.3	1.7	1.5	0.3
(8) Owenduff	18.4	32.6	2.0	4.1	4.1	0	16.3	22.4
(9) Ballynahinch	22.5	67.6	1.4	4.2	0	2.8	1.4	0
(10) Killary	26.6	59.3	6.5	2.3	3.2	1.0	1.0	0.2
(11) Currane	58.4	26.6	8.0	5.5	0.4	0.7	0.4	0
(12) Dowras	24.9	50.3	3.8	5.2	3.8	4.0	4.6	3.3
(13) Drumcliffe	0	0	0	0	14.3	0	42.9	42.9
(14) Argideen	32.7	20.7	1.9	3.8	3.1	3.1	14.5	20.0

Table 4. Distribution of *L. salmonis* on different areas of the body of post smolt sea trout sampled at 14 sites on the west coast of Ireland during May 1992. Sites are located geographically in Fig. 1. Codes are: OG, Owengarve; CN, Clifden; DR, Dowras; KH, Killary; BR, Burrishoole; NP, Newport; GL, Gowla; BA, Ballynahinch; CO, Costello; LC, L. Currane; IR, Inny; AR, Argideen; OW, Owenduff; DC, Drumcliffe

Body region	Site													
	OG	CN	DR	KH	BR	NP	GL	BA	CO	LC	IR	AR	OW	DC
<i>Fins</i>														
Dorsal	23.6	35.0	28.1	30.8	37.9	37.4	37.4	40.9	35.9	57.4	44.0	37.9	26.5	0.0
Caudal	10.4	8.3	8.6	8.0	10.6	8.0	13.2	12.1	10.6	9.7	9.6	1.7	10.2	0.0
Pelvics	9.5	7.4	7.4	7.0	6.8	4.8	9.7	12.1	3.7	8.0	6.7	4.4	10.2	0.0
Pectorals	4.7	1.4	2.9	2.7	3.0	1.2	1.6	1.5	3.2	0.3	2.9	3.8	0.0	0.0
Anal	8.3	12.3	8.4	5.1	8.1	4.3	14.2	4.6	6.3	6.6	5.5	7.7	6.1	25.0
<i>Other</i>														
Head	10.0	7.5	2.9	4.3	0.3	4.3	0.0	1.5	3.0	4.5	3.7	5.5	28.6	0.0
Body	22.4	22.0	28.8	20.8	23.7	29.2	23.4	22.7	23.1	12.5	22.9	36.8	18.4	75.0
Gills	3.6	1.0	5.1	15.1	7.8	8.4	0.5	1.5	1.0	1.0	3.4	1.1	0.0	0.0
Unknown	7.5	5.1	7.8	3.1	1.8	2.4	0.0	3.0	13.4	0.0	2.6	1.1	0.0	0.0

due to infestation by *L. salmonis* (Johnson personal communication). These fish returning from the ocean were infested with an average of 89 (range 9–209) lice, 44% of which were copepodids and 26% chalimi. Some of these fish sampled 100 miles from the coast had heavy infestations, including copepodids, indicating recent infestation in oceanic waters. Epizootics of *L. salmonis* on sea trout in the west of Ireland have now occurred 4 years in succession. Such a systematic occurrence of caligid epizootics is unparalleled and suggests a persistent causal factor(s).

Possible causes of epizootics of *L. salmonis* on sea trout

Increased production of the infective copepodid

In Ireland infestation of sea trout by *L. salmonis* developed to epizootic proportions in 2–3 weeks, indicating high transmission rates of the copepodid. In Norway transmission rates of *L. salmonis* to sea trout in 1992 appeared to be higher than this (Jakobsen personal communication). Elevated transmission rates may come about if the encounter rate between fish and copepodid is increased and/or if the ability of the fish to resist successful invasion by the copepodid is impaired. Because the copepodid of *L. salmonis* is obliged to find a host within days of moulting from nauplius II, its transmission must occur close to the location where the nauplii are produced. Sea trout remain in coastal areas during their marine life (Falkus 1986, Whelan 1989) so the infestation probably occurs in embayments into which the fish migrate. Local production of copepodids may therefore be important in determining the level of infestation that develops. Copepodids of the salmonid-specific *L. salmonis* are produced from farmed and wild Atlantic salmon (*S. salar* L.) and sea trout in the west of Ireland. On a finer spatial scale this production varies in different embayments and is determined primarily by the size of the stock of farmed salmon held at each location (Tully and Whelan 1993). This production may also vary between years both due to changes in the size of the farmed and wild salmon stocks and because of the effects of annually varying sea temperatures on the rate of development and on the number of generations the parasite produces (Tully 1993).

Production of the infective copepodid of *L. salmonis*, in the regions concerned here, has probably increased progressively each year since the advent of salmon farming in the area in 1982. This increase would have been due to increases in the stock size of farmed salmon and consequently in the number of available hosts. These fish are also present throughout the year, in contrast to wild salmon which frequent coastal areas only during periods of migration. Daily production of nauplius I of *L. salmonis* from individual salmon farms in six embayments in 1991, given in Tully and Whelan (1993), ranged from 0 to 4×10^7 per day. Farmed fish contributed over 95% of larval production in the areas where epizootics occurred in 1991. The correlation between production of nauplius I from farmed salmon in six embayments in mid-April 1991 and the subsequent intensity of the infestation of sea trout in these locations during May is shown in Fig. 4. This 3-week time lag was judged to be appropriate given the population structure of lice infesting sea trout at the time of sampling. A linear correlation between production and subsequent infestation levels is apparent up to a production of

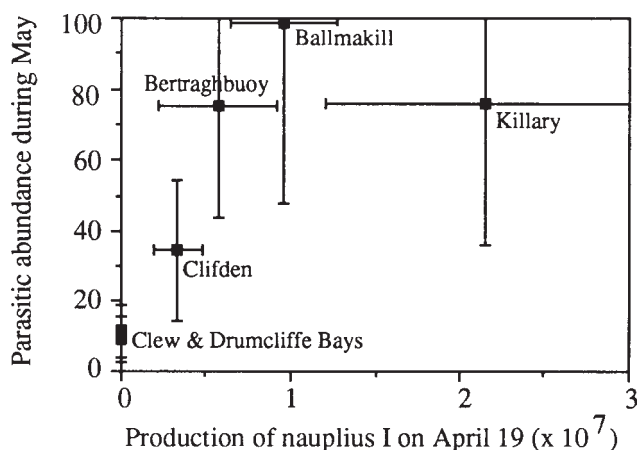


Fig. 4. Correlation between production of nauplius I of *L. salmonis* from farmed salmon in six embayments on 19 April 1991 and parasitic intensity on sea trout during the following May. Vertical and horizontal bars are 95% confidence limits (from Tully and Whelan 1993).

10^7 larvae per day, after which higher production appears to have no additional effect. This correlation was supported by events in 1992 where in three embayments changes in production, compared to that of 1991, led to predicted changes in the intensity of infestations of sea trout in these locations (Tully and Whelan 1993). The similar levels of infestation apparent in different estuaries sharing the same embayment in 1991 and 1992 also suggest that the sea area into which the fish migrates is important in determining the level of infestation that will develop and supports the idea that transmission rates may be correlated with densities of or encounter rates with the copepodid.

Production of larvae was correlated with subsequent infestations only for the period mid-April and early May. No correlation was established at any other time although production of larvae was maintained at April levels during May and June (Tully and Whelan 1993). This production did not, apparently, transmit to sea trout after May as no prematurely returning fish were observed in June. This possibly indicates temporally changing susceptibility to infestation. In 1992 all smolts had migrated from the Burrishoole system by mid-May and no heavy transmission of lice seems to have occurred later than that time. Physiological susceptibility or exposure to larvae may therefore be highest in the initial days after the fish enters sea water.

Increased rate of production of the infective copepodid

Increased production, rate of development and maturation of *L. salmonis* may have occurred in each year between 1989 (the first year in which heavy infestations were recorded) and 1991 relative to the previous 8 years because of higher winter and summer temperatures (Tully 1993). Integration of a temperature development rate function developed from data given in Johnson and Albright (1991b) with daily

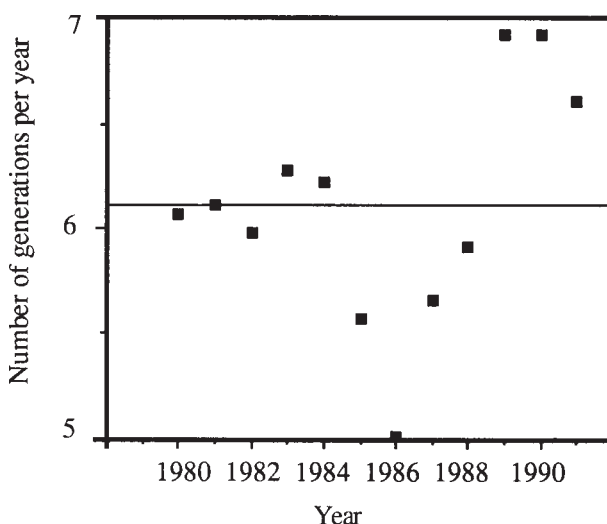


Fig. 5. The number of generations of *L. salmonis* produced in each year between 1980 and 1991, assuming that the rate of development is dependent only on temperature, and the larval production from each generation is retransmitted (from Tully 1993).

temperature data for the Irish north-west coast for the years 1980–1991 by Tully (1993) showed that the potential number of generations per year approached 7 in 1989–1991, compared with 5.5 in 1985–1988 (Fig. 5). Winter generation times in 1980–1991 varied between 95 and 125 days and were approximately 3 weeks shorter in each winter between 1989 and 1991 compared with earlier years. Rates of production and in particular larval release in spring were potentially higher, therefore, between 1989 and 1991. Temperature data for 1992 were not analysed.

Increased host susceptibility to infestation

To date, research into the cause(s) of epizootics of *L. salmonis* on wild sea trout has focused on the relationship between production of lice larvae and subsequent levels of infestation on sea trout (Tully and Whelan 1993). This presumes that infestation levels are determined by the number of copepodids to which the fish are exposed and that resistance to infestation is unimportant. However, in coho (*Oncorhynchus kisutch* (Walbaum 1792)) (Johnson and Albright 1992a) and chinook (*Oncorhynchus tshawytscha* (Walbaum 1792)) salmon (Johnson personal communication) infestations decline with time due to host tissue responses to the developing chalimus larva which impair development and, presumably, causes mortality. These responses are, however, weak in Atlantic salmon, a congener of sea trout, compared with the Pacific salmon species that have been studied (Johnson and Albright 1992a, Jones *et al.* 1990). No data on tissue responses of sea trout to sea lice infestation are available.

If inflammatory tissue responses are important in resisting infestation, then factors such as disease or environmental stressors that suppress the immune system may be important causal factors of caligid epizootics. Johnson and Albright (1992b) increased

susceptibility to infestation in coho salmon by injecting the fish with cortisol, which suppressed the host's inflammatory responses to the parasite. Physiological debilitation, possibly as a result of plankton blooms and high temperatures, are thought to have been responsible for the development of epizootics of *L. salmonis* on sockeye salmon in the Alberni Inlet, British Columbia, in 1990 (Johnson personal communication), although research was not carried out to verify this. The importance of fish health in species such as Atlantic salmon, which has an inherently low resistance to the invading copepodid, is unclear.

Conclusions as to the effects of disease or compromised immunity on susceptibility of sea trout to lice infestation must await experimental testing along the lines established by Johnson and Albright (1992a,b). However, extensive pathological analysis, in three separate laboratories in 1990 and 1991, did not reveal any disease in post smolt sea trout in the west of Ireland that may have increased susceptibility to infestation (unpublished data). Some pathology of the heart and gill hyperplasia were apparent but were not consistently present. Osmoregulatory competence of migrating smolts is thought to vary between years and in different river systems, but it is unknown if this has any effect on susceptibility to lice infestation when the fish enters sea water. Research into the cause of sea lice epizootics on sea trout should now be focused on establishing the natural response of *S. trutta* to invading sea louse copepodids, and if this is significant on identifying the parameters that can impair this response.

ACKNOWLEDGEMENTS

The staff of the Western Regional Fisheries Board and Dr Paddy Gargan of the Central Fisheries Board collected fish samples. This work was partly funded by the Sea Trout Action Group. We express our gratitude to Dr Stewart C. Johnson, Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, for allowing us to quote his unpublished observations on sockeye salmon.

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Part II Control of sea lice

Part IIa Review

17

Review of methods to control sea lice (Caligidae: Crustacea) infestations on salmon (*Salmo salar*) farms

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ABSTRACT

Sea lice, *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordmann (Copepoda: Crustacea), damage, weaken and kill salmon, may transmit microbial pathogens, and farm infestations may impact upon wild salmonids. A comparison of infestations between wild and farmed fish shows a usually higher prevalence and abundance on the latter, indicating enhanced transmission of sea lice in farm conditions. The distribution of the planktonic stages is unknown, and factors determining survival, fecundity and successful establishment on hosts are unclear. The two lice species differ in size, host specificity, geographic distribution, adult planktonic activity, sensitivity to fresh water, and probably other biological features which will influence control strategies.

The choice of a control method depends upon its efficacy, stress to the fish, environmental effects, cost, hazard to staff, marketing implications and ease of application. These factors are discussed for the range of methods considered, namely (a) disorientation of lice by light-traps and shading of cages, (b) hanging of cut onions in cages, (c) chemical baths of dichlorvos, trichlorfon, azamethiphos, carbaryl and hydrogen peroxide, (d) addition to feed of trichlorfon, ivermectin, onions, garlic and diflubenzuron, (e) dips in pyrethrum and dichlorvos, (f) placing cleaner-fish (wrasse) in cages with salmon, and (g) development of other biological control methods including vaccines. Each method has benefits and weaknesses, and will vary in adaptability to different farm conditions. Alternatives are essential to enable control under different farming conditions and prevent development of resistance by lice (as may already have occurred through the use of a single chemical, dichlorvos).

Farm management practices should minimize the likelihood of lice infestation, and include fallowing and use of wrasse. Lessons should be learned from the environmental criticism of Nuvan use in the development of alternative chemotherapeutants. Further research, notably on lice biology, use of wrasse, and improvement of the natural defences of salmon against lice attack, is recommended.

INTRODUCTION

Sea lice (Caligidae: Crustacea) became a problem in commercial Atlantic salmon and rainbow trout (*Salmo salar* L. and *Oncorhynchus mykiss* (Walbaum)) farms as the industry developed in the 1960s in Norway (Hastein and Bergsjø 1976), 1970s in Scotland and Shetland (Rae 1979, Wootten *et al.* 1982), and in Ireland (Tully 1989; see also Chapter 15) and France (Messenger and Esnault 1992). Today they are the most commercially limiting parasite in salmonid culture in northern Europe. With a few exceptions, the more recently developed salmon farms in Canada, and sea bass (*Dicentrarchus labrax* (L.)) and bream (*Sparus aurata* L.) culture around the Mediterranean have not yet suffered commercially significant infestations. A few farms in Canada have had lice problems (Hogans and Trudeau 1989, Stuart 1990), and lice have occurred on farms in Greece (Papoutsoglou personal communication).

In northern Europe and Canada two species dominate salmonid farm infestations, namely *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordmann, with the former being the most significant (Pike 1989). *C. elongatus* has also caused problems in red drum culture in the USA (Landsberg *et al.* 1991). Other sea lice species have also caused significant problems: *Caligus spinosus* Yamaguti on yellow tail (*Seriola quinqueradiata* Temminck and Schlegel) in sea-cages in Japan (Izawa 1969); *Caligus orientalis* Gussev on rainbow trout in sea-cages in Japan (Urawa and Kato 1991); *Caligus epidemicus* Hewitt on Mozambique tilapia (*Oreochromis mossambicus* (Peters)) in ponds in Taiwan (Chapter 1); *Caligus minimus* (Otto) on sea bass in research aquaria in Portugal (Menezes personal communication); and *Pseudocaligus apodus* Brian on mullet (*Mugil cephalus* L.) in ponds in Israel (Paperna 1975). Other species of lice occasionally occur on farmed fish in different parts of the world but in low numbers (Roth *et al.* in press).

Lice are a problem to salmon farmers because infestations (a) kill farmed salmon, (b) weaken the salmon such that they succumb to secondary infections, (c) may transmit microbial pathogens, and (d) can reduce the market value of the fish. Additionally, the production of lice from salmon farms may result in pathogenic infestations on wild fish. The symptoms of infection range from mucus removal, to broken skin and eventually removal of the skin and exposure of the underlying muscle, bone, nervous and other tissues. Most damage is caused by the larger mobile adults, but the sessile chalimus stages can also damage fish (Jones *et al.* 1990, Johnson and Albright 1992a). Lice feed by pressing their tubular mouths onto the host skin and grazing the tissues by a sawing action of the mouthparts (Kabata 1974, 1979, Boxshall 1990b). Fish are sometimes under stress before obvious external lesions are apparent, and die following impaired ion regulation and metabolic efficiency (Tully unpublished). These symptoms may be a general stress response due to irritation caused by the lice. Irritation by protozoan skin infections can kill chum salmon (Urawa 1992).

The transmission by lice of a viral-sized salmon pathogen (agent of infectious salmon anaemia) has been demonstrated in the laboratory, and lice can also carry the bacterium *Aeromonas salmonicida* (agent of furunculosis in salmon) on their body surface (Chapter 28). Considering that preadult and adult lice move between salmon in sea cages (Jaworski and Holm 1992), the transmission of viral and bacterial pathogens by lice appears probable on commercial farms.

While lice are widespread on wild fish (e.g. Boxshall 1974a, Kabata 1979, Panasenکو *et al.* 1986, Nagasawa 1987, Neilson *et al.* 1987), potentially pathogenic infestations of lice have rarely been recorded. Heavy infestations have been found of *C. elongatus* on herring (*Clupea harengus* L.) (over 200 lice on some fish) (MacKenzie and Morrison 1989), of *L. salmonis* on Atlantic salmon returning to rivers (White 1940, 1942), and of *C. minimus* on sea bass (Paperna 1980). An infestation of wild cod (*Gadus morhua* L.) and haddock (*Melanogrammus aeglefinus* L.) by *C. elongatus* was described by Neilson *et al.* (1987), and results suggested that levels on haddock may have been pathogenic. The collapse in numbers of sea trout (*Salmo trutta* L.) returning to rivers in the mid-west of Ireland was linked with an epizootic of sea lice on smolts, and their premature return to the river mouths (Tully *et al.* 1993; see also Chapter 16). Experimental releases of sea trout in Norway suggest that it is the lice infestations that cause the premature return to the river (Jakobsen, personal communication). As it is probable that these lice originate from farm populations (Tully and Whelan 1993; see also Chapter 16), then the control of sea lice on farms will also have benefits for wild salmonids.

Other crustaceans can have pathogenic effects on farmed and wild fish in both marine and fresh-water environments, and lessons learned from efforts to control one species may have applications to others. The copepod *Ergasilus lizae* Krøyer caused significant losses of farmed mullet (*M. cephalus*) in the Middle East (Paperna 1975). In a brackish water farm in Canada, *Ergasilus labracis* Krøyer caused significant mortalities of Atlantic salmon parr (O'Halloran *et al.* 1992). The marine isopods *Gnathia* sp. (Drinan and Rodger 1990) and *Nerocila orbignyi* (Guérin-Meneville) (Douëllou *et al.* 1983, Bragoni *et al.* 1984) have been recorded on farmed salmon in Ireland and sea bass in Corsica, respectively, the latter causing commercially significant mortalities. In fresh water, infestations of the branchiuran *Argulus foliaceus* (L.) on caged rainbow trout caused the closure of a farm in Portugal (Menezes *et al.* 1990), and a natural epizootic of *A. foliaceus* was the most likely cause of the collapse of the perch (*Perca fluviatilis* L.) population in Lake Windermere, England (Pickering and Willoughby 1977, Bucke *et al.* 1979, Craig *et al.* 1979, Elliott personal communication).

It is clear that a range of species of sea lice and other ectoparasitic crustaceans are common on wild fish and will infest cultured fish. Transmission of lice from wild to farmed, between farms, and probably from farmed to wild, can occur. Prevention and control of lice epidemics demand a thorough knowledge of the life cycle, population dynamics and transmission mechanisms of each species.

SEA LICE

Aspects of the life history of sea lice have been described for *L. salmonis* in several studies (White 1942, Hastein and Bergsjø 1976, Johannessen 1978, Wootten *et al.* 1982, Hogans and Trudeau 1989, Tully 1989, Bron *et al.* 1991, Johnson and Albright 1991a,b), and to a lesser extent for *C. elongatus* (Wootten *et al.* 1982, Tully 1989), reflecting its generally lower abundance on salmon farms. Other species of lice, notably *Lepeophtheirus pectoralis* (Müller), have been studied in greater detail (e.g. Boxshall 1974b, Anstensrud 1990a,b,c, 1992). *Lepeophtheirus*

and perhaps *Caligns* species appear to have ten life stages: two non-feeding photopositive planktonic nauplii, one infective swimming copepodid, four feeding chalimus stages sessile on the host skin, two preadults 'mobile' over the fish skin, and one mature and 'mobile' feeding adult. Mobile lice swim rapidly, by a form of jet propulsion over the host surface, and are also agile when free swimming in sea water (Kabata and Hewitt 1971). They maintain position on the host by orientating to face the current, by suction and by the use of posteriorly directed processes (Kabata and Hewitt 1971). Kabata and Hewitt's (1971) observations indicated that caligid loss from a host was more likely when the host was stationary.

The male holds onto preadult females during precopulatory mate guarding (Boxshall 1990a). After the female's final moult into the adult, he deposits a pair of spermatophores over the female's genital apertures (Chapter 12). These spermatophores can supply sufficient sperm for the fertilization of several clutches of eggs extruded (in long, paired sacs) by the female. Female size, egg string length and number of eggs per string vary seasonally, being greater in winter (Tully 1989, Tully and Whelan 1993; see also Chapter 12). The average number of eggs in a pair of egg sacs of *L. salmonis* from farmed Atlantic salmon has been recorded as ≤ 700 (Wootten *et al.* 1982), 1220 (Taylor, 1987), 192 (Hogans and Trudeau 1989), 107–315 (Tully 1989), 345 (Johnson and Albright 1991a), 284–492 (Chapter 12), and 251–715 (Tully and Whelan 1993). However, Tully and Whelan (1993) found that at a similar place and time, ovigerous females on wild Atlantic salmon were bigger and had twice as many eggs (average 977 per female) compared to farmed fish. In *C. elongatus* 178 eggs have been recorded per pair of egg sacs (Hogans and Trudeau 1989). These species of sea lice thus appear to produce a few hundred eggs at one time per female—a not particularly high fecundity for organisms with planktonic larvae.

In *L. salmonis* at 10°C, eggs hatch after c. 8 days' incubation, the nauplius may last 4 days, copepodid 10 days, chalimus stages 25 days, and preadults 17 days (male) to 22 days (female) (Johnson and Albright 1991a). The longevity of adult lice is unknown. Generation time is about 6–12 weeks for *C. elongatus* and 7–13 weeks for *L. salmonis* at summer (10–16°C) temperatures (Tully 1989, Hogans and Trudeau 1989, Johnson and Albright 1991a). Growth rates appear to be largely temperature dependent, such that in warmer years there are extra generations with a potential for an exponential increase in lice abundance (Tully 1989, in press).

The distribution of the nauplii and copepodids in the sea is unknown, although several attempts have been made to find them adjacent to salmon cages (Tully personal communication; see also Chapter 15). The copepodids of *L. salmonis* may live from 2 to 13 days in the plankton while searching for a host (Wootten *et al.* 1982, Johnson and Albright 1991a). In copepodids, positive phototaxis and thigmotaxis (Hogans and Trudeau 1989, Jakobsen, personal communication) have been recorded, but chemosensory responses to host blood, bile, faeces, skin and mucus appear lacking (Chapter 10). Initial host detection is probably by means of mechanosensory setae on the copepodid antennules (Chapters 7 and 9), while chemoreceptors may be utilized when host attachment has occurred. Copepodids of the lernaepodid copepod *Salminicola edwardsii* (Olsson) detect host movements,

and in aquaria more active fish acquire more parasites, which in turn increase host activity (Poulin *et al.* 1991). Copepodids of another parasitic copepod, *Lernaeenicus sprattae* (Sowerby), migrate towards the sea surface at night where their host, *Sprattus sprattus* (L.), congregates (Schram and Anstensrud 1985). In the case of *L. salmonis*, it may be hypothesized that nauplii and copepodids may control their vertical distribution in the water column, perhaps using diel and tidal activity patterns, such that they lie in the path of salmonids migrating to sea. In contrast, *C. elongatus* has many widely distributed hosts (Kabata 1979) and may have adopted a more mobile adult phase to ensure successful host colonization. Adults of the fresh-water branchiuran *Argulus japonicus* Thiele are chemosensitive to hosts (Galarowicz and Cochran 1991), and caligid mobiles may use chemical, physical and visual cues to detect hosts.

Important differences between *L. salmonis* and *C. elongatus* include their size, host specificity, mobility, sensitivity to fresh water, and geographical distribution. The former is widely distributed in northern Atlantic and Pacific waters, but *C. elongatus* appears to have a worldwide distribution. Considering body size, *L. salmonis* is larger (5–7 mm male, 7–18 mm female) and consequently more damaging per individual than *C. elongatus* (4–5 mm male, 5–6 mm female). *L. salmonis* is specific to salmonid fish (Kabata 1979) and has only rarely been recorded on other species in close contact with farmed salmon (Bruno and Stone 1990). It also has less variety of protease enzymes than *C. elongatus*, perhaps reflecting its greater host specificity (Ellis *et al.* 1990). In contrast *C. elongatus* occurs on at least 80 different fish species (Kabata 1979) and appears more mobile than *L. salmonis*. Adult *C. elongatus* have been recorded in the plankton (e.g. Neilson *et al.* 1987) and farm infestations can begin as preadults and adults rather than copepodids (Chapter 19). While attached to a host, *L. salmonis* can survive several days in fresh water (Ashby 1951, Hahnenkamp and Fyhn 1985, McLean *et al.* 1990), whereas *C. elongatus* will only survive for hours (Landsberg *et al.* 1991).

These differences have implications for lice infestations on salmon farms; for example, *C. elongatus* is less likely to occur at pathogenic levels in brackish water, but their arrival may be less predictable owing to the variety of host species from which they may originate and their mobility as adults. Furthermore, studies of *L. salmonis* on salmon farms indicate that both self-infection and cross-infection of adjacent farms are the dominant methods of lice transmission (Wootten 1985, Tully 1989, Jaworski and Holm 1992, Costello unpublished). Hence breaking the lice population cycle at adjacent farms may be more effective for *L. salmonis* than *C. elongatus*.

On wild halibut (*Hippoglossus hippoglossus* (L.)) (Schram and Haug, 1988) and farmed salmon (Jaworski and Holm 1992), sea lice intensity tends to increase with host size. This may reflect increased fish exposure to lice due to greater body size, time at sea and duration of infestation. However, Jaworski and Holm (1992) found that intensity per unit area of (farmed) salmon skin did not change with fish size. This suggests that intraspecific competition for preferred sites on the host may also limit lice densities.

There are marked differences in infestations on wild and farmed fish. Prevalence (number of fish with lice) and mean abundance (average number of lice per fish

sampled) are usually low on wild fish but high on farmed fish (Table 1). Whereas lice abundance generally shows a decreasing curve in wild fish, it often approaches a normal distribution in farmed fish (Fig. 1). Similarly, Paperna (1980) found *C. minimus* had a log-normal distribution on wild sea bass. Such patterns presumably reflect the intensive nature of salmon cultivation whereby parasite transmission is uninhibited. When smolts are put to sea they initially show the pattern of wild fish, but soon the median abundance increases and a normal distribution develops, as shown by examples for Atlantic salmon at 4 and 13 months at sea at a salmon farm in Ireland (Fig. 1). However, whether the variation in intensity within a salmon cage is due to random host colonization or to varying host susceptibility or acceptability to lice is unknown.

SEA LICE CONTROL

Factors to be considered

The choice of a sea lice control method will depend on (a) its efficacy in removing lice, (b) the stress it causes to the fish, (c) financial cost, (d) hazard to staff, (e) environmental effects, (f) marketing implications, (g) availability, and (h) ease of application.

If only mobile lice are removed then further treatment will be necessary within a few weeks when chalimus stages have matured. Short-term treatments, such as chemotherapeutants, should be applied when most mobile lice are at the preadult stage, and egg-bearing females have not yet appeared. Should the proportion of lice removed be < 100% it is likely that the survivors will be the most tolerant individuals. Hence the treatment will select for resistance within the lice population. When continuous methods of control are employed (e.g. cleaner-fish), the rate of lice removal must be balanced against the initial size of the lice population and its subsequent growth.

Both the treatment itself (e.g. pesticide) and its method of use (e.g. bath, dip) may stress the fish. Such stress will reduce feeding and growth, reduce food conversion efficiency, and render fish more susceptible to secondary infections. These effects, if severe, will limit the productivity and commercial viability of the farm.

Pesticides can be harmful to farm staff unless safety precautions are taken. Special training and clothing may be required in the use of the pesticide, and staff handling the pesticide may require regular medical screening. Additionally, in exposed sea conditions, certain sea lice control measures may be physically difficult and hazardous to apply. For example, enclosing a large cage in plastic tarpaulin in strong currents or winds for bath treatment is difficult and can result in billowing of the tarpaulin and possible loss of fish and pesticide (Darwall personal communication).

Marketing of salmon may be adversely affected by reports of negative environmental impacts of farming methods and of pesticide residues in fish tissues. Hence the withdrawal period of a pesticide from the fish must be long enough to ensure that no residues are detectable in the market-place. Furthermore, the farm production methods

Table 1. Proportion of fish with lice (prevalence), and range and mean number of lice per fish (abundance) recorded in wild and farmed fish. Except for Schram and Haug (1988) and Nagasawa (1987), data include chalinus and mobile lice, but numbers of chalinus may have been underestimated

Host	Locality	No. fish	Prev. (%)	Abundance		Reference
				Range	Mean	
WILD FISH						
<i>Lepeophtheirus salmonis</i>						
<i>Salmo salar</i>	Norway	155	94	0-40	7.4	Bristow and Berland (1991)
<i>Salmo trutta</i> ^a	Ireland	76	86	0-91	15.8	Tully <i>et al.</i> (1993)
<i>Salmo trutta</i> ^b	Ireland	56	85	0-436	84.7	Tully <i>et al.</i> (Ch. 16)
<i>Oncorhynchus gorbuscha</i>	Central N Pacific	1757	19	0-9	0.32	Nagasawa (1987)
<i>Oncorhynchus nerka</i>	Central N Pacific	2309	0.6	0-9	0.01	Nagasawa (1987)
<i>Oncorhynchus keta</i>	Central N Pacific	1540	15	0-10	0.26	Nagasawa (1987)
<i>Oncorhynchus kisutch</i>	Central N Pacific	306	11	0-3	0.14	Nagasawa (1987)
<i>Oncorhynchus tshawytscha</i>	Central N Pacific	126	46	0-25	1.67	Nagasawa (1987)
<i>Oncorhynchus mykiss</i>	Central N Pacific	57	28	0-3	0.44	Nagasawa (1987)
<i>Lepeophtheirus hippoglossi</i>						
<i>Hippoglossus hippoglossus</i>	Norway	174	72	0-38	3.4	Schram and Haug (1988)
<i>Lepeophtheirus</i> sp.						
<i>Onus</i> spp.	North Sea	963	8	0-15	2.8	Hartmann (1969)
<i>Caligus elongatus</i>						
<i>Salmo salar</i>	Norway	155	16	0-3	1.4	Bristow and Berland (1991)
<i>Gadus morhua</i>	NW Atlantic	413	—	0-14	6.2	Neilson <i>et al.</i> (1987)
<i>Melanogrammus aeglefinus</i>	NW Atlantic	310	—	0-36	2.1	Neilson <i>et al.</i> (1987)
<i>Caligus minimus</i>						
<i>Dicentrarchus labrax</i>	Mediterranean	480	4-100	0-200	—	Paperna (1980)

Continued

Table 1.1 *Continued*

Host	Locality	No. fish	Prev. (%)	Abundance		Reference
				Range	Mean	
FARMED FISH						
<i>Lepeophtheirus salmonis</i>						
<i>Salmo salar</i>	Ireland	270	44–100	0–7	—	Tully (1989)
<i>Salmo salar</i> ^c	Ireland	480	99.9	0–22	4.5	Costello (unpubl.)
<i>Salmo salar</i> ^d	Ireland	464	100	3–87	24.2	Costello (unpubl.)
<i>Salmo salar</i> ^d	Scotland	145	—	0 ≥ 29	27.5	Wootten (1985)
<i>Salmo salar</i> ^e	Norway	40	100	15–53	33.2	Bjordal (1992)
<i>Salmo salar</i> ^f	Norway	40	100	3–28	14.1	Bjordal (1992)
<i>Caligus elongatus</i>						
<i>Salmo salar</i>	Ireland	270	69–100	0–8	—	Tully (1989)
<i>Caligus spinosus</i>						
<i>Seriola quinqueradiata</i>	Japan	92	—	0–636	28.7	Izawa (1969)

^aWild infestation and ^bsevere epizootic in 1991, ^c3 weeks after removal of wrasse, ^dsubject to regular Nuvan treatments, ^ebefore and ^fafter wrasse added

and particularly the use of biocides must be shown to have no negative environmental impacts.

A control method must be available when needed. Availability is a function of the source of treatment, its distribution within a country, and whether it can be stored in readiness for use. A method of sea lice control should also require minimal staff time and effort, including both purchasing the treatment, training staff in its use, and in the staff time and equipment involved in its application. In considering sea lice control costs, both the initial purchase cost of material and the longer-term costs with respect to fish growth and survival, and marketability of the fish should be evaluated.

Methods considered

Almost all the on-growing of salmonids in sea farms is in cages with volumes commonly ranging from 1000 m³ to 3000 m³, but in a wide range of sea conditions of tidal range, current, swell and wind exposure. While this chapter largely reviews the methods considered for use in sea-cages, these and additional methods could also be used in land-based farms.

A range of methods has been considered for controlling sea lice, and some information is available for the chemicals dichlorvos, trichlorfon, azamethiphos, carbaryl, ivermectin, pyrethrum and hydrogen peroxide (Table 2), and for physical and biological methods.

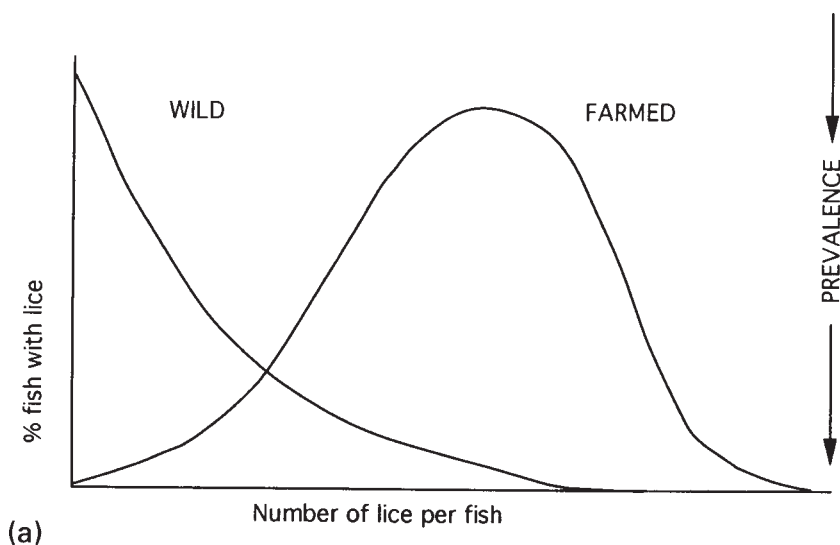


Fig. 1. Relationship between sea lice infestation (percent fish infested) and abundance (number of lice per fish), (a) Diagrammatically. (b) Data for wild halibut (♦, $n=115$) in Norway (from Schram and Haug 1988), and wild sea trout in Ireland (from Tully *et al.* 1993). Sea trout were from localities with mild (□, $n=72$) and severe (■, $n=59$) epizootics in 1991. (c) Farmed Atlantic salmon in Norway (triangles) (from Bjørndal 1992) and Ireland (circles) (Costello unpublished). Farmed salmon were at sea for 4 (○, $n=401$), 6 (triangles) and 13 (●, $n=459$) months. Data for Norwegian salmon are before (▲, $n=40$) and 24 h after (△, $n=40$) the addition of cleaner-fish to the cages.

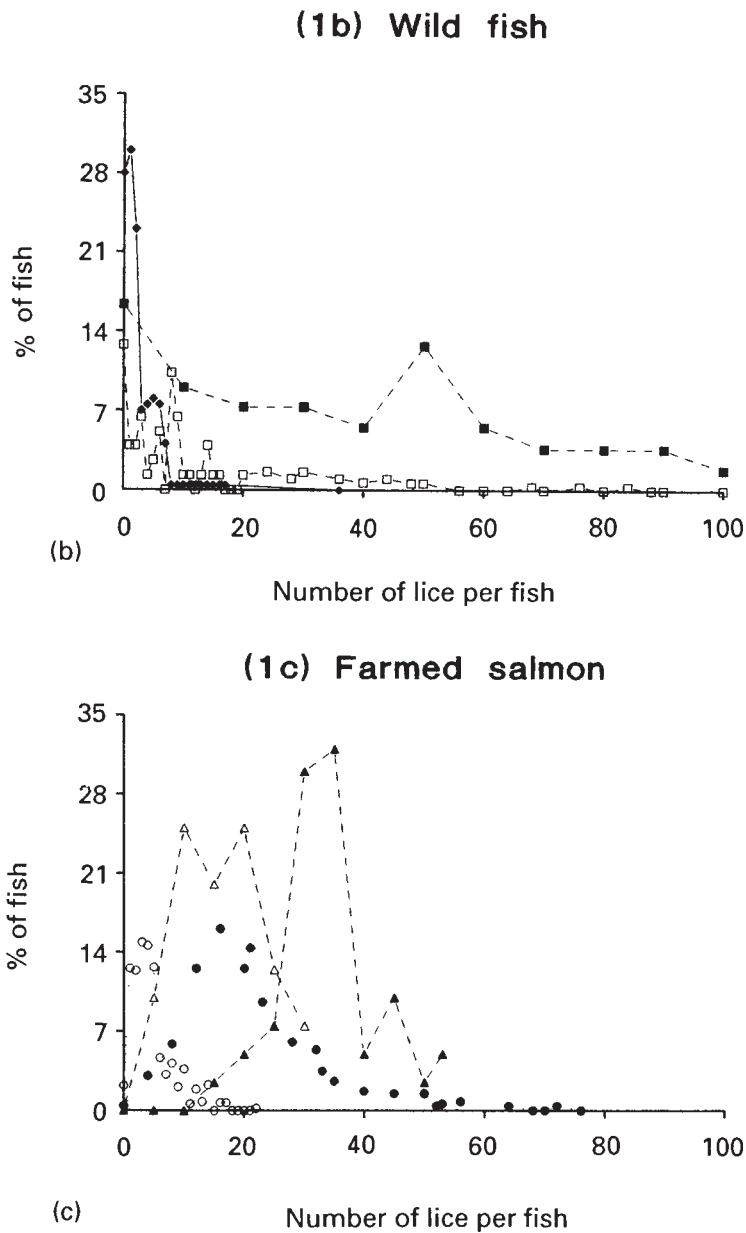


Fig. 1 (b), (c).

Physical methods

Physical methods of lice control may be possible at land-based farms using filtration or other water treatment, and a reduction in cage net mesh size successfully prevented infection by an isopod parasite (Bragoni *et al.* 1984). Such techniques are impractical

Table 2. Features of chemical treatments used and considered for use in the control of sea lice in salmon cages. Values for toxicity range are lowest levels at which significant toxicity was recorded in short-term lethality tests.

Trade name:	Nuvan/Aquagard	Neguvon	Alfacron	Sevin	Ivomec	Py-Sal	Hydrogen peroxide
Active ingredient	Dichlorvos	Trichlorfon	Azamethiphos	Carbaryl	Ivermectin	Pyrethrum (pyrethrin mixture)	Gas embolism
Mode of action	Inhibits AChE	Inhibits AChE	Inhibits AChE	Inhibits AChE	Uncertain	Uncertain	
Treatment Method	Bath	Bath	Bath	Bath	Oral (in feed) 1% body wt d ⁻¹	Dip	Bath
Exposure to active ingredient	1 mg l ⁻¹ h ⁻¹	300 mg l ⁻¹ h ⁻¹	0.01 mg l ⁻¹ h ⁻¹	0.3–0.5 mg l ⁻¹ h ⁻¹	0.05–0.2 mg kg ⁻¹ d ⁻¹	10 g l ⁻¹ 30 s ⁻¹	1.5 mg l ⁻¹ 20 min ⁻¹
Quantity per 1000 kg salmon ^a	67 g h ⁻¹	20 100 g h ⁻¹	0.67 g h ⁻¹	20–34 g h ⁻¹	0.05–0.2 g d ⁻¹	480 g	100 kg 20 min ⁻¹
Frequency (summer)	1–2 mo ⁻¹	1–2 mo ⁻¹	1–2 mo ⁻¹	1–2 mo ⁻¹	< 2 wk ⁻¹	?	0.5 mo ⁻¹
Lethal toxicity range	(> 3.0 mg l ⁻¹)	(> 3.0 mg l ⁻¹)	(> 1.0 mg l ⁻¹)	(> 2.0 mg l ⁻¹)	(> 0.4 mg kg ⁻¹)	(10 g l ⁻¹)	(> 2.0 mg l ⁻¹)
Salmon	3.0	—	1.0	2.0	0.4	> 2 min	2.0
Safety margin ^b	× 3	Variable	< × 100	× 7–× 4	× 8–× 2	× 4	× 1.3
Acceptable human intake (mg kg ⁻¹ d ⁻¹)	0.004	?	?	0.01	0.2 as medication for nematode parasites	0.04	?
Withdrawal period	2 wk (Norway) 4 d (UK)	3 wk (Norway)	?	> 28 d	> 700 ^c d	7 d	0 d
Half-life in sea water	5.1 d at 4.5°C 3.9 d at 13.5°C (pH 8, well aerated)	3.8 d at 4.5°C 1.2 d at 13.5°C (pH 8, well aerated)	?	4 d at 18°C 38 d at 8°C 25 d at 30°C	?	?	?
Toxicity of degradation products	Less	Greater (× 100)	?	Greater to fish and molluscs but less to crustaceans	Less	Less	Less

^aFor bath treatments calculated on basis of stocking density of 15 kg m⁻³. ^bDifference between treatment concentrations toxic to lice and salmon. Abbreviations: d, day(s); °d, degree days; AChE, acetylcholinesterase; ?, no published data.

at sea-cage salmon farms when the water flow required and small size of larval lice (0.5–1.0 mm) are considered. Huse *et al.* (1990) unsuccessfully attempted to reduce infection by the photopositive copepodid by shading salmon pens and placing light-traps beside cages. These physical methods attempt to interrupt parasite transmission. However, infestation patterns (Fig. 1) indicate that transmission is optimized in intensive fish culture, and once established on a farm it will be difficult to interfere with parasite transmission *per se*.

Chemical methods

Most attempts at sea lice control have resorted to the use of chemicals, and their toxicity and pharmacokinetics have been reviewed by Roth *et al.* (1993).

Early efforts to control lice in Norway tried formalin and acetic acid baths with limited success (Hastein and Bergsjø 1976). Landsberger *et al.* (1991) found a 30 min fresh-water dip more effective against *C. elongatus* on red drum in tanks than copper, formalin and trichlorfon. There have been anecdotal reports of reductions in lice infestations following the addition of garlic to salmon feed and hanging of bags of sliced onions in salmon cages (e.g. Anon. 1991a). However, a trial with cut onions hung in cages in Scotland had no significant effect on chalimus and mobile lice (Treasurer personal communication). Furthermore, a diet of 10% onions in Norway showed no effect on lice numbers (Boxaspen and Holm 1992). Salmon fed a diet of 10% garlic had a 17% reduction in lice levels in preliminary trials in Norway (Boxaspen and Holm 1992), but salmon would not eat a 10% garlic diet in a Scottish trial (Treasurer personal communication). Additionally, the possibility of garlic having toxic side effects on the salmon remains to be investigated (Roth *et al.* 1993).

Chemotherapeutants: mode of action

Only two pesticides have been licensed for use on salmon farms, namely the organophosphates trichlorfon (as Neguvon) and dichlorvos (as Nuvan and Aquagard) (in Iceland, Norway and the UK; Schlotfeldt 1992, Buchanan 1992). However, others may be given under veterinary prescription or have been licensed for research trials. Other organophosphates, such as azamethiphos and malathion, and the carbamate carbaryl, all act by inhibiting activity of the enzyme acetylcholinesterase (AChE). Lowered levels of AChE result in an accumulation of the neurotransmitter acetylcholine and, ultimately, death by overstimulation of the nervous system (World Health Organization 1986). All stages of lice have AChE, but for unknown reasons the chalimus stages appear unaffected by dichlorvos (Walday and Fonnum 1989). In Scotland (Jones *et al.* 1992) and Ireland (Tully and McFadden unpublished) evidence suggests that some lice populations have developed a resistance to dichlorvos (as Aquagard and Nuvan, respectively). Cross-resistance to other AChE inhibitors may also have occurred.

Salmon and people also have AChE. Oxygenation during treatment significantly assists salmon in maintaining AChE levels, and salmon must not be treated until AChE levels have been restored following treatment (Raverty 1987, Höy *et al.* 1991). Baths of dichlorvos and trichlorfon (10–30 mg/l for 15–60 min) can affect the immune system of carp (Dunier and Siwicki 1992), and probably that of salmonids. Staff using

dichlorvos should have their own cholinesterase levels regularly monitored (Health and Safety Executive 1987, Wells 1989).

Pyrethrum is a naturally occurring mixture of 2.5% pyrethrins. It is combined with a synergist piperonylbutoxide (4%) and a paraffinic oil solvent (Exxon D100) (92%) for treatment of lice (Jakobsen and Holm 1990, Boxaspen *et al.* 1991, Boxaspen and Holm 1992). While known to affect nerve membranes, its mode of toxicity is not fully understood (Chapter 20). One possible advantage of pyrethroids over the similarly fast degrading organophosphates is that they may have effects on chalimus stages (Jakobsen personal communication; see also Chapter 20).

The mode of toxic action of ivermectin is poorly understood (Turner and Schaeffer 1989), but it does concentrate in the fatty and nervous tissues of salmon (Høy *et al.* 1992). However, the safety margin is narrow, and an overdose causes listlessness, loss of appetite and mortality (e.g. Palmer *et al.* 1987, Høy *et al.* 1991). In calculating the dose an accurate estimate of the fish weight is critical. Considering the variation of both fish weight and appetite in cages, it is possible that both over- and underdosing of fish could occur. The former is harmful to the fish while the latter selects for tolerant parasites (Bjorn 1992). Following oral ivermectin treatment of Atlantic salmon, O'Halloran *et al.* (1992) suggested that the reason the larger fish suffered the greatest mortality was because they ate more and thereby received a higher (and fatal) dose than did smaller fish. It has yet to be clearly demonstrated whether ivermectin removes chalimus stages.

Hydrogen peroxide is a powerful oxidizing agent, and toxicity appears to be due to the formation of gas bubbles within the lice (Chapter 21). There are indications that hydrogen peroxide kills chalimus as well as adult lice (Chapter 21). However, the safety margin between toxicity to lice and to salmon is slight (Table 2) and sublethal effects on salmon gills may occur during treatment (Chapter 21). The safety margin also narrows with temperature, such that hydrogen peroxide is not recommended for use above 14°C (Chapter 21).

Diflubenzuron, which inhibits chitin synthesis and growth, maturation and survival of crustaceans, has also been considered (Roth *et al.* 1993). However, as large quantities (1 kg/kg salmon over 2 weeks) of this highly toxic (c. 0.5 µg/l to some crustaceans) chemical would need to be fed to the salmon, and it would persist for weeks in the environment, with probable impacts on marine crustaceans (Roth *et al.* 1993), its use cannot be seriously considered.

Chemotherapeutants: method of application

Chemicals may be applied as a bath, dip, floating layer, orally, or by injection. It is difficult to achieve a homogeneous solution in a large fish cage, but effective baths can be given if the cage is enclosed in a plastic tarpaulin, the water oxygenated so as to increase circulation and supply oxygen to the fish, and the chemical carefully introduced to the cage (Brandal and Egidius 1979). Special equipment with perforated hoses is available to aid dispersion of solutions in fish cages (Anon. 1989).

Salmon can be transferred into a special treatment cage (enclosed in tarpaulin) held next to the salmon cage, and thus the same solution used for several cages. This has been used in Norway for trichlorfon (Brandal and Egidius 1979). In Scotland and Ireland, fish are not transferred to a treatment cage but their own cage net is drawn up

(to reduce cage volume), surrounded in tarpaulin, oxygenated, and the dichlorvos added (Rae 1979).

The use of only tarpaulin skirts around the sides of the cages, or absence of any method of enclosing the cage as practised by some farms (Ross and Horsmann 1988), will result in variable concentrations of chemicals which can stress the fish but not kill the lice. For example, Wells *et al.* (1990) found dichlorvos ranged from between 0.38 and 3.4 mg/l in a cage with tarpaulin skirts; i.e. both below levels toxic to lice and above those toxic to salmon (Table 2). Additionally, some farms in Norway prepared trichlorfon solutions a day before treatment, which resulted in unpredictable and dangerously high levels of its toxic degradation product, dichlorvos (Boxaspen and Holm 1992).

Bath activities are very stressful to the fish (Bjordal *et al.* 1988), possibly more than any direct effects of the chemotherapeutant. The time-consuming nature of bath treatments often means it can be several days before all the cages on a farm are treated, and there is a temptation not to treat cages with low infestations. It is probable that the mobile lice quickly transfer onto recently treated fish from adjacent cages and the population cycle is maintained. Indeed, despite regular bath treatments which may reduce lice intensity within a cage for a week or two, most farms have a steadily increasing lice population (Wootten 1985, Costello unpublished; see also Chapter 19).

The handling involved in a dip, by which individual fish are passed through a concentrated solution for a few seconds, also stresses fish. However, during size grading fish are passed through chutes and tubes, and it may be practicable to include a lice dip in this procedure. Such use of a 15 mg/l solution of dichlorvos for 1 min has been proposed in France (Messenger and Esnault 1992). Trials in a 5–30 s dip of pyrethrum mixture reduced mobile lice numbers by up to 96% (Boxaspen and Holm 1992). Furevik *et al.* (1993) have noted increased leaping activity of salmon when infected by lice, such that they land on their side, presumably to knock off lice. Initial attempts at using pyrethrum involved floating it in an oil layer on the water surface with the intention that lice on leaping salmon would be affected (Boxaspen *et al.* 1991, Boxaspen and Holm 1992). However, maintenance of the oil layer would be difficult in anything but the calmest sea, collection of the oil layer may be difficult, and reductions in lice numbers were variable (0–74%) (Boxaspen *et al.* 1991).

Oral treatments have the advantage of ease of application in all types of fish cages. However, absorption by the fish may entail longer tissue residue times than external pesticide treatments. Only feeding fish will receive treatment, and dose rates can be complicated if there is a wide range of fish sizes or food consumption rates within a cage. Oral chemotherapeutants often have strong particle-binding properties (to prevent leaching from the food), such that dispersion is limited to the seabed in the immediate vicinity of the farm. However, such properties may also mean that absorption by the fish will be low (so most of the therapeutant is passed out in the faeces) and degradation will be slow in the dark, cold sediments under the fish cage.

No lice treatment has yet been considered for application by injection. Injecting fish with a chemotherapeutant is both very time consuming for staff and stressful to the fish. It can only be considered if it confers long-term (=1 year) protection against lice damage.

Treatment may be intermittent or continuous. Bath and dip treatments are intermittent. While oral treatments may be provided continuously, only physical and biological methods can be applied continuously without concern for increasing environmental impact. The frequency of intermittent treatments will depend on the life stages removed and reinfection rate of lice. As AChase inhibitors only remove mobile lice they must be reapplied (in 2–4 weeks) when chalimus stages have developed to preadults (Table 2).

Chemotherapeutants: environmental concerns

Of the chemicals considered for lice control on salmon farms, the most information on toxicity and environmental effects is available for dichlorvos (Pal and Konar 1985a,b, Mattson *et al.* 1987, 1988, Department of Agriculture and Fisheries for Scotland 1989, Tully and Morrissey 1989, Cusack and Johnson 1990a,b, Horsberg *et al.* 1990, McHenery 1990, McHenery and Francis 1990a,b, McHenery *et al.* 1990a,b, 1991, Murison *et al.* 1990, Raine *et al.* 1990, Robertson 1990, Thain *et al.* 1990, Wells *et al.* 1990, Dobson and Tack 1991, Jackson and Costello 1992, Messenger and Esnault 1992, McHenery *et al.* 1992). However, this was not always the case and the pesticide had been used on salmon farms for about 10 years before most of these studies were undertaken and published. This prompted considerable criticism of salmon farming on environmental grounds (e.g. Ross and Horsmann 1988, Ross 1989).

Dichlorvos degrades within 4–5 days in well-aerated sea water (Samuelsen 1987a,b), does not bioaccumulate, and its degradation products are not toxic (World Health Organization 1989). Field studies indicate that effects on marine organisms will be limited to within 25 m of the fish cages, primarily owing to the rapid dilution of the solution (Murison *et al.* 1990, Wells *et al.* 1990, Dobson and Tack 1991). Based on 96 h LC_{50} and no observed effect concentrations, McHenery *et al.* (1992) recommended an environmental quality standard (EQS) of a 24 h average concentration of 600 ng/l and annual average of 40 ng/l. Such an EQS would consider the dispersal characteristics of the farm site and limit the use of dichlorvos there. Lime could be added to the bath after treatment to raise the pH and thus enhance degradation of dichlorvos before its release to the sea (Brandal and Egidius 1977, Messenger and Esnault 1992), but this has rarely if ever been practised.

Nuvan (and Aquagard) is 50% dichlorvos, the remainder being a carrier (dibutyl phthalate) and emulsifier. Raine *et al.* (1990) found that toxicity to phytoplankton was more related to the carrier and emulsifier than the dichlorvos. Thus a distinction should be made in toxicity studies between the ‘active ingredient’ and other components of the pesticide as used.

A study by Robertson (1990) suggested that an intertidal amphipod, *Hyale nilssoni* (Rathke), near one farm had developed resistance to Nuvan. This and ecological studies by Murison *et al.* (1990) indicate that further field studies are required to determine if subtle effects of Nuvan on marine communities have occurred or not. Fresh-water experiments have found subtle long-term effects at sublethal concentrations; in mesocosms, exposure to the lowest concentration tested, 0.014 mg/l at 7-day intervals for 90 days, reduced plankton and fish production (Pal and Konar, 1985b). The suggestion that Nuvan use in salmon

farms was the cause of cataracts in wild salmon (Fraser *et al.* 1989) was followed by some debate (Dobson and Schuurman 1990, Fraser *et al.* 1990). However, Bruno *et al.* (1991) found no relationship between usage of Nuvan and occurrence of cataracts in farmed salmon that would have been exposed to higher concentrations than wild fish.

The active ingredient of trichlorfon is its first degradation product, dichlorvos (Samuelsen 1987a, Horsberg *et al.* 1989). Trichlorfon was only licensed and widely used for treating lice on salmon in Norway, but is used for other fish species in many countries (Roth *et al.* 1993). Experimentally, an oral treatment of trichlorfon caused blindness in salmon (Brandal and Egidius 1977). It was used commercially as a bath treatment, but at a 300 times greater concentration than dichlorvos (Table 2) (Brandal and Egidius 1979). Degradation to dichlorvos is faster in warm, sunny conditions, and concentrations of dichlorvos have risen such that mass mortalities of salmon (Salte *et al.* 1987, Horsberg *et al.* 1989) and significant effects on marine fauna (Egidius and Möster 1987) have occurred. Treatment exposure (concentration and time) was reduced at higher sea temperatures (Brandal and Egidius 1979, Roth *et al.* 1993). In recent years, there has been a change in use of trichlorfon to dichlorvos in Norway, which is better from clinical, environmental and staff safety aspects (Grave *et al.* 1991a).

In Norway, the recommended treatment concentration of dichlorvos varies with temperature from 0.5 to 2.0 mg l⁻¹ (Grave *et al.* 1991a), but a single treatment concentration 1.0 mg l⁻¹ is licensed for use in the UK (Roth *et al.* 1993). The latter gives an approximately threefold safety margin between dichlorvos toxicity to sea lice and salmon (Table 2). Preliminary laboratory studies with azamethiphos indicate that it may have a considerably better safety margin (Table 2) (Roth and Richards 1992), but malathion did not (Roth *et al.* 1993). However, the benefits of a greater safety margin between lice and salmon need to be balanced against the potential toxicity to wild crustaceans following release.

The degradation products of carbaryl are possibly more toxic than the carbaryl, and will probably bind to sediments and be persistent in the marine environment (Bruno *et al.* 1990, Roth *et al.* 1993). The safety margin is only marginally greater than that of dichlorvos and, as both have similar modes of toxicity, cross-resistance is possible. Hence, on both environmental and clinical grounds, carbaryl appears unsuitable for lice control.

If released, a pyrethrum-oil mixture would be unsightly, and predicting its ecotoxicity may be difficult for such a mixture of pyrethrins, oils and synergist. While some individual pyrethrins, such as lambda-cyhalothrin, may have a narrow safety margin between toxicity to lice and salmon, others such as resmethrin may have more potential (Chapter 20). With a fast degradation rate and different mode of toxicity to organophosphates, pyrethrins may be useful alternatives in lice treatment.

Because hydrogen peroxide rapidly degrades to water and oxygen, its use in salmon cages is unlikely to prove a hazard to marine life beyond the cage. However, as large volumes of this highly caustic chemical would need to be transported to farms, special care would be needed in its transport and handling.

As an effective oral treatment, ivermectin is the easiest chemotherapeutant with which to treat sea lice at present. In salmon, it reaches its maximum tissue

concentration in about 4 days (Höy *et al.* 1992), and from an initial concentration of $150 \mu\text{g kg}^{-1}$ in muscle it takes 350 degree days to reach $30 \mu\text{g kg}^{-1}$, and 700 degree days to reach $1 \mu\text{g kg}^{-1}$ (a tissue concentration of $\leq 10 \mu\text{g kg}^{-1}$ is ineffective against lice) (Chapter 22). To avoid tissue residues its use is thus limited to fish which will not be marketed for several months. Ivermectin shows a strong binding to particulate material (Nessel *et al.* 1989); 36–83% passes unabsorbed through mammals as faeces (Halley *et al.* 1989a), and in agricultural use it may persist in soils for over 240 days (half-life in dark in soil–faeces at 22°C) (Halley *et al.* 1989b). It appears to be excreted unaltered from Atlantic salmon (Höy *et al.* 1992). From the limited information available, it can be expected that ivermectin would accumulate in waste food and faeces on the seabed under salmon cages and persist there for months, with unpredictable effects on marine organisms. Although single oral treatments can be effective (Tully personal communication), some farms have fed ivermectin twice a week for much of the summer. Such continuous treatment may result in environmentally significant concentrations on the seabed. It may also select for resistance in the sea lice, as occurred in soil invertebrates after only three and 11 treatments over 3 years in South Africa (van Wyk and Malan 1988) and 4.5 years in Brazil (Echevarria and Trinidad 1989). Considering the potential for accumulation on the sea bed and development of resistance, oral treatments such as ivermectin should only be used as a last resort (if at all), and then intermittently. The manufacturers oppose any use of ivermectin on salmon farms pending satisfactory ecotoxicology studies (Brewer 1991), and have now decided not to proceed with further development of the compound for use in salmon farming (Chapter 22). However, this does not necessarily mean that ivermectin, or a similar compound, will not find a use in fish farms.

Biological methods

Biological control: cleaner-fish (wrasse)

In Norway, Shetland, Scotland and Ireland native cleaner-fish are increasingly being used to control sea lice on salmon farms (Costello and Bjordal 1990, Costello and Donnelly 1991, Treasurer 1991a,b, Darwall *et al.* 1992, Treasurer 1993). Initial aquarium and cage studies in Norway found that four species of wrasse (Labridae), namely goldsinny *Ctenolabrus rupestris* (L.), rock cook *Centrolabrus exoletus* (L.), corkwing *Crenilabrus melops* (L.), and to a lesser extent (females and juveniles only) cuckoo *Labrus mixtus* L., cleaned lice off salmon (Bjordal 1988, 1990, 1991, 1992) (Fig. 1c). Another common wrasse in northern Europe, ballan *Labrus bergylta* Ascanius, did not show cleaning behaviour in the Norwegian studies, although juveniles may do so (Bjordal 1991, Costello 1991). As cuckoo is not abundant in Ireland, and is popular with sports anglers and scuba divers, it is not used by salmon farms there. Generally, the species used most in all countries is goldsinny, with rock cook and corkwing being used according to their availability. However, field and aquarium observations suggest that rock cook may be the most active cleaner (Costello 1991, Tully unpublished).

Wrasse inhabit inshore rocky coasts with rock and seaweed cover, and feed by browsing on epifaunal crustaceans and molluscs. They are fished using shrimp pots, fyke nets and special collapsible traps (Bjordal *et al.* 1991). Following screening for diseases and parasites, wrasse are placed in the salmon cages at ratios of one wrasse to 50–150 salmon. Escapement increases with cage mesh size (Treasurer 1991a, Bjordal 1992), and wrasse <10 cm will escape from the smallest smolt mesh size of 12 mm×12 mm (square mesh). Cork wing tend to be larger (10–15 cm) than goldsinny and rock cook (5–12 cm) (Costello unpublished). However, unless restocking occurs, most wrasse escape within the first year the salmon are at sea (Costello *et al.* unpublished). Such escapement is normally the reason for apparent failures of wrasse to control sea lice. There are reports of effective lice control by wrasse for the 2 years until the salmon were harvested (Thorburn 1991, Bjordal 1992, Young personal communication). However, regular lice counts on >4kg salmon at known wrasse densities in commercial cages have yet to be conducted. Wrasse mortality tends to be higher within the first 4 weeks after placement in salmon cages, and significant if the wrasse are mechanically size graded with the salmon (Costello *et al.* unpublished). Improved wrasse husbandry, such as transport and temporary holding facilities, or use of hides within salmon cages, may reduce both wrasse mortality and escapement.

Wrasse can be susceptible to salmon pathogens such as *Aeromonas salmonicida* (Treasurer and Cox 1991). Strains of *A. salmonicida* are widespread in marine fish (e.g. Willumsen 1990) and salmonids (e.g. Hussein 1991). An atypical *A. salmonicida* found in wild wrasse was found to be non-pathogenic to salmon (Frerichs *et al.* 1992). Wrasse appear to contain a range of parasite species typical for marine fish (Costello 1991, Costello *et al.* unpublished), but with a notable lack of ectoparasites in the species showing cleaning behaviour. As the metazoan parasites are generally host specific, and/or require intermediate hosts (unavailable in salmon cages) to complete a life cycle, they are unlikely to pose a threat to salmon. The protozoan parasites are less well known, but are being studied (Pike personal communication). Bron and Treasurer (1992) found some chalimus of *Caligus centrodonti* Baird, but an absence of mobile lice, on goldsinny and rock cook. They suggested that the absence of mobile lice may have been due to loss during capture and handling, or emigration of lice from the host. As both interspecific and conspecific cleaning of wrasse has been observed in the field and aquaria (Costello 1991, personal observation), it is probable that such cleaning is a significant reason for an absence of larger ectoparasites on these species. Despite the current absence of any wrasse harbouring pathogens which are known to pose a threat to salmon health, it is prudent that wrasse are screened before use, and that wrasse are not collected from the vicinity of other fish farms.

Wrasse can remove both *L. salmonis* and *C. elongatus* from salmon (Treasurer 1993), including chalimus stages (Costello unpublished, Tully unpublished). Cleaning is apparent within days despite the absence of any invitation posture by the salmon (as observed in other marine species) (e.g. Fig. 1c). No learning period appears necessary (Tully unpublished), but acclimation to the cages may take 1–2 weeks (Donnelly 1992). Observations suggest that a few wrasse conduct most of the cleaning, while fouling on the cage nets provides food for most of the wrasse (Bjordal 1992, Tully

unpublished). Additional food (e.g. crushed mussels) can be placed in cages to supplement the wrasse diet.

Smolts are moved to sea from March to June, but wrasse will not be captured in high numbers until late May or June (Darwall *et al.* 1993). Hence there is a period when wrasse are unavailable and lice infestation may occur. In Shetland, wrasse are not abundant, and populations in Ireland and Scotland are less abundant than in Norway (Lysaght *et al.* unpublished).

Populations of wrasse exploited may suffer local reductions in fish size and abundance (Darwall *et al.* 1993). This will further limit the availability of wrasse to the fish farmer. However, long-term or widespread effects on wrasse populations are unlikely owing to their early maturity, high fecundity, abundance in areas distant from farms, and capacity for rapid recruitment (Johannessen and Gjøsæter 1990, Darwall *et al.* 1993). Furthermore, wrasse culture (Anon. 1993) may provide a certified disease-free stock at almost any time of the year, and reduce impacts on wild stocks.

Biological control: vaccine

Despite the simplicity of the fish immune system in comparison to mammals, the production of effective vaccines to fish pathogens has been slow. Atlantic salmon can produce antibodies to artificially injected *L. salmonis* components, but naturally infected Atlantic salmon and rainbow trout did not similarly respond (Grayson *et al.* 1991). Neither did Atlantic salmon produce an antibody response to naturally infected *C. elongatus* (MacKinnon 1991). In the laboratory, stress and associated reduced immunocompetence increased the susceptibility of Atlantic salmon to lice infestation (Johnson and Albright 1992b). Panasenko *et al.* (1986) found more *L. salmonis* on wild salmonids with saprolegniosis. However, whether host behaviour has a role in fish susceptibility to lice has not been determined.

A host response specific to sea lice may be best transmitted in the blood (Fletcher 1986). Some chalimus and adult *L. salmonis* and *C. elongatus* may ingest host blood (Brandal *et al.* 1976, Johnson and Albright 1992b, Reilly personal communication), but the quantity, regularity and importance of blood in the diet remain to be shown. However, it may also be possible to produce an antibody response to lice in fish mucus (Chapter 23). Considering the limited understanding of immune systems in fish, the development of an effective sea lice vaccine, if feasible, remains years in the future.

The prevalence and intensity of lice infection vary naturally on wild salmonids (Table 1), and experimental studies have found that the host response (on gill and fin tissue) to infestation by *L. salmonis* chalimi is stronger on coho (*Oncorhynchus kisutch* Walbaum) than chinook (*Oncorhynchus tshawytscha* Walbaum) or Atlantic salmon (Johnson and Albright 1992a). MacKinnon (1991) also found little tissue response in Atlantic salmon to *C. elongatus* chalimi. Indeed, in comparison to coho and chinook, the hyperplasia and inflammation of the skin in response to chalimus attachment was weak, loss of lice was less, and lice growth was significantly greater on Atlantic salmon (Johnson and Albright 1992b). Furthermore, different strains of Atlantic salmon may vary in their susceptibility to lice.

Fish have several non-specific defences against external pathogens, including scales, skin, mucus production, compounds in the mucus, and tissue growth and associated cellular response to attack. Taylor (1987) observed that 26% of settling

copepodids became entangled in mucus balls which probably impeded their establishment on the salmon. Individual fish can develop resistance to and reject other ectoparasites (e.g. Kama and Milleman 1978, Shields and Goode 1978, Bauer and Vogel 1987, Shariff and Roberts 1989, Woo and Shariff 1990, Urawa 1992). Comparisons between the responses of different fish species to the same parasites, and between ectoparasite species, may prove fruitful in understanding host–parasite relationships and developing better lice control methods. For instance, what morphological, behavioural or biological features of *C. elongatus* allow it to infest so many different hosts, while *L. salmonis* is restricted to salmonids?

Biological control: lice pathogens

A monogean trematode worm, *Udonella caligorum* Johnston, is commonly found on the dorsal surface of caligids (e.g. Kabata 1973, Schram and Haug 1988, MacKenzie and Morrison 1989; see also Chapter 26). It is believed to feed on the fish, worsening any impact of the lice, without impairing the health of the sea lice. However, it may impair the stability of lice on the fish. A suctorian protozoan, *Ephelota* sp., can similarly occur on both *C. elongatus* and *L. salmonis*, but appears unlikely to affect the lice (Stone and Bruno 1989). Gresty and Warren (Chapter 27) report ciliate protozoans of the genera *Trocholioides* and *Licnophora* from *L. salmonis* obtained from farmed Atlantic salmon in Scotland, and an epistylid ciliate from sea lice from wild chum salmon (*Oncorhynchus keta* Walbaum) from Japan. However, host-specific pathogens and parasites of crustaceans can cause mortality, sterilization, biased sex ratios, or interfere with growth and maturation, be they rhizocephalan cirripedes, bacteria (e.g. Rigaud *et al.* 1992, Rousset *et al.* 1992), protozoans (e.g. Bulnheim and Vávra 1968) or fungi (e.g. Reynolds 1988). The current absence of records of such organisms on sea lice may reflect the limited studies on sea lice biology. Should such parasites be found, it may be possible to aid infection of lice so as to reduce infestations below pathogenic levels. However, it would be necessary for such organisms to be shown to have no deleterious effects on the salmon and other marine crustaceans before release.

CONCLUSIONS

A comparison of methods considered reveals that each has its positive and negative aspects (Table 3). Additionally, treatments will vary in their adaptability to different farm conditions, such as water currents, cage size and staff experience. Considering the differences between lice species, and between location, design and staff resources available at farms, a universal panacea for sea lice is unlikely to be found. Hence a range of methods to minimize lice arriving on a farm (i.e. prevention), limit growth of the lice population, and to reduce pathogenic infestations, will be necessary.

Chemicals such as dichlorvos and ivermectin are less effective, and wrasse are less active at low (<8°C) temperatures. However, the growth and maturation of sea lice also reduce with temperature, so effective lice control before winter should minimize lice impacts until the following spring (Tully 1992).

Table 3. Negative and positive aspects of some methods considered for sea lice control (e.g. '+' = positive; '-' = very negative; '++' = very highly positive)

	Efficacy	Stress salmon	Cost	Hazard to staff	Environment	Marketing	Availability	Ease of use
Neguvon	+	-	-	-	-	-	++	-
Carbaryl	+	-?	?	-	-	-	++	-
Nuvan	++	-	-	-	-	-	++	-
Azamethiphos	++	-?	?	-	-	-	++	-
Pyrethrum	+	-	?	+	+	-	+	-
Ivermectin	++	+	-	-	-	-	++	+
Hydrogen peroxide	++	-?	?	-	+	+	++	-
Cleaner-fish	++	++	+	++	++	++	-	++

Every effort is required to choose farm management policies which will prevent or minimize lice infestations—notably, the avoidance of overlapping generations of fish, maximizing distance between farms, regular (perhaps every 2 years for at least 4 weeks) fallowing of sites, and coordination of these preventative and other control measures between adjacent farms. Importantly, such practices will also have benefits in the prevention and control of other pathogens.

A survey of Scottish salmon farmers in 1991 found that wrasse were the most popular alternative to dichlorvos, and could become the treatment of choice following further development (Anon. 1991c). In addition to being effective, with concomitant benefits to salmon health and growth, they reduce the need for chemotherapeutants, with subsequent benefits in marketing salmon and improving the image of the industry. In Scotland the annual cost of using wrasse is equivalent to four Nuvan treatments, and there may be seven or more Nuvan treatments per year (Chapter 25). In Norway wrasse can halve the cost of lice control (Anon. 1991b), and savings may be greater when benefits to salmon health and growth can be considered.

It is unfortunate that pesticide use on salmon farms has not always been in accordance with manufacturers' and veterinary recommendations (e.g. Ross and Horsmann 1988, Grave *et al.* 1991b, Boxaspen and Holm 1992). This often results in ineffective lice treatment, promotes the development of resistance, and damages the public image of the industry. To avoid the development of resistance in sea lice, similar strategies must be followed as for the use of biocides in agriculture—notably, to use as infrequently as possible but at high (100%) effective doses, avoid underdosing, alternate use with different classes of compounds, and include non-chemical methods in control measures (Bjorn 1992). Underdosing of lice is possible in oral treatments if fish weights are not accurately known or if considerable variation in fish weight and appetite occurs within a cage. Both underdosing of lice and overdosing of salmon are possible in bath treatments if the bath volume is not accurately determined or if mixing of the solution is not thorough. Lice control may be improved by the use of professional lice control teams to visit all farms in an area. This could coordinate control measures, and minimize the need for special training, health screening, and distraction of farm staff from routine duties.

The evidence for resistance to dichlorvos, and the failure of its use to decrease lice populations on farms, emphasizes the need for alternative treatments and importance of minimizing repeated use of any chemical. In the case of chemicals, environmental studies should develop from laboratory to field assessments, and include (a) toxicity to marine plankton, and benthic crustaceans and molluscs, (b) modelling of chemical concentrations in the water column and sediments, (c) partitioning of chemical to particulate matter, sediments, water or marine organisms (bioaccumulation), (d) degradation (persistence) of chemical in sea water and sediments under relevant light and temperature conditions, and (e) toxicity of degradation products. Studies should relate closely to the compound (active and other ingredients) as it is proposed to be applied in farm conditions (i.e. as bath, dip or in diet). The withdrawal period necessary to ensure no trace of the chemical in the marketed salmon must be known. All these data must be published and made available to the public to allay environmental and public health concerns before the chemical is used in fish destined for market. The

lessons from the use of biocides such as TBT (tributyl tin) (as a net antifoulant) and dichlorvos should be learned and acted upon.

FURTHER WORK

It is apparent that despite the economic importance of sea lice, fundamental aspects of their biology and ecology need research. The distribution of planktonic phases and host-finding mechanisms of copepodids and mobiles are unknown. Why sea lice species have different host preferences, and the implications of differences between lice species for their control, are unclear.

Present control methods only consider the removal of mobile and chalimus stages of lice. Methods of inhibiting the settlement, growth, moulting, maturation, mate location, sperm transfer, survival and egg production of sea lice cannot be developed until the physiology and behaviour of lice are better understood.

There is a variable (but poorly understood) generalized response by different salmonid species and individuals to lice attack, and individual fish have an ability to develop resistance to other ectoparasites. Whether immunocompromised fish are more attractive to lice, or the absence of a host response results in a reduced loss of lice, warrants attention. However, the biological, genetic, environmental and behavioural factors influencing host susceptibility and host response to sea lice remain enigmatic. No attempts have yet been made to develop methods to improve the generalized host response to either chalimus or mobile lice attack.

In developing and choosing methods of sea lice control, the long-term and sublethal effects of control methods on salmon stress, disease susceptibility, growth and food conversion remain to be evaluated. Chemotherapeutants need to be thoroughly assessed, taking into consideration the clinical, environmental and public perception problems of current treatments. Before use, the results of ecotoxicological studies (including field studies) must be published and made available to allay public concern.

Non-chemical methods need further development, notably the use of cleaner-fish, vaccines and other potential methods of biological control. In particular, improvement of the availability (in quantity and time of year) and husbandry (reduction of escapement and mortality, interactions between wrasse and salmon) of wrasse could be assisted by studies on fishery potential and culture. Successful commercial culture may also establish certified disease-free stocks, and allow the wider use of wrasse. Biological control can be cost effective. In Australia, biological control in agriculture resulted in a return of \$10.5 per \$1 spent, excluding the improvement of environment quality through reduced pesticide use (Lee 1981).

The fact that salmon farms occur in particularly beautiful, remote and unpolluted areas is an aid to marketing the product as healthy to eat and produced without environmental damage. However, experience with the use of Nuvan to control sea lice has damaged such an image. The concept of clean production, with appropriate environmental auditing, needs to be integrated within the industry. The control of sea lice should focus on preventative and biological measures, with chemotherapeutants being used as a last resort.

ACKNOWLEDGEMENTS

This work was aided by a Commission of the European Communities contract (No. AQ.2.502) under the Fisheries and Aquaculture Research programme. I would like to thank the many persons of whom I have quoted personal communications, allowed me to quote their papers 'in press', assisted with the supply of references, and provided helpful discussions over the past 2 years.

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Part IIb Following

18

The effects of fallowing on caligid infestations on farmed Atlantic salmon (*Salmo salar* L.) in Scotland

Andrew N. Grant and James W. Treasurer

ABSTRACT

Fallowing of salmon farms permits recovery of the seabed and prevents carry-over of infectious agents to the next production cycle. It also breaks the cycle of caligid infestation on mixed year class sites where smolts quickly become infected with sea lice. Fallowing of entire loch systems may also require management agreements between companies, and relevant clauses should include a definition of area, a specification of fallow period, single generation stocking, and monitoring of the lice populations. After fallowing the need to treat the fish is delayed and discharge of chemical reduced. A management agreement in Loch Sunart is cited as an example.

INTRODUCTION

Sea lice (*Lepeophtheirus salmonis* Krøyer, and to a lesser extent *Caligus elongatus* Nordmann) present a serious economic threat to salmon (*Salmo salar* L.) farming in Scotland, Ireland, Norway and Canada. There are several implications for salmon farmers of infestation by *L. salmonis* and of the need to repeatedly carry out chemotherapeutic treatments. Moderate numbers of lice damage fish and may reduce their market value, while severe damage will kill fish. Conventional treatment with chemotherapeutants is labour intensive and weather dependent, and adverse reactions can occur on exposure to dichlorvos and large mortalities have occurred. Also, there is good evidence that lice have become resistant to dichlorvos and presumably to other organophosphates (Jones *et al.* 1992). Appetite may be reduced following exposure to dichlorvos, with resulting loss of growth. It is therefore of great importance to take steps to lessen the degree of infestation and therefore reduce the need to treat fish.

Current control methods rely on immersing affected fish in a parasiticide. In the UK only dichlorvos (Aquagard, Ciba-Geigy) is authorized for this purpose and is licensed as a veterinary medicine under the Medicines Act 1968. In practice fish are enclosed in a known volume of water, the chemical added in a quantity calculated to expose the fish to 1 ppm dichlorvos and the chemical is released after 1 h (Wootten *et al.* 1982). Dichlorvos is only active against mobile preadult and adult lice, and chalimus larvae appear to be unaffected. The decision to treat a population of fish may be determined simply on observation of a 'significant' burden or may be based on continuous monitoring of the numbers and population structure of the parasite. The detection of a predetermined number of mobile lice on a sample of fish will then trigger treatment. Knowledge of the number of chalimus present and of the speed of succession of generations gives advance warning for future treatments.

As part of standard management practice Marine Harvest fallows every farm site between each production cycle. This allows the seabed to recover and removes the risk of carry-over of infectious agents. In addition, the cycle of caligid infestation is broken (see Chapter 10). To achieve maximum effect, as dispersal stages can be transported considerable distances, the area fallowed can be extended to include all farms in a loch area including those operated by other companies, through management agreements. The area encompassed by a management agreement cannot be rigorously determined but could, for example, take the form of a complete sea loch system. The relevant clauses of a management agreement are:

1. The area within a management agreement is defined.
2. The fallow period for the loch is specified.
3. Single-generation (year class) stocking.
4. All fish in—all out.
5. The lice population is monitored and, where possible, treatments on farms are coordinated.

This chapter reports the results of fallowing at two sea lochs in the west of Scotland, one involving a management agreement.

FALLOWING AND MANAGEMENT AGREEMENT CASE HISTORIES

Loch Eil: fallowing

An element of management agreements is that the loch is stocked with fish of the same generation in a production cycle. It is recognized that smolts stocked alongside older fish carrying lice quickly become infested, for example as seen in Loch Eil, west Scotland (56°51'N, 5°8'W) (Fig. 1). Loch Eil farm was stocked in April 1989 with smolts. Lice control became necessary on a routine basis from September and mean numbers of lice rarely fell below 40. On 3 May 1991 a small number (15000) of smolts was stocked alongside the 100000 older 1989 year class (on sea farms, year fish put to sea). Within 3 days copepodids of *L. salmonis* were seen on the smolts and the fish quickly acquired a significant infestation (mean of eight mobiles per fish). Treatment with Aquagard was required within 4 weeks of stocking and thereafter at 4-weekly intervals through the summer (Fig. 1). The farm was fallowed from 15 January

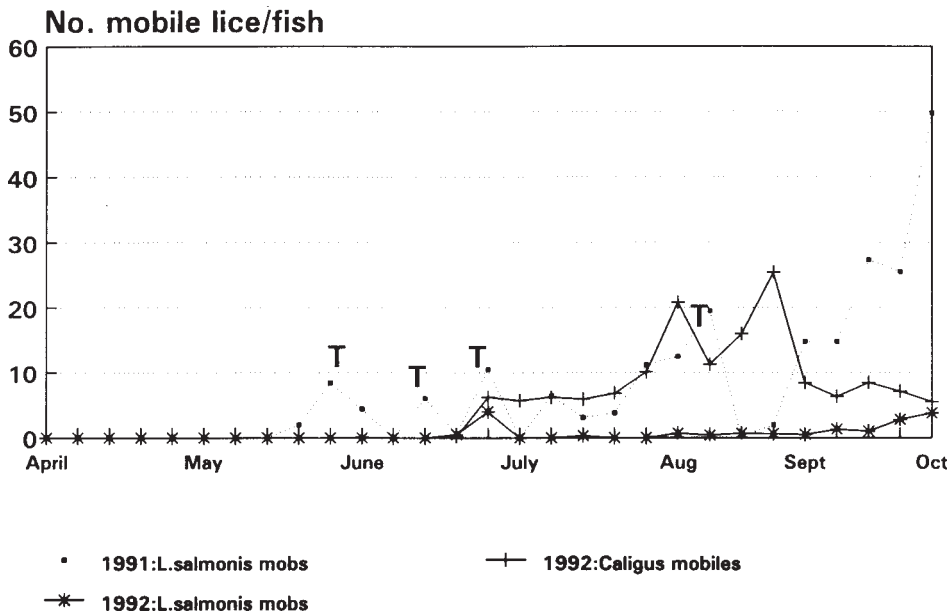


Fig. 1. Sea lice infestation levels in Loch Eil, showing changes in numbers of *L. salmonis* during 1991 when lice were treated with Aquagard, and 1992 when a fallow period had been introduced. The fish were a mixed year class in 1991, smolts being stocked with 1989 year class (=year fish put to sea). T=Aquagard treatment, during 1991 only.

1992 along with the rest of the farms in the loch before restocking during April 1992. No treatment has been required to date. Only *C. elongatus* was present in any number and did not pose a threat.

Loch Sunart: management agreement

The results of a management agreement for a Scottish loch are shown in Loch Sunart, Morvern (56°40'N, 5°38'W) (Figs 2 and 3), and compared with the problems encountered in the previous production cycle. The extent of infestation is based on averaging the number of lice on a representative sample of fish ($n=25$ fish in total, comprising five fish from a selected pen on each of five groups of pens), to give a weekly assessment for the farm. Loch Sunart had been farmed continuously for several years prior to 1991 without a fallow period extending to the whole loch. The three companies farming in the loch, Marine Harvest, McConnell Salmon and Mingarry Fish Farms, agreed to remove all fish from the water in February 1991. After 6 weeks, restocking commenced in April 1991 with smolts of the same generation. The total number of fish stocked in the loch was also reduced. Significant infestation with *L. salmonis* did not occur until June 1992 when the fish were first treated, and treatment has continued since then. Consequently, the use of dichlorvos has been substantially reduced.

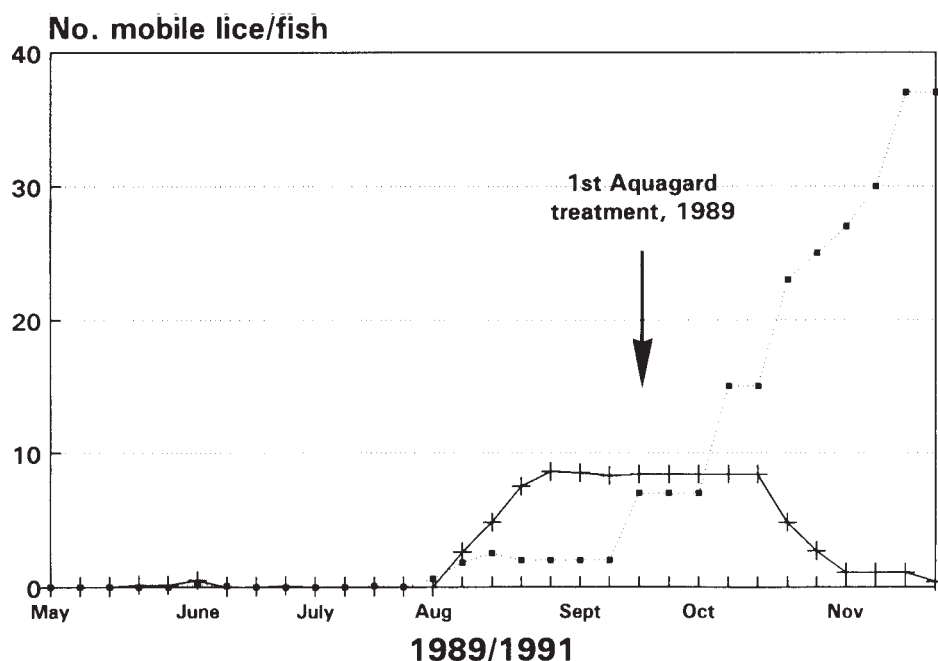


Fig. 2. Sea lice infestation levels in Invasion Bay, Loch Sunart, showing changes in numbers of sea lice during 1989, when lice (dominated by *L. salmonis*) were treated with Aquagard, and 1991 (lice almost solely *C. elongatus*) after a fallow period. ■...1989 *L. salmonis* mobiles +—+—1991 *L. salmonis* mobiles

CONCLUSIONS

1. Incoming smolts will become rapidly infested on mixed year class sites.
2. Following a fallow period, a longer time is taken for a significant infestation to build up from the start of the production cycle. The length of the fallow period should take account of the life cycle of the parasite. In Scottish conditions, the minimum fallow period (=egg incubation time plus maximum longevity of nauplius and copepodid stages) is estimated to be 30 days, that is at water temperatures of 7–8°C in February–March (this may be longer at lower temperatures) (Johnson and Albright 1991), although maximum survival time of females off a host (32 days under laboratory conditions) may also have to be considered (J.Bron, personal communication).
3. Following a fallow period the need to treat the fish is delayed and therefore the discharge of chemical is reduced.
4. Where lice have become less sensitive to organophosphates (Jones *et al.* 1992), imposition of a prolonged fallow period, while having the effects described above, does not appear to eliminate the resistant population (personal observation).
5. Other chemotherapeutants are being tested as alternatives to dichlorvos, including hydrogen peroxide (Chapter 21), pyrethrins (Boxaspen and Holm 1991) and azamethiphos, although lice may also have reduced sensitivity to the latter. Cleanerfish, wrasse, are also being trialled to determine whether they can effectively

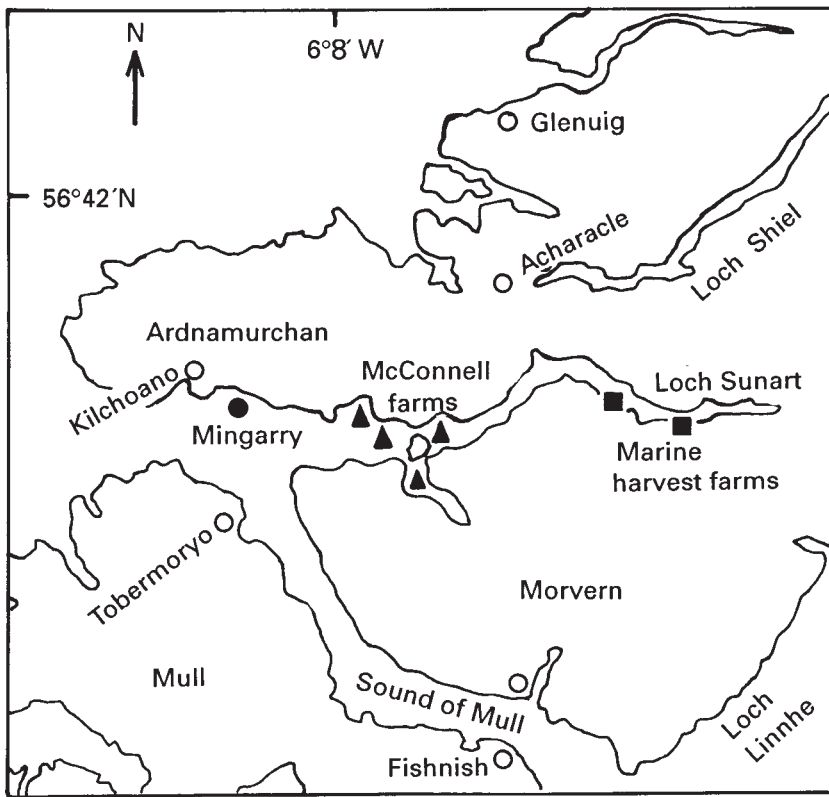


Fig. 3. Map of Loch Sunart showing the location of farms operated by Marine Harvest, McConnell Salmon and Mingarry Fish Farms.

control lice numbers (Chapter 25). All possible measures should be employed to minimize the impact of sea lice infestation.

ACKNOWLEDGEMENTS

We thank staff of the Marine Harvest farms at Invasion Bay and Loch Eil for assistance in the routine monitoring of sea lice dynamics.

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Part IIc Chemotherapy

19

Influence of treatment with dichlorvos on the epidemiology of *Lepeophtheirus salmonis* (Krøyer, 1837) and *Caligus elongatus* Nordmann, 1832 on Scottish salmon farms

J.E.Bron, C.Sommerville, R.Wootten and G.H.Rae

ABSTRACT

This chapter presents information on the effects of dichlorvos treatments on sea lice epizootics of cultured Atlantic salmon *Salmo salar* L. in Scotland and uses data collected from a number of commercial salmon farms over a period of 20 months.

The data collected indicate that treatment of sensitive populations effectively depresses increases in the mean number of mobile (adult and preadult) *Lepeophtheirus salmonis* (Krøyer, 1837) and also reduces larval numbers through effects on recruitment. Where resistance occurs, success of treatments is far more variable. Treatment of *Caligus elongatus* Nordmann, 1832 was found to be highly effective, with no indication of resistance.

Removal of mobile stages through treatment allows the more rapid development of male stages with respect to female stages to be seen in the population and also highlights the apparent disparity between larval and mobile numbers. This latter is explained through rapid development of larval stages and long residence time of adults, leading to a build-up of mobile stages.

Larvae were found to be highly overdispersed over the host population, with peaks in overdispersion frequently coinciding with treatment events. Treatments led to more random dispersal of larval stages. Overdispersal may result from a combination of aggregation of larvae in the water column and heterogeneity of the host population. Higher intensity of infection was particularly associated with disadvantaged fish.

INTRODUCTION

Caligid copepod epizootics attributable to *Lepeophtheirus salmonis* (Krøyer, 1837) and *Caligus elongatus* Nordmann, 1832 are now the most important disease problem recognized for farmed Atlantic salmon *Salmo salar* L. in Scotland. Despite the

widespread use of organophosphate treatments and biological control methods, farmed salmon in Scotland are still subject to persistent outbreaks of sea lice infestation. This situation seems likely to continue, particularly in view of the recent demonstration by Jones *et al.* (1992) that *L. salmonis* is showing resistance to treatment with organophosphates.

Aspects of the epidemiology of caligid epizootics on farmed salmonids have been subject to previous reports both for Scottish populations (Wootten *et al.* 1982, Wootten 1985) and for those in Ireland (Tully 1989), Canada (Hogans and Trudeau 1988, 1989) and Norway (Johannessen 1975, Jaworski and Holm 1992).

Although treatment with the organophosphate pesticide dichlorvos (Aquagard® (Ciba-Geigy Agrochemicals Ltd)) is now widespread (Roth *et al.* 1993) it is only effective against the adult and preadult stages and does not kill attached larvae. Despite its widespread use, little information has been published concerning the effect of dichlorvos treatments on the pattern of sea lice epidemics in cultured fish.

MATERIALS AND METHODS

Five salmon farms on the west coast of Scotland were sampled repeatedly for a period of 20 months between 31 July 1990 and 10 March 1992. The chosen sites comprised one multiple year class farm and four single year class farms stocked with smolts in April/May following variable lengths of fallow period (see Bron *et al.* in press). On each sampling occasion, a number of pens were sampled from each site, with one or two pens being sampled from each cage group on a given site. Samples comprising five fish were taken repeatedly from the same pens on each site at intervals of 2 weeks. For sampling, fish were killed and all parasite stages (copepodids? adults) were removed and returned to the laboratory in 5% neutral buffered formalin for counting and determination of species, sex and stage. In addition to the lice, data were also collected concerning water temperature, salinity and clarity and the recent disease and treatment status of sampled cages.

For the purposes of this study the term 'mobiles' will be used to describe preadult and adult stages which, unlike the attached larvae, are actively mobile over the host surface.

RESULTS

General population profile

Fig. 1 illustrates a typical *L. salmonis* population profile observed for the single year class sites examined in this study. The general pattern seen is one of gradually rising mean intensity of lice ranging from one to two lice in early August to mean peaks of over 45 lice per fish in October and in March of the second year. This rise in mean intensity is perturbed by treatments which decrease the number of lice present on the fish. As numbers build up, there is a tendency for increased larval numbers to occur in distinct peaks, as for example in Fig. 1, where peaks are seen in April, May and August 1991 and in February 1992.

On the multiple year class site (Fig. 2), numbers rose far more rapidly than on

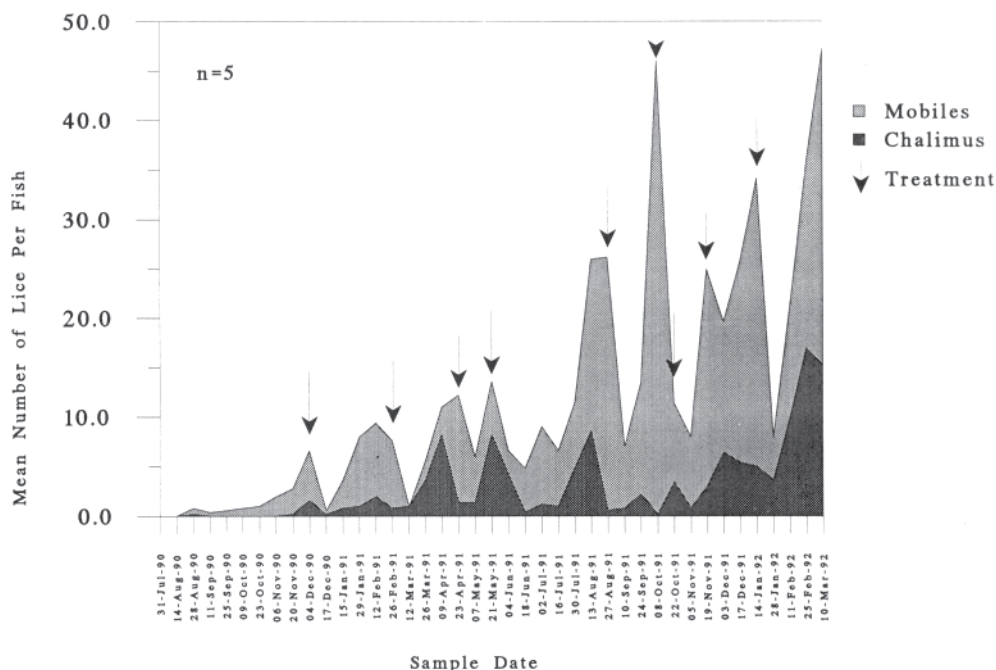


Fig. 1. Mean numbers of *L. salmonis* on a single year class site showing gradual increase in numbers with time.

single year class sites, with mean numbers of lice per fish reaching 40 by August and peaking at over 100 lice per fish in October 1990 and March 1991.

In contrast to *L. salmonis*, *C. elongatus* numbers show a highly seasonally restricted pattern of infection, with highest numbers of lice being recorded principally between July and September (Fig. 3).

The general pattern of *L. salmonis* infection recorded in a single cage is reflected over the whole site. Fig. 4 illustrates the pattern seen on five separate cage groups from a single year class site. While the magnitude of infestation varies from group to group (or cage to cage), the pattern of infection is relatively consistent, with peaks in lice numbers usually occurring concurrently (Fig. 4: March, May, July/August).

Effect of treatments

Following treatment, it can be seen that the number of mobile (adult and preadult) stages is significantly reduced, as is the number of attached larval stages. If the treatment occurs on one sampling date, the effects on larval and mobile numbers are expressed in the population by the next sampling date, although the effect on mobiles alone is apparent on the day of treatment, e.g. Fig. 1: May 1991/June 1991.

Where treatments are carried out against a population sensitive to dichlorvos, they will remove a large proportion of the mobile stages, as may be seen for the single year class site in Fig. 1: December 1990, February 1991. There is also a large reduction in the number of settled larval stages.

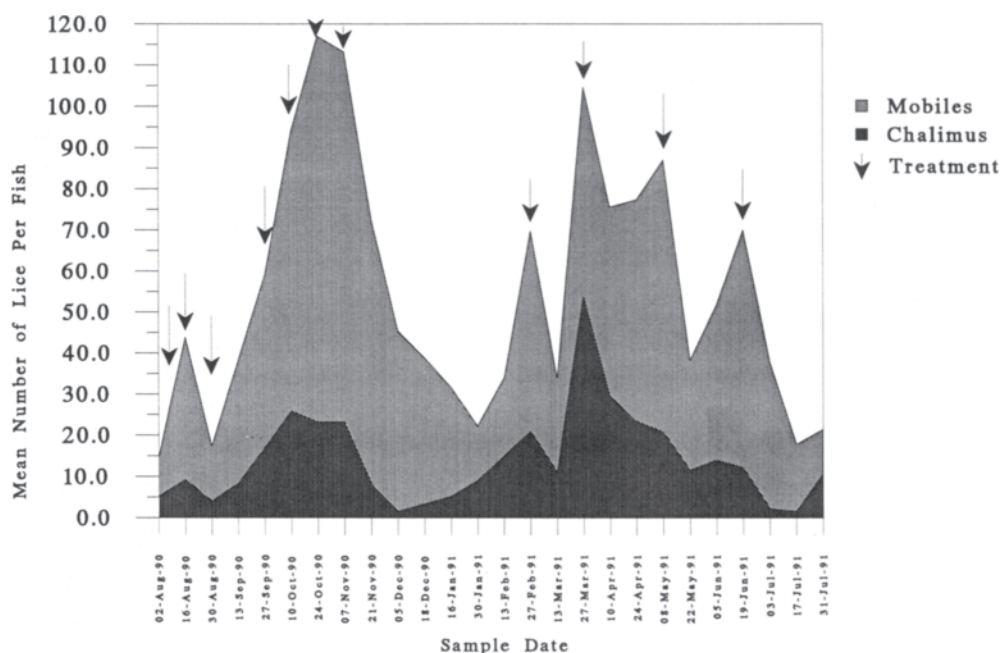


Fig. 2. Mean numbers of *L. salmonis* on a multiple year class site throughout the sampling period, showing high lice numbers and frequent unsuccessful treatments.

Where resistance has developed, as may be seen for the multiple year class site (Fig. 2: October 1990, March 1991) and the later part of the single year class cycle (Fig. 1: November 1991), the success of treatments is far more variable and the proportion of mobiles removed by treatments is relatively much lower. In addition, the reduction in the number of larvae is similarly depressed. As a consequence of low treatment success and subsequent high numbers of lice at this latter site, considerable fish damage occurred.

In comparison with *L. salmonis*, *C. elongatus* demonstrates relatively greater susceptibility to chemical treatment, with a high proportion of mobiles being seen to be removed following any given treatment (Fig. 3). The effect of treatment on larval numbers seems less pronounced, however, than is the case for *L. salmonis*.

Ratio of mobiles to settled larvae

One frequent characteristic of farm epizootics of lice is the appearance of relatively large numbers of mobile stages from an earlier, apparently smaller, reservoir of attached larvae, e.g. Fig. 1: August 1991, October 1991; Fig. 2: October 1990. This gives the appearance of a sudden infection by mobile lice.

Sex ratio

An example of the adult sex ratio for a cage from a single year class site is shown in Fig. 5. It can be seen that the ratio of males to females is usually equal to or greater

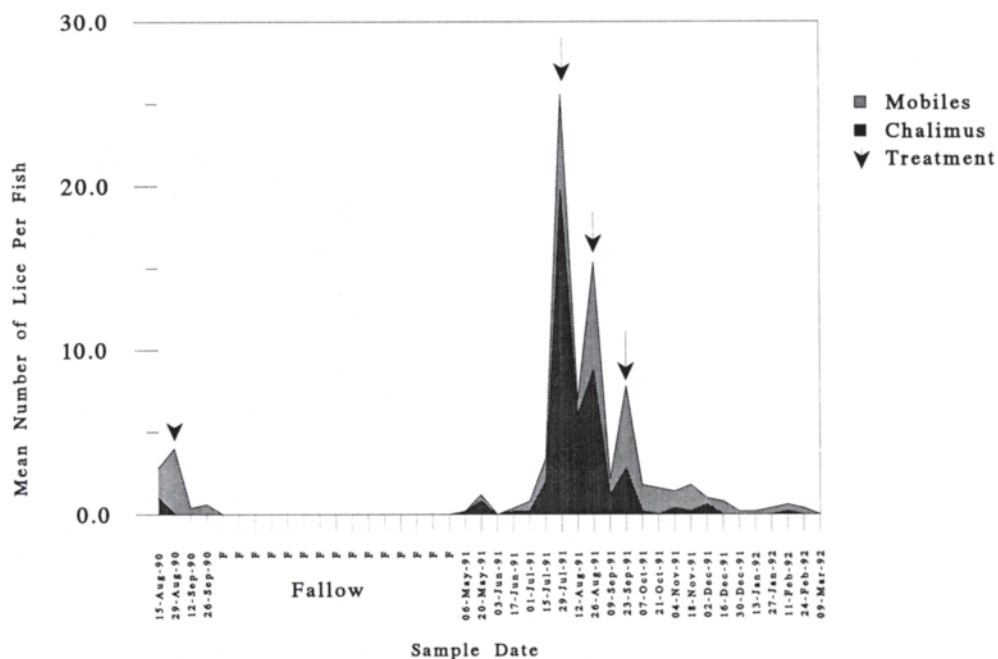


Fig. 3. Mean numbers of *C. elongatus* on a single year class site, showing seasonal infection and effectiveness of treatments.

than 1:1. At certain times, however, the ratio of males to females increases dramatically and it can be seen that this normally occurs subsequent to a treatment or grading event, both of which remove mobile lice stages. Fig. 6 shows the sex ratios for the various mobile stages following a number of treatments. While female numbers are higher than male numbers in the preadult 1 stage, male numbers are higher in the preadult 2 and adult stages.

Dispersal

It was clear from sampling that some individual fish could carry higher parasite burdens than the general population in a given cage, i.e. the lice were overdispersed on the host population. Fish in poor condition, diseased or early-maturing fish were particularly susceptible to high lice infections. By way of an extreme example, a single mature male salmon was found to have a total of 479 settled larval stages, in contrast to a mean of 8.53 larvae per fish for fish sampled in the same and adjacent groups ($n=19$). It was also noted that fish that had been badly descaled during grading or treatment also showed enhanced settlement of larval stages around the damaged skin areas.

When the pattern of dispersion for larval stages alone is considered, the influence of treatments may be observed. Fig. 7 illustrates the dispersion (variance/mean) of total settled larval stages on salmon from a single pen throughout the period sampled. It may be seen that this plot shows a number of peaks of overdispersion (χ^2 , $p < 0.05$)

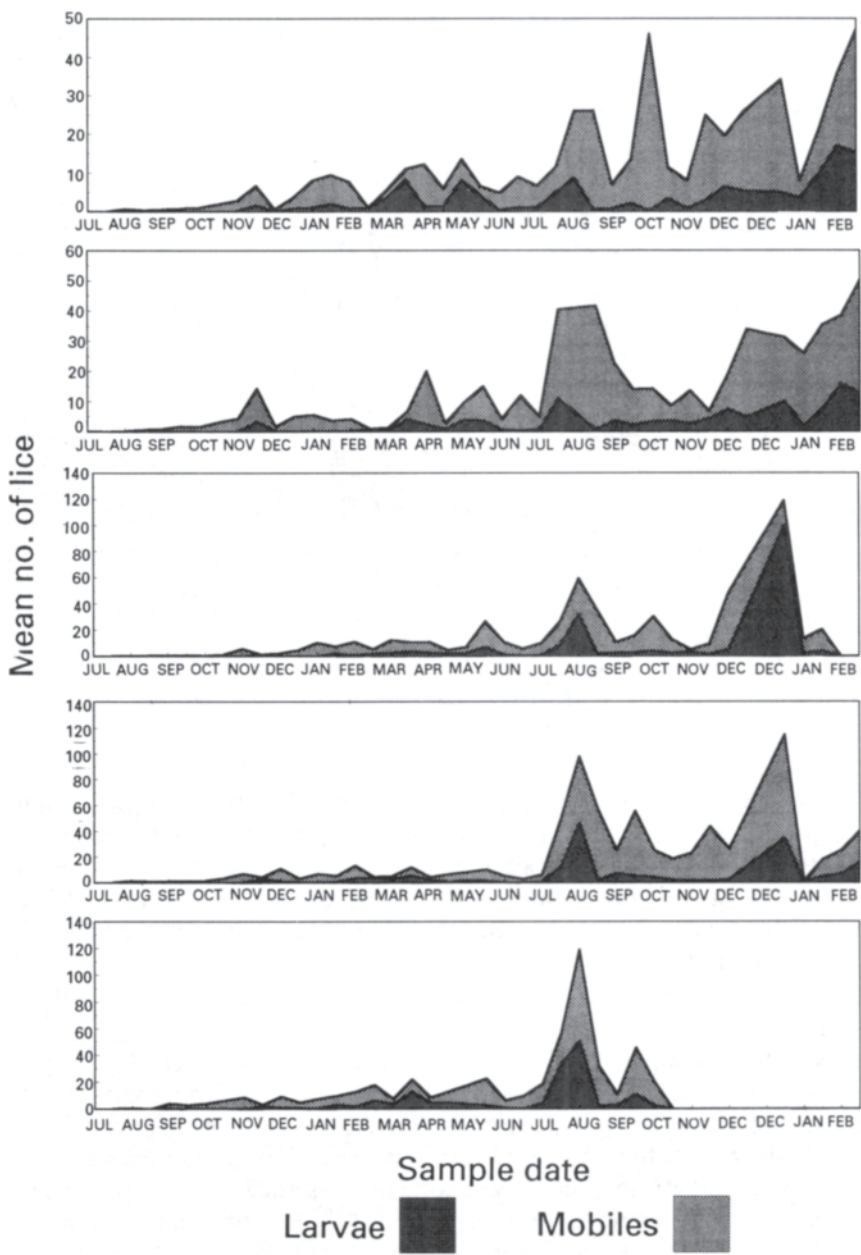


Fig. 4. Comparison of mean numbers of *L. salmonis* on five groups taken from a single year class site, demonstrating the similarity of the pattern of infection throughout the sampling period.

interspersed with troughs which represent an essentially random distribution. It may also be noticed that the peaks in larval overdispersion precede or coincide in general with treatment events and that the troughs usually follow treatments.

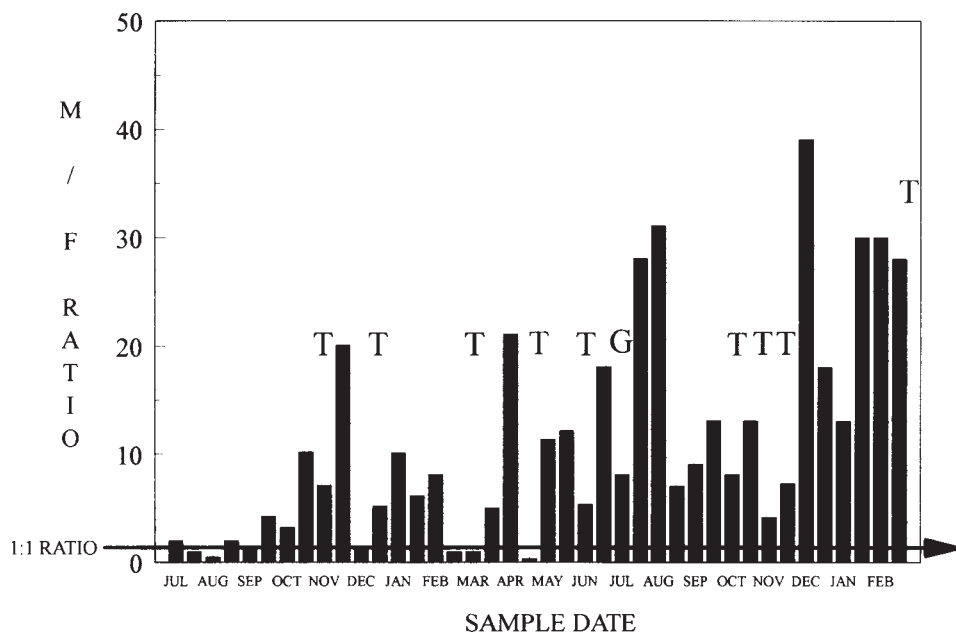


Fig. 5. Adult sex ratio on a cage sampled from a single year class site illustrating the tendency towards male adult predominance. T, treatment; G, grading.

DISCUSSION

The general tendency for numbers of caligid parasites to increase with successive generations seen in the present study has been reported previously by Johannessen (1975) for untreated salmon from Norwegian farms and has also been noted by Wootten *et al.* (1982) for Scottish farmed salmon. In contrast to the findings here, Tully (1989) reported that parasite intensity did not rise cumulatively on the Irish site he studied, and he suggested that low numbers of lice might be sustainable using strategic treatments to remove peak infections. The data presented here suggest that for the sites in this study a lack of treatment, or treatment failure, caused numbers to reach levels that were seriously damaging to fish. It may be that the difference between the present study and that of Tully (1989) is principally one of geographical position. In the latter study preadult females were not found to mature before December, this being attributed to water temperatures, while gravid adult females were present all year round in the present study and in that of Wootten (1985).

The gradual increase in numbers of *L. salmonis* seen here for single year class sites as opposed to the rapid increase in infection of multiple year class sites was attributed to infection of newly introduced fish on the latter sites by fish already *in situ*. This phenomenon is discussed elsewhere in relation to fallowing of sites by Bron *et al.* (in press) and consequently will not be covered here in further detail.

A different pattern of population growth is apparent for *C. elongatus*. In common with the reports of Wootten *et al.* (1982) and Hogans and Trudeau (1989), *C. elongatus*

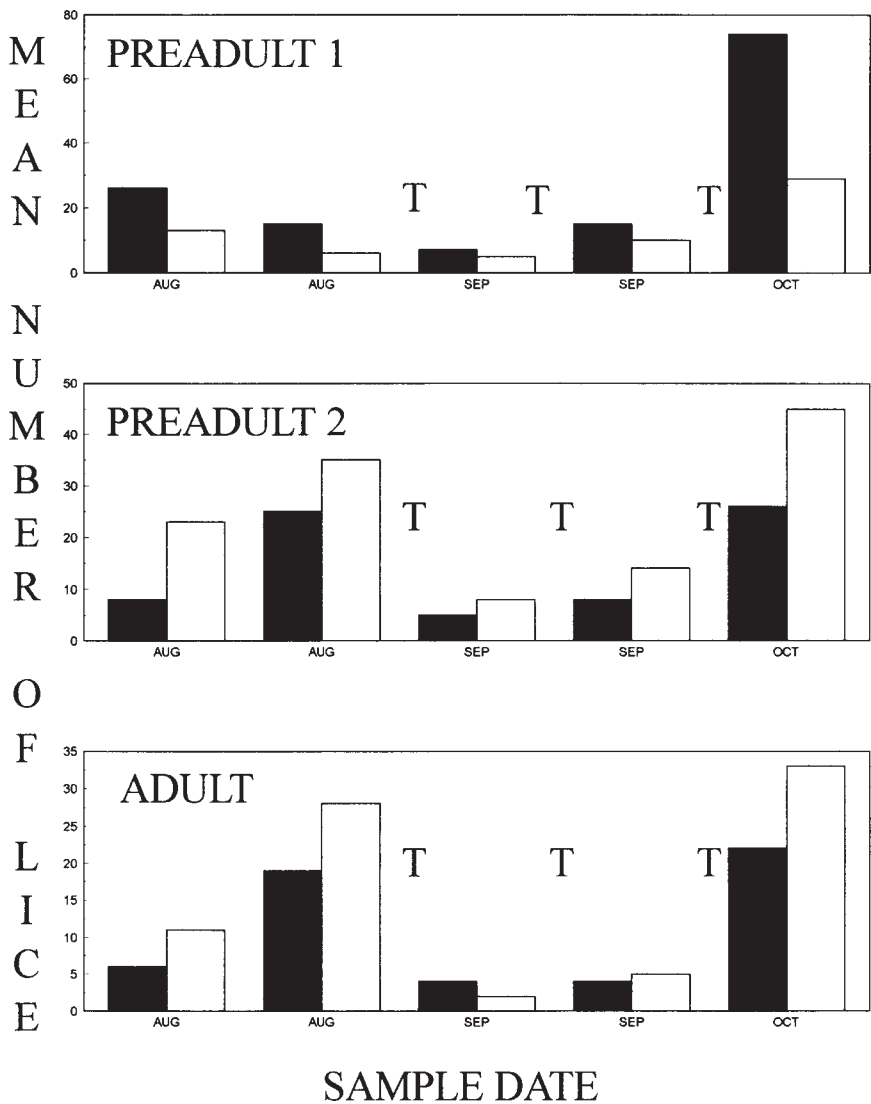


Fig. 6. Sex ratios of preadult and adult stages on a cage sampled from a single year class site, illustrating the faster development of males. T, treatment; black, female; white, male.

was principally a problem of the late summer and autumn for the sites examined in the present study. This temporal restriction in occurrence may be related to the movement of wild fish such as gadoids and scombrids which migrate inshore in the warmer months and which may act as a reservoir of infection (Wootten *et al.* 1982).

The success of treatments against susceptible *L. salmonis* populations is clearly demonstrated by this study. Wootten *et al.* (1982) have reported that dichlorvos does not kill larval stages directly, so that the effect on larval as well as mobile stages seen

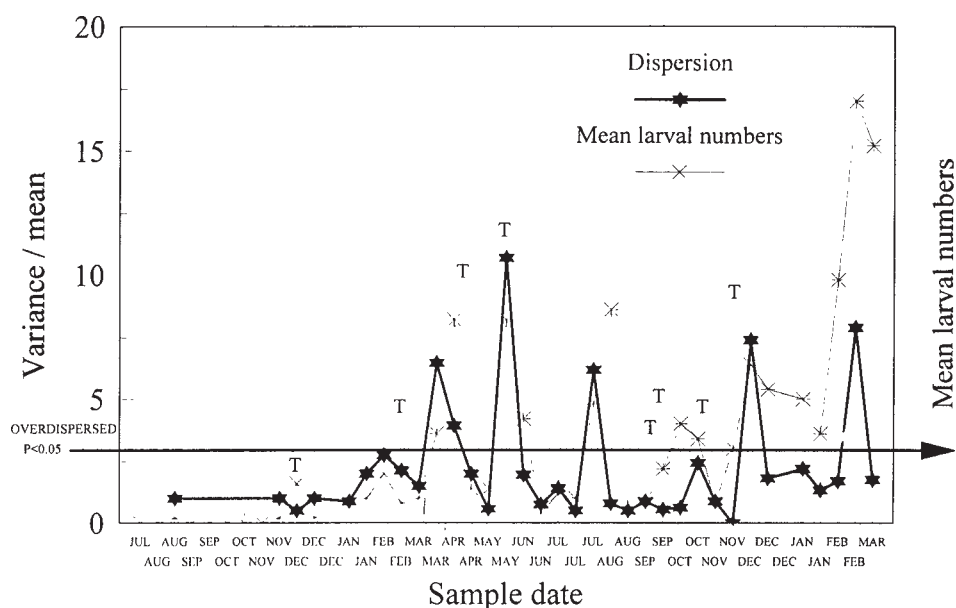


Fig. 7. Dispersion (variance/mean) of total larval numbers on host salmon from a cage sampled from a single year class site, showing the association between population growth and overdispersion and the effect of treatment events. T, treatment.

in the present study must be attributed to recruitment failure following from loss of adult stages.

The apparent resistance to treatment seen at the multiple year class site has serious consequences for the level of site infection. The fact that a high number of mobiles may remain after treatment not only means that fish are subjected to greater louse damage but also that larval recruitment may continue at high levels, causing a further self-amplification of lice numbers on the site.

The present investigation indicates that, at least for the site studied, the general pattern of infection over the whole site, as opposed to the magnitude of infection in individual cages, is similar for all the cage groups on a given site. The fall in numbers of larvae due to recruitment failure following treatment provides evidence that in the case of *L. salmonis* the sites studied are principally self-reinfecting. This latter is in agreement with the conclusions of Wootten (1985) and Tully (1989) for the same species. Taken together, these facts suggest that in order to reduce the pool of infection for a site as a whole, all groups on a farm need to be treated together within a short space of time rather than by a campaign of piecemeal treatment of the single most infected cages through time.

The response of *C. elongatus* to treatment is different from that noted for *L. salmonis* above. The large natural reservoir of infection and pronounced temporal restriction in the occurrence of this species on salmon farms means that resistance to treatment is less likely to develop. This may account for the generally high effectiveness of treatments directed against *C. elongatus*. The relatively small effect of treatment on larval recruitment suggests that for this species a larger proportion of larval infection derives

from outside the site and therefore cannot be controlled through treatment of farm-associated mobile stages.

The appearance of a relatively large number of mobiles of *L. salmonis* following from an apparently smaller number of larvae is explained principally in terms of the rate of development through the various stages. While the adult stages can survive for a number of weeks, individual larval stages develop through to the successive stage in under 7 days. This leads to an accumulation of adult and preadult stages as the attached larval stages quickly develop through to mobiles. A similarly large ratio of mobiles to larvae has also been reported by Johannessen (1975). This phenomenon will be enhanced by higher water temperatures, which speed up the rate of development through the various stages (Johnson and Albright 1991). The difference between larval and mobile numbers will also be increased by the effect of treatments, which will cause a gap in larval recruitment while allowing those larvae already present in the water column and those settled on the fish to develop into mobiles. The possibility that this difference in relative numbers is caused by a sampling error such as the 30% deficit reported by Tully (1989) for counts of chalimus from anaesthetized fish is thought to be unlikely in the present study since killed fish can be examined more closely for larvae.

The sex ratio in adult *L. salmonis* was normally found to be at least 1:1 and was frequently weighted in favour of the males. The data described for the present study indicate that males develop to the adult stage faster than females, which agrees with the findings of Johnson and Albright (1991). This faster development is already evident at the preadult 1 stage, which gives more preadult 2 and adult males than females but leaves a higher number of preadult 1 females than preadult 1 males. Treatments remove all mobiles, so that this rapid male development may be clearly observed in studied populations. Eventually, the greater longevity of adult females will tend to equalize the number of adults, and ultimately adult females will predominate as the male adults die. It is important to note that the situation seen here is, in many respects, artificial and differs considerably from that reported for wild fish by Johannessen (1975), who found a pronounced predominance of adult females, which may be attributable to their greater longevity relative to that of other stages. The latter author reported that the sex ratio on the untreated farmed fish in his survey was approximately 1:1 although this represented both preadult and adult stages. The faster development of males seen in the present study allows the adult males to locate and guard preadult females before they mature. The minimum 1:1 ratio seen in adults ensures that most females reaching maturity will be fertilized, particularly since males may be able to fertilize more than one female. This suggestion is supported by the rarity of unfertilized adult females observed in samples of lice from farmed fish.

The overdispersal of parasites upon their hosts has been suggested to be a characteristic of parasite populations (Crofton 1971), thus it is not surprising to find such overdispersal in the larval stages of *L. salmonis* on farmed fish. Such overdispersal was also noted by Johannessen (1975) at particular times of year and was associated with the presence of mature fish within samples, which carried larger numbers of lice than were found for immature fish in the same samples. Boxshall (1974) has reported similar overdispersal for *Lepeophtheirus pectoralis* at certain times during the year and found more overdispersed distributions to be associated with periods of population mortality (low numbers). In the present study, overdispersal was found to be associated

with periods of population growth (high numbers) and fell drastically following treatments, presumably due to the low numbers of larvae available to reinfect fish as a result of adult parasite mortality. These findings are in accord with the findings of Scott (1985, 1987), who reported that the ectoparasite *Gyrodactylus bullatarudis* Turnbull associated with guppies *Poecilia reticulata* (Peters) showed highest overdispersal at high parasite burdens.

The exact factors leading to the overdispersal of *L. salmonis* larvae upon the salmon are difficult to determine, particularly in view of the number of possible contributory factors which may cause overdispersal (see Anderson and Gordon 1982). Boxshall (1974) has suggested that the overdispersed distribution seen for *L. pectoralis* on plaice resulted from simultaneous infection of hosts by larvae present in randomly distributed aggregations within a heterogeneous environment. Another possible mechanism of overdispersal and one that may be at least partially responsible in the case of farmed salmon is the effect of a heterogeneous host population on settlement of larvae. In this case, it is hypothesized that disadvantaged fish might be more readily infected by larvae from a randomly distributed larval population than their healthy counterparts. This could occur through behavioural modifications such as slower swimming speeds, morphological changes such as damage or change in texture of the epidermis or by compromise of the host immune system through stress. All these factors might affect success of larval settlement or enhance larval survival subsequent to settlement. Heterogeneity of host size and hence area available for settlement and fish swimming speed may also contribute to the overdispersed distribution seen.

ACKNOWLEDGEMENTS

The authors would like to acknowledge funding from the Scottish Salmon Growers Association Ltd, which enabled us to carry out this work. We should furthermore like to thank the companies and staff of the farms we visited for all their assistance, without which this study could not have been undertaken.

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Preliminary studies on the efficacy of two pyrethroid compounds, resmethrin and lambda-cyhalothrin, for the treatment of sea lice (*Lepeophtheirus salmonis*) infestations of farmed Atlantic salmon (*Salmo salar*)

Myron Roth, Randolph H. Richards and Christina Sommerville

ABSTRACT

Pyrethroids (synthetic pyrethrins) represent a large class of recently developed insecticides which are widely used for pest control, but are also well known for their high toxicity to aquatic animals, in particular fish. We report here on the efficacy of two pyrethroid pesticides, resmethrin and lambda-cyhalothrin, in the treatment of adult and preadult sea lice, *Lepeophtheirus salmonis*, infestations of farmed Atlantic salmon, *Salmo salar*. Optimum (90%) efficacy (percent reduction in numbers of lice/fish) with resmethrin (1 h exposure) was achieved at concentrations ranging from 0.01 to 0.1 mg l⁻¹, depending on experimental conditions. Fish tolerated single exposures to resmethrin at concentrations of 1.0 mg l⁻¹ but displayed short-term signs of stress following treatment; thus the therapeutic ratio was estimated to be 10. Lambda-cyhalothrin was found to be highly efficacious at a concentration of 0.005 mg l⁻¹ (1 h exposure). Fish tolerated single exposures to lambda-cyhalothrin at a concentration of 0.01 mg l⁻¹ but not 0.05 mg l⁻¹, which was found to be toxic; thus the therapeutic ratio was estimated to be 5. Optimum efficacy following treatment with resmethrin was achieved 8 h post-treatment. Preliminary observations on the toxicity of resmethrin to larval, or chalmus, sea lice stages indicated a greater tolerance to the compound than observed for preadult and adult stages. These results indicate that some pyrethroid pesticides could be considered as alternative chemotherapeutants for the control of sea lice.

INTRODUCTION

Infestations with caligid copepods (collectively known as sea lice) currently represent the most damaging parasitic problem affecting salmonid farming. Once established

within a farm site, sea lice can quickly overcome stock, causing serious damage and loss (Stewart 1990, Wootten *et al.* 1982). To control sea lice infestations, farmers rely primarily on the use of the organophosphorus pesticides trichlorfon and dichlorvos (Grave *et al.* 1991a, Reyes and Bravo 1983, Rae 1979, Brandal and Egidius 1979). However, owing to high toxicity and unpredictable fish kills following treatment (Horsberg *et al.* 1989, Salte *et al.* 1987) the use of trichlorfon in intensive salmon farming has been largely replaced by preparations based on dichlorvos (Grave *et al.* 1991b).

Sea lice treatments with dichlorvos (marketed under the trade name Aquagard®, formerly Nuvan®, by Ciba-Geigy Agriculture) are affected by difficulties which include low therapeutic ratio, difficulty in estimation of treatment dose and lack of toxic effect to larval stages of lice (Roth *et al.* 1993). Furthermore, the reliance by farmers on the use of a single chemotherapeutant has resulted in reduced sensitivity to dichlorvos in isolated populations of lice (Jones *et al.* 1992).

Several novel compounds have been investigated for suitability as possible alternative chemotherapeutants for sea lice control (Roth *et al.* 1993). Although a number of compounds representing several different classes of pesticides have been investigated, to date there has been very little work on the large group of pyrethroid pesticides.

Pyrethroids (synthetic pyrethrins) represent a large unique class of pesticides commonly used for household, agricultural and public hygiene pests (World Health Organization 1989). Characterization of these compounds has allowed manufacturers to manipulate the design of molecules to meet stringent quality characteristics and reduce the ecotoxicological and environmental impact, resulting in wide acceptance and use (Carter 1989). However, pyrethroids are considered to be highly toxic to fish (for reviews see Clark *et al.* 1989, Haya 1989), suggesting that low therapeutic ratios, as a result of high toxicity to fish, might negate using these compounds for sea lice control. The relatively high toxicity of pyrethroids to fish, relative to mammals, is believed to be related to slower metabolism (Glickman *et al.* 1982), but may also be related to target site specificity (Glickman and Lech 1982). In general, the toxicity of pyrethroids is believed to be primarily due to interference with sodium channels in nerve membranes, resulting in short repetitive firing (known as type I effects), or a long-lasting suppression of impulses (type II effects); both effects are dependent on the structure of the pyrethroid in question (World Health Organization 1989, Baillie 1985).

Pyrethrum, the naturally derived precursor to the pyrethroid compounds, was the first compound in the group reported for use in sea lice control (Jakobsen and Holm 1990). Jakobsen and Holm (1990) described a novel technique which involved coating the surface water of cages with a pyrethrum–oil mixture through which lice-infected fish jumped. The technique achieved variable success and is still being evaluated and modified (Anon. 1991, Boxaspen and Holm 1991). This chapter reports on the results from preliminary trials with two pyrethroid compounds, resmethrin and lambda-cyhalothrin, used as a bath treatment for the control of sea lice under laboratory conditions.

METHODS

General

Two formulations of resmethrin (5-benzyl-3-furylmethyl (1RS, 3RS; 1RS, 3SR)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate) (IUPAC) (Worthing and Hance 1991) were obtained from Border Research Ltd. The first was a 20% active ingredient (a.i.) w/v methanol suspension (MS), the second a 1.0% a.i. w/v emulsified concentrate (EC). Lambda-cyhalothrin ((S)-*a*-cyano-3-phenoxybenzyl (Z)-(1R)-cis-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate and (R)-*a*-cyano-3-phenoxybenzyl (Z)-(1S)-cis-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate) (1:1) (IUPAC) (Worthing and Hance, 1991) was obtained as a 87.8% a.i. w/w technical liquid from ICI Agrochemicals, which was subsequently solubilized in ethyl alcohol (EtOH). Concentrated stock solutions were diluted in chilled, filtered sea water in light, opaque glass containers and are reported as nominal concentrations.

Efficacy trials

Efficacy trials with lice (*Lepeophtheirus salmonis*)-infected Atlantic salmon (*Salmo salar*) were carried out in the laboratory using two protocols which differed with respect to the source of the lice infection. For the first method, lice-infected salmon (for sizes refer to Table 1) were obtained from sea cage grow-out sites from two lochs on the west coast of Scotland (coded lochs 1 and 2). These fish were transferred to the laboratory in polythene bags of aerated sea water and placed into holding tanks with flow-through sea water. Fish were then randomly placed into test groups for each trial (for group sizes refer to Table 1), the water in each of the tanks was adjusted to a known value and an appropriate amount of stock test solution added to achieve the desired test concentrations (for concentrations tested refer to Table 1). For control groups, an amount of solvent was added equivalent to that used for the highest concentration of test compound. Water in the tanks was then allowed to remain static, with aeration, for 1 h to simulate current sea lice treatment exposure regimens (Rae 1979). Following the 1 h exposure, the tanks were flushed with fresh sea water in order to remove the test compound. Fish were then held in the tanks for a further 24 h recovery/observation period. After the 24 h recovery period, fish were removed from the tanks, killed by a blow to the head, weighed and the number of adult and preadult lice counted. For one of the trials, one concentration (resmethrin MS, 0.005 mg l⁻¹) was tested for efficacy following 1 and 2 h exposures.

Only resmethrin was evaluated for toxicity towards sea lice chalimus stages. Effects on the chalimus were documented for three concentrations, which included, 0.01, 0.05 mg l⁻¹ (EC) and 0.5 mg l⁻¹ (MS). Chalimus stages on fish were counted by visual examination with the aid of a low-power microscope. Chalimus stages were identified for developmental stage and whether alive or dead by observing coordinated movements of the appendages and/or body.

The second protocol utilized fish which had been experimentally infected with lice. For these trials, sea-water acclimated fish were obtained from a hatchery on the west coast of Scotland. Lice were collected from a site located in a third loch

Table 1. Experimental parameters and efficacy results for toxicity trials with the pyrethroid compounds resmethrin and lambda-cyhalothrin to sea lice *in vivo*

Trial	T (°C)	Sal. (g l ⁻¹)	Fish Wt ± s.d. (g)	Lice source	Conc. (mg l ⁻¹) (n fish)	Lice count $\bar{x} \pm \text{s.d.}$	Efficacy $\bar{x} \pm \text{s.d.}$
<i>Resmethrin (20% suspension)</i>							
T1	7.0	32.0	≈ 275	Loch 1	PT (10)	10.5 ± 4.5	—
					0.0 (8)	11.4 ± 6.9	—
					0.01 (10)	1.3 ± 1.0	89.4 ± 9.1
T2	7.0	32.0	≈ 275	Loch 1	PT (9)	8.2 ± 2.8	—
					0.0 (6)	7.6 ± 3.6	—
					0.005 ^a (10)	1.5 ± 1.2	80.3 ± 16.7
					0.005 ^b (10)	1.2 ± 1.0	84.2 ± 4.3
					0.01 (6)	0.3 ± 0.5	96.7 ± 3.3
T3	7.0	30.5	638.1 ± 133.3	Loch 1	0.0 (11)	6.2 ± 2.1	—
					0.01 (11)	4.6 ± 2.5	32.9 ± 31.5
					0.05 (10)	0.2 ± 0.4	96.8 ± 6.8
					0.1 (10)	0.0 ± 0.0	100.0 ± 0.0
					0.5 (10)	0.0 ± 0.0	100.0 ± 0.0
T4	15.0	30.0	360.2 ± 68.8	Loch 3	0.0 (10)	11.3 ± 4.1	—
					0.005 (10)	10.8 ± 5.8	29.6 ± 30.7
					0.01 (10)	8.4 ± 3.5	29.6 ± 26.9
					0.025 (10)	5.0 ± 2.3	55.8 ± 21.7
					0.05 (10)	1.7 ± 1.3	85.0 ± 11.3
					1.0 (10)	0.0 ± 0.0	100.0 ± 0.0
<i>Resmethrin (1% EC)</i>							
T1	7.0	35.0	2123.8 ± 896.5	Loch 2	0.0 (6)	24.8 ± 13.9	—
					0.01 (6)	16.0 ± 7.3	37.1 ± 29.8
					0.05 (6)	6.0 ± 4.5	75.8 ± 19.9
<i>Lambda-cyhalothrin</i>							
T1	15.0	30.0	431 ± 56.1	Loch 3	0.0 (12)	13.0 ± 4.2	—
					0.0005 (10)	7.6 ± 3.8	44.6 ± 26.5
					0.001 (10)	2.0 ± 1.5	84.6 ± 12.0
					0.005 (10)	0.1 ± 0.3	92.3 ± 2.4
					0.01 (10)	0.0 ± 0.0	100.0 ± 0.0
					0.05 (10)	0.0 ± 0.0	100.0 ± 0.0

^a1 h exposure; ^b2 h exposure.

on the west coast of Scotland (coded loch 3). Lice were collected during routine harvests from salmon killed by a blow to the head. Lice were carefully removed from fish with forceps and placed into polythene bags pre-filled with fresh sea water. The bags were then placed in an insulated carrier box and transported back to the laboratory.

Salmon were infected with lice by anaesthetizing them (0.075% benzocaine (ethyl-*p*-aminobenzoate) solution) and then placing them into a mesh-lined (64 µm) insulated container filled with fresh sea water. Lice were then placed in the container and allowed

to come into contact with the fish. Fish were removed when a minimum of ten lice could be seen attached to them. Once infected, fish were placed back into their respective holding tanks to recover (24 h). These fish were then assigned to random groups for efficacy testing and treated as described above.

Efficacy of treatment was calculated for each fish in each of the response groups as a percentage reduction from the controls (grouped) using equation 1:

$$100 - ((\bar{x}_i/\bar{x}_c) \times 100) = \text{efficacy (\%)} \quad (1)$$

where $\bar{x}_i$ =mean number of lice/fish on individual fish, and $\bar{x}_c$ =mean number of lice/fish in the control group.

Efficacies were then averaged to estimate the mean efficacy for each of the response groups. However, where the number of lice on a fish exceeded the mean for the control group an efficacy value of 0 was assigned.

All of the tests included a series of test concentrations and a control (detailed below). However, two additional 'pretreatment' groups were included in the first two trials with resmethrin to determine whether or not the treatment procedures were reducing lice numbers on control fish.

Standard Student *t*-tests were used for pairwise statistical comparisons of means. Where efficacies were compared between various groups, data were arcsine transformed according to Zar (1989).

Time response trials

Two trials were conducted with resmethrin to examine the time-to-response of the lice following treatment. Fish in both trials were treated as described above at a concentration of 0.3 mg l⁻¹. For both trials, fish were obtained from loch 1, and were subsequently infected with lice (obtained from the same site) as described above. Following treatment, fish were examined for parasites at hourly intervals for the first 7 h and then at 4 h intervals for an additional 16 h. A final count was made 1 week (168 h) following treatment. Lice counts were made by anaesthetizing fish with benzocaine (0.01%) and inspecting them for parasites by careful visual observation. Only adult and preadult lice were recorded. The trials differed in the number of fish and subsample sizes used. For trial 1 ten fish were used, with a random sample of five being taken for each of the sampling points. For trial 2 five fish were used and all fish were sampled at each of the sampling points. For each trial a control group was used and sampled in the same manner as described for the experimental groups.

Statistical comparisons of each of the groups with respect to time were made using analysis of variance.

Toxicity trials

Toxicity trials were only carried out with resmethrin (MS). Sea-water acclimated juvenile salmon were obtained from a hatchery on the west coast of Scotland. Fish for the trials were divided into two groups: those treated for 1 h, repeated at 24 h intervals; and those treated once for 2 h. Each group consisted of 20 fish. Concentrations tested were 0.0 (control) 0.05, 0.1 and 1.0 mg l⁻¹.

RESULTS

Efficacy trials: resmethrin

Data pertaining to each of the efficacy experiments with respect to compound, temperature, salinity, weight and number of fish, mean number of lice/fish and efficacy are given in Table 1. Comparisons of the mean numbers of lice in the pretreatment (PT) groups and the controls for the initial two trials were not found to be significantly different (T1: $t=0.39$, $p=0.70$; T2: $t=0.34$, $p=0.74$). Similarly, treatments carried out at 0.005 mg l^{-1} (T2) for 1 h and 2 h were not significantly different ($t=0.58$, $p=0.57$).

Trials 1–4 with resmethrin (MS) showed that the compound was highly efficacious at concentrations ranging from 0.01 mg l^{-1} to 0.1 mg l^{-1} . In general, trials 1 and 2 indicated high efficacy at a concentration of 0.01 mg l^{-1} , whereas trials 3 and 4, carried out 11 months later, indicated high efficacy at concentrations ranging between 0.05 and 0.1 mg l^{-1} , depending on experimental conditions (Table 1). Results from experiments 3 and 4 (dose–response trials) are summarized in Fig. 1.

Efficacy appeared to be related to temperature. When lice from lochs 1 and 2 were tested and compared at 7 and 15°C , efficacies were not significantly different at 0.01 mg l^{-1} ($t=0.01$, $p=1.0$). However, there was a significant difference in efficacy between the two temperatures when tested at 0.05 mg l^{-1} ($t=3.56$, $p<0.05$). There appeared to be little, if any, difference between the efficacy of the two formulations when tested at 0.01 mg l^{-1} ($t=0.70$, $p=0.50$). However, the MS formulation was found to be significantly more toxic at 0.05 mg l^{-1} ($t=3.3$, $p<0.05$).

It was difficult to estimate whether resmethrin, in either of the formulations, was effectively killing chalimus stages. Concentrations of 0.01 and 0.05 mg l^{-1} (EC) resulted in 27.1% and 49.7% mortality, respectively, in chalimus stages 24 h following exposure (6.7% control mortality) (Fig. 2a). However, only 32.6% of the chalimus stages exposed to 0.5 mg l^{-1} MS were found dead 24 h following exposure (0.0% control mortality) (Fig. 2b).

Efficacy trials: lambda-cyhalothrin

Lambda-cyhalothrin was only tested once and was found to be highly efficacious at 0.001 mg l^{-1} (85%) and 0.005 mg l^{-1} (99%) (Fig. 3). However, the safety margin was found to be low, with all of the fish dying in the 0.05 mg l^{-1} group; thus no further trial work was carried out.

Time response trials: resmethrin (MS)

Temperatures and salinities for the time response trials were 7.5°C and 34.5 g l^{-1} , respectively. Mean weight of the fish in trial 1 was $835.5 \pm 151 \text{ g}$, and $682.1 \pm 197.8 \text{ g}$ for Trial 2. There were no fish mortalities in trial 1. In trial 2, one control fish died 16 h post-treatment and one fish from the experimental group died 1 week post-treatment.

Results for the percentage reductions (efficacy) following the two treatments are given in Fig. 4. Mean lice numbers on control fish did not appear to vary significantly

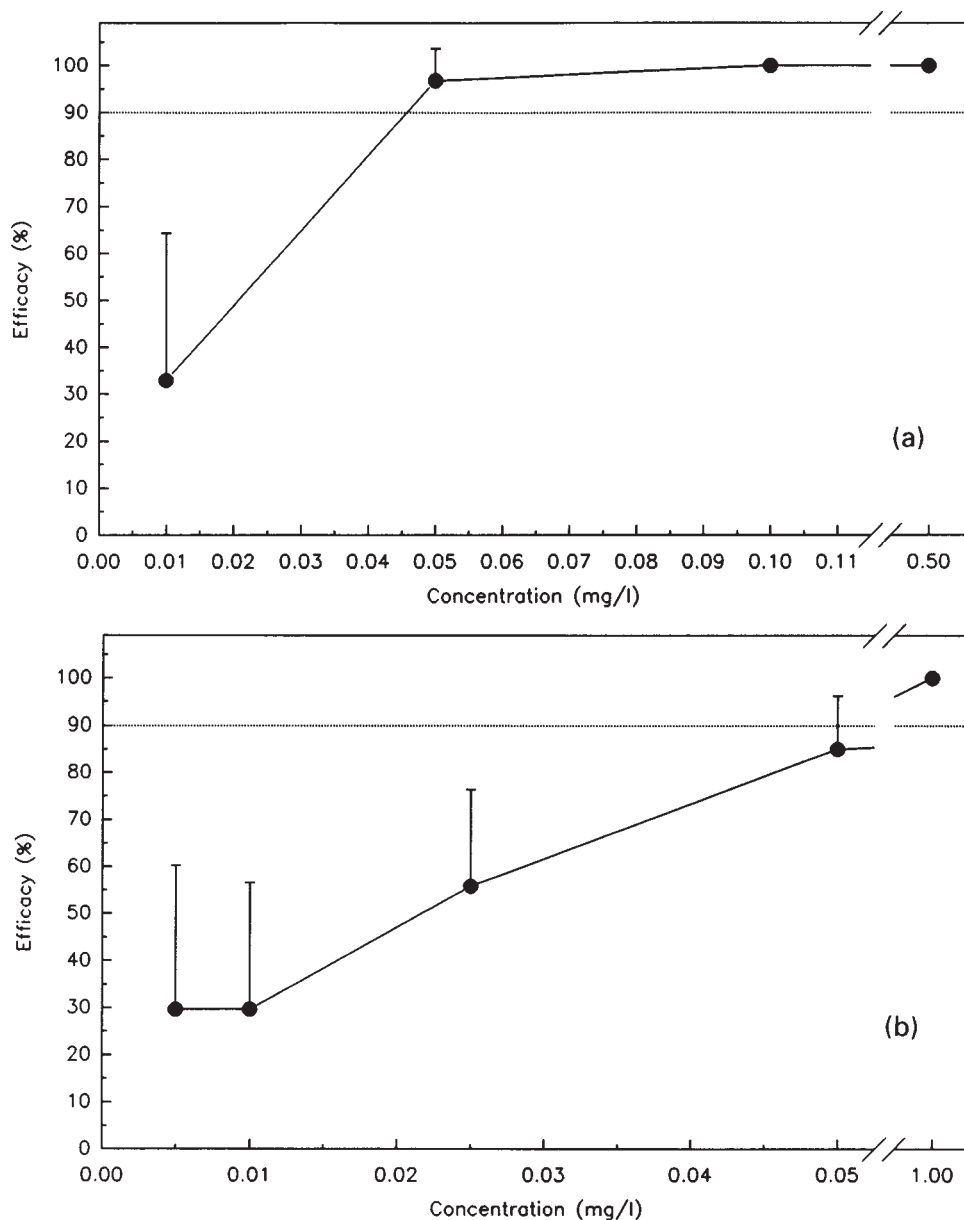


Fig. 1. Efficacy of resmethrin (MS) at removing sea lice from salmon (1 h exposures). Fig 1(a)=Loch 1 lice (T3) (7.0°C). Fig. 1(b)=Loch 3 lice (T4) (15.0°C) (error bars=S.D.).

over the course of the experimental period (T1: $F=1.53$, $p=0.14$; T2: $F=0.14$, $p=1.00$), whereas reductions in lice in both treatment groups over time were highly significant (T1: $F=4.0$, $p<0.05$; T2: $F=7.17$, $p<0.05$). In both trials optimum efficacy (90%) was not achieved until 8 h post-treatment (Fig. 4). A higher degree of variation was

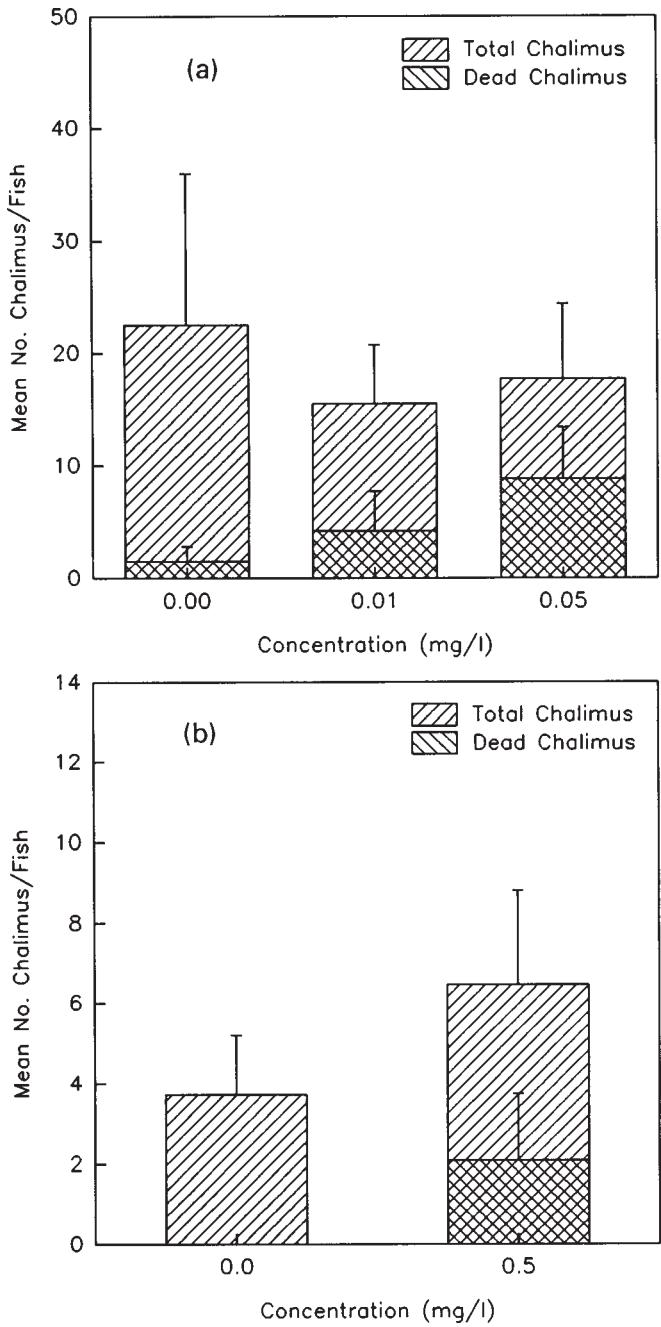


Fig. 2. Acute toxicity of resmethrin to chalcids following 1 h exposures at various concentrations (7.0°C). (a)=EC formulation, (b)=MS formulation (error bars=S.D.).

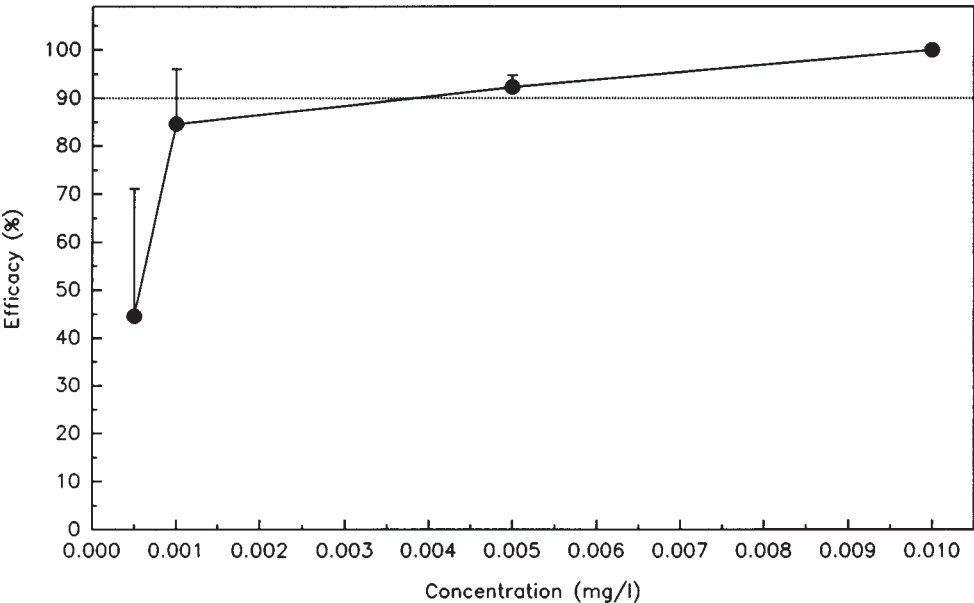


Fig. 3. Efficacy of lambda-cyhalothrin at removing sea lice from salmon following 1 h exposures (Loch 3 lice, 7.0°C) (error bars=S.D.).

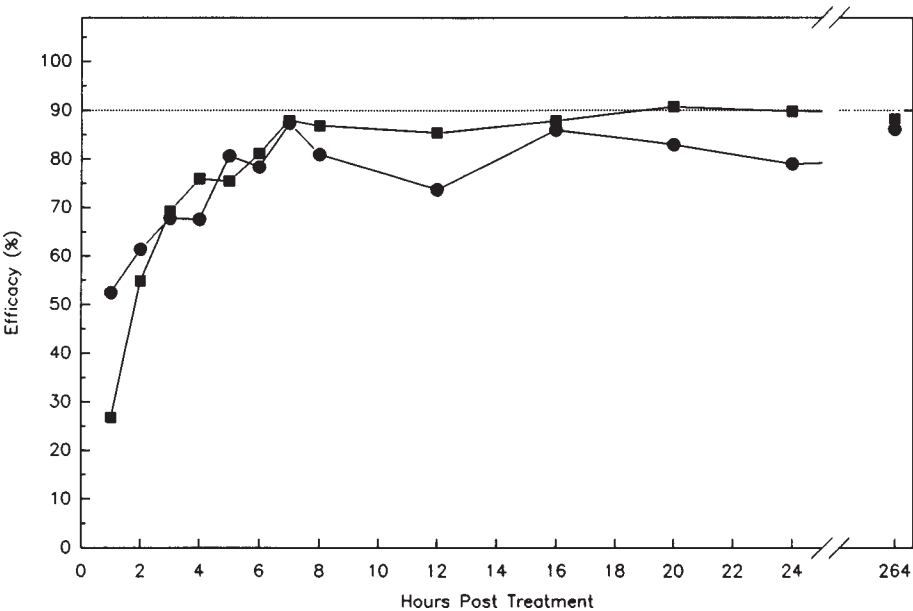


Fig. 4. Cumulative efficacy of resmethrin (MS) at removing sea lice from salmon following 1 h exposure at 0.3 mg L⁻¹ (Loch 1 lice, 7.0°C) (●=T1; ■=T2) (error bars=S.D.).

Table 2. Acute toxicity of three repetitive (24 h intervals) 1 h exposures and one 2 h exposure of resmethrin (MS) to Atlantic salmon (14.0°C)

Concentration (mg l ⁻¹)	No. of fish exposed	Cumulative mortality (%)			
		1 h exposure			2 h exposure
		1st	2nd	3rd	1st
0.00	20	0	0	5	0
0.05	20	0	0	5	0
0.10	20	0	10	50	5
1.0	20	0	70	*	100

*No exposure.

observed in trial 1, which was believed to be due to the subsampling technique used. In both trials efficacy appeared to reach a steady state 8 h post-treatment, with a small proportion of lice remaining on the fish for 1 week. It is possible that these lice represented larval stages, present on the fish obtained from the sea cage site, which had developed into preadult or adult lice.

Toxicity trial: resmethrin (MS)

Average weight of the fish used for the toxicity trials was 63.7±10.0 g. Water temperatures and salinities were 14.0°C and 33.0 g l⁻¹, respectively. Percentage mortalities for both trials (1 and 2 h exposures) are given in Table 2.

During all treatments fish appeared sedated for the first 30 min, with activity slowly increasing over the course of the exposure period. Such behaviour was not observed in the control fish, which showed moderate levels of activity throughout individual exposures. In the 2 h exposure group, fish reacted negatively at the end of the treatment when they were placed into fresh sea water. Fish reacted by frantic swimming in random directions with their heads out of the water, with all fish dying within 3 h. Similar, but less extreme, behaviour was noted in the first 1 h exposure group in which fish recovered and no mortalities were observed. Following the second treatment, fish reacted more violently when placed back into fresh sea water, with 70% of the fish dying within 4 h. A third treatment was not carried out.

DISCUSSION

The results indicate that pyrethroids such as resmethrin or lambda-cyhalothrin are not only toxic to sea lice, but show improved therapeutic margins over currently used sea lice chemotherapeutants. The recommended dose rate for Aquagard (50% w/v dichlorvos) is 2.0 mg l⁻¹ (Ciba-Geigy 1990). However, in cases where resistance to the compound is present, high concentrations and/or prolonged exposures would (hypothetically) be required to achieve optimum efficacy. Field tests with dichlorvos have shown that when used by the method described by Rae (1979), target

concentrations of 1.0 mg l^{-1} (dichlorvos) were found to vary from 0.55 to 3.4 mg l^{-1} when using full tarpaulins, and from 0.32 to 1.89 mg l^{-1} when open skirts were used (Wells *et al.* 1990), indicating a minimal requirement of a 3.5-fold therapeutic margin when sea lice control is carried out at sea. Horsberg *et al.* (1987) found that Atlantic salmon exposed to Aquagard could not tolerate dose rates above 8.0 mg l^{-1} . Thus the therapeutic margin for Aquagard is not more than four. Of the compounds tested in the present study, resmethrin was found to have a therapeutic margin of ten (assuming optimum efficacy at a dose rate of 0.1 mg l^{-1}), whereas the therapeutic margin for lambda-cyhalothrin is estimated to be, conservatively, five (assuming optimum efficacy at a dose rate of 0.005 mg l^{-1}).

The higher toxicity of lambda-cyhalothrin over resmethrin is in general agreement with other toxicity studies, which have shown that lambda-cyhalothrin has a two-fold higher 96 h acute LC_{50} to rainbow trout (*Oncorhynchus mykiss*) ($0.24 \mu\text{g l}^{-1}$) than resmethrin ($0.45 \mu\text{g l}^{-1}$) (Imperial Chemical Industries 1990, Mauck *et al.* 1976). Thus, although lambda-cyhalothrin was found to be highly toxic to lice, it was also highly toxic to fish, indicating the importance of therapeutic margins when assessing potential chemotherapeutants.

It is not surprising that decreased temperature, at effective dose rates, was found to increase the toxicity of resmethrin to sea lice. Increased toxicity with reduced temperatures is a common phenomenon with pyrethroid insecticides. Mauck *et al.* (1976) found that resmethrin was more toxic to several fish species at 12.0°C than 17.0°C . The 96 h LC_{50} for permethrin to rainbow trout is ten times lower at 5.0°C than at 20.0°C (Kumaraguru and Beamish 1981). Similarly, Cutkomp and Subramanyam (1986) found that several pyrethroids were 1.33- to 3.63-fold more toxic at 20.0°C than at 30.0°C to *Aedes aegypti* larvae. The effect of temperature on the efficacy of dichlorvos and trichlorfon is well documented (Messenger and Esnault 1992, Horsberg *et al.* 1987). Our results suggest that, if used for sea lice control, the efficacy of resmethrin may be affected by temperature, and therefore any potential dose regimen would have to be suitably designed.

The relative toxicities of the two formulations of resmethrin suggest that formulation can have a profound impact on the effectiveness of a potential chemotherapeutant. In early trials on the efficacy of pyrethrum in treating *Argulus* infections, Stammer (1959) found that an emulsified formulation was significantly more effective than a powdered formulation. Emulsifying agents have been developed to aid the solubility of many pesticides in aqueous solutions, primarily water. However, the effectiveness of the compound is also determined by the solubility across barriers such as the cuticle which are largely lipophilic. In *Caligus savala* the outer layer of the cuticle is surrounded by a thin epicuticle, primarily composed of lipids (Kannupandi 1976). Resmethrin is not readily water soluble ($<1.0 \text{ mg l}^{-1}$ at 30°C (World Health Organization 1989)) and therefore it might be speculated that the use of a methanol solvent would aid solubility in sea water but would not hinder absorption across the cuticle.

Differences in efficacies, at similar dose rates, between experiments where experimental conditions were compatible are more difficult to explain. For example, lice obtained from loch 1 appeared to be more susceptible in trials 1 and 2 at 0.01 mg l^{-1} (7.0°C) than in trial 3 (see Table 1). One possible explanation may be decreased activity, due to degradation, of the MS formulation in the interval

between the two experiments (11 months). Alternatively, differences in the populations of lice at the time of collection may reflect differences in susceptibility to the compound. Such differences in lice populations were noted by Jones *et al.* (1992), who found reduced sensitivity to dichlorvos in isolated populations. Whether or not the differences reported here are related to population-dependent reduced sensitivity is unknown. This latter hypothesis, although not to be ruled out, is unlikely as the success of pyrethroids in the past has depended on high toxicity to pests which show reduced sensitivity to organophosphates or carbamates (Carter 1989, Sawicki 1975).

Neither of the formulations of resmethrin appeared to be significantly toxic to larval stages at concentrations which were found to effectively kill adult and preadults. However, the dose response in the treated groups suggests that the chalimus stages may be susceptible to higher concentrations. Furthermore, the EC formulation was found to be relatively more toxic than the MS formulation. This difference was not observed in adult or preadult lice and may suggest a possible difference in the structure and/or composition of the larval cuticle or possible differences in lipid storage sites, as seen in calanoid copepods (Kattner and Krause 1987). To date, relatively few compounds have been noted to affect the larval stages of lice (Wootten *et al.* 1982, Brandal and Egidius 1979). Palmer *et al.* (1987) noted that when used orally, ivermectin, representative of the recently developed avermectin insecticides, appeared to impair the development of chalimus stages. Using a similar method of application, Høy and Horbserg (1991) noted a substantial decrease in chalimus numbers following oral treatment with the insect growth regulator diflubenzuron. Earlier work by Jakobsen and Holm (1990) reported efficacies ranging from 68.3% to 82.8% (reduction) against chalimus stages, following topical treatment with pyrethrum in oil. The mechanism by which chalimus larvae show reduced, or lack of, sensitivity to compounds such as dichlorvos, trichlorfon, and in the present study resmethrin, is unknown. Larval toxicity is of considerable importance when one considers that treatment frequency could be greatly reduced if all stages in the life cycle were effectively killed.

Overall, resmethrin showed a therapeutic ratio often. However, the results reported are preliminary, and variations in the efficacy results suggest that the therapeutic ratio could be higher. It was interesting to note that optimum efficacy was not achieved until 8 h following treatment. This would suggest that were compounds such as resmethrin used in clinical situations, effectiveness of treatment could not be evaluated during or directly after treatment.

Given the potential for the development of resistance to dichlorvos in lice populations (Jones *et al.* 1992) and the persistence of resistance mechanisms once selected (Sawicki 1979), there is a definite need to develop a wider range of control strategies, both chemical and non-chemical, for sea lice control to allow the development of integrated pest management schemes. Not only will the development of alternative sea lice chemotherapeutants, such as pyrethroids or avermectins, allow the effective control of dichlorvos-resistant lice populations but they will prolong the usefulness of sea lice chemotherapeutants.

ACKNOWLEDGEMENTS

Thanks are due to Mr J.Braidwood (Border Research Ltd) and Dr M.Hammer (ICI) for helpful comments and suggestions on the manuscript. We also wish to acknowledge financial support for the project from the Scottish Salmon Growers Association.

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21

Hydrogen peroxide as a delousing agent for Atlantic salmon

Jan M. Thomassen

ABSTRACT

The use of hydrogen peroxide as a delousing agent is described. When lice-infested salmon are exposed for 20 min, in an enclosed bath, to a hydrogen peroxide concentration of 1.5 g l⁻¹, 85–100% of lice will be removed. The maximum number of lice fall off within 20 min. Hydrogen peroxide is most effective against preadult and adult stages, but also a large number of chalimus have been observed to fall off. Although hydrogen peroxide is toxic to salmon, and the toxicity increases with increasing concentration, temperature and exposure time, salmon will survive exposures to a concentration of 1.5 g l⁻¹, at temperatures up to 18°C and at exposure times less than 30 min.

INTRODUCTION

Hydrogen peroxide is a strong oxidizing agent that easily breaks down to water and oxygen. Production of hydrogen peroxide is both natural and man made. Industrially produced hydrogen peroxide is used mainly for the production of chemicals, bleaching of cellulose pulp and textiles and for other purposes such as waste treatment. Minor quantities are used in human-related applications, such as food processing, disinfection, drinking-water treatment and hair bleaching. The use of hydrogen peroxide worldwide is increasing, mainly because of its low environmental impact. Although not so efficient, it can substitute for the use of chlorine compounds in many applications.

Hydrogen peroxide is generally more effective as a bacteriostat than as a bactericide (Baldry 1983). It has also, to a lesser degree, a fungicidal effect.

Hydrogen peroxide has been used to treat fish for ectoparasites in fresh water as far back as the 1920s and 1930s (Schperclaus *et al.* 1979), but very little information on its use exists. Hydrogen peroxide has also been used as an oxygen source (Marathe *et al.* 1975, Taylor and Ross 1988).

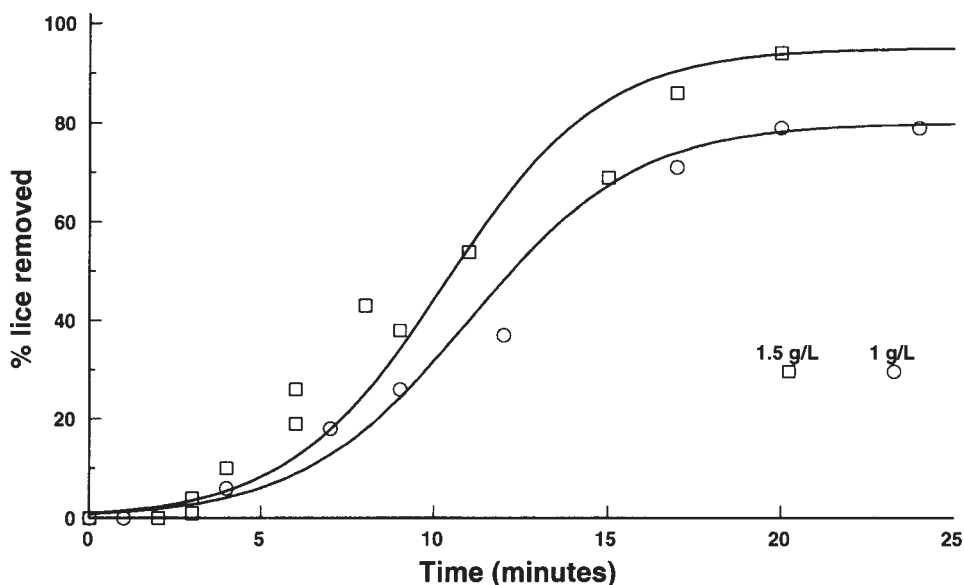


Fig. 1. Percentage of lice falling off salmon against exposure time, at two different concentrations.

At the Agricultural University of Norway, we have for some time been working with new applications for hydrogen peroxide, and one of the applications is the use of this compound as a delousing agent (Thomassen and Lekang 1990, Thomassen 1991). The method is now in use in many places in Norway and the Faroe Islands.

EFFECT ON SEA LICE

The mode of action on sea lice is not yet known. The mechanism for the bactericidal effect of hydrogen peroxide is believed to be through formation of hydroxyl radicals and its attack on DNA (Imlay 1987). Schperclaus *et al.* (1979) suggested that molecular oxygen liberated from hydrogen peroxide as a result of catalase activity was the cause of death for Protozoa and Monogenea when exposed to hydrogen peroxide.

Large amounts of oxygen are liberated inside sea lice exposed to hydrogen peroxide. Gas bubbles can be observed inside the lice, both in the gut and in the haemolymph. As a result of this, most of the lice will float up to the water surface after exposure to hydrogen peroxide. After hydrogen peroxide exposure the lice are totally lifeless, showing no reaction to gentle stimulation or any peristaltic movement. Despite this, if the lice are transferred to fresh sea water some of them will start to move after several hours (3–6 h). It is not known if these lice can attach to salmon again, but we have never seen (or had report of) reinfections after delousing with hydrogen peroxide.

Fig. 1 is from an experiment where single fish were exposed, and lice were counted

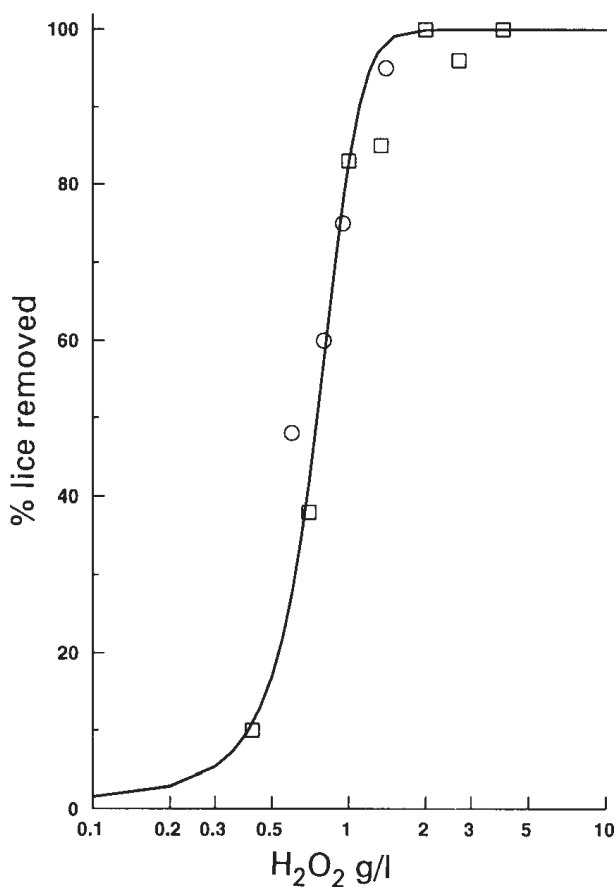


Fig. 2. Percentage of lice removed against hydrogen peroxide concentration. Exposure times 20–30 min.

as they fell off. The remaining lice were counted after exposure. The results indicate that the maximum number of lice will have fallen off within 20 min, and that prolonging the exposure time at lower concentrations has little or no effect. One explanation might be that the lice surviving 20 min have managed to compensate for the build-up of oxygen.

From exposures at temperatures between 6 and 9°C we have calculated a $EC_{50}(20\text{min})$ of 0.8 g l⁻¹. Results from exposures at 12–14°C fit the other results well, and we presumed that temperature has little effect on exposure efficacy (Fig. 2). Later field trials have indicated that it is necessary to increase the peroxide concentration at lower temperatures.

Peroxide is effective on adult and preadult stages, but the effect on chalimus larvae varies. Some exposures showed that up to 60% of the chalimus had been removed, but on other occasions only 5% were removed (Fig. 3).

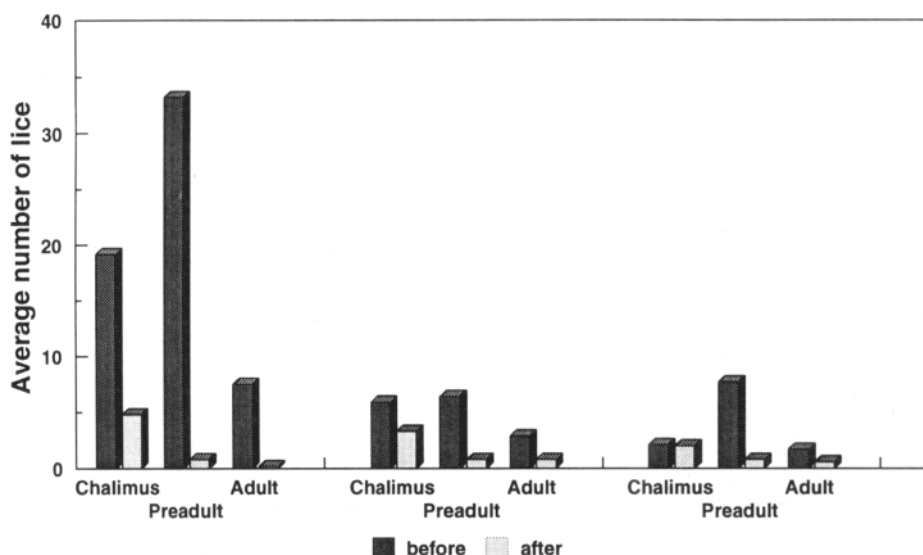


Fig. 3. Average number of lice in the groups indicated before and after exposure to hydrogen peroxide. The results to the left are from an exposure of 2 g l⁻¹ at 8°C; the results to the right are from exposure of 1 g l⁻¹ at 12°C. (Concentration and exposure time for the middle group are unknown.)

EFFECT ON SALMON

Acute lethal toxic effect

Hydrogen peroxide is toxic to salmon. The acute lethal toxicity increases with temperature and exposure time (Thomassen and Poppe 1992). The LC₅₀ (60 min) at 6°C has been estimated as 2.5 g l⁻¹, while LC₅₀ (30min) at 6°C has been estimated as at least 8.8 g l⁻¹. Fig. 4 shows the effect of temperature on toxicity. We did not test at concentrations above 9.7 g l⁻¹ and this concentration did not result in a mortality of more than 0.2. A consequence of these results is that we do not recommend using hydrogen peroxide above 14°C.

Acute sublethal toxic effect

We have investigated the sublethal effect of hydrogen peroxide at 6°C on the gills, cornea and the oesophagus, at concentrations of 1.8, 3.7 and 10 g l⁻¹ and exposure times of 30 min and at a concentration of 1.6 g l⁻¹ for 60 min (Thomassen and Poppe 1992). Compared with a non-exposed control group, there were no effects on the cornea or the oesophagus. The gills were histologically examined and graded on a scale from 1 to 5 (Table 1). There were only minimal effects at 1.7 g l⁻¹ for 30 min, and 3.7 g l⁻¹, and the differences from the control group were not significant. Increasing exposure time to 60 min increased the effect on the gills. At 10 g l⁻¹ for 30 min 25% of

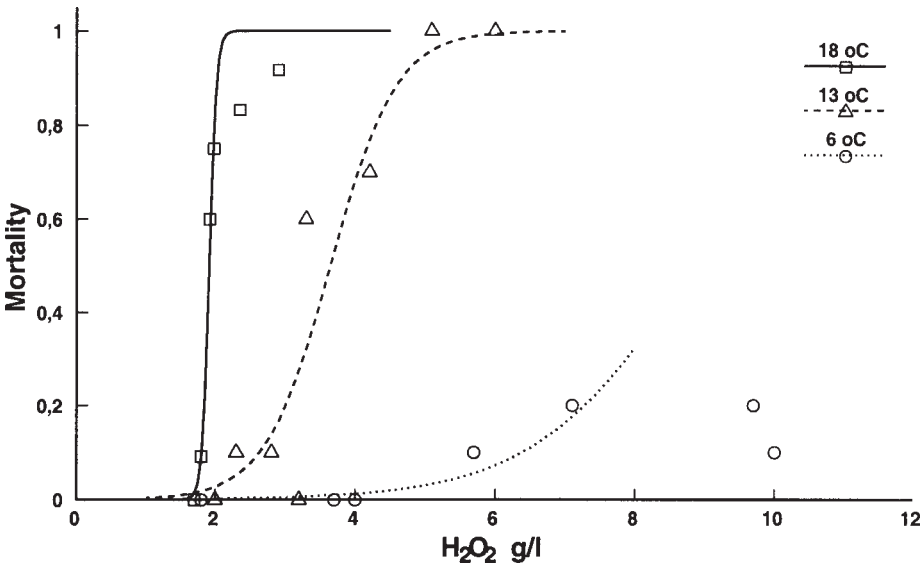


Fig. 4. Mortality for 30 min exposure against hydrogen peroxide concentration, for three different temperatures. (The curve for 6°C has only been drawn as an indication, as there was no concentration producing mortality above 0.2.)

Table 1. Grading of result of histological examination of salmon gills

1.	Normal findings, monolayered epithelial cells and few mucous cells on the secondary lamellae
2.	Minimal hypertrophy and mucous cell hyperplasia without any greater contour disturbances. Signs of hyperplasia at the tip of the secondary lamellae ('clubbing'). The surface of the epithelial cells are wrinkled and folded. This type of change can almost be regarded as a 'normal finding' in salmon from an average Norwegian salmon farm
3.	Moderate hypertrophy and hyperplasia, some fusions of the filaments and changes of the contours. Hyperaemia. Folded or wrinkled surface of the epithelial cells
4.	Extensive hyperplasia, bleeding, necrosis and fusions. Considerable changes in contours
5.	Lifting or desquamation of the respiratory epithelium (subepithelial oedema) ('sloughing') over a sizeable area. Other changes variable

the fish showed severe damage to the gills in the form of 'lifting' or desquamation of the respiratory epithelium (Fig. 5).

ACKNOWLEDGEMENT

This work was commissioned and supported by EKA Nobel AB, Bohus, Sweden.

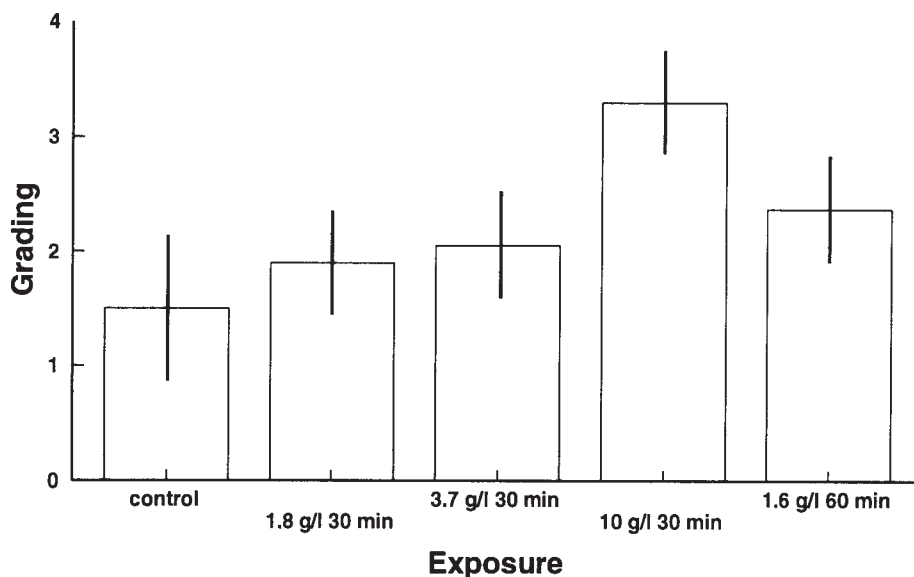


Fig. 5. Average grading of gill samples from each exposure. Vertical bars indicate 95% confidence levels of means.

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The efficiency of oral ivermectin in the control of sea lice infestations of farmed Atlantic salmon

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ABSTRACT

Four long-term (9, 10, 18 and 42 weeks) trials of the efficiency of orally administered ivermectin in controlling lice infestations of farmed Atlantic salmon held under commercial conditions are reported. All dose regimes tested resulted in a significant ($p < 0.001$) reduction in lice numbers. Median percentage reduction in lice per fish of 81% and 97% were recorded following treatment at 0.2 mg kg⁻¹ fish weight every 2 weeks, 91% following 0.075 mg kg⁻¹ twice a week, 76% and 92% following 0.1 mg kg⁻¹ once a week and 93% and 89% following 0.05 mg kg⁻¹ twice a week. Treatment with 0.03 mg kg⁻¹ resulted in an 81% median percentage reduction, but treatment with 0.02 mg kg⁻¹ twice a week only resulted in 47% and 55% reductions. A single dose of 0.2 mg kg⁻¹ administered to fish with an average of over 200 lice each reduced the numbers of lice to an average of two 4 weeks later. Ivermectin therapy was equally effective at controlling *Lepeophtheirus* and *Caligus* species and resulted in larger reductions of adult and gravid female numbers than of juveniles and preadult. No increase in morbidity or mortality was reported in any treated fish, with the exception of one cage which received an accidental overdose at 0.75 mg kg⁻¹. This resulted in a 26% mortality. No data on the ecotoxicology or pharmacokinetics of ivermectin are presented.

INTRODUCTION

The first large-capacity marine salmon cages used in Europe were introduced by the Irish salmon-farming industry in 1984. These cages, with a capacity of 6500 m³ and capable of containing 80 tonnes of salmon each, are now utilized in 25% of the annual production of salmon in Ireland. The use of these cages has presented novel problems for the health management of fish. In particular, the logistical problems of administering bath treatments with dichlorvos to control infestation

by caligid sea lice (Copepoda: Caligidae) were greatly increased (Wootten *et al.* 1982). The existence of these logistical problems suggested that oral administration of chemotherapeutants should be investigated. An additional advantage of oral therapy of caged salmon over bath treatments would be the elimination of the need for net handling required by the latter. This net handling incurs high labour costs for farmers and may also stress the fish, with adverse consequences for their general health.

Brandal and Egidius (1977) reported that oral therapy with trichlorfon was effective in removing lice but that variable feeding responses led to some fish receiving excess dose with resultant toxic effects. Palmer *et al.* (1987) investigated the efficacy of orally administered ivermectin against lice infestation. Ivermectin is a member of the avermectins, a group of 16-membered macrocyclic lactone antibiotics, and is a highly effective agent for the control of ecto- and endoparasites in a wide range of terrestrial host species. The properties of this semi-synthetic, neuroactive antibiotic have recently been reviewed by Campbell (1989). Palmer *et al.* (1987) demonstrated that ivermectin administered at 0.2 mg kg⁻¹ was effective in controlling lice numbers, but they reported that this treatment regime presented a narrow margin of safety and suggested that further work on treatment schedules was necessary.

This chapter reports trials of the use of orally administered ivermectin under commercial conditions using a variety of treatment regimes over prolonged time periods.

METHODS

Fish

All experimental treatments were carried out in the same block of 15m×15m salmon cages owned and operated by a commercial fish-farming company but reserved for experimental trials. At no time did the number of cages receiving ivermectin therapy exceed 50% of the cages in the block. The fish in the remaining cages were used either as controls and received no lice therapy or received standard dichlorvos bath treatments.

All fish husbandry, including determination of feeding rates, was carried out by personnel of the commercial fish farm according to the standard procedures used by the company in their production cages. Monitoring of fish health was carried out on a weekly basis by the company's health management team and all observations were reported to the research group.

Medication of diet

In trials 1–3 a 1% solution of ivermectin (IvermecTM; MSD Agvet) was diluted in cod liver oil by research personnel and mixed with standard feed by farm personnel. In trial 4 the antibiotic was compounded into the diet by commercial feed manufacturers.

Sampling procedure

Each experimental and control cage was sampled, weather permitting, on a weekly basis during the trial. For each day's analysis 15 fish were removed at random from

Table 1. Trial 1: analysis of weekly average ($n=15$) numbers of lice per fish during weeks 4–18

Treatment	Mean ($\pm$ s.d.)	Median	Max.	Min.
Control	6.9 ($\pm$ 4.0)	5	16.2	2.2
0.2 mg kg ⁻¹ 2 wk ⁻¹	1.3 ($\pm$ 1.3)	1.1	4.8	0.4
0.1 mg kg ⁻¹ wk ⁻¹	2.3 ($\pm$ 1.4)	2.0	4.8	0.2
0.02 mg kg ⁻¹ $\times$ 2 wk ⁻¹	3.3 ($\pm$ 2.1)	2.6	6.6	0.8

each cage in the trial. The fish were anaesthetized with benzocaine and the total number of lice on each fish was counted. In addition, in some trials all lice on five of these fish were removed and taken to the laboratory for further analysis.

Analysis of results

In order to interpret the long-term significance of each treatment, lice numbers were allowed to stabilize for 3 weeks immediately following the start of therapy and only data collected from week 4 onwards were analysed. To assess the efficiency of treatments, the mean, median and range of the weekly counts of lice on the control fish and on each treatment group during the trial were calculated. The Wilcoxon signed rank test was used to assess the statistical significance of the numbers of lice counted on treated and control fish. In addition, on each sample day the percentage reduction in lice numbers, compared with the controls, was calculated for each treatment and the range and median values of these percentage reductions were calculated.

TRIAL DETAILS AND RESULTS

Trial 1

The aim of trial 1 was to compare the efficiency of three treatment regimes in controlling lice numbers in smolts. Additional aims were to estimate the differential susceptibility of lice species and of the different life stages of lice to the ivermectin treatments.

Design

The fish used in the trial were smolts in their first year in the sea stocked at 1000 fish per cage. The smolts were transferred to sea at approximately 35–45 g in April and the trial ran for 18 weeks from July to November. Three treatment regimes were used: 0.2 mg kg⁻¹ fish weight once every 2 weeks; 0.1 mg kg⁻¹ once a week; and 0.02 mg kg⁻¹ twice a week. A control group of fish received no treatment to control lice throughout the experimental period. Fish were sampled at weekly intervals during the trial.

Results

A summary of the numbers of lice recorded in each group during this trial is shown in Table 1 and Figs1 and 2. During this trial the control fish experienced a moderate

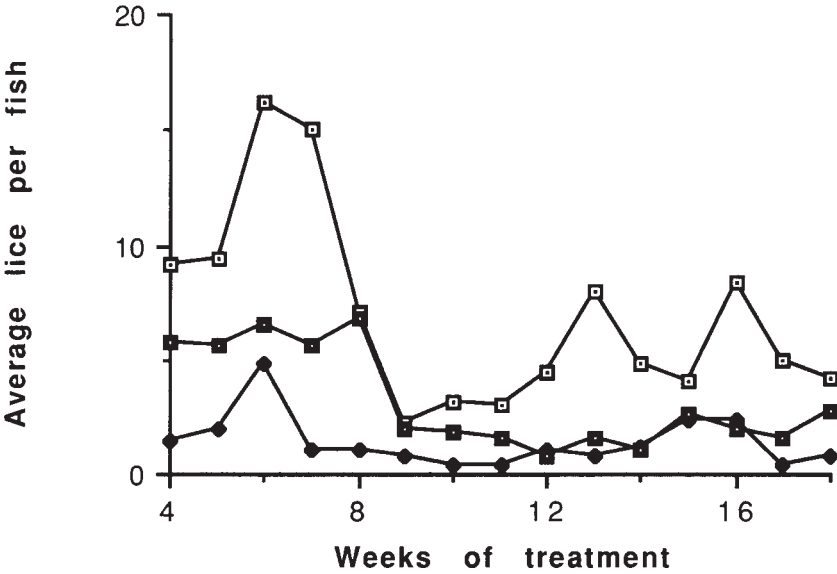


Fig. 1. Trial 1: weekly average ($n=15$) lice numbers per fish during weeks 4–18. Control fish receiving no treatment, fish receiving 0.2 mg kg⁻¹ every 2 weeks—◆—, fish receiving 0.02 mg kg⁻¹ twice a week—■—.

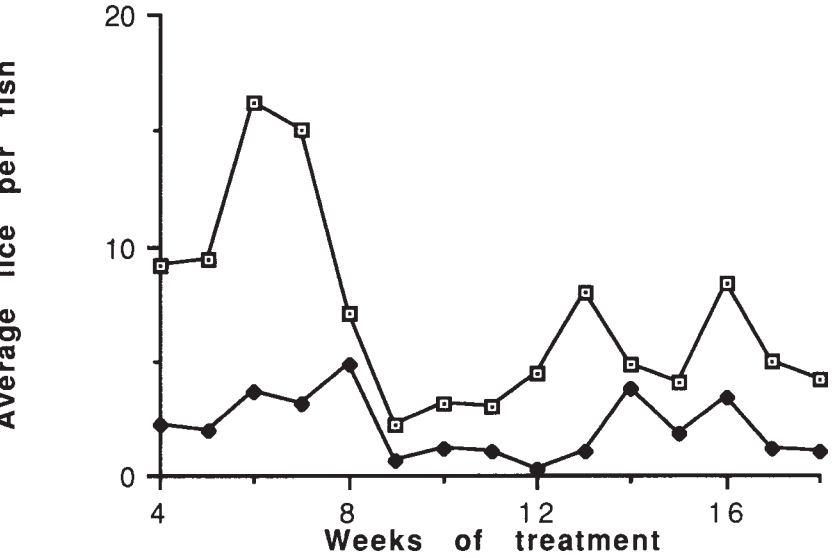


Fig. 2. Trial 1: weekly average ($n=15$) lice numbers per fish during weeks 4–18. Control fish receiving no treatment—□—, fish receiving 0.05 mg kg⁻¹ twice a week—◆—.

Table 2. Trial 1: total numbers of lice on 45 fish (percentage reduction) sampled weeks 4–18

Treatment, females	Juveniles	Preadults	Adults	Gravid
Control	202	134	266	105
0.2 mg kg ⁻¹ 2 wk ⁻¹	51 (72.2)	28 (79.1)	25 (90.6)	8 (92.3)
0.1 mg kg ⁻¹ wk ⁻¹	70 (65.3)	32 (76.1)	58 (78.1)	16 (84.7)
0.02 mg kg ⁻¹ × 2 wk ⁻¹	92 (54.4)	54 (59.7)	98 (63.1)	26 (75.2)

level of lice infestation which reached a maximum of 16.2 lice per fish in the 6th week of the trial. Throughout the trial the number of lice on all fish receiving ivermectin treatment was significantly ($p<0.001$) lower than the number on the control fish. For the same period of the trial the median percentage reduction in lice numbers was 80.9% (range 40–93.3%) for the fish receiving 0.2 mg kg⁻¹ every 2 weeks, 76% (range 20.8–95.5%) for those receiving 0.1 mg kg⁻¹ per week and 46.7% (range 2.8–81.8%) for those receiving 0.02 mg kg⁻¹ twice a week. Throughout the trial no reports were received of morbidity or mortality specifically associated with ivermectintreated fish.

In this trial all the lice on five fish sampled at random from each of the treatment groups each week between week 4 and week 18 were taken to the laboratory for further analysis. During this period a total of 284 *Lepeophtheirus salmonis* (Krøyer) and 403 *Caligus elongatus* Nordmann were detected on the 75 untreated fish examined. The treatment with 0.2 mg kg⁻¹ once every 2 weeks resulted in an 83.4% reduction of *L. salmonis* and an 84.8% reduction of *C. elongatus* numbers. The equivalent reductions for the fish treated with 0.1 mg kg⁻¹ per week were 75.0% and 76.1% and for the fish treated with 0.02 mg kg⁻¹ twice a week, 59.1% and 62.7%.

The percentage reduction in the numbers of the various life stages of lice achieved by each treatment was also determined on the same samples (Table 2). All three treatments resulted in a greater reduction in the numbers of the older life stages than in the numbers of juveniles and preadults.

Trial 2

The aim of trial 2 was to compare the efficiency of three treatment regimes in controlling lice numbers in second summer grower salmon and to test the effect of cessation of therapy on lice numbers.

Design

The fish used in the trial were salmon in their second year in the sea stocked at 400–800 fish per cage. The trial ran for 21 weeks from June to November. Three treatment regimes were used: 0.2 mg kg⁻¹ fish weight once every 2 weeks, 0.1 mg kg⁻¹ once a week and 0.05 mg kg⁻¹ twice a week. Two cages of fish were used to test each treatment and a further two were used as controls. The pairs of cages used for each treatment were randomly distributed in the cage block. Fish were sampled at weekly intervals

Table 3. Trial 2: analysis of weekly average ($n=15$) numbers of lice per fish during weeks 4–9

Treatment	Mean $\pm$ s.d.	Median	Max.	Min.
Control	110 $\pm$ 77.3	105	200	16
0.2 mg kg ⁻¹ 2 wk ⁻¹	2.7 $\pm$ 1.1	3	4	1
0.1 mg kg ⁻¹ wk ⁻¹	6.2 $\pm$ 1.7	6.5	8	4
0.05 mg kg ⁻¹ $\times$ 2 wk ⁻¹	6.2 $\pm$ 1.9	7	8	3

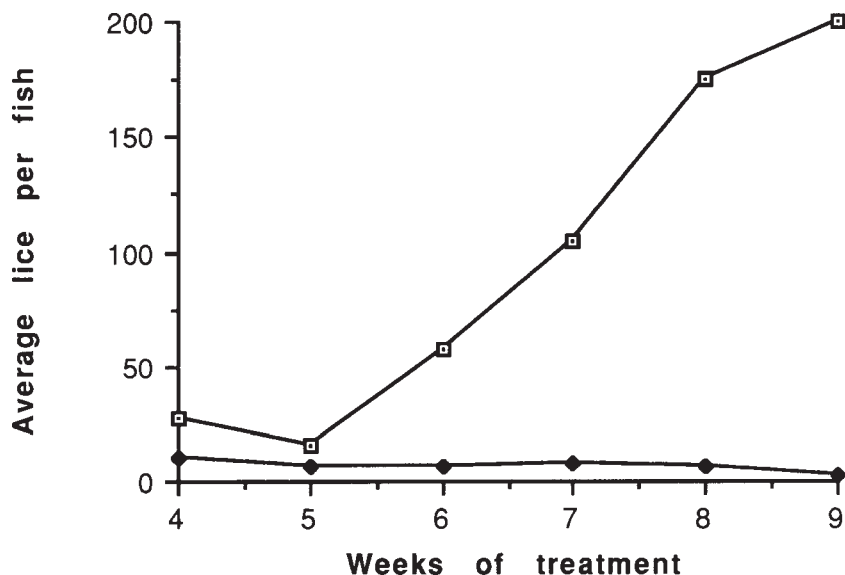


Fig. 3. Trial 2: weekly average ($n=15$) lice numbers per fish during weeks 4–9. Control fish receiving no treatment –□–, fish receiving 0.05 mg kg⁻¹ twice a week –◆–.

during the period of the trial when ivermectin was being fed and at 2-weekly intervals during the withdrawal period.

Results

A summary of the numbers of lice recorded in each group during this trial is shown in Table 3 and Figs 3 and 4. Throughout the trial the number of lice on all fish receiving ivermectin treatment was significantly ($p<0.001$) lower than the numbers on the control fish. The control group of fish were subject to a massive lice challenge during the first 9 weeks of the trial. By week 9 infestation in this group rose to over 200 lice per fish and the fish were in extremely poor health. It was therefore decided to treat the control fish with a single dose of ivermectin at 0.2 mg kg⁻¹. This single treatment in week 9 reduced the average lice numbers to 32 by week 10 and to 14 by week 11. At week 13 these fish had an average of two lice per fish. This treatment of the control fish effectively ended the quantitative aspects of this trial.

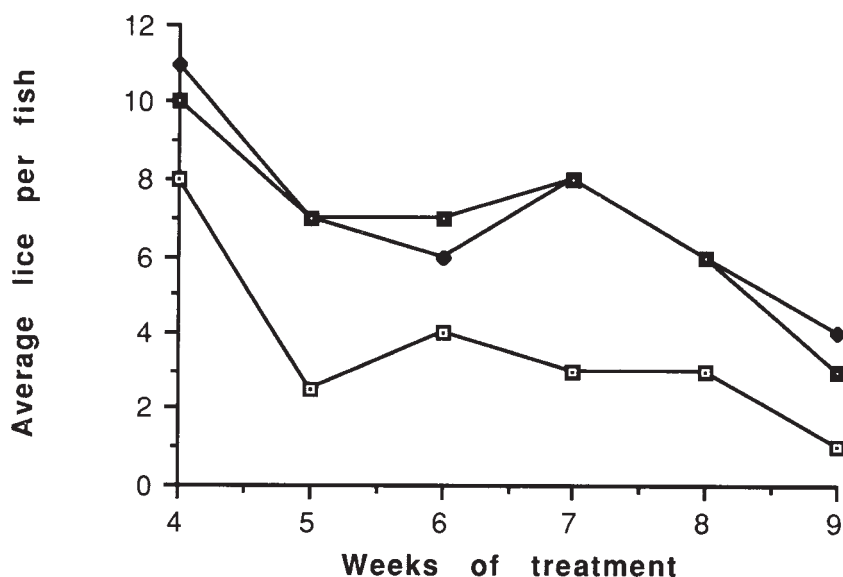


Fig. 4. Trial 2: weekly average ($n=15$) lice numbers per fish during weeks 4–9. Fish receiving 0.2 mg kg^{-1} every 2 weeks —□—, fish receiving 0.1 mg kg^{-1} every week —◆—, and fish receiving 0.05 mg kg^{-1} twice a week —■—. The weekly average lice numbers on the control fish are as shown in Fig. 3.

During the period weeks 4–9 of the trial all three treatments resulted in a significant ($p > 0.05$) reduction in lice numbers. The median of the percentage reductions recorded each week during this period was 97.1% (range 84.4–99.5%) for the fish receiving 0.2 mg kg^{-1} every 2 weeks, 92.4% (range 56.2–98.0%) for those receiving 0.1 mg kg^{-1} once a week and 92.4% (range 56.2–98.5%) for those receiving 0.05 mg kg^{-1} twice a week (Table 3).

Treatment with 0.2 mg kg^{-1} was stopped on week 12 and that with 0.05 mg kg^{-1} was stopped on week 13. Lice numbers on the fish which had received these treatments were counted at fortnightly intervals. The lack of a control group of fish during this period makes it difficult to establish when the therapeutic activity of the residual ivermectin ceased. The two cages of fish which continued to be treated with 0.1 mg kg^{-1} each week had a mean of 3.4 ± 2.8 lice during this withdrawal period. The numbers of lice in the two groups of fish whose treatment had been stopped remained under nine (the mean plus two standard deviations of the lice numbers on those fish continuing to receive 0.01 mg kg^{-1}) for 5–6 weeks after their therapy ceased, suggesting that by this time effective control of lice had ended. Throughout the trial no reports were received of morbidity or mortality specifically associated with ivermectin-treated fish.

Trial 3

The aim of trial 3 was to study the effect of long-term treatment of smolts under commercial conditions with ivermectin at two treatment regimes.

Table 4. Trial 3: average number of lice per fish

Treatment	August–January		February–March	
	Median	Range	Median	Range
Control	6.2	0.2–9.3	37	13.5–62
0.05 mg kg ⁻¹ × 2 wk ⁻¹	0.45	0.1–2.1	1.25	0.8–2.2
0.075 mg kg ⁻¹ × 2 wk ⁻¹	0.7	0.1–2.6	1.8	1.5–2.6

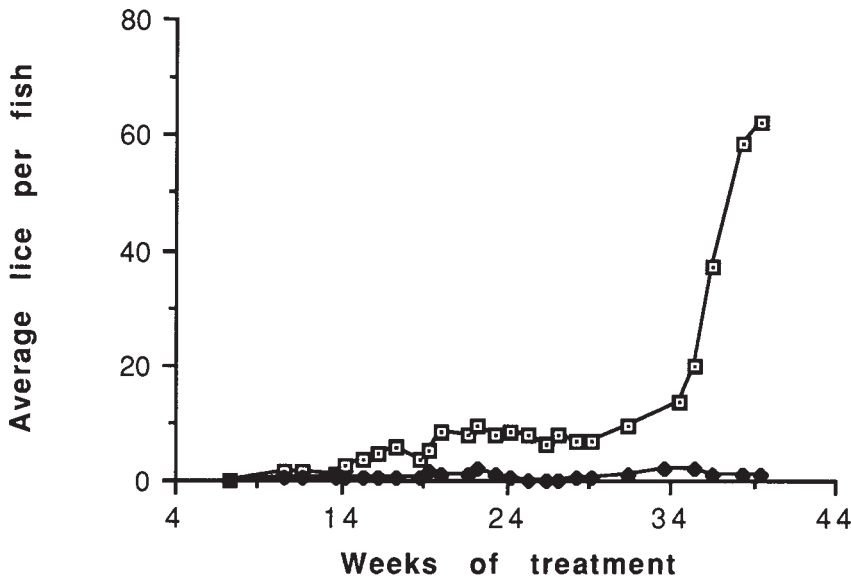


Fig. 5. Trial 3: weekly average ($n=15$) lice numbers per fish during weeks 4–40. Control fish receiving no treatment $\square$ —, fish receiving 0.05 mg kg⁻¹ twice a week $\blacklozenge$ —.

Design

The fish used in the trial were salmon smolts stocked in May at an average weight of 35 g and at a density of 3000 fish per cage. The trial ran for 10 months from June to April. Two treatment regimes were used: 0.05 mg kg⁻¹ fish weight twice a week; and 0.075 mg kg⁻¹ twice a week. Two cages of fish were used to test each treatment and a further two were used as controls. The pairs of cages used for each treatment were randomly distributed in the cage block. Fish were sampled at weekly intervals during the period of the trial.

Results

A summary of the numbers of lice recorded in each group during this trial is shown in Table 4 and Fig. 5. Throughout the trial the number of lice on all fish receiving ivermectin treatment was significantly ($p<0.001$) lower than the number on the control

Table 5. Trial 4: analysis of weekly average ($n=15$) numbers of lice per fish during weeks 4–10

Treatment	Mean $\pm$ s.d.	Median	Max.	Min.
Control	4.0 $\pm$ 1.8	4.2	6.4	1.5
0.02 mg ml ⁻¹ $\times$ 2 wk ⁻¹	1.9 $\pm$ 1.2	2.1	3.4	0.2
0.03 mg ml ⁻¹ $\times$ 2 wk ⁻¹	1.2 $\pm$ 1.2	0.8	3.8	0.2

fish. With respect to the extent of infestation experienced by the control fish the trial can be divided into three phases. In June and July negligible lice numbers were recorded in all groups. From August to January the infestation of the control fish was moderate, with fewer than ten lice being recorded on any sample date. In February and March a significant infestation was recorded which reached a maximum at the termination of the trial of 62 lice per fish.

On the 27 September one of the cages being treated with 0.075 mg kg⁻¹ received one accidental ten-fold overdose. Within a day the fish darkened and became lethargic. In the 4 days following the overdose approximately 800 (26%) mortalities were reported in this cage. All ivermectin treatment of the fish in this cage was stopped and the surviving fish slowly returned to normal swimming and feeding behaviour. No data from this cage were used in assessing the efficiency of the treatment with 0.075 mg kg⁻¹ twice a week. With the exception of the mortalities reported in the cage which received the overdose, no reports were received of morbidity or mortality specifically associated with ivermectin-treated fish.

During trial 3 the percentage reduction in lice numbers on the treated fish was calculated on each sample day. The median of these percentages was 88.7% (range 62.9–98.6%) for the fish receiving 0.05 mg kg⁻¹ and 91.1% (range 7.6–98.8%) for the fish receiving 0.075 mg kg⁻¹.

Trial 4

The aim of trial 4 was to determine efficiency of reduced doses of ivermectin in controlling lice numbers in smolts when the antibiotic was compounded into the diet by commercial feed manufacturers.

Design

The fish used in the trial were smolts in their first year at sea stocked at 5000 fish per cage. The smolts were transferred to sea at approximately 35 g in April and the trial ran for 14 weeks from July to September. Two treatment regimes were used: 0.03 mg kg⁻¹ twice a week; and 0.02 mg kg⁻¹ twice a week. Two cages of fish were used to test each treatment and a further two were used as controls. The pairs of cages used for each treatment were randomly distributed in the cage block.

Results

A summary of the numbers of lice recorded in each group during this trial is shown in Table 5 and Fig. 6. The control group of fish was subject only to a light lice

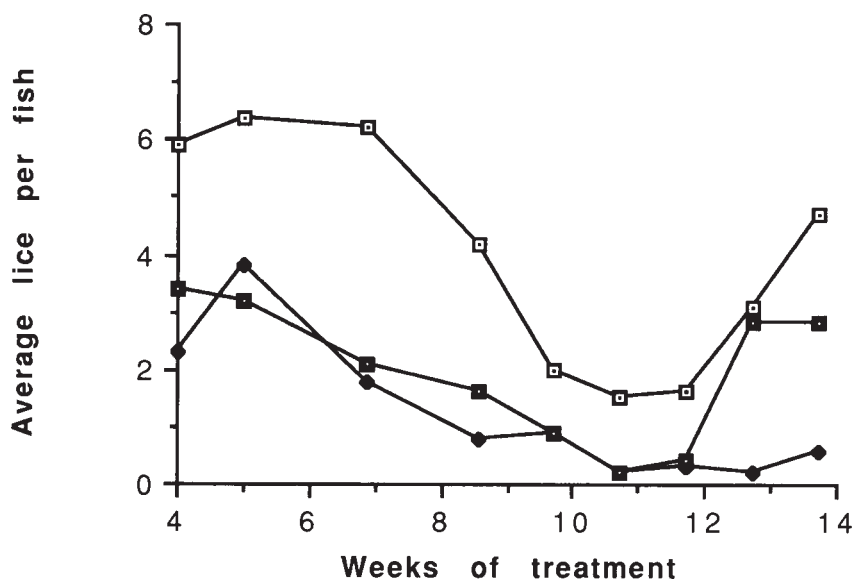


Fig. 6. Trial 4: weekly average ($n=15$) lice numbers per fish during weeks 4–14. Control fish receiving no treatment –□–, fish receiving 0.03 mg kg^{-1} twice a week –◆–, fish receiving 0.02 mg kg^{-1} twice a week –■–.

challenge during this trial, reaching a maximum of 6.4 lice per fish in week 5. Throughout the trial the number of lice on all fish receiving ivermectin treatment was significantly ($p < 0.001$) lower than the number on the control fish. From week 4 to week 14 the median percentage reduction in lice number was 80.9% (range 40.6–93.5%) for those fish receiving 0.03 mg kg^{-1} twice a week and 55% (range 9.6–86.6%) for those receiving 0.02 mg kg^{-1} twice a week. Throughout the trial no reports were received of morbidity or mortality specifically associated with ivermectintreated fish.

DISCUSSION

The primary questions that must be answered with respect to a novel chemotherapeutic technique are those of efficacy and safety. The data presented in this chapter clearly demonstrate that the oral administration of ivermectin at doses of 0.2 mg kg^{-1} every 2 weeks, 0.1 mg kg^{-1} every week or of 0.05 mg kg^{-1} twice a week can reduce lice numbers by between 76% and 97% even during a serious lice challenge, as in trial 2. Further, the data from trial 1 show that the therapy is equally efficacious in controlling both *L. salmonis* and *C. elongatus*. The data from trial 4 showed that when ivermectin was incorporated into the diet by commercial feed manufacturers and fed at 0.03 mg kg^{-1} twice a week, an 81% reduction in lice numbers was achieved. The low lice challenge experienced during this trial, however, means that this result will require further confirmation. The administration of 0.02 mg kg^{-1} resulted in only a 46% reduction in trial 1 and a 55% reduction in trial 4. This suggests that this treatment regime is close to, or

below, the lowest effective dose. However, the similarity in the percentage reduction achieved by 0.02 mg kg^{-1} in trial 1 when the antibiotic was mixed into the food on-farm and by the same dose in trial 4 when it was commercially compounded suggests that compounding by commercial feed manufacturers may not significantly increase the bioavailability of the antibiotic.

One of the main drawbacks of dichlorvos as a therapeutic agent is that it is only active against post-chalimus stages of lice (Rae 1979, Wootten *et al.* 1982). The data from trial 1 (Table 2) show that although continued ivermectin therapy results in a greater reduction in the number of the older life stages of lice than in the younger there is still a significant reduction in the numbers of juveniles recorded. These data are, however, not a clear demonstration that ivermectin is active against juveniles. The reduction in the number of juveniles may be an indirect result of the reduction of the number of gravid females within the stock. On the other hand, the apparent lower efficiency of the therapy against juveniles could be a consequence of continued recruitment of juveniles from outside the treated cage. The design of these trials is such that it is not possible to differentiate between these alternatives.

The safety of a chemotherapeutic treatment must be considered under four headings: safety for the target animal; safety for the farmer; safety for the environment, including non-target species; and, in the case of a food animal, safety for the consumer. The last two of these issues are not addressed in this chapter. With respect to farmer safety, a farmer feeding at 1 % body weight with feed medicated to achieve a dose of 0.05 mg kg^{-1} would need to consume 2.4 kg of the feed to receive the dose of 12 mg used by Dadzie *et al.* (1987) to treat human eye infections.

There has been some concern expressed in the literature concerning the safety of oral ivermectin therapy for the target species. Palmer *et al.* (1987) showed that following the administration of a single dose of ivermectin of 0.4 mg kg^{-1} to salmon mortalities of 17% were recorded, compared with mortalities of 3.6% in untreated fish during the same time period. Hoy *et al.* (1990) followed the distribution of [^3H]ivermectin administered by oral intubation at approximately 0.02 mg kg^{-1} . They suggested that the degree of radioactivity detected in the central nervous system indicated that the blood-brain barrier was poorly developed in Atlantic salmon and therefore that ivermectin would not be a suitable drug for the treatment of parasitic diseases in these fish. Various aspects of the design of the trials reported here were included to address the problem of the safety of the therapy for salmon. In particular the trials were continuous over a prolonged time period, and the administration of the therapy was carried out by commercial farm personnel according to the standard operating procedures of that company. Thus the trials monitored as closely as possible the prolonged application of the therapy under commercial conditions. With the exception of the accidental overdose that occurred during trial 3, no abnormal behaviour or death was reported by the company's health management team to be associated with any of the treatments. The mortalities reported here of 26% following a single dose of 0.75 mg kg^{-1} are of the same order as those reported by Palmer *et al.* (1987).

In conclusion, orally administered ivermectin can effectively control lice infestations of Atlantic salmon, and no significant adverse reactions have been reported during prolonged use under commercial conditions. Thus such therapy is a

realistic alternative to the control of lice by dichlorvos baths. However, ivermectin is not licensed for use in fish in any country and data on the ecotoxicology and pharmacokinetics of the drug would be required before such licensing could be considered.

ACKNOWLEDGEMENTS

This work was in part funded by an EOLAS grant no. HEIC 226/89. The assistance of Ger Meade in the monitoring of the health of fish is gratefully acknowledged.

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Part II d Vaccination

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The extraction and analysis of potential candidate vaccine antigens from the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837)

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ABSTRACT

A vaccine against the caligid parasite *Lepeophtheirus salmonis* remains a high priority for the aquaculture industry. A cohesive search for suitable antigens from such a complex metazoan parasite forms the fundamental basis of any vaccine design programme. The salmon louse, *L. salmonis*, was separated into a number of distinct fractions after column chromatography, and specific enzymatic activities could be associated with both a crude homogenate of lice and the specific separated chromatographic fractions. Candidate functional antigens, three proteases and a lipase, were partially purified and analysed further via electrophoresis. The proteases appeared to be large macromolecules, which remained active as verified by gel overlay assays, in non-reducing conditions. The lipase was also a large molecular complex appearing as three protein bands greater than 200 kDa. Further electrophoretic and immunoblotting assays revealed that the lipase was heavily glycosylated whereas the proteases were not. Immunoblots showed that protein bands in the crude louse homogenate were associated with both these macromolecules, as determined by specific antisera. Immunization studies showed that considerable systemic antibody titres to the proteases and the lipase could be elicited after subcutaneous injection of the macromolecules into mammals.

INTRODUCTION

Current levels of infection of farmed Atlantic salmon (*Salmo salar* L.) in northern Europe with the ectoparasitic caligid copepods *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordmann remain extremely high and often result in large losses of fish stocks due to the direct consequences of louse infection, opportunistic infectious organisms and increased physiological stresses (Grayson *et al.* 1991, Spencer 1992).

Current treatments, such as the use of organophosphate pesticides (Aquagard; Neguvon) have recently been questioned on the grounds of ever-reducing efficacy (increased resistance of the lice to organophosphates) and attendant environmental concern (Spencer 1992). Vaccination of fish stocks against louse infection is potentially the most efficacious strategy of reducing the louse parasite burdens on fish farms (Grayson *et al.* 1991, Reilly and Mulcahy 1992, in press). Ideally, the vaccination regime employed would be applied via the oral route with the aim of preferentially inducing an immune response at the cutaneous surface (Jenkins *et al.* 1993, in press) and avoiding several problems with handling stress and logistical difficulties with immunizing large numbers of fish (Ellis 1988). The design of a vaccination regime is fraught with problems for successful immunization against organisms as complex as *L. salmonis* and *C. elongatus*. However, recent studies on the Australian cattle tick, *Boophilus microplus*, suggest that vaccination of cattle with gut-derived antigens can induce specific immunity that significantly reduces the parasite burden on the cattle and also has an impact on several factors related to the epidemiology of infestation (Opdebeeck *et al.* 1988, Kemp *et al.* 1989, Lee *et al.* 1991).

The design of a rationalized vaccine preparation, and an accompanying delivery system for it, requires extensive characterization of protective antigen(s), the ability of the antigen to induce a relevant immune response, optimization of the route(s), dose and timing of immunization and also the frequency of boost immunization(s) of the target population (Peng *et al.* 1990, Jenkins *et al.* 1992).

Specifically, the overall strategy employed in our studies is aimed at the immunological disruption of an intrinsic functional protein of the gut of the louse and/or immunological damage to some aspect of the structural apparatus of the gut. As a consequence, this preliminary study is specifically aimed at isolating and characterizing potential antigens from only one of the important caligid species (*L. salmonis*).

MATERIALS AND METHODS

Collection of lice and homogenate preparation

Adult stages, as defined by size and motile appearance under a binocular microscope, of *L. salmonis* were collected from sea-farmed Atlantic salmon (*Salmo salar* L.) and stored immediately in liquid nitrogen. The whole adult sea lice were homogenized as previously described (Grayson *et al.* 1991) and the protein content analysed by the method of Bradford (1976) using a commercial kit (Bio-Rad, Watford, UK) prior to analysis by column chromatography, microplate activity assay, enzyme-linked immunosorbent assay, electrophoresis and immunoblotting.

Column chromatography

The whole louse homogenate was fractionated by anion-exchange chromatography using a 1 M NaCl stepped gradient and 50 mM Tris-HCl at pH 8.3 or pH 9.0, at room temperature. A Mono-Q HR 5/5 column was connected to a fast protein liquid chromatography (FPLC) system (Pharmacia, Milton Keynes, UK). Fractions were monitored at 280 nm (UV-1, Pharmacia), peak profiles traced by a chart recorder (not

shown) and the peaks collected (Frac-100, Pharmacia). The protein concentration of the resultant 72 fractions was estimated as above. Fractions were aliquoted and stored at -70°C. Selected fractions were electrophoretically analysed (see below).

Biological activity of louse extracts

Whole adult lice homogenate was positively screened for biological activity at pH 7.2, 7.5 and 8.6 using the following colorimetric enzyme substrates (Sigma, Poole, UK; New Brunswick, Hatfield, UK):

Na-Benzoyl-DL-arginine- β -naphthylamide (BANA), for trypsin activity.

Na-Benzoyl-DL-*p*-nitroanilide (BAPNA), for chymotrypsin activity.

p-Nitrophenylphosphorylcholine chitin for chitinase activity.

p-Nitrophenyl laurate, for long-chain esterase activity.

Homogenates were also assayed using the following fluorometric substrates:

4-Methylumbelliferyl (4-MU) *p*-guanidobenzoate, for lipase activity.

4-MU-*p*-trimethylammonium cinnamate chloride (MUTMAC), for trypsin-like activity.

N-Succinyl-Ala-Ala-Ala-7-amido-4-methylcoumarin, for peptidase activity.

N-Succinyl-Gly-Pro-Leu-Gly-Pro-7-amido-4-methylcoumarin, for collagenase-like peptidase activity.

4-MU-*N*- α -D-neuramide ammonium salt tetrahydrate, for neuraminidase activity.

4-MU-butyrate (MUBUT), for cholesterol esterase activity.

4-MU-elaoate, for lipase activity.

4-MU-haptanoate, for lipase activity.

4-MU-oleate, for lipase activity.

All reactions were carried out in 96-well microtitre plates (Falcon, Becton-Dickinson, Oxford, UK) using 2 μ g louse protein per well and closely monitored for the release of the chromogen or fluorogen. Control wells containing substrate only were present for all reactions. All 72 column fractions were also screened for specific biological activity.

Antisera production

Wistar rats ($n=2$) were injected subcutaneously (s.c.) with 50 μ g of fractionated adult lice protein in Freund's complete adjuvant (FCA), boosted 21 days later in Freund's incomplete adjuvant (FIA) and then sacrificed 21 days after the last injection. Blood was stored overnight at 4°C, the serum removed by centrifugation at 6500 rpm for 5 min and stored at -20°C until required.

Enzyme-linked immunosorbent assay (ELISA)

Selected fractions (those with the highest activities as determined by specific enzyme assay, as above) were tested by ELISA as previously described (Grayson *et al.* 1991) using a rat anti-protease or rat anti-lipase (primary antiserum) followed by a goat anti-rat IgG peroxidase conjugate (secondary antiserum) (Sigma) which was optimized by checkerboard titration at 1:1000.

ANALYSIS OF WHOLE AND FRACTIONATED LICE PROTEIN BY ELECTROPHORESIS

The specificity of the antisera raised to the protease and lipase-positive fractions isolated by column chromatography was analysed electrophoretically and immunologically.

Whole lice homogenate was prepared as previously described and the protein concentration estimated (Bradford 1976) and adjusted to $20 \mu\text{g ml}^{-1}$ in phosphate-buffered saline (pH 7.2). A non-denaturing discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) system (Mini-Protean II, Bio-Rad, Watford, UK), based on the method of Laemmli (1970), was used to separate whole lice homogenate with a 3% stacking gel and an 11% separating gel. Samples ($10 \mu\text{g}$) were incubated for 1 h in sample buffer (with or without mercaptoethanol) and electrophoresed at a constant voltage of 200 V for 45 min. Selected fractions ($10 \mu\text{g}$ per lane) (with maximal positive biological activity, i.e. fractions 11, 16, 30 (proteases) and 37 (lipase) were also analysed by SDS-PAGE (Multiplier, Pharmacia), in reducing conditions, on Exelgel 8-18 pre-cast gradient gels and on 7% discontinuous gels, in non-reducing conditions (Bio-Rad).

In addition, specific fraction samples (11, 16 and 30) that were to be assayed by gel overlay (see below) were also treated with the low molecular weight serine protease inhibitor phenyl methylsulphonyl fluoride (PMSF). A concentration of 10 mM PMSF was added to the samples to attempt to inhibit protease function (Beynon and Bond 1989).

After electrophoresis, gels were fixed and stained either in 0.2% w/v Coomassie brilliant blue R (for the detection of proteins), 0.2% periodic acid followed by Schiff's stain (for the detection of glycoproteins) (Titball 1983) or in acetic acid-acetone solution of Sudan black B (for the detection of lipoproteins) (Bayliss-High 1984). Gels of electrophoresed column fractions (in reducing and non-reducing conditions) with specific protease activity were washed in 2.5% Triton TX 100 for 60 min, to remove SDS, and subsequently washed for 3×10 min in water and 2×10 min in 50 mM Tris 10 mM CaCl₂ at pH 7.8 (activation buffer). Gels were incubated for 1 h in activation buffer and subsequently overlaid with a solution of 1% gelatin plus 1% agarose (in activation buffer) for 24 h. Protease activity was measured by staining with 2.5% aqueous Coomassie blue and subsequent observation of zones of clearance after de-staining, in distilled water.

Analysis of whole and fractionated lice protein by western blotting

Electrophoresed protein was also transferred to $0.45 \mu\text{m}$ nitrocellulose (Schliecher and Shull, Anderman and Co., Kingston upon Thames, UK) according to the method of Towbin *et al.* (1979) in 0.025 M Tris, 0.192 M glycine in 20% w/v methanol at pH 8.3. Blotted (western-blotted) lice protein was immunologically probed on Multiscreen apparatus (Bio-Rad) by sequential incubations with antisera, with optimized dilutions of antisera as follows: rat anti-protease (to fractions 11, 16 and 30) and rat anti-lipase (to fraction 37) at 1:500 followed by rabbit anti-rat IgG (Sigma) at 1:1000. Western blots were developed in 0.05 M Tris 0.15 M NaCl (Tris-saline) containing 0.06% w/v 3'3'-diaminobenzidine tetrahydrochloride (DAB),

Table 1. Biological activities isolated from *L. salmonis* homogenates and column fractions

Positively tested biological activity	Substrate used	Column fraction with maximal activity
Protease (trypsin-like)	BAPNA ^a	11
	BANA ^a	16
Protease (chymotrypsin-like)	MUTMAC ^b	30
Lipase (cholesterol esterase-like)	MUBUT ^b	37

^aColorimetric substrate assay. ^bFluorometric substrate assay.

0.03% w/v NiCl₂ and 0.03% H₂O₂. Western blots were developed for approximately 5 min and the development reactions terminated by thorough rinsing of the blots in distilled water.

RESULTS

Column chromatography resulted in a clear separation of the louse homogenate into 72 distinct fractions (not shown). Analyses of the crude lice homogenates using specific enzymatic substrates revealed that there were distinct activities present in the whole louse that could also be attributed to certain chromatographically derived fractions (Table 1). The most distinct enzymatic activities isolated were of proteolytic (trypsin and chymotrypsin-like) and a lipolytic (cholesterol esterase-like) nature. These proteolytic and lipolytic fractions proved to be considerably immunogenic in rats (Fig. 1) as evidenced by the substantial systemic antibody response raised after the s.c. immunization of equivalent doses of each of the fractions.

Fig. 2 shows a typical electrophoretic separation of unreduced lice homogenate proteins along with identification of the proteins that appear to have polysaccharide or lipid moieties associated with them when stained with Schiff's or Sudan black B stains, respectively (Table 2). Table 3 highlights the apparent molecular weights of these macromolecules (as derived from the interpolation of the relative mobility of the band against a standard curve of log molecular weights of known standard proteins). Fig. 3 shows the electrophoretic separation of certain chromatographic fractions, under reducing conditions, on SDS-PAGE. Fig. 4 shows the electrophoretic separation of the column-derived proteases and lipase. The three proteases all appeared to have a similar electrophoretic profile after SDS-PAGE in non-reducing conditions. The lipase appeared as a high molecular weight complex of three bands, two of which were heavily glycosylated (Fig. 5). There was little glycosylation of any of the three proteases (Fig. 5) but all three exhibited a gelatin clearance capacity (Fig. 4) associated with two bands of an estimated molecular weight of between 200 and 300 kDa.

Both PMSF and reducing conditions prevented gelatin clearance activity of the proteases. Western blotting (Fig. 5 and Table 4) of the electrophoresed louse homogenate

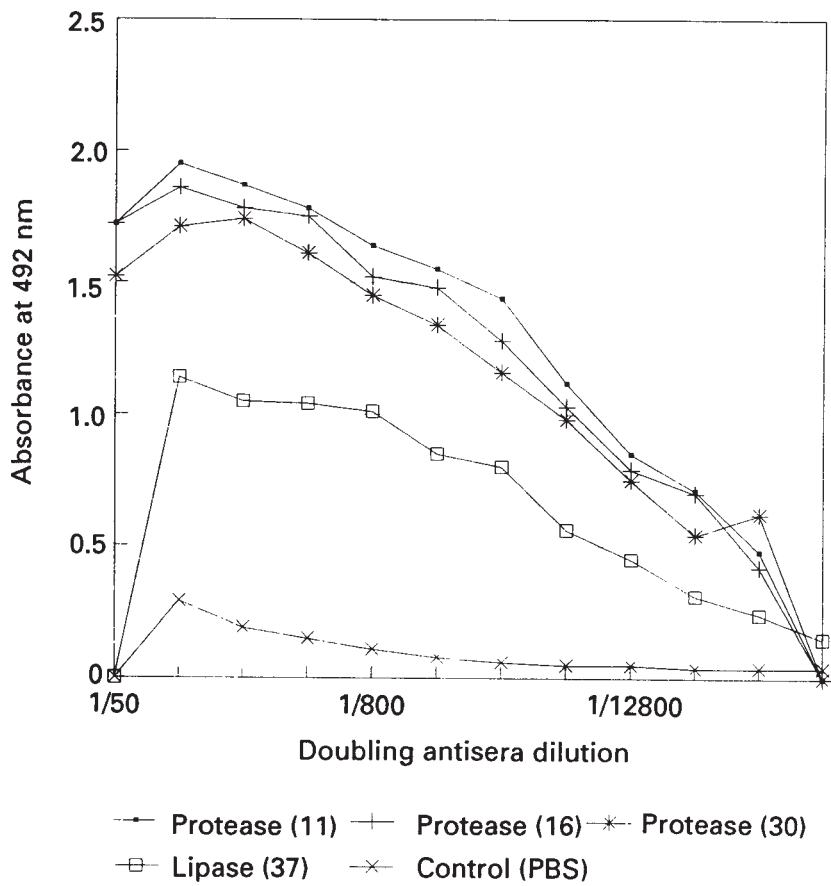


Fig. 1. The immune response of rats immunized with specific salmon lice fractions. Numbers in parentheses are the column fractions in which the molecules were separated.

probed with three pro tease-specific antisera (anti-11P, anti-16P and anti-30P) and one lipase-specific antiserum (anti-37L) revealed that the high molecular weight components of the proteases and lipase were immunologically recognizable.

DISCUSSION

Vaccination of farmed Atlantic salmon would possibly provide an effective means of reducing salmon lice infection levels (Grayson *et al.* 1991). The current study represents data on the formative stage in a strategy which is designed to interfere immunologically with salmon louse infection via disruption of the louse gut. In order to achieve this studies were carried out to examine some fundamental functional properties of the louse and to target these functions for disruption *in vivo*.

The current study shows that the homogenates of whole *L. salmonis* may be separated into distinct fractions by column chromatography, and specific enzymatic

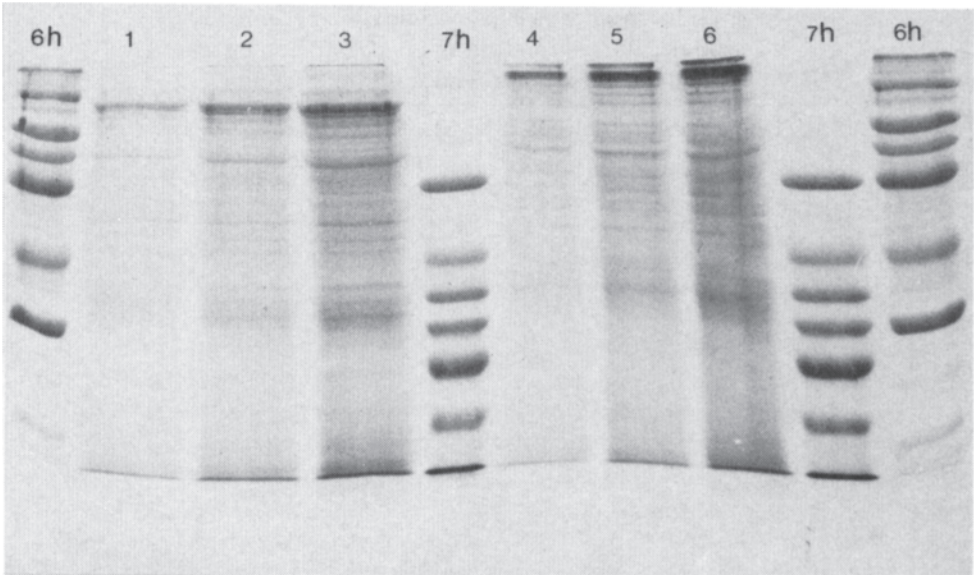


Fig. 2. *L. salmonis* molecular components. A 10% SDS–PAGE gel with approximately 5 µg (lanes 1 and 4), 10 µg (lanes 2 and 5) and 15 µg (lanes 3 and 6) protein. Coomassie blue staining. Lanes 1–3 are reduced samples; lanes 4–6 are non-reduced samples. 6 h and 7 h are designations for high and low molecular weight standards (Sigma), respectively.

Table 2. The apparent molecular weights of the unreduced proteases and lipase and their polysaccharide content

Apparent mol. wt (kDa) of protease molecular components	Level of glycosylation	Apparent mol. wt (kDa) of lipase molecular components	Level of glycosylation
280–300 (2 bands)	–	260–270 (3 bands)	+ + (2 bands)
154.3	+		
119.9	–		
105.0	–	143.1	–
42.4	–		

+, weak Schiff’s staining; ++, strong Schiff’s staining; -, negative staining.

functions may be assigned to specific chromatographic fractions. The use of sensitive fluorogenic and colorimetric substrates for specific enzymatic activities allowed partial purification of several functional macromolecules that may be of interest in the potential elicitation of relevant, protective immunity and, eventually, in the production of a vaccine against *L. salmonis*.

L. salmonis homogenate had a complex separation profile after chromatography (some 72 distinct fractions), as was to be expected from previous studies using column

Table 3. Derived molecular weights of the unreduced macromolecular components of *L. salmonis* homogenate identified as glycoproteins and lipoproteins

Type of macromolecule	Interpolated mol. wt of macromolecule (kDa)
Glycoprotein	180.5
	161.2
	132.2
	118.1
	108.4
	31.2
Lipoprotein	17.2

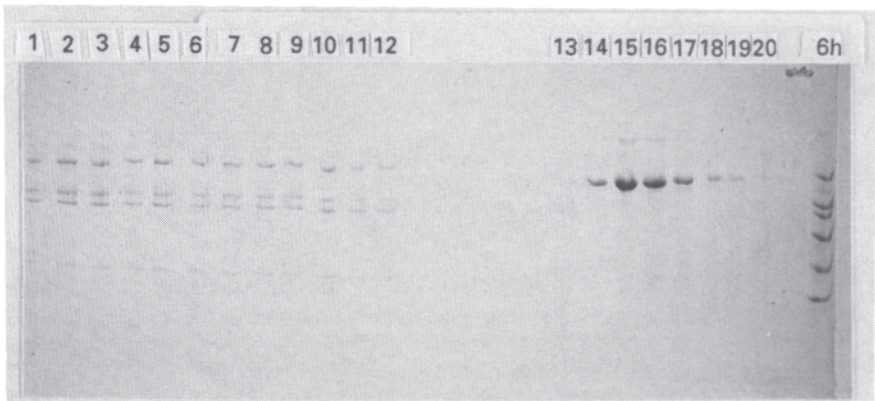


Fig. 3. The electrophoretic profile of fractionated *L. salmonis*. Salmon lice molecular components fractionated on a Mono Q HR 5/5 column and FPLC system. An 8–18% pre-cast gradient SDS–PAGE Excelgel, electrophoresed under reducing conditions with approximately 4 μ g of protein per lane. Coomassie blue stain. Lanes 1–6 are column fractions 2–7; lane 7 is column fraction 10; lane 8 is column fraction 11; lanes 9–12 are column fractions 14–17; lanes 13–20 are column fractions 35–43; 6h are SDS–PAGE high molecular weight markers (Sigma).

separation of antigens from whole and centrifuged preparations of the Australian cattle tick, *Boophilus microplus* (Willadsen *et al.* 1989). Strong proteolytic activity, both of a trypsin and chymotrypsin-like nature and lipolytic activity, of a cholesterol esterase nature was observed in specific fractions, as outlined, and were considered as potential target antigens for immunological disruption, as it would seem logical to assume that such macromolecules would be of fundamental importance in the digestive tract of the louse.

Analyses of these fractions by reducing and non-reducing SDS–PAGE electrophoresis and western blotting revealed that the fractions specific for protease activity were not completely pure, i.e. they contained a number of protein bands of differing molecular

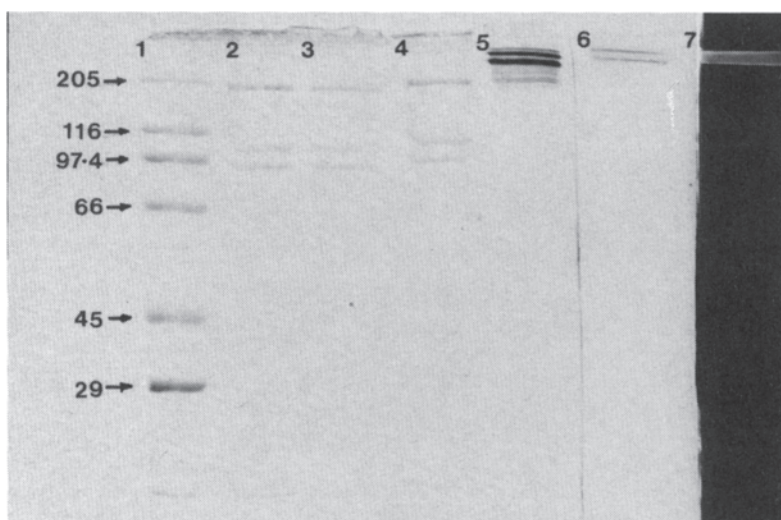


Fig. 4. The electrophoretic separation of the derived proteases and lipase macromolecules from *L. salmonis*. A 7% non-reduced SDS-PAGE gel. Lane 1 represents 6h high molecular weight standard proteins. Lanes 2, 3 and 4 are proteases from fractions 11, 16 and 30, respectively (Coomassie blue staining). Lanes 5 and 6 are the lipase macromolecule from fraction 37 stained with Coomassie blue and Schiff's stain, respectively. Lane 7 is a gelatin gel overlay of one of the protease molecules (fraction 16), showing a zone of clearance corresponding to two protein bands of an estimated molecular weight of between 280 and 300 kDa.

weights. Lipase-active fractions appeared to be formed of a high molecular weight complex (greater than 260 kDa) of three protein bands, in non-reducing conditions, that were of similar molecular weight. Studies also showed that reduction of both the lipase and proteases altered their electrophoretic separation profile, indicating that these macromolecules are dependent, to some extent, on disulphide linkages for their absolute tertiary structure.

The louse homogenate had a typically complex electrophoretic separation profile that was comparable to that observed in previous studies (Grayson *et al.* 1991). There appeared to be several heavily glycosylated proteins separated from lice homogenates, but none of the molecular weights of these corresponded to those associated with the proteases and lipase. There appeared to be only one distinct lipoprotein in the lice homogenate, which appeared to have a low molecular weight. Staining of the chromatographically derived fractions for glycoprotein content revealed that two of the bands forming the lipase complex were also heavily glycosylated, in contrast to the proteases, which revealed only one weakly stained protein band. This may ultimately be of significance as recent studies have shown that the level of the immune response elicited to the mid-gut antigen of vaccine preparations of *B. microplus* is largely due to the polysaccharide moiety of the antigen (Lee *et al.* 1991). In contrast, neither the proteases nor lipase had any appreciable lipid content with the detection methods used in this study. Gelatin overlay clearance revealed that the (or an) active portion of the

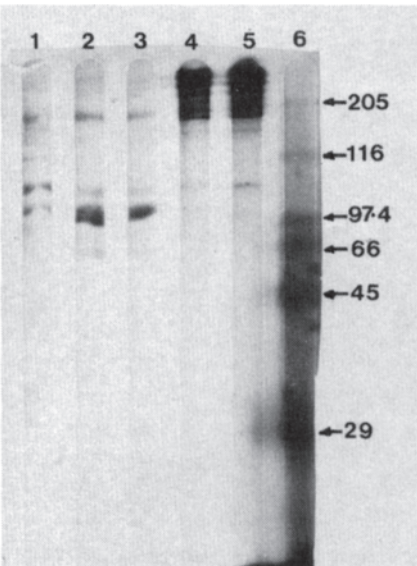


Fig. 5. Western blot showing components of unreduced whole lice homogenate recognized by fraction-specific antisera. Analysis of unreduced whole lice homogenate (7% SDS-PAGE gel) on Multiscreen immunoblot analysis system (Bio-Rad). Lanes 1, 2 and 3 are stained with anti-protease antisera (raised to fractions 11, 16 and 30, respectively); lanes 4 and 5 are stained with anti-lipase antiserum (raised to fraction 37); lane 6 are 6h high molecular weight markers (Sigma), the molecular weights of which are given in kilodaltons (arrows).

Table 4. The molecular weights of the components of unreduced whole lice homogenate identified by fraction-specific antisera

Fraction-specific antisera (and biological activity)	Components identified (mol. wt, kDa)
11P (protease)	Approx. 280
16P (protease)	152.6
30P (protease)	115.0
	105.5
	41.6
37L (lipase)	Complex at approx. 260

protease molecule appeared to be of a high molecular weight (estimated between 280 and 300 kDa), at least under non-reducing conditions, and that it appeared to be inhibited by the low molecular weight protease inhibitor PMSF and by the reduction of its disulphide bonds by β -mercaptoethanol. This is in contrast to a previous study (Ellis *et al.* 1990) in which it was shown that there was a very low serine protease activity in general in *L. salmonis* as compared with *C. elongatus* (on a weight to weight basis), which was also inhibited (largely) by PMSF (i.e. was also, at least partially, of a serine protease nature). This activity was measured utilizing a similar

method to that used in the current study but neglected to state the nature of the electrophoresis used, i.e. whether it was reducing or non-reducing. Protease activity measured by Ellis *et al.* (1990) correlated with bands of 57 kDa, 59.5 kDa and 61.5 kDa, whereas that observed in this study was associated with bands of a higher molecular weight.

Considerable antibody responses could be elicited, in Wistar rats, to both the protease and lipase-positive fractions isolated from *L. salmonis* and that the same antigens are recognized by the antisera in both electrophoresed lice whole homogenates and the chromatographically derived fractions. Immunization with the proteases appeared to elicit a considerably greater antibody titre than immunization with the lipase molecular complex. Together, these data suggest that both fractions may, at least, be considered as immunogens in further studies in both mammals and salmonids. It is worth noting that the individual proteases may also be different, as evidenced by their different chromatographic elution profiles, if subjected to alternative assay methods, e.g. isoelectric focusing electrophoresis.

Studies are currently under way to further analyse the immunogenicity of these candidate antigens, to analyse their recombinantly derived analogues, to analyse structural antigens from the salmon louse gut and, eventually, to combine gut structural and functional antigens into a rationalized vaccine delivery system.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge the assistance of the following: Drs Mike Barrett and Norman Kelly of Unilever; Mr Jim Treasurer of Marine Harvest International; and Mr Stan McMahon of the University of Plymouth, to whom we are indebted.

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Immunohistochemical screening and selection of monoclonal antibodies to salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837)

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ABSTRACT

Antibodies produced by murine hybridoma cells were screened and assessed by two immunohistochemical methods—indirect immunoperoxidase (IIP) and peroxidase anti-peroxidase (PAP) techniques—in order to select monoclonal antibodies which may form the basis for experimental vaccines against the salmon louse, *Lepeophtheirus salmonis*.

Whole adult female louse paraffin sections were used for this purpose and the positive binding to different louse tissues used as a criterion to select the most suitable antibody-producing hybridoma cell cultures. A method has been developed which permits the simultaneous processing of several hundreds of hybridoma supernatants. A novel humid chamber was designed for this purpose.

The hybridoma supernatants screened in this way have shown specific binding to a variety of somatic antigens, including muscular, nervous, connective and epidermal tissues, cuticle, brush border of gut epithelium, haemolymph, ovaries and oviducts.

The PAP technique proved to be much more sensitive than the IIP, the former being able to show positive binding of a monoclonal antibody even when supernatants were used at dilutions of 1:50.

The future identification and production of the protective antigen or antigens by recombinant DNA technology are discussed.

INTRODUCTION

The salmon louse, *Lepeophtheirus salmonis*, is well known as a major problem of intensively reared salmonids in northern Europe, causing considerable damage to the fish. The current treatment for sea lice is largely based on the use of organophosphate pesticides. However, these treatments are expensive and also,

among many other problems related to this treatment, there is an increased concern about the effects of such pesticides on marine and human environments. Thus, alternative control strategies are currently being studied, including the development of a vaccine against salmon lice. Until recently, there has been no evidence for any form of immune response by salmon to *L. salmonis*. However, Stone (1989) demonstrated a serum antibody response to experimental anti-*L. salmonis* vaccination, and Grayson *et al.* (1991) recorded a naturally produced serum antibody response in salmon exposed to heavy chronic infestation. These findings suggest that a protective immune response might be found.

The study presented here forms part of a larger project investigating the possibility of vaccinating salmon against *L. salmonis*. The stimulus behind the project arose largely from the successful experimental vaccination of cattle against the tick *Boophilus microplus* (Willadsen 1980, 1987, Willadsen *et al.* 1988, 1989, Willadsen and Kemp, 1988, Johnston *et al.* 1986, Kemp *et al.* 1989, Opdebeeck *et al.* 1988a,b, Wong and Opdebeeck 1989). Owing to differences in biology and host-parasite interactions, the experience from this work on *B. microplus* may not be directly applicable to the search for a vaccine to *L. salmonis*. A different approach to the principles behind the tick experiments is therefore being applied.

This approach is to use the monoclonal antibodies developed from mice immunized with louse extracts to select individual antigens from a louse recombinant DNA library in the expression vector gt11. These clones can then form the basis of experimental vaccines to be tested in the field or by experimental challenge. As it is impractical to test more than a fraction of the potential antigens in this way, a means of narrowing the range of monoclonal antibodies must be used as an initial step in the selection process.

In the technology of hybridoma antibody production a rapid and reliable screening method is very important (Naiem *et al.* 1982, Aqel *et al.* 1984). Binding assays such as enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) are the screening methods of choice when pure antigen preparations are available. However, immunohistochemical and immunoblotting procedures are the main alternatives when these antigen preparations do not exist, either because their purification is complicated or because the antigen has not been characterized. Both alternatives have been difficult to apply to numerous supernatant samples. There have been successful attempts to overcome this difficulty. One, reported by Verhaert *et al.* (1986), used the plastic microtitre wells themselves for the attachment of histological sections. The most recent method is that of Berghman *et al.* (1989), which permits the use of four conventional 26×76 mm glass microscopy slides, each carrying 24 carefully arranged histological sections, resembling a 96-well pattern. In our hands this technique was difficult to apply owing to the large size of the adult female louse, the longitudinal histological sections of which cannot be fitted into the 3×8 sections pattern on each slide used by these authors.

The immediate aim of this study was therefore to develop an immune tracing method, suitable for fast and reliable screening of large numbers of hybridoma supernatants. The immunoenzyme staining methods chosen were the indirect immunoperoxidase (IIP) adapted for salmon anti-*L. salmonis* sera by Turnbull (1991) and the peroxidase anti-peroxidase (PAP) techniques.

MATERIALS AND METHODS

L. salmonis samples

Adult female *L. salmonis* were collected from farmed Atlantic salmon held in sea cages at several sites on the west coast of Scotland. The lice were placed in plastic bags filled with sea water from the site and transported in an insulated cool-box containing crushed ice. In the laboratory the lice were kept in a refrigerator at 4°C.

Tissue processing

Fixation was carried out within 48 h of sampling using 2.5% glutaraldehyde in either 0.1 M phosphate buffer (pH 7.3) or filtered sea water for 1.5 h. The cuticle around the lateral margins of the cephalothorax and the genital complex was punctured to improve penetration of the fixative and allow better paraffin embedding. Dehydration was through graded alcohols (50%, 70%, 80%, 96%: 30 min each; 100%: 1 h) and chloroform (1 h). Embedding was carried out with paraffin wax kept at no more than 58°C for 1 h. Sections were cut at 5–6 µm on a rotary microtome.

Fractionation protocols

Samples of whole female *L. salmonis* were prepared as described below. Approximately 5 g of lice were used to prepare each extract. The lice were ground with liquid nitrogen to produce a fine powder which was subsequently suspended in phosphate-buffered saline (PBS). The suspension was then homogenized using a motor-driven teflon/glass homogenizer for 2–3 min on ice and the resulting suspension centrifuged at 4000×g for 3 min at 4°C. The pellet was then resuspended in PBS and aliquots were assayed for protein content (Pierce BCA Protein Assay Reagent Kit) before being stored at -20°C.

Polyclonal/monoclonal antibody production

Production of monoclonal antibodies was carried out essentially as described elsewhere (Kennet *et al.* 1980). In brief, BALB/c mice were immunized three times with intervals of 2 weeks between injections using extracts of *L. salmonis*. Each mouse was injected with 50 µg of louse protein diluted in a 50:50 ratio with complete Freund's adjuvant, in a total volume of no more than 0.4 ml. Spleen cells from hyperimmune mice were fused with Ag8.653 myeloma cells in a 4:1 proportion, adding a 50% polyethylene glycol (PEG) 1500 solution. The fusion mixture from one spleen was routinely dispersed into four 96-well plates. Splenocytes from an unimmunized mouse were used as feeder cells. The cells were cultured in DMEM media supplemented with 10% horse serum. The supernatants were screened immunocytochemically as described below. Selected cultures were expanded, frozen and cloned by limiting dilution.

Humid chamber for incubations ('screening box')

A special humid chamber was designed to process several slides at the same time without the need to handle them individually. A perspective view of it is shown in Fig.

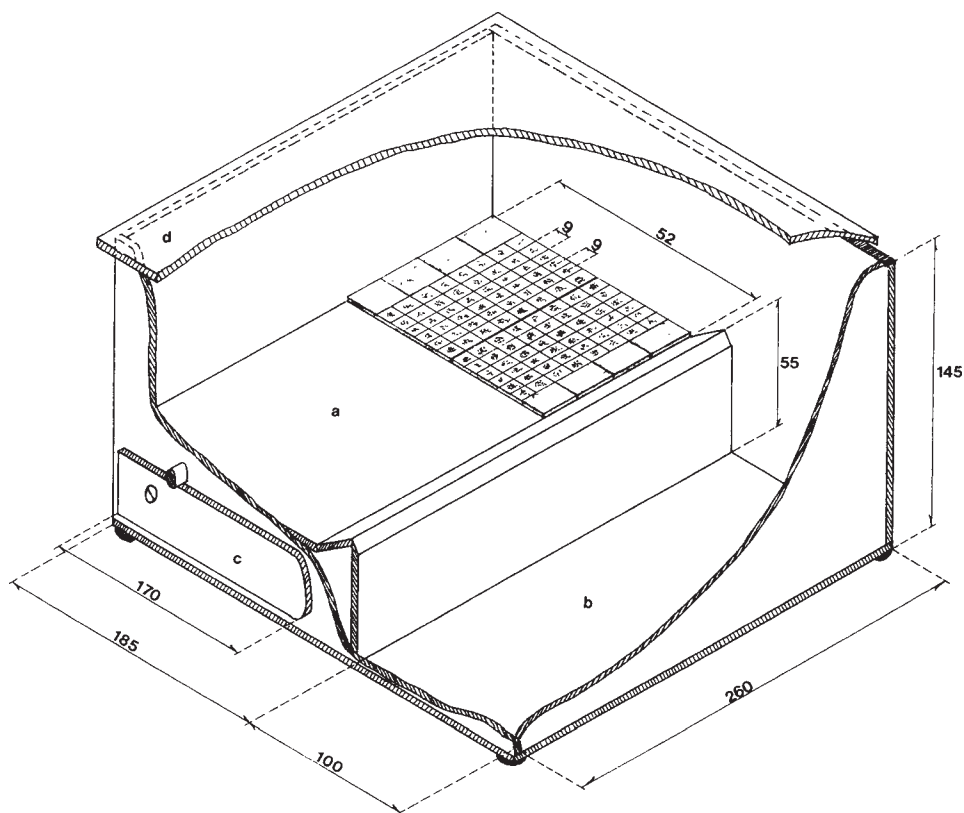


Fig. 1. Diagrammatic view of the incubation box designed for immunohistochemical screening: (a) incubation chamber with total capacity of 20 histological glass slides (eight shown in place); (b) waste chamber; (c) pivoting stand; (d) lid. Dimensions in millimetres.

1. It is made entirely of Plexiglass and consists of an incubation chamber (a), a waste chamber (b), two stands that pivot to incline the box (c) and a lid (d). The incubation chamber holds 20 24×76 mm glass slides (eight slides shown in place in the figure).

Immunohistochemical screening assay

Frosted slides carrying 12 paraffin sections each were prepared in advance. The sections were arranged in a pattern of 6×2. The separation between each section (9 mm, see Fig. 1) corresponded to the separation between the tips of a standard multichannel pipette. A template consisting of a glass slide with 12 distinguishable white spots was used to facilitate the correct and quick positioning of the sections. This template is used by placing it under the slide that is receiving the sections. The paraffin block was trimmed to a rectangle of 10×8 mm prior to cutting to ensure proper section dimensions. The sections were floated in groups of two or three in a water bath, collected and positioned on the slides, dried for 2 h on a hot plate at

40°C and then overnight at room temperature. The dried sections could be stored indefinitely. For every 96-well culture plate with the supernatants to be screened, eight slides were dewaxed and hydrated. Excess water was shaken off and they were then air dried. With a PAP pen (Agar L4197) a grid of water-repellent lines between each section and along the borders of the slide was drawn. This enabled the individual incubation of each section with the different reagents. The slides were then positioned in the incubation chamber of the 'screening box' in two rows of four slides with the frosted side opposite, so having eight columns of 12 sections. Enough TBS-Tween (0.05 M Tris buffer in 0.15 M saline, pH 7.6 with 1% Tween 20) was added to cover the slides for hydration for 2 min, then the TBS was drained by inclining the box with the aid of the two stands which kept it in place. After complete draining, the screening box was returned to a horizontal position and was ready to start the incubations. This operation was done at each of the washing steps of the immunostaining protocol. The immunoreagents were applied to each column of sections by means of a 12-channel pipette (or an 8-channel pipette with six tips). The lid was set in place during the incubations to maintain a humid environment. For jet rinsing after each incubation the screening box was inclined and, using a wash bottle, a jet of TBS was applied two or three times from top to bottom on each column of sections to wash out the immunoreagents.

Indirect immunoperoxidase (IIP) protocol

Endogenous peroxidase activity was blocked with a fresh 0.5% solution of hydrogen peroxide in methanol for 5 min. Sections were blocked to reduce non-specific binding with normal sheep serum (NSS) diluted 10% in TBS for 30 min. Primary antibody incubations were maintained for 30–45 min (supernatants; mouse anti-*L. salmonis* diluted 1:100 in NSS/TBS for positive controls, and TBS and normal mouse serum 1:100 in NSS/TBS for negative controls). Peroxidase-conjugated sheep anti-mouse IgG (1:100 in NSS/TBS) was the second antibody, applied for 30 min. After washing, the peroxidase label was visualized by incubating in DAB-TBS for 8 min (fresh solution of 0.05% diaminobenzidine tetrahydrochloride in TBS with 0.001% hydrogen peroxide).

Peroxidase antiperoxidase (PAP) protocol

Endogenous peroxidase activity was blocked as described above. Blocking for non-specific binding was done before each of the three antibody incubations using normal goat serum (NGS) diluted 10% in TBS for 30 min. This prevented any background staining. The antibody incubations were maintained for 30 min. All antisera and PAP complex were diluted in 10% NGS/TBS. Primary antibodies were the same as for the IIP above. Secondary antiserum was goat anti-mouse polyvalent immunoglobulins (Sigma M-8019), 1:20. Tertiary antiserum was mouse PAP soluble complex (Sigma P-3039), 1:200. The peroxidase was visualized as for the IIP.

Sections were counterstained slightly with haematoxylin, dehydrated, cleared, mounted in Pertrex and examined by light microscopy.

To compare the sensitivity of both techniques, known positive supernatants were diluted 1:1, 1:2, 1:5, 1:10, 1:20, 1:50, 1:100 and 1:200 and tested.

RESULTS AND DISCUSSION

Performance of the screening method

The screening method described here allowed the screening of 240 supernatants by one person within 6 h. Obviously this implies the advance preparation of equal numbers of histological sections in the appropriate pattern. The transfer of the supernatants from the 96-well plates to the sections takes approximately 5–6 min with a 12-channel pipette. After the sections are placed in the box, no further handling of them is necessary until after the DAB incubation and washing, when the slides are transferred to a slide rack for counterstaining and mounting. This represents a considerable economy of time. The economy of reagents is also substantial, as each section can be incubated with as little as 15–20 μ l of solution.

The dimensions of the box can be changed to fit other needs. For example, a box holding 60 slides (four rows of 15 slides) will allow the screening of 720 supernatants in one day.

Results of the screening

Around 5000 supernatants have been screened by this method so far. These supernatants came from the several cloning and subcloning operations of the original hybridomas from the mother fusions.

At first, while using the IIP staining protocol, some supernatants of previously positive clones were shown to be negative and some of their parental hybridomas were also negative, suggesting that the sensitivity of the IIP technique might not be high enough for our system. This led to the trial of another detection system, the PAP technique, which in theory is more sensitive. The PAP technique first had to be adapted to this particular system, testing different incubation times and serum dilutions. After this, the PAP method proved to be more sensitive than the IIP, giving positive staining with supernatants diluted up to 1:50, while the IIP showed negative results after 1:10 dilutions of the same supernatant. Consequently, the PAP was the standard technique used thereafter.

During the production of antibodies against salmon louse essentially seven different immunohistochemical pictures were observed: (1) general binding to all tissues—this is basically the same pattern as that observed with mouse anti-*L. salmonis* sera; discrete binding to (2) oviducts, (3) ovaries, (4) cuticle, (5) haemolymph, (6) the brush border of the gut epithelium (Fig. 2), and (7) binding to more than one tissue where the haemolymph is always common amongst them (i.e. haemolymph and either muscle or ovaries, or both, haemolymph and brush border or cytoplasm of cells).

The binding of antibodies to the brush border and cytoplasm of the gut epithelial cells represented a specially interesting finding since it provided strong immunohistochemical support for the existence of potentially important target antigens for successful vaccination. The gut epithelium would be one of the first tissues contacted by antibodies produced by a vaccinated salmon. In the case of the cattle tick vaccine, the relevant antigen was located on the luminal plasma membrane of the gut epithelial cells (Willadsen *et al.* 1988, Wong and Opdebeeck 1989). Ticks feeding on cattle vaccinated with whole tick extracts showed extensive damage of the gut and leakage of lumen contents into the haemolymph, and this apparently led to damage to other

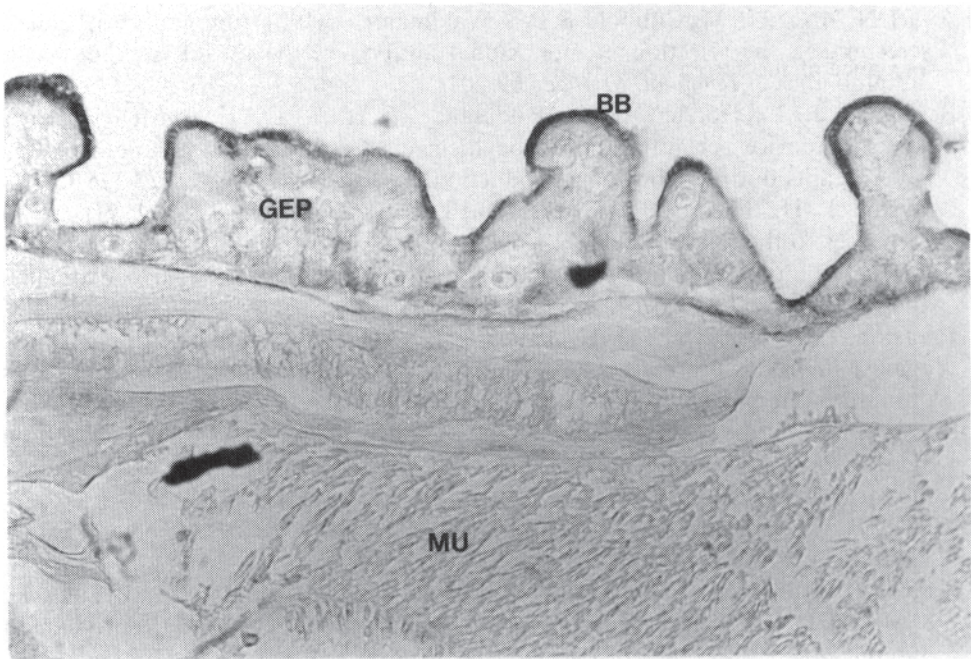


Fig. 2. Sea louse longitudinal paraffin section incubated with culture supernatant from a clone recognizing the brush border of the gut epithelium. Peroxidase indirect technique. Haematoxylin counterstain. GEP, gut epithelium; BB, brush border; MU, muscle. Original magnification 1200 $\times$.

tissues that are antigenic (e.g. muscle, Malpighian tubules) (Agbede and Kemp 1986). Thus the antibodies binding to haemolymph, muscle, ovaries etc. are also important to take into account when considering the possibility of a vaccine which would be a cocktail of several protective antigens.

The information that can be obtained from only one screening in the primary stage of a fusion experiment has thus proved invaluable in the selection and inventory of potentially interesting antibodies.

The monoclonal antibodies which have tested positive and recognize various areas of the louse are being used to screen sea louse DNA libraries. These libraries are constructed in the gt11 phage vector. Fragments of louse DNA are inserted within the lac Z gene, which codes for β -galactosidase. The inserted DNAs are then expressed in phage-infected bacteria cells as fusion proteins. Some positive fusion proteins have been detected which are now being characterized. The hope for the future is that these proteins will elicit a protective immune response in salmonids.

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Part IIe Biological control

25

Management of sea lice (Caligidae) with wrasse (Labridae) on Atlantic salmon (*Salmo salar* L.) farms

James W. Treasurer

ABSTRACT

Validation of control of sea lice, *Lepeophtheirus salmonis* (Krøyer), on farmed Atlantic salmon, *Salmo salar* L., with wrasse (Labridae) is given by comparison of population dynamics of lice in pens with and without wrasse. Although lice recruitment was high, and regular treatments with Aquagard were required on the adjacent production farm, lice on first sea year salmon in an experimental group of 5 m pens were controlled solely with wrasse. Mean mobile lice numbers (range 2–11) were significantly lower in pens with wrasse compared with controls (up to 50 per fish), although chalimus larvae were not significantly different. Wrasse positively selected larger lice stages, adults and second stage preadult females, >5 mm total length. Validation of the technique was supported by observations with underwater camera and analyses of wrasse gut contents. Cleaning activity was less in winter and when nets were fouled. Although means of minimizing difficulties associated with the technique are described, success in the second sea year was restricted owing to mortalities and escapement over winter.

INTRODUCTION

The quest for an alternative sea lice treatment to Aquagard, and environmental concern about its use, have led to the consideration of various forms of biological control comparable with those used in agriculture (Pimentel 1991), including releasing sterilized male lice and finding a disease or organism that would parasitize lice. Although these have not yet been realized, the use of wrasse (Labridae) as cleanerfish has been adopted by salmon farmers in Scotland (Treasurer 1991a), Norway (Bjorndal 1990, 1992, Costello and Bjorndal 1990) and to a lesser extent Ireland (Costello and Donnelly 1990, Darwall *et al.* 1991). The main advantage is environmental but the technique may also reduce stress in salmon and improve growth rates compared

with fish treated with Aquagard. Although cleaner-fish have been known for many years from the Tropics (Feder 1966), Potts (1973) first noted cleaning behaviour in northern European waters when corksiding wrasse, *Crenilabrus melops* (L.), cleaned black bream (*Spondylusoma cantharus* L.), mackerel (*Scomber scombrus* L.) and ballan wrasse (*Labrus bergylta* Ascanius) in the Plymouth Aquarium. Bjordal (1988) recognized the potential of this technique for control of caligids on Atlantic salmon (*Salmo salar* L.) farms and conducted tank trials in 1987 and commercial trials in 1988 (Bjordal 1990, 1992).

Although tank trials gave encouraging results (Bjordal 1988), this does not necessarily indicate that wrasse will control sea lice in salmon cages. Much of the evidence in support of the technique on commercial farms is anecdotal. Field trials have been limited by the lack of replicates (Bjordal 1990, Smith (Shetland trials) personal communication, Treasurer 1991b), or no examination of lice population dynamics (Bjordal 1990, Costello and Donnelly 1990). Scientific evaluation has been limited by the lack of controls as all pens (=cages; pens are used as the common term applied by commercial farms and most aptly denoting the holding of livestock) on a farm are commonly stocked with wrasse to prevent reinfestation of cleaned fish from non-controlled reservoirs. The present study is a validation of the technique by comparison of replicate treatments with wrasse and control pens without lice treatment by monitoring of lice population dynamics, by camera observations, and analyses of wrasse gut contents on an experimental unit on a production farm in Loch Eil, west Scotland.

MATERIALS AND METHODS

The trial group was located in the middle of a production farm containing 100000 salmon of the 1989 production cycle (on sea farms termed 'year class', i.e. the year fish put to sea) in Loch Eil, 15 km west of Fort William (56°51' N, 5°8' W). These older fish had been treated regularly with Aquagard, and lice numbers rarely fell below a mean of 40 mobiles (preadult and adult lice) per fish from April 1991 to January 1992. Salinity was measured daily at the surface and at 4 m, and varied from 30 to 21 ppt. Water temperature was highest in August 1991 at 13°C and declined to 8°C in January 1992. The experimental fish used were 15000 smolts, weight range 120–250 g and 23–26 cm fork length (on 28 August), stocked on 3 May 1991 on the group of 32×5 m² area and 4 m deep experimental pens (water volume=100 m³) with 12 mm mesh net. The experimental group of pens had several advantages: it was possible to have replicates of pens with and without wrasse; as there were only 500–800 smolts per pen, i.e. a stocking density of 5–8 m³, only a small number of wrasse were required in total ($n=440$); the nets could be raised readily and smolts removed for counting sea lice; wrasse mortalities could be removed daily; and wrasse could be transferred between pens easily, if required.

Wrasse were stocked on 28 August 1991 at a ratio of 1:25 smolts, and the mix of wrasse species was three goldsinny, *Ctenolabrus rupestris* (L.) to one rock cook, *Centrolabrus exoletus* (L.). Trials with individual species were not conducted and the stocking ratio was higher than reported (Costello and Bjordal 1990) as, being a mixed year class farm, expected recruitment of lice was high. Eighteen pens were

stocked with wrasse and four controls with no wrasse were randomly chosen. Lice were counted at three levels: (1) every week five salmon were removed from four pens with wrasse and four control pens, anaesthetized in 15 ppm benzocaine, lice identified to species, and total chalimus (fixed) and mobile stages enumerated; lice dislodged during this procedure were counted in the holding container; (2) every 2 weeks five further fish were removed from a pen with/without wrasse, the fish killed by a blow to the head, and all chalimus and mobile stages removed for later identification, counting and measurement of developmental stages: chalimus were identified to stages I–IV and mobiles to preadult (PA) I and II, and adult males and females (Johnson and Albright 1991); the gills of these fish were also examined but no chalimi or mobiles were seen; (3) every 4 weeks lice on five fish from all 22 pens used in the trial were counted. On harvesting all fish on the group (15 January 1992), a larger sample of fish from four pens was taken to assess intensity of infection. Observations of wrasse cleaning activity on a commercial scale were also made at Loch Sunart in 1991 (56°40' N, 5°38' W).

An additional validation was performed by removing all wrasse from one pen where cleaning activity had been verified (pen 17) and transferring these fish to a pen (pen 13) with no wrasse.

Observations of wrasse behaviour were made with a Seamatrix underwater camera located on the bottom of a 5 m pen from 0900 to 1700 h on 30 October 1991. Data were expressed as number of occurrences of a particular behaviour over 8 h. The duration of each activity could not be quantified as individual wrasse were only observed for a brief period before being obscured by the school of salmon. Analyses of gut contents of damaged wrasse were also made, by the occurrence method, i.e. the number of alimentary tracts containing a particular food item.

RESULTS

Wrasse stocking trials

Smolts were stocked on the trial group on 3 May 1991 and, within 3 days, copepodids were noted on these fish and by 16 June there was a mean of eight mobile lice, and treatment with Aquagard was necessary. Three further treatments were required by 26 August 1991, and wrasse were then stocked on 28 August. Analyses of the data showed an overdispersed pattern with a large variance to mean ratio: therefore data were transformed to $\log(x+1)$ and the data presented as geometric means with 95% confidence limits (Fig. 1). Only data for *Lepeophtheirus salmonis* are presented, as *Caligus elongatus* Nordmann numbers did not exceed 5% of total mobiles on any date. No chemical treatments were required on the pens with wrasse from commencement of the trial, although Aquagard was used routinely on the main farm and, as seen from the control group, potential recruitment was high. In pens with wrasse, lice mobiles varied from a mean of 2 to 11 per fish. Mobile lice numbers increased slightly in early December, followed by a slow decline perhaps due to reduced recruitment resulting from lower salinity and harvesting of production fish. There was a corresponding rapid decline in chalimus larvae at this time (Fig. 2).

In contrast, lice numbers on the salmon from control pens increased to 16 per fish 2

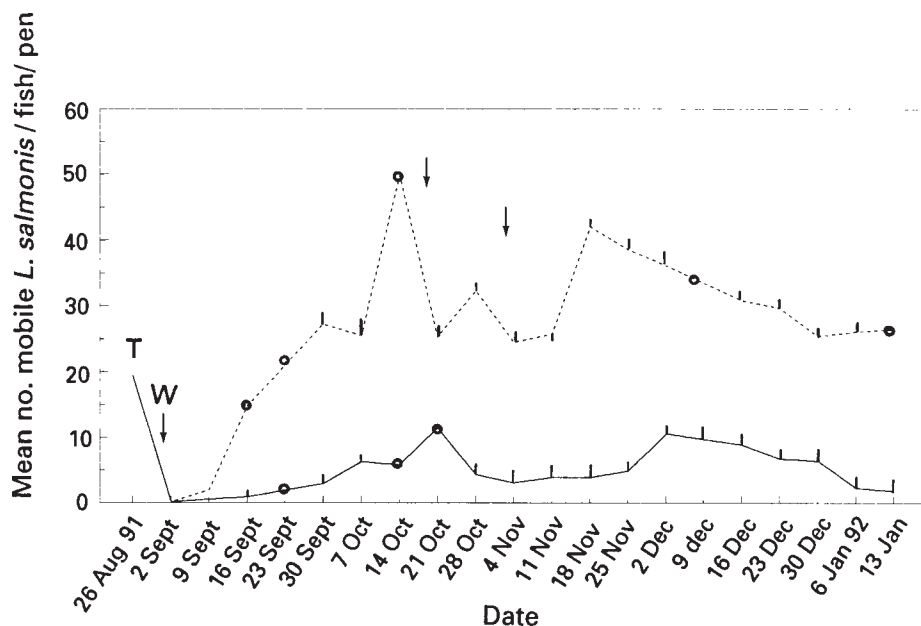


Fig. 1. A comparison of numbers of mobile lice between pens with wrasse and control pens, Loch Eil. Mean, — with wrasse; - - - no wrasse (controls). T, Aquagard treatment; W, wrasse stocked. Second arrow indicates when wrasse were introduced to control pens to reduce lice numbers. Third arrow indicates when wrasse were removed (30 October). Mean is the geometric mean with 95% confidence limits (for pen numbers of more than three only); ○, three or fewer pens.

weeks after Aquagard treatment, to 26 on 30 September, and attained a maximum of 50 by 14 October (Fig. 3). To avoid treating controls with Aquagard, wrasse were transferred from adjacent pens for a period of 10 days on 20 October. Lice numbers immediately halved, but increased following removal of wrasse and then declined slowly as in the fish in pens with wrasse. The trial terminated in mid-January as the pens had to be fallowed prior to restocking of the farm. Mobile lice were compared by Student's *t*-test, and mean numbers were significantly different ($p < 0.05$) at all sampling points.

Mean numbers of chalimi were generally lower in pens with wrasse (Fig. 2), with four to six chalimi per fish throughout the trial. However, there was no significant difference in chalimi between the two groups ($p > 0.05$). Although chalimi were higher on control pens in September, sample size was too small (three pens or fewer sampled) to make a statistical comparison.

Wrasse selected the larger mobiles, namely second stage preadult females and adults (Fig. 4). Adults comprised 6% of all mobile stages on fish with wrasse compared with 49% on controls. In contrast, first stage PAs were 77% on fish cohabiting with wrasse compared with 24% in controls. Wrasse positively selected lice of >5 mm total length, supporting the observation that wrasse did not eat chalimi, perhaps as there was an abundance of mobile lice.

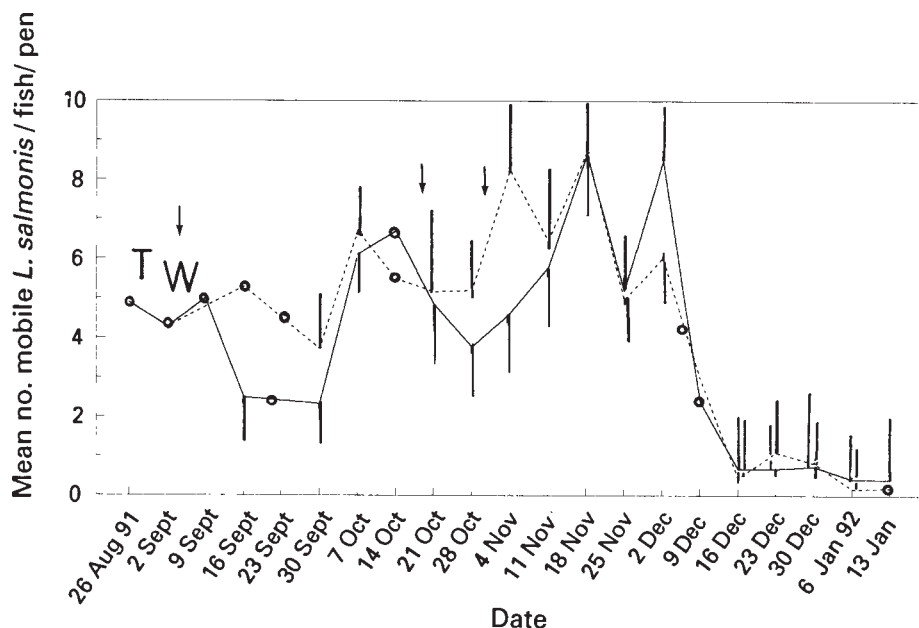


Fig. 2. Mean numbers of chalimus larvae of *L. salmonis* per fish in pens with wrasse and control pens, Loch Eil. Mean, — with wrasse; --- mean, no wrasse (controls). T, Aquagard treatment; W, wrasse stocked. Second arrow indicates when wrasse were introduced to control pens to reduce lice numbers. Third arrow indicates when wrasse were removed (30 October). Mean is the geometric mean with 95% confidence limits (for pen numbers of more than three only); O, three or fewer pens.

Intensity of infection was compared between pens 13 (average of 5.5 mobiles) and 19 (2.7 mobiles) with wrasse and control pen 14 (24.5 mobiles) (Fig. 5). In all cases the distribution was skewed to the right. The distribution in pen 20 was intermediate, as wrasse were removed on 31 October to stock another pen where lice numbers had increased. Various distribution models were fitted to these data and goodness-of-fit tested by χ^2 and Kolmogorov–Smirnov tests: the binomial distribution fitted all four data sets best ($p < 0.001$). The four distributions were also compared by Kolmogorov two-sample test for location and shape and all were significantly different ($p < 0.001$).

On 16 September the mean number of lice mobiles was 19 in pen 13 and two in pen 17. All wrasse from pen 17 were transferred to pen 13. Within a week lice numbers were similar and by 30 September the levels of infestation in the two pens were reversed: 17 mobiles in pen 17 compared with four per fish in pen 13.

Camera observations

Wrasse swam near the net bottom, and salmon, except when feeding, swam within 50 cm of the net floor. The salmon frequently swam slowly at about one body length per second and were sometimes almost stationary. Wrasse swam in an opposite direction to the salmon, slightly outside and below the school. A typical observation

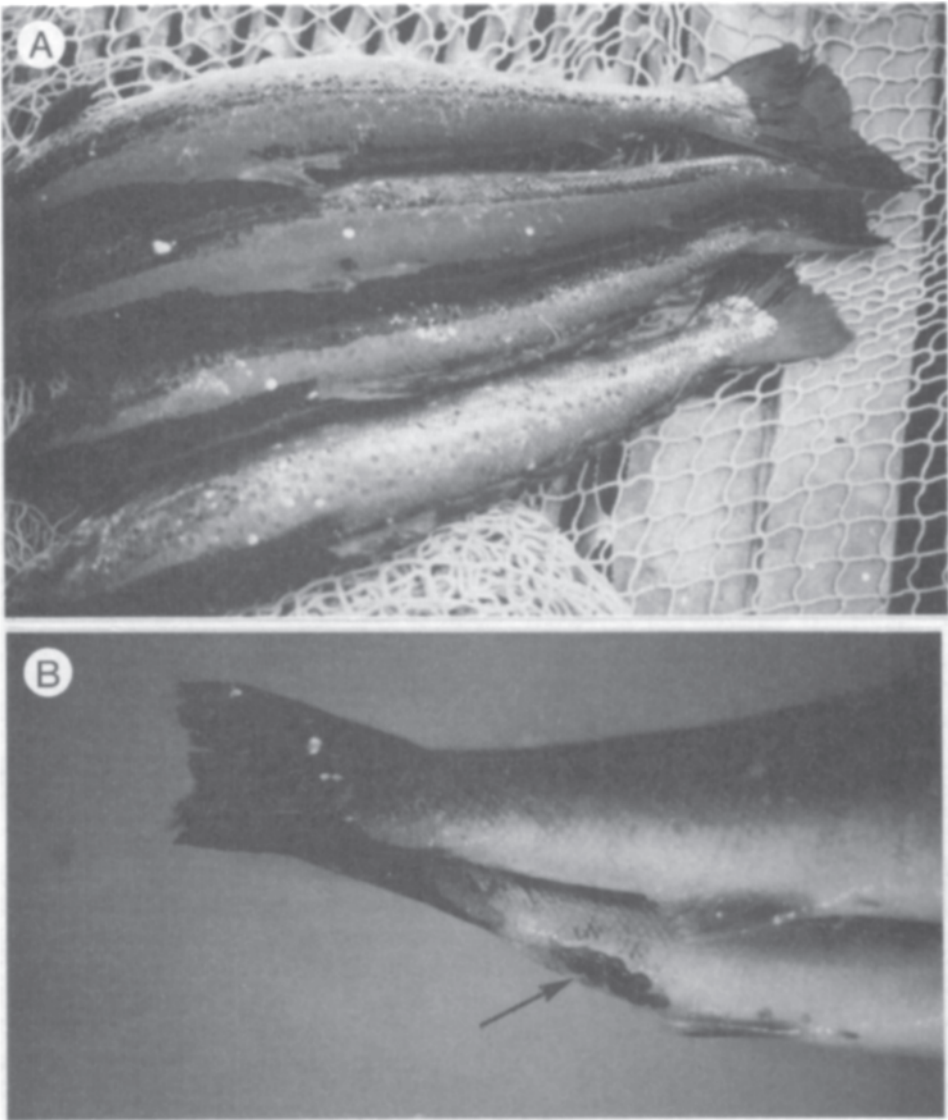


Fig. 3. (A) A comparison of infestation of mobile *L. salmonis* on the dorsal surface of salmon from control pens, particularly between the dorsal fin and adipose fins, and from pens with wrasse (two upper fish). (B) Numbers of mobile *L. salmonis* on the postanal area of a control fish compared with a salmon from a pen with wrasse (upper).

(*n*) was a wrasse swimming under and alongside the salmon (*n*=5), stopping, looking upwards into the school, and then darting upwards to join the salmon. On three occasions a wrasse was seen to swim alongside an individual salmon for several seconds, nudging the salmon, presumably taking a louse, although the movement was too rapid and too distant to confirm removal. Salmon and wrasse did not avoid each

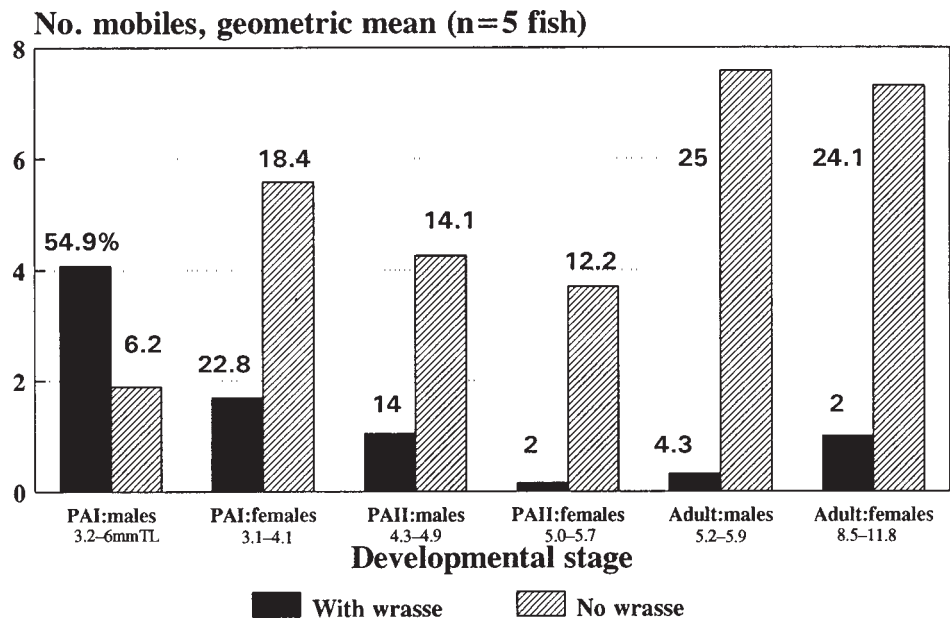


Fig. 4. Composition of developmental stages of *L. salmonis* on salmon pens with/without wrasse. Percentage of total lice numbers that each stage comprised is also shown. Total mobiles, pen 13=7.4, pen 15=30.3. Mobile stages TL (range) mm shown below bars.

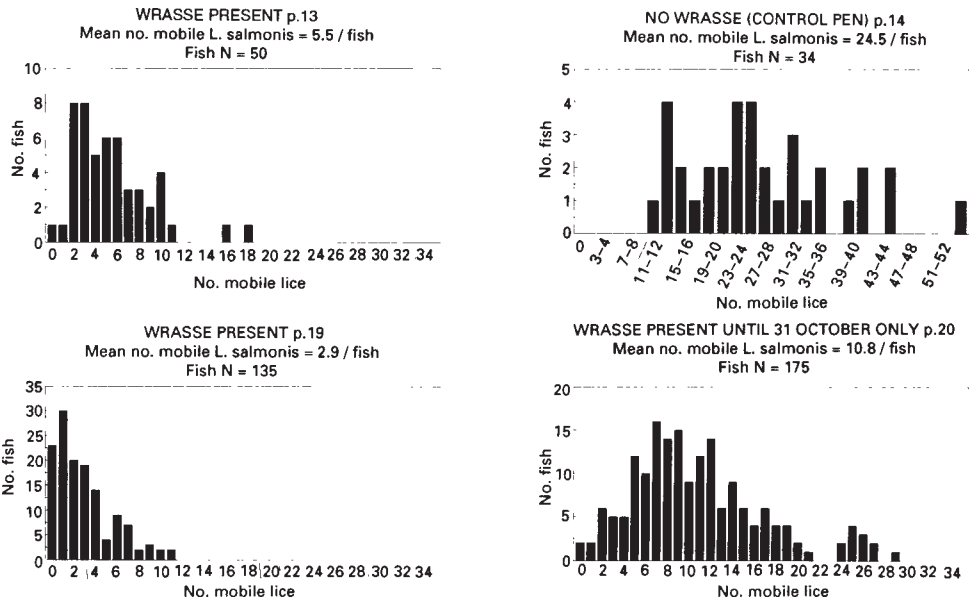
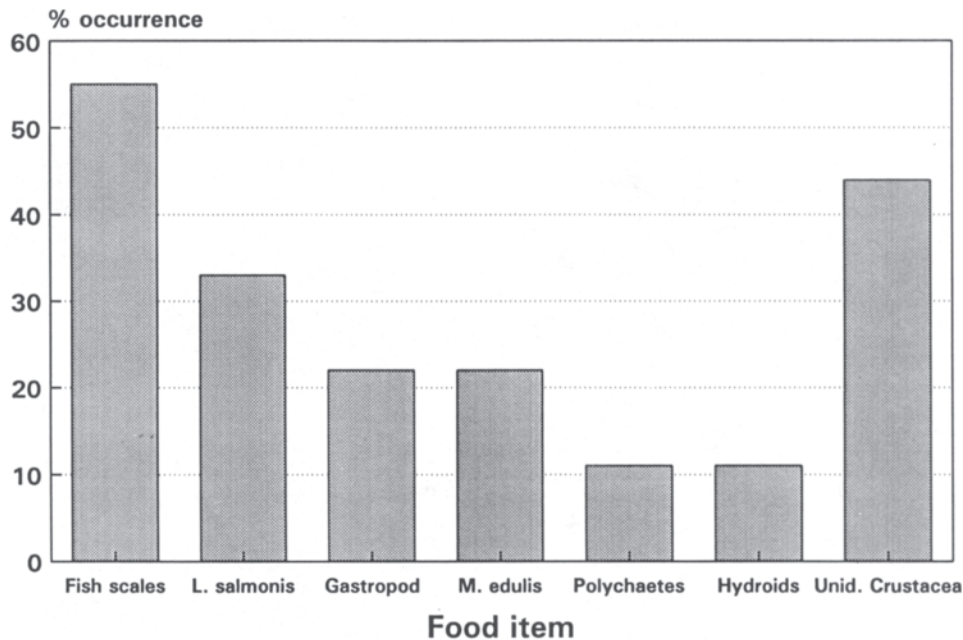


Fig. 5. Intensity of infestation of *L. salmonis* mobiles on salmon in pens with/without wrasse.



Fish N = 9 116-127 mm TL

Fig. 6. Stomach contents of goldsinny wrasse.

other, frequently being in close proximity. Salmon appeared irritated by the presence of lice, frequently jerking, turning over, and rubbing against the net floor.

Stomach contents

There was reluctance to sacrifice wrasse for stomach analyses and, as the exoskeleton of copepods is likely to be intact after passing through the gut, an attempt was made to extrude faeces by gently massaging the stomach of anaesthetized wrasse. This was unsuccessful and a small stomach pump was used. However, the maximum internal bore of plastic tubing inserted into the gut of wrasse was 1 mm and lice could not be removed, with only fragments of two lice recovered. The contents of the alimentary tract of nine wrasse were examined (Fig. 6). A range of invertebrates was eaten, including net-fouling organisms such as mussels (*Mytilus edulis* L.). Wrasse may pick at remains of dead fish, explaining the presence of salmon scales in the gut. Adult *L. salmonis* were identified in three of the nine alimentary tracts.

Mortality of wrasse/damage to salmon

Mortalities/losses over the trial period were low—less than 14% of total wrasse stocked. The main cause of mortality was furunculosis (Fig. 7), a typical strain of *Aeromonas salmonicida* (32 of a total 440 wrasse stocked), and several of the unidentified mortalities were probably also due to this cause, but could not be swabbed because of decomposition. Wrasse were recovered and treated by injection of an antibiotic,

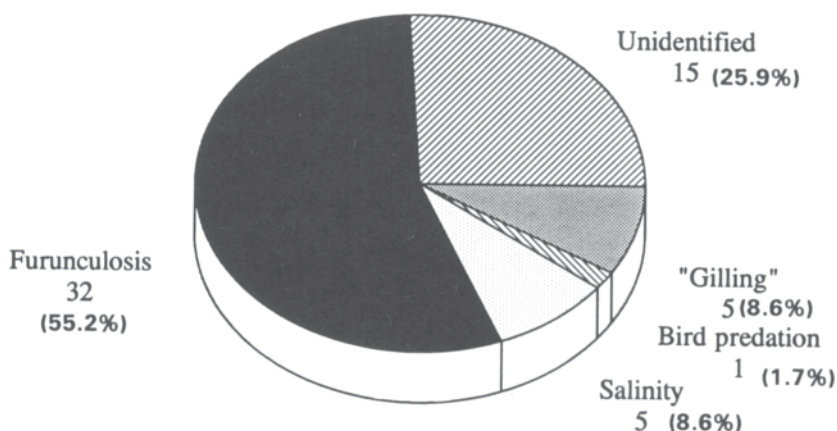


Fig. 7. Causes of mortality of 58 wrasse amongst 440 wrasse stocked in Loch Eil pens, August–December 1991.

ampicillin (brand Penbritin), and no further mortalities occurred. Some wrasse were gilled on the net mesh and some mortalities related to low salinity as these occurred after large volumes of fresh-water run-off. However, the salinity tolerance of wrasse is greater than previously suggested (Treasurer 1991b) and wrasse survived short-term exposure to salinities of 14 ppt and longer periods (3–5 days) down to 21 ppt. Emaciated wrasse were not seen, even when lice numbers were low. Wrasse were in good condition and maintained themselves on invertebrates and fouling organisms on nets.

Salmon were inspected for damage during the trial and all fish (13600) remaining on harvesting. No eye or other damage attributable to wrasse occurred.

DISCUSSION

Cleaning activity

Lice numbers on the group of trial cages were controlled solely using wrasse from August to January despite rapid and high recruitment of lice to control pens and on the production farm. Wrasse selected the larger lice stages, mainly adults, and chalimi were not eaten. Work in Ireland suggested that wrasse will eat chalimi (Costello personal communication) but mobile lice numbers were lower than at Eil and, in a situation where mobile lice are abundant, wrasse may select the largest available food items. Cleaning activity of wrasse diminished over winter, as in Shetland (Smith personal communication), perhaps related to reduced activity with lower water temperature, but reduced day length and rough weather may also be involved.

Production farms

The results of these trials may not be directly applicable to large pens as no sock on nets for collecting mortalities was present in the trials at Loch Eil. Wrasse gravitate to these for shelter and to graze on dead salmon (personal observation,

widely reported by farm staff and salmon scales found in gut contents). However, the provision of suitable hides in pens may reduce this problem. The three major salmon-farming companies in Scotland—Marine Harvest, McConnell Salmon and Golden Sea Produce—stock wrasse. The exception is farms with low salinity and where current speed is too high. Validation is difficult as control pens with no wrasse may not be available. In Loch Sunart an average of <1 mobile *L. salmonis* per fish was recorded through 1991 in the first year of the production cycle, compared with >50 in 1989 (personal observation), although fallowing may have had an influence. Farmers have questioned whether wrasse are effective in cleaning *Caligus* (personal observation). However, significantly fewer *Caligus* were noted on salmon in pens with wrasse at Glenmore Farm, Loch Sunart, compared with a control pen, e.g. one compared with 27.5 mobiles on 9 September 1991 (D. Mitchell, McConnell Salmon, personal communication). Wrasse have been less effective in the second year of the production cycle in Scotland (Treasurer 1991b), with the main problem of keeping wrasse alive over winter and reducing escapement, although Darwall *et al.* (1991) reported continuing cleaning performance in the second year. Special refuges may have to be provided in pens to permit wrasse to overwinter or wrasse removed and kept onshore in ponds or in cages on the loch bed.

These trials demonstrate that the use of wrasse is highly desirable in mixed year situations where smolts become rapidly infested with lice (Chapter 19). Although further developmental work is required in optimizing the use of wrasse, cleaner-fish have an important role in an integrated package to control sea lice, including fallow periods and reduced stocking densities (Chapter 18), vaccines (Chapter 23) and alternative chemicals, such as hydrogen peroxide (Chapter 21).

ACKNOWLEDGEMENTS

I thank the staff of Marine Harvest for their assistance during these trials, particularly Tony Boyd and Donnie MacDonald of Loch Eil Farm, and Trevor Meyer. I am grateful to Mark Costello for his helpful comments on the manuscript.

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26

Udonella caligorum Johnston, 1835 (Platyhelminthes: Udonellidae) associated with caligid copepods on farmed salmon

Dan Minchin and David Jackson

ABSTRACT

Two species of caligid copepod are found on farmed salmon in Irish waters: *Caligus elongatus* and *Lepeophtheirus salmonis*. Both of these species can be infested with the ectoparasitic platyhelminth *Udonella caligorum*. Infestations on *L. salmonis* may have resulted from contacts with *C. elongatus*. In this study *U. caligorum* was recorded from *L. salmonis* on farmed salmon but not from those on wild fish. Results of worm distributions on *L. salmonis* are based on examination of 3635 eggs, 1899 immature and 390 adult worms. The distribution of these stages varies and suggests a specific pattern of movement. Similar results were found for *C. elongatus*. *U. caligorum* was present during the winter and spring on the north-west, west and south-west Irish coasts. No obvious damage to salmon is known as a result of the presence of these worms.

INTRODUCTION

Udonella caligorum Johnston, 1835 is a parasitic helminth whose systematic position has been under debate for several decades and remains unresolved. Following detailed studies on its ultrastructure it has been assigned to the Neodermata by Xylander (1988) and the Turbellaria' by Kornakova (1988). However, according to Ehlers (1985) and Brooks (1985) in their classifications of the Platyhelminthes, the Turbellaria' is not a monophyletic group.

This worm is found attached to caligid copepods, which in turn are normally attached to the surface of various fishes. The worm attaches to the outer surface of its crustacean host and does not appear to harm it.

U. caligorum produces clusters of elongate, pyriform operculate eggs of c. 130 μm , each fixed to the copepod surface by a long spiral stalk with a discoidal attachment

plate. From these the hatchlings, of greater than 200 μm (Dawes 1946), attach to the copepod by means of a single posterior sucker, the caudal haptor. Kabata (1973) suggested that worms, once hatched, move forwards to the cephalothorax and when mature move to the posterior tagmata to lay eggs. It has been suggested that their marginal distribution on the cephalothoracic shield enables feeding on superficial fish tissue and that the presence of worms may aggravate damage to the superficial layers of the fish skin caused by the copepod host, but this has not been shown (Kabata 1973). *U. caligorum* remains attached to its host throughout its life, but transfers of worms may take place during contact between the copepods while on the fish.

This chapter discusses the distribution of *U. caligorum* found on two parasitic copepods, *Caligus elongatus* Nordmann, 1832 and *Lepeophtheirus salmonis* (Krøyer, 1837), found throughout the year on cultivated salmon, *Salmo salar* L.

METHODS

Worms were examined from collections of *C. elongatus* and *L. salmonis* from salmon farms within 11 bays around the south-west, west and north-west coasts of Ireland (Fig. 1). Sampling took place from April to September 1991, and from February to July 1992. In addition, lice on 80 netted wild salmon from the west and north-west coasts were examined.

Lice were removed from 30 salmon from each year class (where available) from each farm. Fish were obtained following hand feeding or seining using a hoop net, placed directly in bins and anaesthetized using ethyl-4-aminobenzoate in acetone. All copepods were removed immediately and placed in a separate phial, containing 70% ethanol, for each fish. Free-moving (preadult and adult) stages were removed in every case, and in 1992 some chalimus stages were also obtained.

Worms were recorded by their stage (eggs, immature and mature) and their position on the copepod was recorded, using the method of Kabata (1973) (Fig. 2). Identification and scoring of attached worms on the dorsal and ventral surfaces were made by means of a light microscope at 40 $\times$ or greater.

Samples are based on over 30000 collections of *L. salmonis* and 1500 *C. elongatus* from 2250 salmon. In all, 3635 eggs, 1899 immature and 390 mature worms were examined from the surface of *L. salmonis*; and 421 eggs, 161 immature and 75 mature worms from the surface of *C. elongatus*.

RESULTS

Worms on *L. salmonis*

Infestations of worms on copepods were highly variable. They were seldom seen from June or July 1992 and were absent in all areas from July to September 1991 (Table 1). High infestations were not dependent on a greater copepod density on fish. Such infestations were recorded from Lough Swilly on the north-west coast in May 1991, from Clew Bay in February 1992 on the west coast and from Kenmare Bay in April 1992 on the south-west coast on one and two sea winter fish (Table 2). None was found from salmon smolts or post-smolts. The greatest worm frequency, 14.6 eggs,



Fig. 1. Distribution of sites where caligid copepods were found associated with farmed salmon.

13.1 immature and 1.9 adults, was from Lough Swilly, and the highest infestation on an individual was also from this area, a female of 11.1 mm body length had 142 eggs, 111 immature worms and nine adults.

Size distributions of both species of infested copepods are shown in Table 3. Males mature at a smaller size and attain 7 mm body length, whereas females reach 14 mm body length. Adult copepods were frequently infested; however, chalimus and preadult stages also carried worms, but at a lower frequency.

Eggs of worms remained attached following collection. Immature and mature worms

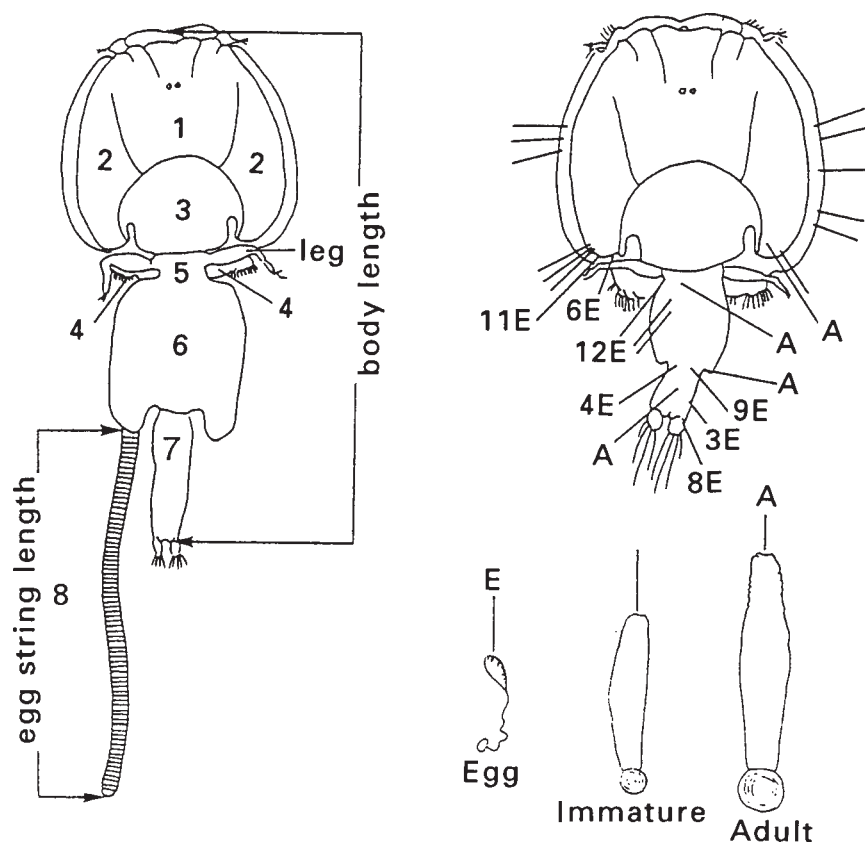


Fig. 2. Left: female *L. salmonis*, 12 mm body length, showing distribution of positions where *U. caligorum* developmental stages were recorded. Right: male *L. salmonis*, 6.2 mm body length, obtained from Lough Swilly on 22 May 1991 from a two-sea winter salmon. Distribution of developmental stages of *U. caligorum* are shown. Bottom right: developmental stages of *U. caligorum* recorded. E, eggs; unlabelled line, immature; A, adults.

normally remained attached; however, some unattached worms were found in sample tubes. Attached eggs were distributed predominantly on the three posterior tagmata, positions 5 to 7 (Table 4). Eggs on positions 2 and 3 were normally located posteriorly (Fig. 2) but were seldom attached to copepod egg strings. Eggs were grouped in clusters ranging from 1 to 34. Greater numbers of clusters ($N=363$) were found on male than female hosts ($N=254$); however, the proportion of the numbers of eggs in each cluster did not differ between sexes. The numbers of clusters per infested copepod ranged from 1 to 15. Eggs on the ventral surface were found only on the posterior three tagmata of both sexes.

Immature worms predominated on position 2 (>80% on female copepods, and c. 50% in males) and were attached to the margin of the cephalothoracic shield, often to the thin marginal membrane (Fig. 2; Table 4). Greater numbers of worms were found

Table 1. Occurrence of *U. caliginos* on *L. salmonis* on salmon during different months

Locality	1991						1992					
	A	M	J	J	A	S	F	M	A	M	J	J
1 Lough Swilly	-	+	-	-	-	-	-	-	-	+	-	-
2 Mulroy Bay	-	0	0	0	0	0	-	-	-	0	-	-
3 Donegal Bay	-	+	0	-	-	-	-	-	-	+	-	0
4 Clew Bay (inner)	-	-	-	-	-	-	-	+	-	0	0	-
5 Clew Bay (outer)	-	0	-	-	-	-	+	-	+	+	+	+
6 Killary Harbour	0	0	0	0	0	0	-	-	0	0	0	-
7 Ballinakill Bay	+	0	0	0	0	0	-	+	+	0	+	-
8 Mannin Bay	0	-	-	-	-	-	-	-	-	0	-	-
9 Bertraghboy Bay	+	+	0	0	0	0	-	+	+	+	0	-
10 Kilkieran Bay	+	+	0	0	0	0	-	-	-	+	+	-
11 Kenmare Bay (inner)	-	+	0	0	0	0	-	-	-	+	-	-
12 Kenmare Bay (outer)	-	+	+	0	0	0	-	-	-	0	-	-
13 Bantry Bay	-	0	-	-	-	-	-	-	-	+	-	-

+, present; 0, absent; -, no sample.

Table 2. Numbers of *L. salmonis* examined on which *U. caligorum* was found, and mean frequencies of worm occurrence for each developmental stage

Locality	<i>L. salmonis</i>		<i>U. caligorum</i>		
	Date	Number	Egg	Immature	Adult
Lough Swilly	5.91	117	14.6	13.1	1.9
Donegal Bay	6.92	9	3.9	1.4	.5
Clew Bay (inner)	3.92	8	.2	1	0
Clew Bay (outer)	2.92	88	10.4	.5	.4
Ballinakill Bay	4.92	62	3.4	1.2	.6
Bertraghboy Bay	4.91	15	1.4	1.1	.3
Bertraghboy Bay	5.91	46	6.9	.4	.3
Kilkieran Bay	4.91	74	2.9	.9	.4
Kenmare Bay (inner)	4.92	15	25.4	3.4	0
Kenmare Bay (outer)	6.91	15	4.0	1	1.4

Table 3. Length frequencies of infested copepods

Body length (mm)	<i>L. salmonis</i>		<i>C. elongatus</i>	
	Male	Female	Male	Female
1	0	0	1	0
2	3	0	1	0
3	15	15	11	1
4	47	4	29	11
5	105	14	3	37
6	121	13	1	7
7	3	0		
8		2		
9		20		
10		17		
11		22		
12		23		
13		18		
14		7		

on the dorsal surface, although some were situated ventrally. beneath the cephalothorax the greater proportion of worms was on male copepods (Table 5).

Adult worms were predominantly found on sections 2 and 3 on female copepods (almost 80%), whereas on males it was about 50%. Worms were normally found attached to the posterior portions of these zones. They occurred in all zones, but less frequently on ventral surfaces and rarely on egg strings.

Table 4. Occurrence (%) of developmental stages of *U. caligorum* according to position on *L. salmonis*. Positions as in Fig. 2

	Sample size	Position								
		1	2	3	4	5	6	7	8	Leg
<i>Females</i>										
Eggs	1397	0.2	26.8	16.4	1	15.8	27.9	11.8	0	0.1
Immature	1145	1.9	81	2.4	1.7	2.6	7.4	2.5	0.2	0.3
Adult	104	0	59.6	23.1	1.9	4.8	8.6	1	1	0
<i>Males</i>										
Eggs	2238	0.5	7.3	18.3	2.9	34.4	26.9	9.1		0.6
Immature	754	2.1	47.9	14.6	8.8	13.6	8.8	2.5		1.7
Adult	286	0.7	20.6	24.2	5.2	15.4	24.8	8.4		0.7

Table 5. Distribution of numbers of immature worms on *L. salmonis*

	Sample size	Position								
		1	2	3	4	5	6	7	8	Leg
<i>Females</i>										
Dorsal	1057	21	923	26	16	25	26	15	2	3
Ventral	88	1	5	1	4	5	59	13	0	0
<i>Males</i>										
Dorsal	681	6	323	106	62	101	54	16		13
Ventral	73	10	38	4	4	2	12	3		0

Worms on *C. elongatus*

U. caligorum was found on chalimus as well as preadult and adult stages of both sexes. In outer Kenmare Bay they were seen only on *C. elongatus*, although *L. salmonis* was present. Almost 75% of the eggs attached to female copepods were found on segments 4 and 5, whereas in males most were recovered attached to the posterior region of segment 3 (Table 6). Immature worms were predominant on the cephalothorax (86.8% females; 64.1% males), as were adults (77.4% females; 68.1% males). Egg clusters ranged from one to seven and comprised 1–34 eggs. No mating pairs of *C. elongatus* were observed in this study.

DISCUSSION

The predominance of infestations during the winter and spring may relate to sea temperature, but increases in moult frequency of the copepod host during the summer may be of some importance in shedding worms. Infestations appear to be sporadic on western Irish coasts. *U. caligorum* was absent from Mulroy Bay, Mannin Bay and

Table 6. Occurrence (%) of developmental stages of *U. caligorum* according to position on *C. elongatus*. Positions as in Fig. 2

	Sample size	Position								
		1	2	3	4	5	6	7	8	Leg
<i>Females</i>										
Eggs	327	0	1.5	7	25.2	49.5	13.8	1.2	1.2	0.6
Immature	113	0	78.8	8	4.4	3.5	3.5	0.9	0.9	0
Adult	53	1.8	54.8	22.6	3.8	5.7	3.8	1.8	5.7	0
<i>Males</i>										
Eggs	94	0	2.1	54.2	7.4	8.5	0	16		11.7
Immature	53	1.8	41.5	22.6	1.8	18.9	1.8	9.4		1.8
Adult	22	0	27.2	40.9	9	9	4.5	4.5		4.5

Killary Harbour and from some farms within other bays. The worm is, however, generally distributed and may well appear in bays where it has hitherto been unrecorded. No significant increase in damage to fish was noted where the copepod parasites were carrying a heavy infestation of *U. caligorum*. There is therefore no evidence from this study to suggest that worms cause damage to farmed salmon.

The observations on the distribution of all worm stages on *L. salmonis* are consistent with those made by Kabata (1973) on *C. elongatus*. It would appear that eggs were laid so that they lie behind the cephalothoracic shield in an area where the copepod is waisted. In this area there may be little drag and some protection from abrasion. Eggs were also laid on the free, fourth pedigerous somite, and predominantly on the anterior and posterior ends of the female genital complex possibly for the same reason. Egg cases, from which worms had hatched, were seen on a number of copepods but were not recorded. Dawes (1946) noted that this species is found mainly on the egg sacs of the female copepod and that the hatching period is synchronous with that of the copepod, although individuals of different sizes could be found on the same egg sac. This was not confirmed in this study or by Kabata (1973).

The distribution of worms may be modified by the presence of patches of stoloniferous hydroids which attach and spread to cover significant areas of the posterior tagmata, egg strings and, less frequently, the central and rear parts of the cephalothorax. Other organisms often found attached to infested copepods included algae, such as *Ceramium* sp., *Ulva* sp., *Vorticella*-like protozoan colonies and recently settled *Mytilus edulis* L. The distribution of immature worms is concentrated about the margin of the cephalothoracic shield and dorsal and lateral surfaces of the posterior tagmata on *L. salmonis*. This is similar to the situation described by Kabata (1973) for *C. elongatus* removed from the buccal cavity of cod, *Gadus morhua* L.

U. caligorum has been described as a parasite present on *C. elongatus* from cod (Kabata 1973, Xylander 1988) on both sides of the North Atlantic, and from herring (*Clupea harengus* L.) (MacKenzie and Morrison 1989) and various caligid species associated with ballan wrasse (*Labrus bergylta* Ascanius), flounder (*Platichthys*

flesus (L.)), hake (*Merluccius merluccius* (L.)), pollack (*Pollachius pollachius* (L.)), ling (*Molva molva* (L.)) and wolf-fish (*Anarhichas* sp.). According to Kabata (1979) *C. elongatus* is known from more than 80 species of fish, including elasmobranchs, and is widely distributed in British and Irish waters. *U. caligorum* may thus be widely distributed, and *C. elongatus* may act as a vector for the transfer of *U. caligorum* to other parasitic copepods. Kabata (1973) referred to it being present mainly on copepods of the family Caligidae. The occurrence of *U. caligorum* on *C. elongatus* on farmed salmon together with *L. salmonis* suggests that worms may be transferred from *C. elongatus* to *L. salmonis* probably by contact. Both copepod species move actively over the fish surface as preadults. *U. caligorum* has not been seen on copepods on captured wild salmon in this study and, furthermore, *C. elongatus* has seldom been captured from wild salmon in Irish waters. Infested free-swimming adult *C. elongatus* have been captured from the plankton during the winter in the Celtic Sea (Minchin 1991), indicating that transfers between fish may indeed be possible.

It is not known how worms remain attached to their hosts during moults. Nevertheless worms have been found attached to late chalimus, all preadult and adult stages. Pairing of copepods, which frequently occurs on the head region of salmon, may be the time when many transfers take place. This supposition is supported by the following observations:

1. Immature worms have been found on females with no evidence to suggest they hatched on the females, because of the absence of eggs and egg cases.
2. Immature worms were predominantly found around the lateral margins of the cephalothoracic shield, a position which would facilitate transfers to females during pairing.
3. The greater proportion of worms found ventrally have been on male *L. salmonis*; this could facilitate transfers during pairing.

ACKNOWLEDGEMENTS

We would like to thank those members of the Irish Salmon Growers Association who enabled and assisted us in sampling.

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Incidence of ciliate epibionts on *Lepeophtheirus salmonis* from salmon in Japan and Scotland: a scanning electron microscopic study

Karen A.Gresty and Alan Warren

ABSTRACT

The ciliate *Epistylis* Ehrenberg, 1830 was found on the carapace fringe and ventral surface of adult sea lice (*Lepeophtheirus salmonis* Krøyer, 1837) removed from Japanese wild chum salmon (*Oncorhynchus keta* Walbaum). Sea lice from Scottish farmed Atlantic salmon (*Salmo salar* L.) also possessed attached ciliates, belonging to the genera of *Trochilioides* Kahl 1931 and *Licinophora* Claparède, 1867. The incidence of attached ciliates was over 70% on the Japanese lice but below 5% on the Scottish lice. This difference may be attributable to the periodic chemical treatment of Scottish farmed salmon which removes the adult lice, thus reducing the time available for a ciliate population to become established on a lice host, and may also directly affect the ciliates.

INTRODUCTION

Ciliate protozoa attach to a wide range of fish and Crustacea of commercial importance (Lom 1973, Esch *et al.* 1976, Hazen *et al.* 1978, Vogelbein and Thune 1988, Conroy *et al.* 1989). Non-commercial organisms have also been examined, such as amphipods (Fenchel 1965b) and marine copepods (Turner *et al.* 1979, Nagasawa 1986, 1988, Stone and Bruno 1989). These ciliate/host relationships have been variously described as phoretic (Nagasawa 1988) where the host merely carries the epibiont; as non-parasitic or ectocommensal (Lom 1973, Hazen *et al.* 1978, Vogelbein and Thune 1988, Stone and Bruno 1989) where the epibiont benefits without damaging the host, for example by receiving fresh oxygenated water or extra nutrients; or finally, as potentially harmful to the individual or

host population (Esch *et al.* 1976, Overstreet and Howse 1977, Turner *et al.* 1979, Harry 1980).

The economic threat posed to the salmon-farming industry by sea lice of the genera *Lepeophtheirus* and *Caligus* has led to a great deal of research into control methods and general biology of sea lice. Fundamental studies on the biology of the Caligidae may lead to new methods for their biological control, and with this in mind Stone and Bruno (1989) described *Ephelota*, a suctorian ciliate found on farmed sea lice. The present study describes the attached ciliate fauna found on the external surfaces of *Lepeophtheirus salmonis* taken from salmon at two sites. Scanning electron microscopy is used to detail the fine structure of the ciliates and their attachment mechanisms.

MATERIALS AND METHODS

Japanese *Lepeophtheirus salmonis* were collected from wild chum salmon off the coast of Hokkaido and fixed in 10% neutral sea water/formalin. Scottish *L. salmonis* were collected from farmed Atlantic salmon on the west coast of Scotland and fixed in 3% glutaraldehyde in a phosphate/sucrose buffer. Both sets of lice were washed in buffer, post-fixed in 1% aqueous osmium tetroxide for 1 h, and dehydrated in a standard acetone series. They were then critical point dried, mounted on stubs and sputter coated with gold/palladium before being observed in a field emission Hitachi-S800 scanning electron microscope, with an accelerating voltage of 8 kV.

RESULTS

Live specimens were not available for study, therefore all data presented here are from fixed material. The Japanese sea lice were examined and 22 of the 31 adults were found to be infected with a species of peritrich ciliate belonging to the genus *Epistylis*.

Epistylis

Epistylis sp. was found mainly on the lower fringe of the carapace and on the ventral surface of the lice, particularly on the second and third legs. Specimens were also present around the oral region and on the antennae. The epistylid was found individually (Fig. 1A), in pairs (Fig. 1B) and as colonies (Fig. 1C), representing different stages of maturity of the ciliate. In some instances, the colonies comprised over 40 individuals, or zooids, in a tight cluster.

Zooids were campanulate to cylindrical in shape and about 30 μm wide (Fig. 1A–C). Examples of contracted, partially contracted and uncontracted individuals were observed (Fig. 1C). In contracted zooids, the cilia were withdrawn into the peristome and enclosed by the peristomial lip. In uncontracted zooids, the peristomial lip was open and the cilia extended (Fig. 1B). The zooids were attached to the host cuticle via a longitudinally striated, segmented stalk which flared at its proximal end into a basal disc about 30 μm in diameter (Fig. 1D).

Twenty-five Scottish sea lice were examined but only one specimen had attached ciliates. The genera found were the spirotrich *Licnophora* and the cyrtophorine *Trochiloides*. These occurred in close association with each other around the genital

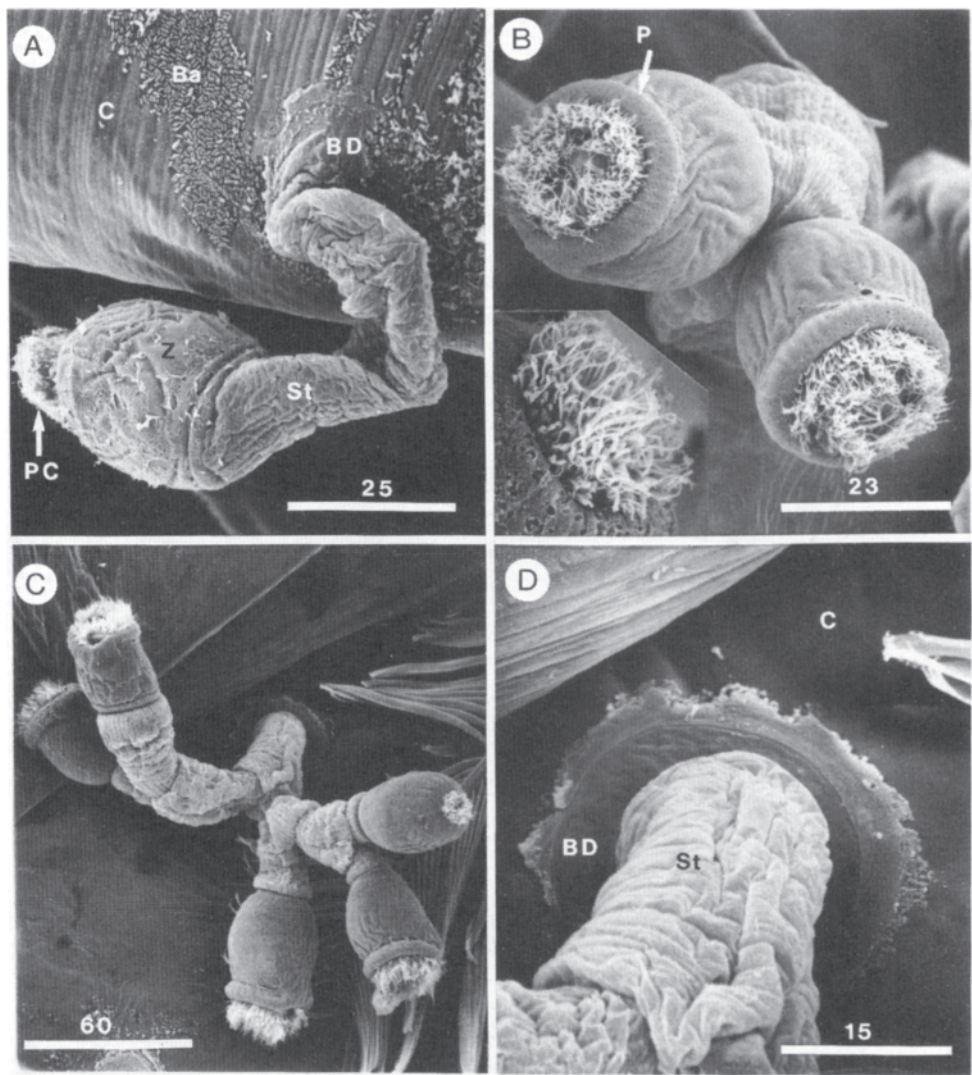


Fig. 1. Japanese sea lice (all scale bars in micrometres). (A) A single attached individual of *Epistylis*, with bacteria around the base of the stalk. (B) Two zooids of *Epistylis*, displaying peristomial ciliature extending from peristome (higher magnification inset). (C) A colony of five *Epistylis* zooids in various states of contraction. (D) *Epistylis* region of attachment showing stalk flared into basal disc on top of host cuticle. Abbreviations: AZM, adoral zone membranelles; Ba, bacteria; BD, basal disc; C, cuticle (of host); CR, ciliary ring; GA, genital aperture; L, *Licnophora*; P, peristome; PC, peristomial ciliature; Po, podite; St, stalk; SC, somatic ciliature; T, *Trochilioides*.

apertures and on the ventral side of the genital region and abdomen (Fig. 2A,B). The host cuticle in this region was also covered by a mesh of bacteria and fungal hyphae, occasionally with other particles (5–10 μm) trapped within (Fig. 2B,D, arrowheads).

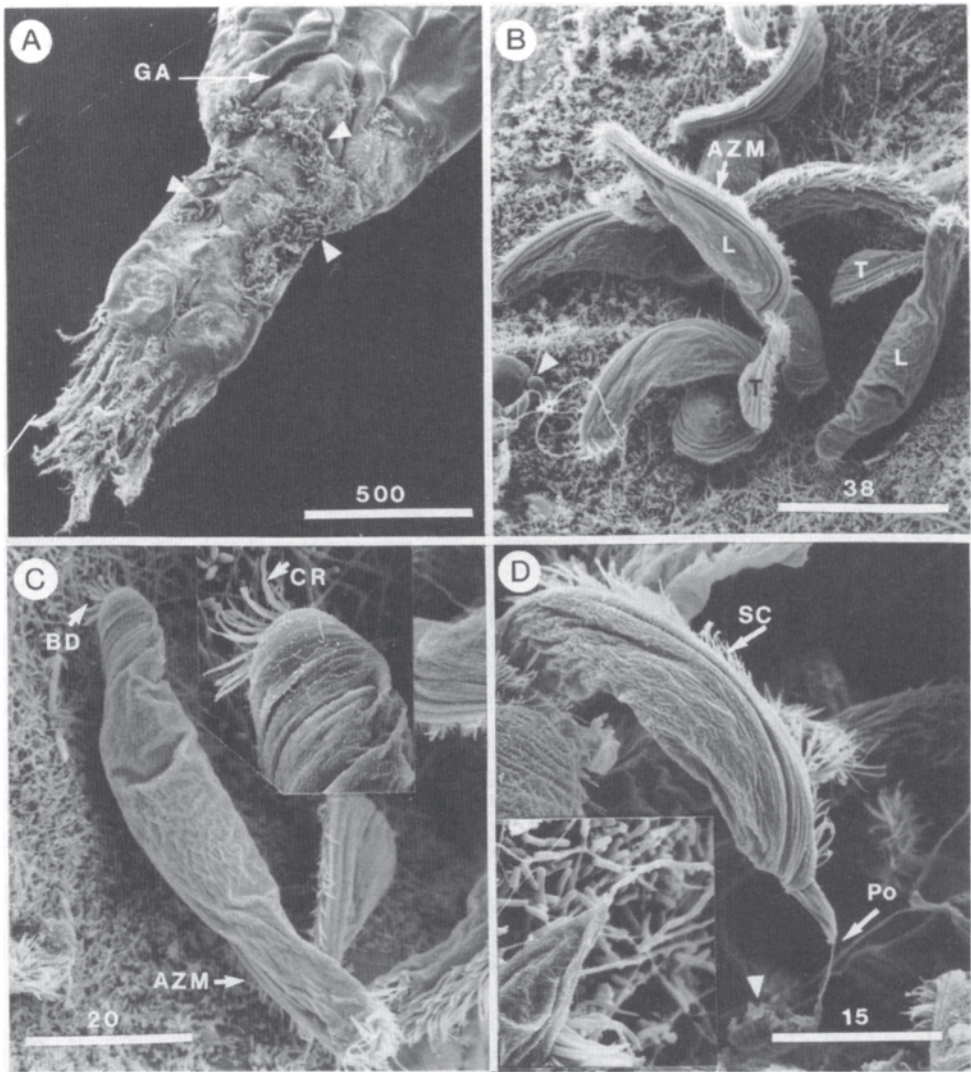


Fig. 2. Scottish sea lice (all scale bars in micrometres). (A) Ciliates (arrowheads) attached to abdomen and around genital aperture of a male sea louse host. (B) *Licinophora* and *Trochilioides* individuals on a layer of bacteria and fungal hyphae, with other particles (arrowhead). (C) A single *Licinophora* specimen, with the attachment area at higher magnification (inset). (D) An individual *Trochilioides* attached to the fungal mesh with other particles (arrowhead) present. Attachment podite shown at higher magnification (inset).

Licinophora

Licinophora sp. was about 70 μm long and 12 μm wide, with a conspicuous adoral zone of membranelles (AZM) winding clockwise around the oral disc (Fig. 2A,B) towards the sunken cytostome. At the posterior end of the body was the basal disc, an organelle of attachment comprising a ventral palette, a flexible flange or vellum and

at least one ciliary ring (Fig. 2C, inset). The basal disc anchored the ciliate to the bacterial/fungal mesh, while the rest of the body was unattached.

Trochilioides

Trochilioides sp. was about 55 μm long and 12 μm wide and with a curved appearance (Fig. 2D). The somatic ciliature was restricted to the ventral surface. The organism was attached to the substratum by means of a cytoplasmic spine or podite (Fig. 2D, inset). The podite extended into the bacterial/fungal mesh, allowing the rest of the organism to hang or rotate free of the host cuticle.

DISCUSSION

Nagasawa (1986, 1988) reported instances of peritrich ciliates from planktonic copepods (*Centropages abdominalis* and *Acartia tonsa*) in Japanese waters, and Stone and Bruno (1989) reported a suctorian ciliate from sea lice in Scottish waters. In neither case was a representative of the genera *Epistylis*, *Licnophora* or *Trochilioides* observed. Nagasawa (1986) stated that peritrich ciliates do not depend upon copepods for nutrition because they are bacteria feeders. During the present study, colonies of bacteria were observed around the basal area of some epistylids from Japanese lice, although it is unlikely that these were a food source for the ciliate since peritrichs are suspension feeders.

Cyrtophorine ciliates are thought to be mainly bacterial feeders, although some are known to be histophagous (Corliss 1979). Fenchel (1965b), however, noted that two species of *Trochilioides* living as epibionts on *Gammarus* had algal remains in their food vacuoles and concluded that they were probably herbivores. The bacterial/fungal web on the Scottish louse had particles trapped in it, possibly diatoms, which could be a potential food source for *Trochilioides*. The spirotrich *Licnophora* is a suspension feeder (Fenchel 1965a) so it is unlikely that any of the microorganisms associated with the host cuticle served as a food source.

The bacteria and fungal hyphae were probably growing on salmon mucus which adheres to the surface of the sea lice. Ciliates are capable of colonizing fish and attaching to their scales (Lom 1973, Esch *et al.* 1976, Hazen *et al.* 1978), so it is possible that ciliates could be transported from fish to fish by the sea lice. However, the salmon hosts of the sea lice studied in this account were not examined for ciliates. The ciliates did not appear to damage the sea lice host and were probably ectocommensal. It is not known if the fungi observed were potentially pathogenic. However, bacteria are sometimes associated with ectocommensal ciliates and are capable of causing disease to the host (Hazen *et al.* 1978).

The salmon from the Scottish fish farms were periodically subjected to chemical treatments of the pesticide Aquagard (an organophosphate) to remove the mobile lice population. This itself may have had an impact upon the ciliate populations, as organophosphates are known to be toxic to ciliates. Saini and Saxena (1986), for example, reported that the growth of *Tetrahymena pyriformis* is markedly inhibited by organophosphates at concentrations between 1.0 and 5.0 ppm, while a concentration above 5.0 ppm is lethal. In farmed salmon the adult lice were

constantly being removed, to be replaced by developing juveniles. By contrast, salmon from Japanese waters were not subject to this chemical treatment and so this adult population of sea lice could reach a greater age. This age difference amongst the adult lice population may account for the large difference in the incidence of ciliate epibionts between the two localities, as the window for ciliate colonization was much smaller in the Scottish lice population (before treatment removed all these lice).

The incidence of ciliate attachment on the chalimus stages of the louse life cycle is not known. Furthermore, moulting may not necessarily result in the loss of the ciliates by the host. Several authors have noted that ciliate epibionts are able to respond to the moult cycle of their crustacean hosts by producing motile stages that are able to resettle on the new cuticle as it emerges (Walker *et al.* 1986). The nature of the stimulus for the evacuation of the exoskeleton by the epibiotic ciliates during moulting of Crustacea is unknown, although various factors have been suggested, including the leakage of exuvial fluid (Bradbury and Trager 1967), changes in urine composition of the host during premoult (Clamp 1973) and the production of ecdysone (Walker *et al.* 1986). The chalimus stages may act as a reservoir of ciliate epibionts, with adults becoming colonized when they come into contact with the juveniles, for example in mate guarding.

Epistylis and *Trochiloides* are generally regarded as ectocommensals since they are not known to cause deleterious effects to their hosts, either through their feeding habits or mode of attachment (Lom 1973, Hazen *et al.* 1978). In the case of *Epistylis*, Vogelbein and Thune (1988) concluded that any harmful effects to its decapod host are probably limited to a decrease in available respiratory surface area and disruption of normal water flow patterns. By contrast, the host/ciliate relationship for *Licnophora* appears to be more variable. Balamuth (1942) and Fenchel (1965b) described *Licnophora* as ectocommensal for its hosts, the sea cucumber *Stichopus californicus* and the mollusc *Thyasira sarsi*. On the other hand, Harry (1980) described *Licnophora auerbachii*, an ectoparasite of the bivalve mollusc *Chlamys opercularis*, as causing mechanical damage to the host's eyes in the form of abrasion and scarring of the eye surface, loss of pigment and surface distortion of the eye. The same author also noted that the presence of large numbers of ciliates on the mantle edge deprives the host of a proportion of its potential food supply.

No evidence of pathogenicity by the ciliates was seen in this study and it would seem likely that the ciliates are harmless commensals. The number of protists known to be associated with Crustacea, particularly decapods, is extensive (Sprague and Couch 1971) and it would appear that the crustacean cuticle is an excellent surface for protists to colonize.

ACKNOWLEDGEMENTS

We would like to thank James Bron (Stirling University) and Jim Treasurer (Marine Harvest International) for help with collection of Scottish lice, and Shigehiko Urawa (Fisheries Agency of Japan, Hokkaido) for collection of the Japanese lice.

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Part II of Pathology

28

The possible role of *Lepeophtheirus salmonis* (Krøyer) in the transmission of infectious salmon anaemia

A.Nylund, C.Wallace and T.Hovland

ABSTRACT

Salmon with wounds caused by *Lepeophtheirus salmonis* have an increased susceptibility to secondary infections. *Aeromonas salmonicida* and infectious salmon anaemia (ISA agent) are believed to be among the pathogens that are secondary invaders. How these pathogens enter the host is not known in detail, but the salmon louse could also function as a vector and a reservoir for these pathogens. The present study shows that *L. salmonis* may transmit the ISA agent from one host to another, and indicates that the louse might be an important vector for this disease. It is suggested that the different delousing strategies should be modified in areas with ISA.

INTRODUCTION

The salmon louse (*Lepeophtheirus salmonis* Krøyer) is a severe pathogen on salmonids in Scottish and Norwegian fish farms (Brandal and Egidius 1979, Wootten *et al.* 1982, Pike 1989). *L. salmonis* feeds on mucus, epithelial cells and blood tissue from the host, causing integumentary wounds which can penetrate deeply into the subcutaneous tissue if present in high numbers. Heavy infestations of salmon lice cause symptoms such as osmoregulatory failure and anaemia (Tully 1991). In addition, wounds caused by *L. salmonis* increase the susceptibility to secondary infections.

There are many fish pathogenic bacteria and viruses that may be secondary invaders; *Aeromonas salmonicida*, *Vibrio salmonicida*, *V. anguillarum*, *Yersinia ruckeri*, IPN virus, and infectious salmon anaemia (ISA agent), to mention a few. How these pathogens invade the host is not known in detail. The salmon lice could function

as a vector and a reservoir for these pathogens (Nylund *et al.* 1991, 1992). *A. salmonicida* has been isolated from the surface of the salmon lice (Horne 1928, Nese 1992) and large amounts of bacteria have been demonstrated both on the surface of the lice and in the gut (Nylund *et al.* 1991, 1992). Mulcahy *et al.* (1990) have isolated infectious haematopoietic necrosis virus (IHNV) from fresh-water leeches (*Piscicola salmositica*) and copepods (*Salmincola* sp.), and they indicated that the leeches could serve as a vector in the spread of IHNV among the spawning adult salmon. Ectoparasites as vectors of viral and bacterial diseases of fish have also been discussed by Ahne (1985), Cusack and Cone (1985, 1986), Cusack *et al.* (1988) and Nylund *et al.* (1991, 1992).

ISA is a disease of farmed Atlantic salmon (*Salmo salar*) in Norway and was recorded for the first time in the late autumn of 1984 (Thorud and Djupvik 1988). It is characterized by severe anaemia and high mortalities. Other symptoms exhibited are exophthalmia, ascites, congestion and enlargement of the liver and spleen, and usually petechiae in the visceral fat. The disease can be transmitted using homogenates of tissue obtained from diseased fish. The infectivity of homogenates is retained after filtration through 100 nm pores, indicating a viral aetiology (Thorud 1991). The infectivity is lost after ether or chloroform treatment of the homogenates, which indicates that the agent contains essential lipids as present in enveloped viruses. The agent causing ISA has not yet been isolated and characterized. This disease was registered in 120 salmon farms in Norway, June 1992, the majority on the west coast of Norway.

The aim of this study is to test experimentally the possibility that *L. salmonis* may function as a vector and a reservoir for the two pathogens *A. salmonicida* and ISA agent.

MATERIALS AND METHODS

L. salmonis specimens were collected from a fish farm on the west coast of Norway. On 28 April 1992, lice (preadults and adults, males and females) were collected with forceps from fish dying from furunculosis (diagnosed by the local veterinarian according to the following procedure: fish with signs of furunculosis were killed and bacterial samples from kidney were inoculated on a blood agar with 2% NaCl and incubated at 22°C for 48 h. The colony morphology was registered. Bacteria from the colonies were further grown on tryptic soy agar (Difco) and Coomassie brilliant blue agar. Also examined was whether the bacteria were catalase and oxidase positive, if they hydrolysed esculin, fermented mannitol and saccharose, and if they produced indole. In addition, biochemical profiles were also determined with API 20E strips.) Two years earlier there had been a serious attack of ISA at the same fish farm, hence this agent could still be present and the clinical signs camouflaged by those of furunculosis, which is more easily diagnosed.

Lice were washed twice in sterile salt water to reduce the amount of bacteria and viruses in the water. Bacterial samples were taken from the dorsal cephalothorax of 24 individuals and inoculated on to two different blood agar plates: (a) containing 2% NaCl and incubated for 6 days at 15°C; and (b) without salt, incubated at 20°C for 6 days. Biochemical profiles were determined with API 20E strips.

Salmon smolts (average weight=114 g, average length=22 cm) in the fresh-water phase, from a farm with no history of disease (licensed by veterinary control as free from furunculosis, bacterial kidney disease, yersiniosis, ISA, whirling disease and without any previous history of infectious disease), were taken into the laboratory and transferred to salt water. They were kept in 0.15 m³ tanks, about 50 specimens in each. The temperature at which they were kept ranged from 8 to 12°C.

In one tank, four lice were manually placed on each of 49 salmon (group I). In addition 50 lice were released into the tank. These lice had adhered to the fish within 1 h of release. Some of the salmon had more than ten lice, but the number decreased, and towards the end of the experiment lice were only present on a few salmon.

The guts of 30 *L. salmonis*, from the fish farm with ISA and furunculosis, were dissected out, homogenized and diluted with phosphate-buffered saline (PBS), pH 7.2, giving a solution of 20 ml. The lice were washed twice for 10 s in 45% ethanol before dissection. Only adult and preadult lice with gut content were chosen for dissection. The homogenate was injected into 48 salmon, 0.2 ml into each. These salmon (here referred to as group II) were kept in a separate tank, isolated from the others in the experiment. Samples from the homogenate were inoculated onto two different blood agar plates: (a) containing 2% NaCl and incubated for 6 days at 15°C; and (b) without salt, incubated at 20°C for 6 days.

A control group consisting of 50 salmon were placed in a tank with 100 salmon lice cohabitants (group III). The lice were restricted from making direct contact with the fish by being placed in a 200 µm plankton mesh bag. As a result fish were exposed to potentially contaminated water. After 42 days fish from the control group were put through a latent carrier test (LCT) (Røttereng *et al.* 1989). The fish were anaesthetized with benzocaine, 0.2 ml prednisolone acetate (10 mg ml⁻¹), injected intraperitoneally, into every specimen, and the temperature was raised to 16°C. The fish were kept at this temperature for 25 days.

In a transmission experiment, blood from moribund salmon in groups I and II was used to challenge another group (group IV) of Atlantic salmon (mean weight about 125 g). The following procedure was used in this experiment. Heparinized blood from three specimens from each group was mixed and gave a total volume of 4.5 ml. The blood was cold centrifuged (2000 rpm for 10 min) to separate the blood cells from the plasma. The blood cells (1.2 ml) were diluted with 6.0 ml EMEM (Eagle modification of minimum essential medium) and sonicated in an Ultra-Turrax 25 for 10 s. The solution was then cold centrifuged at 3700 rpm for 10 min. The supernatant (3.3 ml) and the blood plasma were passed through a 0.2 µm pore-sized sterile filter. They were mixed and kept on ice for 30 min prior to injection into the fish. The fish were anaesthetized with benzocaine and 0.2 ml was injected intraperitoneally into each specimen.

The following measurements were made from fish developing disease, moribund fish and dead fish: weight and length, registration of clinical signs of disease, histological preparations, IPNV test (EEC Directive 1989) and blood samples (haematocrit and blood smear). Each fish was also examined bacteriologically by inoculation of kidney and liver tissue on blood agar plates incubated at 20°C for 6 days, and on blood agar plates containing 2% NaCl incubated for 6 days at 15°C. Biochemical profiles were determined with API 20E strips. The gut contents of the fish in group I were examined and the number of lice on the surface counted.

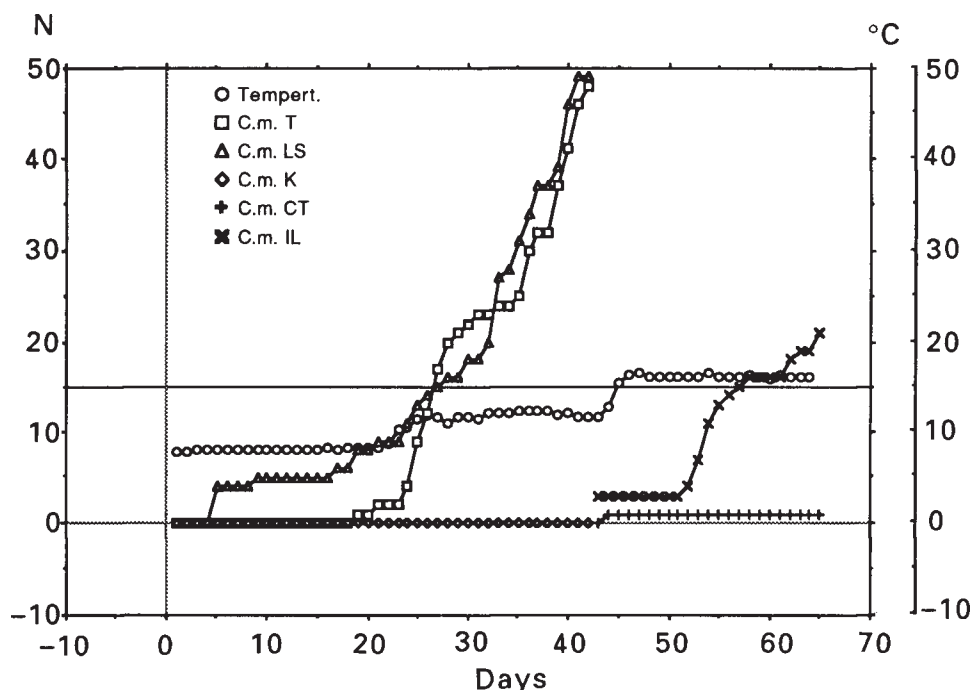


Fig. 1. Cumulative mortality in the different groups and the temperature during the experiment. C.m. T, cumulative mortality in the tank with salmon injected with gut homogenate from *L. salmonis* (group II); C.m. LS, cumulative mortality in the tank with lice on the salmon (group I); C.m. K, cumulative mortality in the control tank (group III); C.m. IL, cumulative mortality in the tank with salmon injected with sterile filtrated blood from salmon in groups I and II; C.m. CT, cumulative mortality in the tank with salmon injected with prednisolone acetate (10 mg ml^{-1}); Tempert., temperature in the tanks; N, number of dead fish; °C, degrees Celsius.

The work was carried out at the Department of Fisheries and Marine Biology, University of Bergen, and at the Industrial Laboratory, Bergen.

RESULTS

More than ten morphologically (growth, colour, haemolysis) different bacterial colony types were found on the blood agar, in the bacterial sample from the dorsal cephalothorax of the lice. The different species were not identified nor were the number of bacteria quantified. The API 20E test showed that none of the isolates had metabolic profiles that resembled any of the known fish pathogens, i.e. *A. salmonicida* was not found. Two different bacterial strains were found in the homogenate from the lice gut, but none of these was among the known fish pathogens in Norwegian fish farms.

The results of the challenge experiments are given in Fig. 1. Mortality was first registered in the group where the lice were placed on the fish (group I). The first four

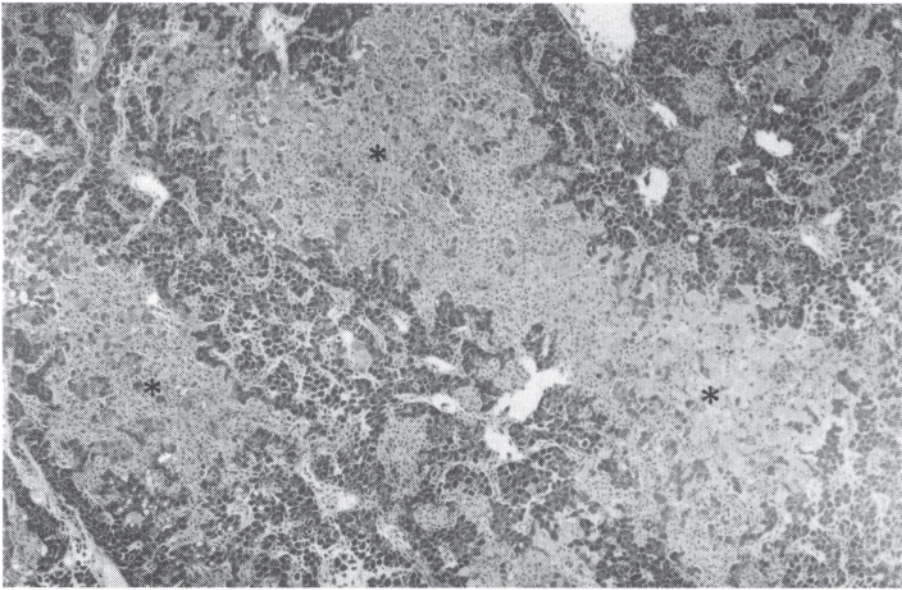


Fig. 2. Section from the liver in one specimen (from group I) that showed signs of ISA (haematocrit=10). The liver was dark and swollen with blood. Note the large grey areas (stars) which are zonal, haemorrhagic, liver necroses, where the dominant cells are erythrocytes and necrotic hepatocytes.

specimens died after 5 days, without any disease signs, supposedly due to handling stress. From day 17 until day 41 there was a fairly constant mortality—one to two specimens every day. All 49 specimens had died in this group after 41 days. The clinical signs were pale gills, haemorrhages in the eye, ascites, congestion of the liver (Fig. 2), enlargement of the spleen, petechiae in the visceral fat, and severe anaemia (haematocrit values as low as 3 were recorded).

The first casualties in group II were noted after day 18, with a peak between days 25 and 30 and another peak between days 36 and 42. All 48 specimens had died in this group after 42 days. The clinical signs were the same as in group I (haematocrit values as low as 9 were recorded).

There were no mortalities in the control group (group III) during the period from day 1 to day 42, i.e. until the start of the latent carrier test (LCT). One specimen died on the first day of the carrier test experiment. This individual did not survive the anaesthetic. The rest survived the LCT and were killed after 25 days. This group showed no sign of disease and the mean haematocrit was 46.5, range 41–53.

In the ISA transmission experiment three specimens died the first day, i.e. they did not survive the anaesthetic. The specimens in this group (group IV) were kept at 16°C and the mortality began after 10 days. All specimens were dead after 23 days. The clinical signs on the dead and moribund fish were the same as observed in groups I and II. Haematocrit values as low as 8 were measured.

No bacteria were isolated from the dead and moribund fish in groups I, II and IV. The IPNV tests were also negative.

DISCUSSION

The results presented in this study indicate that the salmon lice could function as a vector, transmitting the ISA agent. It is also shown that the ISA agent is present in the gut of the lice, but it is not known for how long it can reside inside the lice. Further studies should concentrate on this aspect of the problem.

The mobility of preadults and adults, moving from host to host (personal observation), combined with the lice acting as a reservoir for the ISA agent, could have serious implications for cultured and wild Atlantic salmon. This can also partly explain the spread and maintenance of the disease. A fairly high transfer rate of the preadult and adult males of *L. salmonis* (as has been observed under laboratory conditions) combined with the fact that about four lice per specimen gave high mortalities, suggests that the different delousing strategies should be changed or strongly modified. It is not satisfactory to remove 90% of the preadult and adult lice when a few still remain on the fish. In areas with ISA disease all the lice have to be removed from the fish and killed.

Finally, resources should also be used in the study of the salmon lice as a vector and a reservoir for fish pathogenic bacteria like *A. salmonicida*, *V. salmonicida*, *V. anguillarum* and *Y. ruckeri*. In the present study we were not able to demonstrate that *L. salmonis* can transfer furunculosis. The reason could be that there were too few bacteria present in and on the lice (we were not able to detect *A. salmonicida* in the gut or on the dorsal cephalothorax of the lice examined). However, the method used for isolation of *A. salmonicida* on the surface of the lice is far from optimal. More specific methods (immunomagnetic beads coated with monoclonal antibodies against *A. salmonicida* can retrieve an almost pure culture from very heterogeneous suspensions; Nese 1992) should be used.

ACKNOWLEDGEMENTS

We are greatly indebted to Assistant Professor Heidrun Wergeland who helped us with the bacteriology, and to Espen Raa Nilsen for kind assistance with the virology.

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