A STUDY OF THE BIOLOGY AND BEHAVIOUR OF THE COPEPODID LARVA OF THE SALMON LOUSE *LEPEOPHTHEIRUS SALMONIS* (KRØYER, 1837) (COPEPODA; CALIGIDAE)

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DECLARATION

I hereby declare that this thesis has been compiled by myself and is the result of my own investigations. It has neither been accepted, nor is being submitted for any other degree. All the sources of information have been duly acknowledged.

JAMES BRON

To

My Family

and to

Alison with love

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ABSTRACT

The salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda; Caligidae) is a serious pathogen of wild and farmed salmonids in the marine environment. A study has been carried out to investigate the biology and behaviour of the infective copepodid stage of this parasite.

Copepodids showed a positive phototactic response to a wide spectrum of light wavelengths (300 - 700 nm) with a peak response at 550 nm. Light response was positively correlated to light intensity between 2.4 - 240 lux with immobilisation occurring at 20,000 lux. Copepodids demonstrated a negative geotactic response to increasing pressure. No positive chemotactic or rheotactic behaviour was demonstrated, although copepodids showed a rapid swimming and fast turning response to directed water flow that was considered to be the principal mechanism of host contact. Light and electron microscope studies showed copepodids to be equipped with a wide range of sensory organs including integumental organs, a lensed nauplius eye, an extra-optic photoreceptor (Organ of Gicklhorn), an Organ of Bellonci and antennular elements comprising mechanoreceptive and chemoreceptive components. A new receptor termed the "cauliflower organ" was also described and is suggested to function in host recognition.

Primary attachment was shown to be accomplished through grappling of the host epidermis by the hooked antennae. The maxillipeds were used to further embed the antennae and anterior cephalothoracic shield. Secondary attachment was accomplished through the use of a frontal filament. This was formed internally within the copepodid and may be renewed at each moult. Morphological and histochemical studies demonstrated the frontal filament to be a cuticular extension and showed it to comprise an adhesive "basal plate", a fibrous proteinaceous "stem" and an "external lamina" which was continuous with the cuticle of the anterior cephalothoracic shield. An "axial duct" ran through the stem and terminated at the basal plate. An external "filament duct" protruded from the copepodid rostrum and was continuous with the axial duct. Three major organs, A-, B- and C-glands, were described and their role in filament production discussed. A "ventral filament organ" equating to the "frontal organ" of earlier studies was also present in the copepodid and subsequent stages.

The copepodid alimentary canal comprised a cuticle-lined foregut, a midgut comprising anterior midgut caecum, anterior midgut and posterior midgut and a cuticle-lined hindgut terminating in a slit-like anus. The oral cone conformed to the normal caligid pattern although the labium and labrum were less tightly opposed than those of adults. The strigil was covered by a "labial bar". The midgut comprised three cell types corresponding to the B, R/F and E cells of earlier studies. The posterior midgut comprised only R/F cells. Faeces were enclosed in a

peritrophic membrane. A number of glandular systems were present which were associated with the oral cone. These were termed "mucoid glands" "proximal gland complex" "labial gland" and "distal labral organ" respectively. No glands were observed to interface to the fore- mid- or hindgut.

The free-living copepodid is believed to be lecithotrophic and was demonstrated to carry large vesicles within the epithelial cells of the midgut which are believed to comprise lipid reserves. The attached copepodid was seen to feed on mucus, epidermal / dermal material and other available host components. Host pathology was limited and comprised principally mechanical damage and tissue compression resulting from attachment and feeding. Host tissue response was limited to an increase in mucous cells and occasional influx of eosinophilic granular cells.

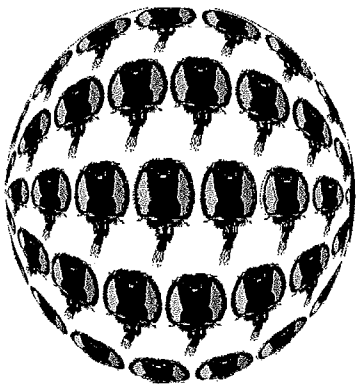
Maximum survival of copepodids under optimal conditions *in vitro* was 23 days following moulting from the second nauplius stage. Survival was not increased by provision of fibrous substrates. Experimental host infections demonstrated that copepodids were host specific and failed to attach to non-salmonid species. Copepodids were demonstrated to infect both marine and freshwater host stages. In experimental infections, larvae were attached principally to the fins and gills. Farmed salmon showed attachment mainly to fins. Spatial distribution was suggested to be principally the consequence of local hydrodynamics. The negative correlation of relative density of infection with size in smolts and positive correlation in larger farmed fish was considered to result principally from differences in swimming-speed and surface area. Disadvantaged, mature and diseased hosts showed higher infection levels. Overdispersal of parasites was considered to be principally the result of host heterogeneity in experimental and farm infections. Attempts to determine the spatial and numerical distribution of copepodids in the wild were unsuccessful and this was suggested to result from the temporally discrete "waves" of infection seen on farmed fish.

The results of this study were discussed with reference to studies of other parasitic and free-living copepod species and suggestions were made as to the implications of these findings for aquaculture.

"To the lice Salar was the earth, a benevolent and inanimate cosmos which yielded nourishment when cultivated and stimulated with chemical injections. Their earth supported them; they knew the pleasures of feeding and sleeping, and the greater joy of perilous love-seeking and satisfaction. For the search for love involved a slow crawl from scale to scale, while their earth was liable to flex into swift movement and the water strike them violently; their bodies were armoured. Salar carried seventeen sea-lice on his body. Most of them were females which had been successfully sought by the smaller males, since each of the females carried twin strings of eggs. Some male lice had lost their grip during the struggle for courtship, and had died of starvation near the Azores."

Henry Williamson, "Salar the Salmon" (1935, p 36)

CHAPTER 1
INTRODUCTION



CHAPTER 1 - INTRODUCTION

1.1 Background

Lepeophtheirus salmonis (Krøyer, 1837) is one of the longest recognised and most familiar of parasitic marine copepods and is also the parasite deemed most inimical to present efforts to culture salmonids in the northern hemisphere.

The generic name of *L. salmonis* comes from the Greek λεπὸς, a scab and φθέιρ, a louse. The genus was established in 1832 by Von Nordmann (Wilson 1905) and the name refers to the flattening of the body and ectoparasitic lifestyle generally displayed by members of the family Caligidae. Its specific name relates to the fact that *L. salmonis* is found almost exclusively on salmonids (Kabata 1979) and it was initially assigned the name *Caligus salmonis* by Krøyer in 1837 (Heegaard 1947) or 1838 (Kabata 1979). The full present-day classification of *L. salmonis* is given below:

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Crustacea (n.b. or Phylum (Manton 1977))
Subclass:	Copepoda Milne Edwards, 1840
Superorder:	Podoplea Giesbrecht, 1882
Order:	Siphonostomatoida Thorell, 1859
Family:	Caligidae Burmeister, 1835
Genus:	*Lepeophtheirus* von Nordmann, 1832
Species:	*salmonis* (Krøyer, 1837)

L. salmonis has a circumpolar distribution in the northern hemisphere and is particularly associated with the marine phase of salmonids of the genera *Oncorhynchus*, *Salmo* and *Salvelinus* (Kabata 1979, 1988). It has been suggested by Kabata that it was included in the composite species *Monoculus* and *Binoculus piscinus* by Linnaeus and Fabricius in the eighteenth century and Heegaard (1947) has suggested that *L. salmonis* is the species referred to by Linnaeus in 1761 in his Fauna Suecica as *Pediculus farionis* since the described species was found on Norwegian salmon. Berland (pers. comm.) further suggests that it is an adult female of this species that was described by Strøm in 1770. Its large size of up to 18.2 mm (Kabata *op. cit.*), obvious colouring and specificity for salmonids undoubtedly mean, however, that it would have been familiar to self-sustaining coastal and riverside communities well before this date as

salmonids have often been a common and important staple food in the past (Davidson 1980). Such was their earlier abundance that in Stirling, Scotland for instance, a statute required that servants and apprentices should not be compelled to eat salmon more than three times a week (Netboy 1980 citing Richard Franck ~1690).

Not only is *L. salmonis* one of the earliest described species of copepod but it has also earned the distinction of being one of the few parasitic species of any group that have been looked upon favourably by humanity (or elements of it). By reason of its association with salmon, which have come, in more recent times, to be regarded as a prestigious and valuable food and sport species (hence the term "King of Fish" for *Salmo salar* L.), *L. salmonis* has also gained recognition. This has occurred because the decline in condition that accompanies maturity in migratory salmonids (Jones 1972, Richards 1980) means that their appearance, stamina and eating qualities are at their best when captured soon after entering freshwater. For this reason, *L. salmonis*, which is intolerant of freshwater (Calderwood 1905, Ashby 1951, Berger 1970, Hahnenkamp & Fyhn 1985, McLean, Smith & Wilson 1990) has long been used as an indicator of how long a salmon has been in a river. Hutton (1923) notes a scheme of events whereby the egg-strings, commonly known as "tails" or "streamers", are lost after 48 hrs and are followed in succession by adult females and finally adult males. The familiarity resulting from this long association with humanity means that *L. salmonis* is one of the rare copepods to have earned a colloquial name ie "salmon louse" or, for caligids generally, "sea-louse". This positive image has continued in the public eye up to the present day and is still evident in the river reports of angling magazines e.g. Salmon, Trout & Sea-Trout (anon. 1991):

> "Maryculter saw two fine, fresh, sea-liced salmon grassed..."

> "On February 5, Peter Willis landed the first clean salmon from the Bengie Pool, a fresh eight-pounder which had lost its lice."

The following excerpt from Mills (pg 53, 1980) underlines this common perception of *L. salmonis*:

> "...its presence on a salmon is an indication of freshness from the sea, and an angler, when referring to the fish he has caught, is quick to remark on the presence of sea lice. It is almost a mark of salmon respectability - like the bloom on a plum"

More recently, comments as to the judging of the freshness of a salmon by its sea lice and the presence or absence of "tails" / "streamers" on them, were made by the Scottish cookery writer Lady Maclean in a dialogue with Keith Floyd on the BBC television programme "Floyd on Fish" (BBC, 1988).

This popular apprehension of *L. salmonis* has, furthermore, allowed it to cross the gap from fact into fiction, a particularly rare feat for an aquatic parasite, such that it is portrayed in the novel "Salar the salmon" (Williamson 1935) which presents a mixture of factual observation and anthropomorphism (see quotation on frontispiece). The poem "The Fish" (Bishop 1983) may also refer to *L. salmonis* in its reference to "sea-lice" although these could be another copepod species.

With such a wealth of public interest, one might perhaps expect a similar scientific interest. As can be seen from Figure 1.1, however, despite occasional publications, interest in *L. salmonis* (as judged subjectively by an approximate estimate of published papers figuring *L. salmonis* as an important or principal component) has only increased measurably since 1971. In the period between 1971-1993, however, interest has increased enormously. The reason for this apparent surge in interest is illustrated in Figure 1.2 which shows the growth of the U.K. farmed salmon industry since 1979 and the accompanying rise in publications[1]. This clearly suggests that research on *L. salmonis* has grown with the production of farmed salmon.

With any type of intensive monoculture, the concentration of large numbers of hosts in a restricted environment is likely to increase the probability of epizootics occurring. If the common assumption, that susceptibility to parasitism increases with host density and the degree of host spatial aggregation is true, as supported by the experiments of Blower & Roughgarden (1989), then the general management of marine fish farms is likely to lead to parasite epizootics. This situation has been exacerbated by the fact that the drive for profit in a burgeoning market has inevitably led to an increase in stocking-density, turnover and area of site coverage.

Table 1.1 shows that the dramatic growth of the aquaculture industry on a worldwide basis has seen a concurrent increase in reports of caligid epizootics on cultured species and these, have in turn, been accompanied by reports of research into caligid biology and into techniques for their control and management. The widespread nature of the caligid problem results from the fact that the Caligidae have a worldwide distribution and that some species e.g. *Caligus elongatus* Nordmann, display a wide range of potential hosts (Kabata 1979). Furthermore, fish species chosen for aquaculture are frequently of local provenance e.g. *S. salar* such that their natural parasites are already present in the farming environment. This said, however, the exposure of

[1]It should be noted that the growth in salmon culture seen in the U.K. reflects a worldwide phenomenon.

Figure 1.1 Approximate number of publications figuring *L. salmonis* as a major component this century

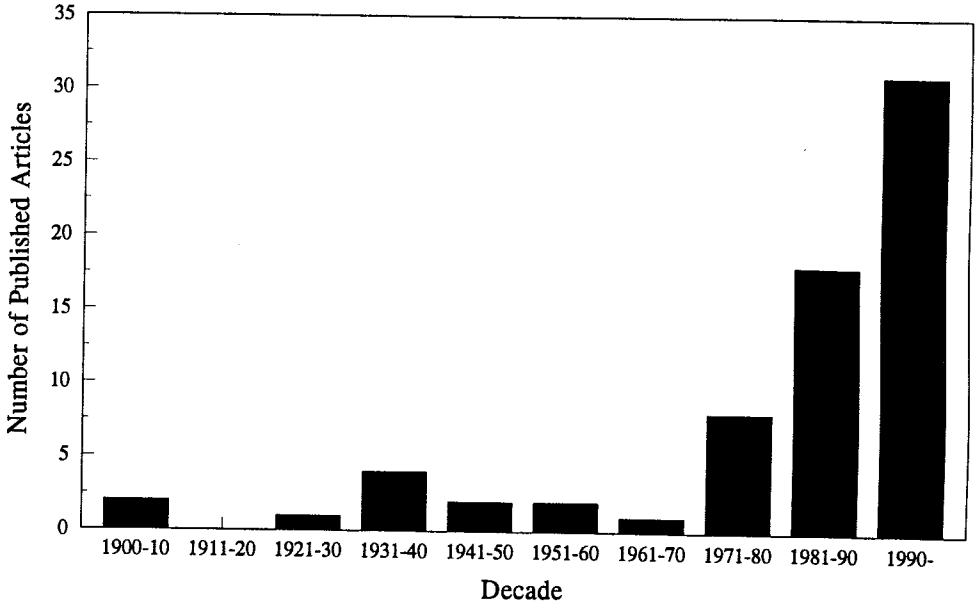


Figure 1.2 Estimated production of farmed salmon in the United Kingdom (Tonnes) graphed against approximate number of articles published on *L. salmonis*

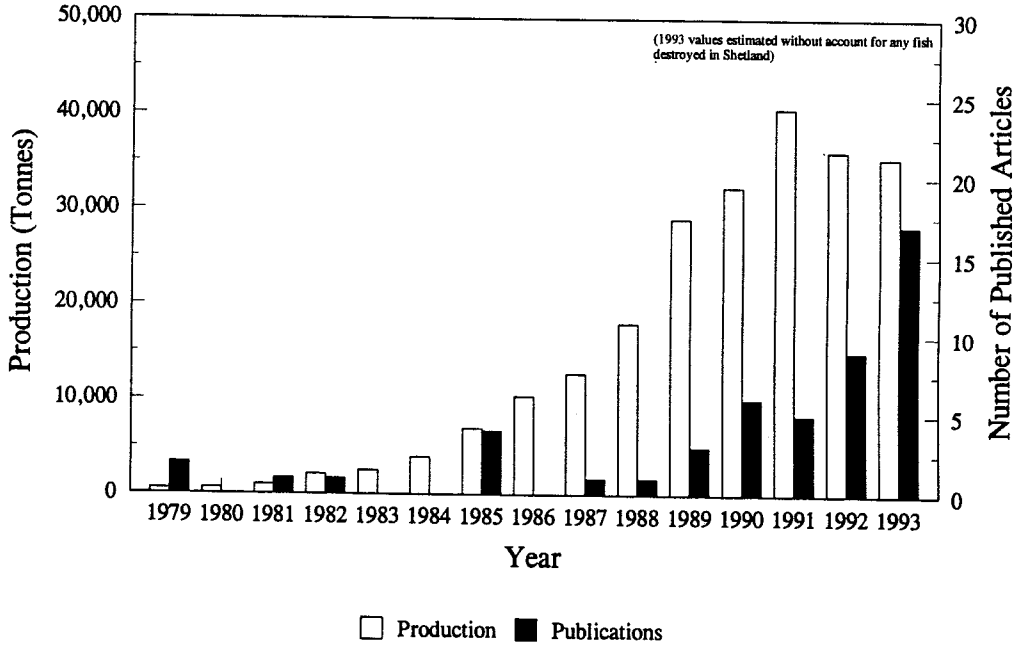


Table 1.1 Chronological listing of published reports of infestations of cultured species by caligid copepods.

Species	Host	Source (chronological order)
C. amplifurcus (= longipedis)	Carangidae	Kubota & Takakuwa 1963
C. orientalis Gusev	Tilapia	Hwa 1965
C. spinosus Yamaguti	Yellowtail	Fujita, Yoda & Ugajin 1968
C. spinosus	Yellowtail	Izawa 1969
L. salmonis (Krøyer)	Salmonidae	Johannessen 1974
C. pageti Russell	Mugilidae	Paperna & Lehav 1974
L. salmonis	Salmonidae	Hastein & Bergsjo 1976
L. salmonis	Salmonidae	Brandal & Egidius 1977
L. salmonis	Salmonidae	Wootten, Smith & Needham 1977
Caligus sp.	Salmonidae	Lundborg & Ljungberg 1977
L. salmonis	Salmonidae	Brandal & Egidius 1979
L. salmonis	Salmonidae	Rae 1979
C. patulus	Milkfish	Jones 1980
C. minimus (Otto)	Percidae	Paperna 1980
L. salmonis	Salmonidae	Chamberlain 1981
L. salmonis *C. elongatus* Nordmann	Salmonidae	Wootten, Smith & Needham 1982
C. teres (Reyes & Bravo)	Salmonidae	Reyes & Bravo 1983
C. epidemicus Hewitt	Prawns	Ruangpan & Kabata 1984
L. salmonis *C. elongatus*	Salmonidae	Wootten 1985
Caligus sp.	Grouper	Leong & Wong 1988
L. salmonis	Salmonidae	Bjordal 1988
C. longicaudatus Brady	Salmonidae	Jones 1988
L. salmonis *C. elongatus*	Salmonidae	Tully 1989
L. salmonis *C. elongatus*	Salmonidae	Hogans & Trudeau 1989
C. elongatus	Red Drum	Landsberg, Vemeer, Richards & Perry 1991
L. salmonis *C. elongatus*	Salmonidae	Richard 1991
L. cuneifer Kabata *C. clemensi* Parker & Margolis	Salmonidae	Johnson & Albright 1991c
L. salmonis	Salmonidae	Johnson & Albright 1991a
C. orientalis	Salmonidae	Urawa & Kato 1991
L. salmonis	Salmonidae	Jaworski & Holm 1992
L. salmonis	Salmonidae	Tully 1992
C. longipedis Basset-Smith	Carangidae	Ogawa 1992
C. epidemicus	Tilapidae	Lin & Ho 1993

non-endemic species to new parasite faunas may lead to even more serious problems as newly introduced hosts will not have co-evolved with endemic parasites to reach the equilibrium required for the long-term maintenance of such a relationship.

Sea lice (comprising for British aquacultural purposes *L. salmonis* and *C. elongatus*) are now the most economically important disease problem for world salmon mariculture. The annual survey of Scottish fish farms for 1992 (SOAFD 1993), has for instance, suggested that "the combination of the bacterial disease furunculosis[2] and lice infestation are the major causes of (salmon) losses". In the worst instances, farms can lose their entire stock through sea lice infestation as reported by Cusack & Johnson (1988)

Sea lice impinge on a number of areas of aquacultural economics. Because of their severe clinical pathology, badly infested fish may be demoted from "Class 1" to "Class 2" as a result of spoiled appearance (Marine Harvest International) and may even require removal of the heads to make them marketable (Taylor 1987). In addition, heavy infestations may lead to loss of appetite, vulnerability to other diseases and consequent loss of growth. Loss of growth also follows from treatment stress and the need to suspend feeding prior to treatment.

In terms of outlay, the cost of the treatment is, in itself, substantial. This results both directly from the cost of pesticides and indirectly from the cost in man-hours lost in administering treatments and preparing for them. In 1988 it was estimated that most salmon farms in Norway and Scotland were being treated for sea lice (Cusack & Johnson 1988). Additional costs accrue from the need for a withdrawal period prior to marketing and the need for safety management resulting from the often high toxicity of chemotherapeutants used.

Because the salmon farming industry in Scotland and elsewhere constitutes a significant part of some local economies (Shearer 1992, SOAFD 1993), the well-being of the industry has assumed national importance. Because of this fact and the contention that fish-farms may be detrimental to the environment, sea lice have incidentally become prominent in the media. Such prominence is perhaps best illustrated by the presence of sea lice on two nationally networked television programmes, the first of which, "The Price of Salmon", was part of a documentary series (Fragile Earth, C4, 1991) and the second of which, "Taggart", was a crime thriller (ITV, 1992). The influential nature of the salmon-fishing lobby, which contains amongst its proponents both the landowners and companies with salmon beats and the anglers who fish them, has ultimately led to the question of sea-lice, their treatment and the effect of fish-farms on local salmonid stocks being introduced for debate in the House of Lords. The latter clearly provides a measure of the status of *L. salmonis*, under private and public scrutiny, at the present time.

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[2]Causative agent *Aeromonas salmonicida* (Lehmann & Neumann)

The history, economic drawbacks and notoriety of *L. salmonis* notwithstanding, very little is actually known of the detailed biology of this species, most effort until recently having concentrated principally on taxonomic concerns. From the above, the need for studies on the basic biology of *L. salmonis* is self-evident.

As a prelude to a description of the objectives of the present study, the following section will attempt to provide an outline of what is known about certain aspects of the biology of *L. salmonis* and related species, although more detailed analyses of the relevant literature will be found in the introductions and discussions of the appropriate chapters.

1.2 Biology

1.2.1. Life-cycle

The life-cycle of *L. salmonis* is divided, in common with most other caligids (Kabata 1972), into 5 phases which comprise in total 10 individual stages separated by moults. The recognised stages consist of two planktonic nauplius stages, one planktonic copepodid stage which is also the infective stage, four chalimus stages characterised by a filament attachment to the host, two unattached preadult stages and one adult stage. Sexual differentiation of the stages has only been reported from the first preadult stage onwards (Johnson & Albright 1991a).

The morphology of the adults has been described by Kabata (1979) and that of the nauplius stages by Johannessen (1975) and Kunz (1985). The copepodid has been roughly described by White (1940a, 1942a) under the term "metanauplius" and by Taylor (1987). The chalimus stages were, until recently, largely neglected, being sketchily described by White (*op. cit*) and Johannessen (*op. cit.*), the latter of whom illustrated them as two stages. More recently all stages have been fully described by Johnson & Albright (1991a). The life-cycle of *L. salmonis* is direct ie reinfection of the host is accomplished without the requirement for an intermediate host.

The life-cycles of a number of other caligid species have also been described and comprise the following: *Caligus centrodonti* Baird (Gurney 1934), *C. curtus* Müller (Heegaard 1947), *L. dissimulatus* Wilson (Lewis 1963), *C. orientalis* Gusev (Hwa 1965), *C. spinosus* Yamaguti (Izawa 1969), *C. clemensi* Parker & Margolis (Kabata 1972), *L. hospitalis* Fraser (Voth 1972), *L. pectoralis* (Müller) (Boxshall 1974d) and *L. kareii* Yamaguti (Lopez 1976). Lin & Ho (1993) have also worked on *C. epidemicus* Hewitt.

1.2.1.1 Eggs

The eggs of caligid copepods are contained within paired egg-sacs extending behind the body of the adult female. In *L. salmonis*, Johannessen (1975) has suggested a figure of between 100 and 500 eggs as the number normally produced in a sac but has reported a maximum of 1445 eggs for a single female. The same author has suggested that lice on wild salmon may produce larger numbers of eggs and this finding is also supported by Kunz (1985). Johnson & Albright (1991b) report a mean egg number of 344.6 with a maximum of 423 and a minimum of 251. Eggs are reported to be laid in repeat batches, with reformation of ovisacs occurring within 24 hrs of hatching of the previous batch (Johannessen 1975). There has been no suggestion that ovulation in *L. salmonis* occurs at any particular time of day, though Voth (1972) found that ovulation and egg-string formation occurred at night in *L. hospitalis*. No report has been made of the maximum number of egg batches that can be produced in a year though Johannessen reports three batches between June and August under laboratory conditions and suggests that even higher numbers could be achieved in the wild.

The egg-development time in *L. salmonis* is highly temperature dependent, being reduced at higher temperatures (Johannessen 1975, Johnson & Albright 1991b). The latter authors found mean development times for *L. salmonis* to be 17.5, 8.6 and 5.5 days at 5, 10 and 15°C respectively. More rapid development may occur for caligids adapted to higher water temperatures as reported by Voth (1972) for *L. hospitalis* who observed a development time of 64 hrs at 20°C.

Under unfavourable conditions such as "increased temperature" or "oxygen depletion" it has been noted that caligid eggs may be aborted through premature release (Heegaard 1947). In *L. salmonis*, low salinities have also been noted to effect the development of eggs and subsequent stages (Johannessen 1975, Wootten, Smith & Needham 1977, Johnson & Albright 1991b) and the latter authors have reported that nauplii only developed at salinities > 20‰ and that copepodid survival was highly variable at 30‰ (0 - 80 %).

With respect to seasonality, White (1942a) considered hatching to occur throughout summer and autumn and Chamberlain (1981) reported a failure of some eggs to hatch in winter (though not all). In the laboratory it seems that egg hatching is not linked to external variables such as light and temperature (Johannessen 1975, Kunz 1985). Shortly before hatching, the embryos within the eggs assume a distinct pigmentation, with highly pigmented areas at proximal and distal ends and in the mid-part of the body (Wilson 1905). This has been used by other workers as a convenient guide allowing isolation of females with eggs on the point of hatching in order to observe the hatching process (Johannessen 1975, Kunz 1985).

When *L. salmonis* have been observed attached to salmon hosts, they have been consistently oriented against the direction of water flow (ie cephalothorax oriented anteriorly) and egg hatching has been seen to be initiated in the distal part of the egg sac in all cases (Johannessen 1975). Without circulation and free from the host, egg sacs are seen to rupture at one or more positions, not necessarily at the distal end (*ibid*). Kunz (1985) obtained similar results in the laboratory for this species but noted that in *C. elongatus*, eggs were released from the distal end even under artificial conditions. Kunz suggested that the reason for initial hatching at the distal end might be that these eggs are the most mature, having been ovulated first. No explanation has, however, been presented for the anomalous results away from the host. Both Johannessen and Kunz report that the excision of the egg-sacs, once they have become pigmented, does not affect subsequent development and hatching. This may be due to a chitin plug which would seem to isolate the sacs, once produced, from the female (Rae pers.comm.) and may hence allow the sacs to maintain their integrity when excised. The loss of the egg sacs has no apparent effect on parental survival (Johannessen 1975) and indeed the sacs may often be deliberately shed by the female, particularly when stressed.

1.2.1.2 Nauplii

The exact mechanism of egg hatching is uncertain. Heegaard (1947) suggested that eggs hatched through increased osmotic pressure leading to rupture. If this is correct, it would agree with proposals for the hatching mechanism of other free-living copepods (Davis 1968, 1981) and of various species of parasitic copepod including the ascidicolid *Haplostomella australiensis* Gotto (Anderson and Rossiter 1969a), the lernaeopodid *Salmincola californiensis* (Dana) (Kabata and Cousens 1973) and the caligoid *Dissonus nudiventris* Kabata (Anderson and Rossiter 1969b). Lewis (1963) observing hatching in *L. dissimulatus* found that the nauplius apparently worked its way out through "sporadic violent movements of its appendages", subsequently resting and swelling before commencing swimming. Kunz (1985) was able to find no evidence of movement within the egg for *L. salmonis* and suggested the eggs to be released from the egg-sac before hatching, whilst Wilson (1905) was, like Lewis, of the opinion that larval movement released the nauplii. Whether hatching occurs or not depends at least in part upon the temperature of acclimation of the female. Regarding this, Johannessen (1975) reported that whilst acclimation temperatures of 9°C gave successful nauplius development only between 8 and 11°C, acclimation at 11.5°C prior to and during the egg-bearing period, gave hatching up to 22°C. Once hatching in *L. salmonis* was initiated, all eggs were reported to have hatched within a period of 40hrs and most within 5-10hrs. More recently, Johnson & Albright (1991b) found hatching times for *L. salmonis* nauplii varying between 18 - 65 hrs at 10°C.

Under laboratory conditions at least, mortality of larvae appears to be fairly high, this being reported by Lopez (1976) and Lewis (1963) for *L. kareii* and *L. dissimulatus* respectively (nb. *L. kareii* = *L. dissimulatus*). In the latter case it was found that highest mortality occurred between the first and second naupliar stages and appeared to be due, at least in part, to an inability to complete ecdysis. Voth (1972) found lower mortalities of 5% and 10% for the first two moults respectively in *L. hospitalis*. Because of the difficulties involved in studying such small organisms in the natural environment, nothing is known about mortality in the wild. The mean duration of the NII stage of *L. salmonis* was found to vary between 52hrs at 5°C to 9.2 hrs at 15°C with an average duration from hatching to moulting to the copepodid stage varying between 222.3 hrs at 5°C - 44.8 hrs at 15°C (Johnson & Albright 1991b).

The caligid nauplius, as with the nauplii of other crustacean groups e.g. cirripedes, is principally a dispersal stage and as such, is provided with an integral food supply (ie it is lecithotrophic). Little work has been deliberately carried out to investigate the behaviour of the nauplii, though various observations have been made in passing. Both nauplius stages have been found to swim actively upwards, this movement being punctuated by phases of passive sinking, with swimming reinitiated by contact with the bottom or sides of the holding vessel or by mechanical disturbance of the vessel. With regard to the reinitiation of swimming, it has been proposed that the two appendages situated at the posterior of the nauplius and suggested to be concerned with the balance/positioning of the larva by Wilson (1905) and Lewis (1963) (hence the term "balancers") may in fact be concerned with mechanoreception since they are the first part of the body to make contact with the vessel substrate. This said, however, Gresty (pers. comm.) has found no evidence for innervation of these structures in ultrastructural studies. The nauplii have also been shown to be highly photopositive, at least with respect to white light, moving towards the illuminated zone of the vessel (Lewis 1963 (*L. dissimulatus*), Johannessen 1975, Wootten, Smith & Needham 1982 (*L. salmonis*)). Lewis also found that in the second nauplius, this response decreased with age.

It has been noted by Wootten *et al.* (1982) that whilst there is little sign of behaviour change before the first ecdysis, prior to the second ecdysis, the nauplii tend to stay near the bottom of the vessel and display little activity until the copepodid is free. Lewis (1963) observing *L. dissimulatus*, noted sporadic movement just before moulting with alternate periods of stopping and vigorous movement. The observations of Wootten *et al.* (*op.cit.*) would appear to agree well with the fact that, in making the transition from nauplius to copepodid, a profound change in morphology is involved which would surely incapacitate the pre-ecdysis larva as ecdysis approached. Both authors reported that the exuviae consistently split open in the front dorsal portion to allow exit of the moulted copepodid.

The above observations have all been made under laboratory conditions, so it is difficult to know how far one can extrapolate them to describe the natural situation. Wootten *et al.* (1982) have suggested that the swimming behaviour and photopositive reaction described might serve to bring the larvae into the upper part of the water column where host fish might be contacted. In support of this, Johannessen reported capture of a single wild first nauplius of *L. salmonis* in a plankton haul on a sunny day with surface temperatures of 13.8°C. Clearly, more accurate data needs to be collected on naupliar behaviour before tangible conclusions may be drawn about behaviour in the wild.

1.2.1.3 Copepodids

Once emerged, the caligid copepodid displays the same photopositive behaviour as the preceding nauplius stages, although this may decrease with age as found for *L. dissimulatus* (Lewis 1963). The swimming of the copepodid is more rapid than that of the nauplius and involves longer swimming periods and shorter rest periods (*ibid*) although these features may solely be the result of scaling considerations. Although Johannessen (1975) was unable to obtain copepodids at temperatures below 8°C this result appears anomalous in that wild copepodids and subsequent chalimus larvae have been collected at temperatures of 5-6°C (pers. obs.). The same author found development to be possible up to 22°C. Even when the development to copepodid has been successfully completed, fairly wide disagreement is found as to the unattached survival period of this stage. Wootten *et al.* (1982) found that copepodids of *L. salmonis* were active for only four days when reared at 12°C whilst Johannessen (1975) on the other hand obtained survival up to a maximum of 30 days at 9°C. Johnson & Albright (1991b) have reported survival of the copepodid to be maximal at 25‰ and 10°C with salinity and temperature both having significant effects on maximum survival. In the latter study, maximum survival was found to be 17 days.

Raibaut (1985) suggests that in general, the duration of the copepodid stage of parasitic copepods is relatively short; of the order of one to two days. It may, however, be worth noting that the copepodids of *Lernaeocera branchialis* L., another parasitic copepod, have been observed to survive up to eighteen days (Whitfield, Pilcher, Grant and Riley 1988) and therefore the extended survival time noted for *L. salmonis* may not necessarily be anomalous.

In Johannessen's experiments (1975), it was found that the copepodids apparently attached to the mesh of the container after a period of about 24 hrs whilst attachment to cotton wool took about three days. One must, however, be wary of the term "attachment", since, unless evidence is presented to indicate that deliberate attachment has taken place, there will always be the possibility that the so-called "attachment" is in fact due to accidental entanglement of the

copepodid's antennae and maxillipeds. This would seem particularly likely in cases of settlement on fibrous or reticulated substrates.

Johannessen (1975) attributed attachment of copepodids to fine-meshed cloth in the upper part of his experimental vessel to a combination of positive phototaxis, negative geotaxis, and possibly also a pressure response whose combined effects were presumed to function in keeping the copepodids in surface water proximal to the host. One might also postulate more specific host-finding mechanisms such as rheotaxis (homing in on water currents created by the host), scototaxis (movement towards shadow such as that created by the passing host) and chemotaxis in response to chemicals emanating from the host or previously settled conspecifics.

Evidence suggests that a shadow response **is** found in some parasitic copepods and in particular this has been noted in *S. californiensis* (Kabata & Cousens 1977). A shadow response has also been reported by Fasten (1913) for the fish parasite *Salmincola (= Lernaeopoda) edwardsii* (Dana) and responses to both shadow and mechanical stimuli have since been demonstrated for this species by Poulin, Curtis and Rau (1990). A negative phototactic response (that is, moving away from a light source) has also been generally supposed for several caligid species at the copepodid stage (e.g. *C. rapax* (= *C. elongatus*), *C. bonito* Wilson, *C. curtus*) this being presumed to be associated with a need to be brought into contact with benthic hosts (Wilson 1905, Heegaard 1947).

So far as can be seen in the literature, little effort has been made to establish the presence or absence of a rheotactic response, though it has been suggested that *Salmincola* may home in on the gill currents of its host, leading to high incidence of settlement around the operculi (Kabata & Cousens 1977). Boxshall (1976) has also suggested that such a response may exist in the copepodids of *Lepeophtheirus pectoralis* which would appear to home in on water expelled from a pipette. Such a response would allow the copepodids to home in on the respiratory currents of the host. Unpublished preliminary experiments by Cabral would also seem to indicate that *C. minimus* (Otto) is attracted by currents caused by respiration and body-movements of *Dicentrarchus labrax* (L.) (see Raibaut 1985). Such a response would clearly be advantageous to this species since it parasitizes the buccal cavity of the bass. Further work by Fraile would also seem to indicate a rheotactic response in the same species (*ibid.*).

Johannessen (1975) has reported an attempted artificial infection of five salmon (*S. salar*) smolts with copepodids of *L. salmonis* using 65 larvae in a 250 litre aquarium. Perhaps not surprisingly in view of the numbers of infectious units and the water quantity involved, no infection of the smolts was recorded. Bron, Sommerville, Rae & Jones (1991) and Johnson & Albright (1991a, 1992a,b) have since accomplished successful settlement of *L. salmonis* on *Salmo* and *Oncorhynchus* spp. Examining other caligids, Lopez (1976) using larvae of *L. kareii*, failed to infect host fish (save for one larva dropped directly on the fish) and concluded that there was

no behavioural evidence for larvae seeking out a host. Boxshall (1974c) has had success in settling *L. pectoralis* on plaice (*Pleuronectes platessa* L.) and Taylor (1987) has managed to elicit settlement of *C. elongatus* larvae on Atlantic salmon smolts.

To date, there has been a general lack of evidence presented to support active "homing" of *L. salmonis* on the host although Hogans & Trudeau (1989) have ascribed a chemotactic ability to *C. elongatus*, having observed direct homing on the host from a distance of 10 cm. Chemoreception at close range has also been described by Fasten (1913) who showed that copepodids of *Lernaeopoda edwardsii* apparently responded to the presence in the water of excised gills of their host brook trout (*Salvelinus fontinalis* (Mitchill) but not to the gills of rainbow (*Oncorhynchus mykiss* (Walbaum) or German brown trout (species not specified in paper) and furthermore, only proceeded to settle on the former species. Experiments by Fraile have also suggested that a distance chemosensory response may exist in *C. minimus* which responds to scales and fresh and freeze-dried mucus from the sea bass host species (*D. labrax*) though not to those of mullet (*Mugil cephalus* (L.)) (see Raibaut 1985). Although contact chemoreception has not been investigated, it seems likely, as suggested by Boxshall (1976) for *L. pectoralis*, that it plays a major part in host recognition by the copepodid stage.

Little is known about the act of settlement itself, particularly with respect to *L. salmonis*. It may be that the copepodid's small size allows it to escape the fast current passing across the fish by utilising the comparatively still water of the boundary layer (Butman 1987) that must exist in contact with fish's surface. Rae (pers.comm.) has witnessed apparent settlement of *L. salmonis* larvae and described clasping of the fish with the hooked antennae coupled with a "pneumatic drill" action commonly seen in settling aquatic organisms. Lewis (1963) describing the settlement of *L. dissimulatus* has also stated that the copepodid attached by use of the antennae. Attachment via the antennae is not surprising, as Kabata (1981) has suggested that this may be the most common type of primary attachment for parasitic copepods in general. The same author has observed *L. branchialis* to attempt settlement on beaker sides and random artefacts, and this settling response has also been reported for *L. dissimulatus*, whose copepodids display "prodding behaviour" when coming into contact with beaker sides and bottom (Lewis 1963). Such behaviour may, perhaps, be interpreted as searching for surface cues or for a secure site for attachment.

Whether or not the copepodid is a feeding stage once attached, would seem a matter for debate (see section on feeding) but nevertheless it would seem that moulting to the first chalimus stage occurs soon after settlement regardless of mode of nutrition. Before moulting, the copepodid of *L. dissimulatus*, according to the report of Lewis (1963), attached itself to the host by means of a "frontal filament". After attachment in this fashion, the activity of the copepodid was reported to decrease until renewed at the onset of moulting. Voth (1972) found that *L.*

hospitalis first clung to the host by means of the antennae for 4-6 days (at 15°C) and then attached by filament whilst Paperna (1980) noted in his study of *C. minimus*, that attachment of the copepodid by the frontal filament was apparently only effective on dental and bony substrata. Kabata and Cousens (1973) have suggested that in the lernaeopodid *S. californiensis* there may exist a "burrowing response" causing the parasite to burrow, on contact with the host, until a hard substrate such as a gill filament, fin ray or fibrous connective tissue is reached. Such an explanation might also be invoked to explain the attachment of *L. salmonis* and *C. elongatus* to scales, gill filaments and fin rays (Bron *et al.* 1991, Johnson & Albright 1992a, Pike *et al.* 1993). The interval before moulting to the first chalimus has been reported to be ~7 days for *L. salmonis* (Johnson & Albright 1991b). For *L. pectoralis* at 10°C, Boxshall (1976) on the other hand found copepodids attached by antennae for only 2-3 days before moulting.

1.2.1.4 Chalimus

Between moults, the chalimus is immobile on the fish, being fixed in one spot by the frontal filament at the anterior end. When moulting it may be either mobile, as suggested by Johnson & Albright (1992a) for *L. salmonis* or alternatively may remain fixed as proposed by Boxshall (1976) for *L. pectoralis*.

White (1940a) suggested that the filament was formed from a mucus secretion from the "anterior mucus gland" and found the anterior expanded end to be well embedded in the host. Scott (1901), looking at *L. pectoralis*, thought the filament to be chitinous, but also found it to pass into the tissues of the host. The end of the filament was said by the latter author to be bluntly pointed or flattened into a disc, although Johannessen (1975) shows a bifurcate end for *L. salmonis* in his illustration of the chalimus stage. Scott stated the filament to be secreted from an anterior "cement gland" via a "filament duct". This latter duct passed across the organ termed by Scott a "median frontal sucker". More recent studies of the filament have been carried out by Bron *et al.* (1991) and Johnson & Albright (1992a). The latter authors have suggested the filament of *L. salmonis* to be internally formed. Pike *et al.* (1993) came to the same conclusion concerning the filament of *C. elongatus* although they could not determine the origin of the filament of *L. salmonis*. These studies, in common with earlier ones did not extensively investigate the filament composition although studies by Kabata & Cousens (1977) and Benkirane (1987) have made more concerted attempts with the bullae of several lernaeopodids.

One major unresolved question is that of whether or not the same frontal filament is retained through each moult as proposed by Lewis (1963) for *L. dissimulatus* or whether instead it is resecreted after each moult as reported for *C. curtus* by Heegaard (1947). Gurney (1934) found an increase of filament length in *C. centrodonti* for each moult, a situation confirmed by

the observations of Lewis (1963) though whether this results from complete replacement of the filament, addition of new material or solely a stretching process of some description remains to be demonstrated. Taylor (1987) has suggested that such lengthening of the filament might act to extend the feeding area of the chalimus and hence it may be of importance to the latter.

Chalimus stages of *L. salmonis* have been reported as being predominantly located on the dorsal fin and its base (White 1942a) though they are also found elsewhere on the body surface. High prevalence on fins has also been reported by Taylor (1987) and Johnson & Albright (1992a). The latter authors also found a high proportion of settlement on the gills in experimental infections and suggested that this might be an important site for initial attachment of copepodids. White (1940a) has suggested that the general absence of chalimus stages on the body surface may coincide with large numbers of mobile stages and that therefore grazing of the latter may preclude the presence of the former although this suggestion has yet to be supported by experimental evidence. Johnson & Albright (1992a) suggested that site preference might correspond, at least to some degree, with the nature of the underlying substrate, particularly with regard to host response to the settled parasite.

The chalimus stage is the first generally recognised feeding stage and has been noted to graze the skin of the host in a small arc around the point of attachment (*ibid*, Jones, Sommerville & Bron 1990). In the species of concern here, three moults bring the chalimus to the first preadult stage. Despite the fact that moulting would presumably occur normally whilst attached to the fish, it has been demonstrated that it may still proceed when contact with the fish has been lost. Moulting away from the host has been reported by Gnanamuthu (1948) for a chalimus of *C. savala* Gnanamuthu taken in a tow net and returned to the laboratory and has also been reported for chalimi of *L. dissimulatus* deliberately removed from the fish (Lewis 1963) though in this case a high mortality was noted. A high mortality for attached stages was also noted for *C. elongatus* on farmed salmon, *S. salar* kept in concrete ponds and cages in Scotland though in this instance it was ascribed to falling water temperatures (Wootten *et al.*1977). Although Lewis (1963) has reported for *L. dissimulatus* that prior to moulting to the first preadult stage, the fourth chalimus detaches from its filament and migrates to a new location, Rae (1979) suggests that for *L. salmonis* at least, the frontal filament may be found on rare occasions on all subsequent stages of the parasite and this has been corroborated by the observations of Johnson & Albright (1991a). The production of a frontal filament by mobile stages has also been noted by Anstensrud (1990) for *L. pectoralis* in which description it was suggested to be produced at the site of the organ ("median frontal sucker" of Scott (1901)) described by Kabata (1974b, 1981) and Oldewage & Van As (1989) for *Lepeophtheirus* and *Caligus* spp.. The exact structure and purpose of this latter organ remains, as yet, unresolved.

1.2.1.5 Preadults / Adults

From the pre-adult stage onwards, caligids are mobile upon the host and indeed may even be able to leave one host in search of another (Kabata 1981). Their ability to change hosts is supported to a certain extent by findings of caligids in the plankton. Wootten *et al.* (1982) report findings of both adult and preadult *C. elongatus* as does Taylor (1987), and the former assume that this indicates such an ability. It has also been reported that both male and female caligids have been frequently captured in plankton nets at night and it has been suggested that reduced fish mobility at night might facilitate reinfection (Kabata and Hewitt 1971). The same conclusion about night-time infection was also drawn by Fryer (1966) for the larvae of another parasitic copepod; *Lernaea bagri* Harding

Paperna (1980) has reported that adult *C. minimus* of both sexes can resettle on new hosts. Because of the difficulty of observing lice in the water column, active movement of lice between hosts (both intra- and inter-species) has not been observed in the wild, though it is clear that accidental transfers in e.g. netted fish are commonplace. Bruno & Stone (1990) have recorded movement of *L. salmonis* between hosts under tank conditions. Indirect evidence for movement between hosts might be drawn from reports of the coincidence of increased levels of infection of farmed salmon by *C. elongatus* with inshore movement of wild gadoids such as saithe (*Pollachius virens* (L.)) (Wootten *et al.* 1982). Whether this is indicative of the movement of adults / preadults from saithe to salmon or solely the dispersal of larvae, remains to be seen, although Wootten (pers. comm.) has noted infection by mobile stages without settled larval precursors. Infection of rainbow trout (*O. mykiss*) on a farm in Nova Scotia has also been attributed to parasites originating in wild fish stocks though again, the nature of infection (preadult/adult or larval) is undetermined (Cusack and Johnson 1988)). Should it prove that caligids do indeed leave their hosts, the question remains as to why such a thing should occur since, as Fryer (1966) points out, "it seems highly improbable that a parasite, once settled will leave its host". It may therefore be found that those lice found in the plankton are ones which have been accidentally displaced or which have been forced to leave by some aspect of the host's immune response. Rae (pers.comm.) suggests that it may also result from a need to locate a mate.

Regarding mobility on the fish itself, it has been noted that the adult males and preadults of both sexes are far more mobile than the adult female, at least in *L. salmonis*, with the latter tending to remain in one locality for a greater length of time (Johannessen 1975). The literature also indicates that site-preference on the fish is quite marked and that certain areas of the fish are regularly chosen over others. Johannessen (1975) found that most sexually mature females of *L. salmonis* seemed to prefer the region behind the dorsal fin and that behind the adipose fin.

The regions behind the anal fin and caudal fin was also frequently settled. The males and preadults are more mobile and hence were to be found all over the fish. Håstein & Bergsjö (1976), Kunz (1985) and White (1942a) agree with these findings though Kunz stated that the preadults were to be found principally on and behind the dorsal fin, on the flanks and on the head. The latter also stated that males were rarely to be found in the same areas as the adult females. Margolis (1958) has stated that *L. salmonis* shows a preference for the perianal region but can be found all over the body and on the operculi of wild *Salmo* and *Oncorhynchus* spp.. A single record has been made of settlement on the gills themselves (Wilson 1905) but it would seem that this is a chance occurrence. The site preferences noted are further backed up by reports of areas of greatest lice damage on fish (*inter alia* White 1940b, 1942a, 1942b, Hutton 1923, Rae 1979, Wootten *et al.* 1982).

The reasons for such precise choice of sites are probably diverse, though it would seem that shelter from the current is probably a factor of primary importance, especially for gravid females (Johannessen 1975, Kunz 1985). Such a requirement might be more important to gravid females because of the fragile trailing egg-sacs they carry, these being liable to premature release if irritated or disturbed (Boxshall 1974b (*L. pectoralis*), Hutton 1923 (*L. salmonis*)). Ease of feeding and attachment may be another factor, and it has been suggested by Johannessen (1974) and Kunz (1975) that skin chosen by adults often has smaller scales and is thinner and hence may facilitate easier penetration by the oral cone, although no evidence has been presented to support these assertions. The latter author has also postulated that competitive exclusion may play a part in the partitioning of sites between different ages and sexes though this would only apply where numbers of lice were such as to give limited space in favourable areas. It has also been suggested that the aggregation of lice in certain areas might be attributable to the fact that the initial lesion made by earlier lice may make feeding by later lice easier (*ibid*). Another feature of positioning on the host is the attitude of the lice with respect to their position on the fish. Kabata & Hewitt (1971) and Kunz (1985) have both commented on the tendency for both adult and preadult caligids to take up position facing in the direction of the host's swimming. This observation has also been made by Boxshall (1974a) for a wide variety of copepods inhabiting the body surface and buccal cavity of marine hosts.

From the first preadult stage onwards the sex of the lice in *L. salmonis* is apparent morphologically (Johannessen 1974, Johnson & Albright 1991a). Taylor (1987) found the sex of *C. elongatus* to be distinguishable from chalimus IV. Although Rae (1979) and Wootten *et al.* (1982) were in agreement in stating that adult *L. salmonis* males will copulate with first and especially second preadult females and also with adult females, it is likely in the light of work by Anstensrud (1990) on *L. pectoralis* that copulation does not occur until the moult to adult female occurs and that instead, males "guard" preadults prior to copulation. Kunz (1985) found

the ratio of males attached to preadults I and II and adult females to be 3:15:1 with no apparent seasonal variation. The low number copulating with adults, as similarly noted in *L. pectoralis* by Boxshall (1974b), was suggested to be due to steric hinderance as was the low number associated with first preadults. Alternatively, the latter could be due to lack of maturation of females at this stage. Wilson (1905) expressed the opinion that a single mating probably serves for all egg production since no remating was observed in *L. dissimulatus*. This results from the fact that the sperm is held in two spermatophores which are implanted by the male on the external posterior ventral surface of the female and which may be utilised through seminal receptacles, over a considerable period of time. Eggs are fertilised as they are extruded from the oviducal opening (*ibid*). It has been noted that in at least one species of parasitic copepod, *Lernaea cyprinacea* L., copulation is a necessary requirement for the continued development of the female (Bird 1968) though the presence of apparently unmated adult females would seem to preclude such a situation in caligids.

One important question of reproduction is that of how the males, these being the most mobile of the two sexes, locate and recognise the females for copulation. One possibility is that they might use some sort of pheromonal system[3], of which many have been recognised in marine crustacea. A review of crustacean pheromones has been published by Dunham (1978) and although caligids have not been studied in this respect, evidence exists for the presence of such pheromones in other parasitic copepods e.g. *Lernaea cyprinacea* (Bird 1971) and in other crustacean groups e.g. *Argulus siamensis* (Wilson) (Sundara Bai 1980). They have also been reported in free-living calanoid copepods (Katona 1973, Griffiths and Frost 1976). This possibility clearly requires much greater in-depth study, although it may prove that the host provides a sufficiently restricted habitat for encounters between sexes to occur through a random process aided by the tendency of individuals to congregate in well defined areas as already mentioned.

1.2.2 Feeding

From the literature there is no evidence that direct ingestion occurs in the parasite until the first chalimus stage is reached, although it may be that the copepodid is a trophic stage once fully settled. Nauplius stages are endowed with a reserve of large cells commonly assumed to be "yolk" cells (Lewis 1963 (*L. dissimulatus*), Johannessen 1975, Kunz 1985 (*L. salmonis*)) and believed to provide sustenance for the planktonic and initial settling stages of the life-cycle. This

[3] Pheromones are defined as substances that, when released by one individual, influence the behaviour of other individuals of the same species (Karlson and Lüscher 1959).

apparent lack of feeding does not, however, preclude uptake of amino acids and other essential nutrients via the body surface, although this has not been investigated.

Whether the copepodid actually feeds or not is uncertain although Håstein & Bergsjö (1976) speculate that the copepodid, on settling, is able to feed on mucus and epithelium. Johannessen (1974) similarly speculated that feeding occurs, suggesting mucus and scales as the substrate although neither of these authors observed feeding directly or noted the contents of the alimentary canal. In *Lernaeocera branchialis* (Linnaeus), copepodids were reported to have no communication open between oral cone and midgut and midgut and hindgut (Pedaschenko 1898 cited in Heegaard 1947) although Capart (1948) has subsequently suggested it to be continuous.

Feeding in the chalimus stage has also received comparatively little attention. White (1942a), describing *L. salmonis*, noted that much of the fin surface tended to be eaten away in heavily chalimus-infected fins and this observation is supported by Tully (1992). Brandal (1977) considered that the chalimus stages feed mainly on mucus around the attachment point of the filament giving light-pigmented spots on the host and these spots or ulcers have also been noted by Wootten *et al.* (1977) and Egidius (1985). More recently, Jones *et al.* (1990) have noted more specific feeding damage beneath the chalimus oral cone and the presence of food material in the chalimus alimentary canal.

The exact nature of the diet of preadult and adult *L. salmonis* is uncertain, although in the first instance the diet has been suggested to be principally epidermis (Wootten *et al.* 1982). Wilson (1905) suggested that the adults feed on the blood of the host and this suggestion is supported by Hutton (1923). White (1942a) proposed that a mixture of skin, subcutaneous tissue, blood and mucus was ingested since he found what was apparently skin with melanophores in the gut of adults. He also found that chopped skin was accepted as a food item. Voth (1972) suggested that the principal food of a related species, *L. hospitalis*, was principally mucus, with occasional blood ingestion in the presence of skin lesions. Chamberlain (1981) looking at *L. salmonis* believed mucus, epithelium and scales to be the likely diet.

Johannessen (1975) and Kunz (1985) both noted the presence of red digestive tracts in certain adult *L. salmonis* and proposed blood-feeding in explanation. It was also noted that this condition was most prevalent in adult females but was also found in male adults and preadults of both sexes. Brandal, Egidius & Romslo (1976) also noted red digestive tracts and confirmed the presence of host blood using spectrophotometry to demonstrate coincidence of haemoglobin peaks in parasite gut and host blood samples. These authors also suggested a possible connection between feeding behaviour and developmental stage, since all egg-bearing females in the experimental sample had red digestive tracts. Johnson & Albright (1992a) note that blood has been observed in the guts of all stages feeding on the gills of hosts but is less common in those feeding elsewhere.

The Siphonostomatoida are characterised by the presence of an oral cone comprising the labrum and labium, the latter being derived from the medial fusion of paragnaths (Huys & Boxshall 1991). Kabata (1974a) has described the morphology of the mouth and the mode of feeding in caligids using a combination of S.E.M. and direct observation and concluded that the toothed strigil probably acts as a sliding rasp with which tissue might be scraped up and subsequently transferred to the mouth via the mandibles. The strigil has been reported as missing in the copepodid stage of *L. salmonis* by Johnson & Albright (1991a). Descriptions of the musculature associated with the oral cone have been made by Kabata (1974b) and more recently in a comparative study by Boxshall (1984) which includes *L. pectoralis*. It has also been suggested that external digestive enzymes and anti-coagulants might also be secreted (Wootten *et al.*1982) although to date no evidence to this effect has been presented and Jones *et al.* (1990) have observed to the contrary that damage in the region of the oral cone of chalimus larvae appears to be of mechanical rather than chemical origin.

The alimentary canal of caligid copepods is relatively simple and mirrors that of free-living species as described by Arnaud, Brunet & Mazza (1978) and Sullivan & Bisalputra (1980). A summarized description of the copepod alimentary canal and associated structures, given by Boxshall (1992), indicates that copepod alimentary canals are fairly consistent and generally comprise an ectodermal, cuticle-lined foregut (oesophagus), an endodermal midgut and a cuticle-lined ectodermal hindgut. The midgut is often further divided into anterior midgut caecum, anterior midgut and posterior midgut. Caligid alimentary canals have been described by Scott (1901), Wilson (1905) and Boxshall (1986, 1990) for *L. pectoralis* and Poquet (1980) for *C. minimus*. Sullivan & Bisalputra (1980) also cite an unpublished description of the alimentary canal of *L. dissimulatus* by Lewis (1961). More recently, the ultrastructure of the alimentary canal of adult *L. salmonis* has been described in some detail by Nylund, Økland & Bjørknes (1992) and the structure of the larval alimentary canal has been described by Bron, Sommerville & Rae (1993c).

1.2.3 Pathology

Because their mode of feeding damages fish, sea lice have long been recognised as potentially serious pathogens. In the wild, mortalities due to sea lice would appear to be comparatively rare (or unrecognised), although White has reported mortality of badly infested salmon ascending the Moser river in Canada during the summer of 1939 (White 1940b) and attributed it to a combination of sea-louse damage and high water temperature.

In the case of farmed fish, the problem is greatly exacerbated (as in many other cases of intensive livestock farming) by factors such as stress, poor fish condition, high probability of

infection/reinfection and a large maintained reservoir of infection. Epizootics of *L. salmonis* and *C. elongatus* have hence been reported from a number of sources. These include sites in Scotland (Rae 1979, Wootten et al.1982), Sweden (Lundborg and Ljungberg 1977), Norway (Braaten 1975, Brandal and Egidius 1977) and Japan (Awakura 1980). On occasion, such infestations may lead to heavy fish mortality as reported in Canada by Cusack and Johnson (1988).

Damaged fish are recognisable through grey or red patches, principally on the back and head, caused through skin abrasion by feeding copepods (White 1940b, Wootten *et al.* 1982, Rae 1979) and physical damage caused by attachment (Jonsdottir, Bron, Wootten & Turnbull 1992). On occasion this damage may be so severe as to expose the skull (Plate 1.1) and may even on rare occasions penetrate it as in a case noted by Kabata (1970) for a pink salmon (*Oncorhynchus keta* (Walbaum)) fingerling. In severe cases the epidermis may slough off leaving the reddish subdermal tissue exposed (hence the term "red-heads"). Skin loosening may be attributable to osmotic effects or exacerbation of ultraviolet radiation effects occurring in damaged areas as suggested by McArdle and Bullock (1987). According to White 1940b, sea-lice are probably also to blame for the so-called "white-spot" disease noted by Calderwood (1905) and observed by M'Gonigle (1931) in Canadian salmon.

Histopathologically, damage manifests itself through oedema of the dermis and sub-dermal tissues with haemorrhage around the feeding site and commonly blood seepage between the scales (Håstein and Bergsjö 1976, Brandal and Egidius 1977, Wootten *et al.* 1977, Jonsdottir *et al.* 1992). Lesions attributable to sea lice are liable to infection by secondary pathogens such as bacteria and fungi and it has been suggested that *Vibrio*-like bacteria may spread from ulcers along connective tissue and may become systemic, leading to death by vibriosis. Johnson & Albright (1992a) have reported variation in the histopathological response of different salmonid species to infestation by *L. salmonis*. Whilst coho salmon displayed well-developed epithelial hyperplasias and inflammatory responses to infection, *S. salar* showed a minimal response and chinook salmon were intermediate between the two. Further work using Hydrocortisol implants (Johnson & Albright 1992b) suggested that non-specific host-responses were important in the resistance of coho salmon to *L. salmonis*.

It is generally considered that the most damaging stages are the adults and preadults (e.g. Rae 1979), these being larger and hence capable of greater physical damage. Although Johannessen (1974) has stated that no harm is done at the chalimus stage, White (1942a) has however, as mentioned previously, reported extensive fin damage in fish heavily infected with chalimi and Wootten *et al.* (1977) have reported that the chalimus removes a small area of epidermis around the point of attachment giving a small ulcer 2-3mm in diameter. A similar observation has been made by Jones *et al.* 1990) who also noted a characteristic melanotic ring around the point of attachment. The most severe damage would appear to be caused by the adult

Plate 1.1 Farmed salmon displaying exposure of the dorsal cranium following damage by *L. salmonis*

female and it has been suggested that this may be due to the relative immobility of this stage on the host (Johannessen 1974). The number of lice required to cause significant pathology is variable, although for *C. elongatus*, it may be that only large numbers are likely to cause significant damage (Rae 1979). The same author has stated that up to 60 *L. salmonis* may be present normally in wild fish, although as few as five adults have been reported to cause significant pathology (Wootten *et al.*1982). Up to 2000 have been recorded on a single fish in Norway (Brandal & Egidius 1977).

Sea lice have been suggested to be associated with outbreaks of vibriosis in Scottish sea cages (*S. salar*) in 1980 though whether the vibriosis was primary or secondary is unsure (Wootten *et al.* 1982). Whether the lice may act as bacterial carriers is also unclear although Kunz (1985) noted the presence of *Vibrio anguillarum* Bergeman on lice taken from infected salmon. Nylund *et al.* (1992) have also considered *L. salmonis* as a possible disease vector, a situation held in common with free-living copepods which have been suggested to carry human pathogens e.g. *Vibrio cholerae* Pacini (Huq, Small, West, Huq, Rahman & Colwell 1983).

Damage is likely to be enhanced in conditions where water temperatures are low, according to Chamberlain (1981), due to a depressed defence response by the salmon. Conversely, Raverty (1987) has noted that gross ulcerative lesions were worse in the summer months though this may of course be a function solely of intensity of infection. Mass mortalities of fish have been reported in Scotland in both winter and summer (Sommerville pers. comm.). Little is known of fish defence mechanisms with regard to caligid attack save that Taylor (1987) believed that at least some settling caligids were destroyed by fish mucus. Few details have been published concerning possible fish antibody responses to copepod infestations, although Thoney and Burreson (1988) found a lack of specific humoral antibody response in *Leiostomus xanthurus* Lacépede attacked by parasitic copepods. Grayson, Jenkins, Wrathmell & Harris (1991) on the other hand noted a serum response to *L. salmonis* in naturally infected *S. salar* and immunized *O. mykiss* (Walbaum). Regardless of the species of caligid parasite, Lüling (1953) pointed out that one characteristic of caligoid attack appears to be the presence of sizeable intra- or sub-cutaneous blood filled lacunae. This is particularly apparent around the vent of fish infested with *L. salmonis* (Kunz 1985) though this observation may be due to ease of visibility in this area (due to light skin pigmentation). There is also an apparent decrease in mucus production in the damaged area (*ibid*). Healing in response to lice damage is apparent in sections of injured tissue with extensive fibrous tissue replacement and formation of blood vessels (though necrotic tissue at the surface may hinder replacement of the dermis (*ibid*). The proliferation of fibroblasts and subsequent fibrous tissue generation has also been noted by Boxshall (1977) in tissue of flounders attacked by *L. pectoralis*. Infiltration of the tissue by macrophages and lymphocytes were also

observed in this species and it was suggested that in this species at least, chalimus stages were the most pathogenic.

1.2.4 Epizootiology

Studies of wild hosts infected with *L. salmonis* have tended to concentrate on fish captured when already in freshwater which has limited studies to times of year when the fish were spawning. Farm studies on the other hand, whilst more detailed through the opportunity for sampling at regular intervals throughout the year, have tended to be affected by factors such as frequent anti-lice treatments which make the dynamics of farm epizootics largely artefactual.

A further problem encountered in elucidating sea-louse population dynamics is that of the sampling error involved in assessing the population of sea lice on the fish. Nagasawa (1985), looking at *L. salmonis* on chum salmon (*O. keta*) captured using two different methods, long-line and gill-net, found that in the gill-net sample the proportion of infected fish in each size-class was depressed with reference to long-line catches. Furthermore, this depression increased with size of fish up to a disparity of 30%. The same situation was also found with mean intensity of infection, with the highest disparity being 76%. Sampling with nets also changed the distribution of lice on the fish, with gill-net samples showing mostly perianal incidence whilst long-line catches showed lice mainly on the back and head region as well as in the perianal area. Johannessen (1975) calculated that 10% of post-chalimus specimens of *L. salmonis* were lost in capture, of which adults of both sexes made up the greatest part. Error also occurs in assessing lice numbers, even when stages are present. Taylor (1987) reported a 40% error in chalimus assessment and a 1.4% error in post-chalimus stage assessment for *C. elongatus* on Atlantic salmon. He also found that a number (up to 10%) were found in the MS222 bath used to anaesthetize the fish examined. In infection experiments, Johnson & Albright (1992a) found up to 65% of previously settled copepodids to be recovered from the anaesthetic bath, with the majority of copepodids being frequently found in the bath rather than still attached to the fish.

Another aspect of sampling error was that highlighted by White (1940a) who commented that female adults, being more conspicuous than their male counterparts were likely to be over-represented in samples, an observation which no doubt extends to other stages such that smaller stages, and in particular the settled copepodid stage, are likely to be under-represented. Keeping these points in mind then, the following discussion will first examine accounts of wild and farmed sea lice (*L. salmonis* and *C. elongatus*) populations and then look at some accounts of other species which, being more detailed, may shed some light on the population dynamics of *L. salmonis*.

Various observations have been made as to the prevalence of *L. salmonis* on wild salmon, although *C. elongatus* is relatively rarer on wild salmon stocks (Wootten *et al.* 1982), occurring more frequently on sea-trout (*Salmo trutta* L.). Recorded prevalences on wild Atlantic salmon range from 100 % (White 1940b, Wootten *et al.* 1982, Kunz 1985) to 65.5 % (salmon captured ascending river, Hutton 1923). Despite the reported high prevalence of lice on wild fish, intensity of infection is often low although Rae (1979), has stated that infestations of up to 60 *L. salmonis* per fish are not uncommon (although numbers of up to 20 are more normal).

Nagasawa, Ishida & Tadokoro (1991), examining *L. salmonis* on ocean-caught wild salmon species, found prevalence to range from 93.1 % on pink to 4.2 % on sockeye with abundance ranging from 5.65 for pink to 0.04 for sockeye. Prevalence in Masu salmon was found to be as low as 2.8 % and abundance as low as 0.03 % in a more recent study by Nagasawa & Takami (1993).

White (1942b), looking at salmon in Nova Scotia, observed that during the early part of the salmon run (June), the stages of *L. salmonis* present were mainly "microscopic young" attached to the fins, with the number of older lice present increasing as the season progressed (as did the intensity of infection). In a study carried out in the same area before he had recognised the young stages, White (1940a) also stated that the few adults that were present during the early part of the season were much larger than those later in the year with the adult females possessing much longer egg-strings. He also noted that these large females appeared to have been in place for some time since they left a large depression in the skin of the host on removal. Later in the season, the number of immature forms of all sizes and of adult stages increased, though at no time were these later adults as large as the earlier ones. From these observations, White suggested that the adult lice must be overwintering on the salmon and that one might hence envisage that the appearance of young lice on the salmon early in the season could be an indication of the resumption of the reproductive / infective cycle with the onset of summer. White (1942b) assumed that the salmon became infested amongst the "barrier of islands and ledges" that runs along the Nova Scotia coast. This barrier, he stated, limits exchange with the sea giving moderately warm low salinity water with little tide to disperse free-swimming stages. White also noted that lice-damage (ie relative density of infection) correlated well with local precipitation, with higher rainfall years leading to a decreased lice burden. He suggested that this situation might arise from heavy river-discharge carrying away larvae before their settlement though it is equally feasible that the observed situation might result from the effect of low-salinity water on larvae/ attached stages. A similar observation of a correlation between high flush rates and low barnacle (*Semibalanus balanoides* (L.)) settlement in bays has been made by Gaines & Bertness (1992).

Hutton (1923), recording sea-lice on rod and line caught fish from Hampton Bishop, England, between the years 1912 and 1923, found an increased prevalence of lice (presumably adults/preadults only) through the summer with an apparent decrease towards autumn. Like White, he also noted an apparent correlation between lice prevalence and rainfall. Alabaster, Gough & Brooker (1991) have since correlated capture of returning *S. salar* grilse having the highest lice numbers with the timing of peak spring tides. It was suggested in this latter study that high lice burdens resulted from a rapid transit time through the estuary and consequently reduced lice mortality before the time of capture.

In the early years of a given farm's development, *L. salmonis* are suggested rarely to be a problem (Wootten *et al.* 1982) and in Scotland at least, it was stated by the same authors that the lice population tends to be greatest in late summer and autumn, this resulting from the accumulation of numbers over several generations during the warmer months. Another paper (Wootten 1985) found greatest numbers of lice from December to March which concurs with the findings of Rae (1979). It is not as yet certain whether reproduction continues throughout the winter or whether a single generation overwinters and then undergoes a rapid expansion in the spring. The former view is supported by Johannessen 1975, Wootten *et al.* (1977), Kunz (1985), Wootten (1985) who found larval stages to be present all year round.

Strong temperature dependency has been noted for generation time (Wootten *et al.* 1977, Kunz 1985, Tully 1989, Johnson & Albright 1991b) with estimated generation times ranging from 4 weeks at higher temperatures (Johannessen 1975) to 13 weeks at lower ones (Tully 1989). Estimates of seasonality in numbers of *L. salmonis* on farm sites vary considerably and are not dependent solely on sea temperatures. Wootten (1985) and Rae (1989) for example, report highest numbers between December and March. Tully (1989) found reproductive output of *L. salmonis* to be greatly reduced in summer due to a general failure of preadult females to mature before November. Despite the suggestion by Kunz (1985) that no seasonal changes in fecundity occur in *L. salmonis*, Ritchie, Mordue & Pike (1993) have observed that mean eggstring length was greater and egg size smaller in winter than in summer which may help explain variability in numbers. Rae (pers. comm.) attributes the rise in lice numbers during December and January to a lack of predation by zooplankton at this time, a view echoed by Kunz (1985).

Generalizations about sea lice numbers must be made cautiously as work at the Institute of Aquaculture has indicated that considerable variability occurs not only between adjacent farms but between adjacent cages. Much variation may therefore result from the use of differing sampling regimes and localities. Conclusions from studies such as that of Jaworski & Holm (1992) which used lice sampled from different cages on each occasion may therefore be tenuous.

More detailed studies of the population dynamics of two further caligid species may augment the scarce information available from the studies on *L. salmonis* cited above. Boxshall (1974b)

studying *L. pectoralis* populations on plaice found fluctuation of prevalence and intensity of infection throughout the study period with the two being closely linked throughout. The main growth of the population occurred from April to August, suggesting a distinct breeding season with rapid population increase at the start. During the early part of the season, more than half of the population was composed of newly settled copepodids which matured and gave rise to the production of several generations over the summer. This breeding season continued up to October, with copepodids found on fish after this time failing to mature. This rapid succession of generations over the summer was followed by a long-lived (10 month) winter adult generation which overwintered and survived to produce the next year's summer generation. The high mortality of males that occurred at the end of summer was explained through their likely death after copulation, with those surviving being uncopulated. At the beginning of the next summer season it was suggested that the winter females produced 2-3 pairs of egg-sacs and then died, restarting the cycle. This seasonal variation was also apparent in the number of females producing egg-sacs at any given time. This alternation of generations was assumed to facilitate rapid population expansion during the summer when breeding was possible.

Paperna (1980), looking at *C. minimus* on sea bass (*D. labrax*) in north Sinai Peninsula, found maximum prevalence / intensity to be in early spring and minimum to be in summer and autumn (high salinity and water temperatures and low oxygen being a feature of the latter period). Ovigerous females were found to be present all year round and hence there was no apparent correlation of reproduction with season. It was also apparent that prevalence of infection by copepodids closely reflected overall adult infection levels and that the highest numbers of larval stages occurred in May. During peak months of larval infection, younger stages outnumbered successively older ones, with this being reversed during months of low infection levels, though it should be noted that such relationships only became apparent when the entire sample was analyzed as a whole. The model of infection suggested by the author in this case was one of several "cohorts" of infection rather than a state of continuous infection. Such a model would seem to be supported by Boxshall (1974c) who noted that, in his study at least, the parasites on the fish tended to be grouped at certain stages, suggesting effectively simultaneous infection by a number of infective larvae.

One important feature that was shared by both of the above studies was the apparent overdispersed distribution of parasites amongst the host population (overdispersal being defined by a variance/mean ratio > 1). Such overdispersed distributions have been suggested to be characteristic of parasite populations (Crofton 1971a) and occur through some hosts having a disproportionately large parasite load and others having a similarly low one.

Boxshall (1974c) was able to model the overdispersed distribution of *L. pectoralis* using a negative binomial model and was further able to determine the nature of distribution using the methods of Iwao (1970) and Iwao and Kuno (1971). The latter approach suggested that individual lice were positively associated (ie had a tendency to clump) and that clumps were apparently randomly distributed throughout the host population. The fact that these lice, are not subject to much (if any) active dispersal once settled (Scott 1901) means that there is an increased probability of a male encountering a female on the same fish in an overdispersed population and so propagating the species. Single-exposure infection experiments gave similar overdispersal ruling out the possibility of a compound Poisson distribution. In the natural population it was found that, as population increased, so prevalence of infection increased giving more dispersed clumps and that, as population decreased, prevalence of infection dropped giving fewer clumps and hence an apparently more contagious distribution. This pattern was explained in terms of an all-or-none type mortality operating on clumps during certain times of the year so that whilst mortality was generally density dependent, at certain times (ie with minimum parasite density during March/April at the end of the overwintering period/start of the summer), loss of clumps occurred due to overwintering females dying from physiological exhaustion after egg production.

Paperna (1980) also found overdispersal of parasites although in this instance it did not conform to a negative binomial model. How the overdispersal is brought about is a fairly complex question with no simple answer although suggestions as to the likely origin of dispersed distributions are given by *inter alia* Iwao (1970), Anderson & Gordon (1982) and Scott (1987). Boxshall (1974c) postulated larvae in a heterogeneous environment aggregating in certain favourable areas and fish moving through such an environment being subject to infection by clumps of larvae. Such a view would seem to be supported by the observation of Paperna (1980) that *C. minimus* overdispersal increase coincides with an increase in diversity of hydrographic conditions. The fact that fish caught in different areas had different prevalences / intensities of infection also supports this idea. Heegaard (1947) suggested that overdispersal in *Clavella adunca* (Strøm) might be associated with hatching of short-lived dispersal stages in clusters.

An alternative explanation for overdispersal of parasites is heterogeneity within the host population itself, although there is some overlap between this explanation and the last, in that host behavioural differences might lead to different utilisation of the environment by different individuals. Boxshall (1974c) found no difference of infection between male and female plaice and the only significant relationship between intensity of infection and host length was explainable through recruitment of smaller uninfected fish to the main population in winter. Paperna (1980) noted that infection was above the expected level in the largest age classes and below it in the smallest and again explained this in terms of different habitat preferences in different age classes.

In *L. salmonis*, an overdispersed frequency distribution has been reported in a number of studies. Johannessen (1975) stated that *L. salmonis* studied on both wild and farmed fish showed an overdispersed distribution that could be fitted by a negative binomial model. He also found overdispersal in *C. elongatus*, though their low numbers make this less reliable. Kunz (1985) also found overdispersal of both species on wild and farmed salmon though did not attempt to fit a binomial curve to the data. Taylor (1987) reports overdispersal of *C. elongatus* on farmed fish introduced in April. Although in the early part of the season (before June) distribution was apparently random, it became overdispersed as the year progressed (July onwards). Again, as with the other species examined, the question must be raised as to how such overdispersal arises. Clearly the environment of a cage on a farm is likely to be far less heterogeneous than the natural habitat of the salmon, but nevertheless, even here there will be variable habitats, particularly at the scale of caligid larvae.

Both Kunz (1985) and Taylor (1987) have noted the tendency for runts/moribund fish to carry higher parasite loads than others in the same population. In this instance it is clearly difficult to determine whether it is the behaviour of moribund fish with respect to the heterogeneous infective environment that leads to higher infection levels or whether it is the heterogeneous nature of the fish population that encourages or allows settlement on certain fish in preference to others. In the case of runts, Kunz ascribes high parasite loads to their tendency to hang around the sides and bottom of the net where he believed larval numbers to be highest. Taylor (1987), however, suggested that moribund fish often swim at the surface giving a higher probability of contacting phototactic larvae. To support this theory of a heterogeneous environment rather than a heterogeneous host population, a settlement experiment was carried out in a tank using copepodids and salmon and it was found that the larvae appeared to settle randomly. Another category of fish found susceptible to higher intensity of infection, would appear to be sexually mature fish. White (1940b) reported that wild grilse were always more intensely infected than larger fish in the same population and Johannessen (1975) found that in farmed fish, sexually mature salmon were significantly worse infected with up to double the number of *L. salmonis* found on other fish. Mature fish also had a significantly lower condition factor than others. Since the mature fish seen already possessed fully adult lice, it was suggested that the increase of infection occurred **during** the maturation process rather than subsequently. The decline in condition of *S. salar* with maturity and its increased susceptibility to disease during this process has been well described by Richards (1980).

The association of degree of infestation by parasitic copepods with length has been previously commented on by Kabata (1981). A positive correlation of *L. salmonis* with host length has similarly been observed by Jaworski & Holm (1992) for farmed fish and Nagasawa (1985, 1987) and Nagasawa, Ishida & Tadokoro (1991) for ocean-caught salmonids. In the

former paper this was suggested to be related to host surface area whilst in the latter paper it was suggested to be related to the time spent in seawater / state of maturity.

1.2.5 Control and management

The control and prevention of *L. salmonis* and other caligids will not be reviewed in detail here as they have already been thoroughly covered in a review by Roth, Richards & Sommerville (1993) and in a thesis by Roth (1992).

At the present time, control of *L. salmonis* is normally accomplished through use of the organophosphate pesticide dichlorvos in the form of Aquagard SLT® [Ciba-Geigy]. The methodology for treatment with dichlorvos was first described by Rae (1979). Treatments are effected by shallowing cages containing fish, surrounding them with a tarpaulin and adding the chemical to give an active dose of 1 ppm dichlorvos for ~1 hr. This chemical replaces the previously utilized and more toxic trichlorfon whose use was described by Brandal & Egidius (1977, 1979). Organophosphates act by inhibiting cholinesterase (ChE) activity in the cholinergic nervous system of the parasite (Baillie 1985) although, more recently, signs of reduced susceptibility to treatment have become apparent in some farmed populations (Jones, Sommerville & Wootten 1992). A more recent development has been the use under test licence of the organophosphate azamethiphos, which, whilst acting in a similar manner to dichlorvos, has a wider therapeutic margin (Roth 1992). Further chemotherapeutants which have been used either under test licences or independently with variable success include Pyrethrum (and the allied pyrethroids), Ivermectin and Hydrogen Peroxide (H_2O_2). None of these compounds have yet been licensed in Britain for use in the aquaculture industry.

The use of vaccines against ectoparasites has had some success in terrestrial agriculture where it has been used to control the cattle tick (Willasden 1980). A similar suggestion has been made for the control of *L. salmonis* and a number of research projects are at present underway to develop a suitable vaccine (Reilly 1990, Smith 1991, Andrade-Salas, Sommerville, Wootten, Turnbull, Melvin, Amezaga & Labus 1993).

Another commonly employed control method is the use of wrasse (*Labridae*) to remove caligids from fish *in situ*. The use of wrasse for control of parasites was first described by Bjordal (1988) although their effectiveness has since appeared highly variable. Management techniques may also be used to help control *L. salmonis* infestations and these include fallowing and the use of single year-class farms (Bron, Sommerville, Wootten & Rae 1993).

1.3 Study objectives

One feature that is particularly notable about the work on caligids undertaken to date is the scarcity of studies describing the general biology of the larval stages and in particular the copepodid stage. Many reasons exist for this lack of data, but amongst the most likely may be counted the following:

1) The small size of larval stages makes them difficult to find and recognise, both on the host and in the water column (note for instance the 50 % and 30 % undersampling error reported for chalimus stages by Taylor (1987) and Tully (1989) respectively and the > 60 % loss of copepodids reported by Johnson & Albright 1991a).

2) The principal stages responsible for host damage (in aquaculture at least) have been perceived to be the preadult and adult stages, such that these stages have therefore merited the greatest attention.

3) The difficulty of obtaining suitable numbers of settled larvae or gravid females may make detailed studies difficult for some species.

4) Problems are frequently encountered in obtaining successful hatching and development of larval stages *in vitro* as with for instance, *L. dissimulatus* (Lewis 1963).

5) In the absence of detailed morphological / taxonomic descriptions of the larval stages of most parasite copepods, there have been considerable problems positively associating larvae with their parent species on naturally infected hosts.

Despite these difficulties, however, the pivotal position played by the nauplius and copepodid stages in particular, in the re-establishment of host contact, should not be underestimated. This fact was recognised by Kabata (1976 pg 2507) who wrote as follows:

> "It is, therefore, increasingly important that more attention be paid to the dispersal stages of these parasites, since it is they that should be prevented from finding and establishing themselves on cultured fish. However, our knowledge of the larval stages of marine parasitic copepods is still scanty"

In 1987, when this study began, little attention had been paid to this crucial area, particularly in terms of caligid larvae.

L. salmonis provides an excellent opportunity for such a study. Because of this species' association with aquaculture, the availability of material is likely to be greater than for comparable species associated with wild hosts. As discussed earlier, the economic consequences of *L. salmonis* epizootics on the salmon-farming industry are also such that there is considerable interest in developing techniques, whether represented by changes in management practices or development of chemotherapeutic or biological control methods, that could reduce the impact of this parasite, and that of other allied species such as *C. elongatus,* in aquaculture. A consequence of such interest is that there is a strong demand for investigations into the basic biology of *L. salmonis* from which techniques for control and management of the problem might be forthcoming.

The purpose of this study has therefore been to carry out a broad-based investigation of the biology of the copepodid stage of *L. salmonis*. It is intended that the information obtained from such a study will not only prompt the development of new methods of control but may also serve as a starting point for further, more detailed studies, at a later date.

Four areas of interest have been focused upon, these corresponding to the four main chapters of this thesis. They are, in order:

1) **Behavioural responses to host and environmental stimuli.**

2) **Settlement and attachment of larval stages.**

3) **Larval nutrition.**

4) **Infection parameters.**

The approach and objectives of each phase of the study are here presented in turn although it should be kept in mind that the results obtained from different phases will have modified the approaches and objectives of other phases:

1.3.1 Behavioural responses to host and environmental stimuli

Almost no work has previously been carried out to investigate either the behaviour of caligid larvae or the sensory organs possessed by the infective stage to transduce information about its environment. The body of work included in this chapter was undertaken to examine the

response of the copepodid to a number of controlled stimuli and to describe the sensory organs that allow such responses to be carried out. The stimuli chosen for investigation were those that could be thought of as most predictable in the natural environment and which might in many cases be simulated by human intervention. Hence the stimuli used were light, pressure, and potentially host-derived chemical and mechanical stimuli whilst such stimuli as salinity and temperature were not investigated.

It was hoped that examination of responses to such stimuli might firstly provide clues as to the principal methods of host-location and might secondly allow predictions to be made as to the likely behaviour of copepodids in the natural environment, so helping to explain the patterns of infection normally observed. It was also hoped that some stimuli might prove effective for use in the field either in attracting or repelling copepodids in order to reduce infection.

By investigating the sensory organs possessed by the copepodid it was also intended that some picture of the range of stimuli that could be transduced by the copepodid be constructed, with results proving potentially useful in predicting which categories of stimuli might prove most important for the copepodid.

Further information to be gained from this phase of the investigation concerns the extent to which the copepodid stage of *L. salmonis* is modified with respect to free-living copepods and indeed in what respects it differs from comparable stages in other parasitic species. As a relatively untransformed species, one might, perhaps, expect little change to have occurred in the transition to parasitism and yet it is clear that the final object of behavioural responses ie re-establishment of contact with the host, has changed markedly. It is therefore possible that the morphology of sensory organs and the precise nature of behaviour will have changed to accommodate this.

1.3.2 Settlement and attachment of larval stages

The settlement and attachment of the copepodid to its host is critical since without success at this stage it will perish (as will its genes). The work described in the second part of this study concerns the question of how the copepodid establishes contact and subsequently how it maintains this contact and accomplishes the permanent attachment by frontal filament associated with the transition to the chalimus stage.

As with the previous area of investigation, the answer to this question was sought using a combination of morphological investigation to define the "tools" available to the copepodid and observational / experimental investigation to examine how those "tools" might be utilised. Despite work on the attachment mechanisms of the Lernaeopodidae mentioned above, no effort

had been made to investigate the mode of attachment of the chalimus stage of *L. salmonis* or any other caligid at the inception of this project.

It was also intended that some idea might be gained of the relationship between the mechanism of larval attachment in *L. salmonis* and the mechanism described for other parasitic species. From this, it was hoped, it might be possible to draw conclusions as to the origin of attachment in a hypothetical semi-parasitic / free-living ancestor.

1.3.3 Larval nutrition

A major question concerning the copepodid stage is one of whether it is a feeding stage, and if so, how and on what does it feed. Although the oral cone of adult caligids is relatively well described, that of the larval stages has been generally neglected. Similarly, although studies exist describing the form of the adult caligid alimentary canal, little work has been carried out to describe the alimentary canal of larvae.

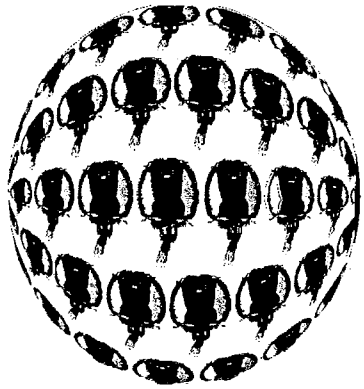
Principally through a combination of morphological and histochemical studies, the work in this section of the study was undertaken to provide answers to the above questions. By examining the form of the alimentary canal it was hoped that it might be possible to determine the nature of any food ingested and the relationship between the copepodid and previously described free-living and parasitic copepods. In particular it was deemed important to describe the copepodid alimentary apparatus using the same descriptive terminology as has been used for free-living species in order that the two might be directly compared.

1.3.4 Infection parameters

Having, in previous sections, formulated some hypotheses as to host-finding, settlement and attachment and utilisation of the host by the copepodid, we are left with a picture of the possible interaction of parasite/host/environment. In order to determine to what extent this image corresponds to reality it was felt to be necessary to investigate the pattern of infection both experimentally in the laboratory and through direct observations of the pattern of infection in the field. In addition an attempt was made to determine the distribution of larvae in the environment. Central to this part of the study was the question of whether variation in the distribution of larval stages results from variation of the external environment or of the host population.

Examination of the temporal and spatial patterns of infection may allow inference of the factors affecting them. Not only should this allow a verification of information gained during other parts of the study but it may also suggest ways in which the settlement might be interrupted so as to minimise the extent of infestation occurring on a farm.

CHAPTER 2
GENERAL MATERIALS AND METHODS



CHAPTER 2 - GENERAL MATERIALS AND METHODS

This chapter outlines the materials and methods that were used throughout this study and are therefore generally applicable to all parts. Where necessary, greater detail will be given under the relevant chapter headings.

2.1 Larval supply and maintenance

2.1.1 Sources of larvae

Gravid adult female *L. salmonis* were obtained from infested farmed salmonids at a variety of sea-farm locations on the west coast of Scotland (Figure 2.1). They were normally taken from anaesthetized fish or from those harvested for market. The principal host species used for this study was the Atlantic salmon *S. salar* since this is the most extensively marine-farmed species in Scotland and therefore the most readily obtained.

Gravid females were removed from fish using coarse forceps and transferred to fresh seawater taken at the site of capture. The following methods were used to transport the lice back to the laboratory:

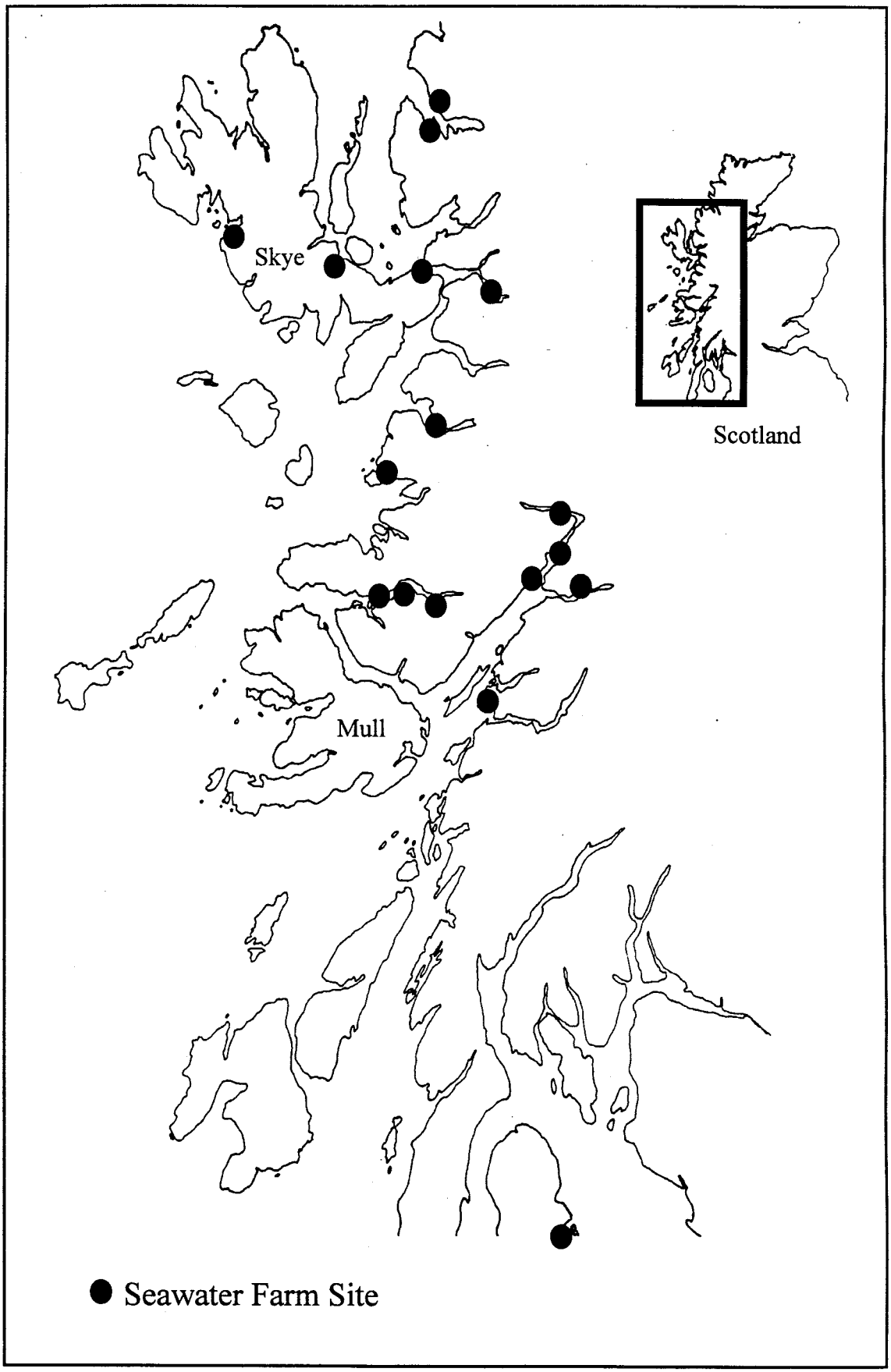
1 Rigid receptacle

Rigid-walled containers with sealing lids such as jars or buckets provided fairly leakproof and easily transported containers. Adhesion to the sides by adults, however, made them difficult to remove without damage when required.

2 Plankton mesh bag

A closed plankton mesh bag (100μ) held in a bucket of seawater or alternatively a mesh sock tailored to fit the rim of a bucket allow easy renewal of seawater. This system was less useful for lice with mature egg-strings since the larvae tended to hatch in transit and get caught and damaged at the rim of the mesh or lost through it.

Figure 2.1 Map illustrating the location of farm sites visited in Scotland



3 Plastic bags

Plastic bags were found to be the most useful transport system and were therefore the preferred method of transport. Not only did the bags allow easy retention of hatched larvae but, by removing the water and splitting the bag, adult females could be easily retrieved from the sides using a spatula or scalpel blade. It was also found to be possible to send lice by post (particularly during the winter months with lower temperatures) by partially inflating plastic bags containing a small quantity of seawater with oxygen and sealing them with wire bag ties before packing them in polystyrene.

Lice were usually transported on ice until they could be transferred to more appropriate containers in the laboratory.

2.1.2 Hatching and maintenance

Where controllable cooling facilities were available, lice were kept at a temperature of between 10°C and 12°C. Where this was not possible, water temperature was dependent upon that of the freshwater used for cooling or the seawater supply on tap. Wherever possible, fresh seawater was used as the maintenance medium since it was found that artificial seawater prejudiced larval survival (as has been found for other crustacean larvae (Walker pers. comm.)).

Salinity was measured using a refractometer and was kept at ambient where possible (ie the salinity of the water in which the lice were originally recovered). When the salinity was particularly low due to high freshwater runoff, water between 30‰ and 32‰ was used where obtainable. The light regime was kept at 12hrs light : 12hrs dark. Water cooling was normally managed using either a Haake D1 circulator bath with a cooling coil or by using ambient temperature fresh or seawater supplies. The two most commonly used cooling systems are illustrated in Figures 2.2, 2.3.

Systems using plankton mesh containers suspended in recirculated or fresh running seawater proved less successful than the systems outlined above. Two principal systems were tested for successful larval rearing (Figures 2.4, 2.5). The problems were firstly that with a mesh size small enough to prevent loss of larvae (100μ), detritus also tended to build up and secondly that there was a tendency for larvae to get stranded in large numbers at the upper rim of the mesh hence increasing larval mortality unnecessarily.

For hatching of larvae, gravid adult females with egg-strings were placed in seawater and the eggs allowed to hatch. Where egg-strings had become detached from the female these were also used, since the presence of the female was not found to be necessary for successful egg

Figure 2.2 Static larval maintenance system (Type 1)

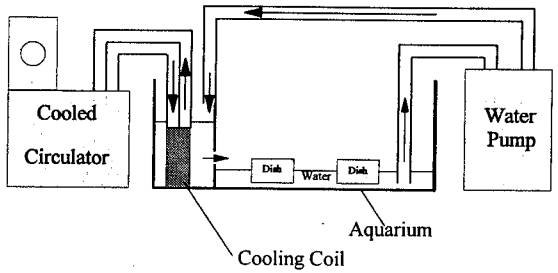


Figure 2.3 Static larval maintenance system (Type 2)

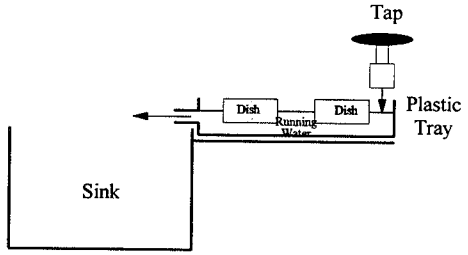


Figure 2.4 Flow-through larval maintenance system (Type 1)

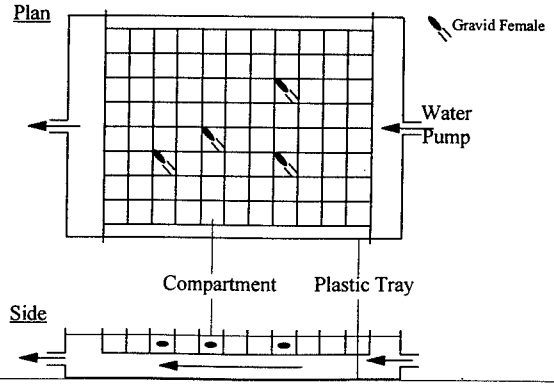
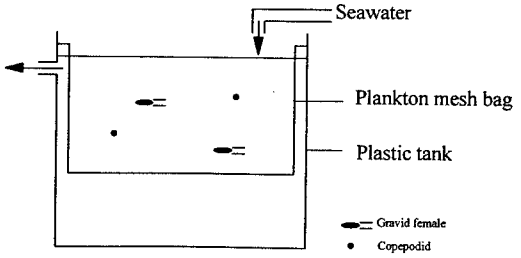


Figure 2.5 Flow-through larval maintenance system (Type 2)



development and hatching. Dark (mature) egg-strings were preferred to light ones since they tended to give a better hatch and survival rate and also took less time to hatch. For behavioural experiments single females were kept in individual containers (crystallising basins) so that larvae chosen for use in the experiments would be approximately the same age (ie hatched at the same time). Throughout the incubation period, the water in static holding systems was changed twice daily, giving adequate water quality for larval survival and development.

Copepodid larvae to be used for experiments were handled using Pasteur pipettes with the tip cut off to give a wider bore and therefore less possibility of larval damage. For behavioural experiments, only copepodids aged between 24 and 72 hours post moulting were used in order to help give consistent results. After initial handling, copepodids were allowed a refractory period of one hour before being transferred to the experimental system.

2.2 Host supply and maintenance

Infected farmed Atlantic salmon *S. salar* were obtained from various salmon farming operations on the west coast of Scotland. For infection and tank experiments, salmon were principally taken from the facility at Lochailort, Inverness-shire. Salmon smolts were maintained in 5 m circular tanks on a flow-through system supplied either with freshwater from the River Ailort, or seawater from Loch Ailort. Transport to the Laboratory was accomplished using large plastic bins.

2.3 Host anaesthesia

Light anaesthesia of salmon for the removal of lice was obtained using a solution of Benzocaine in acetone (10% solution, weight by volume) added below the water surface at a concentration of 0.25 ml l^{-1} of anaesthetic in seawater. The anaesthetic solution had no apparent effect on the sea lice and the anaesthetized salmon recovered rapidly when returned to the cage pen.

2.4 Use of light and electron microscopes

2.4.1 Light microscopy

2.4.1.1 Paraffin wax

Material for wax embedding was fixed in 10% neutral buffered formalin (Humason 1979). Specimens were dehydrated through a series of methanol, ethanol and chloroform in a Reichert-Jung Histokinette 2000 automatic tissue processor. For processing, specimens were placed or pipetted on to fine mesh tissue squares and parcelled to prevent loss of copepodids (~600 microns) during the dehydration procedure. Sections were cut between 4 and 5 μm on a manual Leitz Wetzlar 1212 microtome using disposable metal knives and were picked up from a warmed waterbath at 45°C. After leaving in a 40°C oven overnight, specimens were stained and mounted under coverslips using Pertex mounting medium. The histological stains used are detailed in Table 2.1. For plate headings, wax sections are designated by the initials LMW.

2.4.1.2 Historesin

Historesin is a plastic embedding medium giving superior preservation to wax and high resolution under the light microscope. Fixation of material used 2.5% glutaraldehyde or 10% neutral buffered formalin. Specimens were dehydrated through an ethanol series to 95% ethanol and were then pre-infiltrated in a 1:1 mixture of ethanol : infiltration solution for 12 hours followed by pure infiltration solution for a further 24 hours. Embedding was performed in small plastic moulds and after 24 hours at room temperature these were placed in a 40°C oven to fully polymerize. After removal from the moulds, the bases of the blocks were sanded to give a good mounting surface and mounted on perspex blocks (cut from a perspex rod and reusable after sanding) using a cyanoacrylate fixative (Superglue 3). This mounting technique saved considerable expense, as it obviated the need for the expensive mounting medium and gave equally good results.

The prepared blocks were trimmed down as small as possible using a razor blade in order to give better cutting and were given an orthogonal face. Sections were cut on a Reichert-Jung Supercut 2050 retracting microtome with the blade angle set at 7° and the speed 8-20 mm s^{-1}. Sections were cut at between 3 and 2.5 microns with glass knives produced on a Reichert-Jung histoknifemaker. Ribboning was achieved by brushing the top and bottom edge of the block with a 1:1 mixture of ethanol:infiltration solution which caused successive sections to adhere. Cleaned and dried slides were coated with a thin layer of Glycerin / Albumen by placing a small drop on a slide and smearing it across to ensure that the sections were not lost during staining and sections were placed directly onto distilled water placed on the slide by pipette. The excess water was drained off and the slide dried flat on a hotplate prior to resting overnight in a drying

Table 2.1 Histological stains used to visualize and characterize copepodid and chalimus tissue components

Stain	Purpose / Specificity	Source
Haematoxylin & Eosin	General histology	m. Drury & Wallington 1980
Polychrome stain †	General histology, PS, AMPS (Historesin)	Blackstock (pers. comm.)
Masson's Trichrome	General histology	Drury & Wallington 1980 m. Masson 1929
Mallory's Trichrome	General histology (unsuccessful)	Mallory 1936
Cason's One-step Trichrome	General histology (modified Mallory)	Cason 1950
Periodic Acid-Schiff (PAS)	PS, NMS, Mucoproteins, glycoproteins, glycolipids	Drury & Wallington 1980 m. McManus 1946
Alcian Blue (pH 2.5)	AMPS	Drury & Wallington 1980
Mercuric bromophenol blue	Proteins & peptides	Chapman 1975 in Humason 1979
Performic acid-Alcian Blue	Disulphide (-S-S-)	Adams & Sloper 1956 in Drury & Wallington 1980
Millon Reaction	Tyrosine (protein)	Gomori 1952 in Humason 1979
DMAB-nitrite	Tryptophan	Adams 1957 in Drury & Wallington 1980
Catechol	o-diphenoloxidases	Martoja & Martoja 1967
Acetylthiocholine iodide	Acetylcholine esterase	Gomori 1952 in Jennings & Leflore 1972
Giemsa	Chitinoid material	Cantwell 1981 m. Gier 1949
Diazo	Phenolic compounds	Martoja & Martoja 1967
Modified Gomori (for crustacea)	General histology (unsuccessful)	Cantwell 1981 m. Hubschman 1962
Endoperoxidase	Endoperoxidases	Andrade-Salas (pers. comm.)

m = modified after...
PS = Polysaccharides, AMPS = Acid Mucopolysaccharides, NMS = Neutral Mucopolysaccharides

† Polychrome stain comprises PAS & Heidenhain's haematoxlin counterstained with Acid Fuchsin / Xylidine de Ponceau unless otherwise specified

cabinet at 40°C. Staining and mounting was carried out as described for wax sections. For plate headings, historesin sections are designated by the initials LMH.

2.4.2 Electron microscopy

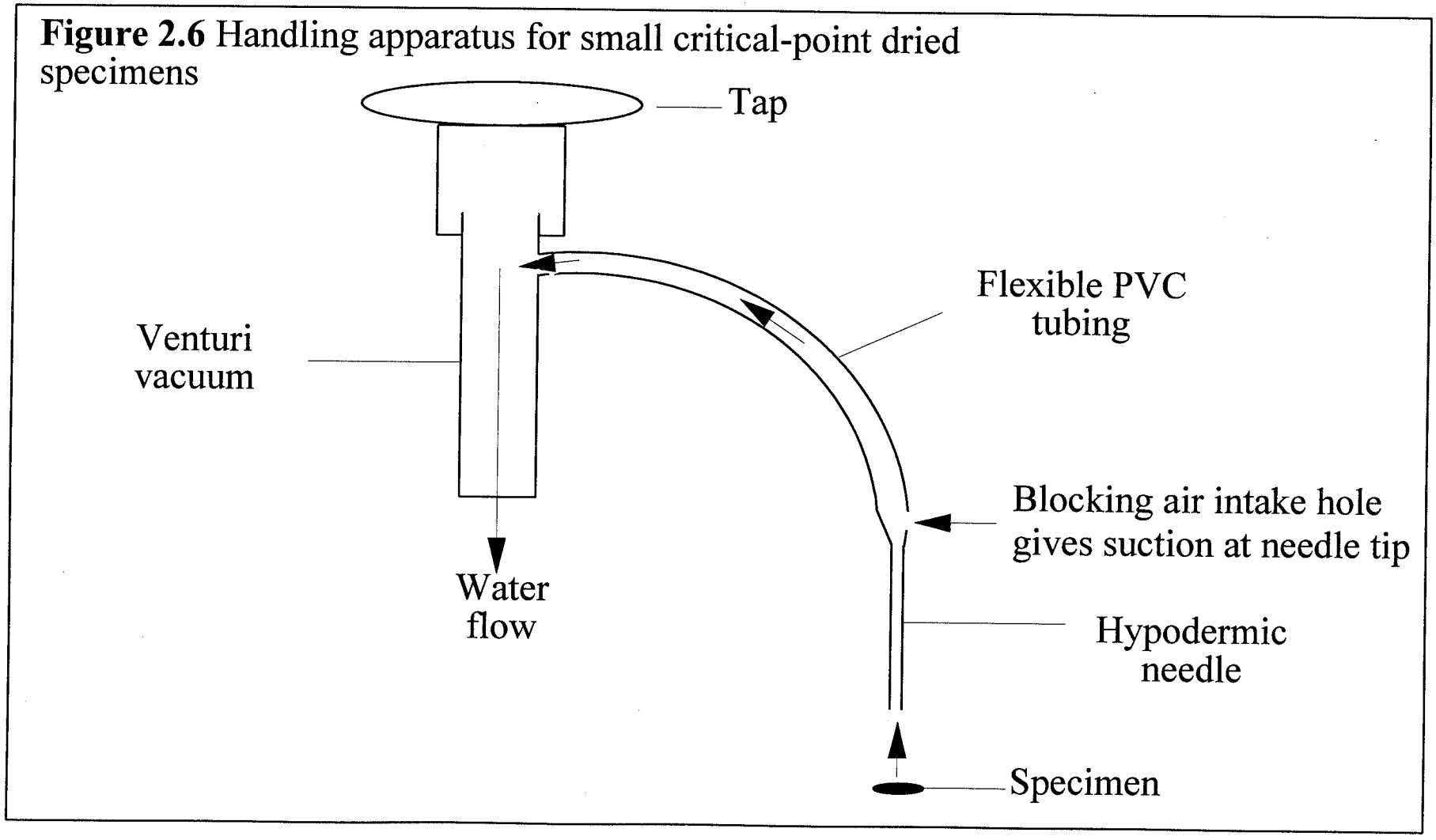
Specimens for scanning electron microscopy were fixed in 2.5% glutaraldehyde, buffered with 0.2 M sodium cacodylate (Glauert 1981). Addition of 0.1 M NaCl and 0.35 M sucrose to the fixative improved results by reducing shrinkage. Good results were also obtained using a 1.3 % paraformaldehyde / 1.6 % glutaraldehyde fixative (Karnovsky 1965).

Specimens were dehydrated through a graded acetone series and critical point dried in a Biorad critical point drier. Handling of dried specimens was performed using the apparatus detailed in Figure 2.6 which was designed to minimize specimen damage during mounting. Specimens were mounted on aluminium stubs using double sided stickers, carbon mountant or Araldite rapide. The former and latter were found to be most effective. The stubs and attached specimens were gold sputter-coated with an Edwards S150B sputter coater using a specimen distance of 3 cm, a current of 40 mA and a voltage of 0.8 KV. Examination was performed using a Philips 500 SEM at running at 12 KV or an ISI60A at 15 KV

Specimens for transmission electron microscopy were fixed in a variety of different glutaraldehyde / cacodylate fixatives, washed in buffer and post-fixed in osmium tetroxide although standard methods gave poor fixation and infiltration, with bad cellular detail and frequent excision of the whole organism or particular areas, e.g. gut, during cutting. The method found to be most successful was a modification of the "low osmium mixed pre-fixative technique" described by Eisenman & Alfert (1982). An extended overnight fixation period was used in contrast to the 1 hr specified in the original paper. Fixation was also improved by bisection of copepodids.

Fixed specimens or fixed specimens subsequently embedded in agar, were dehydrated in acetone and embedded in Taab resin. Trimmed blocks were sectioned using a glass or diamond knife on an LKB ultracut E microtome. Specimens were picked up on uncoated copper grids and stained with uranyl acetate and lead citrate according to the methods of Hayat (1989) and Reynolds (1963) respectively. The uranyl acetate method was modified by the use of methanol rather than water as the solvent. Grids were observed using a Philips 301 TEM running at 80 KV.

Figure 2.6 Handling apparatus for small critical-point dried specimens



2.4.3 Visualization techniques.

Four principal methods were employed in order to improve visualization and thereby understanding, of the three-dimensional structure of certain organs studied:

2.4.3.1 Glass slide technique

Black and white 35 mm photographs of serially sectioned larvae were taken and contact sheets made from them. Glass microscope slides were used to trace structural details from the contact sheet allowing visualization of 3D morphology of simple organs or structures e.g. the alimentary canal (Figure 2.7).

Each successive tracing must be registered with the previous tracing. This is accomplished by placing two small pieces of BluTac at either side of the next photograph in the series to be traced. Above this is held the blank slide to be traced onto and on top, the slide with the previously traced image. The latter, is aligned with the underlying blank slide and is then lined up with the photograph. This position may then be retained by pressing down the blank slide onto the BluTac and removing the top slide with the earlier tracing.

By placing slides prepared in this manner in a standard slotted slide box, it is possible to view the organ sections in an apparently 3D configuration.

The advantage of this technique is that it requires cheap, readily available materials and is relatively fast to perform. Its utility, however, declines with the complexity of the system to be examined.

2.4.3.2 Plasticine reconstruction

By drawing the structures down the microscope using a drawing tube and reducing the drawings to a suitable size with the aid of a photocopier, it is possible to reconstruct the organ system in question using plasticine. This is performed by cutting out the respective organs from the photocopy and using these cut-outs as templates around which to cut plasticine shapes from rolled out sheets of plasticine. By piling these up in the correct sequence and aligning them with the original drawings, it is possible to get a true 3D reconstruction of the structures in question.

As with the previous technique, this method is fairly cheap and easily accomplished and produces good results, even for complex systems.

Figure 2.7 Technique for 3D-reconstruction using glass-slide technique

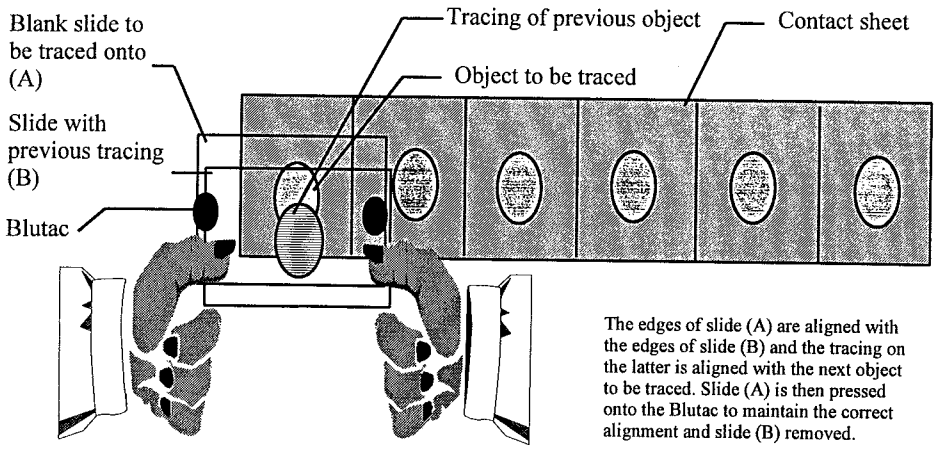
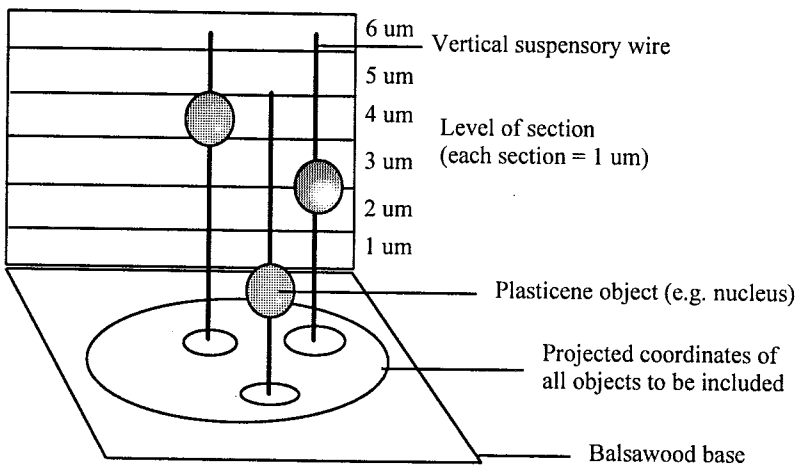


Figure 2.8 Technique for 3D-reconstruction using plasticine and wire



2.4.3.3 Plasticine and wire reconstruction

For certain organs, notably the dorsolateral and ventral ocelli of the nauplius eye, it was particularly difficult to visualize the correct orientation of components with respect to one another. Serial sections of these organs were drawn by use of a drawing tube. These drawings were then superimposed to give a 2-D projection of the organ which was traced onto a balsawood platform (Figure 2.8). Vertical wires marked at regular intervals corresponding to the original section thickness were placed in the position of components of the organ (e.g. retinular cell nuclei). Components modelled in plasticine were then placed at the appropriate height on these wires to give an approximately scale model of the organ in question which could be viewed from any angle to work out the correspondence of components.

Components on section plans modified according to the above reconstruction technique were then converted to XYZ coordinates and a program was written which was then passed to a ray-tracing application (povray.exe) allowing a diagrammatic image of the organ in question to be generated.

2.4.3.4 Electronic Reconstruction

Use of a Vidas 3D reconstruction package produced by Kontron Electronics and implemented on a Kontron 386DX computer, allows full 3D reconstruction of sections entered using a digitising pad, from drawings made using a drawing tube. It was not possible to use automatically captured images since the computer cannot interpret the sections and needs to be told which organ is which and the boundaries thereof. This was more easily done using drawings - hence the use of the drawing tube. Once the sections are entered, the computer can reconstruct them so as to allow viewing from a number of angles and with given organs made transparent or removed to allow visualization of the whole system.

Unlike the previous two methods, this technique is expensive (ie the initial investment in the image-processing equipment) and highly labour intensive (requiring not only the initial tracing using the drawing tube but also subsequent entry of data into the computer). It is, however, ideal for complex systems, allowing the image produced to be easily manipulated for improved visualization of different organs within a system.

2.5 Statistical methods

2.5.1 General overview

For the purposes of this study, data were not automatically assumed to be normally distributed or to possess homogeneous variances and data sets were therefore tested to check these parameters. Much use was made of non-parametric tests such as the Kruskal-Wallis test, Mann-Whitney U-Test, STP and Dunn's tests which were found to be better suited to the analysis of the data produced here. These tests require less stringent conditions for their use and give comparable results to their parametric equivalents. They are also more robust. The arithmetic or sample mean and standard deviation have nevertheless been frequently used to summarize (rather than analyze) data regardless of its normality. This was felt to be justified since it allows comparison with data presented in the literature and gives an understandable overview of the data. Certain other tests were also used to make the results compatible with those given in the literature on a given topic.

2.5.2 Statistical tests used

2.5.2.1 Estimation of the mean

The arithmetic or sample mean $\overline{x}$ gives the best estimate of the population mean μ. This is, however, only true for normally distributed samples.

2.5.2.2 Estimation of the median

The median was selected as the best value for summarizing data sets as it require no *a priori* assumptions. The median is the middle value taken from the data set when it is arranged numerical order. Graphical presentations of the median are given as "box-and-whisker" diagrams. In these, the central line of a box represents the median value, the top and bottom of the box are the interquartiles which describe 25 % of the values above and below the median value and the "whiskers" show the range of values. Points lying above or below the whiskers are "outliers" which are defined as values $> 1.5 \times$ the interquartile range.

2.5.2.3 Homogeneity of variances

F-test

The F-test (Sokal & Rohlf 1981) checks for the departure of the variance ratio of two samples from unity. Where the homogeneity of a number of samples required testing, only the largest and smallest variances of the samples were tested. The calculated value of F_s was compared with the tabled value of F at P = 0.05 (5%). If F_s was less than F, the sample variances were considered to be homogeneous (and therefore suitable for parametric analysis).

2.5.2.4 Normality

Kolmogorov-Smirnov test

The Kolmogorov-Smirnov test (Sokal & Rohlf 1981) was used to test for goodness of fit of sample data to a normal distribution. Where data departed significantly from a normal distribution it was transformed or analyzed using non-parametric methods.

2.5.2.5 Comparison of two samples

T-test

After testing to ensure homogeneity of variance and normality, differences between pairs of means were tested for significance using a t-test (Sokal & Rohlf 1981). If the calculated value for t_s exceeded the tabulated value for t at P=0.05 (5%), the difference between means was considered to be statistically significant.

Mann-Whitney U-test

As a non-parametric analogue of the t-test used for comparison of two samples, the Mann-Whitney U-test was employed on ranked data from independent samples (Sokal & Rohlf 1981)

2.5.2.6 Comparison of multiple samples

ANOVA

Where sample variances were homogeneous and data was normally distributed, one-way analysis of variance ANOVA was employed (Sokal & Rohlf 1981). Because significant differences between individual samples are not determined by ANOVA, the t-test given above was employed to test for significant difference between two samples.

Kruskal-Wallis test

Where sample variances were heterogeneous, a Kruskal-Wallis test (Sokal & Rohlf 1981) was used to make a preliminary comparison between three or more samples.

Dunn's test

Where the Kruskal-Wallis test showed significant differences ($P \leq 0.05$) between samples with unequal numbers of observations or less than 8 observations, Dunn's multiple comparisons procedure was employed (Zar 1984). If the calculated Q exceeded the tabled Q at $P=0.05$ (5%), for a number of groups, k, the difference between the given groups was recognised as being statistically significant.

STP (Studentized range test)

As an alternative to Dunn's test, where group sample sizes were equal, the Studentized range test (Sokal and Rohlf 1981) was employed. In this test, the calculated test value U_s is compared against a calculated critical value. If U_s exceeds this critical value then the tested groups are significantly different.

2.5.2.7 Comparison of proportions

The standard error of a proportion was calculated and confidence limits were then calculated as $\pm$ 1.96 $\times$ S.E (95%) or 2.58 $\times$ S.E. (99%). If the confidence intervals of the two proportions did not overlap, they were considered to be significantly different at the chosen level (Fowler & Cohen 1987).

2.5.3 Sources of statistical tests and computer programs used

The major sources for the statistical tests used in this study were Snedecor and Cochrane (1980), Sokal and Rohlf (1981) Zar (1984) and Fowler & Cohen (1987). Most statistical tests were carried out using the statistical computer program Statgraphics v3.0. For the non-parametric tests (STP and Dunn's) a computer program (stprog3.exe) was specifically written and compiled, as these tests are not available on the normally consulted statistical packages (e.g. Statgraphics, Minitab, SPSS and Systat). This latter program was augmented with a single-way ANOVA, Kruskal-Wallis, F-Test for homogeneity of variance and a non-parametric analogue of the Tukey

Test (Zar 1984) in order to make it more useful as a suite of statistical tools. The program runs on any DOS-compatible computer (PC) and accepts single-column ASCII files from other programs (e.g. minitab, statgraphics etc.). Data typed into the program manually may also be saved for export to other programs or reanalysis at a later date.

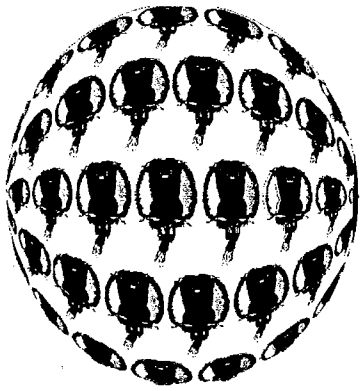
2.6 Photography

Light microscope micrographs were taken either using a Leitz Orthomat equipped with planar lenses and Nomarsky interference filters or an Olympus BH2 microscope with planar lenses and standard interference facilities. The film used for photographs varied, but best results were gained using Ilford Pan-F 50 ASA for black and white, Kodacolor Gold or Ektra 100 / 50 ASA for prints or Agfachrome 60 ASA Tungsten film for diapositives. For black and white photographs a green filter was used to improve contrast and for colour prints, print film was processed for tungsten lighting to remove the excess yellow from tungsten light. SEM micrographs were taken with a large format Rollex camera using continuous large format film. TEM photographs were taken using large format plate film.

All black and white film processing and printing was done *in situ* at the Institute, with photographs being printed on Ilford Multigrade III paper.

A computer program "scabar2.exe" was written to allow the free conversion of measuring units and the calculation of object sizes, scale-bar lengths, magnifications after printing etc. The program runs on any DOS-compatible computer.

CHAPTER 3
BEHAVIOURAL RESPONSES TO HOST AND ENVIRONMENTAL STIMULI



3 - BEHAVIOURAL RESPONSES TO HOST AND ENVIRONMENTAL STIMULI

3.1 Introduction

According to Darwin (1859) all "organic beings" are subject to one general law: "multiply, vary, let the strongest live and the weakest die". The "instincts" of organisms, which may be thought of as congenital patterns of actions in response to stimuli, are also subject to selection according to this law such that "slight modifications" of "instinct" will occur "in any useful direction" (*ibid.*).

Because location of a new host is crucial to the survival of the free-swimming larvae of a parasite such as *L. salmonis*, the most critical period, in terms of survival, is that intervening between eclosion of the nauplius I larva and contact with the host by the infective copepodid stage. Failure to find a host is lethal to any given larva. Any instinctive behaviour that serves to decrease the duration of this interval in the infective stage will therefore be selected for by the improved survival of those larvae possessing it. Prior to the infective stage, however, the requirement for dispersal may lead to behaviour contrary to that required for host location. In a similar manner to free-living organisms (Sukhdeo & Mettrick 1987), infective stages of parasites must be able to respond to cues present in the external environment in order to maximise their chances of survival, host interception and settlement.

According to Crisp (1976) "Marine organisms are essentially opportunists, their pelagic larvae being produced in large numbers each with only a remote expectation of survival". Crisp nevertheless also suggests that random dispersal or settlement of marine larvae should be considered a rare or non-existent event. This would lead one to expect that the larvae of parasitic species such as *L. salmonis* should similarly show non-random dispersal. In the absence of cues as to water movement, "passive drift" has nevertheless been proposed for much of the smaller zooplankton (Hawkins 1985) and similarly Fasten (1913) has stated that the location of its host by *S. edwardsii* is effectively a chance event, although the latter claim is disputed by his own findings and by those of Poulin, Curtis & Rau (1990).

In caligids, the copepodid is the stage principally responsible for location of the host, and success implies the intervention of numerous ecological, behavioural and biochemical factors aimed at improving the probability of host-contact (Raibaut 1985). Kabata (1981) divided the problems facing parasitic copepods in their search for a host into those of long-range location and close-range recognition. This first stage is subdivided by Laing (1937) who states that some parasites may first locate the hosts' habitat and then seek out the host. As Davenport (1955) points out, these two activities may be completely separate and may involve different senses.

Adler (1983) noted the requirements needed for a mobile bacterium to respond to stimuli although they apply equally to copepodids of *L. salmonis*, and a modified version of this scheme is given in Figure 3.1. In terms of the stimuli themselves, the literature concerning the behaviour of parasitic and free-living copepods indicates that the following are, perhaps, the overriding cues available to copepods in the marine environment and are likely to be pivotal in determining the directed movement and behaviour of the infective larvae:

1) Light (e.g. *L. salmonis* Johannessen 1975, Wootten *et al.* 1982).

2) Chemical (e.g. *C. minimus* Raibaut 1985).

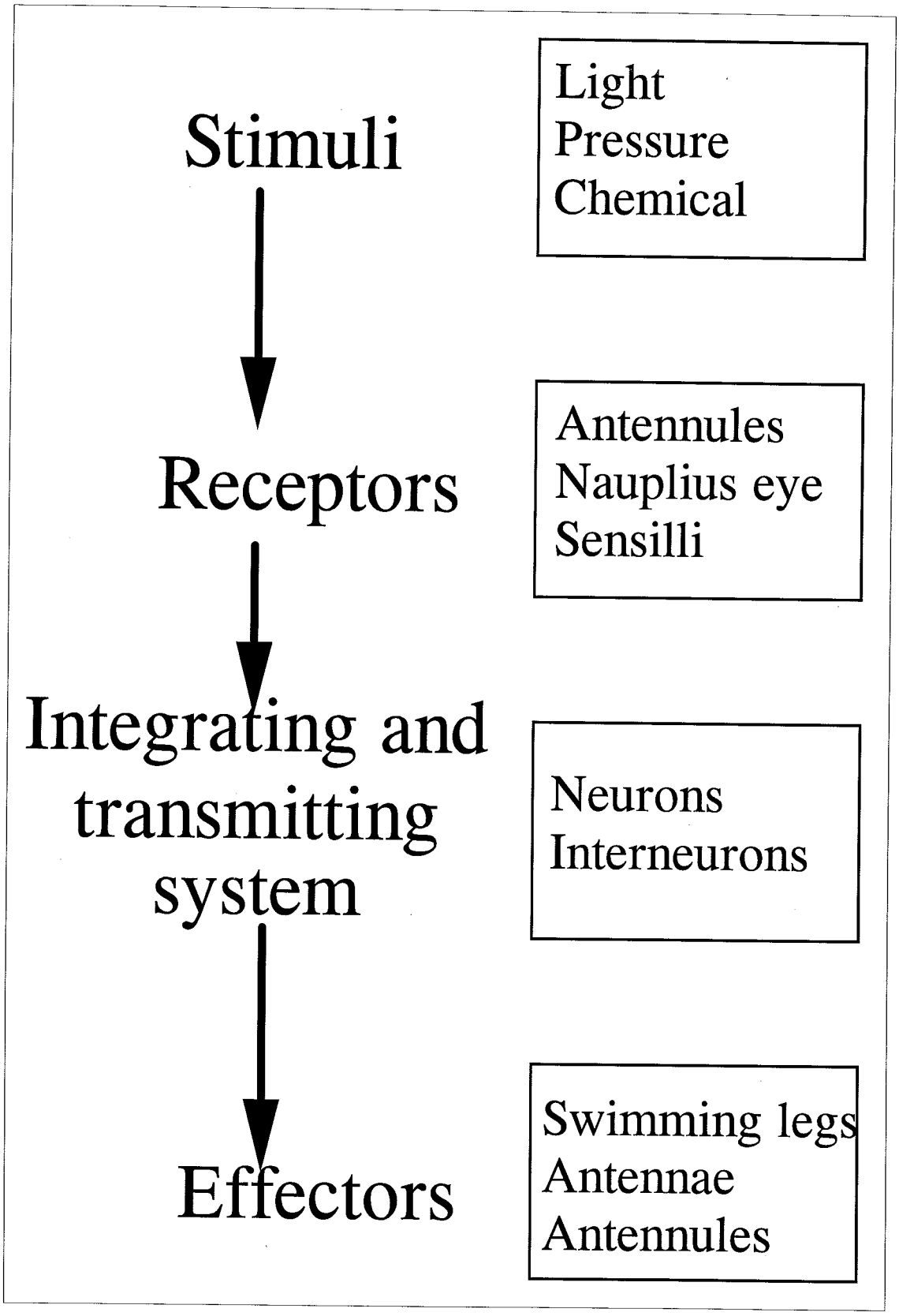
3) Pressure (*Calanus finmarchicus* (Gunnerus) (Hardy & Paton 1947, Rice 1962)).

4) Water flow / vibration (e.g. *L. pectoralis* (Boxshall 1976) and *C. minimus* (Raibaut 1985)).

Many studies have indicated that crustacea in general and copepods in particular are well equipped with a wide range of receptors. These are able to transduce light, chemical, mechanical and gravitational cues as well as pressure and temperature cues although the existence of the latter two receptor types is inferred from responses to the appropriate stimuli (see general discussions by *inter alia* Ache 1988, Laverack 1988, Bush & Laverack 1982, Land 1981, Morgan 1984 and Boxshall 1992). Studies of copepod receptors have, however, been carried out principally on the receptors of adult stages particularly in the case of parasitic copepods although there are notable exceptions such as the work of Dudley (1969, 1972) on the "cephalic sensory receptors" and nauplius eye of the commensal ascidicolous copepod *Doropygus seclusus* Illg.

In caligids, little detailed work on the receptors has been carried out to date. Gresty *et al.* (1993) have described the antennule of copepodids of *L. salmonis* in detail and a similar study has been undertaken on the antennule of adult *C. elongatus* (Laverack & Hull 1993). The nauplius eye of *L. pectoralis* was briefly described by Scott (1901) and that of *C. curtus* (and other caligids) by Wilson (1905). The nauplius eye of *Caligus diaphanus* Nordmann has been described by Heegaard (1947) and Vaissière (1961) described the eye of the same species as well as that of *L. nordmanni*. Boxshall (1992) suggested that the lens and mirror optics of caligids are likely to function in a similar manner to those of the scallop (*Pecten* spp.). Kabata (1974b) suggested that the "median sucker" noted by previous authors in caligids was in fact a sensory organ but this has since been disproved by Oldewage & Van As (1989) and Anstensrud (1990).

Figure 3.1 The requirements of an organism capable of moving in response to stimuli (modified after Adler 1983, Sukhdeo & Mettrick 1987)



The distribution of pores / sensilli on the dorsal surface of copepodids of *L. pectoralis* and *L. salmonis* have been mapped by Boxshall (1976) and Johnson & Albright (1991a) respectively. Although the presumed chemoreceptors termed "cephalic receptors", "frontal organs" or "Organs of Bellonci" have been previously reported in a number of free-living and parasitic copepods (Elofsson 1966, 1971, Dudley 1972) their presence has not been noted in any caligids.

Studies indicate that copepods possess a well-developed central nervous system whose gross morphology is relatively consistent, and this information has been summarized by Boxshall (1992). In *L. pectoralis* the nerve cord is condensed such that in adults it extends only to the first pedigerous somite (Scott 1901, Boxshall 1992). Of particular importance in the response of copepods to stimuli is the presence of a giant fibre system comprising two pairs of giant interneurons (*ibid.*, Park 1966). This system allows for a rapid reflex response to stimuli detected by antennular elements such that, for instance, in the free-living calanoid *Epilabidocera amphitrites* McMurrich stimulation of the antennule on one side has been suggested to cause reflex contraction of the same antennules' remotor muscle, the dorsal trunk muscles on the opposite side and the swimming legs on both sides giving turning of the copepod away from the stimulus (Park 1966).

An organism may respond to a stimulus in a number of ways with the two major classes of response being termed "kinesis" and "taxis". These have been defined as follows (Sukhdeo & Mettrick 1987):

Kinesis — This is an undirected movement response to a stimulus which may be further subdivided into "orthokineses" where the speed or frequency of locomotion is dependent on the intensity of the stimulus and "klinokineses" where the frequency of turning is dependent on the intensity of stimulation. These result in aggregation of organisms in the vicinity of a stimulus but are inefficient and indirect.

Taxis — This consists of movement directed to the source of the stimulus with the longitudinal axis of the body of the organism oriented in line with the source. A taxis may be "positive" (movement towards the source) or "negative" (movement away from the source). Taxes are further divided into "tropotaxes" where the attainment of orientation is direct and "klinotaxes" where attainment of orientation is indirect. These responses are more efficient than kineses but require a greater amount of information about the stimulus.

In addition to the above classes of response, there is also the possibility of trigger cues which may be associated with releaser reactions (Bullock 1957), these latter being defined as preprogrammed, stereotyped, behavioural patterns in response to a short period of specific stimulation (Wilson, 1965). One might, for example, postulate an event whereby detection of a high concentration of host-derived chemicals by a copepodid might release a host attachment response. The instigation of migratory behaviour noted by Neill (1990) in the free-living calanoid copepod *Diaptomus kenai* Wilson responding to the presence of chemical components of their larval midge predator *Chaoborus nyblaei* (= *trivittata*) (Zetterstedt), could be an example of such a response.

Using the behaviour of parasitic and free-living copepods in general as a guide, one might expect the sensory and behavioural repertoire of the copepodid to cater for one or more of the following activities;

1) Avoidance of predation in the plankton.

2) Avoidance of adverse environmental conditions (e.g. high / low salinity, toxic chemicals, high / low temperature etc.).

3) Movement into areas where location of hosts has a higher probability (such areas being similar to areas with high food density for free-living copepods).

4) Detection of possible hosts at a distance (This being comparable with food location in free-living species).

5) Behaviour enabling contact of the host by the copepodid larva (similar to food capture behaviour in free-living copepods).

6) Confirmation of host suitability and attachment (possibly through similar mechanisms to those used in food recognition and acceptance in free-living copepods)

In carrying out these activities, the parasitic copepodid must also conserve its limited energy resources as, being lecithotrophic (Heegaard 1947, present study Chapter 5), it has finite reserves received during egg production. Once attached, however, it may begin feeding.

Despite the recent attention given to the general biology and taxonomy of economically important caligids, little work has been carried out on their 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 larva to light.

A strong positive phototactic response has been reported for copepodids of *L. salmonis* by Johannessen (1975) and Wootten *et al.* (1982). Such a response has also been reported for copepodids of *L. dissimulatus* by Lewis (1963) and of *L. pectoralis* by Boxshall (1976). This positive response decreased with age (Lewis 1963, Boxshall 1976). Negative phototactic responses have been proposed for some caligids, notably *C. curtus*, *C. bonito* Wilson and *C. elongatus* (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 rheotaxic response when subjected to a directional water current (Boxshall 1976). Experiments by Cabral and Fraile (cited by Raibaut 1985) suggest that copepodids of *C. minimus* (Otto) are attracted to currents caused by respiratory and body movements of the fish host *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. *L. kareii* Yamaguti show no evidence of directed host-seeking behaviour (Lopez 1976).

3.2 Study aims

The intention of the studies described in this chapter has been to provide a baseline of information concerning the sensory modalities of the copepodid of *L. salmonis*. The first part of the approach has consisted of a range of experiments designed to examine the response of copepodids to a range of different stimuli. The second part of the approach has been to examine the receptors of the copepodid using a range of light and electron microscopical techniques aided by various reconstructive methods.

Whilst many of the behavioural experiments clearly do not accurately emulate the situation likely to be found in the natural environment, they do give a good indication of the stimuli that can be perceived by the copepodid and the range of larger-scale (ie visible to the unaided observer) responses available to the copepodid to assist both in survival and in host location. The size scale chosen for observations is critical and conclusions drawn from the experiments depend largely upon this. To illustrate the effect of scale on observations, a simple example proposed by Mandelbrot (1967) may suffice. If a coastline is measured with different length rulers it will be found that the smaller the ruler used, the longer the length of the coastline will be, as more of the coastlines' "twists and gnarls" are taken into account, and in fact, the

coastline may be seen to be effectively infinitely long. Similarly, there are an infinite number of size scales at which behavioural responses to a given stimulus can be measured such that responses may not be apparent at one scale (eg millimetric) as they might be at another (eg micrometric). The scale chosen for the present study, principally millimetric → metric and seconds → hours corresponds to the major area of overlap between the copepodid and its' host both in terms of the size of the organisms and their relative movement capabilities. By choosing this scale of observation, behavioural responses at smaller scales, such as those recorded by Strickler (1985) will have been missed.

3.3 Materials and methods

The following experiments were set up to investigate the responses of copepodid larvae to a variety of environmental and host-derived stimuli. All copepodids used in experiments were aged 24 - 72 hours post moulting in order to provide consistency in the results collected between and within different experiments.

3.3.1. "Unstimulated" behaviour

Copepodids were observed swimming in containers of seawater and notes made as to their behaviour in the absence of strong / directional stimuli.

3.3.2. Light

All of the following experiments were conducted in the confines of a darkroom with all light sources, including indicator lights on equipment, extinguished or masked. The complete absence of light within the darkroom was confirmed by exposure of a sheet of photographic paper for a period of 30 minutes and subsequent development and examination of the sheet.

1) Response to varying light intensity

The purpose of these experiments was to investigate the sensitivity of copepodids to different light intensities. Although potentially artefactual with respect to normal responses to light, Forward (1987) has advocated the use of narrow directed light beams for the demonstration of sensitivity with the proviso that results do not necessarily imply anything about normal behaviour. For this reason, an angular light setup was not used in the following experiments.

The light source used for both this and the subsequent light wavelength experiment was a Rank Aldis Tutor II slide projector with a 250W bulb. This was housed in a light-proof wooden cabinet with ventilation holes protected by light-traps to prevent light egress. Exit of the light beam was through a 1 mm slit in one end of the cabinet. Light intensity was varied by use of a circular graded neutral density filter (Barr and Stroud CGN2). Filters were chosen in preference to a change in voltage as the latter has been indicated to change the light wavelength as well as the light intensity (Forward 1988).

The test chamber used (Figure 3.2) was modified from the design described by Forward (1986a). This apparatus consisted of a horizontal perspex trough, along the long axis of which, the light beam could be directed. The dimensions of the trough used were 150 × 38 × 36 mm. Within the trough were four equidistant partitions which could be inserted simultaneously to divide the trough into five equal compartments within which the copepodids could be trapped and enumerated. In contrast to the design of Forward, the edges of the trough partitions were bevelled in order to prevent trapping of copepodids beneath them and minimise turbulence on insertion and removal of the partitions. In addition, alignment of the partitions within the trough was enabled by a spine connecting them and exactly fitting the long dimensions of the trough. Before use, the trough was cleaned in hypochlorite and soaked in running seawater to remove any possible chemical contaminants.

The experimental apparatus was set up as illustrated in Figure 3.3. For each replicate the trough was filled to a depth of 20 mm with cooled seawater at a temperature of 11°C. The filled trough was placed in a square water bath filled with distilled water cooled to the same temperature as the water in the trough. This arrangement was designed to minimise any heating of water in the trough that might occur through exposure to the light source (as advocated by Ryland 1962, Crisp and Ghobashy 1971). Access of the light beam from the source to the trough was facilitated by the provision of a perspex window in the side of the water bath against which aperture the test trough was placed. The inside of the water bath was painted matt black to prevent back-scattering of light from the walls of the bath which might otherwise have led to artefactual behavioural responses. The distance of the trough from the light source was arranged such that the beam width was slightly greater than the width of the face of the trough. This distance was maintained constant by placing all the components of the test system on a wooden platform with blocks to hold their respective positions.

In order to minimise the effects of internal vibration (from the projector cooling fan) or external vibration from equipment elsewhere in the room or building, the projector was isolated from the wooden frame by a foam pad and likewise the wooden frame was isolated from the work-surface by a similar pad.

Figure 3.2 Experimental trough used for light-response experiments

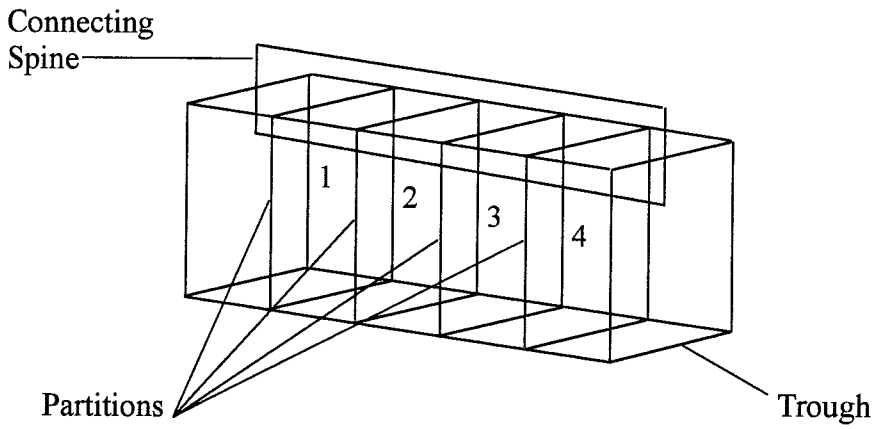
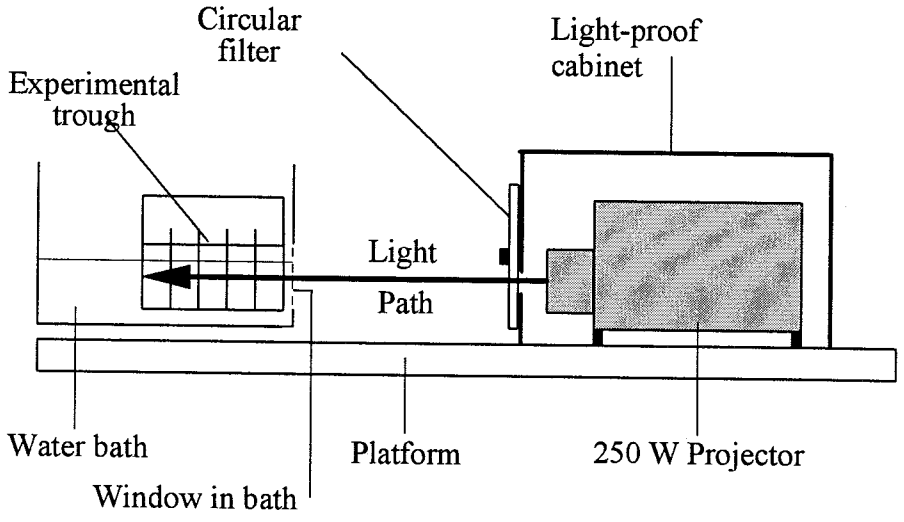


Figure 3.3 Experimental apparatus used for light-response experiments



Five replicates were carried out at each tested intensity. For each experimental replicate, 25 copepodids were transferred into a 5 ml container and dark-adapted for a period of 1 hr (as advocated by Autrum 1981). With the partitions lowered to give five compartments, these copepodids were rinsed into the central compartment and allowed to settle for a period of 60 seconds. The gap between the inserted partitions and the sides of the trough was such as to allow equilibrium of the water-level between compartments to be established without permitting movement of the copepodids from one compartment to the next.

To initiate each experiment, the partitions were raised and the copepodids allowed to settle for a period of 1 minute. After this period, the light source was turned on and the partition left raised for a period of 180 seconds before being replaced. The number of copepodids in each compartment were then enumerated. When more or less than 25 copepodids were recovered at the completion of the experiment due to trapping by the partitions or initial counting errors, the replicate was discarded and repeated. For all replicates, each copepodid was used only once and then discarded. Between each replicate the trough was cleaned with hypochlorite and rinsed in seawater to avoid the effects of possible chemical contamination. Controls were carried out identically to the light experiments but without the light being switched on.

Test values in these experiments ranged between 240 and 2.4 lux (the highest and lowest possible intensities that could be obtained with the equipment available). Intensity was measured using an EEG Luxmeter which was placed at the level of the face of the test trough. Because this device was not waterproof and could not be fitted within the trough itself, actual values could be expected to be slightly lower than the values recorded due to attenuation of the light beam by water and perspex. In addition to the above trials, the behaviour of copepodids exposed to a 20, 000 lux light source was also observed.

2) Response to varying wavelengths

The equipment used for testing the response to varying wavelengths of light was identical to that described for the light intensity experiments, save that a circular graded spectrum filter (Barr and Stroud CS2 bandwidth 8 nm at 550 nm) was placed in tandem with the neutral density filter described above. During use of the graded spectrum filter, light intensity was kept constant by use of the neutral density filter.

The test procedure used for wavelength response was the same as that used for intensity with responses to spectral values of 700 nm, 650 nm, 600 nm, 550 nm 500 nm 450 nm and 400 nm being recorded.

3) Response to shadow and surface albedo / colour

In order to test the response of copepodids to shadow (simulating a passing or static host) and to coloured surfaces (simulating the flanks of the host) a cooled seawater aquarium (320 mm × 610 mm × 410 mm) was set up in the darkroom specified above, within which copepodid reactions to a number of test parameters could be observed.

The aquarium was cooled by a Haake D1 circulator bath with an external cooling coil. Temperature was maintained at 11°C and seawater at a salinity of ~30‰ was used. Lighting was provided by a 15 W fluorescent "Aquaglow" tube mounted in a hood directly above the aquarium. The aquarium was blacked out on three sides to allow better visibility of the copepodids being tested and was also placed on a foam pad to minimise external vibration which might have interfered with the experimental results.

Moving and static shadows were provided by a 15 cm length of plastic tubing suspended on a line above the water-surface and strung between a spindle at one end and a ring mounted on the opposite wall such that the turning of the spindle caused the movement of the tube along the length of the aquarium (Figure 3.4). Mounting of the tube above the water surface prevented vibrations / water currents being set up which might interfere with the shadow response.

In order to simulate responses to different surface colours / albedos, a similar pipe was suspended within the water column and was covered in exposed / unexposed photographic paper (Ilford multigrade) giving a black, grey or white surface or alternatively with aluminium foil giving a silvered surface (Figure 3.5). It was intended that the colours chosen should be roughly analogous to the coloration normally found over various areas of the normal salmon host (ie white = underside, silver = flanks, grey and black = dorsal surface (underside in stressed / runty / grilsing fish)). The use of a tube was intended to simulate the curve of the host underside such that it was intended that light from above might be reflected downwards in a similar way to that found in the host. The choice of photographic paper for the coloration was prompted by its waterproof quality and its ability to give uniform white, grey or black tones with exposure to light.

For each experiment, 1000 copepodids were introduced to the aquarium after a 1 hr period of dark adaptation and their behavioural response observed for a period of 30 minutes under the various test regimes. The shadow cast by the tube could be seen passing over copepodids in its path and subjective observations were made as to the reaction of shadowed copepodids. Responses to the coloured target were also assessed subjectively by observing the response of copepodids in the vicinity of the tubes.

Figure 3.4 Experimental apparatus used for shadow-response experiments

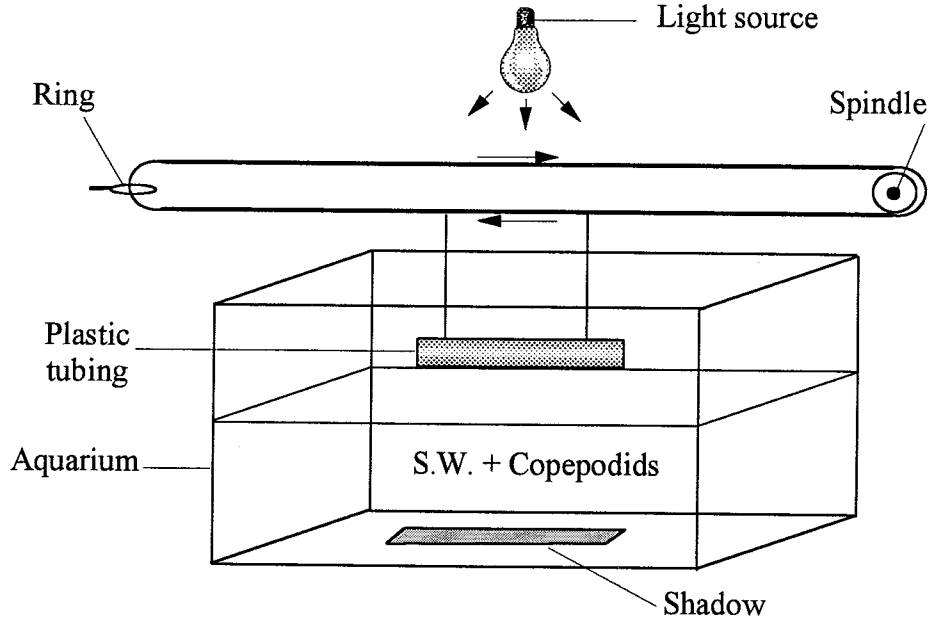
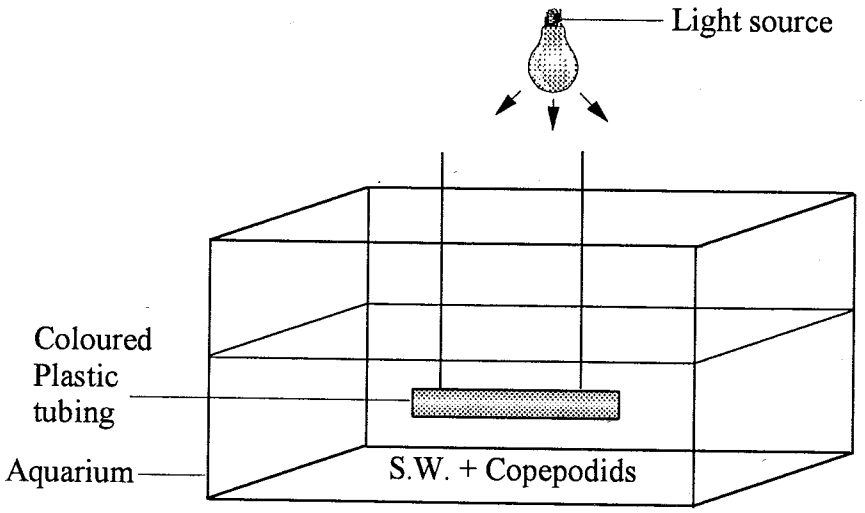


Figure 3.5 Experimental apparatus used for albedo-response experiments



3.3.3. Chemical

Ache (1988) advocated that, in order to determine which substances act as adequate stimuli in the aquatic environment, one should start with the more complex natural stimuli (e.g. "fish extract") and progressively isolate the active components. This top down approach is important as mixtures of chemical substances often act together in eliciting a response (*ibid.*) such that single active ingredients might tend to give artefactual results.

A number of different types of apparatus were used in order to assess the response of copepodids to chemical cues from the host in as many ways as possible. A range of host products was chosen that encompassed the major categories of cue that might be provided by the host. None of these categories was highly specific since it was intended that, should any strong response be seen to one or other of these components, a more detailed study could be initiated to further investigate the responsible active components. The host components used for testing were obtained as follows:

1) Blood

This was obtained by caudal venipuncture of anaesthetized salmon. The hypodermic needle was discarded before expression of the blood to help prevent contamination with skin products and haemolysis. Syringes used to obtain blood were soaked for 24 hrs previously in seawater and were coated with sodium heparin to prevent clotting.

2) Bile

Bile was taken directly from the gall bladder using syringes soaked for 24 hrs previously in seawater.

3) Faeces / urine

After rinsing the area of the anus with distilled water, faecal material / urine were expressed by squeezing the area of the anus / urinary papilla and collecting exuded material on a swab that had been soaked for 24 hrs previously in seawater.

4) Mucus

In order to collect "pure" mucus, a method modified from that of Stabell and Selsett (1980) was followed. The host fish was hung up by the mouth cartilage and the skin surface thoroughly washed with distilled water. Mucus was subsequently collected using a vacuum comprising an aspirator attached to a tap and a length of tubing with rounded edges to avoid skin damage and contamination. Mucus was sucked into this tube and then expressed into a container. Care was taken not to collect mucus from the vicinity of the anus or from any area showing any sign of haemorrhage. As with the other components, all parts of this collection system were soaked for 24 hrs in seawater before commencement of the collection process.

5) Skin

Skin to be used for these experiments was firstly cleaned of mucus in the manner described above. The selected skin was then removed from the fish and the inner surface cleaned with a scalpel to remove muscle insertions. The skin was then cut into small pieces and liquidised with distilled water in a Waring blender. The grey liquid resulting from this process was filtered to remove larger tissue and scale debris and the resulting product was used for the experiments.

6) Whole fish

In addition to the aforementioned products, whole fish were also used for one set of experiments.

The following experiments were used to investigate the response of the copepodids to host chemical cues:

Experiment 1 - Static System 1

This system (Figure 3.6) consisted of a large plastic petri dish marked into four equal quadrants and provided with a removable partition which could be lowered to confine the quadrants into separate compartments. The petri dish was held in a water bath to prevent heating of the water throughout the duration of the experiment and was illuminated from above. All the experiments were carried out in the darkroom already described to remove the possibility of light stimuli external to the experiment. All apparatus were placed on a foam pad to reduce external vibration.

Figure 3.6 Experimental apparatus for testing copepodid chemotactic response: Static system 1

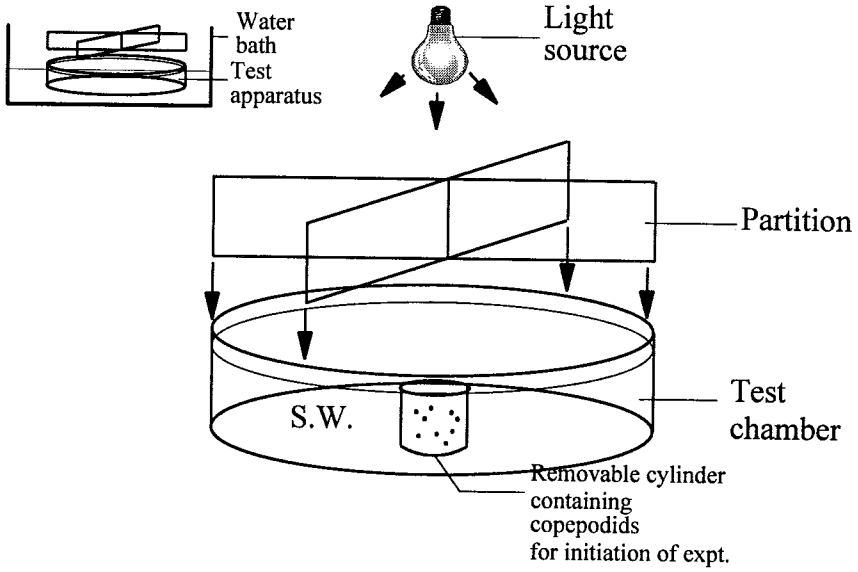
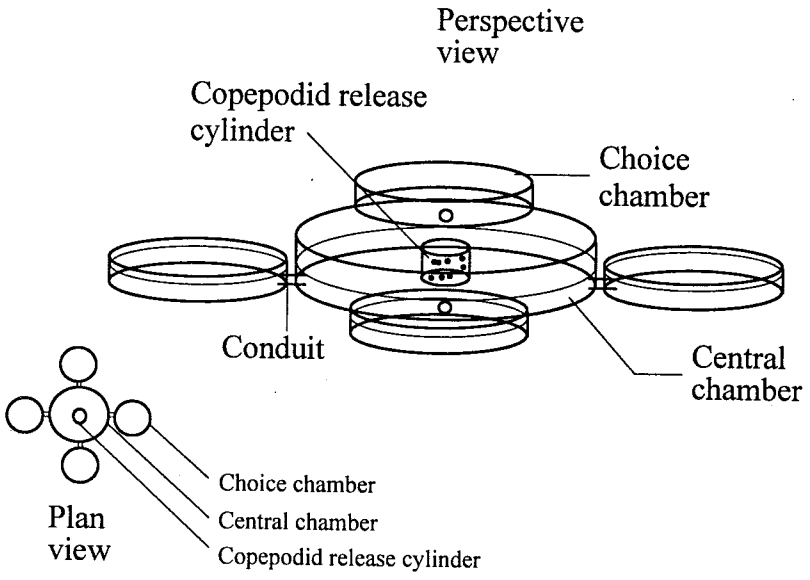


Figure 3.7 Experimental apparatus for testing copepodid chemotactic response: Static system 2



To initiate the experiment, 25 copepodids were introduced into the centre of the system within a cylindrical holder and allowed to acclimatise for 60 seconds. The head of a swab, soaked previously in seawater, and subsequently coated with the chosen test product was then introduced into one quadrant, and 3 others soaked in seawater alone were introduced into the other 3. The system was then left for 15 minutes to allow copepodids to come into contact with the chemical and respond if appropriate. After this elapsed time, the partition was pushed into place and the number of copepodids in each quadrant enumerated. For each different test component, 20 replicates were performed, with the apparatus being cleaned with hypochlorite and soaked in running seawater between each replicate in order to ensure lack of chemical contamination.

Experiment 2 - Static System 2

This system was modified from that described by Bartel and Davenport (1965) and consisted of one large central chamber communicating to 4 satellite choice-chambers through connecting conduits (Figure 3.7). The apparatus was mounted as previously with overhead lighting and a foam pad beneath and was filled with seawater.

To initiate the experiment four swabs were prepared as previously described and one placed in each of the satellite choice-chambers. 25 Copepodids were then introduced into the middle chamber and allowed 30 minutes (a longer time was deemed necessary to allow diffusion of the chemicals from the satellites and active choice by the copepodids) to make a choice of satellite (or stay in the central chamber). After this time had elapsed, the number of copepodids in each choice-chamber was enumerated. As before, 20 replicate experiments were carried out for each test substance with the whole apparatus being cleaned and soaked in seawater between replicates.

Experiment 3 - Flow System 1

This system was identical to the static system above save that there was a gravity-fed input of water from four reservoirs into each of the four satellite choice-chambers causing a general flow toward the central chamber from which the water overflowed down a central sump covered in 100μm plankton mesh (Figure 3.8). This system was designed to produce a more pronounced trail of chemical cue to which the copepodids might respond.

An alternative to this system might have been a "Y-tube olfactometer". It was decided to use the present system in preference because it has been suggested that fast-swimming organisms might tend to overshoot in the former system (Bartel & Davenport) and use of such

Figure 3.8 Experimental apparatus for testing copepodid chemotactic response: Flow-through system 1

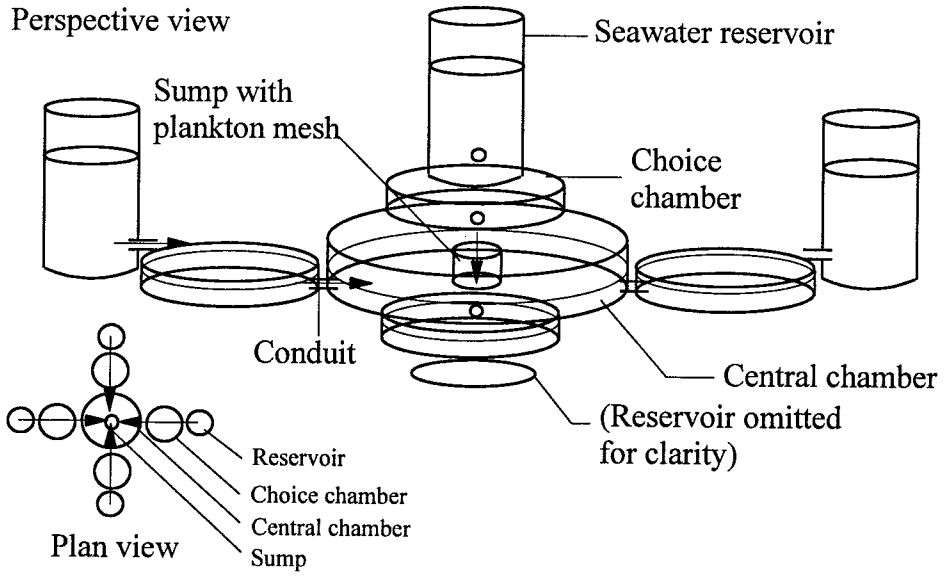
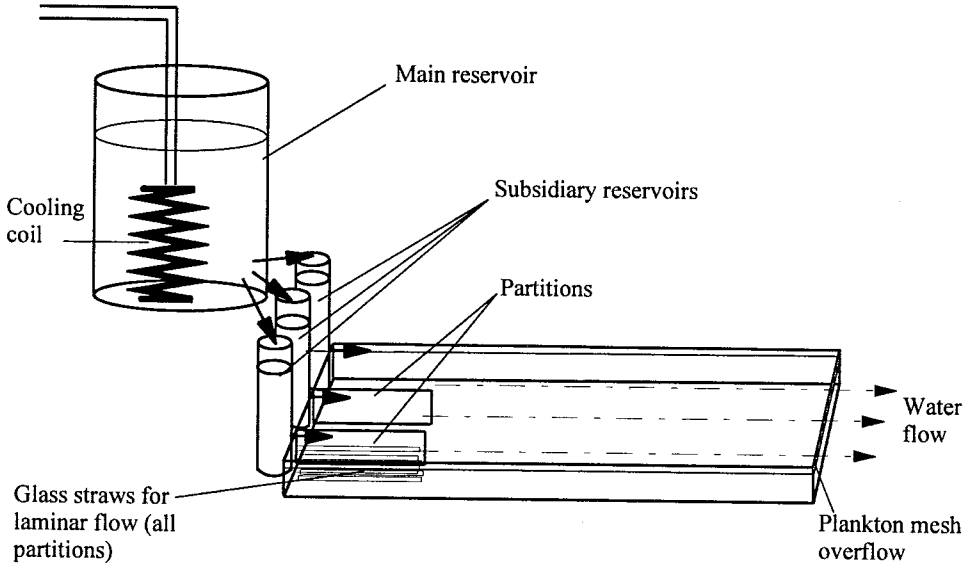


Figure 3.9 Experimental apparatus for testing copepodid chemotactic and rheotactic response: Flow-through system 2



a system with animals of the size of copepodids might also prove difficult, particularly as no tendency to swim against a current was observed in preliminary experiments.

The experiment was carried out similarly to that described before but with a slight flow of 5 ml min^{-1} from the reservoirs (the flow from each being carefully set up such that all gave the same flow rate). Each replicate was only allowed to run for 15 minutes in this case since the chemical trail from any given satellite into the central chamber was assured and hence less time needed to be given to allow a choice of chamber to be made.

Experiment 4 - Flow System 2

This system was modified from that described by Raibaut (1985) who described a system being used by Fraile for the study of the behaviour of copepodids of *C. minimus*. The system used (Figure 3.9) consisted of a large perspex trough 500 mm × 60 mm × 60 mm with 2 equidistant partition walls at the top end which extended 100 mm down the length of the vessel and divided the upstream end into three compartments. At this end, a large seawater reservoir, cooled by a coil from a Haake circulator bath, fed into three smaller reservoirs which in turn fed into the three compartments in the trough. The whole system was gravity fed. Seawater entering the apparatus flowed along the trough and overflowed at the far end through a plankton mesh "catcher" (100μm). In order to reduce turbulence (and hence mixing of the water between lanes) the downstream tips of the partition walls were modified from the design of Fraile by bevelling of their downstream edges and the upstream end of the compartments were filled with bundles of glass straws (haematocrit tubes) which served to ensure that the flow was laminar. The laminarity of the flow was checked using dye trails which showed the water flowing in the three lanes to be distinct for most of the length of the apparatus.

This system was designed to allow the testing of different host components with varying rates of flow. The apparatus was also designed to allow different lanes to carry different components (or for single component lanes to be tested against two control lanes). Such a design would allow copepodids to "choose" between different chemical stimuli presented at the same time.

To initiate the experiments described here, the flow rate was set so that the three lanes had identical flows. Single test components were placed in all three small reservoirs initially to see whether a response could be observed to any of the compounds under varying flow regimes. In each reservoir, a single swab soaked at both ends with the chosen substance, was placed at the initiation of each replicate. A positive response was determined as the movement of copepodids upstream into one or other compartment. To run the experiment, groups of 25 copepodids were poured into a plastic cylinder placed centrally halfway up the trough, allowed

to settle for 60 seconds and then released by lifting the cylinder. The whereabouts of the copepodids was then recorded after 180 seconds. The experiment was run for all the components already mentioned as well as with a single whole fish, placed in the large reservoir, at input rates of 10 ml min^{-1}, 20 ml min^{-1}, 50 ml min^{-1}, 100 ml min^{-1} 200 ml min^{-1}.

After each replicate, the apparatus was washed with hypochlorite and rinsed in running seawater to remove any chemical contaminants.

3.3.4. Water Flow

The apparatus and protocol described above (Flow System 2) was used for testing copepodid responses to different water input rates comprising 10 ml min^{-1}, 20 ml min^{-1}, 50 ml min^{-1}, 100 ml min^{-1} 200 ml min^{-1}. In these experiments, no additional components were added to the system. In order to test for copepodid responses to highly directional and rapid water input, copepodids were placed in a crystallising basin and their response to a high input rate resulting from the rapid expulsion of water from a teat pipette was observed.

3.3.5. Pressure Response

The response of copepodids to increasing and decreasing pressure was tested using a manually raised pressure manometer filled with seawater and having a vertical test chamber (Figure 3.10). The copepodids were placed in a 1.2 m length of tubing with a stop at the upper end comprising a thinner bore tube with a 60 μm plankton mesh stretched over it in order to prevent the copepodids ascending the manometer itself. This lower compartment was marked at 20 cm intervals and was attached to a metal stand to aid stability. Above this was a further length of tubing which, when filled with seawater, could be raised to give a head of water of up to 5 m in height. The entire tube was made of transparent PVC in order to prevent a directional light stimulus affecting the copepodid behaviour. In addition, experiments were carried out on cloudy days in order to limit a directional stimulus from ambient light. Because no quantitative pressure measurement was made, changes are given in terms of head of water (m). Actual pressures might be expected to be slightly lower than calculated from the head of water due to the possible elasticity of components of the system.

In use, 200 copepodids were added to the seawater filled bottom compartment and the plankton mesh bung placed to seal them in. The manometer was then attached with the aid of two jubilee clips and some parafilm to seal the join and seawater added to fill the manometer. This latter was then raised and lowered so as to produce a rise or drop in pressure and the response of the copepodids to such a change noted.

Figure 3.10 Experimental apparatus for testing copepodid pressure response

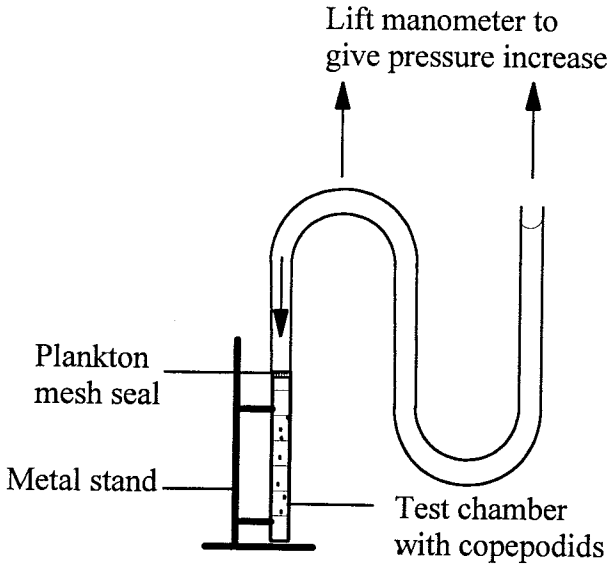
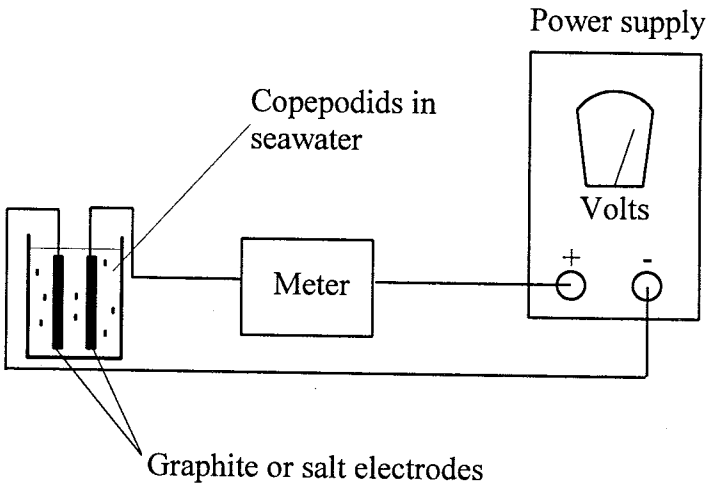


Figure 3.11 Experimental apparatus used for testing copepodid response to electrical stimuli



3.3.6. Response to Electric Current

The response of copepodids to a DC electric current was tested using the apparatus shown in Figure 3.11. A DC power source (Farnell stabilised power supply L30D) with integral volt-meter was fed via two graphite or salt electrodes into a dish containing the copepodids. Power was varied from 1 - 30 V. The current was measured using a Thurlby digital multimeter and values between 80μA - 2 A were tested and the response of copepodids observed.

3.3.7. Response to Mechanical Stimulation

The response of copepodids to mechanical stimulation was observed *in vitro* by subjecting a basin containing copepodids to the vibration caused by a battery-operated handheld fan rotating next to the dish and coming into contact with it via strips of flexible plastic attached to the rotor blades. Copepodids were also stimulated by tapping the holding container with a glass rod and by dropping a 500 g weight adjacent to the container from a height of 200 mm (resulting in more powerful stimuli than that provided by the fan). The response of the copepodids was observed and recorded.

3.3.8. Sensory organs

In order to determine the nature of the sensory apparatus carried by the copepodid, and with a view to determining the cues that dominate its' sensory adaptations, an identification was made of the type and placement of sensory organs possessed by the copepodid.

The process of location and identification of sensory organs was carried out using the light and electron microscopy techniques detailed in Chapter 2. The disposition of the nauplius eye was elucidated using the wire and plasticine technique detailed in the same chapter.

3.4 Experimental results

3.4.1. "Unstimulated" behaviour

During "unstimulated" swimming, copepodids exhibited the "hop and sink" behaviour normally exhibited by free-living copepods. With non-directional illumination copepodids were seen to be distributed throughout the water column. With more directional ambient illumination, copepodids were seen to congregate at the side of the receptacle closest to the strongest stimulus. With increasing time after moulting (days) greater proportions of copepodids were often found on or near the bottom showing active swimming only when stimulated (e.g. by a shock wave). Copepodids were seen to be negatively buoyant, showing sinking when not actively swimming and falling to the bottom of the receptacle when anaesthetized or killed. When sinking passively, copepodids were observed to fall cephalothorax-down.

3.4.2. Light

1) Response to varying light intensity

During the course of both light intensity and light wavelength sensitivity experiments, only 5 out of a possible 1750 copepodids were found in the compartment of the trough lying furthest away from the light source. For this reason, positive phototaxis, that is, movement towards the light source, was chosen over negative phototaxis as an indication of sensitivity to varying light intensities. Copepodids considered positively phototactic for the purposes of analysis were those recovered from the trough compartment closest to the light source.

The mean and median responses of copepodids to the different light intensities tested (with appropriate distribution parameters) are given in Table 3.1 and are illustrated in Figures 3.12 & 3.13.

A Mann-Whitney U-Test (Table 3.2) indicates that all the light-tested responses were significantly higher ($P<0.005$) than the dark controls which allows us to reject the null hypothesis that the tested groups possess the same statistics of location and indicates that the copepodids tested displayed a significant positive phototactic response to all of the intensities tested.

In responding to the highest intensities, the copepodids were seen to swim almost directly (ie in the test case, horizontally) towards the light source with little or no lateral component to the direction of swimming (as contrasted to the more normal sink and swim behaviour seen in the presence of non-directed light sources). At high intensities, copepodids often swam vertically

Table 3.1 Statistics from intensity experiment: Statistics refer to positively phototactic copepodids / 25

Intensity (Lux)	Sample Size	Mean 1 S.D.	Median	Mode	Range
Control[1]	5	1.8 ± 1.5	2.0	2.0	0.0 - 4.0
2.4	5	9.6 ± 3.1	9.0	9.0	7.0 - 15.0
3.75	5	9.6 ± 1.8	10.0	10.0	7.0 - 12.0
7.5	5	9.8 ± 2.6	9.0	9.0	7.0 - 14.0
15	5	13.4 ± 2.1	13.0	12.0	11.0 - 16.0
30	5	18.4 ± 4.5	19.0	19.0	11.0 - 23.0
60	5	14.6 ± 1.5	15.0	16.0	13.0 - 16.0
120	5	18.2 ± 1.8	18.0	18.0	16.0 - 21.0
240	5	23.4 ± 0.5	23.0	23.0	23.0 - 24.0

[1] No illumination

Table 3.2 Mann-Whitney U-Test for significant differences between copepodid responses at tested intensities and in dark controls.

Intensity (Lux)	Z-Value	Significance (P)
2.4	-3.061	$P<0.005$
3.75	-3.061	$P<0.005$
7.5	-3.061	$P<0.005$
15	-3.058	$P<0.005$
30	-3.061	$P<0.005$
60	-3.064	$P<0.005$
120	-3.699	$P<0.005$
240	-3.073	$P<0.005$

Figure 3.12 Mean response of copepodids to varying light intensity with standard error bars

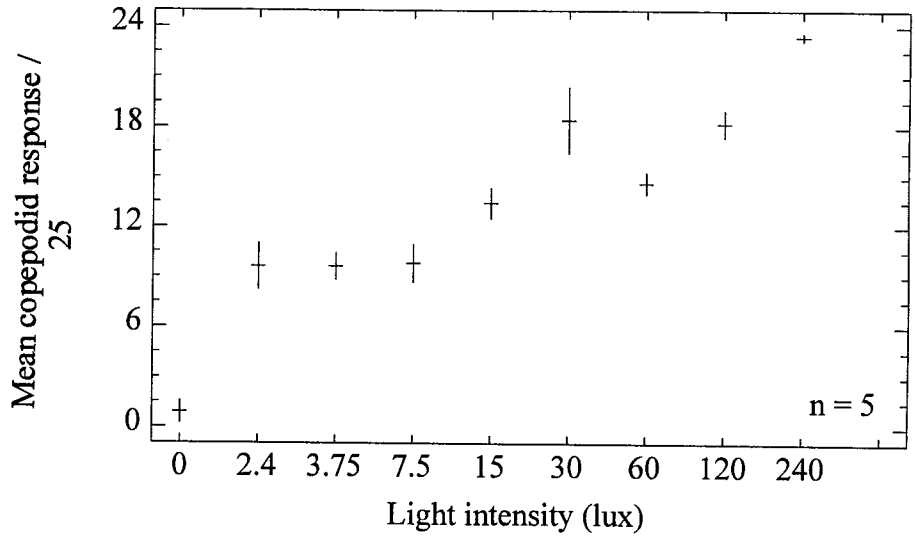
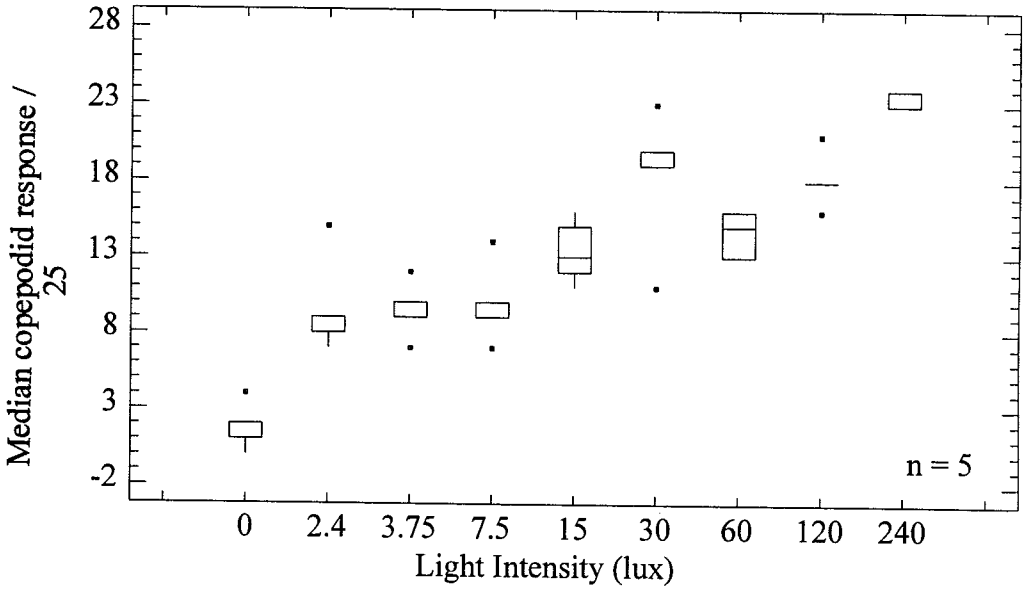


Figure 3.13 Median response of copepodids to varying light intensity presented with range, lower and upper quartiles and outliers > 1.5 x interquartile range



initially and then homed on the light source whilst apparently skipping along the underside of the surface film. At the lower intensities, swimming was seen to be more meandering, with less progress made towards the light source during each swimming burst and a more pronounced lateral component to the general progress.

The data, whose variances were found to be significantly heterogeneous (F-test, $P < 0.05$) was transformed in order to allow a linear regression of the form $y = mx + c$. After adjusting 0 values according to the method of Zar (1984), a calculation of the regression of the data in the form of y = phototactic response (arcsin % positive phototaxis / 100) on x = light intensity (log intensity (lux)) was carried out. This regression is illustrated in Figure 3.14 and shows a highly significant ($P < 0.001$) correlation coefficient (see Table 3.3). This disproves the null hypothesis H0: $\rho = 0$ (ie that there is no correlation between the variables) and indicates that the level of response of the copepodids was highly positively correlated with light intensity.

Dunns test (Table 3.4) indicates that only the response to light at 240 lux is significantly greater than those seen at lower intensities, the response being significantly greater at 240 lux than at 2.4, 3.75 and 7.5 lux.

Exposure of the copepodids to the highest intensity of 20,000 lux caused them to cease swimming and fall to the bottom of the trough.

2) Response to varying light wavelength

As stated above, positive phototaxis as opposed to negative phototaxis was chosen as an indication of sensitivity to the tested wavelengths due to the fact that the majority of copepodids tested responded by moving towards the light source.

The mean and median response to the different wavelengths of light tested are given in Table 3.5 and displayed graphically in Figures 3.15 & 3.16.

A Mann-Whitney U-test applied to the data (Table 3.6) shows that for all wavelengths tested, the copepodid response was significantly higher ($P < 0.01$) than for the dark controls. An STP test (Table 3.7) shows that the response of copepodids to light at 400 nm was significantly less than to that at 550, 600 and 650 nm and the response at 450 nm was also found to be significantly less than that at 550 nm. Between 500 nm to 700 nm the response to light wavelength was found to be fairly uniform. Overall the peak response was to light of 550 nm and the lowest response was to light at 400 nm.

Figure 3.14 Regression of arcsin percentage of positively phototactic copepodids against log light intensity (lux)

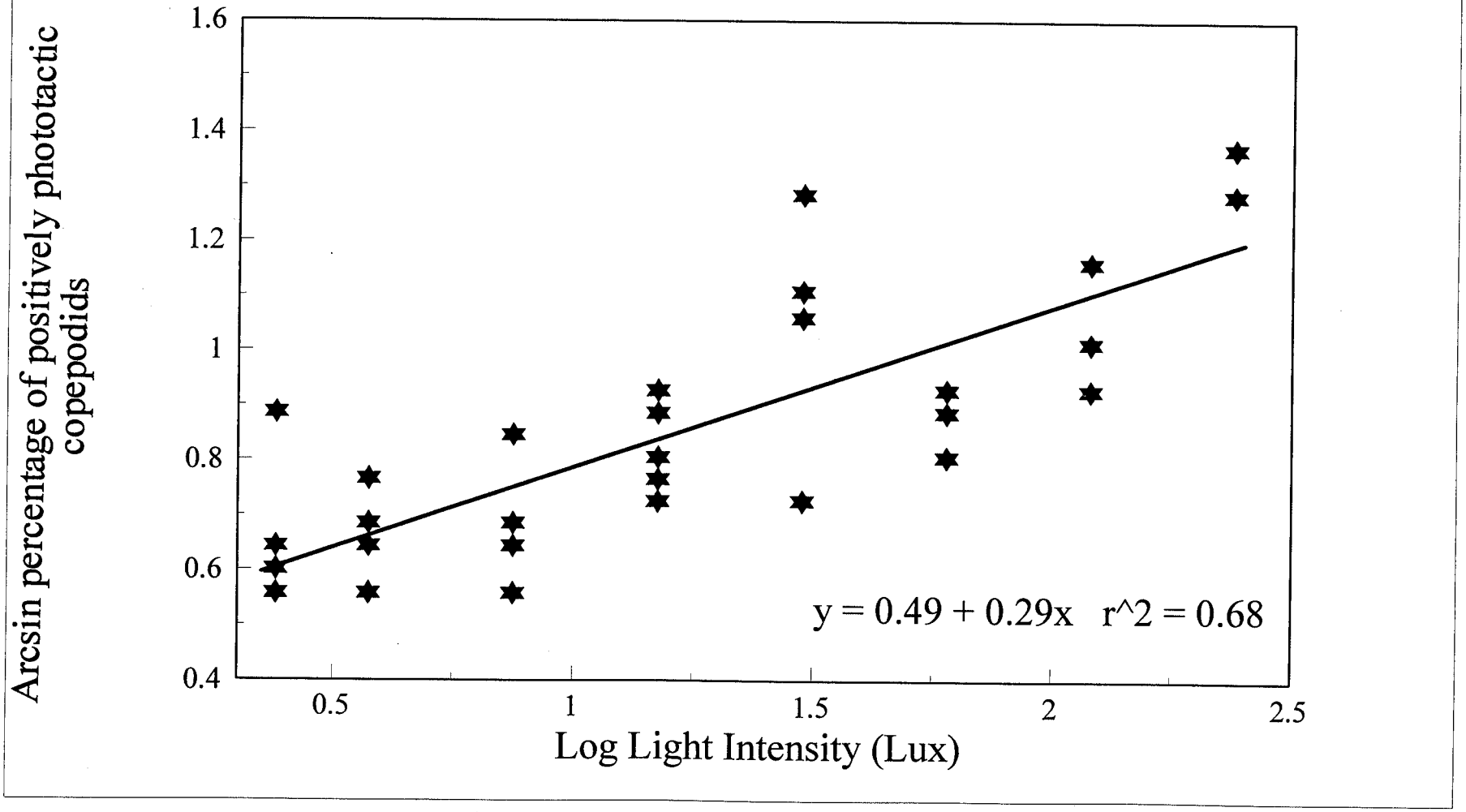


Table 3.3 Table of linear regression of copepodid response against light intensity.

Dependent variable: Arcsin percentage response			**Independent variable:** Log intensity (lux)	
Parameter	**Estimate**	**Standard Error**	**T-Value**	**Probability**
Intercept	0.493	0.048	10.174	P<0.00001
Slope	0.294	0.032	9.088	P<0.00001

Analysis of Variance

Source	**Sum of Squares**	**D.f.**	**Mean Square**	**F-Ratio**	**Probability**
Model	1.549	1	1.549	82.591	P<0.00001
Error	0.713	38	0.019		
Total (Corr.)	2.262	39			

Where H0: $\rho = 0$ ie there is no correlation between copepodid response to different light intensities.

Correlation coefficient r = 0.828 $r^2 = 68.49$
Standard error of estimate = 0.137

Tabled $r_{0.001}$ = 0.519 (35 D.f.)
= 0.490 (40 D.f.)

Therefore P<0.001 for the untransformed r

Using a Z-transformation of r recommended for higher values of r (Sokal & Rohlf 1980):

z = 1.1786
z^*= 1.1506 (improved z for sample sizes <50 (Sokal & Rohlf 1980)).

t_s= 7.19
tabled $t_{0.001\ \infty}$ = 3.291

therefore P<0.001 and the null hypothesis may be rejected ie there is a significant correlation between copepodid response and light intensity.

Table 3.4 Dunn's test for differences in copepodid positive phototactic response to tested intensities

Intensity (Lux)	Control	2.4	3.75	7.5	15	30	60	120
Control								
2.4	1.013 N.S.							
3.75	1.918 N.S.	0.905 N.S.						
7.5	1.025 N.S.	0.012 N.S.	0.892 N.S.					
15	2.267 N.S.	1.254 N.S.	0.35 N.S.	1.242 N.S.				
30	3.413 P<0.05	2.4 N.S.	1.495 N.S.	2.388 N.S.	1.146 N.S.			
60	3.425 P<0.05	2.412 N.S.	1.508 N.S.	2.4 N.S.	1.158 N.S.	0.012 N.S.		
120	3.534 P<0.05	2.521 N.S.	1.616 N.S.	2.509 N.S.	1.266 N.S.	0.121 N.S.	0.109 N.S.	
240	4.788 P<0.001	3.775 P<0.01	2.87 N.S.	3.763 P<0.01	2.521 N.S.	1.375 N.S	1.363 N.S	1.254 N.S.

Tabled Q = 3.197 (P = 0.05), 3.635 (P = 0.01), 3.81 (P = 0.005), 4.191 (P = 0.001)

N.S. denotes a Non-Significant difference between groups (P>0.05)

Table 3.5 Statistics for wavelength experiments: Statistics refer to positively phototactic copepodids / 25

Wavelength (nm)	Sample Size	Mean ± S.D.	Median	Mode	Range
Control[1]	10	2.2 ± 1.3	2.0	2.0	0.0 - 4.0
400	10	18.1 ± 3.4	19.5	16.0	12.0 - 22.0
450	10	20.5 ± .71	21.0	21.0	19.0 - 21.0
500	10	21.0 ± 2.1	21.0	21.0	18.0 - 24.0
550	10	22.9 ± 1.1	22.5	22.0	22.0 - 25.0
600	10	22.2 ± 1.1	22.0	22.0	20.0 - 24.0
650	10	22.0 ± 1.1	22.0	21.0	21.0 - 24.0
700	10	21.3 ± 0.8	21.5	22.0	20.0 - 22.0

[1] No illumination

Table 3.6 Mann-Whitney U-Test for significant differences between copepodid responses to test wavelengths and dark controls.

Test Wavelength (nm)	Z-Value	Significance (P)
400	3.77607	$P<0.0005$
450	3.82922	$P<0.0005$
500	3.78042	$P<0.0005$
550	3.80384	$P<0.0005$
600	3.79208	$P<0.0005$
650	3.79354	$P<0.0005$
700	3.80829	$P<0.0005$

Figure 3.15 Mean response of copepodids to varying light wavelength with standard error bars

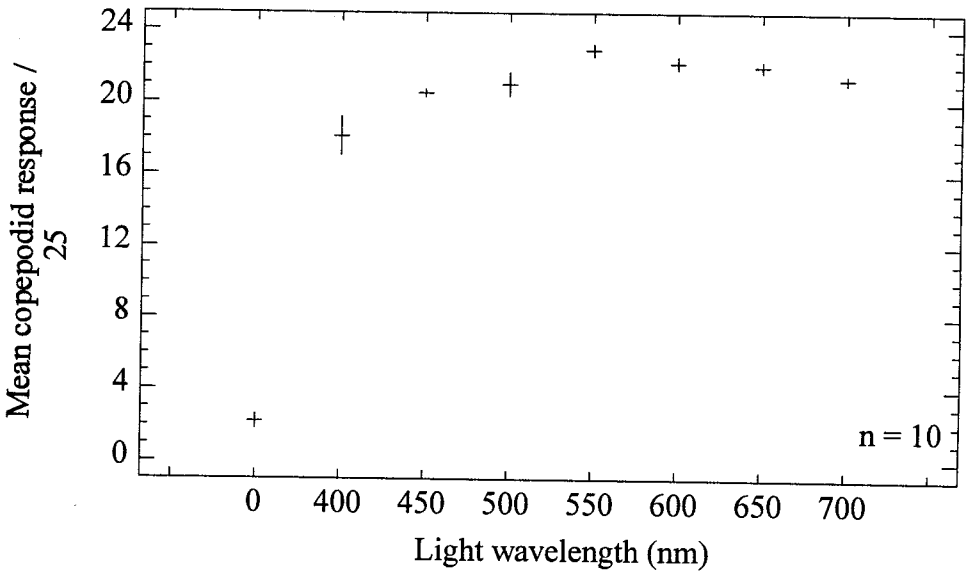


Figure 3.16 Median response of copepodids to varying light wavelength presented with range, lower and upper quartiles and outliers > 1.5 x interquartile range

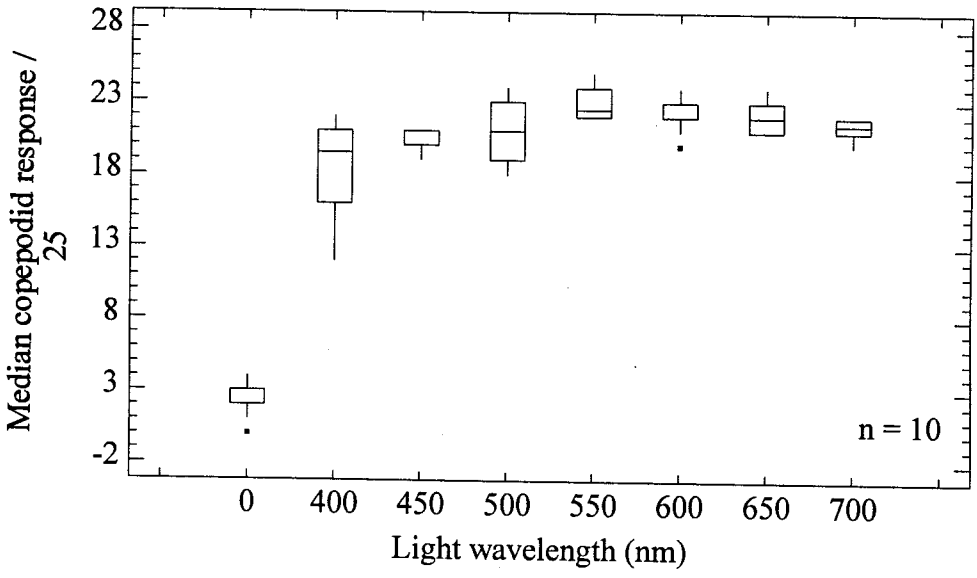


Table 3.7 Standardized range test (STP) for differences in copepodid positive phototactic response to differing wavelengths.

Wavelength (nm)	Control	400	450	500	550	600	650
Control							
400	1.884 N.S.						
450	2.562 N.S.	0.678 N.S.					
500	3.47 P<0.01	1.586 N.S.	0.908 N.S.				
550	5.851 P<0.001	3.967 P<0.005	3.289 P<0.05	2.381 N.S			
600	5.055 P<0.001	3.172 P<0.05	2.494 N.S.	1.586 N.S.	0.795 N.S.		
650	4.704 P<0.001	2.812 P<0.05	2.142 N.S.	1.235 N.S.	1.147 N.S.	0.351 N.S.	
700	3.801 P<0.005	1.918 N.S.	1.239 N.S.	0.332 N.S.	2.05 N.S.	1.254 N.S.	0.903 N.S.

Tabled Q = 3.124 (P = 0.05), 3.57 (P = 0.01), 3.748 (P= 0.005), 4.134 (P = 0.001)

N.S. denotes a Non-Significant difference between groups (P>0.05)

3) Response to shadow / surface albedo / colour

No responsive behaviour was seen in the copepodids exposed to either the moving or the static shadows produced by the tubing suspended above the water surface. There was also no response seen to the reflective or dark / light coloured piping suspended in the water of the aquarium itself.

3.4.3. Chemical

1) Experiment 1 - Static System 1

The mean and median copepodid responses to the various fish components tested are given in Table 3.8 with associated parameters and are illustrated in Figures 3.17 & 3.18.

Using an STP test (Table 3.9) it can be seen that none of the components tested attracted or repulsed the copepodids significantly more than did the seawater controls. The only significant differences were between the response recorded for surface mucus and that observed for blood and faeces. In the table it can be seen that mucus elicited a significantly greater copepodid response ($P < 0.05$) than did the latter two components though it was not, however, significantly different from the controls.

2) Experiment 2 - Static System 2

The mean and median copepodid responses to the tested components are shown in Table 3.10 and displayed in Figures 3.19 & 3.20.

Using an STP test (Table 3.11) it can be seen that none of the tested components showed any significant difference in copepodid response to the seawater controls. There were also no significant differences observed between the different components themselves.

3) Experiment 3 - Flow System 1

The mean and median copepodid responses to the tested components are shown in Table 3.12 and are illustrated in Figures 3.21 & 3.22.

An STP test on the data (Table 3.13) indicates that there was no significant difference between the responses of copepodids in the presence of host components and the responses of those placed with seawater controls. No significant differences existed between the responses of copepodids exposed to the different components.

Table 3.8 Statistics for chemosensory test 1: Statistics refer to positively responding copepodids / 25

Test Component	Sample Size	Mean ± S.D.	Median	Mode	Range
Control (S.W.)	20	7.0 ± 3.0	6.5	4.0	2.0 - 12.0
Bile	20	5.8 ± 2.8	5.0	8.0	1.0 - 11.0
Blood	20	5.5 ± 2.0	5.5	4.0	3.0 - 11.0
Faeces / Urine	20	5.4 ± 2.0	5.0	5.0	2.0 - 9.0
Mucus	20	8.4 ± 3.0	8.0	8.0	3.0 - 14.0
Skin	20	6.3 ± 2.4	6.0	6.0	3.0 - 12.0

Table 3.9 Standardized range test (STP) for differences in positive response to host-components: Chemosensory test 1

Test Component	Control (S.W.)	Blood	Bile	Faeces / Urine	Skin	Mucus
Blood	260.0 N.S.					
Bile	245.5 N.S.	211.5 N.S.				
Faeces / Urine	259.5 N.S.	204.0 N.S.	209.5 N.S.			
Skin	226.5 N.S.	236.5 N.S.	225.5 N.S.	235.5 N.S.		
Mucus	248.5 N.S.	317.5 $P<0.05$	293.5 N.S.	317.5 $P<0.05$	281.5 N.S.	

Tabled U = 305.35 (P = 0.05), 324.35 (P = 0.01)

N.S. denotes a Non-Significant difference between groups

Figure 3.17 Mean chemotactic response of copepodids to tested host components with standard error bars: Static system 1

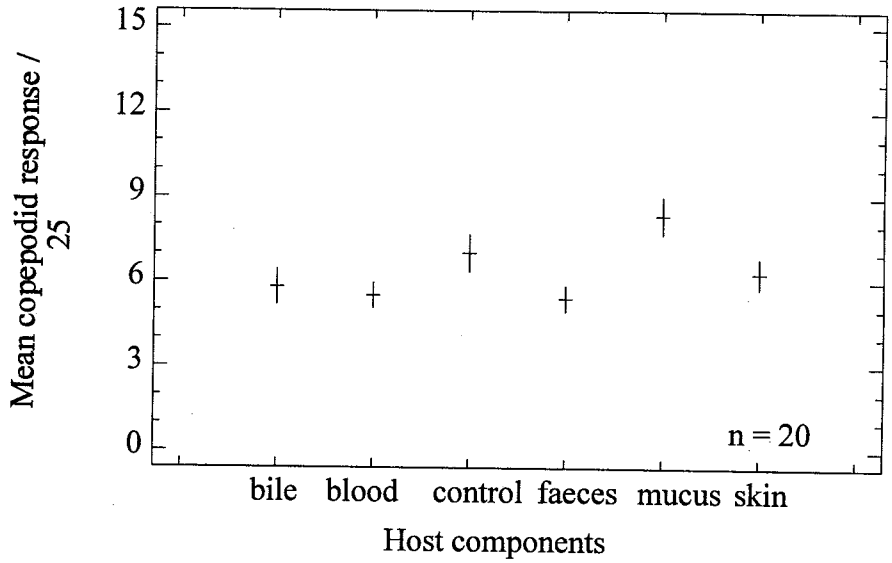


Figure 3.18 Median chemotactic response of copepodids to tested host components presented with range, lower and upper quartiles and outliers > 1.5 x interquartile range: Static system 1

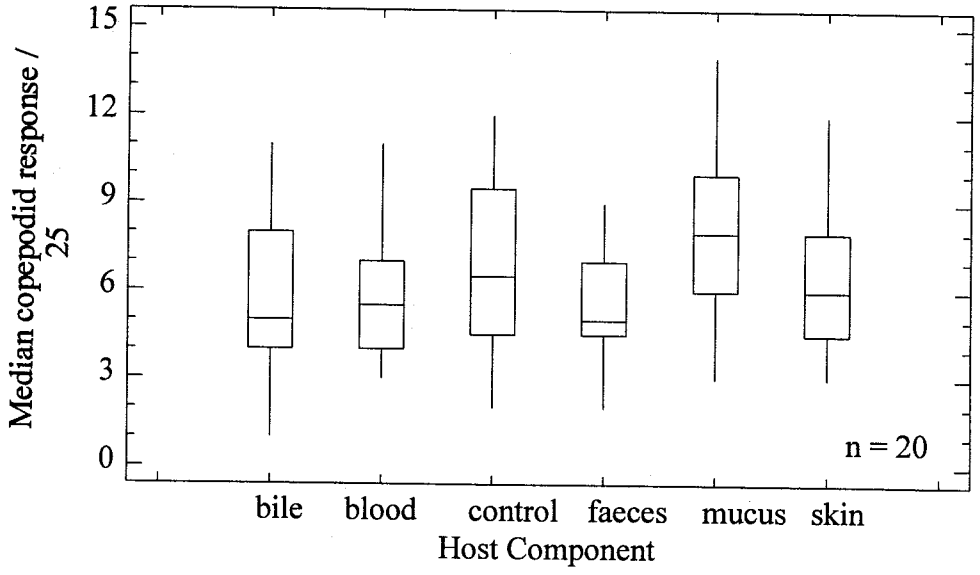


Table 3.10 Statistics for chemosensory test 2: Statistics refer to positively responding copepodids / 25

Test Component	Sample Size	Mean ± S.D.	Median	Mode	Range
Control (S.W.)	20	0.8 ± 0.89	1.0	0.0	0.0 - 3.0
Bile	20	1.1 ± 1.17	1.0	0.0	0.0 - 4.0
Blood	20	1.2 ± 0.94	1.0	1.0	0.0 - 3.0
Faeces / Urine	20	1.0 ± 1.08	1.0	0.0	0.0 - 4.0
Mucus	20	0.8 ± 0.91	0.5	0.0	0.0 - 3.0
Skin	20	0.9 ± 0.85	1.0	1.0	0.0 - 3.0

Table 3.11 Standardized range test (STP) for differences in positive response to host-components: Chemosensory test 2

Test Component	Control (S.W.)	Blood	Bile	Faeces / Urine	Skin	Mucus
Blood	231.0 N.S.					
Bile	225.0 N.S.	202.0 N.S.				
Faeces / Urine	218.0 N.S.	210.0 N.S.	208.0 N.S.			
Skin	216.0 N.S.	216.0 N.S.	212.5 N.S.	204.5 N.S.		
Mucus	208.0 N.S.	238.0 N.S.	232.0 N.S.	225.5 N.S.	224.0 N.S.	

Tabled U = 305.35 (P = 0.05), 324.35 (P = 0.01)

N.S. denotes a Non-Significant difference between groups

Figure 3.19 Mean chemotactic response of copepodids to tested host components with standard error bars: Static system 2

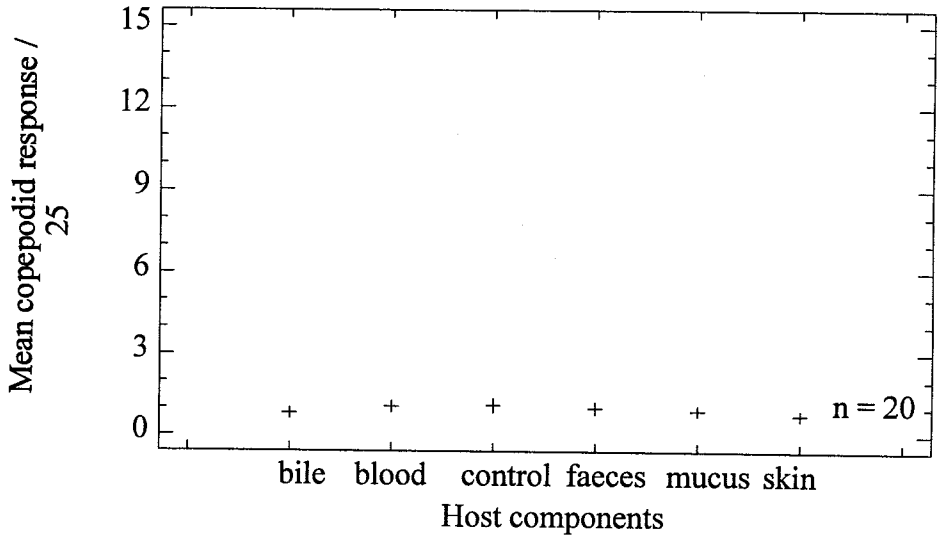


Figure 3.20 Median chemotactic response of copepodids to tested host components presented with range, lower and upper quartiles and outliers > 1.5 x interquartile range: Static system 2

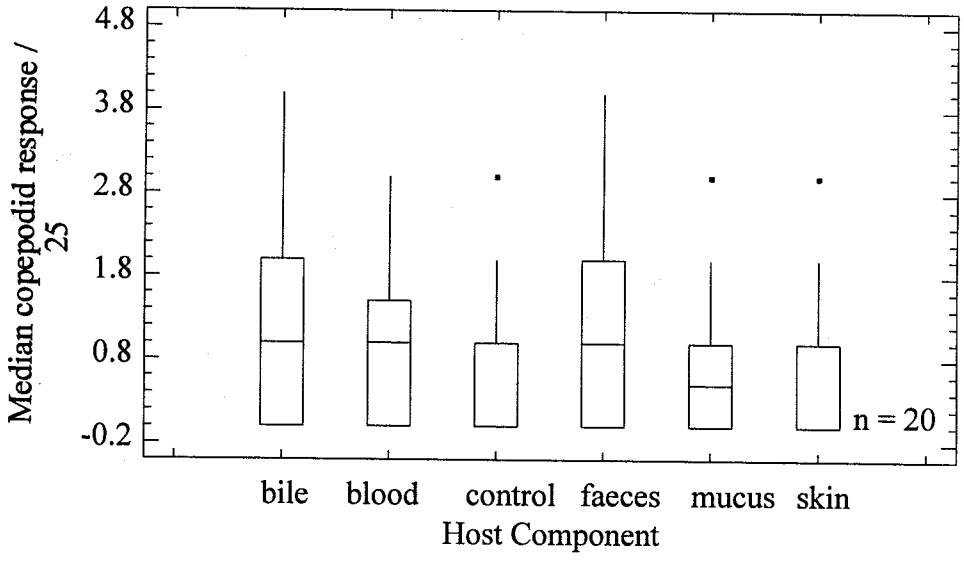


Table 3.12 Statistics for chemosensory test 3: Statisitics refer to positively responding copepodids / 25

Test Component	Sample Size	Mean	Median	Mode	Range
Control (S.W.)	20	1.6 ± 1.4	1.0	1.0	0.0 - 5.0
Bile	20	1.2 ± 0.9	1.0	1.0	0.0 - 3.0
Blood	20	1.6 ± 1.4	1.0	1.0	0.0 - 5.0
Faeces / Urine	20	1.2 ± 1.2	1.0	0.0	0.0 - 4.0
Mucus	20	1.1 ± 1.2	1.0	1.0	0.0 - 4.0
Skin	20	1.5 ± 1.4	1.5	0.0	0.0 - 4.0

Table 3.13 Standardized range test (STP) for differences in response to host-components (chemosensory test 3)

Test Component	Control (S.W.)	Blood	Bile	Faeces / Urine	Skin	Mucus
Control (S.W.)						
Blood	202.0 N.S.					
Bile	224.0 N.S.	227.0 N.S.				
Faeces / Urine	225.0 N.S.	226.0 N.S.	200.5 N.S.			
Skin	207.0 N.S.	206.5 N.S.	219.5 N.S.	219.0 N.S.		
Mucus	247.0 N.S.	244.5 N.S.	226.0 N.S.	218.5 N.S.	233.0 N.S.	

Tabled U = 305.35 (P = 0.05), 324.35 (P = 0.01)

N.S. denotes a Non-Significant difference between groups

Figure 3.21 Mean chemotactic response of copepodids to tested host components with standard error bars: Flow-through system 1

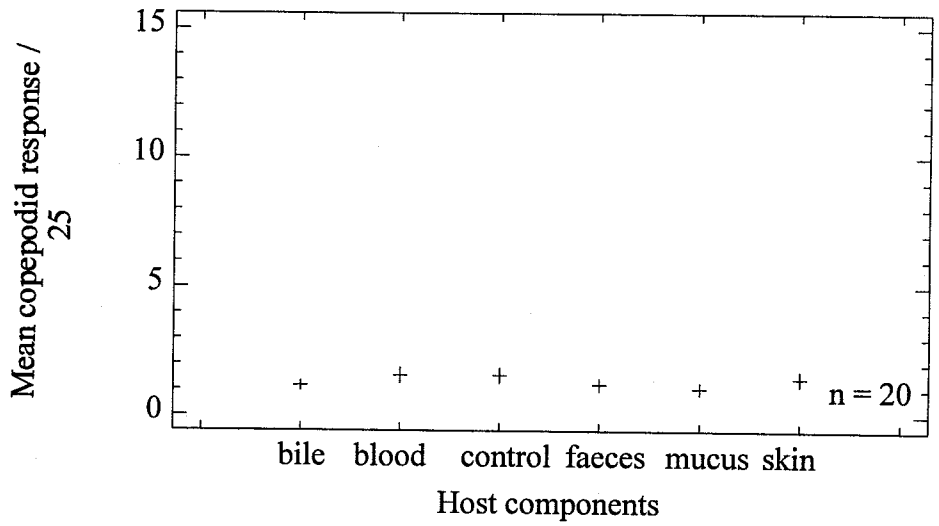
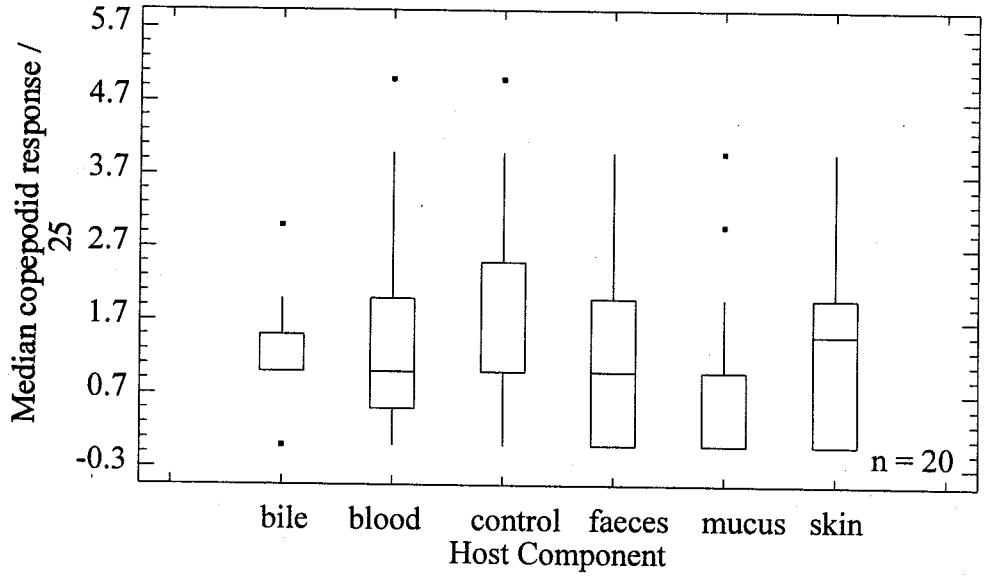


Figure 3.22 Median chemotactic response of copepodids to varying host components presented with range, lower and upper quartiles and outliers > 1.5 x interquartile range: Flow-through system 1



4) Experiment 4 - Flow System 2

No copepodids were found to swim "upstream" under any of the different flow and host component regimes, all being eventually carried "downstream" until they hit the overflow mesh. Occasional individuals swam against the current in short (<2 cm) darts but none was seen to swim for any length of time against the current and none approached the compartments.

No "positive" responses were gained to any of the host components or controls using this system, there being therefore no significant observable difference between them.

3.4.4. Water flow

Using Flow System 2 above, no positive responses were gained when copepodids were exposed to various flow inputs, all moving or being taken "downstream" in the current.

When copepodids were exposed to a fast, directional flow input from a pipette, they executed rapid burst-swimming, often accompanied by "looping" or "spiralling" behaviour composed of what appeared to be tight turns in the vicinity of the stimulus. Some copepodids entered the pipette during the course of such manoeuvres and although it is possible that this occurred through accidental suction it was judged that this was not the case in these instances. On removal of the flow stimulus, behaviour returned to normal almost immediately.

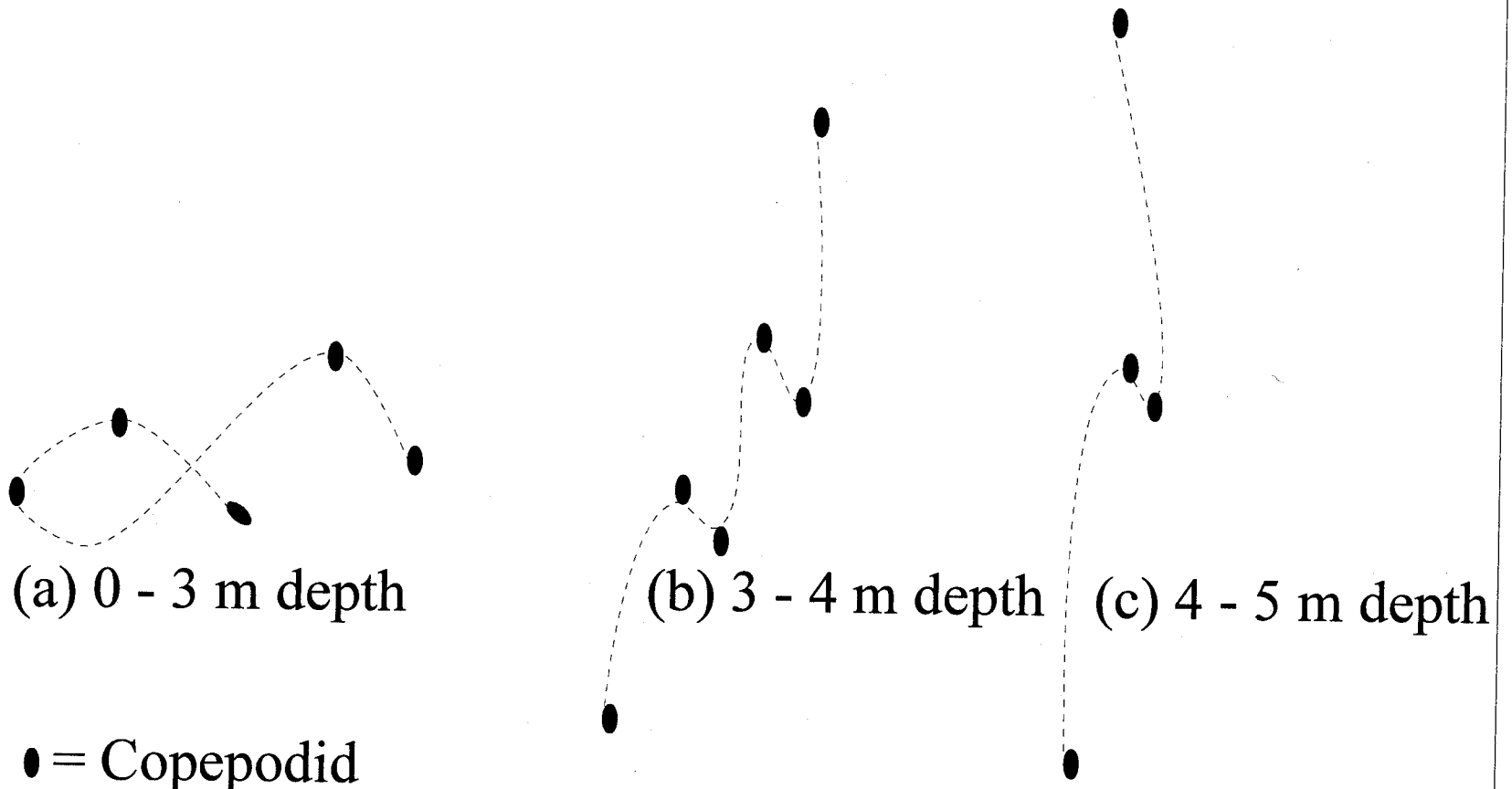
3.4.5. Pressure Response

Whilst setting up the manometer, large numbers of copepodids swam to the surface and became caught in the mesh, suggesting that even the 1.2 m head of the test compartment was enough to cause some degree of geonegative pressure response. Of those copepodids left, a further number stayed, swimming feebly or not at all, in the near vicinity of the bottom of the compartment (bottom 5 cm) and were therefore considered morbid for the purposes of this experiment.

Increase of head of water between 0 and 3 m generated little instant response in the copepodids which showed what appeared to be a holding pattern of sinking passively and then swimming upwards in short 1-2 cm bursts. Over time (5 mins+) a gradual vertical movement was apparent (Figure 3.23a).

At 3-4 m an upward swimming trend was clearly apparent with copepodids swimming upward in 3 cm bursts with short refractory periods between. A fall in head of water was manifested by return to normal sink / swim behaviour with a falling trend (Figure 3.23b).

Figure 3.23 Patterns of copepodid response to pressure stimuli



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 very short refractory periods between (Figure 3.23c).

Increased and decreased pressure therefore resulted in a distinctly active geonegative and passive geopositive responses respectively. Larger pressure changes resulted in more active upward (geonegative) responses.

3.4.6. Electrical Response

No change in copepodid behaviour was noted in response to any of the tested currents. It must therefore be concluded that the copepodids showed no observable response to the electrical fields tested.

3.4.7. Mechanical Stimulation

Mechanical stimulation using the fan did not cause observable responses in copepodid (or nauplius) stages. Stimulation using the glass rod caused the copepodids to dart forward (< 1 cm) at each stimulation, this movement being more pronounced in response to the falling 500 g weight. Stimulated swimming consisted of rapid vertical or horizontal bursts of swimming often interspersed with looping or spiralling events. Nauplius stages exposed to the same mechanical stimuli for comparison responded by swimming upwards. No diminution of the copepodid response was seen during repeated stimulation.

3.4.8. Sensory structures

3.4.8.1 Integumental sensory organs

A number of integumental organs were seen to be arranged bilaterally over the dorsal surface of the cephalothorax and free pedigerous somites although they were apparently absent from the ventral surface (Figure 3.24). These organs were innervated by cholinergic nerves (Plate 3.1) and corresponded closely to those described previously in crustacea as hair (Plate 3.2) and peg (Plate 3.3) sensilli. The dorsal surface of the cephalothorax had 20 sensilli (10 symmetrical pairs) of which 8 pairs were single sensilli and 2 pairs were double sensilli (Figure 3.24). A further pair of single sensilli was found on either side of the midline of the second abdominal somite and a final pair of paired sensilli was located to either side of the midline on the fourth abdominal somite. At the posterior corners of the rostrum, a further pair of sensilli

Figure 3.24 Diagrammatic representation of the distribution of integumental organs over the dorsal surface of the copepodid

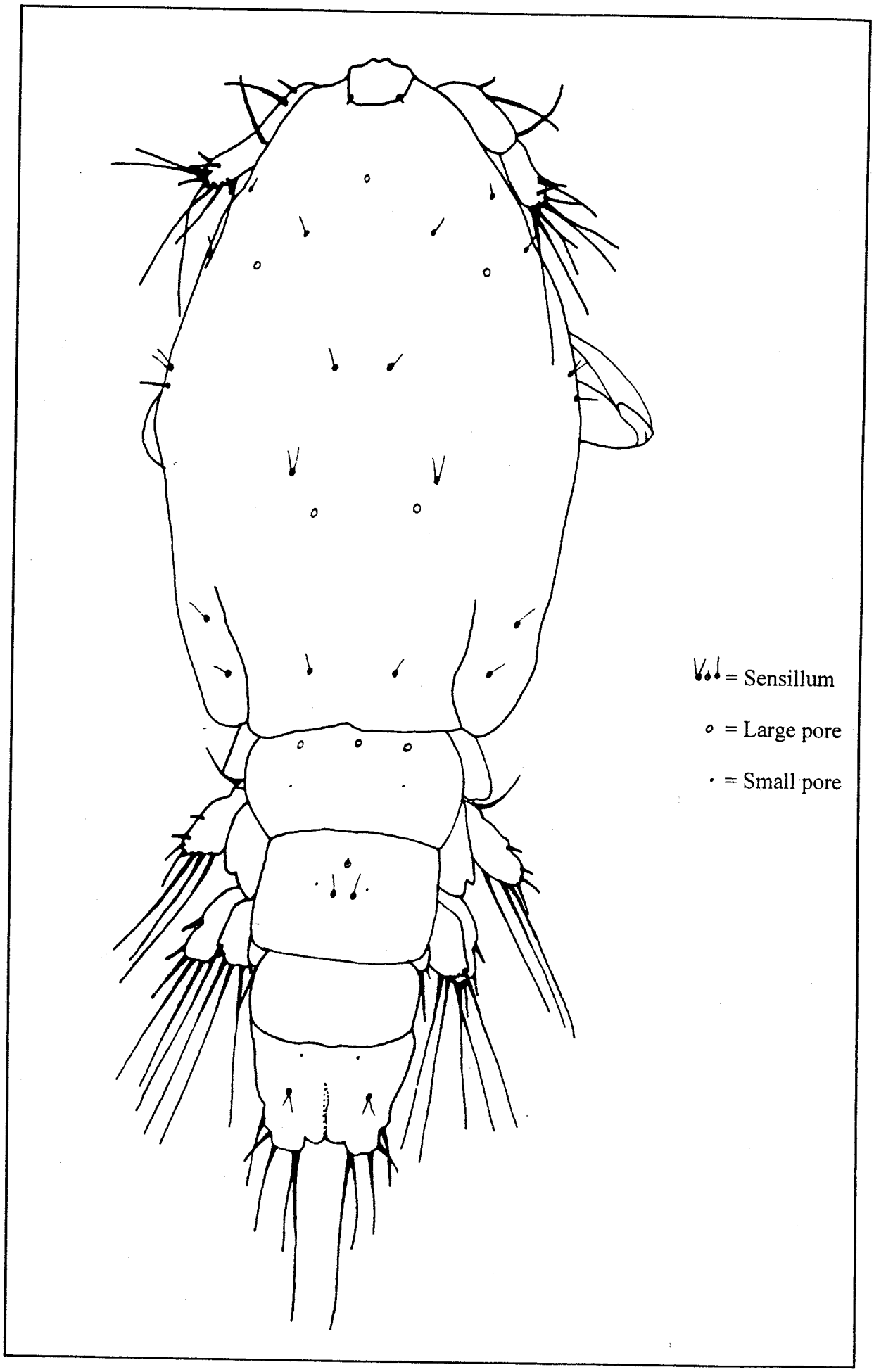


Plate 3.1 Hair sensillum of copepodid stained with acetylthiocholine iodide to show innervation associated with the base (whole mount). c: cuticle; b: basal disc; n: nerve; hs: hair sensillum. Scale = 20 μm

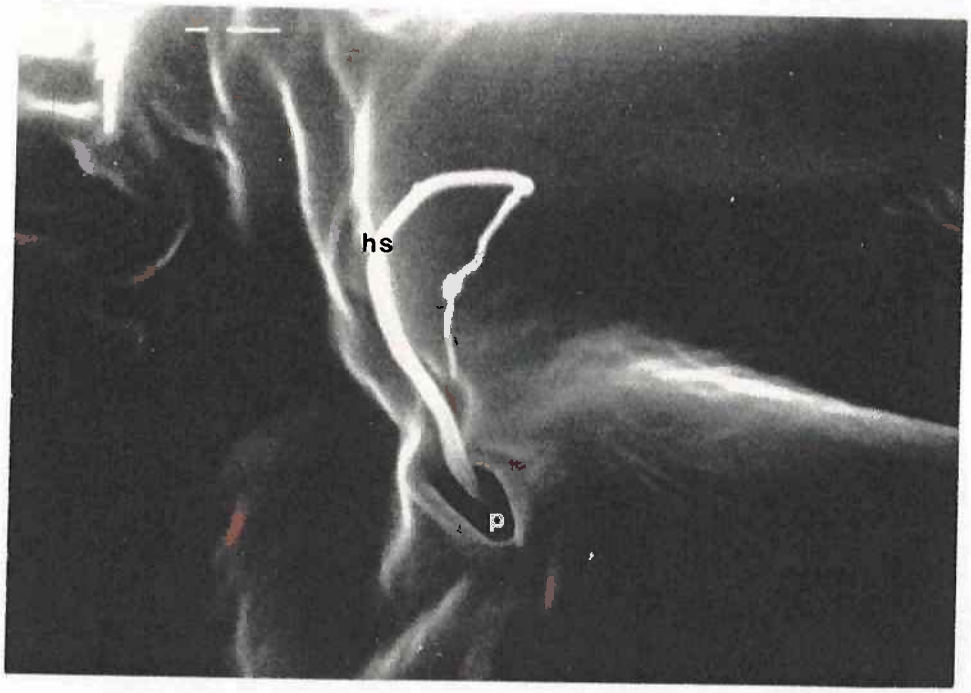


Plate 3.2 Hair sensillum protruding through pore in cuticle (SEM). p: pore; hs: hair sensillum. Scale = 1μm

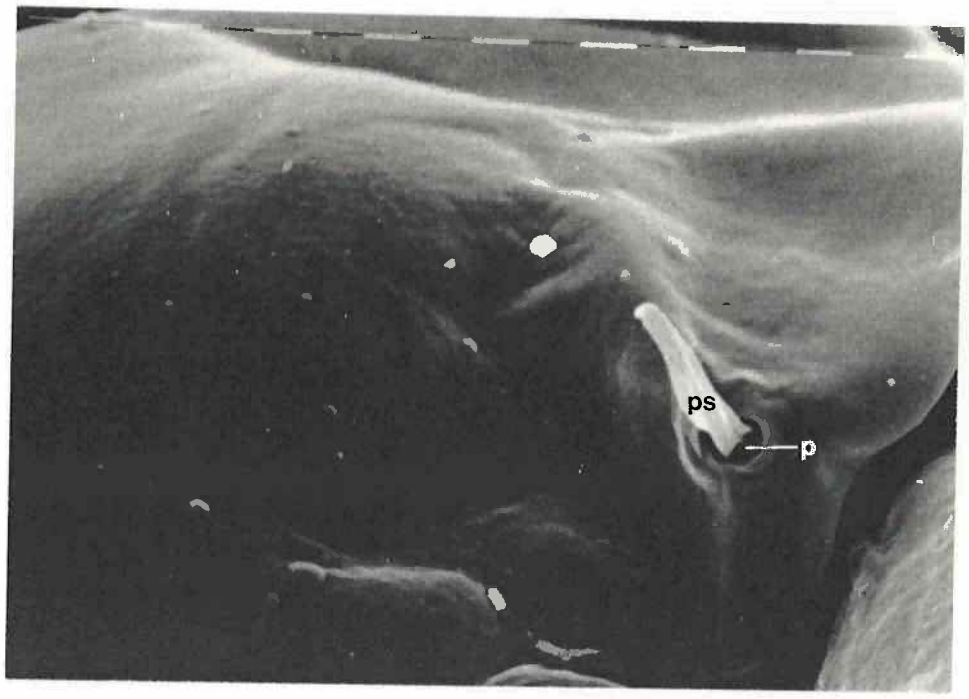


Plate 3.3 Peg sensillum protruding from cuticular pore (SEM). p: pore; ps: peg sensillum. Scale = 1μm

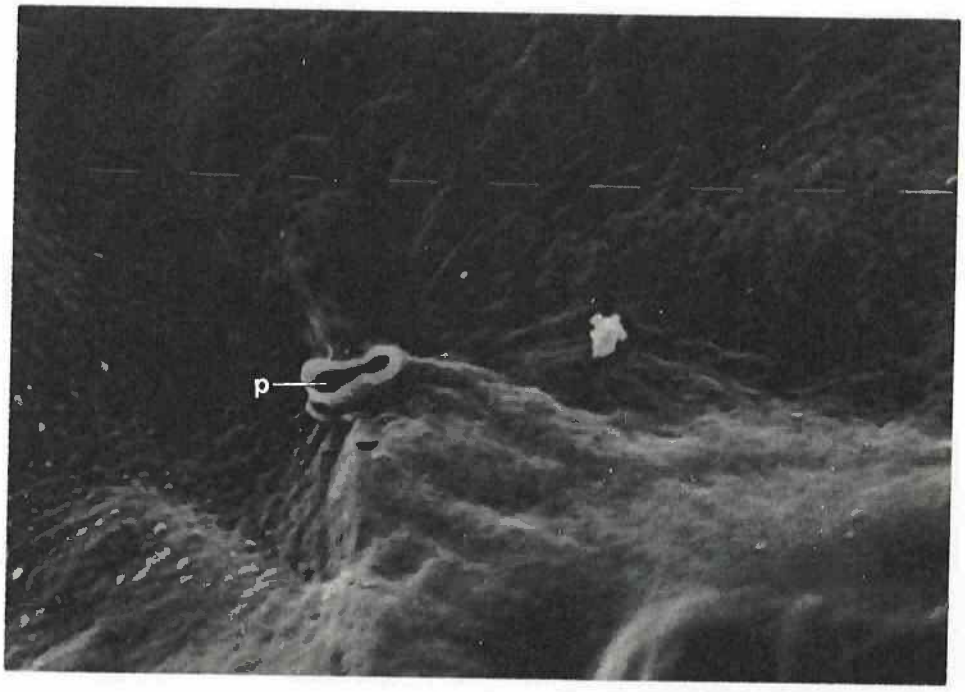


Plate 3.4 Large integumental pore with raised periphery (SEM). p: pore. Scale = 1μm

were located.

In addition to these sensilli, there were a number of pores penetrating the dorsal and ventral integument (Figure 3.24, Plate 3.4).

3.4.8.2 Antennules

The antennules are the primary sensory interface of copepods and have been described in detail for copepodids of *L. salmonis* by Gresty *et al.*(1993) precluding the necessity for a detailed morphological description in the present study.

In general terms, the antennules of the copepodid of *L. salmonis* were large and well-innervated and comprised a large basal segment which carried three anteriorly directed setae and a distal segment equipped with 13 elements. These latter comprised 2 aesthetascs, 5 branched setae, 3 short unarmed and 3 long unarmed setae and in addition, there was a single tube pore (see Gresty *et al. 1993*). In live copepodids, the antennules were held perpendicularly to the sagittal plane with both segments lying within the sphere of a forward projection of the cephalothorax but with the elements of the distal segment projecting without (Plate 3.5). The large antennal nerves passed forward to the antennules from the deuterocerebrum.

3.4.8.3 "Cephalic receptors"

Paired lateral nerves passed forwards to either side of the lateral ocelli of the nauplius eye. These nerves abutted the pigment cells of the eye but showed no connections to the eye itself. The nerves apparently derived from the protocerebrum and passed forwards running laterally and dividing into two nerves. Many fibres ran forward from one of the nerves and terminated anteriorly in an area containing a number of intracellular spaces within the cuticular epithelium. The exact terminations of the fibres were not possible to determine under the light microscope and were not seen under TEM.

The termination of the second nerve could not be observed under the light microscope but under TEM the terminating organ corresponded to a component of the "Organ of Bellonci" described for other copepod species (Boxshall 1992). The anterior part of the nerve subdivided into two bundles of closely-packed cilia (Plate 3.6). These bundles were enclosed by a single large supporting or glial cell which followed them anteriorly. Although this latter was convoluted and appeared in places to have dividing membranes, only a single nucleus was observed and hence it was believed to comprise a single cell (Plate 3.7). The two ciliary bundles were partitioned from one another by a branch of this cell which was thickest at its posterior end and tapered markedly towards its apex (Plate 3.7). This cell, which entirely enclosed the ciliary

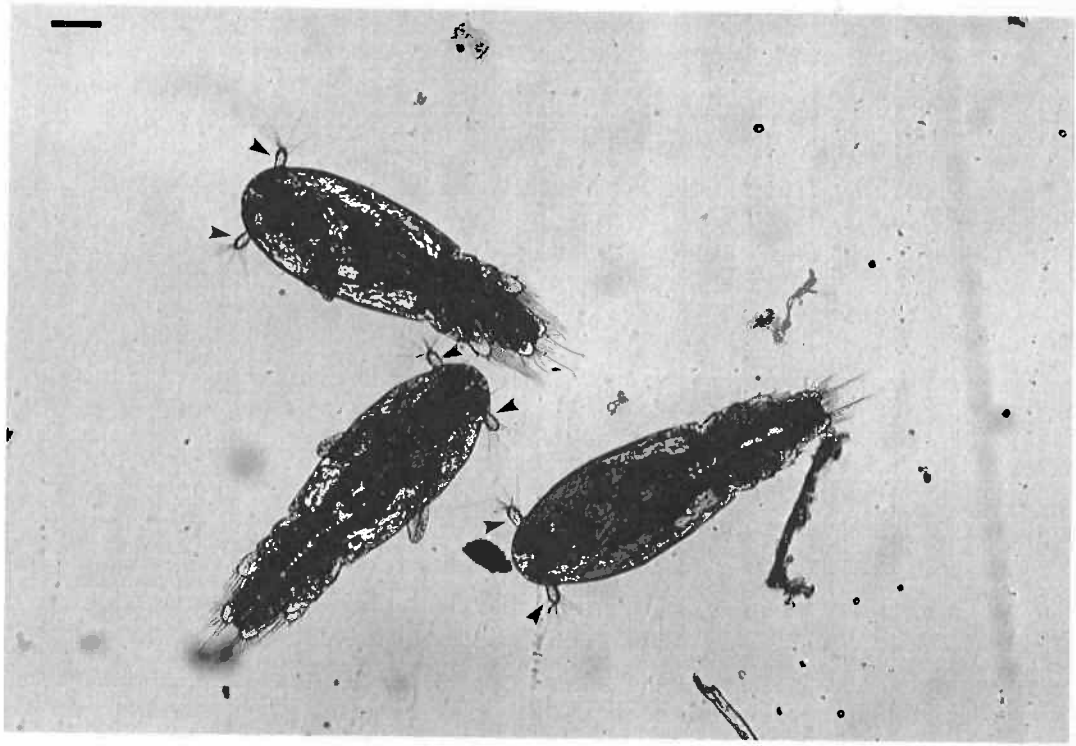


Plate 3.5 Copepodids showing orientation of antennules in swimming. Antennules are held perpendicularly to the direction of movement with elements of the distal segment extending beyond the width of the cephalothorax (live copepodids). Antennules arrowed. Scale = 100μm

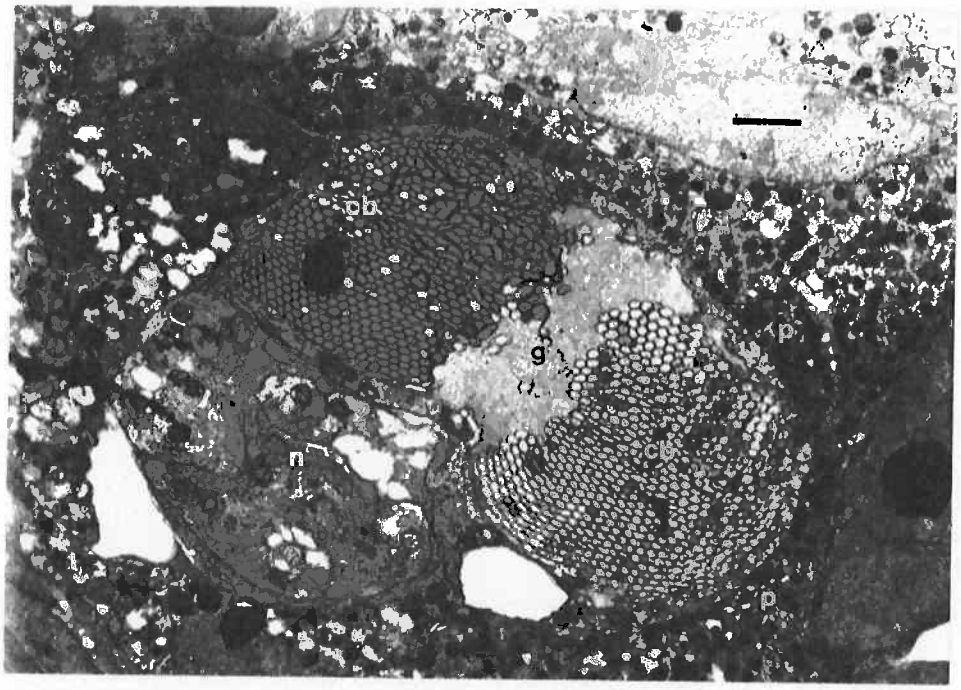


Plate 3.6 Paired ciliary bundles of deep cavity receptor of Organ of Bellonci with glial cell, nerve and surrounding pigment cells. cb: ciliary bundle; g: glial cell cytoplasm; n: nerve; p: pigment cell with osmiophilic crystalline vesicles. Scale = 1μm

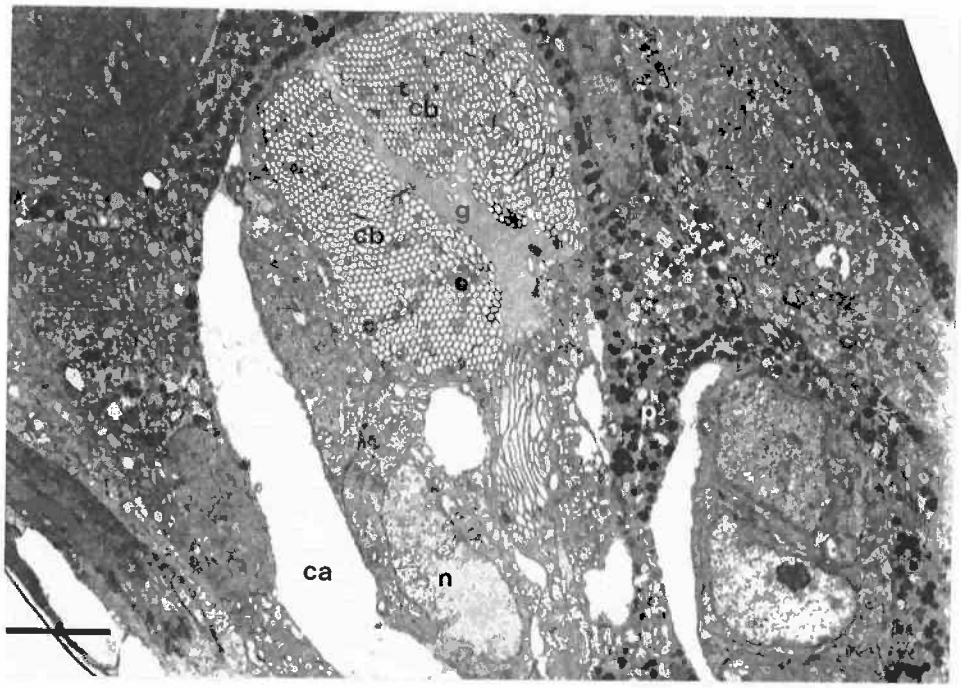


Plate 3.7 Transverse section through anterior area of the deep cavity receptor of the copepodid Organ of Bellonci. ca: cavity (actual or artefactual); cb: ciliary bundle; g: glial cell cytoplasm; n: nucleus of glial cell; p: pigment cell. Scale = 2μm

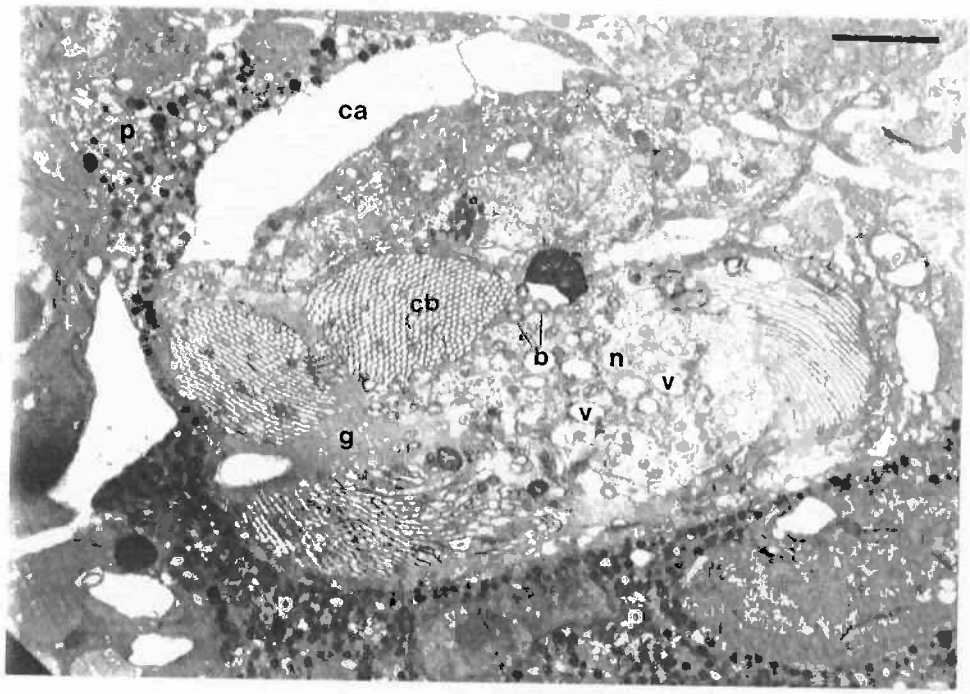


Plate 3.8 Area of origin of ciliary bundles of the deep cavity receptor of the copepodid Organ of Bellonci. b: basal bodies; ca: cavity (actual or artefactual) cb: ciliary bundle; g: glial cell cytoplasm; n: nerve; p: pigment cell; v: electron-lucent vesicle. Scale = 2μm

bundles, was in turn surrounded by a number of large pigment cells corresponding in morphology to those of the nauplius eye (see below). The zone of maximal development (greatest areal coverage) of these ciliary bundles corresponded in transverse section to the area of origin of the antennae / antennules which is to say, posterior to the rostrum. At this point the organ lay at a depth of 6.5 μm from the dorsal cuticular surface.

In the area where the nerve initially subdivided, its cytoplasm contained large numbers of mitochondria and quantities of smooth endoplasmic reticulum and large electron-lucent vesicles (Plate 3.8). The base of each cilia followed the 9 + 0 pattern of double microtubules described for other copepods (Elofsson 1971, Dudley 1972) and these microtubules passed up the cilia in an irregular fashion and declined in number to leave a single microtubule at the distal apex (Plates 3.9, 3.10). The cilia tapered towards their distal extremities, measuring 0.37 μm at their base and 0.12 - 0.051 μm at their tips (measurements taken from circular cross-sections only). Many cilia displayed "myelin figures" but these were likely to be artefactual (Plate 3.10).

The glial cell totally enclosed the cilia, surrounding each individual cilium and partitioning it from its neighbours. The cytoplasm of the cell surrounding the cilia was more electron-lucent than that of surrounding cells and was highly granular and apparently without obvious organelles (Plate 3.10). The cytoplasm of the part of the cell lying peripheral to the ciliary bundles was more electron-dense and was packed with mitochondria and endoplasmic reticulum. In many sections there was a large, apparently empty, space between the glial cell and enclosed cilia and the surrounding pigment cells (Plates 3.7, 3.8). The erratic appearance and the fact that the contours of the space matched those of the glial component within it, suggests that it may have been artefactual. The cilia were apparently totally enclosed by the glial and pigment cells and were not observed to pass outside them.

In addition to the above cephalic receptors, a pair of ciliary bundles were observed under TEM at either side of the dorsal aspect of the rostrum. It was not, however, found to be possible to determine either their area of termination or their provenance. As in the previously described organ, the cilia contained numbers of microtubules. The maximum diameter of these cilia was 0.13 μm and the minimum was 0.053 μm. These measurements correspond closely to those of the aforementioned organ. The location of these bundles was close to the area where the setae were observed on the rostrum under the light microscope. These setae, however, could not be located in the TEM sections examined.

3.4.8.4 "Cauliflower organs"

These complex organs have not been previously described and were therefore named after their gross appearance under SEM. The "cauliflower organs" had a cuticle which was

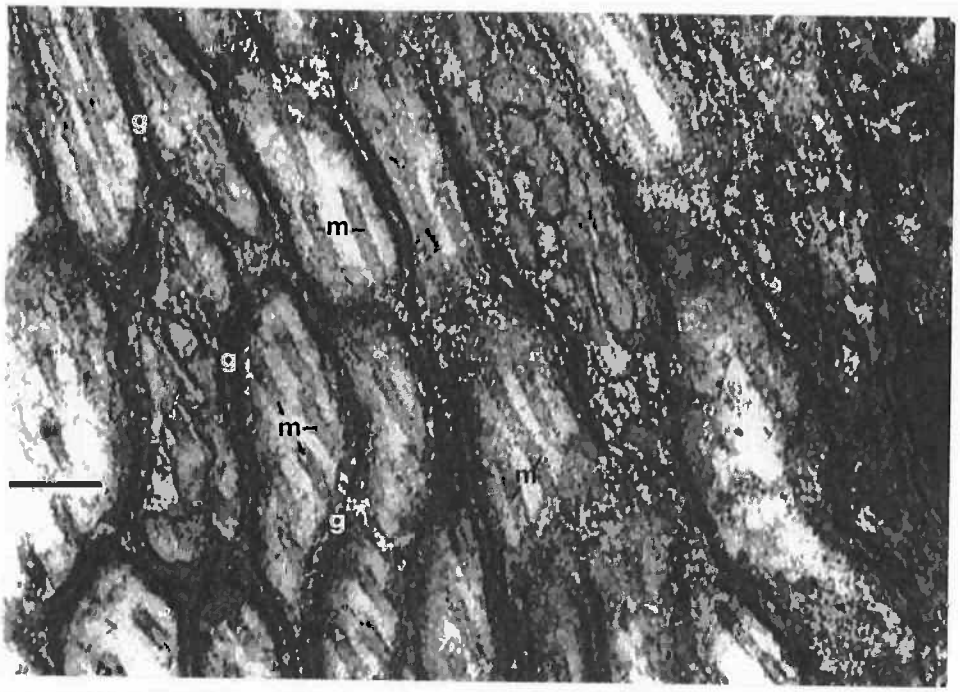


Plate 3.9 Microtubules within the cilia of the deep cavity receptor of the copepodid Organ of Bellonci (TEM, oblique section of cilia). m: microtubules; g: inter-ciliary glial cell ramifications. Scale = 0.1μm

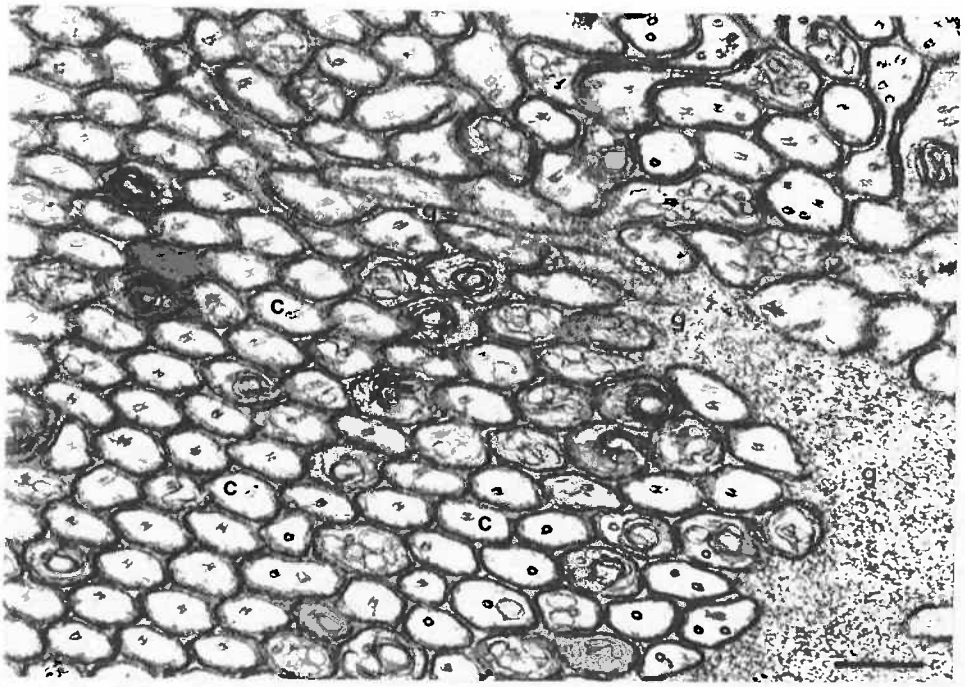


Plate 3.10 Cilia of deep cavity receptor of Organ of Bellonci showing single microtubules in distal part of cilia. Also shown is the glial cell with ramifications surrounding the cilia (TEM, transverse section of cilia). c: cilium with single microtubule. g: glial cell. Scale = 0.25μm

elaborated into a series of ornate knobs resembling florets which were observed under both SEM and TEM (Plates 3.11, 3.12). These organs were located to either side of the base of the oral cone, slightly anterior to the maxillules. They were observed to be present not only in copepodids of *L. salmonis* but also in *C. elongatus*. Under SEM, as well as the abundant ornamentations, it was possible to observe a single element which may be a sensillum although its identity is uncertain at present. Under TEM the organ was seen in transverse section to project ~ 8.5 μm below the ventral body surface thus potentially bringing it into contact with the host substrate in the settled (antenna-attached) copepodid (Plate 3.11, 3.12). Whilst the nearby ventral body cuticle was 0.34 μm thick, the cuticle covering the ornamentation of this organ was extremely thin, measuring 0.014 μm or less (Plate 3.13). The tips of the many of cuticular extensions were often swollen and often contained membrane inclusions, sometimes having the appearance of endoplasmic reticulum (Plate 3.13). The sensillum seen under SEM was not observed using TEM.

The most notable features of these organs, aside from the cuticular ornamentation, were the large bundles of tonofibrils that passed into the cuticular extensions and provided the ventral attachment insertions of a large oblique striated muscle (Plate 3.12, 3.14).

Within the cytoplasm of the organ, in addition to large quantities of tonofibrils / microtubules, mitochondria, vesicles and free ribosomes, there were also membrane-bounded channels that may represent ducts (Plate 3.15). The placement of these organs was such that they may be the recipients of the product of the "mucoid gland" described in Chapter 5 whose presumed external pores could not be elucidated under SEM.

3.4.8.5 Light sensors

Median nauplius eye

The copepodid was equipped with a large median nauplius eye composed of two lensed dorsolateral ocelli and a smaller ventral ocellus. The lensed dorsolateral ocelli were highly visible in the live copepodid, showing a red pigmentation and giving "cats-eye" reflection when viewed at an appropriate angle (Plate 3.16). The eye was located near the dorsal surface and abutted the cerebrum posteriorly and mucous glands anteriorly. Dorsally it lay just below the cuticle. The transverse diameter of each dorsolateral ocellus (excluding pigment cells) was 37.5 μm. The dorsoventral measurement is 34 μm. The transverse diameter of the ventral ocellus is 30 μm and the dorsoventral measurement was 17.5 μm. The whole organ measures 74 μm in transverse diameter and 45 μm dorso-ventrally.

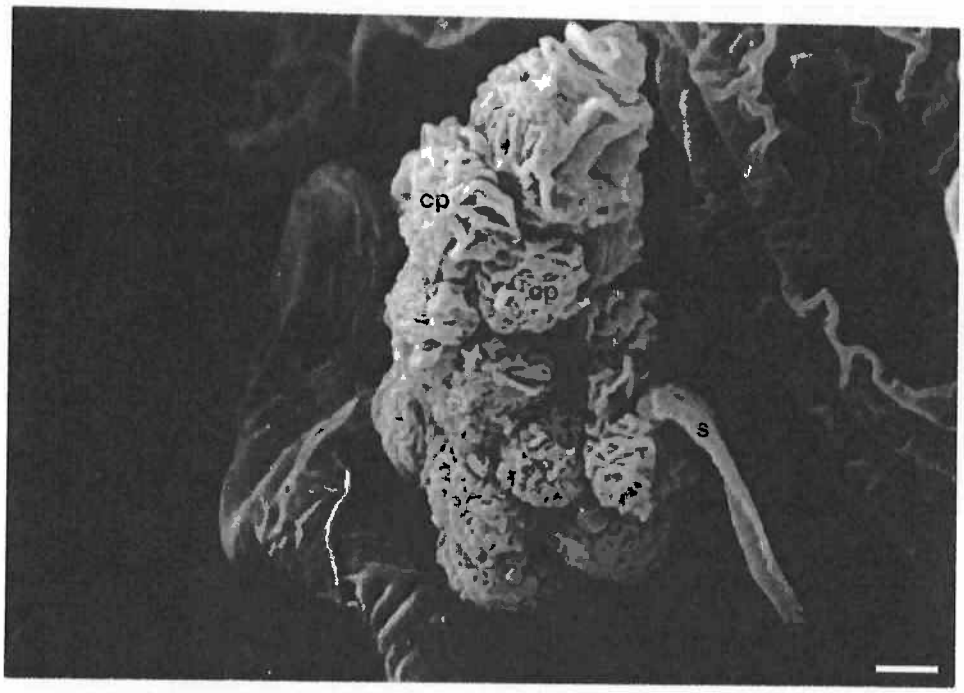


Plate 3.11 cauliflower organ showing elaborate cuticular projections and single sensillum protruding from organ (SEM). cp: cuticular projections; s: sensillum. Scale = 1μm

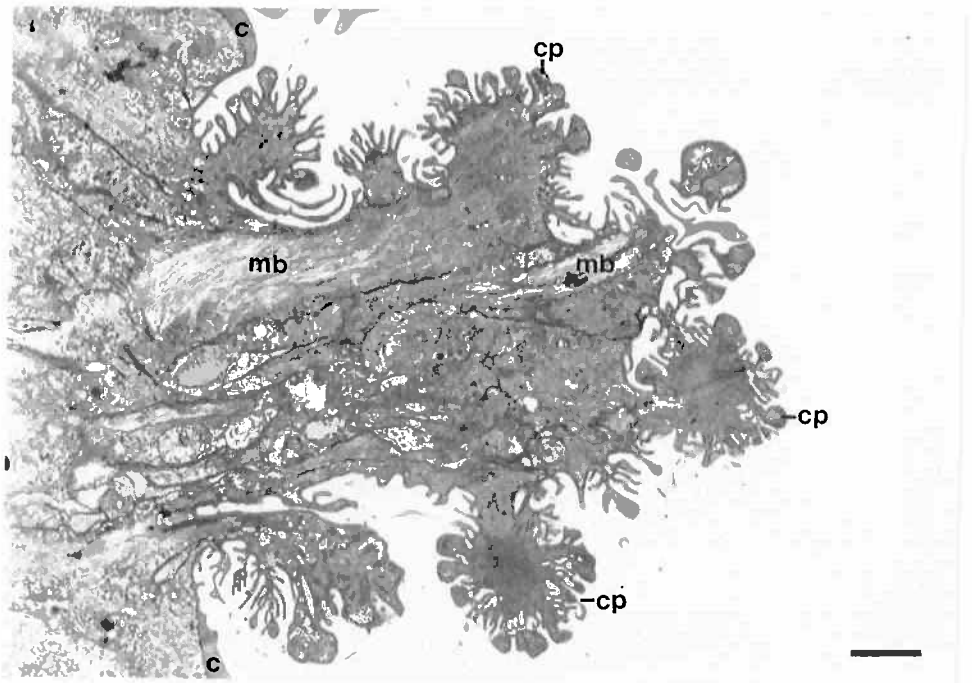


Plate 3.12 Transverse section of cauliflower organ showing microtubule insertions and elaborate cuticular projections with swollen distal apices (TEM). c: normal thickness ventral cuticle; cp: cuticular projections; mb: microtubule bundle. Scale = 1μm

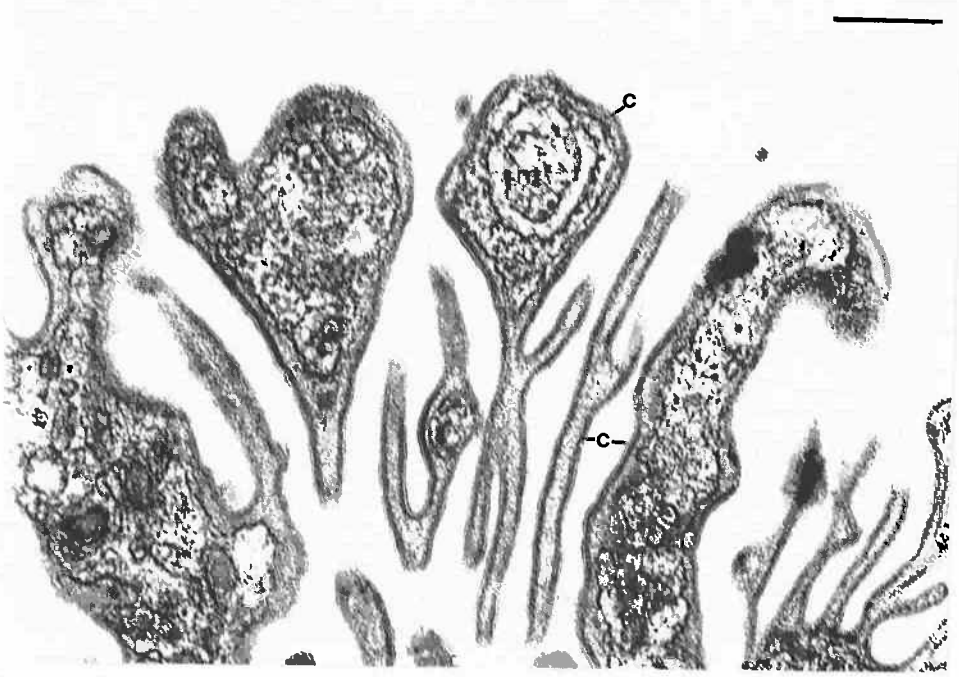


Plate 3.13 Transverse section of cuticular projection of cauliflower organ showing extremely thin cuticle and membraneous inclusions within the swollen distal apices (TEM). c: cuticle; m: membraneous inclusion. Scale = 0.25μm

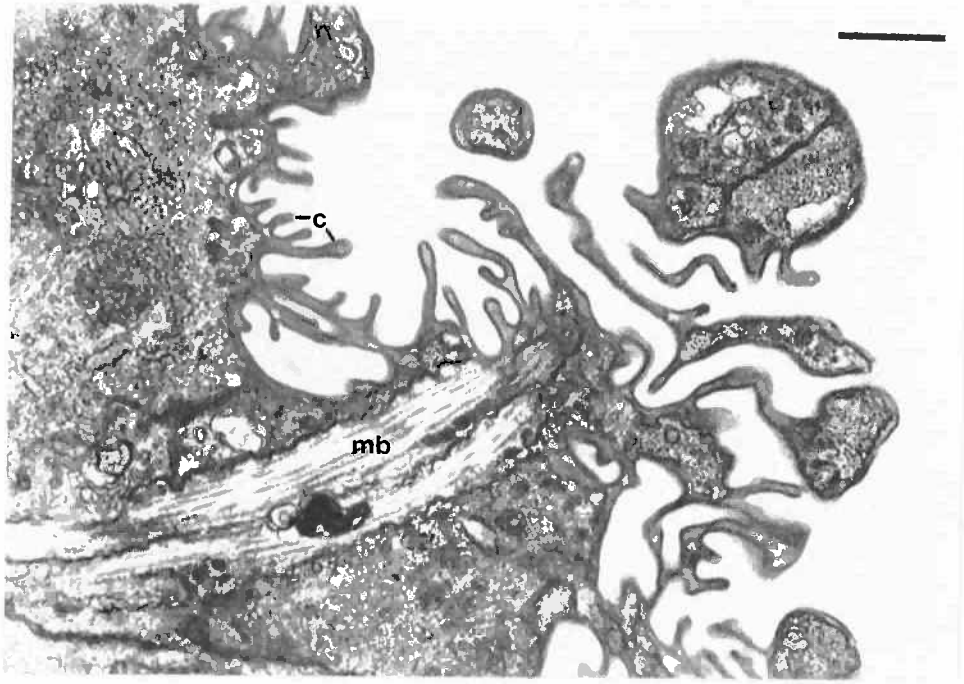


Plate 3.14 Insertion of microtubule bundle on cuticle of cauliflower organ (TEM). c: elaborate cuticle; mb: microtubule bundle. Scale = 0.5μm

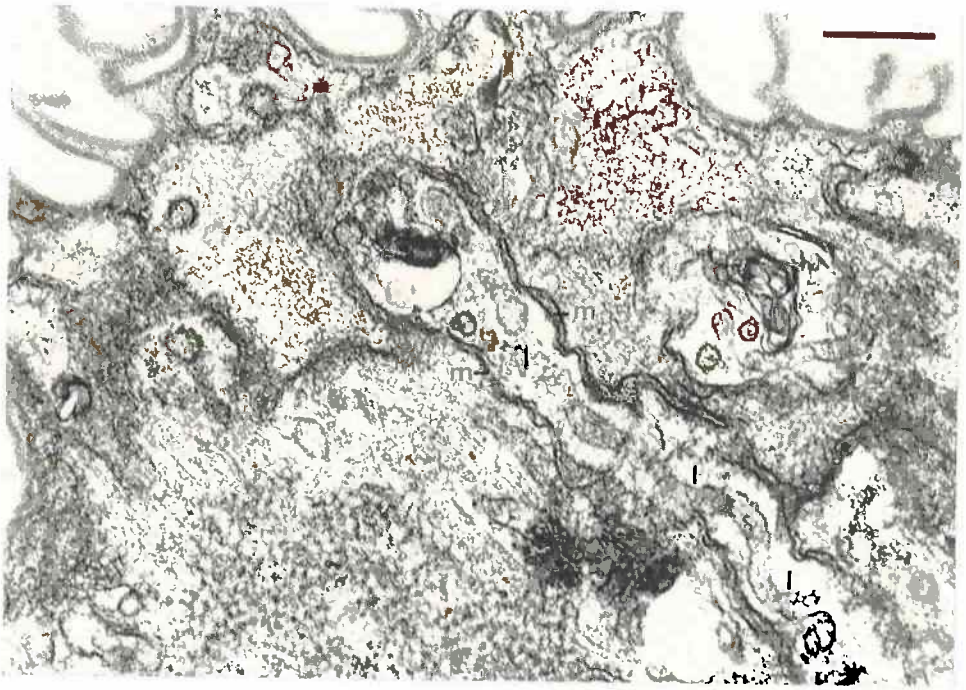


Plate 3.15 Possible secretory channel in cauliflower organ (TEM). l: channel lumen; m: double membrane of channel walls. Scale = 0.25μm

Plate 3.16 Dorsolateral ocelli of copepodid nauplius eye showing the lenses (arrowed) and the red pigmentation of the pigment cells (p) which surround the eye (unfixed whole mount). Scale = 40μm

The eye was surrounded by pigment cells. The number of pigment cells could not be accurately determined due to a lack of full serial TEM sections but from the light microscope observations, a minimum of four pigment cells was present - two anteriorly and two posteriorly, these surrounding the whole eye Plate 3.17). There was a gap between the tips of the anterior and posterior cells that left an unpigmented window above the lens of each dorsolateral ocellus. At its widest, this window was 22 μm and acted as a fixed aperture through which light entered the dorsolateral ocellus (Plate 3.18). The pigment cells abutted adjacent organs but were separated from them by the cell membrane.

Each dorsolateral ocellus was bounded by a capsule of 5 thin conjunctival cells with discoid nuclei and possessed 2 tapetal cells at the posterio-ventral extremity. Within the capsule were 9 retinular cells and a dorsally situated antero-lateral lens (Plate 3.18).

The ventral ocellus appeared bounded by 3 conjunctival cells and possessed 2 tapetal cells and 10 retinular cells. The tapetal cells lay anterior to the retinular cells. The ventral ocellus had no lens.

Within the pigment cells were large numbers of vesicles. These were apparently bounded by a single membrane and often had a highly osmiophilic content. Within the osmiophilic material, irregular needle-like crystals could often be observed (Plate 3.19). The nuclei of the pigment cells were found at their outer periphery. The vesicles appeared to form a sequence, with some vesicles containing a non-crystalline, fairly homogeneous electron-dense material and others containing crystals in all stages of development.

The tapetum of each dorsolateral ocellus was parabolic and opposed the lens starting 15 μm ventral to the dorsal surface of the ocellus and covering the opposing face of the ocellus as far as its ventral surface (Plate 3.20). The tapetum comprised two tapetal cells which met in the sagittal (longitudinal) plane of each ocellus. One of the two ovoid nuclei was positioned centrally and the other at the anterior extremity of the tapetum. The maximum diameter of the tapetal nuclei was 4.9 μm. The tapetum of the ventral ocellus began below the level of the dorsal surface of the ocellus and faced posteriorly (ie lies at the anterior edge of the ocellus) but more ventrally the tapetal cells surrounded the ocellus (meeting in the sagittal plane) and ventrally underlying it. The tapetum of the ventral ocellus was composed of two tapetal cells whose nuclei lay to either side of the sagittal plane of the ocellus. The distance from one rim of the tapetum of the dorsolateral ocellus to the other (maximum) was 30 μm with a maximum depth of 7.25 μm.

About half the depth of the tapetum was filled with reflective plates and the membranes that held them. These plates were rarely seen within the membranes (see discussion) and so their mean dimensions and orientations have been calculated from the spaces left by them. Each plate had an average size of $0.51^2 \times 0.14$ μm. The interstices were 0.1 μm thick and contained dark-

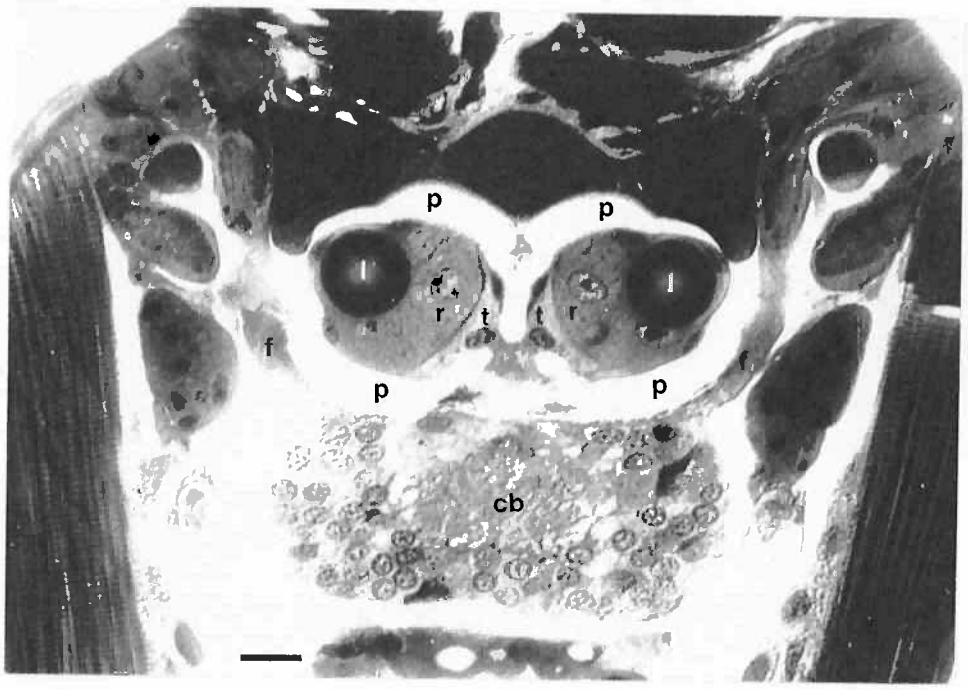


Plate 3.17 Plane section of dorsolateral ocelli showing ocelli and shielding pigment cells. Note the frontal nerves that supply the Organ of Bellonci passing to either side of the nauplius eye (LMH, stained PAS / Heidenhain's / Fast red). cb: cerebrum; f: frontal nerve; l: lens; r: retinular cells; p: pigment cells; t: tapetum. Scale bar = 10μm

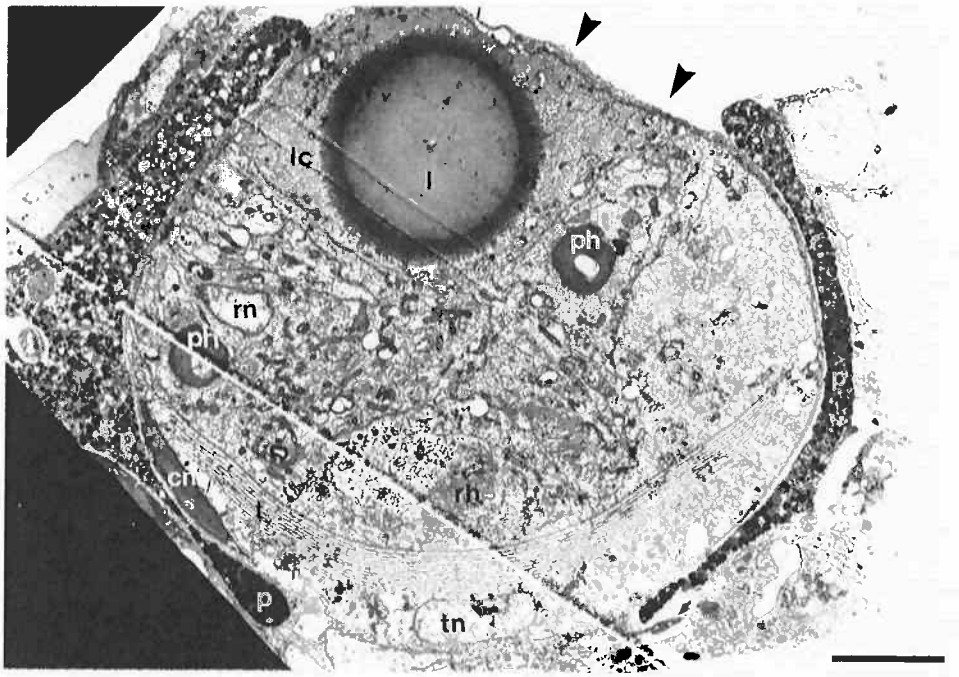


Plate 3.18 Transverse section of dorsolateral ocellus showing dorsolateral aperture between pigment cells (arrowed) and prominent dorsal lens (TEM). cn: conjunctival cell nucleus; l: lens; lc: lens cell p: pigment cell; ph: phaeosome; rn: retinular cell nucleus; rh: rhabdom; t: tapetum; tn; tapetal cell nucleus. Scale = 4μm

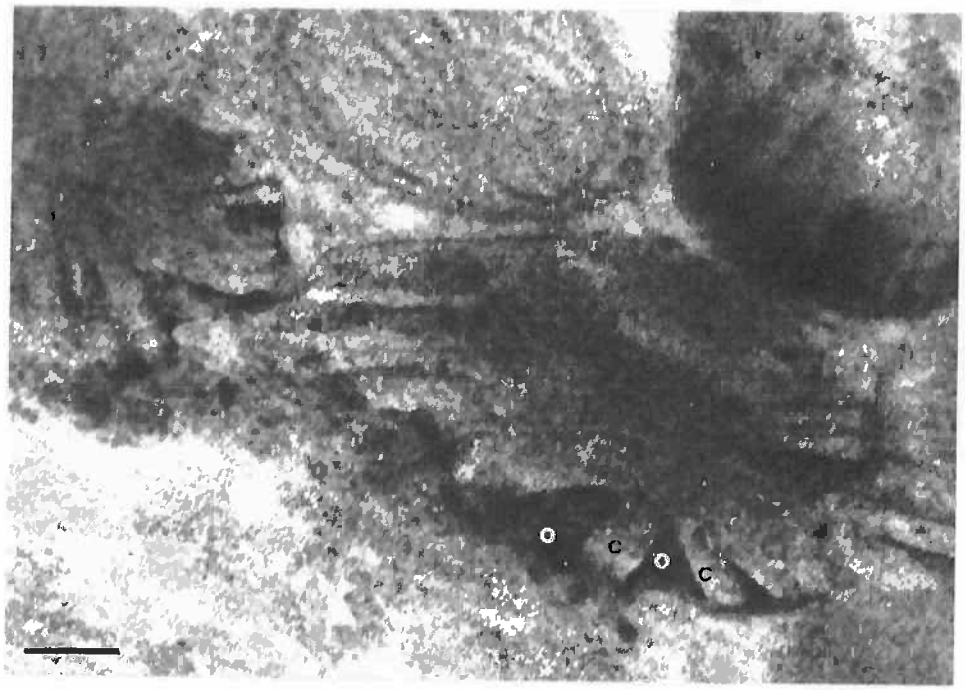


Plate 3.19 Pigment vesicles of pigment cell showing osmiophilic contents and needle-like crystalline inclusions (TEM). c: crystalline inclusions o: osmiophilic contents. Scale = 0.05μm

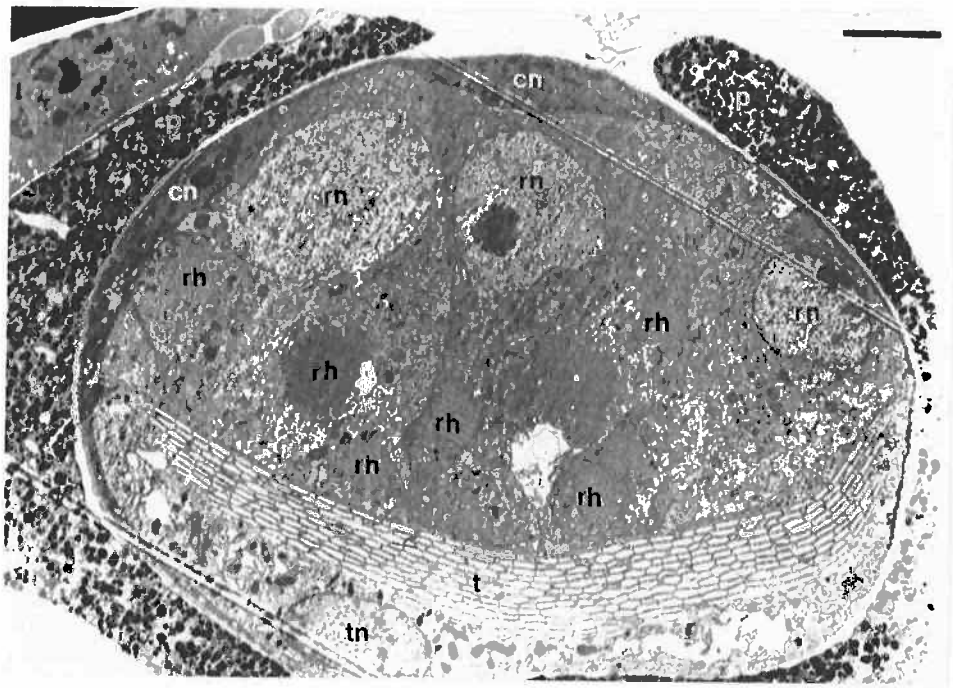


Plate 3.20 Transverse section through dorsolateral ocellus showing rhabdoms arranged in different orientations (TEM). cn: conjunctival cell nucleus; p: pigment cell; rn: retinular cell nucleus; rh: rhabdom; t: tapetum; tn; tapetal cell nucleus. Scale = 3μm

staining cytoplasm. The plates were arranged in rows (~11 rows at deepest point); successive plates being fairly well aligned in the posterior rows but more staggered at the surface. At the lateral edges of the tapetum were smaller plates clearly in the course of production. These latter were not well aligned with the tapetum and were haphazardly positioned (Plate 3.21). Along the lateral edges of the tapetum, the plates were staircased along the diagonal edge. The non-reflective part of the tapetum contained a granular cytoplasm with large numbers of mitochondria and large irregularly-shaped vesicles containing lighter staining material.

The dorsolateral ocelli each contained 9 retinular cells. These abutted against the tapetum at their bases and tapered towards the opposite face of the ocellus. The cell nuclei were found in close proximity to the lens and had maximum diameters of 7.1 μm with prominent nucleoli. The surfaces nearest the tapetum formed a microvillar border (rhabdom). The largest number of microvilli observed in a single section through a rhabdom was 52 with a maximum observed length of 2.6 μm. Microvilli were often observed to contain electron-dense particles although the identity of these was not determined. Sections through the microvilli showed them to be arranged in different orientations relative to the anterodorsal face of the ocelli (Plate 3.22) but principally to lie perpendicular to the lens and therefore to the light path. At the apparent origin of the rhabdomeres, there was often an intracellular space containing large irregular vesicles surrounded by membrane. Within these spaces the cytoplasm showed little staining save for some fine granular aggregations. These spaces correspond to the "bladders" of earlier studies. Within the cytoplasm of the retinular cells were large quantities of endoplasmic reticulum as well as mitochondria and free ribosomes. Tight whorls of membrane which have been termed previously "phaeosomes" (e.g. Fahrenbach 1964) were also prominent in some sections of the dorsolateral ocelli (Plate 3.23). These phaeosomes measured up to 2.4 μm across and were generally curved into a tight arc which was apparently fully closed in some instances (Plate 3.18). Phaeosomes were often associated with quantities of endoplasmic reticulum in neighbouring cytoplasm (Plate 3.23).

Lack of serial TEM sections means that the cell boundaries were often hard to follow but serial sections from the light microscope suggested the following arrangements of retinular cells (Plate 3.24). Moving ventrally from the dorsum of the dorsolateral ocellus there were four planes of cells. The first plane had a single cell with its nucleus lying above and slightly behind the anterior lens. The second plane had two cells with their nuclei below and to either side of the nucleus above. The third and largest plane had four cells with two of the nuclei behind the lens and two to either side of its posterior edge. The final two cells lay below the lens and had nuclei that were more anteriorly positioned. In effect, the nuclei formed a half-bowl or basket around the lens (Plate 3.24) with the major part of the cells lying behind them. Within the

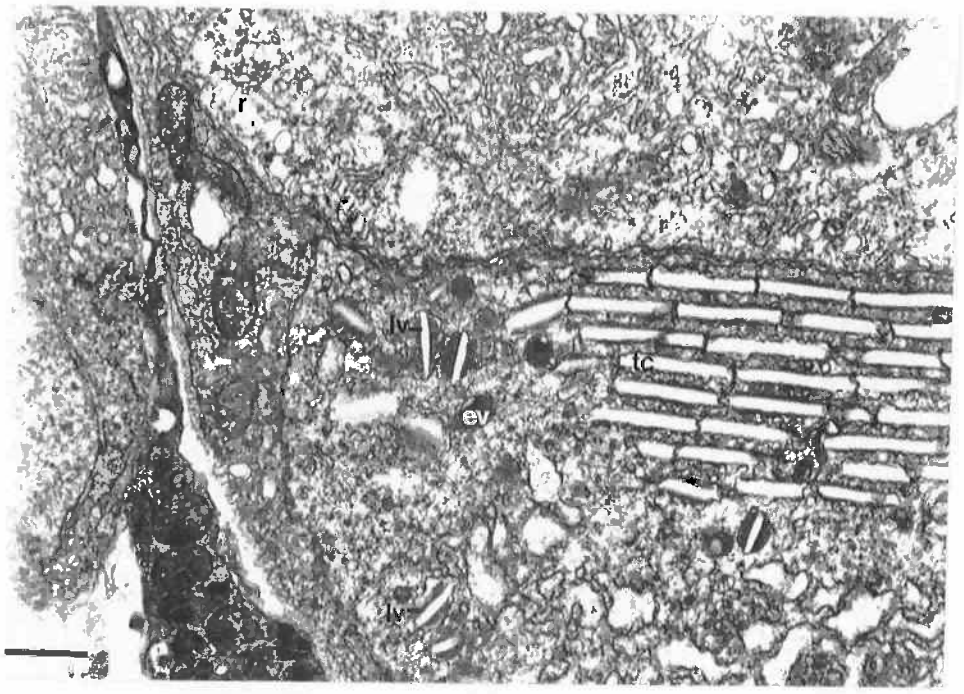


Plate 3.21 Section through edge of tapetum showing production of tapetal crystals within vesicles in the tapetal cell cytoplasm (TEM, transverse). ev: early crystal vesicle without crystal; lv: late crystal vesicle with forming crystal; tc: tapetal crystal; r: retinular cell. Scale = 0.5μm

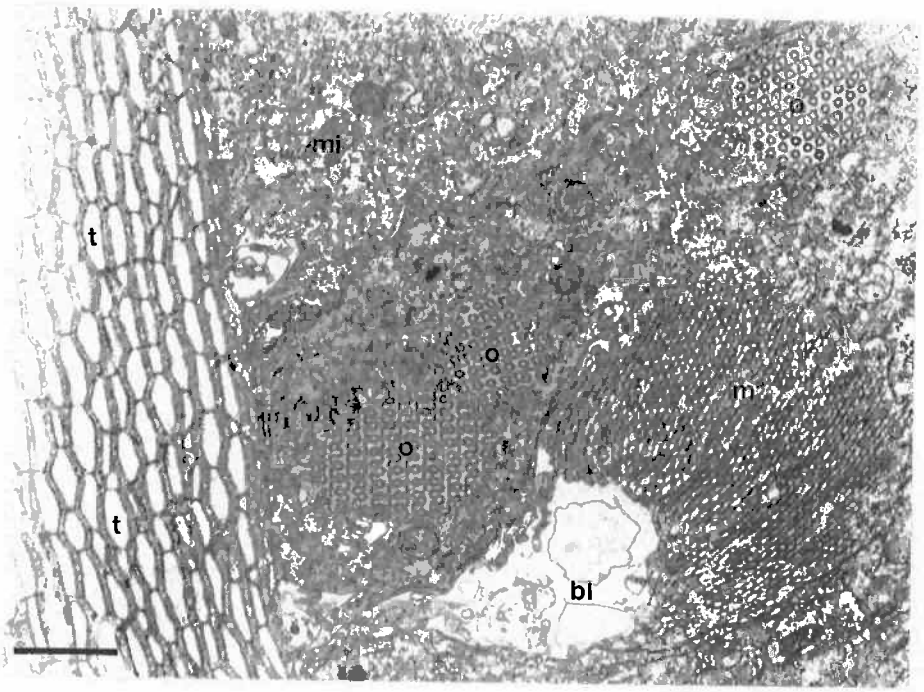


Plate 3.22 Section through rhabdoms showing varying orientations of microvilli (o,m) and "bladder" at base of microvilli (TEM, transverse). bl: "bladder"; mi: mitochondrion; t: tapetum. Scale =1μm

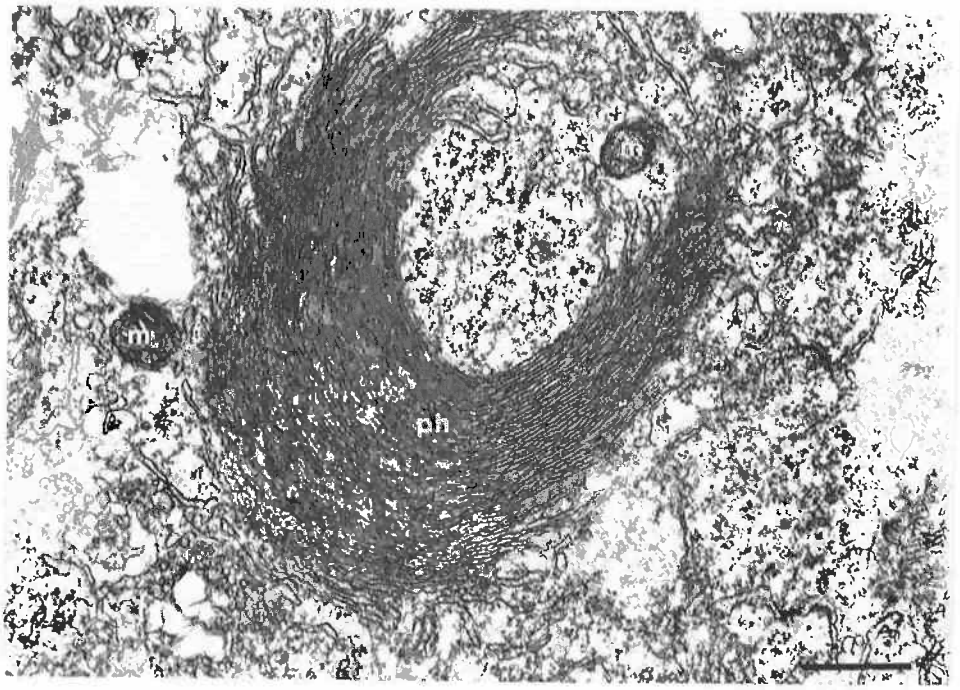
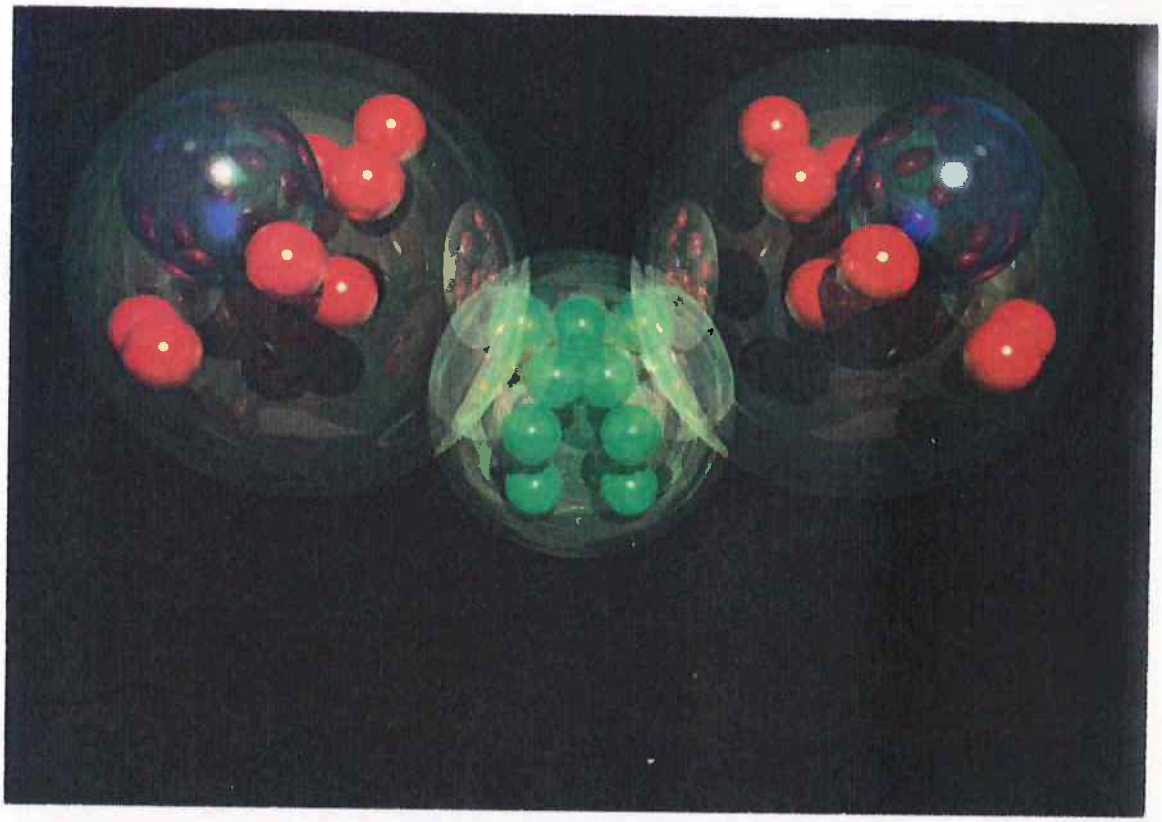


Plate 3.23 Section through phaeosome showing tightly packed membranes of organelle and loose membranes in surrounding cytoplasm (TEM, transverse). ph: phaeosome; m: mitochondrion. Scale = 0.5μm

a



b

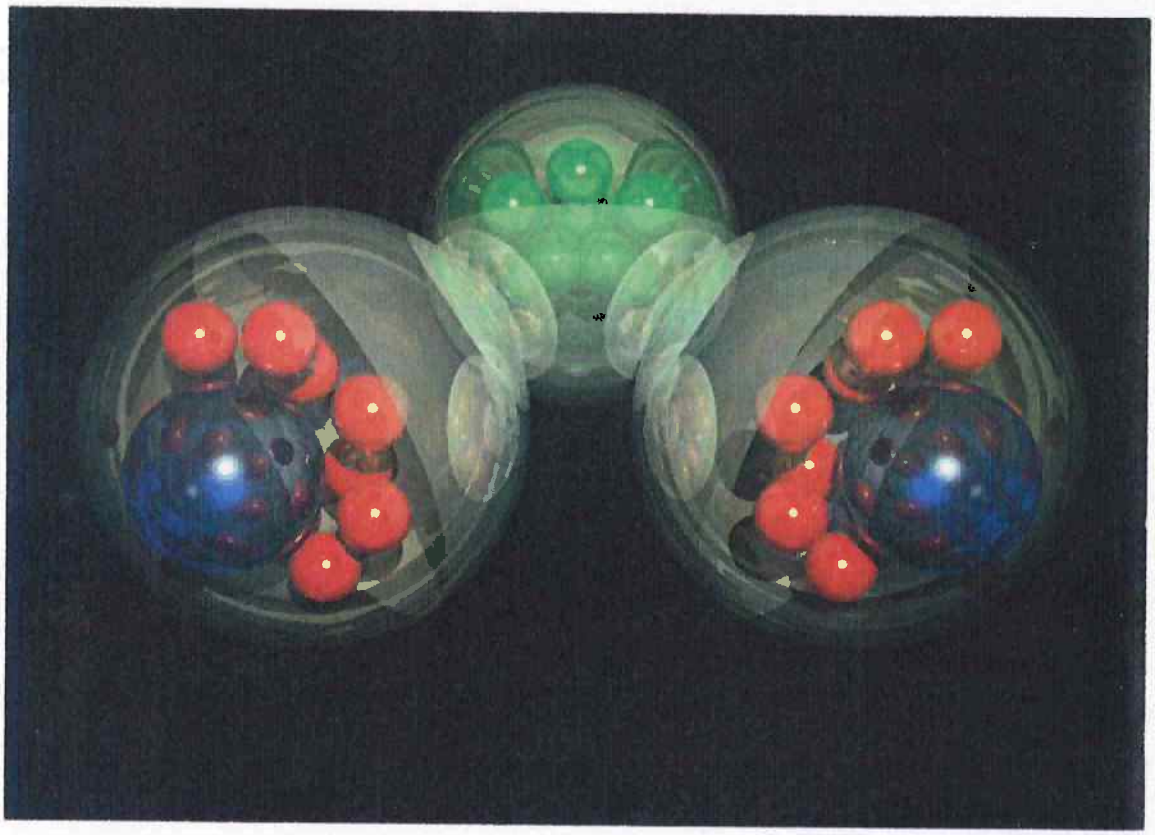


Plate 3.24 Diagrammatic representation of the disposition of retinular cell nuclei in the dorsolateral and ventral ocelli of the copepodid nauplius eye. (a) Anterior aspect (b) Anterodorsal aspect. Blue: lens; Red: Retinular cell nuclei of dorsolateral ocellus; Green: Retinular cell nuclei of ventral ocellus.

cytoplasm of the retinular cells were large quantities of smooth endoplasmic reticulum. There were also large numbers of small mitochondria.

The ventral ocellus had 10 retinular cells and lacked the lens seen in the dorsolateral ocelli (Plate 3.25). Moving from the dorsum, there were three major planes of retinular cells. The first plane was composed of a triplet of cells which were arranged with one straddling the median axis and one on either side of this medial cell. Beneath this were three more cells arranged in much the same manner. The third plane had four cells, the anterior pair being slightly dorsal to the posterior pair. In this group, each pair of nuclei lay to either side of the median axis (Figure 3.24).

The dorsolateral ocelli each possessed a large well-developed spherical lens with a maximum diameter of 18.8 μm. This was positioned anterodorsally and directly opposed the tapetum. Under the light microscope, the lens appeared to have three layers. The outermost layer (*zona externa*) stained similarly to cell membranes and it is suggested that this was the outer-membrane and cytoplasm of the lens cell. Interior to this was another layer (*zona media*) which stained more densely but again stained similarly to the cell membrane. The majority of the lens was composed of a homogeneous hyaline substance which stained differently to the rest of the lens (*zona interna*). With Cason's stain it was clearly distinguished by a homogeneous yellow stain.

Under TEM the lens appeared to be the product of a single cell, since only a single nucleus was observed beneath the conjunctival cell at the anterior of the ocellus. The lens, as defined from light microscope observations, was seen to be composed of only two distinct layers under TEM (Plate 3.26). The outer of these, corresponding to the *zona externa* and *zona media* of the light microscope observations, appeared fibrous (Plate 3.27) but is thought to consist of columns of secretion associated with the production of the body of the lens. The internal zone, corresponding to the *zona interna* of light microscope studies, was highly homogeneous and of medium electron-density (Plate 3.27). Occasional electron-dense particles were seen in this matrix but may have been artefactual or due to faults in the production of the matrix. In the outer periphery of this zone there was evidence of faint darker striations which apparently corresponded to the orientation of the secretory columns of the *zona externa*. No evidence was seen under the TEM for a partitioned or graduated construction that might give rise to differences in the refractive index of different parts of the lens although it is quite possible that such variation might not be apparent under TEM. No indications of the composition of the lens were found under TEM, or indeed, of whether the refractive part of the lens was in solid or liquid phase.

Details of the origins of the axons from the retinular cells were not observed under TEM although it may be that they originated in the intracellular spaces already noted. The dorsolateral

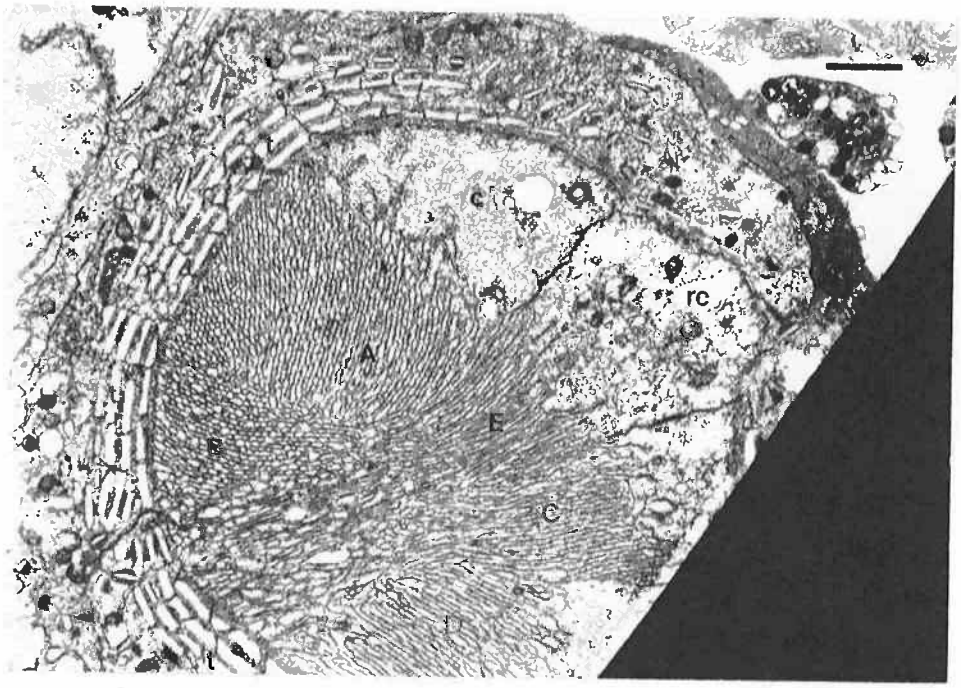


Plate 3.25 Ventral ocellus showing opposed microvilli of retinular cells and tapetum (TEM, transverse). A-E: opposed microvilli of retinular cells; t: tapetum; rc: retinular cell cytoplasm. Scale = 1μm

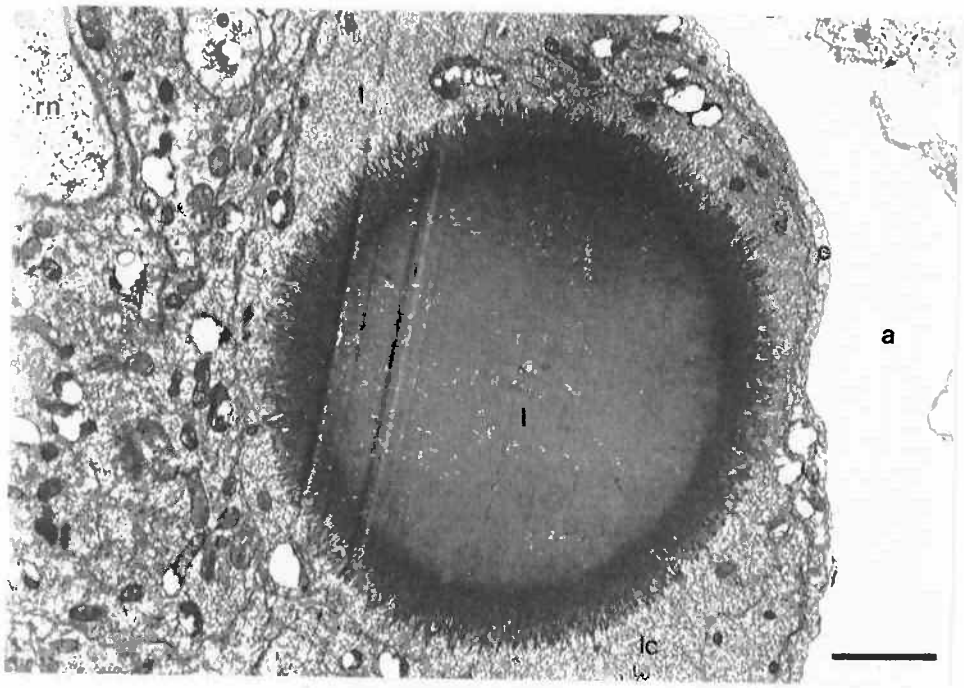


Plate 3.26 Lens cell of dorsolateral ocellus with lens opposing lateral aperture (TEM, transverse). a: dorsolateral aperture; c: overlying conjunctival cell; l: lens; lc: lens cell cytoplasm; rn: retinular cell nucleus. Scale = 2μm

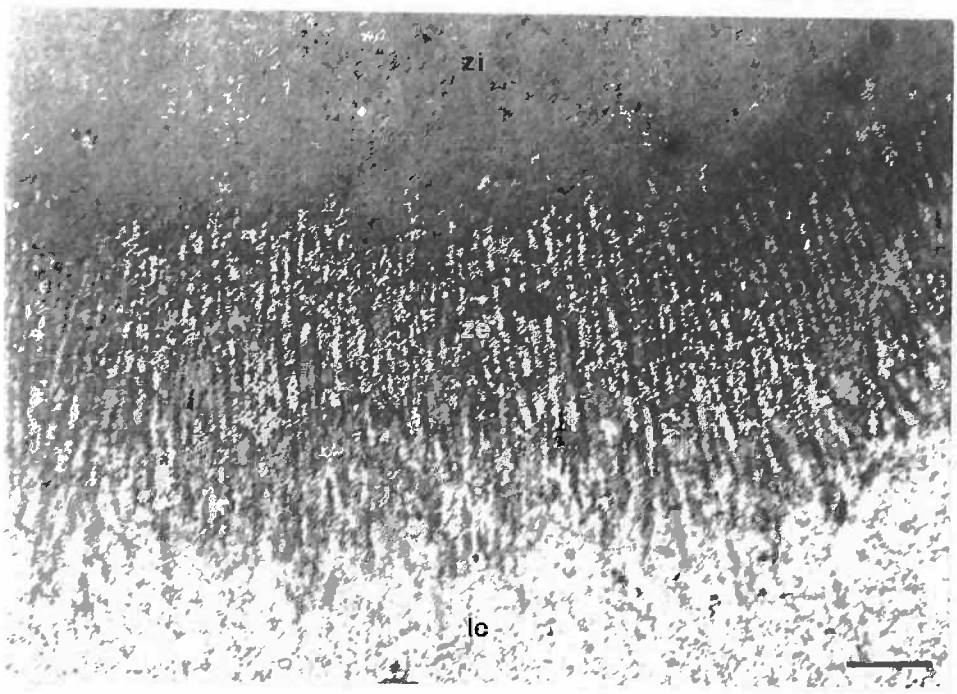


Plate 3.27 High magnification of lens showing the zone of secretion (*zona externa* - ze) and the lens itself (*zona interna* - zi) (TEM, transverse). lc: lens cell cytoplasm. Scale = 0.25μm

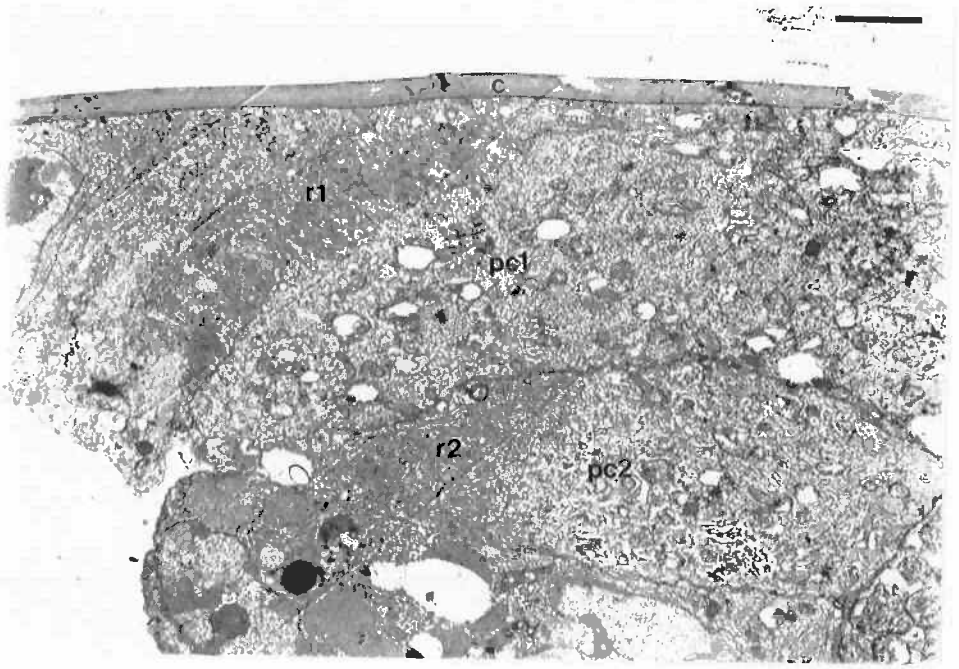


Plate 3.28 Transverse section through extra-optic photoreceptor showing two cells with the characteristic microvillous development of a rhabdom. c: external cuticle; pc1,pc2; cytoplasm of extraoptic photosensory cells; r1,r2: rhabdoms of extra-optic photosensory cells. Scale = 2μm

ocelli sent two major bundles of axons to the protocerebrum, these originating dorsal to the lens and running along the lateral peripheries of the ocellus to pass to either side of the tapetal cells, passing posteriorly directly into the protocerebrum from whence the axons could be traced into its interior. The origin of the axons within the ventral ocellus was similarly unclear. The first clear appearance of the axons was a bundle anterior to the tapetum (ie outside the ocellus) from whence it passed dorsally and joined the bundles from the dorsolateral ocelli to pass into the protocerebrum. No estimate could be made of the total number of axons in each tract.

Extra-optic receptors

A bilateral pair of extra-optic photoreceptors was found just beneath the cuticle of the cephalothorax anterolateral to the nauplius eye under TEM (Plate 3.28). These cells could not be observed under the light microscope. Each receptor was observed to comprise a pair of cells, one below the other, having the appearance of retinular cells. Both cells had a cytoplasm equipped with large numbers of mitochondria and rich in endoplasmic reticulum. The dorsolateral aspect of each cell was composed of a microvillous fringe apparently identifying with the rhabdoms of the retinular cells of the nauplius eye. The exact function of this receptor is unclear.

3.5 Discussion

The ability to respond to the environment is an important and characteristic aspect of the survival of organisms. Although Rohde (1982) notes that some marine parasites, including certain free-swimming ciliates and tapeworm coracidia larvae, infect their hosts in a purely passive manner, responses to the environment which may act to increase the probability of host contact are often present in the infective stages of marine parasites (*ibid.*).

The development in the metazoa of a bilaterally symmetrical body-form from the more primitive (Barnes 1980) radial design has resulted in the concentration of receptor systems at one end of the organism, this being the end where new stimuli are normally first encountered (Barrington 1982). Associated with the concentration of receptors is the concentration of their regulators, both neural and neurosecretory (*ibid*). In arthropods this process of cephalization has been accompanied by movement of the anterior head segments forwards such that, for instance in the Copepoda, the antennules come to lie in anterior to the oral aperture to give a pre-oral sensory system. Such a development has been crucial in that it allows early sensing of information about the environment in the direction of travel. This gives the possibility of avoidance of predators or unfavourable environments or, alternatively, movement into favourable environments and re-channelling of food particles for ingestion (see Strickler 1985).

The fact that the copepodid larva of *L. salmonis* possesses a number of paired sensory systems; notably the dorsolateral ocelli, antennules, sensilli and a cephalic receptor corresponding to the "Organ of Bellonci" as defined by Boxshall (1992), has important consequences for the detection and interpretation of stimuli. Assuming that the state of the two receptors in a given pair may be compared simultaneously by the copepodid, such an arrangement allows for a tropotactic response which is, as stated in the introduction, more direct and efficient than a kinetic or klinotactic response (Sukhdeo & Mettrick 1987). If the receptors can not be compared simultaneously, then the latter two response categories are still possible for the copepodid. It should furthermore be noted that the further apart the members of a pair are, the better is the prospect of resolution of the direction of a stimulus source (Sukhdeo & Mettrick 1987), since the likelihood of a difference in stimulus intensity between the receptors is increased. The release of behaviour by trigger cues require only the detection of the cue at an appropriate intensity and therefore only requires the presence of an unpaired receptor.

The first part of the following discussion will consist of an examination of the functional morphology of the receptors and the nature of responses obtained towards the major stimuli tested. This will be followed by a summary of what the studies presented indicate about the behaviour of the copepodid.

"Unstimulated" behaviour

The tendency for copepodids to sink cephalothorax-down, as opposed to balancer-down as observed for the nauplius stages, has been noted previously for *S. edwardsii* by Fasten (1913) as has the fact that negative buoyancy is a common phenomenon in zooplankton.

The "hop and sink" behaviour displayed by copepodids of *L. salmonis* is well recognised in free-swimming copepods such as *C. finmarchicus*, *Centropages typicus* Krøyer *Cyclops abyssorum* Sars and *Acartia tonsa* (Dana) (reported by Bainbridge 1952, Gauld 1966 Strickler 1975 and Stearns & Forward 1984 respectively) as well as other zooplankton species. Haury and Weihs (1976) have suggested that such behaviour theoretically saves energy relative to continuous swimming at a fixed depth. One might therefore expect such behaviour to be particularly important to a lecithotrophic larva such as *L. salmonis* which must conserve its limited reserves where possible to maximise the probability of host contact. Despite energy considerations, the copepodid must display some spontaneous swimming in order to keep it in the water column where overlap with the host may occur. This behaviour has been previously noted for *L. salmonis* by Wootten *et al.* (1982) and for *L. branchialis* by Whitfield *et al.* (1988). Similar "jerky and erratic" swimming was observed by Lewis (1963) for *L. dissimulatus* and Poulin *et al.* (1990) noted that copepodids of *S. edwardsii* displayed spontaneous activity in the absence of stimuli.

As the copepodids aged, a higher proportion were observed to display reduced activity which may be indicative of exhaustion of energy reserves or senescence. It is also worth noting that the depletion of energy reserves, which are thought to comprise principally lipids (see Chapter 5), might also result in loss of buoyancy thereby making swimming more energetically costly. On the other hand the lessened activity could be indicative of a change of behavioural pattern, e.g. in order to conserve dwindling resources, although there is no evidence available at present to support this. Movement towards light sources which are neither narrow nor directed suggests that the positive phototaxis observed in this species may not be wholly artefactual.

Light

According to Cronin (1988), five principal types of information may be obtained from light in the marine environment: 1) Intensity 2) Temporal changes e.g. motion flicker / photoperiod 3) Spatial distribution 4) Spectral distribution 5) Polarization pattern. The function of any optical system (e.g. the lensed nauplius eye of the copepodid) is to enable at least a crude determination of the spatial distribution of light in addition to the information on intensity and duration that can be obtained using a simple photoreceptor (*ibid.*). To make use of an optical

system requires the organised arrangement of photoreceptors in the form of some kind of retina and for maximum efficiency, a mirrored tapetum which maximises the proportion of the light entering the detector that is perceived by the organism.

Light sensors

The median nauplius eye of the copepodid corresponds to general descriptions of nauplius eyes in copepods by Elofsson (1966) and more specific descriptions by Fahrenbach (1964) for the free-living predatory copepod *Megacyclops albidus* (Jurine) and Dudley (1969) for adults of ascidicolous copepod *D. seclusus* Illg. Each dorsolateral ocellus in the present study has a slightly larger maximum transverse diameter (37.5 μm) than those of adult female *D. seclusus* (37 μm) and is smaller than those of adult *M. albidus* (50 μm). As a proportion of body length ($\sim$ 600 μm) it is 8.33 % relative to 5 - 2.78 % (according to body-length) for *M. albidus* and 1.06 % for *D. seclusus*. Thus *L. salmonis* has a far higher eye : body length ratio. This would seem to suggest that the eye is important for the copepodid or that it is well-developed for a major role in the life of a later stage. The eye of *L. salmonis* was similar to that of *D. seclusus* in that it lacked the basal lamina found to enclose the eye of *M. albidus* and therefore its bounding membranes (those of the pigment cells) were not found to be continuous with the epineurium as was observed for the latter species and the gelatinous zooplankton-associates *Sapphirina* spp. (Elofsson 1969).

The eye of *L. salmonis* closely resembles that of the adult *Caligus acutus* Kirtisinghe which was briefly described by Elofsson (1966) and similarly displayed an antero-lateral lens in each of the dorsolateral ocelli and a total of 9 retinular cells in each dorsolateral ocellus and 10 retinular cells in the ventral ocellus. As in the present study, the retinular cells of the dorsolateral ocelli of *C. acutus* were seen to be arranged in a "semi-sphere" or "bowl". The lens of the present species, as does that of *C. acutus* protrudes somewhat along the long axis of the ocellus. *M. albidus* was seen to have 9 retinular cells in the dorsolateral ocelli and 5 in the ventral ocellus whereas *D. seclusus* had 8 and 8 respectively. It was suggested in the latter case that one of three original retinular cells in the dorsal ocellus of the nauplius must have been lost at the same time as the 6 primordial retinular cells became functional, giving rise to a total of 8 retinular cells. In the present species it would appear that the degeneration of this cell (assuming the naupliar complement of the two species is similar) does not occur, leaving a total of 9 retinular cells. The present study contrasts with that of Vaissière (1961) who found 7 retinular cells in both the dorsolateral and ventral ocelli of *C. diaphanus* and *L. nordmanni*.

The present study indicated all three ocelli to be bounded by "conjunctival" cells similar to those described by Vaissière (1961) and Fahrenbach (1964) and stated to be absent by Dudley.

None of these cells appeared to carry pigment vesicles. All the pigment cells seen in the present study correspond to the description of primary pigment cells in Dudley (1969). Cells corresponding precisely to those described by Dudley (1969) as "accessory pigment glial cells" in adult *D. seclusus* were not observed in that there were no observable intercalations between pigment cells and retinular cells (as was also the case for copepodids of *D. seclusus*) and also no observable unpigmented areas. It would nevertheless, seem likely that some of the pigment cells described in the present study have similar origins to Dudley's glial cells. The pigment was not contained in syncytial tissue as described by Vaissière (1961) for adult caligids.

Dudley found the pigment globules that she reported within the primary pigment cells to contain no carotenoids, melanin or ommochromes. It was suggested in the same study that the osmiophilic material seen in the vesicles might be unsaturated lipids and it was further noted that the vesicles were more heavily staining in naupliar stages than in adults. Fahrenbach, who noted two types of inclusion within the pigment cells found that starved individuals lost fat droplets leaving spaces within one of the observed inclusion types. Vaissière (1961) noted two adjacent pigment layers in caligid adults studied with the outer containing melanin granules and the inner layer containing carotenoids dissolved within lipid vesicles. The vesicles seen in the present study were also highly osmiophilic which suggests that these too might contain lipids. Lipids have a refractive index of $\sim$ 1.5 (Nicol 1989) and their presence has been noted in reflective spherules in the *tapeta lucida* of teleosts (see Table 15.1 in Nicol 1989). In teleosts, the spherules consist principally of the triglyceride glyceryl tridocosahexaenoate (*ibid.*). The vesicles seen in the present study ranged between 588 nm & 1176 nm and would therefore be likely to display "Mie scattering", with all wavelengths across the visible spectrum being reflected equally (Jerlov 1976, Nicol 1989). The fact that the pigment cells show a pronounced red coloration in live specimens is also of interest. In gulls as in most birds, the cones of the retina contain coloured oil droplets although in gulls there is a high proportion of red droplets (Muntz 1972) which have been suggested to block wavelengths below 600 nm such that floating objects in the water would be seen with enhanced contrast (Lythgoe 1979). In the present species then, dissolved carotenoids in the lipid vesicles of the pigment cells would, when combined with the scattering effect of the spherules block wavelengths below 600 nm (ie including the blue part of the spectrum that predominates) and therefore remove most of the incident light. The pigment cells in the present study would therefore tend to act as a diffuse reflector, backscattering a high proportion of incident light and blocking wavelengths $<$ 600 nm. This, in turn, might be expected to give better resolution and directional sensitivity by preventing light entering the eye in any direction except that of the anterior aperture.

The sequence of vesicular morphology and the irregular shape of the crystals contained within the vesicles indicate that the mode of crystal production might be similar to that suggested

for the lower vertebrates by Millot (1923) (cited in Frasson-Boulay 1973). This author suggested that cells produced a solution of guanine such that crystals crystallised rapidly out of solution. In the present study the crystals were present within membrane-bound vesicles. Guanine or another material could be pumped into these vesicles across specific, membrane-bound channels leading to super-saturation within the vesicles and resulting crystallization. It was suggested by Frasson-Boulay (1973) that the guanine of the tapetum of *Pontella mediterranea* Claus might be produced by the breakdown of arginine which was found to be abundant within the nauplius eye of this species. If the pigment crystals are guanine then it may similarly be that high levels of arginine may be located within the pigment cells of *L. salmonis* although this is clearly difficult or impossible to establish. Vaissière (1961) noted melanin granules to be present in the pigment layers of *L. nordmanni* and *C. diaphanus* and this may therefore be the most likely identity of the pigment crystals of *L. salmonis*. The determination of the nature of the pigment cell crystals may be carried out in future by the use of chemical tests such as those described by Vaissière (1961), Dudley (1969) and Frasson-Boulay (1973).

The vesicles of *D. seclusus*, although containing lipid, were not seen to possess large crystalline inclusions. Crystal platelets were noted by Elofsson (1969) in the pigment cells of *Sapphirina* but these were said to be identical to those observed in the tapetum and were hence more regular than the crystals seen in the present study. Both guanine and, more rarely, uric acid are seen in teleost *tapeta lucida* and both constituents have been reported to display a number of different crystal morphologies - needle-shaped crystals and randomly distributed crystallites noted amongst them (Nicol 1989). It would seem most likely that the crystals noted in the pigment cells of *L. salmonis* are most likely to be guanine or melanin and that, these substances having a high refractive index, they act in concert with carotenoids dissolved in the lipid component to scatter incident light and prevent it from entering the ocelli from any direction save the anterior aperture.

The tapetal cells of the copepodid of *L. salmonis* correspond closely to those observed by Dudley and Fahrenbach. The absence of platelets within the membranes may be attributable to a tendency to fall out of thin sections as suggested by the former author or a tendency to sublime in the electron beam as suggested by the latter. The plates in the present study were smaller laterally (0.51^2 μm) than those of *D. seclusus* (0.8^2 μm) but were thicker than the those of the latter (0.14 μm as opposed to 0.1 μm). The plates were smaller in all dimensions than those of *Sapphirina* described by Elofsson (1969). Assuming that all copepod tapetal plates are composed of guanine as has been demonstrated by Frasson-Boulay (1973 in Boxshall 1992) for *Pontella mediterranea* Claus, plates might be expected to be of a similar thickness (¼ λ) to allow maximum reflection of light entering the ocellus thus this difference is puzzling. These deviations may derive from measurement of the spaces left in the membranes by the absent plates rather

than the plates themselves or perhaps from differing ambient light wavelengths and therefore variation in the thickness of plates designed to reflect them. The plates of *L. salmonis* and the other species noted are, however, very much smaller than those of *P. mediterranea* measured by Frasson-Boulay who noted dimensions of 0.5 μm $\times$ 0.35 μm $\times$ 0.4 μm. If the plates of *L. salmonis* are ¼ λ then λ must equal 560 nm. This is close to the tested wavelength giving the highest phototactic response (550 nm) which suggests that this is a reasonable value. One might, however, as previously indicated, suggest this to be a slight overestimate through measurement of the membrane pockets rather than the plates themselves.

Dudley recorded a maximum of 22 rows of tapetal plates for adults of *D. seclusus* whilst Fahrenbach recorded numbers ranging between 20-60 rows and Elofsson (1969) recorded up to 18 layers. The present study noted only 10-11 rows at the deepest point, which corresponds to the number recorded by Dudley for the last nauplius of *D. seclusus* (9 - 11). In the present study, stacks of platelets were found to be generally less well-aligned than those seen by Dudley except for the basal zones. Frasson-Boulay (1973) similarly found the platelets to be offset in *P. mediterranea*. The ventral ocellus showed a maximum of 8 rows as compared with 6-7 in the last nauplius of *D. seclusus*.

The tapetal cells in *L. salmonis* show large numbers of mitochondria in contrast to those of adult *D. seclusus*. There was also evidence of production of numerous new platelets as seen in the nauplii of *D. seclusus*. This all indicates active production of the tapetum in copepodids which would seem to indicate that continuous elaboration or size increment of the tapetum for the use of later developmental stages occurs during the copepodid phase. Theoretically (Nicol 1989) a system of 19 ¼ λ plates, regularly arranged should reflect over 98 % of incident light hence elaboration will tend to increase the efficiency of the reflector in subsequent stages.

The retinular cells observed in the present study correspond to previous descriptions by Dudley, Fahrenbach and Elofsson (*op. cit.*). One feature that was very apparent in the present study was the variation in orientation of the microvilli of the rhabdoms. Dudley suggested that the microvilli often follow the line of the tapetum thus making observation of general orientation difficult. From the present study it would seem that the microvilli tend to be oriented perpendicularly to the light but that adjacent groups often lie at $\sim 90°$ to one another. This may result from rhabdoms following the line of the tapetum but might also be an adaptation to allow the detection of polarized light. Umminger (1968b) noted a similar 90° difference in the microvilli of *Cyclops vernalis* Fischer which were also perpendicular to the path of light and suggested that this arrangement would allow detection of the orientation of polarized light allowing the polarotactic ability observed in this species (*ibid.* 1968a, b). The observation of a similar response in *C. rapax* (= *elongatus*) (*ibid.*) clearly demonstrates that a polarotactic response is also found in at least one caligid.

The axons of the three ocelli enter the protocerebrum directly posterior to the nauplius eye. They are arranged in five bundles as are those of *D. seclusus* with two deriving from each dorsal ocellus and a single bundle from the ventral ocellus. Unlike the latter species, the axons from the dorsal ocelli emerge postero-dorsally and pass laterally to the tapetal cells before passing between (rather than through) the pigment cells posteriorly and into the protocerebrum. The axons from the ventral ocellus, as do those of copepodids of *D.seclusus*, pass dorsally and then move posteriorly with the bundles from the dorsolateral ocelli. The contribution of glial cells to the axon bundles as described by Dudley (*op. cit.*) could not be determined in the present study although there was no sign that either "conjunctival" cells or pigment cells were acting as glial cells or intercalating with the retinular cells as seen in adults and fifth copepodids of the aforementioned species. The intracellular spaces seen at the base of rhabdoms have been previously referred to by Elofsson who termed them "bladders". Their appearance was compared by this author to crystalline cone cells in *Daphnia* sp. and it was suggested that they might be part of a dioptric system. Elofsson also suggested that they might only be present in the "transformation line"[4] starting with the calanoid *Chiridius armatus* (Boeck) and ending in the sapphirinid *Copilia mirabilis* Dana - species commented on by the same author (1966). Within this same "transformation line" was listed *C. acutus* which was found to be similar to *C. armatus* in most respects save for a single lens cell in each dorsolateral ocellus as opposed to the two cells found in the latter species. *L. salmonis* similarly possesses a single lens cell.

The sphericity of the lens described in the present study was to be expected since spherical lenses allow for focusing in the aquatic medium (Lythgoe 1988). The lens in *L. salmonis* appears similar to that described for *Sapphirina* sp. by Elofsson (1969) in that it appears to comprise a single cell. Unlike the lens of adults of the calanoid copepod *Epilabidocera amphitrites*, the lens of *L. salmonis* is not associated with the external cuticle and is not thought to be a product of the cuticular epithelium. There was also no evidence of the lamellae reported for the lens of this former species. Of the three layers seen under the light microscope, the *zona externa* and *zona media* probably represent an area of secretion of the *zona interna*. This latter layer is considered to be the principle refractory component of the lens. From the TEM study there was no evidence of an initial refractory layer with a lower refractive index for the purpose of correcting the expected spherical aberration although it is possible that the *zona externa* functions in this respect. Protrusion of the lens through the anterior aperture as described in this study would be expected to increase the field of view (Sivak 1980).

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[4] A line of development whereby the morphological characteristics of one species appear derived from those preceding it.

The eyes of fish are similar to those of *L. salmonis* in that they possess a protruding spherical lens, hemispherical tapetum (*tapetum lucida*) and an anterior aperture (pupil). The disposition of the lens and retina in fish is usually symmetrical (Nicol 1989) and the two components bear a constant relationship, termed "Matthiesons ratio" whereby the ratio of the focal length R (distance from the centre of the lens to the retina) to the radius of the lens r is normally approximately 2.55r (the relative focal length). If one makes an assumption (as one must without empirical evidence) that the focus of the lens in *L. salmonis* is at the level of the tapetum (or adjacent to it where the greatest proportion of the rhabdoms lie), one may calculate this value for *L. salmonis* to give a value of 2.5r which is very close to the piscine value. It is also possible to calculate the f-number of the system which determines the brightness of the image. This is calculated as A / R where R is the focal length of the lens and A is the diameter of the pupil aperture. In the case of *L. salmonis* this equals 0.96 as compared with 2.1 for man with pupil dilated and 0.89 for a cat (Lythgoe 1988).

Fahrenbach (1964) concluded that the eye of *M. albidus* probably represented a non-imaging light-gathering organ. The eye described here for *L. salmonis* is more complex and corresponds closely to the "type B" lensed eye with reflective tapetum described by Land (1981). Such eyes are believed to function in a similar manner to those of scallops (*Pecten* spp.) in which focusing is accomplished through reflection of light entering the eye from a hemispherical argentea which produces an image concentric with the latter but with half the radius. Land (1965) suggests that a lens would act to reduce the spherical aberration that would result from such a hemispherical reflective surface. A copepod eye of this kind would maximise the retention of light entering the eye with up to 85 % being reflected back towards the rhabdoms by the tapetum. The fact that light would traverse the rhabdoms twice (ie when entering and leaving the eye) means that contrast would be degraded. It has further been suggested that sensitivity would similarly be fairly low since, if the rhabdoms absorbed a high proportion of the light striking them, little would reach the tapetum in the first place (Cronin 1988). The eyes of scallops are suggested to be principally movement detectors and it seems likely from their structure that the eyes of *L. salmonis* might be expected to have similar capabilities. According to Dudley (*op. cit.*) the tapetum of *D. seclusus* may function to maximise sensitivity at low light intensities and may furthermore help to give maximum absorption with a reduced amount of photosensitive pigment (as proposed by Denton & Nicol 1964)).

The orientation of the rhabdoms of *L. salmonis* suggests morphologically that *L. salmonis* could detect the polarization of light and possibly respond to it in the manner of *C. rapax* (Umminger 1968b). Given that the presence of the tapetum in both species should cause light to pass through the rhabdoms twice, this ability would seem to suggest an extraordinary lack of spherical aberration in the ocellus such that light passes through the same rhabdoms on each

traverse. This is presumably accomplished firstly by having the rhabdoms positioned close to the tapetum and secondly by having a lens and tapetum with low spherical / specular aberration such that the light passes back accurately the way it came.

The ventral ocellus will provide information on the level of illumination behind and below the animal ie the areas not covered by the dorsolateral ocelli. Because it lacks a lens and a defined aperture, the ventral ocellus is not likely to have the resolution of the dorsolateral ocelli.

From the above discussion, the morphology described should allow the nauplius eye of *L. salmonis* to detect all of the principal categories of light information available in the marine environment and described at the start of this discussion. The detection of light intensity is enhanced in *L. salmonis* by the provision of the tapetum and by effective pigment shielding of the eye which prevents the loss of light entering the eye. The lower threshold of intensity detection could furthermore be extended by temporal summation of light stimuli although this would degrade the detection of movement (Lythgoe 1988). The eyes of *L. salmonis* should also act as effective spatial detectors through directionality of the dorsolateral ocelli and pigment shielding preventing light from entering from any direction bar the anterior aperture. Spectral sensitivity is likely provided by rhodopsin sited in the rhabdoms and giving (as in free-living copepods) sensitivity to a wide spectrum of visible light with peaks in sensitivity often corresponding to the spectrum available in the local environment. The morphological basis also exists for the eye of *L. salmonis* to act as a polarization detector and, if its similarity to the eye of *Pecten* is significant, it may also function as an efficient movement detector.

The other apparent photoreceptor noted in the copepodid of *L. salmonis* was the pair of cells located beneath the dorsal cuticle of the cephalothorax. These cells correspond to earlier descriptions of "Gicklhorn's organ" as noted by Elofsson (1966) and normally consisting of two binucleated cells connected to the cerebrum by a pair of nerves (*ibid.*, Boxshall 1992). In the present instance, the nuclei were not observed so that the binucleate nature of these cells cannot be confirmed for *L. salmonis*. Although the present cells had microvillar margins they contained no phaeosomes which contrasts to Elofsson's description in which the latter were reported. This may not be anomalous since in retinular cells phaeosomes have been suggested to be transient (Fahrenbach 1964). Because of the reported association of this organ with neurosecretory axons, it has been suggested that it may be concerned with the production of neurosecretory products in response to light (Boxshall 1992) although its function in *L. salmonis* remains to be determined by further studies which may help to elucidate its full structure and identify any product(s).

It cannot be directly inferred, that the theoretical ability to transduce information, as suggested by morphological criteria, necessarily entails that an organism processes / perceives such information and responds to it. The only way to establish such a fact is to examine physiological or behavioural responses. At the present time it is difficult to study real-time physiological responses in organisms such as the copepodid of *L. salmonis* due to restrictions imposed both by the technical difficulties involved in working with such a small, active organism and, more importantly, the financial considerations of solving such technical problems.

The experiments carried out in the present study have therefore sought to examine the larger scale behavioural responses of copepodids to light and other stimuli. These experiments have been principally designed to determine whether copepodids can detect a given stimulus. Response to such a stimulus, denotes, assuming other intervening factors have been controlled for, the detection of the stimulus by the copepodid. Lack of response, however, cannot be taken to result from a lack of sensitivity of the receptor organ concerned, since it may occur through the subsequent intervention of behavioural thresholds, prioritization of responses, requirements for releaser stimuli or other processing-related impediments.

According to Forward (1988) and many others, the demonstration of phototaxis in a narrow light beam is principally a laboratory artefact and it has therefore been suggested that results obtained using such a system should not be extrapolated to the natural environment of an organism. As an example of the dangers of extrapolation, *A. tonsa* tested in the laboratory appeared to respond to all tested light intensities (Stearns & Forward 1984) and might therefore have been expected to occur at the surface during the day. Field observations, however, showed it to display a nocturnal migration pattern and to be low in the water column in daylight hours (Stearns 1986). From a number of studies, Forward (1988) concluded that under natural conditions, positive phototaxis is not common among zooplankton. Despite the latter conclusion, it is worth noting that experiments carried out by Schram & Anstensrud (1985) concerning the behaviour of *Lernaeenicus sprattae* (Sowerby) appeared to indicate a strong correlation between results obtained using a narrow directed light source and those obtained in field capture experiments. Similarly in the present study, copepodids placed in non-directed light appeared to move towards the direction of highest intensity as predicted by narrow beam experiments. Despite the likelihood of artefact, Forward (1987) nevertheless advocates the use of directional light sources for inducing measurable phototaxis since this may shed light on the sensitivity of organisms without necessarily implying anything about normal behaviour.

Photic responses are common in larvae in the marine environment and it has been suggested by Crisp (1976) that the great majority of larvae are, at least initially, strongly positively phototactic, although this statement may need modifying with respect to the comments of Forward (*op. cit.*). Crisp also notes that responses to light are generally reinforced by

responses to gravity. Phototactic responses to light have been noted in a number of caligid and other parasitic species although, as noted previously, these observations have been largely anecdotal and contain few quantitative details of the stimuli or elicited responses. In most of the studies, the likely nature of the taxis (ie klinotaxis or tropotaxis) has not been specifically noted (although the latter has yet to be experimentally demonstrated (Sukhdeo & Mettrick 1987)).

Wilson (1905) made early observations on the positive phototactic behaviour of caligid larvae in general, noting that nauplii swam towards the light on hatching and that nauplii and copepodids were found to swim freely at or near the water surface. Heegaard (1947) reported similar positive phototactic behaviour in the nauplius stage of *C. curtus,* but suggested that the copepodid stage must be negatively phototactic in order to enable contact with benthic hosts. Boxshall (1976) has reported that copepodids of *L. pectoralis* are initially positively phototactic, displaying post-moult swimming activity but that they later become relatively inactive and sink, this being suggested to be a strategy bringing the copepodid into the vicinity of its benthic host. The same author also reports swimming activity to be stimulated by sudden changes in light intensity. Fraile (1986) notes a similarly pronounced positive phototactic response in copepodids of *C. minimus* but makes no comment on the relationship of this response to the age of the copepodid. Hogans & Trudeau (1989) observe that first and second nauplii of *C. elongatus* are also positively phototactic and suggest that this was an adaptation for maintaining the nauplii at the water surface in the vicinity of salmon hosts. These authors also report that the copepodid is positively phototactic and indicate that this response is more pronounced in this stage than in the preceding ones. Johannessen (1975) noted similar behaviour in observations of independent stages of *L. salmonis* which were all observed to exhibit positive phototaxis which was again interpreted in terms of a mechanism for gaining the upper layers of the water column. This latter observation was supported by the observations of Wootten *et al.* (1982). Lewis (1963) observed the same strong phototactic response in independent stages of *L. dissimulatus* as was reported in the other caligid species. In contrast to the report of Hogans & Trudeau (1989) however, he noted that as the age of the second nauplius stage increased, the response grew less pronounced and that copepodids did not respond as strongly as the nauplius stages. Voth (1972) also notes positive phototaxis in the first and second nauplii of *L. hospitalis* although no mention is made of the behaviour of the copepodid.

Fryer (1966) has noted positive phototaxis in larvae of *Lernaea cyprinacea* settled on *Bagrus docmac* (Forskål), a benthic intermediate host and supposed that at some point the sign of this response must change to allow the observed settlement. Considering that *L. cyprinacea* shows a wide range of host specificity including surface feeding species such as *Hypophthalmichthys molitrix* (Valenciennes) (Sommerville pers. comm.), such a change in larval behaviour would appear anomalous.

Whitfield *et al.* (1988) reported that nauplius I of *L. branchialis* were found to be very active on emergence and to show a positive phototactic response. NIIs on the other hand were found to be somewhat lethargic and negatively phototactic, remaining near the bottom until moulting to the copepodid stage occurred. This latter stage was reported to move slowly along the bottom using modified antennules, displaying only sporadic bursts of swimming. No indication was given as to the phototactic response of this stage. Fasten (1913) commented on the response to light of copepodids of *Salmincola edwardsii*, finding them to move towards daylight and to "dart about" in the presence of shadow. He also found them to move towards artificial light, moving in all directions initially but then homing in on the source of illumination. he also noted that the "definiteness" of orientation decreased with decreasing light intensity. It was suggested from this behaviour that the copepodids in the natural environment must move close to the water surface in daylight and sink in darkness this being presumed to match the migration patterns of the trout host. More recent work on *S. edwardsii* by Poulin *et al.*(1990) opposes the suggestions of Fasten. In their study, they found that copepodids greatly increased their locomotory activity when stimulated by visual (ie shadow) or mechanical influences but that in daylight conditions unstimulated copepodids might be expected to be relatively inactive and therefore spend most time on the bottom. This, they reasoned would tend to reduce (as opposed to increase as suggested by Fasten) the spatial overlap between parasite and host, this being possibly justified in terms of a potentially increased lifespan or by the fact that overlap with the host would still occur at night. Kabata & Cousens (1977) working on the related species *Salmincola californiensis* also indicated that shadow and mechanical cues were the chief stimuli initiating swimming activity.

Experiments to investigate the response to light of independent larvae of *Lernaeenicus sprattae* (Schram & Anstensrud 1985) showed that the first nauplius stage gave little or no reaction to light between 5 - 15000 lux. Second nauplius stages were found to aggregate at light intensities > 500 lux but to demonstrate decreased activity below this with no response below 100 lux. Copepodids showed increased activity below 100 lux with aggregation in the upper 20 cm of the holding vessel. As light intensities increased copepodids were stimulated to move towards the bottom and more of them were noted to be immobile. At 5000 lux no swimming could be detected with all copepodids lying on the bottom. The fact that infection prevalence was significantly higher in dark experiments was explained in terms of a negatively phototactic response in the light. It was hypothesized from the data that infection of sprat must occur principally at night in surface waters where they are suggested to be more quiescent.

The above studies are summarized in Table 3.14 in order to aid interpretation. Most of the studies cited above indicate a photopositive response in all independent larval stages. The presence of negative phototaxis is often conjectured by authors as a mechanism whereby infective

larvae might be brought into contact with their benthic hosts, however, it has rarely (if ever) been specifically observed. Many incidences of so-called negative phototactic behaviour do not in fact describe active movement away from the light source (as required by the definition given earlier) but rather passive sinking. With illumination from above, this will incidentally take the larvae in the opposite direction to the light source but this is in fact simply a consequence of the cessation of positive phototaxis or "hop and sink" maintenance of station by a negatively buoyant organism (Forward 1988).

The studies carried out on *L. salmonis* show a strong correlation of positive phototactic behaviour with light intensities at lower intensities $\leq$ 240 lux. At very high intensities (20,000 lux) copepodids were found to sink passively as did those of *L. sprattae* (Schram & Anstensrud 1985). This could not, however be termed negative phototaxis as the light beam was horizontal whilst the copepodids sank vertically. It may be that this latter response at high intensities, serves to prevent the copepodid from being overexposed to UV light in surface waters. It has been shown previously by Dey, Damkaer & Heron (1988) that free-living copepods vary markedly in their UV-B dose-rate thresholds. *L. salmonis* responded to the full range of the visible spectrum with highest responses being recorded to light at 550 nm and lowest to light at 400 nm. This can not be compared with results for other parasitic copepods as the literature seems to indicate that this is the first study to investigate this response in parasitic species. The mode of movement towards the light source is important in that it may be used to give some indication as to the nature of phototaxis in *L. salmonis*. As in *S. edwardsii* observed by Fasten (1913), lower intensities led to less direct, more meandering progress towards the source whilst high intensities gave direct movement. Tropotaxis is similarly direct and requires simultaneous information from a pair of receptors (Sukhdeo & Mettrick 1987). At high intensities, the behaviour observed is suggestive of a tropotactic response. In klinotaxis orientation is indirect and occurs by temporal comparison of stimulus intensities by regular lateral deviations of the whole or part of the body (*ibid.*). At lower light intensities, swimming by copepodids is more sinuous and it is possible that a klinotactic response is responsible. Another possible interpretation is that at low intensities, summation of the light stimulus is required for detection to occur. In this case information may be processed more slowly leading to greater lateral deviations from the expected course. Since both tropotactic and klinotactic responses require a steep gradient of stimulus (*ibid.*) one might also expect the response to be more intermittent at the lower response threshold.

To understand the function of photic (and other) responses in parasitic species of copepod such as *L. salmonis*, the nature of responses in free-living species (both copepods and zooplankton generally) needs to be examined in order to determine which responses derive from

the exigencies of general copepod (or planktonic) existence and which, if any, are modifications or entirely novel constructs selected to fulfil the requirements of the parasitic mode of life.

Light is important to many zooplankton species, including copepods, in that it serves as the principal factor influencing the pattern of vertical migration and also acts as a directional cue (Forward 1988). It has been suggested that most zooplanktonts undergo patterns of daily vertical migration (*ibid.*). Light is clearly also essential to visual predators such as cephalopods and fish. For particle-feeding planktonts, two of the major features of existence in the plankton are food location and predator avoidance. Although the former is not important to lecithotrophic larvae, the latter is still an essential aspect of survival.

In free-living copepods, Buskey (1984) suggests that it is unlikely that vision plays a role in the location of individual food particles particularly since they feed successfully at night. Furthermore he states that the simple structure of the copepod median eye is probably incapable of the sophisticated image formation that would be required for such a task. As already mentioned, a similar view has been proposed by Fahrenbach (1964) for the hunting of moving prey by *M. albidus*. Buskey suggests instead, that food particles are recognised by a combination of chemo- and mechanoreception as demonstrated by the same author for the free-living planktonic copepod *Pseudocalanus minutus* (Kroyer).

According to Boxshall (1981) the principal factors behind vertical migration are those indicated above ie feeding and predator avoidance. Food is more abundant in surface waters since this is the zone in which primary production occurs, however, this is also the zone where a given organism can be most easily seen by visual predators during daylight hours. It is hence hypothesised that many planktonts descend during the day to avoid visual predation and ascend at night to feed (*ibid*). To this end, it is proposed that an increase in activity leads to rising in the water-column whereas a decrease in activity leads to a fall (Forward 1988).

There are two principal hypotheses relating to the spectral sensitivity of free-living species. the "sensitivity hypothesis" (Munz 1958) suggests that the range of maximum spectral sensitivity of an organism might be expected to match the spectral distribution of light in the animals environment. An alternative hypothesis, the "contrast hypothesis" states that the range of maximum sensitivity could be selected so as to maximise contrast between the viewed object and its background (Lythgoe 1968 in Forward 1988). In this case, spectral sensitivity would be matched to or offset from the ambient spectrum according to depth and position of view (McFarland & Munz 1975 in Forward 1988).

Studies of zooplankton in general seem to indicate that their spectral sensitivity does indeed match the ambient spectrum at times of vertical migration as well as that of fish predators (Lythgoe 1985, Forward 1988). Consistent aggregation of spectral sensitivity occurs in the region 460 - 530 nm (with 80 % of values falling between 450 - 700 nm) although most species

demonstrate more than one maximum These figures have been taken to suggest that zooplankton spectral sensitivity is therefore not generally adapted to daytime irradiance (*ibid.*). It has, however, been noted (Swift and Forward 1980, 1982) that spectral sensitivity in larvae may not reflect the requirements of the larval existence but may instead represent ontogenetic development of sensitivity to meet later adult demands.

Light may act as a controlling, an initiating or an orienting cue in vertical migration (Bainbridge 1961). It has been suggested that the following facets could be used for initiation of the migration response: 1) Change in ambient spectrum 2) Change in polarization 3) Absolute change in light intensity 4) rate of intensity change 5) Absolute intensity 6) change in depth of isolumes. It has been suggested by Forward (1988) that the latter three are the most important. In terms of orientation, the most useful cues are 1) Direction of linear polarization 2) Direction of highest light intensity 3) Light-dark contrast at the border of Snell's window (Forward 1988). Zooplankton are usually negatively buoyant (*ibid.*) so that downward migration may easily accomplished by allowing passive sinking.

Estuaries have been reported to transmit light best between 500-600 nm (Jerlov 1976), thus by the "sensitivity hypothesis" one might expect planktonts inhabiting this environment to have wavelength sensitivities reflecting this. Looking at *A. tonsa* (Dana), a free-living copepod, Stearns and Forward (1984) found maximal sensitivity over a fairly wide range (453-620 nm) with response shown to all wavelengths between 380 and 700 nm at high light intensities. The broad response seen was explained in this case in terms of likely overlapping of a number of visual pigments with differing absorption maxima. For *L. salmonis* copepodids in the present study, a fairly uniform response was also observed between 500 and 700 nm with a maximum sensitivity at 550 nm suggesting a similar requirement for wide sensitivity. This peak at 550 nm is slightly higher than the normal peak reported for crustacean larvae in general, which lies at 500 nm (Forward and Cronin 1979). In an experiment carried out by (Forward 1987) brachyuran and anomuran larvae were tested for sensitivity to different wavelengths produced by a directional light source and found peak responses at 500 nm and 400 nm for the former and 400, 480-500 & 580-620 for the anomurans. It was suggested that in surface waters (where predator avoidance is most important), alteration of the spectral characteristics of light would be minimal such that sensitivity to light at 500 nm might be as useful as any other range. It was noted in this study that estuarine and coastal species had similar spectral sensitivities and were therefore assumed to have similar functional requirements for photoreceptors used in vertical migration and predator avoidance. Neither a high peak or a low peak were seen for *L. salmonis*, possibly because the bands tested in this study were too widely spaced to demonstrate some peaks or alternatively because the intensities tested were too high for such distinctions to become apparent. Sensitivity to the 500 nm band has also been suggested to be useful for vertical migration since

this is the region in which maximum quanta are transmitted at twilight (Forward and Douglass 1986) and it may be that the maximal response of *L. salmonis* close to this region derives from the migrating mechanism used by free-living species.

Looking at zooplankton responses to light intensity, it has been noted (Lang, Forward & Miller 1979) that swimming speeds may remain constant or change with intensity and that the sign of the response is affected by the intensity. Most commonly, animals are found to be negatively phototactic at high light intensities and positively phototactic at low light intensities (Forward 1976, Stearns and Forward 1984). The responses of *L. salmonis* would seem to agree with this more generalized free-swimming response although, as noted earlier, the "negative phototactic" response to very high light intensities is more accurately a cessation of positive phototactic or "hop and sink" swimming causing the copepodid to sink. The reasons behind this high intensity response are unclear although it has been suggested for increased swimming speed responses to short duration light pulses in calanoids that such reactions are a photophobic or startle response enabling escape from bioluminescent predators (Buskey & Swift 1985).

"Negative phototaxis" has also been proposed as part of a shadow response used for predator avoidance (Forward 1976, 1977, 1986a) although it has more accurately, been termed a photophobic response by Buskey *et al.* (1986). With respect to this, rapid decreases in light intensity have been noted to cause a response in planktonts. Following adaptation to higher light intensities, small decreases cause a cessation of swimming and result in passive sinking. If intensity declines to a level such that a "negative phototactic" response is initiated, the animal first sinks passively then swims away at high speed (Forward 1988). Forward & Cronin (1977) looked at the light and dark-adapted response of the stage I zoea of the decapod *Rhithropanopeus harrisii* (Gould) and found that the light-adapted phototactic pattern was not induced at intensities that invoked a photonegative response. It was also noted that actual underwater light intensities decrease too slowly to invoke such a response. The authors suggest that the observed reaction must be primarily associated with predator avoidance and would function near the surface during the day as a shadow response. It has been suggested that such responses might be appropriate for the avoidance of vertically-moving, non-visual gelatinous predators such as medusae and ctenophores which inhabit surface waters and therefore are able to cast downward shadows but which can not pursue prey (*ibid.*, Forward 1986b). The free-living copepod *A. tonsa* is preyed upon by the ctenophore *Mnemiopsis leidyi* Agassi and the scyphomedusa *Aurelia aurita* (L.) amongst others and has been shown to have a shadow response whereby a decrease in light intensity prompts a short burst of high-speed, primarily laterally oriented, swimming (Buskey *et al.* 1986). The latter authors conclude that photophobic responses to both rapid increases and decreases in intensity may be the result of a catch-all response principally aimed at avoiding predators. One might also suggest that such a response could trigger similar responses in nearby

planktonts, including conspecifics, through the mechanical disturbance accompanying the response itself. Such a behavioural mechanism would therefore improve survival at a group level as well as at the level of the reacting individual.

L. salmonis copepodids do not demonstrate an observable shadow response. This is particularly anomalous in that both nauplius and mobile (adult and preadult) stages exhibit such a response (personal observations) such that nauplii show increased activity in response to shadows and mobile stages show a host-contact response. It seems likely that despite their vulnerability to predators, this response is absent in copepodids in order that the predator avoidance response does not interfere with host contact. The shadow response in copepodids is thus either inhibited in some manner or is not present and is reinstated in mobile stages. Looking at Table 3.14, it may be seen that none of the larvae of marine parasitic copepods have been observed to display a specific shadow response. In contrast the freshwater species *Salmincola* does display such a response. It is tempting to suggest that this might be due to the absence of gelatinous predators in freshwater, allowing a shadow response to be utilised in host-finding without the likely penalty of predation, although the paucity of evidence on the behaviour of the great majority of parasitic species makes such a conclusion untenable at the present time. The reinstatement of a shadow response in mobile stages of *L. salmonis* may result from the fact that their larger size and stronger swimming abilities make predation by many gelatinous predators less likely.

Little work has been carried out to examine the perception of polarized light in copepods save for the studies by Umminger (1968a, b). In these studies, polarotaxis was demonstrated in 7 out of 15 free-living copepods from a variety of freshwater and marine habitats. In addition, *Caligus rapax* (= *elongatus*) was found to respond to polarized light by swimming at right angles to the plane of polarization. The angle of orientation was found to be the same at all times of day and under all conditions of illumination with no rhythms being demonstrated. The main uniting factor common to 6/7 of the polarotactic copepods was their tendency to be either predators or omnivores. This may relate to the suggestion of Lythgoe & Hemmings (1967) that use of polarized light in clear water might give a better view of distant objects and give sharper intensity contrasts. Nevertheless, it is clear that these species also use polarized light for orientation. It was suggested in this study that the prevalence of polarotaxis in species with complex eyes (4/5) was also associated with their predatory nature.

In the present study polarotaxis in the copepodid was not specifically tested for. It is interesting to note, however, that in the horizontal light beam, copepodids were often observed to swim in a vertical direction initially and then follow the surface film to the light. This may indicate a polarotactic response causing copepodids to swim perpendicularly to the principal axis of polarization as previously observed in *C. acutus*.

Table 3.14 Photic behaviour reported for parasitic copepods.

Species	Reference	NI	NII	Copepodid
Caligidae				
Caligidae	Wilson 1905	+ve	+ve	+ve
C. curtus	Heegaard 1947	+ve	+ve	-ve†
L. dissimulatus	Lewis 1963	+ve	+ve (-)	< +ve
L. hospitalis	Voth 1972	+ve	+ve	
L. salmonis	Johannessen 1975, Wootten *et al.* 1982	+ve	+ve	+ve
L. pectoralis	Boxshall 1976			+ve (inactive)
C. minimus	Fraile 1986			+ve
C. elongatus	Hogans & Trudeau 1989	+ve	+ve	> +ve
	Umminger 1968b	Adults without host, polarotactic (90° to plane of polarisation)		
Others				
S. edwardsii	Fasten 1913			+ve Shadow → Activity
S. edwardsii	Poulin *et al.* 1990			Shadow → Activity
L. cyprinacea	Fryer 1966	+ve	+ve	+ve (-†)
S. sarsi	Carton 1968	Adults without host +ve, with host -ve		
D. seclusus	Dudley 1969	All nauplii +ve		Copepodid I +ve Copepodid II -ve (-)
S. californiensis	Kabata & Cousens 1977			Shadow → Activity
L. branchialis	Whitfield 1988	+ve	+ve	benthic
L. sprattae	Schram & Anstensrud 1985	Nil	+ve > 500 lux	+ve < 100 lux
			Nil < 100 lux	Increasing immob.> 100 lux
				All immobile > 5000 lux

Key:
- +ve signifies a positive phototactic response
- < or > signifies intensity of response < or > that of preceding stage
- ( ) signifies change of sign or nature of response with age
- → signifies consequence of specified stimulus
- † signifies response suggested by author but not observed

Atema (1985) remarked that the sea, or any other given body of water, could be described in terms of an aqueous solution of chemical compounds. From this solution, organisms that need to detect food / hosts / conspecifics must select those compounds which are uniquely associated with their target-organism and which are, furthermore, capable of surviving long enough to be detected. An estimate of the temporal window required for the detection of chemical cues was suggested to lie between ~10 ms and 100 s (Atema 1988). As Ache (1988) notes, in order to discriminate behaviourally relevant cues from the background "noise", aquatic crustaceans tend to be equipped with well-developed chemosensory apparatus.

Atema (1988) states that any given organism, will, in the aquatic environment, be surrounded by a chemical cloud that results from the passive leaking and active excretion of metabolic and other products. This cloud differs in chemistry from the ambient medium and may hence serve to act as a guide to other individuals of the same or different species as to the whereabouts of the source organism. At a scale > 1-10 mm, the extent to which such a cloud will be localised around the organism that created it depends on two major factors, namely the local current speed and the motility of the organism. Motile organisms will clearly be followed by a wake of metabolites (Atema 1988) and similarly, currents passing motile or sessile organisms will tend to create a chemical "trail".

The presence of such chemical "guidelines" might lead one to suppose that the location of a given organism by any other should be a relatively straightforward matter consisting of the recognition of a chemical cue followed by directed movement down an increasing chemical gradient to its source. As has already been pointed out by Crisp & Meadows (1963), however, it is unrealistic to expect small organisms to home in on a chemical source when simultaneously exposed to the vagaries of the aquatic environment. In particular, it is clear that despite originating at a point source, these same chemicals are subsequently removed by various physical, chemical and biological processes (Atema 1988) such that they will eventually disappear or be diluted to such an extent that a gradient cannot be perceived. According to the same author, aquatic systems are likely to be in turbulent motion at size scales higher than 10^{-2} m and hence dispersal by water-movement will be an important factor. It should also be noted that the removal or dispersion of chemicals will not necessarily occur in a regular and therefore predictable manner, thereby making it difficult for organisms to locate a source.

Crisp & Meadows (1963) in their discussion of the probability of homing responses were concerned principally with the responses of larvae derived from sessile organisms seeking a target substrate. In the case of *L. salmonis* there is the further difficulty of a mobile host. Blaxter (1969) estimates salmonids to swim at average cruising speeds of 3-4 body lengths s^{-1}. It is

clearly unlikely then, that copepodid larvae could home on a chemical trail left by a fish swimming at such speeds. To accomplish this, the copepodid would have to sustain a similar or greater speed in order to encounter the source. Such a performance is particularly hard to envision. The size of the copepodid is such that it exists in a world dominated by low Reynolds numbers[5] where the nature of flow is principally viscous. Although copepods can reach relatively high burst speeds e.g. 200 body lengths s^{-1} during escape responses (Strickler 1975) such speeds cannot be maintained. Vogel (1981) indicates that "...'small' almost always means slow and 'large' often means fast.". Carton (1968) suggests that the infective stage of the siphonostomatoid *Cancerilla tubulata* Dalyell which parasitizes the ophiurid *Amphipholis squamata* Della Chiaje cannot use distance attraction because of its small size.

The copepodid of *L. salmonis*, unlike later attached stages, has a limited and non-renewable energy resource and clearly cannot afford to prosecute long-distance "tracking" of potential hosts without a high probability of ultimately making contact; as Carton (1968) remarks, the time available for the copepodid to find a host is severely limited. Calculations by Klyashtorin & Yarzhombek (1973) on the additional energetic cost of active swimming in copepods, suggest that it may represent 20 - 30 % of the basal metabolism. This is clearly a potentially high cost and as Poulin *et al.* (1990) note, the life-span of the copepodid is inversely proportional to energy expended such that use of limited resources in activity must be assumed to aid dispersal or improve host contact.

Chemoreception may be subdivided into "long range chemoreception" and "short range chemoreception". The former may allow movement towards the source of a chemical stimulus along a concentration gradient and requires the presence of low-threshold chemoreceptors (Laverack 1968) such as those used by the blue crab *Callinectes sapidus* Rathbun which can detect taurine down to 10^{-13} M (Pearson & Olla 1977). The latter, often termed "contact chemoreception" concerns higher-threshold receptors (Laverack 1968) used in recognition of food / host / substrate or conspecific on contact or in very close proximity to the source. Both modes

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[5]Reynolds number is a dimensionless number used to describe the forces (viscous and inertial) acting upon a submerged body. This may be calculated as:

$$R_L = \frac{\rho L U}{\mu}$$

where: ρ = density of water
L = length of object
U = Velocity of object
μ = viscosity of water

(see Webb 1975)

of chemoreception could theoretically act to release behavioural responses in a parasite. In the view of Carton (1968) the parasite is brought into immediate proximity with its partner by a chemotactic mechanism and, following contact with the host, undergoes a more discriminating process which supports or refutes the former response. Though it is difficult to draw a clearly defined boundary between these two categories in parasites, the first might be termed "host-location" responses and the second "host-recognition" responses. Experiments in the present chapter have principally concerned the elucidation of chemoreceptive abilities relating to the former activity.

The supposed major sites of chemoreception in many copepods are the aesthetascs of the antennules and possibly the less well understood cephalic receptors such as the Organ of Bellonci (Calanoids and others), cephalic pleural organ (*Pachypygus gibber* (Thorell)) and cephalic dorsal hump (some Calanoids) (see Boxshall 1992 for a brief synopsis). A number of these receptors have been recognised in the present study although, as with other copepods, their exact function is difficult to ascertain due particularly to size restraints on physiological and electrophysiological research (in contrast to the Decapoda).

Antennules

The antennule has been suggested to be the major site for mechanoreception and distance chemoreception in copepods (Griffiths & Frost 1976, Gill 1986)) and has furthermore been suggested to mediate contact chemoreception in caligids by Laverack & Hull (1993). The external morphology of the antennules of *L. salmonis* was seen to correspond to the description given by Gresty *et al.* (1993) for the same species. Ultrastructural studies by the same authors indicate the receptors present in *L. salmonis* to be suitable for distance chemoreception, contact chemoreception and mechanoreception.

The antennules of free-swimming copepods such as the Calanoida are equipped with multiple segments (up to 27 of the 28 found in the ancestral condition) and aesthetascs e.g. adult males of *Eucalanus attenuatus* Dana carry 49 aesthetascs on each antennule (Huys & Boxshall 1991). Gotto (1991) notes an even more extreme example for adult males of the subfamily Haplostominae, family Ascidicolidae which may possess upwards of 100 aesthetascs on the swollen basal segment of the antennule. In this case, the author speculates that such a concentration might be associated with the difficulty of mate-location in these species by chemotactic means. It should be noted, however, that Bresciani (pers.comm.) believes that larger numbers of receptors are not necessarily more effective in chemoreception.

Stock (1991) has noted the apomorphic trend for reduction in the number of segments of the antennule in parasitic and benthic copepods. Following from this trend, the antennules of

siphonostomatoids found on fish hosts show typically reduced segmentation (Huys & Boxshall 1991), showing a maximum of 20 segments (though these are indistinctly separated). The number of aesthetascs is similarly reduced with respect to free-living groups. This pattern of reduction is even more pronounced in the caligids which display a highly specialised 2-segmented antennule and display only three aesthetascs on the antennule of the copepodid stage. This might support a suggestion that the antennules of the copepodid stage are not well equipped for detection of chemical cues at a distance from the host. It should, however, be kept in mind that the reduced nature of the antennule in caligids may simply reflect an evolutionary path taken by an ancestral form and may not reflect the present biological imperatives of the group.

Cephalic organs

Elofsson (1966) made a detailed study of the nauplius eye and cephalic receptors, which he termed "frontal organs", of the non-malacostraca and included within it a careful description of the cephalic receptors of copepods. Information from Elofsson's study and from those of more recent authors have been briefly summarized by Boxshall (1992) and the terminology used in the latter paper will be used here for consistency. The cephalic receptors noted in the present study correspond to the "Organ of Bellonci" described by Eloffsson (1971) in a number of copepods and Dudley (1972) in the ascidicolous copepod *Doropygus seclusus*. It would appear from the literature that the present study provides the first evidence of the presence of an Organ of Bellonci in caligid copepods.

Elofsson (1971) found this organ to be composed of three morphologically distinct subunits which comprised a frontal sensillum, a deep cavity receptor and a superficial cavity receptor. In the present study problems were encountered tracing connections to the frontal sensilli and the superficial receptor although the observations made in the present study suggest that such receptors may be present. The problem of tracing the terminations of these nerves has not been confined to the present study, as Dudley (1972) has previously noted: "many authors have been unable to find the end structures of the frontal organs in copepods although they have found the frontal nerves directed towards the rostrum".

The ciliated receptor described in the present study corresponds closely to the "second unit" described by Elofsson (1971) and the "end organ A" described by Dudley (1972) which represent the deep cavity receptor of the Organ of Bellonci. Unlike the receptor described in the latter study there was no indication in *L. salmonis* that the origins of the superficial receptor passed through the supporting / glial cell of the deep receptor. In this respect the present findings agree more closely with those of Elofsson. The cavity between the glial cell and the surrounding pigment cells is thought to be artefactual in the present study whilst in the study of Dudley it was

considered to be fluid-filled in life. It may be that this question cannot be resolved until the function of this receptor is more clearly understood.

Elofsson (1971) suggested that the function of the deep receptor was homologous to that of the carotid body in mammals such that it functions as a chemoreceptor for the purposes of maintaining internal homeostasis. In Elofsson's study this subunit was suggested to be largely exposed to the haemocoel whilst that of the present study appears surrounded by pigment cells and therefore possibly less well able to monitor the internal environment. The superficial receptor ("end organ B" of Dudley 1972, "third unit" of Elofsson 1971) is generally considered to be an external chemoreceptor and in the study of Dudley (1972) was most developed in the infective copepodid II suggesting a close association with host location. In the present study, extensive intra-epidermal spaces in the anterior cephalothorax suggest that a similarly well-developed organ may exist in *L. salmonis* although this has yet to be properly demonstrated.

The experimental demonstration of chemical attraction in copepods parasitic on fish has been, as noted by Raibaut (1985), rare. Nevertheless, regardless of the ability of parasitic copepods to detect suitable hosts at a distance by their chemical signatures, it is clear that free-living copepods at least, are able to use chemosensors for the detection and recognition of suitable food items or the avoidance of predators. The free-living copepod *Diaptomus kenai* for instance has been demonstrated to display a vertical migration pattern following exposure to water which had been in contact with the predatory midge larvae *C. nyblaei* (= *trivittata*) (Zetterstedt), this being taken as a chemosensory response leading to the reinitiation of predator avoidance behaviour (Neill 1990). Chemosensory ability in free-living copepods has also been noted by Van Alstyne (1986) for *Centropages hamatus* which will feed on sephadex beads only in the presence of added algal chemical components. A further example comes from the work of Buskey (1984) who states that in the species *P. minutus* at least, distance chemodetection definitely occurs. This was demonstrated by the increase in average swimming speed and number of swimming bursts displayed by this species when exposed to water containing phytoplankton-conditioned seawater. Exposed copepods also displayed a less linear path (ie more turning) than copepods in control groups. The same author also demonstrated that this species could detect morpholine and become conditioned to it.

The most detailed investigation of free-living copepod behaviour, particularly with regard to feeding, has been carried out by Strickler (1985). In his experiments, the latter author used high-speed video techniques to observe the feeding of free-living copepods on algae. The algae

appeared to be deliberately re-routed[6] by copepods before contact, which would entail perception of the location and direction of movement of algal particles by the copepods when outside the capture area and beyond the perimeter of flow. In one experiment, an alga was apparently perceived by the copepod at a distance of 1.25 mm (~0.8 body lengths) in front of the capture area. The area within which the copepod can detect particles has been termed its "reactive field" (Holling 1966). In accord with Atema (1988), chemicals have been suggested to accumulate around algal particles providing an "active space"(Bossert & Wilson 1963), this being the zone around the alga where chemical exudates are sufficiently concentrated to promote a behavioural response in copepods (Andrews 1983). This "cloud" will extend in the direction of flow and, where this flow results from the actions of a copepod, will pass ahead of the algal particle such that its presence may be signalled by anteriorly placed chemoreceptors (Andrews 1983). Strickler has demonstrated that calanoid copepods generate their own flow fields which have low Reynolds numbers and are therefore laminar. In his discussion he visualizes the feeding current as being composed of three cores; an inner "motion" core whose contents always passes through the feeding area, a "viscous" core which is the water drawn into the capture area by the copepods feeding currents and finally the sensory core which surrounds the other two and which represents the zone from which perceived algal particles may be re-routed into the capture area where necessary. In this model, different species would have the three cores represented in different proportions and this, as well as the size of the copepod and ambient water conditions would allow effective niche partitioning. This being the case, both the copepod and its feeding current act as the unit of selection.

Assuming that the copepodid of *L. salmonis* located its host through chemosensory means, one might from the above expect the antennules to be enlarged such that the sensory core was extended to allow a greater volume of water to be sampled for host chemicals. The enlargement of the antennules might also allow for expansion in the number of sensory elements and would increase the distance between the elements on each side allowing for better directional homing on a chemical beacon. As discussed above, the antennules of *L. salmonis* are, however, highly reduced both in number of segments and in number of sensory elements. This may be suggestive of the fact that long-range host detection is not a priority for the copepodid stage of this species, as suggested earlier, although it may instead simply reflect an evolutionary "decision" made by ancestral forms.

Despite the suggestion of Crisp & Meadows (1963) to the contrary, there is evidence to suggest that some parasitic copepods can home on hosts from a distance. Using a Y-tube, Carton

[6]"Re-routing" was the term given by Strickler (1985) to the redirecting of algal particles from a path that would pass outside the copepod's capture area to one that would allow capture.

(1968a see 1968) demonstrated that adults of the cyclopoid copepod parasite, *Sabelliphilus sarsi* Clarapéde, could discriminate material in water emitted by its sabellid host, *Spirographis spallanzani* Viviani in 82 % of experimental replicates and was furthermore attracted to a related host species (*S. spallanzani* var. *brevispira* Quatrefages) in 73 % of replicates. Despite this observation, however, it does not follow that related species will necessarily demonstrate similar behaviour. In the case of *Sabelliphilus elongatus* Sars and *Paranthessius anemoniae* Claus for example, no attraction for their hosts (respectively *Sabella pavonina* Savigny and *Anemonia sulcata* (Pennant) could be demonstrated for adults (Briggs 1974 cited in Raibaut 1985, Briggs 1976).

It has been demonstrated (Raibaut 1985, Fraile 1986) that copepodids of *Caligus minimus* show a distance chemotactic response to the presence of scales or fresh / freeze-dried mucus from their host *D. labrax* causing them to ascend a current in their presence and may discriminate these from the mucus of *M. cephalus*.

In support of the suggestion of chemotactic ability in caligid copepodids comes the observations of Hogans & Trudeau (1989) on *C. elongatus*. These authors stated that, when free-swimming within approximately 10 cm of a potential host, copepodids would invariably swim immediately to the body surface, regardless of light projected from a different direction. Claims for such a powerful and accurate ability seem extremely unlikely. For such behaviour to occur, the host in question (*S. salar*) would need to be surrounded by a chemical "cloud" extending 10 cm around the fish on all sides despite, presumably, being on the move. For copepodids to home so accurately and directly towards the host from such a distance would also require them to be able to negate the effects of water turbulence on the direction of the source at any given point in the directional response. As Poulin *et al.* (1990) note, the likelihood of the rapid development of a chemical gradient on the approach of a fish in a large well-mixed body of water is small. Evidence that not all caligids respond to distant chemical cues has been presented by Schuurmans Stekhoven (1934) who noted that adult *L. pectoralis* placed with *Pleuronectes (= Platichthys) flesus* (L.) moved over and close by to the host with no hint of response. This same lack of response has also been noted in the present study for copepodids of *L. salmonis* which, as described in the following chapter, have been observed to come within 1 mm of host fish before swimming off.

Although Fryer (1966) explains the aggregation of copepod and branchiuran parasites on fish from Lake Nyasa in similar terms, positing distance attraction of larvae by conspecific settled parasites, he acknowledges that such small and relatively feeble swimming larvae should theoretically have great difficulty in homing on and attaching to hosts and therefore explains his observations in terms of nocturnal settlement of resting hosts. In view of the fact that, as is demonstrated in later chapters, *L. salmonis* copepodids are able to infect fully active hosts, such

an explanation need not necessarily be invoked to explain the observed ability to settle. Further discussion of this point will be made elsewhere.

Fasten (1913) noted that copepodids of *S. edwardsii* exposed to host *Salvelinus fontinalis* (Mitchill) gills became highly active whilst those exposed to gills of rainbow trout (*O. mykiss*) and German-brown trout (species not given in cited paper) showed no such increase in activity. Such an increase in activity suggests that some distance-chemoreception is in operation although the fact that copepodids did not use the chemical gradient to locate the gills but rather darted about "in every direction" tend to suggest an absence of directional response to chemical stimuli in this species and tend to suggest that chemoreception is acting as a releaser stimulus. Poulin *et al.* (1990) point out that such high activity is only adaptive if the probability of meeting the host is high. This suggests that the reported behaviour should occur only at close range where such a condition might be satisfied.

Gotto (1991) suggests that, should attractive biochemical substances of host origin be present in the ambient water, one might expect invertebrates producing powerful exhalant currents to be favoured by copepod symbionts. This suggestion appears to be supported by the observation that out of 200 known or suspected symbionts in western Europe, 57 are associated with ascidians. Similarly, bivalve molluscs harbour as many as prosobranch and opisthobranch molluscs do together. Again it should be noted that such an observation does not necessarily entail a chemotactic response but may instead suggest that recognition of a chemical stimulus by the infective stage acts as a releaser stimulus causing, for example, a rheotactic response to be initiated.

The experiments carried out in the present study provided no evidence of distance chemoreception in the copepodid of *L. salmonis*. This does not necessarily indicate that copepodids are not able to detect dilute chemical stimuli at a distance but simply that no large scale behavioural responses in terms of taxis or increased activity could be observed using the techniques employed in the present study. Other alternatives, namely that the copepodid **may** react to other cues that were not tested, that responses of the copepodid to the cues could not be observed using the experimental protocols, or that cue reception primes or prompts other behaviours, can also be posited. The resolution of these questions depends on further more detailed analyses of copepodid responses. Ideally, such studies might be carried out using apparatus such as that described by Strickler (1985) which allows an exposure time $< 100\ \mu s$, an observation time of 4 ms or better and additionally provides a long working distance, large depth of field and high optical resolution. Such equipment is beyond the average research budget and an alternative strategy for observing behaviour may be to use the method utilised by Cowles (pers. comm.) which allows the recording of copepod movements in three dimensions using two video cameras.

Electroreception

No indication of a response to electrical stimuli was observed during the present study. Although such a facility is widely reported in elasmobranchs and teleosts (Kalmijn 1988, Zakon 1988), there have been no previous reports of electroreception in invertebrates (Zakon 1988) although it has been suggested (*ibid.*) that this may simply have resulted from a lack of systematic investigation. The results of the present study provide further documentation to support Zakon's former observation.

Mechanical

The term "mechanoreception" describes the perception of a wide range of different stimuli available to an organism in the aquatic environment. Most marine organisms for instance, respond to gravity (Hawkins 1985) and many free-swimming invertebrates are able to detect and respond to pressure changes (Morgan 1984) although the mechanism has yet to be determined in this instance. Many aquatic organisms possess mechanoreceptors projecting from the integument (Hawkins 1985) which are sensitive to shearing forces. Such organs may also, in some cases, be sensitive to the oscillatory motions of the medium generated by sources close to the animal (Land 1981). In a non-turbulent water-mass, moving at constant velocity, such receptors are unlikely to be stimulated and thus an organism could not detect current flow without e.g. magnetic or induced electrical cues and should therefore move with it (*ibid.*). Such organisms would however experience acceleration at any boundary with a current moving at a different velocity. The movement of copepodids of *L. salmonis* downstream in the flow experiments may therefore result from their being unaware of the current such that rheotactic responses are not initiated, although the proximity of the vessel sides and the likely shearing effect that these might be expected to produce make this unlikely. If the lack of cues to current direction is the reason for lack of response then it may be important in future studies to introduce a degree of non-laminarity, perhaps by the use of small obstacles or simply by using unbevelled edges and removing the glass straws used in the present experimental apparatus.

In crustaceans, a wide variety of forms of mechanoreceptor exist including those sensitive to tactile stimuli, water currents, pressure gradients and vibration (particle oscillation) (Land 1981). Some of these may be sensitive to the displacement of the surrounding medium and others to the velocity of its movement (*ibid.*). Because viscous shearing forces have an important effect on determining the movement of smaller receptors, such integumental organs must project beyond the organisms' boundary layer in order to detect movement of the adjacent medium (*ibid.*). It has further been suggested that the possession of superficial units monitoring both the

flow of water across a surface and the agitation / vibration thereof, might be important in the detection of "power sources" swimming in the near vicinity as evidenced by the capture of fish by decapod crustaceans (Laverack 1968).

As with the previous stimuli, an attempt has been made in the present study to investigate both the presence of likely mechanoreceptors and the responses of copepodids to a range of stimuli.

Integumental organs

Copepodids of *L. salmonis*, in common with many other aquatic organisms (Hawkins 1985), were found to have a number of mechanoreceptors projecting from the cuticle, particularly on the dorsal surface. These correspond to the hair and peg sensilli frequently described in a wide range of crustacea (Land 1981). Such processes may enable detection of one or more of the following stimuli: tactile stimuli, water currents, pressure gradients, vibration, water displacement and velocity. Different morphological configurations are likely to determine which stimuli are best transduced by a given organ. Sensilli must be long enough to project past the boundary layer of the organism (Land 1981) since viscous shearing forces determine the action of water movement on the organs. The distribution of these mechanoreceptors in the present study agrees closely with that reported by Johnson & Albright 1991a for the same species and with that given in the descriptions by Boxshall (1974d) of the copepodid stage of *L. pectoralis*. The concentration of sensilli on the dorsal surfaces may be suggestive of the fact that turbulence in the ventral area caused by swimming movements and by the movement of water across the larger ventral structures e.g. antennae / mouth tube prevents the accurate reception and interpretation of stimuli. In attached stages this surface is also the one exposed to the external environment such that receptors on its surface might be important in measuring e.g. current flow / exposure of potential settlement sites.

According to Land (1981), such organs are usually sensitive to water currents and in some cases to the oscillatory motions of the medium generated by sources close to the animal. This therefore makes such organs well suited to the detection of nearby organisms and therefore the possible detection of hosts by the infective stages of parasitic copepods such as *L. salmonis*. The pegs and sensilli of the copepodid of *L. salmonis* were apparently sparsely distributed as was found to be the case in adults of the poecilostome soft coral parasite *Lamippe rubra* Bruzelius observed by Bresciani (1988). This number was far less than the number indicated to be present on adult of the free-living predatory copepod *M. albidus* by Strickler (1975), and Fleminger (1973) similarly found large numbers of sensilli on *Eucalanus* spp.. Large numbers of sensilli are, furthermore, not confined to calanoids as can be seen from the illustration of a female

Asterocheres reginae Boxshall & Huys from the Siphonostomatoida (Huys & Boxshall 1991). It was suggested by Strickler (*op. cit.*) that these sensilli would aid detection of conspecifics for mating and be of use in the avoidance of other species in the plankton. The copepodid of *L. salmonis* is not a mature stage and thus has no requirement for recognising swimming conspecifics. Similarly its need to make contact with a host may preclude the large numbers of sensilli necessary for accurate avoidance reactions. Strickler stated that these sensilli could not be observed using a light microscope, however those in the present study have been observed both in whole and sectioned specimens (principally through observation of their basal articulation).

In addition to the sensilli, numerous pores were noted during this study. Such pores are commonly found in free-swimming copepods (see von Vaupel Klein 1982) and may represent the openings of integumental glands, larger internal glands or even chemosensory pits. From the present study the function of most pores remains undetermined. Where the structure or function of underlying organs has been elucidated, given pores will be discussed elsewhere. As with the sensilli, other species e.g. *Asterocheres reginae* may have large numbers of pores relative to the number carried by the copepodid of *L. salmonis*.

Antennules

The development of distal segments of the antennule tend to have been very conservative in evolution, with setation being maintained in most copepod groups (Boxshall 1992). Such conservatism would seem to indicate an important role for these segments in the life of the copepod and indeed it would appear that the all-important escape response is mediated through receptors on this part of the antennule. It is believed that the distal setae on the posterior margin of the antennule are "hard-wired" to the escape response (Boxshall 1992). This has been demonstrated in *E. amphitrites* by Park (1966) who showed that stimulation of the antennule on one side led to contraction of the dorsal trunk muscles on the other side leading to turning away from the mechanical stimulus. Gill & Crisp (1985) examined the reaction of the free-living planktonic copepod *Temora longicornis* Müller to directed water jets. It was found that the commonest response to stimulation, particularly to that in front of the animal, was an escape response. This response was found to be greatly reduced if a single antennule was amputated and the jet directed to that side and similarly the response was greatly reduced by the complete removal of both antennules. It was found that all areas of the animal were sensitive to direct stimulation with the most sensitive areas being the cephalosome and antennules.

Responses to mechanical stimuli have been previously reported for a number of species of parasitic copepod and Kabata (1981) for instance, has suggested that current plays an important part in the directed movement of parasitic copepodids, a statement echoed by Raibaut (1985). The present study found that vibrational stimuli (shock-waves) caused rapid swimming in copepodids of *L. salmonis* and that directed flows elicited a rapid swimming response with associated turning. Wootten *et al.* (1982) have also observed active swimming in *L. salmonis* copepodids exposed to mechanical disturbance. In view of the general absence of other specific contact responses demonstrated by the present study, it would seem that such behaviour is the principal mechanism mediating host contact in *L. salmonis*. These behavioural responses are assumed to depend, in the field, upon the detection of host movement in the vicinity of the copepodid.

Support for this suggestion comes from the work of Kabata & Cousens (1977) on *S. californiensis* from which it was suggested that one of the chief stimuli initiating swimming in this species was similarly disturbance in water surrounding the copepodid. It was suggested from this that the forward movement of the fish and movement of the operculi and associated respiratory currents might result in increased contact of copepodids with the pectoral fins and operculi. Further work on this species by Poulin *et al.* (1990) indicated that the response of copepodids to artificially generated shock-waves was independent of the magnitude of the stimulus. Such a non-discriminatory response was suggested to possess low intrinsic specificity and this latter conclusion would also appear to be true for the responses of *L. salmonis*.

In the present study, no rheotactic (ie directional) response was demonstrated for copepodids of *L. salmonis*. Many other authors have suggested that a positive rheotactic response may be important in the contact of hosts by parasitic species. Boxshall (1976) looking at copepodid larvae of *L. pectoralis*, reported them to display a positive rheotactic response to directional water currents, with swimming activity being stimulated by general water turbulence. It was suggested by the same author that the copepodids move towards water-currents produced by the host and it was proposed that the distribution of copepodids on fins and around the branchial openings reflected this behaviour. In view of this, the lack of a positive rheotactic response in *L. salmonis* is supported by the fact that copepodids are not found to be concentrated around the operculi, as might be expected were they drawn to opercular currents.

Similar observations of positive rheotaxis have been made for infective females of *Ergasilus lizae* Krøyer attaching to the gills of mullet (Ben Hassine 1983 cited by Raibaut 1985) and for copepodids of *C. minimus* Cabral (reported by Raibaut 1985). The latter author placed copepodids in a compartment on one side of a grille and a specimen of *D. labrax* (the host species) on the other side and found that when the fish faced the grille, there was no copepodid response but that when it faced the other way, copepodids moved through the grille into the host

compartment. This was attributed to a positive rheotactic response to respiratory and body-movement currents created by the host. Repetition of the same experiment in the present study did not cause copepodids of *L. salmonis* to congregate on the same side as the fish (see later chapters). Further work on *C. minimus* carried out by Fraile (Raibaut 1985, Fraile 1986) indicated positive rheotaxis in copepodids tested in a flume apparatus when exposed to currents of 3,300 ml h^{-1} although at lower speeds of 650 ml h^{-1}, they moved down-stream (this latter being suggested to constitute a negative rheotactic response by Fraile 1986). As mentioned earlier for light responses, some care should be taken in describing movement downstream in terms of a negative taxis since this would also be the consequence of lack of activity by the copepodid.

Responses to host-derived currents have also been reported for copepods such as *Paranthessius anemoniae* Claus which are associated with invertebrate hosts (Briggs 1974 cited by Raibaut 1985). In this case, cuticular hairs found on the dorsal surface of the copepod were suggested to function as rheoreceptors of the type described by Laverack (1962) in lobsters. Rheotactic responses need not be invoked as the only mechanism of host contact, and in this respect, Raibaut (1985) suggests that ascidicolous copepods living in the branchial chamber of hosts may locate them initially using chemotaxy and may subsequently use rheotaxy to enter the buccal siphon. As noted before, this behaviour does not necessarily entail a chemotactic response as it might also be explained through chemical recognition acting as a releasing stimulus for rheotactic responses. An important corollary to the suggestion that host contact might result from mechanoreception, is that water turbulence is likely to interfere with host location, an observation made by Gotto (1962). Such an assertion is supported by the observations made by Briggs (1976) on *P. anemoniae* infesting the anemone *Anemonia sulcata*.

The occurrence of mechanoreceptive responses in parasitic species would appear from the above to be fairly well established. Such responses are not, however limited to parasitic species and it is likely that their origins may lie in the feeding and predator-avoidance responses shown by free-living copepods. As noted earlier, the main site of mechanoreception in free-living copepods has been proposed to be the antennules (Griffiths & Frost 1976, Gill 1986). As an example of mechanoreception in free-living species, Gill & Crisp (1985) have reported that *T. longicornis* could apparently detect water disturbance at a distance of < 4.5 mm and Haury *et al.* (1980) have similarly reported that *C. finmarchicus* could detect obstacles at a distance of 7 mm. The strength of the stimulus in the study of Gill & Crisp determined the likelihood of a response, an observation made in the present study with respect to shockwaves (vibrational stimuli). It follows from this that the swimming activity of prey species might be expected to determine their ease of capture and it has been reported that predation rates of *Euchaeta elongata*

Esterly were lower on inactive swimmers than on active swimmers lacking a well-developed escape response (Bailey & Yen 1983).

Further support for the importance of mechanoreception in the feeding of free-living copepods comes from a study by Buskey (1984) who demonstrated that *P. minutus* swam more slowly when exposed to water containing odourless phytoplankton-sized spheres and displayed fewer swimming bursts. This was suggested to be indicative of the use of mechanoreception in the detection of particles in feeding currents. Such observations also extend to the hunting of moving prey such that Fahrenbach (1964) concluded that in hunting *Artemia* nauplii, *M. albidus* only started moving when the prey was so close that tactile rather than visual stimuli were likely to be decisive.

The antennular sensing of particles in calanoid copepods, particularly in those that capture particles from the "sensory" core has been suggested to be susceptible to interference from temporal (and random) changes in the flow field (Strickler 1985). Such interference, it was suggested, might reduce the ability to sense particles and reroute them into the capture zone. This hypothesis is supported by observations that herbivorous copepods which normally glide while feeding, execute many jumps and cease feeding when contained in constantly mixing environments whilst carnivorous calanoids e.g. *Epischura* spp. continue to feed normally under such conditions (*ibid.*). This clearly echoes Gotto's (1962) suggestion that turbulence might interfere with host location in parasitic species although it also suggests that stimulus intensity is important (ie carnivorous species may still detect prey).

From the above observations one can suggest that the responses of copepodids of *L. salmonis* are modifications of the feeding and predator-avoidance responses of free-living species. The response to a "power-source" in the near vicinity mirrors the startle / escape response of free-living species but instead of removing the copepod from predators, in the copepodid of *L. salmonis*, the response serves to promote contact.

Pressure

The present study has demonstrated that copepodids of *L. salmonis* are able to perceive and respond to pressure although the morphological basis for this response has not been identified. Copepodids were observed to rise with increasing pressure and conversely fall with pressure decrease.

Hydrostatic pressure increases by ~ 101325 N m^{-2} (= 1 atm) with every 10 m depth. Crisp (1976) notes the fact that by maintaining themselves at a given depth, pelagic larvae may be able to increase their chances of being carried in a favourable direction, light and gravity providing a reference orientation. In order to accomplish this, pressure is the most consistent and

reliable indicator of depth and the majority of larvae examined show sensitivity to pressure (Rice 1964, Knight-Jones & Morgan 1966). As yet no known pressure receptors have, however, been demonstrated in marine invertebrates (Morgan 1984).

Although there are records of pressure responses in free-living copepods e.g. *C. finmarchicus* (Hardy & Paton 1947, Rice 1962) and *T. longicornis* (Knight-Jones & Morgan 1966) there appear to be no previous observations of pressure responses in parasitic copepods. This may, however, result simply from the fact that such responses have not been previously investigated.

T. longicornis has been reported by Knight-Jones & Morgan (1966) to be sensitive to pressure changes of 10 mb (= $\sim$1000 N m^{-2}). Pronounced behavioural changes were only observed in *L. salmonis* at pressures > 30397.5 N m^{-2} which suggests that it is an order of magnitude less sensitive although this may be an artefact of the relatively crude apparatus used to examine pressure responses in the present study. Such a sensitivity threshold would nevertheless serve to keep *L. salmonis* close to the surface if it is manifested in the natural environment.

"Cauliflower organ"

Discussion of this organ has been left until last because it is difficult to ascribe a function to it and hence it cannot be easily associated with any particular stimulus category. The fact that it is present both in *L. salmonis* and *C. elongatus* copepodids suggests that it may be a feature common to all caligid copepodids. The main features of this organ are its ornate surface morphology, its thin cuticle walls and the multiple attachments of a large striated muscle. It also possesses apparent channels which may allow external expression of material and a prominent sensillum. The channels may represent the external openings of the duct from the "mucoid gland" (see Chapter 5) which could not be otherwise observed under SEM. The function of the swollen extremities of this organ remains unclear. The presence of thin cuticular walls, which are often associated with a chemoreceptive function (Laverack 1968), may suggest such properties, although no other evidence was obtained to support this.

Another possibility for its function is that it might represent a "stickiness receptor" - detecting molecular adhesion between the cuticle of the receptor (or adsorbed material) and the host substrate as postulated by Crisp (1976) for the attachment disk of the antennule of cypris larvae of the barnacle *Semibalanus balanoides* (= *Balanus balanoides*). The suggestion that the function of the attachment disk is that of an adhesion receptor was, however, discounted by the authors of the original description of the structure of the disk (Nott & Foster 1969) who believed that extraneous matter attached to the substrate would interfere with such a mechanism. If this

were true, then the presence of host mucus might similarly be expected to interfere with adhesion detection in *L. salmonis* and it may instead be, that the channels observed within the organ similarly express enzymes acting upon host components to give products recognisable to chemoreceptors located within the organ or elsewhere (e.g. aesthetascs of the antennule or cephalic organs) as suggested by Nott & Foster (1969) for *S. balanoides*.

The cauliflower organ of the present study shares a number of features with the attachment disk of the barnacle cypris. Both apparently possess a large surface area, ducts for secretion, sensory hairs (although only one in *L. salmonis*) and muscle attachments. Nott & Foster (1969) suggested that the attachment disk acted as an adhesive pad with sticky secretions being expelled at its surface and muscles acting to detach it. As noted above, these authors postulated that enzymes secreted via the disk might act upon chemical components of the substrate to give products recognisable to chemoreceptors associated with the disk. Mechanoreceptive sensilli also associated with the disk were suggested to facilitate positioning.

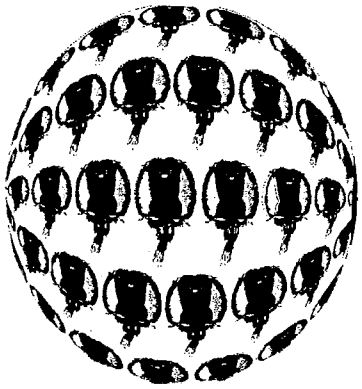
The large surface area of the cauliflower organ and its thin, and therefore possibly flexible, cuticle mean that host-specific adhesion to the substrate, as suggested by Crisp (1976), might be transduced either by cuticular stress detectors or apodeme tension receptors associated with the cuticle, as have been observed in other crustacea (Bush & Laverack 1982) or by muscle receptor organs associated with the attached muscle (*ibid.*). No such receptors have been noted, however, in the present study. It is difficult to envisage how an adhesive pad such as that suggested by Nott & Foster (1969) might aid a copepodid already firmly attached through the antennae. On the evidence of the present study, a definite function cannot therefore be suggested. In order to resolve the function of this organ, it is clear that future studies must concentrate on obtaining and interpreting full serial sections of the organ under TEM and following its presence and / or development in other stages of the lifecycle.

Returning salmonids in estuaries frequently swim near the surface, a fact attested to by netsmen who use this behaviour to allow the capture of individual salmon through use of spotting posts on the shore. Such behaviour in salmon may be associated with the search for odour trails indicative of the "home stream".

If we assume, in the absence of evidence presented to the contrary, that the pressure and light responses reported for copepodids in the present study are not simply artefactual but help to bring copepodids into surface water, then this hypothesized behaviour might be suggested to improve the probability of host contact by increasing the overlap between copepodids and hosts. Although this hypothesis remains unconfirmed in *L. salmonis*, it has been noted by Schram & Anstensrud (1985) that copepodids of *L. sprattae* are generally located in surface water at night.

Once the copepodid has been brought into the zone of maximum probability of host-contact by responses to environmental cues, it is further hypothesized, from the results of the experiments described in the present chapter, that host contact is effected principally through a swimming response to the passage of a host, or other moving object, as detected by mechanoreceptors associated with the antennules and / or dorsal cuticle.

CHAPTER 4
SETTLEMENT AND ATTACHMENT OF LARVAL STAGES



4 - SETTLEMENT AND ATTACHMENT

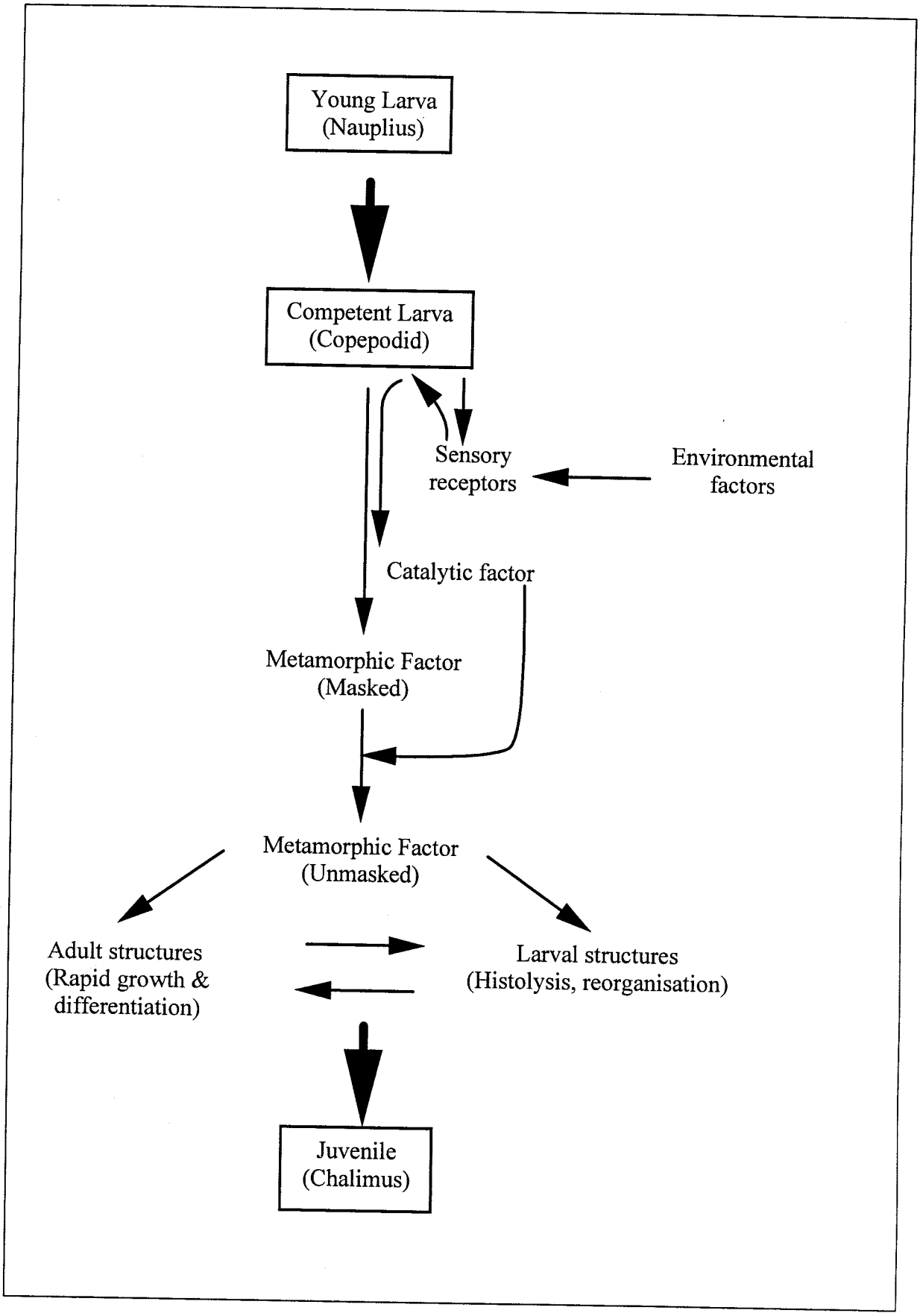
4.1 Introduction

The lack of information concerning the settlement and attachment of parasitic copepods has been previously noted by Kabata (1981) and the situation has changed little in terms of detailed studies save for the excellent work of Benkirane (1987) on attachment in the Lernaeopodidae. More recent work by Jones (1989), Jones *et al.* (1990), Bron, Sommerville, Jones & Rae (1991), Johnson & Albright (1992a) and Pike *et al.* (1993) have also mentioned the frontal filament of *L. salmonis* and the latter study also includes structural details of the filament of *C. elongatus*.

Although there are clear parallels between the settlement of parasitic and non-parasitic organisms, the attachment substrate of the former is likely to be more predictable since it is maintained according to the homeostatic and physiological requirements of the host and should therefore not be expected to be subject to extensive variability (Sukhdeo 1990). Parasites may therefore be observed to develop high specificity regarding both host and site of settlement and hence Boxshall (1974a) for instance, looking at parasitic copepod infections of fish hosts in the North sea, found 70 % of the 39 species studies to be highly host specific.

With regard to marine invertebrates in general, Chia (1978) has proposed a scheme of settlement and metamorphosis which is illustrated in Figure 4.1. This proposed series of events begins with a "young larva" which is unable to settle and metamorphose until it has developed into a "competent larva" whose physiological state allows it to proceed given the appropriate environmental conditions. In the case of *L. salmonis* and other caligids, this latter stage could be considered to be the copepodid and the former, to be the nauplius. Chia proposes that the competent larva may possess a "metamorphic factor" that promotes metamorphosis in the larva but that this factor is unable to function due to "masking" that must normally be removed by a separate catalytic factor. This latter factor is suggested to be produced in response to an environmental cue (e.g. for *L. salmonis* one might propose a component of host mucus) that triggers its production. Once the metamorphic factor is unmasked, it prompts the larva to undergo a period of metamorphosis which probably consists of a combination of the breakdown and reorganisation of existing larval structures and the growth and differentiation of adult (or juvenile) structures. The same author also suggests that the mask of the metamorphic factor may break down over time of its own accord such that most larvae will often metamorphose eventually with or without the appropriate environmental stimuli. Such automatic transformation

Figure 4.1 General events and possible mechanisms of settlement and metamorphosis in marine invertebrate larvae (modified from Chia 1978)



would be lethal to a parasite without a host and under these conditions, such a change would appear to be a waste of dwindling energy resources. One might therefore expect this feature of physiology to be absent in parasitic species, however, Piasecki (1984) has observed premature frontal filament production in *Tracheliastes maculatus* Kollar, which produced filaments in the absence of host material and died during and subsequent to the filament production. Reports of successful moulting to the chalimus stage in the absence of a host have not, however, been recorded in the literature, suggesting that some host factor is indeed required for permanent attachment and moulting to occur.

Crisp (1976) divides settlement responses of marine invertebrates into three principal stages; "attachment" "exploration" and "fixation" with the first stage being deemed reversible such that a larva could delay metamorphosis and re-enter the water-column were the substrate unsuitable. In the light of the statements above, however, one might suggest that metamorphosis is not so much "delayed" as "untriggered". The initial exploration phase was suggested by Crisp to be divided into "wide searching", "close searching" and "site selection" phases, with encounter of appropriate stimuli lowering a settling threshold until a specific stimulus could enable settlement. It was also noted that settlement was more likely with increasing age, since the settling threshold was suggested to decrease with time. Recognition of specific substrates was suggested to occur through close range physical forces rather than by identification of diffused chemical cues over long distances. With respect to this, recognition was therefore postulated to occur only after contact, through an adhesive stimulus resulting from reactions similar to those of the antibody-antigen reaction and suggested to occur between specific sites on the larva and components of the substrate surface. Other cues such as surface roughness were also thought to be important in substrate recognition. The specificity of parasitic copepods parasitizing fish may also result from similar considerations and indeed, the "cauliflower organ" described in the previous chapter may be one candidate for the mediation of such a proposed mechanism. Recognition might also be suggested to occur through the high-threshold chemoreceptors of the antennules or through a chemoreceptive function of the Organ of Bellonci (see Chapter 3).

Sukhdeo (1990) regards location of the host and of the site of settlement once the host is attained as the end-point of a series of "releaser responses" which are defined as "fixed patterns of behaviour that are released by trigger cues or sign stimuli". Such behavioural patterns are postulated to result from the selection, and fixation in the genotype, of behaviour that increases the probability of locating an appropriate host / site for settlement. In the case of *L. salmonis* one could hypothesize that the copepodid larva may be brought into the area of the host by responses to environmental stimuli such as light and pressure and might then contact the host through responses to host-derived cues such as water disturbance. Site location by such "releaser responses" may explain the ability of certain copepods to locate highly specific sites e.g. *L.*

branchialis and *Cardiodectes medusaeus* (Wilson) which invade fish hearts (as described by Kabata 1981) and *Leposphilus labrei* Hesse which invades the lateral line and mucus canals (Quignard, 1968). Such site location in copepods may proceed through mechanisms such as the "reflex" burrowing response implicated in the excavation of a bulla implantation cavity by *S. californiensis* (Kabata & Cousens 1977) although the initiating factors for such behaviour(s) are as yet unknown. The location of specific sites, would by this hypothesis, follow from serial responses to successive cues, allowing gradual focusing on the appropriate attachment site.

Copepod attachment to fish hosts has been stated to consist of "primary" attachment used for feeding / predatory feeding and "secondary" attachment concerned with the maintenance of contact with the host (Kabata 1981). Primary attachment is reversible and therefore equates to the initial "attachment" phase of Crisp 1976). Primary attachment may often be followed by secondary attachment which equates to Crisp's "fixation" phase and is permanent or semi-permanent. The most common and often sole appendages serving primary attachment have been noted to be the antennae (Kabata 1981) and initial attachment via the hooked antennae has previously been described for various caligid spp. by *inter alia.* Wilson (1905), Lewis (1963) and Voth (1972).

Subsequent attachment by means of a frontal filament is a widely distributed trait within the Siphonostomatoida (Huys & Boxshall 1991) and characterizes the "chalimus" larva which is equivalent to copepodids II - IV / V of non filament-producing species. Observations and suggestions as to the origin of production of the filament in caligids in the past have fallen roughly into two categories; those that describe production of the filament from a secretion *in situ* and those that indicate the use of an internally preformed filament for attachment. The former mode of attachment has been described for *L. dissimulatus* (Lewis 1963), *L. hospitalis* (Voth 1972) and various *Caligus* spp. (Wilson 1905) whereby a drop of adhesive material is secreted onto the host tissue and subsequently drawn out into a filament as the larva pulls away from the surface of the host. The alternative method of attachment, whereby the filament is produced entirely within the larva and then everted ready-made and attached to the host has been described by Kabata (1972) who noted a "frontal sac" which was everted on attachment along with an extruded filament in *C. clemensi*. Pre-formed filaments have also been noted by Gurney (1934) for *C. centrodonti* and *C. labracis* Scott, Hwa (1965) for *C. orientalis* and Pike *et al.* (1993) for *C. elongatus*. Pre-formed structures have also been described for the Lernaeopodidae whose attachment structures have been studied in much greater depth than have those of the Caligidae. These attach to their hosts, initially by means of a filament similar to that described for caligids, but subsequently by use a "bulla", attached to the maxillae and comprising a "manubrium" and an "anchor" surrounded by a thin "pellicle". Kabata & Cousens (1972, 1973) and Benkirane (1987) describe copepodids of this family as having an internally constructed

filament, these being extruded and attached to the host by some as yet uncharacterised cement. Benkirane (1987) also found the subsequent bulla to be internally produced and further reported that the manubrium of *Alella macrotrachelus* (Brian) was primarily composed of densely packed sclerotised protein filaments. The same author also suggested that maturation after initial extrusion might be accomplished through the action of a further secretion.

Inter alia, Scott (1901), Wilson (1905) and Lewis (1963) have all noted organs located in the anterior part of the larval caligid cephalothorax and suggested them to be concerned with filament production, although none of these authors have given detailed descriptions or accurately ascribed precise functions to the organs.

4.2 Study aims

The purpose of the studies described in this chapter were threefold:

1) To describe the behaviour of copepodids with respect to live hosts and excised host tissue.

2) To attempt to initiate settlement and metamorphosis on artificial substrates for the purposes of isolating cues responsible and in order to observe the process of filament production and moulting to the chalimus stage.

3) To describe the structure of the frontal filament and determine the origins of its production in terms, both of the organs involved, and the likely affinities of the frontal filament to the normal components of copepod structure.

4) To draw conclusions as to the relationship between the attachment mechanism of caligids and those of other parasitic / "free-living" groups.

4.3 Materials and methods

4.3.1 Source of material

Gravid females were obtained from infested fish at a number of sites and free-swimming copepodid stages were reared from eggs according to the schedule detailed in Chapter 2. Attached copepodid and chalimus stages were obtained either from naturally infected fish or from

fish infected artificially by exposure to copepodid infection (see Chapter 6). Stages were removed from harvested or sacrificed fish either by removing whole fins or scales or by excising the area around the attached parasite using a scalpel.

4.3.2 Experiments to observe settling and attachment behaviour

4.3.2.1. Observation of infection *in vitro*

4.3.2.1.1 Infection of excised host tissue

Material for infection was obtained from freshly killed sea-adapted fish. Whole or part fins and pieces excised from the flanks and operculi were removed using scalpel and fine forceps (in order to avoid handling contamination and removal of mucus).

Observations of copepodid settling were performed on the excised pieces of tissue by placing them in seawater in petri dishes or basins. The seawater was either uncirculated or had circulation induced by the use of an air jet played onto the water surface as described by Crisp (1976) Figure 4.2. The dish was held in a cooled water bath or placed on a cooled substrate to prevent overheating by the microscope light. 25 free-swimming copepodids were placed in this system and were observed using a monocular dissecting microscope with incident tungsten lighting.

Further to these experiments, copepodids of various ages ranging from newly moulted copepodids which had just re-entered the water column to those 7 days old (ie 7 days post-ecdysis) were placed with excised tissue and their ability to settle successfully on the tissue under circulating water conditions observed. Only actively-swimming copepodids were used in these experiments as those remaining on the bottom for extended periods were considered morbid.

4.3.2.1.2 Infection of non-host substrates

This series of experiments was designed firstly to investigate reports that caligid copepodids have been observed to attach directly to fibrous substrates and secondly to attempt to elicit settlement on artificial media for the purpose of observing settlement and possibly development under controlled conditions. The three artificial substrates tested were as follows:

Figure 4.2 Experimental apparatus for *in vitro* infection of excised host tissue

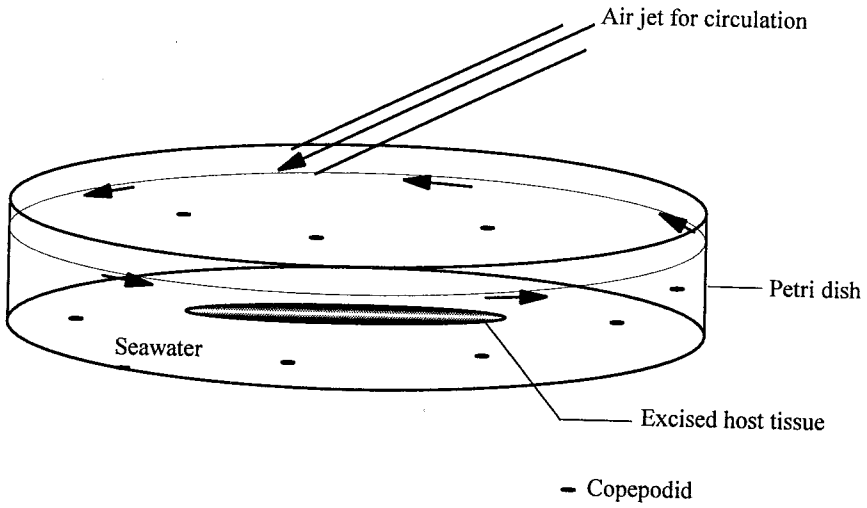
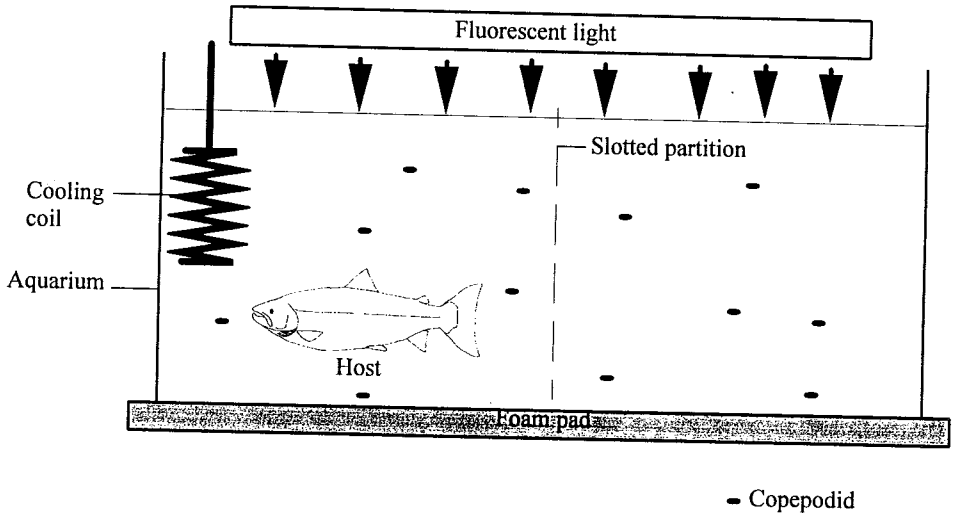


Figure 4.3 Experimental apparatus for *in vivo* infection of salmon hosts



1 Plankton mesh

Strips of 100 μm plankton mesh (~3 mm × 50 mm) were tied into a "spider" with sewing cotton and placed in the test chamber (after soaking in seawater to remove possible contaminants).

2 Swabs

Cotton wool swabs were soaked in seawater for 24 hrs and then presented in the test chamber either floating on the surface or weighted to sink.

3 Host-treated agar

Agar blocks (10 mm × 10 mm × 5 mm) were made up using the liquid resulting from trituration of host skin followed by filtration of the resulting liquid (as described in chapter 3). This liquid was mixed 1:1 with seawater and heated over a bunsen sufficiently to dissolve the agar. The agar was not autoclaved since it was felt to be important to try to leave the host material unaffected (i.e. sterility and lack of bubbles were not important for the purposes of this experiment). The agar blocks, when cooled, were soaked in the same filtered host liquid for 1 hr prior to the experiment.

A further number of blocks made with normal agar were pressed carefully onto the skin of recently killed fish in order to coat them with host mucus as described by Kearn (1967) for experiments with *Entobdella soleae* (Van Beneden & Hesse).

These three types of artificial substrate were subjected to an identical experimental protocol whereby they were placed in a petri dish of seawater maintained at 10°C and circulated with an air jet as described in the above experiments. An infective dose of 25 copepodids was introduced to the system and their behaviour observed for a period of 1 hr. Any copepodids that appeared to have settled were placed in a cooling cabinet (10°C) and observed twice daily until they died or became detached.

4.3.2.2 Observation of infection *in vivo*

The experimental apparatus for observing the behaviour of copepodids exposed to live fish is illustrated in Figure 4.3. The fish chosen were sickly / slow-moving salmon smolts with severe fin erosion so as to allow detailed observations of copepodid settling behaviour (more active fish would disturb the water column and prevent accurate naked-eye observations). These fish had a tendency to rest near the bottom, remaining neutrally buoyant and barely swimming. The observation chamber consisted of a glass aquarium measuring 320 × 610 × 410 mm

surrounded on three sides by black plastic sheeting to improve observation of the copepodids and isolated from external vibration by a foam pad. Lighting was by means of a 15 W fluorescent "Aquaglow" bulb suspended above the tank. The seawater in the tank had a salinity of 30‰ and was cooled to 10°C with the aid of a cooling coil attached to Haake D1 cooled water circulator. The test apparatus was operated in a fully blacked out darkroom to avoid light stimuli from outside the system. The tank was divided into two halves by the use of a slotted plastic partition which precluded movement of the fish from one compartment to the other but allowed movement of the copepodids. All components of the system were soaked in seawater for 24 hrs before the experiment to remove any contaminant chemical traces. Single fish were acclimatised to the system for 20 minutes before commencement of the experiment. Aeration was used while the experiment was not in progress but was turned off before addition of the copepodids to allow observation. The experiment was initiated by the slow addition of 250 copepodids (~ 2 days after moulting) on the opposite side of the partition to the fish. The copepodids were then observed for 2 hrs with notes being taken on their behaviour throughout.

4.3.3 Characterization of attachment

4.3.3.1. Light microscopy

Material obtained as described above was rinsed in seawater and fixed immediately to avoid post-mortem artefacts.

Material for wax embedding was fixed in 10% neutral buffered formalin and dehydrated through methylated spirit, ethanol and chloroform prior to embedding in paraffin wax (see Chapter 2). Material for Historesin embedding was fixed in 2.5% Glutaraldehyde buffered with 0.2M sodium cacodylate and washed in sodium cacodylate prior to dehydration through an ethanol series and embedding in Historesin (see Chapter 2.). Sectioning of material for light microscopy was performed as described in Chapter 2.

Sectioned copepodid and chalimus stages were stained with a variety of histological and histochemical stains, as indicated in Table 2.1, in order both to visualize the morphology and determine the chemical composition of the structures seen. Serial sections were recorded using a drawing tube and reconstructions of the frontal filament and associated organs were made from these drawings using the plasticine and computer 3-D reconstruction techniques described in Chapter 2.

Whole live specimens were also examined under the light microscope as were whole specimens that had been cleared and mounted in Berlese fluid or lactophenol.

4.3.3.2 Electron microscopy

Material was prepared for scanning and transmission electron microscopy as detailed in Chapter 2.

4.3.3.3 X-ray elemental analysis

In order to obtain some estimate of the chemical nature of the filament, x-ray elemental analysis was carried out on filaments excised from attached chalimus stages of *L. salmonis*.

Filaments were removed from chalimus stages using a fine scalpel and were bisected to expose the interior of the filament. The excised filaments were washed 5 times in distilled water to remove host and seawater contamination and were pipetted onto 10 mm diameter carbon stubs which had been previously prepared using emery paper and Velin tissue to polish the surface. The stubs were covered to prevent air-borne contamination and allowed to air-dry. Once dried, the stubs were cemented to clean glass microscope slides using Araldite Rapide. Whole chalimus stages were also prepared in the same manner.

The prepared stubs were coated with carbon using a Nanotech Carbon coater (140 Ma, 120 millitorr, 2×10^{-4} Torr) depositing ~ 12 nm of amorphous carbon and were then analyzed using an Hitachi S2500 / Link AN10 / 55S system, combination energy dispersive X-ray elemental analyzer and scanning electron microscope. Results were processed and printed out using a 386DX computer with an attached printer. A number of other materials were also processed in order to provide examples of known materials. These comprised human nail, prawn (*Macrobrachium rosenbergii* (De Man)) cuticle, prawn exuvium and crab (*Carcinus maenas* (L.)) cuticle chosen to give a range of keratinous and sclerotised / calcified material.

Analysis was carried out using a 1.5 μm beam emitted at a 45 ° angle to the specimen at an acceleration voltage of 15 Kv. The x-ray count was maximised at > 600 counts s^{-1} to avoid background interference. Each point-analysis was carried out for a period of 50 s.

4.4 Results

4.4.1 Experiments to observe settlement and attachment behaviour

4.4.1.1. Observation of infection *in vitro*

4.4.1.1.1 Infection of excised host tissue

Copepodids were found to successfully settle and temporarily attach to excised host tissue both in the uncirculated and circulated seawater. No filament production was obtained under these conditions and it was observed that attachment was more often "successful", ie with copepodids remaining attached after settlement, in the circulated infection environment. The tissue itself was seen to degenerate rapidly making observation of subsequent behaviour and moulting impossible after a few hours.

In the uncirculated system, copepodids made contact with the host material during random swimming events. On contact with the host surface, small scale manoeuvring occurred, with the maxillipeds being held to either side of the copepodid gripping the host surface and providing a fulcrum for probing of the surface with the anterior cephalothorax and therefore the antennules anteriorly and the antennae ventrally. Once an appropriate site had been located, the maxillipeds were used to help lever the antennae into the host using a repeated stabbing movement, the copepodid jabbing forwards with each flexure of the antennae. Once penetrated into the epidermis, the copepodid oscillated from side to side about the antennae, presumably working them deeper into the epithelium and bringing the leading edge of the cephalic shield into contact with the host.

In the circulated system, the behaviour was somewhat different. Initial contact was caused more by the circulation of the water than by the swimming action of the copepodids. Initial contact / attachment was made apparently by the grappling action of the hooked antennae (these becoming caught in the host epithelium) after which event, a concerted effort (in terms of forward jabbing and side to side oscillation) was made to embed them more deeply. In the circulated system, no small-area manoeuvring was observed, with badly grappled copepodids being removed by the current. As before, the antennae were embedded with the aid of the leverage afforded by the maxillipeds and the cephalic shield was brought down and into close contact with the host epidermis.

Settlement at all points of this process was found to be reversible. In the initial stages, copepodids would either leave of their own accord or be removed by the current. During later stages, once the copepodid was more deeply embedded in the host epidermis, mechanical or

other dislodgement still suggested that the process was reversible with the copepodid behaving exactly as it did before initial settlement.

All ages of copepodid tested were apparently able to settle successfully on excised tissue although no estimate could be made of whether such settlement would eventually lead to successful development.

4.4.1.1.2 Infection of non-host substrates

Copepodids were not seen to attach or attempt attachment on either the treated agar or the plankton mesh. Some copepodids appeared to become caught by the antennae and other appendages in the fibres of the cotton swabs but apparently made energetic attempts to escape. Those that remained enmeshed, failed to attach permanently by filament and died within one or two days of getting caught.

4.4.1.2. - Observation of infection *in vivo*

Copepodids were found to distribute themselves evenly throughout the tank with no apparent concentration at the surface or on the side of the partition containing the host. Normal swimming by the copepodids consisted mainly of short darts followed by cessation of swimming and sinking head-down. The fish were fairly still or slow-moving at most times. When rested at the bottom, contact with fish was made apparently accidentally with copepodids often dropping onto them. The undirected nature of this activity seemed to be supported by instances where the copepodid came within ~ 1 mm of the host surface and then darted off in another direction suggesting lack of detection by copepodids of the presence of the host.

Successful attachment usually followed host movement and subsequent copepodid contact. Movement of the host in the vicinity of copepodids initiated a fast-swimming high-turning response which sometimes allowed copepodids to make contact with the fish. Contact was also apparently made through entrainment of copepodids in the turbulence behind fins and other protuberances causing them to contact the host surface. Although detailed, ie high magnification, observation was not possible, it appeared that initial attachment occurred through "deliberate" or accidental grappling of the host by means of the hooked antennae, as was demonstrated for the tissue infection experiments described above. Copepodids were not observed to move from their initial point of attachment.

On two occasions, copepodids taken into the buccal cavity with the inhalant current were exhaled from the buccal cavity during what appeared to be a reflex coughing action by the host.

This suggested that the host was aware of the copepodids in question. No other coughing occurred prior to copepodid entry into the buccal cavity or subsequent to copepodid ejection.

4.4.2 Characterization of attachment

4.4.2.1 Copepodid external morphology

The ventral aspect of the cephalothorax of the copepodid is shown in Plate 4.1. The antennae were sharply hooked and heavily sclerotized appendages which allowed initial snagging of the host and penetration of the epidermis without damage to the copepodid (Plate 4.1, 4.2). The maxillipeds were similarly robust and sharply pointed to give purchase on the host epidermis and allow their use as a fulcrum and source of leverage for the insertion of the antennae.

The anterior of the cephalothoracic shield formed a "rostrum" which hung down over the antennae in the manner of a short flap and thereby obscured the face of the cephalothorax that lay between the antennae (Plate 4.3). The rostrum possessed a short duct ("filament duct") which protruded from its anterior tip through a pore in the rostral cuticle (Plates 4.3, 4.4). This duct terminated in a distal pore and had a tendency to collapse along its length during processing for SEM which supports the suggestion that it was tubular (Plates 4.5, 4.6). The diameter of the duct was circa 3 μm and it was apparently flexible, as the distal tip was often curled ventro-posteriorly between the antennae.

Sections viewed under the light microscope revealed some evidence of external structural detail in the cephalothoracic face underlying the rostrum although this was not seen using SEM despite removal of the rostrum / antennae in some specimens. This structure comprised paired projections of the cuticle lying to either side of the midline of the copepodid. The filament duct could be seen to apparently exit the cephalothorax between these projections (Plate 4.7) and enter the rostrum whence it emerged via a pore in the cuticle as described above. Although the duct, as seen in sections, appeared to cross between the cephalothorax and the rostrum through the external medium, it is thought that this may be artefactual and that the two are normally connected via the cuticular projections.

4.4.2.2. Primary (reversible) attachment - antennae

Initial attachment, as described above under behaviour, occurred through the action of the hooked antennae. When in contact with an artificial substrate such as a slide or petri dish, these were observed to flex repeatedly in the manner of a reflex activity suggesting that this was a fixed action pattern resulting from contact with a surface. Initially the antennae were used to

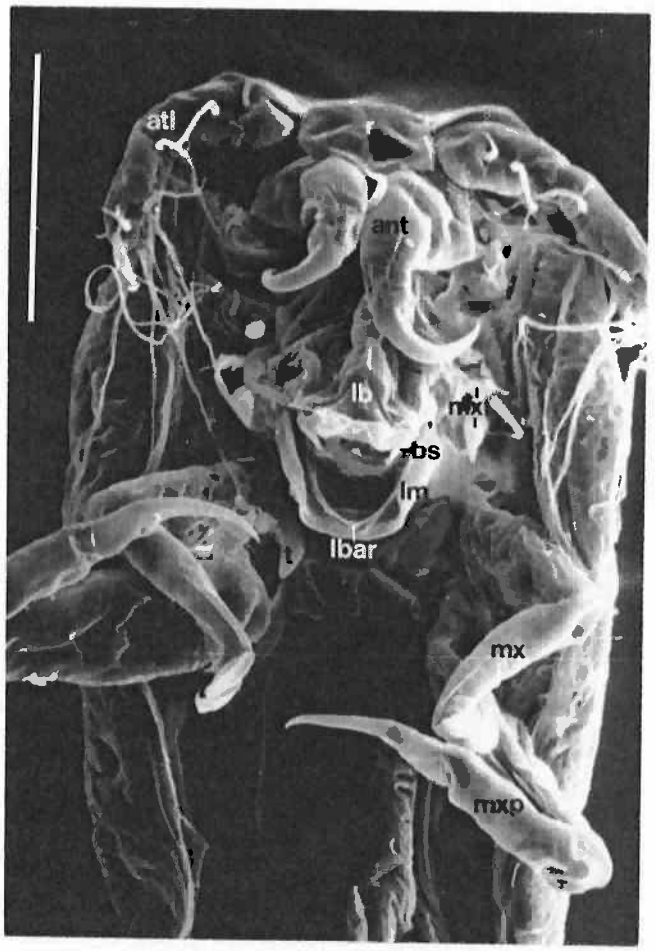


Plate 4.1 Ventral aspect of copepodid cephalothorax showing the principal structures and appendages (SEM). ant: antenna; atl: antennule; bs: buccal stylet; lb: labrum; lbar: labial bar; lm: labium; mx: maxilla; mxl: maxillule; mxp: maxilliped; r: rostrum; t: tine. Scale = 100μm

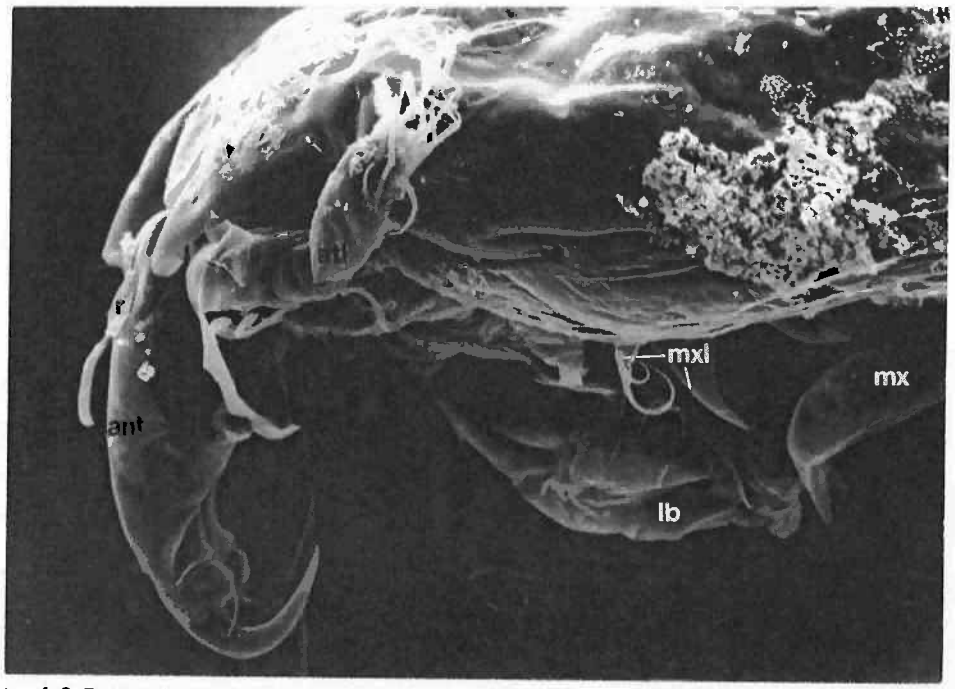


Plate 4.2 Lateral aspect of copepodid cephalothorax showing the ventral projection of the hooked antennae (SEM). ant: antenna; atl: antennule; lb: labrum; mx: maxilla; mxl: maxillule; r: rostrum. Scale = 10μm

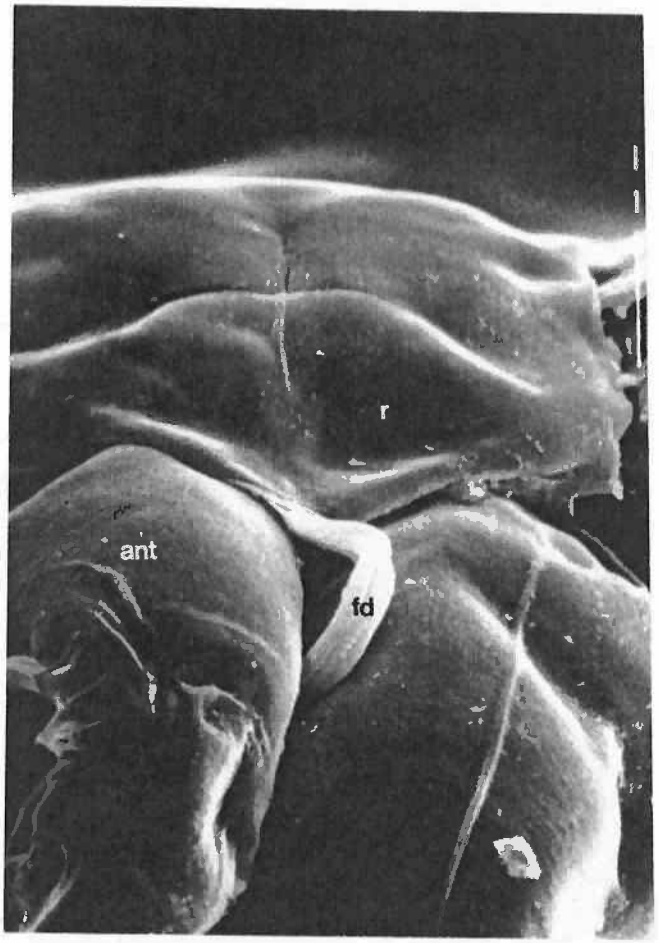


Plate 4.3 Anterior aspect of rostrum showing filament duct protruding from distal tip and recurving posteriorly between the antennae (SEM). ant: antennae; fd: filament duct; r: rostrum. Scale = 10μm

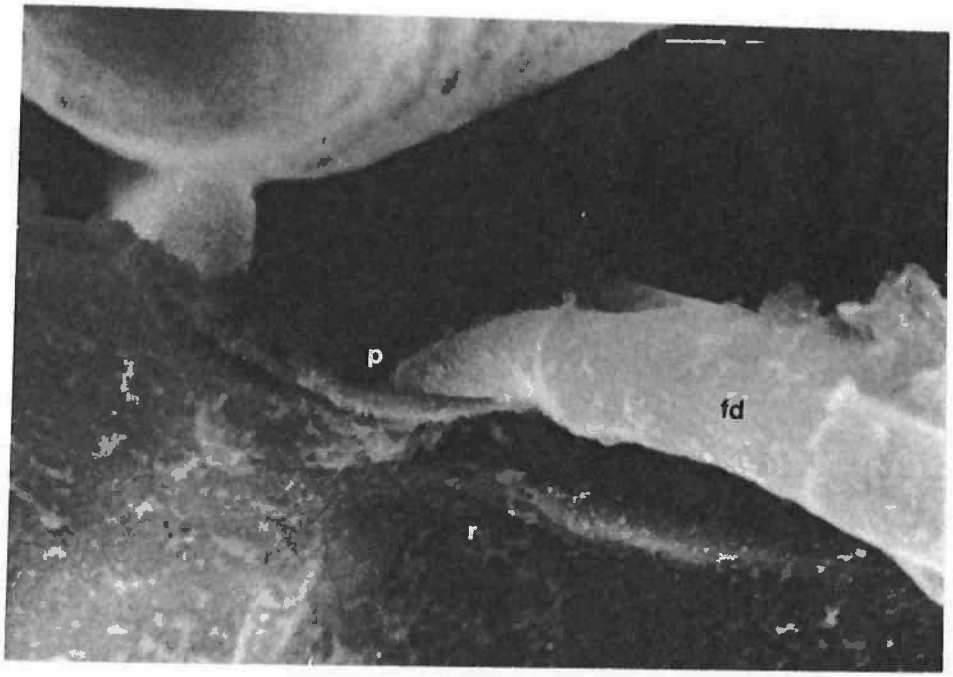


Plate 4.4 Protrusion of filament duct through a pore in the distal tip of the rostrum (SEM). fd: filament duct; p: pore; r: rostrum. Scale = 1μm

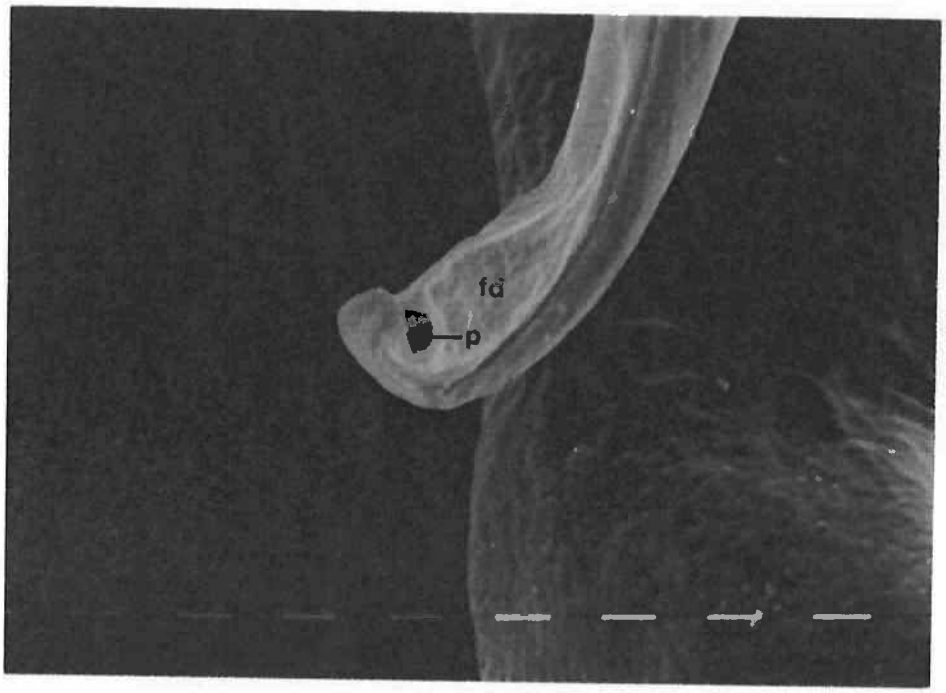


Plate 4.5 Distal tip of filament duct showing pore (SEM). fd: filament duct; p: pore. Scale = 1μm

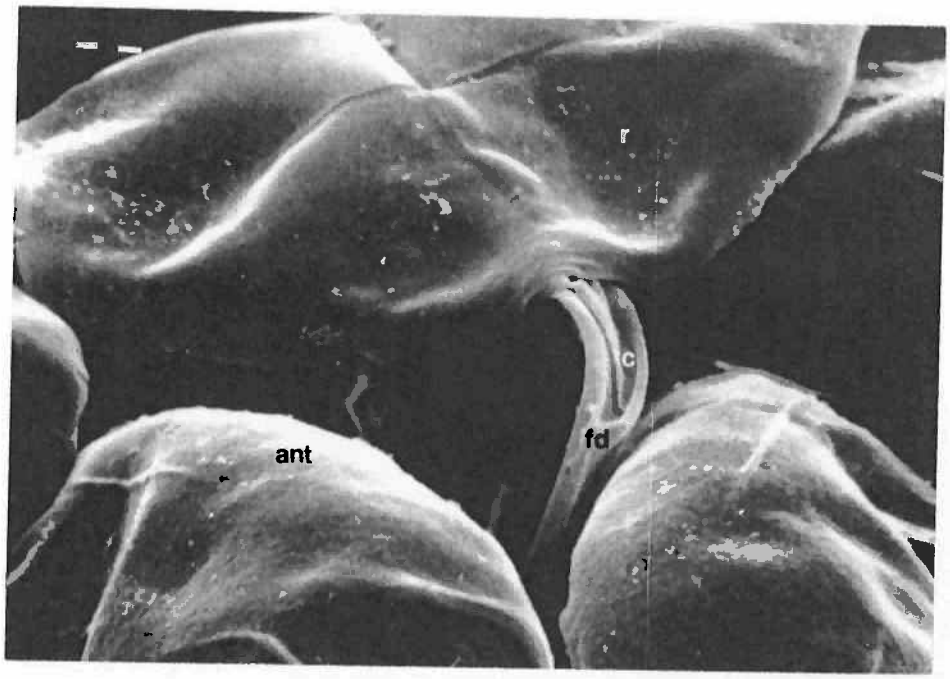


Plate 4.6 Filament duct showing a collapsed area (c) thought to be indicative of a hollow lumen (SEM). ant: antenna; fd: filament duct; r: rostrum. Scale = 1μm

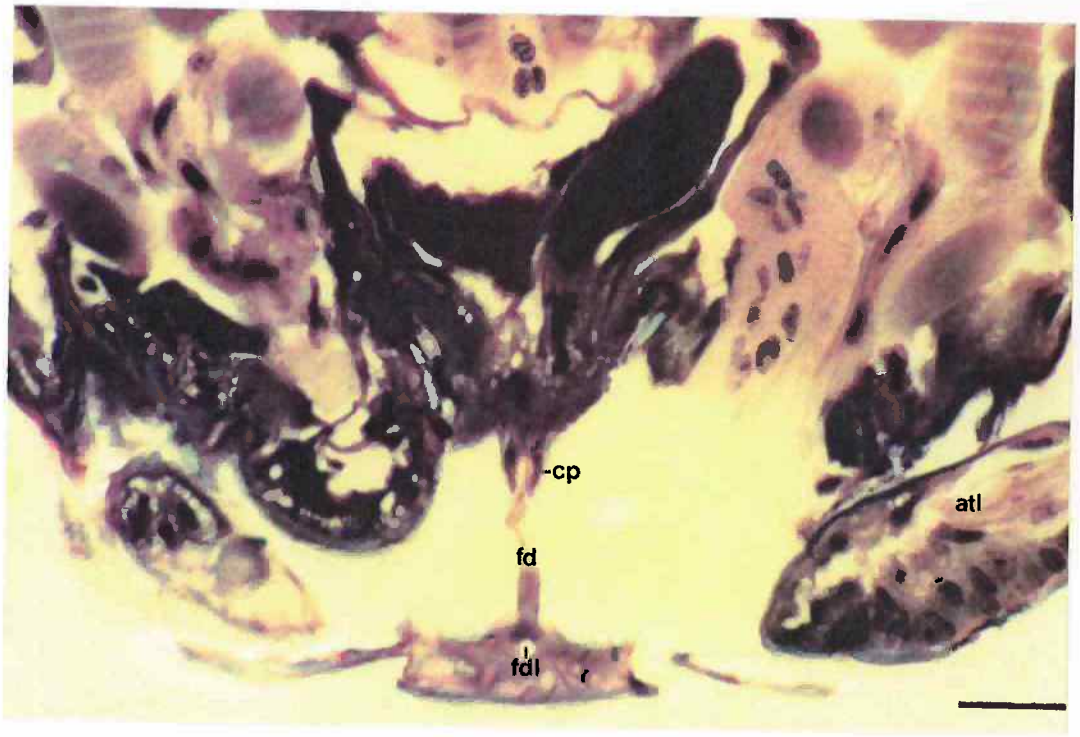


Plate 4.7 Plane section of copepodid showing passage of the filament duct through the anterior face of the cephalothorax via paired cuticular projections (LMH, stained polychrome). atl: antennule cp: cuticular projections; fd: filament duct; fdl: filament duct lumen; r: rostrum. Scale = 20μm

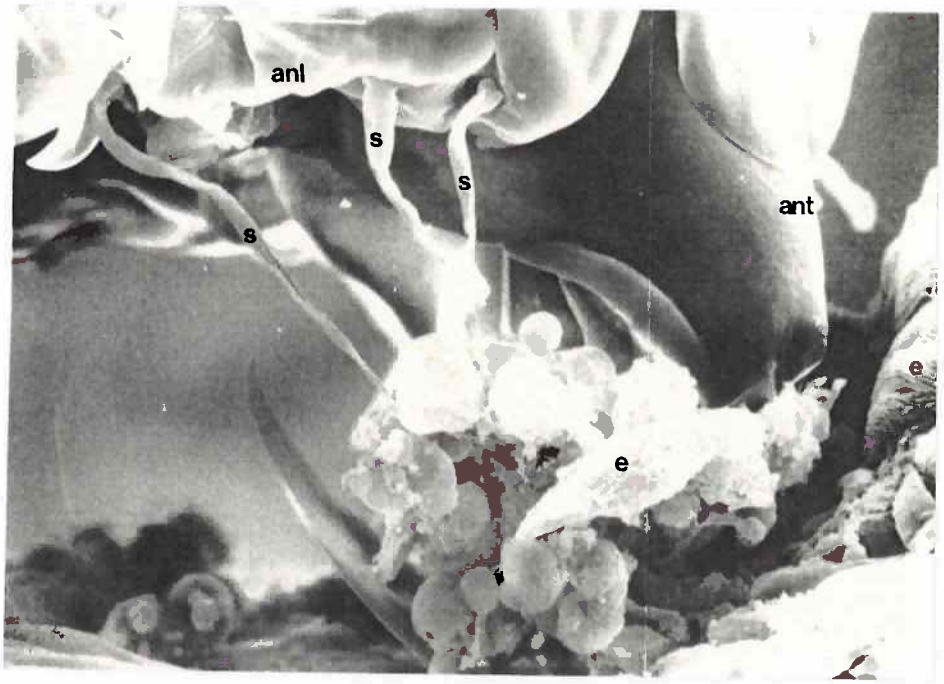


Plate 4.8 Initial grappling of host epidermis by hooked antennae showing contact with host substrate by copepodid setae (SEM, lateral). anl: antennule; ant: antenna; e: host epidermis; s: seta of antennule. Scale = 10μm

snag the host (Plate 4.8) and were then driven deep into the epidermis, penetrating as far as the host basement membrane and sometimes into the underlying dermis (Plate 4.9, 4.10). At all times after initial contact, the antennules or antennular elements would normally be in contact with the host surface (Plate 4.8, 4.11). During the penetration of the antennae, the anterior edge of the dorsal cephalic shield including the rostrum, was drawn down and forward causing the epidermis to lift up and aggregate in front of it, leading to compression of the epidermal cells in the process (Plate 4.11). Host tissue damage was minimal and appeared purely mechanical in origin. Little or no host response was seen to settling copepodids save for occasional amplification of mucous cell and eosinophilic granular cell numbers.

The completion of this process resulted in the copepodid having its antennae completely buried in the host epidermis and the anterior cephalothorax, rostrum, filament duct and antennules similarly lying below the level of the host surface and in contact with host tissue.

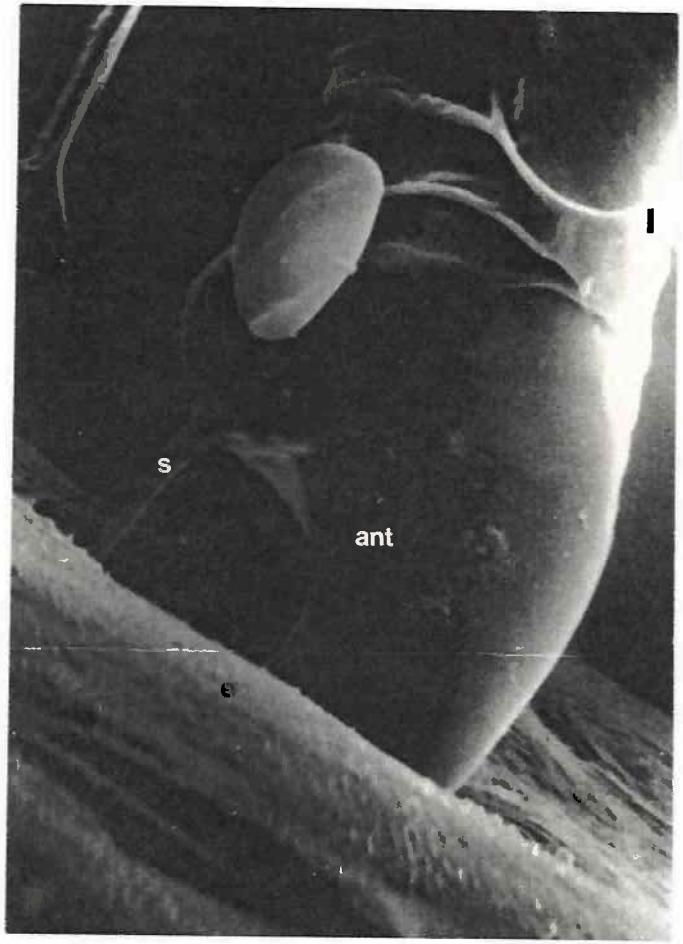
4.4.2.3. Secondary (permanent) attachment - frontal filament

The second phase of attachment involved the production of a frontal filament which anchored the parasite firmly to the host. In order to comprehend the structure and probable origin of the filament it is necessary to describe the structure of the completed chalimus frontal filament and associated organs, and then to compare this state with that existing in the preceding copepodid stage:

4.4.2.3.1 Chalimus

Chalimus stages were found attached to a wide range of sites over the host body. Attachment in experimental infections was observed on scales, fins, operculi, gill filaments, vomer and upper branchials, suggesting a wide range of possible substrata to be suitable for filament attachment. Only the cornea of the eye appeared to be exempt from attachment.

An attached filament is shown in Plate 4.12 from which it can be seen that the filament comprised four distinct components distinguishable by their morphological / compositional characteristics. These comprised the "basal plate", the "stem", the "external lamina" and the "axial duct". In some individuals, (Chalimus I) it was observed that what might normally be thought of as the major part of the filament actually comprised an extension of the chalimus cephalothorax and contained strands of tissue proximally in addition to the more usual stem components found distally (Plate 4.13). The components of the chalimus frontal filament were characterised as follows and their major staining properties as well as those of the cephalothoracic cuticle are summarized in Table 4.1.



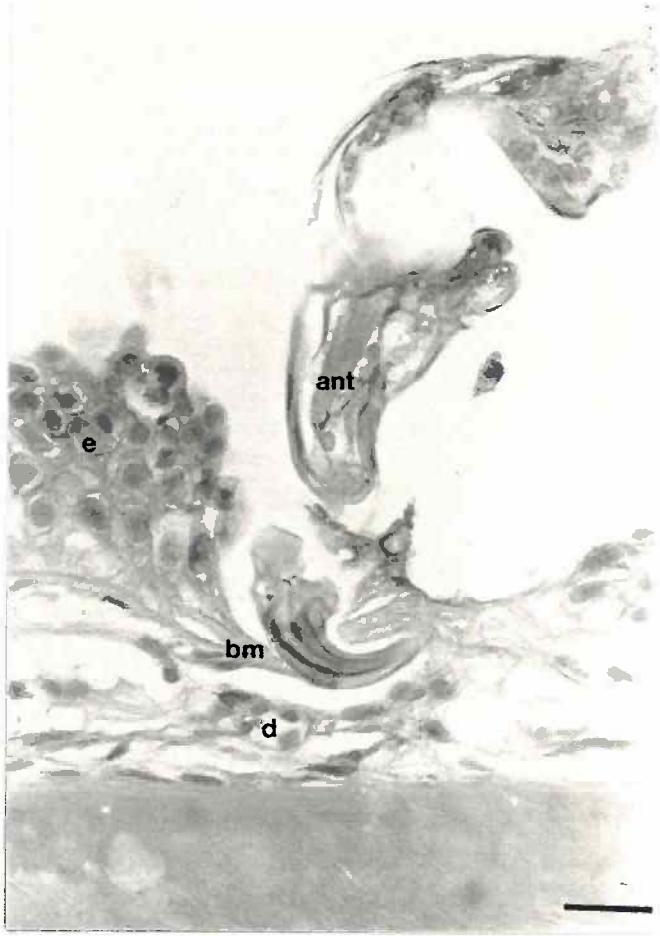


Plate 4.9 Partial penetration of antenna into host epidermis (SEM, lateral). ant: antenna; e: host epidermis; s: spine. Scale = 1μm

Plate 4.10 Sagittal section through copepodid showing penetration of antenna through the host epidermal basement membrane and into the underlying dermis (LMW, sagittal, H & E). ant: antenna; bm: host basement membrane; d: host dermis e: host epidermis. Scale = 25μm

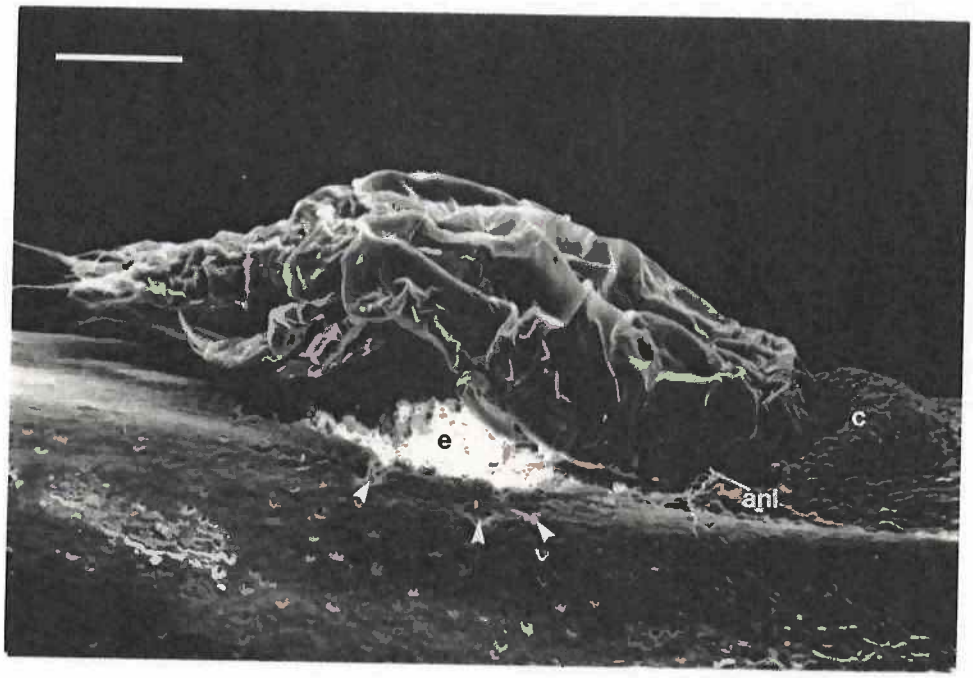


Plate 4.11 Lateral view of copepodid with anterior shield of cephalothorax burrowed into the host epidermis. Note the compression of the epidermis at the leading edge of the cephalothorax (c), the epithelial erosion beneath the copepodid (e) and the peripheral lesions associated with the earlier levering action of the maxillipeds (arrows). Note also the close contact of the antennule (anl) with the host epidermis (SEM). Scale = 100μm

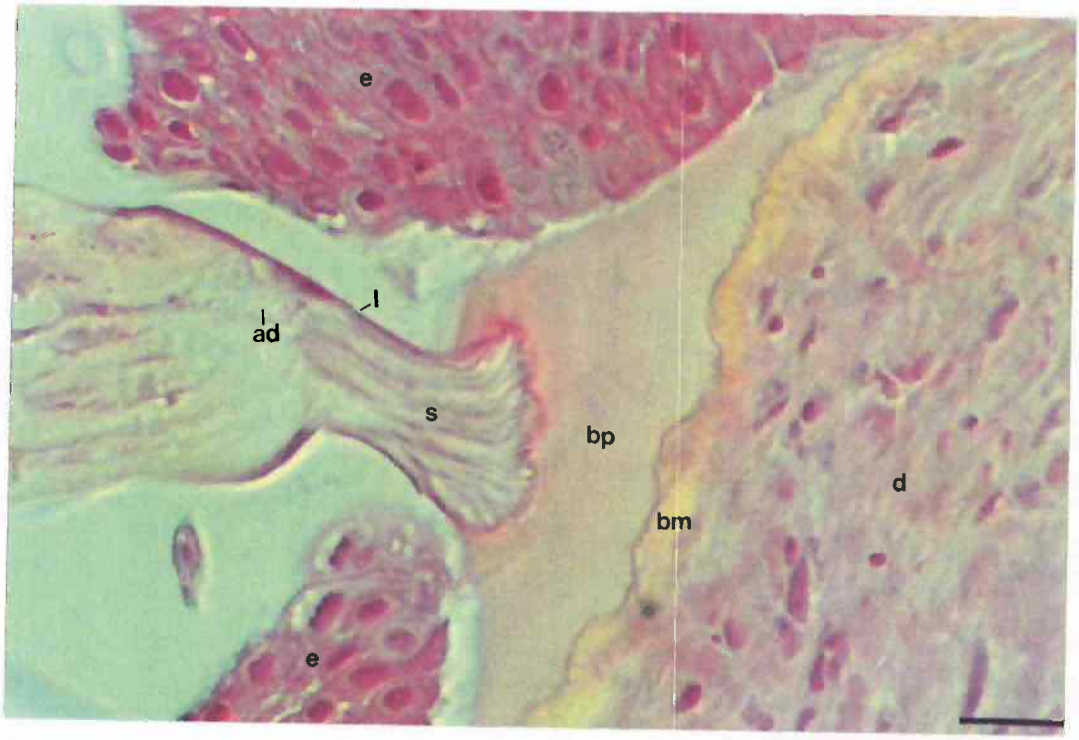


Plate 4.12 Frontal filament of chalimus attached to host basement membrane and showing the four principal components of the filament (LMH, sagittal, Cason's). ad: filament axial duct; bm: basement membrane; bp: filament basal plate; d: host dermis; e: host epidermis; l: filament external lamina; s: filament stem. Scale = 20μm

Plate 4.13 Sagittal section through chalimus illustrating continuity between the filament and the cephalothorax and showing tissue strands associated with the frontal filament (LMH, sagittal, Cason's). d: host dermis; e: host epidermis; oc: oral cone; s: filament stem; ts: tissue strands. Scale = 40μm

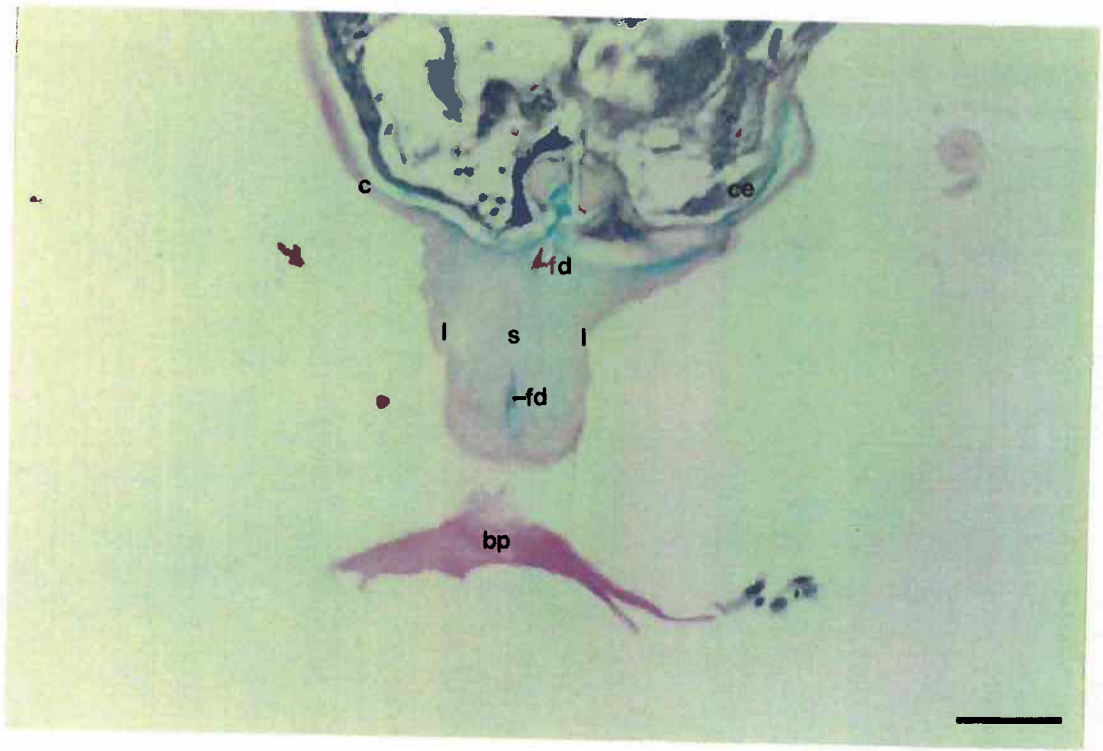


Plate 4.14 Histochemical staining of filament to show positive staining for polysaccharides in the basal plate and cuticle / external lamina (pink) and positive staining for acid mucopolysaccharides (blue) between the cuticle and cuticular epithelium, around the upper periphery of the filament and around the filament duct (LMW, plane, stained Alcian blue / PAS / haematoxylin). bp: basal plate; c: cuticle of cephalothorax; ce: cuticular epithelium; fd: filament duct; l: external lamina; s: stem. Scale = 50μm

Table 4.1 Histochemical reactions of filament and cephalothoracic cuticle prepared with a range of tissue stains.

Stain	Epicuticle	Endocuticle	External Lamina		Stem	Laminated Secretion	Basal Plate	Axial Duct
			outer	inner				
Haematoxylin & Eosin	–	Faint eosinophilia	–	Faint eosinophilia	Faint eosinophilia	Eosinophilic	Eosinophilic	Eosinophilic
PAS	+ve	-ve	+ve	-ve	faint +ve	-ve	+ve	+ve
Alcian Blue	-ve	-ve	-ve	-ve	faint +ve	-ve	-ve	-ve
Tartrazine	–	+ve	–	+ve	+ve	+ve	+ve	+ve
Massons'	red	Blue / green	red	Blue / green	Blue / green	red	red	–
Casons'	–	pale blue (scl. yellow)	–	pale blue (more pink round stem)_	Pale blue	Yellow	Pink	unstained / pink
Mallorys'	–	pale blue	–	–	pale blue	Bright red (some orange)	pale blue (some orange)	–
Mercuric Bromophenol Blue	–	+ve	–	+ve	fibres +ve matrix -ve	+ve	+ve	–
Millons		faint +ve	–	faint +ve	faint +ve	Faint +ve	faint +ve (stronger than stem)	–
Performic acid-Alcian Blue	–	-ve	–	-ve	-ve	-ve	-ve	-ve
DMAB	–	-ve	–	-ve	-ve	-ve	-ve	–
Catechol	–	–	+ve	–	faint +ve	Probable +ve	+ve	–
Acetylthiocholine iodide	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Giemsa	–	dark purple	–	dark purple	dark blue / purple	Dk blue \ Dk purple\ light blue	Light blue (darker periphery)	Purple
Diazo	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

- = component not stained or indistinguishable with stain
scl. = sclerotised cuticle

Filament components

Basal Plate

The parasite was found to adhere to the host substrate via a secretion which formed a plate to which the remainder of the filament was attached. In many preparations the material comprising the plate appeared relatively homogeneous although in some it gave the appearance of radiating fibres. It was not possible to determine which was the natural state although it may be that the homogeneous state results from additions to the plate and / or maturation of its components. This plate was frequently found to be attached directly to the basement membrane separating the epidermis from the dermis (Plate 4.12, 4.13) and typically appeared to have spread along the line of weakness between the basement membrane and the epidermal cells overlying it. Attachment was also seen directly to the surface of scales, as observed under TEM, and to the cartilage of the primary gill filaments.

The basal plate stained positively with PAS and negatively with alcian blue indicating the presence of polysaccharides and a lack of acid mucopolysaccharides respectively (Plate 4.14). With Massons' stain, the basal plate stained red which Martoja & Martoja (1967) suggest is indicative of secreted products and with Cason's it gave a pink stain. Bromophenol blue test for protein gave very dense staining of the basal plate and a Millons' test gave a faint stain for protein. Catechol staining for o-diphenoloxidase used in protein tanning gave a positive result for the basal plate (Plate 4.15). Negative results with Performic acid-Alcian blue show a lack of disulphide (-S-S-) linking of proteins. With a Giemsa stain, the plate stained light blue with a darker periphery. It was not possible to determine from sections whether the differential staining of this latter area was due to a parasite-derived constraining layer or whether it resulted from interactions between the plate material and the surrounding host tissue.

Stem

Partially embedded in the basal plate and comprising the major part of the filament, the stem consisted of a densely-packed bundle of fibres which could clearly be seen under phase contrast (Plate 4.16). The stem was bifurcated at its distal extremity at the point where it made contact with the basal plate (Plate 4.17) and was enclosed for most of its length by the external lamina with only the distal extremity being free of it. At the proximal end its circumference reduced dorsally and finally disappeared to be replaced by the syncytial tissue of the "stem collar".

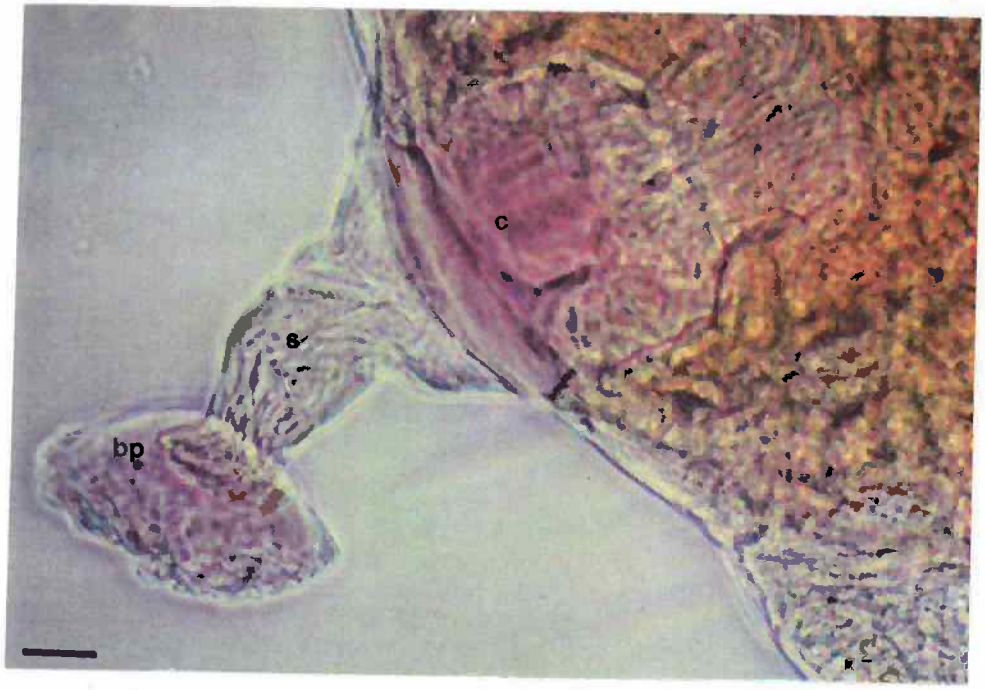


Plate 4.15 Histochemical staining of frontal filament and cephalothorax of chalimus (whole mount, stained catechol) showing localisation of o-diphenoloxidase (brown) within the basal plate of the filament (bp) and anterior part of cephalothorax (c) and lack of strong staining of filament stem (s). Scale = 40μm

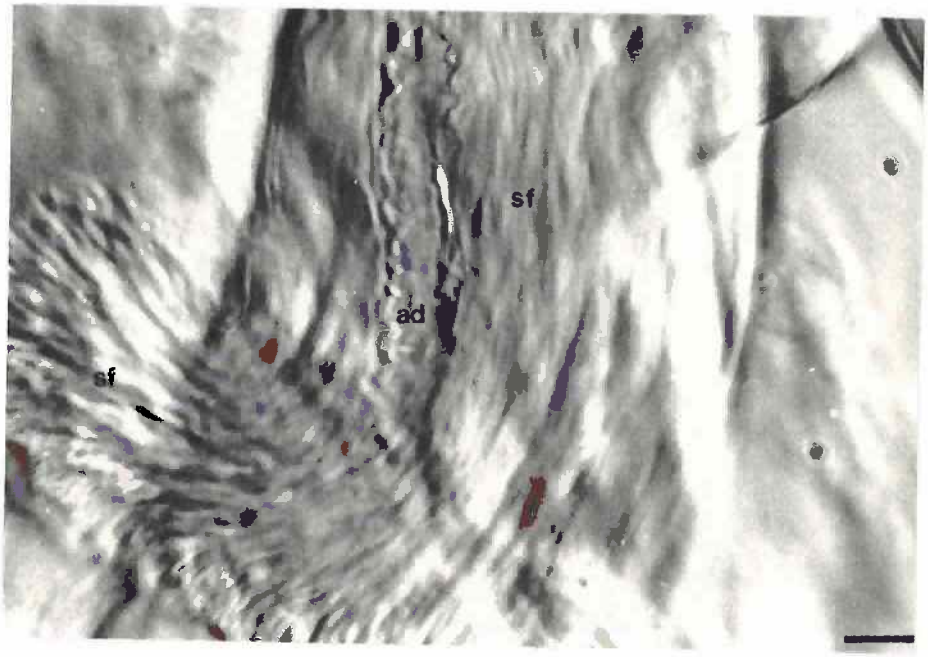


Plate 4.16 Phase contrast illumination of filament stem showing the fibrous structure of the stem and the centrally located axial duct (whole mount). ad: axial duct; sf: stem fibres. Scale = 5μm

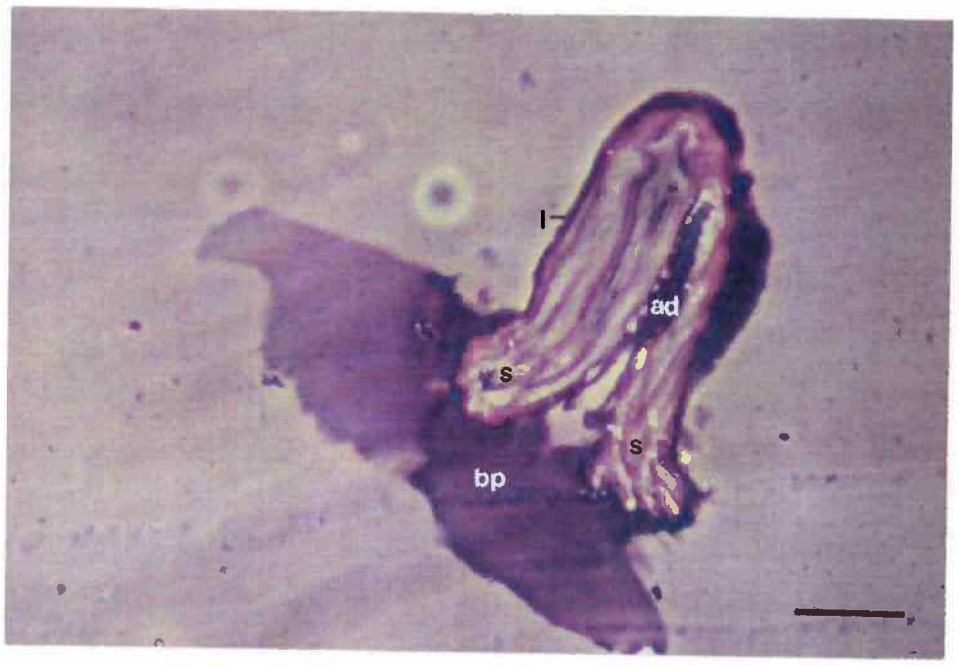


Plate 4.17 Section showing bifurcate nature of filament tip and opening of axial duct into bifurcation (LMH, stained polychrome). ad: axial duct; bp: basal plate; l: external lamina; s; stem. Scale = 20μm

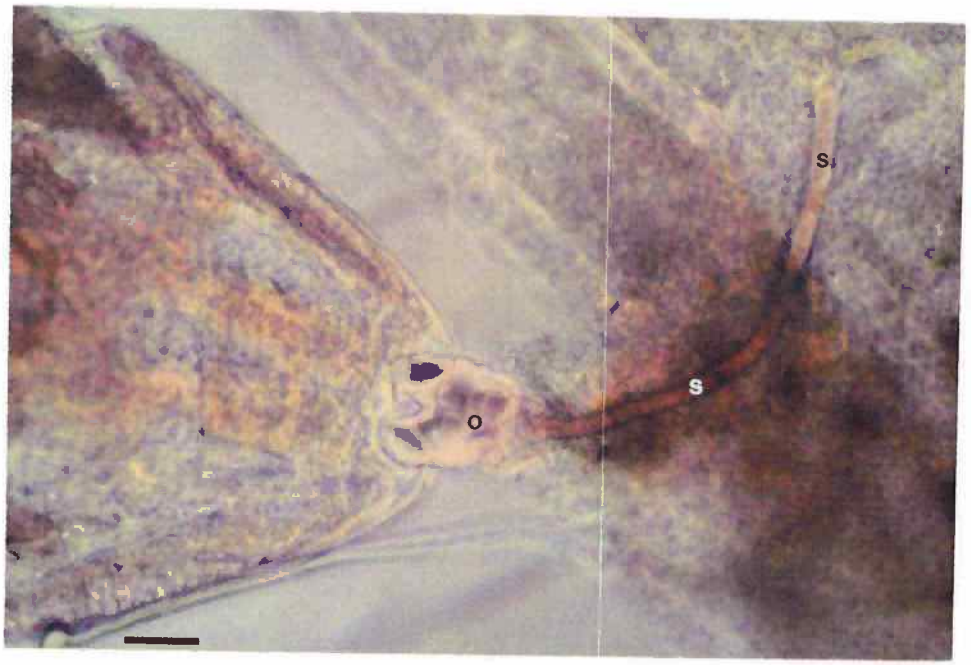


Plate 4.18 Filament of *C. elongatus* stained with catechol to demonstrate presence of o-diphenoloxidase (whole mount). Note strong staining of "stem" (s) and origin / base (o). Scale = 40μm

The stem stained negatively with PAS and very faintly with Alcian blue when stained in tandem indicating the presence of a small quantity of acid mucopolysaccharide (Plate 4.14). This contradicted other instances of staining with PAS alone where the material was entirely PAS negative. This latter may indicate that the Alcian blue staining is artefactual, possibly resulting from trapping of the stain by fibres. The stem gave a positive reaction with bromophenol blue indicating that the fibres were likely to have a proteinaceous component and a faint result with Millon's similarly indicating the presence of protein. The stem stained pink with Cason's and took up tartrazine strongly. Although it gave a slight positive reaction to the catechol test for o-diphenoloxidases, this was very faint and not as pronounced as that shown by the basal plate. In contrast, comparative staining of the filament "stem" of *C. elongatus* for o-diphenoloxidase (Plate 4.18) gave a strong positive reaction mirroring that observed for the basal plate of *L. salmonis*. With Masson's trichrome, the stem fibres of *L. salmonis* stained identically to the endocuticle of the cephalothorax (turquoise). The stem was Performic acid-Alcian blue negative which indicates a lack of disulphide (-S-S-) linkages. Giemsa staining of the stem gave results comparable with staining of the cephalothoracic cuticle.

External lamina

A thin external lamina surrounded the filament stem and appeared to be cuticular in nature. It was continuous with the cuticle of the cephalothorax (Plate 4.17, 4.19) and may hence represent an extension of the latter.

Both the external lamina and the cuticle of the cephalothorax possessed similar staining properties. Both gave a turquoise stain with Masson's and displayed an apparently red-staining epicuticle. Both also showed a PAS positive reaction in the epicuticle indicative of polysaccharides (Plate 4.14). Other stains also gave similar results although the o-diphenoloxidase staining seen in association with the cuticle could not be localised to the external lamina in staining of whole-mounts (possibly due to the fineness of this layer). Giemsa staining indicated the external lamina to be apparently continuous with the cephalothoracic cuticle and in some sections it appeared to cover the periphery of the basal plate although, as noted earlier, this staining may be open to other interpretations.

Axial duct

The axial duct was a ~ 3 μm tube that ran the length of the filament's central axis. It passed down through the stem and opened in the vicinity of the bifurcation of the distal extremity of the stem (Plate 4.17). In longitudinal sections it appeared as a tube or as a line of apparently

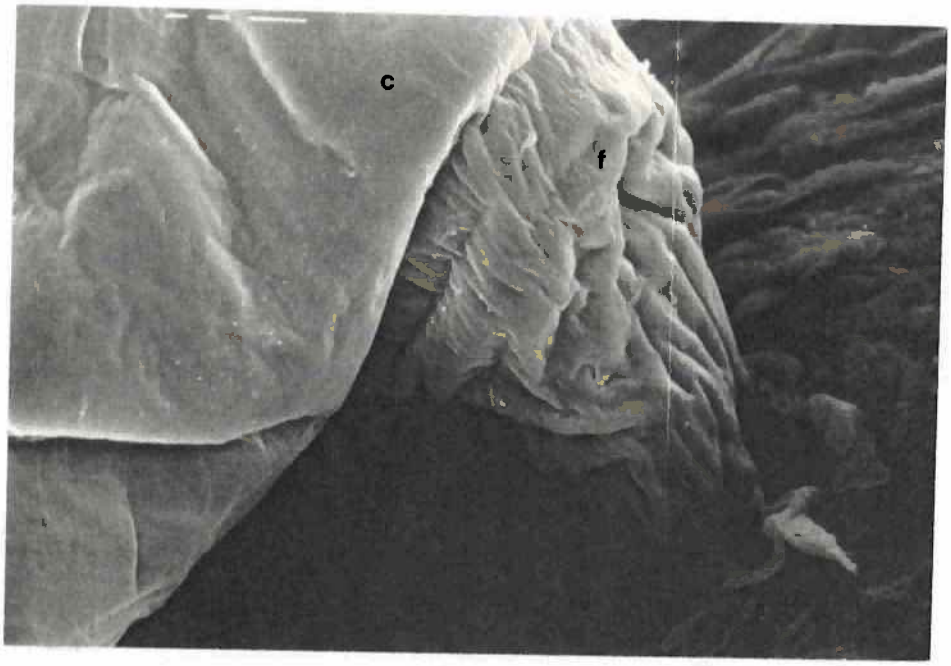


Plate 4.19 SEM of chalimus filament showing continuity of filament (f) with the anterior cephalic shield (c). Scale = 10μm

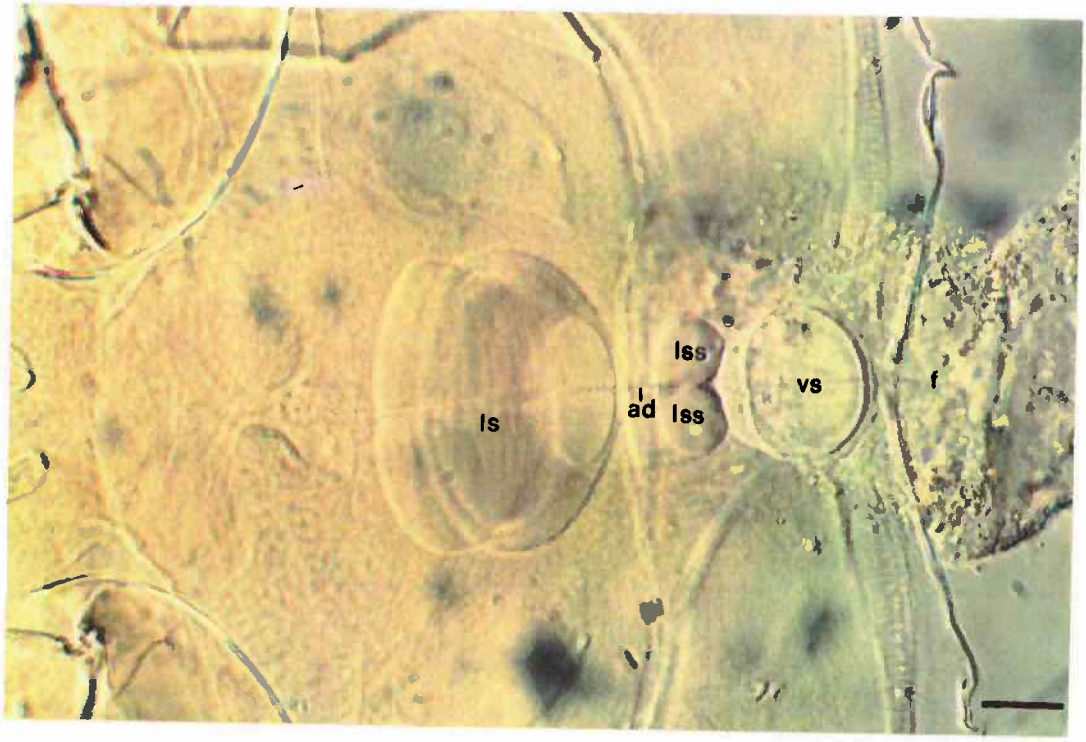


Plate 4.20 Appearance of filament-associated components in whole preparation of chalimus IV seen under phase contrast. ad: axial duct; f: filament; ls: laminated secretion lss: ventral sumps of laminated secretion; vs: area of ventral secretion ("ventral filament organ"). Scale = 40 μm

regularly spaced components (every 0.5 μm) and in transverse sections as rings or as s-shaped components (the different appearances dependent upon different stains and section thicknesses). These observations suggest that the duct may be reinforced in a helical or multiple helical configuration with turns having a 0.5 μm periodicity. Alternatively, rings of reinforcement may be present. Such a proposed structure is also supported by phase contrast observations. Alternatively, these observations may reflect the aggregation of secretion within the lumen of the duct, these being disposed according to the internal structure of the lumen.

Like the basal plate, the axial duct stained positive for PAS indicating the probable presence of polysaccharides. Peripheral to the duct there was also a positive reaction to alcian blue indicating the presence of acid mucopolysaccharides. The structural components of the duct stained positively for protein with bromophenol blue and gave strong staining with Heidenhain's haematoxylin. The duct did not stain positively for the presence of o-diphenoloxidase. The duct (or elements of it) took up tartrazine strongly and was unstained (or slightly pink) with Cason's and red with Masson's stain. The axial duct stained purple with Giemsa.

Filament organs

In addition to the filament itself, there was also a number of organs and structures which were apparently associated with its production or function. The appearance of these components in the intact chalimus is demonstrated in Plate 4.20 and the structure and disposition of these organs within the chalimus have been reconstructed from serial sections and are presented with reference to external appendages in Plate 4.21. A *circa*-sagittal section taken through the reconstruction is given in Plate 4.22

A-Gland

The most posterior organ (A) associated with the filament was a substantial bisymmetric organ comprising two major lobes (A1 and A2) the latter of which was found to extend posteriorly past the eyes and as far as the start of the cerebrum / distal end of the mouth tube (Plate 4.22 4.23). Another large gland, producing mucoid material ("mucus gland") and situated posterior to the A-gland was apparently not involved with filament production (Plate 4.22). Anteriorly, the A1 lobes were found to extend up to a level lying approximately between the bases of the antennules and antennae. The A-gland was enclosed in a capsule of squamous cells and possessed a somewhat granular cytoplasm. The A-gland clearly functions as an exocrine gland, possessing non-staining foci and a system of ducts which fed from the A2 lobes and outer edges of the A1 lobes into a major central collecting duct exiting posteriorly to the gland (Plate

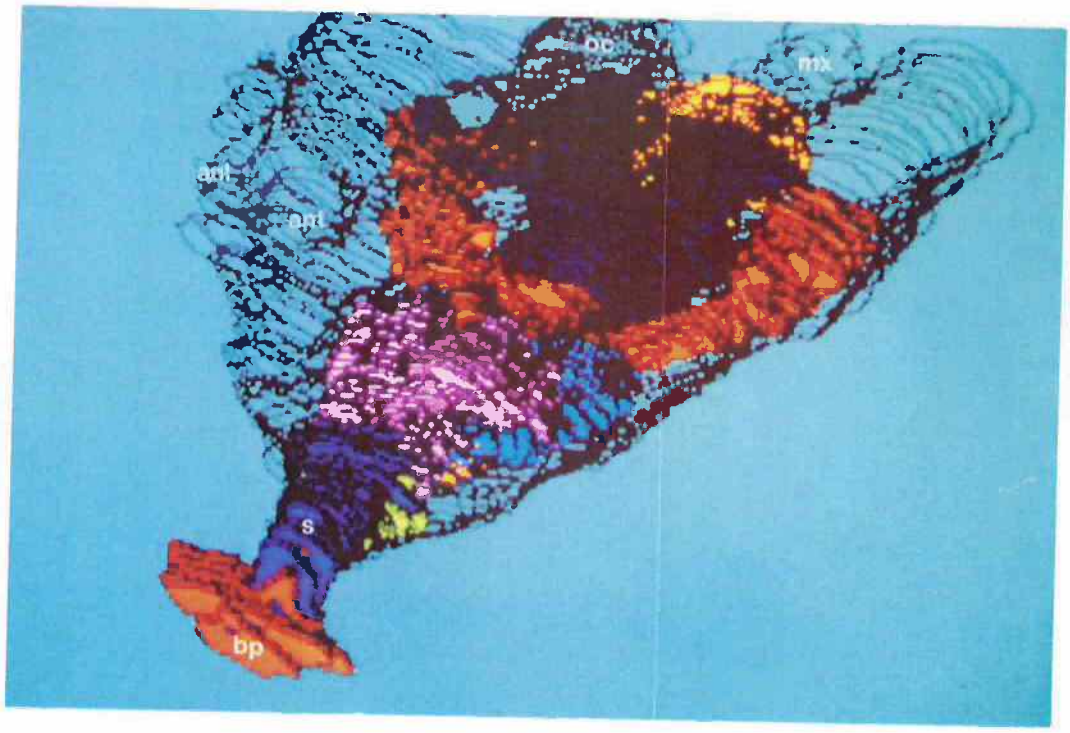


Plate 4.21 3D-reconstruction of chalimus filament and associated organs showing their relationship to the cuticle and external appendages (cuticle = wire-frame, ventral surface = north). anl: antennule; ant: antenna; bp: basal plate; mx: maxilla; oc: oral cone; s: stem; (See Plate 4.22 for identity of organs within the cephalothorax).

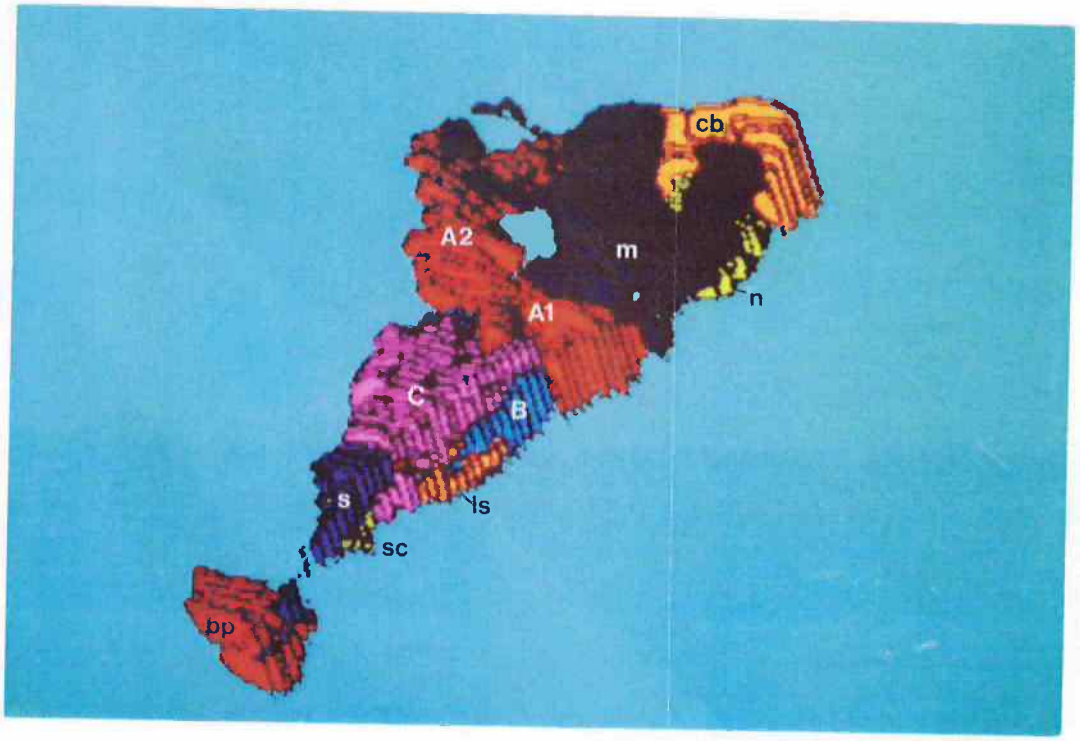


Plate 4.22 3D-reconstruction of chalimus filament and associated organs sectioned in an approximately sagittal plane to illustrate the inter-relationships of the organs and structures (ventral surface = north). A1, A2; lobes of A-gland; bp: basal plate; B; B-gland; C: C-gland; cb: cerebrum; ls; laminated secretion: m: mucous / mucoid organs; n: nauplius eye; s: stem; sc: stem cap.

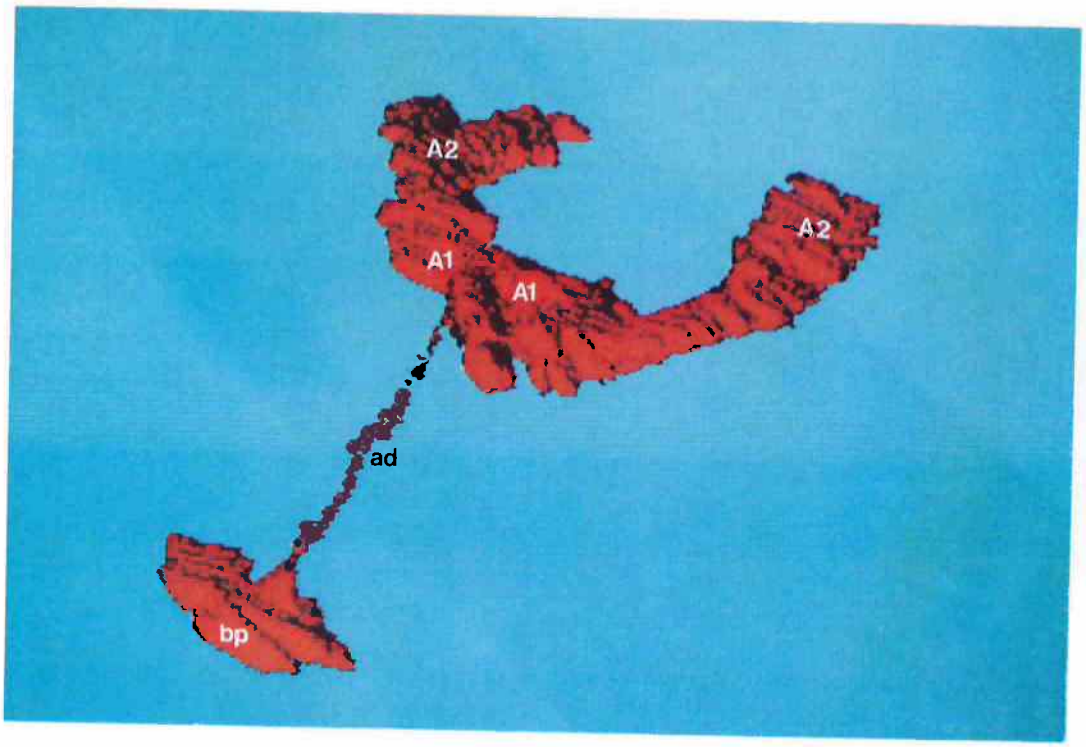


Plate 4.23 3D-reconstruction of A-gland (A1,A2) axial duct (ad) and basal plate (bp) of chalimus. The axial duct arises medially at the posterior of the gland and loops over the top before continuing anteriorly (ventral aspect = north).

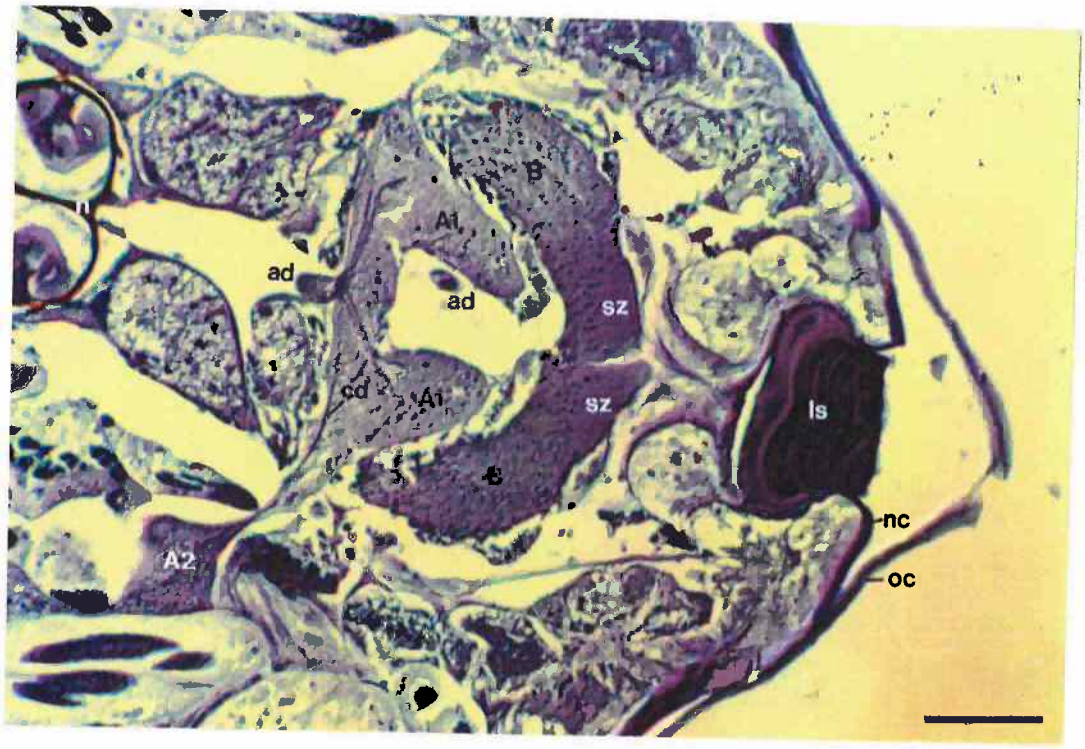


Plate 4.24 Plane section of chalimus stained for protein (bromophenol blue) and illustrating disposition of filament associated organs within the cephalothorax (LMW). A1, A2: lobes of A gland; ad: axial duct; B: B-gland; cd: minor collecting ducts within A-gland; ls: laminated secretion; n: nauplius eye; nc: newly formed cuticle; oc: old cuticle; sz: secretory zone of B-gland. Scale = 50μm

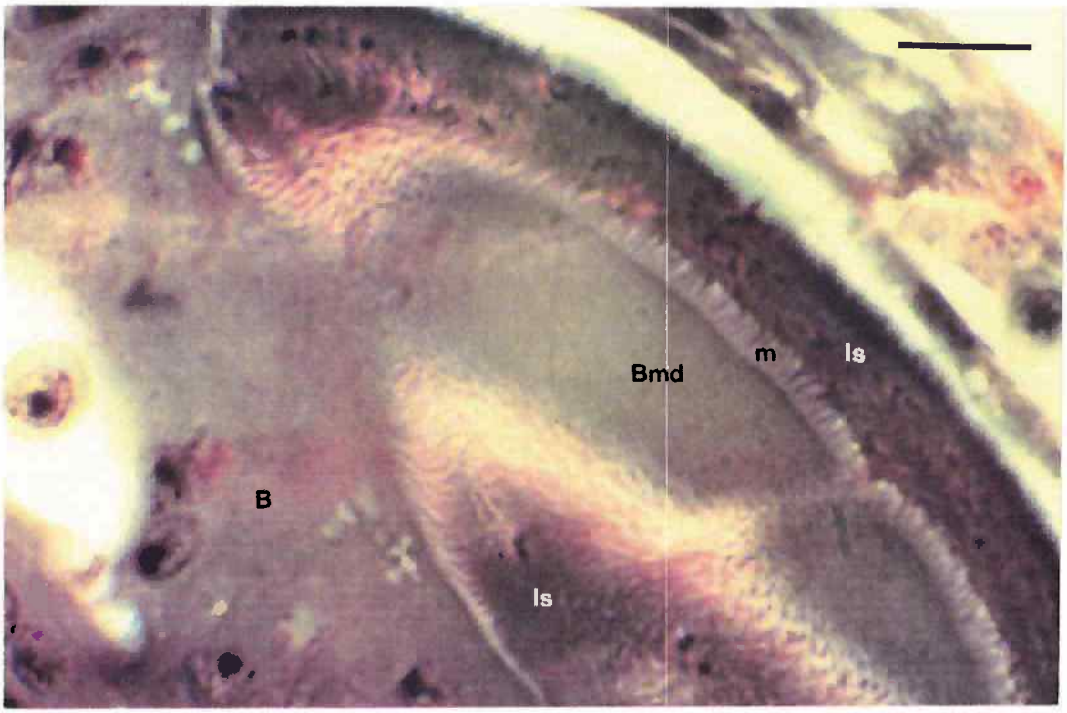


Plate 4.25 Plane section through laminated secretion of chalimus showing helicoidal arrangement of fibres and microvilli and medulla of B-organ (LMH, stained polychrome, ventral = south). B: B-gland; Bmd: B-gland medulla; ls: laminated secretion; m: microvilli of B-gland. Scale = 10μm

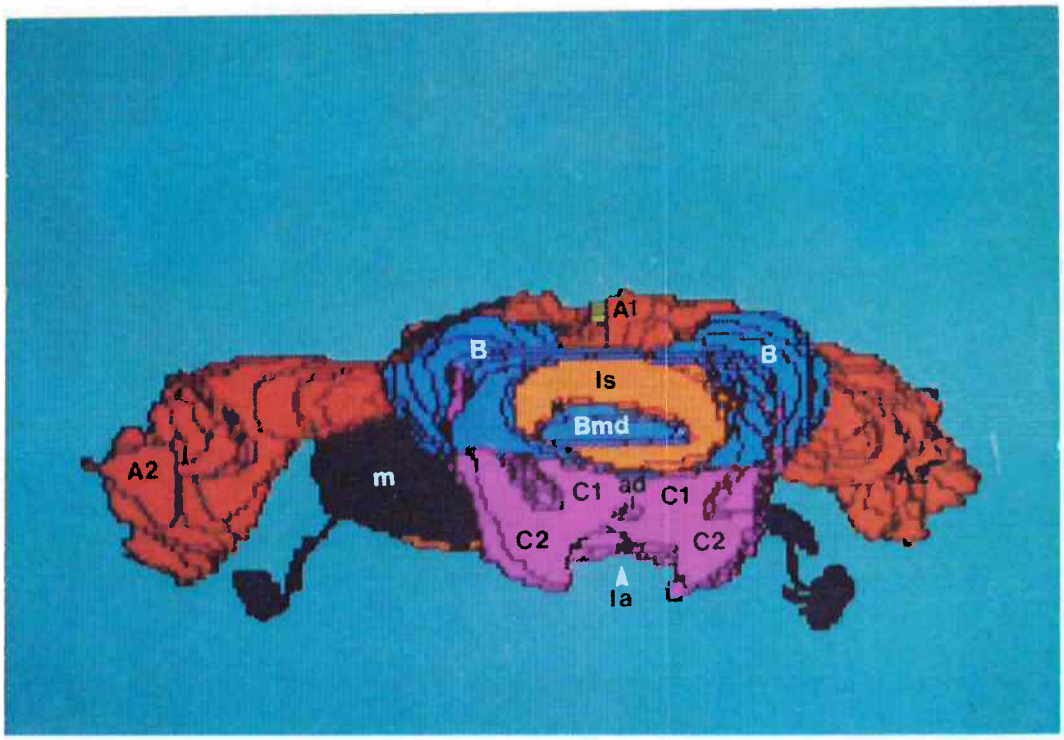


Plate 4.26 Transverse section (anterior view) through 3D-reconstruction of filament organs showing spatial relationships between glands. A1, A2: lobes of A-gland; ad: axial duct; B: B-gland; Bmd: B-gland medulla; C1,C2: lobes of C-gland: la: ventral lacuna (position of "ventral filament organ"); ls: laminated secretion; m: mucoid / mucous gland.

4.24). This collecting duct passed dorsally above the gland and then moved antero-ventrally to become continuous with the axial duct already described (Plate 4.23). The size and structure of the collecting duct indicated that it was identical to the latter and it will therefore be considered as a part of the axial duct.

The ducts ramifying through the A-gland stained positively with PAS and negatively with Alcian blue indicating the probable presence of polysaccharides and an absence of acid mucopolysaccharides. Staining for protein with bromophenol blue also gave a strong positive result (Plate 4.24) with a weak positive result being given by the Millons' reaction. The cytoplasm stained with haematoxylin and contained small PAS-positive granules.

B-Gland

This gland was situated anteriorly to the A-gland although it ran to either side of the latter in its posterior portion (Plates 4.22, 4.24). This gland was found to extend posteriorly as far as the basal segment of the antennae and forward as far as the start of the basal segment of the antennule. The nuclei of this organ were seen to be strongly polarized towards the anterior part of the organ with those at the front seemingly larger and less intensely staining than those found posteriorly, and possessed of larger nucleoli. Like the A-gland, the B-gland was enclosed in a capsule of squamous cells.

Anteriorly, the B-gland was found to be associated with a large quantity of secretory product in the form of a rod (Plates 4.24, 4.25, 4.26). This secretion was clearly visible in the whole chalimus (Plate 4.20). Whether this product was in a solid or liquid phase was impossible to determine from the present study. This secretion had a hyaline appearance and was composed of a series of concentric laminae reminiscent of tree rings which could be observed in transverse section and from which the term "laminated secretion" is derived. In plane section the laminae were apparent running perpendicularly to the antero-posterior axis of the secretion giving the appearance of stripes of material (Plate 4.24). Aside from the laminations, the laminated secretion appeared relatively homogeneous in some sections although others suggested that the layers might be composed of a helicoid arrangement of fibres as observed in arthropod cuticle (Plate 4.25). Within these layers inclusions having the same staining characteristics as nuclei could also sometimes be observed. The B-gland was seen to be cupped around the laminated secretion at its posterior end although a lobe of the gland was noted to project anteriorly into the centre of the rod in the manner of a medulla (Plates 4.25, 4.26). At the interface (both internal and external) between the gland and the rod, the B-gland gave the appearance of a microvillous surface (Plate 4.25). The cytoplasm in the anterior part of the gland was seen to be highly granular and very much more dense than the cytoplasm found more posteriorly and had relatively

few nuclei present, suggesting that it might be a secretory zone (Plate 4.24). The laminated secretion was observed to extend ventrally down a pair of channels having the appearance of "sumps" and ending in the vicinity of the ventral aspect of the C1 lobes of the C gland (see below) (Plate 4.27). These sumps were also visible in whole specimens (Plate 4.20). In some individuals the secreted material was observed to be isolated from the aforementioned organs by an invagination of newly-formed cuticle (Plate 4.28).

The B-gland stained negatively for neutral polysaccharides and acid mucopolysaccharides. A strong bromophenol blue reaction for protein was demonstrated in the anterior part of the gland suggesting that this area was a site of protein production or secretion (Plate 4.24).

The laminated secretion stained bright red with Masson's (Plate 4.28) and showed bright yellow staining with Cason's which was seen otherwise only in heavily sclerotised cuticle and in the central part of the lenses of the dorsolateral ocelli of the nauplius eye. The rod also took up tartrazine strongly and stained positively for Bromophenol blue (Plate 4.24) supporting a principally proteinaceous composition. The rod gave negative results to staining with PAS and Alcian blue indicating an absence of polysaccharides and acid mucopolysaccharides save for a single individual which gave an apparently positive reaction to PAS. This positive reaction in a single individual may signify an addition to the material occurring just prior to moulting. Giemsa staining of this secretion was very inconsistent giving dark blue, dark purple and light blue staining according to the section viewed.

An important feature of the staining reaction of the laminated secretion is the fact that in whole chalimus, the area where it was located stained positively for o-diphenoloxidases (as observed through the cuticle) which corresponded to the staining characteristics of the basal plate. This may therefore suggest that the two structures are related.

C-gland

At its anterior end, the B-gland was observed to merge into a bisymmetric organ comprising two lobes to either side of the midline that became differentiated and encapsulated moving anteriorly (Plates 4.22, 4.26). The origin of these lobes may, however, lie slightly posterior to the B-gland in an area where the B-gland cytoplasm takes up Heidenhain's haematoxylin more strongly.

These lobes fused anteriorly so that it is difficult to say whether each lobe comprises a separate gland or whether they simply represent different aspects of the same gland. The two lobes will be termed C1 and C2 to distinguish them.

The more dorsal C1 lobes were seen to run alongside the axial duct (Plates 4.26, 4.29). Moving anteriorly, their cytoplasm became highly homogeneous and they lost their

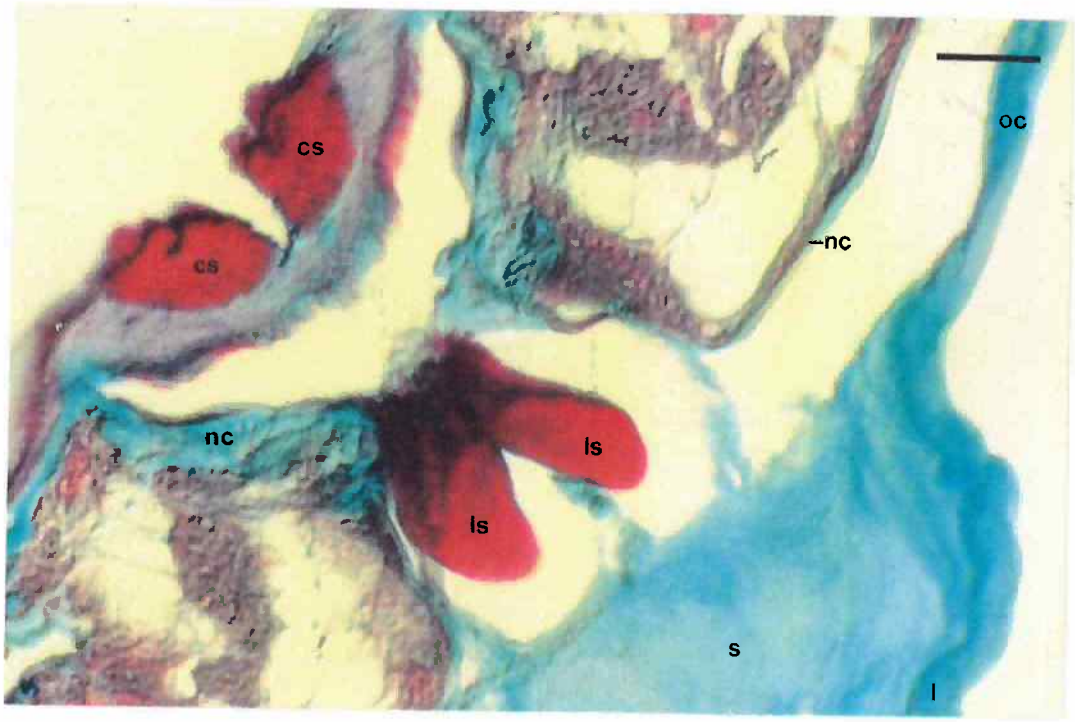


Plate 4.27 Plane section of anterior cephalothorax of chalimus showing paired "sumps" of laminated secretion (ls) anterior to the ventral remnants of the secretion underlying the C-gland (cs). Note also the invagination of the newly formed cuticle (nc) and similar staining properties of old cephalothorax cuticle (oc), external lamina (l) and filament stem (s) (LMW, stained Masson's trichrome). Scale = 20μm

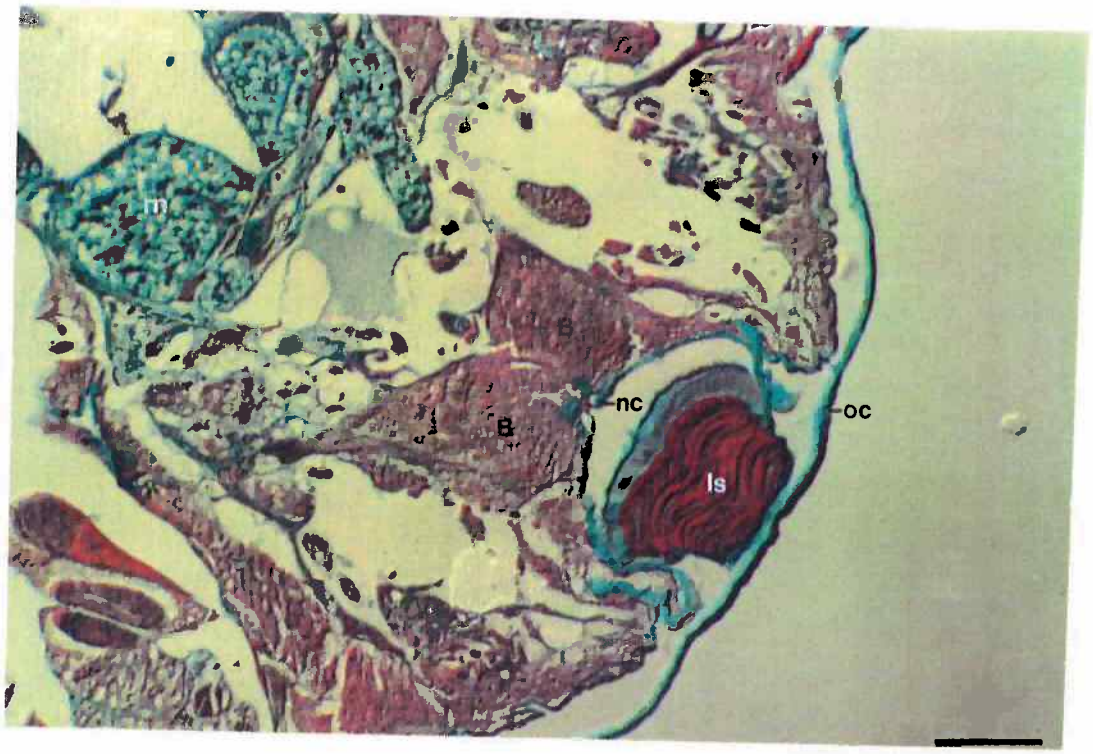


Plate 4.28 Plane section of chalimus showing invagination of newly-formed cuticle around laminated secretion (LMW, stained Masson's). B: B-gland; ls: laminated secretion; m: mucous gland; nc: newly formed cuticle; oc: old cuticle. Scale = 50μm

encapsulation. Further anteriorly, they fused with the C2 lobes and finally lost their identity altogether. In the vicinity of the axial duct, fibre bundles were sometimes seen within the gland (Plate 4.29). Ventral to the C1 lobes, a large lacuna or an area containing secretion was often seen in sections (Plates 4.26, 4.30).

The C2 lobes run latero-ventrally to the anterior portion of the B-gland, becoming encapsulated as they move anteriorly. Further anteriorly they were observed to run latero-ventrally to the C1 lobes before finally losing the encapsulation and fusing ventrally with the C1 lobes. As with the C1 lobes, the C2 lobes subsequently lost their individual identity.

In chalimus III and IV, staining suggested the presence of a possible ventral secretion (seemingly beneath the C1 lobe) associated with one or both of these glands although the secretion was not recognised in other stages. This area of secretion lay dorsal to the ventral cuticle and in chalimus IVs, the cuticle was invaginated dorsally to give a lip around it (Plate 4.30). Moving ventrally, the secretion from this area apparently became continuous with the contents of the paired "sumps" descending from the laminated secretion lying anterior to the B-gland.

The C1 and C2 lobes stained similarly to the B-group proximally but became more protein-positive distally. The C1 lobes stained very strongly with haematoxylin particularly around the edges of the lobes. Both lobes stained negatively for mucopolysaccharides with PAS and Alcian blue.

Stem collar

A collar of tissue was found to extend for a short way, within the external lamina, alongside the proximal tip of the stem in individuals where the cephalothorax was extended to provide part of the filament and has been designated the "stem collar". Proximally, it appeared to merge into the C-gland although within the area of merging, both tissues had no clear identity. Often the stem collar was associated with other tissues in the main part of the chalimus by strands of tissue which travelled up the anterior extension of the cephalothorax. Just before the distal transition from collar to stem, there was an area of apparently disorganised fibres. At the interface between the sides of the collar and the stem, the tissue was seen to be very densely staining with Heidenhain's haematoxylin and somewhat homogeneous. The stem collar was situated within the anterior extension of the cephalothorax (Plate 4.22).

The stem collar stained negatively with PAS and Alcian blue, indicating a lack of polysaccharides. The collar stained positively with bromophenol blue indicating the presence of protein and gave a faint positive result with Millon's.

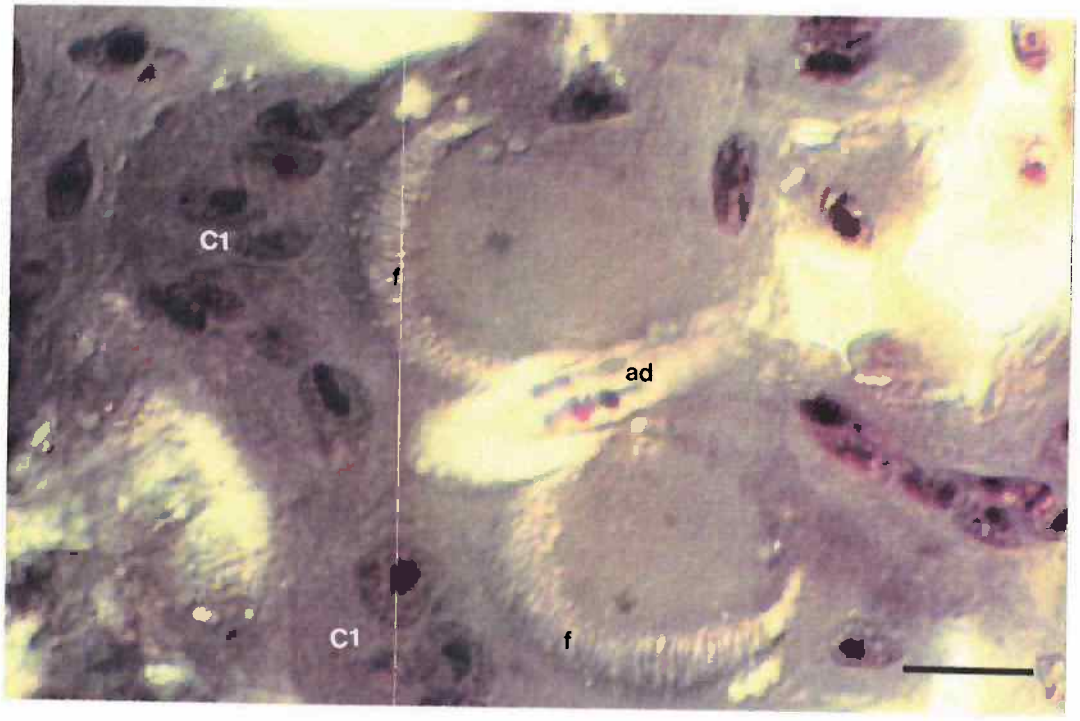


Plate 4.29 Section of chalimus showing the association between the C1 lobes and a fibrous product that possibly represents the stem precursor. Note the axial duct passing between the lobes and medial to the postulated stem (LMH, plane, stained polychrome). ad: axial duct; C1: C1 lobes of C-gland; f: fibrous product of C-gland. Scale = 10μm

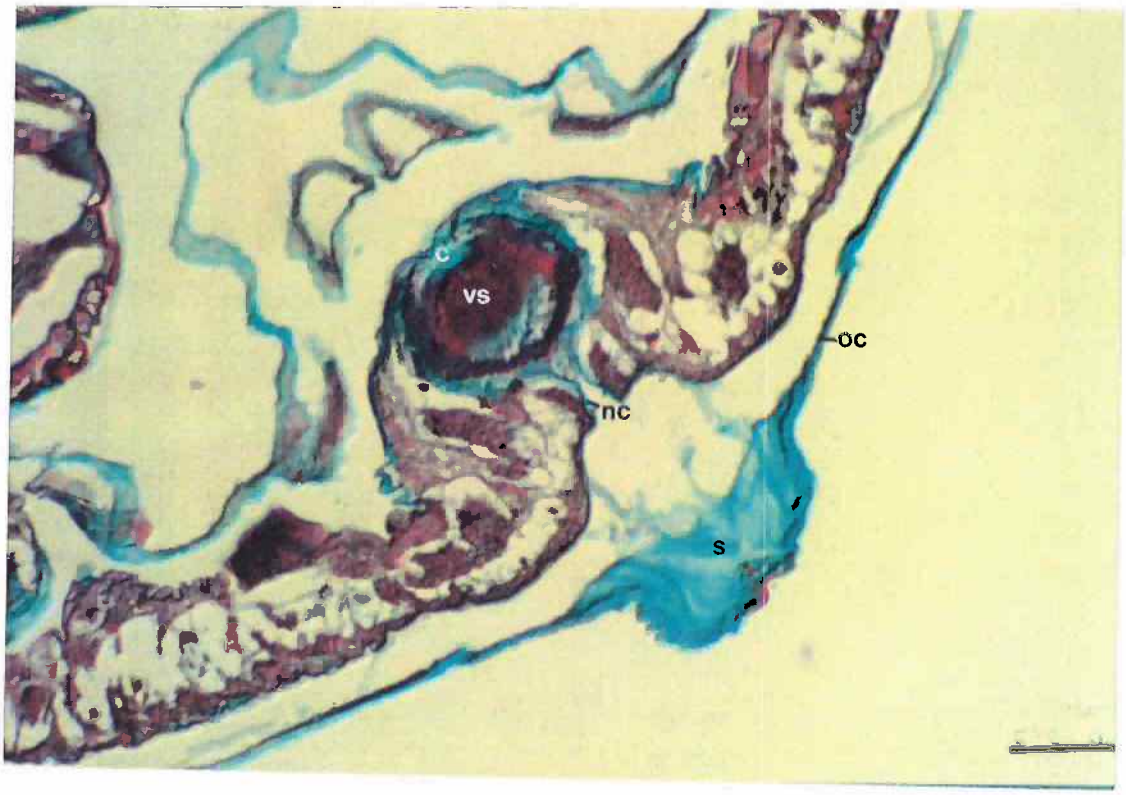


Plate 4.30 Plane section of chalimus showing area of secretion ventral to C1 lobes and surrounded by a cuticular rim projecting dorsally from the ventral surface of the cephalothorax. c: cuticular rim; nc: newly formed cuticle; oc: old cuticle; s: stem; vs: ventral secretion. Scale = 20μm

Cholinesterase staining

None of the filament components gave a positive reaction to the acetylthiocholine iodide stain for acetylcholine esterase, indicating that the filament is not innervated (at least by cholinergic nervous tissue).

X-ray elemental analysis

The X-ray analysis showed a number of elements to be present at a significantly greater concentration than the background level. The results of the x-ray elemental analysis are expressed as relative proportions of elements per 100 g sample. The equipment used cannot measure any elements below atomic number 11 as these are absorbed by the x-ray detector window. The areas analyzed comprised the stem (internal cut surface), external lamina, basal plate (internal cut surface) and anterior cephalothoracic cuticle. Due to the fact that time constraints prevented numbers of replicates being carried out for each area, the results presented here must be taken as a general guide to elemental composition rather than as an exact quantitative estimate.

Tables 4.2 - 4.5 show the values obtained in the analysis and these are presented graphically in Figure 4.4. Of the elemental components specifically analyzed, sulphur and calcium made up the bulk of elements present with Cl usually above the background level and sodium occasionally, though usually non-significant or very low. The basal plate showed the highest sulphur content with the cuticle of the cephalothoracic shield showing the next highest concentration. The external lamina of the filament showed the highest calcium content with the stem and cephalothoracic shield showing roughly equivalent amounts. Of the elements not specifically quantified, there were also consistently large concentrations of bromide in all the areas tested, these being calculated from graphs as representing between 0.505 % (external lamina) and 0.766 % (basal plate) of total elementary composition.

A graph comparing the elemental composition of the chosen biological standards with the values for chalimus cuticle is given in Figure 4.5. The chalimus cuticle showed a higher proportion of sulphur than the other arthropod cuticle samples though this value was less than that of the human nail sample. The proportion of calcium present in the chalimus cuticle was higher than that of crab cuticle and human nail but lower than that of the prawn cuticle and much lower than that of the prawn exuvium. All of the tested cuticles contained S, Ca and Cl as did that of the chalimus. In addition, one sample of crab cuticle and one of prawn cuticle gave evidence of Si and both the prawn cuticle and exuvium contained P. Br was not specifically analyzed for in the chalimus although it was present at relatively high concentrations and was also seen in prawn cuticle although it was absent from the prawn exuvium.

Table 4.2 Elemental composition of the external lamina

Element	External Lamina		
	Mean % Total Composition (n=2)	SD	% of Recorded Elements
Sulphur	0.717	0.121	34.50
Calcium	0.645	0.047	31.01
Silicon	< 0.060	0.001	2.86
Phosphorous	< 0.076	0.002	3.63
Iron	< 0.165	0.002	7.94
Chlorine	0.249	0.182	11.96
Sodium	0.169	0.057	8.11

Table 4.3 Elemental composition of the basal plate

Element	Basal Plate		
	Mean % Total Composition (n=2)	SD	% of Recorded Elements
Sulphur	1.798	0.297	63.37
Calcium	0.492	0.137	17.32
Silicon	< 0.060	0.005	2.11
Phosphorous	< 0.079	0.005	2.77
Iron	< 0.168	0.001	5.90
Chlorine	0.149	0.010	5.25
Sodium	0.093	0.022	3.28

Table 4.4 Elemental composition of the filament stem

Element	Stem		
	Mean % Total Composition (n=1)	SD	% of Recorded Elements
Sulphur	0.712	-	48.11
Calcium	0.362	-	24.46
Silicon	< 0.043	-	2.91
Phosphorous	< 0.059	-	3.99
Iron	< 0.165	-	11.15
Chlorine	0.09	-	6.08
Sodium	< 0.049	-	3.31

Table 4.5 Elemental composition of the cephalic shield

Element	Cephalic Shield		
	Mean % Total Composition (n=1)	SD	% of Recorded Elements
Sulphur	1.316	-	55.46
Calcium	0.584	-	24.61
Silicon	< 0.056	-	2.36
Phosphorous	< 0.075	-	3.16
Iron	< 0.167	-	7.04
Chlorine	0.1	-	4.21
Sodium	< 0.075	-	3.16

Figure 4.4 X-ray elemental analysis of frontal filament composition

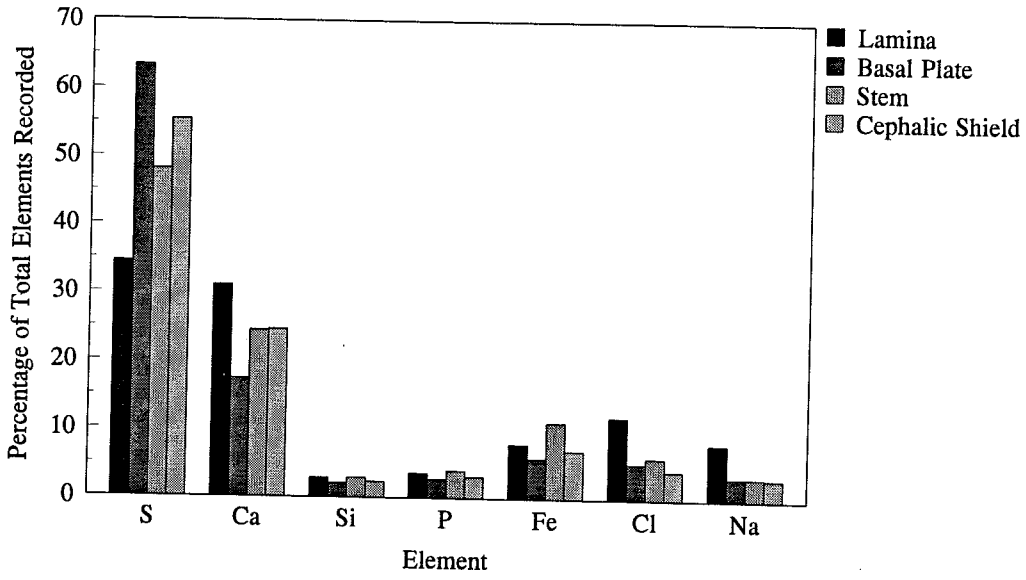
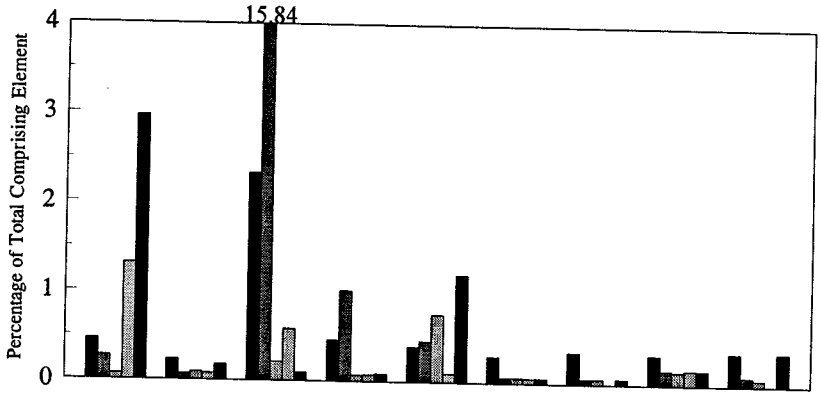


Figure 4.5 Comparative composition of chalimus cuticle and other biological standards determined using X-ray elemental analysis



Element	S	Na	Ca	P	Cl	Si	Br	Fe	K
Prawn Cuticle	0.459	0.228	2.327	0.461	0.391	0.293	0.353	0.330	0.364
Prawn Exuvium	0.269	0.070	15.844	1.019	0.459	0.064	0.059	0.165	0.099
Crab Cuticle	0.066	0.090	0.214	0.069	0.756	0.063	0.058	0.147	0.069
Chalimus Cuticle	1.316	0.075	0.584	0.075	0.100	0.056		0.167	
Human Nail	2.968	0.173	0.096	0.087	1.208	0.059	0.062	0.164	0.371

4.4.2.3.2 Copepodid

Filament components

Basal plate

The copepodid, being without a filament, possessed no basal plate in a recognisable form (ie no structure with the precise morphology or histochemical characteristics of the basal plate). The present study would seem to suggest that the laminated secretion associated with the B-gland of the copepodid and seen also in chalimus stages, comprises at least part of the basal plate, although the staining characteristics and morphology differ in some respects from those of the completed basal plate. As with the chalimus, this material did not give a PAS-positive stain, as was normally displayed by the basal plate itself. The laminated secretion was present in both unattached and attached copepodids although it was larger in the latter (Plates 4.31, 4.32). No ventral sumps were observed in the copepodid stage.

Filament stem

No external structure having the properties of the filament stem was visible in the copepodid. Internally, rare attached individuals were seen to possess structures similar to the completed filament stem. These structures displayed the fibrous appearance and the apparently bifurcate tip of the finished stem (Plate 4.32) and carried a duct which was identical to the axial duct of the finished stem, through the central axis. The majority of the copepodids studied, including those already attached to the host, gave no indication of such a structure being present.

The origin of this structure was difficult to determine. It was often located just posterior to the laminated secretion (Plate 4.32) and was often ventral to the secretion. This position places it in the vicinity of the C1 lobes which would appear to be responsible for the production of these fibres. In a single individual, the stem precursor was apparently attached to or embedded in, the laminated secretion and closely resembled the completed stem seen in chalimus stages (Plate 4.33). A single chalimus larva was similarly observed to possess what appeared to be a fully formed stem attached to the laminated secretion (basal plate) although all others displayed only the presence of the secretion itself.

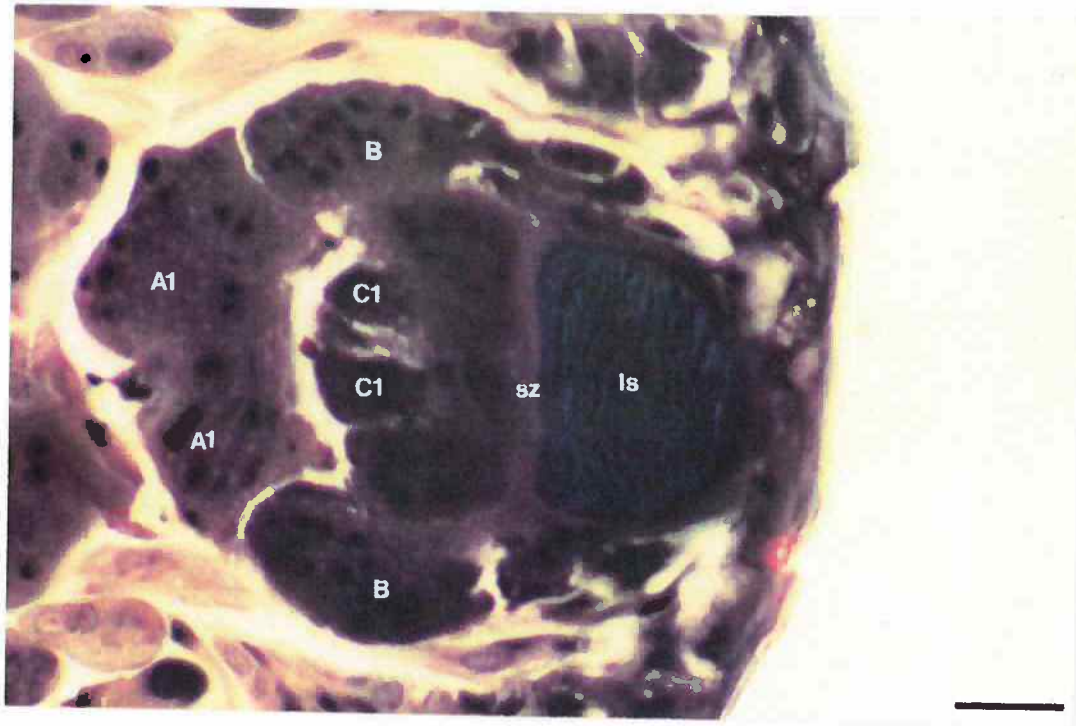


Plate 4.31 Section through anterior cephalothorax of copepodid showing arrangement of filament-associated glands (LMH, plane, stained polychrome). A1: A1 lobes of A-gland; B: B-gland; C1: possibly origin of C1 lobe of C-gland; ls: laminated secretion; sz: secretory zone of B-gland. Scale = 20μm

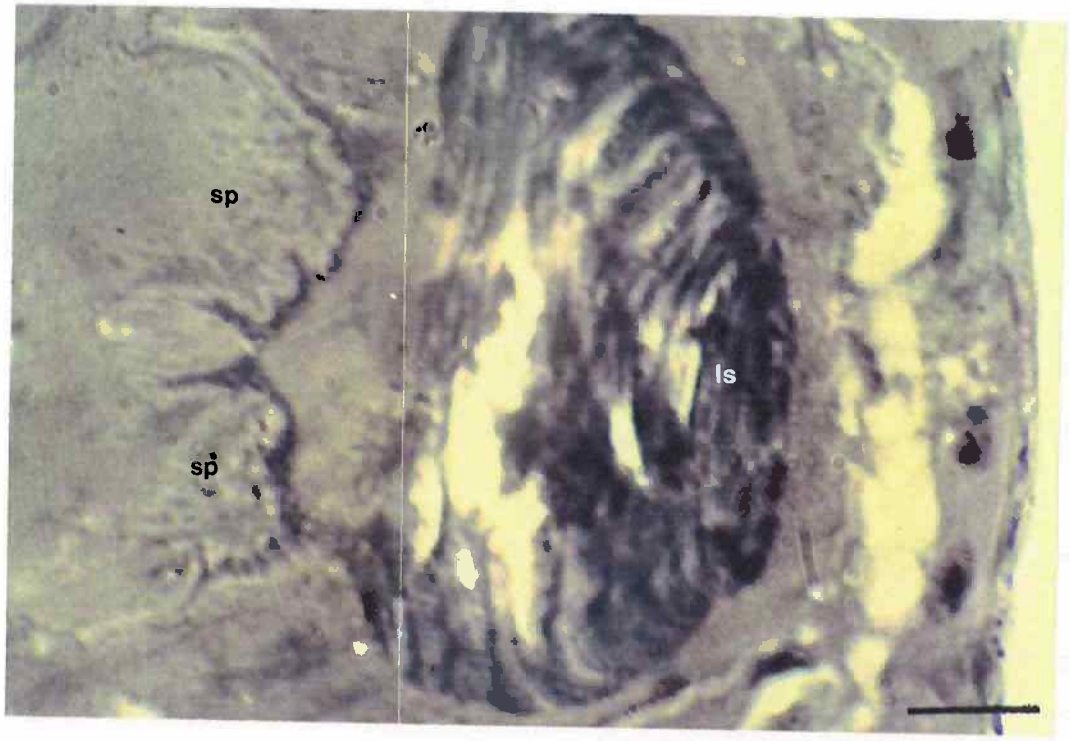


Plate 4.32 Section through anterior cephalothorax of copepodid showing apposition of bifurcate fibrous stem precursor and laminated secretion (basal plate precursor) (LMH, plane, stained Alcian Blue / polychrome). ls: laminated secretion (damaged in sectioning) sp: fibrous stem precursor. Scale = 10μm

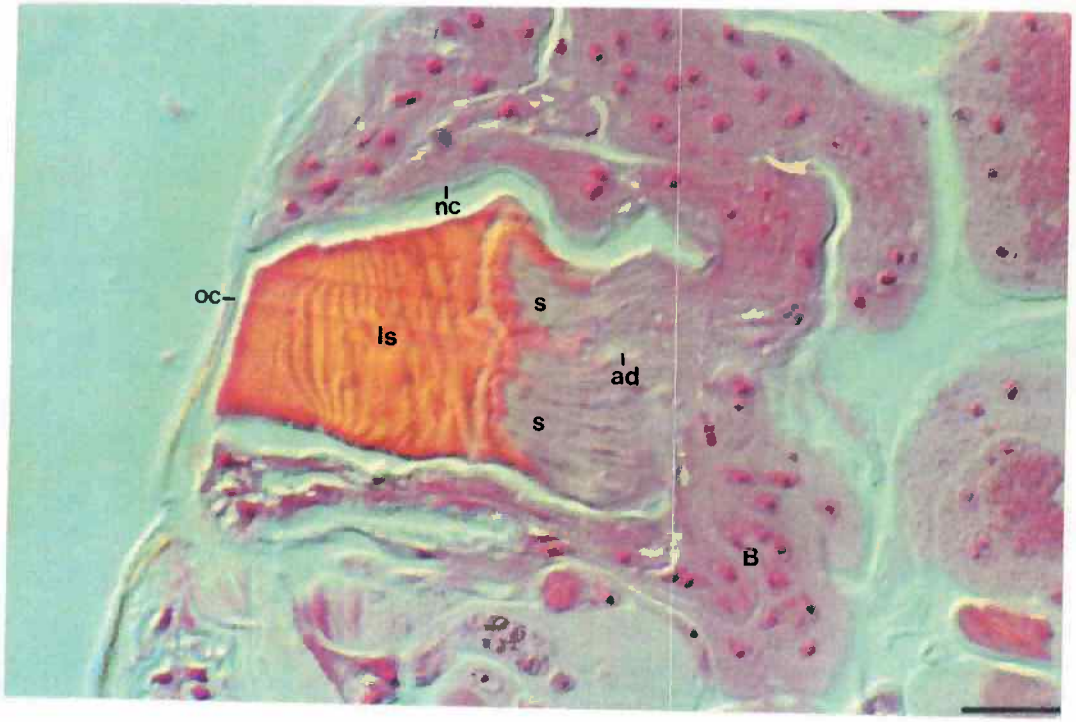


Plate 4.33 Filament precursor within anterior cephalothorax of copepodid. Note the apparent fibrous stem precursor, laminated secretion (basal plate precursor) and axial duct as well as an invagination of the cuticle (LMH, ~plane, stained Cason's). ad: axial duct; B: B-gland; nc: new cuticle; ls; laminated secretion (basal plate precursor) oc: old cuticle; s: stem precursor. Scale = 20 μm

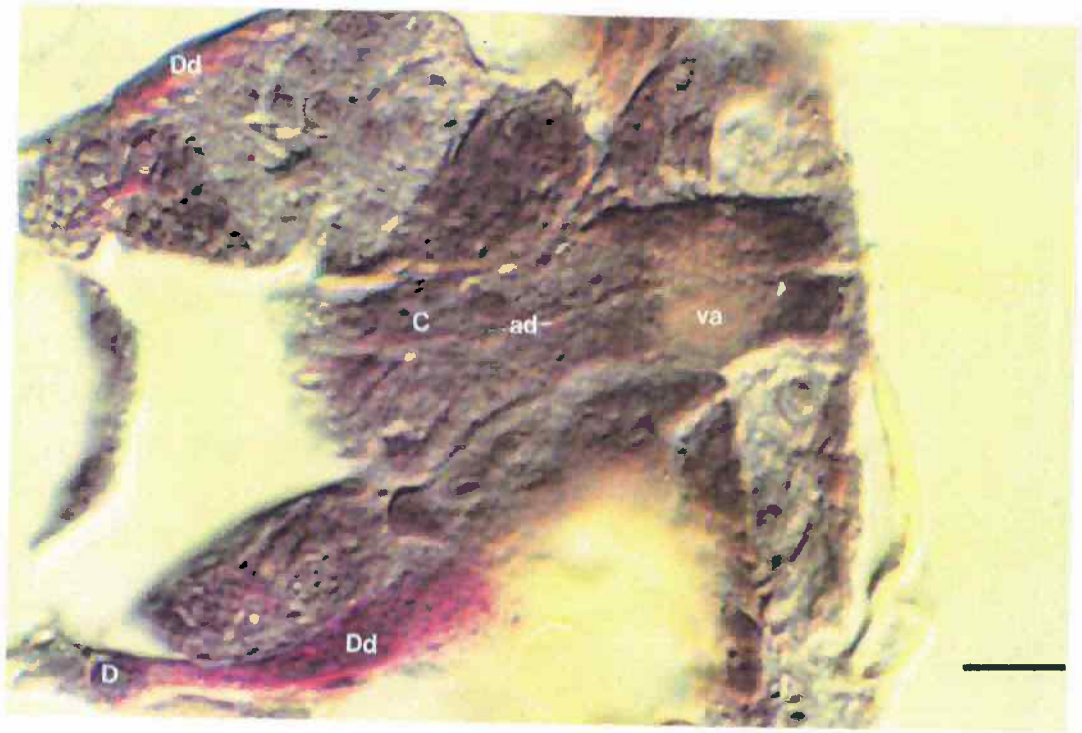


Plate 4.34 Section through copepodid anterior cephalothorax showing D-organ and passage of axial duct through the C-gland to the anterior cuticle of the cephalothorax. Note the faint staining of an area (va) corresponding to the ventral secretion of the chalimus (LMW, plane, stained PAS / Haematoxylin / Tartrazine). ad: axial duct; C: C-gland; D: D-organ; Dd: duct of D-organ. Scale = 20 μm

External lamina

There being no filament in the copepodid, no distinction could be made between a proposed external lamina and the normal cuticle. In the individual copepodid with the stem precursor noted above, however, there was cuticle deposited continuously around the cavity containing the stem and laminated secretion (Plate 4.33). This appeared to have been produced by an invagination of the cuticular epithelium and was likely to be the precursor of the external lamina. It seems likely that this cuticle is laid down just prior to moulting.

Axial duct

The axial duct was present in the copepodid and had the same apparent morphology as that seen in the chalimus stage. It extended from the A-gland where it interfaced with the glands' collecting ducts and, in the individual with the stem precursor, it passed through the long axis of the stem (Plate 4.33). In most individuals where the stem precursor was not seen to be attached to the laminated secretion and where the latter was somewhat ventral to the former, the axial duct apparently passed through the axis of the stem precursor and was continuous with the "filament duct" noted earlier which passes out through the rostrum after exiting the face of the cephalothorax (Plate 4.34).

Filament associated structures

A-gland

The A-gland was fully developed in the copepodid and equipped with the same collecting ducts as were observed in the later chalimus stages (Plate 4.35). As with the chalimus A-gland, the ducts stained positively with PAS and negatively with Alcian blue, indicating the presence of polysaccharides.

B-gland

The B-gland was very much as described in the chalimus stage, enveloping the posterior tip of the laminated rod. In the individual with the apparently complete filament the posterior end of the laminated secretion surrounded the filament stem or was adhered to it via what may be an additional product (Plate 4.33).

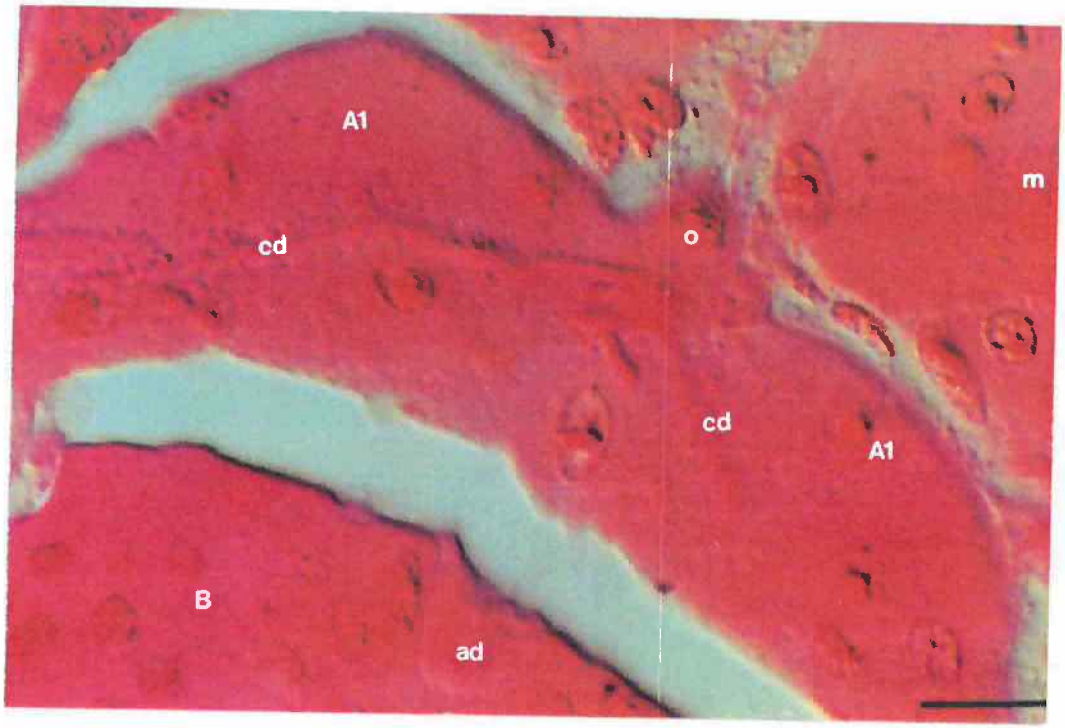


Plate 4.35 Section through A-gland of copepodid showing minor collecting ducts leading posteriorly to join the axial duct (LMH, plane, stained Polychrome with Ehrlich's haematoxylin). A1: A1 lobe of A-gland; B: B-gland; ad: axial duct; cd: minor collecting ducts; m: mucous gland; o: origin of axial duct. Scale = 10μm

The laminated secretion was clearly seen in the copepodid stages at the anterior of the B-gland (see under basal plate above).

C-gland

Both lobes of the C-gland were present in the copepodid stage and were very much as seen in the chalimus. Although the pronounced area of secretion seen in the chalimus stages was not seen in the copepodid, there was an area between the C2 lobes that took up tartrazine in a similar manner to the same area in the chalimus and apparently had a sulcus bisecting it into two semi-circular divisions through which the axial duct passes (Plate 4.34). There was no sign of a contribution from the laminated secretion in the copepodid stage.

D-organ

A fourth structure was seen in the copepodid that was not apparent in the chalimus stages. These organs were bilateral and apparently had ducts which fed into the area lying posterior to the paired cuticular projections of the anterior cephalothorax between which the "filament duct" passes. These organs appeared to be composed of a small number of cells which produced a strongly PAS-positive (Alcian blue negative) product which might therefore be suggested to contain polysaccharide (Plate 4.34). The function of this organ could not be determined from the present study.

4.5 Discussion

The experiments and observations described in the present chapter have been carried out in order to fill in some of the gaps lamented by Kabata (1981) who stated that "next to nothing" was known about the mechanisms initiating or guiding attachment in parasitic copepods. The information presented here contributes to a clearer view of settlement and attachment for at least a single species of parasitic copepod.

Copepodid larvae were found to be able settle on excised host tissue within an hour of completing the moult from the NII stage (although no estimate of subsequent survival or development was obtained during the present study). This agrees with the findings for the same species of Johnson & Albright (1992a) who routinely used "newly moulted" copepodids for their infection experiments. It also agrees with the findings of Lin & Ho (1993) for *C. epidemicus*. Many invertebrate larvae belonging to both free-living and parasitic species are not capable of settling and metamorphosis until they have become "competent[7]". According to Chia (1978) with regards to free-living species, the period required to attain competence is species-specific and varies between a few hours and several months. In *L. salmonis* the period of development and differentiation required to give a competent (infective) larva presumably occurs principally during the preceding nauplius stages such that the copepodid becomes infective immediately the new cuticle hardens and it resumes active swimming following ecdysis. The fact that the copepodid is immediately infective does not, however, infer that it is **fully** competent since work on *Cancerilla tubulata* by Carton (1968) suggests that the highest settlement success in this species occurred 5 days after moulting despite immediate infectivity of the copepodids. This was suggested to result from refinement of the copepodid's sensory faculties allowing greater host location success.

In the *in vivo* experiments, host fish were apparently able to detect the presence of copepodids entering the buccal cavity. This has not been previously reported for any host infection by copepodids, and is important in that it suggests that the host may, for this species, have some active control over success of infection in at least some areas of the epidermis, a fact which may frequently be overlooked in considerations of parasite-host interactions. Despite this, the finding of settled copepodids on the gills of experimentally infected fish indicates that such host responses are not effective enough to be able to entirely prevent settlement.

Further evidence for the detection of copepod parasites by hosts comes from the report of Benkirane (1987) that fish infected with Lernaeopodid parasites may try to remove them

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[7] A physiological state whereby a larva may metamorphose given the right environmental conditions (Chia 1978).

through rubbing and from indications that farmed salmon (*Salmo salar*) infected with *L. salmonis* or *C. elongatus* display evidence of irritation. In these instances, however, the host reaction is probably to the lesions inflicted by adult / preadult stages rather than to settling copepodid stages. In terms of non-accidental detection of copepods by hosts, it has been speculated by Gotto & Threadgold (1980) that adult females of *Notopterophorus papilio* Hesse, a notodelphyid copepod infecting ascidians, utilize a convenient host irritation response to their presence to promote the escape of nauplii in the backwash from the host inhalant syphon and have a cuticle flattened into "alate processes" to help make their presence felt during larval release.

In the present study, observations of copepodid settlement were successfully accomplished using excised pieces of host tissue which elicited settling responses from free-swimming infective stages. Attempts to elicit settling by the use of artificial substrates, however, were unsuccessful despite previous reports of attachment to untreated artificial substrates by other caligids e.g Heegaard 1947 (glass), Lewis 1963 (loose-woven cloth), Johannessen 1975 (plankton mesh, cotton wool). Briggs (1976) has described a similar failure to recognise mucus-coated agar discs in adults of *Paranthessius anemoniae*. From the present study it would seem that the observations of Johannessen (1975) of attachment of copepodids of *L. salmonis* were erroneous and resulted from observation of stranding of copepodids on plankton mesh at the water surface or entangling of the copepodids in the fibres of the cotton wool.

Butman (1987) states that most existing laboratory data suggests that larvae of marine invertebrates must make contact with a surface in order to detect any chemical cues that may be present. Larvae of the red abalone *Haliotis* sp. have for instance been observed to require direct contact with red algae for the initiation of settling and metamorphosis (Morse & Morse 1984). It is quite clear from the reported host specificity of *L. salmonis* (Kabata 1979) that some process of recognition, probably chemosensory, must occur. From the above, it would therefore seem likely that this may occur on contact or in very close proximity to the host.

As Boxshall (1976) states, host chemical cues are likely to be either diffusing from the host or adsorbed onto the host surface. The fact that mucus and other stimuli presented on artificial substrates did not elicit settlement in the present study suggests either that chemically inappropriate stimuli were chosen or that their presentation differs from that to which the copepodid is adapted to respond. Crisp (1974) proposed that recognition of adsorbed chemical factors might act in a manner analogous to an antibody-antigen reaction. It was suggested that larvae might thereby recognise substrates by mechanically detected "stickiness" caused by such a reaction. "Stickiness" may, however be difficult or impossible to detect on a liquid or labile surface such as is provided by the mucus of a fish host. A similar suggestion to that of Crisp has

also been made by Kirchman *et al.* (1982) who proposed that a lectin[8]-glycoprotein reaction might occur in some marine invertebrate larvae between larvae and substrate surface leading to induction of settlement and metamorphosis in the larvae. As noted in Chapter 3, the cauliflower organ of the copepodid may be the mediator of such specificity. Both these suggested reactions may, however, require reactive molecules to be oriented in a particular manner such that removal of host cues and presentation of them on new substrata (as attempted in the present study) might render them unrecognisable to the larva. As noted in Chapter 3, crustacean chemoreception often requires **mixtures** of chemical cues. From this one might also suggest that by removing components from the host environment the fractional proportions of cues change such that the mixture becomes unrecognisable to the copepodid.

In addition to the recognition of chemical cues, copepodids may also require the correct mechanical cues for the initiation of settlement, as do cirripede larvae (Lewis 1978). These cues may not, however, be host specific in themselves, requiring the presence of other cues such as host chemicals to elicit an appropriate response. The correct rugosity etc. is unlikely to be provided by artificial substrata and in addition, the copepodid may be able to detect a lack of mechanical strength e.g. in agar, that would make attachment by the antennae precarious and therefore inappropriate. A final explanation for the failure of copepodids to settle on artificial substrates is the possibility that chemicals adsorbed on to or diffusing from components of the artificial substrates themselves are causing a failure to settle in copepodids.

The *in vitro* and *in vivo* infection experiments described indicate that there is no observable chemotactic response to the host tissue, echoing the conclusions drawn from experiments in the previous chapter and in contrast to the findings of Fraile (in Raibaut 1985, Fraile 1986) for *C. minimus*. Contact with the host or host tissue was brought about either through accidental encounter or more usually, where live hosts were concerned, through the copepodid response to passing "power-sources" involving rapid swimming and tight turns. This agrees with results given in the previous chapter where such behaviour was recorded in response to directed flows and vibrational disturbances. Initial host attachment occurred through grappling / snagging of the host by the sharply hooked antennae of the copepodid which supports the observation of Kabata (1981) that this appendage is the most common mechanism of primary attachment in parasitic copepods. The apparent "reflex" action of the antennae seen in the present study on contact with any substrate regardless of suitability has been previously reported by Kabata (1981) for infective females of *L. branchialis* which were observed to attempt to grasp the walls of cavity glass slides. Raibaut (1985) regards this behaviour as a spontaneous fixation behaviour which occurs in a number of species. On swimming fish and on excised tissue

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[8] Proteins that bind to specific carbohydrates (Goldstein & Hayes 1978).

presented in circulated water, copepodids remained where they initially attached. Settlement of this kind was reversible as copepodids were often removed by the current and continued to behave in the same fashion as observed before settlement. Large-scale active choice of sites (e.g. fins / gills / body surface) during settlement was not seen in the present study and would seem to be unlikely. This agrees to an extent with the comments of Wilson (1905) who was of the opinion that the larva "apparently fastens itself to the first place it happens upon" whilst Kabata (1981) states, perhaps more accurately, that semi-mobile species tend to congregate in sheltered areas providing appropriate food resources.

The minimal nature of the pathology observed to result from attachment of the copepodid probably results from a combination of its small size and the limited duration of its pre-moult existence on the host. The negligible host response to copepodids of *L. salmonis* infesting *S. salar* has been previously noted by Jones *et al.* (1990) and Johnson & Albright (1992a).

The possible hydrodynamic factors affecting settlement site will be discussed in Chapter 6 and the present discussion will therefore concentrate on the nature of the host substrate. From the experiments described here it was apparent that almost any site appeared to be acceptable for initial copepodid attachment, with the exception of the corneal surface of the eye which was never observed to carry attached copepodid or chalimus stages. The reasons for this have not been determined in the present study but probably relate to the structure of the corneal surface. Kearn (1967) noted that the cornea of the sole *Solea* (L.) is devoid of mucous cells or blood vessels and found that oncomiracidia of *E. soleae* failed to attach to corneal samples, suggesting that this was the result of a lack of chemical cue provided by host mucus. This may explain the lack of copepodid attachment in the present study although it is equally possible that this results from the smooth nature of the corneal surface (required to limit aberration of the image) or its toughness relative to normal epidermis. Although females of *L. sprattae* penetrate the eyes of their host, these are much larger than the copepodids of *L. salmonis* (mean 2.61 $\pm$ 0.51 mm Schram 1979) and this greater size may allow easier penetration. Theories concerning smoothness or lack of mucus production of the cornea might perhaps be tested by observing settlement after abrading the surface of excised corneal discs and / or coating them with host mucus. The distribution of *L. salmonis* copepodids mirrors that of larvae of *L. pectoralis* which were similarly found to be present on all body surfaces save the eyes by Boxshall (1974b).

In terms of substrates suitable for filament attachment, Wilson (1905) suggested that caligid filaments were attached only to fin rays, scales and other hard substrates and Paperna (1980) also noted that *C. minimus* appeared to attach only to dental or bony (ie "hard") substrata. Kabata & Cousens (1973, 1977) similarly suggested that copepodids of *S. californiensis* selected sites with solid subdermal supports such as gill filaments, scales, fin rays or bones to allow cementation of the frontal filament. In the present study, copepodids seem to be far less selective

and could apparently attach anywhere, although clearly some areas will provide more secure areas of attachment than others. In most areas the basement membrane appeared to provide a sufficiently robust substrate.

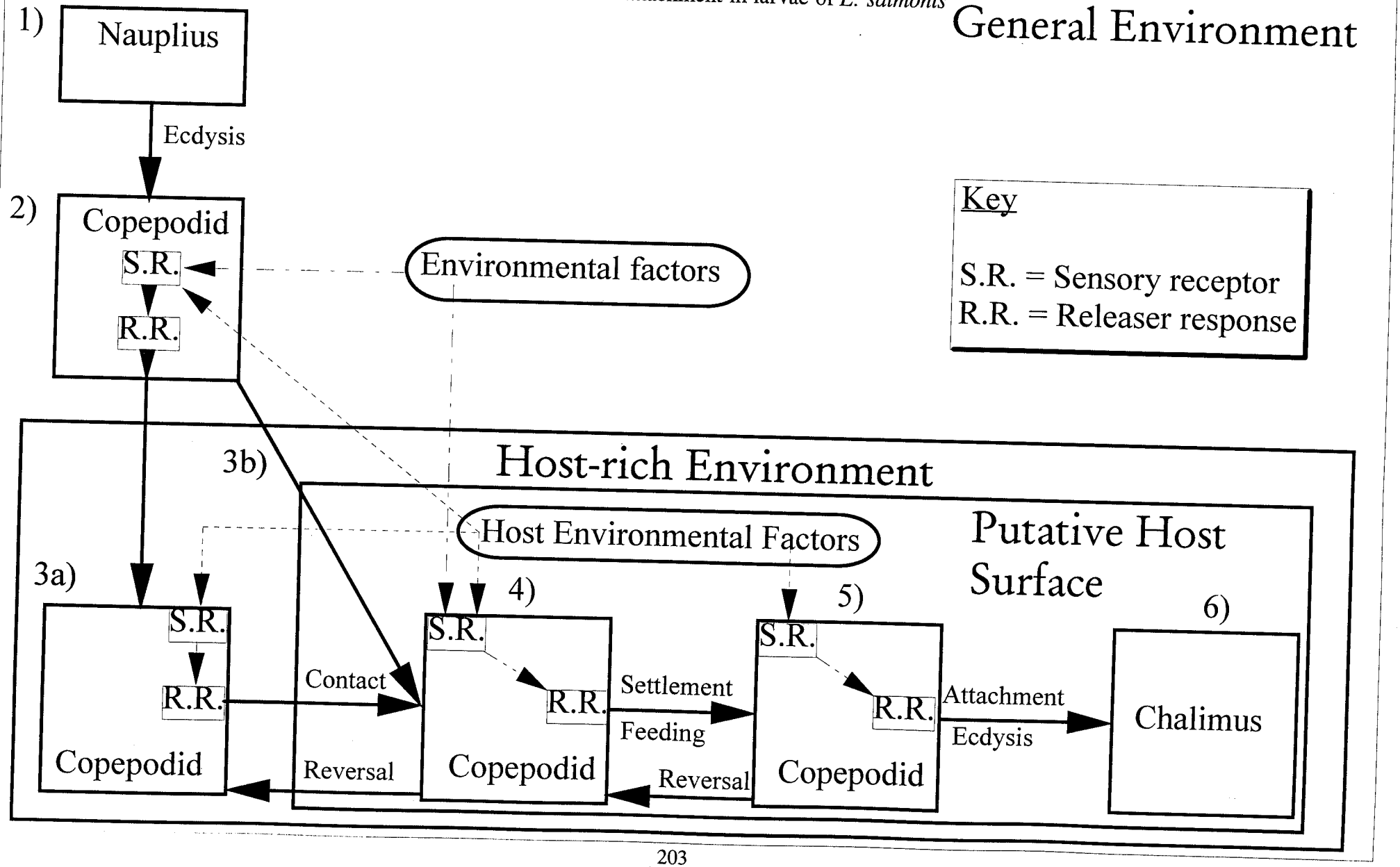
The settlement behaviour observed in this study is characteristic of some non-parasitic marine organisms, displaying the same sequence of "attachment, exploration and fixation" described by Crisp (1976) for marine invertebrates in general. The "wide searching" behaviour described by Crisp for *S. balanoides* does not appear to occur in *L. salmonis* (possibly due to the nature of the substrate) although the "close searching" of a small area described in the same paper was clearly seen to occur on the excised tissue placed in uncirculated water. This latter is, however, likely to be an artefact since it was not observed under flow conditions and seems unlikely in view of the fact that a precariously attached larva probably can not afford the luxury of careful site choice if it thereby risks possible loss of the host. Such searching behaviour has also been reported by Kabata & Cousens (1973) for *S. californiensis* although the flow conditions associated with the observations made were not noted and so these observations may also be artefactual. The prodding behaviour noted during the searching of the copepodid of *L. salmonis* has also been reported by Lewis (1963) for *L. dissimulatus* kept in dishes and by Kabata (1981) who noted a similar action in *L. branchialis* kept under the same conditions. Similar behaviour was also noted by Benkirane (1987) for copepodids of *Alella macrotrachelus* (Brian). The prodding behaviour generally observed in these species is consistent with a bringing into contact of the antennules with the host surface. As noted in the previous chapter, the antennules are probably equipped with contact chemoreceptors which might therefore allow host recognition during such exploratory behaviour. The reversible nature of settlement at this stage similarly agrees with observations made for other parasitic and non-parasitic species and has been suggested to apply for the same species by Johnson & Albright (1992a).

If we take the results of the previous and present chapter into consideration, we may construct a general scheme of settlement and attachment for the copepodid which is illustrated in Figure 4.6. The components of the scheme may be briefly summarized as follows:

1) The nauplius larvae are not "competent" in that they are not able to settle and metamorphose under appropriate environmental conditions. Instead, their main function is that of dispersal, in common with other lecithotrophic marine larvae (Chia 1978).

2) Moulting to the copepodid gives a fully "competent" larva able to immediately infect the host (ie the development of competence occurs during the preceding stages). This larva is fully equipped with sensory receptors (S.R.) which, as demonstrated in Chapter 3, enable it to detect general environmental cues such as light and pressure and host-derived

Figure 4.6 Diagrammatic scheme of host location, settlement and attachment in larvae of *L. salmonis*



cues such as water disturbance. These cues may act as releasers for releaser responses (R.R.) that function to bring the copepodid into or maintain it within host-rich environments where host contact is more likely or, alternatively, function to bring about host-contact (see Chapter 3).

3a) Releaser responses bring the copepodid into the host-rich environment where perception of the host via water disturbance allows the copepodid to make contact with the host. As demonstrated in the present chapter, this contact is maintained by use of the antennae. Despite the receptors and responses possessed by the copepodid to assist the establishment of host contact, such events may also occur purely by chance (3b) where, for instance, a host swims past a copepodid and the latter is brought into contact by entrainment in flow over the host's surface.

4) Once in contact with the putative host, host environmental factors such as host-derived chemical stimuli can be tested by high-threshold receptors (such as those on the antennules) to ensure that the substrate is suitable. If this is the case then the copepodid will remain settled and begin to feed, this behaviour being released by appropriate stimuli such as components of host mucus or host surface texture. Under suitable conditions, small-scale exploration may also occur in an attempt to procure a suitable site for settlement with both host and environmental cues being used as releasers for the settlement response. These stimuli may also prompt the production of components of the frontal filament within the cephalothorax. At this point, settlement is still reversible such that if the substrate is inappropriate, the larva may abandon it. It may also be forcibly removed by local environmental variables such as current flow.

5) Attachment and moulting do not occur immediately, but follow a period of feeding usually lasting between 5-7 days (as described in Chapter 5). It is impossible to elucidate from the present study whether the timing of moulting is simply an adjunct of purely developmental / hormonal processes or whether it is affected by releaser stimuli associated with the host or host-parasite complex. Copepodids that have not settled on a host do not metamorphose or produce filaments, which clearly suggests that some metamorphic factor exists as suggested by Chia (1978) for other marine larvae. As before, settlement at this point appears to be reversible though this may cease to be the case at some point during development. Finally the copepodid attaches using the internally-produced frontal filament (see below) and moults to the chalimus stage.

The scheme outlined above describes the general pattern of host location and initial, reversible, settlement suggested by the present study. The following deals more specifically with the process of settlement and attachment. The general morphology described for the copepodid in the present study agrees well with that described by Johnson & Albright (1991a) for this species, and broadly with that described by Lewis (1963) for *L. dissimulatus* and Boxshall (1974d) for *L. pectoralis*. It also agrees with descriptions by Wilson (1905), Hwa (1965) and Kabata (1972) for *Caligus* spp. The "filament duct" described in the present study may equate with structures previously noted or drawn by a number of authors and usually referred to as a spike, spine or stylet. Such a structure has been described by Boxshall (1974d) for *L. pectoralis*, Gurney (1934) for *C. centrodonti* Baird, Heegaard (1947) for *C. curtus*, Lewis (1963) for *L. dissimulatus*, Hwa (1965) for *C. orientalis* and Pike (1993) for *C. elongatus*. Heegaard suggested that this structure might be used to pierce the exuvium of the NII during moulting and Gurney suggested that it was a chitinous rod possibly used to make a small hole in the host epithelium and initiate an inflammatory reaction. There is no evidence from the present study that the filament duct is related to the "rostral spine" reported for adults belonging to the Vaigamidae (Poecilostomatoida) by Thatcher and Robertson (1983) and similarly associated with the taeniacanthids and bomolochids (which may have 2). Similarly no relationship is apparent to the "rostral filaments" of calanoids described by Huys & Boxshall (1991).

The filament duct appears to pass into the rostrum through a specifically modelled area of cuticle on the anterior face of the cephalothorax underlying it. It exits the rostrum through a distal pore. The filament duct is also tubular and possesses a terminal pore which may indicate that it maintains a precise function rather than being simply a relict ornamentation of the rostrum.

The cuticular processes underlying the rostrum appear to have been illustrated by Gurney (Text-figure 5., 1934) for *C. centrodonti* but have not apparently been commented on specifically by any author. Their only certain function appears to be as a point of exit of the "filament duct" from the interior of the cephalothorax *en route* to the rostrum.

The possession of a frontal filament serving to temporarily attach infective larvae to the host is a feature of many siphonostomatoid families (Huys & Boxshall 1991). Only one of the siphonostomatoid families found on invertebrate hosts possesses a filament, however, this being the Nicothoidae (*ibid.*). Kabata (1981) states that the question of the origin of the frontal filament in siphonostomatoids is particularly problematic and suggests that the Dissonidae may represent primitive forerunners of the Caligidae (Kabata 1979) having no apparent frontal filaments (Anderson & Rossiter 1969b).

In the present study there were no indications of a hole being specifically excavated in the host epidermis for attachment of the filament. Benkirane (1987) also failed to find evidence for cavity excavation and believed the cavity used for filament implantation in *A. macrotrachelus* to be of lytic origin. No evidence of lytic activity was observed, however, in the present study. Excavation of an implantation cavity has been previously reported for *S. edwardsii* (Fasten 1913) using the maxillae whilst Russell (1925), Heegaard (1947) and Kabata & Cousens (1973) have noted excavation for filament implantation to involve the maxillipeds. The latter authors (1977) have suggested that excavation by *S. californiensis* preadult females was brought about by a "burrowing reflex" which was deactivated by contact with tissue of an appropriate firmness for attachment. The initiating factor for this reflex was not, however, determined. The cavity for attachment in the present case appears to be formed by a combination of mechanical disruption by the antennae and introduction of the anterior cephalothorax into the epidermis.

As noted in the introduction, there are two schools of thought regarding the origin of the frontal filament in caligids. Secretion *in situ* has been observed by Wilson (1905) for *Caligus* sp. Heegaard (1947) for *C. curtus* and Lewis (1963) for *L. dissimulatus* whilst the use of pre-formed filaments is suggested for *C. centrodonti* (Gurney 1934), *C. clemensi* (Kabata 1972), *L. salmonis* (Johnson & Albright 1992a) and *C. elongatus* (Pike *et al.* 1993). It is interesting to note that these observations are not, as might be expected, clearly demarcated between *Caligus* spp. and *Lepeophtheirus* spp.. Preformed filaments are also reported to be used by members of the Lernaeopodidae (Kabata and Cousens 1972, 1973, Piasecki 1984, Benkirane 1987) as a temporary attachment mechanism preceding production of the final attachment bulla.

The present study provides evidence that *L. salmonis* produces a fully pre-formed filament in the copepodid stage. Johnson & Albright (1992a) describe a pre-formed filament in pre-moult chalimus stages and note that materials with the same staining properties are present in the copepodid. These authors, however, noted that the internal stem differed from that seen in attached chalimus larvae. Pike *et al.* (1993) on the other hand, were unable to find a pre-formed filament in any *L. salmonis* copepodid though reported the presence of an apparent pre-formed filament in 38 hour copepodids of *C. elongatus*. The present study has also provided the first detailed information concerning the organ systems associated with the filament in caligids although the presence of apparently filament-associated glands in whole specimens has been noted by many of the authors cited above. The ultrastructure of probable filament-producing glands has also been described in detail for the lernaeopodid *A. macrotrachelus* (Benkirane 1987).

The filament of *L. salmonis* clearly differs markedly from that of *C. elongatus* as noted by Pike *et al.*(1993) and is different from others described for *Caligus* species in general. The major difference would appear to be the point of origin of the filament. Whilst that of *Caligus* originates in the area of the structure described as the "frontal organ" by Pike *et al.* (1993), that

of *L. salmonis* is apparently continuous with the cephalothorax. This observation of continuity of the frontal filament in *L. salmonis* concurs with that of White (1940a) for the same species. Since the "rostral sensory complex" which has been indicated to characterize the "rostrum" (Huys & Boxshall 1991) is nowhere represented on the filament (by morphological and histochemical examination) the relationship of the chalimus frontal filament to the rostrum of the copepodid is uncertain although if the former has been everted, it represents an entirely novel structure.

The structure of the completed filament has been described here in terms of four components which have been termed "basal plate", "filament stem", "external lamina" and "axial duct". Descriptions of the filament given by Pike *et al.* (1993) and Johnson & Albright (1992a) agree with those presented here and the latter authors have adopted the terminology suggested by the present study. It is suggested that a more precise name be given to the "frontal organ" previously identified with the "median sucker" of Wilson (1905) and termed the "filament gland" by Anstensrud (1990). According to its likely function in producing the frontal filament of *Caligus* spp. and also the filament of preadult caligids e.g. *L. pectoralis* (Anstensrud 1990) and in order to distinguish it from filament-associated glands, it will be referred to as the "ventral filament organ" for the remainder of this discussion.

The components of the filament of *L. salmonis* suggest that its origins are essentially similar to those of copepod cuticle. The stem is apparently composed of densely-packed fibres surrounded by an external lamina which is continuous with and apparently similar to the cuticle of the cephalothorax. The basal plate appears to comprise both proteinaceous and polysaccharide components and displays an element of protein-tanning indicated by the presence of the enzyme o-diphenoloxidase. The presence of o-diphenoloxidase in the stem of this species was markedly less than was present in the filament stem of *C. elongatus*. This may either suggest that the mode of production of the filament differs or alternatively it may be an artefact due to variation in the time elapsed since a given stem was produced and the time it was tested. In the case of a preformed filament, most o-diphenoloxidase might be exhausted by the time the filament is expressed although Boxshall (pers. comm.) has noted apparent maturation of the filament after attachment for *L. pectoralis* which suggests that such enzymes may still be present or may be subsequently secreted. The different aspects of the basal plate, ie relatively homogeneous or fibrous according to the specimen, may similarly result from a maturation process as has been reported by Benkirane (1987) for the bulla of *A. macrotrachelus*. In this instance an initially fibrous makeup was noted to become more homogeneous with fibres eventually losing their individuality as the bulla became more compact. It was suggested that this might result from the addition of secretions from the maxillary glands (*ibid.*). If such a secretion occurred in *L. salmonis*, it would probably be transported to the site of action by the axial duct (see later).

The results of the x-ray elemental analysis appear to support the contention that the filament is principally a cuticular structure. The two major components recorded for all areas tested were sulphur and calcium. Kannupandi (1976) has reported a calcified layer in the procuticle of *C. savala* and it has been noted (Hackman 1984) that mineralization in crustacea may involve both Ca and Mg carbonates and phosphates. No significant P was noted in the cuticle or frontal filament of the chalimus which contrasts with the situation found in prawn cuticle and suggests that mineralization in *L. salmonis* principally involves carbonates. In normal arthropod cuticles, protein and chitin account for > 90 % of the organic content of the cuticle and it is clear from the present study that the cuticle of the copepodid cephalothorax at least, possesses both helicoidally arranged chitin fibres (as observed under TEM) and protein.

The high sulphur content of the basal plate might normally suggest the possibility of formation of extensive disulphide bonds in the maturation of the cement that attaches the filament to the host although in the present study the Performic acid-Alcian blue test was negative suggesting that such bonds were absent. Although this latter test is specific, it has, however, been noted that it is not particularly sensitive (Drury & Wallington 1980) such that disulphide bonding may have been missed if it occurred at low levels. Of the standards tested using x-ray analysis, only the human nail sample contained a higher sulphur content which is unsurprising since keratin contains extensive disulphide linking. Although Hackman (1984) states that the evidence for such disulphide (-S-S-) linkages in arthropod cuticle is somewhat inconclusive, he suggests that bonding might be accomplished by the action of quinones on sulphydryl (-S-H) groups. The high o-diphenoloxidase activity shown in the basal plate may indicate that the quinones required for such a reaction are present in this area so that it may be that the basal plate in stained specimens was newly formed and that the reaction had not yet had time to proceed. The o-diphenoloxidase might also suggest a quinone sclerotisation process, commonly observed in arthropod cuticles generally, whereby protein chains could be covalently cross-linked by quinones produced through the action of the enzyme on phenols present in the cuticle. The fact that the basal plate is near-colourless, as opposed to displaying the brown colour generated by normal quinone-tanning, suggests that the process of cross-linking may cease before completion of the full chemical reaction, ie at the catechol stage, as suggested by Hackman (1974a).

The fact that the external lamina appeared to contain a slightly higher Ca concentration than other areas may be indicative of higher mineralization of the cuticle in this area which would act to make the cuticle more resistant and less permeable to the free diffusion of ions (Kannupandi 1976). This might function to make the cuticle in this area more resistant to attack or degradation during prolonged contact with the host tissue. The increased mineralization might also explain the concurrent fall in the relative concentration of sulphur seen in this area with respect to the cuticle, there being potentially less "space" available for proteins. This would

agree with the observation of Neville (1975) that "the more calcium, the less protein" is present in arthropod cuticle. Compared with the other arthropod cuticle samples examined, the calcium content of the cuticle of the chalimus was far lower than that of the prawn cuticle samples, particularly the exuvium, whose high proportion of Ca is presumed to result from removal of other components prior to moulting. Calcification of the chalimus cuticle was higher than that recorded for the crab cuticle. The reduced Ca seen in the basal plate with respect to the cuticle and external lamina may derive from a requirement for efficient adhesion in this area since a "brittle cement" is likely to be less effective than a more flexible "glue-like adhesive" (Crisp 1976). Such a tanned but plastic (non-brittle) cement containing high sulphur and o-diphenoloxidase is found in barnacles (Walker 1971) suggesting the parallel development of similar attachment systems. This should perhaps be unsurprising as both groups face the problem of attaching, and remaining attached, to substrates often subject to high current exposure and hence they are likely to be subject to similar selection pressures. Both groups, furthermore, are equipped with a similar array of construction materials and techniques with which to solve the problem.

Comparison of the present results from x-ray analysis with estimates of elemental composition of whole free-living copepods by Vinogradov (1953) indicates a large anomaly in the ratio of P : Ca. In the latter study this was 0.14 % : 0.038 % for whole *C. finmarchicus* whereas in the present study the representation of P in the cuticle and filament components alone was not significantly different from the background level. Although this might result from the P being located elsewhere from the cuticle in free-living species, Vinogradov believed that it was principally derived from the cuticle. In the same study, the highest percentage representation (of the elements examined in the present analysis) was for Cl in *C. finmarchicus*. In the present study the Cl content was lower than the S and Ca contents but again the present study involved only cuticular and filament components.

The presence of high Br levels in all of the filament components tested as well as in the cuticle strongly suggests that the filament components are cuticular in origin. The levels reported in the present study are of the same high order of magnitude (ie n^{-1} %) as reported by Funge-Smith (1992) for the cuticle of the prawn *M. rosenbergii* although Vinogradov (1953) suggested Br in whole crustacea to be present generally at the same order of magnitude as found in seawater. In the former study Br was found to be an essential ingredient of the artificial seawater used for rearing, with larvae showing very high mortality without it. The function of Br in crustacea is uncertain although it has been demonstrated in the cuticle in the form of brominated tyrosines comprising a component of scleroproteins. It has been suggested by Hunt (1984) that the action of oxidase enzymes on quinones may cause bromides and chlorides (and to a lesser extent iodides) to be oxidised to free halogen which may then easily substitute into tyrosine

groups present in the protein. The quinones themselves have also been suggested to be able to carry out this oxidation of halide to halogen (*ibid.*). The incorporation of halogens into scleroproteins has therefore been suggested to be a secondary consequence of the normal tanning process. A second possibility is that Br may actually help facilitate sclerotisation as suggested by Hunt & Breuer (1971) and this was supported by deformity of larvae moulting without bromide observed by Funge-Smith (1992). This was suggested to be indicative of a possible role for bromide as an enzyme cofactor or, alternatively, for molecular bromine in organic compounds facilitating the formation of scleroproteins. Funge-Smith also suggests that halogenation of scleroprotein in crustacean cuticle might increase its water repellency or alternatively give greater elasticity to the procuticle. Assuming that the frontal filament is indeed a cuticular product, the very high Br recorded may act in a similar fashion to make it less permeable and therefore more resistant to attack by the host and possibly also more flexible in order to give greater mobility to the chalimus and reduce the likelihood of stress-fracturing of the filament itself.

The exact composition of the fibres comprising the stem has yet to be positively demonstrated. As noted, the stem gives a positive reaction for proteins which suggests that they are composed of fibrous structural proteins. Such proteins have also been proposed to comprise the bulla of lernaeopodids (Benkirane 1987). In the present study, the fibres were found to stain the same as the endocuticle of chalimus stages suggesting that the same protein components are present in both. Hackman (1984) notes that the proteins present in arthropod cuticle do not have a fibrous configuration although some will form fibres if drawn from concentrated solution. Under these conditions, orientation of the proteins may result directly from shearing forces developed as the fibres are being drawn (*ibid.*). Despite the generally stated lack of fibrous proteins, Krishnan (1969) has noted an amino acid composition resembling collagen in cuticular protein of the branchiopod crustacean *Streptocephalus dichotomus* Baird. Another possibility for the staining pattern observed in the chalimus stage is that chitin microfibrils normally present in the endocuticle and seen in the normal cephalothoracic cuticle in the present study, might be bound to proteins, and the resulting complexes embedded in matrix material as suggested by Blackwell & Weih (1980) for fibres in the ovipositor of the ichneumon fly *Megarhyssa atrata* (Fabricius). With respect to this suggestion, electrophoresis by Benkirane (1987) of protein components of the bulla of *A. macrotrachelus* gave a protein mass of 26 kilodaltons which is comparable to the 27 kilodalton protein extracted from honey bee cuticle by Skerrow (cited in Rudall 1976). This latter was, in turn, suggested to be the same protein as found bound to chitin microfibrils in *M. atrata* by Blackwell & Weih (1980). The apparent large size of the fibres seen in the stem of *L. salmonis* suggests larger units than chitin microfibrils although Hackman (1984) notes that crustacea may have fibre components constructed of bundles of chitin rods measuring

up to 50 - 70 nm in diameter. Measurements taken by Pike *et al.* (1993) suggest that similar fibres in *C. elongatus* are ~ 160 nm which is clearly somewhat larger than might be expected from the bundles previously described for crustacean cuticle. Blackwell & Weih (1980) have suggested that protein-chitin fibrils might show long fibre repetition of 15.3 nm or multiples thereof which might allow for the long fibres observed in the present study and in that of Pike *et al.* (1993). The relatively low Ca content of the stem with respect to the cuticle and external lamina may, similarly to the basal plate, result from a requirement for flexibility that would not be provided by a heavily mineralized cuticle.

The external lamina is continuous with the external cuticle of the cephalothorax and gives apparently identical staining reactions. Whilst the interface between the normal cuticle and the underlying epidermal cells stains positively for acid mucopolysaccharides, the interface between the external lamina and the stem fibres does not. Instead this staining runs along the proximal edge of the finished stem which suggests that the stem and external lamina of the frontal filament may represent an expansion in depth of the normal cuticle with the basal plate representing a novel addition.

Having discussed the morphology and chemical composition of the finished filament, the next problem to be tackled is its origins in the copepodid stage. From this study there is clear evidence that all of the recognised component parts are present as a preformed structure in the anterior cephalothorax of the copepodid. The proto -stem, -external lamina and -axial duct all show similar structures and staining properties to the same portions of the attached filament. The secretion supposed to be equivalent to the basal plate within the copepodid (and chalimus) stage appears, however, to be problematic in that it possesses a laminated and apparently fibrous appearance not seen in the finished basal plate. It also displays different staining characteristics to the finished basal plate, having no apparent PAS-positive component in the unfinished form. It is interesting to note that this secretion stains similarly with Heidenhains' haematoxylin to the "bulla" of the internal frontal filament (more accurately termed by Kabata & Cousens (1973) the proximal plug) of *S. edwardsii* (Fasten 1919) which serves to attach the frontal filament of this species in a manner analogous to the basal plate of *L. salmonis*. This may also serve to suggest that the laminated secretion equates to the external basal plate. The fact that the basal plate stains differently from the internal reservoir of material may result from incorporation of host derived products into the basal plate or alternatively from the addition of new material to the plate prior to or during attachment. This laminated secretion may also be the source of the layer that appears to surround the externally attached basal plate when it is prepared with certain stains e.g. Giemsa.

The basal plate described in the present study apparently contained no cellular inclusions and did not display the dendritic appearance described by Pike *et al.*(1993) for the same species. The present study suggests that the latter description was artefactual and resulted firstly from forceful removal of the basal plate from its substrate giving tissue adhesions and secondly, from long-term exposure of the illustrated filament to attack as part of a normal host reaction to foreign material as described by Jones (1989) which eventually leads to breakdown of the filament.

The axial duct of the copepodid leads anteriorly from the A gland and passes through the length of the proto-stem. It therefore seems likely that this structure antedates the stem and indeed appears to be present in all copepodids examined regardless of the presence of the proto-stem. The proto-stem of the copepodid possesses the parallel fibres of the finished stem. From their appearance under the light microscope and assuming analogy with the fibres in the main stem of *C. elongatus* shown in photographs by Pike *et al.*(1993) it would appear that the fibres are not helicoidally arranged within the stem itself. Such an arrangement of fibres echoes that described by Maugin (1910) and cited in Neville (1988) whereby a nematic[9] liquid crystal of azoxyphenetol was produced by the placement of the liquid between a glass slide and a coverslip. Neville (1984) suggests that the layers of parallel chitin commonly found in arthropods might arise via such a nematic liquid crystal phase. Neville (1988) similarly suggests that the helicoidal arrangement of fibres found in many living systems (including the cuticle of the copepodid) may be self-assembled in the form of cholesteric[10] liquid crystals which may form if secretions are produced such that they are bounded by a constraining layer (which in the case of arthropods is suggested to be provided by the epicuticle). The important feature to note in the formation of both of these systems is the requirement for a constraining layer of some kind.

A tentative scheme of events occurring in the production of the frontal filament of *L. salmonis* has been worked out that appears to fit the various morphological observations made in the present study. Because many of the details, particularly of ultrastructural events, are missing, such a scheme is intended as a working hypothesis that may be used in examining filament production in more detail at a later date. This scheme is illustrated in Figure 4.7 (Diagrams 1-8)

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[9] A nematic liquid crystal is one in which the molecules are arranged in layers but all their axes are parallel (Uvarov, Chapman & Isaacs 1979).

[10] A cholesteric liquid crystal is one in which the molecules are arranged in layers with their axes parallel and in the planes of the layers (*ibid.*).

Figure 4.7 Schematic diagram of proposed scheme of filament formation and attachment (Diagrams 1-4)

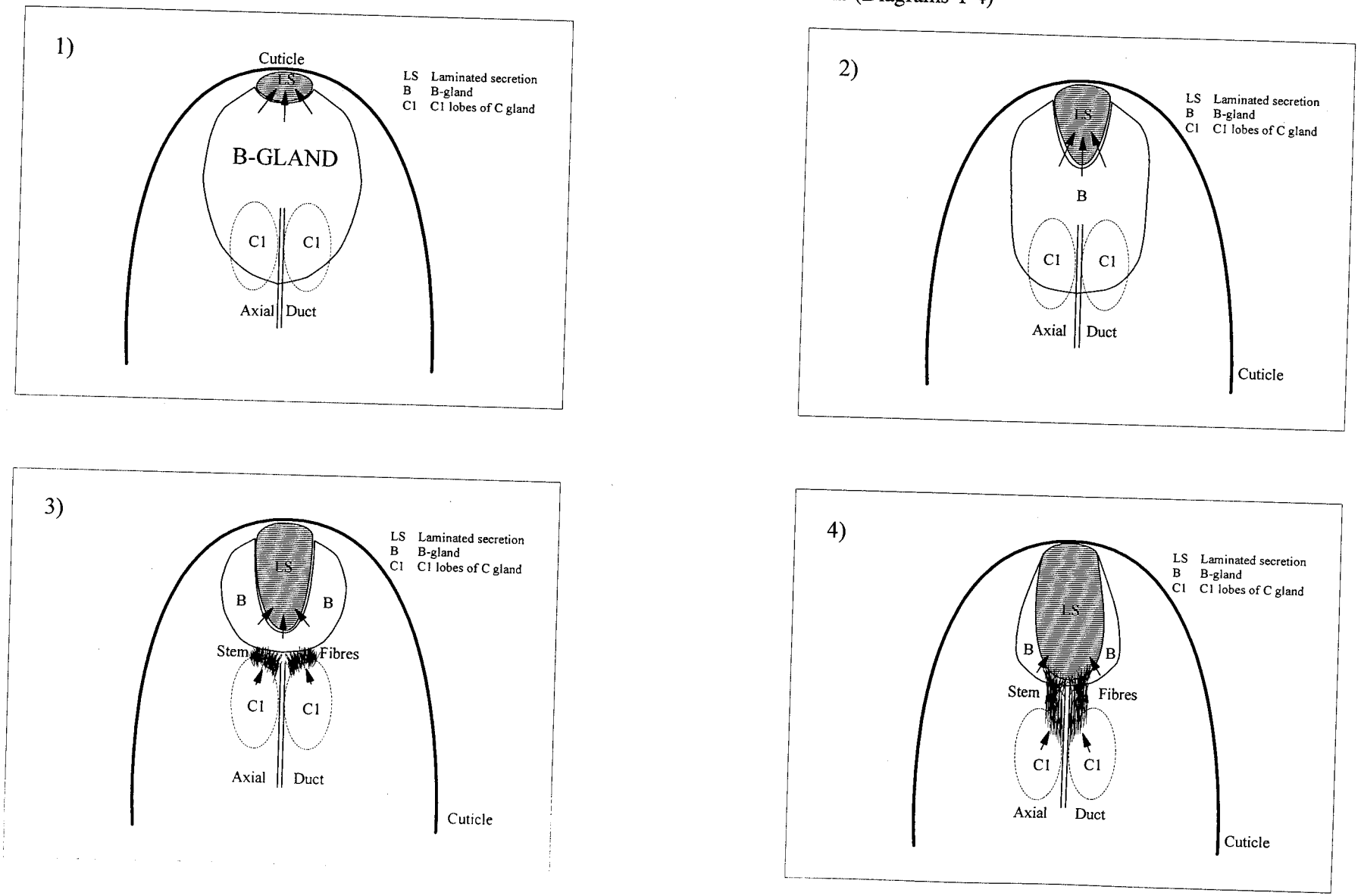
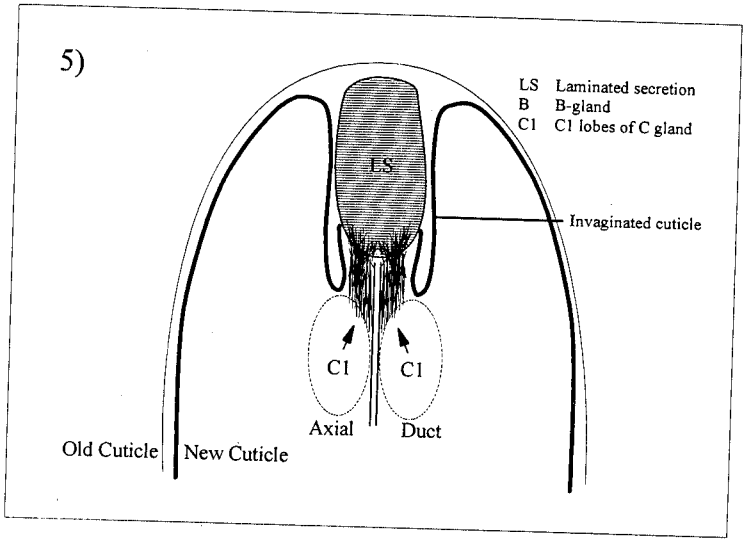
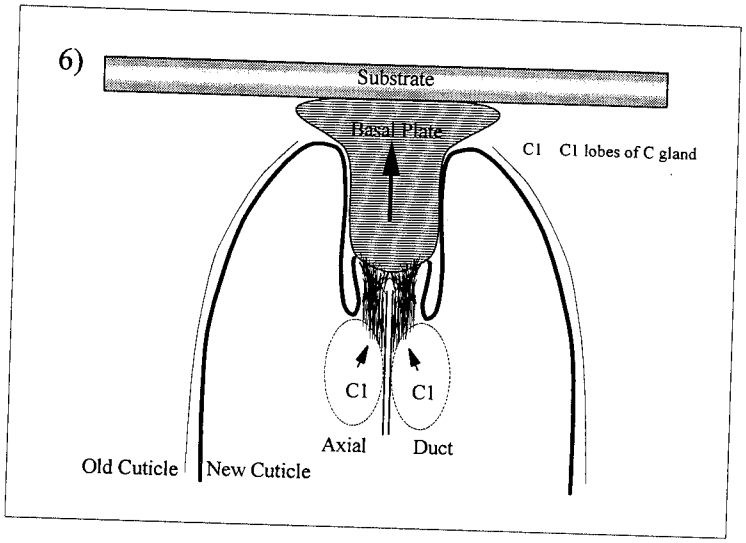
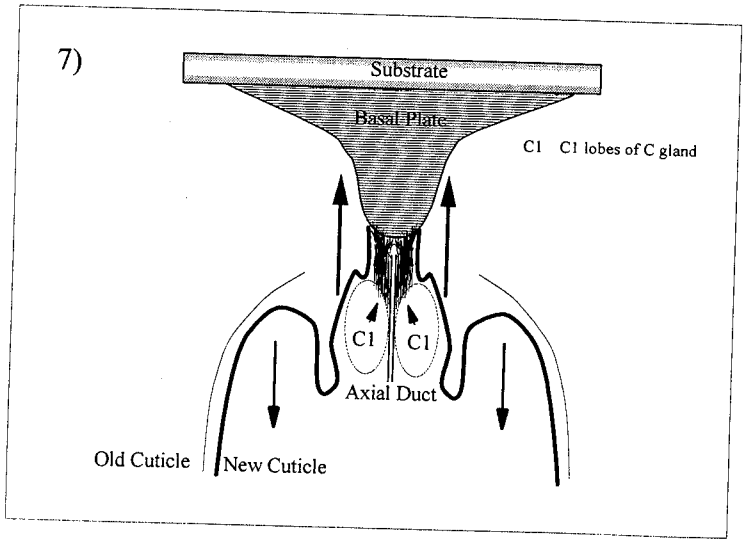
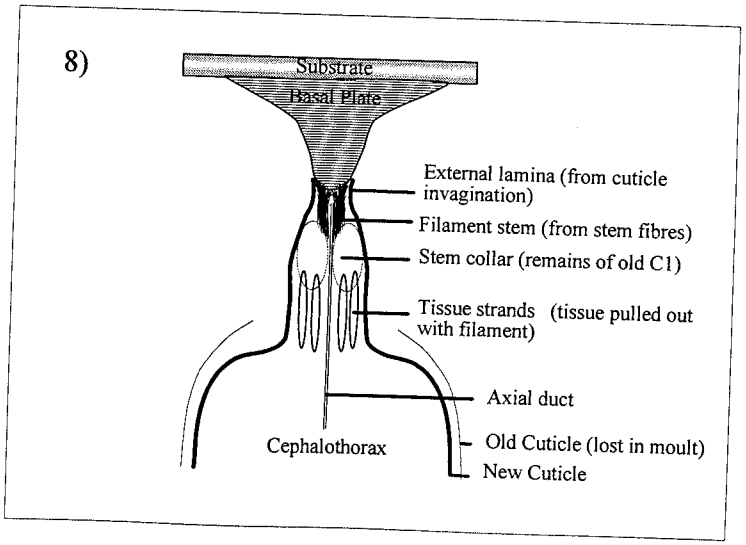


Figure 4.7 Schematic diagram of proposed scheme of filament formation and attachment (Diagrams 5-8)









1) Events are probably initiated by the production of a reservoir of material representing the proto-basal plate. This appears to be produced by the B-gland whose proteinaceous product is either secreted across microvilli at the front of the gland or forms fibres at the interface. The proto-basal plate is secreted initially against a constraining layer e.g. the cuticular epithelium.

2) Successive secretion events, using the previous secretion as a constraining layer cause a laminated / helicoidal structure or reservoir of material (L.S.) to be produced.

3) As the production of the proto-basal plate (L.S.) proceeds, two glandular lobes (C1) produce stem fibres (probably by accretion / self-assembly of products within the cytoplasm) giving rise to the bifurcated, fibrous, distal part of the finished stem.

4) The distal (bifurcate) part of the stem becomes attached to the proto-basal plate, either via an intermediary layer which may derive from the tissue originally separating the two components or by adhesion of the basal plate material to the stem fibres.

5) The stem fibres may continue to be produced. The glandular tissue surrounding both secreted products is replaced by a cavity which becomes lined with cuticle by an invagination of the anterior cuticular epithelium as the moult approaches. This new cuticle may also form the external lamina of the finished frontal filament. Prior to filament evacuation, adhesives / lytic agents may be expressed via the filament / axial duct or the D-organs (not shown) in the anterior cephalothorax.

6) Final attachment of the filament seems likely to occur following the start of the moult to the first chalimus stage. Assuming the initial split in the cuticle occurs in the area of the antero-dorsal cephalothorax as it does in the nauplius, removal of the cuticle will leave the proto-basal plate open to the exterior. With the cephalothorax embedded in the host, the new basal plate will abut the underlying host tissue at the point of maximum penetration and may then be attached. Adhesion and maturation of the basal plate may be aided at this point by secretions passing along the filament / axial duct and possibly by secretions derived from the D-organs.

7) It seems likely that full attachment of the filament must occur before the copepodid leaves the moulted exuvium since the probable soft nature of the new chalimus cuticle would make reattachment via other appendages unlikely prior to cuticular hardening.

Once the basal plate is firmly attached, the copepodid probably backs off causing eversion of the newly formed cuticle and drawing out both the filament stem and the anterior part of the cephalothorax and associated organs.

8) The newly formed external cuticle that previously lined the cavity surrounding the internal filament prevents the loss of haemolymph during this process and the organs formerly surrounding the stem / become the "stem collar" (C1 lobes) and associated tissue strands seen within the newly extended cephalothorax. The cuticle and filament subsequently harden completely, and the chalimus I, uses the maxillipeds and antennae to regain a stable grip on the host and resumes feeding.

It is clear that such a tentative scheme omits or glosses over a number of the key details of attachment. The first obvious omission is the allocation of a precise function to the "filament duct" and the "axial duct". The filament duct appears to be continuous with the axial duct in the copepodid and is morphologically similar. A "tube-like process" described as being connected between the "frontal organ" and the rostrum by Lewis (1963) for *L. dissimulatus* would similarly appear to identify with the presently described "filament duct". The exact function of the duct is, however, difficult to determine. Benkirane (1987) suggests that prior to filament attachment in lernaeopodids, a lytic agent may be released which helps produce a cavity into which the filament may be introduced. In the case of the frontal filament of *L. salmonis*, although evidence for lytic activity was not in itself observed within the host tissue, it may nevertheless be a possibility as the basal plate is often observed to spread along the interface between the host basement membrane and the overlying epidermis. Although such a phenomenon might be explainable in terms of introduction of material under pressure, this would require either a muscular or elastic mechanism which has not been seen in association with reservoirs of material in the present copepodid. Muscles postulated to be associated with the extrusion of the filament or filament material have, however been noted by Fasten (1919) for *S. edwardsii* and Heegaard (1947) for *C. curtus*. Alternatively pressure could occur through the rapid production or insertion of filament material, although this is not suggested to occur in the present model of *L. salmonis* attachment. Invoking the presence of a lytic factor which could spread along a line of weakness or over the surface of less easily lysed material would explain the ability of the basal plate to spread along the basement membrane after secretion. An alternative or possibly simultaneous function of a proposed secretion from the filament duct might be to act as an adhesive or to modify / overlay the host substrate to allow better attachment of the basal plate itself. These proposed functions are at present theoretical and need to be supported by experimental evidence.

The axial duct in both the copepodid stage and the chalimus stage runs between the two lobes thought to be responsible for the production of the stem and, indeed, through the centre of the newly-formed stem itself. It is also clearly visible in the finished frontal filament debouching at the point of bifurcation of the distal stem. Although material secreted along this duct might be responsible for the adhesion of the stem to the proto-basal plate, it would appear more likely that its function involves maturation of the basal plate itself after it has been implanted in the cavity. Secretion of material may occur just before exposure of the basal plate through moulting or alternatively once it is *in situ*. Many arthropods keep the chemical components required for quinone sclerotisation compartmentalised prior to use (e.g. cement glands of cypris larva of *S. balanoides* (Walker 1971) and cockroach colleterial glands (Hackman 1971)) and it may be that the A-gland produces one group of components that is added to facilitate sclerotisation of the basal plate on attachment. As has already been noted, the finished basal plate contains PAS positive material as does the axial duct itself and the minor collecting ducts within the A-gland. This would tend to support the latter hypothesis. Whether material secreted via the "filament" and "axial" ducts is identical or represents temporally discrete products of the A-gland could not be determined from the present study and awaits further experimental elucidation.

A second question is that of the function of the D-organs. These may perform some of the functions allocated above to products expressed from the axial / filament ducts although no evidence exists for their function at present. It is interesting, however to note that the product of these glands is strongly PAS-positive and that the basal plate material also becomes so following expression.

One uncertainty in *L. salmonis* is that of whether chalimus stages produce new filaments at each moult or remain attached by a single filament throughout the duration of the chalimus phase. Other authors have noted reattachment in a variety of caligids including *C. elongatus* (Wilson 1905), *C. curtus* (Heegaard 1947) and *L. dissimulatus* (Lewis 1963). Gurney (1934) on the other hand was of the opinion that *C. centrodonti* and *Caligus labracis* Scott retained the same filament throughout. White (1942a) has further observed that the filament of chalimus IV stages of *L. salmonis* is longer than those of previous stages but this remains to be confirmed. The present study found evidence for a fully formed stem and a large basal plate material reservoir and all the necessary filament-associated organs in the chalimus stage which agrees with the findings of Johnson & Albright (1992a) who similarly report fully formed filaments within the cephalothorax of the chalimus stage suggesting that this stage may produce a new filament at each moult or may add to the earlier filament. In the present study, it should, however be noted, that the presence of both stem and basal plate material was only observed in a single individual; other chalimus stages studied contained only the latter component.

In infection experiments chalimus stages have been found to be consistent in their positions throughout development with no apparent movement over the body surface and no signs of newly abandoned filaments (pers. obs., Shinn pers. comm.). Such observations do not however rule out the possibility of reattachment *in situ*. Hogans & Trudeau (1989) have similarly suggested that chalimus stages of *C. elongatus* do not change position after attachment. Abandoned filaments have, however been noted for *C. curtus* by Heegaard (1947) although there is clearly a problem in distinguishing filaments left by moulting and those remaining following the death of chalimus stages and degradation of less resistant parts of the cuticle to leave the attached filament. With respect to this latter, Kabata & Cousens (1977) believed that the high proportion of "abandoned filaments" present on host fins was indicative of mortality of chalimus stages of *S. californiensis*. Johnson & Albright (1992a) note that the filament of *L. salmonis* is apparently attached to and lost with the moulted cuticle whilst observations made during the present study suggests from exuviae that the cuticle may break around the filament leaving it in place. This latter observation is in agreement with the findings of Jones (1989) for the same species. The fact that *L. salmonis* filaments have not been seen with large quantities of attached epibiota (in contrast to those of *C. elongatus* pers. obs.) lends weight, however, to the suggestion of Johnson & Albright (1992a) that reattachment occurs with each moult although the shortness of the filament in *L. salmonis* might explain this observation as it is mostly embedded in the host epidermis and is probably subject to considerable abrasion etc.. The problem of how the filament could become attached to a new cuticle following separation from the old one also lends support to the hypothesis that a new filament may be produced at each moult. It is possible that the ability to produce a new filament might be facultative which might explain these disparate observations. If this were the case, filament production might then depend on a requirement to change attachment site according to local host tissue reaction or a need to relocate the frontal filament for better attachment. Such site-changes are suggested by Johnson & Albright (1992a) for copepodid stages of *L. salmonis* in the first 1 - 5 days following infection and have been reported by Anstensrud & Schram (1988) who posited migration of copepodids of *L. sprattae* following settlement on the host. Kabata & Cousens (1973) similarly describe exploration by *S. californiensis* prior to bulla implantation. Such a change of attachment would appear to be inherently precarious such that chalimus larvae might be expected to change only in extreme circumstances where the survival of the larva was threatened.

The present study indicates that chalimus larvae can produce a new filament during moulting and the balance of evidence presented above would appear to suggest that a filament change occurs with every moult. Although it is possible that filament renewal is facultative rather than obligatory, in order to accept this hypothesis, evidence needs to be presented that indicates

that reattachment of the same filament occurs or is feasible. Such evidence is best procured either by 1) marking of the attached filament or 2) *in vitro* observation of the moulting process.

Another problem with the presently proposed mechanism of filament production is the positioning of the C1 lobes with respect to the B-gland itself. Often, these were observed to be ventral to the B-gland which makes it difficult to understand how the filament stem becomes attached to the basal plate material. That it **does** become attached is clear from the morphological evidence but the mechanism is unclear and would appear to require the movement of one or other gland or their product. In chalimus the apparently aberrant positioning of the glands might be explainable in terms of the flexibility of the anterior end of the filament / cephalothorax causing misinterpretation of the orientation of sectioning but such a problem should not occur in the copepodid stage. It may well be that in production of the basal plate secretion, the material comprising the gland is continuously depleted causing a change in shape and orientation of the gland. Such depletion has been recorded by Benkirane (1987) for glandular tissue associated with production of the lernaeopodid bulla.

Two further important questions need to be answered concerning the frontal filament. The first of these is the question of the relationship of the present filament to the preadult filament reported for *L. salmonis* by Rae (1979), Wootten & Smith (1982) and Johnson & Albright (1991a) and for *L. pectoralis* by Anstensrud (1990). This question is fraught with difficulty, particularly since no direct observations have been made on the structure of the filament in preadults save for the observation that it is derived from or attached to the ventral filament organ. In the copepodid of *L. salmonis* it was observed in the present study that a ventral area exists that displays different staining properties with respect to the adjoining cytoplasm. This area gives the appearance of two semicircular lobes meeting at the copepodids midline - an appearance that mirrors that of the ventral filament organ seen in caligid preadults and adults. This area underlies or is in the same plane as the axial duct of the copepodid. No sign of this organ is apparent externally in the copepodid. In chalimus stages, particularly chalimus IVs, this area of tartrazine specific staining is very pronounced and associated with a bilateral organ around it (C2). The positioning and morphology of this structure is such as to suggest that it is, in fact, the ventral filament organ of the preadult stage. This is supported by the observations of Pike *et al.*(1993) who note that in chalimus stages of *L. salmonis*, the microvilli corresponding to those of the ventral filament organ of *C. elongatus* are apparent within the cuticle. In the chalimus stage of the present study, two ducts ("sumps") appear to carry the basal-plate secretion ventrally and into the area of the ventral filament organ. A speculative suggestion may be made from this; that the preadult filament is in fact a novel structure either composed of the basal plate secretion or a product produced by tissue surrounding the ventral filament organ (or a combination of the two) and output in the area of

the ventral filament organ. The filament observed by Anstensrud (1990) in *L. pectoralis* consisted of two "twisted strings" corresponding, perhaps, to the two lobes observed here, and was found to be loosely attached in post-moulting preadults. This situation contrasts to that observed for the chalimus frontal filament of *L. salmonis* which is strongly attached. Personal observations of intact attached preadults of *L. salmonis* suggest that the preadult filament of this species is also somewhat elastic - a property not associated with the chalimus filament.

A second question that needs to be discussed is that of the relationship of the frontal filament of *L. salmonis* to the frontal filament of *Caligus* spp. and to the filaments and other attachment structures of other Siphonostomatoids. In *Caligus elongatus* as reported by Pike *et al.* (1993), the point of attachment of the frontal filament in the chalimus is the ventral filament organ, thus its origins appear similar to those of the preadult filament in *Lepeophtheirus* species. The two ventral lobes seen in chalimus stages of *L. salmonis* apparently identify with the "median sucker" (ie ventral filament organ) illustrated by Wilson (1905) for *C. elongatus* and include the proposed "chitinous rod" suggested to be the remains of the filament in that paper but which in *L. salmonis* appears to identify with the axial duct in copepodids and a sulcus between the lobes in chalimus stages.

The structure often suggested to represent an internal frontal filament (or "cementary gland") and illustrated in drawings of copepodids of *inter alia C. elongatus* (*ibid.*), *C. spinosus* (Izawa 1969) and *C. curtus* (Heegaard 1947). would appear to identify with the laminated secretion comprising the copepodid proto-basal plate in the present study. All of these reports similarly show the transverse laminations characterising this structure although it is important to note that such features are **not** apparent in the "stem" of completed *Caligus* filaments. Pike *et al.* (1993) similarly show this structure to be present in copepodids of *C. elongatus*. What this structure **does** identify with in the *Caligus* chalimus filament is the enlarged initial portion that is associated with the ventral filament organ, since this portion demonstrates the laminations and probable helicoid structure of the "basal plate" secretion (as apparent from light microscopical examination and evident in the SEMs and TEMs of Pike *et al* (1993)). Hence, the *L. salmonis* copepodid / chalimus laminated secretion, the *Caligus* spp. copepodid "internal filament" / "cementary gland", and the *Caligus* spp. chalimus filament origin all have the same apparent structure and derivation.

A far more problematic question in regard to the filament of *Caligus* spp. is the origin of the "stem". In the work of Pike *et al.*(1993) there is no indication of any internal structure within the copepodid corresponding to the "stem" portion of the chalimus filament. Similarly no such structure appears to be illustrated by any of the authors including an "internal filament" / "cementary gland" in their copepodid descriptions. Pike *et al.*(1993) have not characterised this part of the filament but note that it is composed of a sheath of fine fibres oriented

perpendicularly to the long axis of the filament surrounding a core of fibre bundles running parallel to the long axis. The present study has demonstrated that this part of the filament in *C. elongatus* gives strong positive staining with catechol indicating the presence of o-diphenoloxidase. The structure at the origin of the filament gives an identical reaction. These reactions equate with those described for the basal plate of *L. salmonis* and seen in the anterior cephalothorax of the same species where it is presumed to represent the internal basal plate reservoir (laminated secretion). One hypothesis for the origin of this portion of the filament in *C. elongatus* is therefore that material equating to the "basal plate material" of *L. salmonis* copepodids might be channelled down to the area of the ventral filament organ (such channels of material are clearly seen in *L. salmonis* chalimus IVs) where it may be augmented by other products and output across the membrane of the organ's microvilli. Although Oldewage & Van As (1989) note that in the specimens they studied (probably adults) the ventral filament organ was covered in cuticle, this would not be present during moulting thus allowing for the proposed method of expression. Many authors have suggested from observations that the filament of caligids is drawn out from a blob of secretion. The present hypothesis also proposes that the *Caligus* filament might be produced in such a manner from the material expressed at the site of the ventral filament organ. Such drawing out of material might also explain the parallel fibres seen in the "stem" since Hackman (1984) notes that arthropod cuticular proteins may orientate with respect to shearing forces as noted earlier. The end-point of this procedure would be a long "stem" attached to the surface of the ventral filament organ. The attachment would be bifurcate as a result of the bilobed nature of the organ. This sequence of events agrees well with the descriptions of the proximal end of filaments of chalimus I stages of *C. centrodonti* and *C. labracis* (Gurney 1934) and *C. orientalis* (Hwa 1965). During intermoults, "basal plate" material would then pass into the area dorsal to the ventral filament organ and at each moult would be secreted as before save for the fact that it would not be drawn out but simply be allowed to remain as an accumulation of material. This then, would give a "stem" attached at its proximal end to a large lump of material ("base unit") which in turn would be attached to the ventral filament organ. This again corresponds to the appearance of chalimus II stages as described by the previous authors and seen in personal observations although it is difficult to explain the two circular ventral plates seen at the base of the filament ie from the present hypothesis one would expect two circular dorsal plates corresponding to the lobes of the ventral filament organ. Such an appearance might be explained if the base unit were formed / stored **above** the ventral filament organ which would then leave the imprint of the ventral filament organ's dorsal surface on the ventral surface of the base unit. If this were the case, the problem would become the explanation of how the new base unit becomes attached to the proximal end of the filament. With each successive moult, a further quantity of material would be attached to the proximal end of

the filament giving the characteristic pattern of one "base" corresponding to each moult, described by Gurney (1934), Hwa (1965) and apparently seen in *C. elongatus* (pers. obs.). These latter observations have, however, been contested by Heegaard (1947) who did not find a perfect correspondence of bases with moults to occur.

The theory proposed here would also explain two other features of the filament of *Caligus*, namely the laminated / helicoidal architecture of the fibres in the upper part of the filament described by Pike *et al.* (1993) and the possibility of reattachment of chalimus stages (Wilson 1905). The helicoidal architecture and lamellar appearance follows, as suggested before, from the formation of a cholesteric liquid crystal against a constraining layer as hypothesized by Neville (1988). We might expect a structure with these properties to form 1) in the initial area of secretion of the "basal plate" material as in *L. salmonis*, 2) in the ventral area above the ventral filament organ and 3) after passage across the microvilli of the ventral filament organ, in the area underlying said organ, with previously secreted filament material acting in this instance as the constraining layer. Laminated structures are observed in all these areas in the present study and in past descriptions.

The reattachment of *Caligus* spp. described in previous reports (Wilson 1905, Heegaard 1947) would be straightforward by this hypothesis, as replacement of a damaged or poorly attached frontal filament would simply entail material being drawn into a filament as for the initial attachment of the chalimus I, instead of being added to the old filament as a single large unit as proposed for later stages. Such a suggestion might also explain the discrepancy between the chalimus stage and the number of basal units counted, as was observed by Heegaard (1947).

The above hypotheses appear reasonably internally consistent, explaining many of the features observed in *L. salmonis* and *Lepeophtheirus* / *Caligus* spp. copepodid and chalimus stages in the present and previous studies. In addition it provides a working hypothesis for further examination of the manner of formation of filaments by preadults.

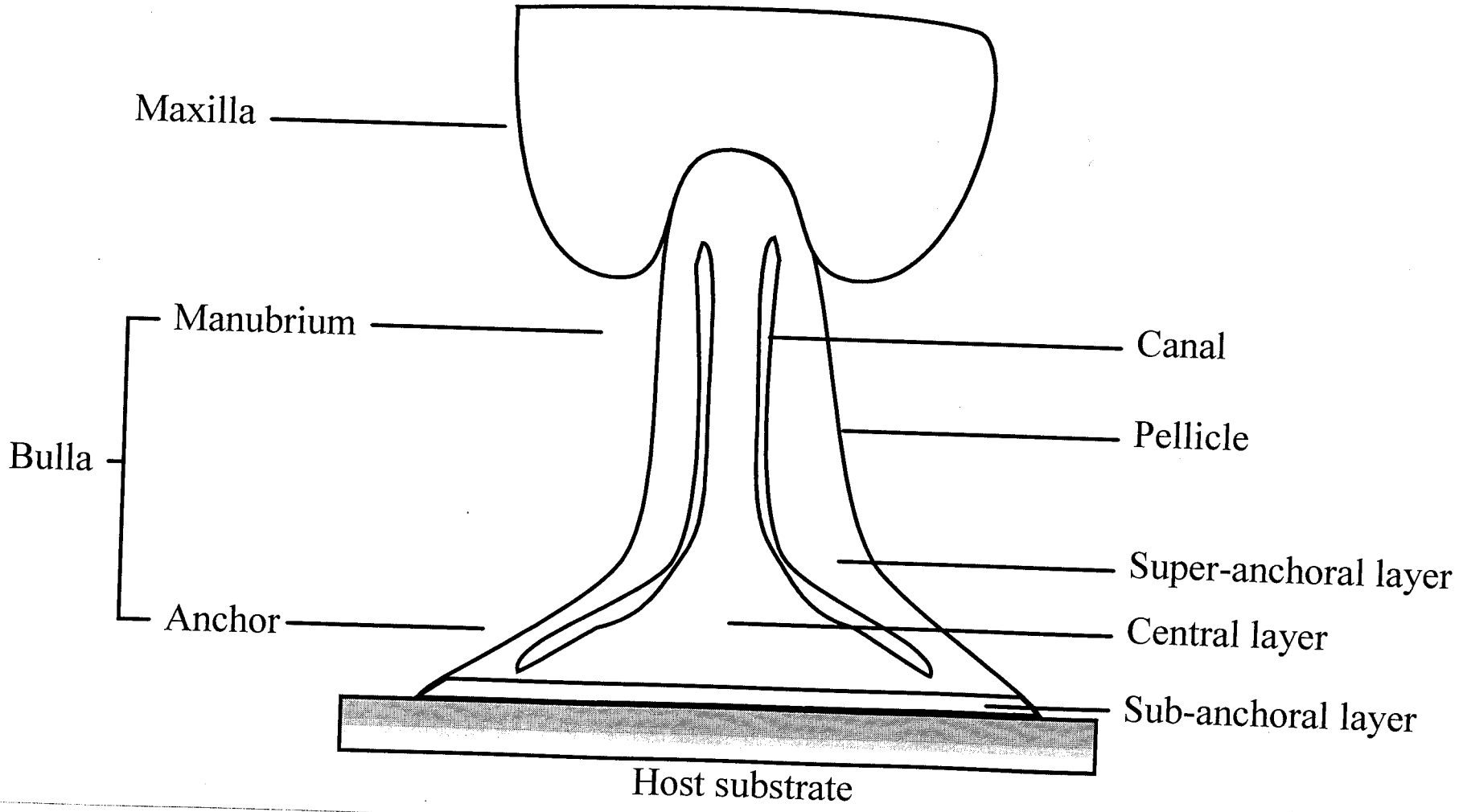
The assumption of a lack of preformed "stem" in the copepodid stage of *Caligus* spp. results from the observed failure to describe one in most reports. A flaw in the previous hypothesis is that it cannot explain the internal structure explicitly noted by Kabata (1972) in *Caligus clemensi* copepodids, which clearly resembles the *Caligus* "stem" rather than the "basal plate" reservoir. In Kabata's description the copepodid possesses a fully formed filament which is everted with a "frontal sac" before the moult to the chalimus I stage. How this is accomplished is not detailed but it is interesting to note that there is no augmentation of proximal filament material described with moults. This would seem to suggest that 1) its mode of filament production more closely resembles that of *L. salmonis* 2) This species does not correspond to many of the other species of *Caligus* so far described suggesting that mode of filament production may vary considerably even within the same genus.

A further question that arises from the present study is one of how, and possibly if, the frontal filament of *L. salmonis* relates to the attachment structures, ie frontal filaments and bullae, of lernaeopodids. Figure 4.8 shows a typical lernaeopodid bulla comprising the manubrium and anchor (the latter representing an expanded part of the former) and the external pellicle which covers both (figure adapted from Benkirane (1987) using the terminology of Kabata & Cousens (1972)). The whole structure is also equipped with canals or ducts. This structure then, would appear highly reminiscent of the frontal filament of *L. salmonis* save for the fact that the filament of the latter remains attached to the cephalothorax whilst the bulla of the lernaeopodids becomes attached to the maxillae.

The filament of *L. salmonis* resembles closely in structure the plaque-like or tegumentary bulla described by Benkirane (1987) and equivalent to the Type II bulla of Kabata & Cousens (1972) which is associated with marine teleosts (the Type III bulla being associated with elasmobranchs and holocephalans). The Type II anchor is normally characterised by being superficially inserted and small (unlike the large deeply buried Type I bullae usually associated with freshwater teleosts). Kabata & Cousens note, however, that Type I bullae attached to the scales of fish and unable to expand under a thick layer of host tissue do not act like the normal deeply buried variety but instead, like that of *Tracheliastes maculatus* Kollar, have a sub-anchoral surface possessing apparent adhesive properties. Type II & III anchors may also possess a hyaline adhesive layer underlying the anchor according to these authors.

Benkirane (1987) has described the histochemistry of the attachment filament and bulla of *A. macrotrachelus*. The bulla was found to stain negatively for polysaccharides (PAS) save for the external pellicle which stained positively. There was also a slight acid mucopolysaccharide content in the external pellicle. The bulla itself was found to be composed principally of dense bundles of protein fibres arranged in an unspecified matrix. The protein fibres were found to stain positively for sulphydryl (-SH) groups and were thought to represent protofilaments of scleroprotein (although this was not tested for specifically). In all these tests then, the bulla of *A. macrotrachelus* corresponds to the filament of *L. salmonis* except for the presence of acid mucopolysaccharides in the area of the pellicle. In *L. salmonis*, acid mucopolysaccharides were found to overlie the cuticular epithelium of the cephalothorax but did not lie at the interface between the stem fibres and the external lamina. The frontal filament of *A. macrotrachelus* was also reported to display the same staining properties as the bulla (*ibid.*). For the same species, Roubal (1989) described the frontal filament as consisting of a long thin cylinder with a narrow central canal and wide amorphous layer of eosinophilic material. Under TEM the filament was seen to consist of dense fibrils surrounding a very narrow central canal. This core was surrounded by a diffuse and amorphous mass of fine fibrils. The filament of

Figure 4.8 Schematic diagram of generalized lernaeopodid bulla (modified from Benkirane 1987).



L. salmonis would therefore appear to resemble both the bulla and frontal filament histochemically and structurally.

Kabata & Cousens (1973) report a frontal sac containing a coiled filament in older free-living copepodids of *S. edwardsii* and a preformed filament has also been seen within the cephalothorax of *A. macrotrachelus* by Benkirane (1987), *Achtheres amblophitis* Kellicot by Wilson (1911) and *Epibrachiella impudica* (Nordmann) by Gurney (1934). Filaments may occur even earlier in the lernaeopodidae, and Piasecki (1984) has reported a filament to be present in the pigmented eggs and the newly-hatched free-swimming nauplius of *T. maculatus* (although this nauplius was reported to moult to the copepodid stage within 30 minutes of hatching). In intact copepodids, these filaments would appear to resemble those described by Kabata (1972) for *C. clemensi* but not those of *L. salmonis* in the present study. Ultrastructural studies by Benkirane (1987) suggest the filament of *A. macrotrachelus* to be principally homogeneous with a central duct or core. The latter feature resembles the axial duct of the stem of *L. salmonis* but the lack of a fibrous stem is at odds with the structure of the finished filament of *L. salmonis* demonstrated in the present study. Benkirane does not note any invagination of the cuticle associated with the filament in the copepodid but reports the frontal filament to evaginate as did Piasecki. In both cases, the filament was reported to evaginate following the exit of a large "plug" from the cuticle overlying it. Benkirane notes a terminal structure underlying the plug (ie possibly analogous to the basal plate) whilst Piasecki suggests the filament to be directly attached to the plug. The latter author envisaged extrusion of the filament as a hydraulic process whereas Benkirane believed it to result from osmotic uptake by the structure underlying the plug. Neither hypothesis has been tested. Whilst Kabata & Cousens (1973) suggest the filament of *S. californiensis* to be attached to the host by a cement produced by an undetermined frontal gland, Piasecki ascribes adhesive properties to a "gelatinous substance" surrounding the filament and Benkirane believes the filament to be implanted through lytic action rather than by adhesion. The filament of *L. salmonis* is clearly strongly adhered to the host substrate (despite the suggestion by Pike *et al.* (1993) that its adherence is tenuous) and must therefore be adhered through its own composition or via some manner of cement. This adhesive may, as already suggested, derive from the filament duct or axial duct or may simply be function of the sclerotisation and general maturation of the basal plate itself.

The bulla of lernaeopodids resembles the filament of *L. salmonis* in that it is fully formed in the cephalothorax, although it is found initially in the chalimus IV rather than in the copepodid. Kabata & Cousens (1973) further note that in *S. californiensis* the bulla occupies exactly the same position as the preceding frontal filament within the cephalothorax. Like the filament of *L. salmonis*, the bulla is also fibrous and, by the end of production similarly sits in a large cavity created by the exhaustion of glandular tissue in its production (Benkirane 1987).

Another important similarity is that this cavity becomes lined with cuticle prior to evagination of the bulla and the bulla itself is coated with a pellicle that strongly resembles the external lamina of *L. salmonis*. The bulla abuts the external cuticle of the cephalothorax as does the basal plate of *L. salmonis* and is presumed to be expelled in this region (although this was not observed). Unlike *L. salmonis* the bulla is transferred to the maxillae before implantation so that it is difficult to compare subsequent events save to note that the bulla, like the frontal filament of caligids, appears to go through a maturation / hardening process following implantation. Implantation in many species studied by Benkirane (1987) and Kabata & Cousens (1972) is suggested to involve an adhesive since, as with the filament of *L. salmonis*, great difficulty was often experienced in separating the bulla from the underlying host tissue.

Two broad theories for the evolution of parasitism in copepods have been proposed by Kabata (1979). The first of these involves free-swimming predatory copepods attacking larger organisms and tearing off pieces. It was suggested that gradual lengthening of this period of temporary association would lead to the development of parasitism. The siphonostomatoids *Pontoeciella* Giesbrecht and *Hyalopontius* have been suggested to attach temporarily to planktonic hosts to feed (Huys & Boxshall 1991) and might therefore be suggested to display the behaviour of the intermediate stages proposed. This theory for the development of parasitism was proposed earlier by Fryer (1957). The alternative theory proposed by Kabata (1979) is that parasitism may have developed in benthic scavengers / browsers exposed to other benthic organisms and eventually becoming associated with them. Benz & Deets (1993) have proposed that siphonostomes parasitizing vertebrates are derived from associates of invertebrates.

The origins of the frontal filament in terms of free-swimming and semi-parasitic copepods, are difficult to determine. It is considered here, that the development of a filament may have occurred initially in parasites of vertebrates, since only in the Nicothoidae are frontal filaments used to attach larvae to invertebrate hosts. Certainly, the need for such an attachment mechanism might be considered most important for larval stages attaching to rapidly moving hosts, although this, in itself, does not preclude invertebrate associates.

We have seen from the present study, that the frontal filament has two principal origins in the copepodid. The main part of the filament, represented by the stem and external lamina, are cuticular in origin and represent an extension of the cuticle of the cephalic shield. The other major component is the basal plate which attaches the cuticular extension to the substrate and is considered to originate as an internally produced secretion. From these observations, two possible scenarios present themselves for the origin of filament attachment.

The first of these considers the cuticular extension to antedate the basal secretion. If, in order to get better purchase or escape flow over the surface of the host, ancestral associates

either burrowed the anterior edge of the cephalic shield into the host surface as seen in the present study of copepodids of *L. salmonis*, or placed it under scales, as demonstrated by Jonsdottir, Bron, Wootten & Turnbull (1992) for *L. salmonis* adults / preadults, then one could envisage a situation whereby modification of this edge might improve attachment. Such a structure is reminiscent of that of lernaeids such as *L. cyprinacea*, fertilised females of which species have been reported to penetrate the host epidermis and become attached via two pairs of processes (one dorsal and one ventral) apparently originating as extensions of the cephalothorax (Grabda 1963). The later development of an adhesive secretion would subsequently serve to improve attachment to the host substrate.

The alternative scenario considers the adhesive secretion to antedate the cuticular extension. By this hypothesis, improved host attachment would initially involve the production of an adhesive secretion. The origin of such an adhesive cannot be demonstrated at the present time, although it is clear that free-living copepods have numerous secretory organs present in the anterior cephalon, that might easily have become modified to produce an adhesive secretion (e.g. see Boxshall 1992). The harpacticoid *Diarthrodes nobilis* (Baird) described by Hicks & Grahame (1979), has paired frontal and lateral reservoirs of secretion which are suggested to contain mucin. This material is expressed via vents in the dorsal cephalothorax and elsewhere, to produce an envelope of mucus. The facility for mucus production in this species is present from the second nauplius onwards. Fahrenbach (1962) makes similar observations for *Diarthrodes cystoecus* Fahrenbach and notes its ability to form a mucus envelope from the 2nd / 3rd copepodid onwards[11]. Following the development of an adhesive system, it is then envisaged that the cuticle of the cephalon might become modified in order to distance the copepod from the point of attachment and thereby allow deeper (and more secure) attachment and greater mobility for feeding.

The observations made in the present study, suggesting that preadult caligids and chalimus larvae of *C. elongatus* are attached by a filament more closely related to the secreted basal plate than to the cuticular stem and external lamina, may favour the second argument, although this suggestion is highly tenuous. If the hypothesised mechanism of production of these latter filaments proves accurate, it may also be important to note, in considering the differences between *Lepeophtheirus* and *Caligus*, that the latter is generally considered to be the more mobile genus.

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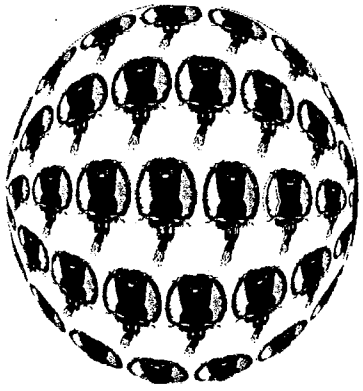
[11]The exact function of these glands in harpacticoids is uncertain at present although Fahrenbach 962) considered that the envelope afforded protection for newly hatched nauplii. An alternative, ·oposed by Hicks & Grahame (1979), suggests that such an envelope might function as a "mucus-ap" allowing aggregation of bacteria and organic particles which might be ingested for food.

Modification of a frontal filament to give a larger more robust attachment is believed to have produced the bulla of the lernaeopodidae which may have avoided problems due to shallow attachment by the attachment of the bulla to the maxillae. The apparently close relationship of the frontal filaments and bullae of the Caligidae and Lernaeopodidae, suggest that much of the proto-filament's structure was fixed before the two development lines diverged.

CHAPTER 5
LARVAL NUTRITION



5 - LARVAL NUTRITION

5.1 Introduction

Following initial settlement of the copepodid as described in the previous chapter, there is a period of several days before the moult into the subsequent chalimus stage (present study, Johnson & Albright 1991a). During this period the copepodid is believed to feed (Jones *et al.* 1990, Johnson & Albright 1992a)) although no studies have been carried out to date to investigate larval nutrition or the functional morphology of the copepodid alimentary canal.

The basic structure of the alimentary canal of both free-living and parasitic copepods is relatively well described and brief overviews have been given by Blades-Eckelbarger (1984) and Boxshall (1992). The structure of the copepod alimentary canal is generally reasonably consistent and has been reviewed in part by Sullivan & Bisalputra (1980). The alimentary canal of copepods normally consists of a cuticle-lined foregut / oesophagus of ectodermal origin, an endodermal midgut which lacks cuticle and a cuticle-lined ectodermal hindgut.

Arnaud, Brunet & Mazza (1978) grouped the four major groups of epithelial cells found in the midgut of the free-living copepod *Centropages typicus* Krøyer under the following four categories which correspond to cell types previously described in the hepatopancreas of decapods. These correspond closely to the cell types described for other free-living species and equate to cell types 1-4 of Sullivan & Bisalputra (1980). These cell types are summarized by Boxshall (1992):

1. Stem Cells (E Cells)

   Undifferentiated epithelial cells replacing senescent cells or those lost in secretion. Often have no contact with lumen.

2. Synthetic Cells (F and D Cells)

   Cubic or columnar microvillous-bordered epithelial cells probably synthesising enzymes (exocytotic and holocrine or merocrine).

3. Vacuolar Cells (B Cells)

Microvillous-bordered cell with quantities of vacuoles and vesicles of various sizes. Absorptive cells showing intense pinocytosis and possibly producing some enzymes for intracellular digestion (acid phosphatase and aryl sulphate activity seen in vacuoles by Arnaud *et al.* (1984a 1984b). These cells display a characteristic sequence of development with 5 recognisable stages culminating in expulsion of cell contents for reabsorption by R cells (Arnaud *et al.* (1978, 1980).

4. Absorptive Cells (R Cells)

Microvillous-bordered cells with long microvilli and numerous small electron-dense apical vesicles. May contain lipid droplets, glycogen particles, and granules of calcium and other elements (Al-Mohanna & Nott 1986).

In free-living copepods the midgut, which is believed to be the principal zone of digestion and absorption in the alimentary canal, has been generally subdivided according to cellular composition, into three zones. In *C. typicus* these zones were suggested by Arnaud *et al.*(1978) to comprise:

Zone I (often including the anterior midgut caecum)

Cells of varying height 6-20 μm. Well-developed microvillous-border. Mainly synthetic and absorptive cells with some stem cells.

Zone II (the anterior midgut)

Principally large vacuolar cells 25-40 μm height. Less developed microvillous-border than Zone I.

Zone III (the posterior midgut)

Regular epithelium of flattened absorptive cells with a well-developed microvillous-border.

This arrangement seemingly applies to other studies including *inter alia* Yoshikoshi (1975) for *Tigriopus japonicus* Mori, Sullivan & Bisalputra (1980) for *Tigriopus californicus* (Baker), Defaye *et al* (1985) for *M. albidus* and Boxshall (1982, 1985) for *Benthomisophria palliata* Sars and *Euaugaptilus placitus* (Scott) respectively.

Arnaud *et al.*(1978) have proposed a sequence of events concerning digestion and assimilation of host material in the copepod *C. typicus* and involving the above cell types. This scheme appears to be generally supported by subsequent work in other species. In this scheme it was proposed that the F cells produced enzyme secretions which were probably secreted into the lumen via a process of macroapocrine and / or microapocrine secretion at the apical surface to act in extracellular digestion of host products. The B cells were suggested to show a continuum of development whereby semi-digested material from the midgut lumen was initially taken into the cell by a process of intense pinocytosis at the apical membrane and then deposited in large vacuoles through the coalescence of pinocytosed vesicles. Into these vacuoles, enzymes would also be deposited, possibly by a process of direct input of the contents of golgi vesicles at the vacuolar membrane. Within these vacuoles, intracellular digestion of materials would then occur, with the endpoint of the process being the extrusion of most of the cell contents into the midgut lumen. The products of intra- and extracellular digestion were then proposed to be absorbed by the R cells which, though present throughout the midgut, were suggested to comprise the entire population of Zone III. The E cells were suggested to be stem cells originating in Zones I & II and replacing those lost through senescence / normal function. It has been further suggested by Al-Mohanna, Nott & Lane (1985) that loss of B cells in the decapod *Penaeus semisulcatus* De Haas coincides with a burst of mitotic activity in the E cells of the hepatopancreas.

The above series of events suggests that the structure of the copepod alimentary canal may vary over time due to the turnover of different cell populations. This was confirmed by Nott *et al.* (1985) who described cyclical changes in the digestive epithelium of *Calanus helgolandicus* (Brodsky) during feeding and non-feeding periods. These changes were relatively rapid, such that obvious changes in the cell populations could occur within a period of a few hours. When not feeding, the cells of the anterior diverticulum became increasingly flattened with long, medium dense, microvilli. The vacuolar B cells in the zone posterior to the diverticulum were observed to become progressively less extensive without food but conversely became more abundant in the presence of food giving encroachment of the vacuolar zone into the anterior diverticulum with many vacuolar B cells extruding their contents into the lumen. Posterior to the vacuolar zone was squamous cuboidal epithelium which showed disintegration (giving necrotic or "N" cells) with feeding and which was thereby suggested to contribute to the production of a peritrophic membrane around the lumen contents. It was suggested that exhaustion of B and N

cells would eventually limit the period of feeding possible, with a refractory period of some kind required for reconstruction of the digestive epithelium.

Despite the extensive work carried out to systematize descriptions of cells and midgut areas in free-living copepods, this trend has been sadly absent from descriptions of the alimentary canals of parasitic copepods, and few authors have attempted to categorize cell populations according to the criteria presently used for free-living species. The few studies undertaken to date on parasitic copepods do, however, indicate that they conform, on the whole, to the same general plan as free-living species, with specializations principally concerning the structure of the mouthparts and the provision of food storage areas within the gut. Most studies carried out to date have concerned the alimentary canals of adult stages only, such that the function and morphology of the alimentary canal of larval stages has been largely neglected. An exception to this general statement is the work of Capart (1948) who noted the stomach (midgut Zone I/II ?) of larval *L. branchialis* to comprise club shaped and microvillous-bordered cells and suggested the latter (probably vacuolar cells) to derive from the former. The intestine (midgut Zone III ?) was noted to have no club-shaped cells but to comprise solely microvillous-bordered cuboid epithelial cells. A similar structure was proposed for the adult females of *Pseudocharopinus dentatus* (Wilson), a parasite of the skate *Raja binoculata* Girard by Rigby & Tunnell (1971). In the adults of *L. branchialis*, the stomach and intestine were reported to be less well demarcated, having an initial zone of cuboid cells followed by an epithelium comprising principally club-shaped cells (Capart 1948). Briggs (1977) found the midgut of *Parenthessius anemoniae* to be undifferentiated into zones and to comprise two cell types corresponding to the vacuolar cells and the secretory (or absorptive) cells of free-living copepods. The hindgut of this species was peculiarly observed to be devoid of cuticle. *P. anemoniae* was also noted to have a spacious foregut which presumably acted for storage, whilst *H. typicus* (a deep sea micropredator / parasite) and *Ergasilus sieboldi* Nordmann were noted to have storage facilities in the form of midgut diverticuli (Boxshall 1990 and Einszporn 1965 respectively). The latter species was observed to have two cell types present in the midgut with a number of intermediate forms. The first type were club-like (probably vacuolar cells) and the second type also had vacuoles but became more cuboid in the posterior midgut. The midgut epithelium of sapphirinids, which are predatory copepods, was similarly suggested to be composed of two cell types, one secretory and one absorptive (possibly vacuolar and secretory / absorptive respectively) (Marino & Onesto 1970). Although many parasitic species would appear to demonstrate few alimentary concessions to the parasitic mode of existence, exhibiting as they do, alimentary canals which are superficially identical to those of free-living species, exceptions would seem to be provided by *Melinacheres (= Saccopsis) steenstrupi* (Bresciani) described by Bresciani & Lützen (1962),

Gonophysema gullmarensis Bresciani & Lützen described by Bresciani & Lützen (1960) and *Parachordeumium* (= *Amphiurophilus*) *amphiurae* (Herouard) described by Goudey-Perrière (1979). The first of these species was reported as having a short chitin-lined tube leading from the terebellid host into a large, muscular "stomach" with multiple chitin-lined diverticuli. This latter was suggested to be an extension of the foregut. From this, a narrow passage led into a second intestinal section lined with an epithelium of rounded cells and with four long diverticuli deriving from it. This species (unlike the related *Melinacheres ergasiloides* Sars (see Bresciani & Lützen 1975)) has no apparent rectum, a feature shared with the dwarf parasitic males of the chondracanthid copepod *Lernentoma asellina* (L.) a parasite of Triglidae (Rousset & Raibaut 1982). The second species, a parasite of ascidians, was observed to have no apparent alimentary apparatus, a situation echoed by the third species, a parasite of ophiuroids. These latter species clearly indicate that the presence of a fully formed alimentary canal is not a prerequisite for nutrition.

One of the few studies that has attempted to assign the cell population of the alimentary canal of a parasitic copepod to the cell groups recognised in free-living species, is that of Gresty (1992) on *Mytilicola intestinalis* Steuer, a parasite of mussels. In this study the midgut was found to consist of a mixture of absorptive (R), synthetic (F) and vacuolar (B) cells. The relative proportions of each group were suggested to vary to some degree and from this it was suggested that the midgut epithelium might be experiencing cyclical changes as observed in free-living species.

The alimentary canal of the caligid *Pseudocycnus armatus* Basset-Smith which was described by John & Nair (1975) differed in some respects from the morphologies described above. The foregut of this species was found to have no cuticular lining in the zone approaching the midgut and the epithelium of all parts of the midgut (stomach and intestine) was found to comprise a syncytium with no apparent microvillous-border (although it would seem in view of the other studies described here that this may be artefactual). The epithelial syncytium of the stomach and mid-dorsal intestine was found to produce globular processes in the presence of food, which broke off into the lumen, ruptured and were thought to release digestive enzymes. In the lateral and ventral walls a second "stumpy" type of process was observed, filled with a lightly staining homogeneous substance, thought to be absorbed material. The exact affinities of these processes to the cell types noted previously are difficult to determine, although the former processes may be allied to the vacuolar cells and the latter to the absorptive cells.

Caligids belonging to the genera *Caligus* and *Lepeophtheirus* have been previously studied by a number of authors although often in limited detail. Scott (1901) and Wilson (1905) describe *L. pectoralis* as having a cuticle lined oesophagus emptying into the midgut posterior to an anterior midgut caecum much as described for free-living copepods. The midgut was also,

in common with those of other studies, divided into a stomach and intestine. Scott also suggested the whole alimentary canal to be lined with cuticle, although in the light of the studies given above, this would seem unlikely and probably represents a misidentification of the epithelial microvillous-border in the midgut. Aside from normal epithelial cells, both authors noted apparently glandular cells which probably correspond to vacuolar cells. These were located in the intestine and posterior portion of the stomach. No valve was found between the stomach and intestine, although Wilson found indications of a non-closeable sphincter. More recently, a description of the morphology of the alimentary canal of this species has been provided by Boxshall (1986, 1990), which indicates the midgut to comprise a narrow tube with a small anterior midgut caecum suggested to be unsuitable for storage of large quantities of food material. Grossly then, the alimentary canal of this relatively untransformed species appears very similar to that of its free-living counterparts.

Lewis (1961) cited by Sullivan & Bisalputra (1980) found two types of cell to be present in the alimentary canal of *L. dissimulatus* which were termed A and B cells and were suggested by the latter authors to correspond to absorptive and synthetic cells respectively. An EM study of the alimentary canal of *C. minimus* by Poquet (1980) revealed three types of cell - A cells having a pronounced microvillous-border and pinocytotic vesicles, B cells having large numbers of vesicles and a short microvillous-border and C cells which were intermediate. A and B cells were found in the same proportions at the start and middle of the intestine (midgut) but type B disappeared at the end of the intestine to be replaced by types A and C. Type C had abundant concretions with a calcareous appearance, a feature that has also been noted for the R cells of decapod crustaceans (Al-Mohanna & Nott 1987). B cells probably correspond to the synthetic cells of other studies and C cells may correspond to R cells. The identity of the A cells is uncertain.

The morphology and ultrastructure of the alimentary canal of adult *L. salmonis* has recently been described by Nylund *et al.* (1992). As with *L. pectoralis*, the alimentary canal was found to consist of a short tubular foregut, a large midgut and a short tubular hindgut and was therefore similar in aspect to that of free-swimming copepods. The midgut was not, however, found to be differentiated into zones with respect to the frequency of different cell types although three types of cell were recognised. Cell types I and II were microvillous epithelial cells and it was suggested from the presence of intermediate cell types that the former developed into the latter. The affiliation of these cells to those of previous studies could not be positively determined by the authors, although they were suggested to be similar to R and F (absorptive and synthetic cells) of free-living species. The type III cells were thought to correspond to the vacuolar (B-cells) previously noted in other species.

In addition to the secretory cells of the alimentary canal, most copepods possess "labral glands" which secrete a variety of products along ducts which normally discharge to the posterior surface of the labrum (Boxshall 1992). These glands are well-recognised in free-living copepods e.g. *inter alia*, *C. finmarchicus* (Lowe 1935), *E. amphitrites* (Park 1966), *T. japonicus* (Yoshikoshi 1975), *Porcellidium fimbriatum* Claus and *P. viride* (Phillipi) (Gharagozlou-van ginneken 1977), *B. palliata* (Boxshall 1982) and *C. typicus* (Arnaud, Brunet & Mazza 1988) but have rarely been mentioned in descriptions of parasitic species save for the observation of glands in approximately the same position by Scott (1901) and Wilson (1905) for *L. pectoralis* and by the latter author for *C. bonito*. Yoshikoshi & Kô (1991) have also noted "labral glands" to be present in a variety of parasitic copepod species studied. In some other species, a lack of glands associated with the alimentary canal has been explicitly noted e.g. *E. sieboldi* (Einzsporn 1964) and *L. branchialis* (Capart 1948).

Other, less well recognised glands, have also been suggested to be associated with the alimentary canal of parasitic species. Scott (1901) and Wilson (1905) noted the presence of a further glandular organ in *L. pectoralis* lying dorsally, approximately at the junction of "stomach" and "intestine" (anterior and posterior midgut). These glands were also described by the latter author to be present in a wide range of other *Lepeophtheirus* and *Caligus* spp studied. A further gland observed by John & Nair (1975) was located close to the proximal end of the mouth tube to either side of the oesophagus and had ducts opening at the angle between the base of the mouth tube and the second maxilla in *Pseudocycnus armatus*. This gland was proposed by these authors to derive from the maxillary gland which is normally associated with excretion in free-living species (see Boxshall 1992).

The feeding of the copepodid stage of *L. salmonis* and the morphology of the alimentary canal have not previously been studied in detail. The free-swimming phase is, however, believed to be lecithotrophic as both first and second nauplius stages have been reported to display what appear to be yolk reserves (Kunz 1985). Settled copepodid and chalimus stages are believed to feed on host surface-epithelium and mucus (Jones *et al.* 1990) whilst adult *L. salmonis* are believed to feed on mucus and epithelium and / or blood (White 1940a, Håstein and Bergsjö 1976, Brandal *et al.*1976). The feeding arena of both chalimus and copepodid stages is restricted to a limited area around the mouth tube since both stages, as demonstrated in Chapter 4, are relatively well-attached to the host. Feeding damage in these stages was described by Jones et al. (1990) and was suggested to result from the action of the toothed strigil as described by Kabata for caligids in general (1974). The strigil was, however, stated to be absent in the copepodid stage of *L. salmonis* by Johnson & Albright (1991a). Damage was found to be principally confined to the epidermis due to the small size and limited penetration of the larval stages. The chalimus stage was slightly more mobile due to the flexibility of its attaching frontal

filament and produced a 360° area of grazing damage around the base of the filament (Jones *et al.* 1990) whilst the copepodid feeding damage was limited to the area directly beneath the oral cone.

5.2 Study aims

Following the description of general behaviour and of settlement / attachment in the previous two chapters, the work in the present chapter sought to describe the interactions characterizing the parasite-host complex. The major part of this work comprised a description of the normal morphology of the alimentary canal and associated organs of the copepodid of *L. salmonis* with a view to determining their function in larval nutrition and thereby define a crucial aspect of the parasite-host relationship. As in the last chapter, such an endeavour could not be successfully carried out by studying the copepodid in isolation, and therefore the structure of the alimentary canal of the chalimus stage was also described.

5.3 Materials and methods

5.3.1 Sources of material

Free-swimming copepodids were reared from eggs as detailed in Chapter 2 and settled copepodid and chalimus stages were obtained from naturally or artificially infected fish.

5.3.2 Light Microscopy

Material was fixed and embedded in wax or historesin as detailed in Chapter 2. Embedded material was cut using glass or metal knives (for historesin or wax) to give serial sections of material in a number of planes. Sections were stained with a variety of histochemical stains (as detailed in Chapter 2) in order to visualize the gut and characterize its secretions and the nature of the material within it.

Live copepodids were also studied, as were specimens cleared and mounted in Berlese fluid or lactophenol, with these being observed under the light microscope.

5.3.3 Electron Microscopy

Material for use with the scanning electron microscope was prepared and observed as described in Chapter 2. Material for TEM was similarly prepared and observed according to the techniques outlined in Chapter 2.

5.3.4 3D Reconstruction

Three dimensional reconstruction of the alimentary canal was carried out using the slide visualization technique and computer 3-D reconstruction package described in Chapter 2. For both reconstructions, every sixth section (each section being 2.5 μm thick) was taken.

5.4 Results

5.4.1 Alimentary Canal

The alimentary canal was clearly visible in live copepodid and chalimus stages. The principal difference between the alimentary canal of the copepodid stage and that of subsequent chalimus stages observed under the light microscope was the size of the alimentary canal and the presence of refractive globules which appeared to be contained within the midgut of the copepodid stage (Plate 5.1). Both stages displayed characteristic peristaltic movements of the midgut and hindgut wall. In the vicinity of the anus, the regular movement of the hindgut caused an apparent two-way movement of fluid (indicated by the movement of small bubbles within the gut). A diagram of the general plan of the copepodid alimentary canal is depicted in Figure 5.1. The shape and structure of the alimentary canal was found to be similar for both copepodid and chalimus stages and comprised four distinct regions: oral cone, foregut (oesophagus), midgut and hindgut these corresponding to components of the same name in previous studies.

The alimentary canal of the copepodid was seen to be very variable in appearance, this being associated with age of the free-living copepodid and possibly with different stages of the feeding / developmental cycle in attached stages.

5.4.1.1 Oral cone

The oral cone was found to conform generally to the pattern normally described for other caligids (Kabata 1974a). The copepodid possessed a highly muscular anterior labrum and a posterior labium (Plate 5.2). No toothed strigil was observed in the copepodid stage under SEM, and its position was apparently occupied by a "ploughshare" or "wishbone" - shaped bar ("labial bar")(Plate 5.3). In sections observed under the light microscope, a structure apparently identifying with the strigil was, however, seen anterior to the bar in the copepodid stage (Plate 5.4). The chalimus stages were equipped with the fully exposed, toothed strigils, normally associated with adult / preadult caligids. Toothed mandibles projected into the buccal cavity through a groove lying at the overlap of the labium and labrum. The larval cone, unlike that of adult *L. salmonis* was not found to be entirely sealed by the overlap of the labium and

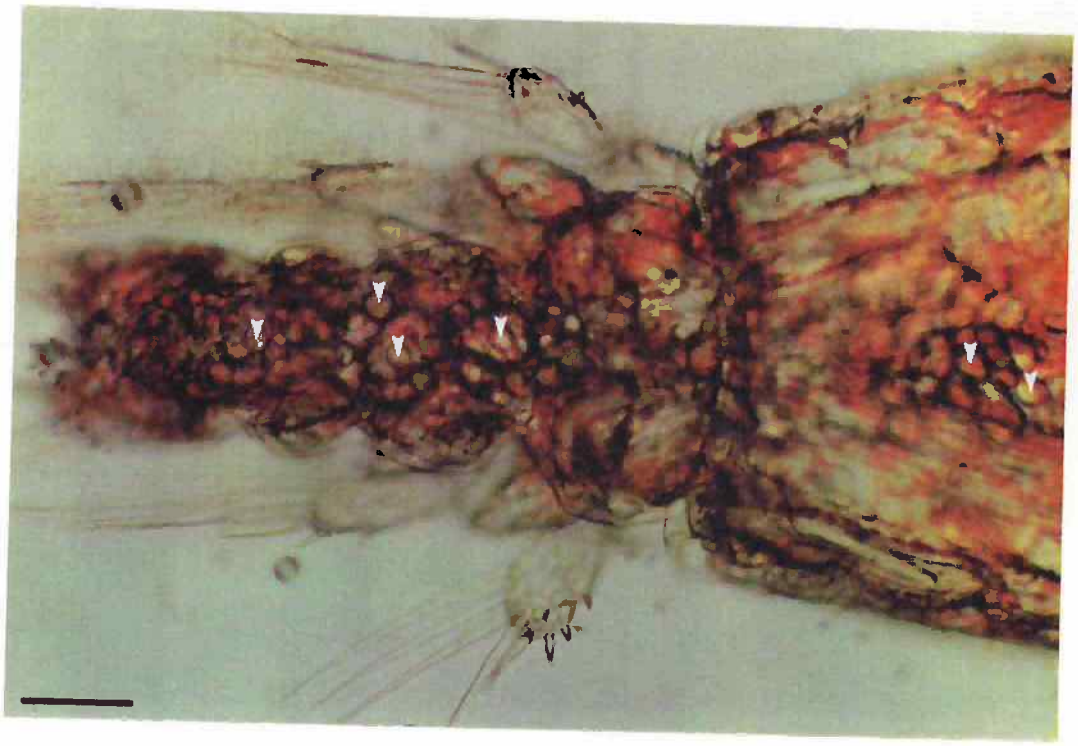


Plate 5.1 Refractive "globules" (arrowed) in copepodid midgut (whole mount, dorsal, unstained). Scale = 50μm

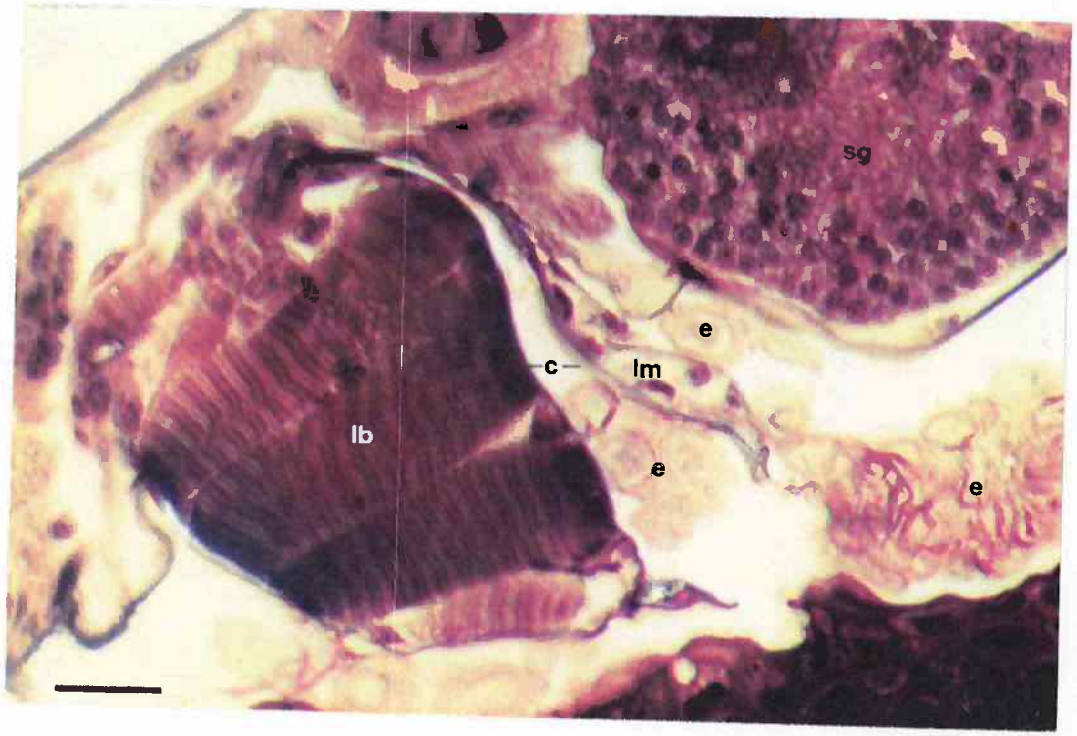


Plate 5.2 Oral cone of copepodid showing muscular labrum, labium and damage to host epidermis at leading edge of labrum (LMH, sagittal, stained polychrome). c: cuticle lining buccal cavity; e: host epidermis; lb: labrum; lm: labium; sg: sub-oesophageal ganglion. Scale = 20μm

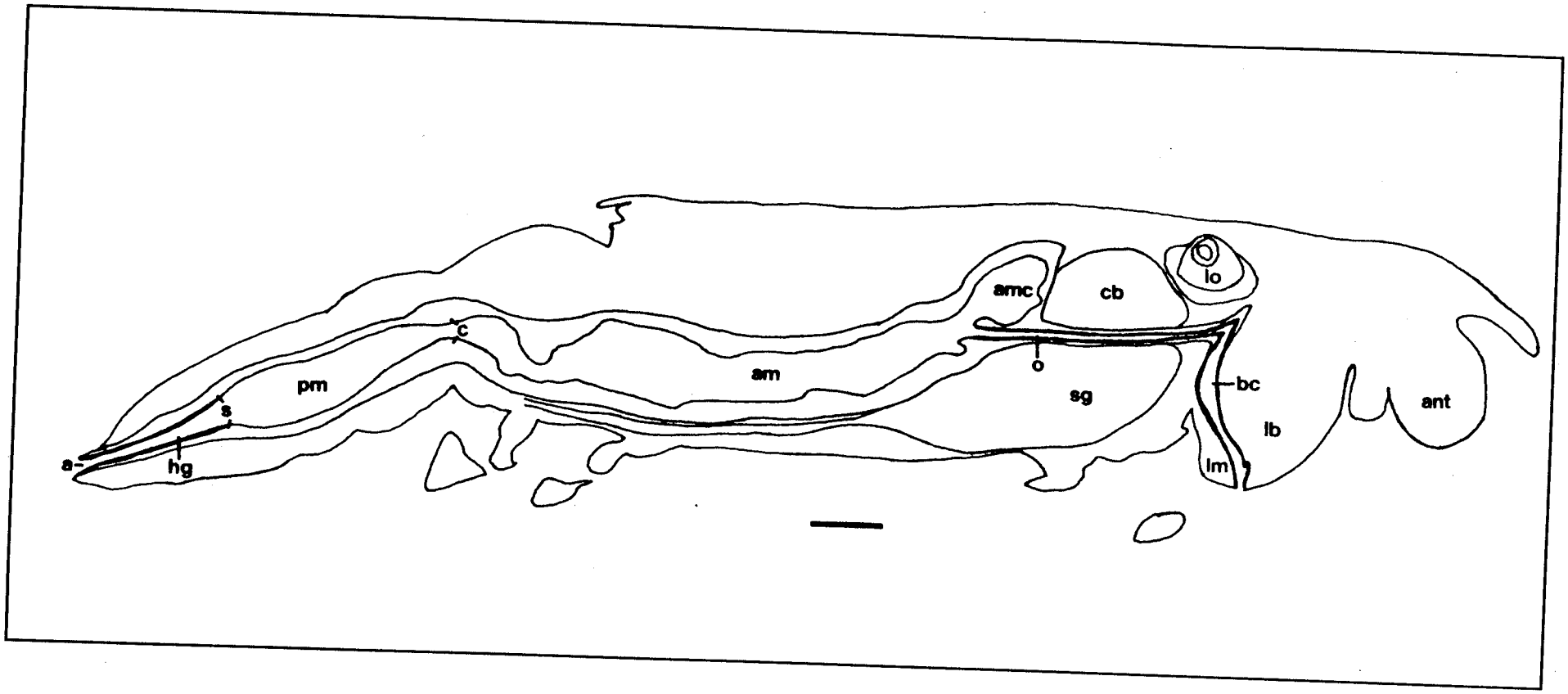


Figure 5.1 Diagram illustrating the general morphology of the copepodid alimentary canal. a: anus; am: anterior midgut; amc: anterior midgut caecum; ant: antenna; bc: buccal cavity; c: constriction or sphincter between anterior and posterior midgut; cb: cerebrum; hg: cuticle-lined hindgut; lb: labrum; lm: labium; lo: lateral ocellus of nauplius eye; o: cuticle-lined oesophagus (foregut); pm: posterior midgut; s: sphincter between posterior midgut and hindgut; sg: sub-oesophageal ganglion. Scale = 40 μm

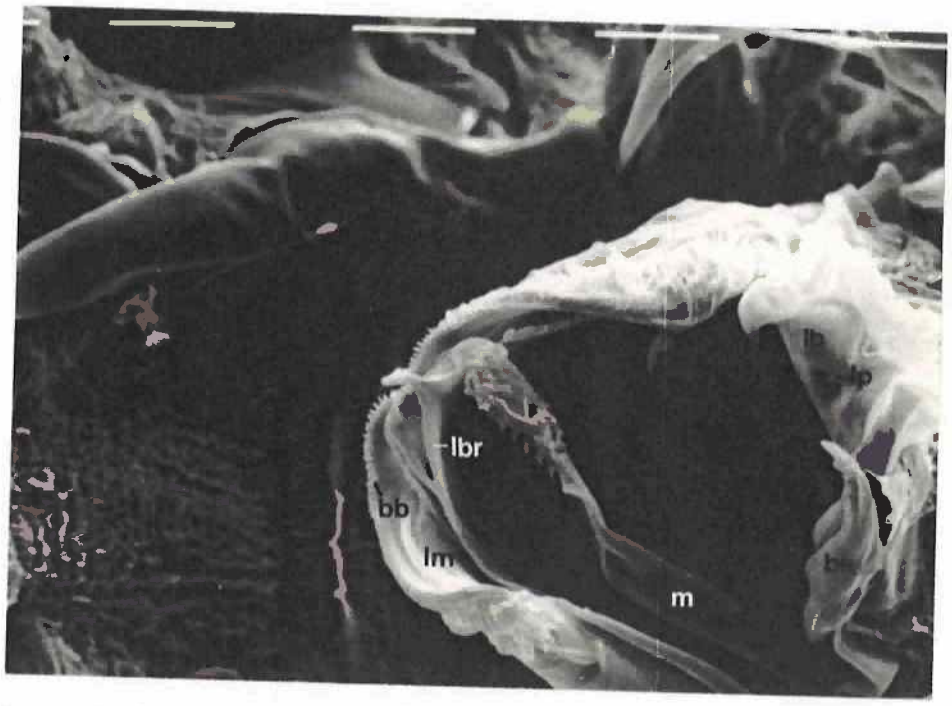


Plate 5.3 Oral cone of copepodid illustrating lack of closure of labium and labrum (SEM). bb; brush border: bs: buccal stylet; lb: labrum; lp: labral pore; lbr: labial bar; lm: labium; m: mandible. Scale = 10 μm

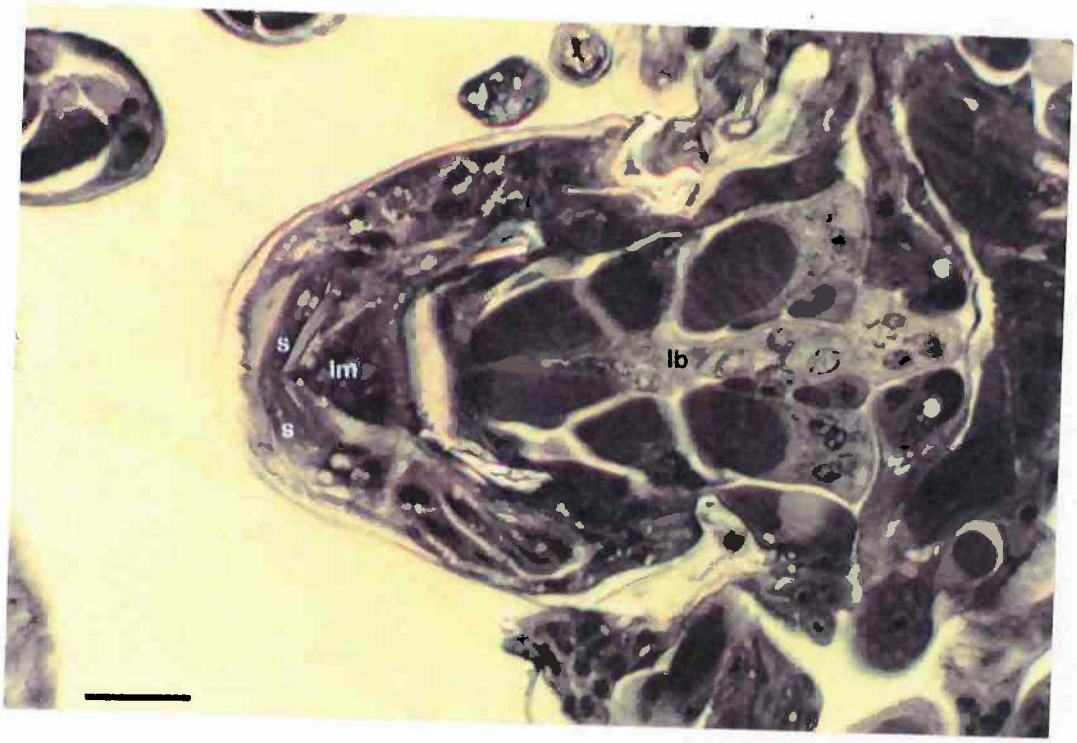


Plate 5.4 Strigil of copepodid (both mandibles are also present in dorsal sections) (LMH, plane, stained Alcian Blue / polychrome). lb: labrum; lm: labium; s: strigil. Scale = 20μm

labrum (Plate 5.3), the latter overlapping outside the former. This apparently gave much greater mobility to the mandibles, particularly in the copepodid stage where their freedom of movement appears, from SEM observations, to be relatively unimpeded by the limits of the buccal cavity. The lumen of the oral cavity was observed to possess a cuticular lining on both labial and labral internal surfaces (Plate 5.2). A pair of presumed sensory palps (termed "buccal stylets" by Kabata 1979), apparently derived from the labrum, projected into the distal portion of the buccal cavity and a brush-bordered marginal membrane was seen to surround the leading (ie distal) edge of both the labium and the labrum (Plate 5.3).

5.4.1.2 Foregut (oesophagus)

The foregut leads posteriorly from the back of the oral cavity, passing through the nerve ring formed by the cerebrum, circumoesophageal commissures and suboesophageal ganglion and meeting with the ventral surface of the anterior midgut. The oesophagus has a thin chitin lining and comprises flattened epithelial cells with no apparent specialized secretory cells. The lumen of the foregut is highly restricted with the epithelial walls extensively folded so as to closely oppose one another. (Plate 5.5). Within the dorsal epithelium of the foregut is carried what appears to be a fine nerve which enters at the start of the oesophagus just dorsal to the anterior extremity of the buccal cavity (Plate 5.5) and which follows the length of the oesophagus, extending almost as far as the interface with the midgut where it disappears briefly and then reappears just below the interface. In the chalimus, this nerve appeared to originate from a pair of cells which might represent neurosecretory organs although this relationship was uncertain (Plate 5.6). The same arrangement may exist in the copepodid but could not be observed because of its smaller size and the closer apposition of its internal organs. Another fine nerve follows the foregut dorsally and may be associated with the nerve carried in the epithelium (Plate 5.5). No muscles could be clearly identified in association with the foregut by use of the light microscope, although small adhesions were noted around the oesophagus that might, under TEM, prove to be muscle fibres. The foregut protrudes ventrally into the midgut in the form of a thick walled papilla formed from somewhat enlarged epithelial cells, and moves posteriorly, terminating in the form of a dorsally open gutter (Plate 5.7, 5.8). The foregut epithelium in this area was thicker than in the earlier part of the foregut and displayed a number of small non-staining vesicles when stained with Heidenhain's haematoxylin (Plate 5.8).

The foregut is accompanied for most of its length by two pairs of buccal levator muscles which originate at the buccal apodemes and eventually attach to the postmaxillary apodemes just ventrally to the interception of the midgut by the foregut. Initially each pair of levators is followed ventrally by a large nerve but these disappear as the foregut enters the sub-oesophageal

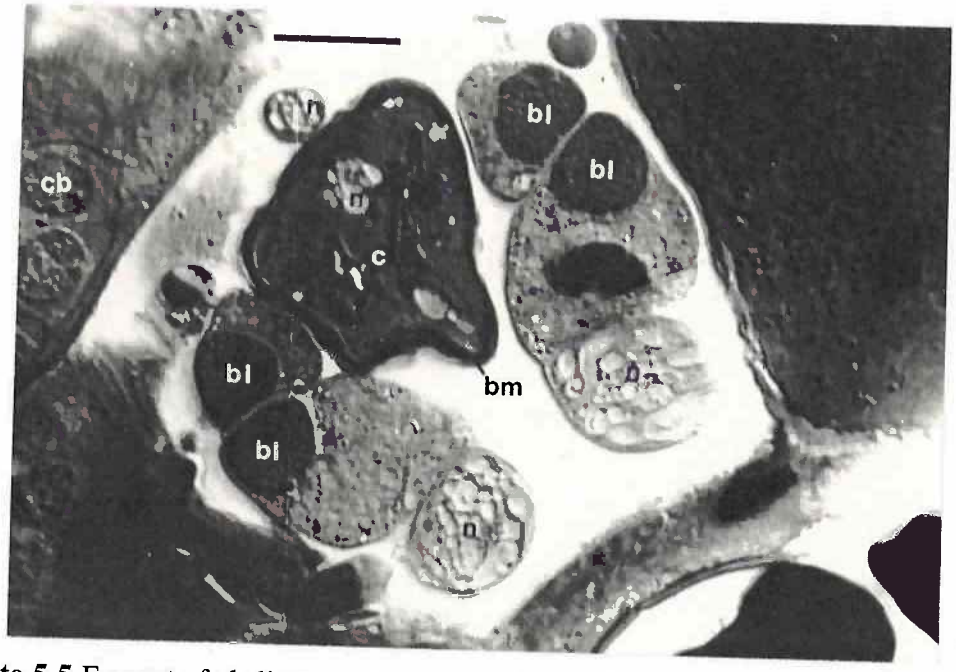


Plate 5.5 Foregut of chalimus running ventral to cerebrum and accompanied by buccal levator muscles. (LMH, transverse, stained polychrome). bm: basement membrane; bl: buccal levator muscles; c: cuticular lining of foregut; cb: cerebrum; n: nerves. Scale = 10μm

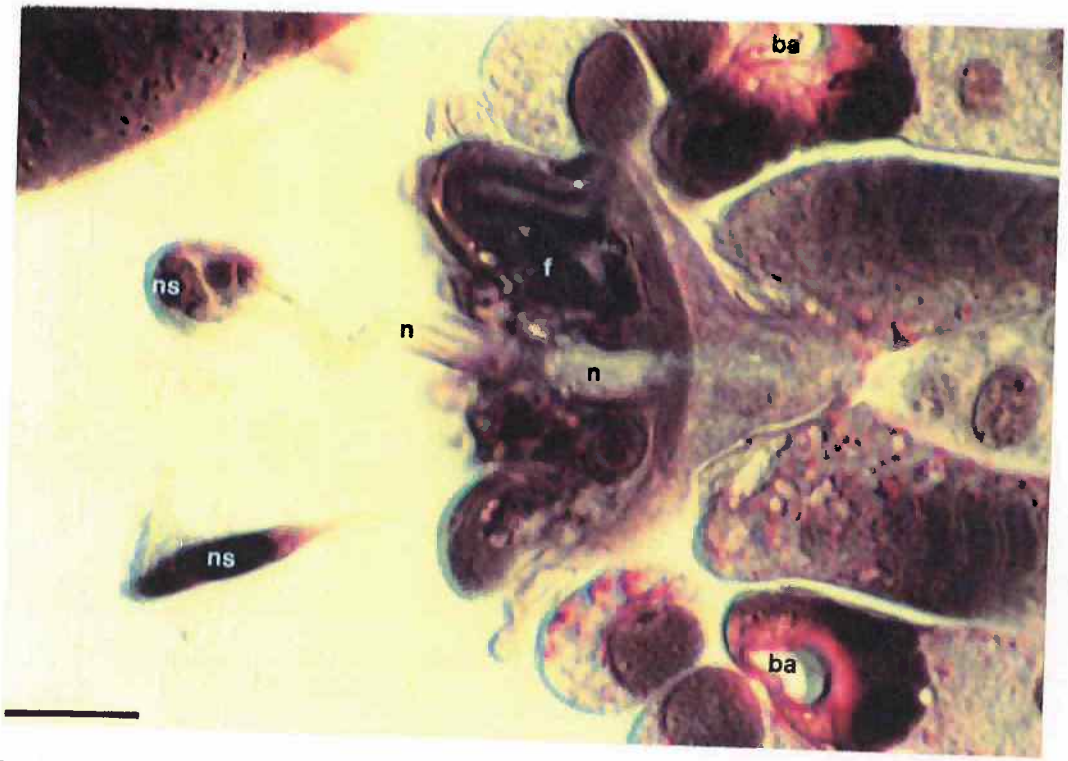


Plate 5.6 Possible neurosecretory cells in chalimus larva. (LMH, transverse, stained polychrome). ba: buccal apodeme; f: foregut origin; n: nerve; ns: neurosecretor. Scale = 10μm

Plate 5.7 Papillate projection of copepodid foregut into midgut lumen (LMH, Plane, stained polychrome using Ehrlich's haematoxylin). c: cuticle; e: epithelial cell of foregut: l: midgut lumen; mc: microvillous cell of midgut; vc: vesicular cell of midgut. Scale = 20μm

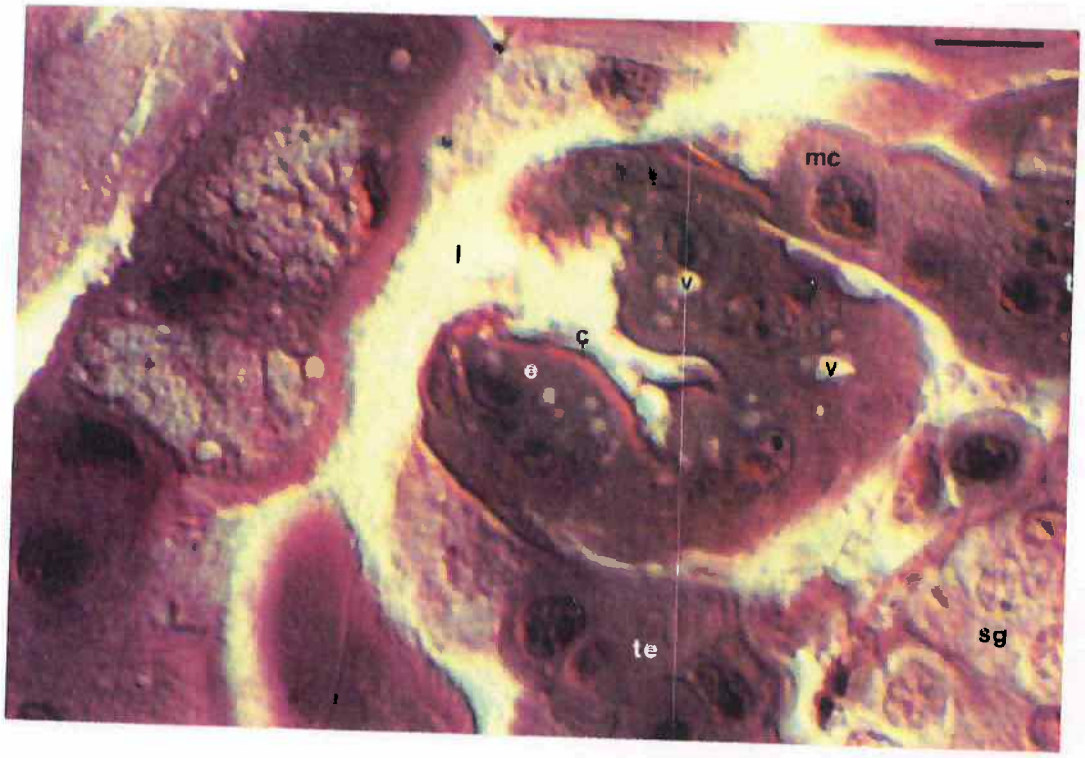


Plate 5.8 Interface of foregut with midgut of chalimus showing gutter-like aspect (LMH, transverse, stained polychrome). c: cuticle of foregut; e: epithelial cell of foregut; l: lumen of midgut; mc: microvillous epithelial cell of midgut; sg: sub-oesophageal ganglion te: thickened epithelium at point of entry of foregut: v: vesicles in foregut epithelium. Scale = 20μm

ganglion (Plate 5.5).

5.4.1.3 Midgut

5.4.1.3.1 Cell Types

The midgut is composed of a number of epithelial cell morphotypes. These comprise (a) microvillous cells (MCs) (b) vesicular cells (VCs) lacking pronounced microvilli and displaying a range of morphologies, often with abundant vesicles (c) smaller, less differentiated "basal cells" (BCs), usually in the vicinity of vesicular cells and often screened from contact with the gut lumen (Plate 5.9).

The MCs possess a pronounced microvillous-border which stains blue with Massons trichrome and positively with PAS indicating the presence of polysaccharides (Plate 5.9, 5.10). The height of the microvilli depends on the area of the midgut concerned though it may vary within a given area. Microvilli in the settled copepodid range in size from ~2.7 μm - 6.2 μm with the longest microvilli being present in the epithelium of the posterior midgut. Microvillus height may characterise two or more cell types within this group although different cell populations could not be determined using the light microscope. The nuclei of these cells are located basally. In older free-swimming copepodids and in some settled copepodids, the height of these epithelial cells was much reduced.

In early free-swimming and recently settled copepodids, there is a profusion of cells containing large vesicles measuring up to 10.7 μm which, judging by the strong osmiophilia displayed under TEM, probably represent lipid stores associated with the lecithotrophic free-living phase (Plates 5.11, 5.12). The suggestion of a lipid contents to these vesicles is also supported by their lack of staining in wax sections which suggests the removal of their contents by the solvents used in processing. The fact that these cells have a pronounced microvillous border suggests that they are a subset of the MCs already mentioned. These apparently vacuolated cells give rise to the globular appearance of the gut seen in live copepodids and occur throughout the midgut (Plates 5.1, 5.11). In older free-swimming copepodids and in some settled copepodids, these vesicles appeared to be largely absent (5.13). These vesicles were generally rarer and less well-developed in chalimus stages.

The VCs show a range of morphologies which probably correspond to a continuum of development. In the earliest recognisable stage of this sequence, these cells show pronounced apical PAS positive vesicles (up to 10 μm) suggesting that they may contain polysaccharides (these vesicles do not stain positively with alcian blue and therefore do not contain acid mucopolysaccharides). They also contain large numbers of other smaller vesicles (Plates 5.9 and

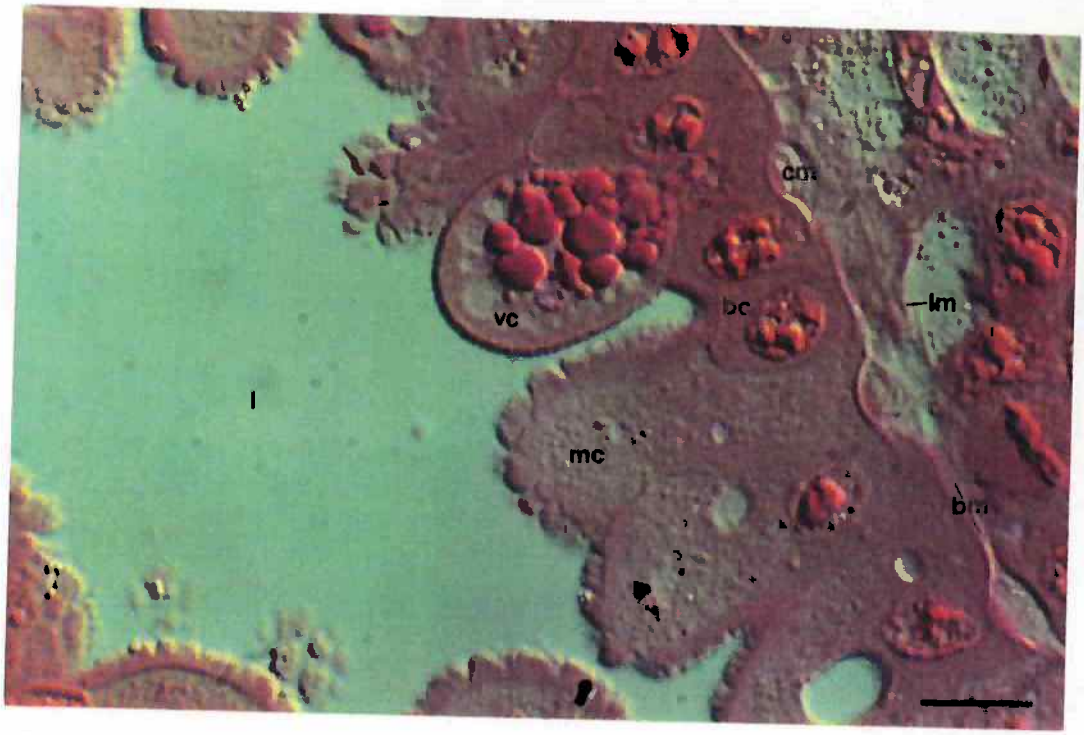


Plate 5.9 Midgut epithelium showing epithelial cell types (LMH, plane, stained polychrome using Ehrlich's haematoxylin). bc: basal cell; bm: basal membrane; cm: circular muscle; l: midgut lumen; lm: longitudinal muscle fibres; mc: microvillous epithelial cell; vc: vesicular cell. Scale = 20μm

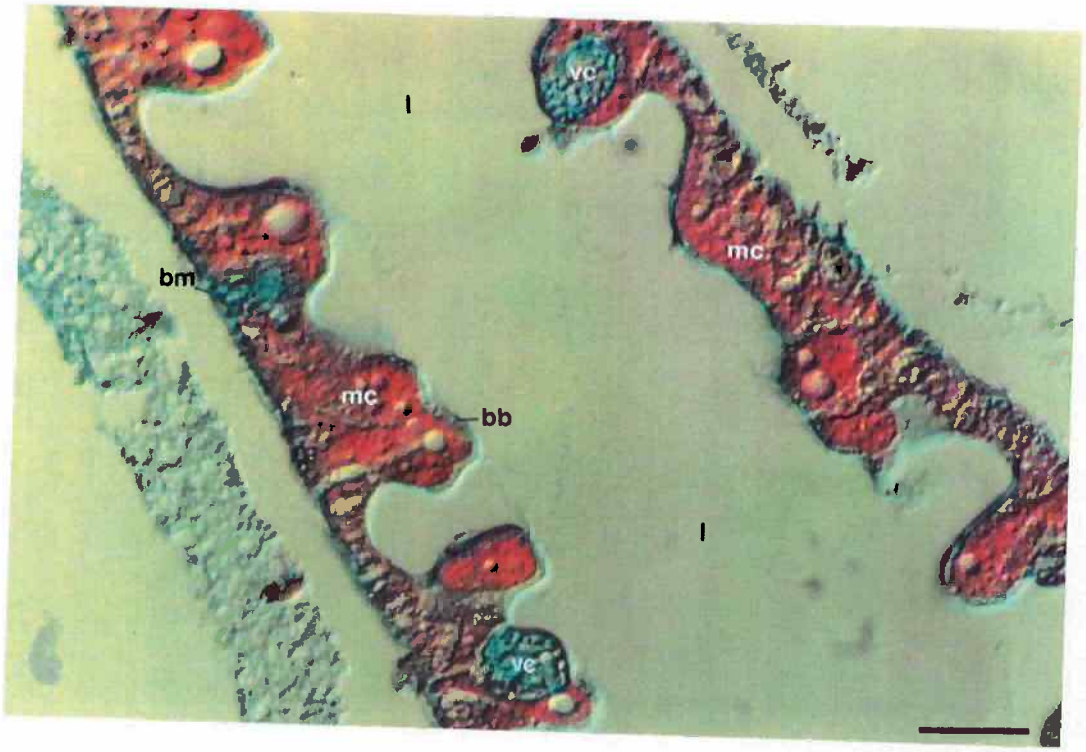


Plate 5.10 Midgut epithelium showing differentiation of microvillous and vesicular cells using Masson's trichrome (LMW, plane). bb: brush border (blue); bm: basement membrane (blue); l: midgut lumen; mc: microvillous epithelial cell (red); vc: vesicular cell (blue). Scale = 50μm

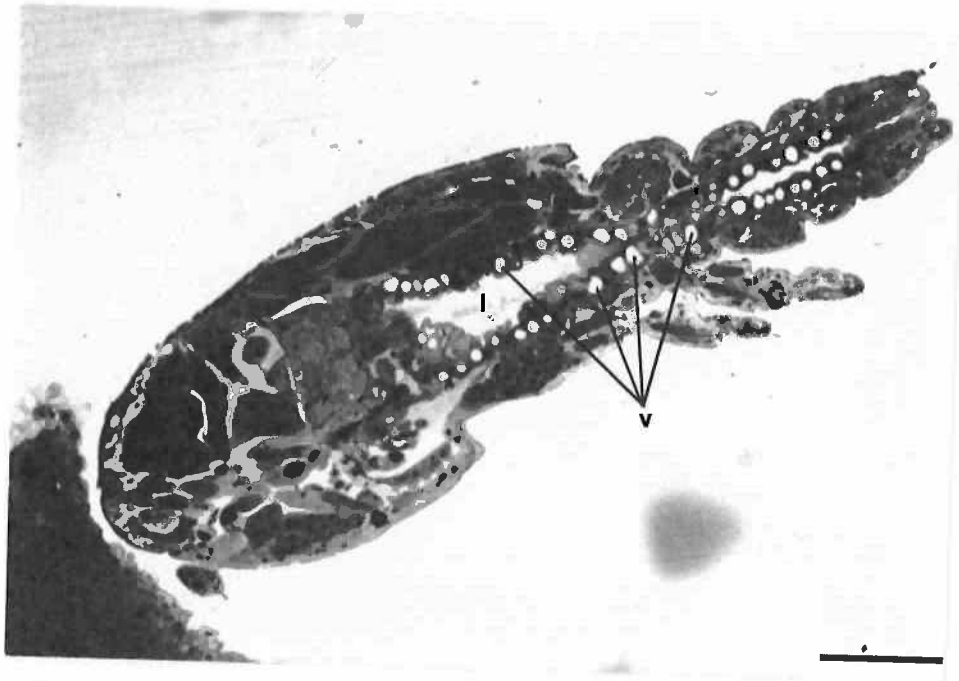


Plate 5.11 Plane section of copepodid alimentary canal showing large vesicles in midgut epithelial cells thought to contain lipid (LMH, ~plane, stained Alcian blue / polychrome). l: midgut lumen; v: vesicles within epithelial cells. Scale = 100 μm

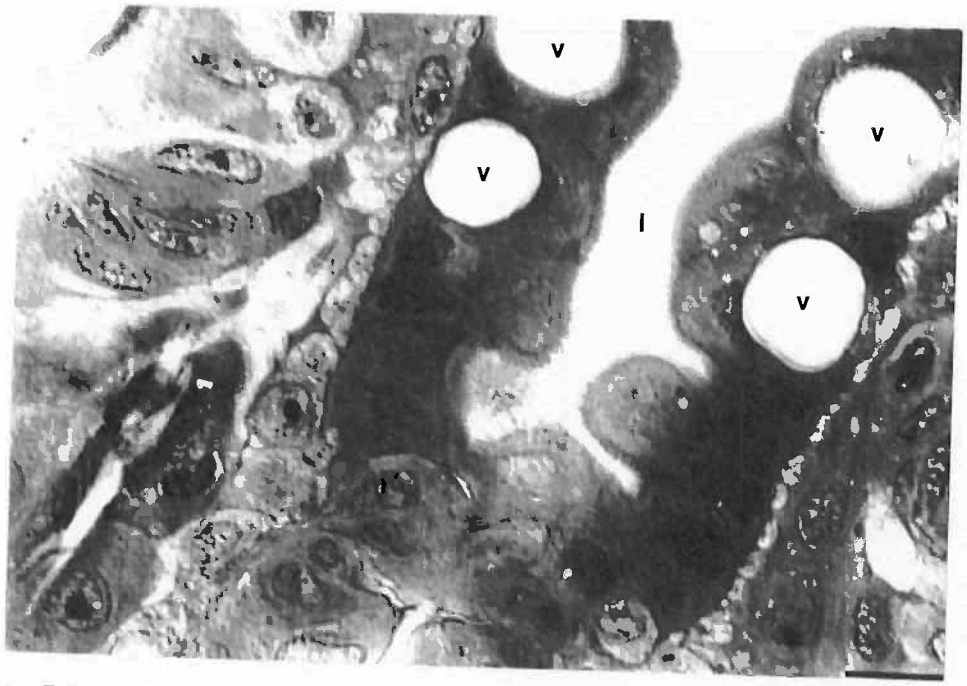


Plate 5.12 Posterior midgut epithelium showing large vesicles within epithelial cells (LMH, ~plane, stained Alcian blue / polychrome). l: midgut lumen; v: vesicles within epithelial cells. Scale = 10μm

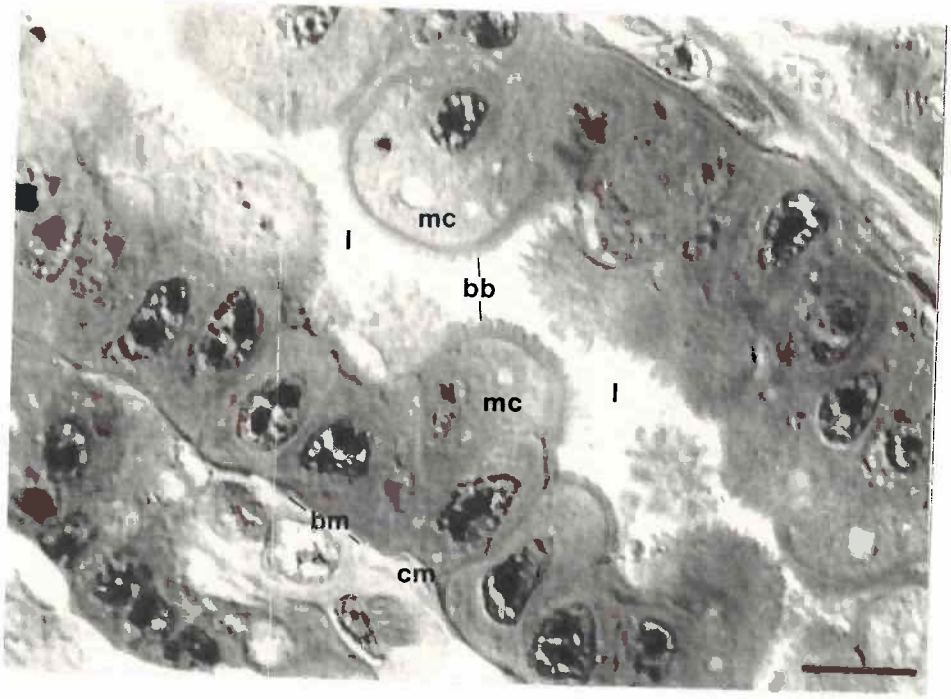


Plate 5.13 Posterior midgut (older settled copepodid) showing microvillous epithelial cells and lack of large vesicles (LMH, plane, stained Alcian blue / polychrome) bb: brush border; bm: basement membrane; cm: circular muscle; l: lumen of posterior midgut; mc: microvillous epithelial cell. Scale = 10μm

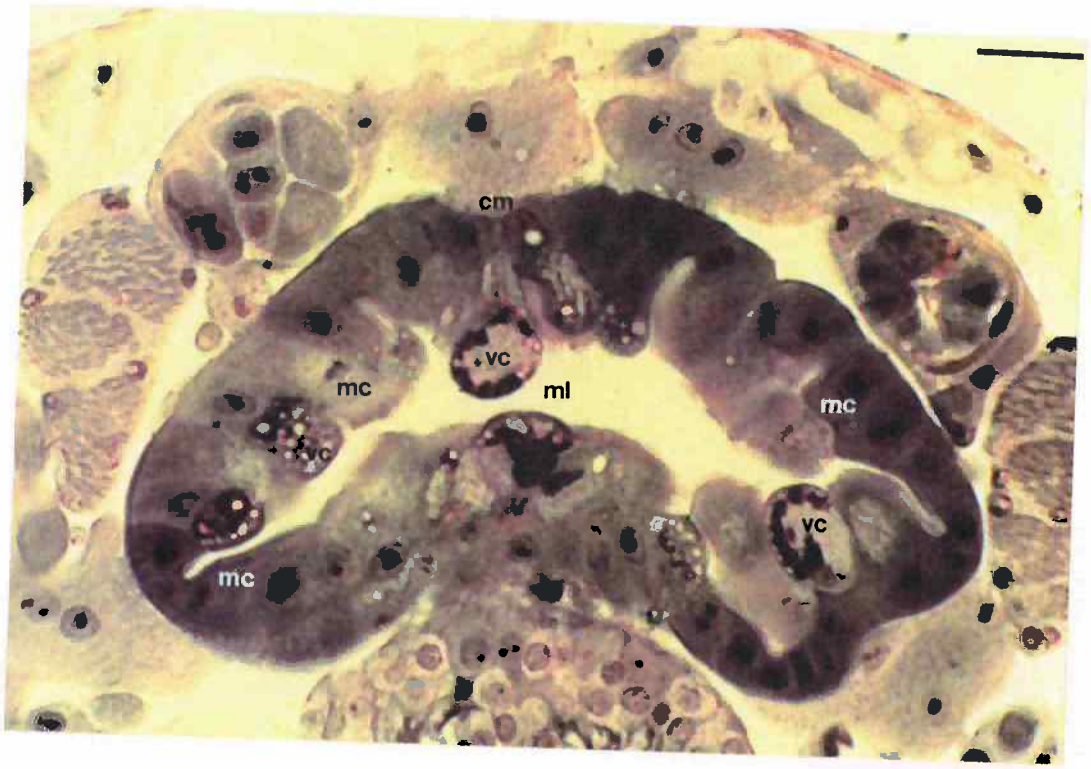


Plate 5.14 Anterior midgut of chalimus showing well developed vesicular and microvillous epithelial cells (LMH, transverse, stained polychrome). cm: circular muscle; ml: midgut lumen; mc: microvillous epithelial cell; vc: vesicular cell. Scale = 20 μm

5.14. VCs stain blue with Masson's trichrome whilst the aforementioned MCs stain red (Plate 5.10). When well developed, the VCs protrude noticeably into the gut lumen, having a finely rugose (possibly finely microvillous-bordered) apical membrane when viewed under the light microscope (Plate 5.9). The depth of this border is ~ 1 μm or less. In well-developed, protruding cells, a larger lighter staining vacuole was often observed basal to the apical vesicles. Disintegrating cells of this or any other type were only rarely seen in sections.

The third category of cell (BCs) are not clearly differentiated under the light microscope but are characterised by a homogeneous cytoplasm, small size and compression between neighbouring cell types, such that they often have no apparent contact with the gut lumen itself (Plate 5.9). These cells are basally situated and, when in contact with the gut lumen, appear to have a fine microvillous-border. They are often found in the vicinity of VCs

5.4.1.3.2 Organisation

The midgut is clearly differentiated from the fore- and hindgut by the lack of a chitin lining and was seen to be divided into three recognisable zones.

The anterior midgut caecum protrudes anteriorly above the cerebrum Figure 5.1. The epithelial lining is composed of all three cell types, MCs predominating, but with a concentration of VCs in the vicinity of the opening of the oesophagus into the midgut. The anterior midgut caecum is not large enough to act as a storage area and has no visible valve-like structures which would allow for it to be sealed off from the rest of the midgut. The ventral epithelium associated with the position of entry of the foregut into the midgut is noticeably thicker than the more anterior epithelium and comprises a depth of several layers of relatively undifferentiated cells thought to correspond to the BCs described above. The wall of the anterior midgut caecum is highly convoluted, presumably to provide a greater area for production of enzymes and absorption of components of the ingested material. The proportion of VCs in the anterior midgut caecum was observed to be highly variable, being rare in some specimens and relatively abundant in others.

The principal portion of the anterior midgut, which continues posteriorly from the anterior midgut caecum (Figure 5.1) also comprises the three major cell types (Plate 5.14), and shows a pronounced constriction at the interface with the posterior midgut which may act as a valve or sphincter to retain food in the anterior part of the gut. Although muscle fibres (circular and longitudinal) are seen in this area it was not possible to determine whether these represent a fully-closing sphincter. This most anterior zone carries a far larger proportion of VCs than the other zones of the midgut.

The posterior midgut (Figure 5.1, Plates 5.13, 5.15) is normally distinguished from the anterior midgut by a lack of VCs and BCs, being apparently composed solely of somewhat cuboidal MCs with a pronounced microvillous border and thick basement membrane. Occasional chalimus specimens were observed to have rare VCs in this area although this was never seen in the copepodid posterior midgut. The posterior midgut is frequently considerably narrower than the anterior midgut and is heavily rugose, particularly where it approaches the hindgut (Plate 5.15).

Fine striated circular and longitudinal muscles surround the midgut throughout its length and are responsible for peristalsis and compartmentation of the gut during food digestion. The circular muscles are relatively evenly spaced, such that the longitudinal and circular muscles form a grid around the midgut (Plates 5.13, 5.15). The circular muscle bands become more closely apposed as they approach the midgut / hindgut sphincter (see "Hindgut") such that they are almost adjoining in this area forming an apparent sphincter (Plate 5.15).

5.4.1.4 Hindgut

The hindgut (Figure 5.1) is separated from the posterior midgut by a collar of enlarged cells ("guard cells") which may act as part of the valve separating the midgut and hindgut or alternatively may be involved in osmoregulation (Plate 5.15, 5.16). Anterior to this collar (ie in the latter part of the posterior midgut) there is a pronounced sphincter comprising multiple rings of circular muscle augmented by large transverse muscles, which attach to the basement membrane of the midgut by means of insertions passing between the circular muscle bands (Plate 5.15).

The hindgut is characterised by a thin cuticular lining that is continuous with the external cuticle. Beneath the cuticle are somewhat flattened epithelial cells. Large transverse muscles are attached between the hindgut wall and the outer cuticle and are responsible for the high gut mobility seen in this area in live specimens (Plate 5.16). The hindgut opens via a slit-like anus situated between the caudal rami (Plate 5.17).

5.4.2 Luminal Contents / Faecal Pellets

Intact host epithelial cells, mucous cells and blood corpuscles are frequently seen in the oral cavity and are, more rarely, recognisably intact in the midgut. Host cells in the oral cavity are often seen to be intact and may be present as large pieces of tissue completely filling the buccal aperture (Plate 5.18). Once in the foregut, however, there is evidence that such tissue has been substantially degraded to give cell fragments and more amorphous material suggestive of

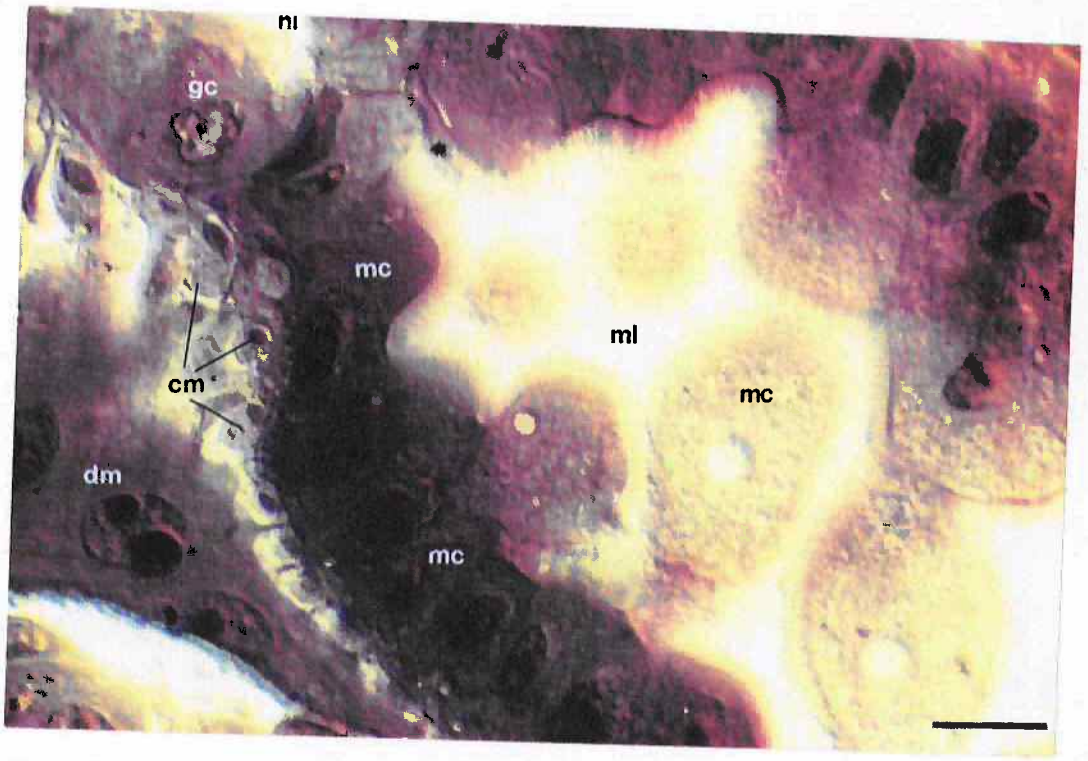


Plate 5.15 Midgut / hindgut interface in copepodid showing pronounced circular muscle and dilator muscle sphincter and rugose construction (LMH, plane, stained polychrome). cm: circular muscle sphincter; dm: dilator muscle: gc: guard cell; hl: hindgut lumen; ml: midgut lumen; mc: microvillous epithelial cell. Scale = 20μm

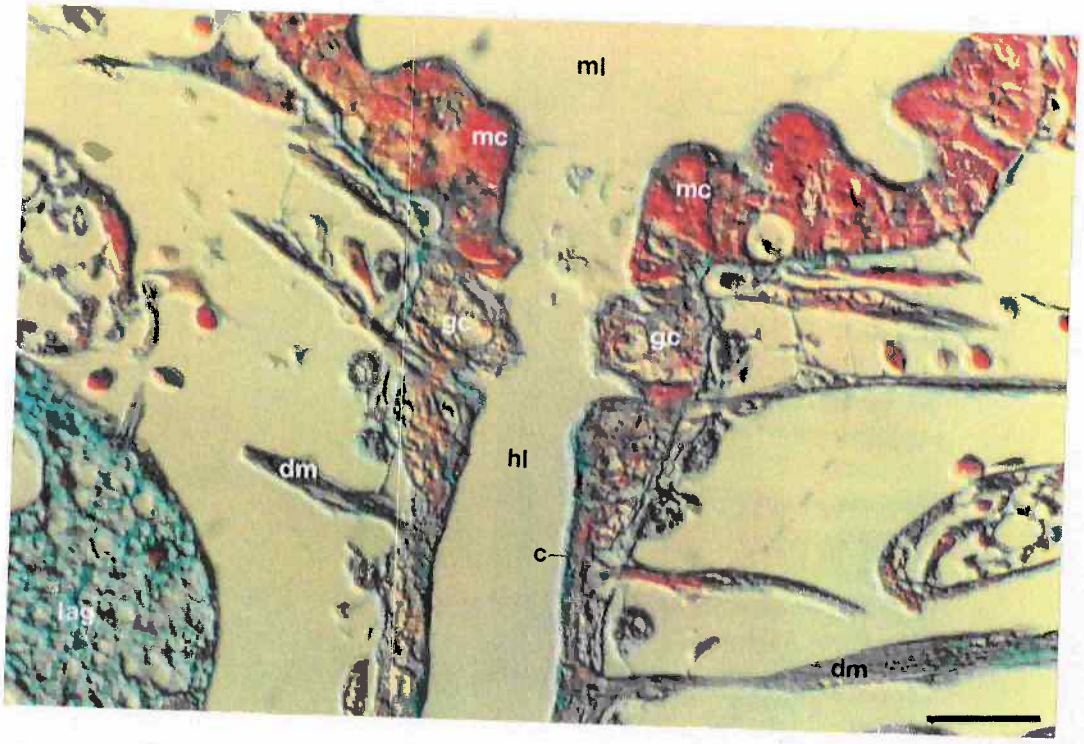


Plate 5.16 Midgut / hindgut interface in chalimus showing cuticle covering of hindgut. (LMH, plane, stained Masson's trichrome).c: cuticle of hindgut; dm: dilator muscle; gc: guard cell; hl: hindgut lumen; lag: lateral anal gland; ml: midgut lumen; mc: microvillous epithelial cell. Scale = 50μm

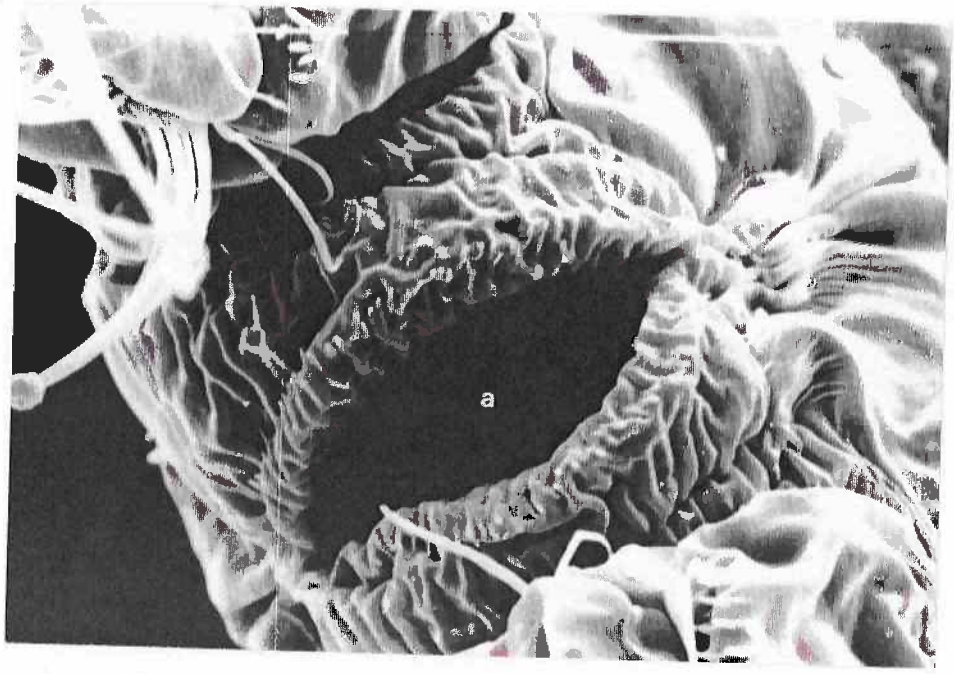


Plate 5.17 Copepodid anus opening between the the uropods (SEM). a: anal aperture. Scale = 10 μm

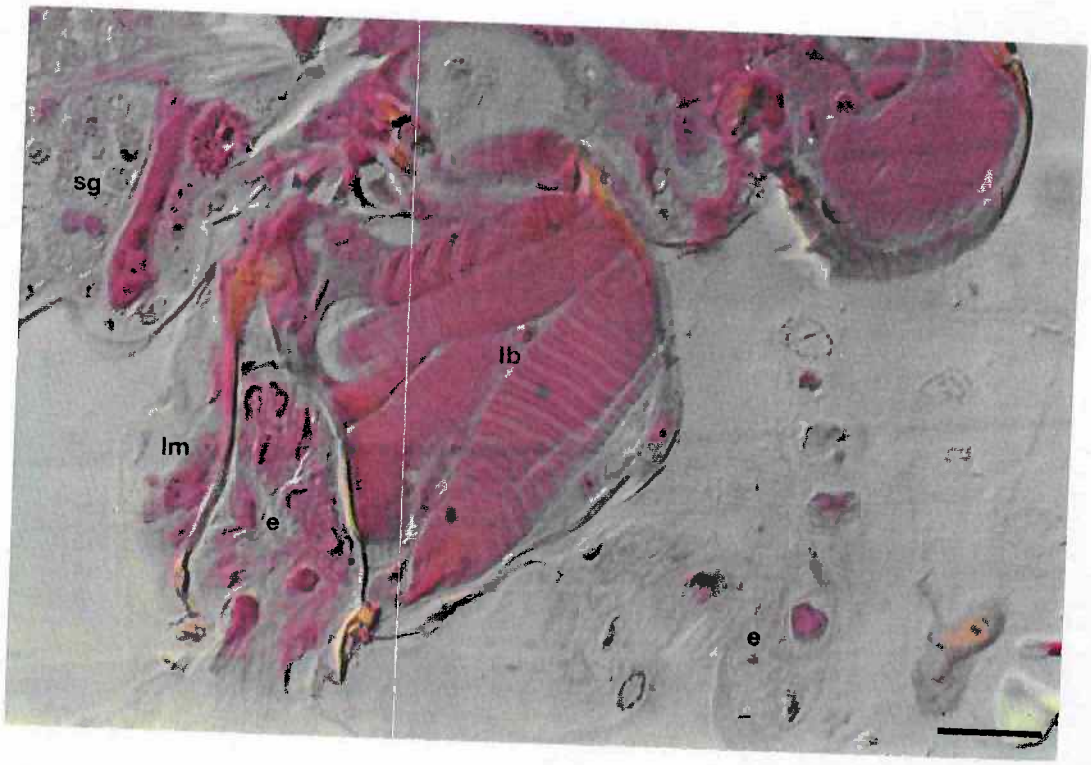


Plate 5.18 Buccal cavity of copepodid with enclosed host epithelial cells (LMH, sagittal, stained Cason's). e: host epithelial cells; lb: labrum; lm: labium; sg sub-oesophageal ganglion. Scale = 20μm

partial digestion. The contents of the midgut normally comprise granular remains staining positively for protein, carbohydrate and acid muco-polysaccharides, positive staining for the latter being apparent only in the buccal cavity and in the anterior part of the midgut (Plate 5.19). In view of the fact that there was no indication of positive staining for acid muco-polysaccharides within any of the cells or organs of the copepodid, save at the interface between the cuticular epithelium and the overlying cuticle, it seems likely that the acid muco-polysaccharides are of host origin. This is supported by the observation that the goblet cells of the host epidermis show strong positive staining.

Within the midgut, large granules were apparent in the lumen under the light microscope. Under TEM large granules were seen which measured up to 8.6 μm, were electron dense, and which displayed extremely regular parallel dark and light banding with an inter-band distance of 0.025 μm (Plate 5.20, 5.21). The origin of these structures could not determined in the present study. Under TEM, the midgut epithelial wall was found to be covered in an electron-dense layer comprising a mixture of cellular debris and indeterminate granular material adhering to the epithelial microvilli. In the central lumen of the midgut was a more dispersed fibrous / granular material resembling mucin (Plate 5.22).

Bacteria were not commonly found in the gut and were present in large numbers only in the guts of senescent \ morbid individuals. Small numbers were occasionally observed in apparently healthy copepodids and were assumed to have been ingested accidentally with host material.

Faecal pellets expelled by the larvae possess a rigid (as opposed to mucous) peritrophic membrane (Plate 5.23). The origin of this membrane could not be determined, since firstly specimens void the faecal material when stressed and secondly, no pellets were observed *in situ* in the midgut / hindgut due to the low resolution of the light microscope.

5.4.3 Glands

A number of organs were observed which were apparently associated with the alimentary canal or possibly with feeding activity.

5.4.3.1 Mucoid glands

A pair of glands was observed which originated anteroventrally to the nauplius eye and ran posteroventrally to either side of the foregut, continuing posteriorly as far as the furthest posterior extension of the nauplius eye and the start of the cerebrum (Plates 5.24, 5.25). These glands were directly posterior to and ventral to the "mucous glands" which separate the A-gland

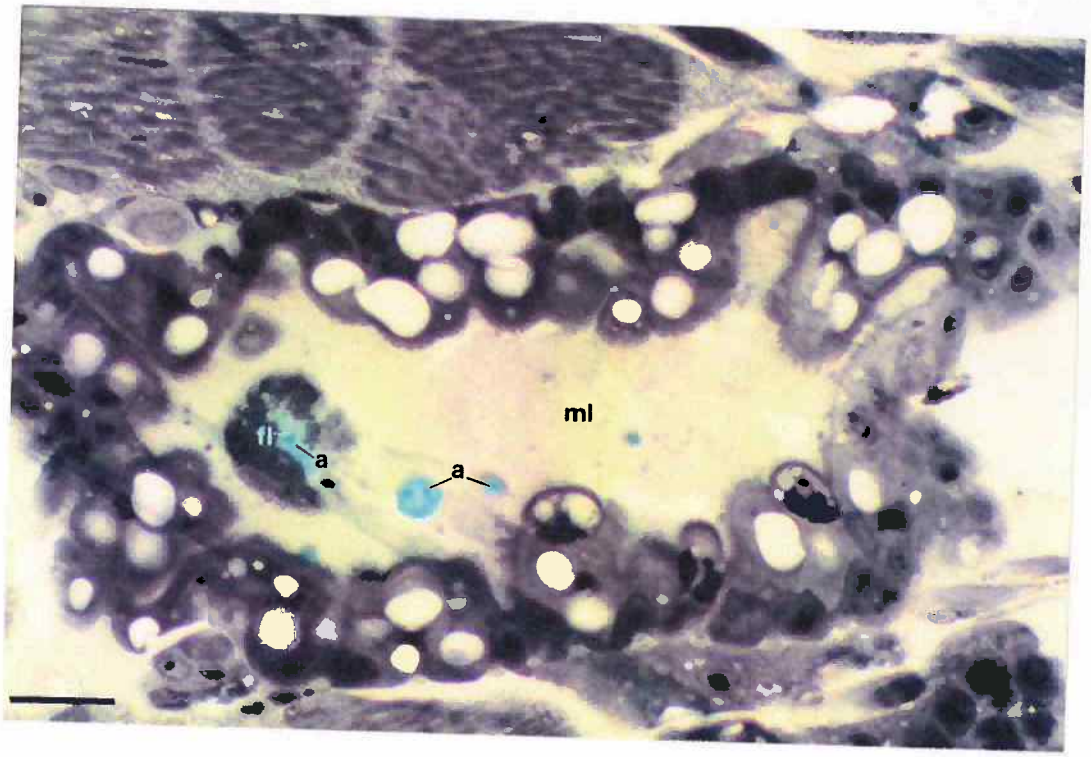


Plate 5.19 Anterior midgut of copepodid showing presence of acid mucopolysaccharides (blue) in foregut and midgut lumens (LMH, plane, stained Alcian Blue, Heidenhain's). a: acid mucopolysaccharide positive material; fl: foregut lumen; ml: midgut lumen. Scale = 20μm

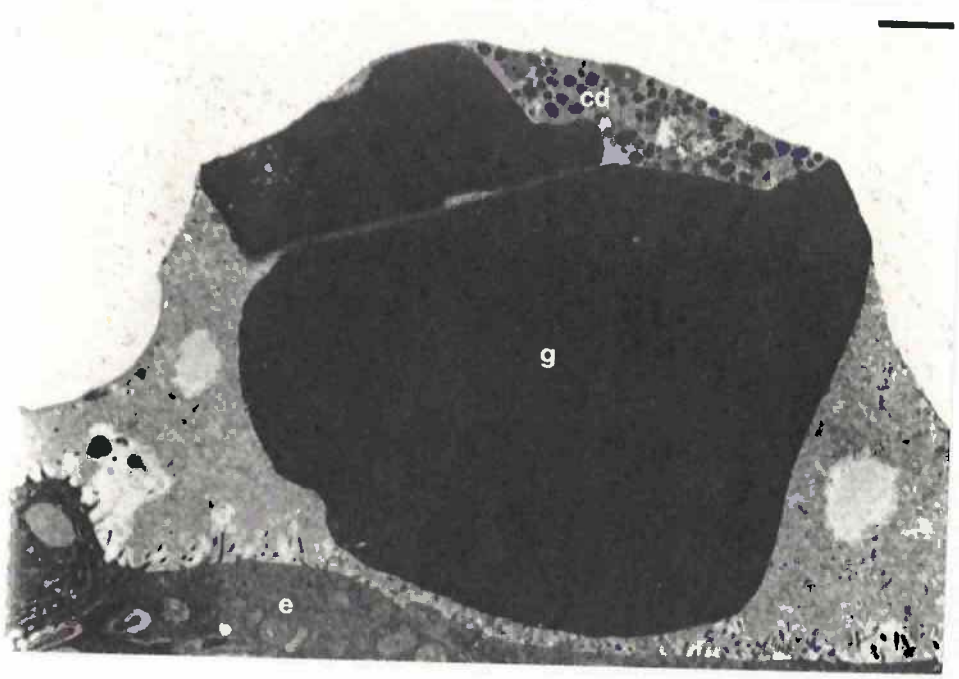


Plate 5.20 Osmiophilic granule in midgut of copepodid positioned within layer of cellular debris in contact with the midgut epithelium (TEM, plane).cd: cellular debris; g: osmiophilic granule; e: midgut epithelium. Scale = 1μm

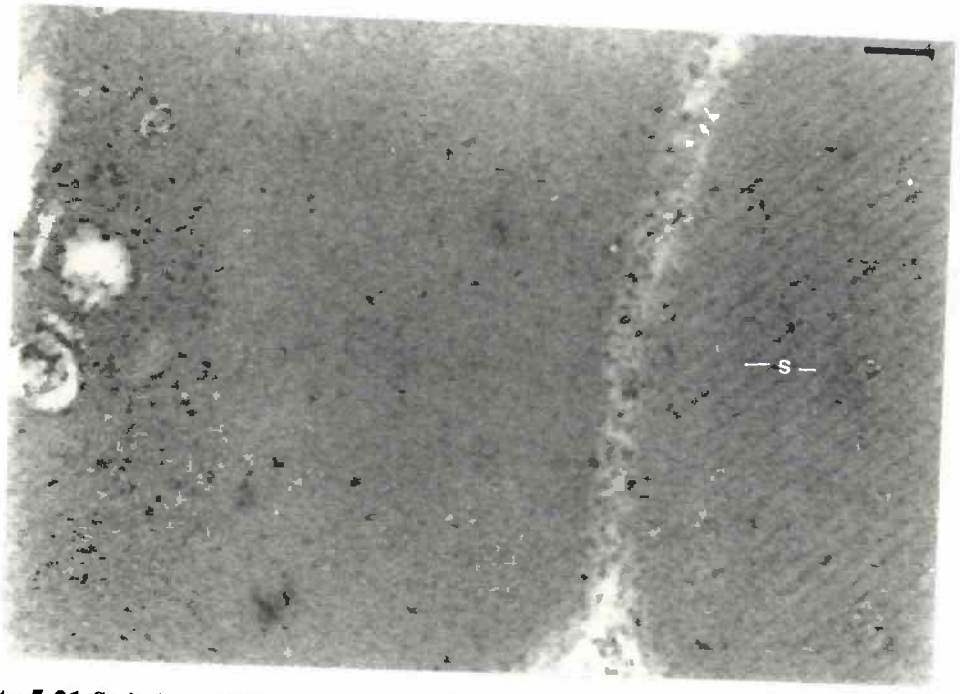


Plate 5.21 Striations visible in osmiophilic granule (TEM, plane). s: striations. Scale = 0.1μm

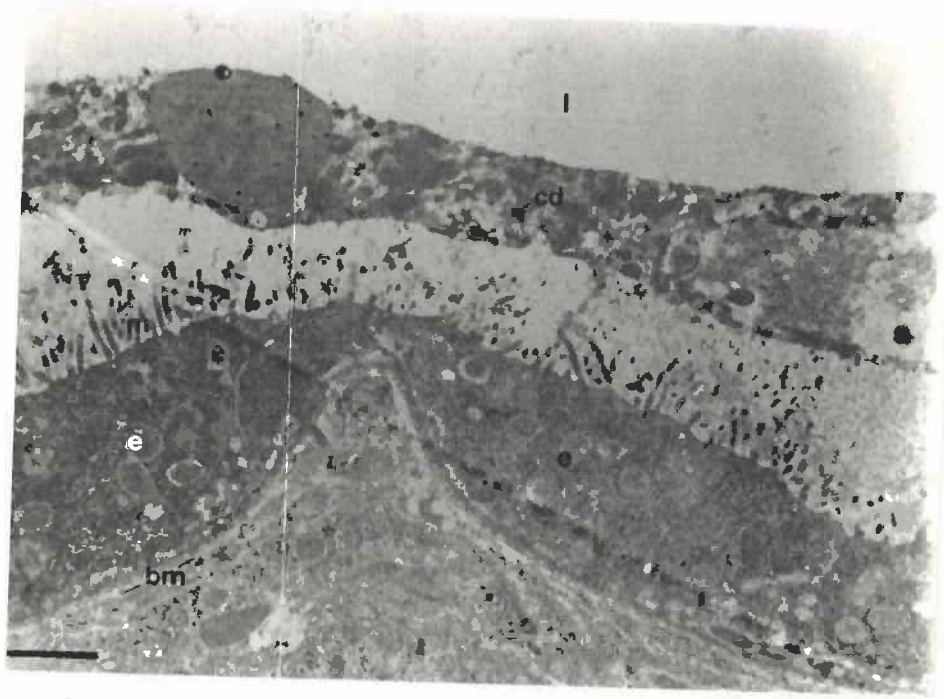


Plate 5.22 Midgut epithelium showing flocculent coating of microvilli, layer of cellular debris and clearer luminal space (TEM, plane). bm: basement membrane; cd: layer of cellular debris; e: midgut epithelium; f: flocculent layer; l: luminal space; m: microvilli. Scale = 1μm

Plate 5.23 Copepodid faecal pellet showing presence of peritrophic membrane (unstained specimen). f: faecal material; p: peritrophic membrane. Scale = 20μm

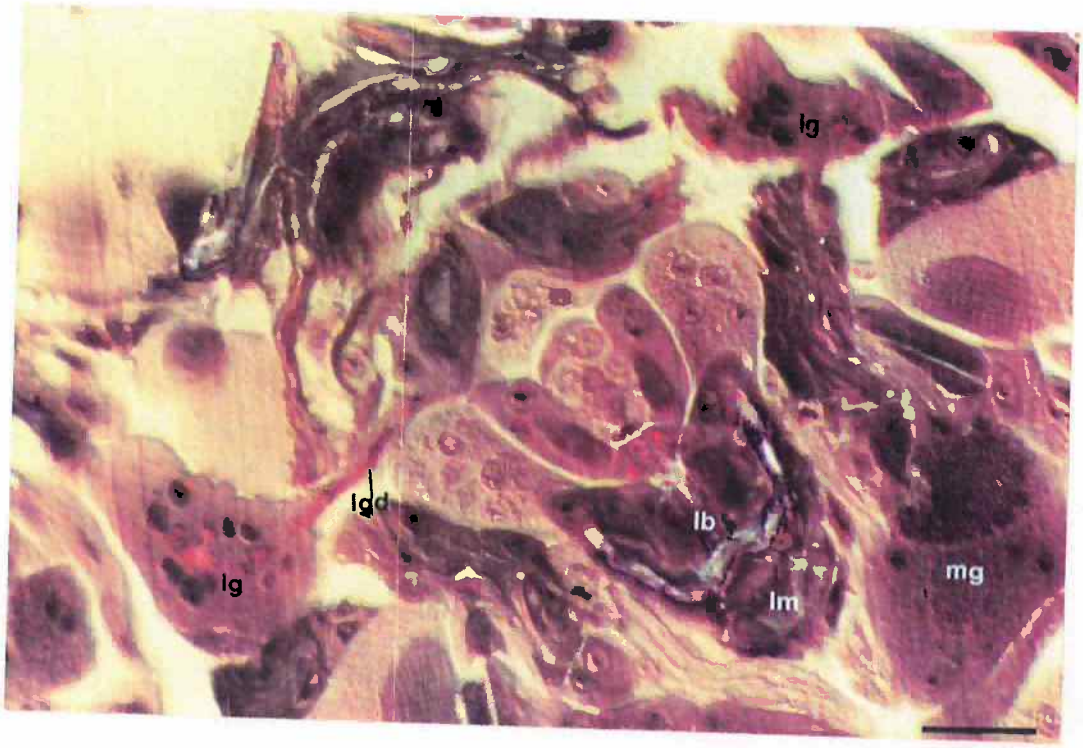


Plate 5.24 Glands associated with the oral cone (LMH, plane chalimus, stained polychrome). lb: labrum; lg: lateral gland; lgd: lateral gland duct; lm: labium; mg: mucoid gland. Scale = 50μm

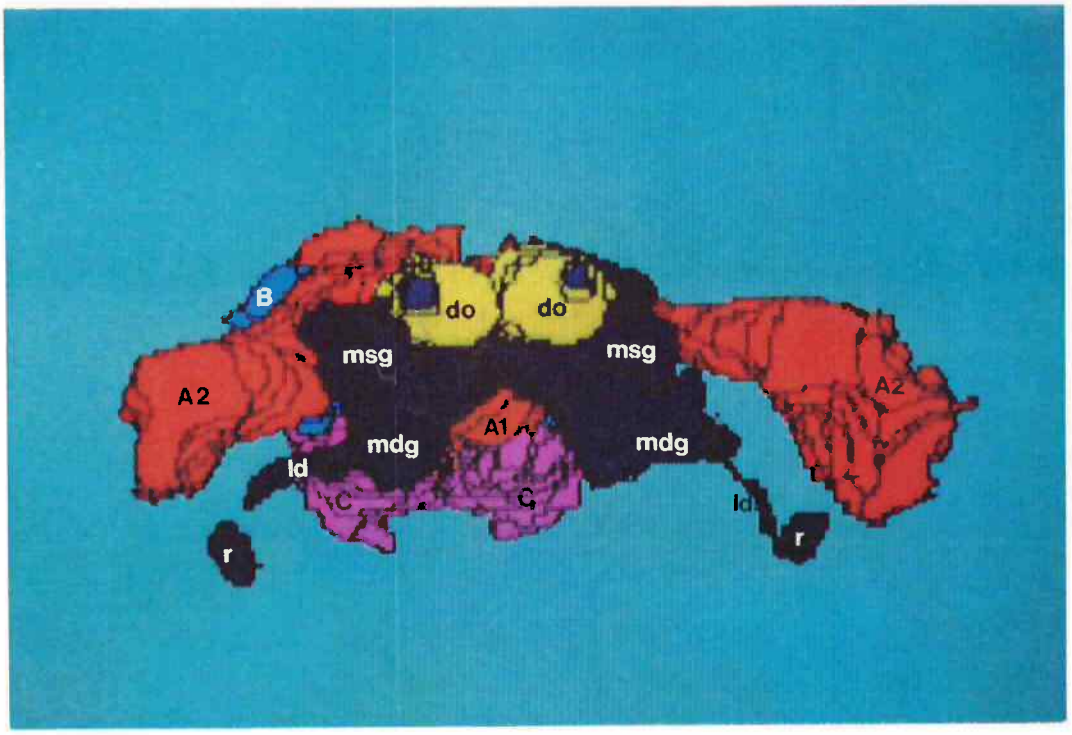


Plate 5.25 3D-reconstruction of mucoid / mucous glands seen from posterior aspect and illustrating lateral ducts with ventral reservoirs (transverse, chalimus). A1, A2: lobes of A gland; B: B gland; C: C gland; do: dorsolateral ocellus of nauplius eye; mdg: mucoid gland; msg: mucous gland; ld: lateral duct of mucoid gland; r: reservoir of mucoid gland.

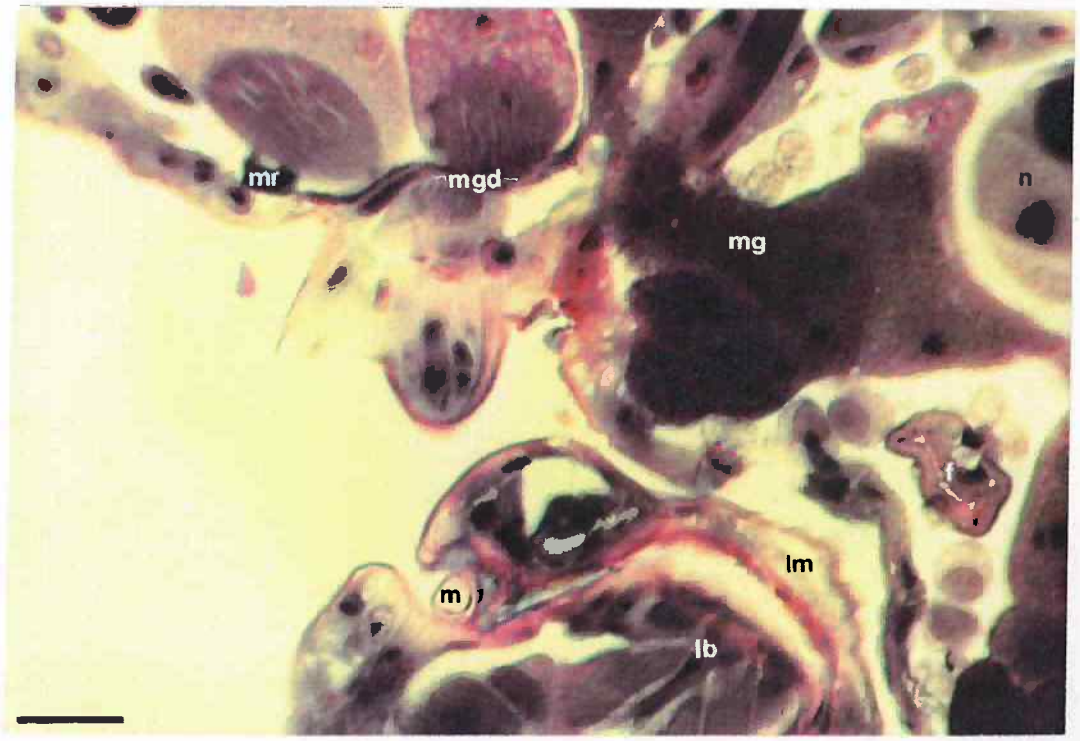


Plate 5.26 Tranverse section through mucoid gland showing focus of PAS-positive granular material in gland and duct which also contains granules and passes ventrally to a reservoir of material (LMH, chalimus, polychrome). f: foregut; m: mandible; mg: mucoid gland; mgd: mucoid gland duct: mr: mucoid reservoir; lb: labrum; lm; labium; n: nauplius eye. Scale = 20μm

of the previous chapter from the nauplius eye described in Chapter 3. Although their structure appears identical to that of the mucous glands, they are separated from these by a membrane and have different foci of product accumulation. The glands are syncytial and possess a cortex of relatively homogeneous cytoplasm with prominent nuclei and scattered PAS-positive (possibly indicative of neutral mucopolysaccharide) granules. The glands show a pronounced medulla with tightly-packed PAS-positive granules identical to those seen in the cytoplasm of the gland. These granules pass into a lateral duct (aboral side) which passes ventrally and terminates in a small reservoir lying just dorsal to the ventral surface (Plate 5.26). From here material is believed to be discharged externally to either side of the oral cone, although the external openings could not be found under SEM.

5.4.3.2 Oral cone - Proximal gland complex

A glandular complex comprising two separate gland types was also observed. The complex comprised paired lateral glands and a single median gland. The former were found to be anterodorsal to the base of the oral cone. These lateral glands were syncytial with a relatively homogeneous dorsal cytoplasm and a more granular ventral cytoplasm containing PAS-positive material and vesicles of various sizes. The lateral glands form a tissue commissure which undercuts the aboral side of the anterior extremities of the buccal apodemes and meets in the median line of the copepodid directly beneath the median gland (Plate 5.27). These glands had ducts which stained positively for PAS and carried the material ventrally through the commissures to join medially. Where the tissue met in the midline it was joined by material from the overlying median gland.

The cytoplasm of the median gland stained only faintly with Heidenhain's, with pale staining nuclei only present around the periphery. Like the lateral glands, it was syncytial. The major part of the gland cytoplasm was observed under the light microscope to be filled with what appeared to be large spherical granules staining positively for PAS and having a range of staining densities. Moving posteriorly, these granules appeared to aggregate into several larger bodies. Small ducts within the median gland passed ventrally to meet with the lateral gland ducts.

A communal duct containing material from both glands passed ventrally and apparently opened onto the anterior surface of the proximal labrum. A single corresponding external pore was seen under SEM opening at the base of the labrum.

The ultrastructure of this gland complex was studied using TEM. Both median and lateral glands were apparently syncytial, the lateral glands having dorsally placed nuclei and the median gland having peripheral nuclei. The ventral zone of the lateral glands displayed a large number of membrane bounded "striate vesicles" containing fibrillogranular material and having what

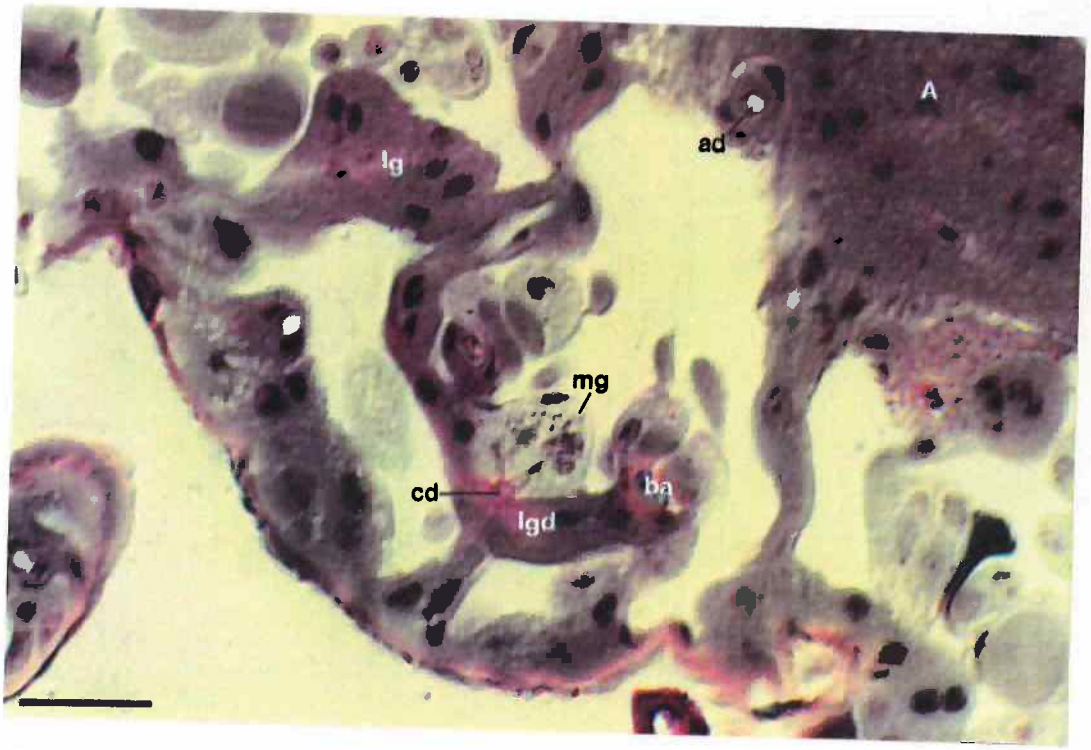


Plate 5.27 Median and lateral glands of proximal gland complex (LMH, transverse chalimus, polychrome). A; A-gland: ad: axial duct; ba: buccal apodeme; cd: common duct; lg: lateral gland; lgd: lateral gland duct; mg: median gland. Scale = 10μm

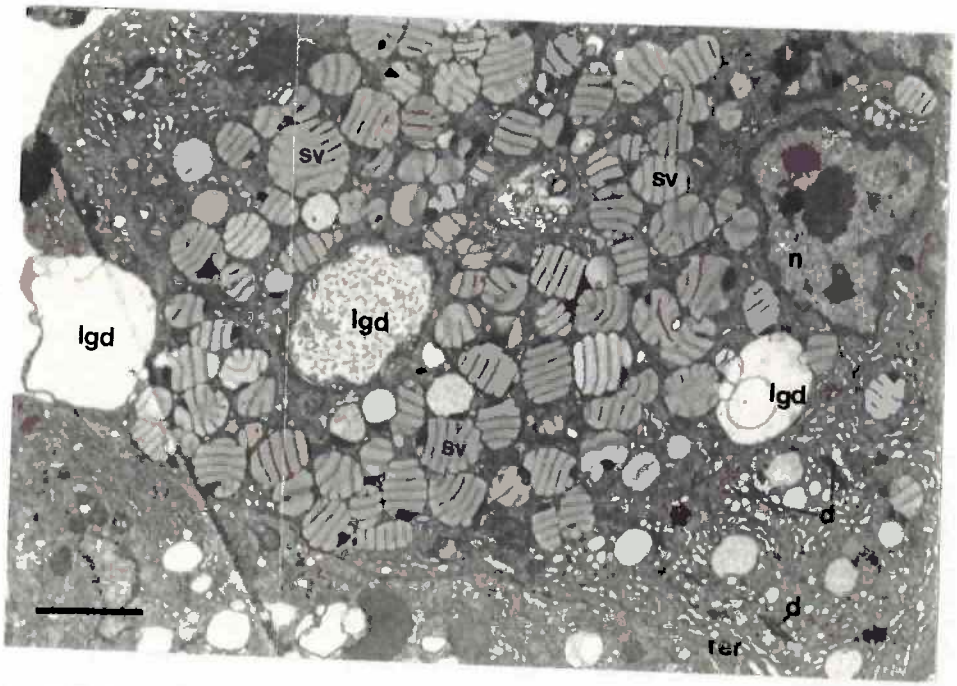


Plate 5.28 Lateral gland of copepodid showing striate vesicles and reservoirs / ducts (TEM, transverse). d: dictyosome; lgd: lateral gland duct; n: cell nucleus; rer: rough endoplasmic reticulum; sv: striate vesicle. Scale = 2μm

appeared to be membrane incursions into the interior of the vesicles giving the appearance of electron-dense stripes (Plate 5.28). The suggestion of a membraneous origin for the stripes is supported by the apparent invagination of the vesicle membrane at the base of the stripes (Plate 5.29). The size range of these vesicles was 0.46 μm - 1.2 μm. The electron dense stripes were often seen to dissociate into the material within the vesicle and were sometimes associated with highly electron dense patches which may represent concentrations of the contained product in the midst of secretion. Within the cytoplasm of the lateral glands, there were also membrane bounded reservoirs of material measuring up to 3.31 μm (Plate 5.29). The material within these reservoirs had a flocculent appearance with particles being more dispersed than in the striate vesicles. These reservoirs were also often observed to have pieces of membrane and "myelin figures" amongst the contained product. Sections of the lateral glands seen under the light microscope suggest that these larger reservoirs represent sections through PAS-positive collecting channels. Within the cytoplasm and particularly around the periphery of the lateral glands there were seen to be large quantities of RER stacked in parallel cisternae as well as dictyosomes and mitochondria (Plate 5.30). It would seem that within the lateral glands, some products are transported across membranes to accumulate in "striate vesicles" and others pass directly into the collecting ducts (these materials being likely to comprise glycoproteins and mucopolysaccharides collectively). The striate vesicles then deposit their contents through fusion with the duct membrane into the collecting ducts (Plate 5.30) to give a combined product which passes ventrally to join that of the median gland.

The structure of the median gland is distinctly different from that of the lateral glands. No striate vesicles are seen within the median gland although most of the cytoplasm appears to be filled with vesicles of varying sizes ranging from 0.09 μm - 2.25 μm (Plate 5.31). These vesicles show a range of electron densities with apparently vacuolated vesicles being more common in the ventral part of the gland. The material in the vesicles is finely granular and on occasion, particularly in the case of the more-electron dense material, appears to have condensed to give only partially-filled vesicles. No ducts were seen within this gland using light or TEM microscopes although one structure seen under the light microscope could represent a fine ventral duct. The cytoplasm of the median gland contained large quantities of RER as did the lateral glands but in this instance it appeared to be far more closely packed, with narrower cisternae and no dictyosomes in contrast to the lateral glands (Plate 5.31).

Stained preparations of whole copepodids indicate that the median gland stains positively for the presence of endoperoxidases whilst the lateral glands do not. In this respect it resembles the paired lateral anal glands and urosomal glands (terminology corresponding to that of Boxshall 1982) of the copepodid, the former of which discharge between the setae at the posterior apex of the uropod and appear to coat the setae with adherent material (Plate 5.32) and the latter of

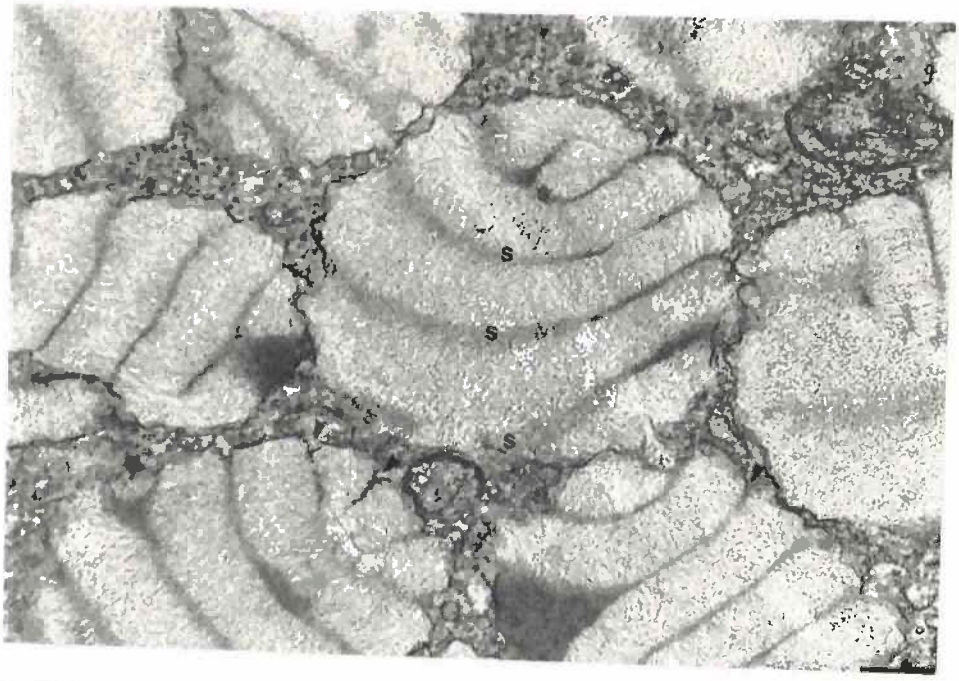


Plate 5.29 Striate vesicles of lateral gland showing invagination of membrane (arrowed) at endpoints of striations (TEM, transverse). s: striation. Scale = 0.25μm

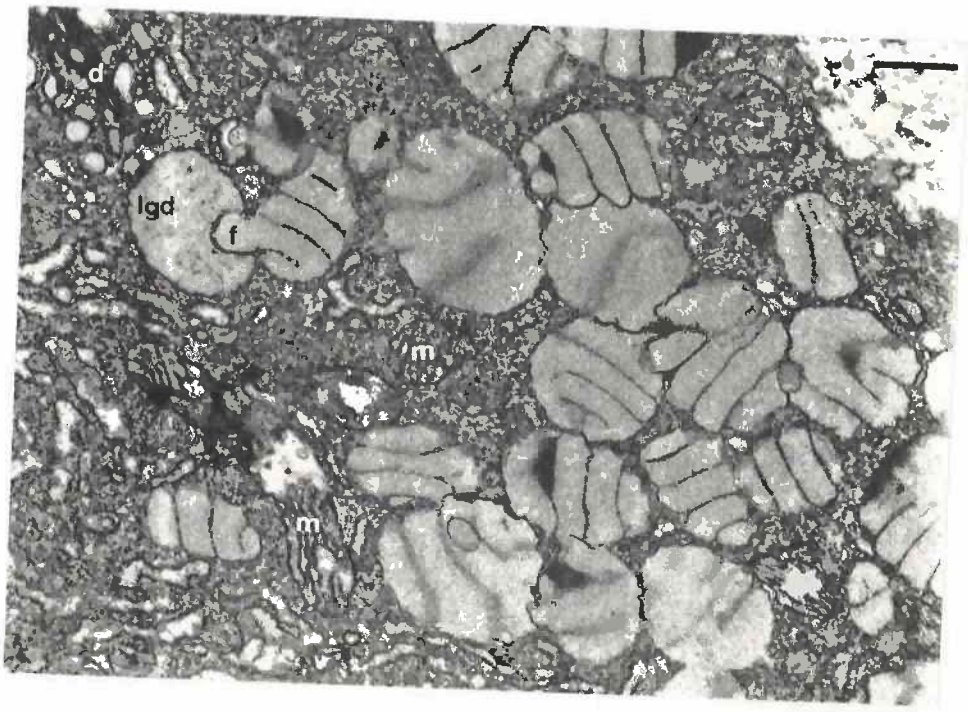


Plate 5.30 Cytoplasm of lateral gland showing organelles and fusion of striate vesicles with ducts / reservoirs (TEM, transverse). d: dictyosome; f: area of fusion between striate vesicle and duct; lgd: lateral gland duct; m: mitochondrion. Scale = 1μm

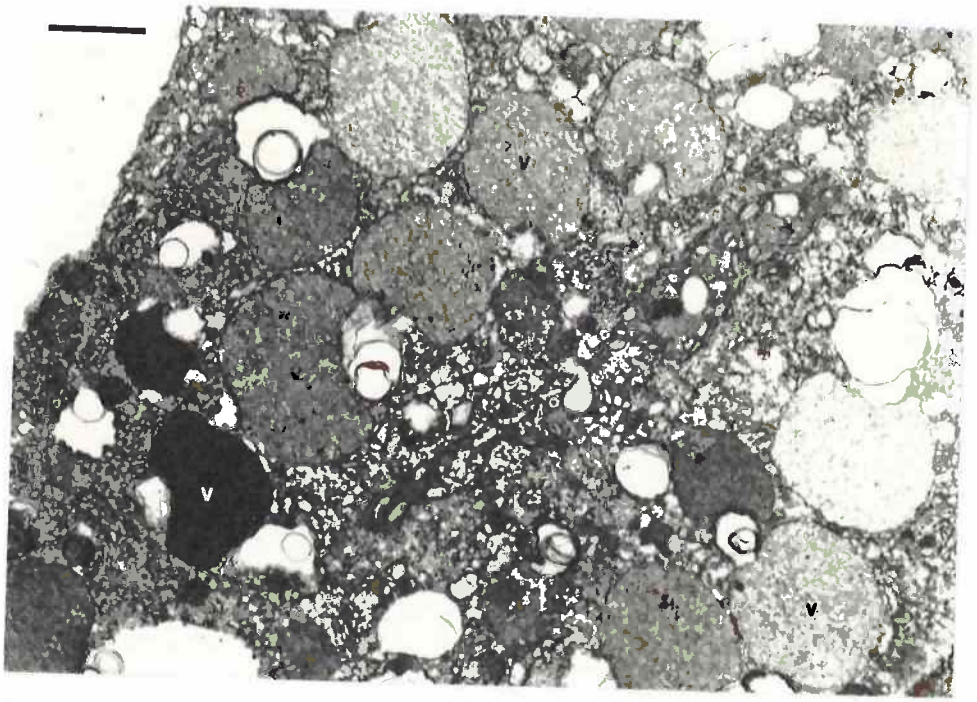


Plate 5.31 Median gland of copepodid with electron-dense vesicles and organelles (TEM, transverse). v: vesicle. Scale = 1μm

Plate 5.32 Lateral anal glands of copepodid with ducts discharging between setae showing peroxidase-specific staining (whole mount). lag: lateral anal gland; lagd: lateral anal gland duct; s: secreted material. Scale = 20 μm

which appear to discharge ventrally. Identical staining is also seen in the numerous glands associated with the sympods, exopods and endopods of the first and second thoracic legs and the interpodal bars between them (Plate 5.33).

5.4.3.3 Labial gland

Within the labium was a third category of organ comprising a small number of dorso-ventrally oriented, somewhat columnar cells (Plate 5.34). The product of these cells stained strongly with fast red and was PAS-positive. Unlike the products of previous glands described, this product was not granular in appearance. The PAS-positive product was occasionally very apparent in attached copepodids, but was normally less obvious. The area of expression of the product of this organ appears to be via a single duct near the distal tip of the labium onto the anterior (intra-oral) surface. No pores were, however, visible (under SEM) in the vicinity of the anterior part of the labium.

5.4.3.4 Distal labral organs

At the distal tip of the labrum were two structures apparently associated via fine fibres or canals to the posterior (intra-oral) surface (Plate 5.35). The function of these structures is enigmatic and clearly requires TEM for its resolution, although a single pore or depression ("labral pore") is apparent on the posterior (intra-oral) labral surface under SEM (Plates 5.3, 5.36).

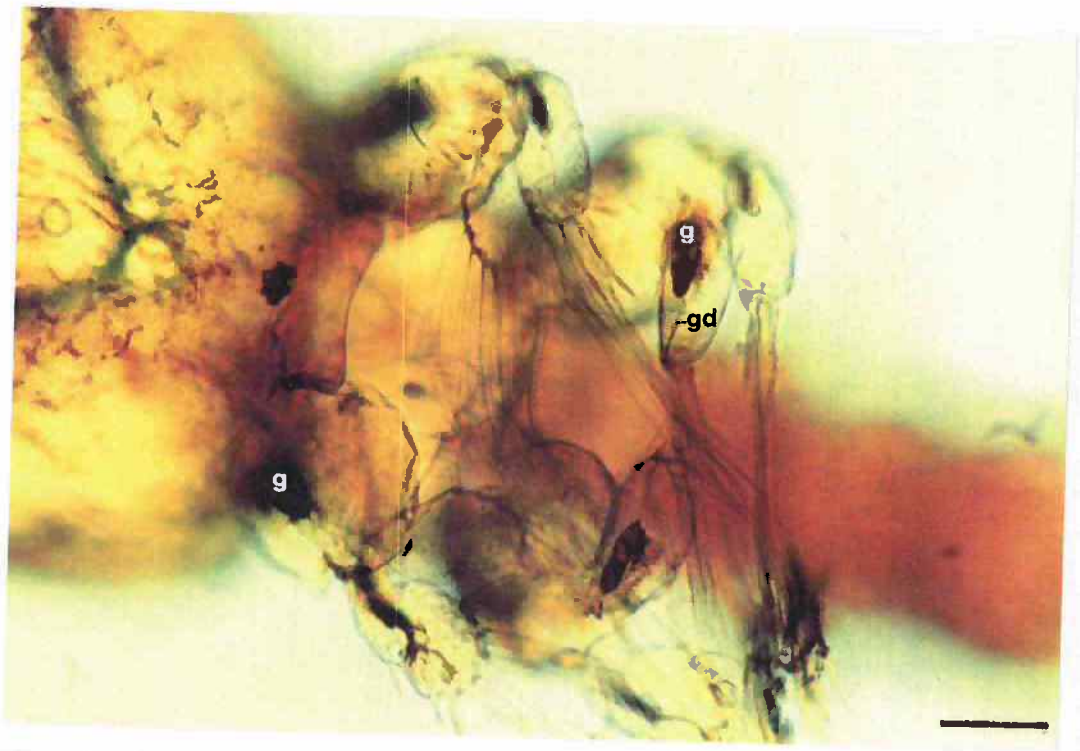


Plate 5.33 Glands associated with the first and second thoracic legs showing peroxidase-specific staining (whole mount). g: gland stained for peroxidase; gd: gland ducts stained for peroxidase. Scale = 32 μm

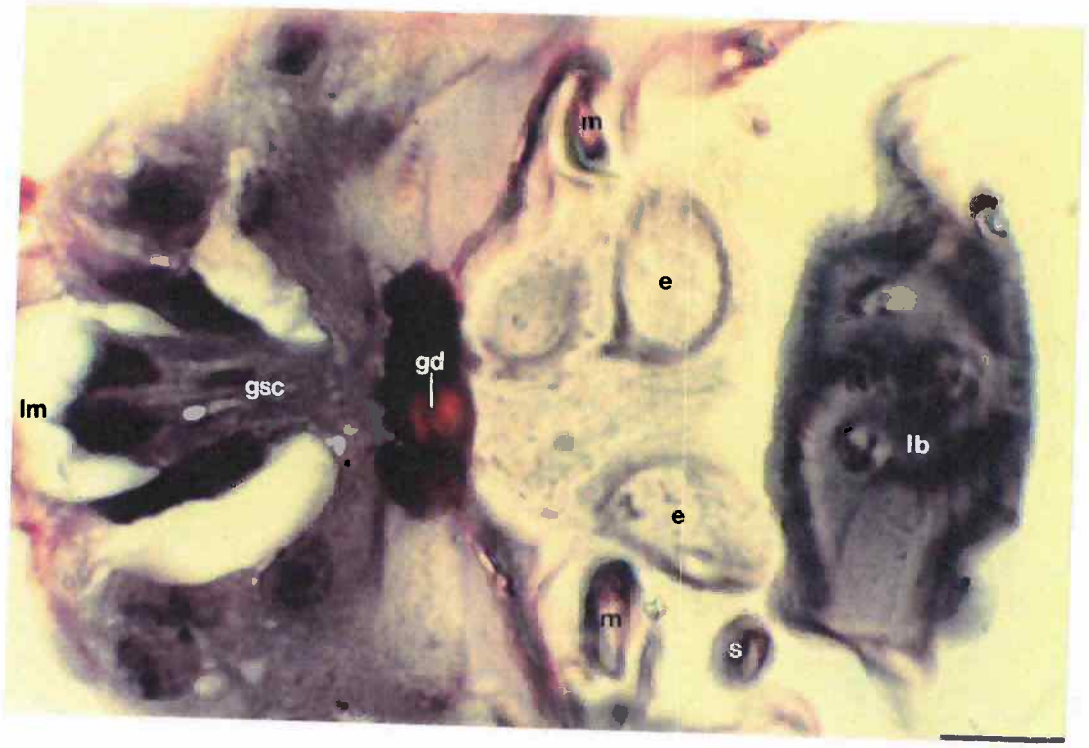


Plate 5.34 Labial gland showing secretory cell group and stained product (LMH, transverse chalimus, stained polychrome). e: host epidermal cell; gd; gland duct; gsc: secretory cells lb: labrum; lm: labium; m: mandible; s: strigil. Scale = 10μm

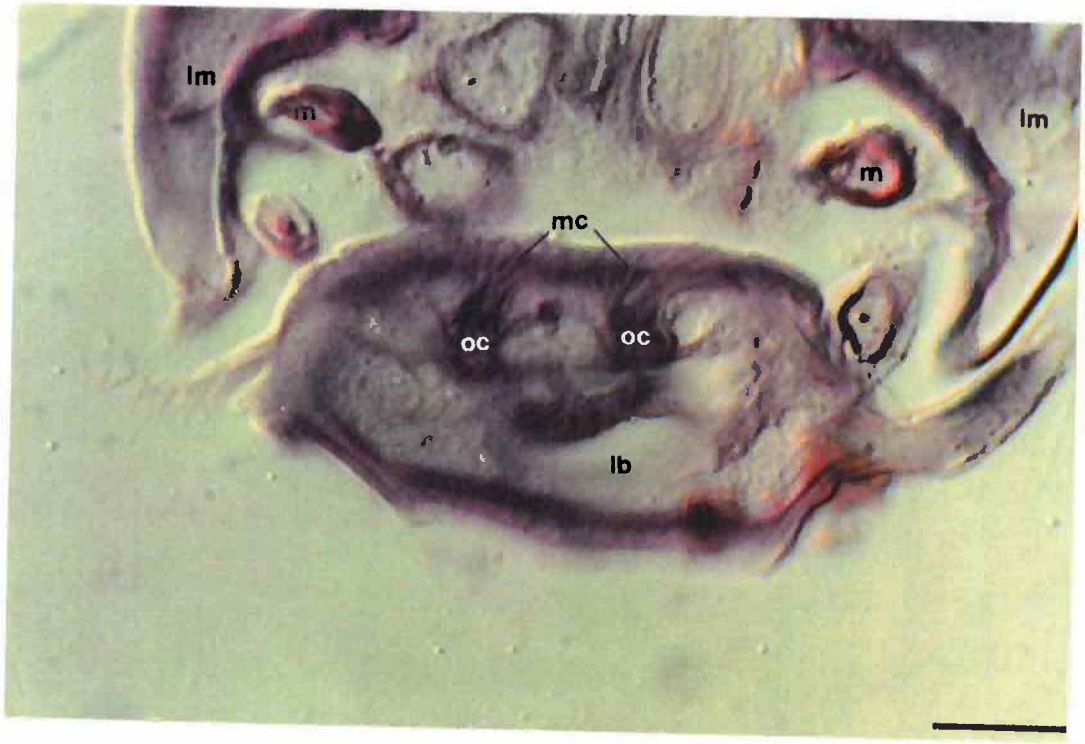


Plate 5.35 Labral organ (LMH, transverse chalimus, stained polychrome). lb: labrum; lm: labium; mc: microchannels; oc: organ components. Scale = 10μm

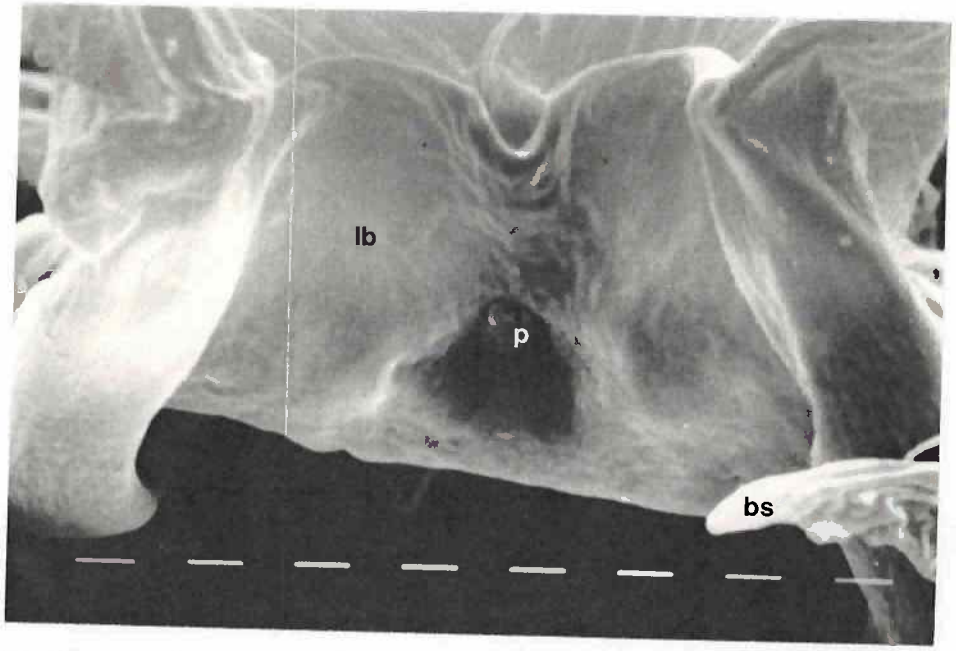


Plate 5.36 Posterior (intra-oral) aspect of copepodid labrum showing depression / pore associated with labral organ (SEM). bs: buccal stylet; lb: labrum; p: labral pore. Scale = 1 μm

5.5 Discussion

The present study indicates that the free-living phase of *L. salmonis* is lecithotrophic, having large numbers of cells possessing vesicles which probably contain lipid. In free-living copepods, early nauplius stages have similarly been reported to possess a high lipid content e.g. *Euchaeta japonica* Marukawa Nauplius II has 43.8% of dry weight as lipid (Lee, Nevenzal & Lewis 1974) and it is therefore not surprising to find nauplius and copepodid stages of parasitic copepods with condensed free-living larval phases to have similarly high lipid levels. Nauplius stages of several caligid species have been reported to have large "yolk" reserves filling much of the body cavity e.g. *C. curtus* (Heegaard 1947) and *C. elongatus* (Kunz 1985). The appearance of "yolk globules" within the gut of copepodid stages has been noted previously by Heegaard (1947) for *C. curtus* and Lewis (1963) for *L. dissimulatus*. In *Caligus* spp. lipid reserves may often be seen as pronounced "droplets" surrounding the gut of post-nauplius stages e.g. *C. savala* (Gnanamuthu 1948), *C. elongatus* (pers. obs.). Observations made in the present study indicate that the lipid stores of the copepodid of *L. salmonis* are intracellularly located within the walls of the midgut rather than occupying the gut lumen as suggested by Heegaard (1947) for *C. curtus*. Intracellular lipid reserves have also been noted in the columnar epithelial cells of the hindgut of the parasitic copepod *P. anemoniae* by Briggs (1977) and in the F cells of *M. intestinalis* by Gresty (1992). Lipid droplets have also been recorded from the midgut of adult *L. salmonis* (Nylund *et al.* (1992) where they were noted to be present in the apical region of "Type II" or "R" cells. The presence of lipid reserves is also a feature of free-living copepods e.g. the copepod *M. albidus* (Defaye *et al.* 1985). A study by Yoshikoshi (1988) demonstrated that when cream was fed to the free-living copepod *Tigriopus japonicus*, the contained lipids were absorbed and accumulated in vacuoles in the non-vacuolar cells of the midgut (these presumably corresponding to the columnar epithelial cells described by Briggs). In the brown shrimp *Penaeus aztecus* Ives, large lipid droplets have also been reported in the cells of the anterior midgut by Talbot, Clark & Lawrence (1972) and Al-Mohanna & Nott (1987) similarly note them to be present in the R cells of the hepatopancreas of *Penaeus semisulcatus*.

Although lipid composition was not analyzed in the present study, a study by Lee (1975) on *L. salmonis*, *L. oblitus* Kabata, *Clavella perfida* Wilson and *Caligus* sp. indicated that the major storage lipids in these species were triglycerides, which contrasts with the situation reported in free-living species whose major storage lipids are normally wax-esters. Host fish skin similarly contained triglycerides as their major neutral lipid. The hydrocarbon fraction of the copepod species studied by Lee was found to give a gas-chromatograph pattern identical to that of skin hydrocarbons of the host species.

From the above studies it would seem probable that copepodids of *L. salmonis* contain the remains of the naupliar lipid reserves within vesicles in the MCs. In the light of previous work, these reservoirs may well consist principally of host-derived triglycerides.

Despite the fact that the extensive lipid supplies of the copepodid seen in this study suggest that it is predominantly lecithotrophic it is possible that certain nutrients may be absorbed directly from the external environment. With regards to such a suggestion, Tester & Turner (1991) have demonstrated using fluorescently labelled dextran that nauplius stages of the free-living copepod *Acartia tonsa* (Dana) are able to uptake water and dissolved solutes. This may support the suggestion that nauplii and / or copepodids of *L. salmonis* could similarly absorb nutrients from the environment to supplement reserves already present at eclosion although this remains to be established.

The alimentary canal of *L. salmonis* copepodids is fully contiguous in both free-swimming and settled larvae in contrast to the situation in *L. branchialis* (L.), whose similarly lecithotrophic copepodids were reported to have no communication open between oral cone and midgut and midgut and hindgut (Pedaschenko (1898) cited in Heegaard (1947)). Capart (1948), however, observed the alimentary canal of larvae of the same species to be fully contiguous as found in the present study for *L. salmonis*. The present observations suggest that, once settled, the copepodid stage of *L. salmonis* ceases to be purely lecithotrophic and begins to feed on the host, this being indicated by the feeding damage observed beneath the oral cone and the host tissue remains observed within the alimentary canal. This agrees with the findings of Jones *et al.* (1990) and Johnson & Albright (1992a). At this stage, the extensive lipid reserves of the copepodid are no longer the only source of nutrition and are presumably largely exhausted by the time the copepodid moults to the chalimus stage, as demonstrated by the relative lack of lipid vesicles in chalimus I and in some settled copepodids. In the early copepodids of the free-living copepods *E. japonica* as reported in Lee, Nevenzel & Lewis (1974) and *Calanus helgolandicus (pacificus)* (Brodsky) as reported in Lee, Nevenzel & Paffenhöffer (1972), the level of lipid reserves may drop to less than a quarter of that of the early nauplius stages before storage resumes at copepodid III/IV. In the case of parasitic copepods such as *L. salmonis,* such resumption of storage is not necessary since they are in permanent contact with their food supply. It is interesting in this respect to note the presence of what appear to be substantial lipid supplies in chalimus, preadult and adult *Caligus* spp. (as already mentioned) which contrasts with the situation seen in *Lepeophtheirus* spp. This may relate to the relatively higher mobility of the former species (pers. obs.) and tendency to re-enter the water column in preadult and adult stages. It is envisaged that accumulation of lipids in *Caligus* spp. may occur through the mediation of "oil sacs" similar to those found in free-living copepods such as *Calanus*

finmarchicus and *Euchaeta norvegica* Boeck (Sargent & Henderson 1986) although no studies have been made of the actual mechanism to date.

The general structure of the alimentary canal of the copepodid and chalimus stages of *L. salmonis* is relatively unmodified in terms of morphological differentiation and cell populations with reference to free-living copepods such as *C. typicus* and *Calanus helgolandicus* Claus (Arnaud *et al.* 1978, 1980), *M. albidus* (Defaye *et al.* 1985) and *T. japonicus* Mori (Yoshikoshi 1975,). Like other minimally transformed species of parasitic copepod such as *L. pectoralis* (OF Müller) (Boxshall 1986, 1990), modifications principally concern the structures associated with food capture although little is known of biochemical / physiological modifications that may exist.

The oral cone closely fits the pattern reported previously for caligids (Kabata 1974a, Boxshall 1986, 1990) although the lack of a full seal between the labrum and labium reported here has not been noted for larvae of other species. Jones *et al.*(1990) have also noted the lack of closure of the copepodid oral cone. Because the copepodid, which is attached by rigid antennae, is more restricted in its feeding arena than the chalimus which possesses a flexible filament attachment to the host, the freedom allowed the mandibles by the lack of closure of the oral tube in the copepodid, might act to extend the possible range of feeding available to this stage. This open structure of the larval oral cone might, however, create problems for the generation of suction within the buccal cavity by the action of the well-developed labral muscles (as described by Boxshall 1990). This would be particularly important in view of the fact that no musculature has been noted in the present study in association with the foregut such that food could not necessarily be carried into the midgut by peristalsis alone (although this latter observation may be an artefact deriving from the limited resolution of the light microscope and the size of the specimens). This would therefore suggest either that the oral cone is "closed" by the proximity of the walls of the groove in the host tissue in which it lies or that its open state is an artefact occurring during processing. If the foregut is equipped with an appreciable muscle complement, it may be able to actively draw material up from the buccal cavity as suggested for adults of *L. branchialis* by Capart (1948) and *Calanus hyperboreus* (Krøyer) by Conover (1966).

Although Kabata (1974a) suggests the strigil to abrade host tissue allowing its movement into the buccal cavity of caligids, copepodids in the present study were often seen with apparently large pieces of tissue filling the entire cavity. The fact that large boluses of tissue were never observed in the foregut or anterior midgut and that, conversely, host cell fragments and amorphous material were, would seem to suggest that large fragments might be taken into the buccal cavity and subjected to preliminary digestion by labral or other glandular secretions. If this is the case (appropriate evidence from the present study being insufficient to confirm this suggestion), then the feeding mechanism of caligids needs to be carefully reconsidered since this

suggests that large pieces of tissue, rather than abraded cell debris, are being taken into the buccal cavity.

The strigil is apparently present in the copepodid stage although not visible under SEM and was therefore not observed in this stage by Johnson & Albright (1991a). It may, however, be that the copepodid in which the strigil was observed in the present study was close to moulting and that this was in fact the pre-formed strigil of the chalimus I. Given its recessed location, it is in any case considered unlikely to be functional in the copepodid. The strigil of the chalimus appears to comprise a single, medially indented structure rather than the divided bar described by Kabata (1974a). This may suggest that it is less flexible than was envisaged by Kabata and therefore that the two sides may not act to abrade tissue through divergence under dorsal pressure.

The general structure of the larval foregut of *L. salmonis* corresponds to that described for adults of other parasitic copepods such as *L. pectoralis* (Boxshall 1986, 1990) and *P. anemoniae* (Briggs 1977) although it is less spacious than that of the latter. Unlike the foregut of *M. steenstrupi* (Bresciani and Lützen 1962), the foregut of *L. salmonis* is not enlarged for storage, being a fairly fine tube, but may nevertheless vary in diameter according to the amount of food ingested as suggested for the H-shaped foregut of *T. californicus* (Sullivan & Bisalputra 1980). Like the foregut of *T. japonicus* (Yoshikoshi 1975), the foregut of *L. salmonis* projects markedly into the lumen of the midgut though does not possess a pronounced muscular sphincter at the opening into the midgut as does that of *B. palliata* (Boxshall 1982) and *E. placitus* (Boxshall 1985). There is, furthermore, no sign of a valve composed of dorsal and ventral flaps of tissue projecting from the roof of the lumen as found in *P. anemoniae* (Briggs 1977). The role of a valve may be assumed by the collar of enlarged epithelial cells surrounding the opening of the foregut into the midgut; these potentially close off the aperture of the foregut when material is not moving from the oesophagus into the midgut. John & Nair (1975) have suggested a similar function for the projecting rim of the foregut of *Pseudocycnus armatus*. No muscles were seen associated directly with the foregut in the present study although they have been observed in adults of the same species by Nylund *et al.* (1992) and are generally reported in other parasitic and free-living species.

The presence of a nerve within the epithelium of the foregut has not been previously noted although Johnson (pers. comm.) has also observed it in *L. salmonis*. This nerve was not observed in adults in the study of Nylund *et al.* (1992). Its function in this area is unclear particularly as it was not observed to extend as far as the junction with the midgut which therefore suggests that it does not concern the action of any muscle sphincter that might be present. The possible association between this nerve and the paired cells (neurosecretors ?)

observed requires further investigation and determination of its function therefore awaits a more detailed ultrastructural study.

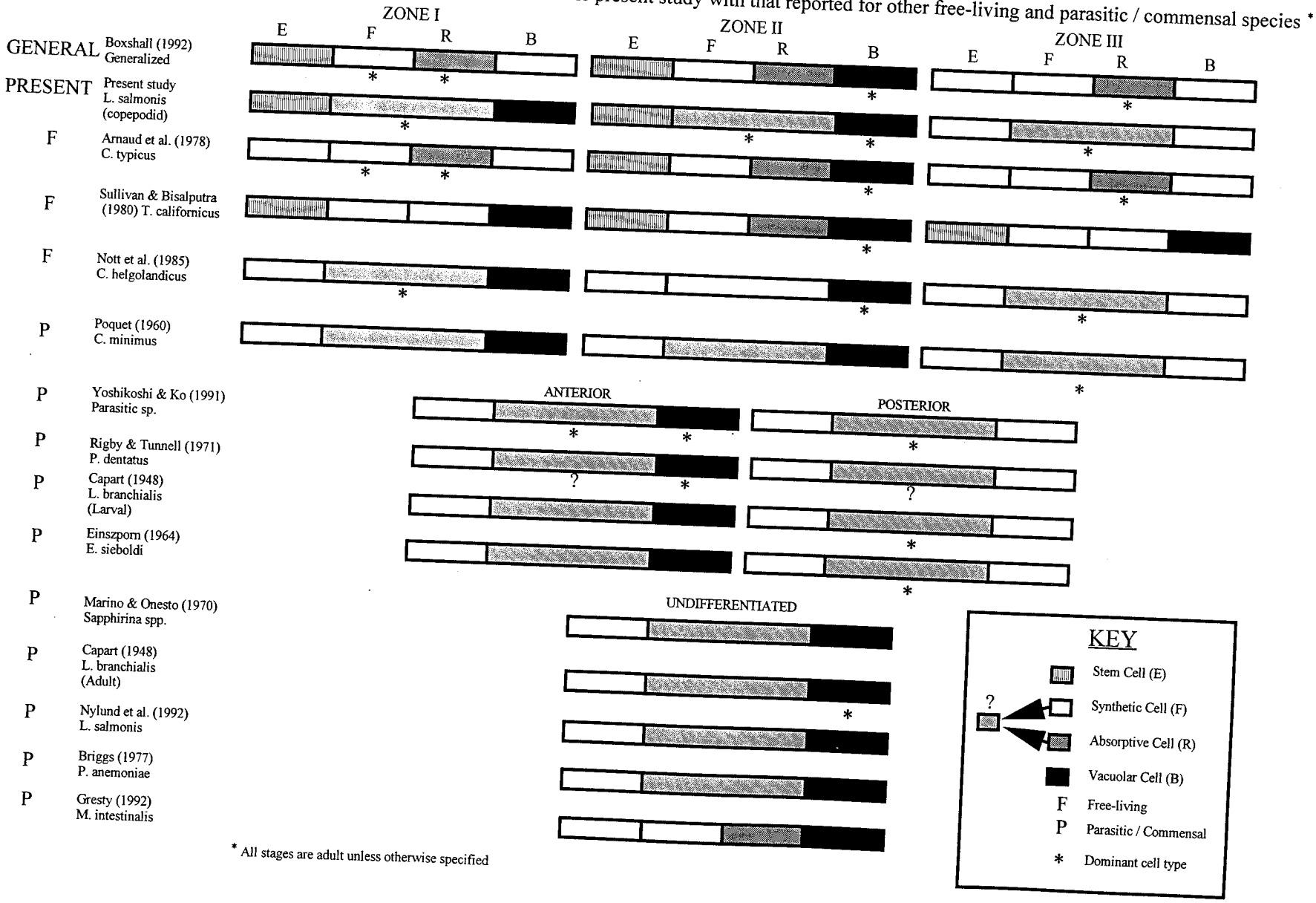
A graphical presentation allowing the comparison of the cell populations of copepod midguts is given in Figure 5.2. This compares the midgut zones and cell populations described by previous authors for other parasitic species and includes a generalized synthesis of the copepod midgut by Boxshall (1992) and a number of free-living copepods for reference. It can be seen from this diagram that the zones and cell types that comprise the midgut of *L. salmonis* closely resemble those previously described for free-living and other parasitic copepods.

The microvillous cells (MC's) of the present study correspond to the R (and possibly F) cells described by Arnaud *et al.* (1978, 1980) although it was not possible to differentiate the cells with the light microscope. R cells of copepods are believed to be principally absorptive (*ibid*) as are the corresponding cells in malacostracans (Al-Mohanna & Nott 1987). F cells, on the other hand, are presumed to secrete digestive enzymes by means of microapocrine or apocrine secretion (Arnaud *et al.* 1978, 1980). In the study of Arnaud *et al.* (1980), cells intermediate between R and F cells were observed in a number of calanoids and notably in *C. helgolandicus* and *Acartia clausi* Giesbrecht. It was suggested in this study that this might indicate that these cells are related and form part of a developmental continuum. Degenerate R cells, as described by Arnaud *et al.*(1978) and Nott *et al.* (1985) were not observed in the present study and a similar lack of the dark-staining degenerative columnar epithelial cells described by Yoshikoshi (1975) in *T. japonicus* was observed.

Most studies of parasitic species (see Figure 5.2) have not distinguished different types of microvillous epithelial cell save for the study of Gresty (1992) on *M. intestinalis*. This may reflect a general lack of ultrastructural study of these species to date or may alternatively stem from the fact that the R and F cells form a continuum of development as noted above for some calanoids. With respect to this suggestion it is therefore important to note that Nylund *et al.* (1992) have made the observation that cell types I & II observed in the alimentary canal of *L. salmonis* are similarly related, as demonstrated by the presence of cells having intermediate morphologies.

The vesicular cells (VC's) described here seem identical to the B or vacuolar cells described by Arnaud *et al.* (1978) which undergo a sequence of development culminating in extrusion into the gut lumen. According to the same authors, B cells are believed to carry out the following functions: (a) ingestion of material from the midgut by pinocytosis (b) development of heterophagosomal structures with intracellular digestion of pinocytosed material and (c) extrusion of digested material for reabsorption by absorptive (R) cells. Briggs (1977) suggests that similar cells in *P. anemoniae* take up host mucus and this might explain the positive PAS

Figure 5.2 Comparison of midgut cellular compostion determined in the present study with that reported for other free-living and parasitic / commensal species *



* All stages are adult unless otherwise specified

reaction of the vesicular cells in the present study. Moore, Lowe & Gee (1978), looking at similar apical vesicles in *M. intestinalis*, found strong staining for β-glucuronidase, acid phosphatase and glucosaminidase similarly suggesting an intracellular lysosomal digestive function. Fahrenbach (1962) in contrast, ascribed a secretory function for similar vacuolar cells in the harpacticoid *Diarthrodes cystoecus* Fahrenbach. In the present study, little indication of vesicular cell degeneration (ie extrusion of vesicular cells into the gut lumen) was seen, although the number degenerating at a **given** point in time might be expected to be small despite a rapid overall turnover. Unlike the B-cells of Arnaud *et al.*(1978), however, the vesicular cells in this study also appeared to be present in the anterior midgut caecum (Zone I). The observation of vacuolar cells in Zone I is not, however, limited to the present study and has also been reported by Poquet (1960) in *C. minimus*, Sullivan & Bisalputra (1980) in *T. californicus* and Nott *et al.* (1985) in *C. helgolandicus*. In the latter study the development of vacuolar cells in Zone I occurred during feeding which suggests that the boundary between zones is, to a great extent, artefactual and that cell populations are extremely plastic in their composition. Nylund *et al.* (1992) found no differentiation of the midgut into zones in adult *L. salmonis* and reported B cells to be distributed throughout. This would appear to support the concept of a degree of plasticity in the composition of the midgut cell population and therefore the boundaries between notional "zones".

Although Arnaud *et al.* (1980) failed to find any relationship between mode of nutrition and structure of the alimentary canal, it may be of importance to note that the alimentary canal of *M. albidus*, a carnivorous freshwater copepod described by Defaye, Such and Dussart (1985) showed an apparent amalgamation of cellular functions within microvillous epithelial cells suggested to correspond to the F cells of Arnaud *et al.* (1978, 1980). Although vacuolar cells were observed in this species, these were not identified with B cells and like F cells were suggested to secrete enzymes. These cells were also believed to carry out pinocytosis of material in the midgut lumen, intracellular digestion within vacuoles with the production of sphaerocrystals, storage / catabolism of lipids, exocytosis of peritrophic membrane material and transport of assimilated material to the haemolymph. These cells would therefore appear to have subsumed the functions of B, R and F cells. The fact that R and F cells may form a continuum in many parasitic species thus far described may represent a step towards the reduction of cell types required for digestion in the same way that the digestive / absorptive elements of the alimentary canals of mammalian carnivores are simplified with respect to their herbivorous counterparts.

From Figure 5.2 it can be seen that previous studies of both parasitic and free-living copepods generally describe the presence of vacuolar cells in Zones I and II of the midgut although with parasitic species, the midgut is often differentiated only into an anterior "stomach"

and a posterior "intestine" or sometimes is undifferentiated with respect to its cell population. In the hepatopancreas of *P. semisulcatus*, vacuolar B cells were suggested to be developed directly from synthetic F cells via intermediate forms (Al-Mohanna, Nott & Lane 1985, Al-Mohanna & Nott 1986). In the present study no cells intermediate between MCs and VCs were seen which suggests that these cell types are not related, a conclusion supported by Nylund *et al.* (1992) in observations of adult *L. salmonis*. Arnaud *et al.* (1978, 1980) reported a similar conclusion for the F and B cells of the midgut of calanoids studied.

The basal cells (BC's) seen in this study correspond in some respects to the D-cells described by Arnaud *et al.*(1978) which were similarly found in the vicinity of vesicular (B) cells, although the cells in the present study often had no apparent communication with the gut lumen. The fact that basal cells are observed both with and without a luminal surface suggests that the former cells may develop from the latter. Arnaud *et al.* (1978) suggested that D cells may possess an absorptive capacity, whilst Boxshall (1992) groups them with the synthetic F cells. An alternative, and possibly more likely suggestion as to the identity of these cells is that they represent the E (stem cells) (*ibid.*) which are undifferentiated cells functioning in replacing senescent cells or those lost in secretion. The observation of such cells in the present study is supported by the study by Yoshikoshi & Kô (1991) of the midgut of a variety of parasitic copepods, which indicated that vacuolar cells in these species developed from similarly undifferentiated cells lying in the midgut epithelium. The facility for development of vacuolar cells from undifferentiated cells may explain the variation in zone allocation seen in studies in general. Studies by Gresty (1992) on *M. intestinalis* and Nylund *et al.* (1992) on adult *L. salmonis*, however, revealed no evidence of E cells.

A study by Yoshikoshi & Kô (1988) suggested that in the free-living copepod *T. japonicus* the midgut was the only site of absorption of nutrients. From the similarity between the alimentary canal of the copepodid of *L. salmonis* and that of free-living species, it may be reasonable to assume that this is also the case in this instance. As can be seen from Figure 5.2, the zones of the midgut of *L. salmonis* correspond to the three zones suggested for free-living copepods, such as *Centropages typicus* and *Calanus helgolandicus* by Arnaud *et al.* (1978, 1980) and Sullivan & Bisalputra (1980). Although the zones described in the present study correspond closely to those reported for the parasitic *Caligus minimus* by Poquet (1960) they are at odds with reports on other species which generally note differentiation of the gut into only an anterior and posterior zone or, alternatively, describe no differentiation. Nylund *et al.* (1992) in their studies of the midgut of adult *L. salmonis* found no differentiation of the midgut in contrast to the present findings.

In the present study, the highly convoluted anterior midgut caecum (Zone I of Arnaud *et al* 1978) is equipped, in the area of entry of the foregut, with a large number of VCs which

suggests high digestive activity. The ventral epithelium of this area shows considerable expansion in its depth and number of cells. These cells may correspond to the E cells of Arnaud *et al.* (1978) in which case they may function in replacing cells lost through rapid turnover during digestion of material entering from the foregut. Since no staining for acid mucopolysaccharides occurs posterior to the anterior midgut (Zone II), it would seem that these are digested, and possibly also assimilated, in this zone. The rarity of large tissue remains in the midgut, suggests that they must be effectively comminuted by the action of the mouthparts and labral / other secretions before entry into the midgut. It may also be that some digestion of material occurs in the preceding foregut through movement of digestive enzymes from the midgut into the foregut as suggested for *T. californicus* (Sullivan & Bisalputra 1980). It may even be, although no evidence has been presented to support this in the present study, that enzymes from the midgut pass down the foregut onto the host tissue in order to effect predigestion of material. Such behaviour would not be unique in arthropods since it has been previously noted in lepidopteran larvae by Felton & Duffey (1991). With respect to this, it has previously been suggested by Halisch (1940) that ergasilids might secrete some enzymatic secretion around the mouth although this suggestion was contradicted by Einszporn (1965) who found no evidence for any glands associated with such production and believed that the dilution that would be experienced by such a secretion when exposed to the external medium would render it useless. Though convoluted, the anterior midgut caecum of *L. salmonis*, like that of *L. pectoralis* and *H. typicus* (Boxshall 1990) is not enlarged to act as a storage area as it is in many siphonostomatoids and in predatory copepods such as *E. placitus* (Boxshall 1985). It is probable that the caecum is not modified for storage because such parasites are likely to be in permanent rather than intermittent contact with their food supply (as previously suggested by Boxshall 1985).

The caecum of *L. salmonis* copepodids was not observed to possess a muscular sphincter to close it off from the rest of the midgut as in adults of *B. palliata* (Boxshall 1982) and *Mormonilla phasma* Giesbrecht (Boxshall 1985) nor is there a constriction apparent in this area as in *E. placitus* (Boxshall 1985). Because the cellular composition of the anterior midgut caecum is similar to that of the anterior midgut itself, it may be that closure for digestion is unnecessary until the point of entry into the posterior midgut.

The anterior midgut of *L. salmonis* (Zone II of Arnaud *et al.* 1978, 1980), like that of free-living copepods (*ibid.*) is fairly tubular and is not split into digestive diverticuli as was observed in *M. steenstrupi* by Bresciani and Lützen (1962) and *Sapphirina angusta* Dana by Marino & Onesto (1970). Like the anterior midgut caecum, the combination of VCs and MCs suggests a digestive / absorptive function for this area. Although there is no facility for storage of material, the midgut of *L. salmonis*, like that of *B. palliata* (Boxshall 1982) is fairly distensible such that "meals" could be loaded into the anterior midgut and left for a period to

digest before evacuation of one "meal" and the loading of another. As with many copepods the passage between the anterior midgut and the posterior midgut is constricted. Whether this constriction acts as a fully closing valve, or results simply from the position of the gut at this point with respect to the exoskeleton and other organs, is not certain.

The wall of the posterior midgut, being composed solely of MCs, may be presumed to be principally absorptive in function. These MCs are likely to be R cells as they possess long microvilli and vesicles which may contain lipids. This corresponds to the situation seen in free-living species e.g. *C. typicus* (Arnaud *et al* 1978) although no such differentiation of the cell population into anterior and posterior midgut was noted by Nylund *et al* (1992) for adults of the same species or Gresty (1992) for *M. intestinalis*. The absorptive capacity of the posterior midgut is enhanced by its narrowness and rugose construction. As with *B. palliata* (Boxshall 1982) the posterior midgut is separated from the hindgut by a strong sphincter composed principally of circular muscle bands with large lateral muscles inserting between them. The large cells, here termed "guard cells" that project into the lumen posterior to this area have also been noted for *B. palliata* (*ibid.*) and similar cells have been noted in adult *L. salmonis* by Nylund *et al.* (1992). Their function remains undetermined from studies thus far, although they may be involved in osmoregulation.

The structure of the hindgut corresponds to that of free-living copepods, lacking the reduction seen in *Pseudocharopinus dentatus* (Wilson) (Rigby and Tunnell 1971) and *M. steenstrupi* (Bresciani and Lützen 1962). The large transverse muscles allow rhythmic contractions of the hindgut. A respiratory function in these movements was suggested by Wilson (1905) for caligids. Gnanamuthu (1948) suggested that the rhythmic amphidirectional contractions in the gut of *C. savala* concerned digestion, osmotic adjustments or respiration and Sullivan and Bisalputra (1980), working with *Tigriopus californicus*, suggested that the thinly chitinised hindgut of this species might also be suited to water transport / osmoregulation, this suggestion being further supported by the presence of mitochondria and the invagination of the luminal surface of these cells.

In free-living copepods such as *C. helgolandicus* the midgut has been shown to undergo cyclical changes within the cell population which correspond to feeding (Nott *et al.* 1985). The onset of enzyme production in free-living copepods through changes in the midgut epithelium, has been suggested to be initiated by filling of the stomach (anterior midgut) which is believed to stimulate secretion by the F cells (Mayzaud 1986). An alternative mechanism could also be suggested although at this point it is purely speculative, whereby the nerve observed within the wall of the foregut of *L. salmonis* in the present study could relay information about entry of material into the buccal cavity to the midgut. If this information consisted of a neurosecretion

(possibly explaining the presence of a possible neurosecretory organ associated with the nerves) then the latter could act upon the large number of apparently undifferentiated cells comprising the epithelium at the point of entry of the foregut into the midgut causing them to differentiate into MCs and / or VCs. The products of these cells would then stimulate differentiation and development elsewhere in the midgut. Mitotic division of the BCs as described by Al-Mohanna *et al.* (1985) for E cells of the hepatopancreas of *P. semisulcatus* following extrusion of B cells (VCs in this study) would then serve to "reprime" the midgut epithelium for the next "meal". For the time being, however, such a theory relies on purely circumstantial evidence.

In the present study, changes of the midgut epithelium were not examined although individuals with large amounts of material within the midgut showed a lessening of the epithelium consistent with the loss of VCs through extrusion into the lumen and loss of MCs in the production of a peritrophic membrane, as described in the study of Nott *et al.* (1985). In this latter study it was suggested that the loss of cells would be such as to require a refractory period for recovery and thus limit the period of feeding. This suggests that feeding in *L. salmonis* might not be continuous. *L. cyprinacea* was observed by Yoshikoshi & Kô (1988) to defecate every 32 $\pm$ 3.8 min (19 $\pm$ 3.1 min with minimal gut content) which would seem to suggest either a very short refractory period or continuous feeding. It may, however, be relevant to note that the latter species has been suggested to be a liquid feeder (Egusa 1991), unlike *L. salmonis* in the present study, which might allow continuous ingestion and digestion of food to occur.

The presence of bacteria in the gut of adult *L. salmonis* has been reported by Nylund *et al.*(1991) who noted that their presence was uncommon. Because bacteria were not usually present in the gut of healthy individuals in the present study and, when present, did not occur in large numbers, it is suggested that they are not commensal as was suggested to be the case with those of *Pseudomyicola spinosus* Raffaele & Monticelli (Yoshikoshi & Kô 1991). It appears that the bacteria observed in this study have no function in digestion but are, rather, accidentally ingested along with host mucus and epidermal material. A similar conclusion of accidental ingestion was drawn by Nott *et al.* (1985) to explain the presence of bacteria in the alimentary canal of *C. helgolandicus*.

The nature of the large electron dense granules seen in the midgut is uncertain, particularly in view of the fact that their origin within the copepodid could not be determined. This latter may in fact signify that they are host derived. Many cellular products show similar banding when viewed under TEM including proteins, lipids / phospholipids, polysaccharides and ferritin granules (Threadgold 1967). Within the arthropoda, banded protein crystalloids have been noted in the fat body vacuolar system of insects (Locke 1984), and intra-cellular and intranuclear protein crystalloids as well as intranuclear ferritin granules have also been reported from the

columnar cells of the insect alimentary canal in several species (Martoja & Ballan-Dufrançais 1984). Crystalline cores have also been noted in vacuoles in the tegumental glands of the free-living copepod *T. longicornis* (Bannister 1993) and in vacuolar cells of the midgut of the parasitic *L. cyprinacea* by Yoshikoshi & Kô (1991) although no description was given of their detailed structure in the latter study.

Ferritin crystals have been reported *inter alia* in the gut caeca of stegocephalid amphipods (Moore & Rainbow 1984) and in the attachment organ of the pennellid copepod *C. medusaeus* (Perkins 1985). In both these studies, production of ferritin was considered to be part of a process of iron detoxification and storage associated with high iron in the diet, provided by host haemoglobin in the case of *C. medusaeus* and possibly by feeding on iron-rich cnidarians in the case of the stegocephalids. The inter-band widths in these latter studies were 0.0046 μm for *C. medusaeus* and 0.001 μm for the stegocephalid *Stegocephaloides christianensis* Boeck which are an order of magnitude smaller than those seen in the present study (0.025 μm). Although copepodids of *L. salmonis* have occasionally been observed to carry host erythrocytes within the gut it is believed that these are likely to be a rare component of the diet of this stage since feeding is very superficial and thus detoxification would not seem to be necessary. Ferritin production might, however, occur as a preadaptation for such a function in later stages as these have often been observed with blood-filled alimentary canals, particularly in the case of adult females, and have been reported by Brandal, Egidius & Romslo (1976) and Brandal (1977). The difference in inter-band distance between the studies of Moore & Rainbow and Perkins and the present one may, however, suggest that the crystalloids are not related.

Faecal pellets produced by *L. salmonis* appear to have a peritrophic membrane, in common with those of free-living copepods such as *Calanus* (Gould 1957) and all of the 25 free-living, commensal and parasitic species examined by Yoshikoshi & Kô (1988). No peritrophic membrane was observed by Briggs (1977) in the gut lumen of *P. anemoniae* and similarly none was observed within the gut lumen of *L. salmonis* in the present study. The failure to find a peritrophic membrane within the alimentary canal concurs with the findings of Nylund *et al.* (1992) for adults of the same species, although it is not believed to be indicative of the fact that the membrane is not in fact present. Peritrophic membranes have been described in a number of copepods parasitizing fish hosts by Yoshikoshi & Kô (1991) and are present in *E. sieboldi* (Einszporn 1965).

Although some peritrophic membranes are described as being mucus-like, such as that of *E. amphitrites* McMurrich (Park 1966), that described here was fairly rigid, like that described by Gnanamuthu (1948) for *C. savala*, who suggested it to be composed of "condensed mucin". A more recent study by Yoshikoshi & Kô (1988) indicates that the peritrophic membranes of the free-living copepod *T. japonicus* and the parasitic species *Panaietis* Stebbing

both consist of chitinous microfibrils in a ground substance containing acid mucopolysaccharides and proteins which concurs with the findings in other arthropods.

The peritrophic membrane is thought to be produced in the midgut and in *E. amphitrites*, it was suggested to derive from vacuolar cells (Park 1966). Yoshikoshi (1975) noted that in *T. japonicus*, a thin membrane was always seen to enclose ingested food in the midgut. A study by Yoshikoshi & Kô (1988) has subsequently revealed that whilst in free-living species studied the peritrophic membrane was present in both the anterior and posterior part of the gut, in most commensal and parasitic species studied it was only present in the posterior midgut. Nott *et al.* (1985), however, believed the peritrophic membrane of the free-living *Calanus helgolandicus* to arise from necrotic R cells ("N" cells) situated posterior to the vacuolar region of the midgut, in the zone that would equate to the posterior midgut of other species. In the study of Yoshikoshi & Kô (1988) it was further noted that the peritrophic membranes of free-living species tended to be thicker than those of their commensal / parasitic counterparts.

Four functions have been proposed for peritrophic membranes in arthropods / copepods, these being discussed by Yoshikoshi & Kô (1988). The four functions suggested are:

1) Protection of the midgut epithelium from abrasion by hard components in food material. This was suggested by Yoshikoshi & Kô (1988) to be more important in particle feeders (free-living copepods) than commensal / parasitic species thus possibly explaining the thicker membranes in the former group.

2) Avoidance of reingestion of food remains through sinking of pellets out of the feeding zone (this being clearly less important for parasitic / commensal species such as *L. salmonis*).

3) Assistance in preventing material passing through the gut too quickly (again possibly less important in species permanently attached to their food supplies).

4) Filtration of ingested material giving more economic and effective use of secreted enzymes. This was suggested to be the function of overriding significance for all the species studied by Yoshikoshi & Kô (1988).

With reference to this latter point, a mechanism has been proposed for insects that is suggested to function in the conservation of enzymes involved in extracellular digestion. It has been suggested (Terra, Ferreira & de Bianchi 1979, Terra & Ferreira 1981) that in the sciarid fly *Rhynchosciara americana* (Wiedemann), the presence of a peritrophic membrane acts to

separate extracellular sites for digestion ie providing endo- and ectoperitrophic spaces. By this theory, digestion occurs in three phases :

1) Food is initially broken down by enzymes within the peritrophic membrane (endoperitrophic space) into units small enough to pass out through it.

2) These smaller units are further broken down by other enzymes in the ectoperitrophic space.

3) The terminal digestion of the products of the previous partial digestion occurs intracellularly or at the apical cell membranes.

In order to conserve enzymes in the endoperitrophic space before they pass out in the faeces, it is suggested that they move out of the membrane in the posterior part of the midgut (no longer being bound to large food molecules) and are carried back up the midgut by water passing through the ectoperitrophic space to be recycled in the anterior part of the midgut. In *Rhynchosciara* sp. water is suggested to be absorbed in the anterior midgut caecum and possibly secreted via a pump in the ventriculus. In copepods liquid in the midgut might be passed along the ectoperitrophic space by the peristaltic waves seen in live specimens and would carry enzymes and products of extracellular digestion into the anterior midgut for recycling of the enzymes and further digestion / absorption of the products of initial digestion. Unlike the situation in insects, the intracellular digestion occurring in the B cells of copepods (VCs of this study) is followed by extrusion of these cells and digested material into the lumen. Whilst larger waste products would remain within the peritrophic membrane for evacuation, the smaller products of digestion would pass out of the peritrophic membrane to be absorbed by the R cells (MCs).

Although Capart (1948) noted a lack of glands associated with the alimentary canal of *L. branchialis* and similarly Einszporn (1965) could find no associated glands in *E. sieboldi*, four groups of putative glands have been described in association with the area of the oral cone in the present study. The PAS-positive glands with ducts appearing to open onto the ventral surface beside the oral cone have granules staining similarly to those in the median gland and are positioned in the same area as the laterodorsal units (LDU) described by Arnaud *et al.* (1988) for *C. typicus*. These authors likewise were unable to discover the area of the ductal openings. These glands would also appear to be very similar in size and disposition to the "maxillary gland" described by John & Nair (1975) for *Pseudocycnus armatus*. This gland was observed

by these authors to be located to either side of the oesophagus with the ventral end terminating in a short duct opening to the ventral surface in the angle between the mouth and the maxilla. In the same study it was suggested that the product of the gland might be an anticoagulant and that the gland was derived from the maxillary gland of free-living copepods (e.g. *Diarthrodes cystoecus* (see Fahrenbach 1962) and *Epilabidocera amphitrites* (see Park 1966)) which is normally described as a coelomic structure associated with excretion. The present study would seem to indicate that such an origin is unlikely for the gland described. Aside from the fact that the gland is clearly actively secreting a PAS-positive product and that a number of features of the free-living maxillary gland are missing ie *inter alia* the coelomic end-sac, podocytes and microvillous-bordered epithelium, the gland presently described appears identical in structure and secretory product to the mucous gland situated anterior to the nauplius eye and posterior to the A-gland described in the previous chapter. Its dorsal surface is furthermore abutted against this gland and separated from it by a membrane partition. This suggests that these two glands have a similar origin. Some harpacticoid copepods e.g. *D. cystoecus* and *D. nobilis* are described by Fahrenbach (1962) and Hicks & Grahame (1979) respectively, as having prominent cephalothoracic mucus reservoirs and associated glands. Such harpacticoid glands may have a similar ancestral origin to those of *L. salmonis* copepodids but differ markedly in the alcian blue positive staining of the product (signifying acid mucopolysaccharides), the lack of obvious granules and the non-syncytial nature of the associated secretory glands. The structure of the "cauliflower organ" described in Chapter 3 suggests that it may be the site of glandular discharge.

The glandular complex located anterior to the labrum and apparently discharging at the base of the anterior labral face closely resemble, in location at least, the "labral glands" suggested to be present in most copepods (Boxshall 1992). The descriptions of labral glands in the literature vary enormously, such that, apart from their similar locations and areas of discharge, it is difficult to ascribe a common identity to them. Glands in the same area have also been noted in adult *L. salmonis* by Turnbull (1991), Andrade-Salas (pers. comm.) and Petruch (1993). The latter author also noted a single large pore to be present in the area suggested for the discharge of these glands in the present study, which was present in adult, preadult, chalimus and copepodid stages of *L. salmonis* but was absent in adults of *C. elongatus*.

The complex described in the present study would appear to more closely resemble those described by Boxshall (1982) for *B. palliata* which similarly discharge their products down a common duct, than those described by Lowe (1935) for *Calanus finmarchicus* (Gunnerus) and Park (1966) for *Epilabidocera amphitrites,* which empty down separate ducts. Boxshall's study of *B. palliata* revealed a pair of multilobed glands comprising six major lobes with varying staining characteristics which were observed to be comparable with those reported in freshwater

cyclopoid and calanoid copepods by Richard (1891) cited in Boxshall (1982). These glands may be related to, but are not identical to, those seen in the present study. Fahrenbach (1962) has reported a similar formation of glands to that seen in the present study in the harpacticoid *Diarthrodes cystoecus* in which the labrum contains a median gland and two lateral glands resting against its anterior surface. Glands which may also correspond to those reported in this study were noted by Scott (1901) for *L. pectoralis* and Wilson (1905) for the same species, both authors noting two large lateral glands and a smaller median gland. In these reports, however, the lateral glands had ducts passing to the median gland and the latter had a single duct which passed into the midgut caecum. Although this would seem to correspond closely to observations in the present study, no duct has been observed passing into the caecum from any gland, and the gland complex reported here appears to empty on to the anterior surface of the proximal region of the labrum instead.

The glands in the present study also resemble in some respects those described by Arnaud, *et al.* (1988) in the free-living copepod *Centropages typicus* (Krøyer, 1849). In this species two symmetric gland clusters on the left and right sides of the labrum are described which, unlike the glands in the present study, open via a number of pores. The first of these clusters is large and located in the anterior distal area of the labrum ("Anterior Unit" - AU), the second is composed of ~ 12 units located in the lateral posterior area (Lateral Posterior Units - LPU). Other secretory units were also observed more dorsal to and posterior to the labrum (Laterodorsal Units - LDU). The main similarities occur in the ultrastructure of these glands which are syncytial as are those of the present study. These glands possess large amounts of RER, numerous dictyosomes and large numbers of secretory "granules" which resemble the vesicles observed in the present study. Some vesicles seen in the lateral glands of *L. salmonis* closely resemble those of the AU and LPU glands of *C. typicus* in terms of their large size and fibrillogranular content although the striate vesicles seen in the present study were not observed in any of the glands described by previous authors. The densely staining material noted in vesicles of the median gland in the present study also resemble those recorded for the LDU of the study cited. Unlike those of this latter study, the glands of the present study are not composed of clusters of units but of separate syncytia with different products.

A major difference between the glands described in the present study and those described for other species is the fact that firstly the external pore is single and secondly that it discharges onto the anterior face of the labrum rather than the posterior face which would bring the secreted products into the interior of the buccal cavity. It may be then, that the median gland at least, rather than being related to the labral glands of other species, is more closely related to the tegumental / sub-cuticular glands frequently reported in other copepods e.g. *inter alia* in *Hemidiaptomus ingens provinciae* (Petit & Schachter) by Brunet, Cuoc, Arnaud & Mazza (1991)

and *T. longicornis* by Bannister (1993). These similarly discharge through single pores and under TEM appear to contain large quantities of RER and numbers of secretory granules (described as vesicles in the present study). In the former study, the glands were suggested to be syncytial as found for the medial gland in the present study whilst in the latter the gland was unicellular (although associated with other cells). Further evidence for supporting the identification of the medial gland of the present study with previous descriptions of tegumental glands comes from the fact that this gland was found to have a similar appearance and staining properties to glands associated with the swimming legs and urosome in *L. salmonis* copepodids. These glands are thought to be likely to correspond to the sub-cuticular glands reported to be associated with the swimming legs of e.g. *T. longicornis* Bannister (1993).

The third glandular group described in the present study may be related to the "labial" or "paragnathal" glands noted by Fahrenbach in *D. cystoecus*. These were described as consisting of two groups of paired cells with ducts extending into the labium although as in the present study, their area of discharge could not be located. The strong PAS-positive reaction seen in the labium in certain individual settled copepods may indicate that activity in these glands is stimulated by host presence (such staining was **not** observed in free-living copepodids).

The nature of the "distal labral organ" is uncertain. Although it may be nervous in origin, no evidence for this was forthcoming in the present study although it might easily be established by the use of suitable stains for nervous tissue. The other possibility for its function stems from the similarity of the fibres / ducts observed to the ducts said to connect small dermal glands to pores in the teeth of the calanoid copepods *Neocalanus spp.* and *Calanus pacificus* Brodsky (Miller, Nelson, Weiss & Soeldner 1990). In calanoids it was suggested that such glands might secrete either toxins or digestive enzymes.

No "digestive glands" were seen associated with the mid- or hindgut in contrast to the observation of such glands by Scott (1901) who noted paired glands between the first and second thoracic legs of *L. pectoralis* with ducts entering near the posterior end of the "stomach", an observation echoed by Wilson (1905) for caligids in general. Two syncytial organs which were noted ventrally to the midgut just anterior to the interception of the latter by the foregut could not be identified and therefore no function could be attributed to them. An absence of digestive glands has been specifically noted for *P. anemoniae* (Briggs 1977) and *T. japonicus* (Yoshikoshi 1975) and their absence is a feature of morphological reports on copepod alimentary systems in general. Presumably the vesicular cells normally found in copepods carry out the functions of the digestive glands found in other crustacean groups.

The function of the glands described in the present study and indeed in previous studies is difficult to determine without detailed biochemical investigation. Labral glands in calanoids which have been suggested to produce mucus to protect the gut from its own secretions (Ong and

Lake 1969) were also believed to lubricate the passage of food through the foregut. Arnaud *et al.* (1988) suggest that the labral glands described for *C. typicus* produce a mixture of mucopolysaccharides and glycoprotein enzymes which serve respectively to agglutinate and digest food particles although Yoshikoshi & Kô (1990) found no evidence of hydrolases in the labral glands of a number of parasitic species tested. Evidence for the presence of mucopolysaccharides in the present study derives from the PAS-positive staining characteristics of products of all the glands described and from the obvious dictyosomes present in the cytoplasm. The contents of many of the vesicles also appears fibrillogranular which further supports the suggestion of a mucopolysaccharide product. Evidence for the possible production of enzymatic or proteinaceous products comes from the presence of abundant RER in the cytoplasm of the glands described. If enzymes are produced by the various glands described then it may be reasonable to ascribe the rapid disappearance of host acid-mucopolysaccharides in the anterior midgut in part to the action of these products. The location of the site of discharge of two of these systems suggests that the products of the glands are applied directly to the host tissue. This may suggest a certain pre-digestion of the host tissue, although no signs of such damage were apparent in the histopathology. It may be that any enzymes present act to digest the host intercellular adhesives making the tissue easier to remove and propel or suck into the buccal cavity. The mucus content of the various secreted products may serve to bind enzymatic products and assure that they maintain contact with host tissue and are less subject to the dilution by the external medium which was suggested by Einszporn (1965) to make the use of external enzyme secretions impractical.

Another possibility for the function of the medial gland in the copepodid is raised by the finding of peroxidase activity apparently associated with the gland. This finding has been confirmed by staining of the adult gland by Turnbull (1991), Andrade-Salas (pers. comm.) and Petruck (1993) who found staining to be localised to the dark granules seen in light microscope sections and presumed to correspond to one of the vesicular types seen in the present study under TEM. Peroxidases have a wide variety of biological functions and have been suggested to be involved in the cuticle tanning of arthropods by Neville (1975) who suggests that they may be utilised in the formation of covalent biphenyl links between the tyrosyl residues of proteins, leading to tanning. The discharge of peroxidase in the vicinity of the oral cone and other areas of the cuticle might therefore be linked to repair of abrasion damage. This may be particularly important in the copepodid of *L. salmonis* since there was no observation of pore canals in the cuticle during the present study. Although pore canals were similarly found to be absent by Bouligand (1966) in the free-living copepods *Acanthocyclops viridis* (Jurine) and *Clausocalanus arcuicornis* Dana, they were present in *Porcellidium* spp. (Gharagozlou-van Ginneken & Bouligand 1975) and *P. anemoniae* (Briggs 1978). Since their function is thought to be in

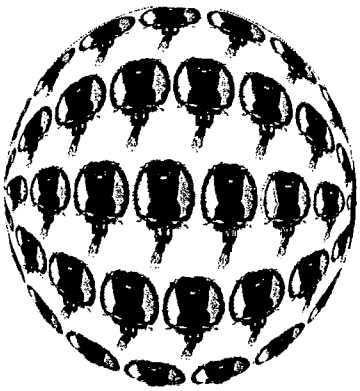
transport of wax for cuticle surface water-proofing and transport of impregnating materials for cuticle tanning (Neville 1975), their absence may leave a requirement for alternative repair mechanisms. Such repairs may be particularly important in the cuticle of structures or appendages showing high mobility such as the oral cone. Such structures are likely to suffer abrasion and may in addition experience an enhanced non-specific or specific host response in the form of an inflammation response following the release of pharmacokinetic amines (e.g. histamine) which would occur following host tissue damage (see Roberts 1989 for details of the inflammation response in teleosts). Such host responses might be expected to degrade the cuticle leading to a requirement for repair.

Peroxidases in biological systems have also been suggested to act in a protective capacity in that they may function to prevent oxidative damage and accumulation of toxic free-radical products through reduction of hydrogen peroxide, lipid hydroperoxides and other organic hydroperoxides (Tappel, Chaudière & Tappel 1982). Free-radicals such as the superoxide anion O_2^- and other products such as hydrogen peroxide are produced as a result of partial reduction of oxygen in the course of the mitochondrial respiratory chain and during various hydroxylation and oxygenation reactions (Lehninger 1981). Such partial products are suggested to occur as transient intermediates on the active sites of enzymes and, being highly reactive, may irreversibly damage various biomolecules (*ibid.*) such that they may be potentially toxic to the copepodid. These products are likely to be abundant in the area of feeding of the copepodid through a combination of the non-specific and specific immune reactions of the host, enzyme release from damaged host tissue and release of lytic enzymes by secondary pathogens such as bacteria. In the case of the first of these, production of superoxide radicals and other oxygen metabolites has been specifically demonstrated for active phagocytes (Ellis 1981). Macrophages have been noted by Johnson & Albright (1992a) to form part of the host reaction to lesions penetrating into the dermis of coho salmon (*Oncorhynchus kisutch* (Walbaum)) following infection by *L. salmonis* copepodids (10+ days post-infection). Although the inflammatory response to copepodids of *L. salmonis* on *S. salar* is limited (present study, Johnson & Albright 1992a), later stages of *L. salmonis* might be expected to induce a more severe inflammatory response through longer term damage and deeper penetration. If toxic products are present in sufficient quantities it may be that the copepodid and subsequent stages require them to be removed before damage to the midgut epithelium / degradation of nutritive materials or enzymes occurs. This might explain the presence of peroxidase in secretions and, if this is the case, might mirror the presence of peroxidases in the midgut tissues and regurgitated secretions of lepidopteran larvae which have been suggested to protect the larvae from hydrogen peroxide toxicity and to prevent the loss of nutrients through decarboxylation and oxidation of thiol groups of amino acids (Felton & Duffey 1991).

If the medial gland is related, as discussed earlier, to the sub-cuticular / tegumental glands of other copepods and crustaceans, then the possible functions of these too must be taken into consideration. Pochon-Masson, Renaud-Mornant & Cals (1975) have suggested that the tegumental glands of mystacocarids might function in cleaning of the cuticle, as an anti-predator mechanism and possibly as a bacteriocide. This latter function might be particularly important in the area of feeding of *L. salmonis* as secondary invasion of areas of feeding damage by bacteria could possibly lead to colonisation and damage of the cuticle. Boxshall (1982), with reference to *B. palliata* has also suggested that products of these glands might be associated with predator deterrence and also with general anti-fouling. Bannister (1993) further suggests that they might, in *T. longicornis*, via their anti-fouling properties or through specific properties of the secretion itself, reduce drag over the copepod. A further suggestion, made for *Tigriopus brevicornis* (Sars), is that they might be involved in sexual activity by way of the production of pheromones (Gharagozlou-van Ginneken 1979). This was supported by the association of the glands with the male genital sternite and the timing of their activity with that of the approach and coupling of males and females.

All the functions proposed above are suggested rather than proven, as is the case with other species studied. This whole area therefore seems to require further more detailed investigation.

CHAPTER 6
INFECTION PARAMETERS



6 - INFECTION PARAMETERS

6.1 Introduction

Kabata (1981) has commented on the need for the "establishment of a solidly based epidemiology of copepod infections" and it is clear that such an endeavour requires a better understanding of the process of settlement on the host by the infective stage. In previous chapters we have examined, using a number of techniques, various aspects of the biology of the copepodid of *L. salmonis*, that suggest how it might locate, attach to and utilise its host. Thus far, however, the information gained has not been directly related to observations of the pattern of larval infection in the field.

The purpose of the work described in this chapter is therefore to afford some insight into the pattern (numerical, temporal and spatial) of larval infection *in vivo* through a combination of infection experiments and observation of the larval population present on farmed salmon and in the farm's environment. It is hoped that the results of this chapter's work may then be used as a benchmark by which to interpret previous observations, and support or refute hypotheses generated in earlier chapters.

One important determinant of the probability of infection is larval survival. It might be expected intuitively, that a longer lifespan would increase the chances of host encounter, although Raibaut (1985) has suggested that the copepodids of parasitic copepods generally have a relatively short lifespan. Previous estimates of the survival of copepodids of *L. salmonis*, however, range widely from values of 4 days (Wootten *et al.* 1982) to 30 days (Johannessen 1975). This situation is further complicated by the fact that the latter author has ascribed increased survival to the attachment of the copepodids to the plankton mesh of their container, an observation seemingly supported by anecdotal reports that net-fouling on farms increases louse infestation (Rae pers. comm.). The experiments described in this chapter have therefore been designed to investigate the survival of the copepodid stage under "optimal" and "sub-optimal" conditions and include an estimate of survival with the addition of a fibrous substrate.

The observation that light may be an important cue to the copepodid of *L. salmonis* (see Chapter 3) has not gone unremarked and has led to attempts to capture lice by means of light traps (Taylor 1987) and to inhibit their settlement on farmed fish by means of shading of pens (Huse, Bjordal, Ferno & Furevik 1990). These experiments have been largely inconclusive although the latter study suggested tentatively that shading did indeed inhibit settlement.

The experiments described in Chapter 3 have established that light is an important environmental cue which is readily detected by the well-developed nauplius eye of the copepodid

and may elicit, at least experimentally, pronounced oriented behaviour. It has also been established that there seems to be an absence of a shadow-response in the copepodid of *L. salmonis*. Part of the work presented in this chapter therefore relates to the experimental examination of the infestation of hosts exposed to infection under illuminated and unilluminated conditions, for the purposes of correlating differences in settlement pattern (or lack of differences) to the earlier *in vitro* observations.

In 1981, Kabata highlighted the fact that copepods parasitising fish hosts do not infect them uniformly and that certain individuals are more susceptible than others to infection. This feature of host-parasite relations, termed "overdispersal", has been suggested to characterize such relationships (Crofton 1971a) and is defined by a variance / mean ratio for distribution frequencies of parasites on hosts that exceeds unity. The shape of the distribution is frequently approximated by that of the negative binomial. Overdispersal has also been termed "aggregation", "contagiousness" or "gregariousness" in recognition of the fact that it entails that certain hosts will have far larger numbers of parasites than the rest of the host population. Fryer (1966), commenting on parasitic copepod infections, has described it in terms of "a marked tendency for a fish which has acquired one parasite to acquire others". Although commonly encountered in parasitic relationships, Anderson & Gordon (1982) suggest that the task of determining the factors causing overdispersal in the natural environment is "fraught with difficulties" and consequently the same authors have proposed that it is initially preferable to carry out laboratory experiments exploring responsible factors.

Overdispersal on farmed salmon has been reported for total adult and preadult *L. salmonis* by Taylor (1987) and Sommerville (pers. comm.). It has also been recognised in studies of *L. salmonis* on wild salmon by Johannessen (1975), Nagasawa & Takegami (1993) and Tully (1992). No work has yet been carried out to look at the dispersal of settled larval stages of *L. salmonis* on the host and as a result little is known of the origins of the overdispersal reported. For this reason a portion of the work described in this chapter aims to investigate the pattern of settlement of larvae on farmed and experimental hosts.

An important step in elucidating how overdispersal comes about is to consider what benefit an overdispersed distribution may have for the parasite. To this end, Iwao (1970) discusses the possible reasons for an aggregated distribution and looks into the underlying causes. One of the major possible benefits for a parasite (or indeed any organism) is that it may serve to increase the probability of male/female encounters for reproduction (*ibid.*, Boxshall 1974c, Heip 1975). Another possible benefit is that it may help regulation of host and parasite populations through density-dependent effects on host mortality (Crofton 1971a, Boxshall 1974c). Although overdispersal may result from active interactions between parasite and host, it is also

clear that it may arise passively. Heip (1975) discussed distribution of organisms (in this case benthic invertebrates) in terms of optimal energy usage and suggested that when energy was not limited organisms might be expected to adopt a random distribution whilst under limited energy conditions a uniform distribution should be more advantageous. The fact that instead, despite the general prevalence of limiting energy conditions, organisms are frequently overdispersed was suggested to be indicative of active distribution and this was supported by evidence that in absence of a need for male / female interaction, distributions were not overdispersed. Overdispersal was therefore portrayed in terms of reduction of the risk of not finding a partner at the expense of the risk of not finding food.

Fryer (1966), examining a variety of copepod infestations, concluded that overdispersed distributions derived either through the facilitation of settlement by already settled parasites, or, alternatively, through chemical (ie pheromonal) attraction. Pheromones are known to exist in free-living species of copepod (see Dunham 1978) and have been suggested to be present in the lernaeopodid *Clavellodes macrotrachelus* (= *A. macrotrachelus*) where male copepodids apparently recognise, and settle in the presence of, already settled females (Caillet & Raibaut 1979, Benkirane 1987). Pheromones have also been suggested to be released by females of the branchiuran *Argulus siamensis* (Wilson) (Sundara Bai 1980). If homing on chemical stimuli is behind the frequently described overdispersal of parasitic copepods then this clearly indicates the intervention of active factors.

Anderson & Gordon (1982) have discussed a number of factors that might generate an overdispersed distribution and it is clear from this that the "mutual attraction" of organisms posited by Taylor (1961) need not be invoked in explanation, and that instead, passive mechanisms may explain distributions. In attempting to outline the mechanisms leading to overdispersion of parasites on their hosts, it was concluded by these authors that the dispersion of parasites results from the interaction of two sets of opposing forces acting respectively to decrease and increase dispersion.

Anderson & Gordon (1982) suggest that the prime causes of overdispersion in host-parasite relationships are "stochastic environmental factors" which comprise firstly, changes in the physical parameters of the environment in time and space, and secondly, differences in host susceptibility to infection. It is suggested that these factors are themselves superimposed over the variability resulting from population events ("demographic stochasticity"). Boxshall (1974c) concluded that overdispersion of *L. pectoralis* came about through aggregation of larvae in the water column arising from spatial variability in the physical cues used by copepodids to locate hosts and Kabata (1981) has similarly suggested that environmental variation was responsible, at least in part, for the variability seen in host infection. The heterogeneity of the host population was also, however, suggested to be a major factor by Kabata, this affecting susceptibility to

infection. Such factors as host immunity and fish size / age were implicated as factors affecting the level of host infection although it is clear that host and environmental heterogeneity may often act together, for instance in cases where host age / maturity causes movement into particular environments. From these discussions, amongst others, it is clear that the interaction of variable elements, postulated by Anderson & Gordon (1982), **are** influencing copepod infections, although the relative contributions of different factors, in the generation of observed distributions, remains unclear.

In contrast to those factors acting to increase overdispersal, density dependent factors frequently move dispersion in the opposite direction ie underdispersal (Scott 1987). One of the major mechanisms suggested for this is the removal of infected hosts through density dependent mortality. This will tend to reduce overdispersion by removing individuals with the heaviest parasite burdens. Other density dependent mechanisms including increased parasite mortality or decreased parasite reproductive potential will also decrease the degree of overdispersal (*ibid.*).

One of the major problems faced in examining the origin of aggregation is that aggregated distributions may occur through the action of a wide variety of different mechanisms. Amongst the mechanisms invoked to explain a negative binomial distribution is the exposure of the host to a series of "waves" of infection which, whilst being themselves random, differ at each exposure (compound Poisson distribution) as noted by Iwao (1970), Crofton (1971a), Elliot (1971) and Janovy & Kutish (1988). Such "waves" of infection clearly occur in copepod infestations, as illustrated by the temporal clumping of particular developmental stages noted in *L. pectoralis* by Boxshall (1974c) and *C. minimus* by Paperna (1980). In view of the questions that exist concerning the distribution of copepod parasites, two approaches have been undertaken in the present study. The first of these has been to infect fish artificially by exposing them to a single wave of infection under highly homogeneous conditions. The second approach has been to examine the distribution of larval stages in a farmed salmon population exposed to "continuous" infection within a relatively heterogeneous environment.

The high host specificity of *L. salmonis* is amply demonstrated by the limited host reports for this species given by Kabata (1979) which indicate a clear salmonid specificity. Because host records generally concern the presence of the larger and taxonomically recognisable adult and preadult stages, however, they provide no indication as to whether such host specificity is associated with the initial phase of larval infection or with post-infection survival / mortality on suitable / unsuitable hosts as was proposed by Lewis, Dean & Gilfillan (1969) for parasitic copepods on pelagic teleosts. With reference to the possible mortality of settled larvae on unsuitable hosts, Takegami (1986) has observed the death of chalimus I's of *Pseudocaligus fugu*

Yamaguti infesting unsuitable species of puffer fish although it was suggested in the same paper that copepodids might leave unsuitable hosts.

In Chapter 3, the experimental evidence suggested, in contrast to the findings of Fraile (1986) and Hogans & Trudeau (1989), that host recognition did not occur through distant chemodetection but instead, was likely to follow host contact. One of the objectives of the work described in the present chapter was therefore to determine whether the cues promoting settlement were present in the parr and first-feeder stages of the salmon host or whether these were associated only with the marine phase of the host. A further aspect of this work was to discern whether the copepodid larva recognises non-salmonid species before or upon contact and avoids infecting them or whether the act of infection itself is an arbitrary process with "success" being controlled by subsequent mortality. Both of these questions were tackled using experimental host infections. The natural infestation of non-salmonid species associated with marine salmon farms was used for comparison with the results from the experimental infection of non-salmonid species.

The observation of the spatial distribution of larvae associated with individual hosts might be hoped to provide further clues as to the nature of the infection process. If, as was suggested in Chapter 4, settlement and attachment can occur **anywhere** on the body surface, then, barring the intervention of other factors, one might expect larvae to be randomly distributed over the host surface. Experimental infections carried out by Johnson & Albright (1992a), however, suggest that this is not the case and that larvae settle predominantly on the fins and gills. Settlement on the fins has also been reported by White (1942a) from observations of wild fish. A number of different, although rarely mutually exclusive, explanations have been proposed for the spatially localised settlement of parasitic copepods although clearly, some copepods, such as the Philichthyidae have a closely defined site requirement that determines their spatial distribution. Kabata & Cousens (1977) have suggested that a rheotactic response causes settlement of *S. californiensis* principally around host pectoral fins and operculi, a suggestion supported by Boxshall (1976) in the case of *L. pectoralis*. Another possibility is that site selection / maintenance might be controlled by local substrate factors such as host response, this being suggested for *L. salmonis* by Johnson & Albright (1992a). In contrast Fryer (1966) has suggested that spatial aggregations of copepods arise through a pheromonal response mirroring the gregarious settling of barnacles reported by Knight-Jones & Stevenson (1950). Local environmental differences such as current speed are also likely to determine distribution on the host.

Part of the work described in this chapter is therefore aimed at the description of spatial patterns of settlement. It is hoped that examination of these patterns will give indications as to

how the copepodid stage finds and settles upon the host and may serve to implicate the major factors involved. This then, may support or refute hypotheses generated as to the nature of host-finding and settlement in Chapters 3 & 4. By comparing the pattern of settlement resulting from artificial experimental infections and natural farm infections it is further hoped that differences, if they occur, may help to explain the observed distribution patterns.

The spatial distribution of free-swimming larval stages of *L. salmonis* in the natural environment is, in common with that of the larval stages of most other copepod parasites, entirely unknown. The principal reasons for this are twofold. Firstly there have been almost no studies specifically designed to investigate the distribution of larval parasitic copepods in the water column, as their relatively small numbers, small size and the difficulty of taxonomic identification make such studies problematical and frequently unproductive. Secondly, larger studies which seek to examine the composition of plankton as a whole, cannot afford to be concerned with unidentifiable, rarely represented, "oddities" such that, although they may be present in investigated samples, the larvae of parasitic copepods are likely to go unremarked. A notable exception to the general lack of studies of the distribution of larval stages has been that of Schram & Anstensrud (1985) which described the pattern of distribution of *L. sprattae* larvae in Oslofjord plankton. These larvae were, on some occasions, found to be relatively abundant in surface water at night. Fryer (1966) has also noted the presence, but not the distribution, of larvae of *Ergasilus* sp. and *Lernaea* sp. in plankton.

The successful determination of the distribution of larvae in the water column has obvious benefits. If a method could be found by which larvae could be reliably sampled, then it would be possible to survey the area in and around fish-farms to determine the environmental preference, if any, of the larvae and site farms appropriately. This might also allow determination of the source of larvae and hence the contribution of fish farms to numbers of lice in the natural environment and *vice versa*. From such information, it is furthermore possible that the determination of factors contributing to the spatial and temporal aggregation of larvae in the environment could be accomplished, allowing for the possibility of changing farm management practices to reduce this. The increased infestation reported within pens having fouled nets for instance, might be explainable in terms of such aggregation. Knowledge of the distribution of larval stages in the water column may also allow the verification or refutation of the suggestions made in Chapter 3 that the light and pressure responses observed could lead to aggregation in certain environments ie notably, surface waters. The ability to sample the number of *L. salmonis* larvae in the water at any given time would also give farm managers a predictive capacity whereby they could be prepared to treat sudden waves of infection in advance of host damage.

In order to address this issue, two of the experiments described in the present chapter have been designed to provide information about the distribution of larvae in the water-column. The first of these involves the use of a light-trap which relies upon the positive phototactic response of the larval stages demonstrated in Chapter 3. Although this method gives no quantitative or spatial estimate (save that it concerns larvae in the vicinity of the trap) it may provide a general measure of abundance. A second method, pump sampling, that allows a good spatial and temporal estimate of distribution, is also described.

6.2 Materials and methods

6.2.1 *In vitro* survival experiments

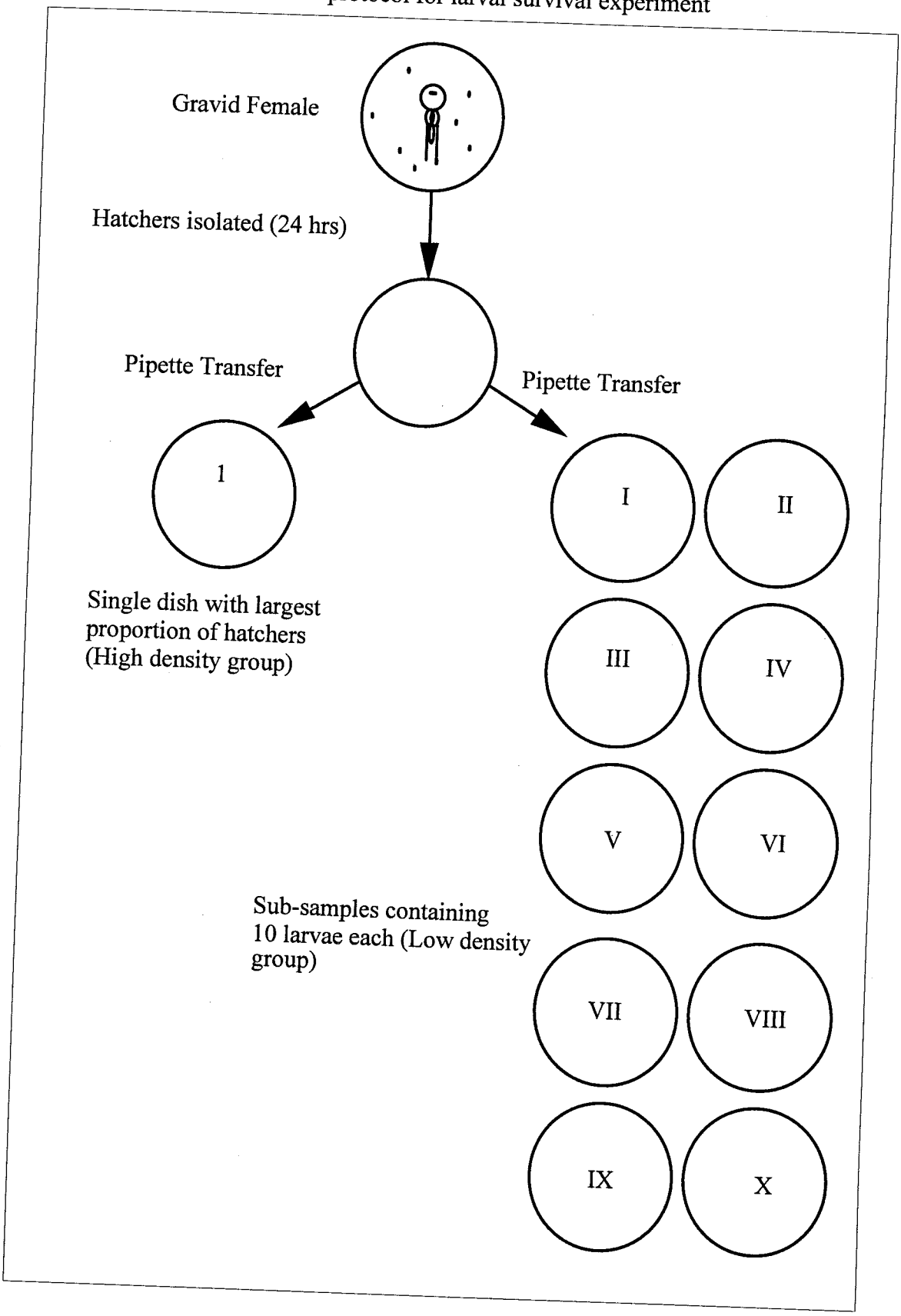
6.2.1.1 Larval survival

In order to examine the maximum infection window open to the copepodid, an experiment was set up to examine the mortality of the free-swimming larval stages between hatching and the death of the last copepodid from a given pair of egg strings. In addition to investigating larval survival, an experiment was designed which would allow comparison of survival in "optimal" and "sub-optimal" maintenance conditions.

The experimental protocol was as follows - adult females with dark (ie mature) egg-strings were placed singly in previously seawater-soaked petri-dishes to allow hatches to be individually monitored. The petri-dishes were kept in a cooling cabinet at 10°C under a 12 hr light : 12 hr dark regime. The salinity of the water was 30‰ and was changed twice daily (~75%) using a pipette. Upon hatching, the number of nauplii successfully hatched and the number which remained unhatched were recorded and after 24 hrs the female and any remaining unhatched / partially hatched eggs were removed to a separate container (in order to maintain hygienic conditions for the successful nauplii). After hatching, a daily record of larval mortality and developmental state was kept, with dead larvae being removed and the water changed as before, to maintain optimum water quality.

Two differing test groups were constructed. The first group, "**low density**", comprised 100 larvae selected randomly from the successful hatchers of a given female and divided into 10 groups of 10 larvae each, which were maintained in separate dishes and whose mortality was recorded over time. The second group "**high density**", comprised the remainder of the hatched larvae from the same female. These were transferred to a single container and mortalities were recorded and removed as indicated above (Figure 6.1).

Figure 6.1 Dish allocation protocol for larval survival experiment



6.2.1.2 Larval survival with plankton mesh

This experiment was constructed to test the report by Johannessen (1975) that survival of copepodids was high in the presence of plankton mesh (i.e. fibrous) substrate. Two groups composed of 100 newly hatched nauplii from the same eggstring pair were taken and split into aliquots of 10 larvae. These were treated in the same manner as the larvae in Experiment 1 above save for the fact that one group had, placed with them, ten 50mm × 3mm strips of 100 μm plankton mesh (soaked for 24 hrs previously in seawater to remove any chemical contaminants).

6.2.2 Experimental infections

A number of experimental infections were carried out in order to investigate the infection process under controlled conditions. For all the infection experiments, only copepodids aged between 24 hrs and 72 hrs post-moulting were used, with those in a given trial having hatched within 12 hrs of one another. Although this limited the number of copepodids that could be produced for any one trial, it was felt to be important for maintenance of consistency within the experiments.

6.2.2.1 Infections in light / dark

Pilot studies comprising the placement of a single fish in seawater for 24 hrs with high densities of copepodid larvae indicated that hosts could be readily infected in an artificial environment.

As a preliminary investigation it was decided to compare the infection of fish in the light and in the dark. This experiment had two purposes - firstly it would indicate whether a light stimulated or visual response plays an important part in the infection process and secondly it would determine whether later infections of large numbers of fish could be usefully carried out in the dark (hence reducing fish stress and thereby prolonging the possible infection time under static tank conditions).

For this experiment, the host population consisted of 20 salmon smolts split into two groups of 10 each. The experimental vessels consisted of two plastic tanks with covers whose dimensions were 60 cm × 60 cm × 77 cm. One of the tanks was wrapped in several layers of black plastic sheeting to exclude the light with the lid in place. Illumination was by overhead lighting and was of an intensity sufficient to elicit phototaxis in the copepodids. Both tanks were

filled with fresh seawater (31‰), aerated, and had 10 fish placed in each and allowed to acclimatise for a period of 1 hr.

At the initiation of the experiment, the water in the tanks was taken down to a volume of 54 litres in order to increase the infective dose as much as possible. To each tank, 500 active copepodids were added via a tube (with the lid closed in the dark tank so that no light should be present when the copepodids entered the container) to give an infective dose of 9.3 copepodids l^{-1}. The infection was allowed to proceed for a period of 60 minutes after which time it was terminated by narcotisation of the fish with Benzocaine. The fish were removed using a hook inserted under the operculum (to avoid accidental removal of copepodids during netting) and the number of copepodids per fish enumerated with the aid of a × 10 dissecting microscope. The positions of the copepodids were plotted on a distribution map for subsequent analysis.

6.2.2.2 Large scale smolt infection

A large-scale infection experiment was performed in order to investigate host infection in an artificial environment and was designed so that both the experimental host population and the environment were as homogeneous as possible in order to observe the resultant dispersal of parasites over the host population.

The study population for this experiment comprised 72 farmed, sea-adapted salmon smolts (mean length 28.7 cm ± 2.15 (1 S.D.). Smolts selected could be expected to have a similar genetic background and were selected so as to omit obviously larger or smaller fish and those showing any signs of distress or damage. The salmon smolts were placed in a 300 litre plastic tank with a flow-through seawater supply (temperature 12°C, salinity 30‰). Live copepodid larvae were reared in seawater at 11°C in a salinity of 30‰.

The experimental smolt population was starved for 24 hrs prior to the initiation of the experiment to reduce water fouling and hence increase the length of the subsequent trial infection period.

Before the initiation of the experimental infection, the tank containing the fish was drained to a volume of 220 litres in order to increase the infection dose. The water supply to the tank was ceased immediately prior to the infection in order to maintain a constant infective dose during the trial. After stopping the water flow, the tank was aerated both to compensate for the lack of fresh seawater input and to thoroughly mix the water and thereby homogenize the environment as much as possible to provide even dispersal of copepodids in the water column.

To initiate the infective exposure, 2250 active copepodids were added via a tube to the experimental vessel, the latter being covered to give a dark environment and thereby reduce fish stress and provide a homogeneous infective environment without a directional light component

(lack of light having previously been shown not to affect settlement in Experiment 1). The exposure was terminated at 1 hr to prevent over-stressing the fish through crowding and oxygen-debt and the water flow was returned to its normal level (the system being allowed to flush directly through to the outflow in order to remove the copepodids and thereby terminate the exposure).

The fish were sampled after a period of seven days rather than immediately. This decision was taken for two reasons. Firstly it allowed the copepodids to moult into the chalimus stage and thereby giving an indication of "successful" settlers comparable with natural (farmed or wild-caught) samples. More importantly, this also meant that fewer larvae would be lost during handling procedures giving a better estimate of settling numbers. This decision carries with it the penalty that unknown numbers would be lost in the interim period through precarious attachment to the host or mortality on the host.

After seven days, the fish were narcotised with Benzocaine and the number of chalimus per fish enumerated with the aid of a low power (× 10) dissecting microscope (the fish being placed in a tray of water during observation). The fork-length of each fish was also recorded and the general appearance of the fish was similarly noted. These observations were taken in order to try and relate settlement patterns to host size and conditions.

6.2.2.3 Infection of salmon parr and fry

These experiment were designed in order to establish whether the recognition factor that apparently allows copepodids of *L. salmonis* to distinguish salmonids is present in the freshwater stages of the Atlantic salmon or is associated with smoltification and the marine phase only. Because this question requires only a positive / negative answer, single fish only were used for these experiments.

Parr and fry were exposed to high concentrations of copepodid larvae in static water systems (both held in cooled external water baths). The parr was infected in a small plastic tank and the fry in a petri-dish. The water used for these experiments had the salinity depressed to 25‰ in order to improve fish survival and was kept at a temperature of 10°C. The parr was exposed to a dose of 100 copepodids for a period of 1 hour and the first feeder was exposed to a dose of 25 copepodids for the same period. Both experiments were observed throughout and notes taken on copepodid behaviour. At the completion of this time, the fish were narcotised with Benzocaine and the presence or absence of settled copepodids noted.

6.2.2.4 Infection of non-salmonid species

Experimental infections of non-salmonid species.

For the purposes of this experiment, a number of principally juvenile fish were captured from the wild by a variety of methods and were exposed to a standard trial to test for infection. The trial infection consisted of individual test fish being placed in 1 litre of water (salinity ~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 a period of 1 hr. After this time, the fish were narcotised with Benzocaine and examined for the presence of settled copepodids using a low-power (x 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 (method of capture listed adjacently):

Cod (*Gadus morhua* L.) (rod and line)
Three-spined stickleback (*Gasterosteus aculeatus (armatus)* L.) (handnet)
Butterfish (*Pholis gunnellus* (L.)) (hand)
Lumpsucker (*Cyclopterus lumpus* L.) (handnet)
Flounder (*Pleuronectes flesus* L.) - yr 0 fish (water pump / bucket)
Eel (*Anguilla anguilla* (L.)) - elver (home-made eel trap)
Saithe (*Pollachius virens* (L.)) (rod and line)

All these species were captured in Loch Ailort or in the River Ailort (elver and stickleback). All fish were examined under a dissecting microscope for ectoparasites at the time of capture (under light anaesthesia save where size was too small to warrant it) in order to preclude infection by copepodids prior to the experiment.

Occurrence of *L. salmonis* on non-salmonid species on farms.

Having examined the settlement of copepodids on non-salmonid species in laboratory experiments, a study of the species found in close contact with farmed salmon was undertaken. Two particular groups of fish examined, these being wrasse which are at present used as cleaner fish on some farm sites and saithe which commonly infest Scottish farm sites.

Wrasse

A number of wrasse species were examined, these comprising *Labrus bergylta* Ascanius (ballan), *Crenilabrus melops* (L.) (corkwing), *Labrus mixtus* L., *Ctenolabrus rupestris* (L.) (goldsinny) and *Centrolabrus exoletus* (L.) (rock cook). Fish were obtained from a number of farmed sites to determine the identity of any caligids infesting them. Fish were examined after narcotisation with benzocaine, with the left side of the fish and all fins being examined under a low power (× 10) dissecting microscope as well as the gills and buccal cavity. If any lice were observed the whole fish was examined and all lice were removed for identification and placed in 10 % neutral buffered formalin before return to the laboratory. Species and stage identifications were made with the aid of Kabata (1979) and Gurney (1934) as well as reference material held at the Institute of Aquaculture.

Saithe

A single sample of 21 saithe (*P. virens*) and two other single fish samples captured from within active salmon cages were examined for caligid infestation according to the protocol described for salmon below.

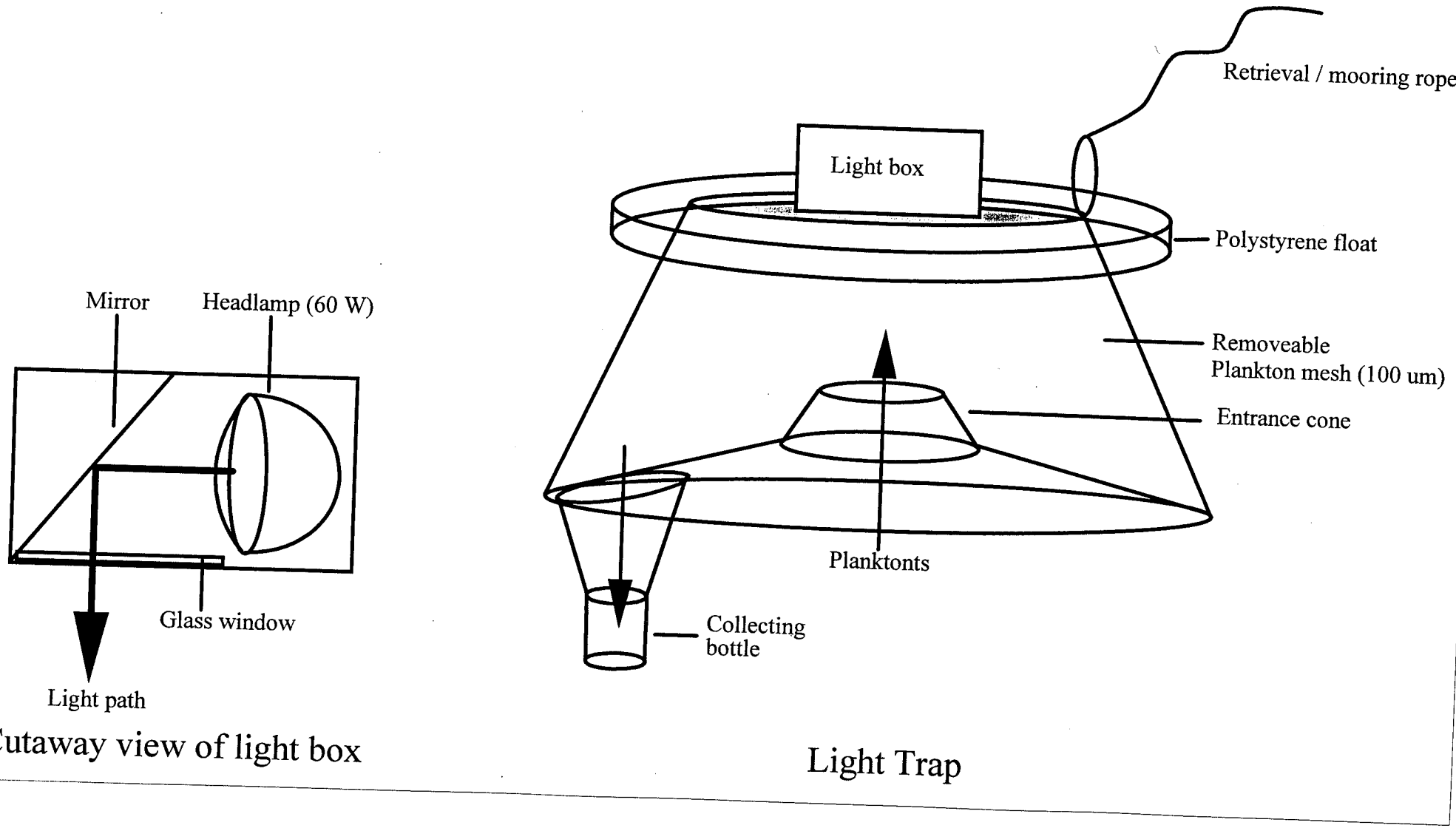
6.2.3 Natural abundance and distribution of copepodids in the water column

These surveys were designed to attempt some estimation of the number of copepodids present in the plankton and their vertical positioning in the water column through the course of 24 hrs. Three plankton / epibiont assessment techniques were used - 1) Light trapping, 2) Pump sampling and 3) Plankton mesh "spider"

6.2.3.1 Light trap

Having already demonstrated a positive phototactic response in copepodids (see Chapter 3), a light trap was constructed according to the general plan given by Jones (1971). The light trap (Figure 6.2) consisted of a welded iron conical frame covered in 100 μm plankton mesh. A waterproof wooden box on top of the frame with a glass plate facing downwards contained a Thorn 60 Watt sealed beam unit which was directed downwards by the use of a mirror. The choice of a white light source was deemed appropriate as a result of the experiments in Chapter 3 which indicated a broad range of spectral sensitivity in the copepodid. The light was powered by a petrol-driven generator connected to the light source via a regulator in order to prevent

Figure 6.2 Schematic diagram of light-trap construction



power surges (and thereby keeping illumination constant). At the base of the net, a smaller mesh-covered cone projected up into the body of the trap in the manner of the aperture of a crab pot. This smaller cone opened at its' uppermost end into the main net (this aperture being covered in 1 cm mesh to prevent the ingress of fish / large predators). The trap was maintained at the water surface by the use of a polystyrene float around its' upper edge. A collecting sock at the rim of the trap furthest from the retrieval rope meant that on retrieval, water draining out of the trap was directed down the sock and into the sample container at the bottom of it.

Before commencing with a full series of experiments, a preliminary trial was performed which indicated that it was able to trap *L. salmonis* larvae (10 × 1 ml aliquots from a total 200ml sample yielding a mean of 0.4 copepodids ml^{-1}.

In order to examine the number of *L. salmonis* larvae present in the water column at a time when infestation on a site was high, the trap was operated inside a fish cage for ten nights with the light switched off every second night to act as a control. Each light on / light off event was used as a pair for the purposes of analysis.

The sampling protocol for each test was as follows. The trap was lowered into the fish cage ~1 hr before dusk, ensuring that the collecting sock and vessel were filled with water and therefore suspended beneath the trap. The light was then switched on and the trap left overnight. Sample collecting was performed after dawn the next day. This involved raising the trap by the edge opposite to the sock and, with the sock still in the water, repeatedly sluicing the trap with seawater from a bucket in order to wash all the captured contents into the sock and collecting vessel. The sock and vessel were then retrieved from the water and the vessel detached and its' contents transferred to a larger vessel. Larger plankton such as fish were removed at this point and their presence noted. Adult / preadult *L. salmonis* caught in the trap were also removed and kept separately. The sample was taken back to the laboratory, filtered through 60 micron plankton mesh and made up to a volume of 200 ml with 4% formalin (this volume giving a countable number of planktonts in each 1 ml aliquot). From this sample, 5 x 1 ml aliquots were taken using a 1 ml Stempel pipette, the sample being agitated by side to side movement of the pipette rather than stirring in order to give a true estimate of the planktonts in the sample (Newell & Newell 1977). All of these aliquots were counted fully in a Sedgewick rafting tray for all planktonts visible under a low power (× 10) binocular dissecting microscope and a record kept of the results. Although Newell & Newell (1977) suggest that a 1 ml sub-sample taken from 200 ml gives a sufficiently accurate estimate of planktont representation, the choice of multiple aliquots was made in order to better estimate the expected low proportion of *L. salmonis* in the samples. All aliquots were discarded after use although *L. salmonis* larvae recovered were retained separately.

6.2.3.2 Pump sampling

6.2.3.2.1 24-Hour sample

In order to examine the numbers and vertical / temporal distribution of copepodids in the water column, a 24-hour pump sampling protocol was carried out. Pump sampling allowed a known volume of water to be sampled from a known depth over a precise interval of time and was further preferred over tow-net sampling in that it could be carried out at night when other authors (Schram & Anstensrud 1985) have noted that copepodids of *L. sprattae* at least are more abundant. Only a single 24 hr sample could be taken as a result of the new safety strictures applying to fish farms in the U.K.

The pump used for sampling was a Jabsco Water Puppy 2000, a 12 V D.C. self priming pump which could be powered from a car battery (in fact, a JCB battery was used for the experiment for improved durability). The time taken for the pump to bring up 100 litres at full battery charge was recorded using a stopwatch and this time used to give the sample volume at each depth. This time was periodically checked to ensure consistent sample volumes. Prior to the commencement of sampling, a 20 litre container was filled with seawater filtered at 60 μm to be used in rinsing equipment after each sample.

The sampling protocol ran as follows. Sampling began at 19:00 hrs on 31/5/90 and was completed at 20:00 hrs on 1/6/90. The samples were taken from the edge of the outer gangway of a fish pen at Ardnish farm in Loch Ailort, the nearby pen containing badly lice-infested second year fish. Each sample was collected in the following manner. The pump tube was first lowered to the appropriate depth (these being 0m, 1m, 2m, 5m, 10m, and 15m with further samples at 5m intervals until the bottom was reached). Prior to the actual sample, the pump was run for 1 minute to ensure that the contents of the tube came from the correct depth and a water sample was taken for salinity analysis. The temperature was also recorded. The pump was then run through a 60 μm plankton mesh sock until a 100 litre sample had been taken. At this time the pump was switched off and the sock rinsed with filtered water into a large container. This sample was then decanted into a labelled sample bottle using a funnel. All the equipment was then rinsed in filtered seawater and the next depth sample taken. Sampling of the whole depth profile normally took approximately 1 hour and was repeated at 3 hourly intervals. A control sample in the form of a bottle of the 60 μm filtrate was also examined to ensure that planktonts were not escaping capture.

During the sampling period, a full record was kept of general environmental variables such as weather conditions, water turbidity and site activity.

At the end of the sampling programme, all the samples were returned to the laboratory and were filtered through a 60 μm plankton mesh and made up to a volume of 50 ml with 4% formalin (this giving suitable numbers of planktonts for counting in a 1 ml aliquot). All the salinities were also measured at this stage using a refractometer.

For counting, 10 $\times$ 1 ml aliquots were taken using a Stempel pipette as outlined above. The first of these aliquots was counted fully using a Sedgewick rafting tray in order to give some idea of the distribution of planktonts in general, whilst the remaining nine were scanned only for the presence of *L. salmonis* (or other caligid) larvae. All aliquots were discarded after use.

6.2.3.2.2 Cage net sample

In order to determine whether copepodid or nauplius stages of *L. salmonis* were capable of attaching to fish farm cage nets, a pump sample procedure was designed to allow samples to be taken from the net sides. The sampling equipment was the same as that described above save that the end 2 metres of the pump tube were tied to a broom handle which in turn had a small aquarium net tied to the tip beside the lower aperture of the tube. This arrangement allowed the tube to be grazed against the net by the operator such that material dislodged by the net would be sucked up the tube. The quantity of filamentous algae and other epibiota precluded the filtering of samples such that single unfiltered samples were returned to the laboratory. After allowing material to settle and observing the plankton catch by eye, an anglepoise light was directed at the surface of the sample and photopositive planktonts were collected by repeated use of a 10 ml pipette. This sample was filtered with a 60 micron plankton mesh and made up to 50 ml with 4% formalin. 10 $\times$ 1 ml aliquots were then taken and scanned for *L. salmonis* larvae in a Sedgewick rafter. The aliquots were discarded after use. Net samples were taken from two farm sites on separate occasions.

6.2.3.2.3 Plankton mesh "spider"

In order to try and examine the possibility of copepodids attaching to fibrous substrates (such as the net sides and their epibiota), strips of shredded plankton mesh (100 μm) were tied together with nylon monofilament and weighted with a large stone tied beneath. This assembly was hung on the outside of a sea cage for a period of seven days and was carefully lifted daily and rinsed into a container with filtered (60 μm) seawater. The sample obtained in this manner was examined for the presence of copepodid larvae.

6.2.4 Farm infections

Despite the usefulness of laboratory studies in examining the pattern of settlement of larval stages, it is clearly important to compare such observations with findings in the field. This allows evaluation of the extent to which laboratory studies reflect the natural situation and the extent to which they are artefactual.

In order to study such parameters, it was necessary to procure a continuous record of the pattern of lice infestation on a "typical" salmon farm, comprising data collected on a regular basis from nominated cages. Such data was obtained from a separate study carried out by the author under the auspices of the Scottish Salmon Grower's Association. This data has hitherto been partially presented in the form of two papers (Bron, Sommerville, Wootten & Rae (1993a) and Bron, Sommerville, Wootten & Rae (1993b)) and two annual reports to the SSGA. The methodology used for the collection of data in this study is described in the following section:

6.2.4.1 Temporal and numerical distribution on farmed salmon hosts

The samples comprising this study were taken from a relatively typical single-yearclass salmon farm at repeated intervals of two weeks for a period of 20 months. On each sampling visit, 5 fish were taken from a single pen on each of the 5 major cage-groups on the site. On each group the sampling pen was initially selected at random, and thereafter, samples were always taken from the same pen. The sample-number of five fish has been previously indicated (Rae 1979) to give an acceptable estimate of parasite abundance.

For each sample, 5 fish from each pen were netted, killed and taken ashore for examination. Each fish was examined for sea lice, and all stages were removed with fine forceps and placed in 5% neutral buffered formalin.

On return to the laboratory all the collected sea lice were identified to stage, and adults and preadults to sex with the aid of a low power ($\times$ 10) dissecting microscope.

6.2.4.2 Spatial distribution on farmed salmon hosts

The pattern of site settlement of the larval stages was recorded for a sample population consisting of 125 fish taken from a number of pens on a single farm site at different times (the samples being again restricted to 5 per pen). In order to plot the distribution of parasites, the fish were given a thorough surface examination which included the gills, nares and buccal cavity. Patterns of settlement were plotted on charts with differentiation of copepodid and chalimus stages.

6.3. Experimental Results

6.3.1. *In vitro* survival experiments

6.3.1.1 Larval survival

Moulting of the majority of nauplius I to nauplius II occurred within 24 hrs of hatching (18-22 hrs) and moulting to the copepodid stage took a further 53-57hrs.

The mean cumulative percentage mortality for the "high density" groups including hatching mortalities is given in Table 6.1 and shown in Figure 6.3. The median hatching mortality was 7.5%, with most of the mortalities being associated with larvae failing to hatch at the proximal end of the egg-strings.

The mean cumulative percentage mortalities excluding the initial hatching mortalities given above, for the "high density" larval batches and their respective "low density" sub-samples, are shown in Tables 6.2 and 6.3 and illustrated graphically in Figures 6.4 and 6.5. From this data it can be seen that the larvae survived better when kept in containers with reduced numbers of larvae.

In the "high density" groups, the first mortalities were, as noted above, hatching-associated, and mortalities continued on day 1 after hatching in 9 out of the ten studied groups. In the "low density" sub-samples the first mortalities occurred on day 3 after hatching, day 13 being the latest onset of mortality in any of the sub-samples (see "minimum" column in Table 6.3). The rate of mortality was also seen to be much higher in the "high density" groups, with more than 50% being dead by day 4 (median value 57%) whereas this did not occur until day 12 for a similar mortality in the sub-sampled "low density" groups (median value 60.8%). Over 95% (median value 95.6%) were dead by day 10 in the "high density" groups whereas in the sub-sampled "low density" groups, 95% mortality was not attained until day 14 (median value = 100%). The last copepodid in each group died on days 25 and 26 respectively for the high and low density groups.

Table 6.4 shows the values for the onset of mortality and for the maximum survival in both the high density groups and their respective low density sub-samples. This data indicates a delay of onset of initial mortalities in the low density group with respect to the high density group. Despite higher mortality rates in the high density groups, as noted above, the maximum survival of copepodids in both high density and low density groups was not found to be significantly different (Wilcoxon's Test, $Z = 1.57$, $P > 0.1$).

Table 6.1 Mean and median cumulative percentage hatching mortalities for high density groups including hatching mortalities.

Time after hatching (Days)	Mean cumulative % mortality (± 1 S.D.)	Median cumulative % mortality	Minimum	Maximum
0	7.6 ± 5.2	7.5	1.8	17.4
1	10.4 ± 8.1	9.2	2.3	30.3
2	18.7 ± 15.4	12.7	6.9	57.4
3	30.1 ±15.7	25.3	16.7	66.8
4	58.2 ± 20.6	58.3	30.9	100.0
5	76.3 ± 18.7	80.2	44.9	100.0
6	83.8 ± 13.9	87.4	60.3	100.0
7	85.9 ±12.3	88.7	64.0	100.0
8	89.1 ± 9.8	90.6	69.9	100.0
9	91.5 ± 8.3	94.1	72.4	100.0
10	93.8 ± 6.8	96.1	76.5	100.0
11	95.8 ±4.2	97.1	85.7	100.0
12	97.5 ± 2.6	98.5	91.9	100.0
13	98.9 ± 1.2	99.3	96.3	100.0
14	99.6 ±0.5	99.9	98.6	100.0
15	99.9 ±0.2	100.00	99.6	100.0
16	99.9 ± 0.2	100.0	99.6	100.0
17	100.0 ± 0.1	100.0	99.8	100.0
18	100.0 ± 0.1	100.0	99.8	100.0
19	100.0 ± 0.1	100.0	99.8	100.0
20	100.0 ± 0.1	100.0	99.8	100.0
21	100.0 ± 0.1	100.0	99.8	100.0
22	100.0 ± 0.1	100.0	99.8	100.0
23	100.0 ± 0.1	100.0	99.8	100.0
24	100.0 ± 0.0	100.0	100.0	100.0
25	100.0 ± 0.0	100.0	100.0	100.0

Figure 6.3 Median cumulative percentage mortality of larvae showing range, lower and upper quartiles and outliers > 1.5 x interquartile range (high density samples inclusive of hatching mortalities)

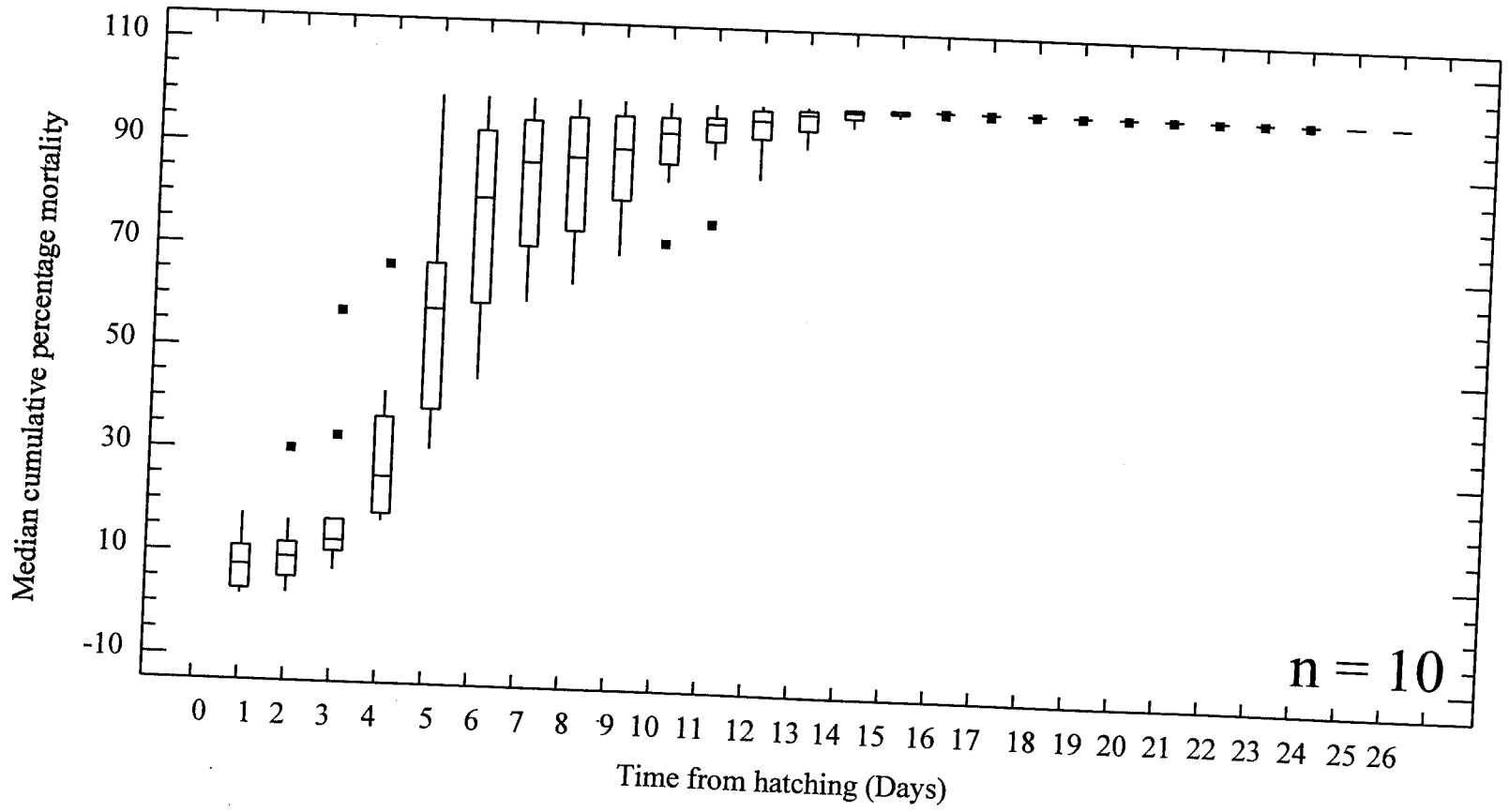


Table 6.2 Mean and median cumulative percentage hatching mortalities for high density groups excluding hatching mortalities.

Time after hatching (Days)	Mean cumulative % mortality (± 1 S.D.)	Median cumulative % mortality	Minimum	Maximum
1	3.1 ± 4.5	2.0	0.0	15.6
2	11.8 ± 16.4	5.2	2.4	56.3
3	24.1 ± 17.0	17.8	0.3	65.9
4	54.5 ± 22.4	57.0	25.7	100.0
5	74.1 ± 20.4	77.1	40.7	100.0
6	82.4 ± 14.9	85.5	57.3	100.0
7	84.6 ± 13.7	86.9	61.3	100.0
8	88.1 ± 10.5	89.4	67.6	100.0
9	90.6 ± 8.9	93.3	70.4	100.0
10	93.2 ± 7.4	95.6	74.7	100.0
11	95.5 ± 4.7	96.9	84.6	100.0
12	97.4 ± 2.9	98.9	91.3	100.0
13	98.9 ± 1.5	99.5	95.6	100.0
14	99.6 ± 0.6	99.9	98.5	100.0
15	99.9 ± 0.2	100.0	99.5	100.0
16	99.9 ± 0.2	100.0	99.5	100.0
17	100.0 ± 0.1	100.0	99.8	100.0
18	100.0 ± 0.1	100.0	99.8	100.0
19	100.0 ± 0.1	100.0	99.8	100.0
20	100.0 ± 0.1	100.0	99.8	100.0
21	100.0 ± 0.1	100.0	99.8	100.0
22	100.0 ± 0.1	100.0	99.8	100.0
23	100.0 ± 0.1	100.0	99.8	100.0
24	100.0 ± 0.1	100.0	99.8	100.0
25	100.0 ± 0.0	100.0	100.0	100.0

Table 6.3 Mean and median cumulative percentage mortality for low density groups.

Time after hatching (Days)	Mean cumulative % mortality (± 1 S.D.)	Median cumulative % mortality	Minimum	Maximum
1	0.0 ± 0.0	0.0	0.0	0.0
2	0.0 ± 0.0	0.0	0.0	0.0
3	0.4 ± 2.0	0.0	0.0	12.5
4	3.3 ± 5.8	0.0	0.0	25.0
5	16.6 ± 28.9	7.7	0.0	100.0
6	23.5 ± 31.0	10.6	0.0	100.0
7	27.5 ± 30.8	16.1	0.0	100.0
8	31.0 ± 30.7	20.0	0.0	100.0
9	34.6 ± 30.3	26.2	0.0	100.0
10	39.6 ± 30.2	32.1	0.0	100.0
11	49.8 ± 29.9	48.1	0.0	100.0
12	61.1 ± 28.4	60.8	0.0	100.0
13	77.3 ± 22.5	80.0	12.5	100.0
14	93.6 ± 11.1	100.0	44.4	100.0
15	98.0 ± 5.2	100.0	75.0	100.0
16	99.4 ± 2.4	100.0	90.0	100.0
17	99.5 ± 2.1	100.0	90.0	100.0
18	99.6 ± 1.8	100.0	90.0	100.0
19	99.6 ± 1.8	100.0	90.0	100.0
20	99.9 ± 1.0	100.0	90.0	100.0
21	99.9 ± 1.0	100.0	90.0	100.0
22	99.9 ± 1.0	100.0	90.0	100.0
23	99.9 ± 1.0	100.0	90.0	100.0
24	99.9 ± 1.0	100.0	90.0	100.0
25	99.9 ± 1.0	100.0	90.0	100.0
26	100.0 ± 1.0	100.0	100.0	100.0

Figure 6.4 Median cumulative percentage mortality of larvae showing range, lower and upper quartiles and outliers > 1.5 x interquartile range (high density samples exclusive of hatching mortalities)

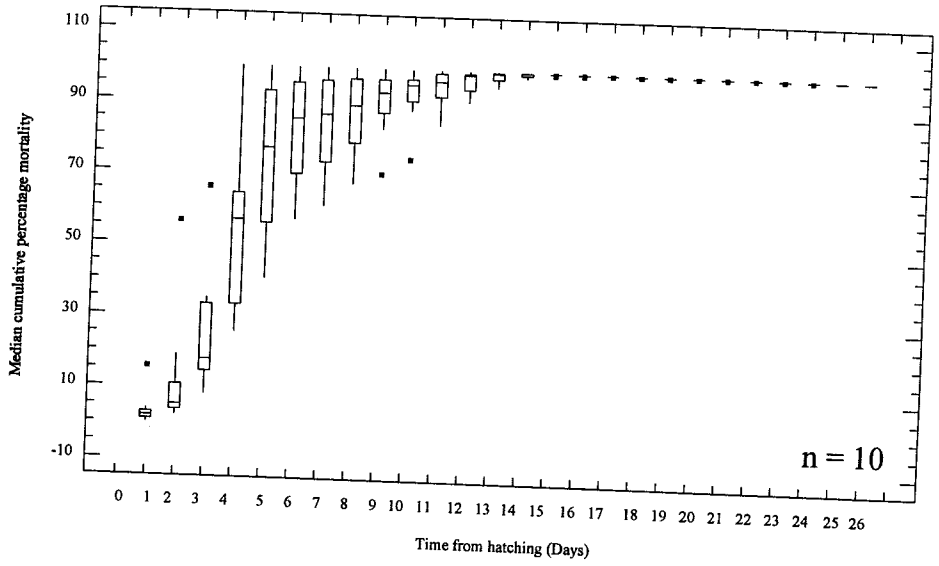


Figure 6.5 Median cumulative percentage mortality of larvae showing range, lower and upper quartiles and outliers > 1.5 x interquartile range (low density samples)

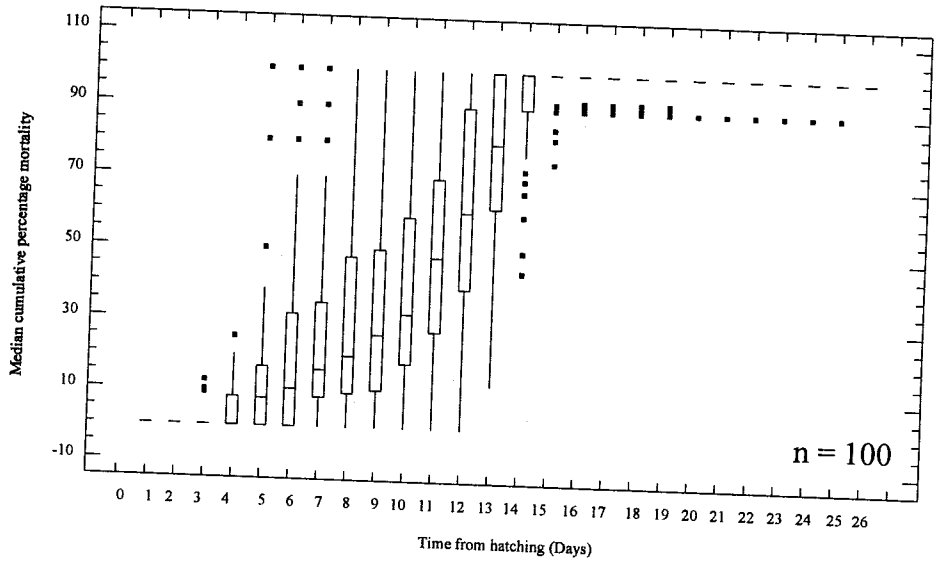


Table 6.4 Differences between timing of initial mortalities and maximum survival for high density groups and their respective low density sub-samples.

Group Number	Onset of mortality (high density group) (days). n = 10	Minimum onset of mortality in low density groups (days). n = 100	Maximum survival in high density group (days)	Maximum survival in low density group (days).	Difference in maximum survival (days).
1	1	5	24	8	-16
2	1	4	15	26	+11
3	1	4	18	17	-1
4	1	4	4	15	+11
5	1	4	14	17	+3
6	1	4	12	14	+2
7	1	3	14	16	+2
8	1	3	14	15	+1
9	1	4	14	19	+4
10	1	5	15	20	+5

6.3.1.2 Larval survival with plankton mesh

Tables 6.5 and 6.6 show the mean cumulative percentage mortality for the test group with added plankton mesh and for the control group without mesh. The mortality curves for both groups are shown in Figures 6.6 and 6.7 respectively and in terms of median values can be seen to be almost identical up to day 10. After this point there is a slight increase in mortality in the plankton-mesh group. The first mortalities in both groups occurred on day 3 after hatching. The last mortality in the test group was on day 15 and in the control group was on day 16. A Mann-Whitney U-test indicates that there was no significant difference between the maximum survival of copepodids with and without plankton mesh ($Z = 1.67$, $P > 0.095$).

6.3.2. Experimental infections

6.3.2.1 Infection of smolts in light / dark

Infection of the smolts was found to be successful in both tanks with a prevalence of infection of 100 % and a relative density of infection of 9.2 $\pm$ 5.5 (1 S.D.) parasites per fish for the dark tested fish and 11.6 $\pm$ 6.3 (1 S.D.) for the light tested fish. The proportion of available copepodids found to have settled was 18.4 % and 23.2 % for the dark and light experiments respectively. In neither group was the infection found to differ significantly from normality (Kolmogorov-Smirnov $P > 0.99$ and $P > 0.44$ for dark and light respectively). Bartlett's test indicated the variances of the two groups to be homogeneous ($P = 0.69$).

A t-test performed between the two groups indicated that there was no significant difference between them ($P > 0.37$) which indicates that light was not of primary importance in successful host infection by the copepodids. This result was further supported by a Mann-Whitney U-test ($Z = -1.11$, $P = 0.27$).

The pattern of infection in terms of settlement site is shown in Figure 6.8 (dark / light, $n = 10$). It can clearly be seen that the predominant sites of infection were the fins, with the general body surface second and the gills last in order of settlement abundance. Table 6.7 shows the total percentages of parasites settled over these three sites for both light and dark tested fish, the confidence intervals for these proportions and the significance of the differences between the two groups. It can be seen from the table that there was no significant difference between the two groups for any of the sites listed using a test for comparison of percentages (Fowler & Cohen 1987).

Table 6.5 Mean and median cumulative percentage mortality with plankton mesh.

Time after hatching (Days)	Mean cumulative % mortality (± 1 S.D.)	Median cumulative % mortality	Minimum	Maximum
1	0.0 ± 0.0	0.0	0.0	0.0
2	0.0 ± 0.0	0.0	0.0	0.0
3	3.2 ± 5.2	0.0	0.0	11.1
4	3.2 ± 5.2	0.0	0.0	11.1
5	4.3 ± 7.6	0.0	0.0	22.2
6	8.6 ± 13.6	5.0	0.0	44.4
7	26.6 ± 31.3	15.0	0.0	88.9
8	33.5 ± 30.4	25.0	0.0	88.9
9	46.3 ± 32.2	56.3	0.0	88.9
10	59.0 ± 24.9	61.3	16.7	100.0
11	74.2 ± 21.6	75.7	41.7	100.0
12	87.6 ± 18.6	95.0	41.7	100.0
13	94.2 ± 8.6	100.0	75.0	100.0
14	94.2 ± 8.6	100.0	75.0	100.0
15	98.8 ± 4.0	100.0	87.5	100.0
16	100.0 ± 0.0	100.0	100.0	100.0

Table 6.6 Mean and median cumulative percentage mortality without plankton mesh.

Time After Hatching (Days)	Mean cumulative % mortality (± 1 S.D.)	Median cumulative % mortality	Minimum	Maximum
1	0.0 ± 0.0	0.0	0.0	0.0
2	0.0 ± 0.0	0.0	0.0	0.0
3	1.0 ± 3.2	0.0	0.0	10.0
4	4.3 ± 5.6	0.0	0.0	11.1
5	4.3 ± 5.6	0.0	0.0	11.1
6	11.6 ± 11.7	10.6	0.0	33.3
7	14.6 ± 13.3	10.6	0.0	40.0
8	26.6 ± 25.7	21.1	0.0	90.0
9	38.7 ± 23.6	31.7	18.8	90.0
10	70.3 ± 18.1	66.7	36.4	100.0
11	93.4 ± 9.7	100.0	72.7	100.0
12	97.1 ± 6.4	100.0	81.8	100.0
13	99.1 ± 2.9	100.0	90.9	100.0
14	99.1 ± 2.9	100.0	90.9	100.0
15	100.0 ± 0.0	100.0	100.0	100.0
16	100.0 ± 0.0	100.0	100.0	100.0

Table 6.7 Settlement of copepodids on hosts under light and dark conditions (n = 10).

	Light (116 total settled)	Dark (92 total settled)	
Settlement Site	Percentage of settled copepodids ± 95 % confidence interval	Percentage of settled copepodids ± 95 % confidence interval	Significance of comparison (range test)
Fins	77.58 ± 7.6	75.30 ± 8.8	P > 0.05
Body	13.79 ± 6.3	17.20 ± 7.7	P > 0.05
Gills	8.62 ± 5.1	7.50 ± 5.4	P > 0.05

Figure 6.6 Median cumulative percentage mortality of larvae maintained with plankton mesh showing range, lower and upper quartiles and outliers > 1.5 x interquartile range

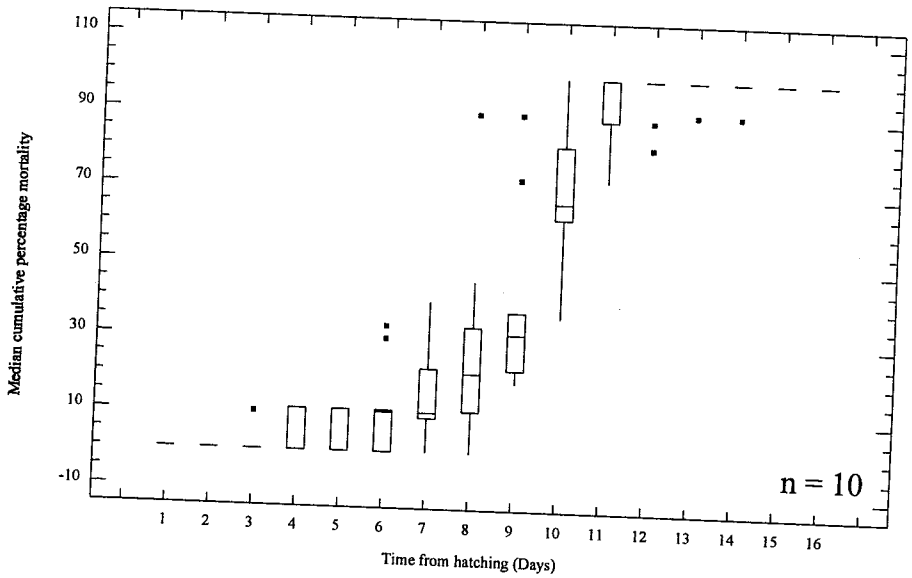


Figure 6.7 Median cumulative percentage mortality of larvae maintained without plankton mesh showing range, lower and upper quartiles and outliers > 1.5 x interquartile range

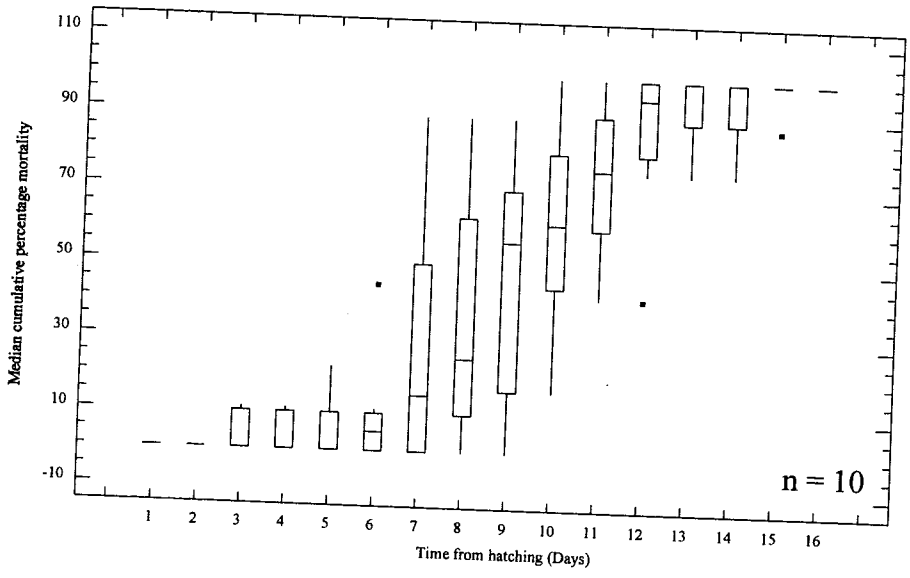
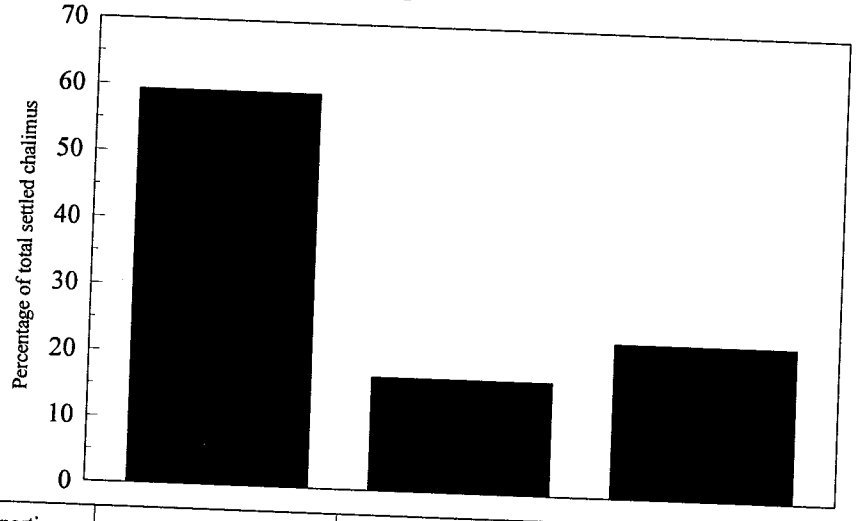


Figure 6.8 Comparison of total percentage settlement of copepodids on different host sites under light and dark conditions

Figure 6.9 Comparison of total percentage chalimus on different host sites following large scale smolt infection experiment



Settled portion	Fins	Body	Gills
Dark (n=72) ■	59.38	17.19	23.44

6.3.2.2 Large scale smolt infection

The smolts survived the infection period well and showed no ill effects or mortalities during the seven days between initial infection and sampling.

Examination of the smolts indicated that the infection had been successful and that the copepodids had moulted to the chalimus stage. The prevalence of infection in this experiment was 59 %, with a mean intensity of infection of 1.66 ± 1.15 (1 S.D.) and a relative density of infection of 0.89 ± 1.18 (1 S.D.). The proportion of available copepodids found to have settled and moulted was 2.84 %. The parasite population was found to be highly overdispersed (ie variance > mean) with a variance / mean ratio of 1.39 / 0.89 = 1.56 ($P < 0.01$ with Chi square) within the host population.

The frequency distribution of parasites within the host population was fitted with a negative binomial curve, the data from this infection experiment having been shown to be significantly different from a normal distribution (Chi square $P < 0.000003$) but not significantly different from a negative binomial distribution ($P > 0.08$). The exponent of the binomial (k) was calculated as 3.05.

The pattern of parasite settlement on the host population was similar to that seen in Experiment 1. (Figure 6.9). Settlement was similarly predominantly on the fins, comprising 59.38 % of settled parasites overall. The next highest site preference was for the gills and buccal cavity which showed an overall percentage of 23.44 % in contrast to the 7.5% seen in the dark experiment seen previously. The body accounted for only 17.19 % of the total number of settled parasites.

The effect of length on the infection parameters described was determined using linear regression of relative density of infection and prevalence of infection against host fork length. For the relative density regression, the fish were divided into 19 classes (ie 1 cm size classes from 20 to 40 cm) and the mean $\log(x+1)$ transformed infection values for each class antilogged and regressed against host length. For the prevalence regression, the regression was performed on arcsine transformed percentage prevalence values (for each class).

Figure 6.10 illustrates the regression of mean number of parasites per fish against host length. The coefficient of correlation for this graph (r) is -0.46 which is not significant (critical tabled value = 0.497 ($P < 0.05$)). The proximity of the test and critical tabled values may suggest a trend towards a negative correlation between host length and relative density ie there are likely to be more parasites on the smaller fish (within the specified host size range) but requires more rigorous experimental confirmation.

The regression of prevalence of infection against host length is illustrated in Figure 6.11. From this it can be seen that there was a highly significant negative correlation ($P < 0.001$)

Figure 6.10 Regression of antilog mean log(x+1) infection number per fish against host length class

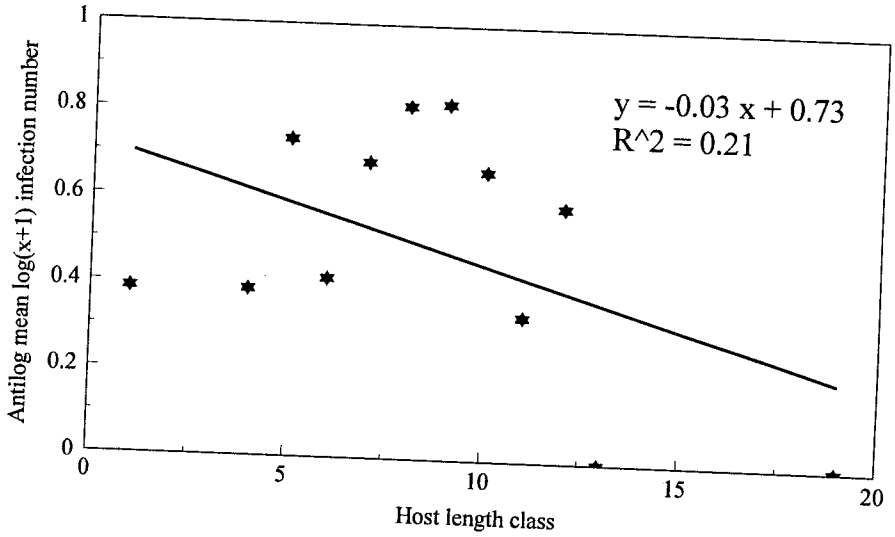
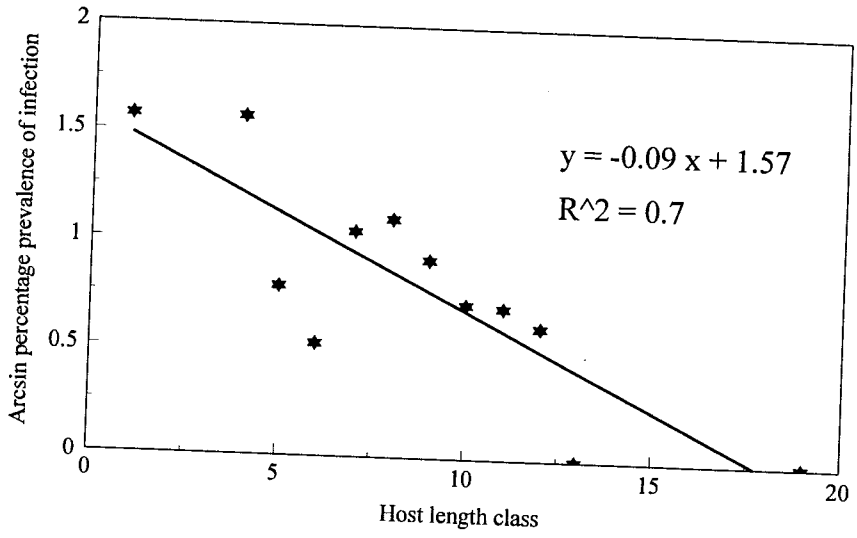


Figure 6.11 Regression of arcsin percentage prevalence of infection against host length class



between fish length and prevalence of infection (r = -0.83). This indicates that there is a significant relationship between the size of fish and its likelihood of infection such that smaller fish are more likely to become infected (within the size range specified).

6.3.2.3 Infection of salmon parr and fry

Both the parr and the first feeder were successfully infected with copepodid larvae, the latter being subject to settlement by the full 25 copepodids that comprised the infective dose.

The behaviour of the copepodids in the presence of both experimental hosts mirrored that described in Chapter 4. This indicates that despite their immature status and lack of smoltification, freshwater stages of Atlantic salmon possess the cues necessary to elicit larval attachment.

6.3.2.4 Infection of non-salmonid species

Experimental infection of non-salmonid species

None of the species tested here elicited settlement by the copepodid larvae. Movement of experimental hosts elicited the same responses described in Chapter 4. but no attempt was made to remain in contact with or settle on the host surface - copepodids leaving immediately on contact.

Occurrence of *L. salmonis* on non-salmonid species on farms

Wrasse

Five species of wrasse (a total of 138 individuals) were examined from seven farmed salmon sites, the prevalence of caligid species found being given in Table 6.8 and the stage breakdown in Table 6.9. It can be seen that whilst *C. elongatus* and *C. centrodonti* were present on wrasse from farmed sites, there was no incidence of mobile or settled stages of *L. salmonis*. This is despite the presence of large numbers of lice on farmed salmon in adjacent cages to the wrasse (e.g. relative density of infection of 20.3 for 10 salmon from Loch Ewe).

It is interesting to note that all the attached caligid stages found on wrasse were located on the fins; a situation comparable with that found in the present study for *L. salmonis* on salmon.

Table 6.8 Prevalence of caligid copepods on wrasse from farm sources.

LOCATION	MAP REFERENCE	WRASSE SPECIES (N INFECTED / N)				
		Ballan	Corkwing	Cuckoo	Goldsinny	Rock Cook
Loch Ewe	57°48′N, 5°45′W	1/3	0/2		0/15	0/1
Loch Eil	56°51′N, 5°18′W				1/58	0/12
Harport, an t-eilean Sgiathanach (Skye)	57°20′N, 6°25′W	1/8			0/7	0/1
Loch Linnhe	56°37′N, 5°30′W	1/2	0/1			
Loch Ailort	56°51′N, 5°40′W	0/2	0/2		0/9	
Loch Sunart (outer)	56°41′N, 5°48′W				0/5	
Loch Torridon	57°33′N, 5°38′W	4/4		0/4	0/1	1/1
FARM TOTAL		7/19	0/5	0/4	1/95	1/15

Table 6.9 Developmental stages of *C. centrodonti* and *C. elongatus* on wrasse from farm sources.

LOCATION	DATE	WRASSE SPECIES	LENGTH (mm) TL	STAGE					
				COP	CH I	CH II	CH III	CH IV M	CH IV F
Torridon	21-8-90	Rock Cook	115	1	1		4	2	1
Torridon	21-8-90	Ballan	164		1	3	5	1	1
Torridon	21-8-90	Ballan	176		3				
Torridon	21-8-90	Ballan	167	8 Chalimus					
Torridon	21-8-90	Ballan	158		1		2	1	1
Ewe	16-8-90	Ballan	167			1*			
Eil	16-9-91	Goldsinny	137		1*				
Harport	7-8-90	Ballan	185	2 Chalimus					
Linnhe	20-9-90	Ballan	210	1 Chalimus					

* *Caligus elongatus* (all others *Caligus centrodonti*)

Saithe

Two saithe from a multi-yearclass farm were taken from a cage containing salmon with a mean intensity of infection of 21.4 *L. salmonis* per fish (n = 5). A single saithe had one adult female *L. salmonis* and five *Caligus elongatus* at various stages of development. The second saithe had three *C. elongatus* but no *L. salmonis*.

A larger sample comprising 21 saithe (mean weight 637.14 ± 151.17 gms (1 S.D.)) was taken from a cage on a single-yearclass farm containing salmon having a relative density of infection of 12.4 ± 13.3 (1 S.D.) *L. salmonis* per fish (n = 5). One fish of 1080 gms carried a single adult female *L. salmonis*. No attached larval stages of *L. salmonis* have been observed on any saithe so far examined.

6.3.3. Natural abundance and distribution of copepodids in the water column

6.3.3.1 Light trap

The trap design proved effective for capturing planktonts and its principal shortcoming was the need to check it daily before placing it in the water, for damage done to the plankton mesh through abrasion on the cage walkways whilst not in the water. The holes so produced (principally in the area of the metal frame) were repaired when necessary with waxed cotton thread.

No difficulty was experienced distinguishing the *L. salmonis* larvae from their free-living counterparts since firstly, familiarity from previous experiments made them more easily recognisable, and secondly, they were noted to be much larger than, and their morphology characteristically different from, free-living species.

The composition of the captured plankton and the mean number of planktonts per 1 ml sub-sample (from a total sample of 200 ml) are given in Table 6.10 and illustrated in Figures 6.12 and 6.13 (with and without free-living copepods respectively). It can be seen from this data that the number of copepodid larvae caught in the trap, whilst being much lower than the number of free-living copepods, was similar in magnitude (during light-on periods) to the number of cirripede larvae and chaetognaths.

For the purposes of statistical evaluation, results from successive light on / light off trials have been regarded as constituting a test pair. Since free-living copepods displayed the most notable differences in abundance between nights with the trap switched on and those with it switched off, they were chosen as a positive control for comparison with the larvae of *L. salmonis*. The mean abundance recorded for these two groups is given in Tables 6.11 and 6.12

Table 6.10 Table of statistics for planktont groups captured in light trap during light on / light off events

Planktont Group	Mean (± 1 S.D.)	Median	Minimum	Maximum
TRAP ON				
L. salmonis larvae	0.68 ± 0.74	1.0	0.0	3.0
Free-living copepods	262.0 ± 182.68	134.0	89	528
Free-living copepod larvae	2.16 ± 2.98	1.0	0.0	10.0
Mollusc larvae	2.88 ± 2.37	2.0	0.0	8.0
Polychaete larvae	1.12 ± 1.39	1.0	0.0	5.0
Cirripede larvae	0.52 ± 1.08	0.0	0.0	4.0
Isopods	0.08 ± 0.28	0.0	0.0	1.0
Chaetognaths	0.88 ± 0.83	1.0	0.0	3.0
Appendicularians	1.64 ± 2.01	1.0	0.0	8.0
Decapod larvae	0.16 ± 0.37	0.0	0.0	1.0
Others	0.88 ± 1.24	0.0	0.0	5.0
TRAP OFF				
L. salmonis larvae	0.4 ± 0.71	0.0	0.0	2.0
Free-living copepods	132.8 ± 164.55	97.0	4.0	789.0
Free-living copepod larvae	1.04 ± 1.24	1.0	0.0	4.0
Mollusc larvae	2.56 ± 2.5	2.0	0.0	9.0
Polychaete larvae	0.48 ± 0.71	0.0	0.0	2.0
Cirripede larvae	0.16 ± 0.37	0.0	0.0	1.0
Isopods	0.0 ± 0.0	0.0	0.0	0.0
Chaetognaths	0.36 ± 0.64	0.0	0.0	2.0
Appendicularians	1.68 ±1.73	1.0	0.0	6.0
Decapod larvae	0.0 ± 0.0	0.0	0.0	0.0
Others	0.32 ± 0.48	0.0	0.0	1.0

Figure 6.12 Mean numbers of planktonts per 1 ml taken from a total light-trap sample of 200 ml (inclusive of non-larval free-living copepods).

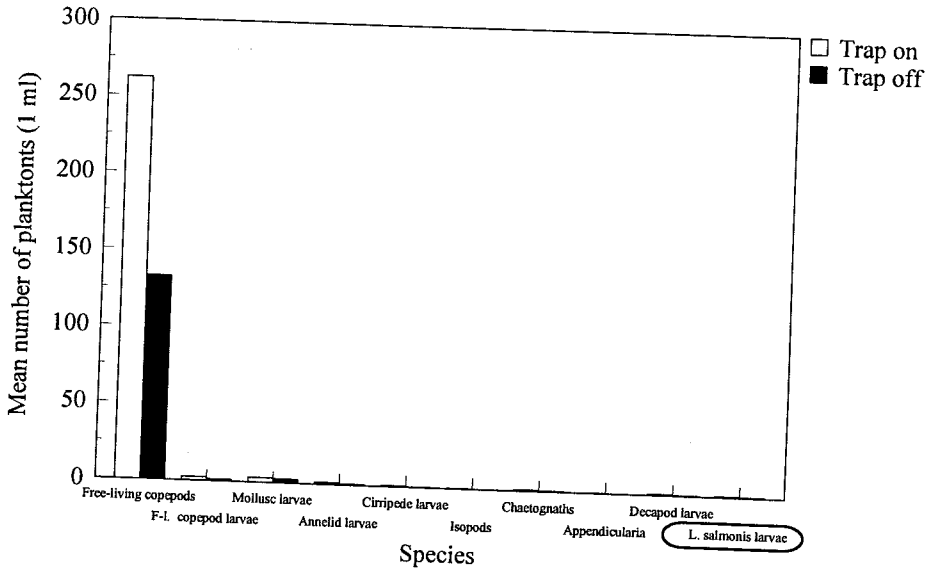


Figure 6.13 Mean numbers of planktonts per 1 ml taken from a total light-trap sample of 200 ml (exclusive of non-larval free-living copepods)

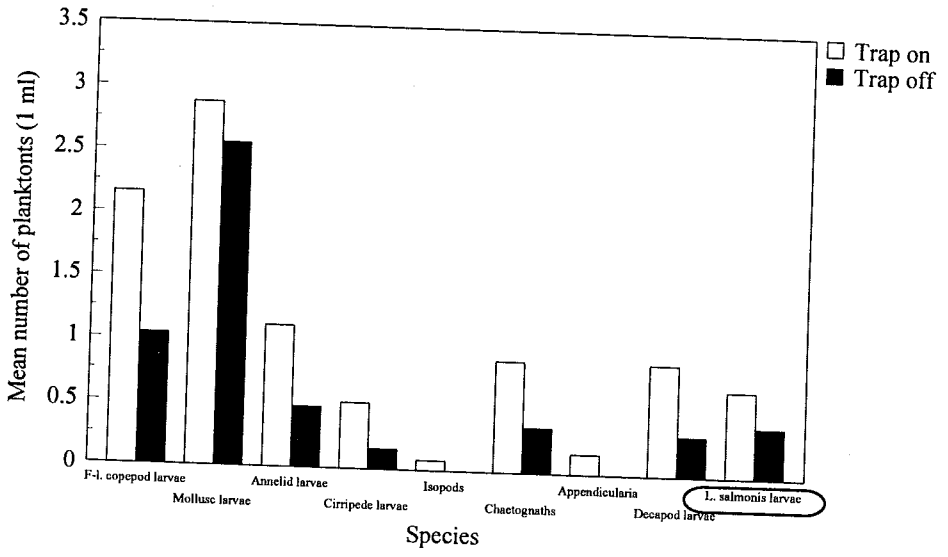


Table 6.11. Table of statistics for free-living copepods captured in light trap during light on / light off events

Sample number	Mean (± 1 S.D.)	Median	Minimum	Maximum
LIGHT ON				
1	485.8 ± 37.8	481.0	432.0	528.0
3	471.8 ± 43.5	477.0	404.0	519.0
5	127.6 ± 0.2	128.0	116.0	139.0
7	115.4 ± 24.7	122.0	89.0	142.0
9	109.4 ± 7.4	109.0	98.0	118.0
LIGHT OFF				
2	263.6 ± 41.2	254.0	212.0	318.0
4	236.0 ± 34.0	97.0	94.0	789.0
6	114.4 ± 16.0	117.0	97.0	139.0
8	40.2 ± 6.8	37.0	34.0	49.0
10	9.4 ± 4.3	9.0	4.0	16.0

Table 6.12 Table of statistics for *L. salmonis* larvae captured in light trap during light on / light off events

Sample number	Mean (± 1 S.D.)	Median	Minimum	Maximum
LIGHT ON				
1	0.6 ± 0.5	1.0	0.0	1.0
3	0.8 ± 0.8	1.0	0.0	2.0
5	0.6 ± 0.5	1.0	0.0	1.0
7	0.2 ± 0.4	0.0	0.0	1.0
9	1.2 ± 1.1	1.0	0.0	3.0
LIGHT OFF				
2	1.0 ± 1.0	1.0	0.0	2.0
4	0.4 ± 0.5	1.0	0.0	1.0
6	0.6 ± 0.9	0.0	0.0	2.0
8	0.0 ± 0.0	0.0	0.0	0.0
10	0.0 ± 0.0	0.0	0.0	0.0

and illustrated in Figures 6.14 and 6.15. Two things are to be noted about the free-living copepod controls - firstly, that they were more numerous when the trap was switched on and secondly that the numbers captured were extremely variable within the test period regardless of the state of the trap (on or off).

Comparing the abundance of free-living copepods with that of *L. salmonis* larvae, it can be seen that whilst the former were significantly more abundant with the light switched on (in three out of the five test pairs ($P < 0.02$)), the latter showed significant differences only in the fifth paired sample (Table 6.13). Numbers of copepodids were higher with the light on in 3/5 of the samples.

In addition to the larval stages mentioned, the trap also caught four preadult and one adult sea lice, all of which were male. A number of other mobile *L. salmonis* stages were also seen to adhere to the outsides of the trap when it was still in the water but were lost during recovery of the trap.

6.3.3.2 Pump sampling

6.3.3.2.1 24 hour sample

The equipment used functioned perfectly throughout the experiment. Pump clogging only occurred when sampling close to the benthos and was not a serious problem (requiring only that the tube be raised a little and the sample retaken). During the course of sampling, a total of 7000 litres (7 tonnes) of water was filtered for plankton.

As in the case of the light trap samples described above, the free-living copepods were the most numerous component of the plankton sampled and their distribution in the water column throughout the sampling period is given in Figure 6.16 The data collected for the free-living copepods indicates that the sampling strategy was successful in capturing planktonts at different depths and showing their distribution in the water column with time.

No larval stages of *L. salmonis* or any other caligid were captured during the 24 hours of sampling. Since the pump successfully captured other adult and larval copepods, this suggests that they were not escaping from the pump but were, rather, present in such low numbers, that the sampling strategy used failed to detect them.

Figure 6.14 Mean numbers of free-living copepods per 1 ml taken in light trap during light on / light off events

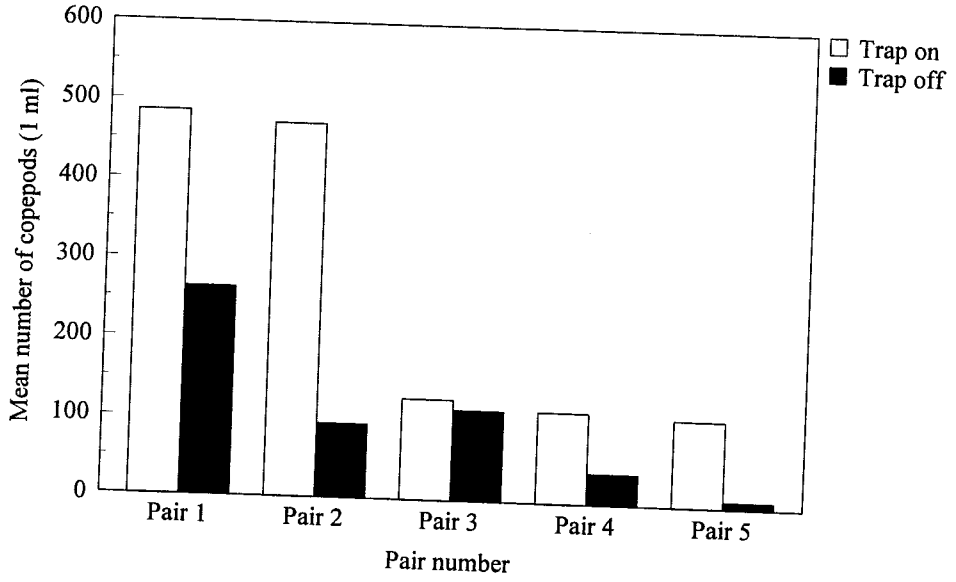


Figure 6.15 Mean numbers of *L. salmonis* larvae per 1 ml taken in light trap during light on / light off events

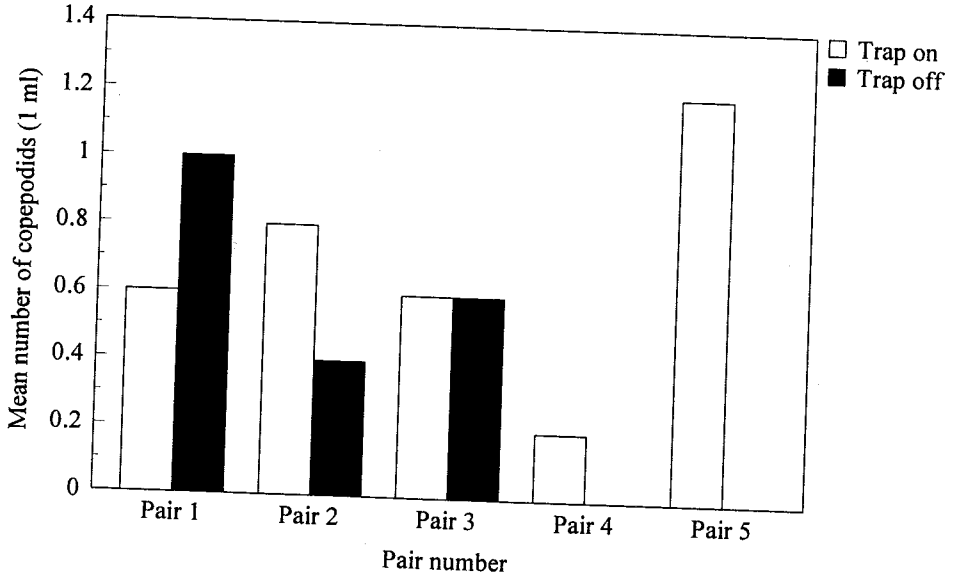
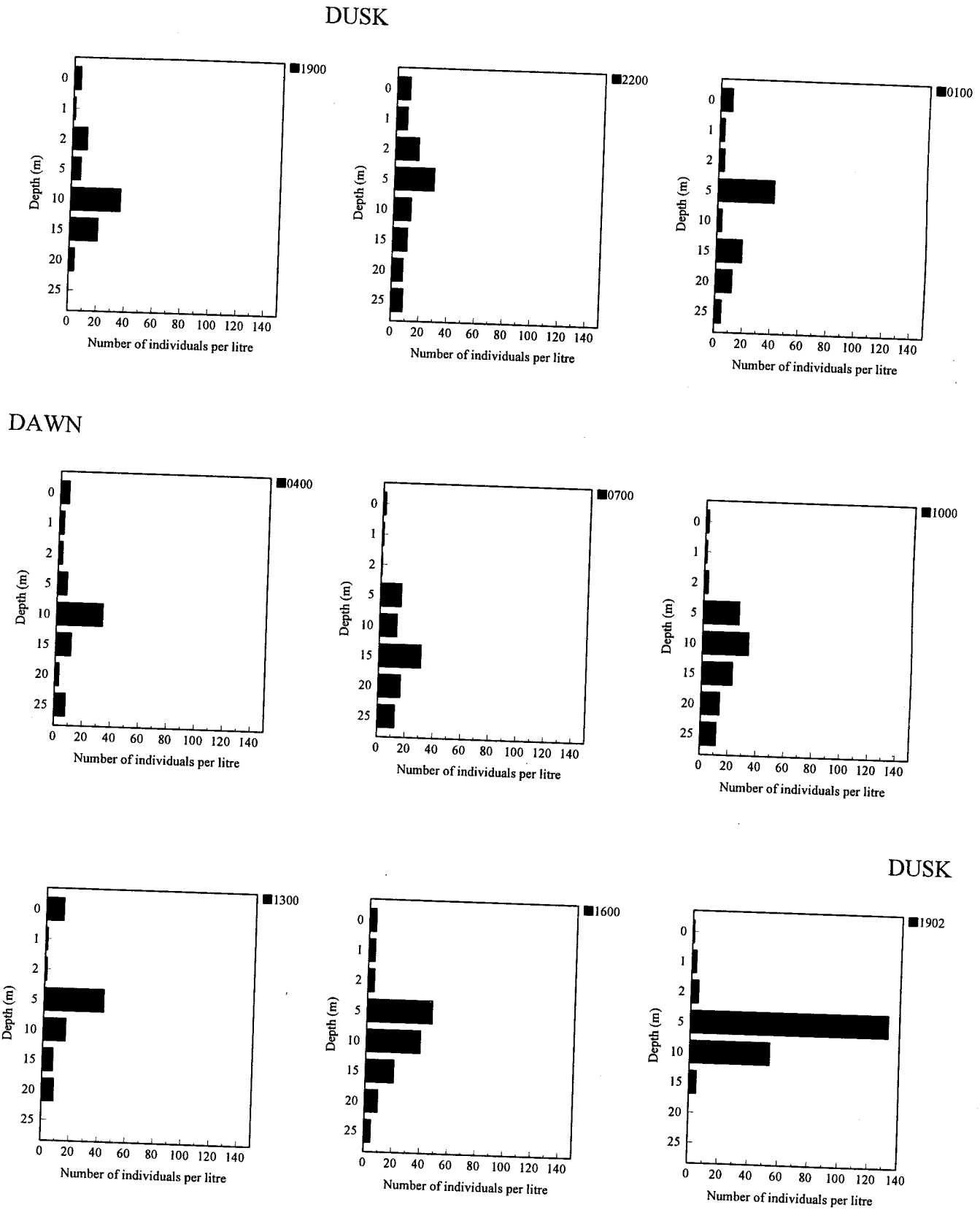


Table 6.13 Table of significance for differences between test (light on) and control (light off) pairs from light-trap catches (Mann-Whitney U-test).

Pair Number	Free-living copepods	*L. salmonis* larvae
1	Z = 2.51 P = 0.012	Z = -0.56 P = 0.576 N.S.
2	Z = 1.46 P = 0.142 N.S.	Z = 0.69 P = 0.488 N.S.
3	Z = 1.15 P = 0.247 N.S.	Z = 0.11 P = 0.908 N.S.
4	Z = 2.51 P = 0.012	Z = 0.80 P = 0.424 N.S.
5	Z = 2.51 P = 0.012	Z = 2.27 P = 0.023

N.S. signifies non-significant at $P < 0.05$ level

Figure 6.16 Free-living copepod distributions obtained by timed pump-sampling at various depths through the water column on a sea-farm site



6.3.2.2.2 Cage net sample

No *L. salmonis* or other caligid larvae were observed either in the live sample or the preserved sample. There was therefore no evidence for their attachment to the cage nets or epibiota associated with them.

6.3.2.2.3 Plankton mesh "spider"

No *L. salmonis* or other caligid larvae were recovered from the plankton mesh spider. This concurs with the lack of evidence for attachment to netting or net epibiota found using the pump-sampling technique already mentioned and observed in previous chapters and in the survival experiments described above.

6.3.4. Farm infections

The small number of fish in each sample and the variability between cages mean that some analyses (e.g. fitting of negative binomials to the data) of the population of parasites with respect to the host population could not be carried out. Nevertheless, the information gained is useful for comparison with the data sets already obtained from the experimental studies covered previously in other chapters and in the present one.

6.3.4.1 Temporal and numerical distribution on farmed salmon hosts

The number of attached copepodids on any given sampling date was normally low. For this reason the standing population of all larval stages is considered as it provides a more useful picture of the larval dynamics on a farm.

Figure 6.17 shows the mean number of attached larvae (all stages) per fish for five separate cages over a period of 20 months on a marine salmon farm. The most noticeable declines in larval abundance often follow organophosphate treatments - numbers of larvae often falling substantially subsequent to a successful treatment (see e.g. Figure 6.18). Figure 6.17 also illustrates the tendency for sudden peaks of infection on all or some of the pens.

Another feature of the graphs of mean larval abundance is the agreement between the different pens sampled. Figure 6.17 shows the superposed patterns of the five pens sampled, moving from top to bottom, away from the sea. Despite the clear differences in the magnitude of peaks between pens, there is nevertheless considerable concordance in the number and timing of peaks between the pens. This suggests that the entire site is subject to the same overall pattern

Figure 6.17 Comparison of simultaneously sampled mean larval numbers from five pens on a marine salmon farm

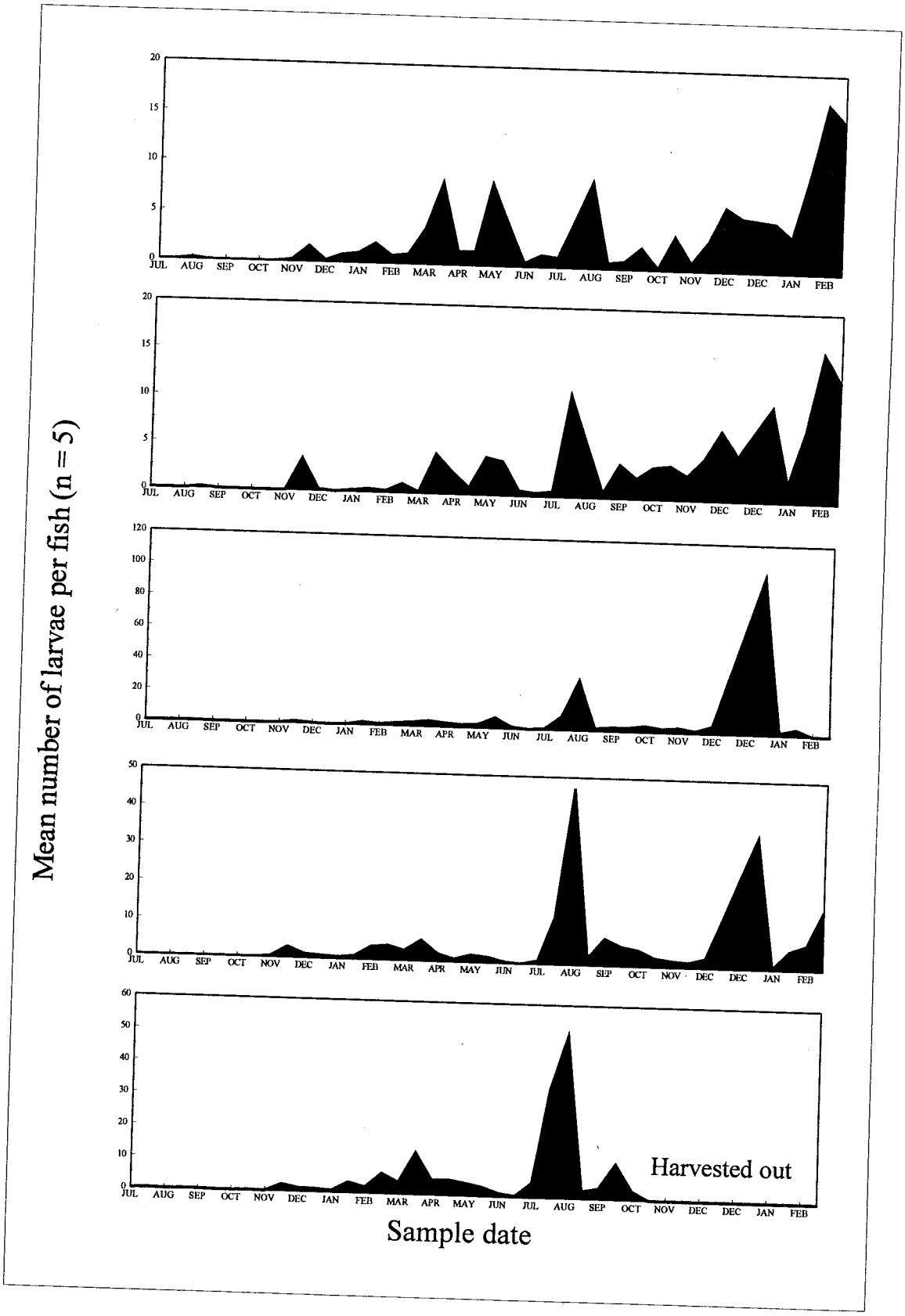
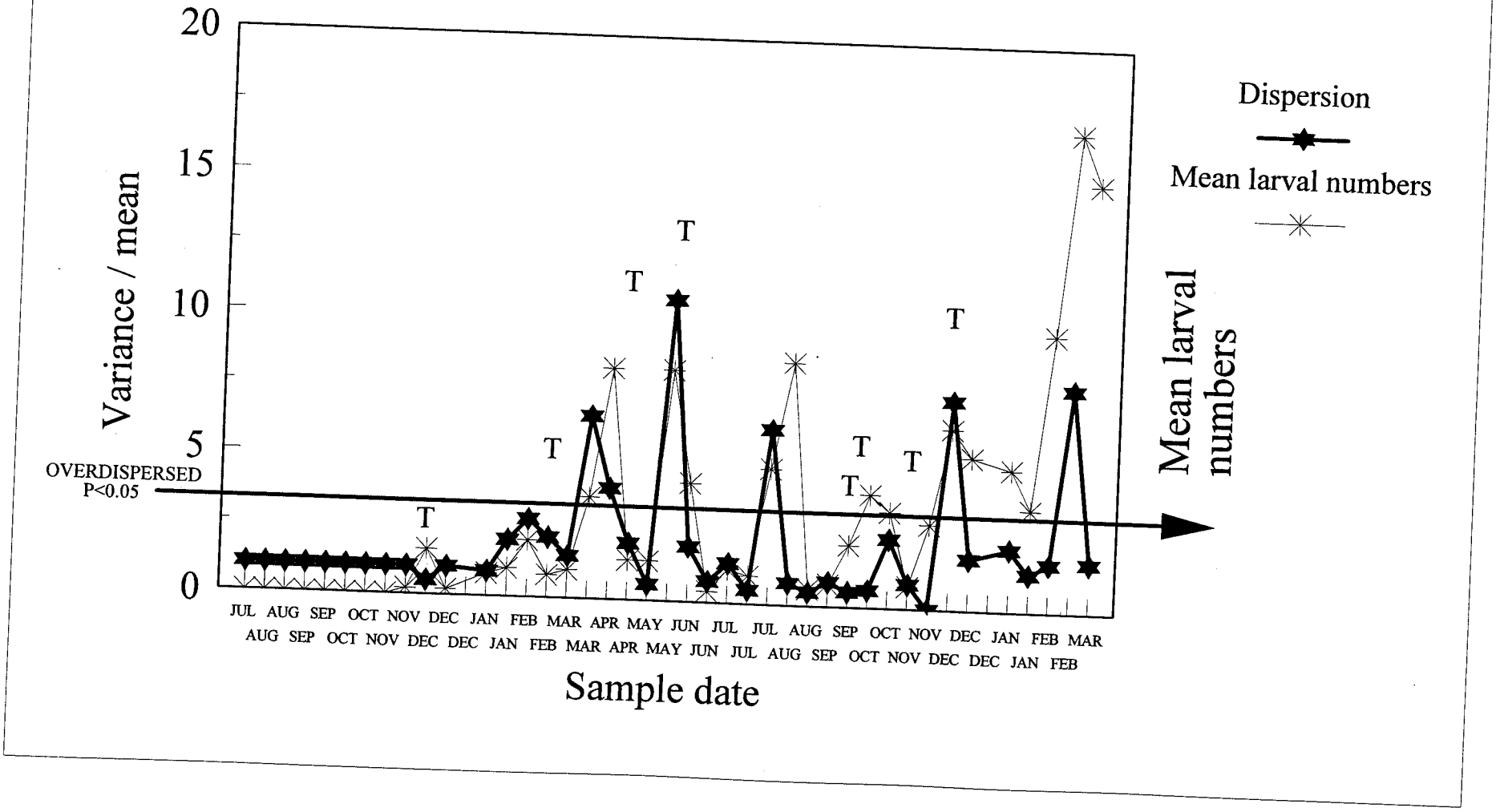


Figure 6.18 Dispersion (variance / mean) and mean number of larvae on farmed salmon from a single pen sampled bi-weekly



of settlement, with local differences superimposed upon a more general pattern. It may also be noted that adjacent groups resemble one another more strongly than do groups which are further apart.

Although a negative binomial curve was not fitted to the data, an estimate of dispersion may be gained through the calculation of the variance / mean (relative density) ratio using tables for small sample sizes to provide confidence intervals (Elliot 1971). Table 6.14 gives the means and variances for one of the sampled pens and the variance / mean ratio. The results are also shown graphically in Figure 6.18 with the mean number of larvae per fish added for reference. From this it may be seen that the population is intermittently overdispersed throughout the year and that significant overdispersal generally coincides with high larval numbers. A regression of $\log(x+1)$ mean number of larvae against $\log(x+1)$ variance (shown in Figure 6.19) confirms the relationship between mean and variance to be significant ($P < 0.001$). Significant overdispersal often precedes or coincides with treatment events (Figure 6.18).

In all pens there is an apparent tendency for escalation in larval numbers throughout the period although this tendency is somewhat obscured by the twin perturbations of treatments and sudden infection peaks (Figure 6.17).

6.3.4.2 Host susceptibility

The effect of host size on infection parameters was investigated using the same technique as described for the large scale smolt infection experiment. Since the farmed fish displayed considerable variation in size and, more importantly shape, weight was chosen as an indicator of size rather than fork length. Because larvae were not normally abundant enough to obtain a good estimate of host-size effects and because of the small fish sample sizes, a single group of fish ($n = 25$) was pooled from five cage samples taken on the same date from adjacent groups and showing, collectively, a relatively high abundance of larval stages. This data was divided into 13 weight classes (1000 - 3400 g in 200 g classes) transformed log $(x+1)$ and the means of the transformed values antilogged and regressed against the host weight. The regression was carried out for chalimus I stages only which makes the results comparable with those obtained from the large experimental smolt infection. Because of the ease of loss of copepodids during netting and handling of hosts it was felt that copepodid numbers on fish would be highly artefactual. Regression of data concerning chalimus II, III & IVs was also felt to be inadvisable in that post-settlement mortality would play an increasingly large role in the determination of numbers present on the host with respect to time post-settlement.

Table 6.14 Table of dispersion (variance / mean) for all larval stages from a single pen over a 20 month sampling period.

Sample Month	Mean	Variance	Variance / Mean	Sample Month	Mean	Variance	Variance/ Mean
JUL	0.00	0.00	1.00	**JUN**	4.20	8.20	1.95
AUG	0.00	0.00	1.00	**JUN**	0.40	0.30	0.75
AUG	0.00	0.00	1.00	**JUL**	1.20	1.70	1.42
SEP	0.00	0.00	1.00	**JUL**	1.00	0.50	0.50
SEP	0.00	0.00	1.00	**JUL**	4.80	29.70	6.19
OCT	0.00	0.00	1.00	**AUG**	8.60	6.80	0.79
OCT	0.00	0.00	1.00	**AUG**	0.60	0.30	0.50
NOV	0.00	0.00	1.00	**SEP**	0.80	0.70	0.88
NOV	0.20	0.20	1.00	**SEP**	2.20	1.20	0.55
DEC	1.60	0.80	0.50	**OCT**	4.00	2.50	0.63
DEC	0.20	0.20	1.00	**OCT**	3.40	8.30	2.44
DEC	N.S.	-	-	**NOV**	0.80	0.70	0.88
JAN	0.80	0.70	0.88	**NOV**	3.00	0.00	0.00
JAN	1.00	2.00	2.00	**DEC**	6.40	47.30	7.39
FEB	2.00	5.50	2.75	**DEC**	5.40	9.80	1.81
FEB	0.80	1.70	2.13	**DEC**	N.S.	-	-
MAR	1.00	1.50	1.50	**JAN**	5.00	11.00	2.2
MAR	3.60	23.30	6.47	**JAN**	3.60	4.80	1.33
APR	8.20	32.20	3.93	**FEB**	9.80	16.70	1.7
APR	1.40	2.80	2.00	**FEB**	17.00	134.00	7.88
MAY	1.40	0.80	0.57	**MAR**	15.20	26.70	1.76
MAY	8.20	87.70	10.70				

N.S. = No sample taken from pen on date

Figure 6.19 Regression of log(x+1) relative density of larvae against log(x+1) variance for farmed salmon from a single pen sampled bi-weekly

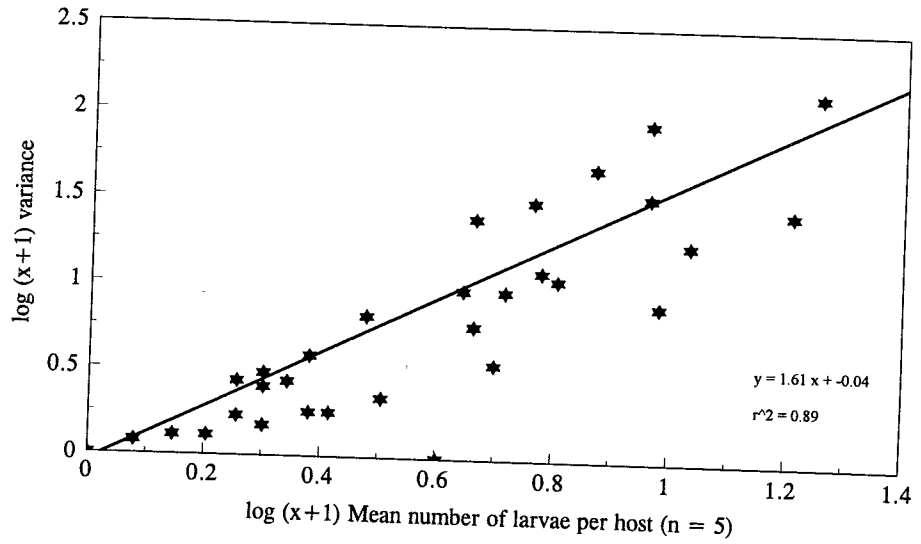
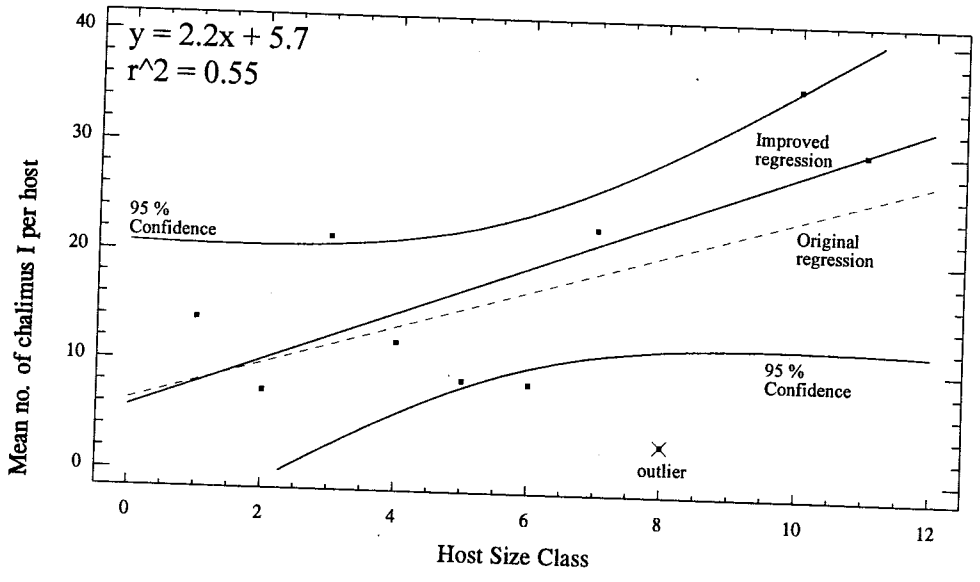


Figure 6.20 Regression of number of chalimus I (x+1 transformed, meaned and antilogged) against host size class (1000-3200 g, 200g classes)



A regression of mean numbers of chalimus I against weight gave a significant ($P < 0.05$) correlation ($r = 0.54$). Removal of a single outlier (Figure 6.20) improved significance ($r = 0.74$, $P < 0.005$). This suggests that, the larger the fish, the more chalimus I's they carry.

In addition to the above findings concerning host susceptibility to infection, it was clear that disadvantaged fish had a much higher incidence of infection (the samples being random, however, disadvantaged fish were unfortunately rarely included). Diseased fish such as those displaying pancreas disease or furunculosis also displayed a greater incidence of infection. Grilsing (mature) fish were also prone to a high level of infection as demonstrated for instance by a single heavily grilsing male which showed a total of 479 settled larval stages in contrast to a mean intensity of 4.25 ± 2.23 (1 S.D.) for the other fish sampled from the same pen ($n = 4$) and 16.6 ± 24.81 (1 S.D.) for the fish sampled from three other groups (pooled $n = 15$).

Aggregations of lice around a particular stage of development were common, especially amongst the larvae. Table 6.15 shows the results of linear regressions performed on the data set from a single pen to investigate the relationship of the abundance of each stage with that of successive stages. The number of copepodids was found to be significantly correlated with the numbers of all the succeeding attached larval stages. The abundance of each individual larval stage was also significantly correlated with the stages following. The number of adult females was not, however, found to be correlated with the number of copepodids.

6.3.4.3 Spatial distribution on farmed salmon hosts

The distribution of larvae was similar in all of the samples observed, with the principal site of settlement being the fins as was the case in the experimental settlements. Figure 6.21 shows the percentage of total settled stages (copepodids and chalimus from pooled $n = 125$) located in different host sites. Both stages show a similar distribution with settlement principally on the fins although it should be noted that a proportion of the attached copepodids may have been lost in host handling. Table 6.16 and the accompanying Figure 6.22 give a more detailed overview of site distribution of copepodid and chalimus stages. Although these stages do not differ significantly in terms of overall settlement on body or fins, there was a significantly higher proportion of chalimus stages on the dorsal fin and operculum. The adipose and caudal fins showed appreciably fewer settled stages, particularly copepodids, than the other fins, this difference being heightened by the relatively large area of the latter.

The principal difference found between the pattern of settlement on the farmed fish and that described for the experimental settlements, was the lack of **any** settled stages on the gills or the buccal or branchial cavity linings of the former group.

Table 6.15 Table of regressions of larval stages and adult females against successive stages.

	Stage			
	Copepodid	**Chalimus I**	**Chalimus II**	**Chalimus III**
Chalimus I	R = 0.92 P < 0.005			
Chalimus II	R = 0.75 P < 0.005	R = 0.84 P < 0.005		
Chalimus III	R = 0.75 P < 0.005	R = 0.65 P < 0.005	R = 0.58 P < 0.005	
Chalimus IV	R = 0.51 P < 0.005	R = 0.53 P < 0.005	R = 0.43 P < 0.005	R = 0.57 P < 0.005
Adult Female	R = 0.01 P > 0.05	-	-	-

Table 6.16 Table of comparison of settlement sites of copepodid and chalimus larvae from a pool of 125 farmed salmon. Figures indicate percentage of total settled stage ± 95 % confidence limits.

Settlement site	**% total settled copepodids (± 95 % conf.)**	**% total settled chalimus (± 95 % conf.)**	**Significance (95 % conf.)**
Dorsal fin	20.83 ± 11.5	42.75 ± 4.9	P < 0.05
Adipose fin	2.08 ± 4.0	2.54 ± 1.6	N.S.
Caudal fin	2.08 ± 4.0	3.05 ± 1.7	N.S.
Pectoral fin	14.58 ± 10.0	6.36 ± 2.4	N.S.
Ventral fin	8.33 ± 7.8	7.38 ± 2.6	N.S.
Anal fin	16.67 ± 10.5	5.6 ± 2.3	N.S.
Operculum	0.0 ± 0.0	3.82 ± 1.9	P < 0.01
Body surface	35.42 ± 13.5	29.77 ± 4.5	N.S.
Total body surface	35.42 ± 13.5	33.59 ± 4.7	N.S.
Total fins	64.58 ± 13.5	66.41 ± 4.7	N.S.

Figure 6.21 Comparison of total percentage settlement of copepodids and chalimus on different host sites on farmed salmon (n = 125)

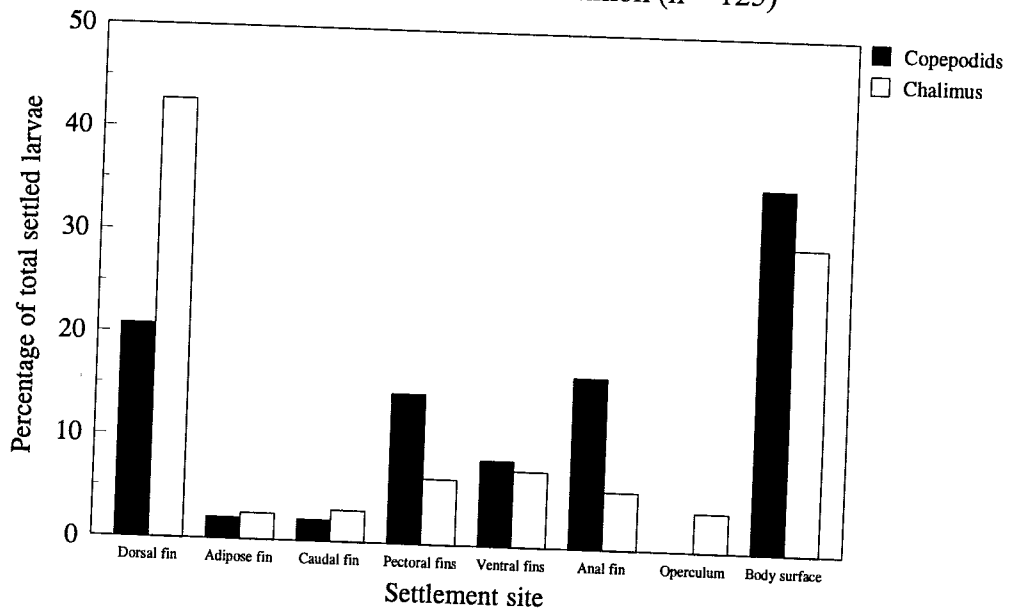
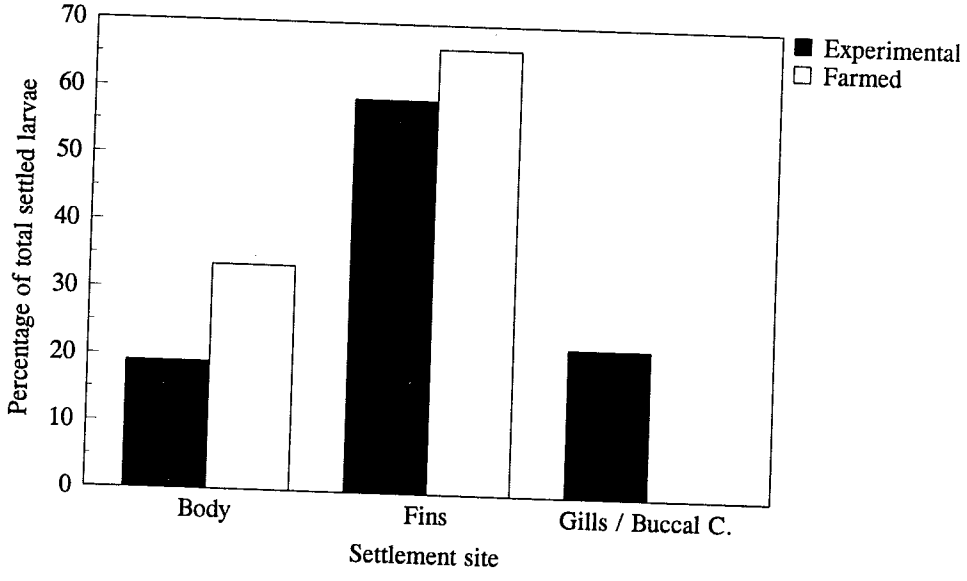


Figure 6.22 Comparison of total percentage settlement of chalimus on different host sites on experimentally infected smolts (n = 72) and farmed salmon (n = 125)



6.4 Discussion

One might expect that the greater the survival time of the copepodid, the greater is the opportunity for its making contact with the host. The initial hatching mortalities in the present study were minimal (median 7.5 %) and were followed by a relatively low mortality between hatching and moulting to NII (median 9.2 %). This compares, for instance, with a mortality of 55.8 % for *L. branchialis* up to NII (Whitfield *et al.* 1988). It is believed that the mortality in the present study might be even further reduced by the assistance to hatching provided by current flow in the natural situation and therefore that mortality can clearly be considered to be low at this stage.

Best survival in the laboratory was obtained under conditions which maximised water quality. In contrast to the findings of Johannessen (1975), the presence of fibrous substrates did not enhance survival. Because the water quality of a static laboratory system is likely to be lower than that of the natural environment, it might be reasonable to suggest that survival in the wild is likely to be even greater. Furthermore, the fact that copepodids in the laboratory will come into frequent contact with the experimental vessel means that they may be stimulated into avoidance / swimming activity more frequently than might be the case in open water, resulting in a premature depletion of energy reserves. This improved survival in the wild would, however, be offset by factors such as predation in the plankton.

Although Raibaut (1985) suggests that the copepodid stages of parasitic copepods generally have a short life-time (1-2 days) the present study indicates that *L. salmonis* may survive up to a maximum of 23 days *in vitro* and that 50% may survive up to 8 days after moulting to the copepodid stage. The values for developmental times in the present study are of the same order as those given by Johnson & Albright (1991a), Johannessen (1978) and Wootten, Smith & Needham (1982) (Table 6.17) although the time taken to moult to NII is lower than the estimates given by the former two authors. The copepodid survival times are relatively large in comparison with those given for other caligids (Table 6.17) and for a number of other parasitic species (Table 6.18) although little indication is normally given of water quality used in rearing which may explain low values such as the 4 day survival given for *L. salmonis* by Wootten *et al.* (1982).

Despite the apparent extended survival of *L. salmonis* copepodids, the present study has not investigated their infectivity throughout this time and it is highly likely that this will change with time. In common with *C. epidemicus* (Ho pers. comm.), copepodids of *L. salmonis* are infective immediately they enter the water column although no estimates have been made of success following attachment. Carton (1968) found that copepodids of *C. tubulata* parasitizing *A. squamata* increased their settlement success from 34% immediately following moulting to

Table 6.17 Table of survival and development times for a range of *Lepeophtheirus* and *Caligus* species.

Species	Duration of NI (hours)	Duration of NII (hours)	Duration of settled copepodid (days)	Maximum survival of free-swimming copepodid	Temperature (°C)	Authority
L. salmonis	18-22	53-57	< 7	23 (30‰)	10	Present study
"	52	170.3				
	30.5	56.9	~7 (50 %)		5	Johnson & Albright (1991)
	9.2	35.6		17 (25‰)	10	
"	35	42			15	
	12	63 (inc. NI)		30	9.2	Johannessen (1978)
		33 (inc. NI)			11	
"	18	46			15.5	
					19	
L. hospitalis	24	40	5.5-8	4	12	Wootten (1982)
	7.5	11				
"				3-8	15	Voth (1972)
		120-150 (inc. NI)			20	
L. dissimulatus	4.5-13	9.5-19			15	Lopez (1976)
L. pectoralis				3	23	Lewis (1963)
			2-3			
Caligus spinosus	4-6	12-18		3	10	Boxshall (1976)
C. minimus				5-7	20	Izawa (1969)
				13	15	Paperna (1980)
C. longipedis	2.1-2.5	16.5-17.2	<3	4	29	
C. curtus	~24	~24		7	22.5 ± 2	Ogawa (1992)
C. epidemicus	6	14.5	2	4-5	6-8	Heegaard (1947)
C. elongatus	15-30 (range for diff. temps)	35 (10°C)		4-5	24-25	Lin & Ho (1993)
				2 (13°C)	ambient	Hogans & Trudeau (1989)
C. pageti	~24	~24				Ben Hassine (1983) cited in Johnson & Albright (1991)

Table 6.18 Survival and development times for selected non-caligid parasitic copepods

Species	Duration of NI (hours)	Duration of NII (hours)	Duration of settled copepodid (days)	Maximum survival of free-swimming copepodid	Temperature (°C)	Authority
Lernaeenicus sprattae	23-27	24		5-6	15	Schram & Anstensrud (1985)
Cancerilla tubulata		~48 (total inc. NI)		11		Carton (1968)
Trochicola entericus		~72 (total inc. NI)				Bocquet, Stock & Kleeton (1963) cited in Carton (1968)
Lernaeocera branchialis	24	24	2	18	10	Whitfield *et al.* (1988)
Salmincola salmoneus				1-1.5		McGladdery & Johnston (1988)
Salmincola californiensis	-	-	3.5 hrs - 2.5 days	2	10	Kabata & Cousens (1973)
Alella macrotrachelus			1.75 (inc. "pupa")			Benkirane (1987)

72% at 5 days and then fell to 60% at 9 days. It was suggested that the increase of success resulted from refinement of sensory faculties and that the falling success followed a progressive loss of vitality. Such a decline in infectivity is well recognised in other parasites e.g. the digenean *Transversotrema patialense* (Soparker) described by Anderson & Whitfield (1975) and a similar pattern might therefore be envisaged for *L. salmonis* although this has yet to be examined.

In the present study, no significant difference in infection was noted between fish infected in the light and those infected in darkness. This would seem to support the findings presented in Chapter 3 which suggested that host contact was mediated through mechanoreception rather than through responses to shadow as has been demonstrated for e.g. *S. edwardsii* by Poulin, Curtis & Rau (1990). In contrast to the present study, Schram & Anstensrud (1985) noted a significantly lower prevalence and intensity of infection for sprats infected with *L. sprattae* in the light as opposed to those infected in darkness. It is not, however, possible to infer from this experiment that light has no effect on host infection in the natural environment since within the water column it may, as stated in previous chapters, be used as a cue to increase spatial overlap between parasite and host. Within the relatively small tanks used for the present series of experiments, such overlap is relatively well guaranteed such that the contribution of light may not be detectable (particularly with the small host sample sizes used here). These results may be supported by findings in Norway (Alderson pers. comm.) that covering (shading) of farm pens with tarpaulins had no effect on sea lice infections, although in a farm environment copepodids will be continuously brought through a cage by horizontal water movement such that shading might be expected to have little effect in any case. The findings of the present study throw into question the likely effectiveness of shading as a control measure for lice as proposed by Huse *et al.* (1990) whose conclusion that experimental shading was effective, appears to be based on an inappropriate statistical analysis of their results. The results of this experiment have allowed subsequent experiments to be carried out in darkness, thus reducing host stress and extending the possible period of infection.

The relative density of infection in the large smolt infection was lower than that of the light / dark experiment despite the fact that infection doses were similar. This may result either from the fact that there were more copepodids available per fish in the former experiments or alternatively it may be a factor of turbulence caused by the larger number of fish per litre in the second experiment. This latter factor has been suggested to inhibit host location and infection by Gotto (1962) and Briggs (1976) and may similarly be important where fish are in fast or turbulent currents.

The success of attempts to infect parr and fry indicate that whatever the factor required for host recognition by the copepodid, it exists in these freshwater stages and is not therefore a novel component generated by Atlantic salmon in the course of smoltification and migration into the marine environment. Conversely, the experiments described here demonstrate that the host-specific factor is **not** apparently carried by the non-salmonid species tested or is present at a different concentration. These species were therefore rejected on contact by the copepodid despite the fact that mechanical cues provided by their movement in the water column apparently elicited the same copepodid pre-contact behaviour as described in Chapter 3 for salmonid hosts.

In agreement with the experimental infections discussed above, examination of non-salmonids kept in contact with infected salmon indicates that they do not display any tendency to be infected by larval *L. salmonis*. Although Bruno & Stone (1990) considered saithe (*P. virens*) to be a host for *L. salmonis* by reason of the presence of adult and preadult stages of this species on fish kept in contact with salmon, the lack of feeding damage or larval settlement observed would suggest that "infection" is purely accidental and that no true relationship, beyond a possible phoretic one, exists between "host" and parasite.

These findings indicate that the general failure to observe *L. salmonis* on non-salmonid hosts (Kabata 1979) probably results from active rejection of such hosts by the infective stage in the wild. This contradicts the suggestion of Lewis *et al.* (1969) that initial larval settlement on hosts is random and that host specificity is thereafter enforced by differential mortality of attached larvae.

Similarly high host-specificity has been well demonstrated in *L. pectoralis* by Boxshall (1976) and recognition of host mucus has been observed for *C. minimus* by Fraile (1986). Other parasitic copepods show similar specificity of copepodid stages e.g. *L. sprattae* presented with sprat (*Sprattus* (L.) and herring (*Clupea harengus* L.) in experiments carried out by Anstensrud & Schram (1988) and *S. edwardsii* presented with a number of salmonid species in addition to its brook-trout host (Fasten 1913). As indicated in Chapter 3, such host specificity is likely to be mediated via chemoreceptors on contact with the host surface.

As part of the attempt to elucidate infection parameters in the field, a number of techniques were used to try and assess the prevalence and distribution of larval *L. salmonis* stages in the vicinity of cages. The first of these was a light trap which relied on the fact that *L. salmonis* larvae had been demonstrated to be positively phototactic. The light trap used in the present study gave comparable results to that used by Jones (1971) in terms of capture of zooplankton in general. The general lack of significant difference in the number of *L. salmonis* larvae captured with the light on / off indicates that the light was ineffective in attracting

additional copepodids. Although far fewer *L. salmonis* than free-living copepods were captured, they were nevertheless approximately as abundant, with the light on, as cirripede larvae and chaetognaths which suggests relatively high numbers to be present in the vicinity of cages. Multiplying up from the sub-samples, the highest mean capture for a night (1.2 ± 1.1, $n = 5$) gives a total trap contents of 240 larval *L. salmonis* which seems high considering the small ventral opening and relatively constrained light source. The success of the present trap seems far greater than that of smaller light traps utilising cyalume sticks used by Taylor (1987) which captured a total of 3 NI *L. salmonis* during three nights of sampling although this may be more a factor of larval abundance than trap effectiveness. The fact that relatively large numbers of *L. salmonis* larvae were captured near the surface at night may suggest that, like *L. sprattae* (see Schram & Anstensrud 1985), they migrate to the surface after dusk. Such a suggestion must, however, remain tenuous without further investigation since firstly, no successful estimate has been made of diurnal numbers and secondly the depth from which larvae are attracted by the light is unknown.

A fortuitous result from the light trap was the capture of a number of male preadult and adult *L. salmonis*. This supports a contention that *L. salmonis* adults are liable to enter the plankton - a trait shared with the *C. elongatus* which has been reported from plankton samples by Pearson (1904), Hardy (1956) and Neilson, Perry, Scott & Valerio (1987). *Caligus coryphaenae* Steenstrup & Lutken and *Caligus productus* Dana have also been recorded in plankton (Heegaard 1972) as has *C. epidemicus* (Ruangpan & Kabata 1984). The finding of adults and preadults attached to the light trap may also support observations of positive phototaxis in these stages under laboratory conditions (pers. obs.).

The second method of collection was by the use of pump-sampling. This method appeared to provide a good representation of the zooplanktonts present at different depths in the water column and at different times but failed to capture any *L. salmonis* (larvae or adult). Samples obtained from pen sides also failed to produce larval stages as did the "plankton spider" which may, when taken with the failure of copepodids to attach to fibrous substrates in the laboratory as demonstrated in Chapter 4, indicate that the increased infection associated with fouling of nets (Rae pers. comm.) is not a result of a reservoir of infection attached to the net sides. Alternative explanations for the association with heavy fouling with higher infestations may be firstly that fouling may reduce turbulence and thereby enhance the prospect of host location and settlement (see earlier) and secondly that fouling reduces current speed and thereby makes settlement and attachment easier (see below for discussion of hydrodynamics). It is also possible that the relationship between fouling and infestation reflects reduced flushing of nauplius stages from within the pen, although in view of the 3 days required for the moult to copepodid and the

fact that flow through the cage must nevertheless be sufficient to keep fish alive, this suggestion seems less likely.

The light-trap and pump-sampling strategies gave different indications as to the numbers of larval stages of *L. salmonis* present in the water column with the former giving high and the latter low (ie 0) estimates. It is suggested, however, that rather than representing an artefact of the respective sampling strategies, this probably reflects the variability of numbers of larvae in the water column over time. This hypothesis, however, clearly needs to be validated in future by the use of simultaneous sampling using both methods although the fact that the light trap will draw larvae from an unquantifiable area makes direct comparison difficult. High variability presumably leads to the observation of "waves of infection" on farms (as supported by clustering of stages) and may in fact result from "generation waves" on farm sites. This phenomenon is likely to be exacerbated by repeated removal of adult and preadult lice through treatment on farm sites which will further reduce larval production to a number of relatively discrete waves.

The present study has failed to demonstrate that either of the two methods tested are suitable for monitoring larvae in the water column. A better estimate of the efficacy of these two methods requires longer term studies carried out contemporaneously in order to compensate for the variability inherent in the planktonic larval population. Such studies may be difficult to arrange because of the stringent safety requirements required on farm sites which normally preclude manned overnight sampling and certainly make it extremely difficult to organize sampling within the short space of time required to intercept a "peak" of copepodid infection.

Studies of the distribution of the larvae of parasitic copepods within the water column are scarce in the literature. An excellent study by Schram & Anstensrud (1985) of larvae of *L. sprattae* demonstrated that larvae were up to 6 × more abundant in neuston samples taken at night. Although Nansen and Bongo net hauls resulted in few specimens being captured, a specially designed tow-net gave good results although it was only used to a depth of 90 cm. During the day numbers of larvae increased from shallow to deep tows whilst at night the larvae were found to have migrated upwards in the water column and were maximally abundant at a depth of 50 - 70 cm. It was suggested that copepodids undertook greater vertical migrations than nauplius larvae as a result of greater swimming potential and that this behaviour allowed spatial overlap with the host at night when 0-group sprats were to be found at the surface. Despite the capture success demonstrated on a number of occasions in this latter study, it should be noted that capture rate was highly variable with some tows capturing a total of only 1 copepodid in a total sample of 125 m^3. No explanation was given for this variability although it concurs with a concept of temporally and spatially discrete waves of infection in some copepod parasites.

It has been suggested that the level of infestation of salmon (*S. salar*) with *L. salmonis* is negatively correlated to rainfall / river discharge (White 1942b). This assertion is supported

by anecdotal evidence from farm sites in Scotland. Although this is often considered to be an effect of low salinity, it may also be attributed in part to the flushing of larvae from the area of infection. In this context, it may be possible to draw parallels with the findings of Gaines & Bertness (1992) who recorded a high correlation between settlement rates and flushing times for larvae of the barnacle *S. balanoides*. Such a situation could, in part, explain the variability of recruitment in *L. salmonis*.

Heegaard (1947) reported that nauplii of *C. curtus* were recovered in plankton hauls during the day but were never found higher than a depth of 40 m. If it is also the case that larvae of *L. salmonis* are similarly restricted to greater depths during the day then infection of farmed fish must occur principally at night since the cages normally extend to a depth of only 12 m. In some lochs, however, the shallow nature of the water could nevertheless extend the spatial and temporal overlap of copepodids and farmed salmon and it is noteworthy that anecdotal evidence suggests that Norwegian farms have had some success in the prevention of infection by pumping seawater into salmon cages from depths of $>$ 100 m (something that is rarely possible on British farm sites).

Fryer (1966), as noted in the introduction, has also noted the presence of larval stages of *Lernaea* sp. and *Ergasilus* sp. in plankton in Lake Nyasa but was of the opinion that they would be unable to make contact with benthic hosts in the water column and might therefore alternate phases of dispersal with phases of host seeking, the latter suggested to be principally nocturnal.

The frequency distribution of settlement under controlled laboratory conditions was found to be significantly overdispersed (ie variance greater than the mean) and to correspond to a negative binomial distribution. In contrast, a similar infection experiment carried out by Taylor (1987) for the same species gave a random (Poisson) dispersion as measured by variance / mean ratio. This latter result appears to run counter to the assumption that parasites are generally overdispersed over the host population. Such a result might be explainable if not only the infection environment, but also the host population itself, were highly homogeneous such that aggregation of parasites in the environment or on the host did not occur. The establishment of such a homogeneous environment, even within the strictures of a controlled experiment, seems, however, improbable.

The fact that the single-wave infection experiment described in the present study produced an overdispersed distribution clearly indicates that such a distribution can arise without multiple waves of infection. This finding is supported by similar results from infection experiments described by Boxshall (1974c) and Poulin, Curtis & Rau (1991) for *L. pectoralis* and *S. edwardsii* respectively. The value for k of the negative binomial, suggested by Crofton

(1971b) to represent a measure of overdispersion, was 3.05 for the large scale smolt infection in the present study. This comes at the upper limit of values for k said to be characteristic of parasitic populations (*ibid.*). It was suggested by Crofton (1971b) by use of a mathematical model of host-parasite relationships that at values of $k < 1$ the parasite population could be effectively suggested to have no effect on the host population whilst for values of k between 1 - 3 an equilibrium would be reached between host and parasite. Similar conclusions for k were drawn in a model by Holling & Ewing (1969) in which at very low k, attacks were so contagious that only a few individuals would be affected whilst at $k > 3$ oscillations of increasing amplitude resulted in the extinction of the parasite population. In this latter paper, k was considered to be constant for any given species and yet in more recent work (e.g. Boxshall 1974c, Scott 1987) it has become apparent that, when one observes host-parasite relationships over time, the dynamics of aggregation are firstly not static and secondly, may rise to values in excess of the "transitional point" of Holling & Ewing ie $k = 3$, without the extinction of the population (for instance *L. pectoralis* reached $k = 9.8$ in Boxshall's study). The observed dispersion pattern therefore reflects a balance between opposing forces (see introduction) at any given point in time and therefore, as suggested by Boxshall (1974c), it is "unwise to ascribe fundamental properties to k".

Before embarking on a discussion of the results from the analysis of numbers of larvae on farmed fish, it is important to note that because of the small sample sizes involved, the conclusions drawn must of necessity be tentative. It has been pointed out that the probability that the sample mean is an underestimate is always > 0.5 because of the asymmetric distribution of a negative binomial about the mean and that this probability will increase with decrease in sample size or increase of aggregation (Pacala & Dobson 1988). Unfortunately, a corollary of working with a high-value commercial species over an extended period of time is that sample sizes are likely to be low. The results presented, however, allow hypotheses to be generated which may be more rigorously and explicitly tested at a later date.

Population parameters of *L. salmonis* on farmed fish are likely to differ markedly from those of their wild counterparts as a result of a number of factors. These include the age structure, genetic composition, and density of the host population, the effect of management strategies such as grading and treatment on the parasite population and the constrained spatial configuration of the site such that hosts are confined within a particular environment which in itself is likely to be more homogeneous than the natural environment. Many of these features, such as the relatively homogeneous nature of the environment and the consistent age of hosts, may in fact be helpful to the understanding of population parameters since this suggests that the parameters of certain factors which might influence parasite populations are constrained within certain limits. Other features, such as treatment of the parasite population, make interpretation

of results far more difficult as the population structure and dynamics that result are clearly highly artefactual.

Despite the observation of continuous recruitment throughout the year, as reported also by Johannessen (1975), Wootten *et al.* (1982) and Ritchie, Mordue & Pike (1993), numbers of settled copepodids were generally low. This may be explained by the relatively short duration of the settled copepodid stage such that the numbers observed at any given time do not accurately reflect the rate of infection. There were, nevertheless, peaks of larval abundance which seemed to correspond to waves of infection, although the origin of these waves is debatable. One possibility is that they represent generation waves following earlier peak infections of farmed fish whilst the major alternative is that some peaks may correspond to the movement of wild fish into the lochs prior to entering rivers. In particular, the high peak seen in August followed the observation of high numbers of lice on wild fish at a time when few lice were to be found on the farmed fish in the same location. Because detailed records of lice on wild fish captured in the vicinity of farm sites are not available at the present time, it is difficult to gauge their impact on farmed populations (or *vice versa*). Although a seasonal component such as that described by Ritchie *et al.* (1993) is generally reported, its true effects are likely to be obscured to a great extent by the treatment regime normally employed.

The agreement of infection parameters between different cage groups is perhaps not surprising, as most sites are relatively constrained spatially and therefore all groups are likely to be subject to similar environmental variables. The apparent fall in larval numbers following treatment may result either from recruitment failure due to loss of adults / preadults or from the development of large numbers of larvae into the preadult / adult population which in itself may prompt the treatments. If recruitment failure is the cause of the fall in larval numbers on a site, as was suggested to be a possible consequence of Ivermectin treatment in Ireland (Smith *et al.* 1993) then this provides good evidence for the fact that sites are principally self-reinfecting as otherwise treatments would have little effect on the ambient population of free-swimming larvae. With respect to this, Wootten (1985) has suggested that the bulk of infective larvae on Scottish farms appear to be derived from farmed fish and Tully (1989) has indicated that on Irish farms the transfer of parasitic stages from outside the farm are believed to be minimal. Further evidence for the fact that farm epidemics are self-amplifying comes from the increase in infestation frequently observed over a farming cycle despite regular treatments throughout. The suggestion that parasitic abundance rises throughout the farm cycle has also been made by Jaworski & Holm (1992) although Tully (1989) found that parasitic intensity failed to increase with succeeding generations. This latter observation may, however result from the fact that in Ireland preadult females were not found to mature at certain times of year.

Whilst examination of the dispersion of *L. salmonis* using small sample sizes is somewhat insecure (Elliot 1971), the results obtained would appear to reflect more general observations made *in situ* on cages. Some disagreement, however, exists in the literature as to the suitability of the ratio of variance / mean as a measure of aggregation. Heip (1975) suggests that the ratio will approach unity as density decreases and that therefore it is not a good indicator of aggregation and proposes that the calculation of k is preferable. Scott (1987) argues on the other hand that because the value of k is not corrected for the mean (as is the former value) comparisons in magnitude between samples are not useful since, if the sample means differ, lower values of k will not be indicative of a higher degree of aggregation. In addition she notes that the value of the variance / mean ratio will be particularly sensitive to the presence of single heavily infected hosts whereas k is more useful in the description of the spread of values about the mean. Because the prevalence of infection on the farm studied tended to be constant ($\sim$ 100 % after a short period of initial infection) the observation by Scott (1987) that calculation of the two values should yield similar results under constant prevalence suggests that the use of variance / mean in the present context is acceptable as an indicator of aggregation. Whatever statistic is used to investigate distribution, however, it is wise to note the observation of Iwao (1970) that descriptions of distributions using mathematical models can only be "superficial and approximate".

A general tendency for overdispersal has been reported for host-crustacean relationships so far described (Van Damme & Hamerlynk 1992). The results of the present study show that the settled larval population on farmed fish was intermittently overdispersed as was also found to be the case for total *L. salmonis* by Taylor (1987), a finding which contrasted with the Poisson distribution reported by the same author for experimental infections. Overdispersal has also been recognised in studies of *L. salmonis* on wild salmon by Johannessen (1975) and by Nagasawa & Takami (1993) who reported that the distribution of *L. salmonis* on wild pink salmon corresponded to a negative binomial distribution. Although Tully (1992) has also described a highly overdispersed population of *L. salmonis* on wild salmon, he reported that parasites on farmed salmon were approximately normally distributed.

Overdispersal has been demonstrated to occur in both the experimental infection and in the farmed population in the present study. The crucial difference between these two host populations is that the experimental population was exposed only to a single wave of infection under highly homogeneous conditions (both host and ambient environment). Anderson & Gordon (1982) indicate that a simple process of immigration of larvae (infection) should theoretically lead to a Poisson (random) distribution of larvae over the host population in the absence of superimposed environmental factors. Such a random fallout of larvae was suggested to occur in copepod parasites of pelagic teleosts by Lewis, Dean & Gilfillan (1969) and although such

distributions are rare in nature, a good agreement with the Poisson distribution was noted for *L. branchialis* infecting 0+ whiting (Van Damme & Hamerlynk 1992) and was suggested to be indicative of a single random wave of infection. As already noted, Taylor (1987) similarly obtained a random distribution in an infection experiment using *L. salmonis* and it may be that in the latter case, this resulted from a greater degree of homogeneity of hosts and environment than realized in the present study.

The fact that overdispersion occurred in the present study appears to signal that superposed environmental variables are likely to be involved, particularly as the infection experiment described here used only a single short-term infection and therefore overdispersal is not a result of a compound Poisson distribution. The most likely cause of overdispersal in the present case is differential infection resulting either from aggregation of infective stages in the environment with fish passing through the aggregations at random as suggested by Boxshall (1974c) for *L. pectoralis* or alternatively, differential susceptibility of hosts to infection. Another possibility might be that of differential mortality of settled larvae resulting, from e.g. heterogeneity in host immune response.

Aggregation of larval stages in a farm environment, although perhaps reduced in comparison with a more heterogeneous natural environment, is nevertheless to be expected. The possibility of such aggregation within the controlled environment of the infection experiment is, however, considered doubtful for the present study and so an alternative explanation must be found for the observed overdispersal. Anderson & Gordon (1982) consider host susceptibility to be the prime cause of overdispersed parasite-host relationships and it seems likely that this is also the case in the present study. Although Fryer (1966) was not of the opinion that differences in host susceptibility could explain aggregation of copepods, his suggestion was based on a contention that the effects of host differences need be expressed either through differential chemotaxis of settling larvae or by active copepodid desertion of unsuitable hosts. Other mechanisms may, however, be invoked to explain variation in host susceptibility.

Although an attempt was made to use relatively homogeneous hosts, the negative correlation between host size and prevalence of infection indicates that this was not successful in the present infection experiment. These findings would, however, seem to confirm that host heterogeneity is an important factor in the settlement of copepodids. In the farmed host population there is a contrasting **positive** correlation between host length and prevalence / relative density of infection. These results are supported by the experiments of Poulin *et al.* (1991) which investigated single wave infections in *S. edwardsii* and similarly found that the principal factor involved in overdispersion was host length and that host behaviour, as measured by level of activity and spatial positioning, was not important. It must be kept in mind, however,

that host length itself could result from other factors, leading to poor growth and making hosts more susceptible to infection.

The findings of the present study suggest that significant overdispersion coincides with increased larval numbers. This observation was similarly made by Taylor (1987) for total *L. salmonis* on farmed populations in Ireland. In contrast, Boxshall (1974c) found in *L. pectoralis* that dispersion of the population, as measured by k, became more random during periods of population growth and conversely became more aggregated during periods of population decline. A similar result was presented by Paperna (1980) for *C. minimus* on *D. labrax* who found that in both larvae and adults the variance / mean ratio declined to unity or below when hosts were heavily infected but remained above unity when hosts carried a low infection.

Taylor (1961) has suggested that variability is directly linked to abundance so that the significant relationship found between variance and mean in the present study is not unexpected and indeed it has been reported elsewhere (e.g. Scott & Anderson 1984). This linear relationship has been noted to be a strong tendency in parasite-host relationships in general and was held to reflect the effects of demographic stochasticity (Anderson & Gordon 1982). As a consequence it was suggested that the relationship might therefore be species-specific (*ibid.*). Without further data sets it is not possible to establish from the present study whether this is the case for *L. salmonis*.

In *L. pectoralis*, Boxshall (1974c) attributed the increase in aggregation associated with decreased density to the decline in numbers or size of parasite "clumps" present in the host population. The situation found in the present study, however, more closely resembles that reported for *Gyrodactylus turnbulli*[12] Harris infesting guppies *Poecilia reticulata* Peters by Scott (1987). In this latter study it was suggested that the increase in aggregation associated with increase in parasitic density / prevalence was a result of increasing host heterogeneity due to variation in the time individual hosts had been infected. In Scott's study heterogeneity was eventually suggested to decrease as the history of individual's past exposure to parasites became more similar and this was followed by a "crash phase" in which density dependent mortality of hosts or parasites caused a decrease in aggregation. Although this explanation might be helpful in explaining the results for *L. salmonis* in the present study there are important differences. As shown in the present study and in work by Johnson & Albright (1992a) Atlantic salmon show little response to attached stages in contrast with other species such as coho (*Oncorhynchus kisutch* (Walbaum)) (*ibid.*, Johnson & Albright 1992b). Host response is therefore unlikely to be a major factor leading to increased heterogeneity in the present study. Heterogeneity could,

[12]Reported as *G. bullatarudis* in the original report, but subsequently reidentified (Harris 1986).

however, be promoted by physical damage and secondary infection which may both lead to host behavioural changes or to changes in host resistance.

Although it is commonly stated that high parasite density may cause host mortality (e.g. Crofton 1971a, Anderson & Gordon 1982, Scott 1987), the artificial situation on the farm, where treatments are used to remove parasites, will tend to preempt this. Treatment will, however, mimic host / parasite mortality by making infection more homogeneous. If host mortality does occur in farmed or wild fish, it is also perhaps important to note that adults and preadults of *L. salmonis* are able to abandon the host and may still reinfect others. Thus the general assumption that host death = parasite death may not hold true in this instance, although it may lead to some reduction in aggregation according to the nature of reinfection by mobile stages. In the study of Scott (1987) it was suggested that as peak densities approached, the degree of aggregation decreased as density dependent effects became apparent. The fact that there was no sign of such a reduction in the present study may reflect the management practices of fish-farms and the consequences of the development of critical densities being curtailed by treatments.

If we conclude, from the above discussion, that host heterogeneity may affect the probability of successful settlement of the copepodid stage of *L. salmonis*, then it is important to investigate the mechanisms by which this could occur. Two aspects of the host population may be examined at this juncture. Firstly is the division of the host into a number of different habitat types (ie fins, body-surface, gills etc.), the differential settlement of which by the copepodid may tell us something more general about the nature of infection. Secondly is the effect of host size, a visible expression of host heterogeneity, on the settlement of larval stages.

The predominance of copepodid settlement on the fins and fin bases seen under controlled laboratory conditions is a common pattern amongst parasitic copepods and indeed amongst many groups of aquatic parasites e.g. *Gyrodactylus* sp. This characteristic is particularly pronounced given the low surface area of the fins with respect to the area of the body surface as a whole. Similar results have been reported for *L. salmonis* by Johnson & Albright (1992a) for experimental infection of a number of salmonid species including Atlantic salmon and by Taylor (1987) in an experimental infection of Atlantic salmon. Taylor (1987) also notes predominance of attachment of chalimus and preadult stages on farmed salmon to be on the pelvic and anal fins. Tully *et al.*(1993) has suggested that infection may normally occur through attachment of copepodids to the dorsal fin, and if this is true, it may also explain the severe damage caused to the dorsal fin of Norwegian sea trout smolts in the wild (Jakobson 1993). Settlement on farmed fish in the present study shows a similarly high settlement on the dorsal fin (Table 6.14).

High copepodid settlement on the fins and fin-bases has also been a feature of experimental infections with a number of other species of parasitic copepods. Amongst these are *S. californiensis* infecting fry of *Oncorhynchus nerka* (Walbaum) (Kabata & Cousens 1977), *S. edwardsii* (Poulin, Curtis & Rau 1991) which was found to settle principally the gills and base of the pectoral fin and *L. cyprinacea* (Shariff 1985) which was found to have a general preference for the periorbital area and the base of fins in general. The present study has also indicated incidentally that all stages of *C. elongatus* and *C. centrodonti* observed on wrasse were found attached to fins. In experimental infections with *L. pectoralis*, Boxshall (1976) found copepodids to be concentrated on the dorsal surface of experimentally infected fish, principally on the fins and the opercular side of the lateral line.

Despite the frequent predominance of settlement of copepod and other ectoparasites on fins there is little information available to suggest as to why this should be the case. Kabata (1981) has previously noted that most parasitic copepods favour specific sites and postulated as yet undetermined morphological and physiological factors. It is nevertheless clear that many species such as *L. salmonis* in the present study and *S. californiensis* in the study of Kabata & Cousens (1977), whilst predominating in certain areas can survive and mature in less preferable ones.

One explanation for the predominance on fins is that copepodid larvae might migrate following initial attachment. In *L. sprattus*, attachment to fins has been reported by Anstensrud & Schram (1988) in which instance it was noted that copepodids were initially randomly distributed and subsequently migrated to the fins. El Gharbi, Rousset & Raibaut (1985) have also reported preferential attachment of chalimus of *L. sprattae* to the base of the pectoral fin of *Sardina pilchardus* (Walbaum) whilst attachment elsewhere was extremely rare. Movement following settlement has also been proposed by Johnson & Albright (1992a) for *L. salmonis*, although in the present study there has been no observation of extensive movement from the point of initial attachment and similarly Boxshall (1976) considered that, once settled, copepodids of *L. pectoralis* did not change position on the host. Intuitively it would appear unlikely for copepodids to risk detachment by migration across the host surface particularly as the body-surface would seem to provide little protection against the current. It must be presumed that the only justification for such behaviour might be the perceived likelihood, either of imminent detachment or of an inability to permanently attach / feed at the point of initial attachment.

Another possible explanation for the spatial distributions observed is that copepodids are in some way able to "home" specifically on host fins or other areas. It has been suggested by Boxshall (1976) that the distributions observed for *L. pectoralis* resulted principally from copepodids homing on respiratory and swimming currents localised around the operculum and fins and indeed, the fact that copepodids may display rheotactic behaviour has already been

discussed in a previous chapter. According to the results of Chapter 3, however, it would appear likely that rather than "homing" on currents, *L. salmonis* copepodids would react with a fixed pattern of behaviour (e.g. looping) when a current is encountered and are therefore more likely to make contact with the host in areas where such currents are generated or most pronounced. Thus the expected distribution would be the same as that suggested by Boxshall although the mechanism for its generation would be slightly different.

A third consideration as to the origin of this settlement pattern is the nature of the substrate itself. It is clear that many facets of the host epithelium such as thickness of the epidermis / dermis, nature of underlying substrates (e.g. fin rays or scales), blood supply, number of mucous cells etc. will vary according to specific location. This then may tend to make certain sites easier to attach to or more attractive chemically. Alternatively, some sites may provide better facilities for nutrition or permanent attachment. There are also likely to be local differences in host response to the parasite. The work of Johnson & Albright (1992a) supports these suggestions and indicated that local differences in host response to *L. salmonis* did appear to occur in infection experiments. In these experiments it was noted for instance that development was slower on the gills than the fins of Atlantic salmon and it was suggested that this might concern non-specific humoral factors of the host or, alternatively, nutritional differences. It was also suggested that settled stages might change position in order to avoid host responses, this accounting for the variability seen in the magnitude of host response observed with respect to individual copepodid stages.

One other reason, which may be suggested for completeness, although it would not seem to apply in the present case, is that some parasites may pass directly from the fin of one host to the fin of another since the fins may often extend some way from the body of the fish and may therefore be the normal point of host contact with conspecifics. This situation has been observed to occur in *G. turnbulli* on guppies (Shinn pers. comm.) and may therefore explain the predominance of attachment on fins for some monogeneans / other groups.

The fifth explanation principally concerns the hydrodynamics of contact, attachment and maintenance of position on the host. This hypothesis, that the spatial distribution of copepodids depends to a great extent upon where they manage to gain a purchase and maintain it in the face of local currents, will be discussed in some detail, as it has previously been largely neglected in the literature. It nevertheless has interesting implications for the prediction, not only of site of attachment, but also of which individuals are likely to be most susceptible to infection.

Crisp (1976), commenting on the settlement of marine invertebrates in general, noted that in a given environment there are likely to be periods of slack water or sanctuaries from the effect of wave action where settlement will always be possible. He further drew attention to the crucial importance of larval size in the avoidance of ambient flow effects. That settlement may occur

on a wide range of substrates, regardless of their speed or smoothness, is attested to by the constant and apparently unavoidable fouling by epibionts of man-made substrates such as the keels of boats. The same considerations are likely to apply to parasitic copepods as is reflected by the comment of Paperna & Zwerner (1982) that the final pattern of attachment of *E. labracis* on the gills of *M. saxatilis* was likely to be determined by the parasite's ability to hold on in the ventilating current.

In order to appreciate the factors affecting the attachment of the copepodid, it is necessary to postulate some model of the likely fluid dynamics of the host's surface. This is particularly difficult to do as the successful visualization of flow around any fish (let alone salmonids) has proven impossible to date (Wardle 1985) and thus tentative suggestions must be made in the light of observations carried out on fairly regular solid objects.

Vogel (1981) notes that "at the interface between a stationary body and a moving fluid, the velocity of the fluid is zero" and that consequently there must be a zone of transition where the velocity of the fluid increases until it equates to that of the free-stream velocity. This zone of transition is termed the "boundary layer" and is of importance to a settling organism in that it may aid in the establishment and maintenance of contact with the substrate (e.g. the host) by providing shelter from the ambient water flow. The depth of this layer, whose thickness is normally considered to be bounded by the height at which the flow velocity is 99 % of the ambient flow, depends on the viscosity of the fluid and the time for which the viscous forces act (Webb 1975). The calculation of this depth depends on the Reynolds number R_L which for a moving body is dependent upon the length of the body in question and its swimming speed. In general, the greater the Reynolds number, the smaller the boundary layer and the greater the steepness of the velocity gradient or "shear rate" (Vogel 1981). Most observations of fish (Webb 1975) have been made in the narrow R_L of 3×10^4 to 10^6. The Reynolds numbers of salmon (*S. salar*) measuring 0.15 m, 0.45 m and 0.6 m have been estimated by Tang (1993) to be approximately 6.6×10^4, 3.3×10^5 and 5.5×10^5 respectively.

The boundary layer takes two recognisable forms - "laminar" and "turbulent" with the transition between them occurring between Reynolds numbers of 10^6 and 3×10^6. A laminar boundary layer may be thought of as a "semi-stagnant" region which exchanges its contents with the ambient flow only slowly. A turbulent boundary layer is deeper and well mixed such that the velocity profile represents a mean value rather than a continuum as in the laminar layer. Turbulent boundary layers will nevertheless possess a so-called "laminar sub-layer" adjacent to the substrate surface. Factors leading to the generation of a turbulent boundary layer include surface roughness elements (protrusions) of a greater height than the laminar boundary layer, turbulence in the free-stream and distance from the leading edge of the surface. In practice it has been suggested (Webb 1975) that most fish, e.g. those in shoals / turbulent streams, will be

exposed to external turbulence at a level likely to cause the boundary layer to be turbulent over their surface although he also suggests that transitional boundary layer conditions may prevail such that fish may possess a laminar boundary layer to the shoulder and a turbulent boundary layer downstream. According to Wardle (pers. comm.) the large amplitude swimming motions occurring in certain areas such as the caudal peduncle are likely to result in enormous variation in the depth of the boundary layer with consequent increases in the steepness of the velocity change (shear). As a result, settlement might be expected to be largely confined to areas of the fish anterior to the caudal peduncle.

It was noted above that roughness elements of greater magnitude than the boundary layer will tend to make the layer turbulent. According to Yaglom (1979), if the roughness elements prevent flow through the cavities between them, then the flow will not penetrate into the bottom of these cavities and this will thereby give rise to "stagnant" zones. It was suggested that these must be excluded from the so-called "real flow region" such that the boundary layer is located, if the roughness elements are dense enough, above them (ie is displaced upwards by an amount equal to the height of the roughness elements).

If the above statements hold true for the salmonid host (and, as I have indicated, whether they do or not must remain for the time being, a matter of conjecture) then we may perhaps make suggestions as to what might be expected in terms of the distribution of larvae on the host. Clearly, the overriding feature of their distribution is that they will be found in areas where they can attach initially and remain attached. Thus they require areas with either a deep boundary layer or, if possible, stagnant zones. Areas of turbulence or those associated with large roughness elements (projections) may therefore be favoured whilst highly smooth areas will not, since in these areas the boundary layer is likely to be shallow and in addition there may be a lack of projections for catching of the antennae on initial contact. The dorsal surface of the head and the adipose fin and general body surface are likely to fall into the category of "smooth" areas and in particular the cornea of the eye which must be entirely smooth in order to preclude visual distortion. As mentioned above, the head in itself may experience a certain degree of laminar flow, although areas between the branchiostegal bars and at the edges of the operculum might be expected to provide sanctuary. In addition, areas posterior to the caudal peduncle may experience serious fluctuations in the depth of the boundary layer as noted earlier, thereby preventing settlement. If the fin rays / spines can be regarded as roughness elements, lying as they do relatively perpendicular to the current in all fins bar the caudal fin and the adipose fin (which lacks them altogether) then the spaces between them may represent areas where water flow is significantly reduced allowing not only initial attachment but, perhaps more importantly, the maintenance of contact until the permanent attachment of the chalimus stage is established. In addition the bases of fins and the area downstream of them may also experience turbulence

and a consequent increase in the depth of the boundary layer and decrease in velocity allowing settlement. A further important note is the observation that the area behind wounds has similarly been noted to be turbulent (Allan 1961).

If we compare this predicted spatial distribution with that found on farmed or experimentally infected fish we find that it compares favourably, with few larval stages found on the open body surface where the boundary layer is likely to be thinnest and most found in the lee of protuberances such as fin rays, fin bases and other irregular hollows such as the area between the branchiostegals. Larvae are rarely found on the leading slope of the head as predicted above and, taking into account its surface area, are relatively sparse on the caudal fin posterior to the caudal peduncle. The dorsal fin is often particularly heavily settled (as noted earlier) and this may result from a combination of large fin rays (with deep hollows between them) and from the fact that these are normally held highly perpendicularly to the current. In addition, observations suggest that chalimus frequently attach to the downstream side of the fin ray surface and may often be seen to be attached to the body surface in areas where damage has occurred through abrasion (e.g. during grading). In this latter case, chalimus are seen to be attached to the periphery of the damaged area, where turbulence will increase the thickness of the boundary layer. The adipose fin shows few settlers in accord with its smooth surface and the cornea showed no settling of larvae in the present study although rare settlement on the cornea was noted by Johannessen (1975). A lack of settlement on the eye was also noted by Boxshall (1974b) for *L. pectoralis*. Hydrodynamic considerations would therefore appear to be an important factor in the settlement of larval stages although it must be remembered that the hydrodynamics are not the only factors which vary from site to site (e.g. scales) so that this conclusion cannot be certain.

A further consideration may arise from the observations of Yaglom (1979) of the effects of density of roughness elements on the boundary layer. If we can consider settled parasites as roughness elements then the more that settle over a given area, the easier it should make further settlement through provision of a deeper boundary layer and greater protection from the free-flow. This may lead to greater aggregation of parasites on already infected hosts and therefore to greater overdispersal of parasites. Certain observations in the literature may provide evidence of this effect. Fryer (1966) for instance has observed that on *Oreochromis variabilis* Bouleng and *Oreochromis esculenta* Graham, larvae of *L. cyprinacea* were found to settle near previously established adults and other larvae. Although this may be evidence of chemical attraction as suggested by Fryer, the above hypothesis might also explain it. Clustering has also been noted for the poecilostomatoid *Taeniacanthodes gracilis* Wilson on the fins of *Paralichthys squamilentus* Jordan & Gilbert by Dojiri & Cressey (1987) where 170 females, 15 males, 3 copulating pairs and three copepodids were noted in a single cluster. Judging by the photographs

presented in that paper, all the individuals figured are attached between the fin rays or in folds in the fin so that it may be a combination of fin and parasite roughness that has served to allow settlement in this instance. Another copepod, the pandarid *Pandarus satyrus* Dana, a parasite of sharks has also been noted to form dense clusters on the pectoral fins (Benz 1981, Rokicki & Bychawska 1991) which similarly, from a photo in the former paper, appear to be aligned within folds in the fin surface (and are not present / clustered elsewhere on the fin).

The major difference between the settlement sites in the farmed fish and the experimentally infected fish was the presence of large numbers of settled larvae on the gills of the latter and apparent complete absence of such on the former. It is felt that this reflects the speed of flow of water over the gills with slower-swimming experimental fish in tanks having a slower passage of water. High numbers of settlers were also noted on the gills of experimentally infected salmon by Johnson & Albright (1992a, 1992b) and it was similarly noted that these were absent from pen-reared fish in Canada. Johnson & Albright (1992a) have, however, noted attached stages on the gills of wild sockeye salmon (*O. nerka*) in British Columbia. Taylor (1987) observed no settlers on the gills in a similar infection experiment.

If we conclude that hydrodynamic factors are important to settlement then we can clearly see that fish speed ought to have an important effect on larval attachment. This follows from the fact that it is an important component of the Reynolds number cited above and will therefore affect the depth of the boundary layer as well as the ambient current which must be resisted to remain attached. Theoretical considerations would thus suggest that faster fish have a thinner boundary layer and greater free-flow velocity and should thus be more difficult to settle upon. Allan (1961) noted in this context that fast fish had an apparently laminar boundary layer whilst that of slower fish was considerably more turbulent.

This observation brings us back to the effect of host size on settlement and thereby also to the possible mechanisms by which host heterogeneity may affect dispersal. Size is normally considered to affect infestation through a combination of two aspects. Spatially, a larger length or weight implies a larger surface area for settlement so that the often observed correlation of parasite density with length is frequently suggested to stem from a larger available area for settlement (see Kabata 1981). Temporally, larger fish are often older and thus may have been exposed to infestation for a greater period, leading to an accumulation of parasites with time. In addition fish of different age groups may inhabit different ecological areas some of which may have greater spatial overlap with infective stages than others, such that fish of a given size / age may be exposed to a greater probability of infection (*ibid.*). Cressey & Collette (1970) have further suggested for copepods associated with needlefish that whilst specialist copepods (ie those with holdfasts and displaying high specificity) were found to be associated with hosts of larger than average size through a requirement for particular areas of attachment, non-specialists (free-

moving and less host-specific) had a distribution unrelated to size of host. A similar lack of relationship to size of host has been noted by Collard (1970) for copepod parasites of mesopelagic fish and by Marcogliese (1991) for *L. cyprinacea*. A further suggested effect of greater size, suggested by Poulin *et al.*(1991), is that larger fish might be expected to create larger stimuli and so perhaps provoke greater numbers of settling larvae.

Because all the fish, in both farmed and experimental host populations, were of identical or near-identical ages and were exposed to infection for the same length of time, we should expect no age-related differences in accumulation of parasites. What we should, perhaps expect from the above statements, is the positive correlation of larval density and prevalence with size (and therefore surface area). This was indeed observed in the distribution of chalimus I's in the farmed population. Such an association of relative density of *L. salmonis* with host length has also been reported for wild salmonids (*Oncorhynchus* spp.) by Nagasawa (1985, 1987) and Nagasawa, Ishida & Tadokoro (1991) and has been noted for farmed Atlantic salmon by Jaworski & Holm (1992) although, in contrast to the present study, only mobile stages were considered.

What is more difficult to explain in the light of the above, is the negative correlation observed in chalimus I's in the experimental smolt infection. Kabata (1981) lists a number of studies in which such a relationship was observed in wild populations but in these cases the ages of fish differed. One possibility offered by Noble, King & Jacobs (1963) for *Ergasilus auritus* Markevich on *Gillichthys mirabilis* Cooper was of a build-up of immunity to the parasites over time. For pennellids it was suggested that such a situation might arise through differences in availability of intermediate hosts (Kabata 1958, Moser & Taylor 1978). Neither of these explanations, however, can explain the situation observed in the present study. Another possibility might be that the negative correlation is an artefact caused by loss of larval stages during host capture. Because larger fish are generally more difficult to land and handle it may be that copepodid stages at least could be lost prior to examination although chalimus stages are firmly attached such that such loss is far less likely.

One facet of host size that is normally overlooked in such studies is that fish swimming speeds normally increase with length (Blaxter 1969, Webb 1975). One might therefore expect settlement of the copepodid to become more difficult as host length increases as this will affect the depth of the boundary layer and the velocity of free-stream flow. Although little investigation has been carried out on swimming speeds of *Salmo salar*, work on other salmonids suggests that growth to ~ 30 cm leads to a linear increase in swimming speeds expressed as body-lengths s^{-1} which might therefore explain the negative correlation seen for experimental infection of smolts. It has been noted, however (Brett 1965, Blaxter 1969, Webb 1975), that as the fish get larger there is, at some point, a relative **fall** in cruising performance with **increase** in size, coupled with

a tendency for a fall in stamina. It is therefore suggested that the positive correlation of infection with size seen in the larger farmed salmon, results from a different balance between surface area and swimming speed to that seen in smolts. In addition larger fish may also provide a greater number of sites which are protected enough to allow attachment due to e.g. the increase of depth of fin rays.

Swimming speed has been previously suggested to be a possible factor in the susceptibility of salmonids to *L. salmonis* by Nagasawa *et al.* (1991) who noted that the greater susceptibility of wild pink than sockeye salmon might be associated with slow swimming speed of the former. He also suggested that cage salmon might similarly be susceptible as a result of slow swimming speed. Low swimming speed was also suggested to be the reason for the increased susceptibility of farmed arctic char kept side by side with salmon, by M^{c}Kinnon (pers. comm.) although the higher densities at which this species is normally stocked could also explain the disparity. A further piece of evidence for the effect of swimming speed (or rather current speed) is the observation by Johannessen (1975) that the installation of current regulators at a site in Norway greatly reduced the severity of lice infestations at a later date. This said, however, there has as yet been no correlation of local current speeds (normally judged subjectively) with level of infection with *L. salmonis* on Scottish salmon farm sites to date.

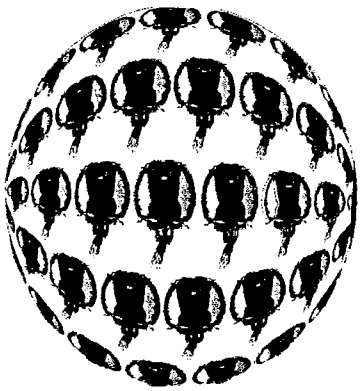
If low swimming speed is a factor in the increase of susceptibility to infection then this may explain some of the other observations made in this and other studies. It was apparent as noted above that disadvantaged / mature fish were more susceptible to infection and these latter have been previously observed to be more susceptible by Johannessen (1975). Reports of higher numbers of *L. salmonis* on diseased fish have been previously made by M^{c}Vicar (1987) who suggested that salmon with pancreas disease had higher infestations and by Bruno & Stone (1990) who noted "transfer" of lice from healthy to moribund salmon. Mature fish have also been suggested to seek out slower currents (and presumably swim more slowly) in order to conserve energy by Johannessen (1975) whilst disadvantaged fish can be observed to swim more slowly than healthy fish presumably through loss of stamina / energy through disease or inadequate food intake. Disadvantaged fish are also frequently smaller than their conspecifics and will therefore swim more slowly in any case. As might be expected, fin damage, as is often noted for badly infested fish, could also result in lower swimming speeds as demonstrated by Radcliffe (1950) and indeed the infection itself could also affect swimming speed as shown in the case of parasitization of *Osmerus eperlanus* (L.) and *A. anguilla* by nematodes (Sprengel & Lüchtenberg 1991). The fact that caligids may similarly affect performance of hosts is also attested to by the observation that infestation of herring larvae by *C. elongatus* led to deterioration in the number of successful strikes at prey (Rosenthal 1967). External damage to fish is also likely to provide more settling sites through increase of sheltered sites and fostering of turbulence as noted earlier.

Behavioural traits such as swimming near the surface, displayed by disadvantaged fish, may also be important, particularly if the infective copepodids are not homogeneously distributed in the environment. The significance of such behaviour has not, however, been evaluated in the present study.

It is clear that swimming speed is not the only factor making disadvantaged fish more susceptible to infection. Stress through disease, damage or farm management procedures e.g. treatment / grading will all tend to make fish more susceptible to infection since it has been noted by Esch, Gibbons & Bourque (1975) that stress in any animal will tend to reduce resistance, whether natural or acquired, to parasites. The likely effect of stress on naive coho salmon (*Oncorhynchus kisutch* (Walbaum)), as realised by cortisol implants, has been clearly demonstrated by Johnson & Albright (1992b) who found that subsequent to infection there were significantly fewer *L. salmonis* on controls than tested salmon at any given time. Although the response of naïve Atlantic salmon is lower than that of the former species (present study, Johnson & Albright 1992a), one might assume a similar situation to apply. A change in behaviour may also make distressed fish prone to higher infection. This was suggested to be the case for sticklebacks experimentally infected with *Argulus canadensis* Wilson by Poulin & Fitzgerald (1987) where it was apparent that already infected sticklebacks were far more likely to pick up further parasites. It was suggested in this paper that the erratic behaviour of infected fish made them more vulnerable.

Wootten (1985) notes that, following treatment on farms, there were often heavy reinfestations by larvae. This may be a simple consequence of there being large numbers of mature stages which prompted treatment in the first place and which will have simultaneously released large numbers of larvae into the water column. It has been demonstrated, however (Blaxter 1969), that short-lived strenuous exercise may have marked and long-lasting biochemical effects leading to poorer locomotory performance. Paulik, Delacy & Stacy (1957) have demonstrated for instance that 5 hrs might be required for complete recovery of exhausted coho salmon (*O. kisutch*). Since treatment and active grading may cause acute stress and lead to strenuous fish activity, it may be reasonable to expect slower swimming during recovery during which time they might also be more susceptible to infection.

CHAPTER 7
SUMMARY AND CONCLUSIONS



7 - SUMMARY AND CONCLUSIONS

The work presented in this thesis has sought to investigate a wide variety of aspects of the biology and behaviour of the infective copepodid stage of the parasitic copepod *L. salmonis*. The justifications for such a study are both those of purely scientific interest, which follow from the general lack of knowledge of the biology of larval stages of caligid copepods, and those of wider concern that result from the pathological effects of this species on wild and farmed salmonids. In addition, by providing answers to biological questions, it was hoped that this project might suggest applications whereby infection of farmed salmonids by copepodid stages might be reduced.

The first part of this study aimed to examine the "unstimulated" behaviour of the copepodid followed by its response to a number of simple stimuli. In addition, an inventory and description of the sensory organs of the copepodid was carried out in order to better define the environmental stimuli that the copepodid is adapted to perceive.

During "unstimulated" swimming, the copepodid displayed the "hop and sink" behaviour commonly seen in free-living planktonts. By this means the copepodid may conserve its finite energy supplies whilst remaining in the water-column where host contact must occur.

The copepodid larva was shown to possess a well-developed lensed nauplius eye which corresponds well to previous descriptions of the eyes of adult caligids. Future work may establish the nature of the pigment(s) associated with the eye by straight-forward chemical tests and similarly determine the chemical nature of the lens. Use of neuro-staining techniques would also establish the path of neural connections to the eye.

Theoretical considerations, based on the structure demonstrated in the present study, suggest that the nauplius eye is well adapted for the perception of all five principal types of light information suggested to be available in the marine environment by Cronin (1988). The present study suggests, therefore, that the eye of the copepodid functions to optimize image brightness and possibly also in the detection of movement and the determination of direction of light polarization. Confirmation of the latter function would be an interesting subject for future work. An extra-optic photoreceptor, thought to correspond to the Organ of Gicklhorn of other studies, has also been reported here for the first time in caligids. Its function remains to be confirmed by future ultrastructural and histochemical examination.

The copepodid larva was demonstrated to display a distinct light response under experimental conditions, responding to a wide spectral range with a peak response around 550 nm. This suggests that the larva is well-adapted to respond to the full spectrum of light available

in shallow / surface waters. The copepodid showed a positively phototactic response to light, with this being linearly related to light intensity within the tested parameters. The apparent immobilization of the copepodid at very high light intensities may serve to keep it away from highly insolated surface water where excess ultraviolet radiation could be detrimental. Further experiments might establish the interaction of intensity and spectral properties of light in terms of the copepodid response and furthermore, provide upper and lower intensity and spectral limits for the phototactic response observed.

No experimental evidence was obtained in the present study of a shadow or albedo response that might serve, as it does in some other parasitic species, to promote contact with the host. Repetition of all light-associated experiments, using apparatus giving an angular light distribution, would establish whether the responses observed are at all artefactual.

This study has shown that the copepodid possesses a full complement of chemoreceptors including both antennulary aesthetascs and an Organ of Bellonci corresponding to those described for other species. The Organ of Bellonci is described for the first time in caligids, although its detailed ultrastructure now requires further study. The results of the present study showed that there was no chemotactic response by the copepodids to host-derived chemical stimuli, despite earlier reports of positive responses in other species of caligid copepodid. It is still considered that chemoreception is the most likely candidate for host recognition, but it is probable that it occurs once contact has been achieved rather than being used for distant host detection, although this will be difficult to prove experimentally. The chemosensory experiments described all relied on the detection of a principally chemotactic response. Another approach might be to examine behaviour at a smaller size-scale, possibly using video / image analysis equipment, in order to determine whether there are other behavioural responses or whether chemoreception of appropriate stimuli might release later settlement responses.

The results of the present study suggest that mechanodetection of the passage of the host is the principal mechanism eliciting host contact. The copepodid was shown to be equipped with a wide range of integumental mechanoreceptors and, in addition, to possess a number of sensory elements on the antennules. Work described here showed that the copepodid is responsive to shock-waves and directional water flow and responds by fast swimming and tight turning. It was suggested that such behaviour may function to bring the copepodid into contact with a passing host. No response was seen in larger scale flow experiments and it was suggested that, without references, the copepodid might not be able to detect such flow, requiring some element of shearing for the flow to be detectable. The mechanoreception experiments described here, although effective in demonstrating a copepodid response, were not designed to quantify such responses. These experiments may be observed in future at a smaller size scale using recording

and analysis equipment. Recording apparatus may also allow the observation of acclimation and refractory periods for these and other stimuli to be observed.

A clear demonstration of a pressure response was shown in the copepodid causing it to rise in the water column with increasing pressure and fall with decreasing pressure, however, no morphological basis for the detection of the stimulus was observed. No response was seen to electrical stimuli. Use of, e.g. a barometric chamber, would make the quantification of both response and hydrostatic pressure possible in future experiments.

Scanning and transmission electron microscope studies have revealed for the first time the enigmatic structure termed here the "cauliflower organ". The function of this structure remains unclear although it is suggested that it may be concerned either with the chemosensory recognition of the host, or the tactile recognition of the host through specific binding of surface proteins. Ultrastructural and histochemical studies may contribute to the elucidation of the function of this organ.

The responses displayed by *L. salmonis* in the presence of environmental stimuli in the present study correspond very closely to those displayed by free-living copepod species. From this it has been suggested that, whilst in this parasite the responses have changed little, their function has become modified so as to promote host contact. The responses described in this study demonstrate the detection of a number of stimuli *in vitro* and suggest that the copepodid is brought into the host vicinity by a combination of light and pressure responses and makes contact through a rapid swimming response elicited by mechanical detection of the host as it passes.

Another major focus of this study has been upon the act of settlement and the following phase of attachment by means of the frontal filament. This part of the study has been prosecuted through a combination of *in vivo* and *in vitro* observations of settlement and the use of a wide variety of morphological and histochemical techniques for examination of both the copepodid and the following chalimus stages.

Observations of *in vivo* infection supported the suggestion that contact was mediated by host movement and no evidence was seen for chemotactic host location. *In vitro* studies demonstrated that primary attachment occurred through grappling by the copepodid antennae. Secondary attachment via the frontal filament did not occur on host-derived or artificial substrates, thus subsequent conclusions were drawn from morphological / histochemical observations only. Copepodids were found to be capable of immediate infection once ecdysis was completed and were observed to follow a scheme of "attachment, exploration and fixation" analogous to that described by Crisp (1976) for settlement of marine invertebrates in general. The "exploration phase" was, however, curtailed under precarious attachment conditions.

Copepodid attachment was found to occur on all host substrates save for the cornea of the eye. Under the SEM and light microscope it was apparent that the antennae were strongly hooked and heavily sclerotised to maintain a purchase on the host; the distal segment of the maxillipeds was similarly armoured. At the distal tip of the "rostrum" a short tube ("filament duct") with a distal pore was observed.

This study conclusively demonstrated that the major components of the frontal filament are all substantially pre-formed within the cephalothorax of the copepodid and that the filament is probably attached to the host during the moult to the first chalimus stage. The frontal filament has been described in the present study as consisting of four components, comprising: "basal plate", "filament stem", "external lamina" and "axial duct". It was concluded from x-ray analysis and structural / histochemical evidence that the frontal filament of *L. salmonis* represents a specialised portion of the anterior cuticle.

The external lamina has been demonstrated to be continuous with the cuticle of the cephalothorax and appears to have a similar structure and composition. The filament stem was seen to be composed of bundled proteinaceous fibres which may be partially sclerotised and provides the major part of the filament itself. The attachment of the frontal filament to the host substrate occurs through the intervention of the basal plate. This structure, which is thought to be modified on extrusion, either adheres to the host substrate itself or is attached via a cement. The basal plate contains both protein and polysaccharide components and is highly sclerotised. An axial duct passes down the central axis of the filament and is thought to deliver either the attachment cement or materials forming part of the plate itself.

Unlike previous descriptions of the caligid frontal filament, some attempt was made in the present study to investigate the manner of filament formation and the organs involved. The work presented here has proposed functions for an A-gland associated with the axial duct, a B-gland associated with production of the basal plate and a C-gland thought to be concerned with the production of the filament stem fibres. The presence of a large reservoir of material ("laminated secretion") thought to identify with the basal plate and an apparent proto-stem have been recognised both in the copepodid stage and in chalimus stages. It is suggested that all chalimus stages have the faculty to produce a new filament at moulting, although whether such an ability is obligate or facultative was uncertain from the present study.

From the above information, a full scheme of events for the production of the filament in *L. salmonis* has been proposed, which is the first such description for a caligid copepod. Suggestions have also been made as to the relationship between frontal filaments in chalimus stages of *L. salmonis* and those of *C. elongatus*. It was suggested that the filament of the latter is closely related in origin to the basal plate of the frontal filament of *L. salmonis* and that, furthermore, the frontal filaments of preadult *L. salmonis* and those of other *Lepeophtheirus*

preadults (e.g. *L. pectoralis* as described by Anstensrud 1990) derive from a similar source (and are separate entities to the original filament). It was also suggested from work in this study that the frontal filament of *L. salmonis* is closely related to the frontal filament and bulla of lernaeopodid copepods. A brief attempt has also been made to speculate on a possible scheme by which a structure such as the frontal filament might arise in a free-living / semi-parasitic ancestor.

In addition to the establishment of a more detailed description of filament composition, it is hoped that future work may concentrate on the comparative description of the filaments and bullae produced by different siphonostomatoid groups and may thereby contribute to an understanding of the phylogenetic relationships existing between them.

The third part of the work described in this thesis concerned the alimentary canal and mode of nutrition of the copepodid. As with previous areas, earlier studies have concentrated on adult caligid stages only.

The alimentary canal of the copepodid was found to closely resemble that of adult caligids and indeed, was similar to that described for free-living copepods in general. The foregut and hindgut were both cuticle lined and the midgut was, as in other species, divided into an anterior midgut caecum, anterior midgut and posterior midgut. The anterior midgut was possibly separated from the posterior midgut by a sphincter and the posterior midgut was separated from the hindgut by a pronounced sphincter. Three types of epithelial cell were described from the midgut and were thought likely to correspond to the B, R/F and E cells of earlier studies. The precise identities of these cell types require ultrastructural resolution. As a means of comparing the cell population of the present study with those of previous studies, a method of diagrammatic annotation was devised that allowed similarities and differences to be more easily recognised.

In the planktonic phase, the microvillous epithelial cells of the midgut (R cells of free-living species) were observed to contain large vacuoles which were believed to be filled with lipid. This supports the suggestion that the copepodid is a lecithotrophic stage. These reserves were observed to decline with time spent in the plankton and following successful host infection. Future studies should allow characterisation of the vacuolar contents and may further allow more precise observation of progressive changes occurring within the epithelium of the alimentary canal.

The oral cone of the copepodid varied from that of the adult stages by the lack of closure of the labium and labrum. The strigil, although seen in light microscope sections, lay under a "wish-bone"-shaped structure termed here the "labial bar" and was considered likely to be non-functional. In contrast to the normal view of feeding, copepodids were frequently observed with large pieces of tissue filling the oral cavity rather than the abraded tissue debris that might have

been expected. Since such large pieces were not seen in the gut, it was suggested that some digestive activity might occur within the buccal cavity.

A number of glandular systems were described that were thought to be associated either with feeding / digestion or with repair of the cuticle. The function of these glands remains to be determined, although this area of investigation is amenable to further histochemical studies which may determine the nature of any products / enzymes produced both by these glands and by the epithelium of the alimentary canal. In contrast to earlier studies, no glandular systems were seen to discharge to the foregut, midgut or hindgut although the interface between the latter two zones had a collar of large cells ("guard cells") whose function remains to be determined. Faecal matter was enclosed in a peritrophic membrane.

The small size and limited penetration of the copepodid meant that damage to the host was minimal. Host pathology consisted of compression of the epidermis by the cephalic shield and antennae and of mechanical damage attributable principally to the antennae, oral cone and maxillipeds. Host response to the copepodid was also limited, with increased numbers of eosinophilic granular cells and mucous cells occasionally observed in the vicinity of the parasite, but very little evidence of epithelial hypertrophy or hyperplasia.

The final area of work carried out for this project was an *in vivo* investigation of the pattern (temporal and spatial) of copepodid infection, carried out both under laboratory conditions and using data from a farmed salmon site. It was intended that the information gained from such a study would function to support or refute the hypotheses generated during other phases of the work.

Experiments to examine the maximum survival of copepodids indicated that optimal survival occurred under conditions of low larval density and presumed high water quality. Maximal survival under these conditions was 26 days post-eclosion (23 days post-moult from nauplius II). No improvement in survival was observed in the presence of a fibrous substrate (plankton mesh). Further information on the maximum survival of copepodids needs to be determined for a range of temperatures and salinities under otherwise optimal water quality conditions. In addition, the infectivity of the copepodids needs to be assessed throughout such experiments, in terms both of settlement and of subsequent survival.

Attempts to infect freshwater stages of the host (fry and parr) were successful, indicating that the host recognition factor used by the copepodid is not associated with smoltification and migration of the host to the marine environment. In contrast, no copepodids settled on a variety of non-salmonid species presented, although they responded to their swimming in an identical manner to that seen for salmonid hosts. In addition, no larval stages were seen to be attached to non-salmonid species associated with infested farmed salmon. This evidence appears to support

the contention that whilst contact occurs through a mechanosensory response, host identification involves contact chemoreception, and specificity is expressed by the settling copepodid.

Tank infection experiments indicated that there was no difference in infections carried out under light and dark conditions, which supports the earlier contention that light is not involved in host contact. A large scale single-wave infection of smolts undertaken under homogeneous conditions gave an overdispersed distribution with prevalence of infection negatively correlated with host size. It was suggested from this that host heterogeneity played an important role in the pattern of infection.

Little success was experienced in the attempt to capture larval stages in the plankton. The light trap showed little significant difference between numbers captured with the trap switched on or off and the pump sampling produced no larval stages at all. No evidence was produced to support the theory that copepodid stages can attach to the sides of farm pens. It is considered that the pump sampling method provides the best opportunity for monitoring larval numbers in plankton and, if this is the case, it is felt that a long-term pump-sampling project is the most likely way to gain an estimate of larval numbers. It is felt that the combination of sampling for larvae and daily profiling of aquatic parameters on a farm site (ie current speed, temperature, salinity, turbidity, rainfall etc.) may provide useful additional information.

Examination of larval population data from a farm site indicated high variability both in numbers and in the pattern of dispersal over time. The pattern of settlement was, however, similar across the whole site. Larval numbers responded to treatment events although it was not possible to be certain whether this arose from recruitment failure or from the moulting of larvae to the mobile phase prompting treatments. The level of overdispersal was found to be positively correlated with larval numbers, significant overdispersal being associated with high numbers of larvae. In contrast to the situation found in the experimental smolt infection, numbers of larvae were positively correlated with host size. It was suggested that this difference resulted from slower swimming speeds giving higher infestation of smaller individuals, with this effect being diminished in larger farmed fish by the possession of a larger surface area and by a slower increase of swimming speed with growth. The effect of size on infestation was proposed to provide further evidence for the importance of host heterogeneity on infestation levels. This was supported by the observation of higher infection levels on disadvantaged / diseased / mature fish.

Whilst experimental infections gave high numbers of larvae on gills and fins, larvae in farm infections predominated on the fins and were never observed on the gills. In both instances few larvae were found over the general body surface. It was suggested that this spatial distribution resulted principally from the hydrodynamic characteristics of the host surface.

Explanations for the patterns of infection observed have relied on relatively small sample sizes. Future investigations need, ideally, to use larger sample sizes and to be designed so as to

examine specific aspects of infection e.g. the effect of size on settlement. The methods of analysis of infection data in general need to be re-examined and statistical methods tailored to the distributions observed. New techniques of data analysis and faster computers should make such analyses more straightforward in the future.

One of the reasons for initiating this project was the possibility that it might provide insights into methods for reducing the level of infestation of farmed salmonids. The long survival of the copepodid demonstrated in this study shows why fallowing between yearclasses is essential. The present work has demonstrated a maximum survival of the copepodid stage of 26 days from eclosion. Added to the maximum survival time recorded for adult females off the host (31 days pers. obs.) and the time taken for an egg string to mature (10 days pers. obs.) a tentative figure of 67 days or ~ 10 weeks is obtained. It is suggested that this may be an appropriate period for the fallowing of a site in order to reduce reinfection on introduction of a new yearclass.

In view of the observation that infection probably results from mechanodetection of the host, it may be possible to interrupt this process by the use of methods producing turbulence within cages. The most likely candidate for such a mechanism would be the production of bubble curtains within the cage. From the results of this work, prospects for preventing settlement by the use of chemo-attractants, light-traps and shading do not appear good. If copepodids are present in surface waters, pumping water from depth might prove a good solution. This may, however prove costly and assumes a source of deep water.

According to the work presented here, the recent trend for high-grilsing stocks will tend to promote sea lice. This will occur as a consequence both of the presence of more susceptible mature fish in cages and of the need to grade them out, which damages and stresses the remaining fish, thereby making them more susceptible to infection by copepodids. Passive grading should improve this situation. Putting larger smolts to sea may reduce initial infection as they should be faster swimmers which, from the results of the present work, may make them harder to infect. At present the trend is for the introduction of smaller fish. "Poor-doers" are likely to be more susceptible to infection as are diseased fish.

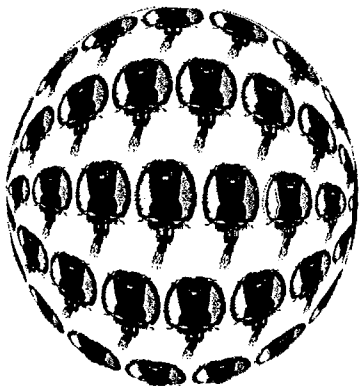
As the copepodid is an active feeding stage, once settled it may be susceptible to oral pesticides. Such pesticides must, however, be present at an inhibitory / toxic level in the epidermis / mucus as skin penetration by the copepodid is limited and blood is an infrequent part of the diet. Although vaccines might be developed against the copepodid, the latter's limited depth of penetration may mean that serum antibodies would have limited effect. Antibodies in the mucus (either serum derived or via cutaneous pathways) could, however, be more damaging. Although the copepodid midgut epithelium may be susceptible to attack by antibodies, the frontal

filament is not thought to be highly vulnerable in view of its strong attachment and sclerotised composition and because it may be renewed with each moult.

The salmon louse, *L. salmonis*, presents an ideal opportunity for the in-depth study of a parasitic copepod. Among its most important attributes are its availability, the large size of the adult stages, its lack of extensive transformation relative to free-living species and its potential for economically important damage to wild and farmed salmonids.

To date there has been a general shortage of work on the biology of the larval stages of *L. salmonis*, perhaps because these are less visible and are not considered to be responsible for serious pathology of the host. It is hoped that the present study may provide a baseline for future studies of the larval stages and a justification for further work on their biology.

REFERENCES



REFERENCES

Anon. (1991). In Goodchild, D. (ed.) *Salmon, Trout & Sea Trout*. Gamefishing Publications Ltd. Peterborough.

Ache, B. (1988). Integration of chemosensory information in aquatic invertebrates. Chapter 15 in Atema, J., Fay, R.R., Popper, A.N. and Tavolga, W.N. (eds): *Sensory Biology of Aquatic Animals*, 387-402. Springer-Verlag New York Inc.

Adams, C.W.M. (1957). A p-dimethylaminobenzaldehydenitrite method for the histochemical demonstration of tryptophane and related compounds. *Journal of Clinical Pathology* **10**, 56-62.

Adams, C.W.M. & Sloper, J.C. (1956). The hypothalamic elaboration of posterior pituitary principles in man, the rat and dog; histochemical evidence derived from a performic acid-Alcian blue reaction for cystine. *Journal of Endocrinology* **13**, 221-228.

Adler, J. (1983). Bacterial chemotaxis and molecular neurobiology. *Cold Spring Harbor Symposium of Quantitative Biology* **48**, 803-804.

Al-Mohanna, S.Y. & Nott, J.A. (1987). R-cells and the digestive cycle in *Penaeus semisulcatus* (Crustacea: Decapoda). *Marine Biology* **95**, 129-137.

Al-Mohanna, S.Y. & Nott, J.A. (1986). B-cells and digestion in the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda). *Journal of the Marine Biological Association of the United Kingdom* **66**, 403-414.

Al-Mohanna, S.Y., Nott, J.A. & Lane, D.J.W. (1985). Mitotic E- and secretory F-cells in the hepatopancreas of the shrimp *Penaeus semisulcatus* (Crustacea: Decapoda). *Journal of the Marine Biological Association of the United Kingdom* **65**, 901-910.

Alabaster, J.S., Gough, P.J. and Brooker, W.J. (1991). The environmental requirements of Atlantic salmon *Salmo salar* L., during their passage through the Thames Estuary, 1982-1989. *Journal of Fish Biology* **38**, 741-762.

Allan, W.H. (1961). Underwater flow visualization techniques. *U.S. Navy Ordnance Testing Station Technical Publication* **2759**, 28pp.

Anderson, D.T. & Rossiter, G.T. (1969a). Hatching and larval development of *Haplostomella australiensis* Gotto (Copepoda, Fam. Ascidicolidae), a parasite of the ascidian *Styela etheridgii* Herdman. *Proceedings of the Linnean Society of New South Wales* **93(3)**, 464-475.

Anderson, D.T. & Rossiter, G.T. (1969b). Hatching and larval development of *Dissonus nudiventris* Kabata (Copepoda, fam. Dissonidae), a gill parasite of the Port Jackson shark. *Proceedings of the Linnean Society of New South Wales* **93(3)**, 476-481.

Anderson, R.M. & Whitfield, P.J. (1975). Survival characteristics of the free-living cercarial population of the ectoparasitic digenean *Transversotrema patialense* (Soparker, 1924). *Parasitology* **70**, 295-310.

Anderson, R.M. and Gordon, D.M. (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373-98.

Andrade-Salas, O., Sommerville, C., Wootten, R., Turnbull, T., Melvin, W., Amezaga, T. and Labus, M. (1993). Immunohistochemical screening and selection of monoclonal antibodies to the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837). In Boxshall, G. & Defaye, D. (eds.) *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood Ltd. London, 323-331.

Andrews, J.C. (1983). Deformation of the active space in the low Reynolds number feeding current of calanoid copepods. *Canadian Journal of Fisheries and Aquatic Science* **40**, 1293-1302.

Anstensrud, M. (1990). Moulting and mating in *Lepeophtheirus pectoralis* (Copepoda:Caligidae). *Journal of the Marine Biological Association of the United Kingdom*. **70(2)**, 269-281.

Anstensrud, M. and Schram, T.A. (1988). Host and site selection by larval stages of the parasitic copepod *Lernaeenicus sprattae* (Sowerby) (Copepoda, Pennellidae) in the Oslofjord. *Hydrobiologia* **167/168**, 587-595.

Arnaud, J., Brunet, M. and Mazza, J. (1978). Studies on the midgut of *Centropages typicus* (Copepod, Calanoid). I Structural and ultrastructural data. *Cell and Tissue Research* **187**, 333-353.

Arnaud, J., Brunet, M. and Mazza, J. (1980). Structure et ultrastructure comparées de l'intestin chez plusiers espèces de Copépodes Calanoides (Crustacea). *Zoomorphologie* **95**, 213-233.

Arnaud, J., Brunet, M. & Mazza, J. (1984a). Détection d'une activité arylsulfatasique dans les cellules B de l'intestin moyen de *Centropages typicus* (Copépode, Calanoida). *Comptes Rendus de l'Académie des Sciences de Paris* **296(III)**, 727-731.

Arnaud, J., Brunet, M. & Mazza, J. (1984b). Cytochemical detection of phosphatase and arylsulphatase activities in the midgut of *Centropages typicus* (Copepod, Calanoid). *Basic and Applied Histochemistry* **28**, 399-412.

Arnaud, J., Brunet, M. & Mazza, J. (1988). The labral glands in *Centropages typicus* (Copepoda, Calanoida). II. Sites of secretory release. *Journal of Morphology* **197**, 209-219.

Ashby, A.B. (1951). Sea lice on salmon. *Salmon and trout magazine* **131**, 82-85.

Atema, J. (1985). Chemoreception in the sea: Adaptations of chemoreceptors and behaviour to aquatic stimulus conditions. In Laverack, M.S. (ed.): *Physiological Adaptations of Marine Animals. Symposia of the Society for Experimental Biology* **39**, 387-423.

Atema, J. (1988). Distribution of chemical stimuli. Chapter 2 in Atema, J., Fay, R.R., Popper, A.N. and Tavolga, A.N. (eds): *Sensory Biology of Aquatic Animals*, 29-56. Springer-Verlag New York Inc.

Autrum, H. (1981). Light and dark adaptations in invertebrates. In Autrum, H. (ed.) *Handbook of Sensory Physiology - Vision in Invertebrates C: Invertebrate Visual Centers and Behavior II*, **VII/6C**, 1-91. Springer-Verlag, Heidelberg.

Awakura, T. (1980). On the parasites and parasitic diseases of salmonid fish in Hokkaido. *Fish Pathology* [In Japanese with English summary] **14**, 207-209.

Bailey, K.M. & Yen, J. (1983). Predation by a carnivorous marine copepod, *Euchaeta elongata Esterly*, on eggs and larvae of the Pacific hake, *Merluccius productus*. *Journal of Plankton Research* **5(1)**, 71-82.

Baillie, A.C. (1985). The biochemical mode of action of insecticides. In Janes, N.F. (ed.): *Recent Advances in the Chemistry of Insect Control. 11. The Royal Society of Chemistry, Special Publication* **53**, 1-23.

Bainbridge, R. (1952). Underwater observations on the swimming of marine zooplankton. *Journal of the Marine Biological Association of the United Kingdom* **31**, 107-112,

Bainbridge, R. (1961). In Waterman, T.H. (ed.) *The Physiology of Crustacea, Vol II*, 431-463. Academic Press, New York.

Bannister, N.J. (1993). Distribution and structure of sub-cuticular glands in the copepod *Temora longicornis*. *Journal of the Marine Biological Association of the United Kingdom* **73**, 97-107.

Barnes, R.D. (1980). *Invertebrate Zoology* (4th edition). Holt-Saunders Japan, Tokyo

Barrington, E.J.W. (1982). *Invertebrate Structure and Function* (Second Edition). Van Nostrand Reinhold (UK) Co. Ltd, Wokingham.

Bartel, A.H. and Davenport, D. (1965). A technique for the investigation of chemical responses in aquatic animals. *British Journal of Animal Behaviour*. **4(3)**, 117-119.

Ben Hassine, O.K. (1983). Les copépodes parasites de poissons Mugilidae en Meditérranée occidentale (Côtes francaises et tunisiennes). Morphologie, bio-écologie, cycles évolutifs. Thèse d'État, Université de Montpellier **2**, 452pp.

Benkirane, O. (1987). Recherches sur l'organe de fixation des Lernaeopodidae (Copépoda Siphonostomatoida). Thèse d'État, l'Université des Sciences et Techniques de Languedoc.

Benz, G.W. (1981). Observations on the attachment scheme of the parasitic copepod *Pandarus satyrus* (Copepoda: Pandaridae). *Journal of Parasitology* **67(6)**, 966-967.

Benz, G.W. & Deets, G.B. (1993). Phylogenetic relationships of siphonostomes parasitizing vertebrates. *Abstracts of Fifth International Conference on Copepoda, Baltimore 1993* 14.

Berger, V. Ya (1970). The effect of marine water of different salinity on *Lepeophtheirus salmonis*, ectoparasite of salmon. *Parazitologia* [in Russian with English Summary] **4**, 136-138.

Bird, N.T. (1968). Effects of mating on subsequent development of a parasitic copepod. *Journal of Parasitology*. **54**, 1194-1196.

Bird, N.T. (1971). Investigation of some factors affecting reproduction of *Lernaea cyprinacea* L., a parasitic copepod. *Dissertation Abstracts International B Science and Engineering*. **31(9)**, 5707.

Bishop, E. (1983). *Complete Poems 1927-1979*. Chatto and Windus, London.

Bjordal, Å. (1988). Cleaning symbiosis between wrasses (Labridae) and lice infested salmon (*Salmo salar*) in mariculture. *International Council for the Exploration of the Sea*. **C.M. 1988/F17** Mariculture Committee.

Blackwell, J. & Weih, M.A. (1980). Structure of chitin-protein complexes: ovipositor of the ichneumon fly *Megarhyssa*. *Journal of Molecular Biology* **137**, 49-60.

Blades-Eckelbarger, P.I. (1984). Aspects of internal anatomy and reproduction in the copepoda. In Schriever, G, Schminke, H.K. and Shih, C (eds): *Proceedings of the Second International Conference on Copepoda, Ottawa, Syllogeus* **58**, 26-50.

Blaxter, J.H.S. (1969). Swimming speeds of fish. *F.A.O. Fisheries Report* **62(2)**, 69-100.

Blower, S.M. and Roughgarden, J. (1989). Parasites detect host spatial pattern and density: a field experimental analysis. *Oecologia* **78**, 138-141.

Bocquet, C., Stock, J.H. & Kleeton, G. (1963). Copépodes parasites d'invertébrés des côtes de la Manche. XI Sur le développement de Trochicola entericus Dollfus, 1914, Copépode cyclopoïde de Trochidae. *Archives de Zoologie Expérimentale et Générale* **102**, 49-68.

Bossert, W.H. & Wilson, E.O. (1963). The analysis of olfactory communication among animals. *Journal of Theoretical Biology* **5**, 443-469.

Bouligand, Y. (1966). Le tegument de quelques Copépodes et ses dependances musculaires et sensorielles. *Mémoires du Muséum National d'Histoire Naturelle (Paris) Série A, Zoologie* **XL**, 189-206.

Boxshall, G.A. (1974a). Infections with parasitic copepods in North Sea marine fishes. *Journal of the Marine biological Association of the United Kingdom* **54**, 355-372.

Boxshall, G.A. (1974b). The population dynamics of *Lepeophtheirus pectoralis* (Müller): seasonal variation in abundance and age structure. *Parasitology* **69**, 361-371.

Boxshall, G.A. (1974c). The population dynamics of *Lepeophtheirus pectoralis* (Müller): dispersion pattern. *Parasitology* **69**, 373-390.

Boxshall, G.A. (1974d). The developmental stages of *Lepeophtheirus pectoralis* (Müller 1776). Copepoda : Caligidae. *Journal of Natural History* **8**, 681-700.

Boxshall, G.A. (1976). The host specificity of *Lepeophtheirus pectoralis* (Müller, 1776) (Copepoda: Caligidae). *Journal of Fish Biology* **8**, 255-264.

Boxshall, G.A. (1977). The histopathology of infection by *Lepeophtheirus pectoralis* (Müller) (Copepoda: Caligidae). *Journal of Fish Biology* **10**, 411-415.

Boxshall, G.A. (1981). Community structure and resource partitioning. Chapter 11 in Forey, P.L. (ed.): *The evolving biosphere - Chance change and challenge*. 143-156. British Museum (Natural History), Cambridge University Press.

Boxshall, G.A. (1982). On the anatomy of the misophrioid copepods, with special reference to *Benthomisophria palliata* Sars. *Philosophical Transactions of the Royal Society London B* **297**, 125-181.

Boxshall, G.A. (1985). The comparative anatomy of two copepods, a predatory calanoid and a particle-feeding mormonilloid. *Philosophical Transactions of the Royal Society London B* **311**, 303-377.

Boxshall, G.A. (1986). The comparative anatomy of the feeding apparatus of representatives of four orders of copepods. In Schriever, G, Schminke, H.K. and Shih, C (eds): *Proceedings of the Second International Conference on Copepoda, Ottawa, Syllogeus* **58**, 158-169.

Boxshall, G.A. (1990). The skeletomusculature of siphonostomatoid copepods, with an analysis of adaptive radiation in structure of the oral cone. *Philosophical Transactions of the Royal Society of London B* **328**, 167-212.

Boxshall, G.A. (1992). Copepoda. Chapter 7 In *Microscopic Anatomy of Invertebrates Volume 9: Crustacea* 347-384. Wiley-Liss Inc.

Braaten, B.R. (1975). Recent Norwegian experience in fish farming. *Proceedings of the Society for Underwater Technology Conference "Oceanology International 1975", Brighton* 168-172.

Brandal, P.O. (1977). Neguvon (O,O-dimethyl-1-hydroxy-2,2,2-trichlorethyl-phosphonate), an organophosphate for treatment of salmon, *Salmo salar*, infected with salmon lice, *Lepeophtheirus salmonis*, Krøyer (Copepoda: Caligidae). [translated from Norwegian]. Thesis in Fish Biology, University of Bergen.

Brandal, P.O. and Egidius, E. (1977). Preliminary report on oral treatment against salmon lice *Lepeophtheirus salmonis* with Neguvon. *Aquaculture* **10**, 177-178.

Brandal, P.O. and Egidius E. (1979). Treatment of salmon lice (*Lepeophtheirus salmonis* Krøyer, 1838). *Aquaculture* **18**, 183-188.

Brandal, P.O., Egidius, E. and Romslo, I. (1976). Host blood: a major food component for the parasitic copepod *Lepeophtheirus salmonis*, Kröyeri, 1838 (Crustacea: Caligidae). *Norwegian Journal of Zoology* **24**, 341-343.

Bresciani, J. (1988). The integumental ultrastructure of *Lamippe rubra* Bruzelius and *Enalcyonium rubicundum* Olsson (Copepoda, Poecilostomatoida). *Hydrobiologia* **167/168**, 539-543.

Bresciani, J. & Lützen, J. (1960). *Gonophysema gullmarensis* (Copepoda parasitica): An anatomical and biological study of an endoparasite living in the ascidian *Ascidiella aspersa*. *Cahiers de Biologie Marine* **1**, 157-184.

Bresciani, J. and Lützen (1962). The anatomy of a parasitic copepod *Saccopsis steenstrupi* N. Sp. *Crustaceana* **3**, 9-23.

Bresciani, J. & Lützen, J. (1975). *Mellinacheres ergasiloides* M. Sars, a parasitic copepod of the polychaete *Melinna cristata*, with notes on multiple infections caused by annelidicolous copepods. *Ophelia* **13**, 31-41.

Brett, J.R. (1965). The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). *Journal of the Fisheries Research Board of Canada* **22(6)**, 1491-1501.

Briggs, R.P. (1974). Aspects of the biology and structure of the associated copepod *Paranthessius anemoniae* Claus. Ph.D. Thesis, Queen's College, University of Belfast.

Briggs, R.P. (1976). Biology of *Paranthessius anemoniae* in association with anemone hosts. *Journal of the Marine Biological Association of the United Kingdom* **56**, 917-924.

Briggs, R.P. (1977). Structural observations on the alimentary canal of *Paranthessius anemoniae* - a copepod associate of the snakelocks anemone, *Anemonia sulcata*. *Journal of Zoology* **182**, 353-368.

Briggs, R.P. (1978). Structure of the integument in *Paranthessius anemoniae* Claus, a copepod associate of the Snakelocks anemone *Anemonia sulcata* (Pennant). *Journal of Morphology* **156**, 293-315.

Bron, J.E., Sommerville, C., Jones M. and Rae, G.H. (1991). The settlement and attachment of early stages of the salmon louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae) on the salmon host *Salmo salar*. *Journal of Zoology (London)* **224**, 201-212.

Bron, J.E., Sommerville, C., Wootten, R. & Rae, G.H. (1993a). Fallowing of marine Atlantic salmon, *Salmo salar* L., farms as a method for the control of sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837). *Journal of Fish Diseases* **16**, 487-493

Bron, J.E., Sommerville, C., Wootten, R. and Rae, G.H. (1993b). Influence of treatment with dichlorvos on the epidemiology of *Lepeophtheirus salmonis* (Krøyer, 1837) and *Caligus elongatus* Nordmann, 1832 on Scottish salmon farms. In G.A. Boxshall and D.Defaye (eds): *Pathogens of Wild and Farmed fish: Sea Lice*. Ellis Horwood Ltd. London, 263-274.

Bron, J.E., Sommerville, C. & Rae, G.H. (1993c). The functional morphology of the alimentary canal of larval stages of the parasitic copepod *Lepeophtheirus salmonis*. *Journal of Zoology (London)* **230**, 207-220.

Brunet, M., Cuoc, C., Arnaud, J. & Mazza, J. (1991). Tegumental glands in a copepod *Hemidiaptomus ingens*: structural, ultrastructural and cytochemical aspects. *Tissue and Cell* **23(5)**, 733-743.

Bruno, D.W. and Stone, J. (1990). The role of saithe, *Pollachius virens* L., as a host for the sea lice, *Lepeophtheirus salmonis* Kroyer and *Caligus elongatus* Nordmann. *Aquaculture* **89**, 201-207.

Bullock, T.H. (1957). The trigger concept in biology. In Bullock, T.H.(ed.): *Physiological Triggers. American Physiological Society, Washington D.C.* 1-8.

Bush, B.M.H. and Laverack, M.S. (1982). Mechanoreception. Chapter 9 in Atwood, L. & Sandeman, D.C. (eds), Bliss, D.E. (o.ed.): *Biology of Crustacea Vol. 3 - Neurobiology: Structure and Function*. 399-468. Academic Press, New York.

Buskey, E.J. (1984). Swimming pattern as an indicator of the roles of copepod sensory systems in the recognition of food. *Marine Biology* **79**, 165-175.

Buskey, E.J. & Swift, E. (1985). Behavioral responses of oceanic zooplankton to simulated bioluminescence. *Biological Bulletin (Woods Hole Mass.)* **168**, 263-275.

Buskey, E.J., Mann, C.G. & Swift, E. (1986). The shadow response of the estuarine copepod *Acartia tonsa* (Dana). *Journal of Experimental Marine Biology and Ecology* **103**, 65-75.

Butman, C.A. (1987). Larval settlement of soft-sediment invertebrates: the spatial scales of pattern explained by active habitat selection and the emerging role of hydrodynamical processes. *Oceanography and Marine Biology Annual Review* **25**, 113-165.

Caillet, C., & Raibaut, A. (1979). Observations expérimentales sur la sexualité du Copépode Caligoide *Clavellodes macrotrachelus* (Brian,1906), parasite branchial du Sar *Diplodus sargus* (Linné, 1758). *Comptes Rendus de l'Académie des Sciences (Paris), Série D* **288**, 223-226.

Calderwood, W.L. (1905). Experiment to test the length of time sea-lice remain attached to salmon in freshwater. *Appendices to Twenty-fifth Annual Report of the Fishery Board for Scotland* **25(App.5)**, 75-77.

Cantwell, G.E. (1981). Methods for Invertebrates, Chapter 9 in Clark, G. (ed.): *Staining Procedures Used by the Biological Stain Commission* (4th edition). Williams & Wilkins, Baltimore.

Capart, A. (1948). Le *Lernaeocera branchialis* (Linné, 1767). *Cellule* **52**, 159-212.

Carton, Y. (1968a). Spécificité parasitaire de *Sabelliphilus sarsi*, parasite de *Spirographis spallanzani*: III. Mise en évidence d'une attraction biochimique du copépode par l'annelide. *Arch. Zool. Exp. Gen.* **109(1)**, 123-144.

Carton, Y. (1968b). Comportement et spécificité parasitaire chez les copepodes parasites essai d'analyse expérimentale. *Archives de Zoologie Expérimentale et Générale* **109(4)**, 545-589.

Cason, J.E. (1950). A rapid one-step Mallory-Heidenhain stain for connective tissue. *Stain Technology*. **25(4)**, 225-226.

Chamberlain, D. (1981). Controlling sea lice in salmon. *Freshwater Catch* **11**, 8.

Chapman, D.M. (1975). Dichromatism of bromophenol blue, with an improvement in the mercuric bromophenol blue technique for protein. *Stain Technology* **50**, 25-30.

Chia, F. (1978). Perspectives: Settlement and metamorphosis of marine invertebrate larvae. In Chia F-S. & Rice, M.E. (eds) *Settlement and Metamorphosis of Marine Invertebrate Larvae*, 283-285. Elsevier, New York.

Collard, S. (1970). Some aspects of host-parasite relationships in mesopelagic fishes. *American Fisheries Society Special Publication* **5**, 41-56.

Conover, R.J. (1966). Feeding on large particles by *Calanus hyperboreus* (Krøyer). In Barnes, H. (ed.): *Some Contemporary Studies in Marine Science* 187-194. George Allen and Unwin Ltd., London.

Cressey, R.F. & Collette, B.B. (1970). Copepods and needlefishes: a study in host-parasite relationships. *Fishery Bulletin* **68(3)**, 347-432.

Crisp, D.J. (1976). Settlement responses in marine organisms. In Newell, R.C. (ed.): *Adaptations to environment: essays on the physiology of marine animals*. 83-84. Butterworths, London.

Crisp, D.J. and Ghobashy, A.F.A.A. (1971). Responses of the larvae of *Diplosoma listerianum* to light and gravity. In Crisp D.J. (ed.): *Fourth European Marine Biological Symposium*, 443-465. Cambridge University Press.

Crisp, D.J. and Meadows, P.S. (1963). Adsorbed layers: the stimulus to settlement in barnacles. *Proceedings of the Royal Society B* **158**, 364-387.

Crofton, H.D. (1971a). A quantitative approach to parasitism. *Parasitology* **62**, 179-193.

Crofton, H.D. (1971b). A model of host-parasite relationships. *Parasitology* **63**, 343-364.

Cronin, T.W. (1988). Vision in Marine Invertebrates. Chapter 16 in Atema, J., Fay, R.R., Popper, A.N. and Tavolga W.N. (eds): *Sensory Biology of Aquatic Animals*, 403-418. Springer-Verlag, New York Inc.

Cusack, R. & Johnson, G. (1988). A study of dichlorvos (Nuvan; 2,2 dichloroethenyl dimethyl phosphate), a therapeutic agent for the treatment of salmonids infected with sea lice (*Lepeophtheirus salmonis*). *Aquaculture* **90**, 101-112.

Darwin, C. (1859). *The Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life*. Pub. John Murray, London. Page numbers from reprint of first edition published in 1983 by Penguin Books, Harmondsworth.

Davenport, D. (1955). Specificity and behaviour in symbioses. *Quarterly Review of Biology*. **30**, 29-46.

Davidson, A. (1980). *North Atlantic Seafood*. Penguin Books Ltd. Harmondsworth

Davis, C.C. (1968). Mechanisms of hatching in aquatic invertebrate eggs. *Oceanography and Marine Biology Annual Review* **6**, 325-376.

Davis, C.C. (1981). Mechanisms of hatching in aquatic invertebrate eggs. II. *Oceanography and Marine Biology Annual Review* **19**, 95-123.

Defaye, D., Such, J. and Dussart, B. (1985). The alimentary canal of a freshwater copepod *Macrocyclops albidus* and some other Cyclopoida. *Acta Zoologica*. **66(2)**, 119-129.

Denton, E.J. & Nicol, J.A.C. (1964). The chorioidal tapeta of some cartilaginous fishes (Chondrichthyes). *Journal of the Marine Biological Association of the United Kingdom* **44**, 219-258.

Dey, D.B., Damkaer, D.M. and Heron, G.A. (1988). UV-B dose/dose rate responses of seasonally abundant copepods of Puget Sound. *Oecologia* **76**, 321-329.

Dojiri, M. & Cressey, R.F. (1987). Revision of the Taeniacanthidae (Copepoda: Poecilostomatoida) parasitic on fishes and sea urchins. *Smithsonian Contributions to Zoology* **447**, 250pp.

Drury, R.A.B. & Wallington, E.A. (1980). *Carleton's Histological Technique* (5th edition). Oxford University Press, Oxford, New York, Toronto.

Dudley, P.L. (1969). The fine structure and development of the nauplius eye of the copepod *Doropygus seclusus* Illg. *Cellule* **68**, 7-42.

Dudley, P.L. (1972). The fine structure of a cephalic sensory receptor in the copepod *Doropygus seclusus* Illg. (Crustacea: Copepoda: Notodelphyidae). *Journal of Morphology* **138**, 407-432.

Dunham, P.J. (1978). Sex pheromones in the crustacea. *Biological Review* **53**, 555-583.

Egidius, E. (1985). Salmon lice, *Lepeophtheirus salmonis*. Fiches d'identification des maladies et parasites des poissons, crustacés et mollusques. *Conseil International pour l'Èxploration de la Mer, Copenhagen, Denmark*. **26**, 4pp.

Egusa, S. (1991). *Infectious Diseases of Fish*. Oxonian Press PVT. Ltd.: New Delhi, Calcutta

Einszporn, T. (1965). Nutrition of *Ergasilus sieboldi* Nordmann. I. Histological structure of the alimentary canal. *Acta Parasitologica Polonica* **13(7)**, 71-78.

Eisenman, E.A. and Alfert, M. (1982). A new fixation procedure for preserving the ultrastructure of marine invertebrate tissues. *Journal of Microscopy* **125(1)**, 117-120.

El Gharbi, S., Rousset, V. & Raibaut, A. (1985). Biologie du copépode *Lernaeenicus sprattae* (Sowerby,1806) et ses actions pathogenes sur les populations de sardines des côtes du Languedoc-Roussillon. *Revue des Travaux de l'Institut de la Pêche Maritime* **47**, 191-201.

Elliot, J.M. (1971). Some methods for the statistical analysis of samples of benthic invertebrates. *Scientific Publication of the Freshwater Biological Association* **25**.

Ellis, A.E. (1981). Non specific defence mechanisms in fish and their role in disease processes. *Developments in Biological Standardization* **49**, 337-352.

Elofsson, R. (1966). The nauplius eye and frontal organs of the non-malacostraca (crustacea). *Sarsia* **25**, 53-128.

Elofsson, R. (1969). The ultrastructure of the nauplius eye of *Sapphirina* (Crustacea: Copepoda). *Zeitschrift für Zellforschung* **100**, 376-401.

Elofsson, R. (1971). The ultrastructure of a chemoreceptor organ in the head of copepod crustaceans. *Acta Zoologica* **52**, 299-315.

Esch, G.W., Gibbons, J.W. and Bourque, J.E. (1975). An analysis of the relationship between stress and parasitism. *The American Midland Naturalist* **93(2)**, 339-353.

Fahrenbach, W.H. (1962). The biology of a Harpacticoid Copepod. *La Cellule* **62**, 301-376.

Fahrenbach, W.H. (1964). The fine structure of a nauplius eye. *Zeitschrift für Zellforschung* **62**, 182-197.

Fasten, N. (1913). The behaviour of a parasitic copepod *Lernaeopoda edwardsii* Olsson. *Journal of Animal Behaviour* **3**, 36-60.

Fasten, N. (1919). Morphology and attached stages of first copepodid larva of *Salmincola edwardsii* (Olsson) Wilson. *Publications of Puget Sound Biological Station* **2**, 158-181.

Felton, G.W. & Duffey, S.S. (1991). Protective action of midgut catalase in lepidopteran larvae against oxidative plant defenses. *Journal of Chemical Ecology* **17(9)**, 1715-1732.

Fleminger, A. (1973). Pattern, number, variability and taxonomic significance of integumental organs (sensilla and glandular pores) in the genus *Eucalanus* (Copepoda, Calanoida). *Fisheries Bulletin* **71**, 965-1010.

Forward, R.B. Jr. (1976). A shadow response in a larval crustacean. *Biological Bulletin (Woods Hole)* **151**, 126-140.

Forward, R.B. Jr. (1977). Occurrence of a shadow response among Brachyuran larvae. *Marine Biology* **39**, 331-341.

Forward, R.B. Jr. (1986a). Behavioral responses of a sand-beach amphipod to light and pressure. *Journal of Experimental Marine Biology and Ecology* **102**, 55-74.

Forward, R.B. Jr. (1986b). A reconsideration of the shadow response of a larval crustacean. *Marine Behavioural Physiology* **12**, 99-113.

Forward, R.B. Jr. (1987). Comparative study of crustacean larval photoresponses. *Marine Biology* **94**, 589-595.

Forward, R.B. Jr. (1988). Diel vertical migration: Zooplankton photobiology and behaviour. *Oceanography and Marine Biology Annual Review* **26**, 361-393.

Forward, R.B. Jr. & Cronin, T.W. (1977). Crustacean larval phototaxis: possible functional significance. In Mclusky, D.S. and Berry, A.J. (eds): *Physiology and Behaviour of Marine Organisms, Proceedings of the 12th European Symposium for Marine Biology*, 253-261. Pergamon Press, Oxford.

Forward, R.B. Jr. & Cronin, T.W. (1979). Spectral sensitivity of larvae from intertidal crustaceans. *Journal of Comparative Physiology* **133**, 311-315.

Forward, R.B. Jr. & Douglass, J.K. (1986). Crustacean larval visual sensitivity during diel vertical migration. In Klekowski, R.Z. (ed.): *Proceedings of the 21st European Marine Biology Symposium*, 59-66.

Fowler, J. & Cohen, L. (1987). *Statistics for Ornithologists - British Trust for Ornithology Guide*, **22** 176pp..

Fraile, L. (1986). Experimental demonstration of an attraction of the copepodous and adult stages of the parasite *Caligus minimus* (copepoda: caligidae) for the Bass *Dicentrarchus labrax* (teleostei: serranidae). Vivares, C.P., Bonami, J.-R. & Jaspers, E. (eds): *Pathology in Marine Aquaculture, European Aquaculture Special Publication No. 9. Bredene, Belgium*, 185-186.

Frasson-Boulay, M. (1973). Les cellules a guanine de l'ocelle median de *Pontella mediterranea* Claus (Crustacé; Copépode). *Comptes Rendus de l'Académie des Sciences (Paris)* **276**, 3323-3325.

Fryer, G. (1957). The feeding mechanism of some freshwater cyclopoid copepods. The *Proceedings of the Zoological Society of London*. **129**, 1-25.

Fryer, G. (1966). Habitat selection and gregarious behaviour in parasitic crustaceans. *Crustaceana* **10**, 199-209.

Fujita, S., Yoda, M. & Ugajin, I. (1968). Control of an ectoparasitic copepod, *Caligus spinosus* Yamaguti on cultured adult yellowtail. *Fish Pathology* **2**, 122-127.

Funge-Smith, S.J. (1991). Ionic aspects of the physiology and biology of *Macrobrachium rosenbergii* (de Man) 1879. PhD Thesis, University of Stirling.

Gaines, S.D. & Bertness, M.D. (1992). Dispersal of juveniles and variable recruitment in sessile marine species. *Nature* **360**, 579-580.

Gauld, D.T. (1966). The swimming and feeding of planktonic copepods. In Barnes, H. (ed.) *Some Contemporary Studies in Marine Science*. Allen and Unwin Ltd., London.

Gharagozlou-van Ginneken, I.D. (1977). Contribution a l'étude infrastructurale des glandes labrales de quelques Harpacticoides (Crustacés Copépodes). *Archives de Biologie* **88**, 79-100.

Gharagozlou-van Ginneken, I.D. (1979). Étude ultrastructurale et cytochimique de l'activité temporaire des glandes tegumentaires d'un crustacé copépode. *Annales des Sciences Naturelles, Zoologie, Paris*, serie **13(1)**, 205-212.

Gharagozlou-van Ginneken, I.D. & Bouligand, Y. (1975). Studies on the fine structure of the cuticle of *Porcellidium*, Crustacea, Copepoda. *Cell Tissue Research*. **159**, 399-412.

Gier, H.T. (1949). Differential stains for insect exoskeleton. *Journal of the Kansas Entomological Society* **22**, 79-80.

Gill, C.W. (1986). Suspected mechano- and chemosensory structures of *Temora longicornis* (Copepoda: Calanoida). *Marine Biology* **93**, 449-457.

Gill, C.W. and Crisp, D.J. (1985). Sensitivity of intact and antennule amputated copepods to water disturbance. Marine Ecology Progress Series **21**, 221-227.

Glauert, A.M. (1981). *Fixation, Dehydration and Embedding of Biological Specimens*. North-Holland Publishing Company, Amsterdam.

Gnanamuthu, C.P. (1948). Notes on the anatomy and physiology of *Caligus savala*, a parasitic copepod from Madras plankton. *Proceedings of the Zoological Society of London*. 118, 591-606.

Gomori, C. (1952). *Microscopic Histochemistry*. University of Chicago Press, Chicago 273pp..

Gotto, R.V. (1962). Egg-number and ecology in commensal and parasitic copepods. *Annals and Magazine of Natural History* **5(13)**, 97-107.

Gotto, R.V. (1991). Some aspects of host- and mate-finding in the symbiotic copepods of invertebrates. In Uye, S.-I, Nishida, S. & Ho, J.-S. (eds): *Proceedings of the Fourth International Conference on Copepoda; Bulletin of the Plankton Society of Japan, Special Volume*, 19-23. The Plankton Society of Japan, Higashi-Hiroshima.

Gotto, R.V. & Threadgold, L.T. (1980). Observations and speculations on the alate processes of the ascidicolous copepod *Notopterophorus papilio* (Cyclopoida: Notodelphyidae). *Journal of Zoology, London* **190**, 337-363.

Goudey-Perriere, F. (1979). *Amphiurophilus amphiurae* (Herouard), Crustacé Copépode parasite des bourses genitales de l'ophiure *Amphipholis squamata* Della Chiaje. Echinoderme; morphologie des adultes et études des stades juveniles. *Cahiers de Biologie Marine* **20**, 201-230.

Gould, D.T. (1957). A peritrophic membrane in calanoid copepods. *Nature* **179**, 325-326.

Grabda, J. (1963). Life cycle and morphogenesis of *Lernaea cyprinacea* L. *Acta Parasitologica Polonica* **11**, 169-199.

Grayson, T.H., Jenkins, P.G., Wrathmell, A.B. & Harris, J.E. (1991). Serum responses to the salmon louse *Lepeophtheirus salmonis* (Kroyer, 1838), in naturally infected salmonids and immunized rainbow trout, *Oncorhynchus mykiss* (Walbaum), and rabbits. *Fish and Shellfish Immunology* **1(2)**, 141-155.

Gresty, K.A. (1992). Ultrastructure of the midgut of the copepod *Mytilicola intestinalis* Steuer, an endoparasite of the mussel *Mytilus edulis* L. *Journal of Crustacean Biology* **12(2)**, 169-177.

Gresty, K.A., Boxshall, G.A. & Nagasawa, K. (1993). Antennulary sensors of the infective copepodid larva of sea lice (Copepoda: Caligidae). In Boxshall, G.A. & Defaye, D. (eds): *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood Ltd. London, 83-98.

Griffiths, A.M. & Frost, B.W. (1976). Chemical communication in the marine planktonic copepods *Calanus pacificus* and *Pseudocalanus* sp. *Crustaceana* **30**, 1-8.

Gurney, R. (1934). The development of certain parasitic Copepoda of the Families Caligidae and Clavellidae. *Proceedings of the Zoological Society of London* **2**, 177-217.

Hackman, R.H. (1984). Cuticle: Biochemistry. In: Bereiter-Hahn, J., Matolsky, A.G. & Richards, K.S. (eds) *Biology of the Integument: 1 Invertebrates*, 583-610. Springer-Verlag: Berlin, Heidelberg, New York, Tokyo.

Hackman, R.H. (1971). The integument of arthropods. In Florkin, M. & Scheer, B.T. (eds): *Chemical Zoology: Arthropoda Part B*, 1-62. Academic Press: New York & London.

Hackman, R.H. (1974). Chemistry of the insect cuticle. In Rockstein, M. (ed.): *The Physiology of Insecta* (2nd edition) **VI** 216-270. Academic Press, London, New York.

Hahnenkamp, L. and Fyhn, H.J. (1985). The osmotic response of the salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae), during the transition from sea water to fresh water. *Journal of Comparative Physiology. B.* **155**, 357-365.

Halisch, W. (1940). Anatomie und biologie von *Ergasilus minor* Halisch. *Zeitschrift für Parasitenkunden* 11, pages not given.

Hardy, A. (1956). *The Open Sea - Its Natural History. Part 1: The World of Plankton*. Collins, London 391pp.

Hardy, A.C. & Paton, W.N. (1947). Experiments on the vertical migration of plankton animals. *Journal of the Marine Biological Association of the United Kingdom* **26**, 467-526.

Harris, T.D. (1986). Species of *Gyrodactylus* von Nordmann, 1832 (Monogenea Gyrodactylidae) from poeciliid fishes, with a description of *G. turnbulli* sp. nov. from the guppy, *Poecilia reticulata* Peters. *Journal of Natural History* **20**, 183-191.

Håstein, T. and Bergsjö, T. (1976). The salmon lice *Lepeophtheirus salmonis* as the cause of disease in farmed salmonids. *Rivista Italiana Piscicoltura e Ittiopatologia* **11(1)**, 3-5.

Haury, L.R. & Weihs, D. (1976). Energetically efficient swimming behavior of negatively buoyant zooplankton. *Limnology and Oceanography* **21**, 797-803.

Hawkins, A.D. (1985). The mechanical senses of aquatic organisms. In Laverack, M.S. (ed.): *Physiological Adaptations of Marine Animals. Symposia of the Society of Experimental Biology* **39**, 425-458.

Hayat, M.A. (1989). *Principles and Techniques of Electron Microscopy - Biological Applications* (3rd Edition). Macmillan Press Scientific and Medical, Basingstoke and London

Heegaard, P. (1947). Contribution to the phylogeny of the arthropods. Copepoda. *Spolia Zoologica Musei Hauniensis* **VIII**, 1-227.

Heegaard, P. (1972). Caliginae and Euryphorinae of the Dana expedition (Crustacea: Copepoda: Caligidae). *Steenstrupia* **2**, 295-317.

Heip, C. (1975). On the significance of aggregation in some benthic marine invertebrates. In Barnes, H. (ed.): *Proceedings of the 9th European Marine Biological Symposium*, 527-538. Aberdeen University Press.

Hicks, G.R.F. and Grahame, J. (1979). Mucus production and its role in the feeding behaviour of *Diarthrodes nobilis* (Copepoda: Harpacticoida). *Journal of the Marine Biological Association of the United Kingdom* **59**, 321-330.

Hogans, W.E. and Trudeau, D.J. (1989). Preliminary studies on the biology of sea lice, *Caligus elongatus* and *Lepeophtheirus salmonis* (Copepoda: Caligoida) parasitic on cage-cultured salmonids in the lower Bay of Fundy. *Canadian Technical Report of Fisheries and Aquatic Sciences* **1715**, iv + 14pp.

Holling, C.S. (1966). The functional responses of invertebrate predators to prey density. *Memoirs of the Entomological Society of Canada* **48**, 1-86.

Holling, C.S. and Ewing, S. (1969). Blind man's buff: exploring the response space generated by realistic ecological simulation models. In Patil, G.P., Pielou, E.C. and Waters, W.E. (eds): *Statistical Ecology vol. 2: Sampling and Modelling Biological Populations and Population Dynamics*, 207-230. Pennsylvania State University Press, University Park and London.

Hubschman, J.H. (1962). A simplified azan process well suited for crustacean tissue. *Stain Technology* **37**, 379-80.

Humason, G.L. (1979). *Animal Tissue Techniques* (4th edition). W.H. Freeman and Company, San Francisco.

Hunt, S. (1984). Halogenated tyrosine derivatives in invertebrate scleroproteins: Isolation and identification. In Wold, F. & Moldave, K. (eds): *Methods in Enzymology*. Vol 107, *Posttranslational modifications, Part B*. Academic Press, New york.

Hunt, S. & Breuer, S.W. (1971). Isolation of a new naturally occurring halogenated amino acid: monochloro,monobromotyrosine. *Biochimie et Biophysique Acta.* **252**, 397-400.

Huq, A., Small, E.B., West, P.A., Huq, M.I., Rahman, R. & Colwell, R.R. (1983). Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. *Applied and Environmental Microbiology* **45(1)**, 275-283.

Huse, I., Bjordal, A. Ferno, A. & Furevik, D. (1990). The effect of shading in pen rearing of Atlantic salmon (*Salmo salar*). *Aquacultural engineering* **9**, 235-244.

Hutton, J.A. (1923). The parasites of salmon. *Salmon and Trout Magazine* **34**, 301-312.

Huys R. & Boxshall, G.A. (1991). *Copepod Evolution*. Ray Society, Natural History Museum, London. **159**, 468pp.

Hwa T.-K. (1965). Studies on the life history of a fish-louse (*Caligus orientalis* Gusev). *Acta Zoologica Sinica* [In Chinese with English Summary] **17**, 48-57.

Iwao, S. (1970). Problems of spatial distribution in animal population ecology. In Patil, G. (ed): *Random Counts in Scientific work*, vol. 2, 117-149. Pennsylvania State University Press, University Park & London.

Iwao, S. and Kuno, E. (1971). An approach to the analysis of aggregation pattern in biological populations. In Patil, G.P., Pielou, E.C. & Waters, W.E. (eds): *Statistical Ecology*. Vol 1. *Spatial Patterns and Statistical Distributions*, 461-513. Pennsylvania University Press, University Park & London.

Izawa, K. (1969). Life history of *Caligus spinosus* Yamaguti, 1939, obtained from cultured yellowtail, *Seriola quinqueradiata* T. and S. (Crustacea: Caligoida). *Reports of the Faculty of Fisheries Prefectural University Mie Tsu* **6**, 127-157.

Jacobsen, P.J., Nylund, A. & Alexandersen, S. (1992). Salmon louse infections on wild population of salmonids - A view of the problem in Norway. *Pathogens of Wild and Framed Fish : Sea Lice*. Presentation at First European Crustacean Conference.

Janovy, J. Jr. & Kutish G.W. (1988). A model of encounters between host and parasite populations. *Journal of Theoretical Biology* **134**, 391-401.

Jaworski, A. and Holm, J.C. (1992). Distribution and structure of the population of sea lice, *Lepeophtheirus salmonis* Kroyer, on Atlantic salmon, *Salmo salar* L., under typical rearing conditions. *Aquaculture and Fisheries Management* **23**, 577-589.

Jerlov, N.G. (1976). *Optical Oceanography*. Elsevier, Amsterdam 94pp.

Johannessen, A. (1974). Oppdrett av laksefish i Norske kystfarvann. Lakselus. *Fisken og Havet Series B* [In Norwegian] **2**, 21-33.

Johannessen, A. (1975). Salmon louse, *Lepeophtheirus salmonis* Krøyer (Copepoda, Caligidae). Independent larval stages, growth and infection in salmon (*Salmo salar* L.) from breeding plants and commercial catches in west Norwegian waters 1973-1974. Thesis in Fish Biology, Norway's Fisheries High School, The University of Bergen 113 pages.

Johannessen A. (1978). Early stages of *Lepeophtheirus salmonis* (Copepoda Caligidae). *Sarsia* **63**, 169-176.

John, S.E. and Nair, N.B. (1975). Functional morphology of the digestive system in *Pseudocycnus armatus* (a parasitic copepod). *Forma et Functio* **8**, 361-386.

Johnson, S.C. and Albright, L.J. (1991a). The developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). *Canadian Journal of Zoology* **69**, 929-950.

Johnson, S.C. and Albright, L.J. (1991b). Development, growth and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. *Journal of the Marine Biological Association of the United Kingdom* **71(2)**, 425-436.

Johnson, S.C. and Albright, L.J. (1991c). *Lepeophtheirus cuneifer* Kabata, 1974 (Copepoda: Caligidae) from seawater-reared rainbow trout, *Oncorhynchus mykiss*, and Atlantic salmon, *Salmo salar*, in the Strait of Georgia, British Columbia, Canada. *Canadian Journal of Zoology* **69**, 1414-1416.

Johnson, S.C. & Albright, L.J. (1992a). Comparative susceptibility and histopathology of the response of naive Atlantic, chinook and coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Diseases of Aquatic Organisms* **14**, 179-193.

Johnson, S.C. & Albright, L.J. (1992b). Effects of cortisol implants on the responses of naive coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Diseases of Aquatic Organisms* **14**, 195-205.

Jones, D.A. (1971). A new light trap for plankton. In Crisp, D.J. (ed.): *4th European Marine Biology Symposium*, 487-493: Cambridge University Press

Jones, J.B. (1988). New Zealand parasitic copepoda; genus *Caligus* Müller, 1985 (Siphonostomatoida: Caligidae). *New Zealand Journal of Zoology* **15**, 397-413.

Jones, J.B. (1980). A redescription of *Caligus patulus* Wilson, 1937 (Copepoda: Caligidae) from a fish farm in the Philippines. *Systematic Parasitology* **2**, 103-106.

Jones, J.W. (1972). *The Salmon*. Collins, London 192pp.

Jones, M. W. (1989). An investigation of the relationship between the attached stages of the "salmon louse" *Lepeophtheirus salmonis* (Copepoda; Caligidae) and its Atlantic salmon host *Salmo salar*. MSc Thesis, University of Stirling

Jones, M.W., Sommerville, C. and Bron J. (1990). The histopathology associated with the juvenile stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **13**, 303-310.

Jones, M.W., Sommerville, C. and Wootten, R. (1992). Reduced sensitivity of the salmon louse *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. *Journal of Fish Diseases* **15**, 197-202.

Jonsdottir, H., Bron, J.E., Wootten, R. & Turnbull, J.F. (1992). The histopathology associated with the adult stages of *Lepeophtheirus salmonis* on the Atlantic salmon *Salmo salar* L. *Journal of Fish Diseases* **15**, 521-527.

Kabata, Z. (1958). *Lernaeocera obtusa* n. sp.; its biology and its effects on the haddock. *Marine Research, Department of Agriculture and Fisheries for Scotland* **1958(3)**, 1-26.

Kabata, Z. (1970). Crustacea as enemies of fishes. In Snieszco, S.F. & Axelrod, H.R. (eds): *Diseases of Fishes, Book 1*. T.F.H. Publications, Jersey city, N.J. 171pp.

Kabata, Z. (1972). The developmental stages of *Caligus clemensi* (Copepoda: Caligidae). *Journal of the Fisheries Research Board of Canada*. **29**, 1571-1593.

Kabata, Z. (1974a). Mouth and mode of feeding of Caligidae (Copepoda), parasites of fishes, as determined by light and scanning electron microscopy. *Journal of the Fisheries Research Board of Canada* **31**, 1583-1588.

Kabata, Z. (1974b). Two new features in the morphology of caligidae (Copepoda). In *Proceedings of the 3rd International Congress of Parasitology*, 1635-1636.

Kabata, Z. (1976). Early stages of some copepods (crustacea) parasitic on marine fishes of British Columbia. *Journal of the Fisheries Research Board of Canada* **33**, 2507-2525.

Kabata, Z. (1979). *Parasitic Copepoda of British Fishes*. The Ray Society **152**, 468pp + plates. British Museum (Natural History), London.

Kabata, Z. (1981). Copepoda (Crustacea) parasitic on fishes: problems and perspectives. *Advances in Parasitology*. **19**, 2-63.

Kabata, Z. (1988). Copepoda and Branchiura. In Margolis, L. & Kabata, K. (eds): *Guide to the Parasites of Canadian Fishes, Part II. Crustacea. Canadian Special Publications in Fisheries and Aquatic Sciences* **101**, 3-127.

Kabata, Z. and Cousens, B. (1972). The structure of the attachment organ of Lernaeopodidae (Crustacea:Copepoda). *Journal of the Fisheries Research Board of Canada*. **29**, 1015-1013.

Kabata, Z. and Cousens, B. (1973). Life cycle of *Salmincola californiensis* (Dana, 1852) (Copepoda:Lernaeopodidae). *Journal of the Fisheries Research Board of Canada* **30(7)**, 881-903.

Kabata, Z. and Cousens, B. (1977). Host parasite relationship between sockeye salmon *Oncorhynchus nerka* and *Salmincola californiensis* (Dana, 1852) (Copepoda:Lernaeopodidae). *Journal of the Fisheries Research Board of Canada* **28**, 1143-1151.

Kabata, Z. and Hewitt, G.C. (1971). Locomotory mechanisms in Caligidae (Crustacea: Copepoda). *Journal of the Fisheries Research Board of Canada* **28**, 1143-1151.

Kalmijn, A.J. (1988). Hydrodynamic and acoustic field detection. Chapter 4 in Atema, J., Fay, R.R., Popper, A.N. and Tavolga, W.N (eds): *Sensory Biology of Aquatic Animals*, 83-130. Springer-Verlag New York Inc.

Kannupandi, T. (1976). Cuticular adaptations in two parasitic copepods in relation to their modes of life. *Journal of Experimental Marine Biology and Ecology* **22**, 235-248.

Karlson, P. & Lüscher, M. (1959). "Pheromones": A new term for a class of biologically active substances. *Nature* **183**, 55-56.

Karnovsky, M.J. (1965). A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology* **27**, 137A.

Katona, S.K. (1973). Evidence for sex pheromones in planktonic copepods. *Limnology and Oceanography* **81**, 574-583.

Kearn, G.C. (1967). Experiments on host-finding and host-specificity in the monogenean skin parasite *Entobdella soleae*. *Parasitology* **57**, 585-605.

Kirchman, D., Graham, H., Reish, D. & Mitchell, R. (1982). Lectins may mediate in the settlement and metamorphosis of *Janua (Dexiospira) brasiliensis* Grube (Polychaeta: Spirorbidae). *Marine Biology Letters* **3**, 131-142.

Klyashtorin, L.B. & Yarzhombek, A.A. (1973). Energy consumption in active movements of planktonic organisms. *Oceanography* **13**, 575-580.

Knight-Jones, E.W. & Morgan, E. (1966). Responses of marine animals to changes in hydrostatic pressure. *Oceanography and Marine Biology, Annual Review* **4**, 217-299.

Knight-Jones, E.W. and Stevenson, J.P. (1950). Gregariousness during settlement in the barnacle *Elminius modestus* Darwin. *Journal of the Marine Biological Association of the United Kingdom* **24**, 281-296.

Krishnan, G. (1969). Nature of the cuticle protein of the branchiopod crustacean *Streptocephalus dichotomus*. *Experientia* **25(6)**, 658-659.

Kubota, S.S. & Takakuwa, M. (1963). Studies on the diseases of marine-culture fishes-I. General description and preliminary discussion of fish diseases at Mie Prefecture. *Journal of the Faculty of Agriculture of the Prefectural University Mie Tsu* [in Japanese] **6**, 107-124.

Kunz, E.W.M. (1985). Some aspects of the biology and population dynamics of *Lepeophtheirus salmonis* Kroyer, 1838 and *Caligus elongatus* Nordmann, 1832, Copepoda, Caligidae, parasites of *Salmo salar* L. MSc thesis, University of Stirling

Laing, J. (1937). Host-finding by insect parasites. 1. Observations on the finding of hosts by *Alysia manducator*, *Mormoniella vitripennis* and *Trichogramma evanescens*. *Journal of Animal Ecology* **6**, 298-317.

Land, M.F. (1965). Image formation by a concave reflector in the eye of the scallop *Pecten maximus*. *Journal of Physiology (London)* **179**, 138-153.

Land, M.F. (1981). Optics and vision in invertebrates. In Autrum, H. (ed.) *Handbook of Sensory Physiology - Vision in Invertebrates B: Invertebrate Visual Centers and Behavior I.*, **VII/6B**, 471-592. Springer-Verlag, Heidelberg.

Landsberg, J.H., Vermeer, G.K., Richards, S.A. & Perry, N. (1991). Control of the parasitic copepod *Caligus elongatus* on pond-reared red drum. *Journal of Aquatic Animal Health* **3**, 206-209.

Lang, W.H., Forward, R.B. Jr., Miller, D.C. (1979). Behavioral responses of *Balanus improvisus* nauplii to light intensity and spectrum. *Biological Bulletin (Woods Hole)* **157**, 166-181.

Laverack, M.S. (1962). Responses of cuticular sense organs of the lobster *Homarus vulgaris* (Crustacea). I - Hair-peg organs as water current receptors. *Comparative Biochemistry and Physiology* **5**, 319-325.

Laverack, M.S. (1968). On the receptors of marine invertebrates. *Oceanography and Marine Biology Annual Review* **6**, 249-324.

Laverack, M.S. (1988). The diversity of chemoreceptors. Chapter 11 in Atema, J., Fay, R.R., Popper, A.N. and Tavolga, W.N. (eds): *Sensory Biology of Aquatic Animals*, 287-312. Springer-Verlag New York Inc.

Laverack, M. & Hull, M.Q. (1993). Sensory innervation of the antenna of the pre-adult male *Caligus elongatus*. In Boxshall, G.A. & Defaye, D. (eds): *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood Ltd. London, 114-122.

Lee, R.F. (1975). Lipids of parasitic copepods associated with marine fish. *Comparative Biochemistry and Physiology* **52B**, 363-364.

Lee, R.F., Nevenzel, J.C., and Lewis, A.G. (1974). Lipid changes during life cycle of marine copepod *Euchaeta japonica* Marukawa. *Lipids* **9**, 891-8.

Lee, R.F., Nevenzel, J.C. and Paffenhöfer, G.A. (1972). The presence of wax esters in marine planktonic copepods. *Naturwissenschaften* **59**, 406-11.

Lehninger, A.L. (1981). *Biochemistry* (2nd edition). Worth Publishers Inc.

Leong, T.S. & Wong, S.Y. (1988). A comparative study of the parasite fauna of wild and cultured grouper (*Epinephelus malabaricus* Bloch et Schneider) in Malaysia. *Aquaculture* **68**, 203-207.

Lewis, A.G. (1961). A contribution to the biology of caligoid copepods parasitic on Acanthurid fishes of the Hawaiian islands. Ph.D. Thesis, University of Hawaii, Honolulu, Hawaii.

Lewis, A.G. (1963). Life cycle of the caligid copepod *Lepeophtheirus dissimulatus* Wilson, 1905 (Crustacea:Caligoida). *Pacific Science* **17**, 195-242.

Lewis, A.G., Dean, J. & Gilfillan, E. (1969). Taxonomy and host associations of some parasitic copepods (Crustacea) from pelagic teleost fishes. *Pacific Science* **23**, 414-437.

Lewis, C.A. (1978). A review of substratum selection in free-living and symbiotic cirripeds. In Chia F. & Rice M.E. (eds): *Settlement and Metamorphosis of Marine Invertebrate Larvae*. Elsevier: New York 290pp.

Lin, C. & Ho, J. (1993). Life history of *Caligus epidemicus* Hewitt parasitic on the Tilapia (*Oreochromis mossambicus*) cultured in salt water. In Boxshall, G.A & Defaye, D. (eds): *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood Ltd. London, 5-15.

Linnaeus, C. (1761). *Fauna suecica sistens animalia Sueciae regni: mammalia, aves, amphibia, pisces, insecta, vermes. Distributa per classes et ordines, genera et species*. Editio altera, auctior. Stockholm. 578.

Locke, M. (1984). The structure and development of the vacuolar system in the fat body of insects. In: King, R.C. & Akai, H. (eds.) *Insect Ultrastructure* Volume 2. Plenum Press, New York and London

Lopez, G. (1976). Redescription and ontogeny of *Lepeophtheirus kareii* Yamaguti, 1936 (Copepoda: Caligidae). *Crustaceana* **31**, 203-207.

Lowe, E. (1935). On the anatomy of a marine copepod *Calanus finmarchichus* (Gunnerus). *Transactions of the Royal Society of Edinburgh* **58**, 561-603.

Lüling, K.H. (1953). Gewebeschäden durch parasitäre Copepoden, besonders durch *Elytrophora brachyptera*. *Zeitschrift für Vergleichende Parasitenkunde* **16**, 84-92.

Lundborg L.E. and Ljungberg, O. (1977). Attack of *Caligus* sp. on salmon and rainbow trout in brackish water floating cage management. *Nordic Veterinary Medecine* [Norwegian] **29**, 20-21.

Lythgoe, J.N. (1968). Visual pigments and visual range underwater. *Vision Research* **8**, 997-1012.

Lythgoe, J.N. (1979). *The Ecology of Vision*. Clarendon, Oxford.

Lythgoe, J.N. (1985). Aspects of photoreception in aquatic environments. In Laverack, M.S. (ed.): *Physiological Adaptations of Marine Animals Symposia of the Society for Experimental Biology* **39**, 57-82.

Lythgoe, J.N. (1988). Light and vision in the aquatic environment. Chapter 3 in Atema, J., Fay, R.R., Popper, A.N. and Tavolga, W.N. (eds): *Sensory Biology of Aquatic Animals*, 57-82. Springer-Verlag New York Inc.

Lythgoe, J.N. & Hemmings, C.C. (1967). Polarized light and underwater vision. *Nature* **213**, 893-894.

M'Gonigle, R.H. (1931). Pathological and other investigations. *Annual Report on the Work of the Biological Board of Canada for the Year 1930*, 20-21.

Mallory, F.B. (1936). The anilin blue collagen stain. *Stain Technology* **11**, 101.

Mandelbrot, B.B. (1967). How long is the coast of Britain ? Statistical self-similarity and fractional dimension. *Science* **156**, 636-8.

Manton, S.M. (1977). *The Arthropoda: Habits, Functional Morphology, and Evolution*. Clarendon Press, Oxford

Marcogliese, D.J. (1991). Seasonal occurrence of *Lernaea cyprinacea* on fishes in Belews Lake, North Carolina. *Journal of Parasitology* **77(2)**, 326-327.

Margolis, L. (1958). The identity of the species of *Lepeophtheirus* (Copepoda) parasitic on Pacific salmon (genus *Oncorhynchus*) and Atlantic salmon (*Salmo salar*). *Canadian Journal of Zoology* **36**, 89-892.

Marino, D. & Onesto, E. (1970). La nutrizione dei saffirinidi (Copepodi). Struttura del canale alimentare di *Sapphirina angusta* Dana. *Pubblicazioni della Stazione Zoologica di Napoli* **38**, 355-363.

Martoja, R. & Ballan-Dufrancais, C. (1984). The ultrastructure of the digestive and excretory organs. In: King, R.C. & Akai, H. (eds) *Insect Ultrastructure* Volume 2. Plenum Press, New York and London.

Martoja, R. & Martoja M. (1967). *Initiation aux Techniques de l'Histologie Animale*. Masson et Cie, Paris 345pp.

Masson, P. (1929). Some histological methods. Trichrome stainings and their preliminary technique. *Journal of the Technical Methods Bulletin of the International Association of Medical Museums* **12**, 75.

Mauguin, M.C. (1910). Sur les cristaux liquides de Lehmann. *Bulletin de la Société Française de Minéralogie et Crystallographie* **34**, 71-117.

Mayzaud, P. (1986). Digestive enzymes and their relation to nutrition. In Corner, E.D.S. & O'Hara S.C.M. (eds): *The Biological Chemistry of Marine Copepods*. Clarendon Press, Oxford

McArdle, J. and Bullock, A.M. (1987). Solar ultraviolet radiation as a causal factor of "summer syndrome" in cage reared Atlantic salmon *Salmo salar* L.: a clinical and histopathological study. *Journal of Fish Diseases* **10**, 255-264.

McGladdery, S.E. & Johnston, C.E. (1988). Egg development and control of the gill parasite *Salmincola salmoneus*, on Atlantic salmon kelts (*Salmo salar*) exposed to four different regimes of temperature and photoperiod. *Aquaculture* **68**, 193-202.

McLean, P.H., Smith, G.W. and Wilson, M.J. (1990). Residence time of the sea louse *Lepeophtheirus salmonis* K., on Atlantic salmon, *Salmo salar* L., after immersion in fresh water. *Journal of Fish Biology* **37**, 311-314.

McManus, J.F.A. (1946). Histological demonstration of mucin after periodic acid. *Nature (Lond.)* **158**, 202.

McVicar, A.H. (1987). Pancreas disease of farmed Atlantic salmon, *Salmo salar*, in Scotland: epidemiology and early pathology. *Aquaculture* **67**, 71-78.

Miller, C.B., Nelson, D.M., Weiss, C. and Soeldner, A.H. (1990). Morphogenesis of opal teeth in calanoid copepods. *Marine Biology* **106**, 91-101.

Millot, J. (1923). Thèse de Médecin (cited in Frasson-Boulay op. cit.).

Mills, D. (1980). *Scotland's King of Fish*. William Blackwood. 52-53.

Moore, M.N., Lowe, D.M. and Gee, J.M. (1978). Histopathological effects induced in *Mytilus edulis* by *Mytilicola intestinalis* and the histochemistry of the copepod intestinal cells. *Journal du Conseil Permanent International de l'Èxploration de la Mêr* **38**, 6-11.

Moore, P.G. & Rainbow, P.S. (1984). Ferritin crystals in the gut caeca of *Stegocephaloides christianensis* Boeck and other Stegophalidae (Amphipoda: Gammaridea): A functional interpretation. *Philosophical Transactions of the Royal Society London B* **306**, 219-245.

Morgan, E. (1984). The pressure-responses of marine invertebrates: a psychophysical perspective. *Zoological Journal of the Linnean Society* **80**, 209-230.

Morse, A.N.C. & Morse, D.E. (1984). Recruitment and metamorphosis of *Haliotis* larvae induced by chemicals uniquely available at the surfaces of crustose red algae. *Journal of Experimental Marine Biology and Ecology* **75**, 191-215.

Moser, M. & Taylor, S. (1978). Effects of the copepod *Cardiodectes medusaeus* on the lanternfish *Stenobrachius leucopsaurus* with notes on hypercastration by the hydroid *Hydrichthys* sp. *Canadian Journal of Zoology* **56**, 2372-2376.

Muntz, W.R.A. (1972). Inert absorbing and reflecting pigments. In Dartnell, H.J.A. (ed.): *Handbook of Sensory Physiology - Photochemistry of Vision* **VII/I, 529-565**. Springer-Verlag, Heidelberg.

Munz, F.W. (1958). The photosensitive retinal pigments of fishes from relatively turbid coastal waters. *Journal of General Physiology* **42(2)**, 445-459.

Munz, F.W. & McFarland, W.N. (1975). Part I: Presumptive cone pigments extracted from tropical marine fishes. *Vision Research* **15**, 1045-1062.

Nagasawa, K. (1985). Comparison of the infection levels of *Lepeophtheirus salmonis* (Copepoda) on chum salmon captured by two methods. *Japanese Journal of Ichthyology* **32(3)**, 368-370.

Nagasawa, K. (1987). Prevalence and abundance of *Lepeophtheirus salmonis* on high-seas salmon and trout in the North Pacific Ocean. *Nippon Suisan Gakkaishi* **53(12)**, 2151-2156.

Nagasawa, K. & Takami, T. (1993). Host utilization by the salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) in the Sea of Japan. *Journal of Parasitology* **79(1)**, 127-130.

Nagasawa, K., Ishida, Y. and Tadokoro, K. (1991). Occurrence of salmon lice *Lepeophtheirus salmonis* on longline-caught salmon in the North Pacific Ocean and Bering Sea in the summer of 1991. *Document submitted to the Annual Meeting of the International North Pacific Fisheries Commission, Tokyo, Japan, October 1991*, 15pp.

Neill, W.E. (1990). Induced vertical migration in copepods as a defence against invertebrate predation. *Nature* **345**, 524-526.

Neilson, J.D., Percy, R.I., Scott, J.S. & Valerio, P. (1987). Interactions of caligid ectoparasites and juvenile gadids on Georges Bank. *Marine Ecology - Progress Series* **39**, 221-232.

Netboy, A. (1980). *Salmon - The World's Most Harassed Fish*. Ebeneazer Baylis & Son Ltd., The Trinity Press, Worcester & London 304pp.

Neville, A.C. (1988). The need for a constraining layer in the formation of monodomain helicoids in a wide range of biological structures. *Tissue and Cell* **20(1)**, 133-143.

Neville, A.C. (1975). *Biology of the Arthropod Cuticle*. Springer-Verlag: Berlin, Heidelberg, New York 448pp.

Neville, A.C. (1984). Cuticle: Organization. Chapter 31 in Bereiter-Hahn, J., Matoltsy, A.G. & Richards, K.S. (eds): *Biology of the Integument: 1 Invertebrates*, 611-625. Springer-Verlag; Berlin, Heidelberg, N.Y.

Nicol, J.A.C. (1989). *The Eyes of Fishes*. Clarendon Press, Oxford.

Noble, E.R., King, R.E. & Jacobs, B.L. (1963). Ecology of the gill parasites of *Gillichthys mirabilis* Cooper. *Ecology* **44**, 295-305.

Nott, J.A. & Foster, B.A. (1969). On the structure of the antennular attachment organ of the cypris larva of *Balanus balanoides* (L.). *Philosophical Transactions of the Royal Society of London B* **256**, 115-134.

Nott, J.A., Corner, E.D.S., Mavin, L.J. & O'Hara, S.C.M. (1985). Cyclical contributions of the digestive epithelium to faecal pellet formation by the copepod *Calanus helgolandicus*. *Marine Biology* **89**, 271-279.

Nylund, A., Økland, S. and Bjørknes, B. (1992). Anatomy and ultrastructure of the alimentary canal in *Lepeophtheirus salmonis* (Copepoda: Siphonostomatoida). *Journal of Crustacean Biology* **12(3)**, 423-437.

Ogawa, K. (1992). *Caligus longipedis* infection of cultured striped jack *Pseudocaranx dentex* (Teleostei: Carangidae) in Japan. *Gyobyo Kenkyu* **27(4)**, 197-205.

Oldewage, W.H. and Van As, J.G. (1989). On the sensory structure (?) between the frontal plates of *Caligus* O.F. Müller, 1785 (Copepoda, Caligidae). *Crustaceana* **57(1)**, 72-78.

Ong, J.E. and Lake, P.S. (1969). The ultrastructural morphology of the midgut diverticulum of the calanoid copepod *Calanus Helgolandicus* (Claus) (Crustacea). *Australian Journal of Zoology* **18**, 9-20.

Pacala, S.W. & Dobson, A.P. (1988). The relation between the number of parasites/host and host age: population dynamic causes and maximum likelihood estimate. *Parasitology* **96**, 197-210.

Paperna, I. (1980). Study of *Caligus minimus* (Otto, 1821), (Caligidae Copepoda) infections of the Sea Bass (*Dicentrarchus labrax* (L.) in Bardawil lagoon. *Annales de Parasitologie (Paris)* **55(6)**, 687-706.

Paperna, I. & Lehav, M. (1974). Mortality among grey mullets in a sea-water pond due to caligid parasitic copepod epizootic. *Bamidgeh* **26**, 12-15.

Park, T.S. (1966). The biology of a calanoid copepod *Epilabidocera amphitrites* McMurrich. *La Cellule* **66**, 129-251.

Paulik, G.J., DeLacy, A.C. & Stacy, E.F. (1957). The effect of rest on the swimming performance of fatigued adult silver salmon. *Technical Report of the School of Fisheries University of Washington*. **31**, 1-24.

Pearson, J. (1904). A list of the marine copepods of Ireland. Part 1 - Littoral forms and fish parasites. *Scientific Investigations, Fisheries Branch of the Department of Agriculture and Technical Instruction for Ireland* **III** 30pp.

Pearson, W.H. & Olla, B.L. (1977). Chemoreception in the blue crab, *Callinectes sapidus*. *Biological Bulletin* **153**, 346-354.

Pedaschenko, D. (1898). Development of the embryo and metamorphoses of *Lernaea branchialis*. *Travaux de la Société (Impériale) des Naturalistes de St. Petersbourg - Section de Zoologie et de Physiologie* **26(4)**, No. 7.

Perkins, P.S. (1985). Iron crystals in the attachment organ of the erythrophagous copepod *Cardiodectes medusaeus* (Pennellidae). *Journal of Crustacean Biology* **5(4)**, 591-605.

Petruch, A. (1993). BSc Thesis, University of Stirling

Piasecki, W. (1984). Extrusion of the filamentum frontale in copepodids of *Tracheliastes maculatus* Kollar, 1835 (Siphonostomatoida: Lernaeopodidae). In Schriever, G, Schminke, H.K. and Shih, C (eds): *Proceedings of the Second International Conference on Copepoda, Ottawa, Syllogeus* **58**, 589-593.

Pike, A.W., MacKenzie, K. and Rowand, A. (1993). Ultrastructure of the frontal filament in chalimus larvae of *Caligus elongatus* and *Lepeophtheirus salmonis* from Atlantic salmon, *Salmo salar*. In G.A. Boxshall and D.Defaye (eds): *Pathogens of Wild and Farmed fish: Sea Lice*. Ellis Horwood Ltd. London, 99-113.

Pochon-Masson, J., Renaud-Mornant, J. & Cals, P. (1975). Contribution a la connaissance des glandes tegumentaires metameriques d'un crustacé meiobenthique interstitiel (Crustacea: Mystacocarida). Cytologie structurale et infrastructurale. *Archives de Zoologie Experimentale et Generale* **116**, 123-146.

Poquet,M. (1980). Contribucion al estudio ultrastructural del aparato digestivo de *Caligus minimus* Otto, copepodo parasito de la lubina. *Boletin de la Real Sociedad Espanda de Historia Natural (Biologica)* **78(3)**, 459-471.

Poulin, R. & FitzGerald, G.J. (1987). The potential of parasitism in the structuring of a salt marsh stickleback community. *Canadian Journal of Zoology* **65**, 2793-2798.

Poulin, R., Curtis M.A. and Rau, M.E. (1991). Size, behaviour and acquisition of ectoparasitic copepods by brook trout, *Salvelinus fontinalis*. *OIKOS* **61**, 169-174.

Poulin, R., Curtis, M.A. and Rau, M.E. (1990). Responses of the fish ectoparasite *Salmincola edwardsii* (Copepoda) to stimulation, and their implication for host-finding. *Parasitology* **100**, 417-421.

Quignard, J.P. (1968). Rapport entre la présence d'une "gibbositè frontale" chez les *Labridae* (Poissons, Téléostéens) et le parasite *Lepospilus labrei* Hesse, 1866 (Copépode *Philichthiyidae*). Annales de Parasitologie (Paris) **43(1)**, 51-57.

Radcliffe, R.W. (1950). The effect of fin-clipping on the cruising speed of goldfish and coho salmon fry. *Journal of the Fisheries Research Board of Canada* **8(2)**, 67-73.

Rae, G.H. (1979). On the trail of the sea lice. *Fish Farmer* **25**, 22-23.

Raibaut, A. (1985). Les cycles évolutifs des copépodes parasites et les modalités de l'infestation. *Année Biologique* **XXIV(3)**, 233-274.

Raverty, S.A. (1987). Epidemiology of the salmon louse, *Lepeophtheirus salmonis* on Booker McConnell farm sites and the clinicopathology and enzymology of repetitive Nuvan EC500 treatments in *Salmo salar*. MSc thesis, University of Stirling.

Reilly, P. (1990). Sea lice control: an immunological approach. In Joyce, J.R.(ed.): *Bradan '90 Proceedings. Irish Salmon Grower's Association, Dublin* 16-17.

Reyes, X.P. & Bravo, S.S. (1983). Nota sombre una copepodosis en salmones de cultivo. *Investiaciones Marinas Valparaiso* **11**, 55-57.

Reynolds, E.S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208.

Rice, A.L. (1962). Responses of *Calanus finmarchicus* (Gunnerus) to changes of hydrostatic pressure. *Nature* **194**, 1189-1190.

Rice, A.L. (1964). Observations on the effects of changes in hydrostatic pressure on the behaviour of some marine animals. *Journal of the Marine Biological Association of the United Kingdom* **44**, 163-175.

Richard, J. (1991). Sea lice in North America: Experiences and Concerns. *Report for the British Columbia Aquaculture and Development Council* 34pp.

Richard, J. (1891). Recherches sur le systéme glandulaire et sur le systéme nerveux des Copépodes libres d'eau douce suivies d'une revision des espèces de ce groupe qui vivent en France. *Annales des Sciences Naturelles B* **12**, 113-260.

Richards, R.H. (1980). Studies in perispawning mortalities in brown trout (*Salmo trutta* L.) from Loch Leven, Kinross, Scotland. Ph.D. thesis, Institute of Aquaculture, University of Stirling.

Rigby, W.D. and Tunnell, N. (1971). Internal anatomy and histology of female *Pseudocharopinus dentatus* (Copepoda Lernaeopodidae). *Transactions of the American Microscopical Society* **90(1)**, 61-71.

Ritchie, G., Mordue, A.J. and Pike, A.W. (1993). The reproductive output of *Lepeophtheirus salmonis* adult females in relation to seasonal variability of temperature and photoperiod. In Boxshall, G.A. & Defaye, D. (eds): *Pathogens of Wild and Farmed fish: Sea Lice*. Ellis Horwood Ltd. London, 153-165.

Roberts, R.J. (1989). The pathophysiology and systemic pathology of teleosts. In: Roberts, R.J. (ed.) *Fish Pathology*. Baillère Tindall: London, Philadelphia, Sydney, Tokyo, Toronto.

Rockicki, J. and Bychawska, D. (1991). Parasitic copepods of Carcharhinidae and Sphyridae (Elasmobranchia) from the Atlantic Ocean. *Journal of Natural History* **25**, 1439-1448.

Rohde, K. (1982). *Ecology of Marine Parasites*. University of Queensland Press, St Lucia, Queensland.

Rokicki, J. & Bychawska, D. (1991). Parasitic copepods of Carcharhinidae and Sphyridae (Elasmobranchia) from the Atlantic Ocean. *Journal of Natural History* **25**, 1439-1448.

Rosenthal, H. (1967). Parasites in larvae of the herring (*Clupea harengus* L.) fed with wild plankton. *Marine Biology* **1**, 10-15.

Roth, M. (1992). Studies on aspects of the chemotherapeutic control of the salmon louse *Lepeophtheirus salmonis* Kroyer 1837 (Copepoda: Caligidae). PhD thesis, University of Stirling.

Roth, M., Richards, R.H. and Sommerville, C. (1993). Current practices in the chemotherapeutic control of sea lice infestations in aquaculture: a review. *Journal of Fish Diseases* **16**, 1-26.

Roubal, F.R. (1989). Pathological changes in the gill filaments of *Acanthopagurus australis* (family Sparidae) associated with the post-settlement growth of a lernaeopodid copepod, *Alella macrotrachelus*. *Journal of Fish Biology* **34**, 333-342.

Rousset, V. & Raibaut, A. (1982). Intégration de nouveaux caractères structuraux a la systematique des Chondracanthidae (Copépoda, Poecilostomatoida). *Bulletin de la Société Zoologique de France* **108(1)**, 115-127.

Ruangpan, L. and Kabata, Z. (1984). An invertebrate host for *Caligus* (Copepoda, Caligidae). *Crustaceana* **47(2)**, 219-220.

Rudall, K.M. (1976). In Hepburn, R.H. (ed.): *The Insect Integument*. Elsevier Scientific Publishing Co.: New York

Russell, F.S. (1925). A new species of *Caligus* from Egypt. *Annual Magazine of Natural History* **9**, 15.

Ryland, J.S. (1962). The effect of temperature on the photic responses of polyzoan larvae. *Sarsia* **6**, 41-8.

Sargent, J.R. & Henderson, R.J. (1986). Lipids. In Corner, E.D.S. & O'Hara, S.C.M. (eds): *The Biological Chemistry of Marine Copepods*. Clarendon Press, Oxford

Schram, T. (1979). The life history of the eye-maggot of the sprat, *Lernaeenicus sprattae* (Sowerby) (Copepoda, Lernaeoceridae). *Sarsia* **64**, 279-316.

Schram, T.A. and Anstensrud, M. (1985). *Lernaeenicus sprattae* (Sowerby) larvae in the oslofjord plankton and some laboratory experiments with the nauplius and copepodid (Copepoda, Pennellidae). *Sarsia* **70**, 127-134.

Schuurmans Stekhoven, J.H. Jr. (1934). Zur sinnesphysiologie der parasitären copepoden *Lepeophtheirus pectoralis* (O.F. Müller) und *Acanthochondria depressa* (T.Scott). *Zeitschrift für Parasitenkunde* **7**, 336-362.

Scott, A. (1901). *Lepeophtheirus* and *Lernaea*. *L.M.B.C. Memoirs* **6**, 1-54.

Scott, M.E. (1987). Temporal changes in aggregation: a laboratory study. *Parasitology* **94**, 583-595.

Scott, M.E. and Anderson, R.M. (1984). The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology* **89**, 159-194.

Shariff, M. (1985). Studies on the biology, host-parasite interactions and distribution of *Lernaea* spp. in West Malaysia. Ph.D. thesis, Institute of Aquaculture, University of Stirling.

Shearer, W.M. (1992). *The Atlantic Salmon: Natural History, Exploitation and Future Management*, 244pp. Fishing News Books, Oxford.

Sivak, J.G. (1980). Accommodation in vertebrates: a contemporary survey. In Zadunaisky, J.A. and Davson, H. (eds): *Current Topics in Eye Research* **3**, 281-330. Academic Press, New York.

Smith, P. (1991). Advances in vaccine research. *Fish Farmer* **14**, 22.

Smith, P., Moloney, M., McElligott, A., Clarke, S. & O'Brien, F. (1993). Studies of the efficiency of oral Ivermectin therapy in the control of sea lice infestations of farmed Atlantic salmon. In Boxshall, G.A. & Defaye, D. (eds): *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood Ltd. London, 296-307.

Snedecor, G.W. & Cochran, W.G. (1980). *Statistical Methods* (7th edition). Iowa State University Press, Ames, Iowa 507pp.

SOAFD (Scottish Office Agriculture and Fisheries Department) (1993). *Report of the SOAFD Annual Survey of Fish Farms for 1992.*

Sokal, R.R. & Rohlf, F.J. (1981). *Biometry* (2nd edition). W.H. Freeman and Company, New York.

Sprengel, G. & Lüchtenberg, H. (1991). Infection by endoparasites reduces maximum swimming speed of European smelt *Osmerus eperlanus* and European eel *Anguilla*. *Diseases of Aquatic Organisms* **11**, 31-35.

Stearns, D.E. (1986). Copepod grazing behavior in simulated natural light and its relation to nocturnal feeding. *Marine Ecology Progress Series* **30**, 65-76.

Stearns, D.E. and Forward R.B. Jr. (1984). Copepod photobehaviour in a simulated light environment and its relation to nocturnal vertical migration. *Marine Biology* **82**, 91-100.

Stearns, D.E. and Forward, R.B. Jr. (1984). Photosensitivity of the calanoid copepod *Acartia tonsa*. *Marine Biology* **82**, 85-89.

Stock, J.H. (1991). Some reflections on the antiquity of the copepod lineages. Uye, S., Nishida, S. & Ho, J. (eds) *Proceedings of the Fourth International Conference on Copepoda, Bulletin of the Plankton Society of Japan Special Volume* 1-7. The Plankton Society of Japan, Higashi-Hiroshima.

Strickler, J.R. (1975). Intra- and interspecific information flow among planktonic copepods: Receptors. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **19**, 2951-2958.

Strickler, J.R. (1985). Feeding currents in Calanoid copepods: two new hypotheses. In Laverack, M.S. (ed.): *Physiological Adaptations of Marine Animals Symposia of the Society for Experimental Biology* **39**, 459-485.

Sukhdeo, M.V.K. (1990). Habitat selection by helminths; a hypothesis. *Parasitology Today* **6(7)**, 234-237.

Sukhdeo, M.V.K. & Mettrick, D.F. (1987). Parasite behaviour: Understanding platyhelminth responses. *Advances in Parasitology* **26**, 73-144.

Sullivan, D.S. and Bisalputra, T. (1980). The morphology of a harpacticoid copepod gut: A review and synthesis. *Journal of Morphology* **164**, 89-105.

Sundara Bai, A. (1980). Tests for sex-pheromone in Argulus siamensis (Wilson). *Bulletin of the Ethological Society of India*. **2(1)**, 1-6.

Swift, M.C. & Forward, R.B. Jr. (1980). Photoresponses of *Chaoborus* larvae. *Journal of Insect Physiology* **26**, 365-371.

Swift, M.C. & Forward, R.B. Jr. (1982). Photoresponses of second-instar *Chaoborus* larvae. *Journal of Insect Physiology* **28**, 183-187.

Takegami, T. (1986). Difference of host specificity between larva and adult in *Pseudocaligus fugu* Yamaguti (Copepoda, Caligidae). *Zoological Science* **3(6)**, 1111.

Talbot, P., Clark, W.H. & Lawrence, A.L. (1972). Fine structure of the midgut epithelium in the developing brown shrimp *Penaeus aztecus*. *Journal of Morphology* **138**, 467-486.

Tang, J.W. (1993). An investigation into the energetics of swimming fish with special reference to Atlantic Salmon (*Salmo salar* L.). Ph.D. Thesis, University of Aberdeen.

Tappel, M.E., Chaudiere, J. & Tappel, A.L. (1982). Glutathione peroxidase activities of animal tissues. *Comparative Biochemistry and Physiology* **73B(4)**, 945-949.

Taylor, L.R. (1961). Aggregation, variance and the mean. *Nature* **189**, 732-735.

Taylor, R.S. (1987). The biology and treatment of sea lice on a commercial Atlantic salmon farm. MSc thesis, University College, Cork.

Templeman, W. (1967). Distribution and characteristics of Atlantic salmon over oceanic depths and on the bank shelf areas off Newfoundland, March-May, 1966. *International Commission for the Northwest Atlantic Fisheries Research Document* 67-65 Serial No. 1865, *Annual Meeting, June 1967*

Terra, W.R. & Ferreira, C. (1981). The physiological role of the peritrophic membrane and trehalase: digestive enzymes in the midgut and excreta of starved larvae of *Rhynchosciara*. *Journal of Insect Physiology* **27(5)**, 325-331.

Terra, W.R., Ferreira, C. & Bianchi, A.G. de (1979). Distribution of digestive enzymes among the endo-and ectoperitrophic spaces and midgut cells of *Rhynchosciara* and its physiological significance. *Journal of Insect Physiology* **25**, 487-494.

Tester, P.A. & Turner, J.T. (1991). Why is *Acartia tonsa* restricted to estuarine habitats ?.In Uye, S.-I, Nishida, S. & Ho, J.-S. (eds): *Proceedings of the Fourth International Conference on Copepoda; Bulletin of the Plankton Society of Japan, Special Volume*, 603-612. The Plankton Society of Japan, Higashi-Hiroshima.

Thatcher, V.E. and Robertson, B.A. (1983). The parasitic crustaceans of fishes from the Brazilian Amazon. 11. Vaigamidae fam. nov. (Copepoda: Poecilostomatoida) with males and females of *Vaigamus retrobarbatus* gen. et sp. nov. and *V. spinicephalus* sp. nov. from plankton. *Canadian Journal of Zoology* **62**, 716-729.

Thoney, D.A. & Burreson, E.M. (1988). Lack of a specific humoral antibody response in *Leiostomus xanthurus* (Pisces: Sciaenidae) to parasitic copepods and monogeneans. *Journal of Parasitology* **74(1)**, 191-194.

Threadgold, L.T. (1967). *International Series of Monographs in Pure and Applied Biology Volume 37: The Ultrastructure of the Animal Cell*. Pergamon Press: Oxford, London, Edinburgh.

Tully, O. (1989). The succession of generations and growth of the caligid copepods *Caligus elongatus* and *Lepeophtheirus salmonis* parasitising farmed Atlantic salmon smolts (*Salmo salar* L.). *Journal of the Marine Biological Association of the United Kingdom* **69**, 279-287.

Tully, O. (1992). Predicting infestation parameters and impacts of caligid copepods in wild and cultured fish populations. *6th ICIR Plenary session III: Pest control and parasitism.*

Tully, O., Poole, R. & Whelan, K. (1993). Origins and infestation parameters for epizootics of *Lepeophtheirus salmonis* parasitic on post smolt sea trout (*Salmo trutta*) in the West of Ireland. In Boxshall, G.A. & Defaye, D. (eds): *Pathogens of Wild and Farmed Fish: Sea Lice*. Ellis Horwood Ltd. London, 202-213.

Turnbull, T. (1991). Development of an immunocytochemical technique to assess the localisation of specific antibodies within tissue sections of the salmon louse *Lepeophtheirus salmonis*. MSc Thesis, University of Stirling.

Umminger, B.L. (1968a). Polarotaxis in copepods. I An endogenous rhythm in polarotaxis in *Cyclops vernalis* and its relation to vertical migration. *Biological Bulletin* **135**, 239-251.

Umminger, B.L. (1968b). Polarotaxis in copepods. II The ultrastructural basis and ecological significance of polarized light sensitivity in copepods. *Biological Bulletin* **135**, 252-261.

Urawa, S. and Kato, T. (1991). Heavy infections of *Caligus orientalis* (Copepoda: Caligidae) on caged rainbow trout *Oncorhynchus mykiss* in brackish water. *Gyobyo Kenkyu* **26(3)**, 161-162.

Uvarov, E.B., Chapman, D.R. & Isaacs, A. (1979). *The Penguin Dictionary of Science* (5th edition). Penguin Books, Harmondsworth 488pp.

Van Alstyne, K.L. (1986). Effects of phytoplankton taste and smell on feeding behaviour of the copepod *Centropages hamatus*. *Marine Ecology Progress Series* **34**, 187-190.

Van Damme, P.A. and Hamerlynk, O. (1992). The infection dynamics and dispersion pattern of *Lernaeocera branchialis* L. on 0+ whiting (*Merlangius merlangus* L.) in the Oosterschelde (SW Netherlands). *Journal of Fish Biology* **41**, 265-275.

Vinogradov, A.P. (1953). The elementary chemical composition of marine organisms. *Memoir of the Sears Foundation for Marine Research: Yale University* **2**, 647pp.

Vogel, S. (1981). *Life in Moving Fluids - The Physical Biology of Flow*. Willard Grant Press, Boston.

Von Vaupel Klein, J.C. (1982). Structure of integumental perforations in the *Euchirella messinensis female* (Crustacea, Copepoda, Calanoida). *Netherlands Journal of Zoology* **32(3)**, 374-394.

Voth, D.R. (1972). Life history of a Caligid copepod, *Lepeophtheirus hospitalis* Fraser, 1920 (Crustacea:Caligoida). *Dissertation Abstracts International B. Science and Engineering* **32(9)**, 5547-5548.

Walker, G. (1971). A study of the cement apparatus of the cypris larva of the barnacle *Balanus balanoides*. *Marine Biology* **9**, 205-212.

Wardle, C.S. (1985). Swimming activity in marine fish. In Laverack, M.S. (ed.): *Physiological Adaptations of Marine Animals. Symposia of the Society for Experimental Biology* **39**, 521-540.

Webb, P.W. (1975). Hydrodynamics and energetics of fish propulsion. *Bulletin of the Department of the Environment Fisheries and Marine Service Ottawa* **190**, 152pp.

White, H.C. (1940a). The parasitic copepod, *Lepeophtheirus salmonis*. *Atlantic Salmon and Trout Investigations*. Report no. **14**.

White, H.C. (1940b). Sea lice (*Lepeophtheirus*) and death of salmon. *Journal of the Fisheries Research Board of Canada* **5(2)**, 172-175.

White, H.C. (1942a). Life history of *Lepeophtheirus salmonis*. *Journal of the Fisheries Research Board of Canada*. **6(1)**, 24-29.

White, H.C. (1942b). Severe injuries from *Lepeophtheirus salmonis* occur during drought years. *Atlantic Salmon and Trout Investigations*. Report no. **21**.

Whitfield, P.J., Pilcher, M.W., Grant, H.J. and Riley, J. (1988). Experimental studies on the development of *Lernaeocera branchialis* (Copepoda:Pennellidae): population processes from egg production to maturation on the flatfish host. *Hydrobiologia* **167/168**, 579-586.

Willasden, P. (1980). Immunity to ticks. *Advances in Parasitology* **18**, 293.

Williamson, H. (1935). *Salar the Salmon*. Faber and Faber Limited. (page numbers cited refer to 1987 edition, Webb & Bower (Publishers) Ltd.:Exeter).

Wilson, C.B. (1905). North American parasitic copepods belonging to the family Caligidae. Part 1. The Caliginae. *Proceedings of the U.S. National Museum* **28**, 479-672.

Wilson, C.B. (1911). North American parasitic copepods. Part 9. The Lernaeopodidae. *Proceedings of the U.S. National Museum* **39**, 189-226.

Wilson, E.O. (1965). Chemical communication in the social insects. *Science* **149**, 1064-1071.

Wootten, R. (1985). Experience of sea-lice infestations in Scottish salmon farms. *International Council for the Exploration of the Sea, Mariculture Committee* **CM1985/F:7/Ref.M.**

Wootten, R., Smith, J.W. and Needham, E.A. (1977). Studies on the salmon louse, *Lepeophtheirus*. *Bulletin Officiale de l'International Epizootiologie* **87**, 521-522.

Wootten, R., Smith, W. and Needham, E.A. (1982). Aspects of the biology of the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. *Proceedings of the Royal Society of Edinburgh* **81B**, 185-197.

Yaglom, A.M. (1979). Similarity laws for constant-pressure and pressure-gradient turbulent wall flows. *Annual Review of Fluid Mechanics* **11**, 505-540.

Yoshikoshi, K. (1975). On the structure and function of the alimentary canal of *Tigriopus japonicus* (Copepoda; Harpacticoida) - I Histological structure. *Bulletin of the Japanese Society of Scientific Fisheries* **41(9)**, 929-935.

Yoshikoshi, K. (1988). The morphology of absorption of nutritive substances in the gut of *Tigriopus japonicus*. *Nippon Suisan Gakkaishi* **54(2)**, 203-208.

Yoshikoshi, K. & Kô, Y. (1991). Histochemical localization of hydrolase activities in the alimentary canal of some parasitic copepods. *Nippon Suisan Gakkaishi* **57(4)**, 613-618.

Yoshikoshi, K. & Kô, Y. (1988). Structure and function of the peritrophic membranes of copepods. *Nippon Suisan Gakkaishi* **54(7)**, 1077-1082.

Zakon, H.H. (1988). The electroreceptors: diversity in structure and function. Chapter 32 in Atema, J., Fay, R.R., Popper, A.N, and Tavolga, W.N. (eds): *Sensory Biology of Aquatic Animals*, 813-850. Springer-Verlag New York Inc.

Zar, J.H. (1984). *Biostatistical Analysis*. Prentice-Hall Inc. Engelwood Cliffs N.J.