STUDIES ON THE BIOLOGY AND ECOLOGY OF THE FREE SWIMMING LARVAL STAGES OF *LEPEOPHTHEIRUS SALMONIS* (KRØYER, 1838) AND *CALIGUS ELONGATUS* NORDMANN, 1832 (COPEPODA: CALIGIDAE)

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by

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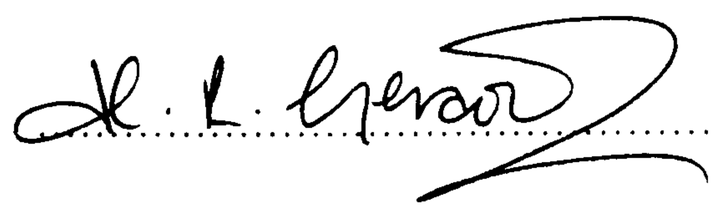
DECLARATION

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



Abstract

The study investigated biological and ecological parameters controlling and influencing the production and distribution of the free-swimming larval stages of *Lepeophtheirus salmonis* (Krøyer, 1838), and to a lesser extent *Caligus elongatus* Nordmann 1832, in the natural environment

The reproductive output of *L. salmonis* was influenced by seasonal effects. The number of eggs produced per brood showed an inverse relationship with increasing temperature. The number of eggs per brood was also influenced by adult female body size (cephalothorax length), which in itself exhibited an inverse relationship with increasing temperature. Photoperiod had no significant effect upon the number of eggs produced or on adult female size. Mean egg size of *L. salmonis* varied significantly over the year; larger eggs were produced during the summer months and smaller eggs over the winter. However, factors controlling the size of the eggs were not elucidated. The proportion of viable eggs per *L. salmonis* ovisac remained constant throughout the year. Large variations in egg number per egg string were found in both *L. salmonis* and *C. elongatus* populations sampled at one point in time. These were attributed in part to phenotypic variation in adult female size and also the number of broods individual females had produced. Egg viability was not correlated with brood size, but mean egg size was related to the number of eggs per brood.

Experimental studies indicated that hatching and development of *L. salmonis* was highly variable. The percentage of eggs hatched and the time period over which hatching occurred varied markedly, even when held under constant and optimal environmental conditions. Temperature did not affect hatching success or viability of the nauplius I stage, although at higher temperatures the period over which hatching

occurred was reduced. Low and medium salinities caused a significant decrease in both hatching success and nauplius viability. Photoperiod had no effect on initiation of hatching. Hatching occurred in a manner similar to that observed in free-living copepods. The nauplii were enclosed by two egg membranes, the outer one bursting within the ovisac, the inner one after the ovisac membrane has split. Swelling of the egg and its subsequent hatching was attributed to osmotic effects, with water being taken up from the external environment.

Development was also highly dependent upon both temperatuure and salinity. At 5°C, nauplius II stages failed to enter the moult to the copepodid stage. At 7.5°C, although moulting was initiated, in a large proportion of cases it was not successfully completed. At 10°C, development to the copepodid stage was successful. Nauplii only developed successfully to the copepodid stage at salinities of 25‰ or greater. Copepodids raised under optimal conditions then exposed to a range of salinities had a greater salinity tolerance than nauplii.

Biochemical analysis of the eggs of *L. salmonis* revealed that lipids constituted a large proportion of their dry weight. Naupliar stages contained a discrete area containing lipid which decreased in size over time, suggesting that the free-swimming larval stages utilised this as an energy reserve. Rate of depletion was faster in nauplii held at higher temperatures. Longevity, activity and infectivity of the infective stage decreased with age. However, both spontaneous and stimulus dependent activity ceased many hours before death and both activity and longevity were affected by temperature. Infectivity of 1 day old *L. salmonis* copepodids was higher than 7 day old larvae, and was considered to be related to the size of the energy reserves. The

settlement and distribution pattern of copepodids did not change with age of copepodid, the majority being recorded from the fins.

All three *L. salmonis* free-swimming larval stages demonstrated a "hop and sink" swimming pattern. The velocity and duration of both passive sinking and active swimming was recorded for both nauplii and copepodids. Although greater periods of time were spent passively sinking, the speeds obtained during both upward spontaneous and stimulated swimming meant that a net upward movement of larvae in the water column occurred. At higher temperatures spontaneous swimming activity increased, whilst low salinities caused a cessation of such ability. *L. salmonis* larvae were postively phototactic and negatively geotactic. As well as their positive responses to light intensity, the nauplius II and copepodid stages reacted positively to blue-green spectral wavelengths. Moulting times were relatively short, although the larvae were not able to swim during such periods. No relationship was found between the level of lipid reserves and the overall buoyancy of the larvae.

Naupliar stages of both *L. salmonis* and *C. elongatus* were obtained from the water column as a result of a plankton sampling programme at a commercial Atlantic salmon farm. No copepodid stages of either species were found. There was no difference in the vertical distribution of the two *L. salmonis* naupliar stages. Live larvae tended to aggregate between 0 and 5m in depth, with no diurnal vertical migration. Dead nauplii, and those with low lipid reserves, were found deeper in the water column. Naupliar stages, and in particular the first larval stage, were concentrated in number within cages indicating that the cages have a retentive characteristic. A novel control method in the form of a commercially available light lure was tested. Though increasing the numbers of free-living copepods captured, it

had no effect on the numbers of *L. salmonis* naupliar or copepodid stages obtained in plankton samples.

The present study has therefore provided valuable data concerning the biology and ecology of the free-swimming larval stages of sea lice, in what was a comparatively poorly understood area.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1. General Introduction

1.1. Infections in Atlantic salmon, *Salmo salar L.*

The most serious disease of marine cage cultured Atlantic salmon in Scotland is widely accepted to be that of infection by sea lice. Sea lice are ectoparasitic copepods of the family Caligidae. Two species are present in Scotland belonging to two separate genera. The predominant species on farmed fish is *Lepeophtheirus salmonis* Krøyer, 1838 though *Caligus elongatus* Nordmann, 1832 has also been recorded, but to a lesser extent (Wootten, Smith & Needham, 1982; Pike, 1989). *L. salmonis* has also been reported from farmed salmon in Ireland (Tully, 1989; Tully & Whelan, 1993), Norway (Johannessen, 1978; Brandal & Egidius, 1979), Canada (Hogans & Trudeau, 1989) and recently for the first time in farmed salmonids in Japan (Nagasawa & Sakamoto, 1993). *C. elongatus* has also been reported from Ireland (Tully, 1989) and Canada (Hogans & Trudeau, 1989), and although much smaller in size than *L. salmonis* can cause significant pathology if abundant enough (Wootten *et al.*, 1982).

The parasitic stages of sea lice feed on the skin and mucus of the host (Kabata, 1974) and also on the blood (Brandal, Egidius & Romslo, 1976). Mortality of the salmon is due to osmotic shock (Wootten, Smith & Needham, 1977; Wootten *et al.*, 1982; Jónsdóttir, Bron, Wootten & Turnbull, 1992) or potentially, indirectly due to secondary infections such as vibriosis (Wootten *et al.*, 1982), furunculosis (Nese & Enger, 1993) or infectious salmon anaemia (Nyland, Wallace & Horland, 1993).

Sea lice became a problem in both commercial Atlantic salmon and rainbow trout (*Oncorhynchus mykiss* Walbaum) farms during the 1960s in Norway as the fish farming industry expanded (Hastein & Bergsjo, 1976), and also as expansion occurred in Scotland during the 1970s (Rae, 1979; Wootten *et al.*, 1982), and later on in Ireland

(Tully, 1989). According to Costello (1993) they are the most commercially limiting parasites in salmonid culture in northern Europe and, at present, the more recently developed salmon farming industry in Canada has been suffering losses attributed to sea lice infestations (Hogans & Trudeau, 1989; Piasecki & MacKinnon, 1995).

1.2. Taxonomy

The taxonomy of parasitic copepods has been extensively reviewed by Kabata (1979), and this guide is generally accepted as the definitive key for species present on British fish. Parasitic copepods belong to one of three sub-orders of the order Copepoda. These are the Poecilostomatoida, the Cyclopoida and the Siphonstomatoida. The sub-order Siphonstomatoida encompasses the family Caligidae.

Both *Lepeophtheirus salmonis* and *Caligus elongatus* belong to the family Caligidae which consists of 27 genera and 350 species (Yamaguti, 1963; Kabata, 1988). According to Kabata (1979), all members of the Caligidae cling to their hosts' surface with the aid of prehensile appendages and are capable of free movement over these surfaces. They are strikingly flat and, in addition to this, the body of a caligid copepod is characterised by being composed of four tagmata (Kabata, 1979).

The genus *Caligus* Müller, 1785 is one of the most successful genera of parasitic copepods, consisting of approximately 200 species distributed throughout the oceans and seas of the world (Kabata, 1979). According to Kabata (1979), the genus appears to be better represented in tropical and subtropical waters when compared to higher latitudes, with only 13 species present in British waters. *Caligus* spp. can be considered to be almost exclusively marine, with only one species, *Caligus lacustris* Steenstrup and Lütken occuring in freshwater, although a few, such as *Caligus epidemicus* Hewitt have been occasionally reported in brackish waters (Kabata, 1979).

Members of the genus parasitise many fish species, the vast majority being teleosts, although some have also been reported from elasmobranchs, such as *Caligus willungae* Kabata. Although some species have only been reported from a single host species (for example *Caligus zei* Norman and T. Scott), some, such as *Caligus elongatus* have very low host specificity being recorded from a broad range of species. *C. elongatus* has been recorded from over 80 species of fish from most regions of the world's oceans, and it is probably the most common species of parasitic copepod in British waters (Kabata, 1979). It has been observed to parasitise salmonids, being recorded from both *Salmo salar* and *Salmo trutta* L. (Kabata, 1979).

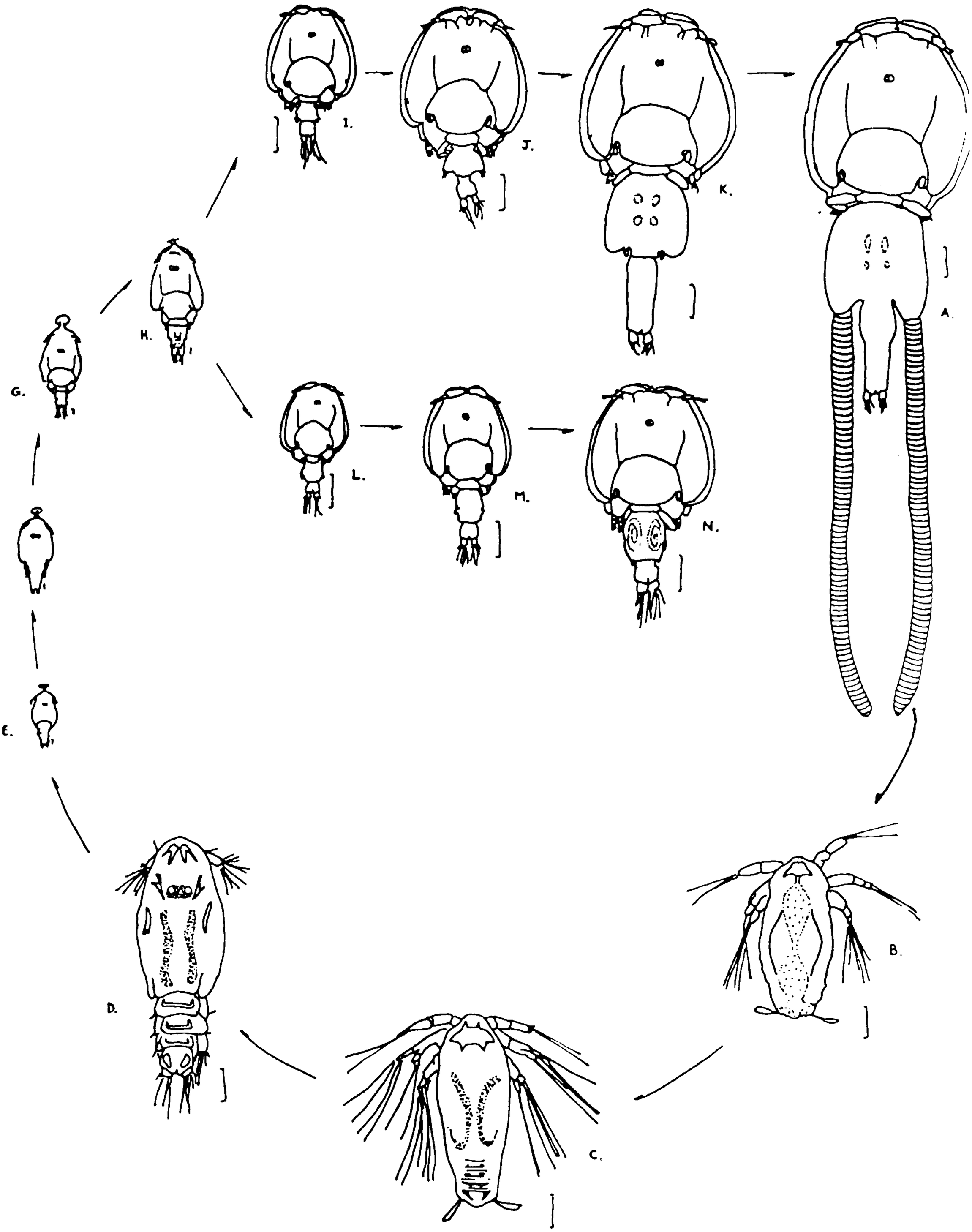
The genus *Lepeophtheirus* von Nordmann, 1832 in contrast to *Caligus* is particularly prevalent in temperate latitudes or cooler waters, with approximately 90 species having been reported (Kabata, 1979). The species of this genus are parasitic exclusively on marine fishes and occur mainly on teleost hosts, with 7 species being found in British waters. Again, like *Caligus*, species of *Lepeophtheirus* can either demonstrate a wide or a narrow host specificity. *Lepeophtheirus salmonis* occurs on most species of the genera *Salmo* and *Oncorhynchus*, and appears to be restricted to the salmonids, occurring commonly on British species. Kabata (1979) stated that the occurrence of *L. salmonis* on non-salmonid hosts must be considered unusual, and that development and survival only occurs when parasitising salmonids. Bruno & Stone (1990) however did record *L. salmonis* from *Pollachius virens* L., reporting it as a new host for the parasite. It is unclear though as to whether *L. salmonis* can complete its life cycle on a non-salmonid host, or whether this report simply represents parasites that had accidentally transferred onto another, unsuitable, fish species.

1.3. Life cycle

The life cycle of *L. salmonis* is generally accepted to consist of ten stages, each separated by a moult (Wootten *et al.*, 1982; Pike, 1989; Johnson & Albright, 1991; Schram, 1993) and is shown in fig. 1.1. Gravid adult females present on the host produce paired ovisacs containing eggs. Upon hatching, these eggs release the first of two free-swimming naupliar stages into the water column. The first larval stage moults to produce the second naupliar stage, which, after the second moult becomes the infective copepodid stage. The copepodid is also free-swimming in the water column and infects a host by the use of its two large hook-like second antennae (Bron, Sommerville, Jones & Rae, 1991).

Once established on a host, the copepodid produces a frontal filament anchoring the larva to the host (Bron *et* al. 1991) and according to these authors is probably rapidly followed by the moult to the first of four larval chalimus stages. These four stages are also permanently attached to the fish by a frontal filament, which penetrates beneath the host epidermis to the basement membrane (Bron *et al.*, 1991). It is not yet known however whether a new filament is produced for each of the chalimus stages or not. The fourth chalimus stage then moults to the first of the three stages which are mobile over the surface of the host. These three stages consist of two pre-adult stages and a final adult stage. It is at the first of the two pre-adult stages when the sexes can be distinguished, although it is possible to differentiate at the chalimus IV stage. Adult males are frequently observed to form pre-copula relationships with both pre-adult 1 and, predominantly, with pre-adult 2 females (Wootten *et al.*, 1982). Upon the moult to the adult female, copulation occurs, with a spermatophore sealing the females genital pore. Eggs are then fertilised upon extrusion into the ovisacs.

Fig. 1.1. General life cycle of sea lice with diagrams showing *Lepeophtheirus salmonis* (after Johnson, 1993): (A) gravid adult female; (B) nauplius I; (C) nauplius II: (D) copepodid; (E) to (H) chalimus I to IV; (I) preadult 1 female; (J) preadult 2 female; (K) adult female; (L) preadult 1 male; (M) preadult 2 male; (N) adult male (A, I to N, scale bars = 1mm; B to H, scale bars = 0.1mm).



Although it is generally accepted that *C. elongatus* has a similar life cycle to that of *L. salmonis* (Pike, 1989; Wootten *et al.*, 1982), there is some confusion in the literature concerning the numbers and types of life cycle stages of *Caligus* spp., and according to Wootten *et al.* (1982), although the developmental sequence of *C. elongatus* appears to be identical to *L. salmonis*, it is difficult to distinguish between pre-adult stages. A recent study by Piasecki & MacKinnon (1995) stated that there were no pre-adult stages in the life cycle of *C. elongatus*, the parasite therefore only possessing eight stages, contradicting the usual ten (Kabata, 1972).

The life cycle of *L. salmonis* and *C. elongatus* is temperature dependent. Wootten *et al.* (1982) stated that the generation time of *L. salmonis* would take approximately 6 weeks at temperatures between 9 to 14°C, though Johnson & Albright (1991) estimated a longer time of between 7.5 and 8 weeks at 10°C, and Tully (1989) reported generation times between 7 and 13 weeks depending on the water temperature. Hogans & Trudeau (1989) reported that the life cycle of *C. elongatus* took approximately 5 weeks at 10°C, with this time being directly related to water temperature, and Piasecki & MacKinnon (1995) found that at 10°C, the generation time was 43.3 days (6-7 weeks).

As already stated, both *L. salmonis* and *C. elongatus* are exclusively marine parasites, and Berger (1970), Hahenkamp & Fyhn (1985) and Johnson & Albright (1991) have demonstrated that development of *L. salmonis* can be compromised or halted at low salinities, with death occurring in freshwater. Although there have been no studies on the effect of salinity on *C. elongatus*, since it is not found in fresh or brackish water, it seems likely that it also cannot survive or complete its life cycle in such environments.

1.4. Background to the present study

Due to the increasing problem caused by sea lice in the fish farming industry, early studies, predominantly on *L. salmonis*, concentrated on life cycle work (Johanessen, 1978) and the development of organophosphate control measures (Brandal & Egidius, 1979). Subsequently, later studies have included many aspects of the parasite's biology and control, many of which are represented in Boxshall & Defaye (1993). However, most attention has been concentrated on the parasitic stages, and the free-swimming larvae, the nauplii and the copepodid stages, have been largely neglected with the exception of Bron *et al.* (1991) and Bron, Sommerville & Rae (1993a, 1993b), where the infection process, the behaviour and the gut morphology of the copepodid stage was studied.

Little is known though about the ecology of the copepodid stage, and especially the early naupliar stages. Research into such areas would make available information that could assist in the management of the disease. The control of sea lice without recourse to chemical treatment is also highly desirable and necessary for a combination of reasons; there exists a differential susceptibility by different stages of sea lice to treatment (Walday & Fonnum, 1989), and there is also the possibility of a development of resistance to drugs (Jones, Sommerville & Wootten, 1992) along with environmental problems associated with treatment (Egidius & Møster, 1987). Information regarding the early planktonic stages of sea lice may assist with parasite management.

Recently, controversy has developed over the role of *L. salmonis* in the collapse of wild sea trout and wild Atlantic salmon populations from the west coast of Ireland and Scotland. It has been suggested by the public press (Clover, 1994; Wilkie, 1995) that copepodids originating from fish farms in large numbers are responsible for

infecting wild sea trout and salmon, and populations of the fish have reduced dramatically. However, to date, there is little scientific evidence with which to clarify this issue along with data regarding the distribution of the free-swimming larval stages.

Research directed towards the free-swimming larval stages of *L. salmonis* by Tully & Whelan (1993) attempted to estimate the production of the first naupliar stage from *L. salmonis* present on farmed and wild salmon and correlate this with future infestation levels of wild sea trout. However, due to the lack of information on the behaviour and ecology of the first free-swimming larval stages, attempts to model the dynamics of whole populations are considerably constrained and their usefulness reduced.

The approach of the present study was therefore to provide more information regarding the ecology, biology and behaviour of the naupliar and copepodid stages, in order to elucidate such issues as (a) the factors that control the production of the first larval stages of sea lice, (b) the development of the free-swimming stages, (c) their behaviour and infectivity characteristics, and (d) their distribution in the natural environment. Results from such research would hopefully provide much needed information that would assist in the understanding of the population dynamics of sea lice and in their management and control.

CHAPTER 2

REPRODUCTIVE OUTPUT

2.1. Introduction

At present *Lepeophtheirus salmonis* is observed to infect Atlantic salmon, *Salmo salar* throughout the year on commercial Scottish marine fish farms (Wootten *et al.* 1982; Bron 1994). *Caligus elongatus*, although largely absent over the winter on such farms, can also occur in relatively high numbers over the summer. The levels of infection on sites vary widely, both seasonally and within individual cages on rafts at one time (personal observation). Although there are many parameters, both abiotic and biotic which influence such levels of parasites on fish farms, understanding the reproductive output of a species, and the factors that influence it are key factors in the understanding of the population dynamics.

Currently, there is a paucity of information concerning the reproductive output of sea lice, the data available being largely restricted to investigations on the number of eggs per ovisac (Wootten *et al.*, 1982; Tully, 1989; Hogans & Trudeau, 1989; Jackson & Minchin, 1992; Johnson, 1993; Pike, Mordue & Ritchie, 1993). However, the results within these reports vary widely, and there is little, or no information available on the number of broods produced or the viability of eggs within the egg sacs.

There have however, been many studies on numerous crustacean groups (for example, Fish, 1975; Evans & Diaz, 1978; Sheader 1981, 1983) which attempt to identify the endogenous and exogenous factors, both singly and collectively, that control and modify the fecundity of populations and hence explain the variations observed within so many species worldwide. The majority of this work has understandably been performed on free-living species. Nevertheless, it is possible to compare trends across different crustacean groups, and more especially between different species of copepods. Such a comparison may enable identification of the

areas where research is needed with respect to the reproductive output of sea lice and also may highlight valuable similarities or differences between these species and their parasitic counterparts.

Temperature has been shown to have a marked effect upon the reproductive output of both free-living and parasitic copepods, although the form of its effect is not universally agreed upon. Different authors have shown that it can act upon fecundity both directly and indirectly. For example, it has been demonstrated that temperature can influence fecundity through body size (Evans & Diaz, 1978). At low temperatures, when developmental rates are reduced, the time between moults is consequently increased and a larger body size is often attained with a concomitant increase in fecundity observed. Although there are other variables that have been invoked to explain changes in body size of free-living copepods such as food quality and availability and the effects of size selective predation (Coker, 1933; Deevey, 1960; McLaren, 1963, 1965; Huntly & Boyd 1984; Runge, 1985), these would appear to be of little importance to parasitic copepods as, once established on a host, they have a year round supply of food in the form of their host and are less exposed to predation as they are no longer free-swimming in the water column. A relationship between temperature and body size has been demonstrated for *L. salmonis* by Tully (1989) and Ritchie, Mordue & Pike (1993), with the body size of both males and ovigerous females being observed to increase with decreasing temperature. Ritchie *et al.* (1993) showed that these were statistically correlated. However, this is in contrast with a report by Johnston & Dykeman (1987) on the parasitic copepod *Salmincola salmoneus* L., who demonstrated that a decrease in temperature under experimental conditions was accompanied by a reduction in the body size of the parasite. Despite this contradiction, both Tully (1989) and Johnston & Dykeman (1987) describe an increase in reproductive output with an increase in body size,

and Ritchie *et al.* (1993) demonstrated that a higher number of viable eggs which they described as those showing normal colouration and arrangement (total numbers of eggs were not recorded), is carried per ovisac by larger adult females. However, only Ritchie *et al.* (1993) tested this relationship statistically, finding a significant correlation between body size and the number of viable eggs produced, indicating that body size does modify the fecundity of *L. salmonis*.

Given that temperature was demonstrated by the same authors to be correlated to body size, it may be assumed that temperature would also be correlated to the number of viable eggs for *L. salmonis*. However, Ritchie *et al.* (1993) showed that differences in size did not directly account for variability in the number of viable eggs per egg string. This appears in contrast to Elbourn (1966), who found that the number of eggs produced by the free-living copepod *Cyclops strenuus* Fischer was determined equally by both temperature and the size of the adult female. However, it is not possible from the data of Ritchie *et al.* (1993) to determine whether this is the case for the total number of eggs carried by *L. salmonis* since only numbers of "normal eggs" were recorded, and thus the results from this study cannot be compared to other trends in crustacean biology.

For *C. elongatus*, there are few reports on the reproductive output of the species, with these being limited only to mention of the number of eggs per ovisac (Hogans & Trudeau, 1989; Pike *et al.*, 1993). Although a relationship between size and temperature has been postulated by Tully (1989), no statistical details were given and, in his report and that of Hogans & Trudeau (1989), it was stated that temperature did not affect fecundity, with the latter showing no apparent seasonal change. However, again there were insufficient details, either from field observations or experiments, given to support the

conclusions. If these conclusions were based on small samples, or ineffective sampling programmes any pattern between temperature, body size and fecundity may be missed.

Despite temperature and body size appearing to be the two major controlling factors of reproductive output in free-living copepods, and perhaps also being the case for their parasitic relatives, high variation in clutch sizes within individual samples of free-living copepods has been reported (Elbourn, 1966; Hopkins, 1977; Maly, 1983; Lawrence & Sastry, 1985; Crawford & Daborn, 1986) that is not explained by these. Such intrinsic variation has also been observed for species of parasitic copepods (Schram, 1979; Tully & Whelan, 1993). Other factors therefore have been investigated in order to explain this and have been seen to modify and influence the brood size of free-living copepods, such factors possibly also being applicable to the reproductive output of *L. salmonis* and *C. elongatus*.

The age of the adult female has been demonstrated to effect the egg production rate of both those free-living copepods that continuously release eggs (Parrish & Wilson, 1978) and the brood size of those species which produce discrete clutches of eggs (Smyly, 1970; Hopkins, 1977; Maly, 1983; Crawford & Daborn, 1986). Older individuals either showed a reduction in the rate of egg production or a diminishing number of eggs laid in successive broods. *L. salmonis* females have been reported to produce more than one brood, with Johannessen (1978), cited by Anstensrud & Schram (1988), being the first to observe a second pair of ovisacs. Ritchie, in unpublished observations cited by Ritchie *et al.* (1993) states that up to six broods are produced by *L. salmonis*, but no further details were given. There are no available reports on the number of broods produced by *C. elongatus*, and there is no information on this species or for *L. salmonis* on the number of eggs contained within successive broods, and its relationship with age, if any. However, there have been reports for other species of *Lepeophtheirus*, although these again tend to contain very little detail.

De Meeüs, Raibaut & Renaud (1993) found that first and last clutches in both *Lepeophtheirus thompsoni* Baird and *L. europaensis* Zeddam, Berrebi, Renaud, Raibaut & Gabion contained fewer eggs than intermediate broods. However, no information was given on the number of broods produced or the number of eggs contained within the ovisacs. If there does exist a significant change in brood size either with age, or between successive broods, then this may help to explain part of the high variations observed in fecundity for *L. salmonis* as reported by Tully & Whelan (1993), and also provide more information upon the factors controlling the reproductive output of sea lice, since age or brood number have not been factors which have yet been considered for *L. salmonis*.

Although there are factors that affect the reproductive output of free-living copepods that will be of negligible effect for parasitic species, such as food abundance and quality, there are factors that will conversely affect only parasitic copepods, with these being factors associated with the host. It has been observed that the fecundity of *L. salmonis* infecting Atlantic salmon was approximately twice that of this parasite on Chinook salmon, *Oncorhynchus tshawytscha* Walbaum (Johnson, 1993), and the author also stated that from personal observations, higher numbers of eggs were also carried by *L. salmonis* adult females infecting adult Coho salmon, *Oncorhynchus kisutch* Walbaum compared to immature fish. As well as effects due to species of host, Jackson & Minchin (1992) observed that lice obtained from wild Atlantic salmon carried more eggs than those from farmed fish of the same species. Johnson (1993) observed that current investigations of differences in the reproductive output of *L. salmonis* showed that this was most commonly related to seasonal environmental conditions, but suggested that host immunological factors may also play an important role in controlling the reproduction of the parasite, and thus this

aspect of host influence should not be ignored when considering the reproductive output as well as host species.

As well as recording variations in the fecundity of sea lice, personal observations by Ritchie, cited by Ritchie *et al.* (1993) stated that there appeared to more areas of disorganised or discoloured eggs present in *L. salmonis* egg strings during winter, suggesting a seasonal variation in the condition of eggs. These were unpublished observations, and no details were given so that the relationship between season and the viability of *L. salmonis* or *C. elongatus* eggs is not known. There have been some reports concerning the viability of eggs produced by free-living copepods, with the majority of these investigating the relationship between seasonal food availability and quality, and its subsequent effect upon the development of the egg (Ianora, Mazzocchi & Grottoli, 1992). As parasitic copepods possess a year round food supply in the form of their hosts, it is seems unlikely that diet will be a major factor in controlling the viability of eggs produced by sea lice. However, there have been reports for free-living copepods that indicate that diet is not the only factor influencing the viability of the eggs, but that other parameters such as sperm depletion, remating and age of the female will also act to modify the viability of the reproductive output (Parrish & Wilson, 1978), and these are also possible influential factors for sea lice.

Another factor to be considered in relation to the reproductive output of sea lice is that of egg size. This has been shown by Ritchie *et al.* (1993) for viable *L. salmonis* eggs to have a seasonal nature, with larger eggs being obtained in the summer compared to smaller eggs which were recorded over the winter months. This is in contrast to reports for free-living copepods, where egg size has been shown to be larger in winter, and smaller in summer, being controlled by temperature (Crawford & Daborn, 1986). The factors

controlling the egg size of *L. salmonis* were not made clear by Ritchie *et al.* (1993) but they considered that photoperiod appeared to exert a greater influence over it than temperature. However no relationship between egg size and other reproductive variables such as the number of eggs produced was tested, despite the authors stating that there appeared to exist two "generation" (*sic*) types of *L. salmonis*. These consisted of a winter type producing many small eggs, and a summer type where fewer larger eggs tended to be produced. Again it is difficult to compare these authors' data with other records as they only consider eggs that appeared viable, the total number of eggs not being recorded. A relationship has been described between egg size and number for free-living copepods by Maly (1983), where the clutch size appeared to control the size of the eggs as also for other crustacean groups (Steele & Steele, 1975a; Kerfoot, 1977). The factors that influence the size of the egg in *L. salmonis* however, and in other species of sea lice are largely unknown, and since the existing data contrasts with the patterns seen for free-living copepods, such factors need to be elucidated. Also, Ritchie *et al.* (1993) only recorded eggs showing normal development, and thus few comparisons can be made with the data available for free-living copepods since this is based on the total number of eggs.

Other reports concerned with the fecundity of sea lice, such as those of Jackson & Minchin, Johnson (1993) and Tully & Whelan (1993) have indicated that non-environmental factors may also control the reproductive output of *L. salmonis*, with Johnson showing a difference in reproductive output of the parasite depending on what host species it infects. Jackson & Minchin (1992) and Tully & Whelan (1993) both demonstrated that lice from wild Atlantic salmon were more fecund than those from farmed fish, with the latter authors suggesting that the use of chemotherapeutants may possibly cause this. However, it seems unlikely that until all the environmental factors controlling reproductive

output in sea lice are elucidated, other factors such as host and treatment effects, will be fully understood. By achieving a better understanding of the control of reproductive output, it may prove possible to forecast future infection levels on farms, and also more fully understand the patterns of infection that are observed.

The present study was therefore undertaken in an attempt to elucidate the abiotic and biotic factors that control the reproductive output of both *L. salmonis* and *C. elongatus*, both in terms of brood size and egg size. As well as looking for any seasonal patterns that may exist, variations of reproductive output within populations sampled at one point in time were also studied, in order to find any other controlling factors that may not be apparent, or indeed obscured by seasonal patterns.

2.2. Materials and Methods

2.2.1. Collection of *Lepeophtheirus salmonis* and *Caligus elongatus*

Both *Lepeophtheirus salmonis* and *Caligus elongatus* were obtained from commercial Atlantic salmon farms in Scotland. Lice were predominantly collected when the farms were harvesting salmon as this usually meant both the smallest inconvenience to the farmers and access to a large number of fish. As harvesting techniques varied from farm to farm it was obviously impossible to standardise a collection technique. However it is possible to summarise the three main methods employed.

i. Atlantic salmon were removed from the harvest cage by means of a mechanically operated winch with a net attached and placed in a cylindrical plastic tub approximately 1.5m in diameter and 2m high containing seawater from the site. Fish were then individually removed from the tub by the use of a hand net and killed by a blow to the head with a wooden stick. Sea lice were gently removed from the fish using a pair of curved forceps.

ii. Atlantic salmon were individually removed from the harvest cage by the use of a long handled hand net, and killed with a blow to the head by a wooden stick. Lice again were removed by curved forceps, taking care not to damage them.

iii. Atlantic salmon were killed using carbon dioxide at the harvest site. Fish were placed in a purpose built device designed to deliver CO_2 by the use of a mechanically operated winch with a net attached. Fish were then gassed using carbon dioxide until they were either dead or "docile" enough to be handled. They were then removed from the carbon dioxide and their gills were cut on one side by the fish farmers, and the fish deposited into 1 tonne harvest bins measuring 1m x 1m x 1.5m, usually containing seawater

and/or ice. Fish were then individually picked out of the bins and lice removed using curved forceps and the salmon returned to the bins.

Once the sea lice had been removed from the salmon, they were placed in a plastic bag (approximately 45 x 30cm) half filled with clean seawater from the same site. Care was taken not to contaminate the water with mucus or blood from the fish, so keeping the water as clean as possible. Approximately 200 lice were put into each plastic bag. The bags were then sealed by a rubber ring and placed inside a second plastic bag which was sealed in the same manner to prevent any leakage. Bags were then put inside cool boxes with a small amount of ice to keep the temperature at a low and maintained level. Seawater from the site was collected in plastic containers each holding 45 litres for future maintenance of the parasites *in vitro*.

2.2.2. Seasonal variations

Monthly samples of gravid adult female *L. salmonis* were collected from the same commercial Atlantic salmon farm on the west coast of Scotland. Between 20 to 30 gravid adult females were removed from fish on each occasion prior to harvesting and fixed in 10% buffered formalin. The number of fish sampled and the final number of parasites obtained was dependent upon the infection levels present. All fish harvested were year class 2 fish. The surface water temperature was taken at the site and the photoperiod was obtained from published sources, based on the ambient photoperiod at Glasgow, Scotland, this being the nearest geographical location to the site where such measurements were recorded.

The gravid adult females were then measured, using a dissecting microscope (Olympus SZ30) at x40 magnification for their cephalothorax length, taken as the distance between the joint of the cephalothorax to the anterior tip of the frontal organ.

Each right hand egg string (still attached to the female) was measured, using a dissecting microscope (x40) for its length, the number of viable eggs, and the number of abnormal eggs. Abnormal eggs were classed as those not showing normal development, being either discoloured or disorganised. They were easily distinguished from those that appeared to be viable by the absence of the characteristic pigmented appearance of mature eggs, or, in the case of immature eggs, having a disorganised "spongy" appearance, compared to eggs which, from separate laboratory investigations (Chapter 3), were seen to go on to show normal development. These are illustrated in fig. 2.10a. The size of eggs was obtained by measuring three randomly selected sections of twenty eggs within each egg string, and calculating the mean egg size for each of the three regions and then the overall average of this figure. The term "egg size" therefore refers to the lateral diameter of the unhatched egg (fig. 2.10a).

2.2.3. Infrapopulational variation

The results of the seasonal studies revealed that there were large variations within samples. Because of this, a large sample was taken in order to determine the causes of this variation. 83 gravid adult female *L. salmonis* and 102 gravid adult female *C. elongatus* were collected from Atlantic salmon prior to harvesting at the same farm in a single sample on one day during September and fixed in 10% buffered formalin. This number was the maximum number of gravid females which could be collected during the same sampling session and was dependent upon the infection levels on the fish being harvested. Gravid adult females from both species were again measured for cephalothorax length, egg string length, egg number (both viable and non-viable) and egg size. The relationships between female body size and egg number, viability and egg number and egg size and egg number were then analysed statistically.

2.2.4. Numbers of broods produced

From standard infection trials taking place at the Institute of Aquaculture's field station (Marine Environmental Research Laboratory, Machrihanish), it proved possible to collect consecutively produced egg strings from *L. salmonis* gravid adult females. Atlantic salmon were routinely infected by staff at the Laboratory with copepodids and development monitored every second day by anaesthetising fish with Ethyl p-Amino Benzoate (benzocaine) and observing the stages present under a dissecting microscope (x40). Once egg strings were observed to be produced by adult females each left egg string was removed and fixed in 10% buffered formalin. These samples were then collected from the Marine Station and the number of eggs in each egg string counted under a dissecting microscope at x40 magnification. In adult females that produced more than one brood, it was observed that the remaining right egg string was always shed before the next pair of ovisacs was extruded, so it was always possible to know how many broods each adult female had produced. Due to the low levels of infections resulting from the controlled infections, there was no confusion between individual adult females present on the salmon.

2.2.5. Statistical Analysis

All data were tested for normality before applying analysis of variance tests (ANOVA). If a significant level of variance was identified, then a Dunn's test was used to identify the samples that differed significantly from the overall mean.

2.3. Results

2.3.1. Seasonal variations in reproductive output

The monthly surface water temperatures at the site sampled are shown in fig. 2.1. The minimum temperature observed was 4°C in February, with the maximum being 14°C during the months of July and August. Seasonal photoperiod for Glasgow, the closest geographical location to the site where such measurements are recorded, is shown in fig. 2.2.

2.3.1.1. Female body size

The variation in mean cephalothorax length of *L. salmonis* adult females sampled monthly over one year is shown in fig. 2.3. The maximum mean length was observed to be 5.74mm (± 0.027), achieved in March, with this declining to a minimum mean size of 5.05mm (± 0.024) in October. An analysis of variance demonstrated that a significant variation in mean cephalothorax length did occur over the twelve monthly samples ($p < 0.001$). The relationship between temperature and mean cephalothorax length is shown in fig. 2.4. A Dunns test revealed that all samples were significantly different from each other, with the exceptions of the February and March samples, the April and May samples, the August and September samples and the July and November samples.

It was found that temperature and mean cephalothorax length were negatively correlated with each other ($r^2 = 0.685$, $p < 0.001$) over the whole sampling period. However, the maximum and minimum temperatures did not coincide with the minimum and maximum mean cephalothorax lengths, but instead a lag was observed. The minimum temperature experienced (4°C) was in February, but the maximum mean cephalothorax length was observed in March. Likewise, the maximum temperature

Fig. 2.1. Seasonal surface water temperature (C°) of the site sampled recorded on the sampling day.

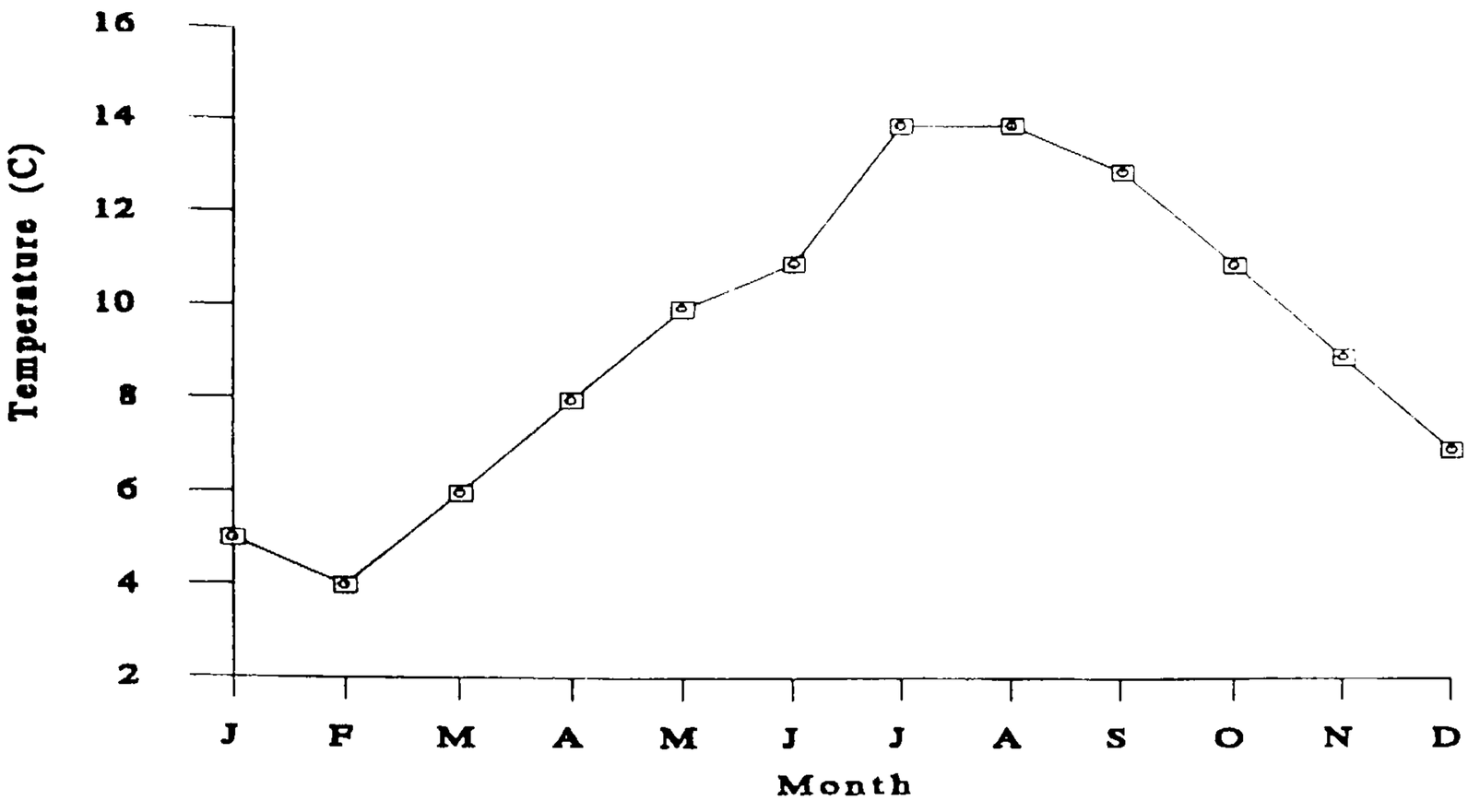


Fig. 2.2. Seasonal photoperiod of Glasgow: hours of light from sunrise to sunset

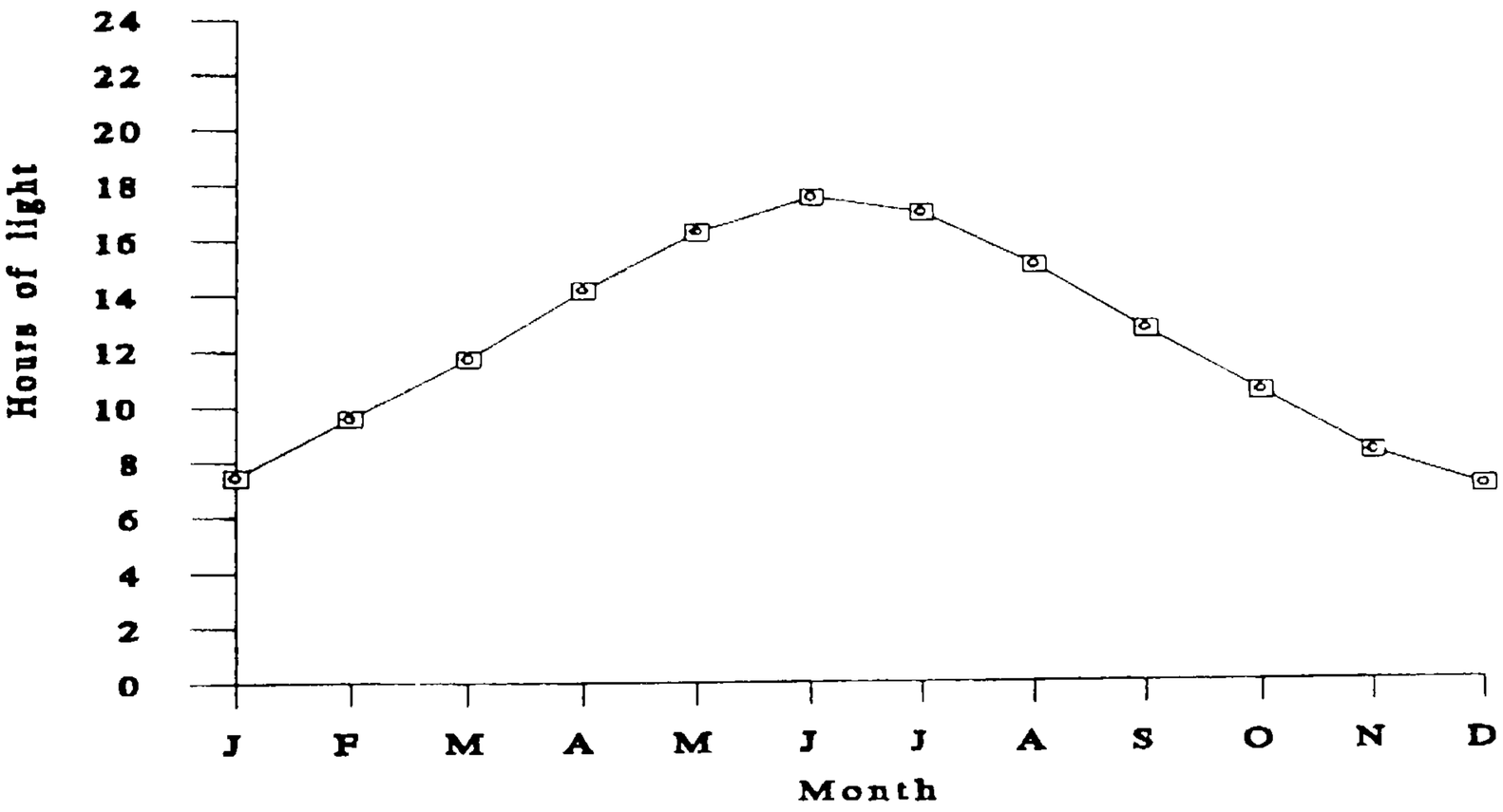


Fig. 2.3. Seasonal variation of the mean cephalothorax length (mm ± 1 SD) of *L. salmonis* gravid adult females

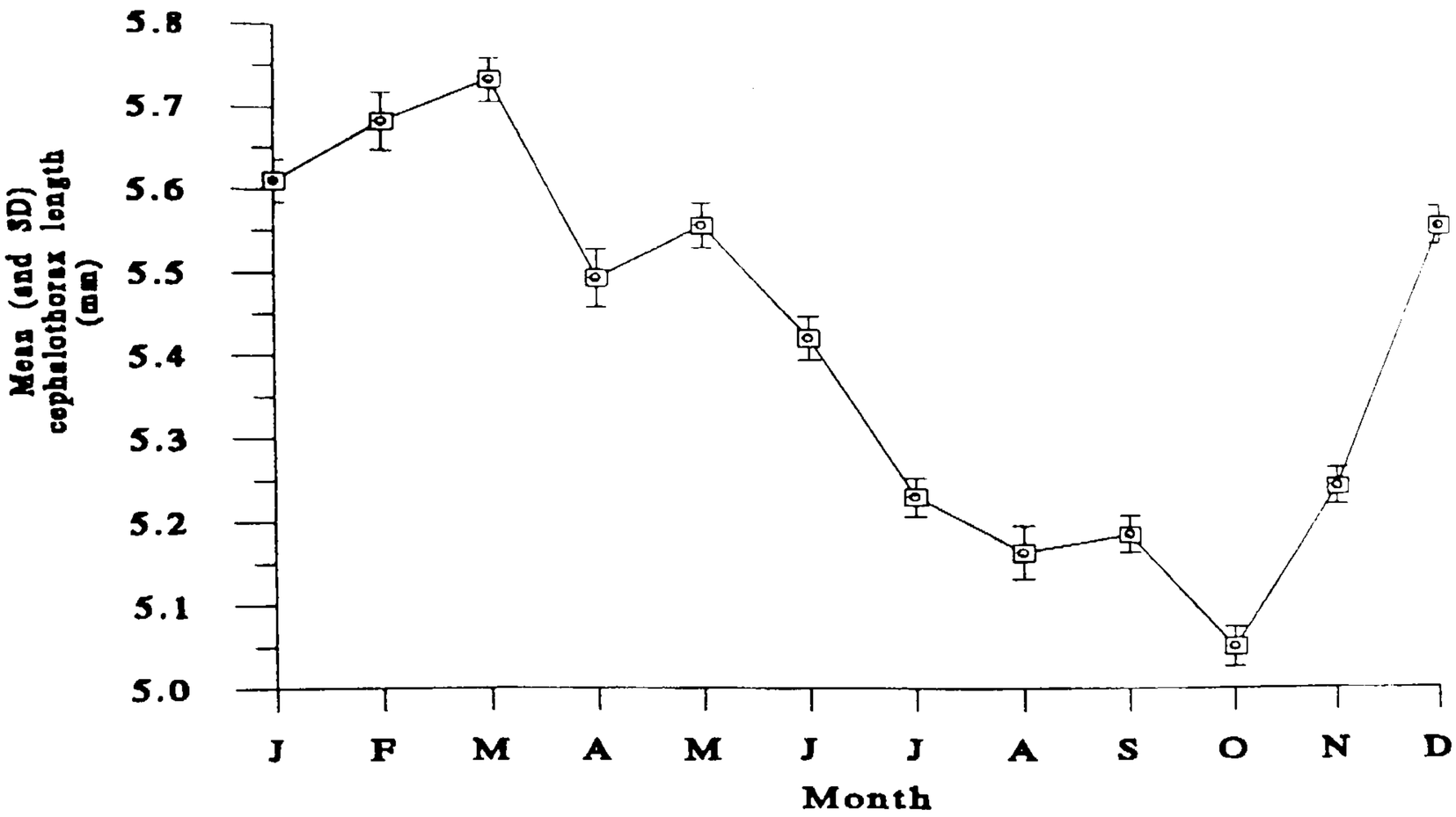
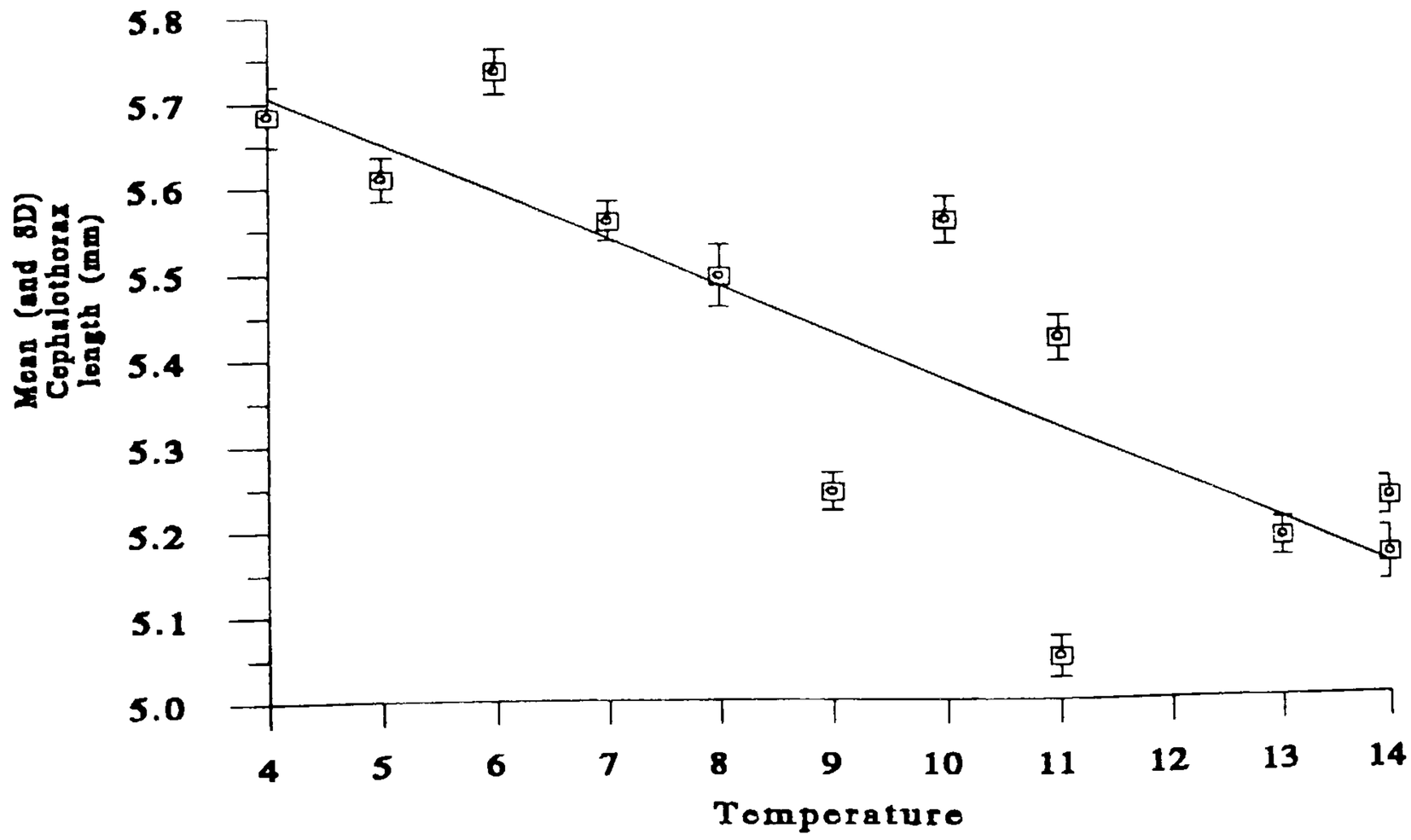


Fig. 2.4. Relationship between mean cephalothorax length (mm) of *L. salmonis* gravid adult females and the seasonal surface water temperature



over the sampling period (14°C) was during July and August, although the minimum mean cephalothorax length was observed in October. It was found that that temperature from the previous month and mean cephalothorax length were more strongly negatively correlated with each other ($r^2 = 0.874$, $p < 0.001$).

The relationship between photoperiod and mean cephalothorax length is shown in fig. 2.5. No correlation was observed between the body length of *L. salmonis* females and photoperiod over the sampling period ($r^2 = 0.051$, $p = 0.479$), indicating that seasonal variations in photoperiod cannot explain any of the variation observed in mean cephalothorax length.

2.3.1.2. Brood size

The mean number of eggs per *L. salmonis* egg string over the sampling period is shown in fig. 2.6. An analysis of variance demonstrated that there was a significant difference in the number of eggs per egg string between the twelve samples ($p < 0.001$). The maximum mean number of eggs per egg string was 286.9 (± 36.9), which was observed during the month of March, and the minimum mean number of eggs being 194.1 during October. A Dunns test showed that the maximum March and minimum October samples were significantly different from the remainder of the samples, as was February.

Fig. 2.7 shows the relationship between surface water temperatures at the site sampled and the mean number of eggs per egg string. Temperature and brood size were found to be negatively correlated with each other ($r^2 = 0.709$, $p < 0.001$) over the sampling period. Again, there was a lag between maximum and minimum surface water temperatures and the corresponding minimum and maximum mean number of eggs per egg string. The minimum number of eggs, 194.1 (± 66.8) in October, was

Fig. 2.5. Relationship between mean cephalothorax length (mm) of *L. salmonis* gravid adult females and the seasonal photoperiod

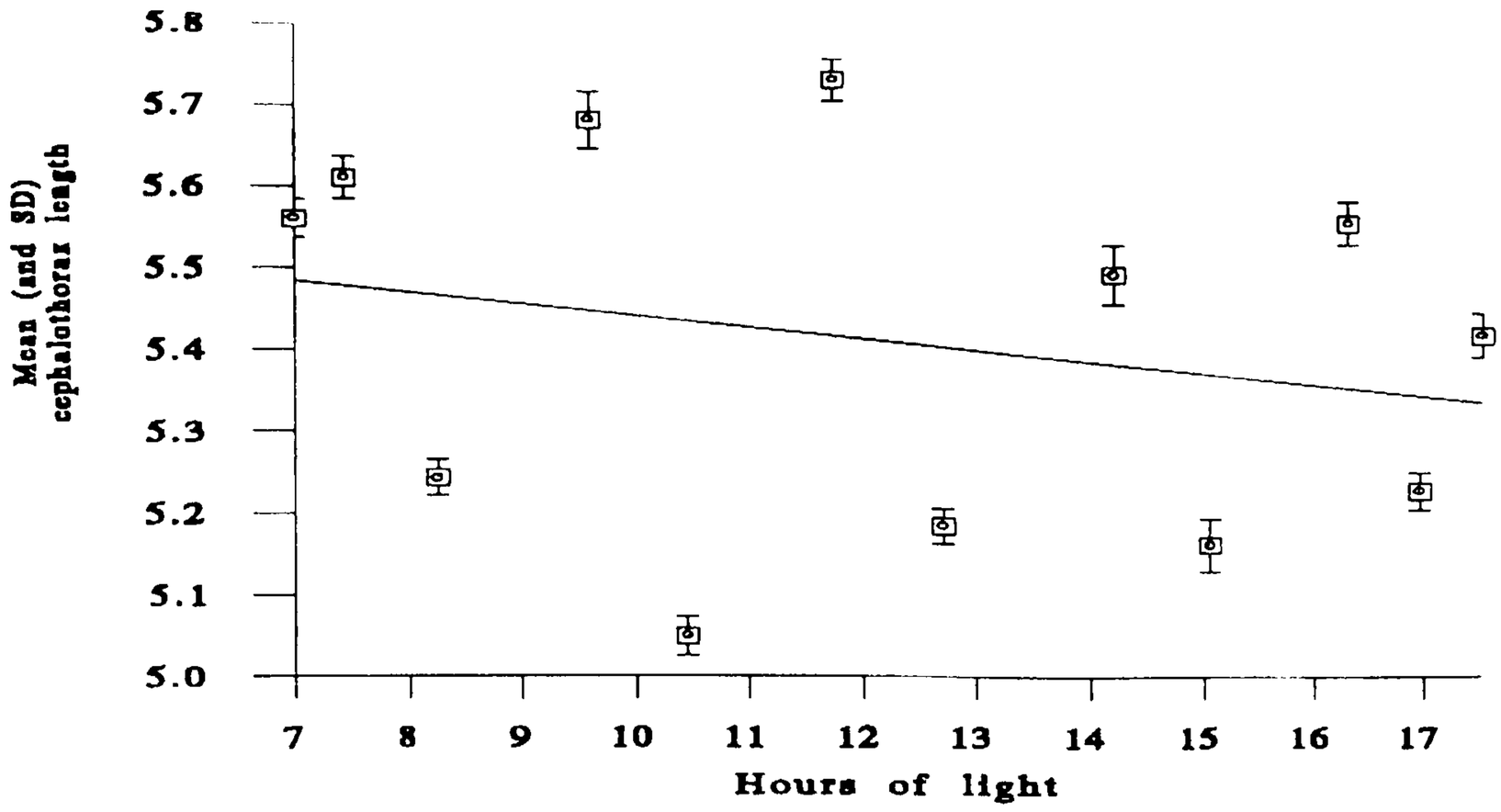


Fig. 2.6. Seasonal variation in the mean number of eggs (± 1 SD) per *L. salmonis* egg string.

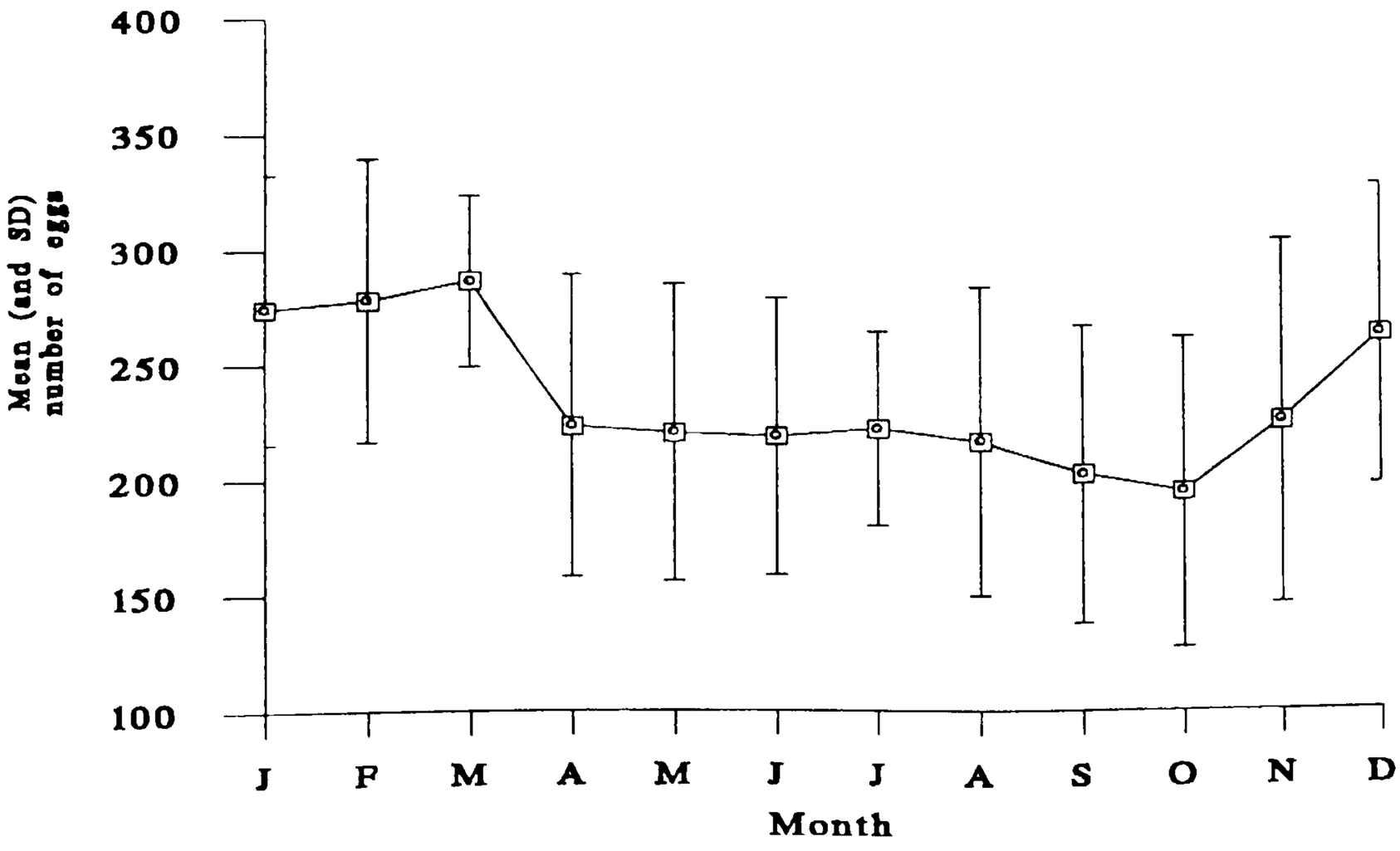


Fig. 2.7. Relationship between mean number of eggs per *L. salmonis* egg string and the seasonal surface water temperature

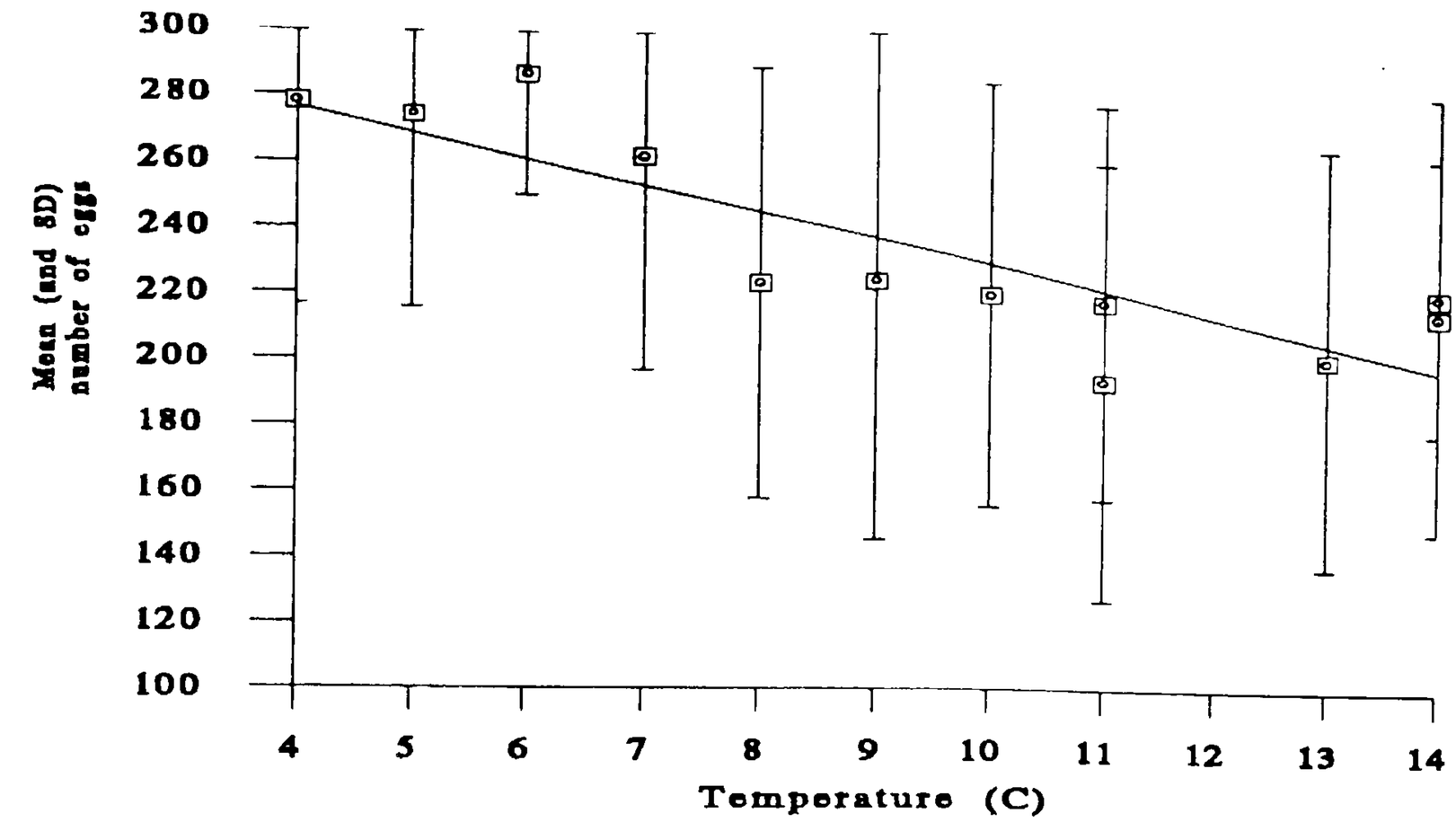
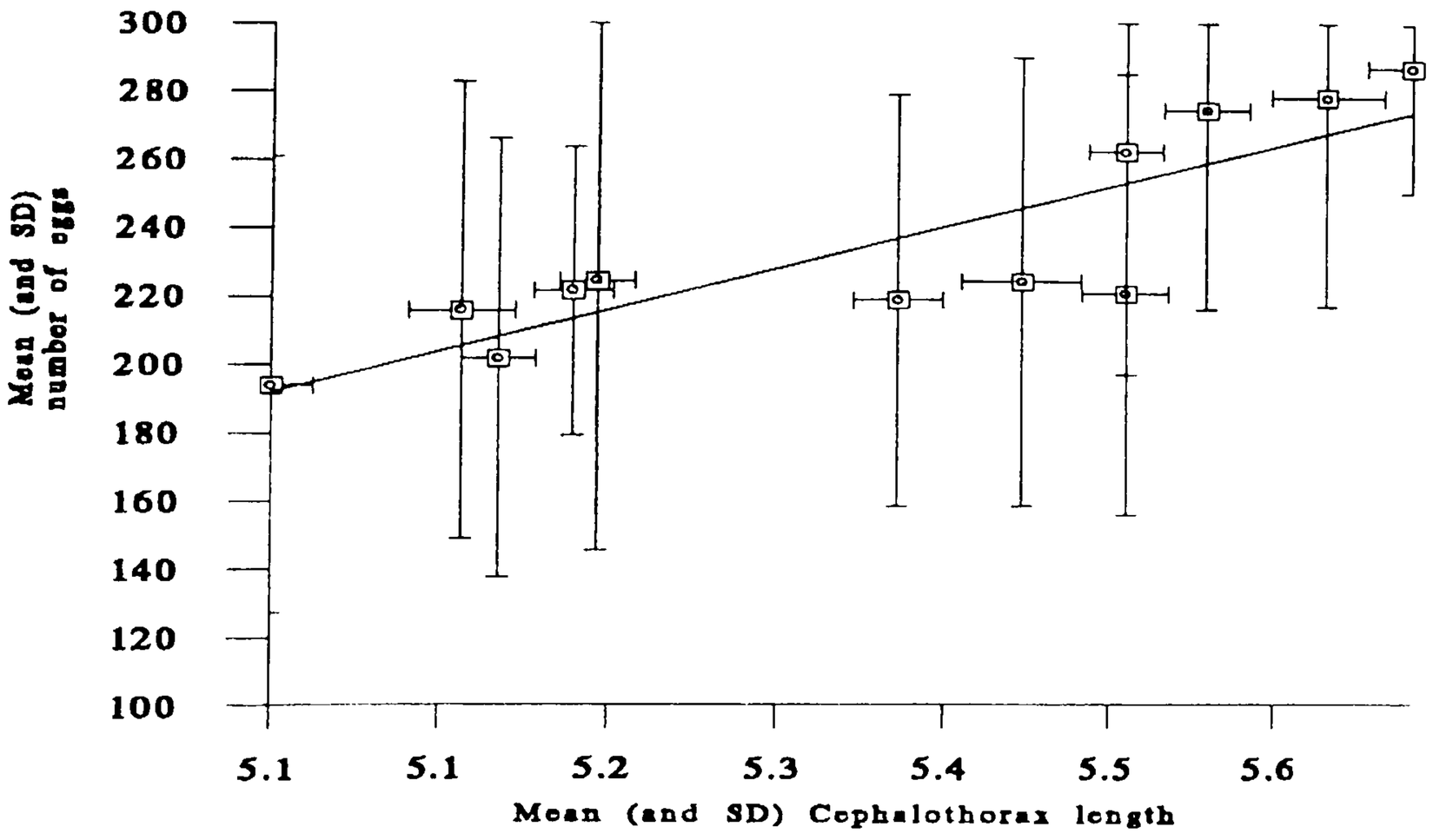


Fig. 2.8. Relationship between mean number of eggs per egg string and mean cephalothorax length (mm) of *L. salmonis* gravid adult females



observed to occur two to three months after the highest water temperatures, July to August, and the maximum number of eggs, 286.9 in March, was recorded one month after the lowest water temperature was experienced in February. However, it was found that mean brood size and temperature from two months previously was not as strongly negatively correlated ($r^2 = 0.247$, $p < 0.05$) as the temperature from the same month, and that mean brood size and temperature from three months prior was not negatively correlated at all ($r^2 = 0.04$, $p = 0.837$).

The maximum number of eggs per egg string however was seen to coincide with the largest mean cephalothorax length (March), and the lowest number of eggs was observed at the time when the mean cephalothorax lengths were also at a minimum (October). Fig. 2.8 shows the relationship between the monthly mean cephalothorax sizes over the sampling period and the mean number of eggs per egg string for each of the twelve monthly samples. The mean number of eggs per egg string was found to be positively correlated with the mean cephalothorax length ($r^2 = 0.747$, $p < 0.001$).

The relationship between the mean number of eggs per egg string and seasonal photoperiod is shown in fig. 2.9. It was demonstrated that photoperiod has no effect upon the brood size ($r^2 = 0.256$, $p = 0.093$), no correlation between the two factors existing.

2.3.1.3. Viability of eggs

Fig 2.10a illustrates the difference between eggs classed as viable and those classed as abnormal. The seasonal variation in the mean proportion of viable eggs per egg string for *L. salmonis* is shown in fig. 2.10b. Analysis of variance demonstrated that there was no seasonal variation in the mean proportions of viable eggs per egg string over the sampling period ($p < 0.01$), indicating that the percentage of eggs

Fig. 2.9. Relationship between mean number of eggs per *L. salmonis* egg string and the seasonal photoperiod

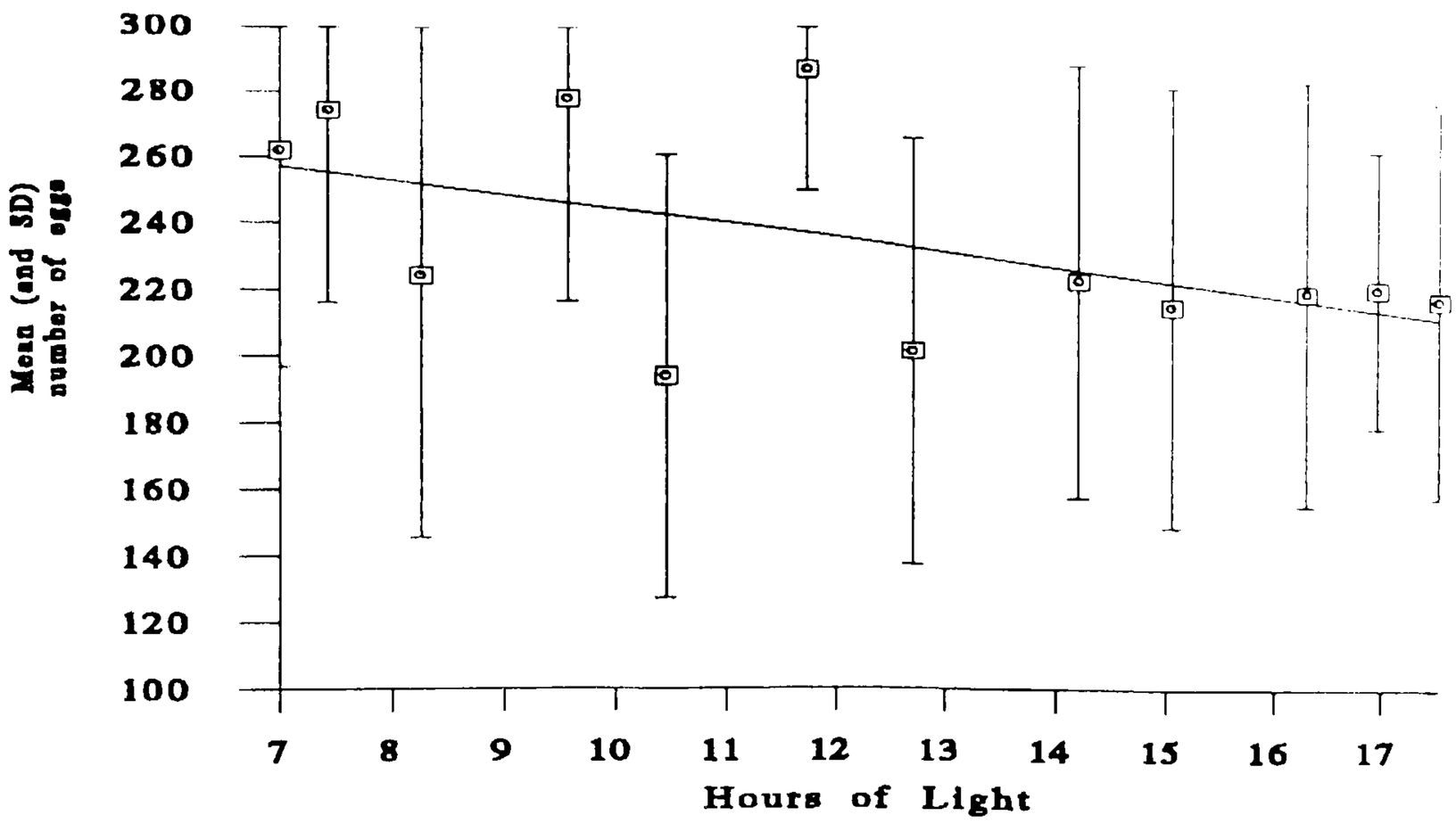


Fig. 2.10b Seasonal variation of the mean proportion of viable eggs (± 1 SD) per *L. salmonis* egg string

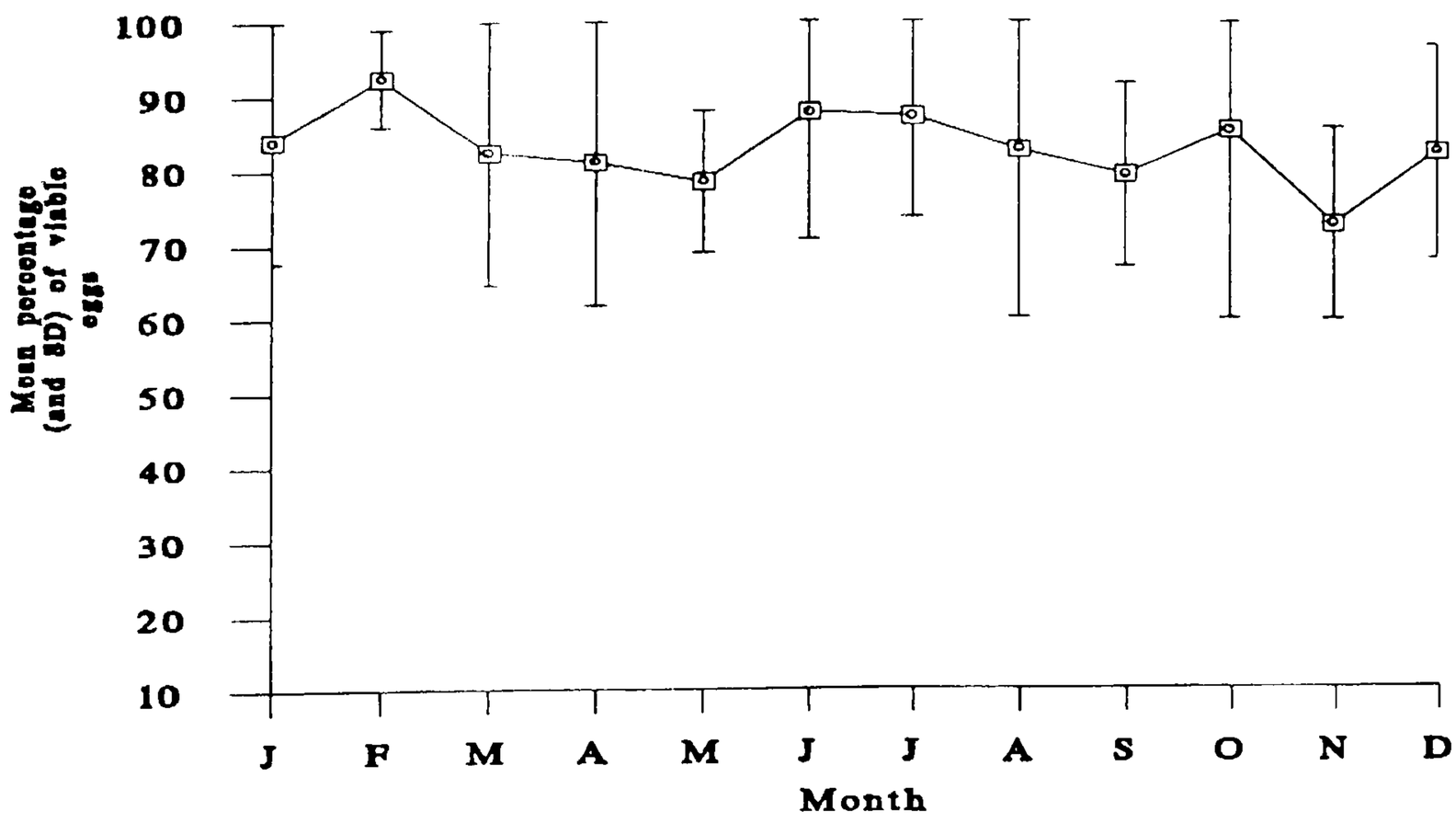
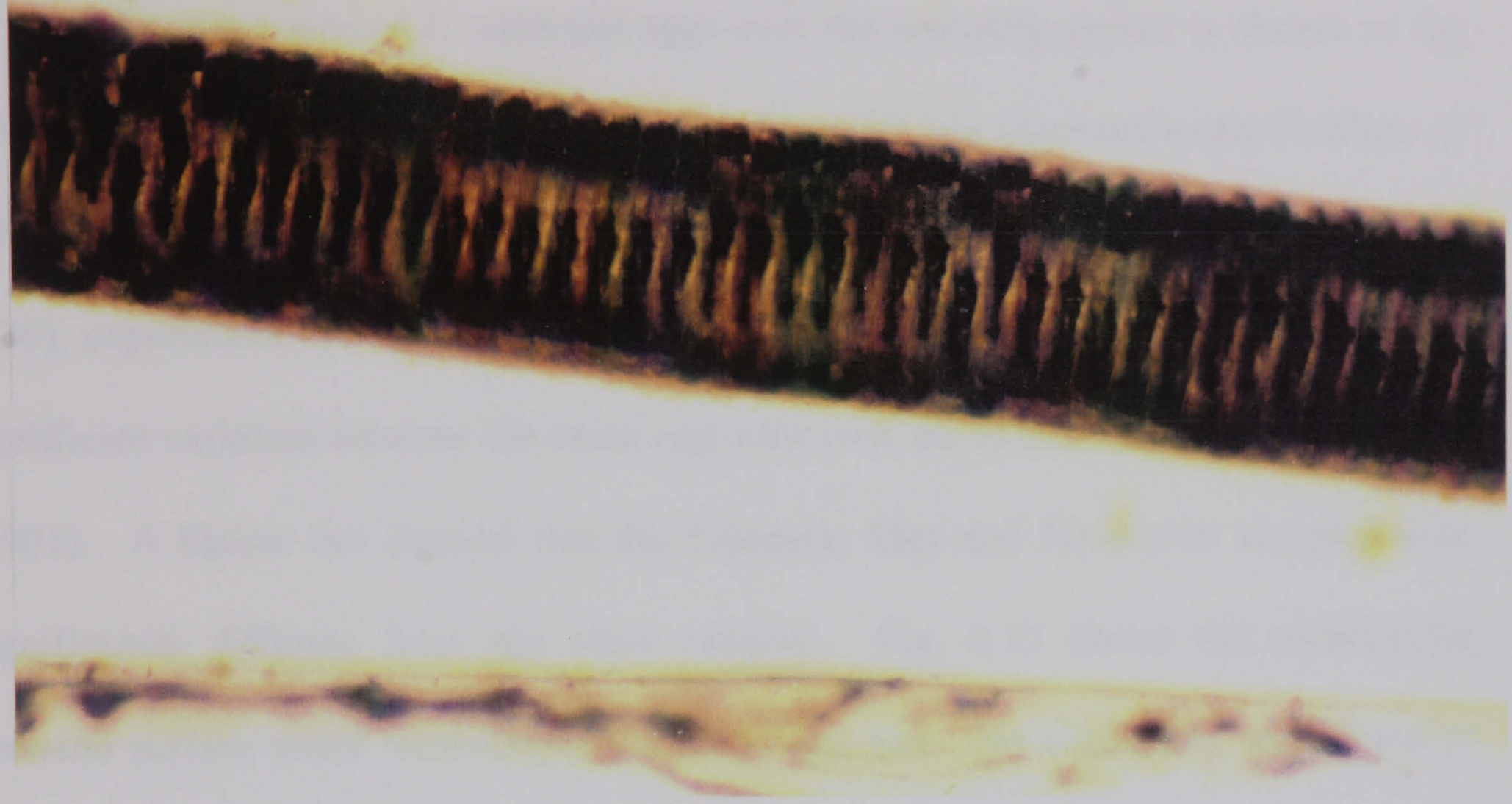
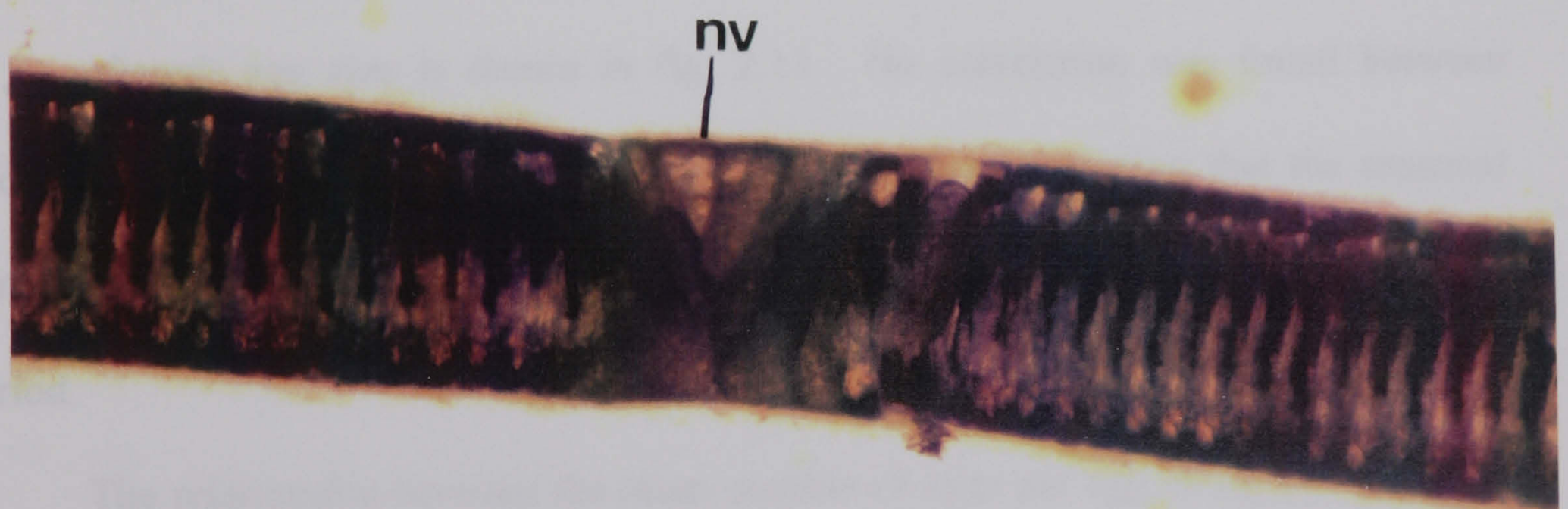


Fig. 2.10a. (i) *Lepeophtheirus salmonis* egg string containing viable eggs (x40 magnification). Note regular arrangement of nauplii within egg string, all aligned in the same direction.



(ii) *Lepeophtheirus salmonis* egg string containing both viable and non-viable eggs (nv) (x40 magnification). Note irregular arrangement of non-viable eggs, lack of pigmentation and development.



showing normal development remains at a constant level over the year. The deviation around the mean varied from a minimum of ± 6.54 in February to a maximum of ± 25.48 in October.

2.3.1.4. Egg Size

The mean size of *L. salmonis* eggs over the sampling period is shown in fig. 2.11. The maximum mean egg size (lateral diameter) was observed to be 73.03μm (± 10.57), recorded during September, with the minimum mean size being 57.50μm (± 7.26), experienced during April. Analysis of variance demonstrated that there was a significant variation between the mean egg sizes over the twelve monthly samples ($p < 0.001$). A Dunns test showed that the February, May and November samples were significantly different from the other samples. Fig. 2.12 shows the relationship between surface water temperature and mean egg size. No correlation was found to exist between temperature and the mean egg sizes ($r^2 = 0.085$, $p = 0.359$), suggesting that temperature and perhaps therefore seasonality has no significant direct effect upon egg size of *L. salmonis*.

The relationship between the seasonal variation in photoperiod and the variation in *L. salmonis* egg size is shown in fig. 2.13. No correlation was found between photoperiod and egg size ($r^2 = 0.111$, $p = 0.289$), again suggesting that the seasonal variation in photoperiod does not influence the variances in egg size over the sampling period.

The relationship between the mean number of eggs per egg string and the mean egg size is shown in fig. 2.14. Again, no correlation was found between the mean brood number and egg size ($r^2 = 0.017$, $p = 0.683$). Thus it appears that the mean number of eggs per egg string does not directly effect the size of the eggs.

Fig. 2.11. Seasonal variation in the mean size (μm ± 1 SD) of *L. salmonis* eggs

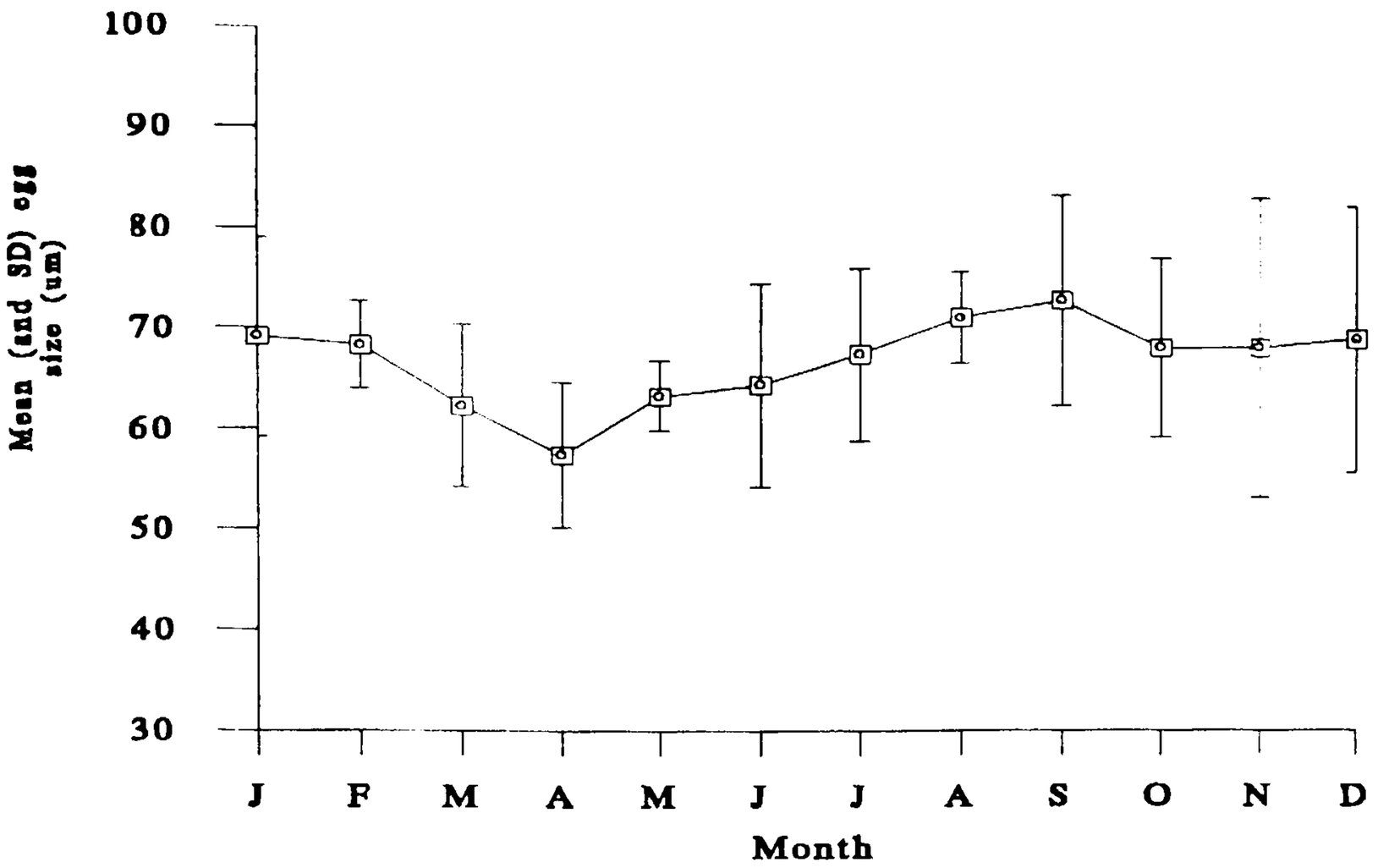


Fig. 2.12. Relationship between mean size (μm) of *L. salmonis* eggs and the seasonal surface water temperature

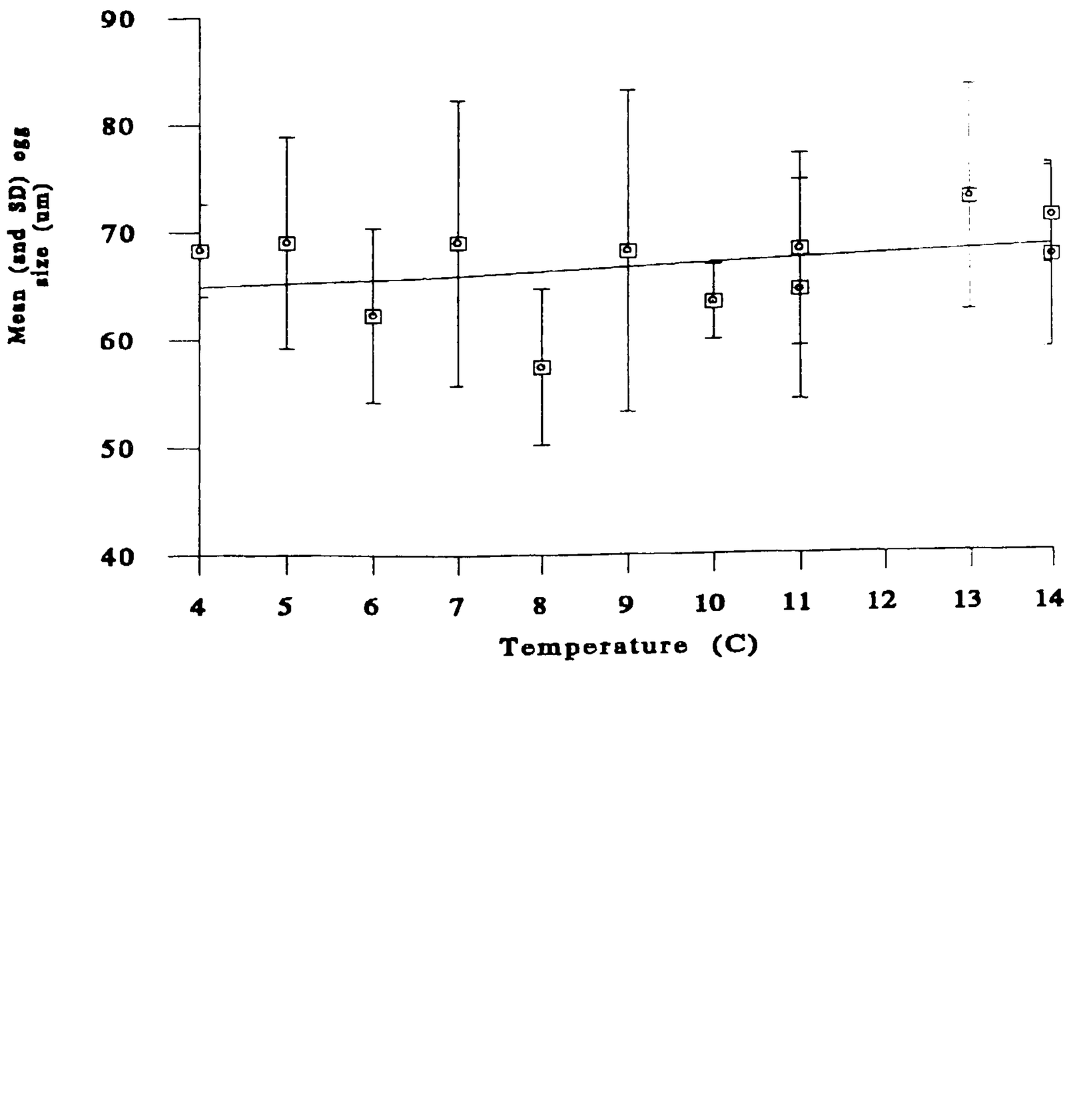


Fig. 2.13. Relationship between mean size (µm) of *L. salmonis* eggs and the seasonal photoperiod

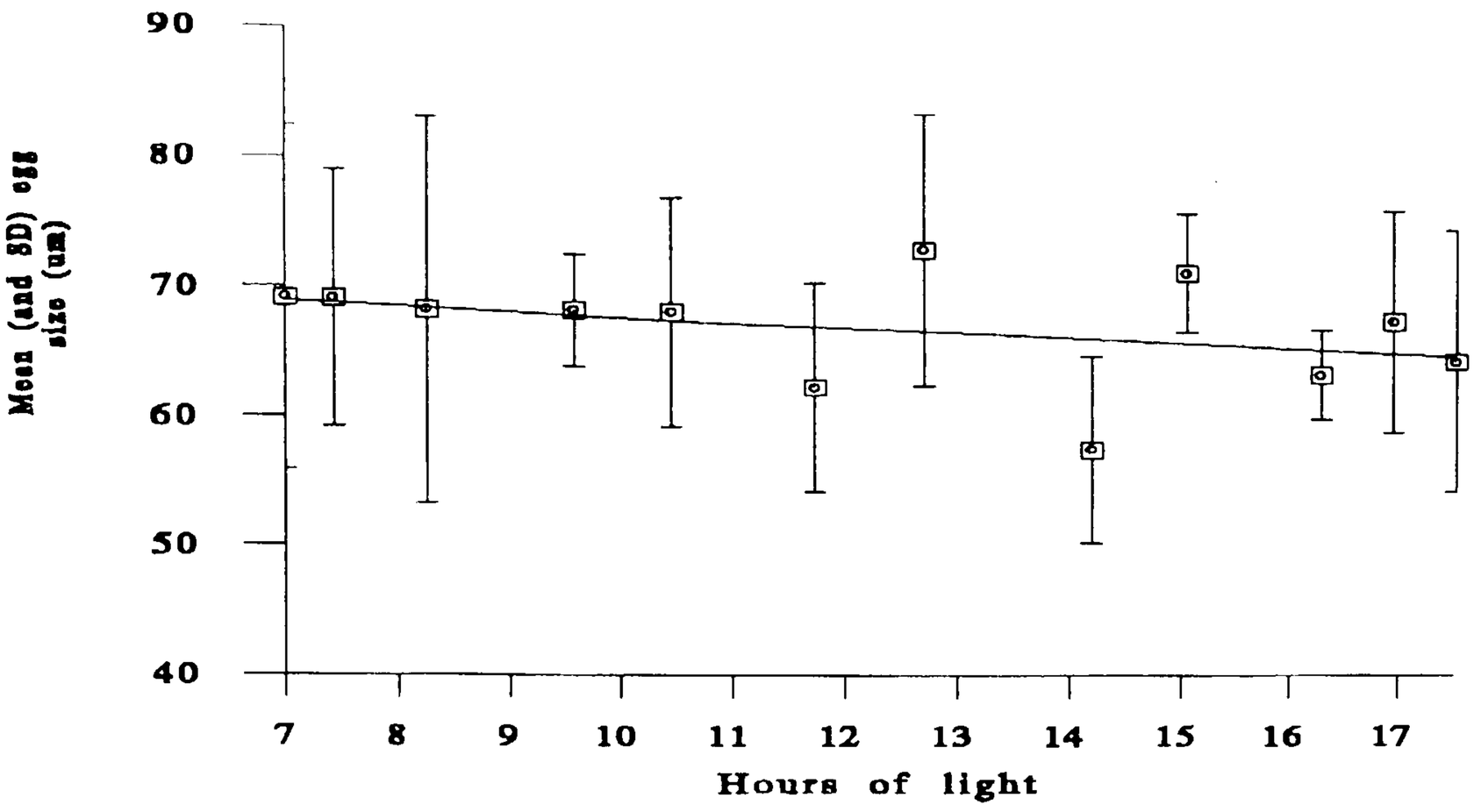


Fig. 2.14. Relationship between mean size (µm) of *L. salmonis* eggs and the mean number of eggs per egg string

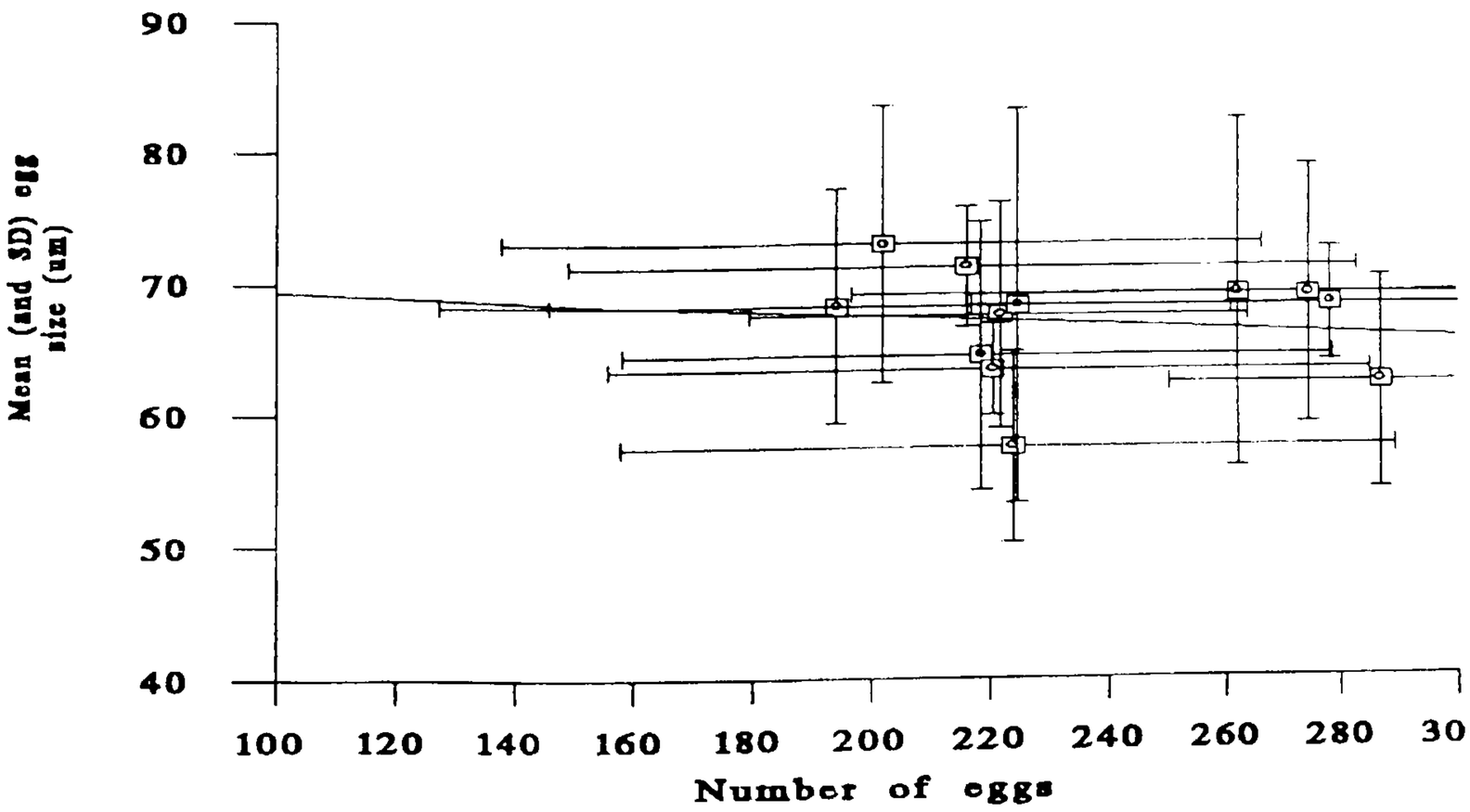


Fig. 2.15 shows the relationship between the mean cephalothorax length of *L. salmonis* adult females and the mean egg size over the sampling period. The mean cephalothorax length did not significantly affect the mean egg size ($r^2 = 0.214$, $p = 0.13$), that is, no correlation could be found between the two.

2.3.2. Infrapopulational variations in reproductive output

2.3.2.1. Fecundity

The mean number of eggs per egg string for both *L. salmonis* and *C. elongatus* in the large sample taken in August is shown in table 2.1. For *L. salmonis*, the mean number of eggs per ovisac was 206.2 (range, 76 to 402), and for *C. elongatus*, the figure was 52.62 (range, 6 to 87). For both species, wide standard deviations were observed, 74.09 eggs per egg string for *L. salmonis* and 17.08 eggs per egg string for *C. elongatus*. The frequency distribution of the number of eggs per egg sac for both species can be seen in figs. 2.16 and 2.17. Both appear to show a normal distribution.

2.3.2.2. Egg viability

The relationship between the number of eggs per egg string and the proportion of viable eggs produced for both *L. salmonis* and *C. elongatus* in the extended sample are shown in table 2.2 and figs. 2.18 and 2.19.

For *L. salmonis*, the mean number of viable eggs per egg string was 168.17 ± 47.00, with this being equivalent to 82.34% (± 23.01) of eggs within an egg string showing normal development. An analysis of variance on the data showed that there was no significant variation in the number of eggs showing normal development from egg strings containing different brood sizes ($p = 0.860$), demonstrating that there is no relationship between the number of eggs produced per brood and the subsequent

Fig. 2.15. Relationship between mean size (μm) of *L. salmonis* eggs and the mean cephalothorax length (mm) of gravid adult females

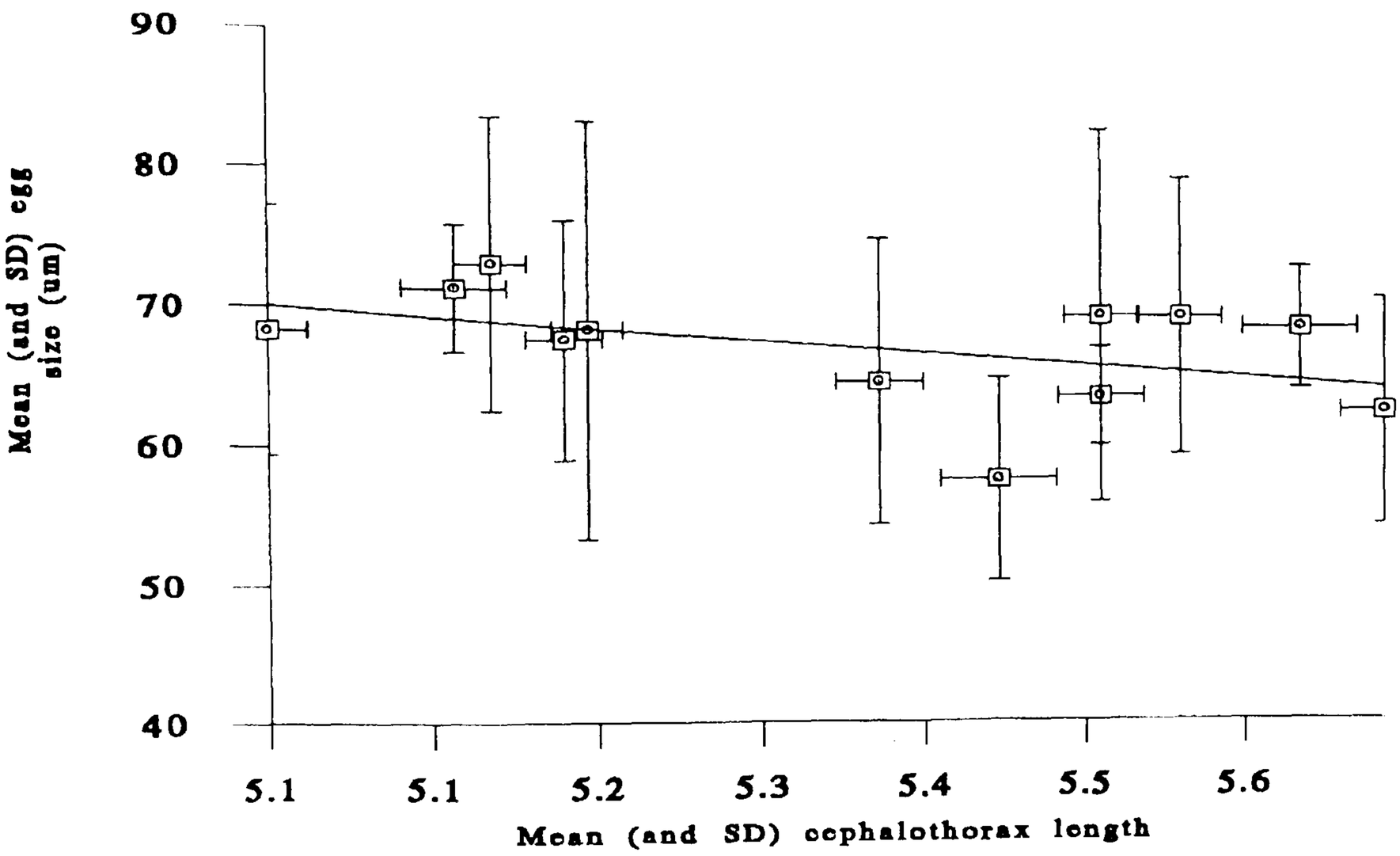


Table 2.1. Mean number of eggs per ovisac for *L. salmonis* and *Caligus elongatus* for individual populations sampled at one point in time

Species	Mean number of eggs per ovisac	Standard deviation	Range
Lepeophtheirus salmonis	206.20	74.09	76-402
Caligus elongatus	52.62	17.08	6-87

Table 2.2. Proportion of ovisacs with eggs showing normal development for *Lepeophtheirus salmonis* and *Caligus elongatus*

Number of eggs per ovisac	Percentage of viable eggs per ovisac ± 1SD
Lepeophtheirus salmonis	
52-101 (n=5)	93.99 ± 7.53
102-151 (n=14)	82.43 ± 17.81
152-201 (n=30)	82.08 ± 26.83
202-251 (n=11)	87.83 ± 9.21
252-301 (n=12)	78.32 ± 28.82
302-351 (n=8)	79.36 ± 31.46
352-402 (n=3)	83.39 ± 5.59
Caligus elongatus	
1-10 (n=1)	00.00
11-20 (n=8)	62.50 ± 51.57
21-30 (n=6)	75.64 ± 39.24
31-40 (n=15)	65.41 ± 42.37
41-50 (n=10)	82.66 ± 30.94
51-60 (n=41)	67.68 ± 42.59
61-70 (n=21)	76.71 ± 34.48
71-80 (n=17)	81.13 ± 28.43
81-90 (n=1)	90.80

Fig. 2.16. Infrapopulational variation in the number of eggs per egg string for *L. salmonis*

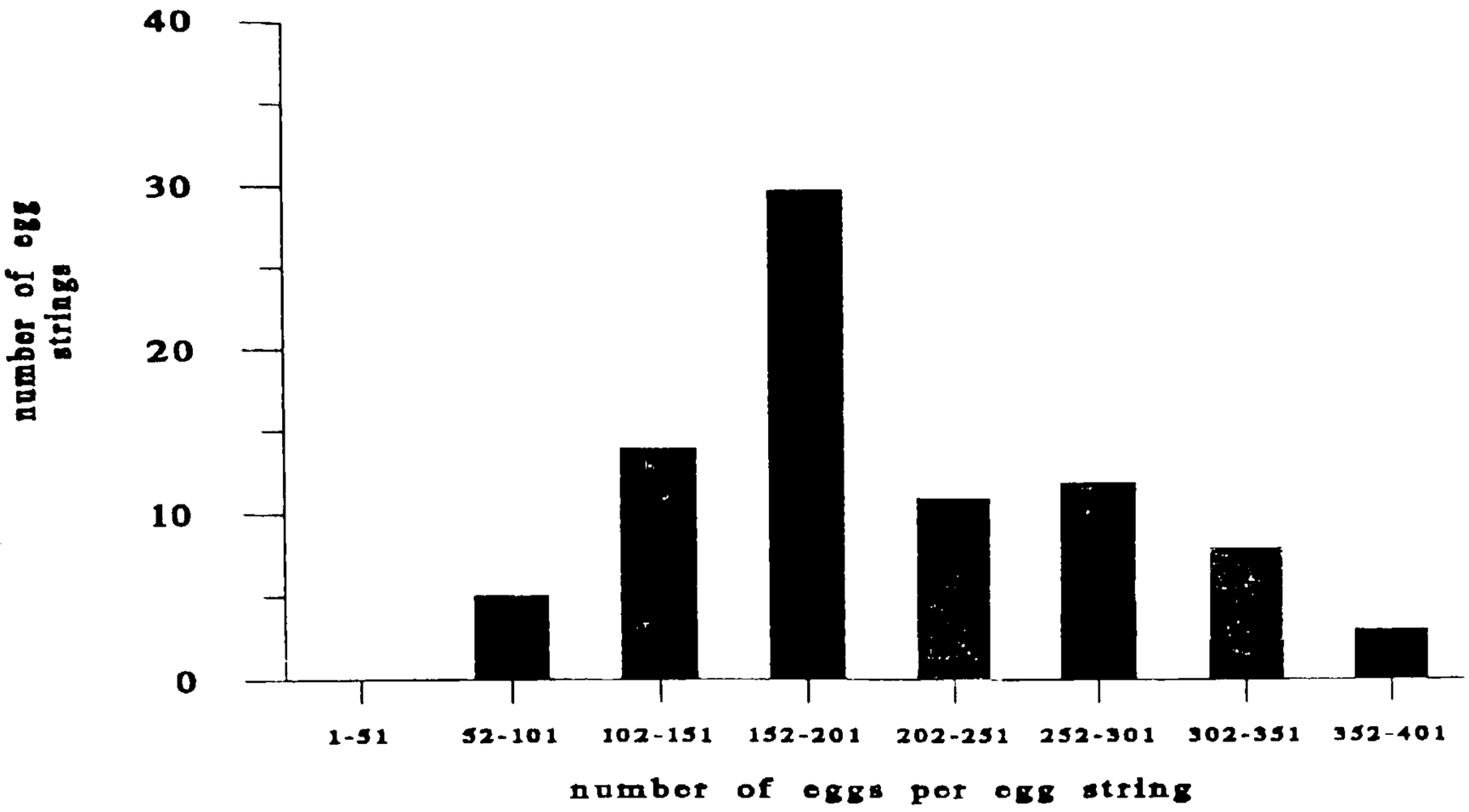


Fig. 2.17. Infrapopulational variation in the number of eggs per egg string for *C. elongatus*

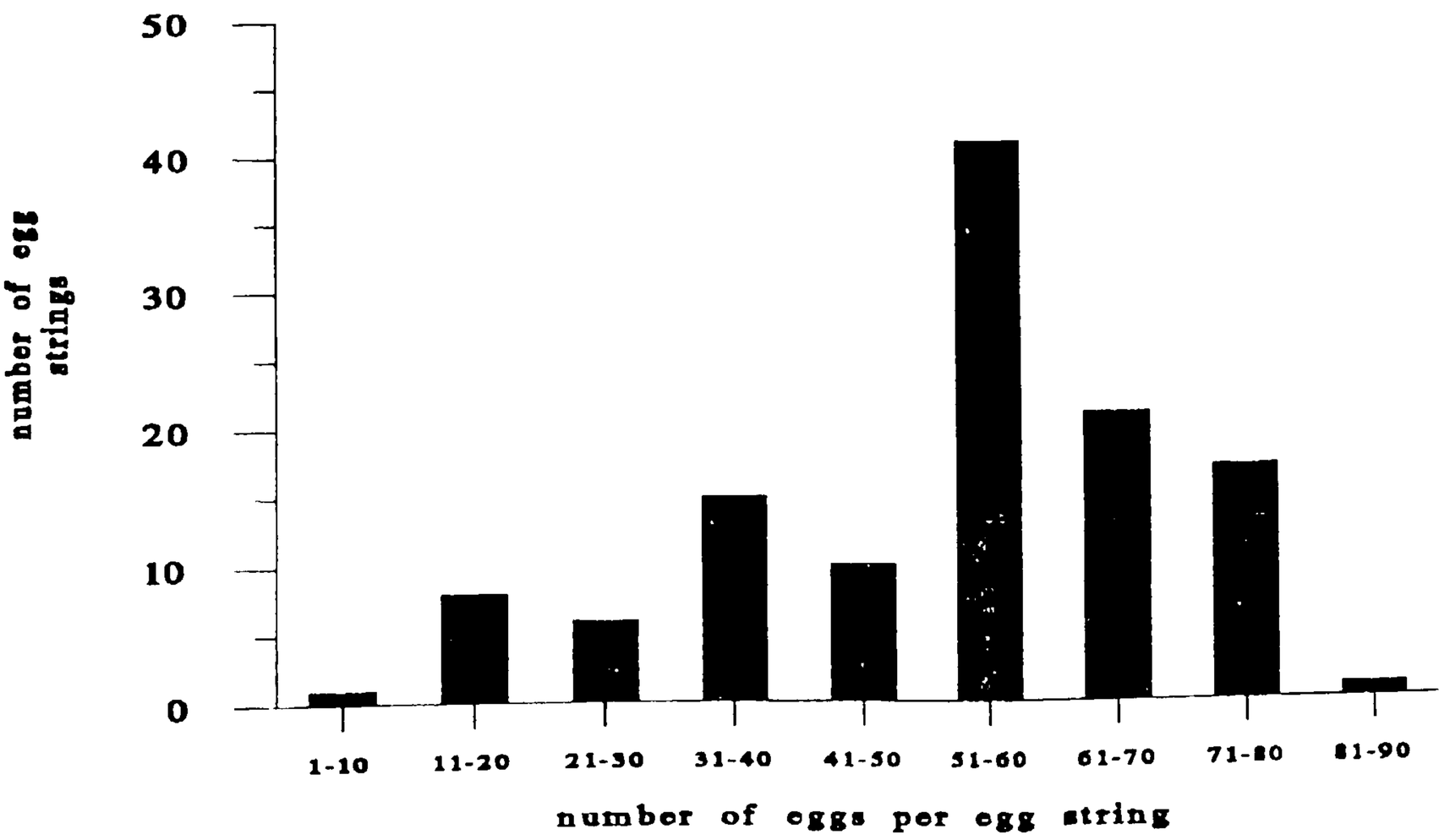


Fig. 2.18. Relationship between the number of eggs per egg string and the number of viable eggs for an *L. salmonis* population sampled at one point in time

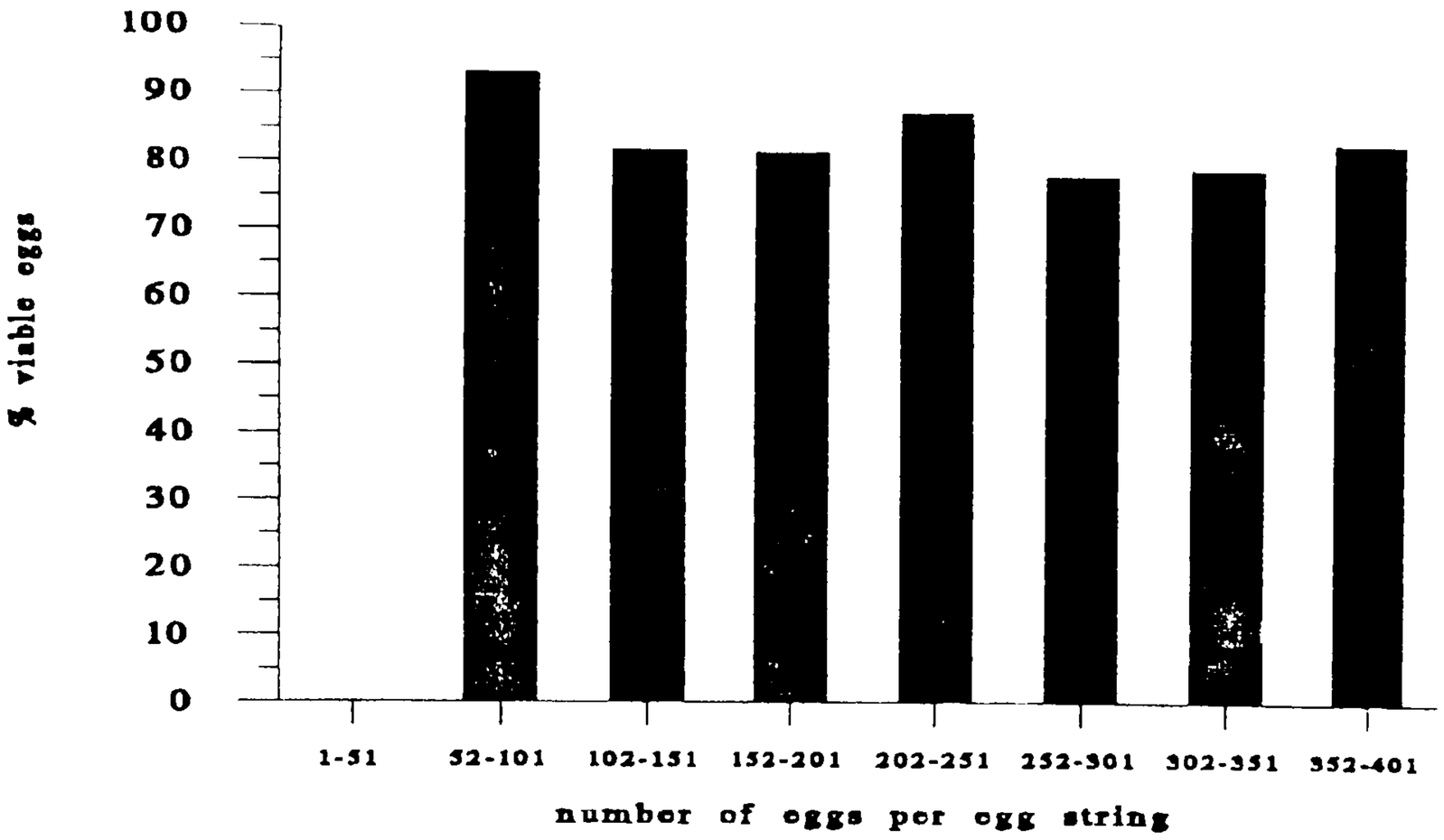
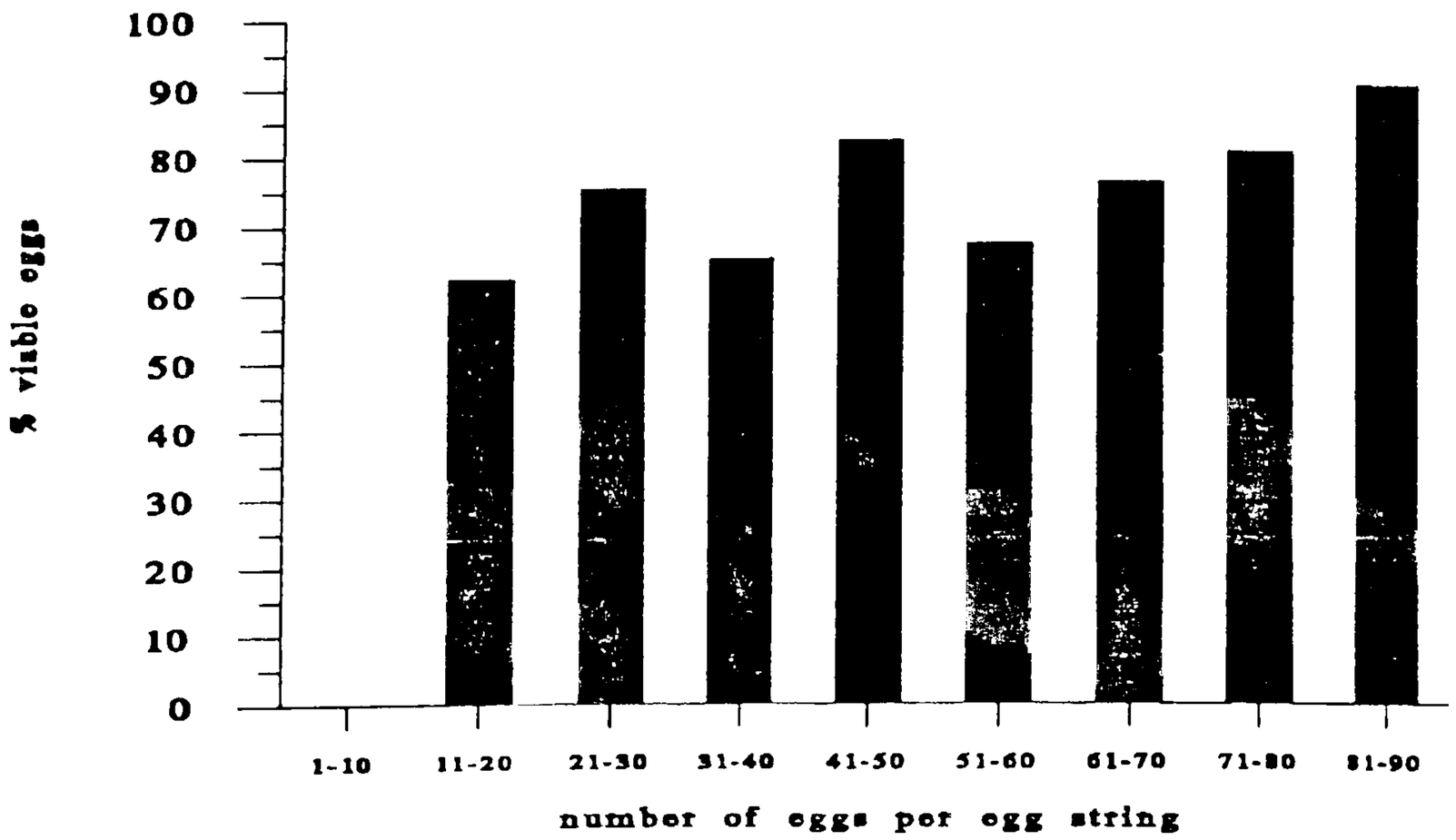


Fig. 2.19. Relationship between the number of eggs per egg string and the number of viable eggs for *C. elongatus* population sampled at one point in time



viability of these eggs. Only 2.41% of *L. salmonis* egg strings sampled were found to contain wholly undifferentiated eggs that did not demonstrate any normal development.

For *C. elongatus,* the mean number of eggs per egg string showing normal development was 34.06 ± 24.81, with this being 71.81% (± 24.81) of eggs within an ovisac. Analysis of variance again indicated that there was no relationship between the number of eggs produced by female *C. elongatus*, and the viability of the eggs ($p = 0.604$), demonstrating that the viability of eggs is not dependent upon the brood size. High numbers of *C. elongatus* egg strings were observed to contain wholly undifferentiated eggs that did not subsequently show any development, with such egg strings accounting for 18.33% of ovisacs sampled.

2.3.2.3. Cephalothorax length

The relationship between the cephalothorax length of *L. salmonis* adult females and the number of eggs in each egg string in the larger September sample is shown in fig. 2.20. The mean cephalothorax length was 5.29mm (± 0.23), ranging from 5.00 to 5.71mm. A correlation was found between adult female cephalothorax length and the number of eggs contained within each ovisac ($r^2 = 0.521$, $p < 0.001$) demonstrating that the size attained by an individual adult female will have a subsequent effect upon the brood sizes produced.

However, interestingly, no correlation was found between *L. salmonis* cephalothorax length and the number of viable eggs within each egg string. Such a correlation may have indicated that immature females (in the case of a negative correlation) produce less viable eggs than older females, or in the case of a positive correlation, older perhaps senile females prodded more abnormal eggs ($r^2 = 0.13$, $p = 0.681$).

Fig. 2.20. Relationship between the number of eggs per egg string and the cephalothorax length (mm) of gravid *L. salmonis* adult females from one population sampled at one point in time

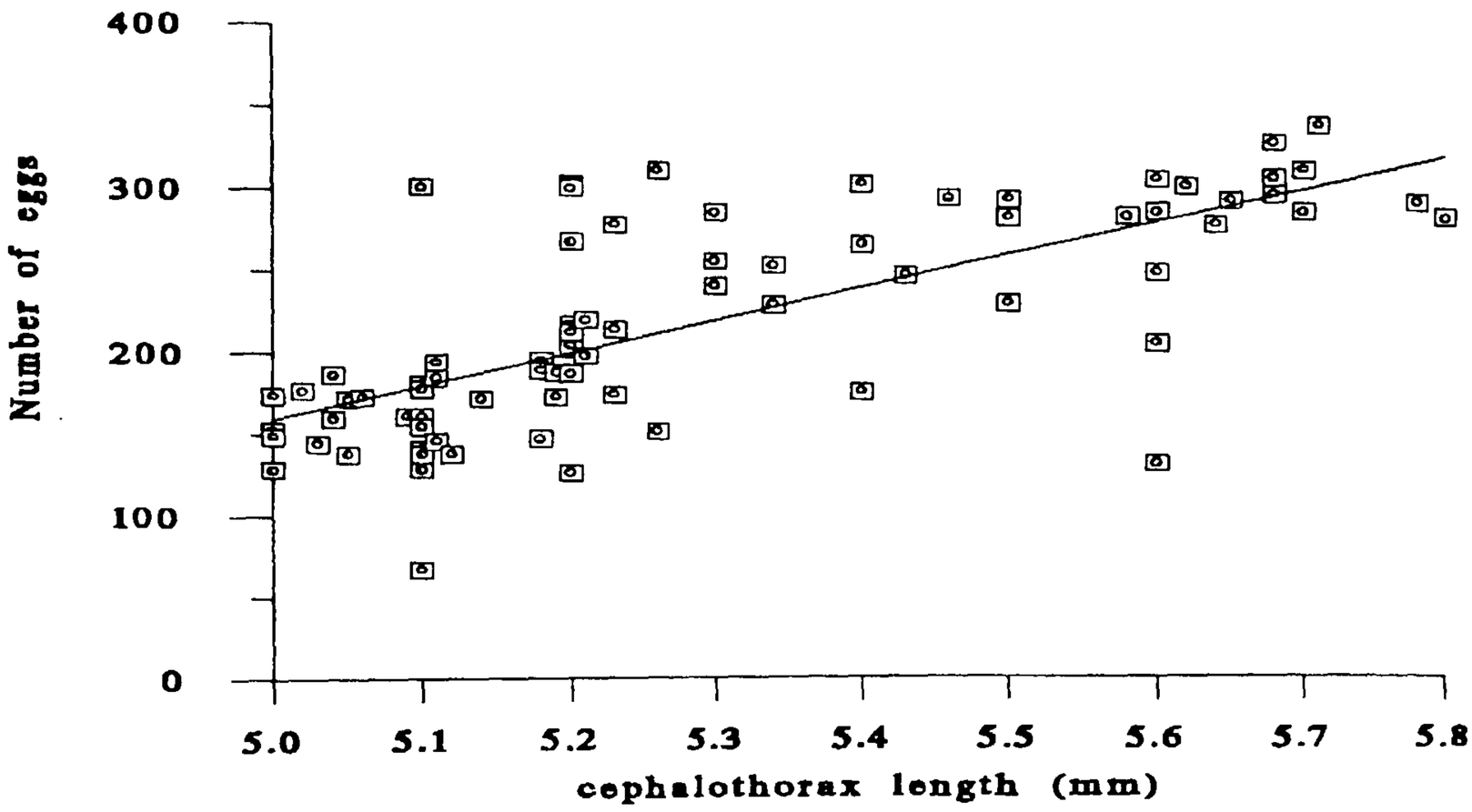
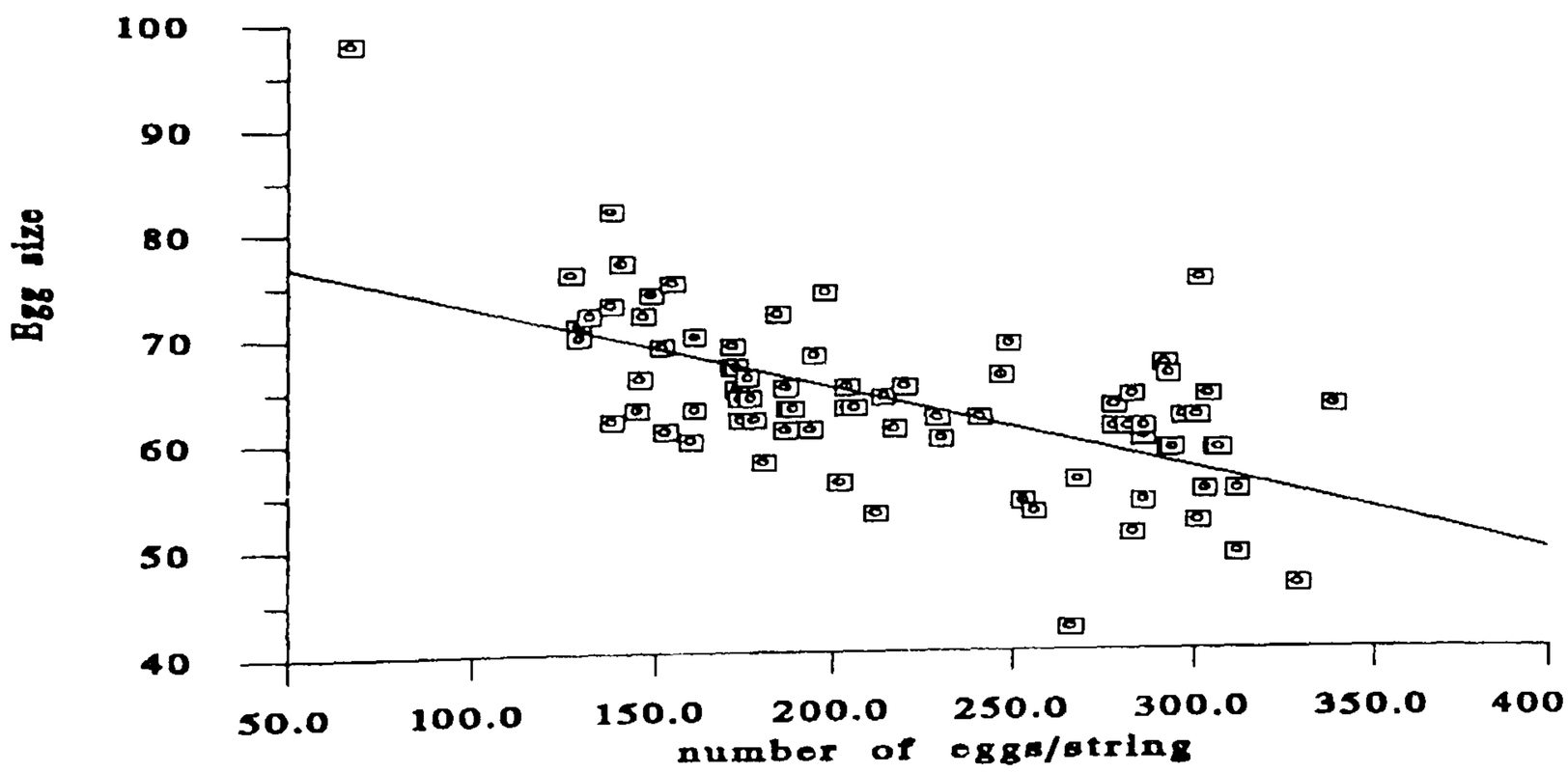


Fig. 2.21. Relationship between the number of eggs per egg string and the mean egg size (μm) for a *L. salmonis* population sampled at one point in time



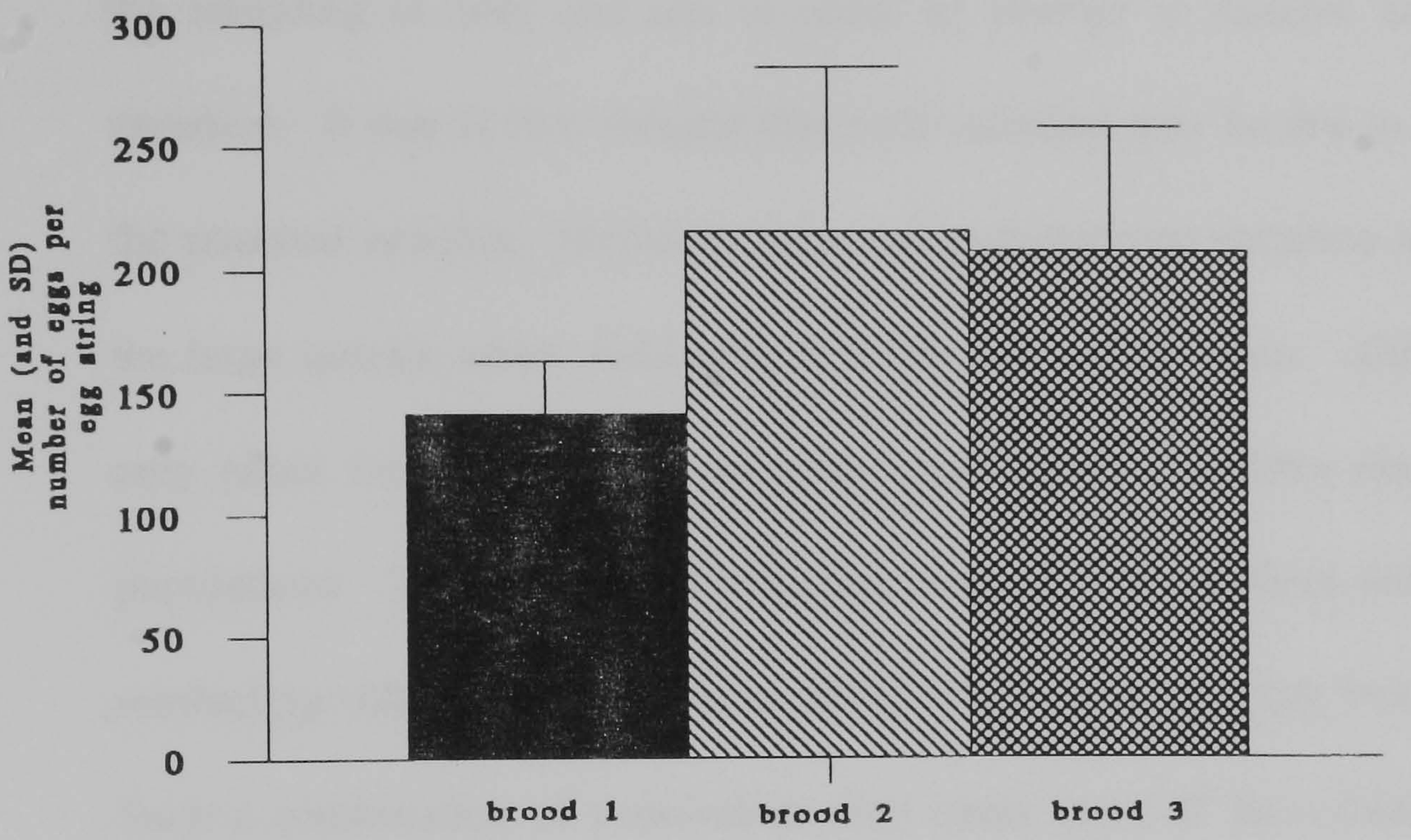
2.3.2.4. Egg size

The relationship between the number of *L. salmonis* eggs per egg string and the mean egg size is shown in fig. 2.21. Egg size ranged from 42 to 98μm, with the overall mean being 63.60μm (± 8.07). In contrast to the seasonal data, a correlation did occur between the mean size of eggs within an ovisac and the number of eggs contained within it ($r^2 = 0.381$, $p < 0.001$), indicating that the number of eggs produced will effect their mean size, explaining some of the variation observed in the size of *L. salmonis* eggs.

2.3.3. Brood number

The mean number of eggs produced per egg string per brood by *L. salmonis* adult females is shown in fig. 2.22. The lowest number of eggs per egg string was observed in the first brood produced, the mean number of eggs being 141.09 (± 22.19), with this rising to a maximum of 216.4 (± 67.59) during the second brood, and then declining slightly to 208.2 (± 50.97) in the third and final brood observed. Analysis of variance indicated that there existed a significant variation in the number of eggs from different broods ($p < 0.001$), although a Dunns test revealed that this difference was limited to broods 1 and 2, no significant differences occurring between broods 1 and 3 and broods 2 and 3.

Fig.2.22. Mean number of eggs per egg string produced per brood for *L. salmonis*



2.4. Discussion

The investigations into the reproductive output of *L. salmonis* were characterised by a high degree of variation in the results. This was despite carrying out the sampling at only one site in order to attempt to remove any interpopulational variation. It was at first thought that such variation may be due to low sample sizes in the seasonal samples. However, large infrapopulational variation was also observed in the large sample taken, indicating that this was not the case. Although samples were only taken from one site, it must be remembered that there may still be a mix of populations. The majority of parasites in an established farm will be due to the site reinfecting itself, there will be an influx of larvae originating from outside the farm. Such a combination of populations may cause some of the variation observed. This will always be a feature in field sampling and a factor that is probably impossible to remove.

2.4.1. Seasonal Factors

The study revealed that a number of parameters such as adult female body size, egg number and size varied significantly over a continuous sampling period of twelve months. Such occurrences are not unusual; in a number of studies on both free-living and parasitic crustaceans, seasonal factors have been shown to have a marked effect upon the reproductive output of a wide range of species. Temperature appears to be the most influential abiotic factor regulating fecundity but, despite its importance in controlling metabolic processes, there have only been a few studies investigating the relationship between temperature and fecundity of parasitic copepods. Though previous links between temperature and fecundity for both free-living and parasitic

species have been documented, some have tended however to concentrate upon the effect of temperature upon body size, and the then subsequent effect of body size on fecundity.

2.4.1.1. Seasonal body size

The mean size of *L. salmonis* adult females was found to be negatively correlated with temperature, varying significantly over the sampling period. The minimum size attained, 5.05μm, was observed in October, this rising to a maximum of 5.74μm in March. However, although a negative correlation was observed, the maximum and minimum sizes attained did not coincide with the maximum and minimum temperatures experienced over the sampling period, with a time lag between the two being apparent. The maximum mean cephalothorax length as already noted was recorded in March, one month after the lowest water temperature of 4°C. The minimum mean cephalothorax length occurred in October, with the maximum surface water temperatures (14°C) being experienced in July and August. This time lag can be expected, since the ambient water temperature during development will affect the developmental rate of the parasite and the subsequent size attained. According to Kurata (1962), the increase in generation time at low temperatures is due to the reduction in the moult frequency of crustaceans causing a larger size increment to occur between moults. Increasing temperatures will therefore cause an increase in the developmental rate of the parasite, with a consequent decrease in the time period between moults, and thus the size gained. It is therefore likely that the ambient water temperature of previous months will be more highly correlated with body size than the respective temperature of the month sampled. In support of this, a regression analysis

between the surface water temperature of the previous month and the mean cephalothorax length of *L. salmonis* revealed that these were more highly correlated than the water temperature of the month when the sample was taken (r^2 = 0.874 compared to 0.685). Photoperiod however was not found to have any significant direct effect upon the cephalothorax size of ovigerous *L. salmonis* females, and thus cannot explain any of the seasonal variation observed.

This relationship between water temperature and body size, as observed in the present study, appears to be generally one of negative correlation across a number of crustacean groups, with body size increasing with decreasing temperature. The variables that are usually invoked to explain changes in body size include temperature, food quality and availability, and the effects of size selection predation. Crawford & Daborn (1986) studied the seasonal body size variations of the free living calanoid copepod *Eurytemora herdmani* Thompson & Scott. By studying *E. herdmani* in a macrotidal, highly turbid estuary which had a year round food supply, lack of competitors and visual predators, the authors anticipated that an examination of body size of *E. herdmani* would indicate whether food supply, temperature or size-selective predation was the cause of body size variation in copepods. They found a significant relationship between body size and temperature, indicating that developmental temperature is the primary determinate of mature body size, with this having also been suggested by Coker (1933), Deevy (1960), McLaren (1963, 1965).

Evans and Diaz (1978) considered that a negative correlation between mean individual sizes and temperature was widespread within free living planktonic copepods, and indeed there have been many reports of this relationship in the available

literature which is summarised in table 2.3. Although the majority of these reports are concerned with measurements taken from field obtained specimens, extensive evidence of an inverse relationship between water temperature and the size of the planktonic copepods reared under laboratory conditions is set out by McLaren (1974).

There have also been previous descriptions of a negative correlation between temperature and body size for parasitic copepods including sea lice that support the findings of the present study. Tully (1989) found that the mean body sizes (no details of how this was measured was given) of both *L. salmonis* and *C. elongatus* increased with decreasing surface water temperatures. For *L. salmonis,* the mean length of the adult female stage was approximately 7.5mm in samples taken in August, with this increasing to approximately 12mm for samples taken in January when water temperatures would be cooler. The time lag between temperature and size observed in the present study can also been seen in the data of Tully (1989). The maximum body length of adult males over the period sampled by Tully (July to January) occurred one month after the minimum surface water temperatures experienced, these being January and December respectively with this also being observed for *C. elongatus* ovigerous females in the same period. Minimum body length for *C. elongatus* adult males and ovigerous females were also observed to occur after the maximum surface water temperature, these months being September and August respectively. However Tully (1989), unlike the present study, did not test for a correlation for this lag. Ritchie *et al.* (1993) also described a negative correlation between the mean cephalothorax length of ovigerous *L. salmonis* and the ambient water temperature. Again from the data of Ritchie *et al.* (1993) a time lag between temperature and size can be observed with the

Table 2.3. Free-living copepods whose body size is negatively correlated with temperature.

Authors	Species
Hartmann (1917)	*Cyclops strenuus* Fischer
Digby (1950)	*Oithona similis* Digby
Deevy (1960)	*Acartia tonsa* Giesbrecht *Acartia clausi* Dana *Calanus finmarchicus* (Gunnerus) *Centropages hamatus* (Lilljeborg) *Centropages typicus* (Krøyer) *Labidocera aestiva* Wheeler *Microcalanus pygmaeus* (G.O. Sars) *Paracalanus parvus* (Claus) *Pseudocalanus elongatus* Boeck *Pseudodiaptomas coronatus* Williams *Temora longicornis* (O.F. Müller)
Gaudy (1962)	*Temora stylifera* (Dana)
Deevy (1964)	*Pleuromamma piskei* Farran *Pleuromamma abdominalis* (Lubbock) *Pleuromamma xiphias* (Gisbrecht)
Vucetic (1965)	*Calanus helgolandicus* Claus
El-Maghraby (1965)	*Acartia latisetosa* Kriczagin *Centropages kroyeri* Gisbrecht *Oithona nana* Gisbrecht *Euterpina acutifrons* (Dana)
Deevy (1966)	*Centropages auklandicus* Krämer *Calanus australis* Brodsky *Pleuromamma gracilis* (Claus)
Elbourn (1966)	*Cyclops strenuus* Fischer
Eriksson (1973)	*Acartia longiremis* (Lilljeborg)
Moraitou-Apostolopoulou (1975)	*Temora stylifera* (Dana) *Calanus minor* (Claus)
Evans (1977)	*Oithona similis* Digby *Acartia longiremis* (Lilljeborg)

maximum mean length occurring one month after the minimum surface water temperature and the minimum mean length occurring two months after the highest temperatures. Like the present study, Ritchie *et al.* (1993) found that seasonal photoperiod had no effect upon the size attained by ovigerous *L. salmonis* females.

This negative correlation between temperature and body size at maturity however, is not always universally seen in the crustaceans. Evans and Diaz (1978) described a direct relationship between seasonal sea temperatures and the adult size of the planktonic copepod *Microstella norvegica* (Boeck) with size increasing with temperature. However, they offered no explanation for this deviation from the apparent normal inverse relationship between temperature and body size in planktonic copepods.

More recently, Johnston and Dykeman (1987), studying the parasitic copepod *Salmincola salmoneus* from the gills of Atlantic salmon, found that temperatures of 7°C significantly reduced the body size of mature female parasites when compared to temperature regimes of 12°C. Again, no explanation or hypothesis was proffered for this reduced growth of the copepod at low temperatures. Perhaps reduced temperature would cause a reduced somatic growth in some species due to a decrease in metabolism.

2.4.1.2. Seasonal fecundity

Despite the discrepancies between temperature and body size, there does however seem to be little doubt about the relationship between body size and fecundity, with a larger body size at maturity apparently permitting the crustacean to be more fecund than a smaller mature female. This reproductive output, though, may appear to be intrinsically linked with temperature due to the control of this parameter over

growth, and some studies have linked temperature directly to fecundity rather than with body size.

In the present study, a seasonal variation was observed in the total number of eggs per string carried by *L. salmonis*, with the maximum mean number recorded being 289.6 in March and the lowest, 194.1 in October. The mean number of eggs was found to be directly correlated with both temperature and female cephalothorax size. This was in the form of an inverse correlation for temperature, with the number of eggs per ovisac increasing as the ambient surface water temperature fell. Again, a time lag was observed with the minimum mean number of eggs being recorded in October, two to three months after the maximum water temperatures in July and August. The maximum number of eggs was observed in March, this being one month after the lowest ambient temperatures. However, in contrast to body size and temperature, a correlation was not observed to exist between temperature and fecundity when a lag factor of one, two or three months was introduced. Egg number was found to positively correlate with mean cephalothorax size, with larger females producing a greater number of eggs. The maximum and minimum cephalothorax sizes coincided with the periods when the greatest and least numbers of eggs per ovisac were being produced respectively, indicating that a time lag did not occur.

Crawford & Daborn (1986) in contrast to the present study demonstrated the absence of a significant relationship between temperature and clutch size for the calanoid copepod *Eurytemora herdmani*. However, other authors have recorded a direct finding, supporting the present study, which perhaps indicates that the importance of temperature on egg number varies from species to species (McLaren,

1963; Woodward & White, 1981). Elbourn (1966) found a seasonal variation in the number of eggs per egg sac for *Cyclops strenuus,* with a maximum over winter and a minimum over summer, like that for *L. salmonis* which has also been demonstrated by Rzoska (1927), Ravera (1955), Røen (1957) and Elgmork (1959). Elbourn, like the present study, concluded that the number of eggs produced by *C. strenuus* was correlated positively with both the size of the adult female, and also negatively with water temperature; both of these factors he considered to be equally important in determining the number of eggs carried in an ovisac.

Most studies on free-living species have concentrated only on the effect of female size on fecundity for various crustacean groups and positive correlations between female size and egg number have been shown for freshwater diaptomids (Ravera & Tonolli, 1956; Davis, 1959a), cyclopoids (Margalef, 1953, 1955; Elgmork, 1959; Elbourn, 1966) and for marine species of calanoids (Marshall, 1949; McLaren, 1963, 1965). Ohno, Takahashi & Yaki (1990) concluded from published reports that in general, the body size of adult females correlates positively with fecundity in copepods (Landry, 1978; Uye, 1981).

The reasons for this increase in fecundity with increasing body size are unknown. Lawrence & Sastry (1985), whilst studying variation in egg production in the free-living copepod *Tortanus discaudatus* Thompson & Scott suggested that small size may impose physical limits on fecundity, or that such a decrease in size may be associated with a higher metabolic rate (no doubt caused by an increase in temperature) as is evident for other crustaceans (Vernberg, 1959), consequently leaving less energy available for reproduction.

There have only been a few published reports on the relationship between temperature and/or adult female body size and ensuing fecundity in parasitic crustacean species, and some of these are more akin to passing observations. Tully (1989) observed that during the winter, fecundity of *L. salmonis* parasitic on Atlantic salmon on the west coast of Ireland was higher than in the summer, finding an average of 315 eggs per egg string in January compared to only 107 eggs per egg string in August, compared with 274.4 and 216.0 for these two months respectively from the present study. From Tully's data, as in the present study, the number of eggs carried appeared to be positively linked to a seasonal variation in the mean body size of adult females. Tully recorded greater sizes in January than August, which as shown in the present study is due to water temperature. However, Tully (1989) did not test for the presence of correlations between egg number and either temperature or female body size. It is unfortunate that Ritchie *et al.* (1993) only recorded the number of viable eggs within *L. salmonis* egg strings and not the total number present. From their data it is not therefore possible to determine whether the actual total number of eggs does vary seasonally and whether it is related to body size. Although their data represent an important contribution to the knowledge and understanding of sea lice biology, it is impossible to compare their results with other trends in crustacean reproductive biology. Johnston & Dykeman (1987) looked at the egg production of *Salmincola salmoneus* and found that reduced body size and a reduced number of eggs were related and initiated by a drop in temperature, again indicating the close relationship between temperature, body size and fecundity.

Temperature however may not be the only factor controlling fecundity whether directly or indirectly through body size. It has been suggested (Moore & Francis, 1986) that changes in reproductive output may be based on a combination of temperature and photoperiod, and since temperature and photoperiod are closely associated with one another, the effect of their combination on reproductive parameters cannot be discounted. In the present study however, no relationship was found to occur between photoperiod and fecundity of *L. salmonis* and, as already noted, there was also no correlation between photoperiod and body size. McGladdery & Johnston (1988) looked at egg development of *Salmincola salmoneus* exposed to different regimes of temperature and photoperiod. However, when the relative effects of temperature and photoperiod on the egg development were compared, temperature appeared to be the dominant environmental variable of the two, with parasites held at 12°C in different photoperiod regimes producing very similar egg development patterns. Johnston & Dykeman (1987), in their study on the effects of temperature and photoperiod on *S. salmoneus*, also demonstrated that temperature had a more dominant effect on reproduction than photoperiod, supporting the present study, but suggested that photoperiod may have some effect upon the trunk length of adult females. However, as already discussed, photoperiod appears to have no significant control over the size attained by *L. salmonis* ovigerous females. If Johnston & Dykeman's (1987) observations that the size of the adult female does affect fecundity in *S. salmoneus,* it could be argued that photoperiod will therefore have some effect upon fecundity of this parasite through its control over body size. However, Johnston & Dykeman do not do this, attributing the reduction in egg numbers observed to cooler water temperatures

instead of body size. Ritchie *et al.* (1993) in a more detailed investigation into the effects of temperature and photoperiod on the reproductive output of *L. salmonis* found, in contrast to the present study, that photoperiod did affect the output of the parasite, but in different ways to that of temperature. They suggested that temperature had a greater effect on female size and the number of eggs produced than did photoperiod, but that egg size was affected more significantly by photoperiod than by temperature. However, closer examination of the data of Ritchie *et al.* (1993) suggests that photoperiod may have a more dramatic effect on reproductive output than just being confined to influencing egg size. The reproductive output of *L. salmonis* was studied at two farm sites. At farm 1, the mean cephalothorax length was found to be influenced by both temperature and photoperiod. Looking at the mean number of eggs per string, Ritchie *et al.* by stepwise regression analysis found that photoperiod was negatively correlated with mean egg number, this accounting for 79% of the variability, with temperature having no significant effect upon brood size. At farm 2, variations in mean egg number were found to be negatively correlated with temperature, which accounted for 77% of the variability, whilst photoperiod had no significant effect upon egg number. It seems from the results of farm 1 that the effect of photoperiod on cephalothorax length and subsequent egg numbers per brood cannot be discounted as Ritchie *et al.* (1993) appear to have done in their discussion of the results, and that the relationship between temperature and *L. salmonis* brood size may not necessarily be such a straight forward one as first assumed.

Although from the present study and from published reports there appears to be a positive correlation between fecundity and body size and/or a negative correlation

between fecundity and temperature, the form of this relationship and the amount of influence it exerts in relation to other factors, if any, such as photoperiod is less clear. Indeed, according to Moore & Francis (1986), the conditions that cause a reduction in reproductive output may be different to those that increase it, and so the overall relationship between abiotic factors and fecundity may be complex and worthy of further studies of this kind.

C. elongatus, on the otherhand, does not seem to conform to the same pattern as other free-living and parasitic copepods already described, as there does not seem to be any relationship between temperature and/or body size and reproductive output from the literature available. Tully (1989) found that although the size of the parasite was seen to change inversely with temperature, the fecundity was not observed to change. Indeed, Hogans & Trudeau (1989) stated clearly that temperature did not affect the number of eggs produced by *C. elongatus*. However, in both cases, little or no details were given. *C. elongatus* has been described as having a world-wide distribution (Kabata, 1979), but in the present study it was observed that this species was only present in significant numbers during the summer months, and was absent from the majority of fish farms sampled over the winter. It was therefore impossible to obtain year round samples with adequate sample sizes and hence it was not possible to try and clarify the relationship between the fecundity of *C. elongatus* and body size and/or temperature. It may be also the case that both Tully (1989) and Hogans & Trudeau (1989) had difficulty in obtaining enough gravid adult females to demonstrate a seasonal variation in reproductive output.

2.4.1.3. Latitudinal variation

Although at all sites temperature and photoperiod vary seasonally, each site will have its own distinct temperature and photoperiod pattern, such abiotic factors being primarily dictated by the latitude of that area and subsequently seasonally modified. The effect of latitude on different populations of the same species has been occasionally commented upon but no real evidence is available for specific crustacean species. Sheader (1981) stated that "generally with increase in latitude, gammarid species show an increase in the minimum and mean size of females at maturity with the associated size-related increase in fecundity," and Fish (1975), was tempted to suggest an increase in gammarid body length and brood size occurs with increasing latitude, but did not have the amount of data available to allow this. This is presumably due to the presence of cooler average temperatures at higher latitudes, causing an increased generation time and subsequent increase in the intermoult period, resulting in an increased body size and consequent fecundity.

From the available reports of the reproductive output of *L. salmonis* parasitic on Atlantic salmon (table 2.4), there does not seem to be a cline in brood size with latitude. Although the egg numbers per ovisac reported by Hogans & Trudeau, 96 at approximately 45.0° latitude seem vastly different to the 700 eggs per ovigerous female reported by Wootten *et al.* (1982) at a latitude of approximately 57°, Johnson (1993) reported the mean reproductive output of *L. salmonis* as being 879.2 eggs per gravid adult female at a latitude of approximately 49°, the highest figure reported. Again it is impossible to compare the data of Ritchie *et al.* (1993), to any other data as they only counted viable eggs showing normal development, and hence total egg numbers produced are not known. There may however, be a number of reasons why a cline, if

Table 2.4. Comparison of the reproductive output of *Lepeophtheirus salmonis* and *Caligus elongatus* parasitic on *Salmo salar* from published reports and the present study.

Authors	Species	Mean number eggs per ovisac (± 1 SD)	Range	Location	Approximate latitude
Wootten *et al.* (1982)	*Lepeophtheirus salmonis*	-	<100-700 (per ovigerous female)	North-East Scotland	57.2°
Present study, January	*Lepeophtheirus salmonis*	274.30 ± 58.40	114-421	Argyll, West Scotland	56.2°
Present study, August	*Lepeophtheirus salmonis*	216.00 ± 66.80	89-352	Argyll, West Scotland	56.2°
Tully (1989), January	*Lepeophtheirus salmonis*	315.00 ± 35.00	-	Mid-West Ireland	53.0°
Tully (1989), August	*Lepeophtheirus salmonis*	107.00 ± 19.00	-	Mid-West Ireland	53.0°
Jackson & Minchin (1992), offshore farm	*Lepeophtheirus salmonis*	758 (per ovigerous female)	-	West Ireland	53.0°
Jackson & Minchin (1992), inshore farm	*Lepeophtheirus salmonis*	297	-	West Ireland	53.0°
Johnson (1993)	*Lepeophtheirus salmonis*	897.2 ± 112.5 (per ovigerous female)	-	Strait of Georgia, British Columbia	49.0°
Hogans & Trudeau (1989)	*Lepeophtheirus salmonis*	96.00	-	Bay of Fundy, Canada	45.0°
Pike *et al.* (1993)	*Caligus elongatus*	80.00	-	North-East Scotland	45.0°
Present authors, September	*Caligus elongatus*	56.62 ± 17.08	6-87	Argyll, West Scotland	56.2°
Hogans & Trudeau (1989)	*Caligus elongatus*	89.00	-	Bay of Fundy, Canada	45.0°

present, is obscured. It may of course be argued that seasonal factors must be taken into consideration, and unless it is stated when a sample took place, as is the case for Tully (1989) and the present study, where both January and August brood sizes are given in table 2.4, data may be misleading, and obscure any pattern that might exist. It also may be argued that host effects will influence the reproductive output of sea lice, as has been shown by Johnson (1993) for *L. salmonis* infecting *Oncorhynchus tshawytscha* and *S. salar*. However, all of the reports listed in table 2.4 are from sea lice parasitic on Atlantic salmon, and thus differences due to host species can be discounted. However, Jackson & Minchin (1992) and Tully & Whelan (1993) demonstrated a brood size difference between lice infecting farmed and wild Atlantic salmon, with lice from wild salmon carrying more eggs per ovisac than farmed salmon. This, though, seems to be explained by the larger sizes of ovigerous females present on wild fish as shown by Jackson & Minchin (1992), although why this should occur is not known. Tully & Whelan (1993) suggested that chemical treatments may compromise the growth potential of sea lice on farmed salmon, and indeed, Jackson & Minchin (1992) found that *L. salmonis* from salmon on an offshore farm, where no treatments were used were larger and more fecund than lice from an inshore farm where a comprehensive programme of lice treatments was followed. All the reports in table 2.4 represent specimens obtained from commercial fish farms, but the history of the control programmes undertaken is unknown, and therefore what effect these may have on the reproductive output of such parasites. The larger size and greater fecundity of lice from wild salmon suggests that there must be other factors, such as perhaps different population characteristics rather than just the effect of chemotherapeutents controlling

fecundity. Egg numbers from lice recovered from wild Atlantic salmon recorded at the Institute of Aquaculture are higher than those from farmed salmon (Sommerville, pers. comm.), and it may be the distribution of wild salmon in deep ocean waters that cause this effect. Such waters will be cooler, causing a subsequent increase in body size and fecundity in contrast to lice infecting fish kept in cages near the water surface. The differences in fecundity observed from the published reports may instead be due to the species distribution of *L. salmonis*. The distribution of *L. salmonis* has been described as northern circumpolar (Kabata, 1979), and Johannessen (1978) suggested that the optimal temperature for "maximum overall survival" for the parasite as being between 9°C and 11°C. It could therefore be suggested that *L. salmonis* may be at the southern end of its range in the Bay of Fundy and consequently its biology may be compromised, explaining the low fecundity observed by Hogans and Trudeau (1989).

Hogans & Trudeau (1989) and Tully (1989) as already noted, showed no seasonal variation in fecundity for *C. elongatus*, despite the latter author demonstrating that a seasonal variation in body size occurred. Indeed the figures for fecundity recorded for this species (present study, Pike *et al.*, 1993, Hogans and Trudeau, 1989) are all very similar, suggesting no cline of fecundity with latitude. If temperature was the major abiotic factor affecting fecundity through body size as has been suggested, it would be expected that the colder temperatures of the northern sites such as Scotland would result in higher fecundities, and this is not observed. Hogans & Trudeau (1989) stated that temperature did not have any effect upon fecundity, though gave no details. However, no explanation was given by Tully (1989) or Hogans & Trudeau (1989) for this phenomenon, and indeed the reasons for this seem unclear. Perhaps, because *C.*

elongatus has not been of such economic importance as *L. salmonis*, it may merely be a reflection of the fact that its biology and life cycle have not been studied in enough detail to reveal all seasonality traits, and this together with difficulty in obtaining year round samples may cause artefacts to be observed from small scale sampling.

2.4.1.4. Seasonal viability of eggs

In order to determine whether a seasonal variation occurred in the proportion of viable eggs produced by *L. salmonis*, the numbers of eggs showing normal colouration and organisation and those showing abnormalities were recorded. Although both the number of viable and non-viable eggs varied over the twelve month sampling period, increasing in numbers over winter months as already discussed, the ratio of normal to abnormal eggs did not show any significant changes. Ritchie *et al.* (1993), as already noted, also found that the number of viable eggs varied seasonally in the same manner as the present study. However, since the authors did not record the total number of eggs per ovisac or the number of abnormal eggs, it is not apparent from these authors' data whether this is just a reflection of the increased number of eggs produced by adult females at cooler water temperatures, as already discussed, or, whether the proportion of viable and non-viable non-developing eggs changes over the year. Unpublished observations by Ritchie, cited by Ritchie *et al.* (1993) did however state, that more areas of disorganised and discoloured eggs were observed during the winter, but no data was made available.

The viability of eggs of free-living copepods has been shown to fluctuate strongly. Ambler (1985) reported that egg viability of *Acartia tonsa* varied with season, from almost 100% in the spring to less than 70% in the autumn, and Ianora *et*

al. (1992) found that mortality of eggs (as opposed to unfertilised eggs) of *Centropages typicus* fluctuated, but was not related to variations in breeding intensity and environmental variables such as chlorophyll *a* and temperature. The authors also noted that the proportion of viable eggs produced by *Temora stylifera* was positively correlated to season, but again unrelated to chlorophyll *a* production. They considered that remating was not a factor that could explain eggs not being viable, as the eggs had been fertilised, but suggested that egg mortality may be caused by a nutritionally inadequate diet due to food availability and quality varying seasonally for free-living copepods. It is unknown as to whether the abnormal eggs observed in *L. salmonis* and *C. elongatus* ovisacs are unfertilised or, like *C. typicus* (Ianora *et al.*, 1992), have aborted for some reason. Diet, however seems unlikely to be a major factor in controlling the reproductive output of *L. salmonis* and *C. elongatus*, since these parasites, once established on a host, are unlikely to experience seasonal variations in food availability or quality. This may explain the lack of seasonal variability in egg viability observed for *L. salmonis* and is worthy of investigation.

2.4.2. Infrapopulational variation in fecundity

Large standard deviations were observed in the fecundity of *L. salmonis* during the seasonal sampling programme and thus a larger sample of the parasite was taken from the same site on one sampling day during September. Due to the relatively high water temperatures present at the site, there were comparatively high numbers of *C. elongatus* also present, allowing some analysis of the fecundity of this species. Large variations in fecundity were also observed within these samples for both species. The mean number of eggs per ovisac for *L. salmonis* was 206.2, and the standard deviation

observed was 74.09. For *C. elongatus*, a mean of 52.62 eggs per egg string was recorded, with the standard deviation being 17.08. This infrapopulational variation in brood size has been noted by other authors for both parasitic and free-living copepods (Elbourn, 1966; Hopkins, 1977; Schram, 1979; Maly, 1983; Lawrence & Sastry, 1985; Crawford & Daborn, 1986 and Tully & Whelan, 1993), and indeed this variation in brood size on "any one day sampled" appears to be a familiar feature of many studies on copepod fecundity. The most common explanation for this variation in free-living copepods is the availability of food and the quality of the diet but this factor is of little or no importance for parasitic copepods. Also, it has been shown by some workers (Lawrence & Sastry, 1985; Durbin, Durbin & Campbell, 1992) that food, although important, was not the only factor causing variations in fecundity in populations of free-living copepods. There are a number of other factors aside from food abundance and quality that may help to explain this phenomenon, and the consequent variation observed in infection patterns of *L. salmonis* and *C. elongatus* present on fish farms.

2.4.2.1. Phenotypic variation in size

As well as the discussed seasonal variations in size and their subsequent effects upon fecundity, it might be expected that within any one population of sea lice at any single point in time, a variation in adult female size will occur, as with any population within a species. It may therefore be natural to expect that with this size variation, there will exist a corresponding variation in individual fecundity, explaining some of the large variations observed for *L. salmonis* and *C. elongatus*. In the present study, it was demonstrated that there existed a wide variation in the cephalothorax length of ovigerous *L. salmonis* females sampled at one point in time, ranging from 5.00 to

5.71mm (mean, 5.29mm, standard deviation, 0.23). This was found to positively correlate with the number of eggs per egg string within the population, and hence variation in individual female body size can explain some of the variation recorded in brood size from one sampling point. Although it is not possible to compare the two correlations of seasonal size and brood size and individual size and brood size due to the different sample sizes, these findings do appear to strengthen the argument that body size and not temperature has the most direct effect upon the fecundity of *L. salmonis*.

Tully & Whelan (1993) also found significant differences in the fecundity of *L. salmonis* within sites sampled. They stated that variations in egg number could partially be explained by differences in "louse" size, with a positive linear relationship existing between cephalothorax length and egg number. However, it was not shown whether these variations in fecundity within sites were seasonal or were variations that existed at one point in time. Maly (1983) studied the brood size in calanoid copepods, and found positive correlations between brood size and female length within sites sampled, suggesting that the phenotypic variation in female size seen in all populations will cause a subsequent phenotypic variation in clutch size. However Maly (1983) went on to state that the correlations between size and fecundity varied widely between sampling sites, indicating that variation in female body size is not the only factor affecting reproductive output. If it were, it would be expected that the correlations of size with fecundity at different sampling sites would be the same.

2.4.2.2. Age and brood number

At present, there are no published reports of the number of egg batches produced by either *L. salmonis* or *C. elongatus*, though Ritchie *et al.* (1993) cited unpublished observations where up to six broods had been seen to be produced by *L. salmonis*. Johannessen (1978) cited by Anstensrud & Schram (1988), was reported to have observed the extrusion of a second pair of ovisacs 24 hours after the previous pair had been lost in *L. salmonis*, but no details were given regarding the maximum or mean number of broods produced. Schram (1993) stated that younger *L. salmonis* females had shorter egg strings than older gravid females. Since the number of eggs in an ovisac is highly correlated to its length (Ritchie *et al.*, 1993), it appears, according to Schram's comments, that older females produce more eggs per ovisac. However, Schram (1993) gave no details regarding either egg string length or egg numbers and therefore there appears to be no statistical evidence for this.

The number of broods produced by *L. salmonis* under experimental conditions was seen to total three during the present study. The first brood produced the least number of eggs (mean, 141.09), the second the most (mean, 216.40), and the third slightly less (mean, 208.2) than the second with the differences in brood size between the first brood and the second and third broods being found to be significant. Since this study, observations at the Institute of Aquaculture have revealed that as many as five broods are produced by the parasite (Petrie, pers. comm.). Such differences in the number of eggs between different broods will partially help to explain some of the variations in fecundity observed within one sample from a single population.

One of the few reports published of fecundity variations between different clutches in parasitic copepods is that of de Meeüs *et al.* (1993), although, like Schram,

(1993) no specific details were given. Studying the comparative life histories of two species of sea lice, *Lepeophtheirus thompsoni* and *L. europaensis*, de Meeüs *et al.* (1993) looked at the size of both natural and experimental clutches of eggs in both species. For both *L. thompsoni* and *L. europaensis* it was found that first and last clutches contained fewer eggs than intermediate clutches (though the actual number of clutches or the number of eggs was not reported), this being similar to the findings of the present study. The authors, as is also the case in the present study, concluded that this "may explain the large variances observed in natural clutches." However, de Meeüs *et al.* (1993) went on to state that experimental clutches (eggs obtained from parasites held in laboratory conditions) contained fewer eggs than natural ones (eggs from parasites obtained from the natural environment), with this difference being very significant, and warned that experimental results must therefore be interpreted with care. It is may seem difficult therefore to treat their results with anything but scepticism, but rationally, it is impossible from copepods collected from the natural environment, to determine whether a clutch is the first produced, the last, or an intermediate. This can only be determined from infecting fish under laboratory conditions and following the females through their development.

Multiple egg sac production occurs in parasitic copepods of fish with both direct and indirect life cycles and, according to Whitfield, Pilcher, Grant & Riley (1988) enhances the overall reproductive potential of the parasite *Lernaeocera branchialis* L. Schram (1979) also reported that *Lernaeenicus sprattae* (Sowerby) is capable of multiple ovisac production. Producing offspring in a number of broods does carry certain survival advantages. If environmental conditions are not optimal when one

brood is ready to hatch, then releasing eggs in discrete broods will favour a higher survival. With abiotic and biotic factors changing constantly in the marine environment, such as salinity, temperature, currents and host distribution, the release of all eggs at one point in time would be a highly risky strategy.

Although there are advantages on multiple egg sac production as mentioned, this does not explain why successive broods may contain significantly different numbers of eggs. Differences in egg production has been studied in a number of free-living copepods, and it is worthwhile considering these reports since, as has already been shown, there are a number of reproductive traits that are common in both free-living and parasitic copepods.

Lawrence and Sastry (1985), whilst studying the seasonal variation of egg production in the free-living copepod *Tortanus discaudatus* Thompson & Scott found that several factors could be related to the variation in egg production by females. Temperature effects were found to account for only approximately 35% of the variance in egg production rate for *T. discaudatus* under experimental conditions, and the authors found that the analysis of variance for the temperature experiments contributed 26% of the variation in egg production differences among individuals. The authors went on to suggest that an important difference among individuals, where food was not a limiting factor, was that of age.

The relationship between age and ensuing fecundity was also considered by Durbin *et al.* (1992) It was hypothesised that the egg production of *Acartia hudsonica* (Pinhey) was regulated by the age distribution of the adult population, and not by temperature and food abundance. Declining egg production was suggested to be due to

senescence of autumn hatched overwintering copepods, and that a marked increase in egg production was consistent with a shift to a younger age structure, with a higher intrinsic production rate. The data of Durbin *et al.* (1992) suggests that age frequency distribution may significantly affect production by adult copepods. The authors concluded that copepod egg production rates are age dependent, declining during the latter part of the adult life span. This was also suggested by Parrish & Wilson (1978) and Uye (1981).

Crawford & Daborn (1986) attributed great variation in egg numbers of the calanoid copepod *Eurytemora herdmani* from one sampling date to the next to a decrease in reproduction output in older individuals. Maly (1983), as already noted, studied the body size and clutch size relationships in calanoid copepods taken from lakes and ponds near Montréal. Although the author found a highly positive correlation between clutch size and female length in populations as was seen in the present study for *L. salmonis*, like Crawford & Daborn (1986), correlations varied widely between sampling sites and dates. Possible explanations put forward by Maly (1983) included one where it was suggested that fecundity may be related to age, and that older individuals could not produce clutches that were as large as their younger counterparts.

If age does regulate the number of eggs a female can produce, then it would be expected that age contributes to a large part of the variation observed in the fecundity of both *L. salmonis* and *C. elongatus* from single populations sampled at one point in time. However, the present study also showed that it was the first brood produced by *L. salmonis* rather than later broods that contained fewer eggs, in contrast to the findings of Maly (1983), Durbin *et al.* (1992) and Crawford & Daborn (1986), though in

agreement to the observations of Schram (1993) on the same species. Simply put, it may be the case that when the first brood is produced, *L. salmonis* females have not attained their maximum size. A size increase following this would cause the increase in egg numbers in the second brood and the onset of senesence would then cause a decline later on.

Parrish & Wilson (1978) conducted a four year long study on the fecundity of the free-living copepod *Acartia tonsa*, demonstrating that the typical egg production pattern for *A. tonsa* consisted of a general increase in egg-laying over the first 2 to 3 days, with a relatively high production then being maintained for the next 10 to 14 days, thereafter the rate began to decline. Such a pattern, although it is for a species that has continuous rather than discrete egg production, has similar characteristics to that observed in the present study for *L. salmonis* and those observed by De Meeüs *et al.* (1983) for *L. thompsoni* and *L. europaensis*. For *A. tonsa*, after the decline in the daily rate of production, infertile eggs began to appear and, if females were not remated, the laying of infertile eggs often continued at a moderate or low rate for several days, whereupon egg-laying dropped abruptly or ceased altogether, and was followed shortly afterwards by the death of the female. Statistics from *A. tonsa* individuals show a clear cut onset of infertility, with the majority of individuals subsequently developing total infertility.

Smyly (1970) looked at the rate of development, longevity and fecundity of the cyclopoid copepod *Acanthocyclops viridis* (Jurine) in relation to the type of prey items that was available. This species, like *L. salmonis* produces discrete batches of eggs. It was observed that the number of eggs laid in successive broods (the mean number of

broods being 8.6) tended to diminish with age, and that individuals fed on *Artemia* nauplii laid more eggs in the second and third broods, especially in the third, than in the first or later broods. For *A. viridis*, over one quarter of the total number of eggs laid were laid in the second and third broods, and nearly half in the first four. Again, this pattern shows similarities to *L. salmonis*, where the first egg strings produced had a significantly lower number of eggs when compared to the second and third broods. Hopkins (1977) demonstrated that as well as seasonal variability in clutch size for the copepod *Euchaeta norvegica* Boeck in females of the same size class, there also existed, like the reports of Maly (1983) and Crawford & Daborn (1986), quite large variations in the number of eggs per sac between females of the same size class from the same plankton sample, and concluded that there were certainly non-seasonal factors concerned in fecundity. One of the suggestions was that fecundity is determined by the number of broods previously produced and the physiological condition of the individuals (Hopkins, 1977). Obviously it was not possible to determine the physiological condition of sea lice collected at fish farms, and the egg strings of lice from experimentally infected fish at the Marine Station, Machrihanish were collected as a "by-product" from standard infection trials in which the condition of the ovigerous females was not of importance. Therefore although the condition of females may well have a significant effect upon their fecundity, it is not possible to quantify what its magnitude will be. Indeed, Hopkins (1977) did not qualify his hypotheses with data. It seems fair to assume that in nature, the adult age distribution, though generally unknown, will potentially affect mean production (in terms of fecundity) rates, and this, from the information available from studies performed on free-living copepods appears

to be the case. Such dependence of egg production upon age may therefore explain some of the variations observed in the mean fecundities of single populations sampled at one point in time of both *L. salmonis* and other species of sea lice.

2.4.2.3. Individual variation in egg viability

Within samples in the present study, it was not uncommon in either species for all the eggs within the egg strings to be non-viable, for example for *C. elongatus*, 18.33% of all egg strings had totally non-viable eggs compared to only 2.41% for *L. salmonis*. It is not known what causes the presence of these non-viable eggs. There are two possible causes worth considering. Firstly, sperm depletion may have occurred, since the spermatophore deposited on the female sealing the genital opening holds a finite store of spermatozoa. Depletion would result in the eggs deposited in ovisacs being unfertilised. This was observed for *A. tonsa* by Parrish & Wilson (1978). Secondly the eggs deposited may have been fertilised but subsequently aborted, normal development no longer continuing. It is not known whether it is just one of these two explanations that cause eggs to be non-viable or both.

It is common to find groups of normally developing eggs alongside non-viable eggs within an egg string. If it were the case that the eggs were fertilised but subsequently aborted, it may be hypothesised that the number of non-viable eggs for *L. salmonis* and *C. elongatus* may be related to the number of eggs in the egg strings, due to a trade-off existing between producing a smaller number of possibly more viable eggs and a larger number of less viable eggs. However, from the present study this was shown not to occur, as there was no significant difference in the proportion of eggs showing normal development from egg sacs with a higher number of eggs compared to

egg sacs containing a low number for both species. This may suggest that eggs that appear non-viable (that is, not showing normal development) are possibly unfertilised eggs. If the sperm from the spermatophore in *L. salmonis* and *C. elongatus* adult females is depleted and they begin to produce unfertilised eggs, the question that immediately arises is why adult females do not remate and so continue to produce fertilised viable eggs, as happens in the case of *A. tonsa* (Parrish & Wilson, 1978)? According to Ritchie *et al.* (1993), Anstensrud (1990) showed that the egg string length and hence the number of *Lepeophtheirus pectoralis* (Müller) eggs (egg string length was shown by Ritchie *et al.* (1993) to be highly correlated with egg number, allowing an accurate estimation of egg number from egg string length) was affected by the time of mating, being significantly greater in adult females that had mated immediately after moulting from the pre-adult 2 stage, compared to those females that had remained virgins for a longer period of time. However, this is not entirely clear from Anstensrud's 1990 paper and it appears that this conclusion may be due to Ritchie *et al*'s. evaluation of Anstensrud's results. However, if Ritchie *et al.* (1993) are correct, it may be the case that older virgin females are not as fecund as younger virgin females. If, therefore, males have an evolutionary vested interest in producing as many offspring as possible, then they would tend to form precopula pairs with as many young females as possible. It has been observed (Johnson & Albright, 1991) that *L. salmonis* adult males commonly establish precopula with pre-adult 1 and pre-adult 2 females, in "preference" to adult females. Upon the moult to the adult female, copulation occurs and the female genital opening is sealed with spermatophores, and therefore it seems advantageous for males of a population to mature earlier than females, to ensure

survival of the individual males genetic material. It appears therefore, that there will be no individual advantage gained by adult males in mating with an "old" adult female, as a greater potential reproductive success (that is, more eggs per egg string) for the males is gained by mating with a recently moulted female. This could in theory, partially explain why large numbers of inviable eggs are sometimes observed in egg sacs of both *L. salmonis* and *C. elongatus*. If the eggs are unfertilised, it will most likely to be due to the sperm depletion, caused by a lack of spermatozoa in the spermatophore, and the possible lack of remating of adult females by adult males due to the males preference for early mating in virgin females. However, Ianora, Scotto di Carlo & Mascellaro (1989) studying the free-living copepod *Temora stylifera*, found that even after females were remated once they had begun to produce unfertilised eggs, egg viability never exceeded 80%, suggesting that either remated females do not produce such viable clutches as females that have only mated once, or that other factors (such as age, as already discussed) are responsible for the continued production of viable clutches. If this is generally the case, then again there would be no evolutionary advantage in *L. salmonis* males mating with females that had already been mated, as viability of their potential offspring would be reduced. More data are obviously needed upon the number of viable eggs per brood of *L. salmonis* and *C. elongatus*, and the status of the non-viable eggs, that is whether they are fertilised or not, before any final conclusions can be drawn on the causes of non-viability in eggs.

2.4.2.4. Host effects, immune responses and reproduction

Johnson (1993) stated that the host's nutritional and/or immunological factors may also play an important role in controlling the reproduction of the parasite. The

effects of the host on the biology of ectoparasitic copepods cannot therefore be eliminated from the explanation of the variation in fecundity observed within the same population of *L. salmonis* and some effects have already been documented for parasitic copepods. Johnson (1993) demonstrated that the number of eggs produced by *L. salmonis* was dependent upon the host species. Gravid adult females taken from Atlantic salmon, *Salmo salar*, carried on average twice as many eggs within their ovisacs as those produced by *L. salmonis* infecting Chinook salmon, *Oncorhynchus tshawytscha*; 879.2 (± 112.5) compared to 430.0 (±100.0). This difference was suggested to be due to either host nutritional or immunological factors, or both. The size of ovigerous *L. salmonis* females on *O. tshawytscha* and *S. salar* was not significantly different (Johnson, 1993), and hence host effects upon body size cannot explain the difference in fecundity observed.

Other host effects on the biology of ectoparasitic copepods have also been documented with these including the direct effect of the host on the reproduction and the fecundity of the parasite. Woo and Shariff (1990) studied the dynamics of resistance in recovered and naive kissing gourami, *Helostoma temmincki* Cuvier & Valenciennes, after infection with the parasitic copepod *Lernaea cyprinacea* L. The authors attempted to determine the effect of the host on rejection of the parasite and viability of egg sacs and eggs. Fish that had been previously infected with *L. cyprinacea* and had since recovered and naive *H. temmincki* were experimentally infected with *L. cyprinacea*. It was demonstrated that the number of egg sacs lost during the first week of infection was significantly higher in previously infected fish than from the naive fish infected for the first time. Most of the losses from previously

infected fish occurred in the first two weeks whilst major losses from parasites infecting naive fish for the first time were in the late second and third week. The difference in the number of egg sacs lost between previously infected and previously naive fish also proved to be significant. The fish were then infected again (second generation) and again it was observed that egg sacs were lost in larger numbers from the fish twice previously infected compared to the group that was initially naive and had subsequently only been infected once, and from this group when compared to a third group of (new) naive fish. It was suggested that egg sacs were rejected in larger numbers from the naive groups in the third and fourth weeks of infection due to the primary activation of the immune system, giving a protective mechanism. It was also found that eggs from parasites on recovered fish either failed to hatch, or if hatching did occur, the copepodids were either non-infective or that they caused a low transitory infection in naive fish. Woo & Shariff (1990) suggested that the recovery of fish affects the viability of the parasite by immunological lesion starvation. Immunological lesions can be caused by complement fixing antibody (Jones & Woo, 1987) and cellular reactions against parasitic copepods have previously been described (Shariff & Roberts, 1989). It has also been suggested that the starvation of the parasite may also be caused by immunological lesions and/or blockage (for example, immune complex) of the feeding and digestive system, with this resulting in the reduction of the longevity of the parasite and its reproductive capability - for example poorly developed egg sacs which are readily lost and low viability of eggs and larvae (Woo & Shariff, 1990).

Paperna & Zwerner (1982) studied the host-parasite relationship of the striped bass, *Morone saxatilis* (Walbaum) and the ergasilid copepod *Ergasilus labracis* Krøyer.

It was noticed that there was a cessation of egg sac production from *E. labracis* attached to *M. saxatilis* on one side of the branchial basket, whilst ovigerous females were present on the other side of the branchial basket. Paperna & Zwerner (1982) gave no explanation for this loss of egg sacs (and females) from one side. The only explanation that they give is that it could be a similar mechanism to that suggested by Woo & Shariff (1990) who stated that the protective mechanism in recovered fish (probably due to primary activation of the immune system, the piscine immune system being very responsive to antigenic stimulation) was also directed against parasite reproduction, through starvation of the parasite.

These two reports clearly demonstrate the lack of research directed towards the relationship between fish immunity and parasitic development and reproduction. There are no reports of the effect of the piscine immune system on fecundity apart from Woo & Shariff (1990) and Paperna & Zwerner (1982) on the loss of egg sacs, and in the former, subsequent effects on the eggs (failure to hatch, non-infective or very low infectivity). It is not known whether the fecundity of a parasite may be reduced if the fish host is exhibiting an immune response, although Johnson & Albright (1992a) demonstrated that cortisol-implanted coho salmon, *O. kisutch*, were more susceptible to infection by *L. salmonis* due to chronic immunosuppression than control fish. However, as some effects such as egg sac loss, reduced infectivity exist, the possibility of reduced fecundity cannot be eliminated. The parasites obtained for this fecundity study were obtained from commercial Atlantic salmon farms, and hence was impossible to ascertain the history of infection of the host. Therefore it is not possible to estimate

the effect that the host will have on the reproductive output of *L. salmonis* and *C. elongatus*, and what effect this has on their subsequent reproductive output.

2.4.3. Variation in egg size

As well as the seasonal and infrapopulational variations in adult female size and fecundity, it has also been documented that egg size within crustacean groups varies, both seasonally and within populations. However, many of these reports are contradictory with no clear pattern emerging as to the controlling factors or the evolutionary reasons for this.

2.4.3.1. Egg size and temperature

In the present study, mean *L. salmonis* egg size was found to range from 57.70 and 73.03μm depending upon the time of the year, with largest egg sizes being recorded in September, and the minimum mean size being obtained in April. Neither temperature or photoperiod was found to account for any of this variability. It was also demonstrated that no correlation existed between the mean number of eggs per ovisac and their mean size.

Ritchie *et al.* (1993) also demonstrated a distinct seasonal variation in viable egg size; eggs length decreasing from October to February (73.65 to 62.70μm), and then increasing again, and remaining relatively constant over the summer months. In contrast to the present study, the authors found that this change in egg length was positively correlated with photoperiod, though, as was the case in this study, temperature was not found to exert any influence. Ritchie *et al.* (1993) noted that variations in environmental conditions are expressed more rapidly in egg length, than in

egg string length or in brood size and, as a result, a poor relationship is obtained between these parameters.

Although it can therefore be suggested that there appears to exist two "generation types" of *L. salmonis*, as has been suggested by Tully (1989) and Ritchie *et al.* (1993) - a winter generation being characterised by producing more eggs of a smaller size, compared to a summer generation, which tends to produce less eggs, but which are of a greater mean size, this may be misleading. It may allow the assumption that egg size is controlled by seasonal factors and/or egg number, when, from the present study, neither temperature, photoperiod or egg number appear to exert any influence on it whatsoever.

The lack of a relationship between *L. salmonis* egg size and environmental/seasonal parameters is in contradiction to other reports on egg size and season. Sheader (1983) studying reproduction in the amphipod *Gammarus duebeni* Lilljeborg, reported that where reproduction in a species continues throughout most or all of the year, as is the case for *L. salmonis*, egg size may be greater during the winter months, and an inverse relationship was shown between size and temperature. This was also suggested by Steele & Steele (1975a). A greater egg size during the winter contrasts to the findings of the present study, and the data of Ritchie *et al.* (1993) for *L. salmonis,* since for the parasite smaller eggs are recorded over the winter months.

2.4.3.2. Egg size and egg number

Sheader demonstrated that there was no relationship between egg size and egg number, the two varying independently. This at first, may appear in contrast to the data

of Ritchie *et al.* (1993), who described two "generation" types, one producing a greater number of smaller eggs and a second producing a smaller number of larger eggs. However these authors did not test the relationship between the number of eggs and size. They did test for correlations between the number of eggs and temperature and photoperiod and between the size of eggs and temperature and photoperiod, but this in itself would not indicate whether there exists a relationship between egg size and number.

Seasonal studies on the egg size of *L. salmonis* in the present study showed, like Sheader (1983), that there was no relationship between egg size and the number of eggs per brood. However within one population of *L. salmonis* that was sampled at one point in time, it was found that there was a correlation between egg number and egg size, indicating that within a population, the number of eggs within an ovisac has an effect upon the size of the eggs produced. It therefore seems surprising that since the number of eggs within an ovisac from one population of *L. salmonis* sampled at one point in time does appear to effect the mean size of the eggs, that there does not exist a correlation between the seasonal mean number of eggs and their seasonal mean size. It may be the case that due to other existing relationships between brood size and factors such as surface water temperature and cephalothorax length, a relationship between seasonal egg number and size, if present at all, may be obscured, especially if it is not a strong correlation. A similar situation was reported by Maly (1983). Maly reported that although the mean egg diameter of calanoid copepods with larger clutches tended to be somewhat smaller than egg diameters from smaller clutches, this relationship was not always found, and suggested that other factors were also influencing egg size.

There has been some evidence to show the existence of an inverse correlation between egg size and the number of eggs per brood in other crustacean groups, with Steele & Steele (1975a, 1975b), Kerfott (1977) and Van Dolah & Bird (1980) demonstrating that for amphipods, egg size is correlated with egg number at any given female size.

2.4.3.3. Energy investment

The concept of energy investment in reproduction differing seasonally is an interesting one. Crawford & Daborn (1986) demonstrated that a decrease of 10% in the egg diameters of *E. herdmani* from winter to summer represented a 27% reduction in egg mass, and a corresponding decrease in energy invested. This, combined with the fact that a lower number of eggs are produced in the summer results in *E. herdmani* possessing a seasonal variation of reproductive investment - in winter there are many large eggs compared to summer when smaller and fewer eggs are produced.

It is not known whether such a seasonal investment occurs for *L. salmonis* (or indeed *C. elongatus*), as the situation is not the same. A greater investment per egg does occur in the summer since each egg is larger compared to the size attained over the winter. However since the parasites produce a greater number of eggs in the winter and a lower number in the summer, although a decrease in egg size in winter may initially represent a decrease in reproductive investment, the increased number of eggs produced at the same time will represent a concurrent increase.

Sheader (1983) demonstrated that the egg size of the amphipod *Gammarus duebeni* was directly related to the size of the resulting juveniles, and evidence suggests

that larger juveniles are fitter, with a greater chance of survival than smaller juveniles (Smith & Fretwell, 1974; Steele & Steele, 1975b). Sheader suggested that larger fitter juveniles are likely to have a greater chance of survival, and that this may explain why larger eggs are produced. Since both the infective stages of *L. salmonis* and *C. elongatus* are dependant on finite energy reserves, not feeding whilst in the water column, it might be expected that larger eggs would contain greater amounts of energy reserves, allowing a potentially longer survival time in the water column before depletion of the energy reserves, and thus a longer time in which to locate and infect a suitable host. Larger eggs, and therefore perhaps larger nauplii are produced during the summer by *L. salmonis*, when temperatures are higher. However, this does not mean that *L. salmonis* nauplii have a longer period to find a host than their "winter" counterparts. Metabolic rates would be increased due to the higher temperatures, and this may cause a faster rate of lipid depletion, consequently decreasing the time for the parasite to infect a suitable host. By producing larger eggs in the summer, there may be a subsequent increase in the amount of lipid reserves available, making allowances for the faster rate of depletion, and thus extending the life of the larval stages. Metabolic rates are lower at colder temperatures, allowing a longer period of time over which depletion of the energy reserves would occur. Therefore, the eggs could be of a reduced size in winter, as is the case for *L. salmonis*. The hypothesis is explored in Chapter 4.

From the present study therefore, the variations observed in the reproductive output of *L. salmonis* can be explained by a combination of both seasonal and infrapopulational factors. Both temperature and body size appear to strongly control

the fecundity of the parasite, with this, then being modified by the age of the female. From previously published reports such reproductive output may also be influenced by immune effects of the host and chemical treatment programmes used on farms. In addition to the influences identified in the present study, more work now needs to be undertaken to elucidate the effects of the host and chemotherapeutants on egg production. Viability of eggs appears to be linked to egg size within a population, but this is not seen over a seasonal sample, with the factors controlling egg size being unclear. Further studies on size, viability, and possibly other parameters should clarify the relationship. Once these are elucidated, and combined with the results and conclusions of the present study, a greater understanding of the population dynamics of the species will be acheived.

CHAPTER 3

HATCHING & DEVELOPMENT

3.1. Introduction

Between 1989 and 1990 the sea trout population off the west coast of Ireland collasped together with the premature return of smolts and kelts to estuarine and freshwater habitats being observed. Badly debilitated and emaciated fish were present in these returns, with a proportion of them carrying heavy infestations of *Lepeophtheirus salmonis* (Tully & Whelan, 1993; Tully, Poole, Whelan & Merigoux, 1993; Bass & Murphy, 1995).

Due to the importance of the sea trout industry in Ireland, a large research programme was set up devoted to studying the parameters and possible causes of epizootics of *L. salmonis* infesting sea trout. The majority of the subsequent findings published from this research attempted to establish a causal link between the development of the salmon farming industry along the west coast of Ireland and the collapse of the sea trout populations, with particular emphasis on the role of sea lice in the relationship (Tully & Whelan, 1993; Tully *et al.*, 1993). One aspect of this research was an attempt to correlate the production of the nauplius I stage of *L. salmonis* from farmed and wild salmon with infection levels observed in wild sea trout (Tully & Whelan, 1993). Tully & Whelan (1993) estimated that farmed salmon contributed 95% of the total production of nauplius I stages of the parasite in the mid west coast region of Ireland. Using data obtained from six "embayments," the authors then correlated the nauplius production with the parasite intensity of *L. salmonis* on wild sea trout three weeks later.

This attempted link of the production of nauplii of *L. salmonis* with such future infection levels and the subsequent inference of the ability to predict future parameters and impact is indeed a large step to take considering the paucity of information available on the biology of the free swimming stages of this parasite. Data on hatching, survival and

development of the species along with information concerning the dispersal and distribution is limited, and, in some cases, completely unknown.

To understand the importance of why research into the biology of the free swimming stages is necessary, it is probably best to look at the inherent problems of Tully & Whelan's (1993) model for the prediction of infection parameters of *L. salmonis* from the production of the first naupliar stage.

Tully & Whelan (1993) admitted that within their model, there exists various sources of error for the estimation of the production of larvae. These include the difficulty in obtaining a representative estimate of the average numbers of ovigerous females per fish and the variation in the number of eggs per adult female, which have already been discussed in Chapter 2. Despite acknowledging that these inherent problems were present, the authors did not appear to allow for the difficulty in estimating the subsequent hatching success and viability of the larvae. Indeed, they assumed that all eggs counted with a normal appearance were viable, and hence would hatch to produce a live nauplius I stage.

Descriptions in the literature of the process of hatching in sea lice are however brief, with most being more incidental. Because of this, data on the hatching process, success and duration are limited. The level of hatching success of parasitic copepod eggs and its variations with respect to environmental parameters is of clear importance in attempting to predict future infection levels of a parasite on cultured fish. Estimation of the fecundity of a parasite may be a poor predictor of the production of the first larval stages in view of the reports outlining hatching details of caligid copepods. From these it can be seen that, despite a high number of eggs hatching, overall hatching was generally not found to be 100%. Johnson, Constible & Richard (1993) reported an average hatching success of *L. salmonis* (percentage of eggs hatched from the total number present) of 93%, with the range being

between 79 and 99%. Pike *et al.* (1993) found that over 85% of *Caligus elongatus* nauplii hatched from the egg strings, however neither of these two reports comment upon whether the hatched nauplii were viable or not. Johnson & Albright (1991) did look at the viability of the first naupliar stage and found that, although at high salinities (25 to 30‰) a hatching success of 100% was obtained, there existed a large variation in the viability of the first stage, with as few as 9.7% of the nauplius I stages being active up to a maximum of 95% under the same conditions. The counting of eggs which appear to be normal and assuming that they will all hatch and subsequently release viable naupliar stages, does not therefore seem to be a valid assumption.

The effect of constantly changing abiotic factors such as temperature and salinity as occurs in the natural environment, on the hatching ability and success of sea lice is also relatively unknown. Again, there are a few reports on such environmental effects on the production of the first naupliar stage of sea lice, but the data they herald is limited.

The relationship between temperature and hatching has been studied but this seems to have been limited mainly to the effects upon the duration of the hatching period (Johannessen, 1978; Wootten *et al.*, 1982; Johnson & Albright, 1991). Pike *et al.* (1993) however found that temperature had no significant effect upon the overall hatching success of *C. elongatus* ovisacs but unfortunately no information was provided concerning the effects upon the viability of the subsequent nauplius I stage.

Salinity was demonstrated by Johnson & Albright (1993) to have a pronounced effect upon the hatching success of *L. salmonis* eggs. No hatching occurred below 15‰ salinity, and it was compromised from its high levels (100%) at salinities of 25 to 30‰, when held between 15 and 20‰, falling to 70 and 78% respectively. Also, the authors

found that the percentage of nauplii exhibiting post-hatch activity decreased with falling salinity, from a mean of 65.9% in 30‰ to 0% in 15‰.

Thus, as well as there existing a natural variation in hatching success and viability of the first nauplius stage of sea lice, environmental variables act also to modify the production of this stage. It therefore seems highly unlikely that the production of viable nauplius I stages of *L. salmonis* can be estimated from the fecundity of the parasite without further information being made available concerning the ecology of hatching.

Tully & Whelan (1993) admit that the estimates of the production of *L. salmonis* nauplius I stages from fecundity is probably a poor predictor of subsequent transmission rates since it is not the infective stage. Nevertheless, the authors proceeded to state that if susceptibility to infection by the host is not variable in time and space, a correlation between the production of larvae and subsequent infection levels is "logically expected." There are a number of inherent problems however with this hypothesis.

Firstly, Tully & Whelan (1993) themselves conclude from their results that temporally varying susceptibility to infection may have a role in determining the levels of infection, and indeed, host effects (such as the immune status of the host) upon the biology of ectoparasitic copepods have already been previously documented (Shariff, 1981; Paperna & Zwerner, 1982; Woo & Shariff, 1990; Johnson & Albright, 1992a, 1992b). Johnson & Albright (1992b) demonstrated that cortisol-implanted coho salmon, *Oncorhynchus kisutch*, were more susceptible to infection by *L. salmonis* than control coho salmon. Although the authors stated that their results could not be taken as proof that stress or disease-induced elevations of plasma cortisol would lead to increased susceptibility of naive coho salmon to infection with *L. salmonis*, it is clear that the assumption that there is no variability in host susceptibility to infection is highly likely to be an inaccurate one. Indeed there is a school of

thought that anthroprogenic changes (for example, pollution, flow changes) have increased the susceptibility of salmonids and other fish to disease.

Also, the conclusion of Tully & Whelan (1993), that a correlation can be made between the production of the nauplius I stage and infection levels, in turn means that there will exist a correlation between the production of the first larval stage and the subsequent numbers of the infective copepodid stage in the water column. Little work has been published on details of the development, survival and mortality of the first three larval stages, with that available tending to concentrate only upon the effect of temperature on the duration of the first two naupliar stages, and the effect of salinity upon survival. This is no doubt due to the majority of research being directed towards the biology and control of the later, more damaging, stages.

The effect of temperature on the duration of the naupliar stages has been described by Johannessen (1978), Wootten *et al.* (1982) and Johnson & Albright (1991) for *L. salmonis*, and by Hogans & Trudeau (1989) and Pike *et al.* (1993) for *C. elongatus*, all concluding that the development time is directly dependent upon temperature. Pike *et al.* (1993) also looked at the effect of temperature on survival and mortality levels, and demonstrated that low temperatures could cause higher mortalities in *C. elongatus* naupliar stages when compared to higher temperatures. From this one report it can therefore be seen that, although it is known that the duration of stages is inextricably linked to temperature, this may not be the only effect temperature has upon development, and hence it may not be possible to predict the subsequent numbers of the infective stage from the production of the first larval stage until more data is available on the response of the free swimming stages to this parameter.

Salinity has also been shown briefly to have an effect upon the development and survival of the larval stages of sea lice. Berger (1970), Johannessen (1978) and Johnson & Albright (1991) all demonstrated that exposure to low salinity water caused a decrease in survival of the larval stages of *L. salmonis*. Johnson & Albright (1991) stated that it may be possible that *L. salmonis* may be excluded from low salinity areas (of less than 15‰) due to the reduced hatching success and subsequent survival of the naupliar and copepodid stages. In the natural environment however environmental parameters such as salinity are rarely constant, but instead fluctuate. There is no data available though upon the effects of constantly changing salinities on the development and survival of free swimming larval stages of sea lice, or upon the effect of duration of exposure to low salinities. Until such gaps in the literature are filled by future investigations, it again seems implausible that predictions of future numbers of copepodids can be made from estimates of the production of nauplius I stages of *L. salmonis*.

It is clear that, if a prediction is to be made of future numbers reaching the infective stage, the effect of abiotic factors upon the development, survival and mortality of the first two naupliar stages as well as the copepodid must be studied. At present there are only two reports available on the developmental success to the copepodid stage in *L. salmonis* (Johnson & Albright, 1991; Johnson *et al.*, 1993), and one for *C. elongatus* (Pike *et al.*, 1993).

Johnson & Albright (1991) found that virtually no active copepodids (less than 0.01%) were obtained from egg strings kept in salinities below 30‰ at 10°C. They found that an average of 35.2% of eggs within an ovisac became active copepodids when held in static water and 26.8% in flowing water at a salinity of 30‰. However, the variation in this figure observed was high, with the ranges of these two means being 0 to 80.6% and 0 to

59.5% respectively. Johnson *et al.* (1993) found that on average, 16% of eggs successfully developed to the copepodid stage, but again a high variation was seen to exist as this ranged from 0 to 47%. However, Johnson *et al.* (1993) did not clarify whether this figure was for active copepodids or for all copepodids obtained, whether alive or dead.

Pike *et al.* (1993) demonstrated a considerably higher developmental success for the infective stage for *C. elongatus*. The authors state that the survival rate of nauplii to the copepodid stage was 90% at 15°C (approximately 76.5% of the total number of eggs within the ovisacs), with this falling to 60% (approximately 51.0% of eggs within ovisacs) at 5°C.

The developmental success of the infective stage of sea lice can therefore be seen, not only to be highly variable for *L. salmonis*, but also dependent upon salinity and temperature for *L. salmonis* and *C. elongatus* respectively. Thus, for a model to work effectively in estimating future numbers of infective stages, more information must be made available on the variability of developmental success and the effect of abiotic factors, such as temperature and salinity on such development post-hatching.

Tully & Whelan (1993) admit that estimates of the abundance of copepodids in the water column rather than the production of nauplius I stages, would be a better predictor of subsequent transmission rates of *L. salmonis*. There has been no published research however, that has investigated the relationship between the numbers of copepodids present and the subsequent infection rate of the parasite on the host. It cannot be assumed that once the copepodid stage has been reached, 100% infection will occur. According to Johannessen (1978), the time of development of *L. salmonis* will give information upon the timespan from hatching to when a theoretical infection with copepodids may occur. Data on the effects of parameters such as salinity on the longevity of the copepodid stage is therefore important.

Previous reports on the survival of the infective stage are brief. Johannessen (1978) stated that the copepodid of *L. salmonis* could live for up to one month if a suitable host is not available, and hence be "of a danger" for a long time. Wootten *et al.* (1982) found that the infective stage of the parasite remained active for only 4 days at 12°C, and Johnson & Albright (1991) demonstrated that maximum survival was 17 days at 10°C in 25‰ salinity but on average it ranged from 2 to 8 days. When newly hatched copepodids were transferred to low (5 to 10‰) salinities, survival was less than 24 hours, but was prolonged at higher salinities (15 to 30‰). Statistical analysis of their data by Johnson & Albright (1991) showed that the survival rates of the copepodid stage were affected significantly by salinity.

In summary therefore, it is evident that there remains relatively little known about the first three free swimming stages of sea lice. Information on the hatching and development of the free swimming stages would obviously help to improve the accuracy of such models as Tully & Whelan's (1993). The accurate estimation of future infection levels would be of obvious help in predicting infection parameters on fish farms, which can only be of benefit for management strategies and chemotherapeutic treatments. It may also help to identify if there are causal links between fish farming and the collapse of wild fisheries, such as that of *Salmo trutta*.

This present study was thus undertaken in an attempt to provide such data. By studying the natural processes involved in hatching, its duration, success and subsequent viability of larvae alongside the effect of abiotic factors such as temperature and salinity further information will be made available regarding the production of the first nauplius stage. Data on the subsequent development, survival and mortality of the naupliar and copepodid stages will also hopefully help in the understanding of what occurs in the window

between nauplius I production and the subsequent transmission rates of the infective copepodid stage. Such information may help to explain some of the variations observed in infection parameters and patterns on fish farms.

3.2. Materials and Methods

3.2.1. General Maintenance of Lice

L. salmonis gravid females were collected according to the procedures described in Chapter 2, section 2.2.1. Once transported to the laboratory, lice were removed from the plastic bags and placed into round 4 litre containers. This was achieved by cutting the corners of the bags and filtering the contents through a 60μm filter. Lice were either contained by the filter or remained attached to the surface of the plastic bag. They were then either removed by forceps from the filter or by sliding a scalpel blade underneath the lice and lifting them from the bag. They were then placed into the containers with twice filtered (through a 60μm filter) fresh seawater obtained from the site of origin, the water being gently aerated by the use of an air stone, and kept at 10°C in a constant temperature room. Often egg strings became detached from the females. Where this occurred, the egg strings were kept in a separate container from gravid females, but under the same conditions. Water was changed daily and replaced with twice filtered seawater. Lice which died in transit or due to handling were removed and their egg strings detached from the genital segment by the use of watchmakers forceps.

Once eggs had extruded from the egg strings and hatching had occurred, nauplii were removed from the large plastic containers and placed into 500ml beakers containing 400ml of seawater (full strength salinity at 10°C unless otherwise stated). This "holding" water was always filtered twice, aerated prior to use, and changed daily, unless stated otherwise. Aeration did not occur in the glass beakers since there was a tendency for larval stages to be removed from the water in vapour droplets created by the action of the air stone. Dead larvae were removed from the system daily.

In some hatching experiments, it was also necessary to hold egg strings in 500ml beakers rather than larger containers. In such cases, these procedures outlined above regarding maintenance were followed, egg strings having been removed from the adult female with watchmakers forceps.

3.2.2. Hatching of larvae

3.2.2.1. Observations on hatching

Whole egg strings containing pigmented eggs were observed for hatching in a petri-dish containing full strength (35‰) seawater under a dissecting microscope (Olympus SZ30) at x40 magnification. The egg strings had been removed from gravid females by a pair of watchmakers forceps to allow constant observation. Live females show frequent swimming motions and these would naturally cause interruption of observations. In egg strings with hatching eggs, individual eggs were removed from the ovisac by the use of a pair of fine watchmakers forceps and a pipette. These individual eggs were then placed in a welled microscope slide containing full strength seawater, and hatching observed at x100 magnification in a welled microscope slide (volume of well = 1ml) under a compound microscope (Olympus CH2). Egg strings that were seen to be hatching in some areas but not in others, were cut in non-hatching regions with a scalpel blade and examined under x100 magnification, in order to observe the initiation of the hatching process.

3.2.2.2 Effect of environmental conditions on hatching

i. Temperature

In order to look at the effect of temperature upon hatching, 30 gravid females (60 egg strings in total) were divided into groups of 10 and placed in one of three temperatures, 5, 7.5 and 10°C (20 egg strings in each temperature). Each gravid female was individually

placed in a 500ml glass beaker containing full strength seawater. The individual gravid females were then monitored daily and the number of live or dead nauplii hatched each day recorded and then removed from the system. The percentage daily hatch (number of eggs hatched in each 24 hour period as a percentage of the total number of eggs contained in each individual egg string) was then calculated, along with the duration of the hatching period.

ii. Salinity

80 gravid females (160 egg strings) were placed in one of eight salinities, 0, 5, 10, 15, 20, 25, 30 or 35‰, 10 gravid females in each. Each gravid female was placed in a 500ml glass beaker containing one of the test salinities and the lice held at 10°C. Again, the egg strings were monitored daily, with the number of dead or alive nauplii being counted and removed from the system. The percentage daily hatch and the overall hatching duration was again calculated.

3.2.2.3. Photoperiod

i. Control experiments

In order to determine whether both egg strings of a pair possessed the same inherent hatching pattern, the following procedure was used. Pairs of pigmented egg strings were removed from 20 gravid females (40 egg strings) and each half of the pair was placed into a separate 500ml glass beaker containing full strength seawater at 10°C. Each pair of egg strings was examined to check they both had approximately the same number of viable and non-viable eggs. Both egg strings of a pair were then kept under the same light conditions, ten of the pairs being held in one constant light regime, and the other ten being held in 23 hours of darkness and 1 hour of light daily. This provided the ideal control, as both egg strings of a pair are of the same age, have the same genetic origin and have the same past

and developmental conditions. The total number of eggs per string was recorded, and then each ovisac was monitored daily, with the number of dead or live nauplii hatched every 24 hours being recorded and removed from the system. The daily hatching rate and duration was then calculated for each group.

ii. Experimental procedure

In order to see if egg strings demonstrate different patterns of hatching under different environmental regimes, or if hatching is initiated by light or by dark conditions, egg strings within a pair were again separated and placed in 500ml glass beakers containing full strength seawater at 10°C. 12 pairs (24 egg strings) were used. However, this time one half of each pair was placed in a different light regime to the other, as opposed to section 3.2.1.3 (i) where egg string pairs were separated but kept in the same light regime. The following four regimes were tested:

	Left Egg String	Right Egg String
Photoperiod Regime	23L:1D (n=12)	1L:23D (n=12)
Photoperiod Regime	24L:0D (n=12)	1L:23D (n=12) *

* It was not possible to examine the daily rate of hatching in egg string kept in complete darkness, because the use of a dissecting microscope and light was necessary to record the number of larvae hatched daily.

The total number of eggs in each egg string was counted, and again the number of live or dead nauplii hatched were recorded every 24 hours, with any hatched larvae being removed from the system. The daily hatching rate and duration were then calculated.

To see if complete darkness had an effect upon hatching, 20 egg strings were removed from 10 gravid females, with one half of each pair (the left egg string) being held in constant light, and the other (the right egg string) placed in constant darkness. The egg strings kept in constant light were monitored daily and hatched larvae removed from the system, until no more hatching was seen to occur. The egg strings kept in complete darkness were then examined, with the total percentage hatch being calculated for both regimes.

3.2.2.4. Natural variation in hatching success and duration

In the light of the results from these experiments, the hatching pattern and variation of a larger group of egg strings was observed in order to assess the daily hatching rate, overall hatching success and duration of hatching of *L. salmonis* egg strings. The total number of eggs in each of 64 egg strings (32 pairs) showing areas containing mature eggs was recorded. Each individual gravid female was kept in a 500ml glass beaker in constant light at 10°C in 35‰ salinity. Hatching was monitored daily, with the number of hatched live and dead nauplii were recorded every 24 hours and subsequently removed from the system. The daily hatching rate, the final hatching success (number of eggs hatched as a percentage of the total number of eggs) and the duration over which hatching was observed to occur was calculated.

3.2.3. Development of larvae

3.2.3.1. Effects of environmental conditions upon development

i. Temperature

30 gravid females with mature egg strings were each placed in a 500ml glass beaker containing full strength seawater. After counting they were then immediately transferred to

one of three temperatures, either 5, 7.5 or 10°C, 10 females in each. The gravid females held at 5 and 7.5°C were held in incubators and the females held at 10°C were kept in a constant temperature room. Each beaker was monitored daily, and after the first 24 hours any larval stages that had hatched were removed from the "maternal" container. Live stages were placed into a separate "holding" container, and dead stages were removed from the system and disposed of. After another 24 hours (48 hours from when experiment commenced), any live larvae that had hatched since the last examination (24 hours from commencement) were again counted and removed from the "maternal" container and placed into a new separate holding container and any dead larvae again removed from the system. The age of the larvae was thus known, as their maximum age when removed to the holding container was 24 hours.

The live larvae that were removed the previous day (24 hours) were checked for any mortalities and moulting that had occurred. A sub-sample of 25 live larvae was examined from the holding container and the stage recorded. This procedure for following hatching and of recording the larvae was continued every 24 hours in all the "maternal" and "holding" containers until no more hatching occurred in any of the 30 "maternal" containers and there were no more live larvae left in any of the "holding" beakers.

ii. Salinity

a. Progression of development

The above procedure was repeated for the examination of the effect of salinity upon development. 40 gravid females with mature egg strings were each placed into 500ml glass beakers containing water at one of eight test salinities, 0, 5, 10, 15, 20, 25, 30 or 35‰ at 10°C. Newly hatched larvae were transferred from the maternal containers into separate

holding beakers every 24 hours. Again, every 24 hours, a sub-sample of 25 live stages were removed from each of the holding containers and the stage of each recorded. Dead stages were removed from the system.

b. Ontogenetic variation to salinity

To test if the copepodid stage was more tolerant of salinity than the earlier naupliar stages, mature egg strings were placed in 500ml glass beakers containing full strength seawater at 10°C and development allowed to proceed to the copepodid stage. Immediately this had been reached, the copepodids were removed from this salinity and were placed in 500ml glass beakers containing water at one of the following salinities, either 0, 5, 10, 15, 20, 25 or 30‰, with 100 copepodids being placed in each. 100 copepodids were left in the full strength seawater as controls. All copepodids were held at 10°C. The copepodids were then monitored every 24 hours and the number alive and dead in each salinity recorded.

3.3. Results

3.3.1. Hatching of larvae

3.3.1.1. Observations on hatching

Egg strings of *L. salmonis* were observed to demonstrate a progression of development, with undifferentiated material closer to the oviduct opening, whilst at the distal end of the egg string, differentiation of eggs and pigmentation of the resultant "enclosed" nauplius was observed. All eggs in the ovisac were orientated in the same direction. The anterior end of each nauplius was orientated towards the outer side, rather than the medial side of the ovisac.

Individual eggs separated from the egg strings and observed under x100 magnification in full strength seawater (35‰ salinity) were seen to hatch. Mature pigmented nauplii within the egg membranes were observed to exhibit agitated movements of their body and of their appendages. The movement of the bodies of the nauplii were in the form of longitudinal contractions and relaxations. This movement of the body was observed to be the first motions performed when the nauplius became active within the egg membrane, followed, shortly after, by the twitching of the appendages. Despite these twitching movements of the appendages which appeared to be synchronous in conjunction with the body movements, the appendages appeared to be held down at either side of the body. However, it could not be determined as to whether the cuticle of the body was joined or fused with the appendages. The balancers at the posterior end of the body were seen to be held in a position perpendicular to the length of the body, rather than in line with the long axis of the body, as is seen when the nauplii are free swimming (fig. 3.1).

Fig. 3.1. *Lepeophtheirus salmonis* nauplius I within the egg membrane. Balancers (b) are held at a perpendicular angle to the body of the nauplius whilst within the egg. Appendages are held at the side of the body. Scale bar = 100µm

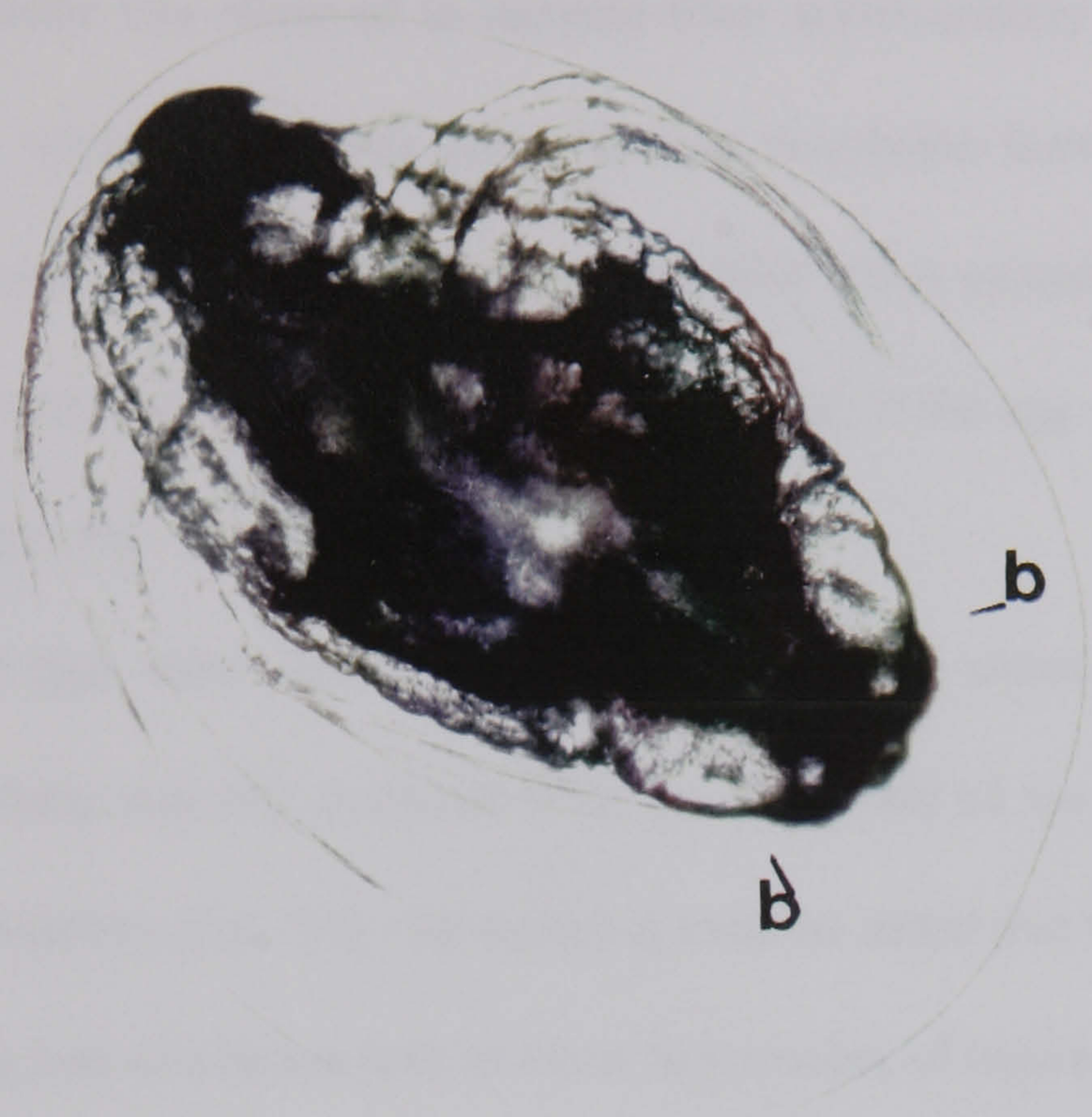
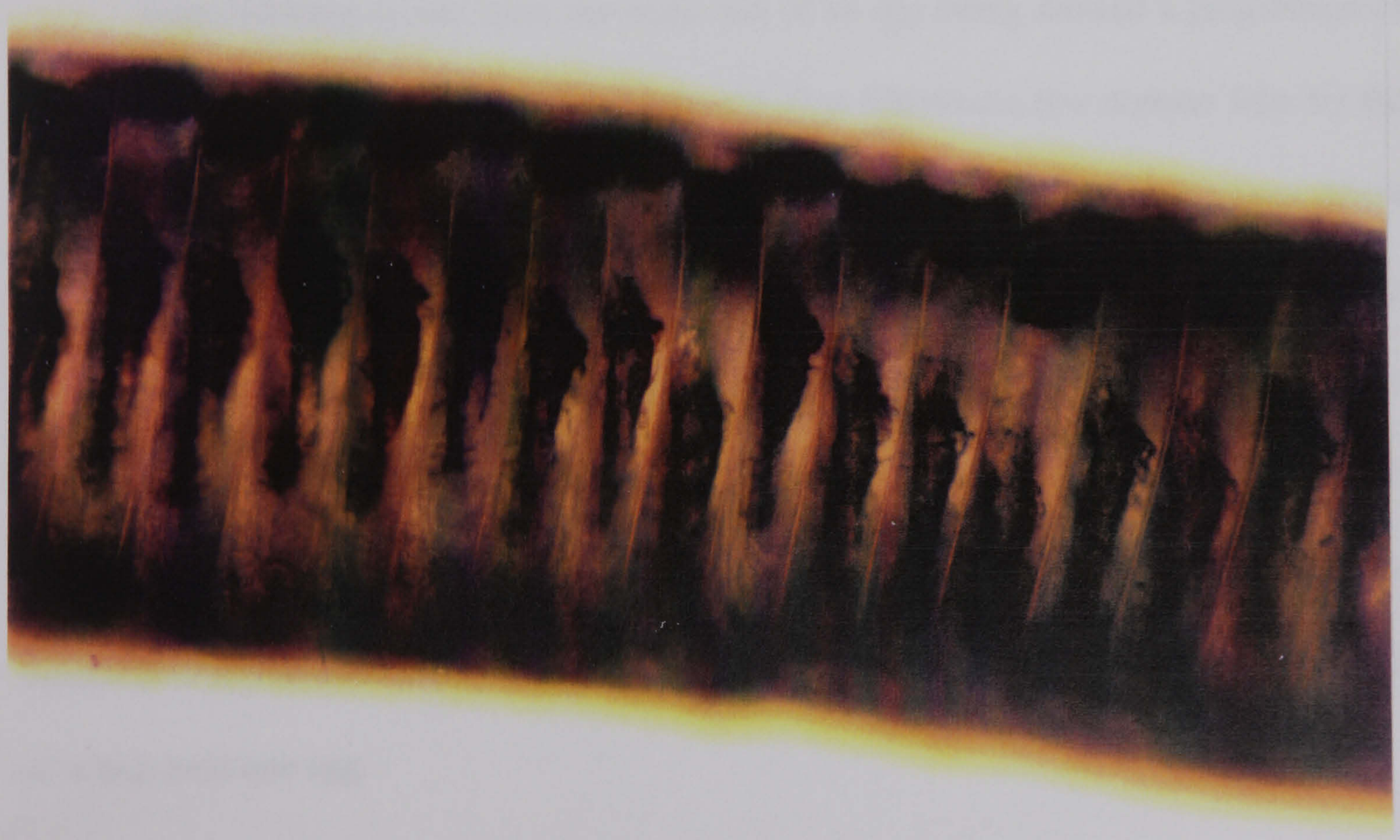


Fig. 3.2. (a) *Lepeophtheirus salmonis* eggs within the egg string. Due to compression, the eggs take on a coin shaped appearence (x 100 magnification).

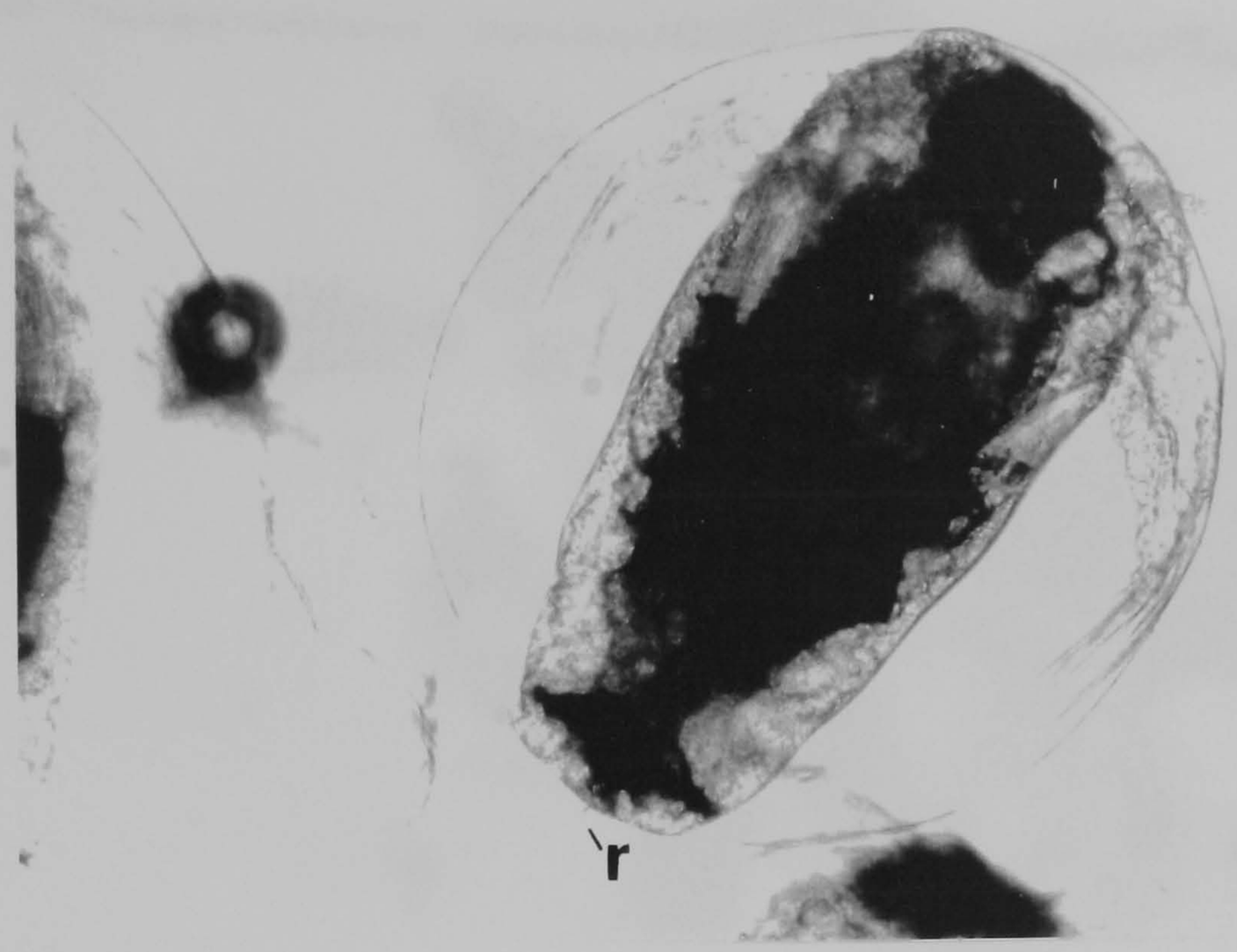


Eggs removed from the ovisac prior to hatching were seen to swell laterally, causing the egg to changed from a "coin shaped" flattened structure (fig. 3.2) to a spherical egg (fig. 3.3a). The diameter was observed to increase from approximately 65.53μm to 455.85μm before hatching occurred at which point the egg membrane burst. Although only one membrane was ever observed to surround the nauplius, there appeared to be the remains of another, outer, membrane attached to the posterior end of the egg membrane surrounding the nauplius (fig. 3.3b).

Where eggs were observed hatching whilst still in association with the maternal egg string, hatching typically occurred from the distal end of the egg string, proceeding proximally in sequence (fig. 3.4). However, it must be stated that this was not always the case, as hatching was sometimes seen to occur in a number of regions along the length of an egg string at any one time (fig. 3.5). This was usually in conjunction with sacs that contained areas of eggs containing mature larvae that were interspersed with large areas of undifferentiated ova.

Eggs hatching *in situ* from the distal end of an egg string showed a progression of swelling, with the terminal egg increasing in size first followed a few minutes later by the next adjacent egg. This tended to cause a collection of hatching eggs at the terminus where the ovisac had split with eggs subsequently hatching within the ovisac. The terminal eggs remained attached to the ovisac by some method (fig. 3.6a). Fig. 3.6b shows the presence of transverse membranes within the ovisac. These membranes were usually observed post-hatching, but were occasionally seen in egg strings that were not fully filled with eggs. The membranes appeared to separate the egg string at regular intervals into compartments, each of which held one egg.

Fig. 3.3. (a) *Lepeophtheirus salmonis* egg after release from the egg string. The egg has swollen due to an intake of water, allowing it to become spherical. Note small remnant of membrane material at posterior apex of the egg membrane (r). Scale bar = 100μm.



(b) Membrane-like remnant (r) attached to the posterior apex of the egg membrane surrounding an *Lepeophtheirus salmonis* nauplius I stage. Scale bar = 100μm.

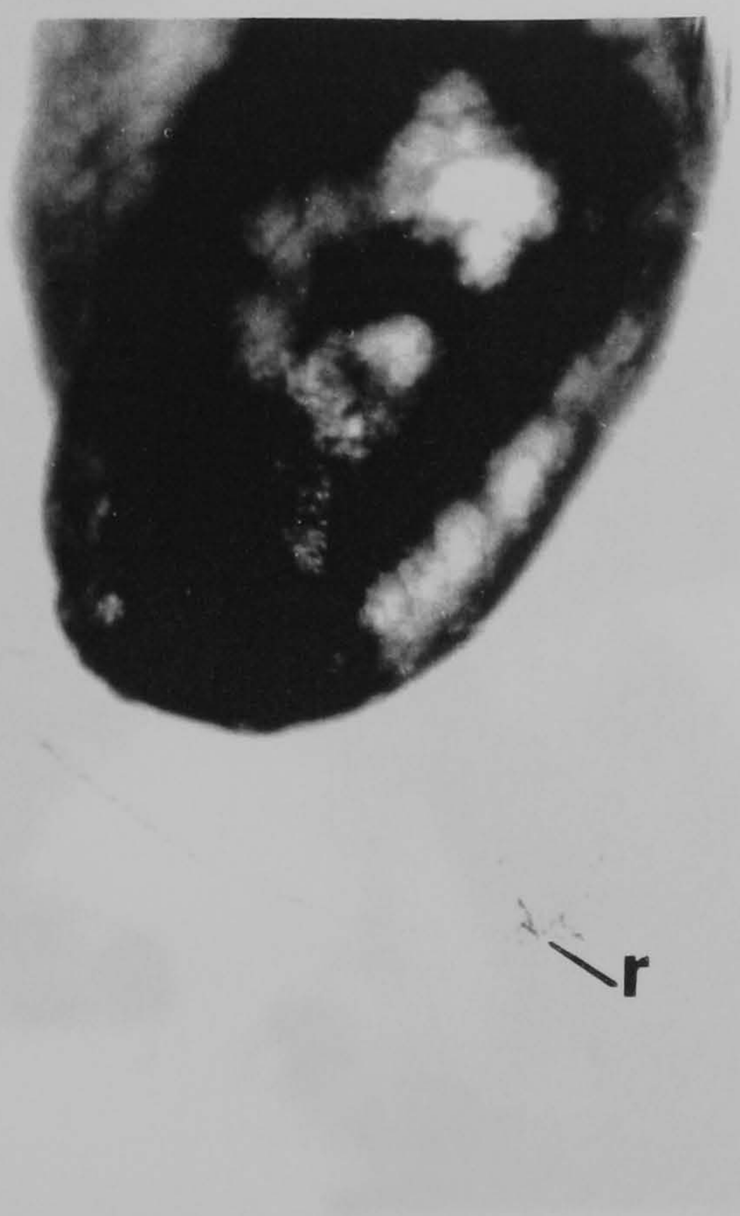


Fig. 3.4. *Lepeophtheirus salmonis* egg string hatching proximally in sequence (x40 magnification).

Fig. 3.5. *Lepeophtheirus salmonis* eggs hatching from an egg string in a number of different places (x40 magnification).

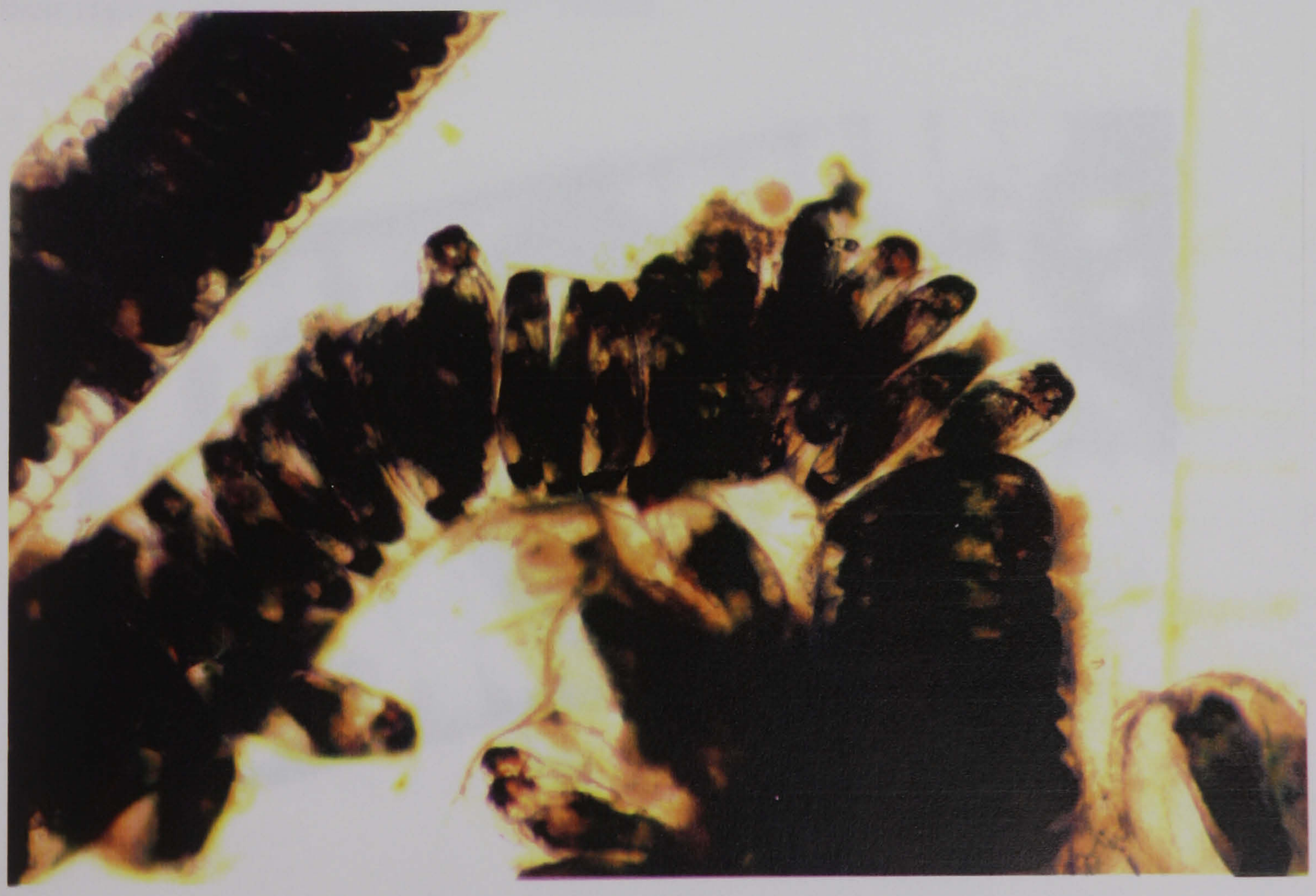
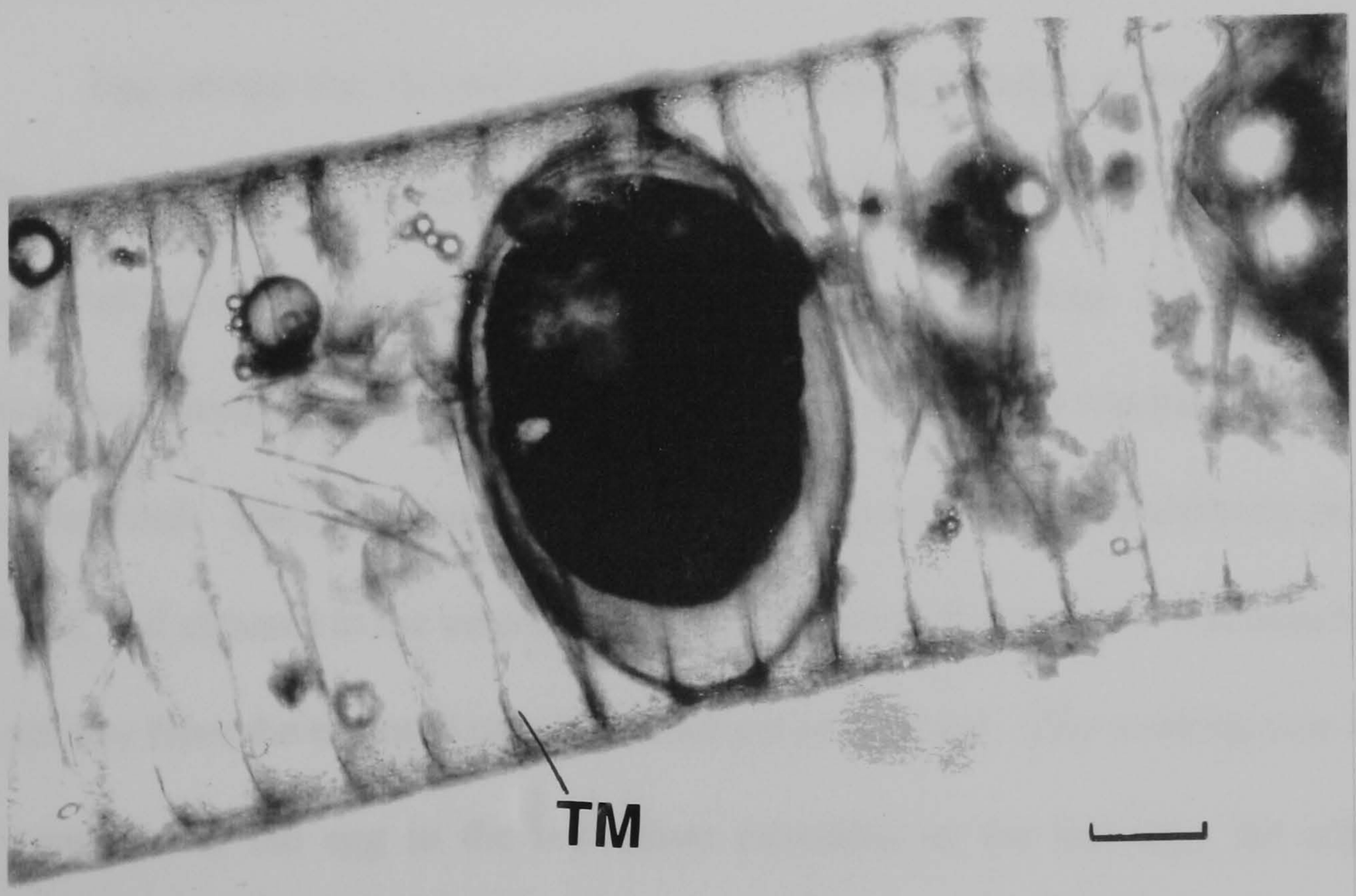


Fig. 3.6. (a) *Lepeophtheirus salmonis* eggs swelling and hatching within an egg string. Scale bar = 100μm. Eggs appeared to remain attached to the egg sac membrane due to some adhesive mechanism.

(b) Transverse membranes (tm) within an *Lepeophtheirus salmonis* egg string. Note their regular appearance. Scale bar = 100μm.



There were however eggs that were observed not to swell to such an extent as those containing live active nauplii. Such eggs would contain an abnormally shaped nauplius. These tended to be one of two shapes, either a kidney-shaped curved nauplius, or, a rounded spherical (compared to the normal elongate larvae) shape, with the cuticle appearing shrivelled rather than the smooth appearance of a normal nauplius. The nauplii showing these characteristics did not exhibit any movement within the egg membrane and appeared dead (fig. 3.7).

In those eggs either removed from the ovisac prior to hatching, or hatching at the very distal end of the egg sac (that is, those that first hatch, and are not obscured by other eggs) the egg membrane was observed to suddenly burst, with the resulting live nauplius being "catapulted" out of the membrane head first. The nauplii did not remain attached to the membrane, but instead became immediately clear of it after it burst. Nauplii that hatched from eggs still contained within the egg string did not immediately move into the external environment, but took a few minutes before actively working their way out of the ovisac by the usage of their appendages.

Egg strings that showed mature nauplii starting to hatch at the distal end were cut with a scalpel blade in regions containing larvae with a pigmented mature appearance, but which had not yet shown any internal movement, or any swelling. Upon severance of the ovisac, no immediate activity within the egg membrane or any swelling of the membrane was observed. Thirty minutes later, slight convexing of the egg immediately present at the cut end, and exposed to the external medium (full strength seawater), was observed, though no activity from the enclosed nauplius could yet be detected. This swelling was observed to be restricted to the egg in the immediate proximity to the seawater, the adjacent eggs

Fig. 3.7. (a) Abnormally shaped *Lepeophtheirus salmonis* nauplius I stage. This kidney shape was regularly seen in nauplii that were dead after hatching. Scale bar = 100µm.

(b) Dead nauplius I stage just released from the egg membrane. The wrinkled appearance of the cuticle (c) was often observed in dead nauplii, especially those exposed to low salinity water. Scale bar = 100µm.

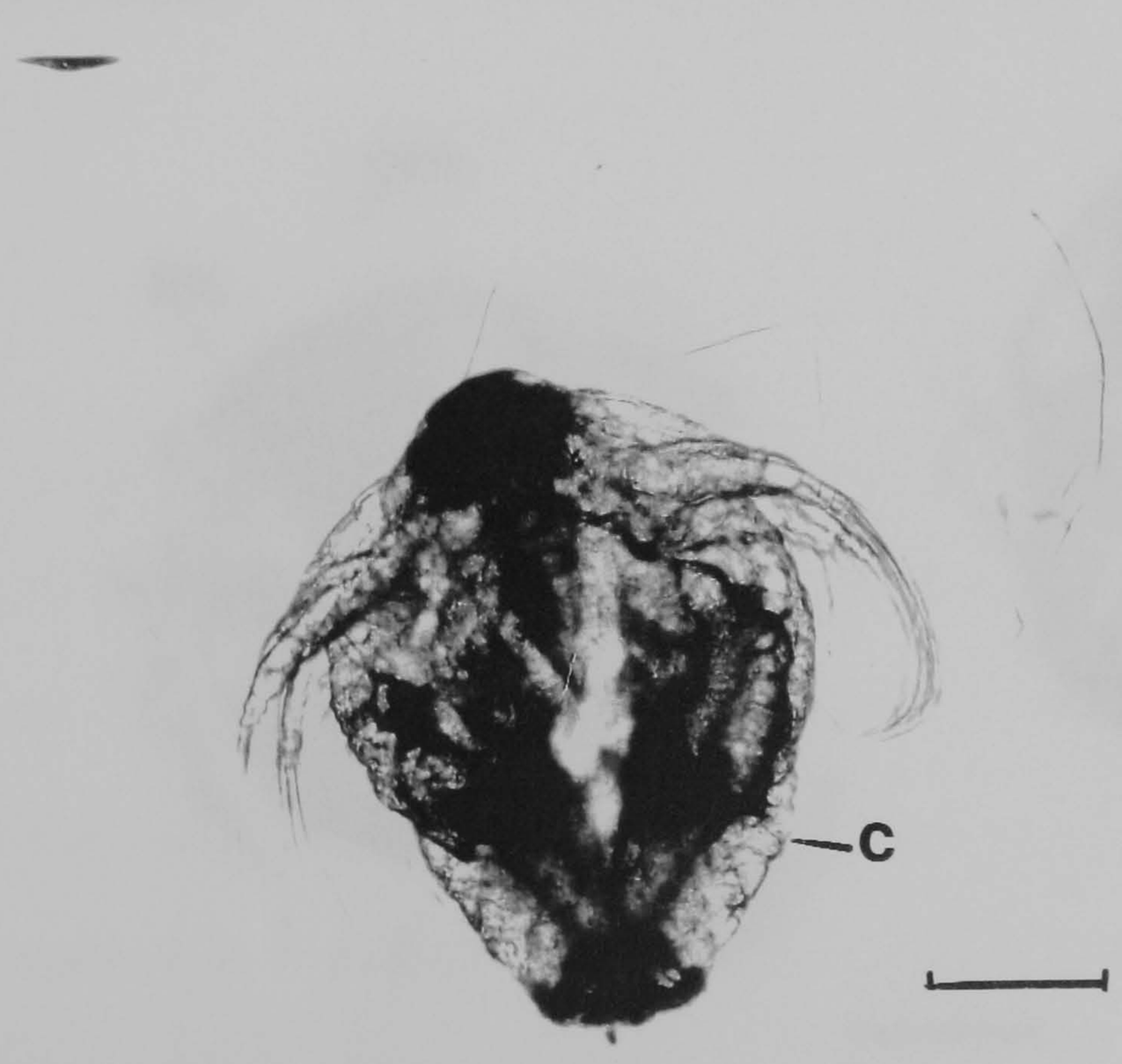


Fig. 3.7. (c) Normal appearance of an *Lepeophtheirus salmonis* nauplius I stage immediately post-hatching. Scale bar = 100μm.

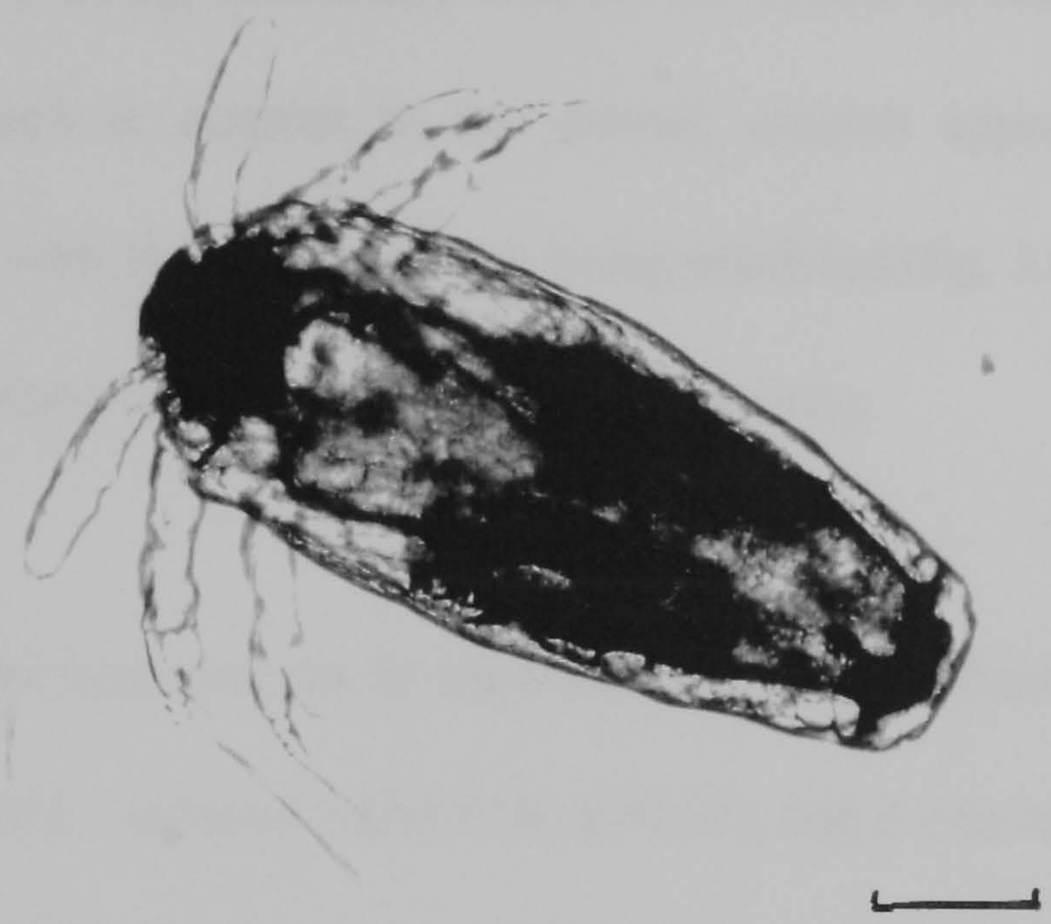
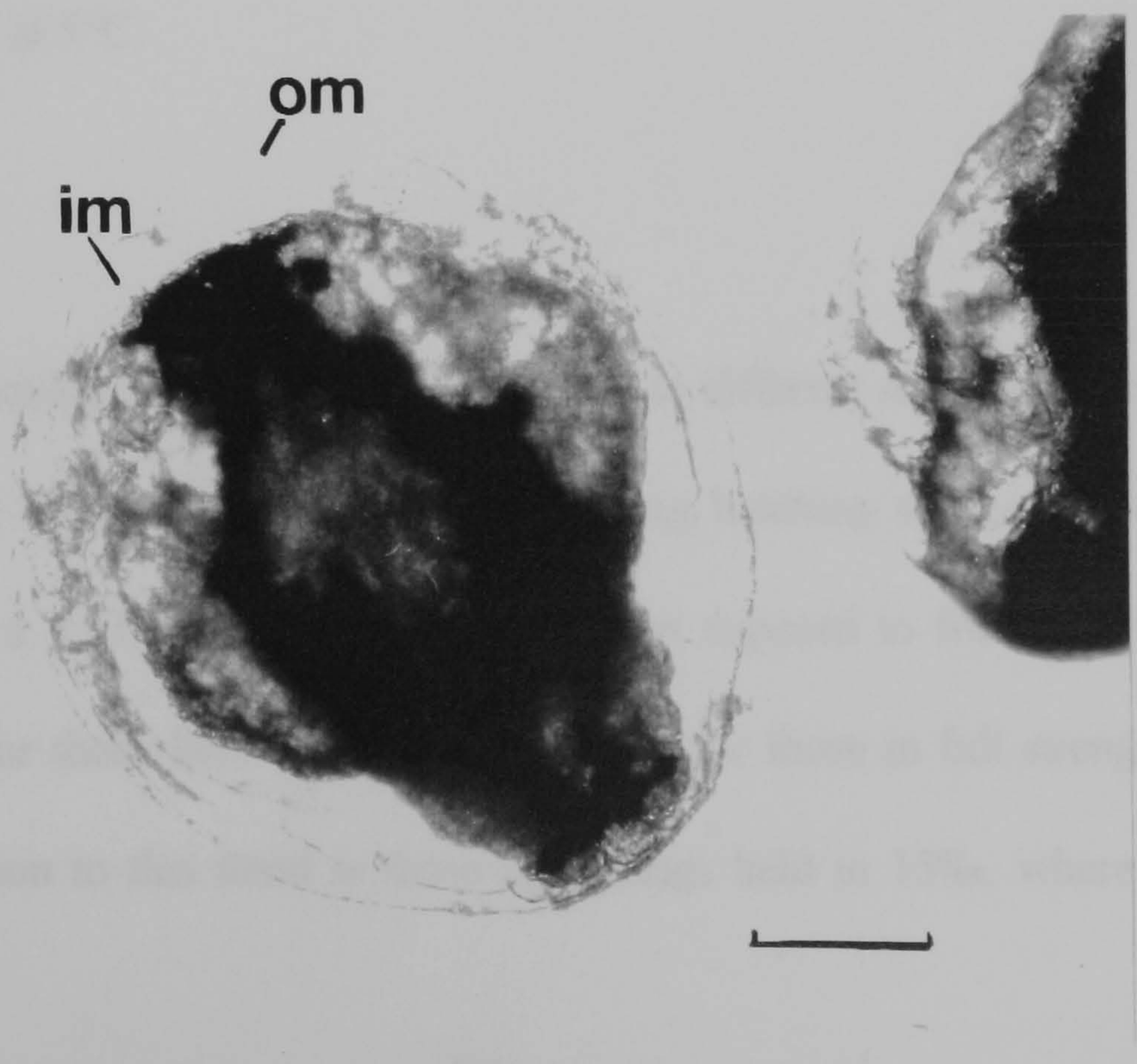


Fig. 3.8. *Lepeophtheirus salmonis* nauplius I stage surrounded by an inner (im) and outer (om) egg membrane. Two membranes were only ever observed in eggs exposed to the external environment prior to natural hatching by severing the egg string. Nauplii released from these eggs tended to be dead, having taken on a swollen and wrinkled appearance. Scale bar = 100μm.



showing no sign of change. This egg hatched approximately forty minutes after the initial cut, though the shape of the extruded nauplius was abnormal. It was a more rounded spherical shape than the normal elongate nauplii released during the natural hatching process. As well as being abnormally shaped, the cuticle of these nauplii had a crinkled, shrivelled appearance in contrast to the normal, smooth appearance. Generally, these nauplii were dead, with no movement ever being exhibited (fig. 3.8).

3.3.1.2. Effect of environmental conditions upon hatching

i. Temperature

Temperature was seen not to significantly affect the final overall level of hatching success (fig. 3.9) of *L. salmonis* (ANOVA, $p>0.05$), but it was seen to effect the daily rate of hatching (fig. 3.10). Egg strings held at 5°C showed a decreased rate of hatching compared to egg strings held at 7°C, with those held at 10°C exhibiting the highest daily hatching rate, these differences being significant (ANOVA, $p<0.05$). As a consequence of this, the overall hatching duration was longest for those egg strings held at 5°C, and shortest in those held at 10°C. At 10°C the hatching duration was 144 hours, increasing to 192 hours at 7°C and 240 hours at 5°C.

ii. Salinity

a. Daily hatching

The daily hatching rate of egg strings exposed to different salinities is shown in fig. 3.11 It can be seen that there is a trend of increasing hatching success with increasing salinity, rising from a 3.27% total hatch in egg strings exposed to freshwater (0‰) to a success of 86.36% for those kept in 30‰ and 80.61% for those in full strength seawater (35‰). The exception to this trend is those egg strings held in 15‰, where the rate of

Fig. 3.9. Cumulative percentage hatch of *L. salmonis* egg strings held at (a) 5°C, (b) 7.5°C and (c) 10°C.

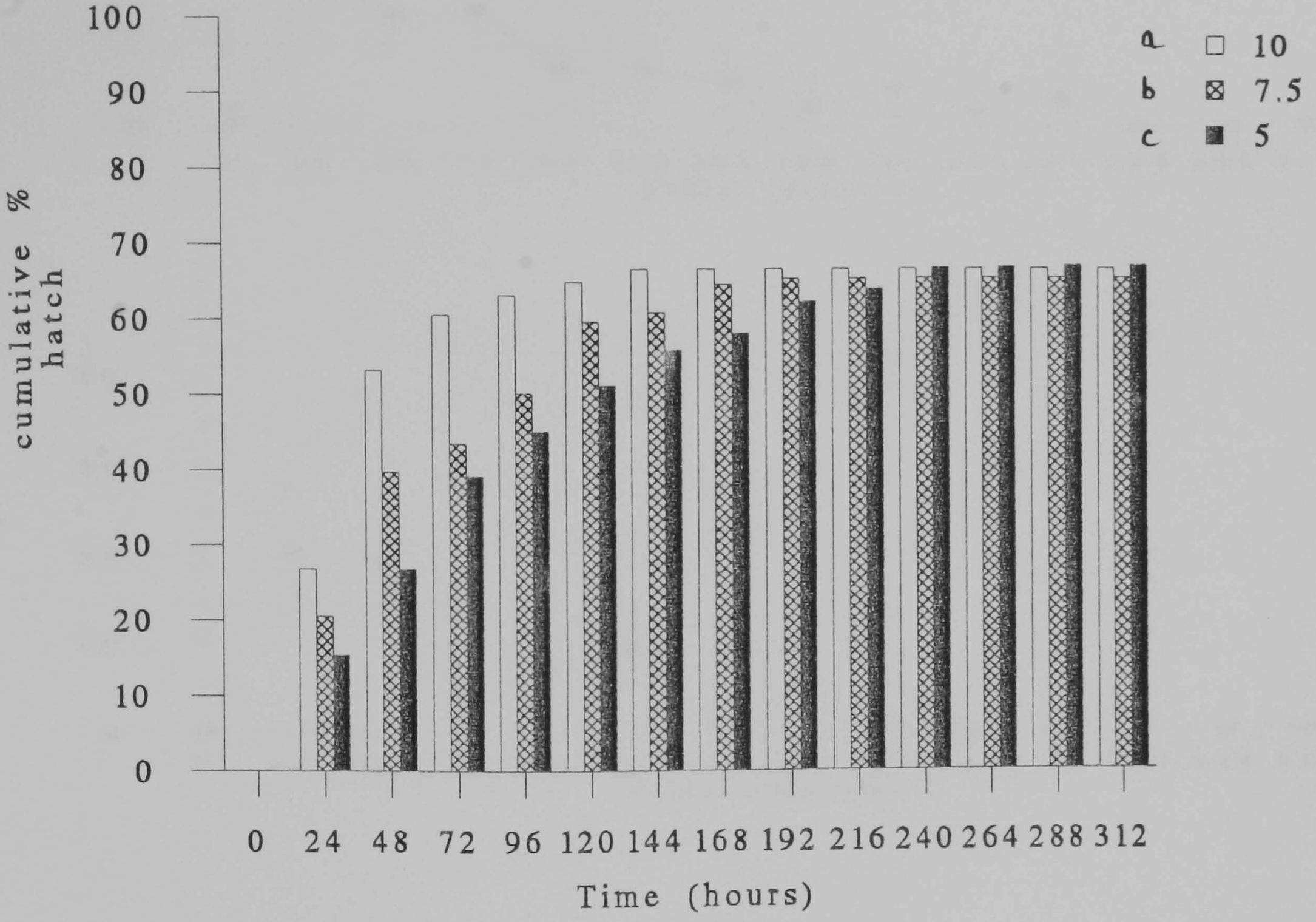


Fig. 3.10. Mean daily percentage hatch (± 1 S.E.) of *L. salmonis* egg strings held at (a) 5°C, (b) 7.5°C and (c) 10°C.

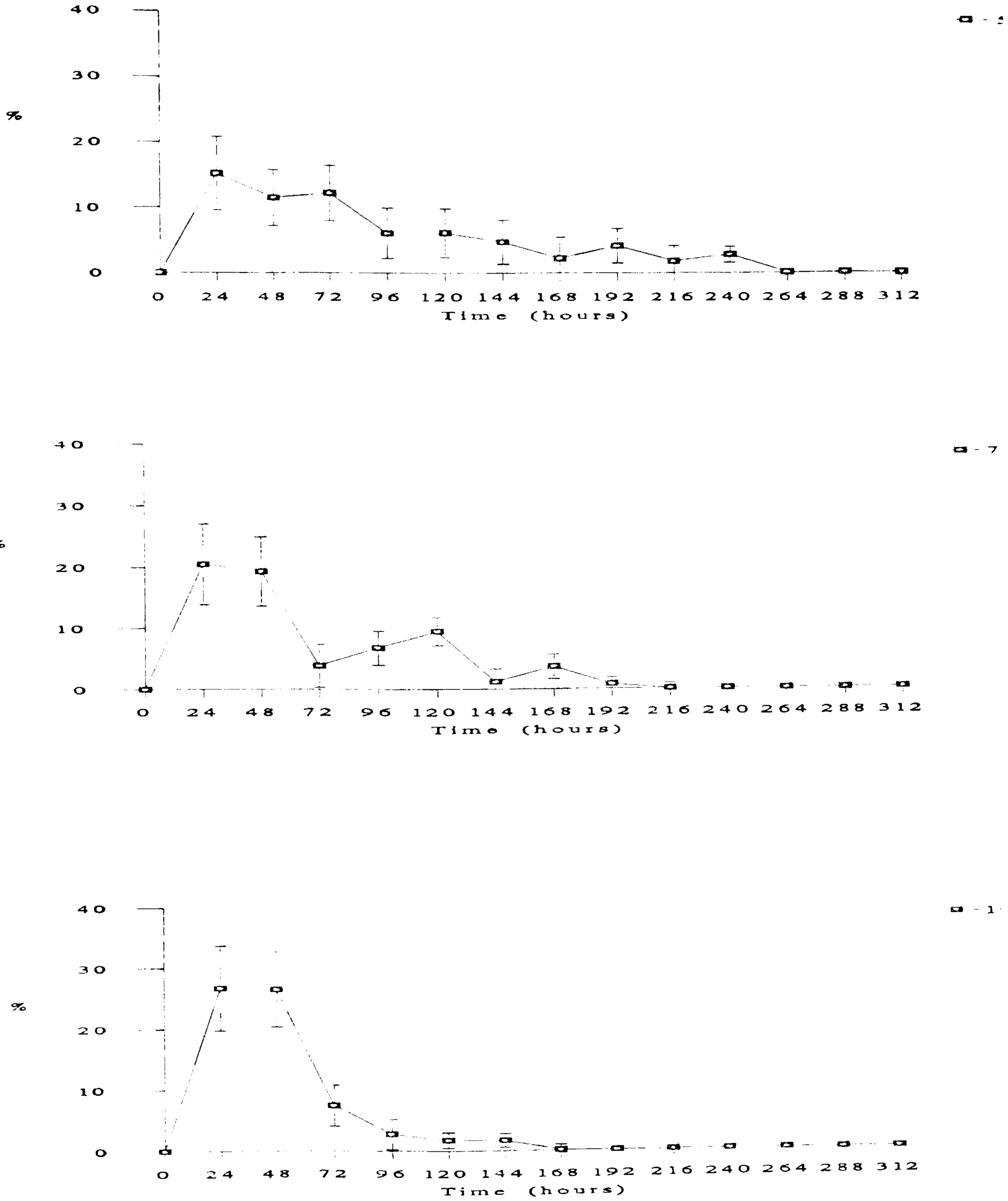
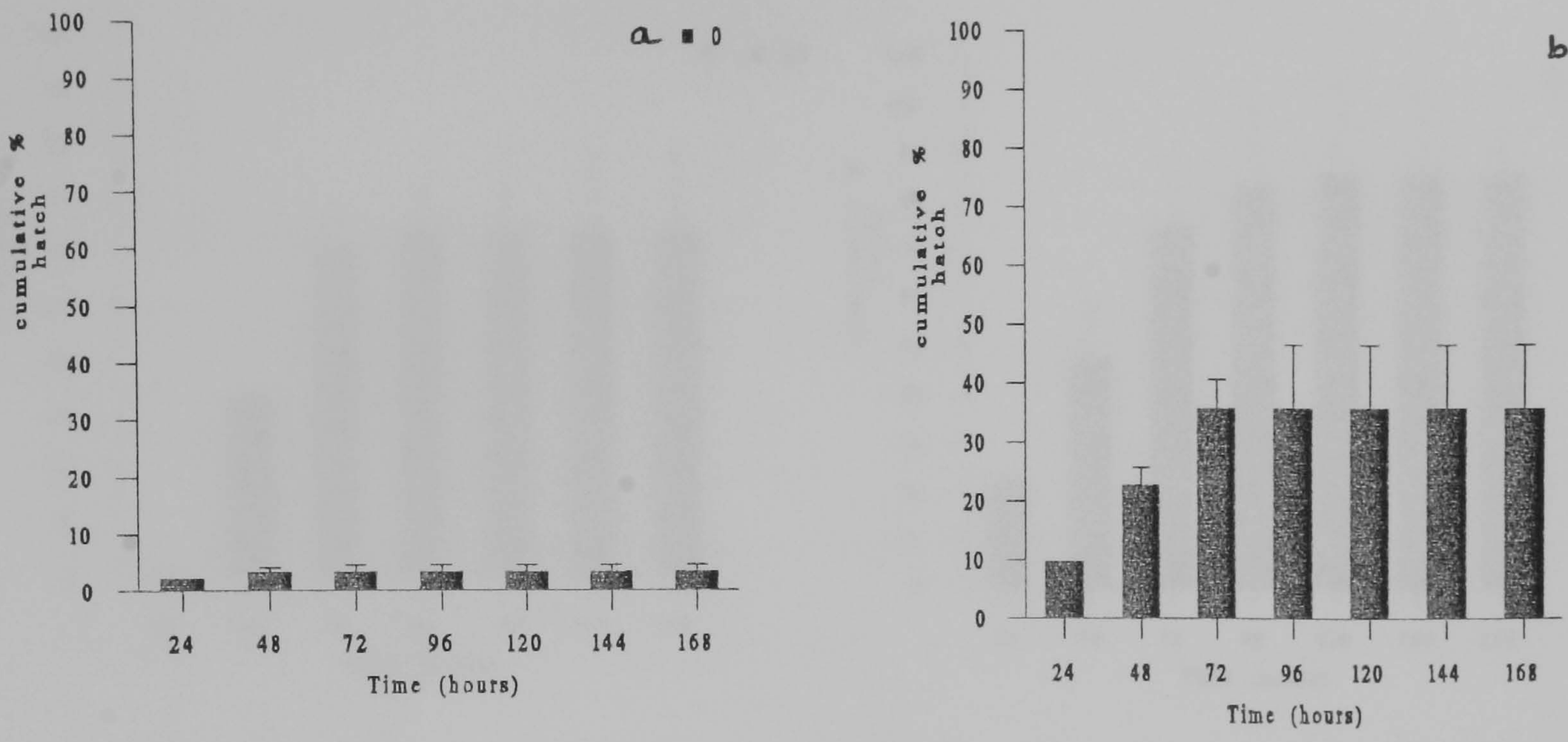
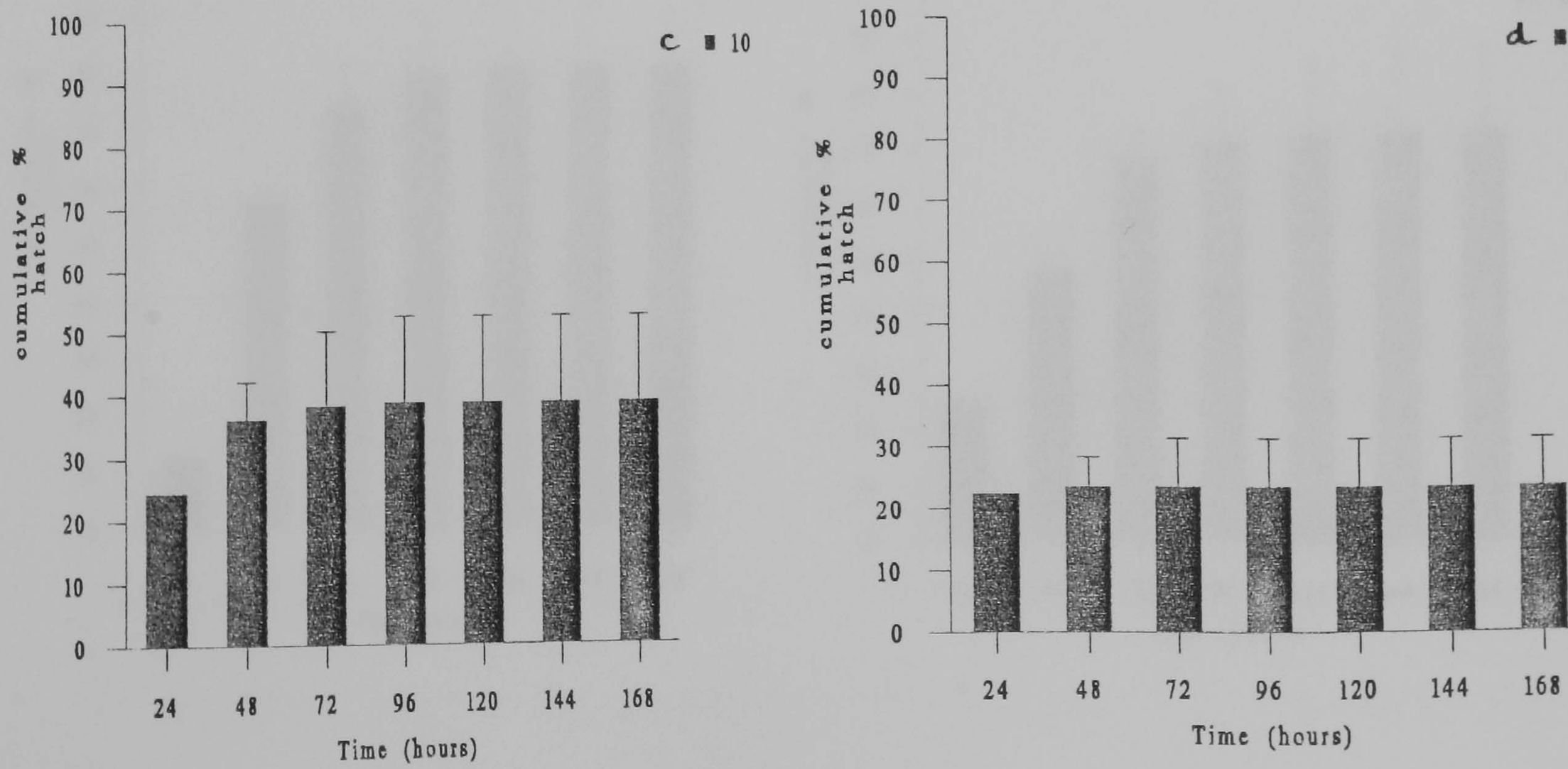
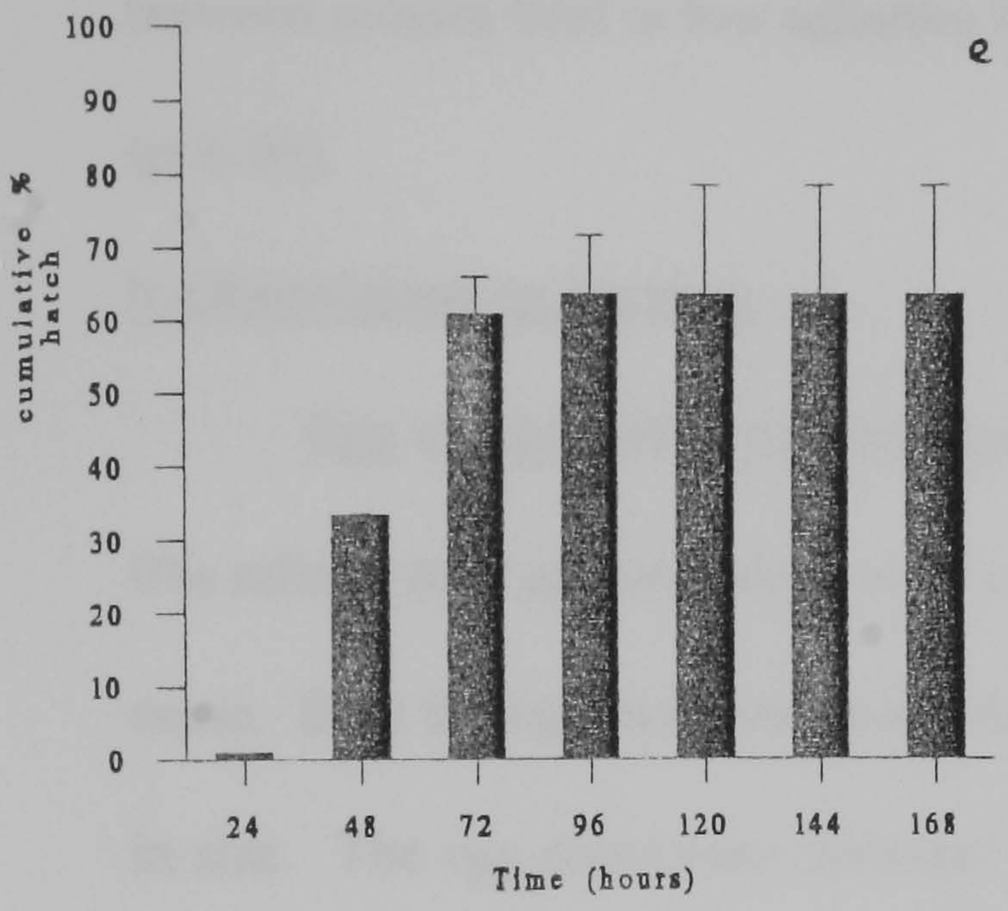


Fig. 3.11. Mean daily percentage hatch (± 1 S.E.) of *L. salmonis* egg strings held in salinities of (a) 0‰, (b) 5‰, (c) 10‰, (d) 15‰, (e) 20‰, (f) 25‰, (g) 30‰ and (h) 35‰.






e ■ 20
cumulative % hatch
100
90
80
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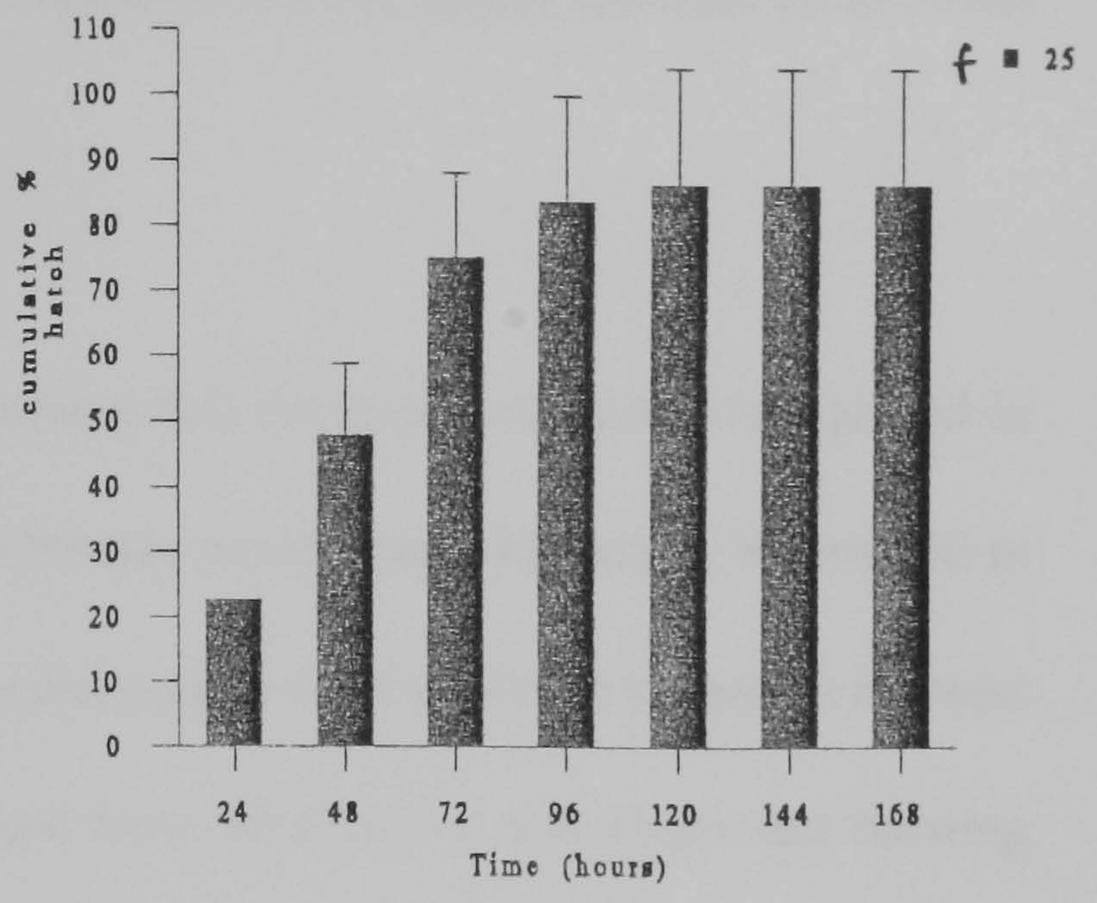
0
24
48
72
96
120
144
168
Time (hours)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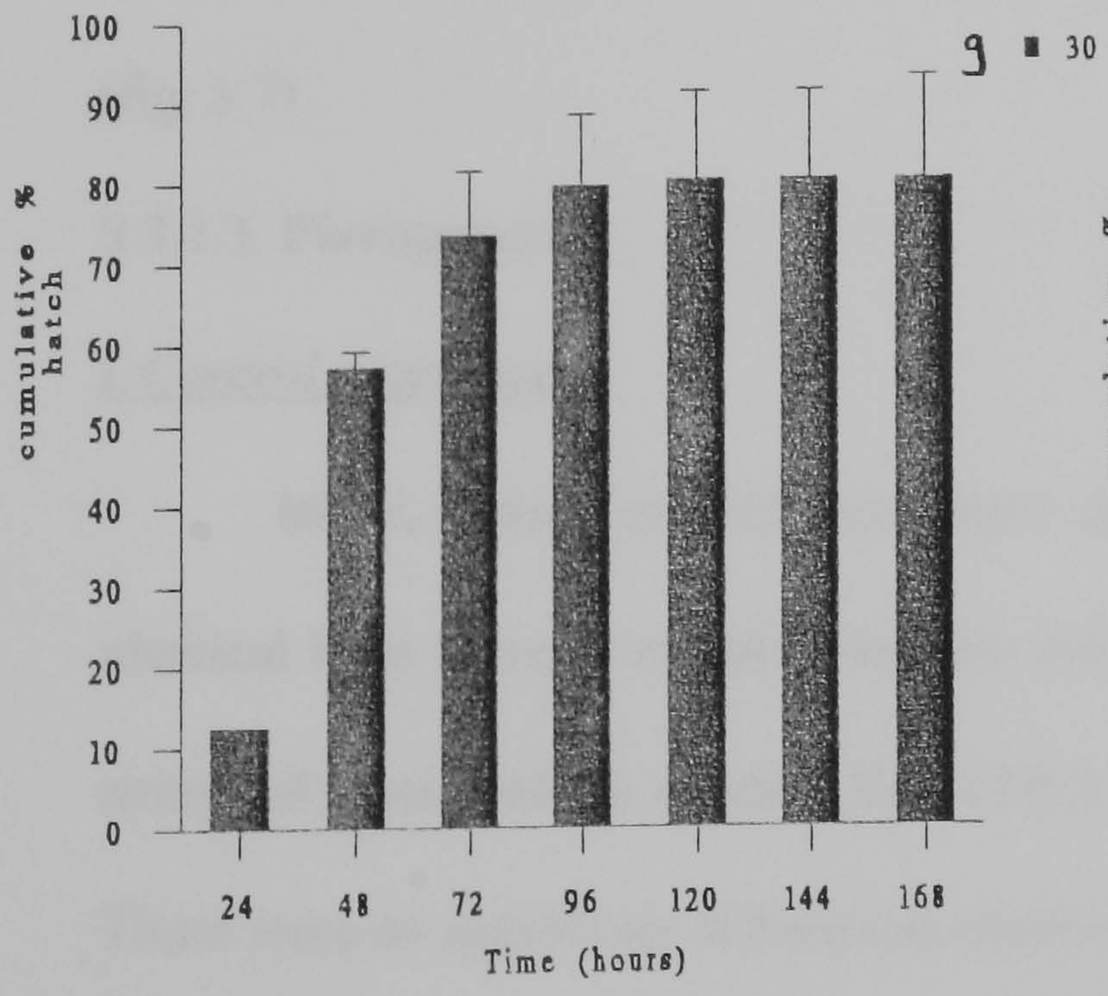
Time (hours)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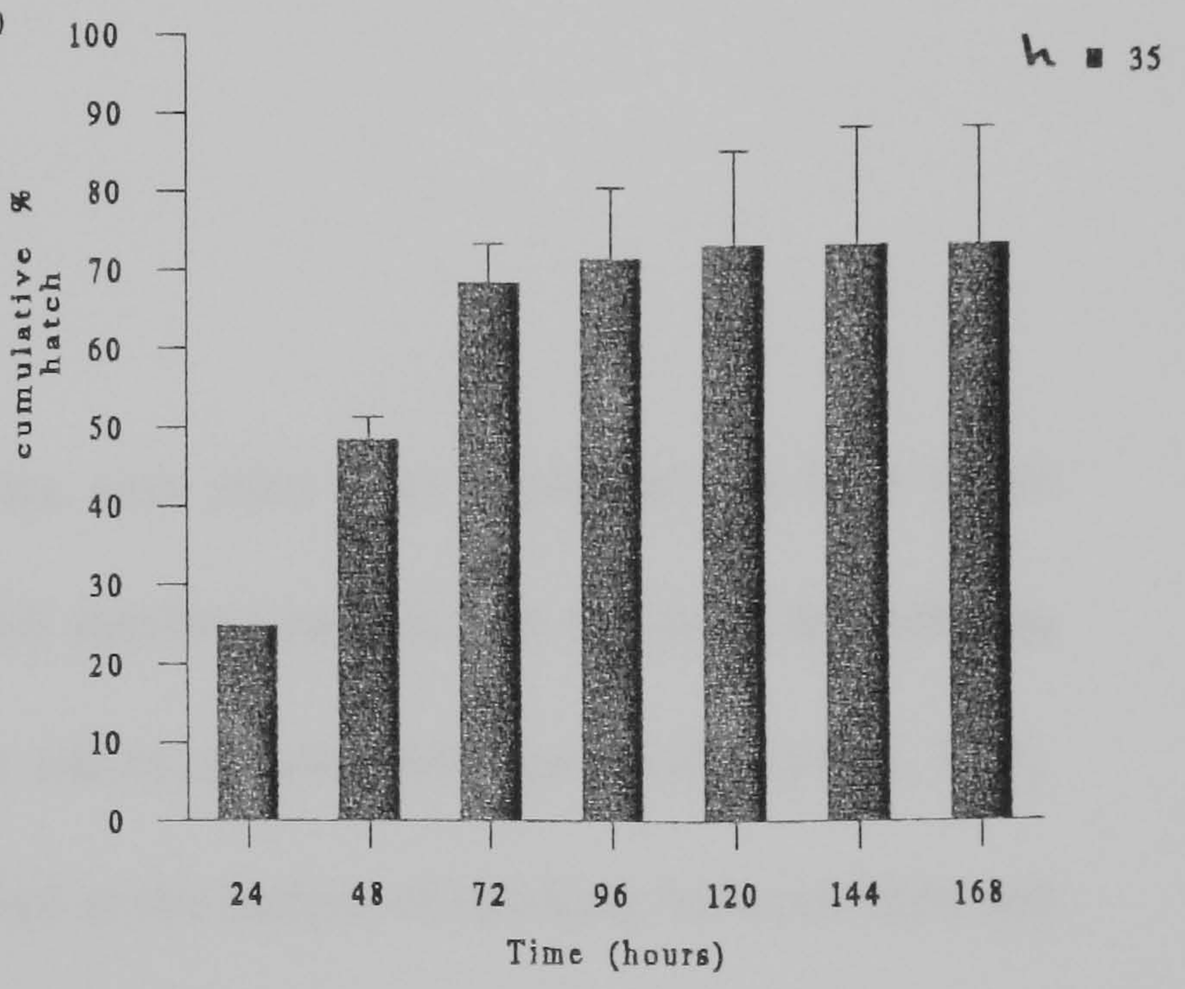
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144
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Time (hours)

hatching was much lower than those in 5 and 10‰, 23.38% compared to 35.74 and 38.70% respectively. Analysis of variance demonstrated a significant difference in hatching levels between ovisacs held in low salinities (0 to 15‰) and those in higher salinities (0 to 30‰) ($p<0.05$).

b. Observations on hatching

Egg strings with extruding eggs containing live developed nauplii, when placed in 0‰ salinity took approximately sixty seconds for any movement exhibited by the nauplii to cease. Both the egg membranes and the enclosed nauplii were observed to greatly increase in size. The egg membrane diameter increased from 60.53µm to 658.45µm and bursting occurred approximately four minutes after the initial exposure to freshwater, releasing dead and swollen nauplii. The resulting larvae were almost spherical in appearance (fig. 3.12), the dimensions being 415.33µm for the length, and 334.29µm for the width as compared to the average length and width for a successfully hatched nauplius, 516.63µm and 192.47µm (fig. 3.7).

3.3.1.3. Photoperiod

i. Control experiments

Initial control experiments where egg sacs pairs were separated but kept under identical light regimes revealed that the daily hatching pattern was the same in both egg strings of a pair, both in constant light (24L), and in majority darkness (23D:1L) (fig. 3.13). There were no significant differences observed in the pattern of hatching between right and left egg strings for either the light regime or the dark regime (two-tailed paired *t*-test, $p<0.01$).

Fig. 3.12. Released *Lepeophtheirus salmonis* nauplius I stage after exposure to freshwater whilst surrounded by an egg membrane. Such larvae tended to take on a spherical appearance and were dead. Scale bar = 100μm.

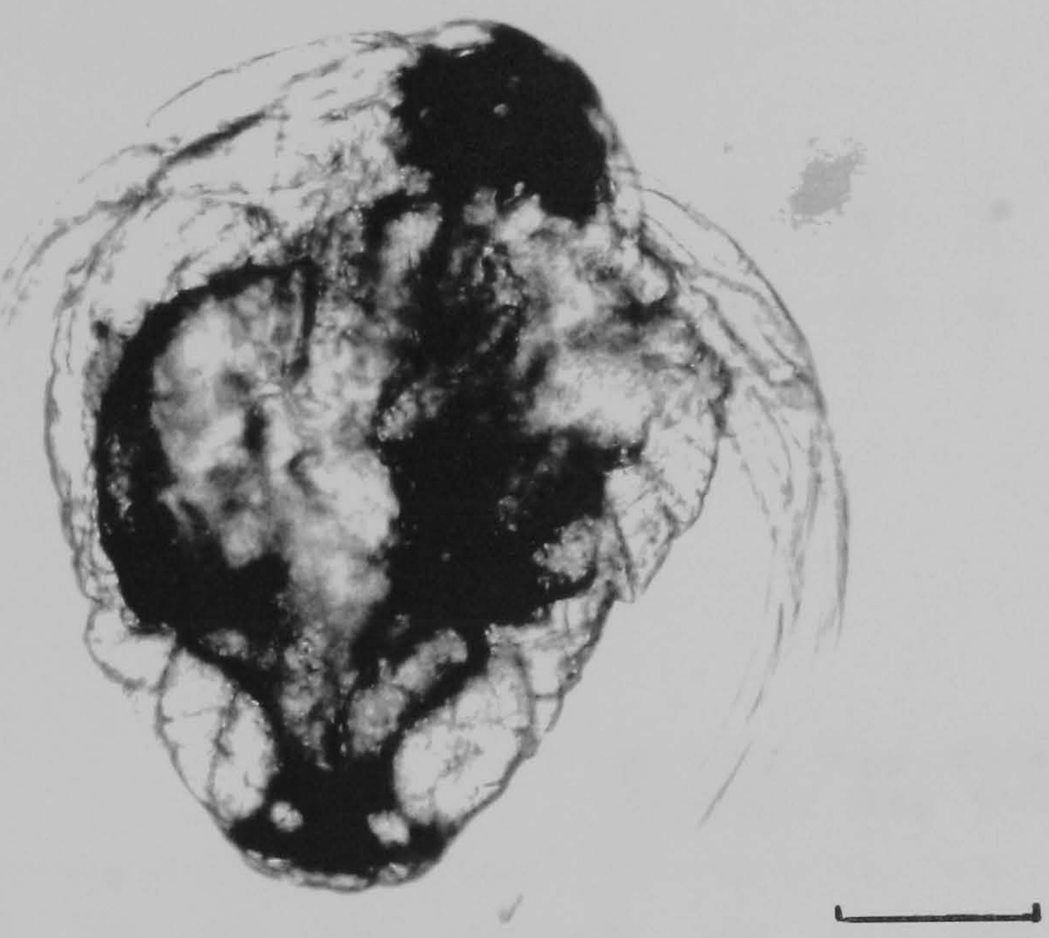
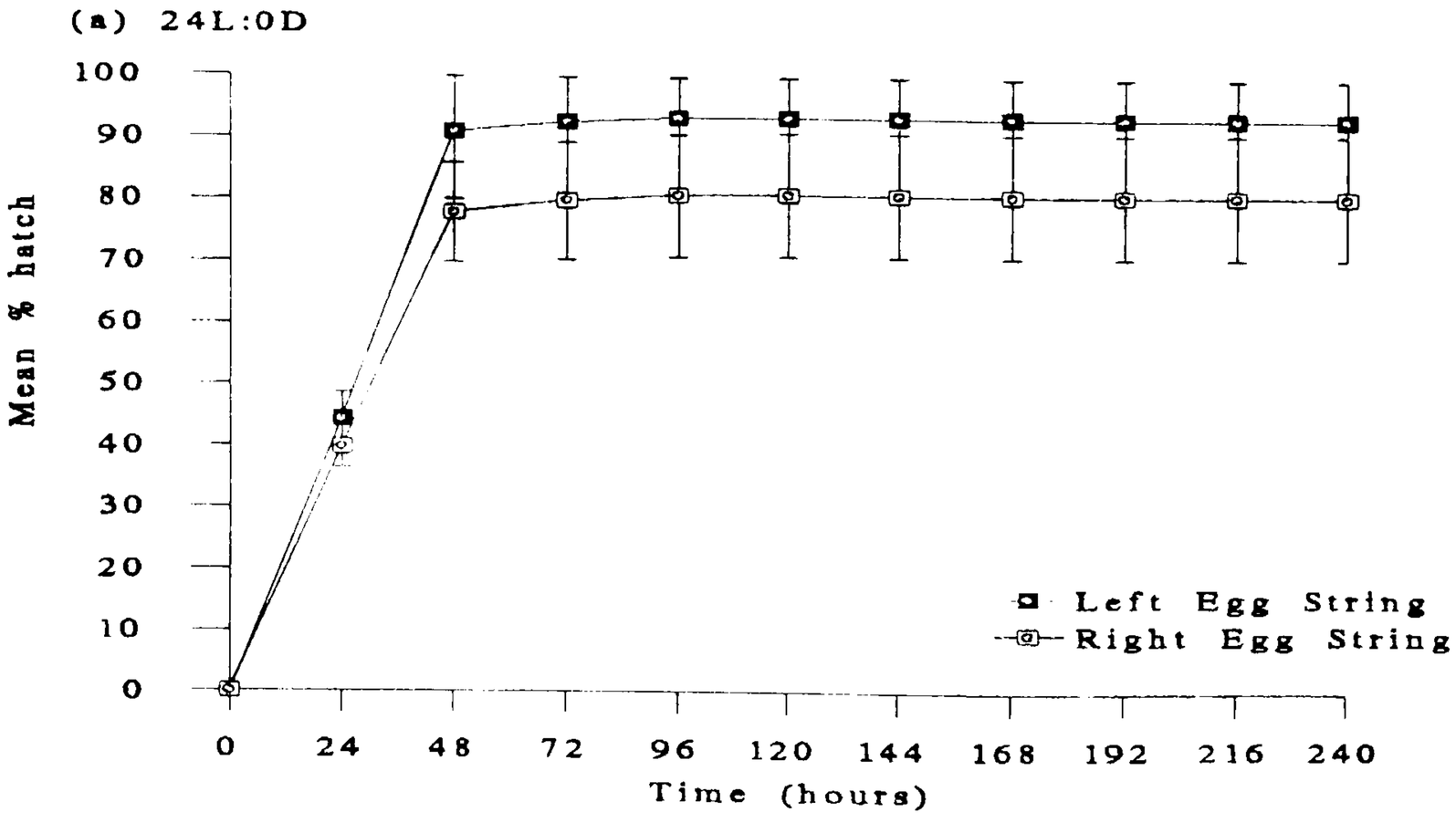
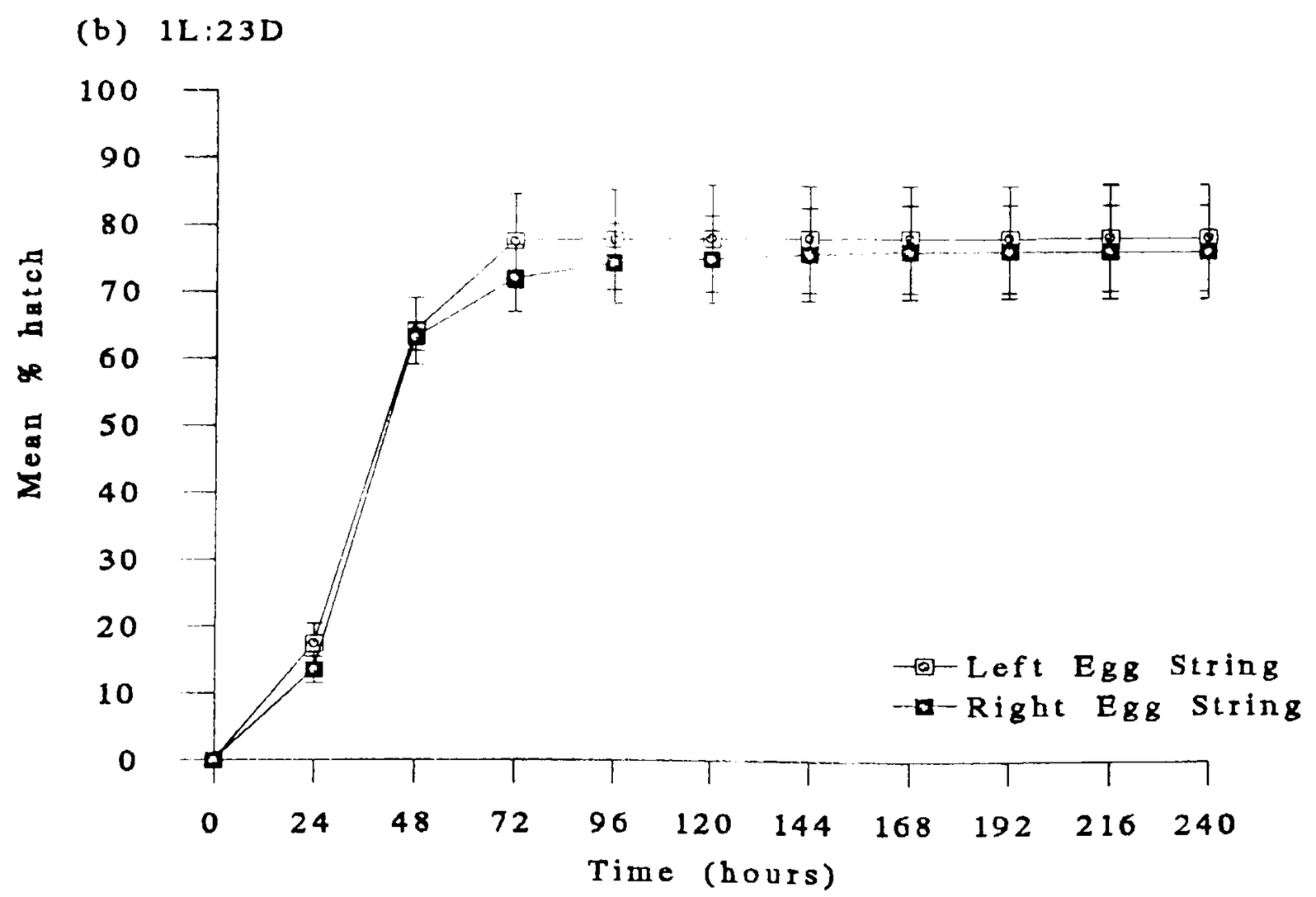


Fig. 3.13. Mean hatching patterns (± 1 S.E.) of *L. salmonis* egg strings under control light conditions: left egg strings and right egg strings separated but held under the same photoperiod regime (a) 24L:0D, (b) 1L:23D.





For each individual egg string, the time when hatching was first observed was termed 24 hours, since, 24 hours previously, no hatching had been observed. Each individual pair of egg strings that had been separated from each other, were observed to start hatching on the same day (table 3.1) with the exception of one pair in the light regime and two in the dark. However this difference was never greater than 24 hours.

ii. Experimental results

The mean percentage hatch for each of the left egg strings held in one regime was compared with the mean percentage hatch for their right egg string counterparts. The results are shown in fig. 3.14. There were no significant differences between the patterns of hatching observed from egg strings held in constant light (24L) and the other half of the pairs held in majority darkness (23D:1L), or between those held in majority light (23L:1D) and those in majority darkness (23D:1L) (two-tailed paired *t*-test, $p<0.05$).

In addition no significant differences were observed between the mean percentage hatch of eggs kept in constant darkness (0L:24D) compared to those held under constant illumination (24L:0D) (two-tailed paired *t*-test, $p<0.01$) as can be seen from fig. 3.15.

3.3.1.4. Natural variation in hatching success and duration

The results of the mean hatching success and hatching duration for 64 egg strings are given in table 3.2 and figs. 3.16 and 3.17. The mean percentage hatch observed in these egg strings was 59.90%, with the range of eggs hatched being 0.00 to 100%. The recording of numbers hatched for each individual egg string started as soon as hatched nauplii from that egg string were observed, this time being assigned as 24 hours (since, the previous observation, 24 hours before, had showed no hatching). The mean percentage daily hatches are given in fig. 3.17. It can be seen that the majority of hatching occurs within the first 48

Table 3.1. Hatching pattern of individual pairs of *L. salmonis* egg strings under control light conditions (24L:0D or 1L:23D).

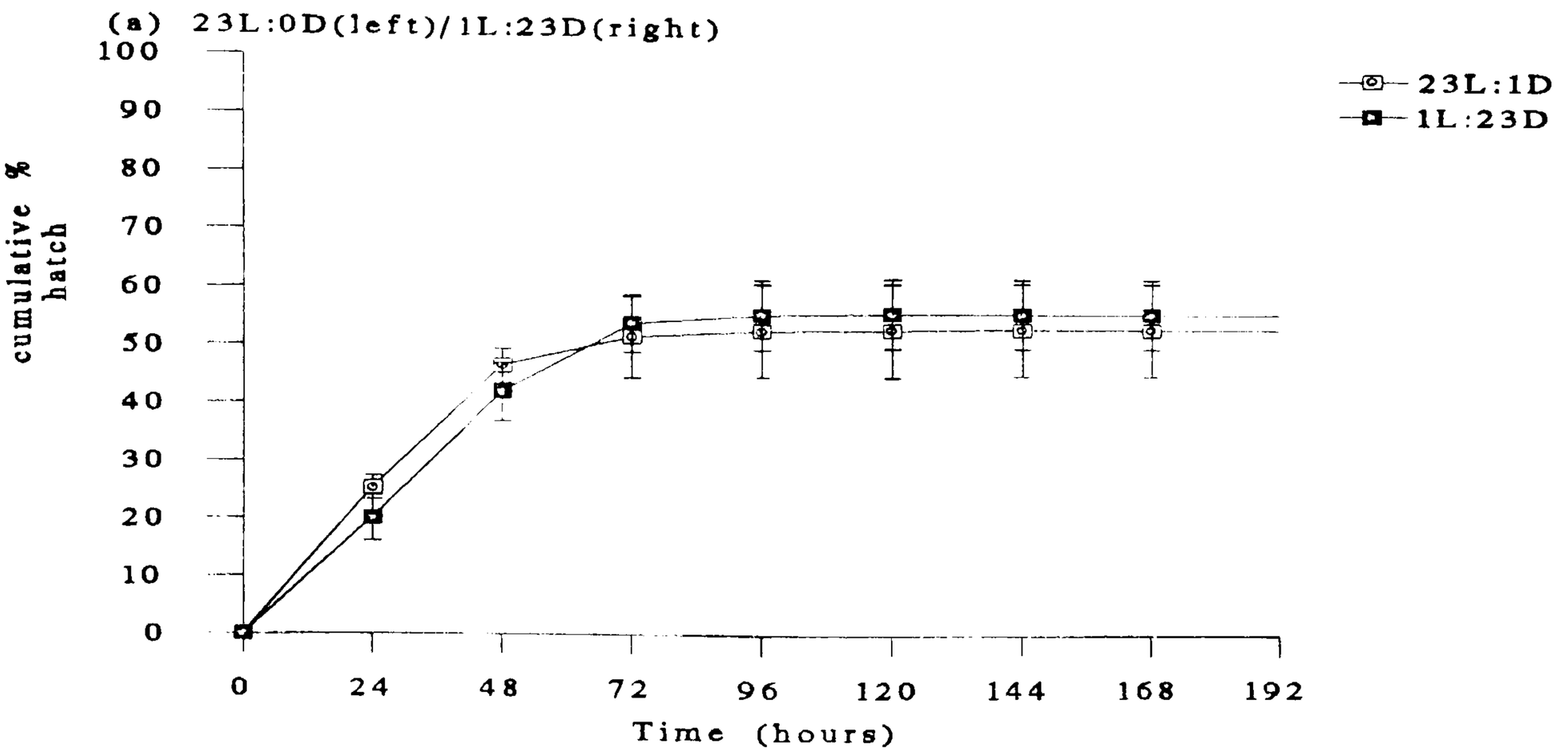
Day on which hatching commenced 24L:0D regime		Day on which hatching commenced 1L:23D regime	
Left Egg String	Right Egg String	Left Egg String	Right Egg String
4	4	1	2
1	1	1	1
6	6	3	3
6	6	3	4
1	2	1	1
1	1	1	1
3	3	4	4
2	2	2	2
4	4	3	1
1	1	1	1

Table 3.2. Hatching pattern of *L. salmonis* egg strings: duration, daily percentage hatch and cumulative percentage hatch.

Time (hours)	Mean percentage daily hatch	S.E.	Range	Cumulative percentage hatch
24	20.69	3.51	0-89.20	20.69
48	26.09	3.42	0-80.09	46.78
72	9.48	2.03	0-71.50	56.26
96	2.94	1.29	0-78.28	59.20
120	0.39	0.11	0-5.35	59.59
144	0.20	0.08	0-3.68	59.79
168	0.09	0.04	0-2.23	59.88
192	0.01	0.01	0-0.56	59.89
216	0.01	0.01	0-0.90	58.90

Total number of egg strings studied (n) = 64
Total number of eggs = 13,835
Mean number of eggs per string = 216.17 (S.E. = 4.80, range = 134-315)

Fig. 3.14. Mean hatching patterns (± 1 S.E.) of *L. salmonis* egg strings under experimental light conditions: (a) left egg strings held in 23L:1D regime, right egg strings held in 1L:23D regime. (b) left egg strings held in 24L:0D regime, right egg strings held in 1L:23D regime.



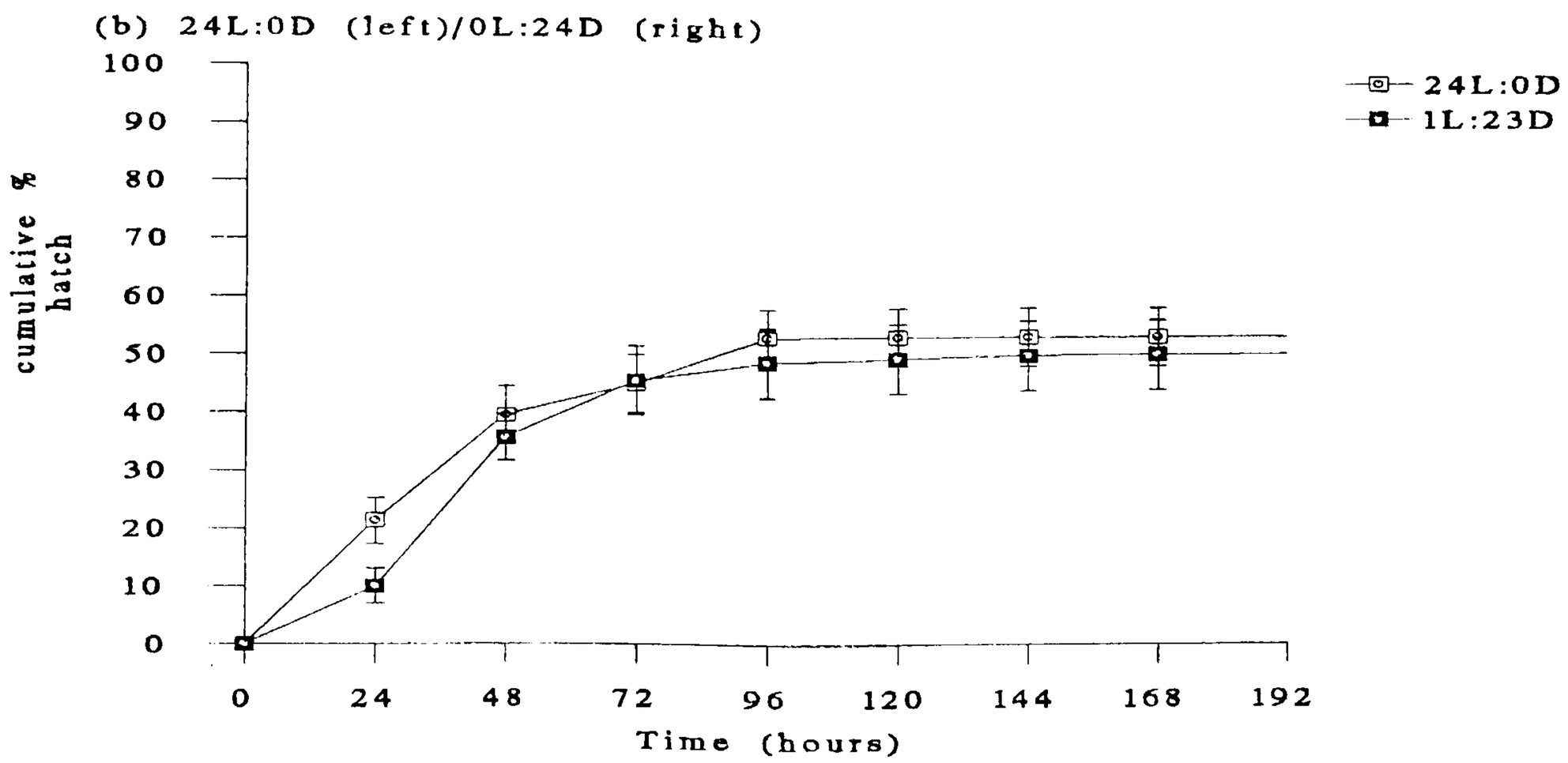


Fig. 3.15. Cumulative percentage hatch (± 1 S.E.) for left egg strings held in 24L:0D regime and final percentage hatch (± 1 S.E.) for right egg strings held in 0L:24D regime.

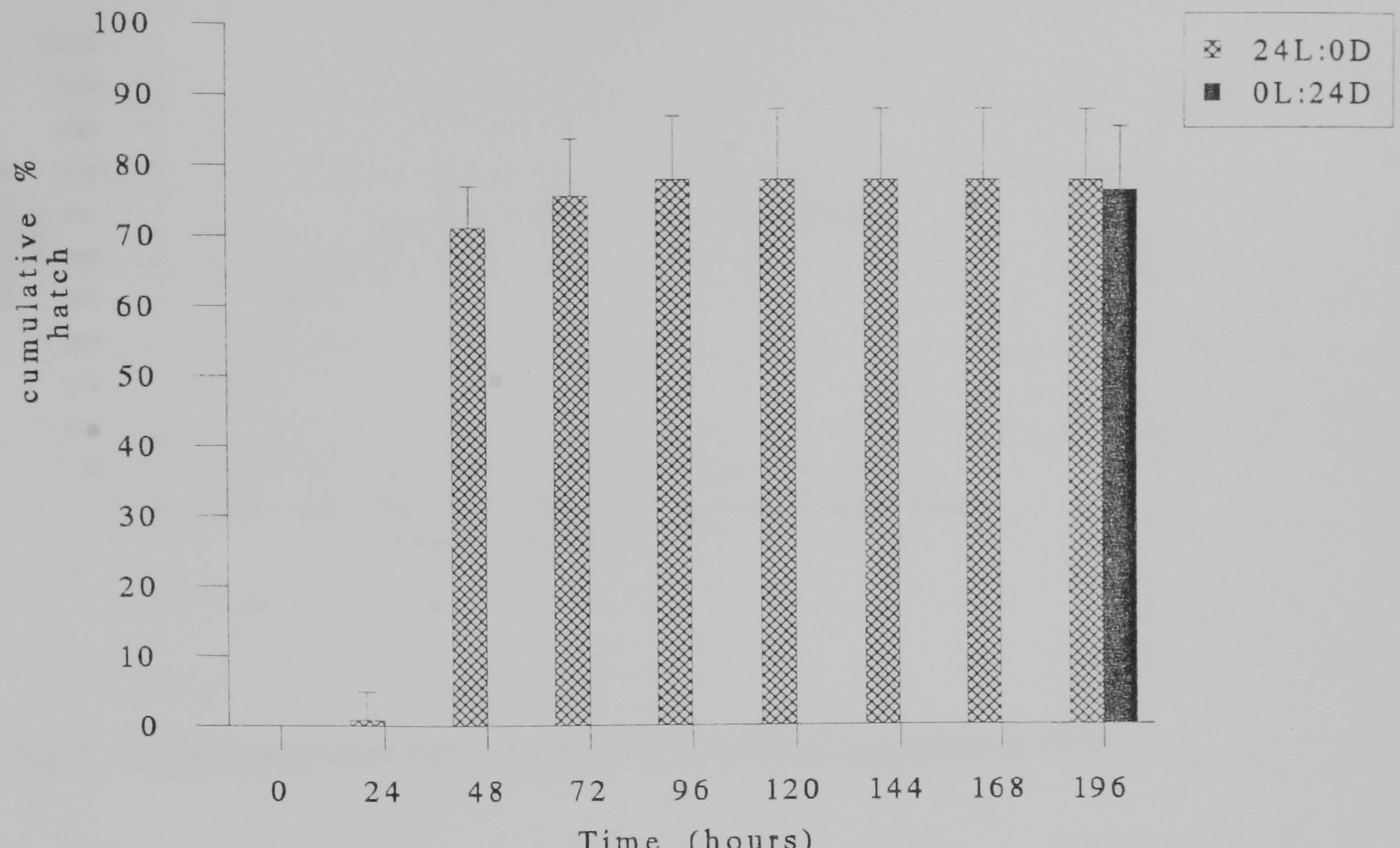


Fig. 3.16. Cumulative percentage hatch of *L. salmonis* egg strings at 10°C.

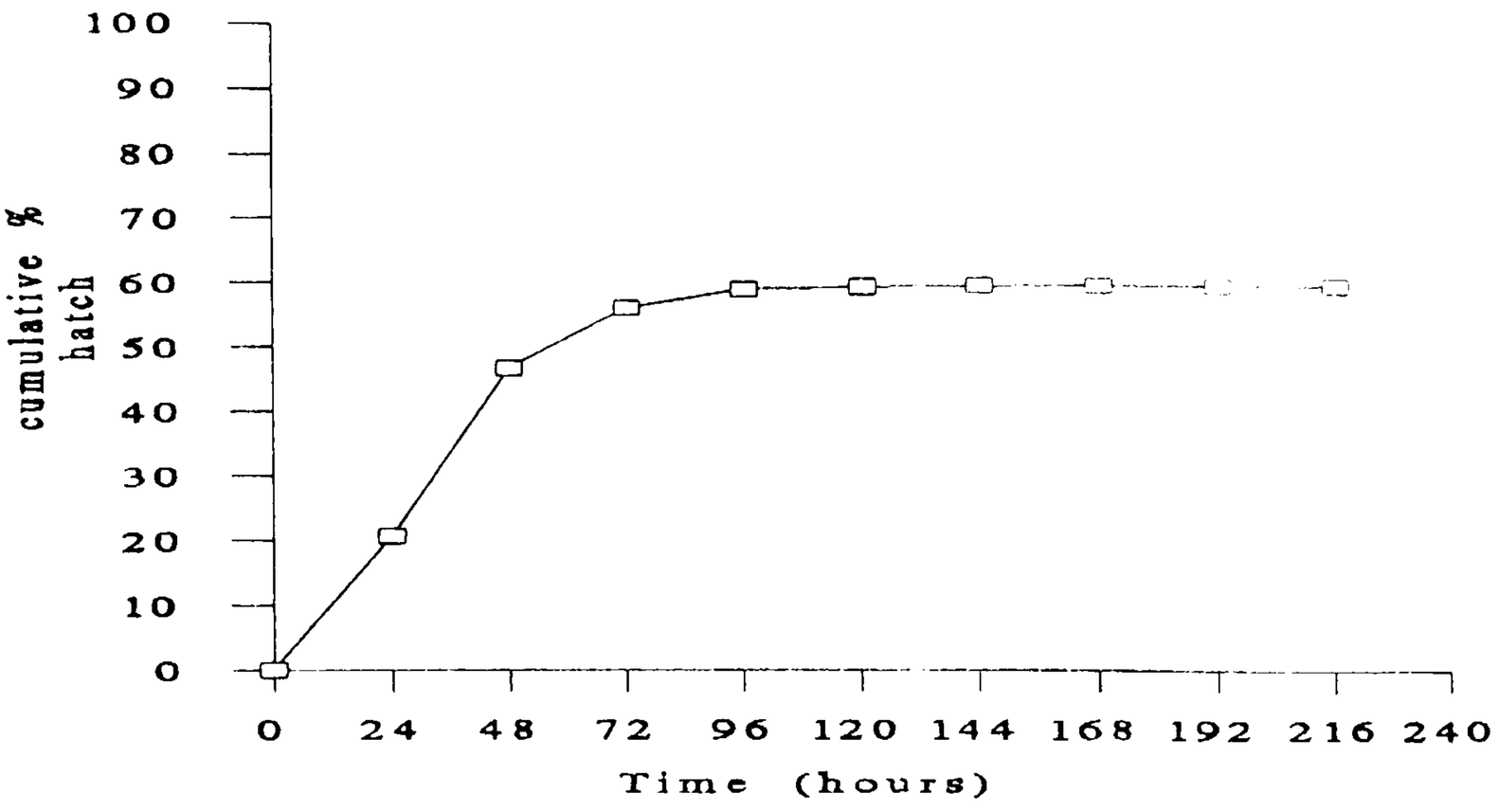
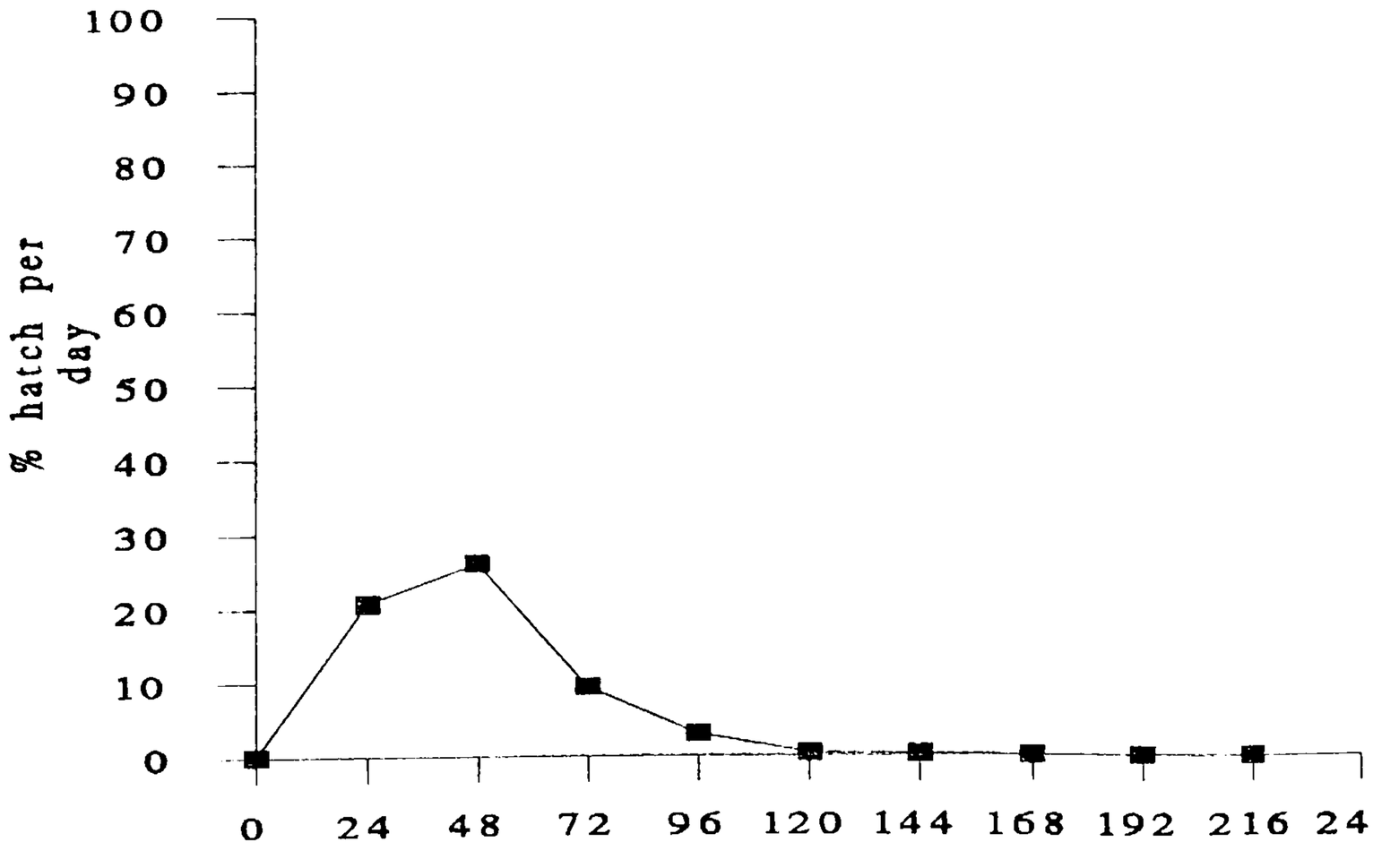


Fig. 3.17. Daily percentage hatch (± 1 S.E.) of *L. salmonis* egg strings at 10°C.



hours, with the difference in the percentage hatch in the first two 24 hour periods being non-significant (two-tailed paired *t*-test, $p<0.05$), 20.69% for the period of 0 to 24 hours, compared to 26.09% for 24 to 48 hours. After 48 hours the percentage of eggs hatched per day was seen to decrease, until no more eggs were released. The mean number of days over which hatching was seen to occur was 9 days (216 hours) at the test temperature of 10°C, although the range was highly variable (24 to 312 hours). However, over the last four days, only 0.309% of the total number of eggs was observed to hatch (this being equivalent to 0.516% of the eggs that did hatch), whilst 78.098% of the eggs that did hatch, hatched in the first 2 days.

3.3.2. Behaviour of larvae

3.3.2.1. Behaviour immediately post-hatching

Post hatching, released nauplii were propelled completely clear of the egg membrane. The power for propulsion was most likely to be due to the large internal pressure from within the egg membrane. The membrane was not seen to remain attached to the nauplius. The nauplii then appeared to be quiescent compared to their earlier active movements within the egg. The contractions of the body observed before hatching, although still occurring, were greatly reduced in their frequency after they had been freed. The earliest movements of the free nauplius were from the appendages which showed sporadic, jerky twitches, which appeared to be co-ordinated with the occasional sporadic wave of the body. They were still held close to the side of the body although no attachment could be observed. However, with gradual jerking movements, the appendages were seen to gradually move away from the sides of the body, eventually being held perpendicular to the body. These movements continued until the appendages reached their normal position,

with the characteristic swimming behaviour then occurring. During the period of rest, nauplii were observed to swell a small amount and became more elongate. This behaviour generally took between 120 and 300 seconds. However, nauplii that hatched and appeared spherical and corpulent, despite showing some activity, did not appear to be viable, and died shortly afterwards.

3.3.3. Development of larvae

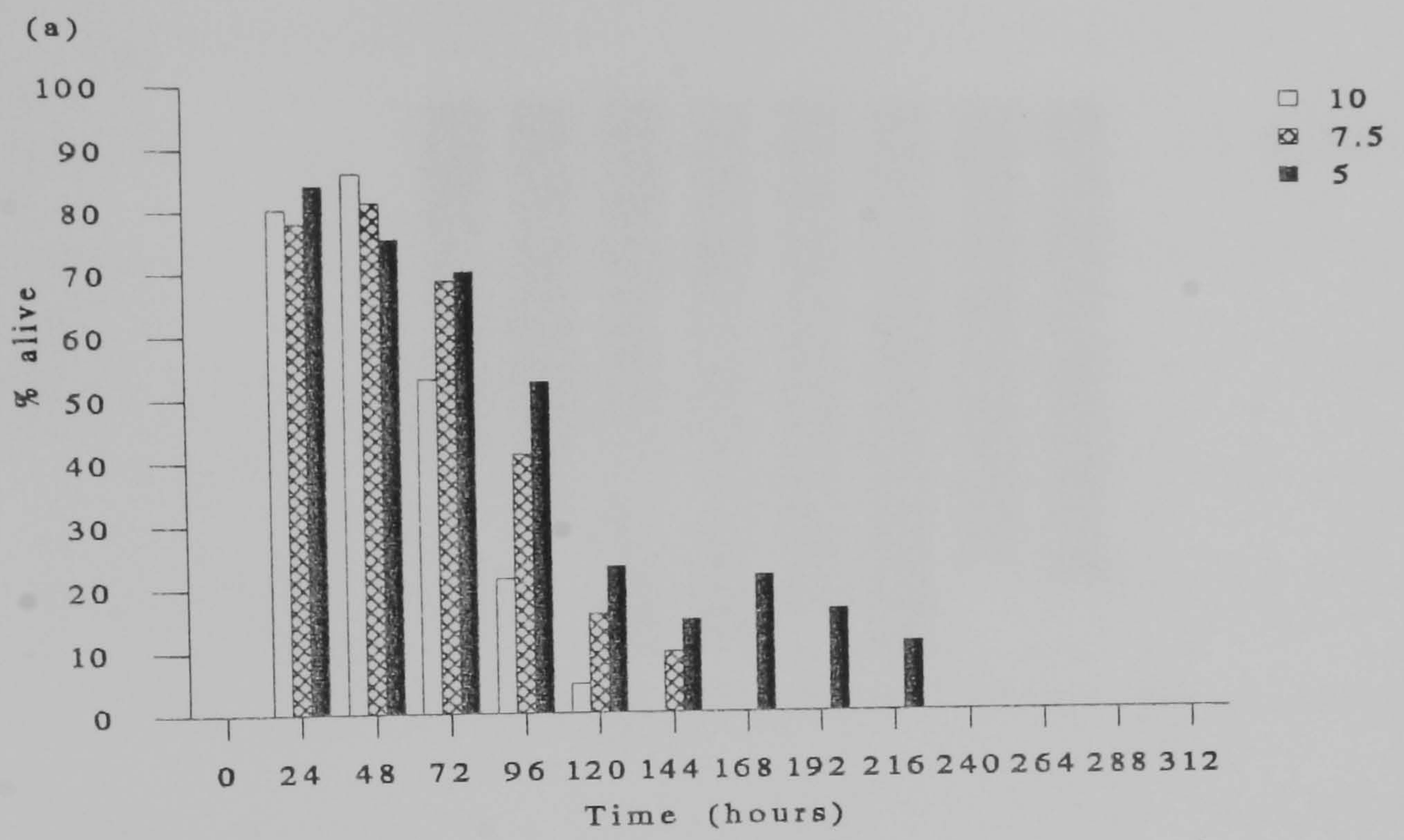
3.3.3.1. Effects of environmental conditions on development

i. Temperature

The viability of hatched larval stages was observed not to be constant, but instead decreased over time (fig. 3.18). During the period when high numbers of eggs are hatching, viability is high. However, this then decreases as hatching rates decrease to a minimum. Due to the differences in hatching duration of the three temperatures, low temperatures possessed an extended hatching period as can be seen by the percentage daily hatch in fig. 3.18. Thus the viability was observed to be extended in 5 and 7.5°C compared to 10°C. However, as can be seen from fig. 3.18, at all three temperatures, this decrease in viability occurs over the periods during which the number of eggs hatching falls from its initial high level.

Temperature was seen to have an effect upon the development of the larvae. Fig. 3.19 shows the proportion of the different stages (NI, NII and copepodid) present each day sampled for the three temperatures (5, 7.5 and 10°C). At 5°C, development was only seen to proceed as far as the second naupliar stage, with this first appearing at approximately 46 hours. No copepodids were attained, the NII stages dying before they entered the moult. At 7.5°C, development was observed to proceed to the copepodid stage, but only relatively low

Fig. 3.18. (a) Mean proportion (± 1 S.E.) of live nauplius I stages present in the daily hatch of *L. salmonis* egg strings held at 5°C, 7.5°C and 10°C. (b) Daily percentage hatch rate at 5, 7.5 and 10°C.



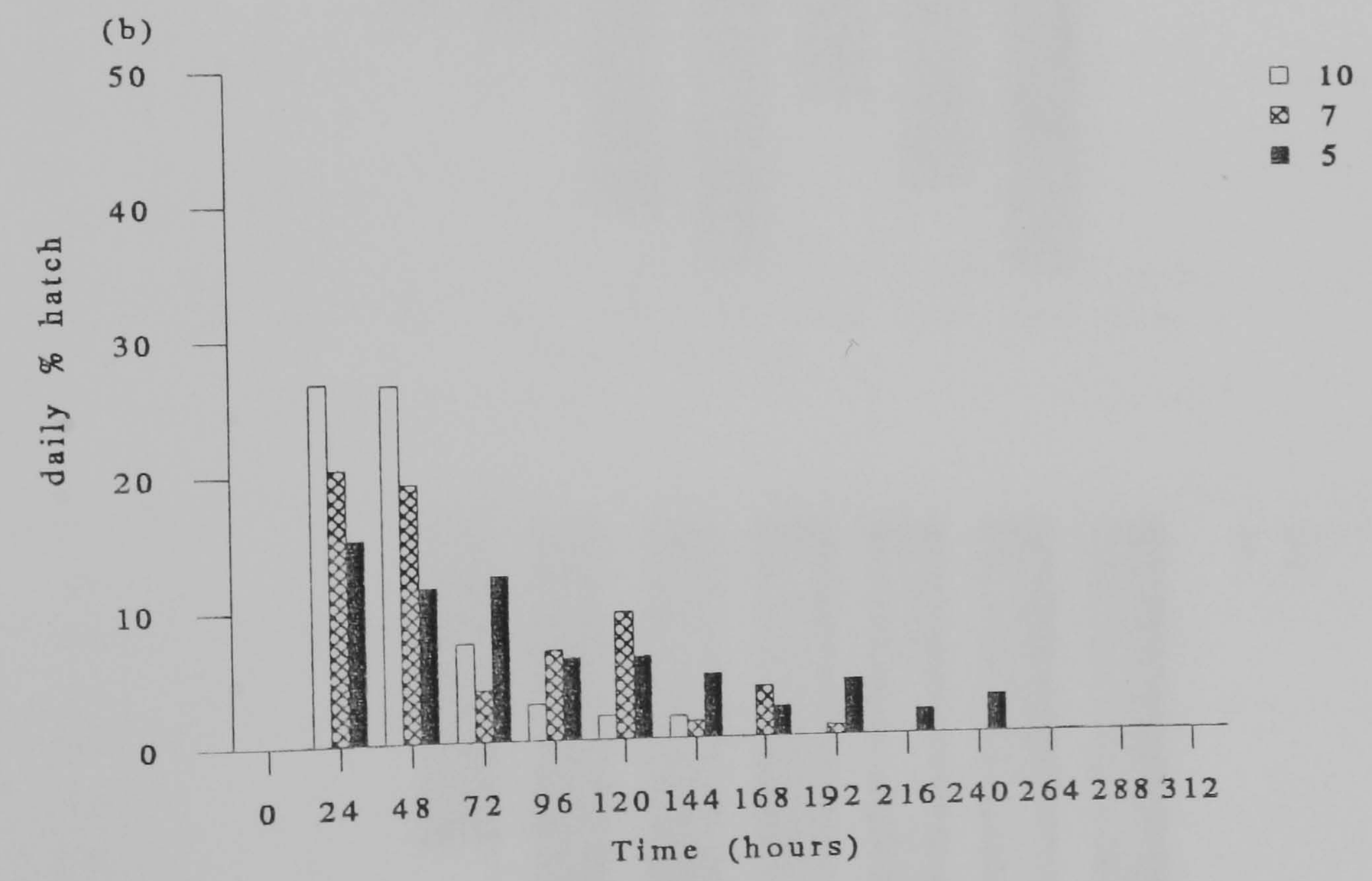
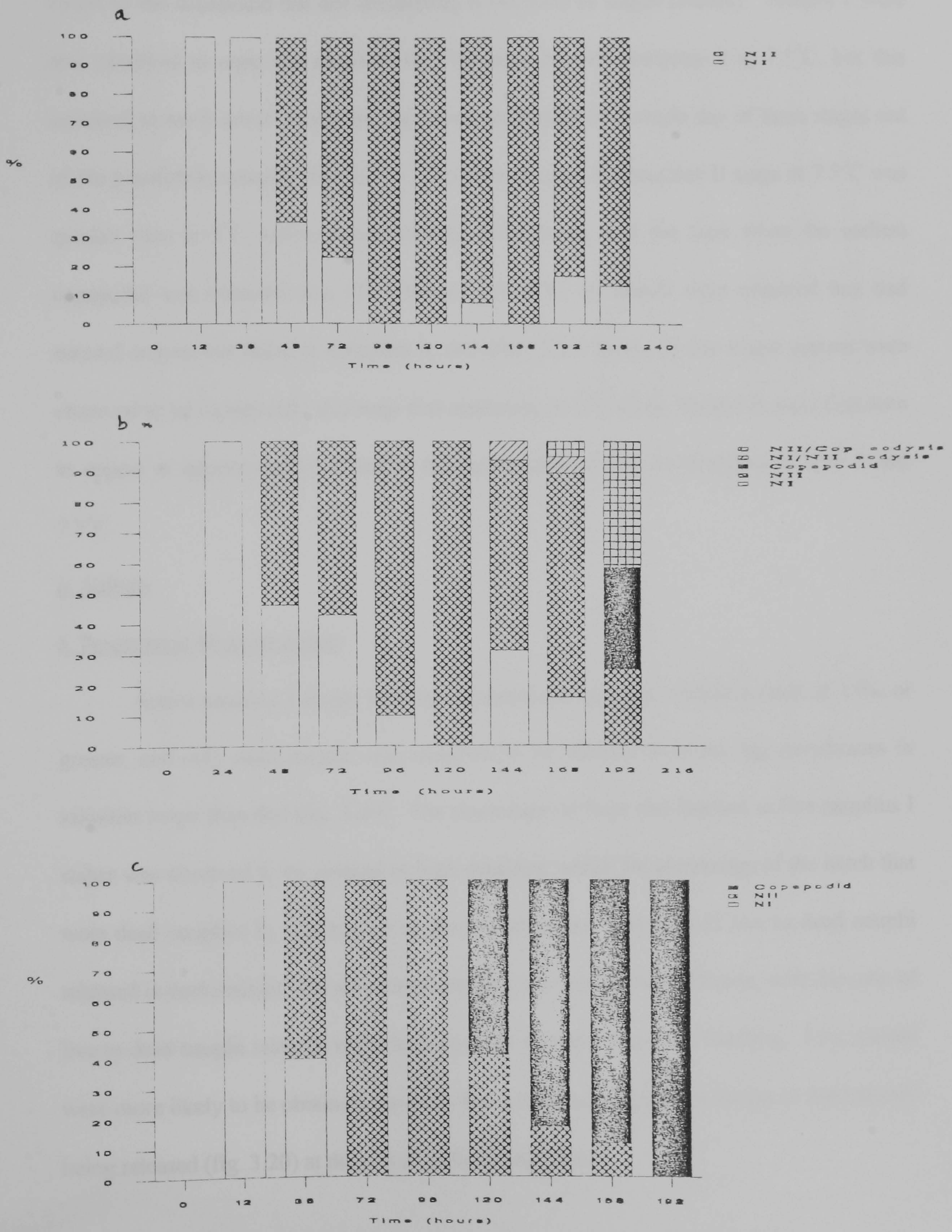


Fig. 3.19. Proportion of nauplius I, II and copepodid stages present daily during the free swimming phase of the lifecycle held at (a) 5°C, (b) 7.5°C and (c) 10°C.



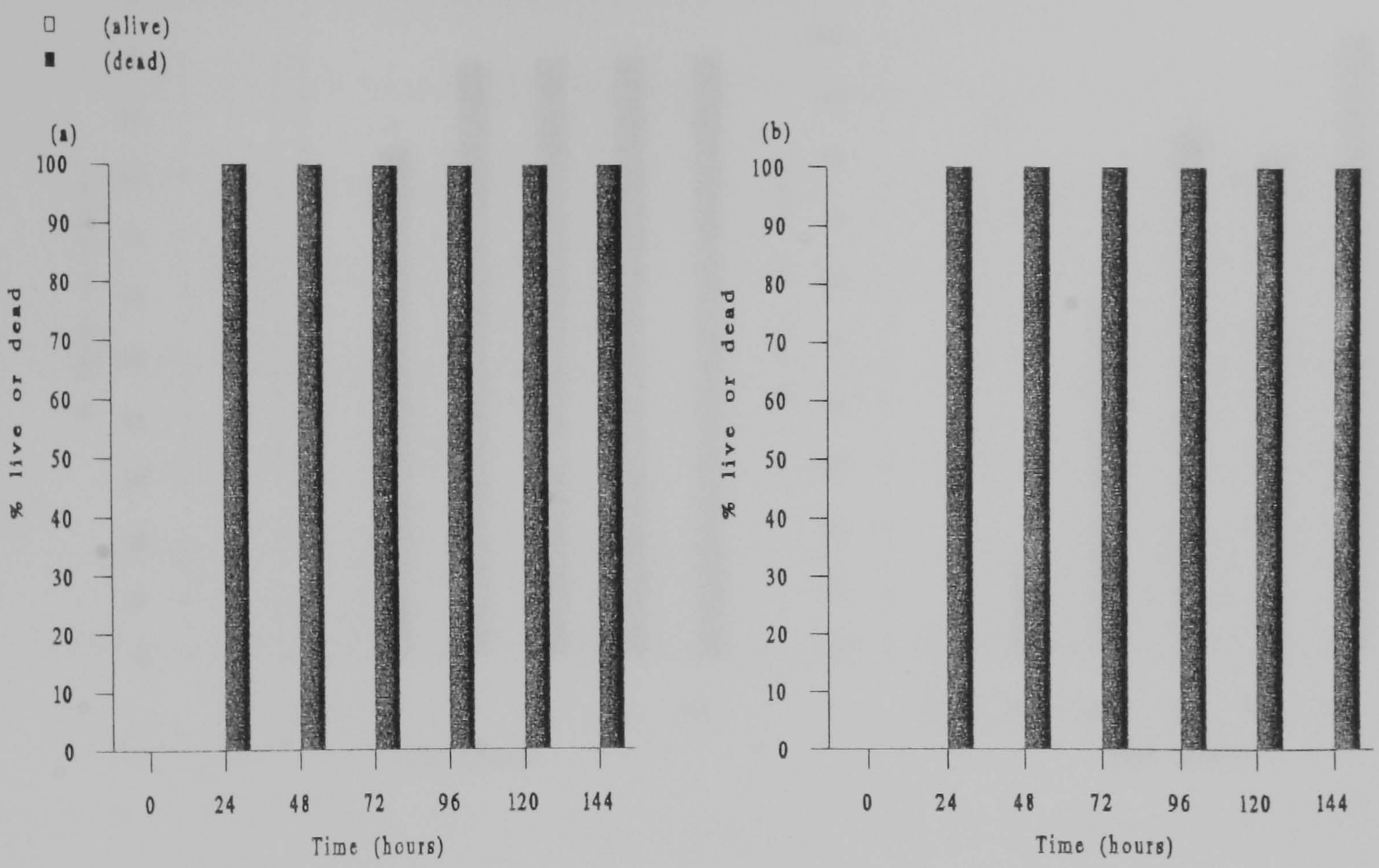
numbers were obtained (the maximum proportion of copepodids in the sample did not exceed 33.33%), with stages tending to remain either as nauplii II (25%), or entering the moult to the copepodid but not completing it (41.67% of stages present). Nauplii I were also observed to enter the moult to the NII stage but not complete it at 7.5°C, but this occurred at much lower levels; the maximum proportion on a single day of these stages out of the population sampled was 6.25% The time to reach the nauplius II stage at 7.5°C was quicker than at 5°C, taking approximately 43.25 hours, and the time when the earliest copepodid was obtained was 187.5 hours. At 10°C, no nauplii were obtained that had entered ecdysis but failed to complete it, and after 175.5 hours, all live stages present were observed to be copepodids, this stage first appearing at 111 hours. Nauplii II were first seen to appear at approximately 36 hours, this being quicker than development at both 5 and 7.5°C.

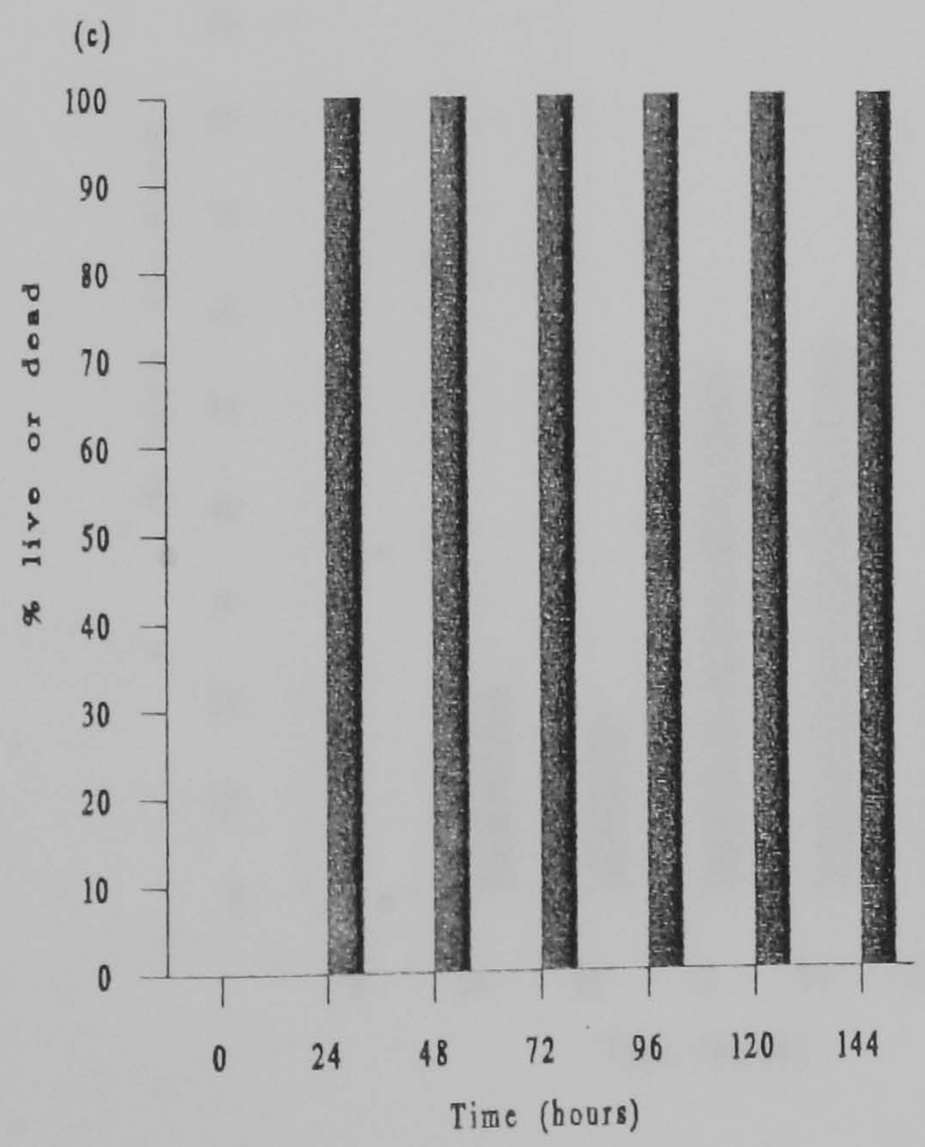
ii. Salinity

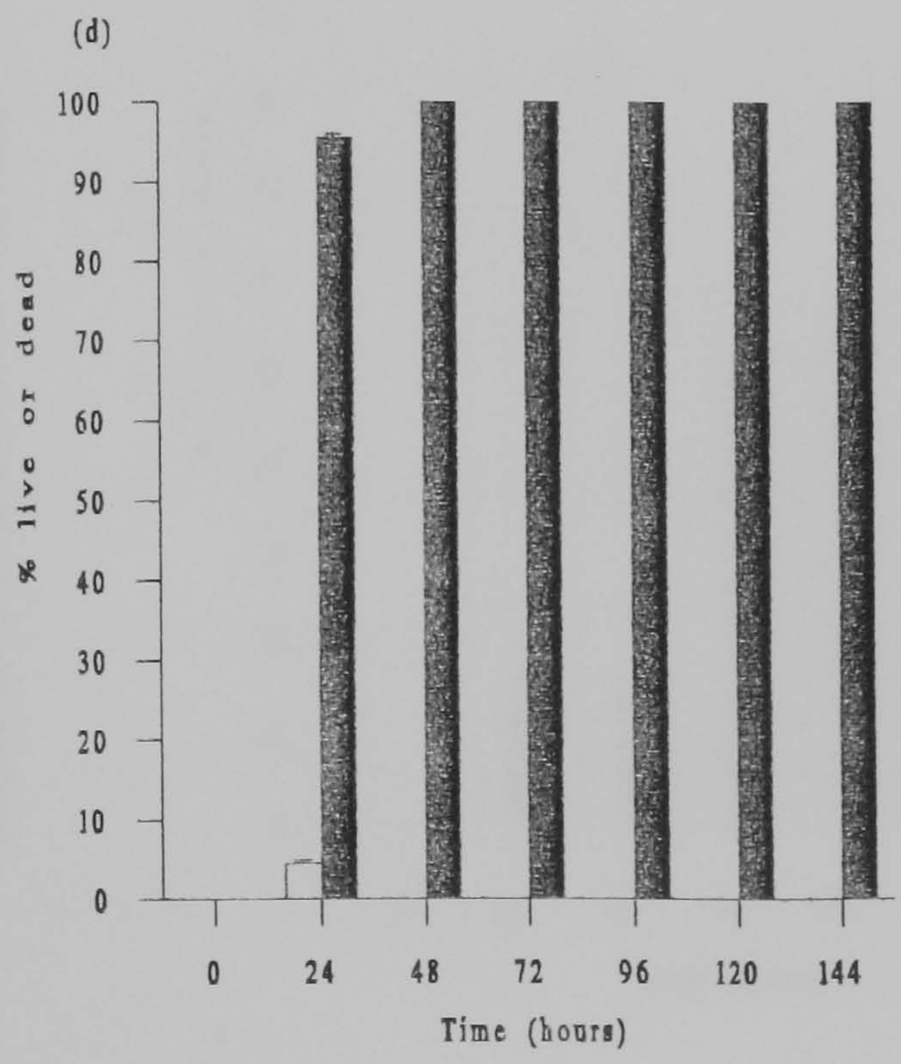
a. Progression of development

Active nauplius I stages were not obtained until salinity reached a level of 15‰ or greater, and only dead nauplii were observed to be released from the egg membranes in salinities lower than this (fig. 3.20). The percentage of those that hatched as live nauplius I stages was observed to be greatest in high salinities, whilst the percentage of the hatch that were dead nauplius I's was highest in the low salinities. The ratio of live to dead nauplii released in each salinity was also seen to change over the hatching duration, with the ratio of live to dead nauplii released being highest in the first few days over hatching. Live nauplii were more likely to be obtained, the ratio then becoming weighted in favour of dead nauplii being released (fig. 3.20) at the end of the hatching period.

Fig. 3.20. Mean proportions (± 1 S.E.) of live and dead nauplius I stages present in the daily hatch of *L. salmonis* egg strings held in salinities of (a) 0‰, (b) 5‰, (c) 10‰, (d) 15‰, (e) 20‰, (f) 25‰, (g) 30‰ and (h) 35‰.







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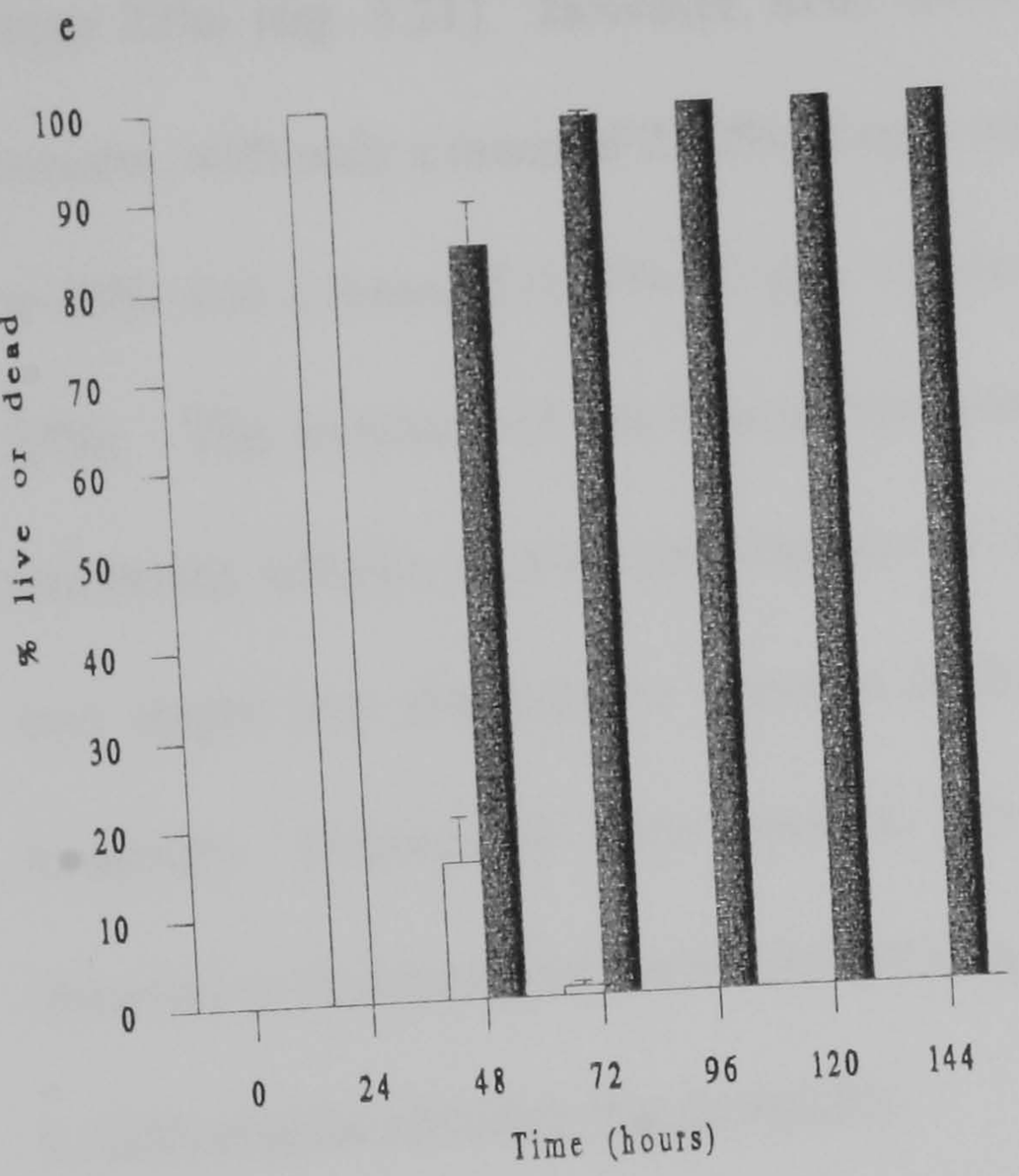
% live or dead
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48
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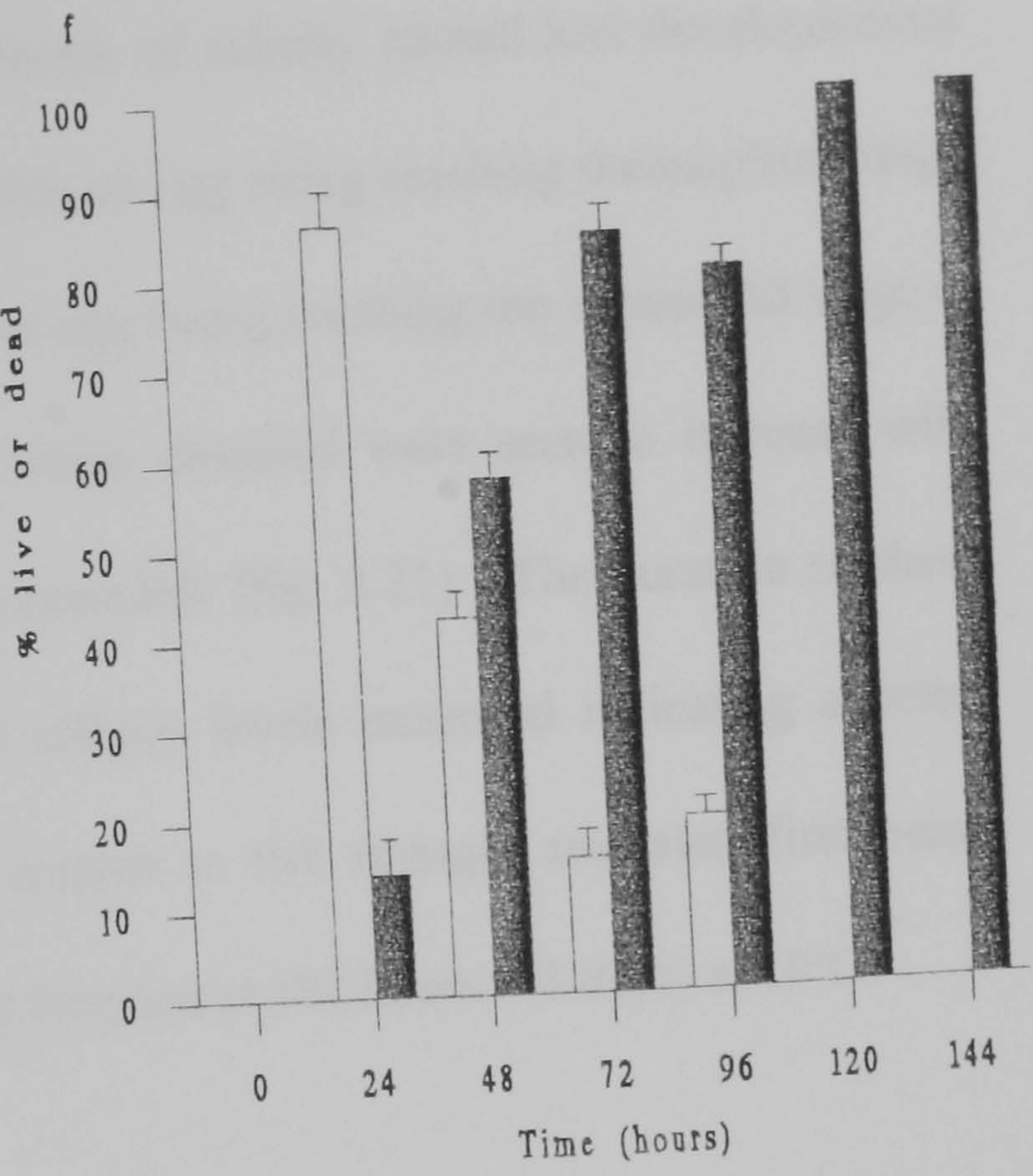
144
Time (hours)

f


% live or dead
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Time (hours)

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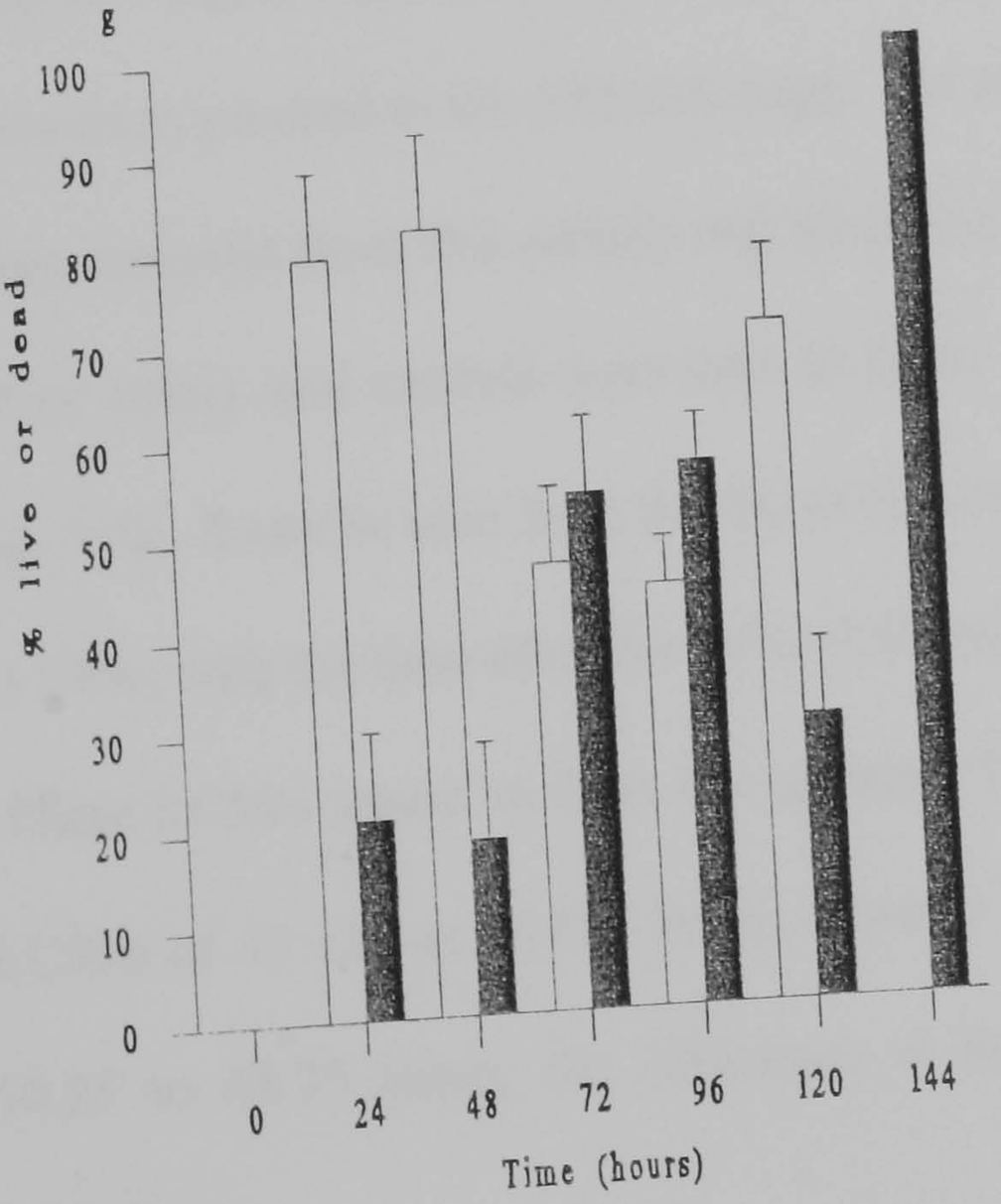
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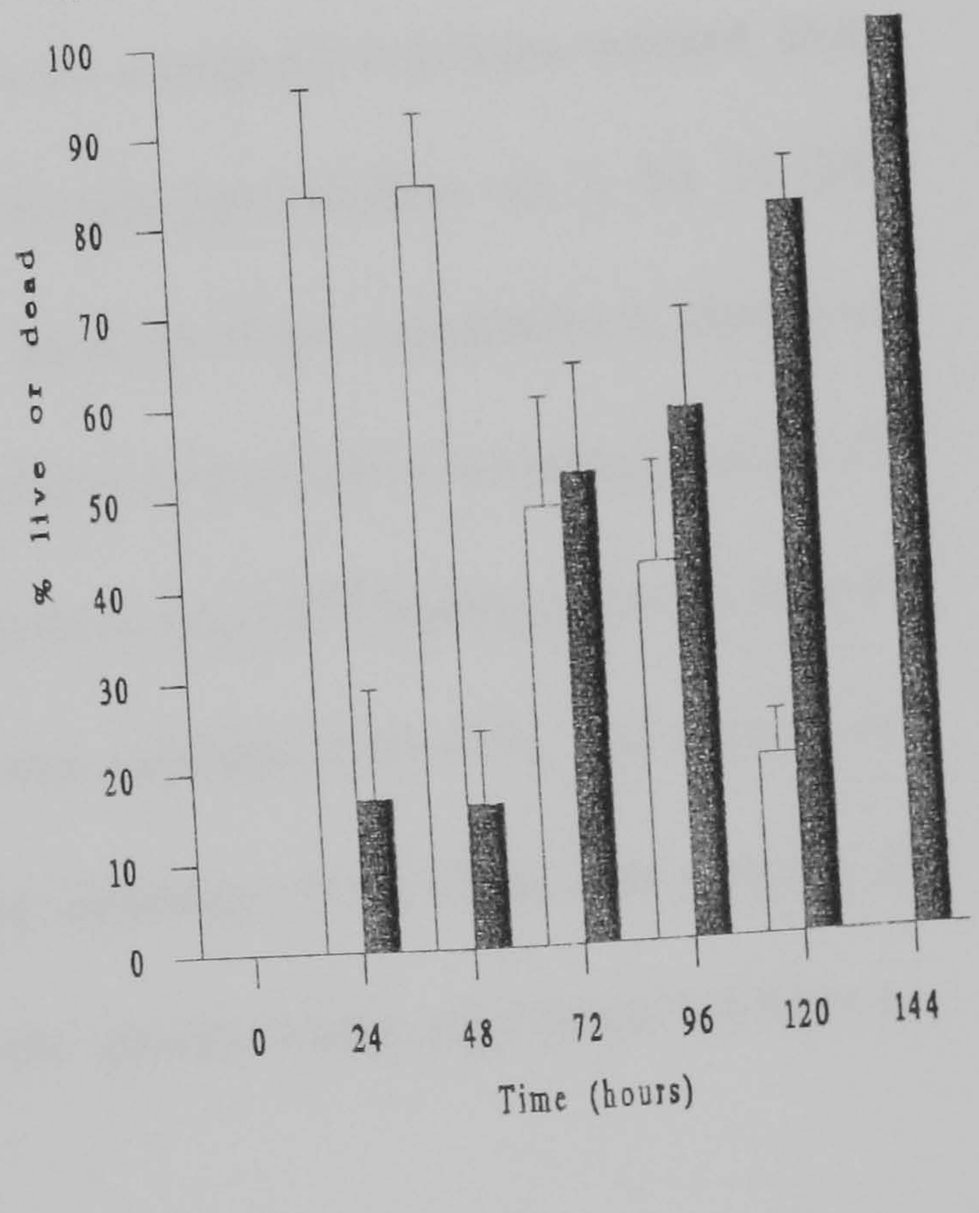
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Time (hours)

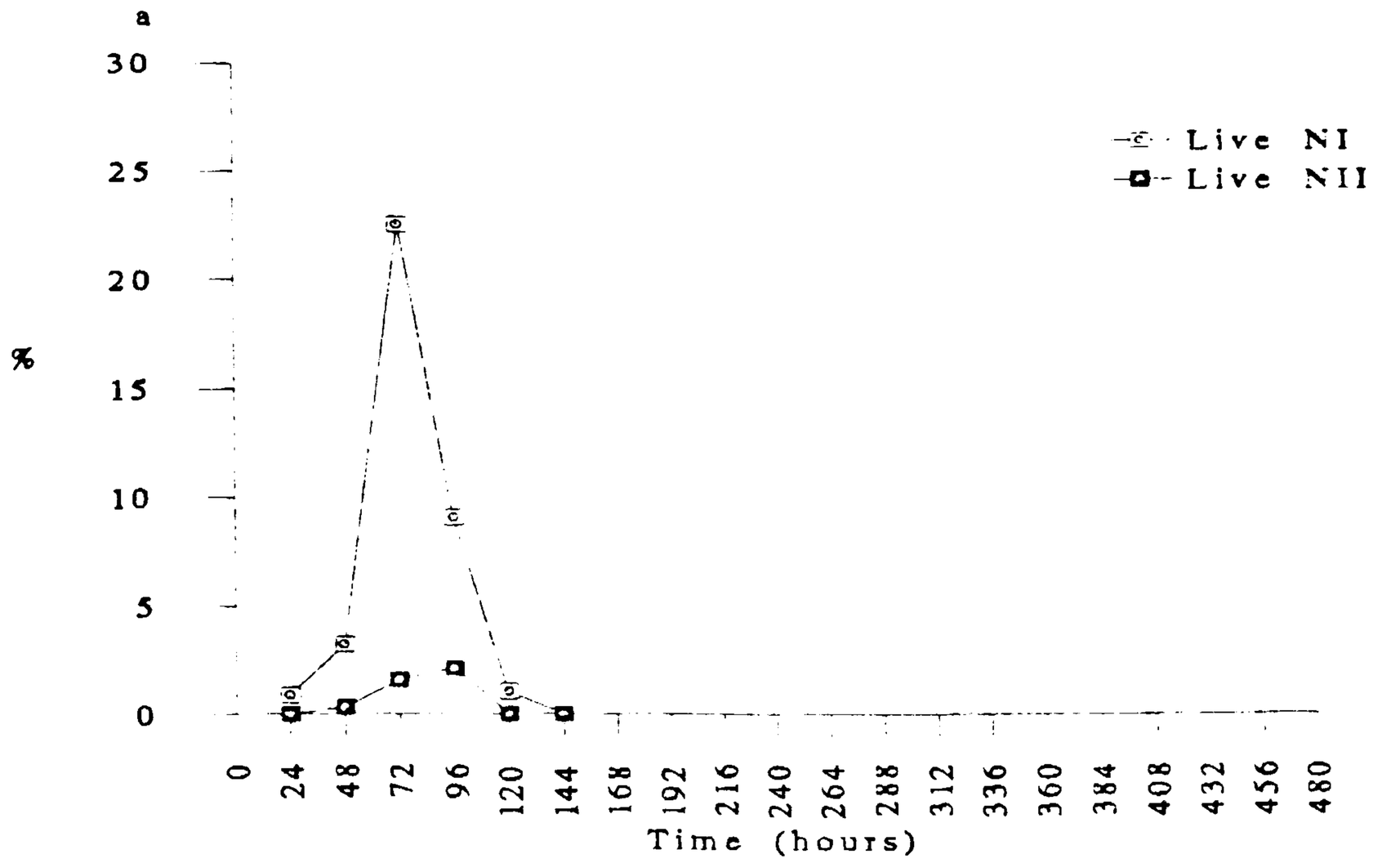
Development was seen to proceed to the nauplius II at 20‰ and to the copepodid stage 25‰ (fig. 3.21). However, even these levels of salinity caused low developmental success, with only a mean of 2.12% of eggs within an egg string reaching thenauplius Istage at 20‰ and a mean of 0.03% of eggs within an egg string reaching the copepodid stage at 25‰. The numbers of the second naupliar stage obtained were seen to increase with increasing salinity, as were the numbers of copepodids (fig. 3.21). The duration of these two stages was also seen to increase as the salinity levels increased indicating a better longevity. Copepodids were found to live longest in full strength seawater, first being obtained at 96 hours post hatch, and last being detected at 432 hours (18 days) at 10°C.

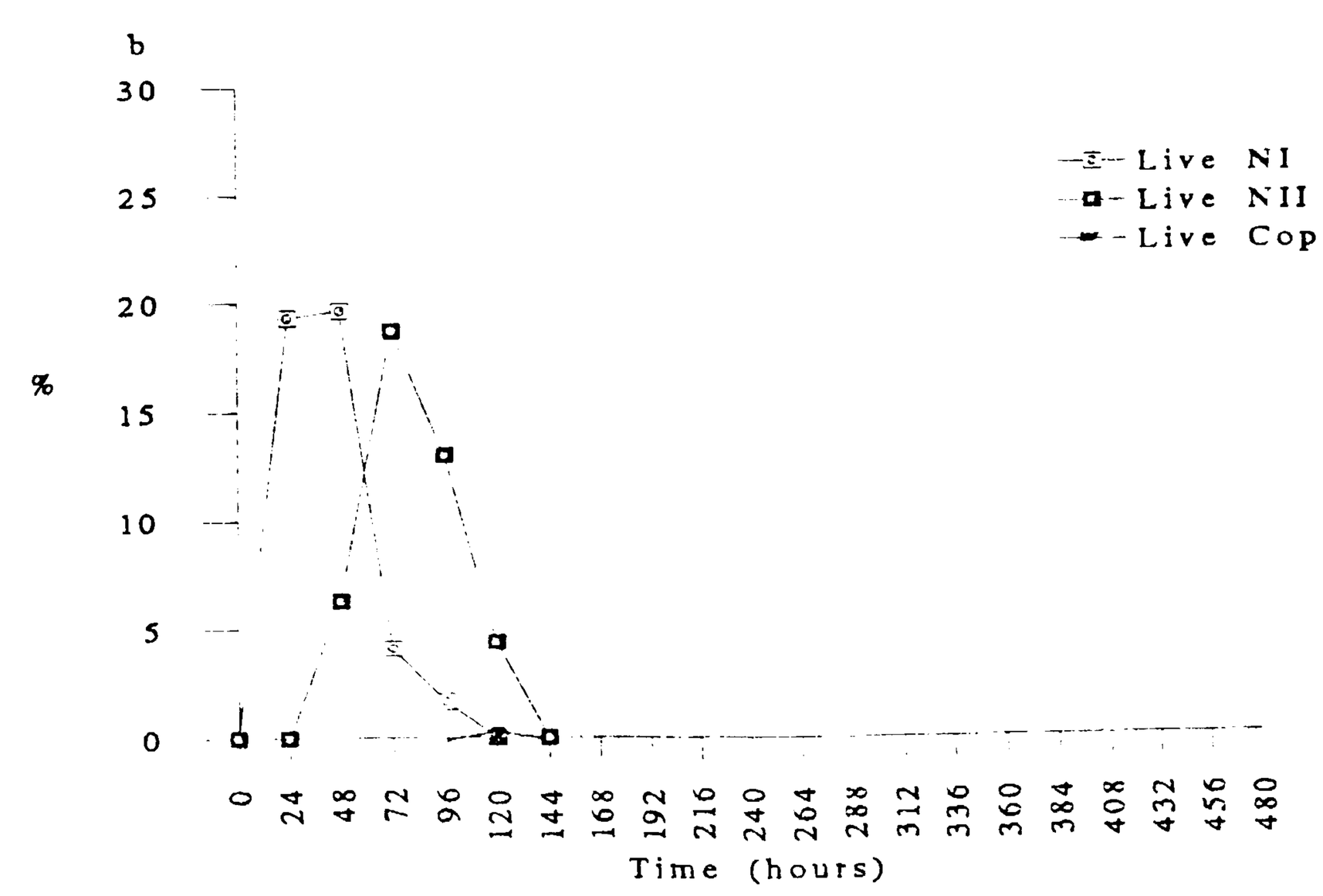
b. Ontogenetic variation due to salinity

To see if the copepodid was more tolerant to salinity levels than the first two naupliar stages, egg strings were placed in full strength seawater, and development was allowed to proceed to the infective stage. As soon as the copepodid had been reached, they were removed from this salinity and placed in one of eight test salinities (0, 5, 10, 15, 20, 25 or 30‰), and controls were kept in 35‰. The survival of the copepodids is shown in fig. 3.22. It can be seen from the figure that copepodids survive well in the salinity range 15 to 35‰, with the time taken for 50% of the copepodids to die (LC50) being given in table 3. These LC50's appear to form two groups. The lower salinities, 0 to 10‰, had a range of LC50's of 13.125 to 14.875 hours, whereas higher salinities, 15 to 35‰, had a range of 54.25 to 68.25 hours, the difference in these two groups being significant (ANOVA, $p<0.01$).

3.3.3.2. Mortality

Fig. 3.21. Development of *L. salmonis* in salinities between (a) 20‰ (b) 25‰. (c) 30‰ and (d) 35‰. Figures indicate percentage of eggs within the ovisacs that reach each stage.





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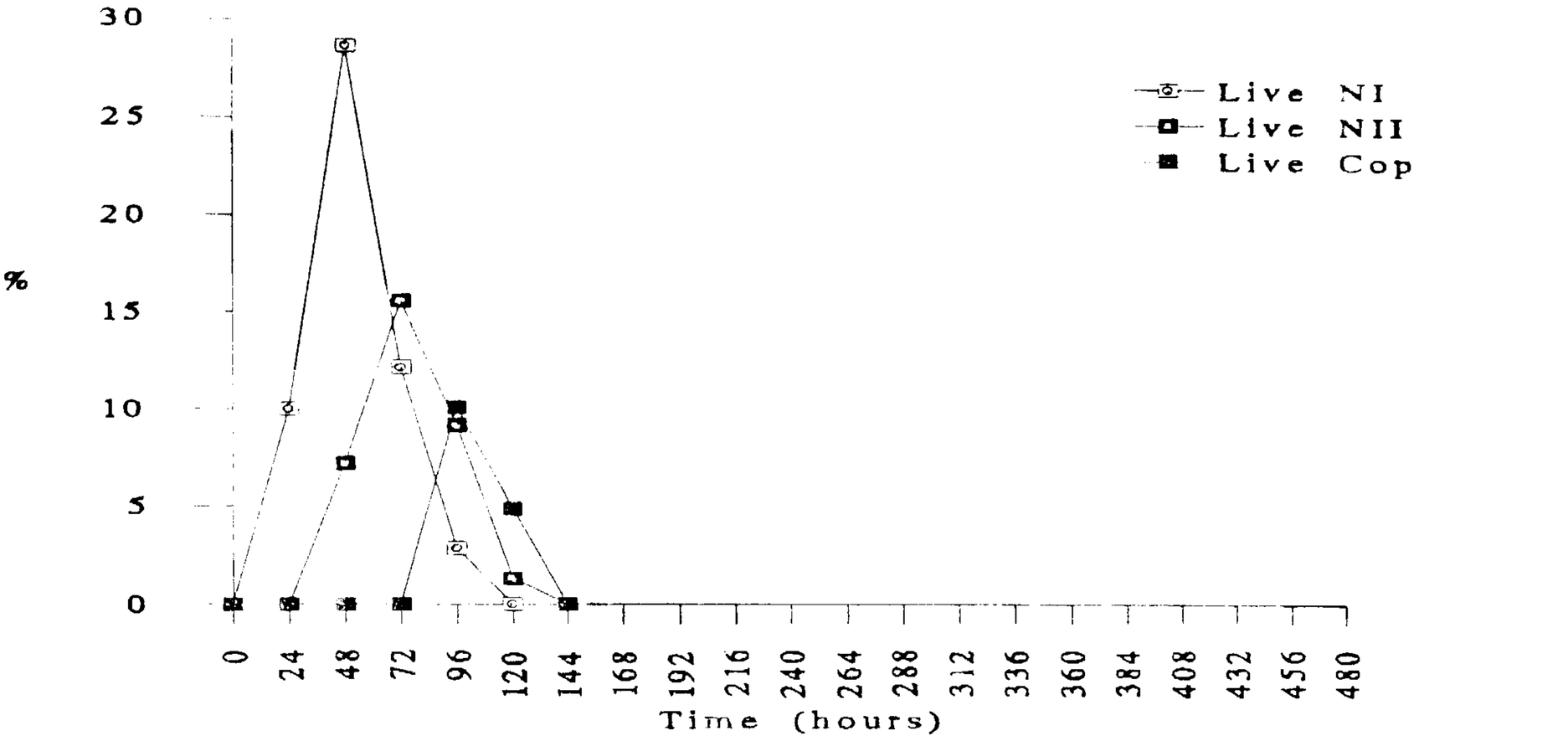
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Time (hours)
Live NI
Live NII
Live Cop

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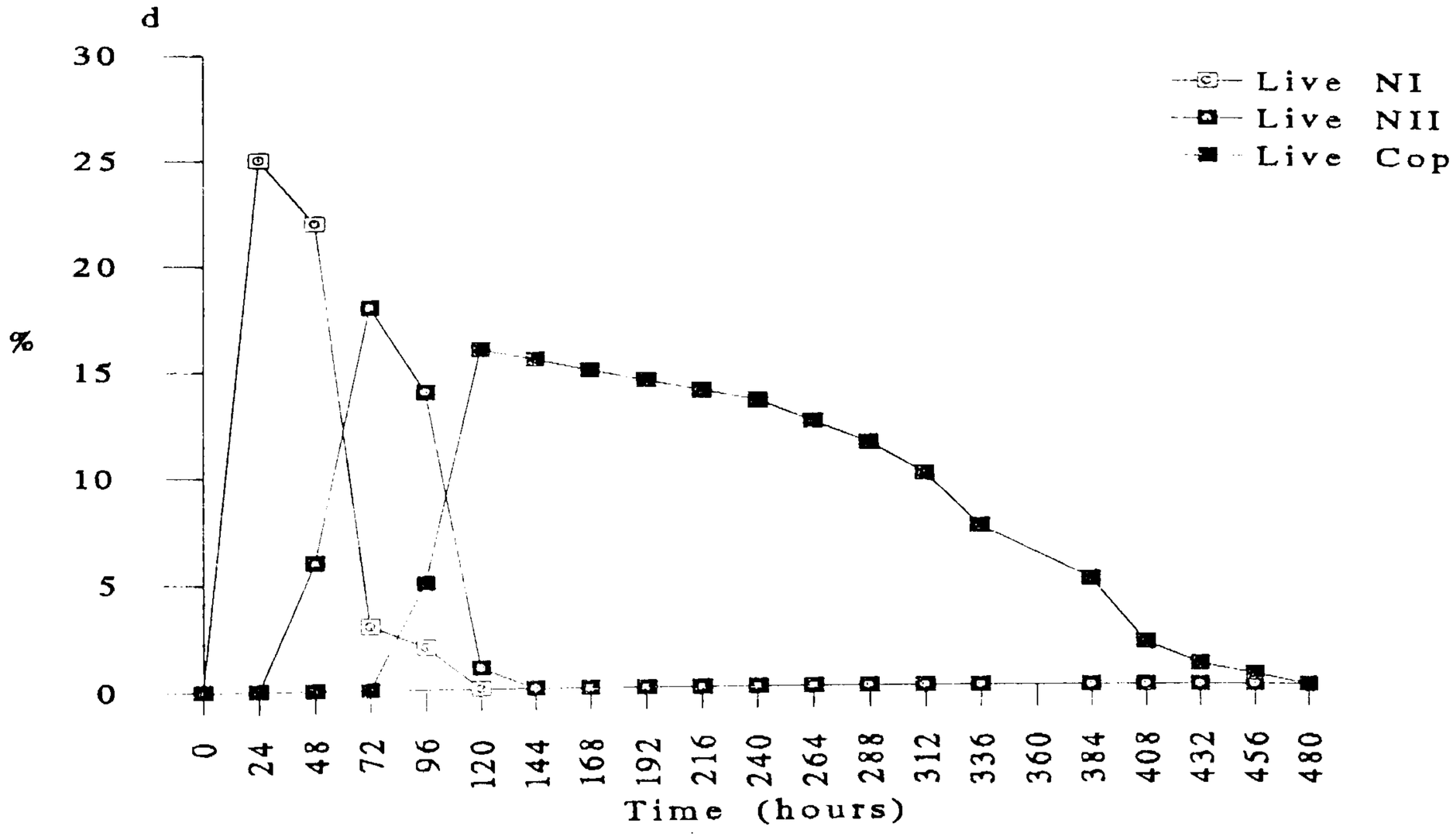
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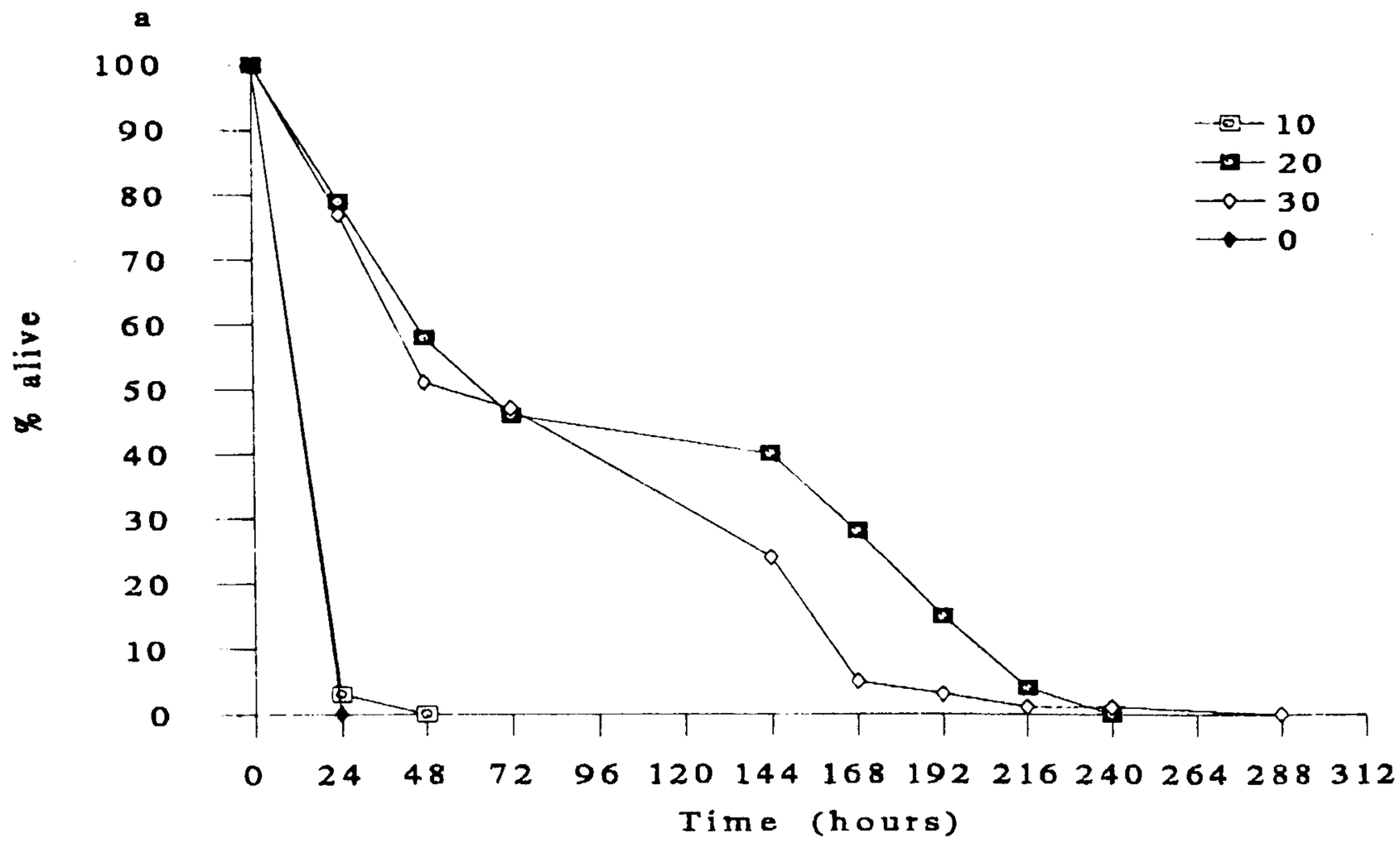
Time (hours)
Live NI
Live NII
Live Cop

Fig. 3.22. Survival of copepodids raised in full strength seawater and then transferred to salinities between 0 and 35‰. (a) 0, 10, 20 and 30‰. (b) 5, 15, 25 and 35‰.



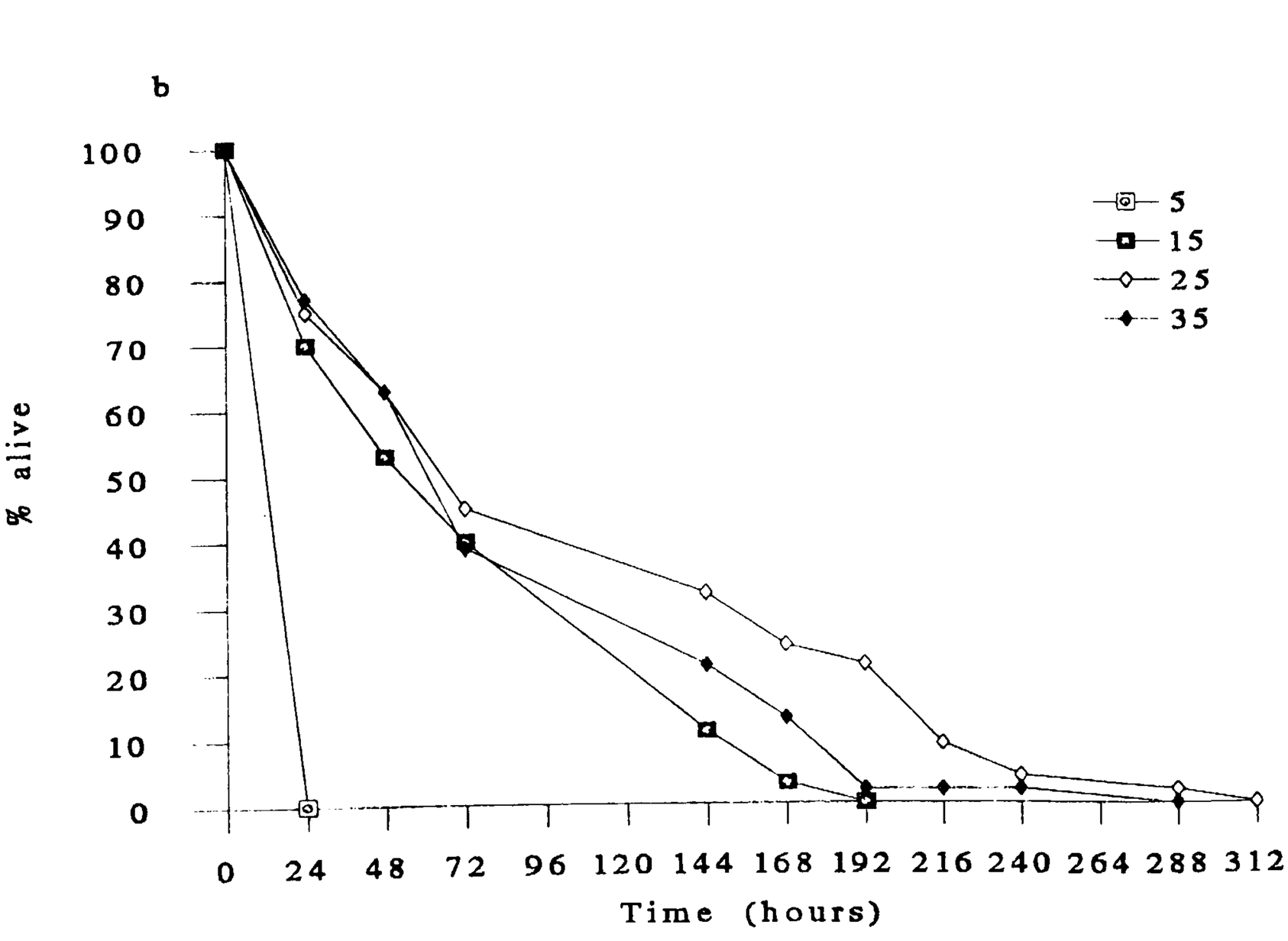
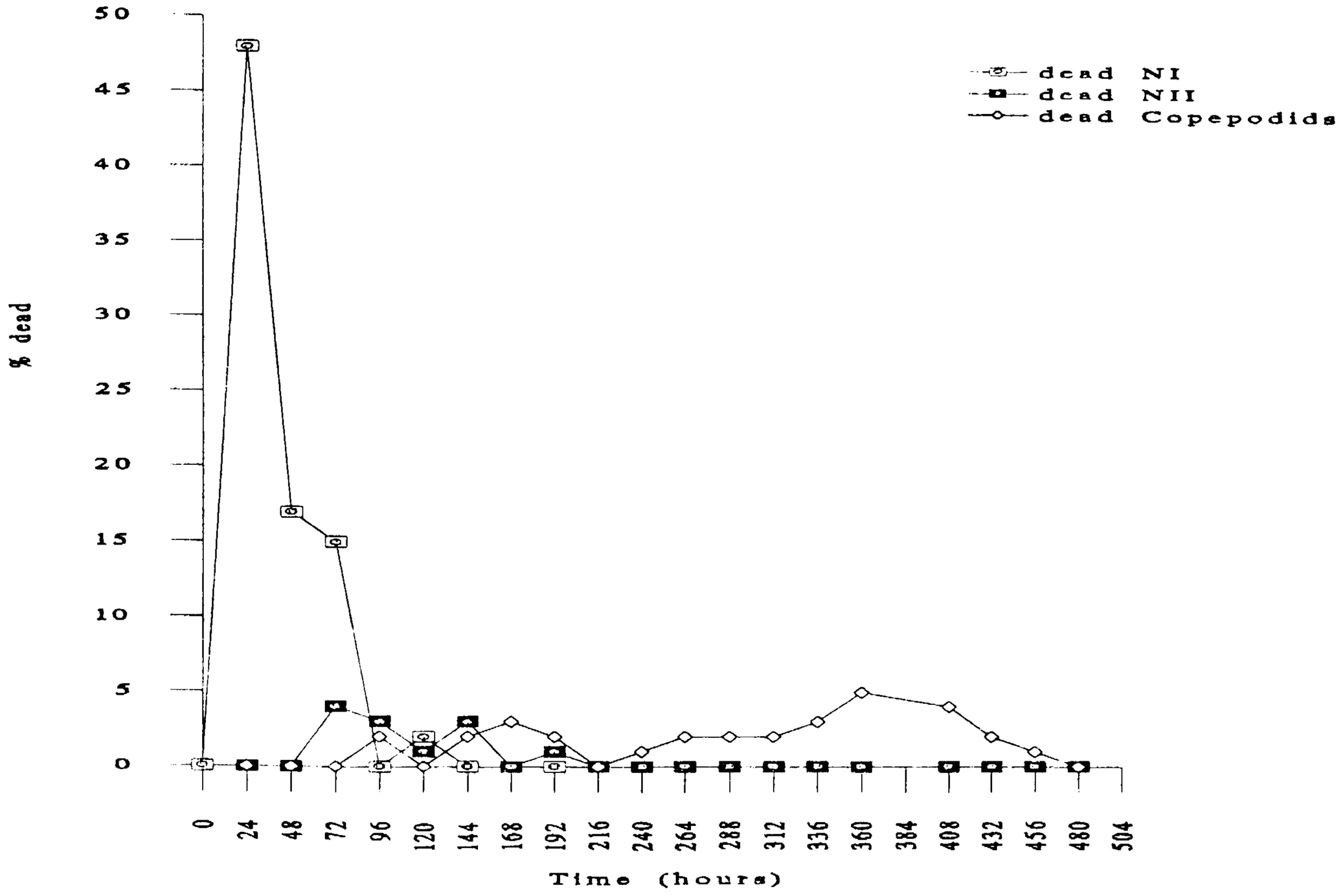


Table 3.3. Time taken for 50% mortality to occur in *L. salmonis* copepodids raised in full strength seawater and then transferred to salinities between 0 and 35‰.

Salinity (‰)	Time for 50% mortality (hours)
0	14.87
5	13.12
10	14.87
15	54.25
20	66.50
25	68.25
30	55.12
35	63.87

Mortality levels of *L. salmonis* were studied at 10°C, larvae being held in 35‰. Fig. 3.23 shows the pattern of mortality in the different stages, the graph showing the percentage of hatched larvae subsequently dying. As can easily be seen from the graph, the period when deaths occur in their greatest numbers is immediately post hatch, with almost 50% of nauplii I stages hatched dying before they enter the moult to the second naupliar stage. The NII and the copepodid stages both then demonstrate comparatively low levels of mortality. Due to the initial high mortality observed, the number of eggs within an ovisac reaching the copepodid stage is low, with only 18.33% attaining infective status.

Fig. 3.23. Mortality pattern for *L. salmonis* nauplius I, II and copepodid stages at 10°C.



3.4. Discussion

The study has shown that the hatching and development of the early stages of *L. salmonis* is highly variable in nature even under constant environmental conditions. These are further influenced by relatively small changes in the temperature and salinity of the environment, which highlights the complex nature of the parasite.

3.4.1. Hatching

3.4.1.1. Appearance and structure of egg strings

The development of eggs of caligid copepods, in common with the majority of parasitic copepods, has not been extensively described. Observations from the present study revealed that a progression of development occurs along the egg string. Eggs immediately below and close to the oviduct contained undifferentiated cell material whilst terminal eggs contained fully developed larval stages ready to hatch. Such a progression has also been reported by Hogans & Trudeau (1989) for *C. elongatus* and by Lin & Ho (1993) for *C. epidemicus*.

Another feature observed in the present study was that of the eggs taking on a pigmented appearance. Eggs that were pigmented had visible naupliar morphological characteristics, and those with the most advanced colouration were observed to move. This pigmentation has also been described for other caligid species as well as free-living copepods. Probably the most detailed description available on the development on a caligid copepod egg string is that of Lin & Ho (1993), observed whilst studying the life history of *C. epidemicus* Hewitt, parasitic on the Mozambique tilapia, *Oreochromis mossambicus* (Peters). The authors observed that, whilst the cytoplasm of newly formed eggs was uniformly distributed at first, it almost immediately concentrated on the medial side

(towards the central axis of the body of the parasite), with a change in colour from colourless to green. Approximately six hours later, dark pigment on both the medial and the outer sides of the eggs appeared in the distal end of the sac. This development of pigmentation appears to be similar to that observed in *L. salmonis* eggs.

The earliest report regarding pigmentation is that of Wilson (1905), who observed that eggs of species within the Caligidae in general changed with advancing development, from a colourless to a pigmented appearance. Johannessen (1978) also observed pigmentation, stating that it was easy to distinguish *L. salmonis* larvae near to hatching due to the presence of this dark pigmentation. Such colour changes appear to be a feature of copepods in general with Marshall & Orr (1954) describing changes in the nauplii of free-living *Calanus, Pseudocalanus, Euchaeta* and *Acartia* species as being an indication of the first sign of hatching. The alignment of eggs symmetrically along the egg string observed in the present study has also been observed for the eggs of *Caligus curtus* (Müller) (Heegaard, 1947), and may well be a characteristic common in caligid copepods.

In the present study membranes were observed within the ovisacs of *L. salmonis*. These structures though not discernible when the ovisacs were "packed" with eggs were readily observed post-hatching, especially when the ovisac had split along its length, revealing "coin-shaped" flat membranes attached transversely to the ovisac membrane at regular intervals. On rare occasions, egg strings were obtained where eggs had not been extruded to fully fill the sac. In such cases, these "transverse" membranes were observed, with empty spaces between neighbouring ones, where an egg should have been deposited. Even rarer, some egg strings were obtained with no eggs in at all. In these cases, no

transverse membranes were observed, the transparent ovisac being completely empty and unpartitioned.

The presence of membranes similar to those observed in *L. salmonis* ovisacs post-hatching have been described by Lin & Ho (1993) for *C. epidemicus*. They termed these structures "transverse membranes" and, like the present study, were only discernible after the egg had hatched. Lin & Ho (1993) attributed this to the egg membrane pressing against the transverse membrane, the two thus appearing as one thick membrane. Structures similar to those seen in *L. salmonis* ovisacs have also been reported by Heegaard (1947) for *Caligus curtus*. Heegaard stated that the egg sacs were "divided by partitions into a number of chambers each enclosing an egg." Schram (1979) observed that the egg strings of *Lernaeenicus sprattae* were partitioned every 50µm giving the egg string a box like appearance with lateral slats, and Roth (1988) noted a similar structure in *Haemobaphes intermedius* Kabata, stating that chambers for individual eggs could be seen in the empty egg cases. Wilson (1905) attributed the production of these "partitions" to the cement gland of the Caligidae, stating that "...the cement gland .... pours out a layer of its secretion between every two adjacent eggs. This forms a membranous partition and divides the egg tube into a series of narrow compartments, in each of which is a single egg...," with this method also being described by Heegaard (1959) for *Caligus rapax* M. Edwards.

However the presence of such "transverse membranes" in parasitic copepods appears not to be universal. Even within the Caligidae, it is not a consistent feature. A study of the development of *Lepeophtheirus pectoralis* by Boxshall (1974a) did not confirm that the ovisacs were partitioned into discrete sections. The presence of such structures in the egg strings of parasitic copepods can therefore not be accepted as a general structure,

with the function of such membranes in parasites which possess them being unknown. Membranes associated with eggs are discussed in further detail in section 3.4.1.3.

3.4.1.2. The hatching process

Movements of *L. salmonis* embryos were observed within egg membranes whilst still in the ovisac. These movements were small in magnitude, the appendages not being held out but, instead, remaining at the side of the body. Such early movement was also noted by Lewis (1963) for *L. dissimulatus* and by Lin & Ho (1993) for *C. epidemicus*. The movement described by Lin & Ho (1993) appears to be more exaggerated that that observed for *L. salmonis*. According to the authors, the appendages of *C. epidemicus* were spread out and their vigorous movements caused ejection of the nauplius from the egg membrane. Such use of the appendages in causing hatching was not observed in the present study, the appendages of *L. salmonis* remaining close to the side of the body for some time even after hatching. Instead, hatching occurred after a period of swelling. This swelling only appeared to occur once the ovisac had split, and the eggs came into direct contact with the external medium. Once an egg became spherical, it burst, releasing the nauplius, anterior end first. The nauplius was ejected at speed, indicating that a large internal pressure had built up. Hatching occurred from the distal end first so that those eggs that had become pigmented and mature first tended to be exposed to the external medium and hatch first. Once an egg had been extruded, though it sometimes remained adhered to the ovisac, the space was quickly filled by expanding neighbouring eggs. Such extrusion and adhesion was also observed by Schram (1979) for the eye maggot, *Lernaeenicus sprattae*. Schram (1979) described the egg membrane as being sticky with the egg consequently remaining attached to the ovisac for some time before it "dropped" off or hatched to release a nauplius. Like

the present study, *L. sprattae* eggs were also coin shaped before emission from the ovisac, expanding after extrusion suggesting the hatching mechanism is similar in both parasites.

The swelling reported here for *L. salmonis* eggs has also been observed for other parasitic copepods. However, information on hatching is scanty, and limited to brief descriptions supplied by, for example, Wilson (1905) and Izawa (1969). An exception to this is the report of Lin & Ho (1993) regarding *C. epidemicus*. In their detailed description of hatching they, like the present study, also noted that the process commenced distally, proceeding proximally in sequence. They attributed the splitting of the egg sac to the swelling of the eggs within as did Piasecki (1989), studying the life cycle of the lernaeopodid copepod *Tracheliastes maculatus* Kollar, who stated that the swelling of the eggs caused the egg sac to rupture. Swelling prior to the ovisac splitting was not observed for *L. salmonis* in the present study and, indeed, the report of Boxshall (1974a), like the present findings, described expansion and subsequent rupture of eggs of *L. pectoralis* to occur after the splitting of the egg sac. Lin & Ho (1993) go on to state that the eggs hatch whilst in their individual "compartments" due to the action of the appendages, again not observed in the present study.

Of the briefer reports, Lewis (1963) also observed expansion of the eggs of *L. dissimulatus*, though it is not completely clear whether this was before or after the splitting of the ovisac. Unlike the present study, *L. dissimulatus* is described as ejecting its way out of the egg membrane by "sporadic violent movements of its appendages." Wilson (1905) described hatching in Caligidae in general, stating that once the ovisac had split, the nauplius could "wriggle out." From the observations of the present study it appears that this is not the case in general, as the appendages of *L. salmonis* nauplii within egg membranes remain

close to the side of the body and are restricted to "twitching" motions. Instead, it appeared that internal pressure was the major contributing factor to the hatching of the egg.

Nauplii released from eggs were adhered to the and sometimes were unable to be "free" of membranous material, whether it originated from the egg sac membrane or the egg membrane. In such cases, only when the appendages were able to move freely after hatching did they remove themselves clear of the ovisac.

Lin & Ho (1993) attempted to group authors whom they considered had alluded to hatching in parasitic copepods as being a one step process (Wilson, 1905; Lewis, 1963; Hwa, 1965; Izawa, 1969; Johannessen, 1978) or as a two step process (Boxshall, 1974a; Schram, 1979; Piasecki, 1993 and their own (1993) description). However, from the descriptions of published reports and from observations of hatching of *L. salmonis* in the present study, it seems highly probable that what Lin & Ho (1993) report as either one step or two step processes, are in fact one and the same. There appears to be no major differences between the two, with both movement prior to swelling and swelling and subsequent hatching occurring. The only differences in the two groups of reports appear to be the amount of detail included in the descriptions, "one steps" tending to contain less detail than the slightly more extensive "two step" reports. From this, and the present study, it is apparent that the pattern in hatching in parasitic copepods is very similar.

3.4.1.3. Egg membranes

In this present study of *L. salmonis*, only one membrane was ever observed to surround the differentiated egg/formed nauplius. However, in the majority of extruded differentiated eggs examined under x100 magnification, there appeared to be the remains of another membrane attached to the outside of the egg membrane. This remnant was always

present on the posterior area of the egg membrane. The origin of this membrane was not ascertained and so it is unclear as to whether it was some form of attachment between the egg membrane and the ovisac wall, or the egg membrane and the transverse membranous structures observed within the ovisac.

Despite the varying amount of detail in the published reports concerning hatching in parasitic copepods, the majority of descriptions, like the present study, report the presence of only one egg membrane, surrounding the differentiated egg/formed nauplius. There have however been a few execeptions. Piasecki (1989) observed both an inner and an outer membrane around each egg produced by *T. maculatus*. The outer membrane of *T. maculatus* remained within the ovisac, while the inner membrane subsequently burst once the egg containing the nauplius had been released.

In a number of other published reports on both parasitic and free-living copepods, the presence of two egg membranes has also been described. In his two reports on the hatching of aquatic invertebrate eggs, Davis (1969, 1981) is in no doubt about the existence of the two membranes. Despite differences in the opinion of the actual mechanisms of hatching, Davis (1969) cites a number of workers who also describe both an inner (also termed larval or blister) membrane and an outer chorionic membrane (Marshall & Orr, 1954, 1955; Davis, 1959b; Elster, 1966) for a number of free living copepod species (*Heterocope, Calanus* and *Diaptomus* spp. respectively). The presence of these two membranes has also been described for the parasitic copepod, *Haplostomella australiensis* Gotto. *H. australiensis* is parasitic on the ascidian *Styela etheridgii* Herdman, and Anderson & Rossiter (1969a) reported its hatching mechanism in which the "outer egg membrane ruptures, revealing the transparent inner membrane." These two membranes could not have

been mistaken by the authors for one egg membrane and the egg sac membrane as Anderson & Rossiter (1969a) go on to state "...the simultaneous increase in volume of the inner membranes, in addition to rupturing the outer membranes, generated a collective pressure sufficient to rupture the ovisac longitudinally." However, in another report on hatching in a parasitic copepod (Anderson & Rossiter, 1969b), the caligoid *Dissonus nudiventris* Kabata, they presented no evidence for the existence of the two membranes. If the presence of two egg membranes is a common phenomenon in copepods, it may seem surprising that it has not been reported in more parasitic species than just *H. australiensis* (Anderson & Rossiter, 1969a) and *T. maculatus* (Piasecki, 1989). However, if, as Piasecki (1989) and Anderson & Rossiter (1969a) observed, the outer membrane ruptures within the ovisac, it may be difficult to distinguish the presence of it. The remnant of material attached to *L. salmonis* eggs in the present study, is indicative that the egg membrane was in close relation to another membrane at some point in the ovisac. It may be possible that the transverse membranous structures observed in the ovisac in the present study of *L. salmonis* are the remains of an outer egg membrane that burst within the ovisac and remained attached in some way.

3.4.1.4. Mechanisms of hatching

The actual mechanism causing *L. salmonis* eggs to hatch has not been previously described. Nauplii are "propelled" out of the egg membrane when it bursts, indicating that a large internal pressure has built up. The pressure build up must be due directly to the swelling of the eggs. Such swelling must be due to an increased amount of fluid present within the egg membrane presumably permeating osmotically across the egg membrane(s) from the external medium. Swelling was observed to occur once nauplii were active and the

ovisac had split. This suggests that a concentration gradient exists between the internal environment of the egg and the external medium. Eggs containing nauplii that were not observed to be active did not show immediate swelling of the egg membrane when emitted from the ovisac, instead taking a much longer period of time to hatch. This suggests that the concentration of the internal fluid of eggs containing active nauplii is greater than those containing dead, motionless larvae. This suggests that active nauplii must in some way contribute either to the concentration of the internal fluid or, in some way, increase the permeability of the membrane to water. It may be the case that when the nauplius becomes active within the egg membrane it starts to excrete. Such a function would cause an increased solute concentration within the egg membrane(s). This build up may then cause a concentration gradient across the membrane(s), which, if selectively permeable to water, would cause an influx of water across and the subsequent swelling and bursting observed. If this is the case, then it may also be possible that, if an outer chorionic membrane does exist around *L. salmonis* eggs, it may burst whilst the eggs are still within the ovisac leaving behind the transverse structures observed. It may only take a small concentration gradient caused by excretion of the nauplius and hence a small amount of swelling to burst the outer egg membrane within the ovisac. Such pressure may also be the cause of the ovisac splitting and the subsequent extrusion of the eggs. No noticeable swelling was observed of eggs within the ovisac though this does not necessarily mean that none takes place. Once in contact with the external medium, increased swelling may then occur due to an increased concentration gradient, as observed in the present study until the pressure is great enough to cause the inner larval egg membrane to burst. *L. salmonis* eggs placed in freshwater (0‰) were observed to swell faster, and to a greater extent than those in full strength seawater

(35‰), suggesting a higher concentration gradient present. In higher salinities, and hence higher levels of solutes, it would take longer for a concentration gradient to be set up when and if a nauplius begins to excrete within an egg membrane(s). This may explain the slower swelling and hatching observed in *L. salmonis* eggs held in full strength seawater compared to freshwater. From dead eggs it can be seen that the membranes are already permeable to water, as some swelling does occur. It seems likely that a membrane will either be permeable or non-permeable to water, with the degree of permeability being unlikely to change. It is therefore suggested that nauplii do excrete solutes to increase the concentration gradient across the egg membrane(s), causing an influx of water by osmosis resulting in the swelling observed.

Davis (1959b) and other investigators claimed that such swelling indicated that the method of hatching in copepod eggs is osmotic, and that "no other explanation comes readily to mind" (Davis, 1969). Marshall & Orr (1954) also suggested that it "might be (that) a sudden increase of excretion by the embryo leads to an increased content of salts and the imbibition of water," the authors studying hatching in *Calanus finmarchicus*.

Due to the lack of detailed descriptions pertaining to hatching in parasitic copepods, it is not at all surprising that the information available for the mechanisms of hatching are limited compared to their free-living counterparts. However, all appear, in their brief reports, to agree with the findings of the present study. Schram (1979) attributed hatching of *L. sprattae* nauplii to internal (osmotic) pressure, and Piasecki (1989) concluded that the swelling of eggs of *T. maculatus* was due to an uptake of water by osmosis. Heegaard (1947) cited by Roth (1988) believed that the nauplii of *C. curtus* became active due to osmotic forces, but no real details to sustain this hypothesis are given in these reports. One

of the few more detailed reports is that of Anderson & Rossiter (1969a) for the ascidicolidean copepod *Haplostomella australiensis*, a parasite of the ascidian *Styela etheridgii*. The authors stated that hatching was by an osmotic mechanism, and that the sequence of events in the process and the operation of the osmotic hatching mechanism in *H. australiensis* were comparable to those previously described by Marshall & Orr (1954) for marine planktonic copepods and for freshwater copepods by Davis (1959b). Although no descriptions for hatching in *H. australiensis* were given as such, the authors suggested that osmotic stress is set up either in the secretion of an osmotically active substance, or in a change in the structure and permeability of the inner membrane, or indeed both. Anderson & Rossiter (1969b) also reported hatching for a dissonid parasitic copepod, *Dissonus nudiventris*, a gill parasite of the Port Jackson shark, *Heterodontus portjacksoni* (Meyer), finding that the egg strings of the parasite were sensitive to the osmotic concentration of the external medium, hypotonic seawater causing quick synchronous hatching as was observed for *L. salmonis* in the present study, whereas hypertonic seawater inhibited further hatching of the same egg string once transferred. They inferred that as in other copepods (Marshall & Orr, 1954; Davis, 1959b) already described, the swelling of the egg membrane during hatching of the eggs of *D. nudiventris* had an osmotic basis.

The mechanisms of hatching in the Copepoda according to Davis (1969) may all follow one method, citing that the best accounts were those of Elster (1936) for the freshwater calanoid *Heterocope borealis* Fischer, Marshall & Orr (1954, 1955) for the common marine species *Calanus finmarchicus* (Gunnerus) and Davis (1959b) for three north American species of *Diaptomus, D. siciloides, D. ashlandi* Marsh and *D. oregonensis*. All of these workers agree that an outer egg membrane, the chorion, splits by pressure from

within. Davis (1959b) observed that the initiation of hatching in *Diaptomus* species was indicated by the appearance of a fluid filled space between the nauplius and the egg membranes, followed by a bulging of the egg surface, this "bulging" being attributed to the expansion of the inner (larval) egg membrane. The aforementioned authors are also in agreement over the observation that the membranes then subsequently expand until they are much larger than the original egg, and that within this blister membrane the unhatched nauplius begins to swim actively around, as was observed for *L. salmonis* in the present study and for *L. dissimulatus* (Lewis, 1963), *T. maculatus* (Piasecki, 1989) and *C. epidemicus* (Lin & Ho, 1993). The movement of *L. salmonis* was first observed in the present study when the mature eggs were still inside the ovisac prior to it splitting. At this point in time, no space could be distinguished between the nauplii and their corresponding egg membrane(s), due to their packed "coin-shaped" appearance whilst within the egg string. Once the ovisac membrane had split, swelling was observed to occur and a space between the nauplius and the egg membrane became visible, similar to that described by Davis (1959b). However, no "bulging" of the egg membrane(s) was seen, in contrast to Davis (1959b). If there were a second outer egg membrane present around *L. salmonis* nauplii it appears likely that it bursts within the ovisac. Hence the "bulging" described by Davis (1959b) would be very unlikely to be observed.

In contrast to Davis (1969) however, Ziegelmayer (1927), studying 17 species of *Cyclops* thought that the outer membrane swelled rather than the inner one, causing a pressure to develop between the two egg membranes, with as a result, the outer membrane subsequently bursting. It seems implausible that an outer egg membrane, if present in *L. salmonis* could have swollen in this manner within the ovisac. No "gap" was observed to

occur between eggs, which would be expected if there was a swelling between a second outer egg membrane and the inner egg membrane.

After the breaking of the outer membrane, the second inner membrane became visible according to Davis (1959b) and Marshall & Orr (1954) for *Diaptomus* species and *C. finmarchicus* respectively. As the inner membrane continued to expand in both cases, the outer membrane receded back, allowing the emergence of the inner membrane plus nauplius. Nauplii of *L. salmonis* were observed to have their appendages held close to the body prior to hatching. Davis (1959b) also recorded typically no movement of the unhatched *Diaptomas* nauplius, which was concluded to be due to the appendages being "fixed" to the side of the body. As with the report of Davis (1959b), Marshall & Orr (1954) also observed that the nauplii of *Calanus finmarchicus* had their limbs folded closely to the body. However, as already described, movement in the form of *L. salmonis* bodily contractions was discernible prior to even the splitting of the ovisac which may well set up the initial concentration gradient across the egg membrane(s). It may be the case that Davis (1959b) and Marshall & Orr (1954) only recorded obvious appendage movements and did not look at the rest of the body.

The spherical appearance following swelling observed in the present study was also noted by Marshall & Orr (1954). The time taken for these events to take place for a single *L. salmonis* egg hatching naturally was on average between 10 to 20 minutes, a time that closely corresponds to that given by Marshall & Orr (1954) for the hatching time of individual *C. finmarchicus* eggs. They reported a time of 10 to 15 minutes for hatching of individual eggs. Both of these reports are in striking contrast to the 14 hours or longer reported by Ziegelmayer (1927). However, since it is suggested that hatching does have an

osmotic basis, the time may dependent on whether the eggs are in a marine or freshwater environment. According to Marshall & Orr (1954), hatching takes place along the same general plan for copepods belonging to several different groups: the rigid outer membrane being burst by pressure from within the inner membrane, and the latter then emerging from the outer membrane, with the time taken and the change in volume dependent upon the species. For those not held in egg sacs (for example, *Calanus* spp.), this change in volume is approximately fivefold, according to Marshall & Orr (1954) before the nauplius hatches, but for eggs which are carried within egg sacs, the increase is usually smaller, and may be little more than necessary to "crack" the outer shell.

3.4.1.5. Initiation of the hatching process

Although the hatching sequence of copepods appears to be osmotically controlled, there have been suggestions made in the literature concerning environmental factors that change the osmotic conditions, initiating hatching (Anderson & Rossiter, 1969a; Roth, 1988; Poulin, Conley & Curtis, 1990b; Conley & Curtis, 1990, 1993). Such reports tend to concentrate on the role of photoperiod or light controlling hatching, though others have investigated tidal influences.

In the present study various photoperiod experiments revealed that not only did *L. salmonis* egg strings of a pair demonstrate the same hatching pattern, but also showed that hatching was not controlled or affected in anyway by light or dark regimes. This lack of control by photoperiod or light on the hatching of *L. salmonis* is in contrast to other reports concerning parasitic copepods. Anderson & Rossiter (1969a) induced and observed hatching of the *H. australiensis* under bright light stimulation and concluded that the vigorous pre-hatching movements of the embryo were induced by this bright light

stimulation. They suggested that this was the first of two components involved in the hatching mechanism, with this setting up the second, already discussed, osmotic component.

There are possible evolutionary reasons for this apparent control by light. By starting to hatch in the presence of bright light the ovisac would have already been released by the adult female parasite and had sufficient time to be carried quickly through the gut of the ascidian host and out of the exhalant siphon. Such hatching after "expulsion" would cause the parasite to avoid the digestive enzymes of the gut of the host. However, the authors stated that they did not observe hatching without bright light stimulation and so the experimental hatching cannot be compared with a control group. They did note though that in laboratory held specimens hatching from ovisacs occurred overnight and the authors "timed" this to the nauplii hatching synchronously at dawn. This, though, seems a little tenuous, as they did not explain how they calculated the timing of hatching. Presumably they must have been able to age the nauplii to determine the time of hatching, but no details of their methods were given.

The same authors (Anderson & Rossiter, 1969b) also studied the hatching of *D. nudiventris* but the process for this species was not found to be promoted by light. This is probably, if one accepts the evolutionary hypothesis, because there is no evolutionary advantage to hatching in response to light, as being a gill parasite of the Port Jackson shark, hatching would not bring the resulting nauplii into a region containing digestive enzymes or other chemicals which would cause death. Such evolutionary reasons may explain why *L. salmonis* eggs showed no increased hatching response to light. The eggs are released directly into the water column not having to pass through the host at all. It may be hypothesised that if hatching is initiated by an environmental trigger, the parasite may

maximise its chances of infecting a host by releasing its larval stages when hosts are more likely to be abundant. However due to the developmental pattern of *L. salmonis*, the first stage released is not the infective copepodid stage. Instead there are two free-living stages before the copepodid is reached. It therefore seems highly unlikely that there will exist such a trigger as the infective stage will not be reached for many days. Johannessen (1978), like the present study, also found that hatching of eggs of *L. salmonis* took place at all times of the day and night and Pike *et al.* (1993) found that there was no correlation between the time of hatching of *C.* elongatus (a parasite also possessing two free-living naupliar stages) and the time of day.

It may be more likely however that parasites whose eggs release the infective stage, rather than non-infective nauplii, may have a cue triggering hatching to release these stages into the external environment when hosts are more likely to be present, increasing the chances of infection. *Salmincola edwardsii* (Olsson) eggs hatch to release infective copepodids (Conley & Curtis, 1990, 1993; Poulin *et al.*, 1990b). Work by Poulin, Curtis & Rau (1990a) suggested that the intrinsic activity of *S. edwardsii* copepodids is generally low, and that they probably spend the majority of their time resting or swimming just above the bottom. Poulin *et al.* (1990b) suggested that such behaviour may cause the host, the brook trout *Salvelinus fontinalis* (Mitchill), to be more vulnerable to infection at night as this is when the trout rest near the bottom. In order to create such a spatial overlap of the parasite and host, maximising their chances of infection they hypothesised that *S. edwardsii* eggs would need to hatch at sunset. The authors tested two cues associated with nightfall - the onset of darkness (tested by different photoperiod regimes) and a decrease in temperature - on the initiation of hatching, and the rate of hatching of the copepods eggs. It was found

that there was no evidence to suggest that the initiation and rate of hatching of the eggs of *S. edwardsii* were stimulated by photoperiod and/or by fluctuations in temperature.

Roth (1988) studying the parasitic penellid copepod *Haemobaphes intermedius* hypothesised that tidal influences may initiate the hatching sequence for this particular parasite as it infects intertidal fish such as the tidepool sculpin, *Oligocottus maculosus* Girard, and the sharpnose sculpin, *Clinocottus acuticeps* Gilbert, both of which take refuge in tide pools where, at low tide, environmental conditions fluctuate. Roth (1988) suggested that the physical or chemical environmental changes characteristic of the tide pool act to stimulate mature nauplii within their egg membranes and initiate the rapid hatching sequence. This would allow the release of nauplii, which are capable of moulting into infective copepodids within 12 hours. These infective stages would then be in an environment with near and abundant hosts for quick infection before the tide returns to the high pools, thus securing a position for further development. However Roth (1988) found that parasite egg cases did not show such hatching patterns.

It may not be surprising that parasites with a direct life cycle and a relatively long developmental time to the infective stage, such as *L. salmonis*, do not appear to hatch in relation to an environmental trigger. However, it would seem likely that those parasites that release the infective stage directly such as *S. edwardsii* or those with a short developmental time to such a stage (for example *H. intermedius*) would have developed a strategy with respect to hatching as *H. australiensis*. Future research into environmental cues with a greater range of parasite species may well herald different results.

3.4.1.6. Hatching success

The hatching success of parasitic copepod eggs from egg strings and their subsequent survival is of obvious importance in any attempt to try and predict future infection levels on fish farms. Tully & Whelan (1993), in trying to establish a relationship between the production of nauplii of *L. salmonis* from farmed and wild salmon and the infection of wild sea trout off the west coast of Ireland appeared to assume that hatching occurred in all normal eggs. From the present study, such assumptions were seen not to be valid.

Hatching success of *L. salmonis* was observed to vary greatly in the 64 egg strings held at 10°C in 35‰ and monitored. Although egg strings of a pair were observed to demonstrate the same hatching pattern, duration and success, the hatching success overall was seen to vary widely between pairs. The mean percentage hatching success was 59.9%, but this ranged from 0% (no eggs hatching within an ovisac) to 100% (all eggs hatching). The majority of eggs were observed to hatch within the first 48 hours, with 46.8% (78.1% of eggs that hatched) of eggs within the ovisac hatching during this time. Although the mean duration of the hatching period was 9 days (216 hours) at 10°C (range 24 to 312 hours), in the last 5 days (120 hours), only 0.7% (1.2% of hatched eggs) of eggs within an ovisac hatched. The duration of *L. salmonis* hatching in the present study, although highly variable, is longer than previously reported for the parasite. Johannessen (1978) reported that the hatching period of *L. salmonis* egg strings was less than 40 hours at 5 to 12°C, with the majority of hatching occurring within 5 to 10 hours. Johnson & Albright (1991) also reported a highly variable hatching duration for *L. salmonis*, with this ranging from 18 to 65 hours (mean, 31.7 hours) for ovisacs held at 10°C in 30‰ static water. These reduced durations may be due to the authors discontinuing observations early, not having noticed and

therefore not recording the small percentage of eggs that hatch over the last few days of the hatching period. Obviously it is difficult to compare hatching duration between *L. salmonis* and hatching in other parasitic copepods, due to species differences. However it can be seen that hatching in other species can occur over long periods, with *Lernaeocera branchialis* egg strings taking 12 days (268 hours) to hatch at 10°C (Whitfield *et al.*, 1988).

Viability of the nauplius I stage was also examined in the present study. At 10°C in full strength seawater, viability of the nauplius was not constant, but dependant upon when hatching occurred during the hatching period. Viability of hatched nauplii was observed to be highest in those which hatched within the first 48 hours of hatching coinciding with the time when the majority of hatching occurred. Approximately 83% of the nauplii that hatched (49.7% of total eggs within an ovisac) in this time period were alive, with this number declining to 0% after 120 hours, when only dead nauplii were seen to be released. This slow hatching in dead eggs is most probably simply due to the small osmotic difference between the internal and external medium, as the dead nauplius cannot contribute any solutes by excretion. The period when dead nauplii were released coincided with the period when hatching had declined to very low numbers. Johnson & Albright (1991) did not state whether the viability of *L. salmonis* nauplii I altered over the hatching period unlike the present study, though they did observe high variability of the numbers of active nauplii, 9.7 to 95% of hatched nauplii being active, with the mean number of active nauplii obtained being 65.9% at 10°C in 30‰.

There is a lack of detailed studies on the hatching success of parasitic copepods, despite the obvious importance of understanding the population dynamics of each stage. Johannessen (1978) in one of a small number of reports on hatching success of *L. salmonis*

described it as being "very high." However no details were given regarding the proportion of eggs that remained undeveloped and did not hatch. Johnson *et al.* (1993) looked briefly at the normal hatching success of eggs reporting an average hatching success of 93% with a range of 79 to 99%, whilst Pike *et al.* (1993) found that over 85% of *C. elongatus* nauplii hatched from egg strings that matured 0 to 3 days after collection (at 5, 10 and 15°C), but in egg strings that matured later, poor hatching was observed. However no exact figures were given. The majority of information on hatching in copepods is therefore concerned with free-living copepod species. Knowledge of the production of these species has been of importance with respect to primary and secondary plankton masses and food chains, but data on hatching may give a better understanding of the general processes involved for parasitic copepods.

Ianora *et al.* (1992) found that the hatching success of the planktonic copepod *Centropages typicus* ranged from 41 to 94% depending upon the time of year and the year studied. However hatching success was found not to be related to environmental variables such as chlorophyll and temperature. Those eggs which did not hatch were seen to rapidly deteriorate with time, indicating that they were not eggs in diapause. Ianora *et al.* (1992) concluded that eggs that did not hatch were non-viable eggs that represented eggs that had either not been fertilised or, alternatively, fertilised eggs in which development was incomplete or had failed due to unknown physiological causes. To test if these unhatched eggs had been fertilised, the authors stained them with the fluorescent stain Hoescht 33342. The fertilised eggs would thus show two visible "female and male" pronuclei in eggs stained immediately after deposition, and many visible nuclei for those incubated for 24 hours and in an advanced state of cleavage. Unfertilised eggs would show one nucleus

Fluorescence demonstrated that the unhatched eggs of *C. typicus* had been fertilised, with large numbers of visible nuclei being observed, indicating that in most cases development had proceeded to an advanced stage before cessation and death of the embryo. Only eggs that continued normal development appeared to be able to hatch, whilst those that had "aborted" in some way did not. If this is the case for *L. salmonis*, then dead nauplii that were observed to be released must have developed to a very advanced stage and died shortly before hatching.

Low egg viability, and its subsequent knock-on effect upon hatching success for *C. typicus* was observed in the summer and early autumn period and higher viability and thus higher hatching success was observed from January to May, explaining the seasonal variations observed by Ianora *et al.* (1992). The findings that higher egg viability and hence hatching success was observed over the winter for *C. typicus* by these authors is in contrast to Ritchie *et al.* (1993), who cites unpublished observations that "...more eggs and regions of viable egg strings appeared discoloured and disorganised in winter generations of *L. salmonis*." However, Ritchie *et al.* (1993) do not comment upon any possible relationship between egg viability and their hatching success. The results of Ianora *et al.* (1992) demonstrated that the majority of unhatched eggs had undergone cleavage post fertilisation, but due to unknown causes, they had failed to develop to hatching. Explanations in the literature for this have dealt principally with the inadequate supply of diets for free living copepods, which have been shown to have an effect on maternal fecundity and survival and development of juveniles (Huntly, Ciminiello & Lopez, 1987 for *Calanus pacificus* Brodsky; Nassogne, 1970 for *Euterpina acutifrons* (Dana); Provasoli, Shiraishi & Lance, 1959 for *Tigriopus brevicornis* Mueller; Arnott, Brand & Kos, 1986 for *Gladioferens*

pectinatus (Brady)) but there does not seem to be a link between diet and egg fertilisation and viability, and hence hatching success. Although it may appear that food quality and diet will have little effect with respect to parasitic copepods and thus there must be other factors that help to account for eggs not developing or hatching, one must take into account the fact that mucus quality and production may well be affected by seasonal factors. This in turn may affect the physiological condition of gravid females and the subsequent quality of her eggs.

3.4.1.7. Environmental conditions affecting hatching

It can be expected that hatching, like many biological functions, will be influenced by certain environmental parameters. The duration of *L. salmonis* hatching was seen to be inversely related to temperature, although the final percentage of hatching was not affected, the final percentage of eggs hatched being 67.1, 65.6 and 66.8% at 5, 7 and 10°C respectively. It might however be expected that egg strings held at lower temperatures may produce less viable nauplius I stages, since the viability of hatched larvae was seen to be low during the later hatching period. This though was not observed, the viability of the nauplius I stages remaining higher for longer at lower temperatures. Viability was however seen to decrease once the majority of eggs had hatched, falling to 0% over the last few days of hatching, when few eggs are released. The proportion of live nauplii hatched therefore appears to decrease once the majority of hatching has occurred, with eggs that hatch late in the hatching period being highly unlikely to release live larvae.

Temperature has been demonstrated to have an effect upon the duration of the hatching period for other species of copepods, though unlike the present study, viability of the subsequent larvae was not studied. Poulin *et al.* (1990b) observed an earlier onset of

hatching and a shorter hatching duration at higher mean temperatures for the parasitic copepod *S. edwardsii*. However, in contrast, Conley & Curtis (1992) found that the onset of hatching of the eggs of *S. edwardsii* was directly related to increasing water temperature (that is, a shorter developmental period), but had no effect upon the duration of hatching. In a later report, Conley & Curtis (1993), studying hatching in the same parasitic copepod, again found that the onset of egg sac hatching was directly related to increasing water temperature, but that the duration of hatching was not affected by temperature. Additionally, they reported that the hatching success was not affected by temperature as was also seen in the present study for *L. salmonis*. Pike *et al.* (1993) in contrast to Conley & Curtis (1992, 1993), but like the present study, found that increasing temperature caused a decrease in the mean duration of the hatching period, the difference in time between 5 and 15°C being significant. The effect of decreasing hatching duration observed for *L. salmonis* is no doubt associated with the increased metabolic rate and subsequent developmental rate due to higher temperatures, which have been reported by other authors (McGladdery & Johnston, 1988) for the parasitic copepod *Salmincola salmoneus*, and in free living copepods (Burgis, 1970; Bottrell, 1975; Sarvala, 1979 and Palmer & Coull, 1980).

As well as increasing temperature affecting some aspects of the hatching process such as duration, Heegaard (1947) suggested that other unfavourable conditions will often lead to abortion.

One of the principal environmental factors affecting sea lice other than temperature will be salinity. Hatching was seen to occur in *L. salmonis* in the present study in salinities ranging from 0 to 35‰. However, it was found to be compromised in the present study in low salinities, with only 3.3% of eggs hatching in freshwater (0‰). This figure

rose to 80.6% for egg strings held in full strength seawater. However, despite reaching the hatching stage in all salinities, live nauplius I stages were not obtained in salinities of less than 15‰, with only dead larvae being observed to be released from egg membranes at these low salinities. Dead larvae will be released from egg membranes despite the fact that they will not be contributing, probably by excretion, to the concentration gradient across them. At low salinities the gradient will be so large that the nauplii would not have to add to the solute concentration in order for the egg to swell. The same occurred with *L. salmonis* eggs when Johannessen (1978) placed them in reduced salinity water of 11.5‰; only a few nauplii hatched which subsequently died and Berger (1970) determined the lower limit of optimal salinity for *L. salmonis* to be 16‰. However even at 15‰ in the present study, only 4.4% of *L. salmonis* nauplii I larvae released at 24 hours into the hatching period were observed to be active. Johnson & Albright (1991) also found that no active nauplii were released in 15‰ despite a high hatching success (70%). This success is in contrast to the levels of hatching observed at this salinity in the present study (23.38%).

In salinities of 20‰ or greater, high levels of active viable nauplii were again seen to be released from the egg membranes in the present study, with this again decreasing, until, at the end of the hatching duration, only dead larvae were obtained. Development of *L. salmonis* therefore does not seem likely to occur in the natural environment in areas where sea lice are exposed to constantly low salinities, due to this low hatching success, and the subsequent reduced viability of the larvae.

3.4.2. Behaviour post-hatching

L. salmonis nauplii in the present study were observed to be "ejected" clear of the egg membrane after it had burst and it was concluded that this was due to the large internal

pressure that had built up. If they were impeded by other eggs or the ovisac, the nauplii tended to remain within the burst membrane. In both cases, nauplius I stages appeared quiescent compared to their earlier activity levels within the egg membrane, with only minor movements of the appendages being observed. Once the appendages had assumed their normal position, characteristic swimming was observed. For *L. salmonis* nauplii that were unable to become clear of the egg membrane due to other hatching eggs, this movement of the appendages to their normal position allowed the nauplii to become free of the egg membrane.

There have been a number of brief descriptions on the appearance and immediate behaviour of the released nauplius I stage of caligid parasites. Lin & Ho (1993) also observed that once *C. epidemicus* nauplii were ejected from the broken egg membrane, they were motionless for a short period of time after which they started to swim with their typical jerky movement. A similar description was recorded for *L. dissimulatus* by Lewis (1963).

In the present study, during the period of relative inactivity immediately post-hatching, *L. salmonis* larvae were observed to swell slightly, subsequently becoming a more elongate shape. Hatched live nauplii that were more rounded in shape died shortly afterwards, despite showing some activity. Johannessen (1978) also reported that more corpulent nauplii seemed to be less viable than slimmer longer ones. Similarly, the nauplii of *C. spinosus* according to Izawa (1969) are ovoid when newly hatched, but as time passes, they become more elongated. Such changes were also noted by Pike *et al.* (1993) for *C. elongatus.* Schram (1979) noted that the newly hatched larvae of *L. sprattae* were diamond shaped, shrunken and had irregular "skin." This phenomenon was also observed to occur in

newly hatched *L. salmonis* in the present study, but the shape of the nauplius became more smooth and elongate during the period when the larvae were quiescent.

3.4.3. Development

The development and mortality of early stages of sea lice and the effect of abiotic and biotic factors upon these parameters has not been extensively studied, and indeed some of the literature is confined to anecdotal reports. However, because of the significant effects that temperature and salinity had on hatching, it is equally important to fully understand their influence over development.

3.4.3.1. Temperature and development

During the present study it was observed that not only was the rate of development inversely related to temperature, but that moulting to the copepodid stage was also affected. At 7.5°C relatively low numbers of copepodids were obtained, with the larvae tending to either remain in the NII stage, or enter the moult but not complete it. At 5°C no copepodids were obtained with no nauplius II larvae entering the moult. This compromising of development was also reported by Lewis (1963) who suggested that the mortality of *L. dissimulatus* was high because the nauplii were unable to shed their old cuticles and by Hogans & Trudeau (1989) and Pike *et al.* (1993) for *C. elongatus*.

Pike *et al.* (1993) noted that mortalities of *C. elongatus* were high at 5°C, with a reduced number of nauplii subsequently attaining copepodid status although the authors noted that internal development still continued in individual *C. elongatus* larvae despite this blocking of moulting by low temperatures. Like Pike *et al.* (1993) the present study found that those *L. salmonis* nauplii that were observed to enter a moult but not complete it still exhibited movements. Kinne (1970) claimed that for several species of crustaceans, the

shedding of the cuticle is blocked at low temperatures, even though the life processes are still continuing but gave no further explanation for this.

This effect of temperature on development will have a significant effect on the numbers of infective stages present in the water column around fish farms at different times of the year. At 5°C development will only proceed as far as the NII stage, and hence reinfection on fish farms may be limited. This, however, does not mean that there will be no lice present on fish within a farm, as adult females can live for a number of weeks on a host. During this time, the female can produce a number of batches from one insemination, or possibly be remated and produce an even greater number. Thus these multiple clutches will hatch at different times, with some hatching at more favourable temperatures than others, and so may go on to develop to the copepodid stage. At 7.5°C, although development does occur to the infective stage, the proportions seem to be compromised, with a reduced number attaining infective status than at 10°C. Hence, at this temperature, although reinfection can occur in the natural environment on fish farms, it may be expected that reduced levels may be observed.

Obviously this would cause variation in the proportion of nauplius I stages that successfully complete development to the infective stage, and it is probably highly unlikely that an accurate estimation of the abundance of copepodids in the water column can be made without taking into account the actual or probable ambient water temperature at that time. Without this, it would be assumed that a constant proportion of copepodids is obtained from the first larval stage. This, as has been demonstrated, is not the case, and will no doubt explain some of the variations observed in infection levels on fish farms seasonally.

In the present study, *L. salmonis* nauplius II stage was were reached at 46, 43.3 and 36 hours post hatch at 5, 7.5 and 10°C whilst copepodids were first observed at 187.5 and 111 hours post hatch at 7.5 and 10°C. Determinations of development times have been made for other species of *Lepeophtheirus* species confirming the inverse relationship between temperature and development. Johannessen (1978) also studied the time of development for *L. salmonis*. At 15.5°C, the duration of the first nauplius stage was 12 hours, whilst at 9.2°C, this had increased to 35 hours, a similar figure to the present study. The duration of the nauplius II stage however was only investigated by Johannessen in one case, and was found to be 42 hours at 9.2°C, considerably shorter than the duration in the present study at 10°C (generally between 60 and 75 hours) However in two separate cases, the author found the time of development from hatching to the copepodid stage to be 63 and 33 hours at 11 and 9°C respectively, again much shorter than observed in the present study. Johannessen (1978) observed that copepodids were occasionally obtained at temperatures up to 22°C, but the duration of development was not recorded, and it is not known as to whether a plateau is reached where development time no longer decreases with increasing temperatures.

Wootten *et al.* (1982) recorded that moulting to the second stage of *L. salmonis* took 18 hours at 12°C, and to the infective copepodid, 63 hours at 11°C and 33 hours at 19°C, again somewhat shorter than that reported here. Johnson & Albright (1991) also demonstrated the temperature dependence of the developmental rate of *L. salmonis*. The moult to the second nauplius stage took 30.5 and 9.2 hours at 10 and 15°C, and development to the copepodid stage, 87.4 and 44.8 hours at 10 and 15°C, these being more similar to those reported in the present study.

It may seem difficult to directly compare the development of the free-swimming larval stages of sea lice with free-living copepods since the vast majority of the literature concerning the development of free-living copepods take into account food availability. Sea lice are lecithotrophic whilst in the water column, not externally feeding. Despite this however, there has been some research into the effects of other factors on the development of free-living copepods such as temperature.

According to Thompson (1982), temperature is one of the greatest influences on developmental rate. In addition to this, Paffenhöfer & Harris (1976), Harris & Paffenhöfer (1976) and McLaren, Walker & Corkett (1968) have shown for free-living copepods that although food density and salinity are important, they have a lesser effect than that of temperature. Sarvala (1979) stated that in the presence of abundant food, temperature seems to govern the rate of post embryonic development, though in contrast, Jamieson (1986) when looking at the effects of temperature and food on naupliar development, growth and metamorphosis in three species of the copepod *Boeckella*, *B. triarticulata* (Thomson), *B. dilatata* Sars and *B. hamata* Brehm, demonstrated that temperature had the major effect upon developmental times, with food level affecting only the length of the first copepodid stage. McLaren *et al.* (1968) and Paffenhöfer & Harris (1976) suggested that salinity and the type of food were relatively unimportant as far as the developmental rate of some free-living species were concerned and hence the importance of temperature as a governing and regulating function cannot be ignored.

Many free-living copepod species have been studied in an attempt to describe the form of the relationship between development and temperature (table 3.4). From such reports this appears to be in the form of curvilinear relationship and Sarvala (1979) and

Table 3.4. Species of free-living copepods and cladocerans that have been tested for a temperature-development relationship.

Author	Species
Vijverberg (1980)	*Daphnia hyalina* Leydig *Daphnia cucullata* Sars *Bosmina coregonis* Baird *Bosmina longirostris* Mueller *Chydorus sphaericus* Muell *Ceriodaphnia pulchella* Sars *Diaphanosoma brachyurum* Lieve *Leptodora kindtii* (Focke) *Acanthocyclops robustus* (Sars) *Mesocyclops leuckarti* (Claus) *Diacyclops bicuspidatus* (S.A. Forbes) *Cyclops vicinus vicinus* Uljanin *Eurytemora affinis* (Poppe)
Sarvala (1979)	*Paracyclops fimbriatus* (Fischer) *Canthocamptus staphylinus* (Jurine) *Attheyella crassa* (Sars) *Moraria brevipes* (Sars) *Moraria mrazeki* T. Scott *Bryocamptus echinatus* (Mrázek) *Paracamptus schmeili* (Mrázek)
O' Doherty (1985)	*Bryocamptus zschokkei* Coker
Jacobs & Bouwhensis (1979)	*Eudiaptomas vulgaris* Schmeil
Thompson (1982)	*Pseudocalanus elongatus* Boeck

Vijverberg (1980) concluded that such models are an accurate reflection of the relationship. To determine whether such a relationship exists for *L. salmonis* further studies on a wider range of temperatures would have to carried out. This may then allow the inclusion of a temperature function in a model such as that of Tully & Whelan (1993).

3.4.3.2. Salinity and development

The present study showed that salinity had a significant effect on the number of active *L. salmonis* NI stages obtained after hatching. At salinities of between 0 and 10‰ no active NI stages were obtained, whilst at 15‰ very low levels were observed. However at salinities between 20 to 35‰ significantly higher levels were recorded (19.7 to 28.7%). The influence of salinity is not restricted to the viability of the first larval stage. Indeed development to the second naupliar stage at 20‰ was seen to also be compromised, with only 2.1% of hatched nauplius I larvae moulting successfully to the second naupliar stage. The numbers of the NII stage obtained from hatched nauplius I larvae were found to increase with increasing salinity, as were the numbers of copepodids obtained. *L. salmonis* copepodids were only obtained in salinities of greater than 25‰, though even at this level, less than 0.1% of hatched NI larvae attained infective status. As well as numbers increasing with increasing salinity levels, longevity of the stages was also seen to increase, indicating that low salinity not only compromises the life cycle by preventing development, but also shortens the lifespan of the infective stages, and consequently impedes its probability of finding a host. The longevity of the copepodid stage of *L. salmonis* is investigated in further detail in Chapter 4.

Salinity has been previously shown to have an effect on the development of the larval stages of sea lice as well as other ectoparasitic copepods. Johannessen (1978) reported

that in 11.5‰ salinity water, the nauplii hatched from *L. salmonis* eggs (most had already aborted) only lived for a short time. This corresponds well with the present study, where live *L. salmonis* nauplii I stages were only obtained in a minimum salinity of 15‰, with subsequent survival being less than 24 hours. It is also similar to the Russian report of Berger (1970), cited by Johnson & Albright (1991) who reported that the nauplii of *L. salmonis* survived for 32 and 48 hours when transferred to water with salinities of 8 and 12‰ respectively. Johnson & Albright (1991) did not obtain active *L. salmonis* nauplii in salinities of less than 20‰, with this being very similar to the results of the present study. At 20‰, Johnson & Albright (1991) reported that only 19.8% (calculated from the total number of eggs) of nauplii were active with this increasing to a maximum of 65.9% in static 30‰ water. In flowing 30‰ water this value decreased to 54.6%, and it may be the case that aeration may damage nauplii, so explaining the reduced numbers in the flowing 30‰ sample. Although the results from the present study and Johnson & Albright's 1991 study differ, the latter authors reporting a higher percentage of active stages obtained, it is clear that the two do follow a similar pattern. Negligible numbers of active nauplii were observed in salinities of 0 to 15‰ which rose to an average of 25% in levels of 20 to 35‰. Like the present study, Johnson & Albright (1991) found that development to the copepodid stage only occurred at high salinities. Active copepodids were only obtained in 30‰, though at 25‰ a few were recorded but they were dead (Johnson & Albright, 1991).

When newly moulted copepodids were transferred to low salinities in the present study, it was found that the copepodids survived for similar lengths of time in salinities between 15 and 35‰, with the time for 50% mortality to occur ranging from 54.25 to 68.25 hours. However, the longevity of this stage appeared to be compromised in low salinities,

with the time for 50% mortality to occur in 0 to 10‰ ranging from 13.125 to 14.875 hours. The copepodid stage therefore appears to be more tolerant of salinity than its earlier two stages. This corresponds well to the report of Johnson & Albright who found that though maximum survival occurred at 25‰ (10°C) (as was also the case in the present study), survival did occur in salinities between 15 to 30‰ again showing the greater tolerance of the later stage.

Schram & Anstensrud (1985) looked experimentally at the development of the pennellid copepod *Lernaeenicus sprattae* and found that no development of nauplii and copepodids took place below salinities of 15‰. In addition, they also found that the nauplius stages were much more sensitive to extreme environmental conditions than copepodids. The authors concluded that *L. sprattae* may be excluded from low salinity areas (less than 15‰) due to reduced hatching success and the survival of the naupliar and copepodid. From the present study, although it appears that copepodids are able to tolerate a wide range of salinities, only in levels of 20‰ or greater does a consistent level of production of live nauplius I stages occur. Even at this concentration, numbers and longevity of the subsequent stages are still reduced. It seems unlikely therefore, that *L. salmonis* is adapted to low salinity habitats, such as estuaries, as has been suggested (Bass & Murphy, 1995), but instead reproducing populations will probably be excluded from such areas due to the failure of development to successfully occur.

3.4.3.3. Differential development and mortality

Mortality levels of *L. salmonis* larvae hatched and held at 10°C in full strength seawater were observed in the present study. It was found that the period when mortality was at its highest was immediately post-hatch, with almost 50% of the first larval stages

dying before they entered the moult to the second naupliar stage. However, it was not ascertained as to whether these died simply because this is the stage that is the least tolerant, or, if the individuals that died were in some way less "fit" than those that survived. According to Johannessen (1978), *L.salmonis* nauplii spending much longer than average in any stage generally did not develop into the next stage and Pike *et al.* (1993) also observed that specimens of *C. elongatus* with longer duration times than average generally did not develop into the next stage. This caused mortalities to be being highest at the first naupliar stage, particularly during the first ecdysis (Pike *et al.*, 1993). Also, Pike *et al.* (1993) stated that nauplii that developed faster or slower than average showed the same differential in the next stage. It may be be the case therefore that the high mortality observed in the present study removes "unfit" larvae rather than the NI stage being more sensitive than the subsequent stages. If "unfit" larvae are removed initially, then it would be expected that the mortality rate would be reduced significantly afterwards.

In contrast however, Trujillo-Ortíz & Arroyo Ortega (1991) analysed mortality and expectation of life for the calanoid copepod *Acartia californiensis* Trinast under laboratory conditions. During the development of the copepod, the maximum mortality rate was observed in the naupliar stages rather than in the subsequent copepodid stages. This was attributed, by the authors, to the hatching process and the exhaustion of the vitellus as had been previously observed by Marshall & Orr (1972) for *Calanus finmarchicus.* *C. finmarchicus* and *A. californiensis* are dependent upon the vitellus until the nauplius II stage is reached. If this is exhausted before it reaches this stage when it can filter and obtain its own food, the copepod will die. This is clearly a similar situation to *L. salmonis*, where the free swimming stages are dependent upon finite energy reserves until infection occurs.

Trujillo-Ortíz & Arroyo Ortega (1991) concluded that in a population of *A. californiensis*, this naupliar mortality rate observed was due to the naupliar stage being the one at greatest risk out of all the stages.

Lopez (1991) however gave an alternative explanation for this phenomenon of high naupliar mortality - a continuous culling of what the author termed "laggards" during ontogeny, causing only the fastest developing individuals to survive. In Lopez's (1991) study on mortality and moulting in naupliar stages of *Calanus pacificus*, it was demonstrated that moulting success and survivorship increased during the development of the six naupliar stages in the laboratory, this giving the appearance of earlier stages being more sensitive than later ones. However, it was demonstrated that individuals with slow developmental rates (i.e. those that remain longer within each stage) have poorer chances of surviving and advancing to the next stage, like that suggested by Johannessen (1978) and Pike *et al.* (1993). According to Lopez (1991), while such nauplii may eventually moult, they remain at a disadvantage by entering the subsequent stage at a later age. Therefore, the early developmental rates of individuals can influence their developmental rates in later stages. This is consistent with the observations of Peterson (1986) and Peterson & Painting (1990) where the variance in the developmental rate for *Calanus marshallae* Frost, *C. australis* Brodsky and *Calanoides carinatus* Krøyer decreases in later stages.

To determine whether the mortality rate observed in the present study is due to differential sensitivity or to "laggards" being removed from the system, further work would have to be performed studying individual larval developmental rates. Until this is done, it can only be accepted that there is a large initial NI mortality, that will obviously have a significant effect on the model of Tully & Whelan (1993).

Therefore, in order to estimate where reproducing populations of *L. salmonis* will occur in the natural environment, the abiotic factors of sites must be taken into account. Any model used to predict the hatching, development and resulting numbers of the free-swimming stages must take into account these variables as they strongly influence the early life history of the parasite up to the copepodid stage.

CHAPTER 4

ENERGY LEVELS & LONGEVITY, ACTIVITY & INFECTIVITY

4.1. Introduction

The free-living stages of sea lice, both the nauplii and the copepodid, are apparently lecithotrophic ("yolk-feeding"), not feeding whilst in the water column (Pike *et al.*, 1993). During these early stages, sea lice must be dependent upon stores of energy laid down during egg production and available within the body of the first nauplius stage. This store must be finite since nutrition from external sources begins only upon infection of a host by the copepodid stage. This suggests that, if these internal energy stores are depleted prior to infection, the death of the larva will occur. The potential importance of such a dependence on finite energy reserves on the population dynamics of *L. salmonis* has not been investigated, though it is clearly another parameter that will influence infection of fish. Indeed, although it is generally accepted that the free-swimming larval stages are lecithotrophic, this appears not to have been demonstrated in the available literature. There has also been a lack of research directed towards the biochemical composition of the eggs and nauplii. Such studies may yield valuable information concerning proportions allocated to energy reserves and the effect of such stores upon the longevity, activity and infectivity of the parasite.

Many studies have been carried out on the biochemical composition of whole eggs of free-living marine invertebrates, however. A review by Holland (1978) shows that, in general, protein forms the major constituent of eggs, followed by lipid and then carbohydrate. According to Holland (1978), the high levels of protein and lipid compared to carbohydrate in these eggs clearly demonstrates that carbohydrate does not act as a major energy reserve in egg development when no external food source is

available. In the vast majority of cases, lipid is the major energy reserve, and it seems likely that this would be the case for the free-living stages of *L. salmonis*.

There have also been some studies into the energy reserves of larval stages of free-living crustaceans which may also provide some insight into the area of larval nutrition of parasitic copepods. The cyprid stage of barnacles, like the early stages of *L. salmonis*, do not feed and the survival and success at metamorphosis and through metamorphosis to the adult stage is dependent upon stored energy reserves. Holland & Walker (1975) demonstrated that total lipids accounted for 14% of the dry weight of *Balanus balanoides* L. cyprids. This fraction was shown to fall dramatically in organisms that were prevented from settling, indicating that lipids were the main energy reserve. Protein and carbohydrate fractions were not observed to fall to such a great extent, and Raymont, Srinivasagam & Raymont (1969) concluded that, in general, the larvae of planktonic crustaceans tended to utilise lipid as their major energy store.

Information on the energy reserves of parasitic copepods is minimal and those reports available do not descibe detailed investigations. Wilson, as early as 1905, described the posterior portion of nauplii of Caligidae in general, as containing yolk granules by which the nauplius is nourished until it infects a host and obtains its own food source. Heegaard (1947) of *Caligus curtus* Müller stated that the body of the nauplius contained yolk, and Lewis (1963) observed that large globules of material, "presumably yolk," were evident within the body of the first nauplius of *Lepeophtheirus dissimulatus* Wilson. The newly hatched nauplius of the parasitic copepod *Lernaeeniscus sprattae* was described by Schram (1979) with a similar lack of detail, as having the greater part of the body filled with yolk. These few reports show clearly the lack of research into the area of energy reserves in parasitic copepods.

There are no data available on the energy reserves of *L. salmonis* and consequently there is also a lack of information on the effects of the depletion of such reserves on the biology and population dynamics of *L. salmonis*. The longevity of the copepodid stage of *L. salmonis* and other parasitic copepods has been studied, but, with the exception of a minority of cases, there has been no attempt to relate this to the depletion of the energy source. The importance of the longevity of the infective stage was highlighted by Johannessen (1978), who stated that whilst copepodids of *L. salmonis* were alive, they would be of a potential danger to salmonid species. Johannessen (1978) concluded that results from experimental data indicated that *L. salmonis* copepodids could live for up to one month. However, Wootten *et al.* (1982) commented that it would seem unlikely that copepodids that remain free-swimming (Johannessen's were apparently attached to a substrate) would have sufficient food reserves to survive for such a long period and, in these authors' experiments, the *L. salmonis* copepodids remained active for 4 days at 12°C, though they did not give the actual longevity of the parasite. Johnson & Albright (1991) demonstrated that the average survival of copepodids of the same parasite was between 2 and 8 days, with this dependent upon temperature and salinity. However, despite these figures for longevity, none of these authors studied the depletion of energy reserves and the effects upon not only the longevity of the infective stage, but on the subsequent development of all three of the free-swimming larval stages.

The responses of the parasitic copepod *Salmincola edwardsii* Olsson and the implications for host-finding was investigated by Poulin, Curtis & Rau (1990a). The authors concluded that since copepodids of *S. edwardsii* did not feed until they located a host, their lifespan was probably inversely proportional to the rate at which the energy

reserves were spent. Heegaard (1947), in an experiment designed to keep the nauplius I stage of *C. curtus* constantly swimming, noted that depletion of the "yolk mass" did occur within 2 days, causing the nauplii to no longer actively swim, but instead quoted "feeble efforts to stay afloat." No details are given by Heegaard though as to how he measured this depletion. Due to this depletion of a finite source of energy, Heegaard (1947) stated that the nauplii's reserve of strength was too small to manage a moult, and that the larvae died. In a report on free-living barnacle larvae, Holland & Walker (1975) suggested that cyprids of *B. balanoides* that were prevented experimentally from settling, could have so depleted lipid reserves that metamorphosis to the adult stage may not be achieved. From these two reports it can therefore be seen that, not only does the limited energy store determine the longevity of the infective stage of parasitic copepods, but also that it may actually also prevent development from early stages to later ones. Since there exists two moults between the first nauplius stage and the infective copepodid of *L. salmonis* and *C. elongatus*, the depletion of energy reserves may play an important role in the population dynamics of sea lice.

Despite the suggestion of Johannessen (1978) that whilst *L. salmonis* infective stages are alive they will be of potential danger to hosts, it seems more likely that the actual longevity of infective stages of parasites may not necessarily be equated with the infectivity of the parasite, due to the depletion of energy reserves and the effect this has upon the activity of the parasite. Conley & Curtis (1993) emphasised that once *S. edwardsii* copepodids were motionless, the chance of infection occurring was very unlikely, and therefore although copepodids continued to live long after they had lost the ability to swim, they could be considered functionally non-viable. Whitfield *et al.* (1988) studied the population processes of the parasitic copepod *Lernaeocera*

branchialis and, although the authors commented that copepodid infection success would be enhanced by the prolonged survival of this non-feeding free-swimming stage, they suggested that an age-dependency of copepodid infectivity may exist.

The relationship between longevity, activity and infectivity was addressed by Anderson & Whitfield (1975) for the free-living cercarial populations of the digenean *Transversotrema patialensis* (=*T. patialense* Soparkar). In a thorough investigation it was observed that both activity and infectivity of the parasite dropped to low levels many hours before death actually occurred, in agreement with the suggestions made by Conley & Curtis (1993) and Whitfield *et al.* (1988). Anderson & Whitfield (1975) attributed the age-dependent death and activity rate to the progressively diminishing energy reserves of *T. patialensis*. The period of transmission of the parasite would therefore be much shorter than the longevity of the infective stage.

This age dependant infectivity has also been observed for the miracidial stage of digenean parasites, and according to Whitfield, Anderson & Bundy (1977) is the case in general for both digenean miracidial and cercarial stages. Waadu (1991) demonstrated that as the age of the non-feeding miracidial stage of the strigeid *Diplostomum spatheceum* Rud increased, the viability and host-finding capacity declined. However, Waadu (1991) did not attribute this decline to activity levels and/or diminishing energy reserves. Anderson, Mercer, Wilson & Carter (1982) also found that the infectivity of the miracidia of *Schistosoma mansoni* Sambon declined rapidly with larval age. However unlike Waadu (1991), they did ascribe this to the exhaustion of a finite energy store and its subsequent effect on the rate of movement.

Hence, it can clearly be seen that energy reserves, as well as controlling the development and longevity of the free-swimming stages of parasites, also strongly

influences their infectivity through the control of activity levels. According to Chernin (1970) and Mason (1977), both studying *S. mansoni*, the rate of movement, and thus distance covered, is an important determinant of the likelihood that an infective stage will locate and successfully infect a suitable host. This was confirmed by Miller & McCoy (1930) who found that cercariae of *Cercaria floridensis* McCoy were able to infect fish only when active enough to reach them.

Since spatial overlap is necessary for the infection of a host by a parasite to occur, as well as the actual process of infection, the depletion of energy reserves and the subsequent effect upon activity and thus infectivity is of clear importance for *L. salmonis*. Since Atlantic salmon are pelagic fish, free-swimming larval stages of *L. salmonis* and in particular the infective copepodid stage may need to remain in the surface layers of the water column in order to maximise their chances of spatial overlap and therefore infection with potential hosts, as well as the mechanisms of actually attaching to the host. The depletion of energy reserves and the subsequent effect upon the activity and infectivity of the parasite will therefore be an important key in understanding the population dynamics of the species, and in explaining infection levels and patterns observed. For these reasons, the present study was undertaken in order to determine the biochemical composition of the eggs and thus the free-swimming stages of *L. salmonis*, the energy usage of these larvae, and the relationship between larval age, energy levels, activity and infectivity.

4.2. Materials and Methods

4.2.1. Egg string composition

The composition of *L. salmonis* egg strings was analysed in both October and May in order to identify any seasonal traits using the following biochemical methods.

4.2.1.1. Wet weight analysis

A minimum sample of 3.0g of egg strings was used to allow further dry weight analysis (Porter, pers. comm.). Egg strings were detached from adult females of *L. salmonis* and blotted on paper towels in order to absorb excess seawater. The sample was then placed in a pre-weighed foil tray and weighed before being placed in a drying oven overnight. The sample was then reweighed and the percentage dry weight calculated.

4.2.1.2. Dry weight analysis

4.2.1.2.1. Total Carbohydrate

In order to determine the amount of carbohydrate in *L. salmonis* egg strings, a colorimetric method was utilised (Burrin, 1986).

Four replicate 3.0mg samples of the dried egg strings were each placed into pyrex test-tubes, and 2.5ml of deionised water was added to each. The following test-tubes were prepared in duplicate for a calibration curve.

i.	2.5ml deionised water	(0mg blank)
ii.	0.5ml glucose standard + 2.0ml deionised water	(0.5mg glucose)
iii.	1.0ml glucose standard + 1.5ml deionised water	(1.0mg glucose)
iv.	1.5ml glucose standard + 1.0ml deionised water	(1.5mg glucose)
v.	2.0ml glucose standard + 0.5ml deionised water	(2.0mg glucose)
vi.	2.5ml glucose standard + 0.0ml deionised water	(2.5mg glucose)

All the test-tubes (four test samples plus six calibration samples) were then thoroughly shaken and 1ml of 5% phenol was added to each. The tubes were shaken again and placed on ice for 5 minutes. After this period, whilst still on ice, 8ml of concentrated sulphuric acid was quickly added to each test-tube. The optical density of all tubes was then read at 520nm in a spectrophotometer (Kontron Uvikon 810) against the 0mg glucose blank. A calibration curve of optical density against glucose concentration was then plotted using the six calibration samples, and the glucose concentration of the four test samples was then read off and the average calculated. Total carbohydrate was calculated by expressing the amount of glucose in the samples as a percentage of the initial sample weight (3.0mg).

4.2.1.2.2. Folch Lees lipid extraction

Although the extraction of lipids from biological materials is complex, the Folch Lees procedure is accepted as a satisfactory method (Wharton & McCarty, 1972). This method uses the triglycerides in biological specimens as they are the easiest to work with, not complexing with carbohydrates or proteins. Such lipids can be readily extracted with lipid solvents such as acetone or diethyl ether. After initial extraction with such solvents, the lipids are usually contaminated with many non-lipid components. The Folch Lees procedure equilibrates the lipid solution with an aqueous salt solution. Many of the non-lipid components are more soluble in the salt solution than in the organic solvent, and thus can be removed in the resulting partition.

Three replicate 100mg samples of dried egg strings were each weighed into a large glass teflon homogeniser tube. 10ml of methanol was added to each tube, and then each homogenised mechanically (Ultra turrex) for 60 seconds. 20ml of chloroform was then added to each tube, and each again homogenised for 60 seconds.

The contents of each tube were then filtered through prewashed (in a chloroform:methanol 2:1 mixture) Whatman number 1 filter paper into a quickfit 60ml boiling tube. 10ml of chloroform:methanol (in a 2:1 mixture) was then added to an homogeniser tube, and homogenised for a few seconds. This was then added equally between the three filtering samples. After filtration was completed, the funnels were removed, and 10ml of 0.25% potassium chloride aqueous solution was added to each sample. The samples were then each stoppered and shaken thoroughly, and then each poured into a separating funnel before being allowed to stand for several hours until separation had occurred. The upper layer of each sample was then removed and discarded, and the chloroform blown off the lower phase using a stream of air in a fume cupboard whilst warming the tubes up to 40°C in a water bath. When almost dry, the lipid from each sample was then transferred to a preweighed small glass vial, using a few millilitres of chloroform:methanol (2:1) mixture. The samples were then each evaporated to dryness, dessicated overnight and then reweighed. The lipid content of the dried egg strings could then be calculated by the weight of the lipid in the vial at the end of the experiment being expressed as a percentage of the initial sample weight (100mg).

4.2.1.2.3. Modified Lowry Protein Assay

The assay for protein is also colorimetric, based on the same colorimetric theory as that for carbohydrate. The phenolic group of tyrosine residues in a protein will react with a reagent, Folin-Ciocalteau, to produce a colour change that can be read on a spectrophotometer (Wilson, 1986). Again, known quantities of protein are used to produce a calibration curve in order to estimate the quantity of protein present in the test sample.

3x60mg of dried *L. salmonis* egg strings were placed into 3 Kjeldahl tubes 0, 5, 10, 20, 30, 40, 50 and 60mg of Bovine Serum Albumen (BSA) were weighed into 8 labelled Kjeldahl tubes (for calibration). To each, 5ml of 1M Sodium Hydroxide containing 0.25% of Sodium Dodecyl Sulphate was added. The tubes were then covered with parafilm and placed in a water bath at 60°C for 2 hours. The tubes were then cooled with tap water before adding 95ml of distilled water to each using 100ml burettes. The contents of the tubes were then mixed thoroughly, and 1ml from each used to perform a Lowry assay. To all tubes (11 in total), 1ml of Lowry reagent was added (Sigma Protein Assay kit) and thoroughly mixed, and then allowed to stand at room temperature for 20 minutes. 0.5ml of Folin-Ciocalteau Phenol reagent was then rapidly added to each tube and mixed. The tubes were then left to stand for 30 minutes to allow the development of colour. After this time the optical density at 720nm of each tube was read using a spectrophotometer (Kontron Uvikon 810). A calibration curve was then plotted of mg of BSA read from the 8 calibration tubes against optical density at 720nm. The weight of protein (in mg of BSA) for each test sample was read from the curve, and the percentage of protein in the sample weight calculated.

4.2.2. Food Depletion

The first three stages of *L. salmonis* have a discrete area containing vesicles in the body of the nauplii, which is believed to contain the stored food reserves. If these reserves are lipids, the area will stain with lipid stains such as Sudan Black B.

4.2.2.1. Staining method

In an attempt to demonstrate these reserves, nauplii and copepodids were stained with the lipophilic stain Sudan Black by dissolving 0.3g of Sudan Black B in 100ml of 70% ethanol. The method was adapted from that given by Hartman (1940). Approximately 2ml

of the stain was placed in a watchglass and 4 live nauplii or copepodids were removed from full strength, twice filtered, seawater and placed into the stain with the minimum amount of water necessary. The watchglass was then covered with a piece of glass for approximately 2 minutes to avoid evaporation of the ethanol, and then the stain carefully drawn off using a pipette. The watchglass was then flooded with 70% ethanol, and the larval stages immediately removed with a pipette and placed in a cavity microscope slide with a small drop of ethanol, and a coverslip placed on top. The larvae were immediately measured for the length and width of the lipid area at x100 magnification under a compound microscope. If lipid was present, the sudanophilic granules would stain a dark blue.

4.2.2.2. Depletion of energy reserves

20 mature pigmented egg strings from 10 *L. salmonis* females were placed in 500ml beakers containing 400ml of twice filtered full strength seawater that was aerated prior to use and changed daily at 10°C in a constant temperature room. 24 hours after the first hatched nauplius was observed, the egg strings were removed from the system leaving only larvae that were between 0 and 24 hours old. Twenty five of these individuals were then randomly selected, removed and stained using the above procedure and the length and width of the stained area recorded. Twenty four hours later, 25 further larvae were again removed and stained, with this procedure continuing until no more larvae remained alive.

This procedure was repeated at two lower temperatures, 5 and 7°C, to look at the effect of temperature upon energy usage. For this purpose, egg strings were held in incubators, where the temperature was monitored twice daily.

4.2.2.3. Seasonal variation in food reserves

Twenty egg strings were each sampled in January, May, August and October, with each sample being obtained from the same fish farm as described in Chapter 3. Mean egg

size was determined by the method described in Chapter 2. The mean size of eggs within ovisacs, the subsequent size of the first nauplius stage, and the initial size of the yolk sac was measured in order to determine if there existed a seasonal variation in food reserves within individual larvae. The same method for staining the energy store as described in 4.2.2.1. was carried out, with larvae being stained and measured 12 hours after hatching was first observed. The length and width of the nauplius I stages was measured in addition to the dimensions of the yolk sac already described.

4.2.3. Copepodid age and activity

4.2.3.1. Longevity

Mature *L. salmonis* egg strings were placed in 500ml glass beakers containing 400ml of twice filtered full strength seawater that had been aerated prior to use, and which was changed daily, at 10°C in a constant temperature room. The time at which hatching was observed to commence was noted and the egg strings were removed 12 hours later leaving nauplius I stages that were between 0 to 12 hours old. Development was then followed to the copepodid stage, the stages being checked every 24 hours. Once the moult from the nauplius II stage had successfully occurred, copepodids were removed from the system and placed into three separate 500ml glass beakers containing 400ml of twice filtered, aerated full strength seawater that had been aerated prior to use, and held at either 5, 10 or 15°C. The number of live copepodids was counted every 24 hours until all had died.

4.2.3.2. Activity

The above procedure for obtaining copepodids was repeated at 5, 10 and 15°C. Copepodids were then observed every 24 hours for their activity status, by placing them in a petri-dish containing twice filtered aerated, full strength seawater and examining them for signs of activity under x40 magnification under a dissecting microscope. Copepodids were

classed as either active without stimulation, active only by stimulation or totally inactive. Copepodids that did not require stimulation were those that exhibited spontaneous swimming movements, whilst those classed as individuals requiring stimulation only swam in response to the expulsion of water from a pipette. Those that were inactive showed no swimming actions at all. Although examination of the copepodids took place under a dissecting microscope in a laboratory at room temperature, it was possible to study them at the test temperatures (5, 10 or 15°C). In order to prevent a rise in water temperature, the time for examination was reduced by placing only 10 copepodids in the petri-dish for examination at a time, the remainder being kept at their test temperatures. The petri-dish was placed on top of an upturned petri-dish in order to avoid heat transfer from the microscope's light source (pers. comm. Ferraz D'Olivera).

4.2.4. Copepodid age and infectivity

4.2.4.1. Infection experiments

The success of infection of Atlantic salmon by *L. salmonis* copepodids of different, known ages was investigated. Copepodids of known ages were obtained using the method described above for the relationship between age and activity. 2000 active copepodids that were either 1 (0-24 hours old) or 7 (145-168 hours old) days old (4000 in total), obtained from egg strings collected from one site on one sampling day were placed in an aquarium containing 25 litres of full strength, aerated seawater in the dark at 10°C with 10 naive post-smolt *S. salar* for 8 hours. During the period of exposure of the fish to the copepodids, filtration and circulation of the system were withheld, keeping all the infective stages within the system. After the 8 hour exposure period, the fish were killed using an overdose of benzocaine and examined under a dissecting microscope at x40 magnification for any attached copepodids. The number of copepodids attached were counted and the site of each

infection was recorded. The water and handling nets were examined in order to record any copepodids that may have become dislodged due to handling.

4.2.4.2. Copepodid energy reserves

Histological examination of longitudinal sections of *L. salmonis* copepodids was undertaken in order to observe the relative proportions of energy supplies remaining (in the form of food reserves) in individuals of different ages. A sample (approximately 25) of 1 and 7 day old copepodids were fixed overnight at 4°C in Karnovsky's fluid and then placed in two changes of Cacodylate buffer for a minimum of 3 hours each at 4°C. The samples were then dehydrated through 30, 60, 90 and 100% ethanol, each at 40 minutes at room temperature. After dehydration, copepodids were infiltrated with L.R. White Resin at room temperature in a fume cupboard. Infiltration required four changes into fresh resin, the first after 1 hour, the second after the samples had been left overnight in the resin, and the third and fourth again each after 1 hour. The samples were then embedded in Gelatin capsules which were filled to the brim with L. R. White resin, and then polymerised in an oven for 24 hours at 60°C.

Polymerised blocks were trimmed and 0.50μm longitudinal serial sections cut, dried onto glass microscope slides and stained with 1% Toluidene Blue in 1% borax solution. The diameters of twenty randomly selected yolk sac vesicles surrounding the gut of the copepodids (identified from Bron *et al.*, 1993b) were then measured under a compound microscope. For each individual vesicle chosen, the diameter was measured from the section where it was at its maximum.

4.3. Results

4.3.1. Egg string composition

The moisture, lipid, carbohydrate and protein composition of *L. salmonis* egg strings are given in table 4.1 and figs. 4.1 (wet weight analysis) and 4.2 (dry weight analysis). Although the moisture content was found to be lower, 73.15% in May compared to 83.69% in October, there were no significant variations observed in the proportions of protein, lipid, carbohydrate or ash between the two dry weight samples (t-test, $p<0.05$). Therefore the results for the dry weight were not treated separately but instead pooled.

The organic fraction of the dry weight of the ovisacs was 91.54%, with this comprised of proteins, lipids and carbohydrates in a ratio of 5.39 : 3.07 : 1.00. Protein was found to be the major organic component of the dry weight of egg strings, accounting for 52.17% of the weight. 29.69% of the dry weight was due to the presence of lipids, whilst carbohydrates accounted for 9.68%. The inorganic fraction of the ovisacs, in the form of ash therefore contributed to only 8.46% of the dry weight.

4.3.2. Energy depletion

Staining of the naupliar stages of *L. salmonis* with Sudan Black B revealed that it formed a discrete region within the body of the nauplius (fig. 4.3). This region tended to extend almost the full length of the nauplius, from above the ocular region to just above the balancers. The presence of the lipids in one discrete area, made the measurement of the length and maximum width of the lipid area possible providing a basis for quantification.

The staining of *L. salmonis* copepodids with Sudan Black B proved difficult as the gut was formed at this stage. Because of the morphological changes, the discrete lipid area no longer existed and the lipids appeared as inclusions in the epithelial cells of the gut. It

Table 4.1. Wet and dry weight biochemical composition of *L. salmonis* ovisacs. For both May and October samples, 3.0g of egg strings were used for the analysis.

Month	% moisture	% protein	% lipid	% carbo-hydrate	% ash
Wet weight					
May	73.15	13.91	8.04	2.15	2.75
October	83.69	8.57	4.80	1.85	1.09
Mean	78.42	11.24	6.42	2.00	1.92
Dry weight					
May	-	51.80	29.94	8.00	10.26
October	-	52.54	29.44	11.35	6.67
Mean	-	52.17	29.69	9.68	8.46

Fig. 4.1. Biochemical composition of *L. salmonis* egg strings: wet weight analysis. May and October samples pooled.

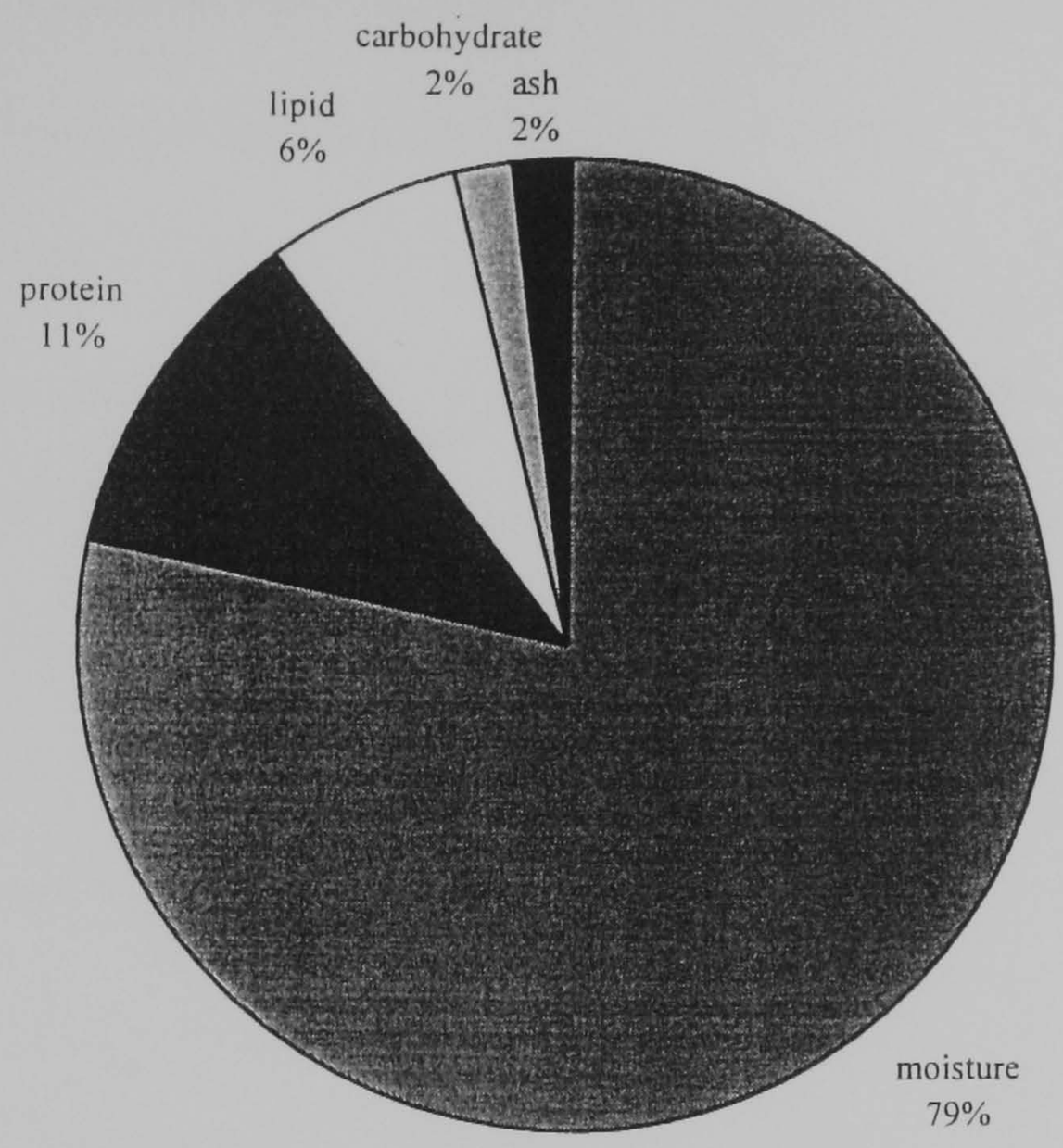


Fig. 4.2. Biochemical composition of *L. salmonis* strings: dry weight analysis. May and October samples pooled.

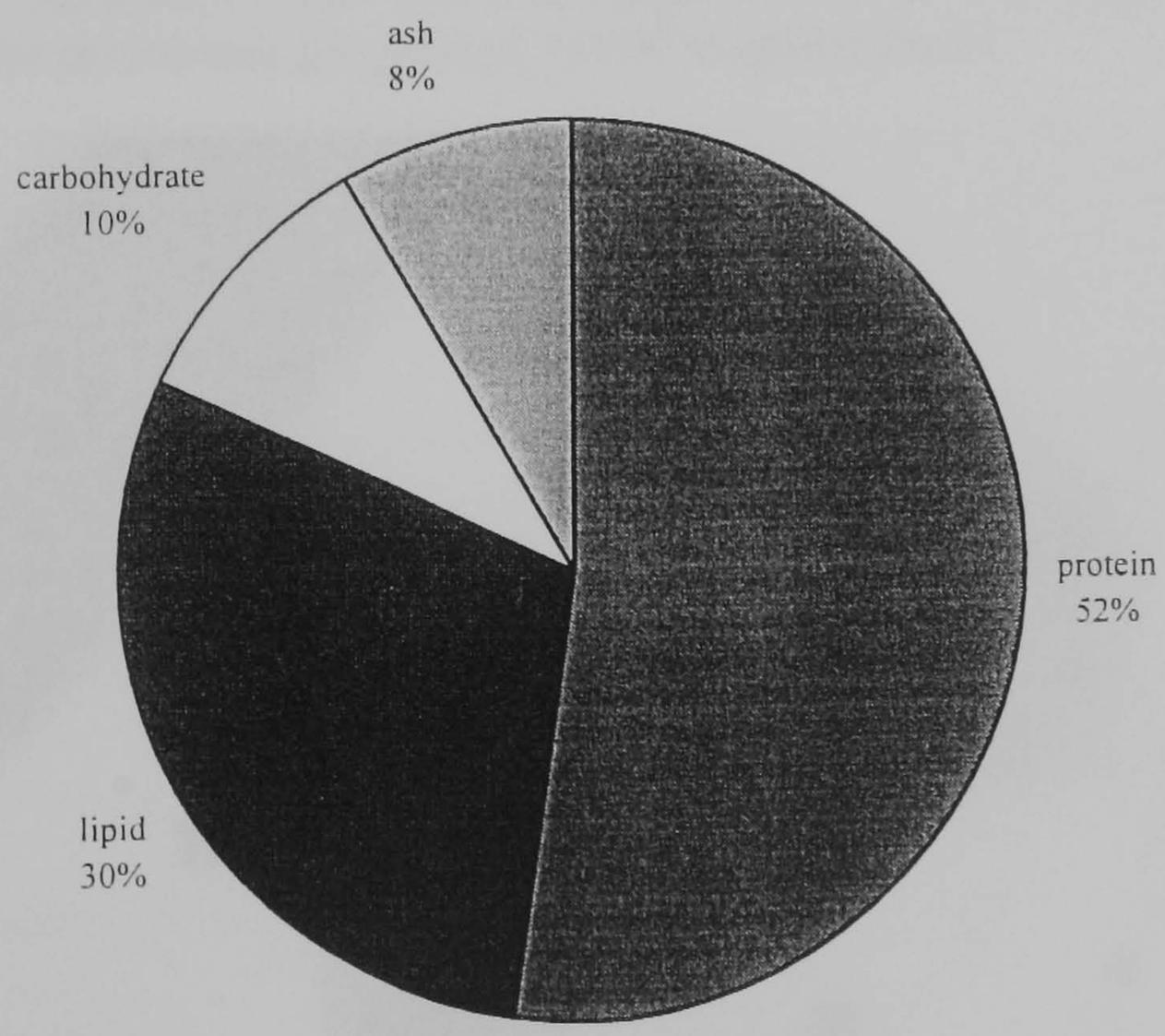
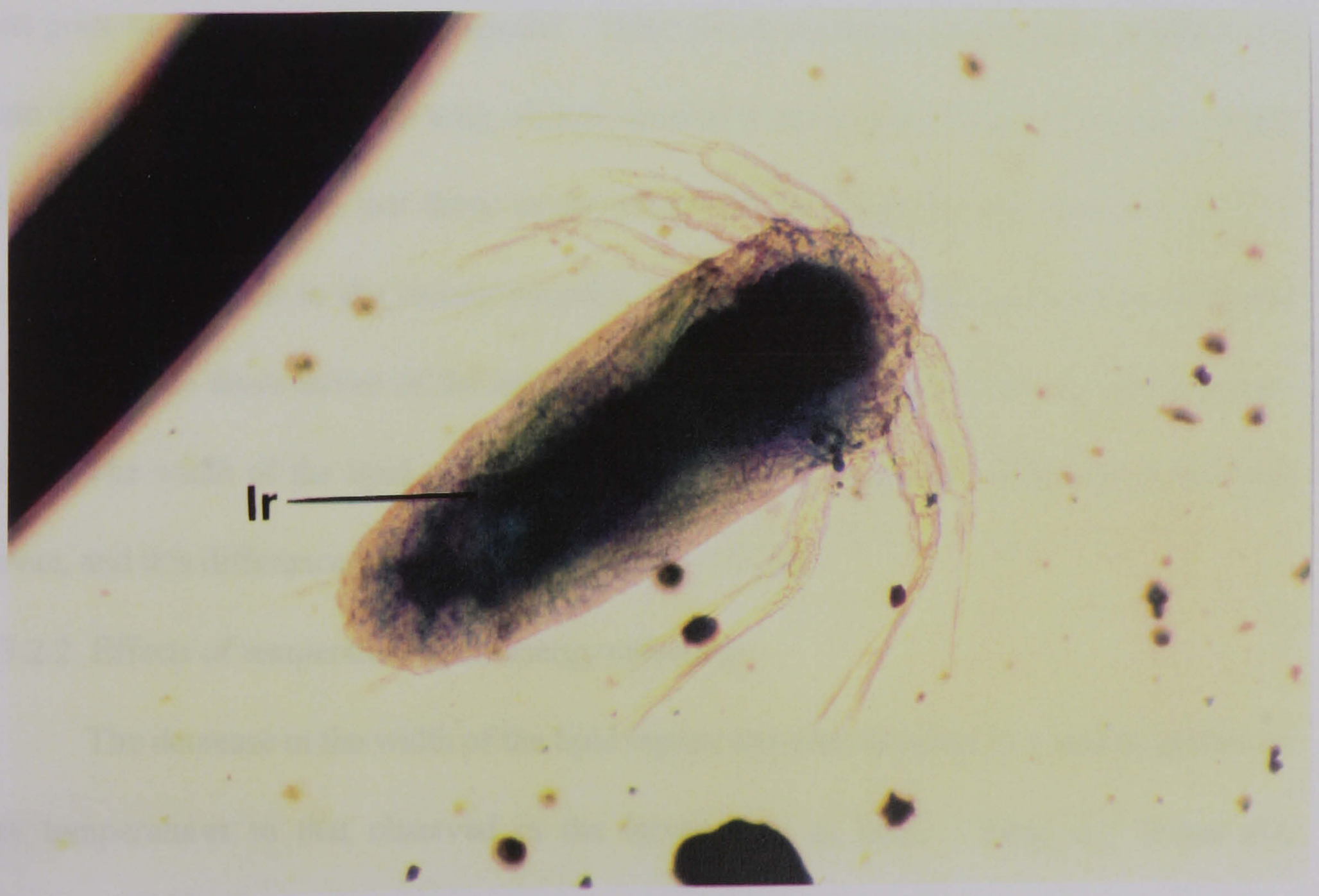


Fig. 4.3. (a) *Lepeophtheirus salmonis* nauplius I stage stained with Sudan Black B to reveal an area of lipid reserves (lr) (x100 magnification).

(b) *Lepeophtheirus salmonis* nauplius II stage stained with Sudan Black B to reveal an area of lipid reserves (lr) (x100 magnification). The width of this area is less than that observed in nauplius I stages due to its depletion. N.B. A precipitate of the dye forms as drying of the wet mount progresses. (x100 magnification).



was not possible therefore to measure the length and width of one discrete region in the copepodid, and hence the following results are for the naupliar stages only.

4.3.2.1. Age and energy utilisation by naupliar stages

The results of the naupliar staining are shown in table 4.2 for larvae held at 10°C. Analysis of variance revealed that the length of the lipid sac did not decrease significantly over the duration of the naupliar stages, with the length tending to stay the same ($p = 0.056$). This may be reflected in the distribution of the lipid inclusions in the gut epithelium of the copepodid which tended to appear along the whole length of the gut. Analysis of variance demonstrated that the width of the area of the lipid reserves was seen to significantly decrease over time ($p < 0.001$), with older nauplii having a lipid area with a much decreased width, as can be seen in fig. 4.4, and a high correlation between age and width of the lipid region was found to exist ($r^2 = 0.966$, $p < 0.001$).

The decrease in the width of the lipid "sac" of nauplii can be seen from fig. 4.4. as a gradual decline, falling from 154μm in 24 hour old larvae to 56μm in 144 hour old nauplii just prior to the moult to the copepodid. There are no readings available for nauplii older than 144 hours since, after this time, all had moulted to the infective copepodid stage. From the graph it can be seen that those larvae which are 48 hours old and have successfully completed the moult to the second nauplius stage have less energy in the form of lipids remaining than those larvae of the same age that have not moulted from the first naupliar stage. The width of the lipid area in moulted larvae is 116μm and 127μm in unmoulted larvae, and this difference was significant (t-test, $p < 0.05$).

4.3.2.2. Effects of temperature upon energy utilisation

The decrease in the width of the lipid region was seen to occur in a similar pattern at low temperatures to that observed in the larvae held at 10°C. Table 4.3 shows the

Table 4.2. Mean (± 1 S.D.) width and length of the lipid area in *L. salmonis* naupliar stages at different ages at 10°C. 25 nauplii measured per day.

Stage		Maximum age (hours) (n = 25)		Width of lipid area in nauplii (μm)	
NI		24		154 ± 18.42	
NI	NII	48	48	127 ± 15.78	116 ± 5.56
	NII		72		107 ± 20.91
	NII		96		84 ± 20.34
	NII		120		65 ± 18.37
	NII		144		56 ± 18.67
Stage		Maximum age (hours) (n = 25)		Length of lipid area in nauplii (μm)	
NI		24		324 ± 31.70	
NI	NII	48	48	374 ± 23.92	364 ± 21.86
	NII		72		314 ± 17.25
	NII		96		374 ± 33.52
	NII		120		289 ± 26.87
	NII		144		314 ± 32.65

Table 4.3. Mean percentage of lipid remaining in *L. salmonis* naupliar stages at different temperatures as a proportion of the intial width when first measured at 24 hours of age. 25 nauplii sampled per temperature per day.

Maximum age (hours)	10°C (n = 25)		7°C (n = 25)		5°C (n = 25)	
	NI	NII	NI	NII	NI	NII
24	100.00		100.00		100.00	
48	82.47	75.32	96.15	79.92	85.72	
72		69.48	87.41	82.41	80.82	70.32
96		54.55	61.19	63.37	80.82	71.08
120		42.21	61.19	50.69	68.58	65.22
144		36.36	61.19	56.33	59.31	48.32
168			34.96	49.27	60.01	55.24
192				34.96	55.01	48.32
216					46.16	34.29
240					34.32	34.29
264						30.77

Table 4.4. Mean (± 1 S.D.) seasonal variation in egg size (results from Chapter 2), nauplius I size and lipid width of *L. salmonis*. Mean egg size calculated from 20 egg strings (3 regions measured per egg string). 25 nauplii stained and measured for each month.

Month	Egg size (μm) (n = 60)	Length of nauplius I stage (μm) (n = 25)	Width of nauplius I stage (μm) (n = 25)	Width of lipid area in nauplius I (μm) (0-24 hours old) (n = 25)
February	68.32 ± 4.36	536.70 ± 28.44	197.74 ± 14.82	113.69 ± 14.42
May	63.40 ± 3.49	529.88 ± 11.97	197.96 ± 8.87	112.98 ± 11.59
August	71.25 ± 4.58	466.98 ± 30.28	214.05 ± 13.76	135.84 ± 19.43
October	68.25 ± 8.93	549.57 ± 17.70	209.91 ± 12.71	117.51 ± 7.65

Fig. 4.4. Decrease in the width of the lipid region over time for *L. salmonis* naupliar stages held at 10°C.

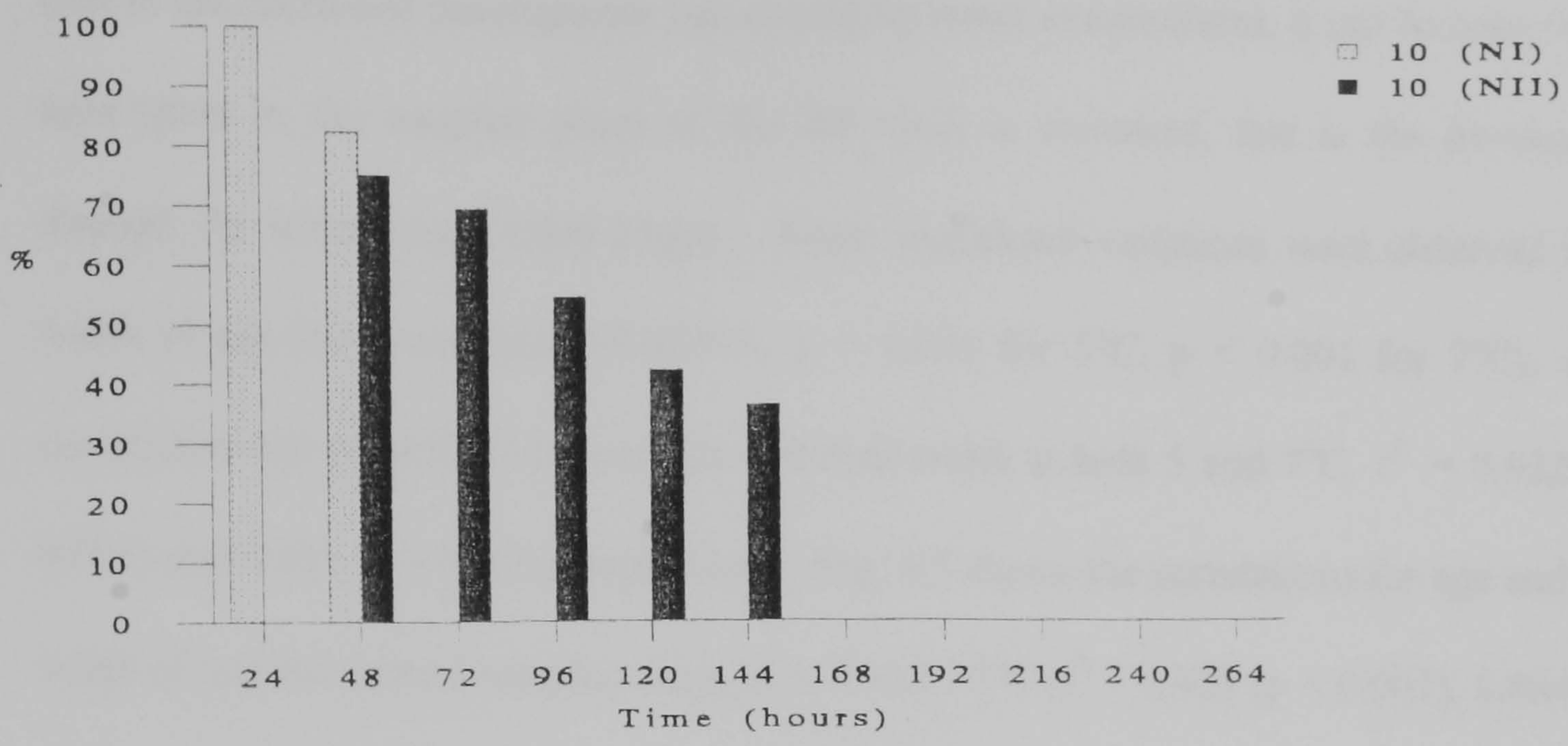
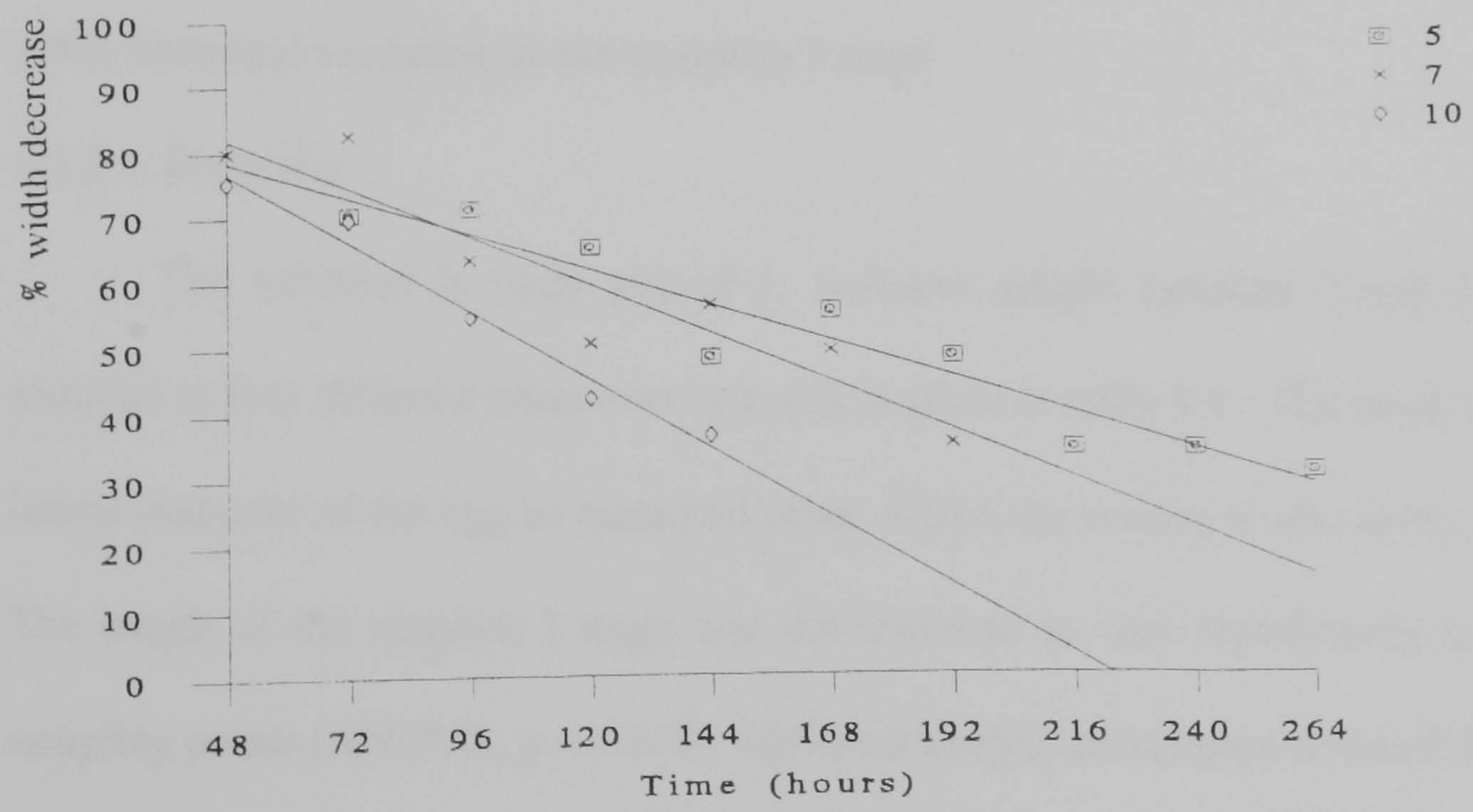


Fig. 4.5. Fitted regression curves for the decrease in width of the lipid region of *L. salmonis* nauplius II stages held at 5, 7 and 10°C.



percentage decrease in the width of the lipid of *L. salmonis* naupliar stages over time at 5, 7 and 10°C, with the width of the lipid region in 24 hour old nauplii I representing 100%. Due to the decreased development rate caused by lower temperatures, it can be seen that the time spent in the naupliar phase of the life cycle is increased, that is the development through the larval stages takes longer. Again significant variations were observed in the width of the lipid over time (ANOVA, $p < 0.001$ for 5°C, $p < 0.001$ for 7°C), and a correlation was observed between age and lipid width at both 5 and 7°C, $r^2 = 0.922$ ($p < 0.001$) and 0.831 ($p < 0.001$) respectively. Fig. 4.5 shows the correlations for age and lipid width of just the second nauplius stage at 5, 7 and 10°C ($r^2 = 0.920$ ($p < 0.001$), 0.884 ($p < 0.001$) and 0.977 ($p < 0.001$)) respectively. It can be seen from these correlations that the time taken for the width of the lipid area to reach 50% of its initial value (initial value being 100% in nauplii between 0 and 24 hours old) is shortest at 10°C, this time being approximately 109.8 hours. At 7°C, this time increased to approximately 153.0 hours, and was longest at 5°C, taking approximately 173.4 hours.

4.3.3. Seasonal variation in the nauplius I stage

4.3.3.1. Body size

The variation in body size of *L. salmonis* nauplii between 0 and 24 hours old sampled at four different times over one year is given in table 4.4. The mean egg size (the lateral diameter of the egg as measured when within the ovisac) is also given in the table. The length of the nauplius I stage was not observed to vary significantly over the four sampling points (ANOVA, $p = 0.915$). However analysis of variance revealed that the body widths of the hatched larvae at different times of the year were significantly different ($p < 0.05$) with the largest mean width recorded being 214.05μm in August when the mean egg size within the ovisac was 71.25μm. The February and May samples however were not

found to differ significantly from each other, the body widths being 197.74 and 197.76μm respectively (ANOVA, $p > 0.01$).

The relationship between egg size and nauplius body width remained relatively constant over the four periods sampled as can be seen from Fig. 4.6, with the lateral diameter of the egg as expressed as a percentage of the body width being 34.55, 32.03, 33.29 and 32.51% in February, May, August and October respectively.

4.3.3.2. Lipid size

The mean widths of the lipid reserves of *L. salmonis* nauplii I stages between 0 and 24 hours old are also shown in table 4.4. These widths were demonstrated to vary significantly between the four sampling times (ANOVA, $p < 0.05$), ranging from a mean of 112.98μm in May to 135.84μm in August, with the largest mean lipid width, 135.84μm, being recorded at the same time as the largest mean body width, 214.05μm, in August. However no significant differences were observed between the February and May samples, the mean widths being 113.69 and 112.98μm respectively (ANOVA, $p < 0.01$).

The relationship between body width and lipid width remained relatively constant over the four periods sampled as can be seen from Fig. 4.6, with the width of the lipid expressed as a percentage of the body width being 57.49, 57.07, 63.46 and 55.98% in February, May, August and October respectively. Although the August figure appeared to be slightly elevated compared to the other three, an analysis of variance showed that it did not differ significantly ($p = 0.648$).

4.3.4. Copepodid longevity

The survival pattern of *L. salmonis* copepodids held at 5, 10 and 15°C is shown in Fig. 4.7, and the time for 100% mortality to occur is given in table 4.5. The longevity was highest at 5°C, the time taken for all copepodids to die being 408 hours (17 days), with this

Fig. 4.6. Relationship between egg size and nauplius I size and between nauplius I size and width of the lipid region for *L. salmonis* at 4 points in time. Egg size and width of the lipid region are both expressed as percentages of the width of the NI stage.

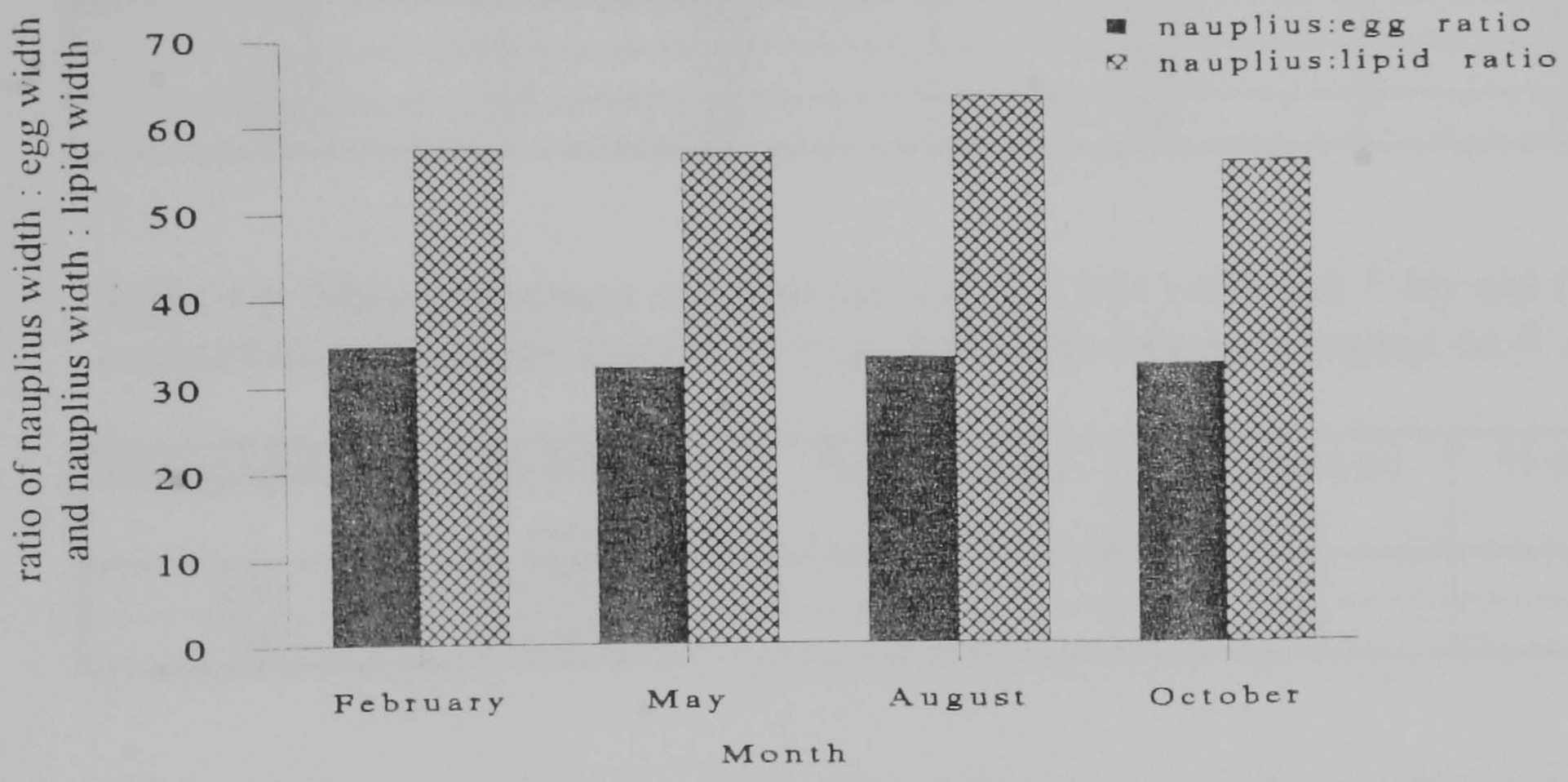


Fig. 4.7. Longevity of the copepodid stage of *L. salmonis* at 5, 10 and 15°C.

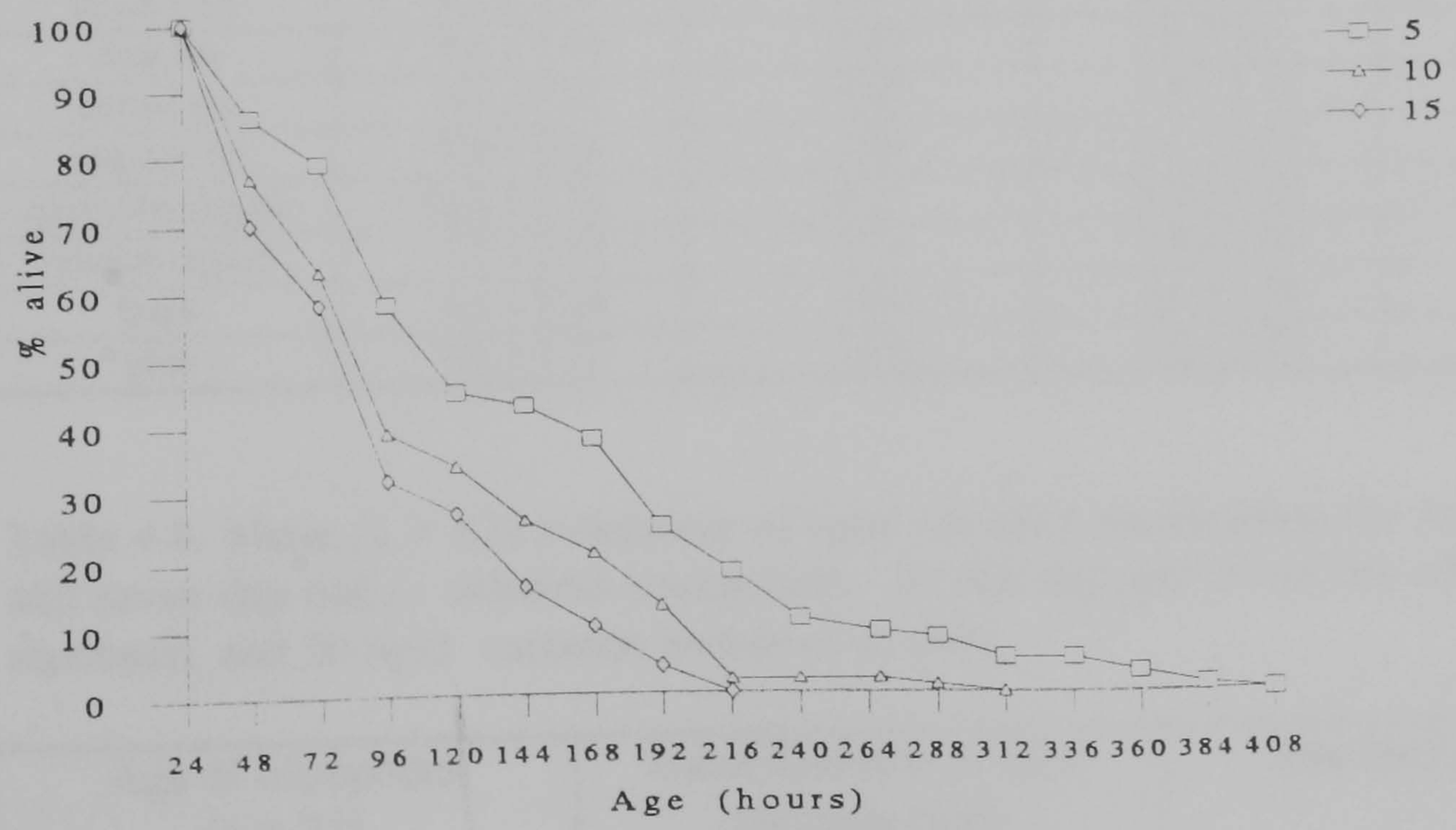


Table 4.5. Time taken for 50% of *L. salmonis* copepodids to swim only in response to external stimulation and 100% to become inactive and to die.

Temperature	Swimming (50%) in response to stimulation (hours)	Age when 100% totally inactive (hours)	Age at which 100% mortality occurs (hours)
5°C	200	312	408
10°C	168	264	312
15°C	80	168	216

Table 4.6. Mean percentage infection success (± 1 S.D.) of 1 and 7 day old *L. salmonis* copepodids, and the percentage of copepodids in the four main regions on *S. salar*.

Copepodid age	% infection success	% present on fins	% present on body	% present on gills
1 day	22.22 ± 8.32	59.00	37.40	3.60
7 days	14.00 ± 8.71	59.15	37.28	3.57

Table 4.7. Mean numbers (± 1 S.D.) of *L. salmonis* copepodids per fish and percentage of copepodids in different regions on *S. salar*: 1 day old and 7 day old copepodids.

Site of infection	1 day old copepodids		7 day old copepodids	
	mean number	percentage	mean number	percentage
pectoral fins	5.8 ± 3.70	13.06	3.4 ± 2.97	11.97
pelvic fins	5.0 ± 2.55	11.26	3.4 ± 1.95	8.45
caudal fin	8.4 ± 5.59	18.92	5.6 ± 3.29	19.72
anal fin	4.4 ± 1.34	9.91	3.0 ± 3.0	10.56
dorsal fin	2.0 ± 2.55	4.05	1.8 ± 1.64	6.34
adipose fin	0.8 ± 0.84	1.80	0.6 ± 0.55	2.11
opercular region	11.2 ± 5.54	25.23	6.2 ± 4.27	21.83
cranial region	1.2 ± 1.10	2.70	0.8 ± 0.84	2.82
flank	4.2 ± 1.10	9.46	3.6 ± 3.78	12.68
gills	1.6 ± 1.52	3.60	1.0 ± 1.22	3.52

Table 4.8. Mean (± 1 S.D.) diameter of lipid vacuoles surrounding the mid-gut of one and seven day old *L. salmonis* copepodids. 25 one day and seven day old copepodids sectioned, and 20 lipid vacuoles measured in each.

Age of copepodid (n = 25)	mean diameter of lipid vacuoles (μm) (n = 20 for each copepodid)	Standard deviation
1 day	5.066	1.207
7 days	4.130	1.088

falling to 312 hours (13 days) at 10°C and 216 hours (9 days) at 15°C. The time taken for 50% mortality to occur can also be observed from fig. 4.7 and is also inversely related to temperature, taking 116.08, 89.57 and 82.04 hours at 5, 10 and 15°C respectively.

4.3.5. Copepodid activity

The activity patterns of *L. salmonis* copepodids held at 5, 10 and 15°C are given in table 4.5 and figs. 4.8 and 4.9. The time taken for 50% of copepodids to swim only in response to external stimulation and then to become totally active was inversely related to temperature, being shortest at 15°C (80 and 168 hours respectively), compared to 168 and 264 hours at 10°C and 200 and 312 hours at 5°C. Fig 4.8 demonstrates the cumulative increase in the proportions of *L. salmonis* no longer showing any signs of activity, and the subsequent decrease in those exhibiting swimming movements, whilst fig. 4.9 shows how the proportions of both copepodids showing spontaneous swimming without stimulation and those only swimming in response to stimulation change over time.

During these observations it was also noted that copepodids held at 15°C appeared to have a higher intrinsic level of activity than those held at 5 or 10°C, spending longer periods of time actively swimming, before passive sinking occurred, with the time spent in this being shorter in duration when compared to copepodids at the two lower temperatures.

4.3.6. Copepodid infectivity

4.3.6.1. Infection experiments

4.3.6.1.1. Age and infection success

The mean percentage infection success of 1 and 7 day old *L. salmonis* copepodids and the distribution of the infective stage on the fish are given in tables 4.6 & 4.7. The mean percentage infection success of 1 day old copepodids was 22.2% (± 8.32), and that of

Fig. 4.8. Relationship between age and activity of *L. salmonis* copepodids held at 5, 10 and 15°C: Activity includes both stimulatory and non-stimulatory movements.

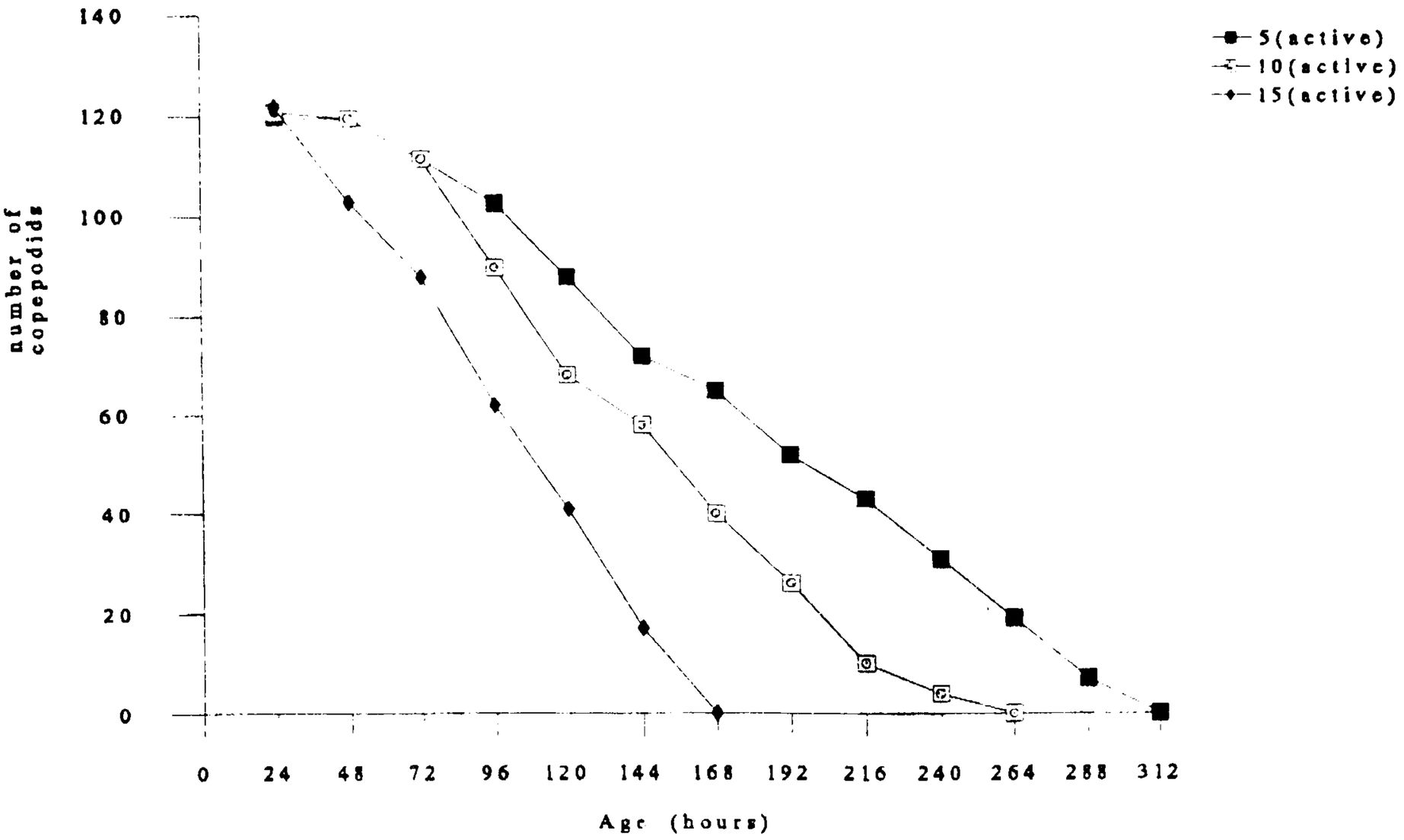
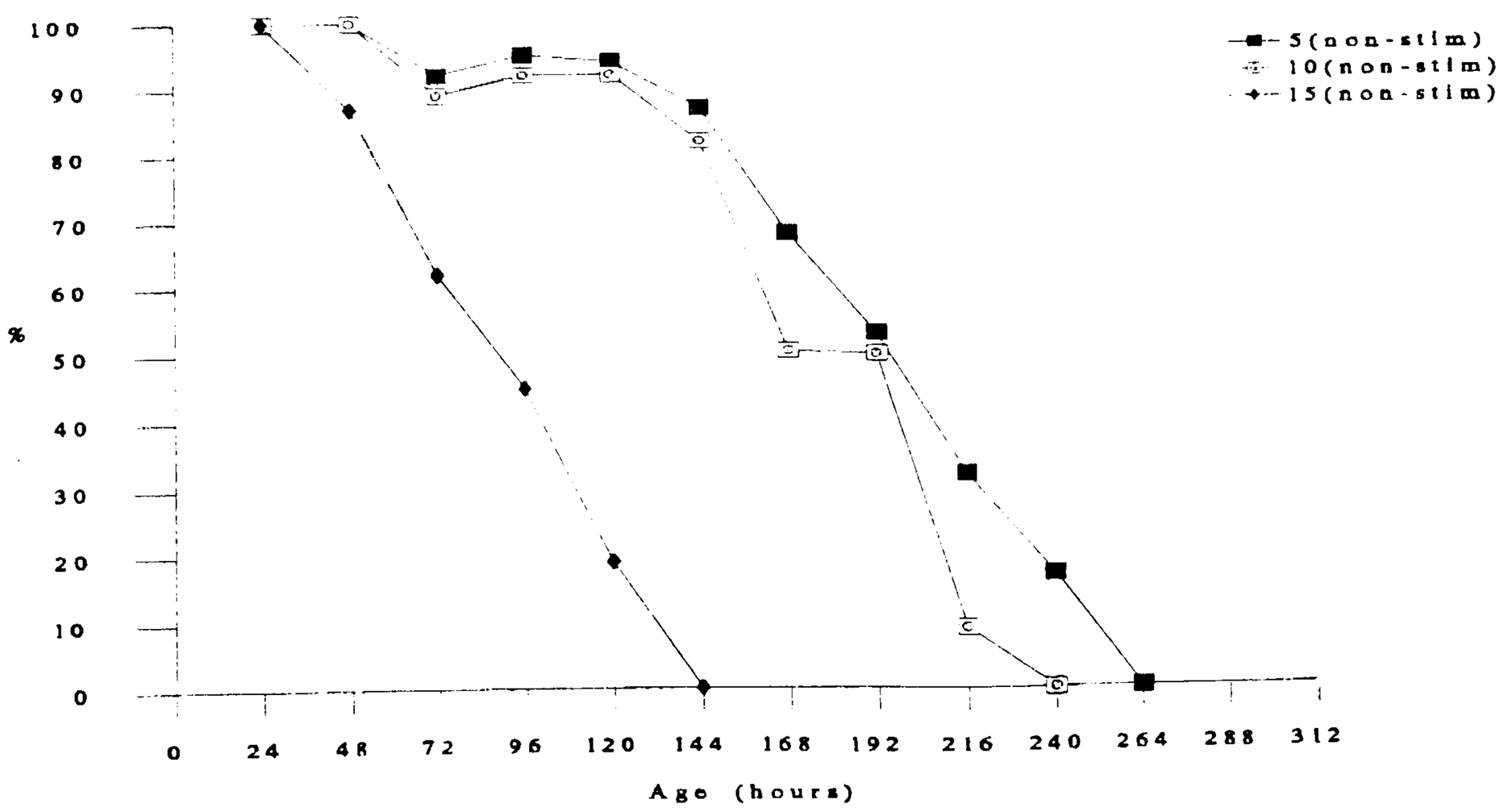


Fig. 4.9. Relationship between age and spontaneous (non-stimulatory) activity exhibited by *L. salmonis* copepodids held at 5, 10 and 15°C.



7 day old copepodids being 14.2% (± 8.71). This difference was found to be significant (t-test, $p < 0.05$), with 7 day old copepodids being less infective than 1 day old stages.

4.3.6.1.2. Settlement pattern

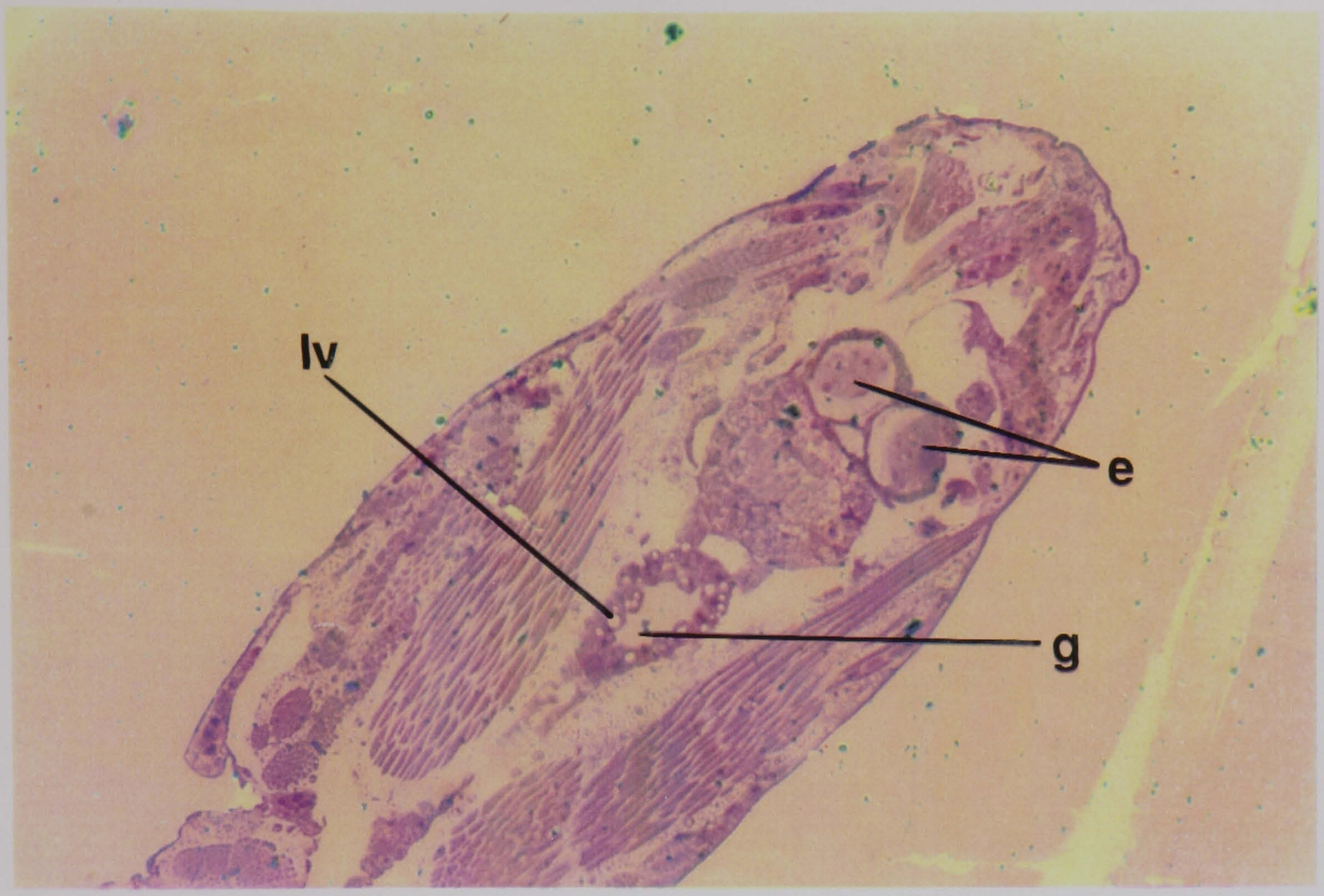
The distribution of the copepodids after infection is shown in tables 4.6 and 4.7. A t-test revealed that there were no significant differences in the settlement pattern between 1 and 7 day old copepodids ($p = 1.00$). Copepodids recorded on the fins accounted for 59.2 and 59.0% of the infections in 1 and 7 day old copepodids respectively, whilst 37.4 (1 day old) and 37.3% (7 day old) of infective stages settled on the body surface, which is comprised of the flanks and the opercular and cranial regions. Only 3.6 and 3.6% of 1 day and 7 day old copepodids respectively were found to have attached to the gills.

In both cases, the caudal fin was found to have the most infective stages attached out of all the fins, and these accounted for 18.9 and 19.7% of all the 1 day and 7 day old attached copepodids respectively, and 32.0 and 33.3% of attached stages on the fins alone. The body region found to have the greatest numbers of attached stages was that of the operculae, accounting for 67.5 and 58.6% of 1 and 7 day old copepodids attached to the body surface, and 25.23 and 21.83% of all the copepodids.

4.3.6.2. Energy depletion and age

The lipid vesicles present within the gut of copepodids can be seen in fig. 4.10. The maximum diameter of twenty randomly selected lipid vesicles in the mid-gut of each of ten 1 day old and ten 7 day old copepodids was measured. The results are give in table 4.8, which shows that the mean diameters of the lipid vesicles are 5.07μm and 4.13μm for 1 and 7 day old copepodids respectively. A t-test demonstrated that a significant difference existed between the mean size of these vesicles in the copepodids of different ages ($p <$

Fig. 4.10. Semi-thin section (0.50µm) of a *Lepeophtheirus salmonis* copepodid stained with Toluidene Blue. Lipid vacuoles (lv) around part of the gut (g) can be observed, posterior to the eyes (e) (x200 magnification).



0.001), indicating older infective stages have a decreased amount of lipid stored compared to newly moulted copepodids.

4.4. Discussion

The present study attempted to investigate the energy supplies of the free-swimming larval stages of *L. salmonis*, and how these influenced the early life history of the parasite. Results from such studies should provide information on the critical transition from a free-swimming to a parasitic mode of life.

4.4.1. Biochemical composition

The biochemical analysis of *L. salmonis* egg strings revealed that the majority of the egg string is composed of water, moisture accounting for 78.42% of the wet weight. Dry weight analysis showed that protein constituted the major fraction (52.17% of dry weight) followed by lipid (29.69%), with carbohydrate accounting for only 9.68% of the dry weight, and ash 8.46%. The ovisac is a membranous structure surrounding the eggs, each of which is comprised of a developing nauplius enclosed by an egg membrane(s). Since there is no further input from the maternal female after the ovisacs have been produced, it can be assumed that the biochemical composition of the egg strings can be used as a fairly accurate estimation of the composition of individual eggs and, therefore, of the first non-feeding larval stage.

The domination of protein as the major organic fraction has been demonstrated for free-living marine zooplankton (Raymont, Austin & Linford, 1966; Raymont & Linford, 1966; Seguin, 1968; Raymont, Austin & Linford, 1967; Raymont *et al.*, 1969). Raymont *et al.* (1969) found that for decapods, protein as a percentage of total organic matter varied from 65 to 85%, with lipid ranging from 13 to 33%. In the present study, protein and lipid constituted 57 and 32% of the total organic fraction comparing favourably with Raymont *et al.* (1969). These authors found that carbohydrate

accounted for only a very small proportion of the total organic fraction, only 2%, compared to the 11% in the present study. Holland (1978) reviewed the studies performed on the biochemical composition of eggs of marine invertebrates and found that protein formed the major constituent of the eggs followed by lipid and then carbohydrate, as is also the case for the present study. The inorganic fraction, that is the ash content, of *L. salmonis* egg strings was found to be 8.46%, which is, according to Holland & Walker (1975), within the comparatively wide range of ash weights, 2 to 21% of the total dry weight, quoted for several species crustacean zooplankton (Raymont, Austin & Linford, 1963; Raymont *et al.*, 1967; Raymont, Srinivasagam & Raymont, 1971).

4.4.2. Energy reserves

Due to the lecithotrophic nature of the free-swimming stages of *L. salmonis*, the energy store will have a finite "lifespan." Though descriptions of the energy source of parasitic copepod larval stages have tended to be brief, Wootten *et al.* (1982) recognised the importance of energy reserves for *L. salmonis*, the authors stating that the swimming activity and longevity of the infective copepodid stage may be curtailed due to the lack of sufficient food reserves.

The area stained by Sudan Black B was found to extend from just above the occular region to just before the posterior end of the nauplii and was therefore assumed to be lipid. By staining the naupliar stages daily, it was observed that, despite the fact that the length of this lipid region did not decrease significantly over time, the width of this "sac" was seen to decline, suggesting that this lipid is used as an energy supply during the lecithotrophic phase of the life cycle. Such an area had been previously

described for other parasitic copepods - *Caligus curtus* (Heegaard, 1947), *Lepeophtheirus dissimulatus* (Lewis, 1963) and *Lernaeenicus sprattae* (Schram, 1979) and for the Caligidae in general (Wilson, 1905), but had not been confirmed by staining.

Though the length of the vesicle tended to remain constant in *L. salmonis*, Heegaard (1947) found that the anterior boundary of this "yolk" in *C. curtus* nauplii receded more and more, until, shortly before the transition to the copepodid stage, the yolk only filled the posterior third of the body. In the present study, some *L. salmonis* nauplii were observed to have a "longitudinal" decline in the area of lipid. However this decrease was found, by analysis of variance, not to significantly change over time, whilst the width, by the same statistical method, was shown to decline significantly.

The use of lipid as an energy reserve has been demonstrated for free-living crustaceans. Holland (1978) reviewed the information available showing that that in the eggs of marine invertebrates, including crustaceans, the nutrient reserves are in the form of lipid vesicles and proteinaceous yolk granules, with the latter being thought to be made up of aggregates of lipoproteins termed lipovitellins. These, according to Holland (1978) will allow species to produce larvae with enough energy to survive without feeding, as is the case for the naupliar and copepodid stages of *L. salmonis* whilst in the water column. Holland & Spencer (1973) demonstrated that the lipid levels of the developing larvae of the oyster *Ostrea edulis* L. changed over time. These larvae feed during their pelagic life prior to settlement, in contrast to *L. salmonis*. However, in common with *L. salmonis*, the energy accumulated by the feeding larvae was in the form of lipids. By removing the source of food of the larvae, Holland &

Spencer (1973) showed a decrease in the lipid levels in *O. edulis* larvae over time, indicating the utilisation of it as an energy reserve. Also, they stated that during the early stages of metamorphosis, the larvae were unable to feed, and again would therefore be forced to rely on this stored energy supply. They demonstrated this by showing that the levels of lipids in non-starved larvae accumulated during larval development, but then fell dramatically at metamorphosis. Indeed, Holland (1978) found that often a considerable part of the larval energy reserve of marine invertebrates was utilised during metamorphosis. From the present study it was seen that the dimensions of the lipid reserves in the first two naupliar stages of *L. salmonis* decreased over time, indicating their use as an energy store. Also, it was observed that those larvae that were 48 hours old and had successfully completed the moult to the second nauplius stage had a smaller discrete area of lipid remaining than those larvae of the same age that had not moulted from the first naupliar stage, the width of the lipid area being 116 and 127μm respectively. It can therefore be seen that, despite there being no major metamorphosis occurring between the first and second naupliar stage, a greater amount of energy is needed for a successful completion of the moult than compared to that utilised by non-ecdysing larvae over the same time scale.

The moult from NII to the copepodid is accompanied by major morphological changes. It might therefore be expected that due to such changes occurring between these stages, a greater amount of energy will be required than from nauplius I to nauplius II. If this energy is not available, due to the depletion and exhaustion of the lipid stores, it seems unlikely that the second moult will occur. Holland & Walker (1975) suggested that, although the energy cost of metamorphosis in the barnacle *Balanus balanoides* is not

known, it may be the case that a non-feeding cypris larva which had been prevented from settling would have its lipid reserves so depleted that a successful metamorphosis could not be achieved. It was not possible to measure the width of the lipid area in copepodids due to the formation of the gut. To see whether there is an increased depletion at the nauplius II to copepodid transition, compared to the nauplius I to nauplius II moult, all three stages would have to be examined histologically and measured for the diameter of their lipid vesicles.

From the results in Chapter 3, it was seen that the moult to the copepodid was reduced and even halted at low temperatures. However, it seems unlikely that this was due to the exhaustion of the lipid reserves since, from the present studies on utilisation, it can be seen that larvae held at lower temperatures demonstrate a lower rate of lipid depletion, and therefore do not have a reduced amount of energy when the time for metamorphosis occurs at lower temperatures. The control of temperature over moulting is therefore still not clear, and further studies are needed to understand its influences.

From the results of Chapter 2, it was observed that smaller eggs are produced by *L. salmonis* females during the winter, whilst in the summer months there is an increase in size. From such results, it appears that there may be a seasonal pattern of energy investment into individual eggs. In the nauplius energy studies, although there was no significant variation in the length of the nauplius I larvae in the February, May, August and October samples, there was a significant difference in the width of the larvae. This corresponded well to the observed increase in the width of the egg (termed "egg size" in Chapter 2 and "egg length" by Ritchie *et al.*, 1993; this is a measurement equatorially across the body of the enclosed nauplius I, which is equivalent to its width whilst in the egg membrane). The maximum mean width of the nauplius was found to be 214.05µm

in August, when mean egg size was 71.25μm, and the minimum mean widths of the nauplius were 197.74μm in February and 197.93μm in May when the egg sizes were 68.32μm and 63.40μm respectively.

Ritchie *et al* (1993) also suggested that there might exist a seasonal variation in the amount of energy invested into individual eggs of *L. salmonis*, having also shown a variation in egg size over a twelve month period. Crawford & Daborn (1986) demonstrated that, for the free-living copepod *Eurytemora herdmani*, a 10% reduction in egg diameter represented a 27% reduction in egg mass (volume), and thus a corresponding decrease in energy invested. Ritchie *et al.* (1993) hypothesised that larger eggs would produce nauplii with a higher survival rate, with this no doubt being due a larger body size containing an increased amount of energy reserves. However, the authors were unable to confirm this as they did not measure the size of the resultant nauplii or their energy reserves as did the present study.

The increased width of nauplius I stages between 0 and 24 hours old was also found to be accompanied by a significant increase in the width of the lipid area during August. The maximum width of the lipid region was 135.84μm in August, and the minimum lipid width was 112.98μm, being observed in May. Although this was not when the minimum nauplius width was recorded, which was 197.74μm in February, there were no significant differences found between the mean width of the naupliar stages or the width of the lipid reserves in February and May, these being 197.74 and 197.93μm and 113.69 and 112.98μm respectively. It is assumed that the larger nauplii in the summer will have a larger energy store than their winter counterparts. According to the hypothesis of Ritchie *et al.* (1993), larger nauplii would have an increased

survival rate, presumably due to their higher levels of energy reserves allowing them more time in which to locate and successfully infect a suitable host. However, this is not necessarily the case. At higher temperatures during the summer, metabolism will be greater, and therefore the energy reserves will be depleted at a greater rate. Also it has been suggested (Prah & James, 1977) that lecithotrophic larvae are more active at higher temperatures and this would also add to the faster depletion of the lipid store. It is shown in Chapter 5 that nauplii and copepodids are more active at higher temperatures than at lower temperatures. Thus, the larval stages held at increased temperatures spend a greater proportion of time swimming spontaneously, and a lesser amount of time in rest periods. This, combined with a higher intrinsic metabolic rate at increased temperatures might explain the faster utilisation of lipids by nauplii at 10°C than at either 7 or 5°C in the present study. Since this faster rate of depletion does occur, then it is not surprising that *L. salmonis* nauplii produced in the summer require larger energy supplies than winter larvae. By producing larger nauplii in the summer, with a consequent increase in the amount of lipid reserves available, the parasite is making allowances for this faster rate of depletion, and thus ensuring that the life expectancy in terms of energy reserves is the same as that at colder temperatures.

Although it seems likely that there is a seasonal variation in energy investment in individual *L. salmonis* eggs, it is unclear as to whether there is an overall seasonal variation in reproductive investment and therefore output. Biochemical analysis of two samples of *L. salmonis* egg strings, one obtained in the late spring (May) and one in the autumn (October) showed that there were no significant differences in the proportion of lipid, protein or carbohydrate at different times. This is expected, as although the eggs

and hatched larvae are larger in the summer and early autumn, and so will presumably each have a higher protein, lipid or carbohydrate content than smaller eggs or larvae, there is no reason why the overall proportions of the three organic fractions should change. However, to determine if there exists a variation in the total amount of energy invested into egg strings in winter compared to summer, quantitative analysis is necessary.

An overall seasonal reproductive investment by *Eurytemora herdmani* was shown by Crawford & Daborn (1986). The free-living copepod produced a fewer number of smaller eggs over the winter compared to summer months. For *L. salmonis* however, since the total number of eggs produced increases when the size of eggs is declining (present study, Chapter 2), in contrast to *E. herdmani,* a net change in reproductive investment is not so simple to determine. Seasonal investment tends to occur in free-living copepods in order to accommodate unfavourable environmental regimes such as food supply (Durbin, Durbin, Smayda & Verity, 1983). However the food supply for parasitic copepods such as *L. salmonis* is in the form of the host, the availability of which is unlikely to have a seasonal nature like that of planktonic food sources, and therefore it is unlikely that a seasonal investment in total reproductive output does occur.

4.4.3. Longevity

Information on the infectivity of the copepodid stage of sea lice, as well as other ectoparasitic copepods has tended to remain restricted to the measurement of the longevity of the infective stage. The infectivity of a parasite may not be necessarily linked to its lifespan (Anderson & Whitfield, 1975) and, indeed, it is not possible to

estimate the ability of a stage such as the copepodid to infect and establish on host from simply measuring its longevity. The length of survival of an infective stage does, however initially allow the calculation of a maximum duration of time during which it has the potential to infect a fish host. Measuring the longevity of *L. salmonis* copepodids makes it possible to compare the results to other data obtained regarding energy levels and activity and therefore observe which may be the best indicator of the infection status of a parasite.

The longevity of the copepodid stage of *L. salmonis* has been reported by a number of authors. The earliest report for *L. salmonis* is that of Johannessen (1978), which indicated that the infective stage may live for up to a month if attached to a substrate, thus being "... a potential danger of infestation to salmonid species for a long time." The attachment of *L. salmonis* copepodids to an artificial substrate has not been reported elsewhere in the literature and, indeed, in the present study this behaviour was never observed. Wootten *et al.* (1982) considered a month an unlikely amount of time given their finite food reserves, but gave no information on the duration of the infective period. The survival of the copepodid of *L. salmonis* under laboratory conditions was investigated by Johnson & Albright (1991), and was found, not surprisingly, to be dependent upon environmental conditions such as salinity (as discussed previously in Chapter 3) and temperature, with the average survival of the stage ranging between 2 to 8 days. However, it is difficult to determine from the data of Johnson & Albright (1991) the survival of the copepodid at different temperatures, and these are not given in the text. Temperature is likely to have a profound effect upon the longevity of the

infective stage due to its influence on the metabolic rate of depletion of the larvae's finite energy reserves.

In the present study it was found that the longevity of the copepodid stage of *L. salmonis* was temperature dependent, ranging between 9 and 17 days, a much greater length of time than that reported by Johnson & Albright (1991) for the same stage. At the three temperatures tested, 5, 10 and 15°C, it was observed that the survival span of the infective stage was longest at the lowest temperature, 5°C, and shortest at 15°C, 9 days (216 hours) and 17 days (408 hours) days respectively. The time for 50% mortality to occur was also inversely related to temperature, with these times being 116.08, 89.57 and 82.04 hours at 5, 10 and 15°C respectively. This pattern of longevity is no doubt due to relationship between temperature and metabolic rate. The rate of utilisation of the lipid reserves will be greater at higher temperatures compared to the rate at lower levels. This consequently will cause a faster depletion of the available energy, and therefore once there is no more energy left to fuel the essential life processes, death will occur, resulting in the temperature-longevity results observed.

The dependence of the longevity of the infective stage upon temperature has been reported for other parasitic copepods, but most of the reports seem to concentrate upon the digeneans. Prah & James (1977) demonstrated that the survival span of the snail-infecting miracidial stage of *Schistosoma haematobium* and *S. mansoni* was shorter at higher temperatures and Farley (1962) demonstrated the same phenomenon for *Schistosomatium douthitti*, with miracidia having a 50% survival period of 11 hours at 8°C decreasing to 1.5 hours at 35°C. Anderson *et al.* (1982) stated that their results were in agreement with those of Prah & James (1977), the former authors finding that

the instantaneous *per capita* death rate of *S. mansoni* miracidia increased at higher temperatures, with this indicating that the lifespan of the infective stage has decreased compared to miracidia held at lower temperatures.

This dependence of survival on temperature has also been seen for the parasitic copepod *Salmincola edwardsii*. Conley & Curtis (1992), like the present study, demonstrated that temperature had a significant effect upon the longevity of the infective copepodid stage, with the mean survival time being longest at 8°C, decreasing from approximately 30 days to 20, 13 and 8 days at 12, 16 and 20°C respectively. However, Conley & Curtis do not offer any explanation as to the cause of this, though it seems likely to be due to the quicker depletion of the finite energy reserves at higher temperatures. According to the authors, no similar study had been performed on sea lice, despite the obvious importance of obtaining such data for these economically important parasites. However, since their report, a study has been published concerning the effect of temperature upon longevity for a species of sea louse (Hallet & Roubal, 1995). However, the results contrasted with the findings of the present study, those of Conley & Curtis (1992) and the data discussed for digenean parasites. Hallett & Roubal (1995) looked at the overall infection dynamics of *Caligus epidemicus* on perchlets, *Ambassis marianus* and demonstrated that the longevity of the infective copepodid stage was not temperature dependent, with copepodids surviving for 7, 8 and 5 days at 19, 22 and 26°C respectively. This does appear strange, and the authors do state that survival might be expected to be some function of the normal seawater temperature. However, they give no alternative explanation as to why this is not reflected in their data. It may be the case that the temperatures in the region of 26°C are very high for a

marine parasite and may be lethal. Perhaps a lower temperature range may have heralded the same results as the present study.

4.4.4. Activity and energy levels

As has already been suggested, although the longevity of the infective stage may give an indication of the maximum period of time when infection can possibly occur, the presence of life does not necessarily reflect the ability to infect. Indeed, it can be expected that the energy required to maintain life will be much lower than that needed to infect and successfully establish on a host. It may therefore be more of more value to look at parameters other than the lifespan of infective stages in order to estimate the infective potential of a parasite.

According to Chernin (1970) and Mason (1977), the rate of movement, and thus the distance covered is an important determinant of the chances of an infective stage locating and successfully infecting a host. Both these authors were studying the activity of the digenean parasite *Schistosoma mansoni*, and Anderson & Whitfield (1975) studying the same parasite, found that older miracidia exhibited decreased rates of movement and were also less infective than their newly hatched counterparts. Miller & McCoy (1930) had previously also found that for *Cercaria floridensis*, infestation of fish was only possible when the cercariae were active enough to reach them. Testing the hypothesis that activity is correlated with infectivity appears to have been mainly confined to the digeneans, and the theory has only occasionally been suggested for other groups of parasites besides these. According to Conley & Curtis (1993), the infective stage of the parasitic copepod *Salmincola edwardsii* could be considered functionally dead when it was no longer capable of infecting a host, and although

copepodids did continue to live long after they had lost the ability to swim, the chances of infection once they were motionless were very low.

4.4.4.1. Age dependent activity

Since the activity of infective stages of parasites such as cercariae, miracidia and copepodids is dependent upon a finite energy store, it can be considered a function of the age of the stage.

The activity of the infective stage of *L. salmonis* was observed in the present study to decline over time, demonstrating an age-dependent nature and considered to be due to the utilisation of finite energy reserves. From the present study, it was observed that *L. salmonis* copepodids demonstrated three different types of "activity" patterns. The first, observed in newly moulted copepodids, was of swimming in spontaneous bursts without any external stimulation. This was followed by a period when the copepodids entered a non-spontaneous period, 50% of individuals becoming active only by stimulation at 8 days (168 hours) after the moult to the copepodid at 10°C. After 8 days, swimming only occurred when stimulated with a current of water expelled from a pipette which may perhaps mimic the current flow associated with the passing of a fish. The stimulus-dependent activity continued until 264 hours post moult at 10°C. After this phase, the infective stage exhibited no activity, even when stimulated with water flows. It therefore appears that *L. salmonis* copepodids may adopt a strategy of energy conservation after a certain period of time after which they have not located and infected a host. By swimming only in response to stimulation indicating the presence of a host, copepodids will utilise their remaining energy store at a decreased rate

compared to continuous spontaneous swimming. This will prolong the life of the copepodid, thus allowing more time in which to infect a suitable host.

Examples of age dependent activity have been reported and, again, the majority of these are associated with the infective stages of digeneans. Anderson & Whitfield (1975) demonstrated that the proportion of time spent swimming by the cercariae of the digenean *Transversotrema patialense* to be markedly age dependent, with this time declining appreciably as cercariae aged, reaching zero many hours before death occurred. They attributed this age-dependence of activity to the utilisation of a non-replaceable energy reserve, and hence, later in the cercarial lifespan, little swimming activity will occur. The authors used a model to test this hypothesis, declaring that the model incorporated the assumption that the rate of change of activity was directly proportional to the quantity of energy reserves present and remaining. The model was demonstrated to provide a good fit to the authors' experimental data. Chapman (1974) also demonstrated that the cercariae of *Cryptocotyle lingua* Creplin spend a decreasing amount of time in bursts of swimming, and an increasing proportion in inactivity as the age of the parasite increased. However, these reports are in contrast to the observations of the present study and the hypothesis that *L. salmonis* may adopt two strategies concerning activity. If *L. salmonis* copepodids conformed to Anderson & Whitfield's (1975) model, then one would not expect to observed two such strategies. Swimming would simply stop when no more energy was available, and swimming in response to stimulation would not be observed.

However, Whitfield *et al.* (1977), also demonstrating an age dependent activity in *T. patialense* cercariae, attributed the increasing proportion of time spent inactive by

the cercariae as they age, not to the concomitant decline in energy reserve, but to a direct neural control of swimming by the "brain in the cercarial head" bringing about the observed age dependent decline in swimming frequency. The decline in activity according to the authors is probably a means of maximising the likelihood of infection encounters with a fish host during the utilisation of a finite diminishing nutritive source. Swimming bursts by *T. patialense* must, according to Whitfield *et al.* (1977) consume more energy than dropping or resting, the other two activities exhibited by the cercarial stage, and therefore the changing values of the frequency of swimming events observed will correspond to the changing rate of energy (in the form of glycogen for cercariae) utilisation. The authors, like the present study, also put forward the hypothesis of two alternate strategies employed by the infective cercarial stage. The first strategy, like *L. salmonis*, would be a high constant rate of energy utilisation characterised by a high swimming ability. If at the end of this period a successful infection encounter had not occurred, a second strategy would be adopted. This would prolong the infective period by a decreasing the amount of time spent swimming and increasing the proportion of time being inactive. Thus the remaining energy reserve would be conserved, only utilising it at high rates when external stimuli signal the chances of an infective encounter are temporarily increased. This, and the present study therefore contrast with the hypothesis of a constant depletion of the finite energy reserve suggested by Anderson & Whitfield (1975). However depletion occurs though, it is obvious that there clearly exists an age dependent activity in larval stages of some parasites.

4.4.4.2. Temperature dependent activity

As well as age dependency, the present study demonstrated a temperature dependent activity. The overall duration of activity, both non-stimulated plus stimulated, of *L. salmonis* copepodids was longer at lower temperatures (5°C) compared to those at 10 and 15°C, 13 (312 hours), 9 (264 hours) and 7 (168 hours) days respectively. The period of time over which non-stimulated swimming occurs was shortest at 15°C, with copepodids switching to their second "strategy" before those held at 5 and 10°C, with the time taken for 50% of copepodids to "switch" being 80, 168 and 200 hours at 15, 10 and 5°C respectively. This is again probably caused by the different rates of energy utilisation by copepodids at different temperatures. From the present study it was seen that the energy levels in the form of lipids in the first two naupliar stages of *L. salmonis* were depleted at a greater rate at 10°C compared to nauplii held at either 5 or 7°C, with the percentage rate of depletion of the finite energy store being lowest at 5°C, and it can be assumed that this pattern also occurs in the copepodid stage. The reasons for this faster depletion of energy reserves are speculative, with no data being available in the published literature. As has already been discussed, the primary reason that comes to mind is that of an increase in the metabolic rate associated with an increase in temperature, causing the observed faster depletion of lipids in *L. salmonis* nauplii and the temperature dependent activity rate in *L. salmonis* copepodids.

There have been other reports of temperature-dependent activity in parasites. Whitfield *et al.* (1977), like the present study, demonstrated that activity periods of the cercarial larval stage of *T. patialense* decreased over time quicker at higher temperatures. Through the temperature range tested, 25 to 32°C, the spontaneously

active life of cercariae declined from 18 hours to approximately 6 hours at the higher levels. Prah & James (1977) demonstrated that temperature also directly affected the activity levels of miracidial stages of digeneans, using both *Schistosoma haematobium* Bilharz and *S. mansoni*, with the period of time these two species were active decreasing at high temperatures. Prah & James (1977) suggested that the decline in the degree of activity is sharper at high temperatures and more gradual at moderate and low temperatures. This suggests that at high temperatures, the rate of metabolism is increased compared to the rate at lower temperatures.

This dependence of activity of lecithotrophic infective stages on temperature has also been shown for parasitic copepods. Conley & Curtis (1992) found that temperature not only had a significant effect upon the longevity of the copepodid stage of *S. edwardsii*, but also upon the duration of its swimming activity. The duration of activity, like *L. salmonis*, was found to be inversely related to temperature, being longest at 8°C than at 12, 16 or 20°C. Within each temperature band however, activity was observed to decrease over time, as was also seen in the present study, again showing an age dependent activity of the parasite. Despite these results, the authors, unlike the present study, did not attribute either the age dependent decline or the temperature dependent decline in activity to the exhaustion of a finite energy resource, although they did recognise the importance of activity as being essential for predicting critical time periods during which infection could occur. Conley and Curtis (1993) also demonstrated that the duration of swimming ability and copepodid survival of *S. edwardsii* were inversely related to age and temperature, but the data from this paper appear to be the same as that of Conley & Curtis (1992), and yet again the authors do

not attribute the results to the depletion of energy levels, but merely stating that once activity had halted, infection was unlikely. The authors (Conley & Curtis, 1992) did however suggest that, to their knowledge, no similar study had been performed on sea lice, specifically *L. salmonis*. The present study has therefore attempted to rectify such a situation.

As well as an increased rate of utilisation, Prah & James (1977) also suggested that miracidia of *S. haematobium* and *S. mansoni* were actually more active at higher temperatures. Increased activity would cause a faster depletion of the energy reserves, and hence would also explain why lecithotrophic stages show a decreased period of activity at higher temperatures. During the observations in the present study on *L. salmonis* copepodids, and those in Chapter 5 on *L. salmonis* nauplii and copepodids, it was observed that whilst active, stages held at higher temperatures spent more time in spontaneous active swimming than spent in passive sinking. This would no doubt add to the faster depletion of energy reserves due to an associated increased demand for energy, again explaining the temperature-dependent activity levels observed for *L. salmonis* copepodids. However, it is not known in what proportions an increased metabolic rate and an actual added increase in activity levels combine to cause the temperature dependent activity nature observed in the present study.

In contrast to the present study, and the other reports for infective stages already discussed, Hallett & Roubal (1995) in their study of infection dynamics *C. epidemicus* Hewitt, on perchlets found that like their longevity results discussed earlier, no pattern was observed in the activity of the copepodids of *C. epidemicus* in relation to temperature, with there appearing to be no significant differences in copepodid activity

at 19, 22 or 26°C, but those held at 22°C appeared to be the most active, suggesting no direct or inverse relationship between activity and temperature. As was suggested earlier, it may be the case that 26°C may have been too high a test temperature for marine parasite larvae, and thus an abnormal behavioural pattern may have been observed.

4.4.5. Infectivity and energy levels.

It has been suggested by Chernin (1970), Anderson & Whitfield (1975) and Mason (1977) that infectivity is more likely to be linked with the activity status of an infective stage of a parasite than with its overall survival span. This may be intuitive for parasites of pelagic fish since, if an infective stage of a parasite remains motionless, then it will passively sink eventually remaining on the bottom of the water column. Its chances of infecting a pelagic fish will then be dramatically reduced. It may be argued that by merely sinking in the water column, larvae will also encounter pelagic fish, and that sinking maybe the behaviour employed to encounter a host. However, even if this were the case, infective stages would need to be active in order to ascend the water column prior to sinking and then "climb" it again if a successful encounter did not occur. Therefore, by remaining active, the infective stage will increase the chances of a spatial overlap occurring between itself and potential hosts, thus increasing the chances of an infection encounter. Since activity has been shown to be age dependent, with the exhaustion of energy reserves being responsible for this phenomenon, it can also be expected that infectivity will be closely correlated with this activity, and also exhibit a strong age dependence. According to Maldonado & Acosta-Matienzo (1948), Chernin

(1968) and Prah & James (1977), infectivity is often a function of the age of the organism.

4.4.5.1. Age dependent infectivity

Seven day old *L. salmonis* copepodids in the present study were found to have a decreased infection success compared to those that were newly moulted (1 day old), the infection success of the two groups being 14 and 22% respectively. As already noted, activity was seen to be highest in 1 day old copepodids, with 100% of individuals exhibiting spontaneous swimming activity without the need for external stimuli. This percentage of individuals showing this type of swimming fell over time. Approximately only 20% of 7 day old copepodids at 10°C swam spontaneously without stimulation and, overall, the total percentage of infective stages that could still actively swim, with or without stimulation was only 40%. Because of this decrease in activity, the chances of a copepodid coming into contact with a host is probably greatly reduced, explaining the decrease in infection success between 1 and 7 days. Activity and infectivity therefore seem to be inextricably linked.

Thus the amount of time spent swimming is controlled by the levels of energy remaining, the ability to infect should therefore be linked to the amount of lipid remaining. The diameter of the lipid vesicles present in the gut of *L. salmonis* copepodids was found to be 18.5% greater in 1 day old copepodids confirming the correlation between the amount of lipid and infection success. Rather than using the longevity of the parasite as an indication of the infective status of *L. salmonis* copepodids, the measure of activity of the parasite does indeed appear to be a more accurate estimator of infection success.

Using a mathematical model, Anderson (1978) concluded that the ability of larval digeneans, both cercarial and miracidial stages, to encounter and infect a potential host invariably declines as the larvae age. This was linked again to the utilisation of the larval energy stores. Due to this existence of age-dependent infectivity, Anderson (1978) suggested that the age of the infective stage must be determined prior to infection experiments. Only then can accurate estimates of rates of infection be obtained. The conclusions of this model have also been demonstrated in the laboratory for a number of digenean species; Miller & McCoy (1930) for *C. floridensis,* Anderson & Whitfield (1975) and Anderson, Whitfield & Mills (1977) for *T. patialense*, Anderson *et al.* (1982) for *S. mansoni* and Waadu (1991) for *Diplostomum spathaceum*. All noted that infectivity was age dependent.

Despite the studies that have been performed on infectivity and age on trematodes and the association with the energy stored in the larvae, none of these reports has quantified the depletion of the energy reserves. The biological processes involved in both activity and infectivity, for digenean cercariae, are thought to be non-replaceable and are present primarily in the form of glycogen in the tail musculature of the larvae (Erasmus, 1959; Coles, 1973). Although Anderson & Whitfield (1975) state that biochemical determinations of the quantity of glycogen in *S. mansoni* cercariae have been made, showing a constant rate of depletion, these data were from a personal communication, and no data have been made available for study.

There have been very few reports upon the age dependent infective characteristics of parasitic copepods. However, Hallett & Roubal (1995) did look at the infection dynamics of *C. epidemicus*, infecting perchlets with copepodids of known

ages. Active *C. epidemicus* copepodids that were between 1 and 6 days old were placed separately with a single fish and left for 24 hours at either 19 or 26°C, when the fish were anaesthetised and examined for copepodid attachment. At 19°C, attachment success was found to be highest for copepodids that were 4 days old, 32.5% attachment, compared to only 5% attachment success for 1 day old copepodids. At 26°C, attachment was highest for 3 day old copepodids, with 35% successfully infecting the perchlets, compared to 25% of 1 day old copepodids. Hallett and Roubal (1995) suggest that due to the high activity observed in "young" copepodids and the lower attachment success compared to older infective stages, there appears to be a dispersal strategy employed by the newly moulted copepodids, with activity and infectivity not declining in a similar manner. Such a pattern was not observed for *L. salmonis* although only two ages were tested it may be argued that the time spent as nauplii would allow for sufficient dispersion. Boxshall (1976), in contrast, reported that the copepodids of *Lepeophtheirus pectoralis* are initially positively phototactic, but then sink to the bottom where the flatfish host is located. This again suggests a dispersal phase of the parasite, since there appears to be no other value in copepodids of *L. pectoralis* remaining in the water column. They would be unlikely to encounter any potential demersal hosts.

From the data in the study, it was concluded that the time available for infection is likely to be governed by the quantity of energy remaining. Energy reserves and their effect on infectivity were not addressed by Hallet & Roubal (1995) or Conley & Curtis (1992, 1993), despite the fact that the latter recognised the importance of an age-

dependent longevity and activity in the population dynamics of the parasitic copepod *S. edwardsii*.

5.2. Temperature dependent infectivity

Since infection experiments with *L. salmonis* copepodids were carried only at 10°C, the effect of temperature upon the success of infection is therefore unknown. However there have been studies performed on digenean parasites which may highlight important information regarding the relationship between temperature and infectivity.

Temperature was found to have a marked impact on the rate at which infectivity declines with age for the miracidia of *S. mansoni* according to Anderson *et al.* (1982). The importance of temperature to the infection success of miracidia has also been previously documented by Standen (1952), Stirewalt (1954), De Witt (1955), Wen (1962), Purnell (1966) and Prah & James (1977). It would be expected that, due to the strong influence of temperature upon activity, infectivity will consequently be affected. Increasing temperatures caused a decrease in the period of time spent actively swimming by *L. salmonis* infective stages dependent on energy stores, and therefore it would be expected that the amount of time during which successful infection could occur would decline in parallel. Again though, it is not known whether this inverse relationship between the duration of infectivity and temperature is caused simply by an increase in the metabolic rate of the parasite at elevated temperatures, a higher intrinsic activity rate at increased temperatures, or a combination of the two.

Prah & James (1977) found that the influence of temperature upon the infectivity of *Schistosoma haematobium* and *S. mansoni* was profound. Infection rates of snails were observed to increase for both *Schistosoma* species with increasing

temperature. Infection was found not to take place at temperatures below 10°C, but at 15°C the success was approximately 40% for both species with this rising to between 60 and 75% at 20°C. This suggests that despite the probable decreased period of time over which infection can occur, the actual success is higher at increased temperatures. This direct relationship between infection success of newly hatched miracidia and temperature cannot be due to just the increase of the metabolic rate of the miracidia at higher temperatures. This would only cause a decrease in the amount of time spent active and thus the period of time when infection could occur. It may not determine the infection success. An increase in the rate of activity of the larvae at higher temperatures would however not only cause faster depletion of energy reserves and thus the observed shortened periods of activity and infectivity, but also would promote a higher infection success rate. Higher activity levels may increase the likelihood of a chance encounter with the host, and so explain the infection success levels observed by Prah & James (1977). Indeed the infection success was attributed by these authors to an increase in activity of the miracidia accompanying a rise in temperature. By being more active at higher temperatures (that is, spending a greater proportion of time actively swimming rather than passive sinking), a trade-off between increasing longevity of infection status of sea lice and increasing the chances of encountering a host appears to exist.

Despite Hallett & Roubal (1995) appearing to show no clear relationship between activity, infectivity and age for the parasitic copepod *C. epidemicus*, from their results it is possible to see that infectivity does appear to be controlled by temperature to some extent. Hallett & Roubal (1995) infected perchlets with copepodids of known ages at 19 and 26°C. Infection occurred with copepodids up to 6 days old at the lower

temperature but only with copepodids up to 4 day old at 26°C. This suggests that the infective status of a parasite is longer at decreased temperatures thereby increasing the window when hosts are in potential danger. However, infection success was greater in 1 day old copepodids at 26°C (25%) compared to 1 day old copepodids held at 19°C (5%) suggesting that the higher temperatures are advantageous.

It may be expected that *L. salmonis* copepodids would follow a such pattern, having increased infection success over a shorter period of time at higher temperatures. It was observed in the present study (pers. obsv.) that copepodids held at 15°C spent a greater proportion of time actively swimming compared to those held at lower temperatures, though this relationship is explored in greater detail in the behavioural studies of Chapter 5. However, it must be remembered that although there are similarities between miracidia and copepodids, such as their dependence on stored energy, there are also differences. The activity of miracidia is a constant swimming motion, whilst that of *L. salmonis* copepodids is more akin to a "hop and sink" pattern (discussed in greater detail in Chapter 5). Therefore it may first be necessary to determine which part of the copepodid swimming pattern is used for encountering hosts before information concerning other parasitic groups can be extrapolated for sea lice.

Whatever the behavioural pattern, since infectivity appears linked to the activity of *L. salmonis*, with both these functions being age and temperature dependent, it may be possible to estimate the ability of an infective stage to successfully infect a fish from the age of the parasite and the ambient temperature, rather than the maximum lifespan of the stage.

4.4.6. Settlement pattern

The most common site of infection by *L. salmonis* copepodids in the present study was the fins, with approximately 59% of 1 day and 7 day old copepodids attached to them after infection. Hallett & Roubal (1995) also found a similar proportion (61%) of copepodids of *C. epidemicus* settling on the fins of the perchlet *A. marianus*. This "preference" by infective stages for the fins was suggested by Bron, Sommerville, Jones & Rae (1991). However, these authors did not look at the initial distribution of the infective stage, but instead sampled once the larvae had moulted to the chalimus stage. Bron *et al.* (1991), in contrast to the present study, therefore gives a picture of the distribution of successful settlers rather than the number and distribution pattern of initial larvae. Hence from the data of Bron *et al.* (1991) one could not draw any conclusions with respect to the overall distribution of the copepodid stage at infection.

The aggregated settlement on the fins observed in the present study has been reported by other authors for the copepodid stage of parasitic copepods. Kabata & Cousens (1977) showed that copepodids of *Salmincola californiensis* Dana tended to settle on the fins of the sockeye salmon, *Oncorhynchus nerka* Walbaum, and also on the opercula. In the present study, comparatively high numbers of *L. salmonis* copepodids were also observed to attach around the opercula of Atlantic salmon, when compared to those settling on the flanks and cranial region of the fish, with 25% and 21% of 1 day and 7 day old copepodids being observed on the opercula compared to only 9% and 12% on the flanks and cranium. Anstensrud & Schram (1988) found that copepodids of *Lernaeenicus sprattae* were initially randomly settled on the host, but that the distribution of this stage changed within 2 hours of infection, with the copepodids

migrating towards the fins. For the present study, and that of Kabata & Cousens (1977), it is not known as to whether a migration of the copepodid stages occurs. Bron *et al.* (1991) attribute the migration observed by Anstensrud & Schram (1988) to the possible homing of the copepodid to water currents, and indeed Kabata & Cousens (1977) explain the disproportionate settlement of *S. californiensis* in terms of rheotaxis by the copepodid, with the currents being caused by respiratory and fin movements. Bron *et al.* (1991) considered that rheotaxis would explain the site selection observed for *L. salmonis*, but also that it may just be a question of the ability of the copepodid to "hold on" to the fish rather than site selection, since fin rays lie perpendicular to the water current, and may therefore provide shelter and allow easier settlement.

In summary, from the present study, it can be seen that the measuring of just a single parameter such as the longevity of an infective stage, especially for those species where this stage is dependent upon finite energy reserves, may not allow an estimation of the infectivity of a parasite, and thus the time period when hosts will be at their most vulnerable. Activity appears to be a far better predictor of the infective status of such stages since the chances of an encounter with a potential host are greatly reduced in non-active larvae. By being able to estimate the likelihood of infection, more information will be available to identify those periods when fish are most likely to become infected with *L. salmonis*. This will also aid in the understanding of the variations observed in infection experiments and challenge trials. A combination of mortalities within a population of infective stages combined with age dependent and temperature dependent infectivity rates will decrease the rate of infection observed. These results suggest that unless experimental data are available concerning larval

survival characteristics, age-dependent activity and infectivity, the use of infection experiments to determine infestation patterns in the study of population dynamics of the infective copepodid stage of *L. salmonis*, or for that matter any parasitic lecithotrophic infective stage, will be of little, if no value.

CHAPTER 5

BEHAVIOURAL STUDIES

5.1. Introduction

The completion of the life cycle of sea lice is clearly dependent upon the survival of all stages of the parasite. However, there are certain points during the life history of caligid copepods that are viewed as more critical than others. The principal one of these is the transition from a free-swimming phase to a parasitic mode of life via the infection and successful establishment on a host by the copepodid stages. Bron *et al.* (1993a) suggested that in order to maximise the chances of survival and successful host interception and settlement, copepodid larvae must be able to respond to cues present in the external environment. However, such behaviour is unlikely to be restricted to the infective stage due to its relatively short duration of infectivity (present study, Chapter 4). It seems highly unlikely that the naupliar stages will demonstrate host-finding capabilities because of their inability to infect the fish, but behaviour that would bring or keep the larvae in areas inhabited by hosts would increase the chances of a future host-parasite encounter due to the spatial overlap of the two.

Bron *et al.* (1993a) reported that despite recent attention upon other aspects of the general biology of *Lepeophtheirus salmonis*, little work had been carried out on the behaviour of the parasite. Many of the published observations are largely anecdotal, and relate principally to the response of *L. salmonis* larvae to light (Johannessen, 1975, 1978; Wootten *et al.*, 1982), as is also the case for other caligid parasites (Wilson, 1905; Fasten, 1913; Heegaard, 1947; Hogans & Trudeau, 1989; Piasecki & MacKinnon, 1995). Such anecdotal observations were made in reports concentrating on life cycle and taxonomic studies of sea lice prior to the advent of fish farming, or when sea lice were first observed to be an increasing problem. With the increasing interest in sea lice in fish farms, more comprehensive studies on the behaviour of the

larval stages of sea lice have been made but these have tended to concentrate on the infective copepodid stage (Bron *et al.*, 1993a; MacKinnon, 1993), and its behavioural responses with respect to infection (Bron *et al.*, 1993a). Because of this, there still remains a lack of detailed information regarding the behavioural ecology of all the free-swimming stages and the implications of this with regards to the distribution of sea lice in the natural environment.

Data available regarding the behaviour of the first three free-swimming stages of sea lice is largely confined to their responses to light. As early as 1905, Wilson observed that caligid nauplii, once hatched, swim towards light, and Heegaard (1947) reported that the first nauplius stage of *Caligus curtus* was markedly phototactic, ascending in the water column immediately after hatching. However, Heegaard (1947) suggested that the copepodid stage of *C. curtus* was negatively phototactic in contrast to nauplius I, though he did not actually present any observations to show this. The first two naupliar stages of *Lepeophtheirus dissimulatus* were also observed by Lewis (1963) to exhibit a strong positive phototactic response in the laboratory, though he found that "older" nauplius II stages and the subsequent infective copepodid stage possessed reduced responses compared to nauplius I and younger nauplius II stages. Johannessen (1978) considered that all free-swimming larval stages of *L. salmonis* were positively phototactic, as did Wootten *et al.* (1982); the latter authors stating that such behaviour appeared to have value in seeking out a free-swimming host present in the upper layers of the water column. Hogans & Trudeau (1989) believed that such behaviour exhibited by nauplii and copepodids of *Caligus elongatus*, is an adaptation by the larvae to keep them in the vicinity of salmon, which also frequent the surface waters. MacKinnon (1993) reported that *C. elongatus* copepodids demonstrated in the laboratory, an

increasing photopositive response with increased light intensities, as did Bron *et al.* (1993a) for *L. salmonis* copepodids.

However, these observations on the responses of caligid larval stages to light have all been tested in aquaria with artificial lighting which does not simulate the natural distribution of light underwater but, instead, is presented as a narrow directed beam of light. Forward (1988) suggested though, that the demonstration of positive phototaxis in zooplankton is an artefact in many species, resulting from this artificial light environment. Indeed, it has been shown by Stearns & Forward (1984b) that in free-living copepods under conditions of natural light distribution simulated in the laboratory, the phototaxis was reversed, demonstrating that a negative photoresponse was the natural response. Bron *et al.* (1993a) recognised this problem, but did not test the light responses of *L. salmonis* copepodids under "natural" light distribution and therefore it is not known as to whether the responses of the copepod are reversed in the same way. MacKinnon (1993) tested the light responses of *C. elongatus* also under artificial lighting, ignoring the possibility of results being artefactual, and found a photopositive response.

Bron *et al.* (1993a), showed that the copepodid larva of *L. salmonis* exhibited similar responses with respect to its spectral sensitivity to that of free-living copepods. This may be especially valid for the first two naupliar stages as they are more likely to behave as their free-living counterparts due to their inability to infect a host. However, to date, no studies have been performed on the responses of *L. salmonis* nauplii to either light intensity or wavelength under artificial or natural light. The responses to light are obviously a key factor in helping to determine the distribution of larvae in the water column.

Another abiotic factor that has been shown to determine the distribution of larval stages of free-living crustaceans in the water column is that of salinity. Salinity in the natural environment is not held at a constant level but, due to the nature of tidal flushing, freshwater run-off and vertical water exchange, is a consistently changing parameter. Cronin (1982) suggested that crustacean larvae could avoid being flushed out of an estuary by ascending or descending in the water column, such migrations being determined by responses to water of varying salinities. Cronin (1982) cited that this behaviour is probably employed by other marine larvae such as the oyster larvae, *Crassostrea virginica* Gmelin, Woods & Hargis (1971) having previously showed that the larvae of this species avoided estuarine flushing. Cronin (1982) attributed these behavioural migrations to changes in both the phototactic and geotactic responses of larvae. Latz & Forward (1977) found that the photo- and geo-taxes of the estuarine crab larvae, *Rhithropanopeus harrisii* Gould were altered by salinity changes of only 1 to 2%, with a negative phototactic responses being induced in the normally photopositive larvae by sudden exposure to low salinity. It may be argued that *L. salmonis* larvae, and those of sea lice in general, have no evolutionary advantage in reversing such taxes. A reversion may seem important only for those free-living species with sessile or relatively immobile adults and planktonic larval stages that may be transported to unfavourable habitats. However due to the sensitivity of *L. salmonis* nauplii stages (present study, Chapter 3) to low salinity levels, it may be expected that some sort of avoidance behaviour will be exhibited, thus influencing their distribution in the water column. Indeed, downward movements in the form of either directed swimming or passive sinking in relation to low salinity has been observed in 17 copepod species (Lance, 1962; Grindley, 1964; Harder, 1968). Latz & Forward (1977)

suggested that it may be a general characteristic of pelagic larvae, removing them from areas of low salinity.

A positive geotaxis was observed by Bron *et al.* (1993a) for *L. salmonis* copepodids, but these authors did not attempt to see if this or the phototaxis were altered by external environmental conditions such as changing or fluctuating salinity levels. Barnes (1953) suggested that it might be expected that in animals with planktonic larvae, the ability of the early larval stages to withstand changes in salinity would be of considerable importance in determining the distribution of the species. If salinity affected the behaviour of *L. salmonis* larvae as well as their survival, it can be expected that their distribution in the natural environment may be different under different conditions, and that their potential distribution in the water column may not be determined by their responses to just light and perhaps gravity.

One of the determinants of the distribution of larval stages of sea lice in the water column is the swimming ability of each of the three free-swimming stages. According to Kabata (1981), because locomotion has been considered unimportant for parasitic copepods (due to the great majority being immobile as adults), it has never been studied seriously. The movements of nauplii and copepodids have been assumed to be effected in a manner similar to that used by free-living copepods, and, also according to Kabata (1981), have usually been dismissed with brief and uninformative statements. From the few reports available, it appears that different species of parasitic copepods possess different swimming behaviours. Anderson & Rossiter (1969b) observed that the movements exhibited by the copepodid of *Dissonus nudiventris* were slow and "demersal," whilst Wilkes (1966) reported that the copepodid of *Nectobranchia indivisa* Fraser darted around rapidly. Copepodids of *Clavella adunca*

(Müller) on the other hand, within the same family as *N. indivisa,* were described by Shotter (1971) as being poor swimmers, using both their antennae and swimming legs to propel themselves through the water whilst Kabata (1981) observed the "vigorous" swimming of *Salmincola californiensis* copepodids.

As well as the lack of detailed descriptions regarding the swimming behaviour of the copepodid stages of parasitic copepods, there is virtually no information regarding the swimming abilities of the naupliar stages of such species. Wootten *et al.* (1982) described the swimming pattern of the free-swimming stages of *L. salmonis* very briefly, stating that the nauplius and copepodid stages swim actively upwards with this then being followed by a passive sinking phase. This "sink and swim" behaviour was observed by Bron *et al.* (1993a) and, in the present study (Chapter 4), to be the normal behaviour of *L. salmonis* copepodids. However, there are no details available in the literature on the proportion of time spent in active swimming and in passive resting periods by parasitic copepods, and hence it is not possible to estimate the ability of *L. salmonis* larvae to either remain in, or swim to particular areas of the water column.

The distribution of *L. salmonis* nauplii and copepodids in the water column will also be affected by the time spent in moults. From general observations over the present study, it has been noticed that the moulting larvae lost the ability to swim; ecdysing nauplius I and nauplius II stages were found at the bottom of experimental containers. As a result, any position attained in the water column would be lost, and to regain such a position would require active directed swimming by the resultant NII or copepodid stage. The distances to be traversed are obviously dependent upon the amount of sinking that occurs during the moult, with this being dependent upon the time spent within the moult. However, again there is no information available

regarding the length of time the two ecdyses take and hence it is not possible to estimate the effect of moulting on the distribution of *L. salmonis* free-swimming larvae in the water column.

The swimming patterns of free-swimming larval stages of parasitic copepods have been seen to be affected by stimuli present in the external environment. Wootten *et al.* (1982) reported that if *L. salmonis* nauplii or copepodids were mechanically disturbed during a passive sinking phase, a new bout of swimming activity was initiated. Bron *et al.* (1993a) and the present study (Chapter 4) found that *L. salmonis* copepodids exposed to a highly directional flow of water resulting from the rapid expulsion of water from a pipette, demonstrated a fast "looping" or "spiralling" behaviour. According to Wootten *et al.* (1982) such behaviour would appear to have value in seeking out a free-swimming host. However, these reports on the swimming activity of *L. salmonis* are purely observational, and have not attempted to quantify such behaviour. Indeed, Kabata (1981) stated that quantitative studies on the effects of stimuli on copepodid behaviour are rare, with this appearing to be limited to those studies performed on light responses (Schram & Anstensrud, 1985; MacKinnon, 1993; Bron *et al.*, 1993a). Poulin *et al.* (1990a) on the other hand did attempt such a calculation and found that copepodids of *Salmincola edwardsii* greatly increased their locomotory activity when stimulated by visual and mechanical cues. It can be expected that naupliar stages of sea lice will also exhibit responses to mechanical stimulation, as was suggested by Wootten *et al.* (1982).

Due to the lack of information available regarding the distribution of the free-swimming larvae of *L. salmonis* in the water column in the natural habitat, the present study was undertaken in order to attempt to elucidate the behavioural responses of the

nauplii and copepodid stages that control and modify such distributions. By elucidating the responses of the free-swimming larvae to light, salinity and other stimuli, and calculating the time spent swimming and sinking, both between and during moults, vital information regarding the distribution of such stages in the natural environment may be gained. Such data can only be of assistance in understanding the population dynamics of the free-swimming larvae of sea lice.

5.2. Materials and Methods

5.2.1. Behavioural responses to light

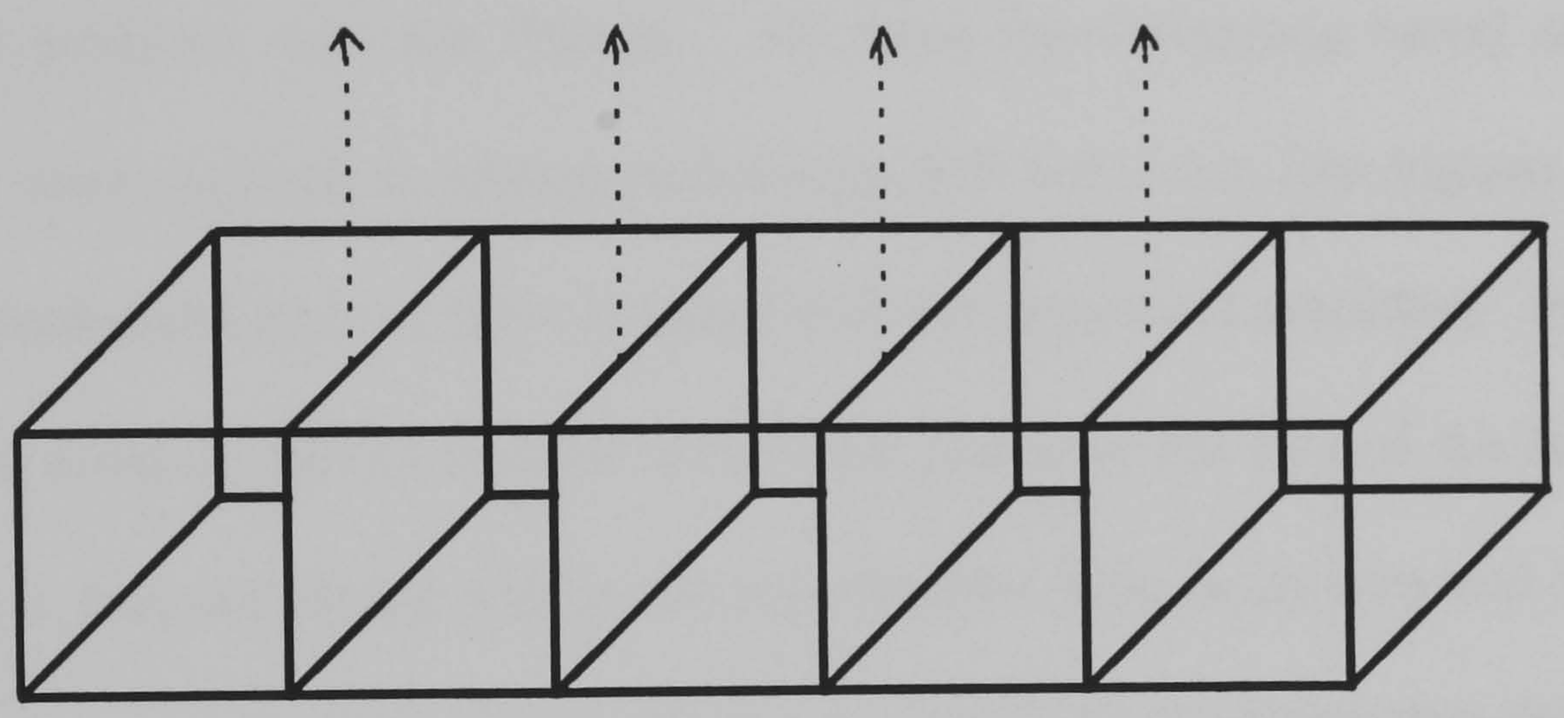
5.2.1.1. Responses to artificial lighting

The responses of both *L. salmonis* naupliar and copepodid stages to the intensity and wavelength of artificial lighting, using a narrow field beam of light in a horizontal plane, were tested using equipment based on the description of Forward, Cronin & Stearns (1984) and similar to that of Bron (1994) (Fig 5.1.). The light source was a Radis Aldis Tutor II slide projector with a 250W bulb, that was housed in a light proof box. Exit of the light beam was through a 1mm slit at one end of the box. Both light intensity and wavelength were regulated using neutral density and spectral filters respectively. The test chamber consisted of a rectangular perspex cuvette (measuring 15 x 5 x 5cm), which was divided into five equal sections along the longitudinal axis. The sections were separated by thin partitions constructed so that all could be moved vertically in unison. The effects of stray light were eliminated by enclosing the chamber in a black container which permitted entrance only of the stimulus light.

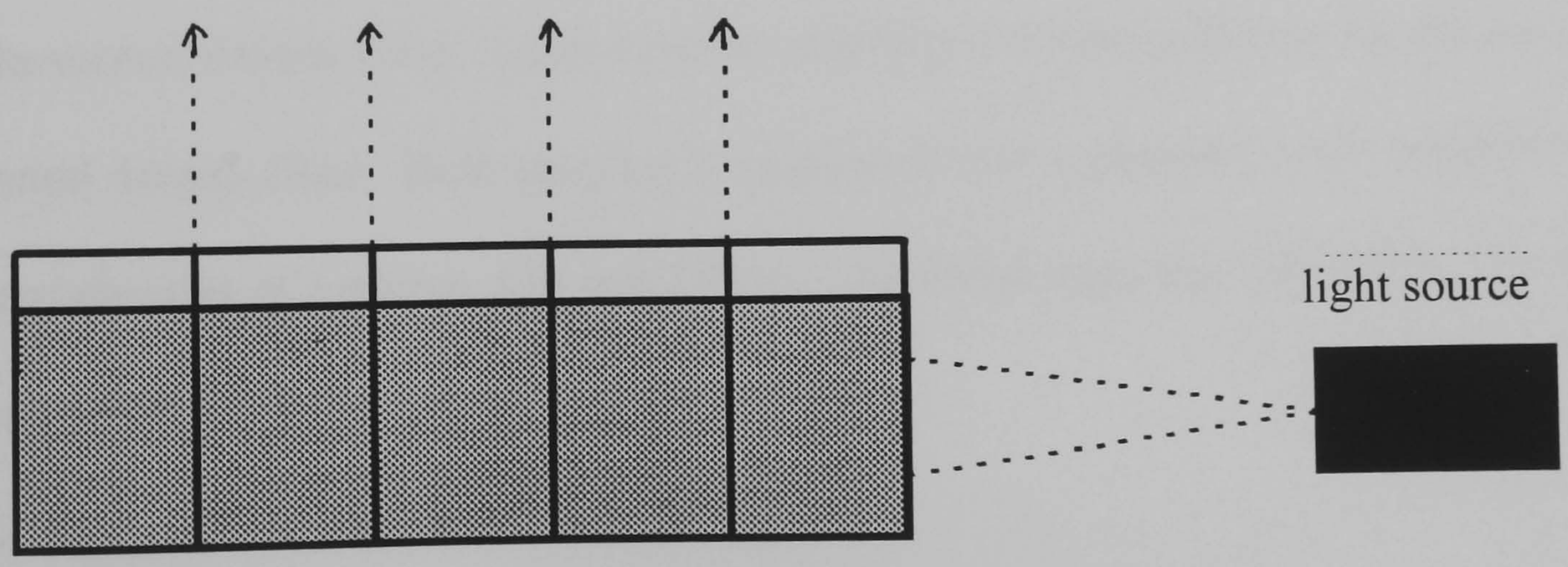
To determine phototactic responsiveness, groups of 50 *L. salmonis* nauplius I, nauplius II or copepodid stages were introduced into the middle section of the chamber, containing cooled water at 10°C, and dark adapted for 30 minutes. After this time, the partitions separating the sections were raised. The larvae were then stimulated with a horizontal light beam from the slide projector for 180 seconds and then the partitions lowered. The distribution of the larvae among the five compartments was then recorded, after which all the stages were removed, new cooled water was added to the chamber, and another group of larvae was tested. Control groups of both nauplius I,

Fig 5.1. Experimental apparatus used to test the photoresponses of *L. salmonis* nauplii and copepodids to a horizontal narrow beam of light.

(a) Test chamber



(b) apparatus setup



nauplius II and copepodid larvae were tested following the same procedure, except the larvae remained in the dark for the whole trial. Each trial was performed in triplicate.

i. Light intensity

Light intensity was regulated using a graded neutral density filter (Barr and Stroud CGN2) placed between the light source and the test chamber. Whilst testing the responses of larvae to differing light intensities, the wavelength of the light emitted from the projector was not filtered. All three free-swimming larval stages of *L. salmonis* were exposed to intensities between 805 and 3 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 face of the test chamber. Because it was not placed within the test chamber (due to its size and it not being waterproof), actual values of intensity can be expected to be lower than those recorded by the light meter due to attenuation of the light beam by water and perspex (Bron, 1994).

ii. Spectral wavelength

The equipment used for testing the response to different wavelengths of light was identical to that used for the light intensity experiments, except that a graded spectral filter (Barr and Stroud CS2, band width 8nm at 550nm) was placed in series with the neutral density filter. Light intensity was kept constant at 805nm by the use of the neutral density filter. Both nauplius I, nauplius II and copepodids were exposed to light wavelengths of between 439 and 700nm, calculated from the calibration data on the spectral filter.

5.2.1.2. Responses to simulated "natural" light

In the second method of measuring photoresponsiveness, the free-swimming larvae of *L. salmonis* were tested using equipment designed to produce an angular light distribution (ALD) similar to that occurring underwater during the day, based on the apparatus of Forward *et al.* (1984). A perspex chamber (measuring 5 x 5 x 15cm) was filled with cooled seawater at 10°C and positioned at the centre of a much larger perspex water bath (50 x 50 x 25cm) which had its walls and bottom painted matt black and contained cooled 10°C seawater. The test chamber was elevated using a support stand so that its surface was level with that of the water bath. The height of the chamber was marked every 3cm, "dividing" the chamber into five regions. The size of the water bath was such that its walls were outside of the critical angle (zenith ± 48.6°) as viewed from the bottom of the test chamber. The light source was as described previously for the experiments testing horizontal light intensities. The light source was aligned to enter the top of the bath, and the beam expanded to a size larger than the bath and reflected down by a mirror (fig 5.2.). As with the previous test, light intensity was regulated using the neutral density filter and intensities within the water bath measured. Either 10 nauplius I, nauplius II or copepodid stages were placed in the test chamber and dark adapted for 30 minutes. After this time they were stimulated for 180 seconds and, at the end of this time, their position in the water column in the test chamber was recorded by the use of the five marked regions along the height of the chamber. Control groups of nauplius I, nauplius II and copepodid larvae were tested following the same procedure, except that the larvae remained in the dark for the whole trial. Each test was performed in triplicate.

The photoresponses of the larvae to under an artificial light field and an angular light field were then compared to see if the behaviours differed in any way.

Fig 5.2. Experimental apparatus used to test the photoresponses of *L. salmonis* nauplii and copepodids to angular light distribution.

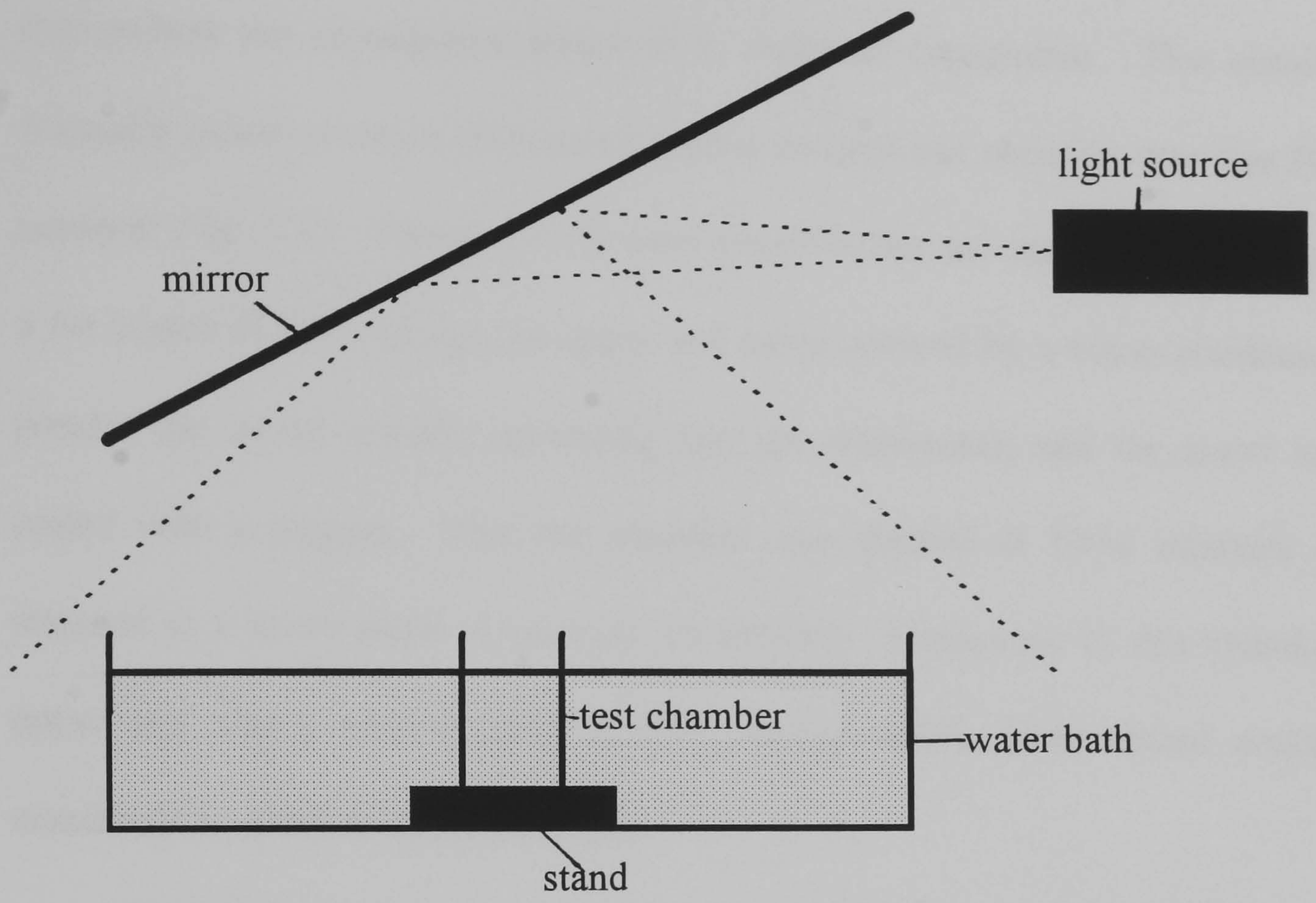
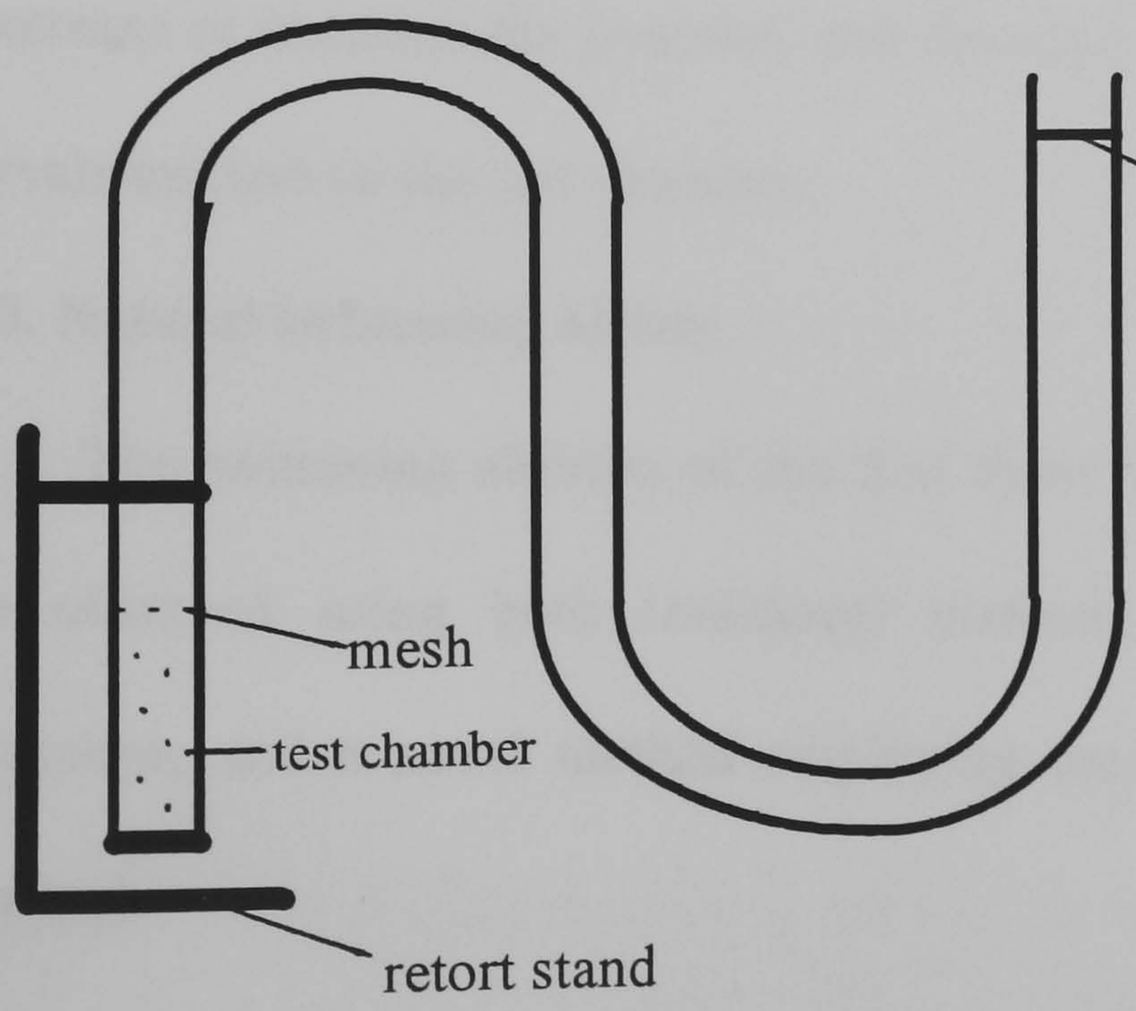


Fig 5.3. Manometer used to test responses of *L. salmonis* nauplii to increasing and decreasing pressure.



5.2.2. Behavioural responses to gravity

The response of the first two stages of *L. salmonis* to increasing and decreasing pressure was tested using the method laid out by Bron (1994) for that used to demonstrate the geonegative nature of *L. salmonis* copepodids. This consisted of a manually raised pressure manometer with a vertical test chamber that was filled with seawater (fig. 5.3.). Nauplii I or II were placed in the test chamber which consisted of a 1m length of PVC tubing, the upper end being covered by a 60µm plankton mesh to prevent the larvae actually ascending into the manometer, and the lower end being sealed with a stopper. This test chamber was marked at 10cm intervals and was attached to a retort stand to increase its stability. Connected to this chamber, at its upper end was a second piece of PVC tubing which, when raised could give a maximum 5m head of water in height.

Nauplii I and II stages were tested separately as it is impossible to identify the two stages once in the tubing. 100 of each stage were placed in the bottom test chamber with sea water and the plankton mesh sealed on to prevent escape. The manometer was then attached using a jubilee clip and parafilm to prevent leakage from the join between the two tubes. The manometer was then raised and lowered in order to increase or decrease the pressure, and the position of the nauplii recorded using the intervals marked on the test chamber.

5.2.3. Natural swimming ability

The swimming abilities of the first three free-swimming stages of *L. salmonis* were observed using both traditional methods, observations under a dissecting microscope, and a novel method employing the use of an Image Analysis System (Kontron).

5.2.3.1. Microscope observations

The naupliar and copepodid stages of the parasite were placed in a petri dish containing fresh twice filtered seawater that had been aerated prior to use. This petri dish was placed on another, upturned, petri dish to avoid excessive heat transfer from the light source of the microscope. The natural horizontal swimming movements of the three stages were recorded.

5.2.3.2. Image analysis observations

The vertical swimming movements of the free-swimming larval stages were also observed using an Image Analysis System. Larvae were placed in a narrow perspex chamber (5 x 1 x 10cm), in which the back "wall" had been painted matt black. On the front "face" of the chamber, a series of white dots had been painted to form a grid of 1 x 1cm. The chamber was illuminated with fibre optic lights and the movements of the larvae were recorded using a closed circuit video camera (JVC KYF30B) connected to the Image Analyser. Larvae were easily picked up by the camera due to their appearance as bright white "specks" (caused by the fibre optic lights) against the black background of the chamber. The velocity achieved by each of the stages caused by direct swimming movements and by passive sinking could then be calculated by controlling the number of frames recorded per second by including a "wait cycle" in the image analysis computer programme and subsequently overlaying their images, and by using the grid marked on the front of the chamber.

5.2.3.3. Buoyancy and lipid reserves

The results of Chapter 4 showed that older nauplii possess a decreased amount of lipid compared to their younger counterparts. Since the amount of lipid might affect buoyany, a simple experiment was carried out to determine if the amount of lipids

remaining affected their passive sinking rate. A range of ages of nauplii I and II stages were killed using 5% buffered formalin. Each individual larva was carefully placed into a 2000ml measuring cylinder filled with seawater and the time taken for it to reach the bottom recorded. The stage of the larva was then identified and its lipid reserves stained and measured with Sudan Black B using the method given in Chapter 4.

5.2.4. Behavioural responses to temperature

To test the behavioural responses of the free-swimming stages of *L. salmonis* to temperature, each stage was "reared" at 10°C, and then tested for variations in swimming behaviour at 5, 10 and 15°C. Larvae were reared at the same temperature in order to avoid differential lipid depletion as had been observed in the present study (Chapter 4). Egg strings were placed in 500ml glass beakers containing 400ml of twice filtered full strength seawater. Once hatching was observed to have commenced, the egg strings were removed from the system, so that all the larvae were of approximately the same age. Water was changed daily with fresh filtered seawater that had been aerated prior to use. Once the stage to be tested had been reached (nauplius I, nauplius II or copepodid), 60 individuals were placed in 3 petri dishes (20 in each) with full strength seawater, and placed in an incubator or a constant temperature room at 5, 10 or 15°C for at least 30 minutes.

After this acclimation period, an individual larva was removed from one of the petri dishes and placed in a small (3.5cm) petri dish, along with sufficient water at the test temperature (5, 10 or 15°C) on top of an upturned petri dish. The number of spontaneous swimming movements of the larva were then counted under a dissecting microscope at x40 magnification.

Because of the gradual rise in temperature of the water in the petri dish due to observations being carried out at room temperature, observation periods were restricted to a period of 120 seconds only.

5.2.5. Behavioural responses to salinity

To look at the effect of salinity upon the behaviour of the free swimming larval stages, the nauplius I, II and copepodids of *L. salmonis* were transferred from 500ml glass beakers containing 400ml of twice filtered full strength seawater that had been aerated prior to use, at 10°C by a pipette into a welled microscope slide at room temperature containing water of a lowered salinity.

5.2.5.1. Effects of concentration

The activity and behaviour of the larvae were then examined under x100 magnification under a compound microscope, and the time taken for swimming activity in all three stages and the characteristic gut movements in the copepodid stage to cease was recorded for a range (between 0 and 35 ppt) of salinities. After the observations were complete, the final test salinity in the well was recorded using a refractometer.

5.2.5.2. Exposure time and subsequent recovery

To see if recovery was possible after exposure to reduced salinities, both naupliar stages and the copepodid stage were individually exposed to a range of lowered salinities (between 0 and 35ppt) for either 120 or 240 seconds using the same methodology as described above. Larvae were then removed from the welled slide containing the test salinity by a pipette, and placed in a petri dish containing full strength seawater. The larvae were then placed with a small amount of this full strength seawater by a pipette into a second welled slide, and their activity once again observed under x100 magnification under a compound microscope.

5.2.6. Moulting times

Egg strings were hatched and the resulting *L. salmonis* nauplius I stages were placed in small petri dishes containing twice filtered full strength seawater that had been aerated prior to use. Ten stages were placed in each of the petri dishes which were kept at 10°C in a constant temperature room, the water being changed daily. Development was followed by observing the larvae within the petri dishes using a stero dissecting microscope at x40 magnification. Larvae were checked every hour between the hours of 0800 and 1800, and once at 2200 hours. Once individual nauplii were observed to have entered the moult to the second nauplius stage, they were isolated into individual marked small petri dishes, and the time taken for the moult to take place recorded by observing the larvae under an inverted compound microscope (Olympus IMT-2) at x 100 magnification until the second nauplius stage was observed to actively swim. The observations were repeated in the same manner for calculating the time taken to moult from the second nauplius stage to the infective copepodid stage. Twenty five nauplii I stages, and twenty five nauplii II stages were used. The characteristic used to determine whether moulting had commenced was the withdrawal of appendages away from the cuticle surrounding them.

5.3. Results

5.3.1. Photoresponses to artificial light

5.3.1.1. Light intensity

The distribution of the naupliar and copepodid stages of *L. salmonis* between the five compartments of the test chamber when kept in the dark (control) is given in table 5.1. No significant variations were observed between the numbers of each of the three stages recorded from each compartment (ANOVA, $p < 0.001$), demonstrating that random swimming both horizontally and vertically must occur whilst in the dark. If there was no movement in the absence of light or merely vertical upward swimming followed by passive sinking ("hop and sink"), all stages in the control experiment would have been recovered from the central compartment.

For this reason, the threshold figure chosen for a positive photoresponse occurring was 40%. Due to the random distribution of all three larval stages between the five compartments of the test chamber in the dark, it can be assumed that, on average, 20% of each stage will be found in each compartment during control experiments. A Chi-squared contingency test on the numbers found in each compartment in the control experiments demonstrated that this was an acceptable hypothesis ($p < 0.005$). Therefore, for a positive photoresponse to be recorded, over 40% of the total numbers of larvae must be recorded from the two compartments nearest the light source.

i. Nauplius I stage

A positive photoresponse was only observed in *L. salmonis* nauplii I at intensities of over 200 lux, as can be seen from fig 5.4. Analysis of variance revealed that there was a significant difference in the percentage of nauplius I stages exhibiting a

Table 5.1. Mean percentage (± 1 S.D.) of *L. salmonis* stages present in the five compartments (artifical lighting experiment controls) (3 trials for each stage, n=50 for each trial).

Compartment	Nauplius I	Nauplius II	Copepodid
1	18.7 ± 1.53	18.7 ± 0.58	20.0 ± 1.00
2	22.6 ± 0.58	21.3 ± 0.82	21.3 ± 0.58
3	18.7 ± 1.15	20.0 ± 1.73	18.7 ± 0.58
4	18.7 ± 0.58	18.7 ± 1.15	18.7 ± 1.15
5	21.3 ± 0.58	21.3 ± 1.53	21.3 ± 1.53

Table 5.2. Mean percentage (± 1 S.D.) of *L. salmonis* stages present in the upper portion of the test chamber (ALD experiment controls) (3 trials for each stage, n=10 for each trial).

Stage	Nauplius I	Nauplius II	Copepodid
mean ± s.d.	53.33 ± 0.58	50.00 ± 1.00	53.33 ± 1.15

Table 5.3. Effect of lowered salinity upon the frequency of *L. salmonis* copepodid gut movements.

Salinity (‰)	Mean number (± 1 S.D) of peristaltic movements per minute
240 second exposure to test salinity	
0	0 ± 0.00
4	6 ± 2.34
10	13 ± 5.36
15	34 ± 3.78
21	47 ± 12.80
23	65 ± 10.45
32	59 ± 6.20
35	73 ± 14.89

Table 5.4. Time for natural recovery of *L. salmonis* copepodids after exposure to lowered salinity.

Salinity (‰)	Time for cessation of swimming (seconds)	Time for swimming to recommence (seconds)
0	30	never
4	60	never
10	120	never
14	120	never
21	240	300
23	240	210
30	did not stop swimming	-

Fig. 5.4. Photoresponse of *L. salmonis* nauplius I stage to varying light intensity: below dashed line indicates no directed response.

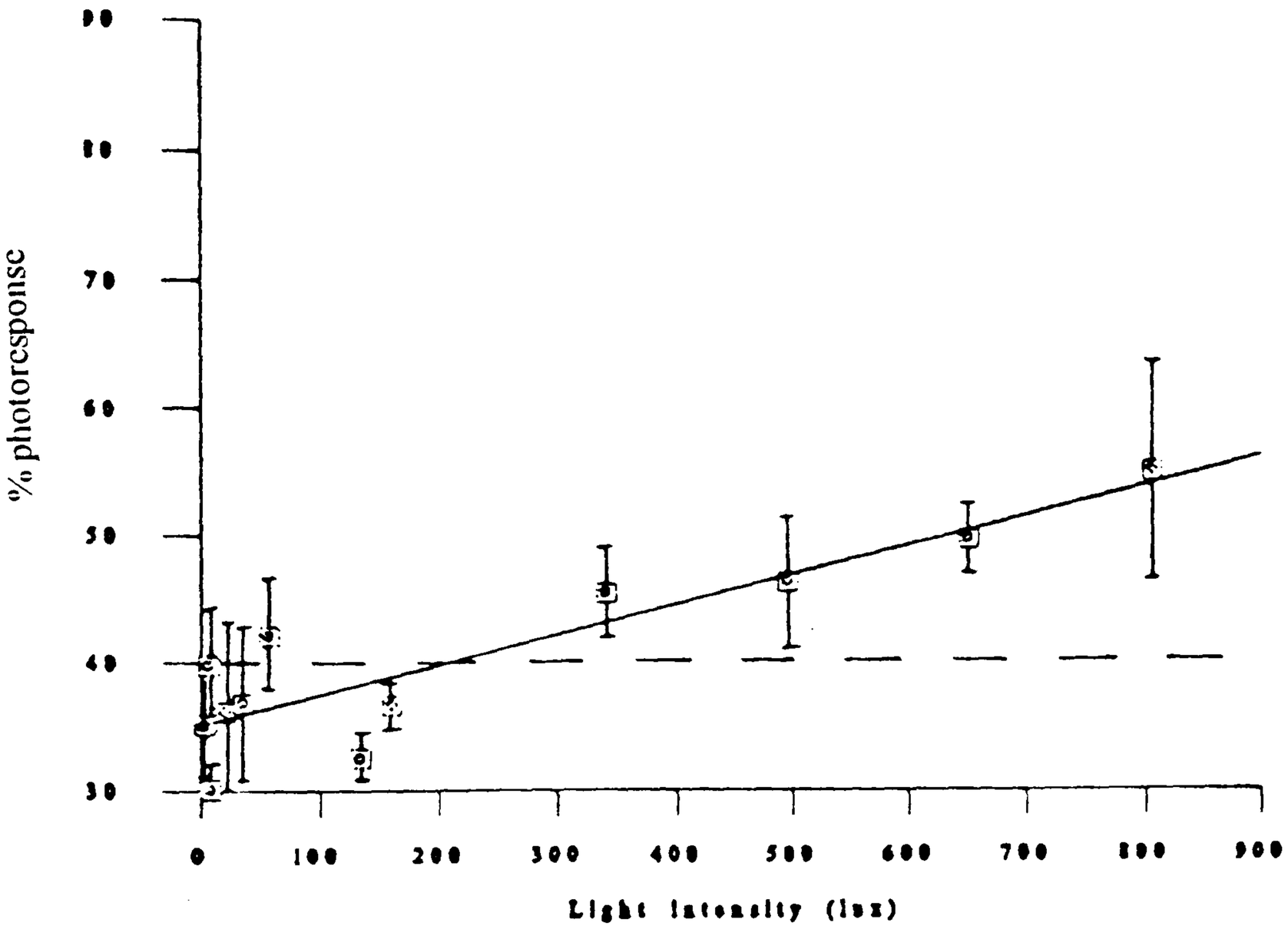
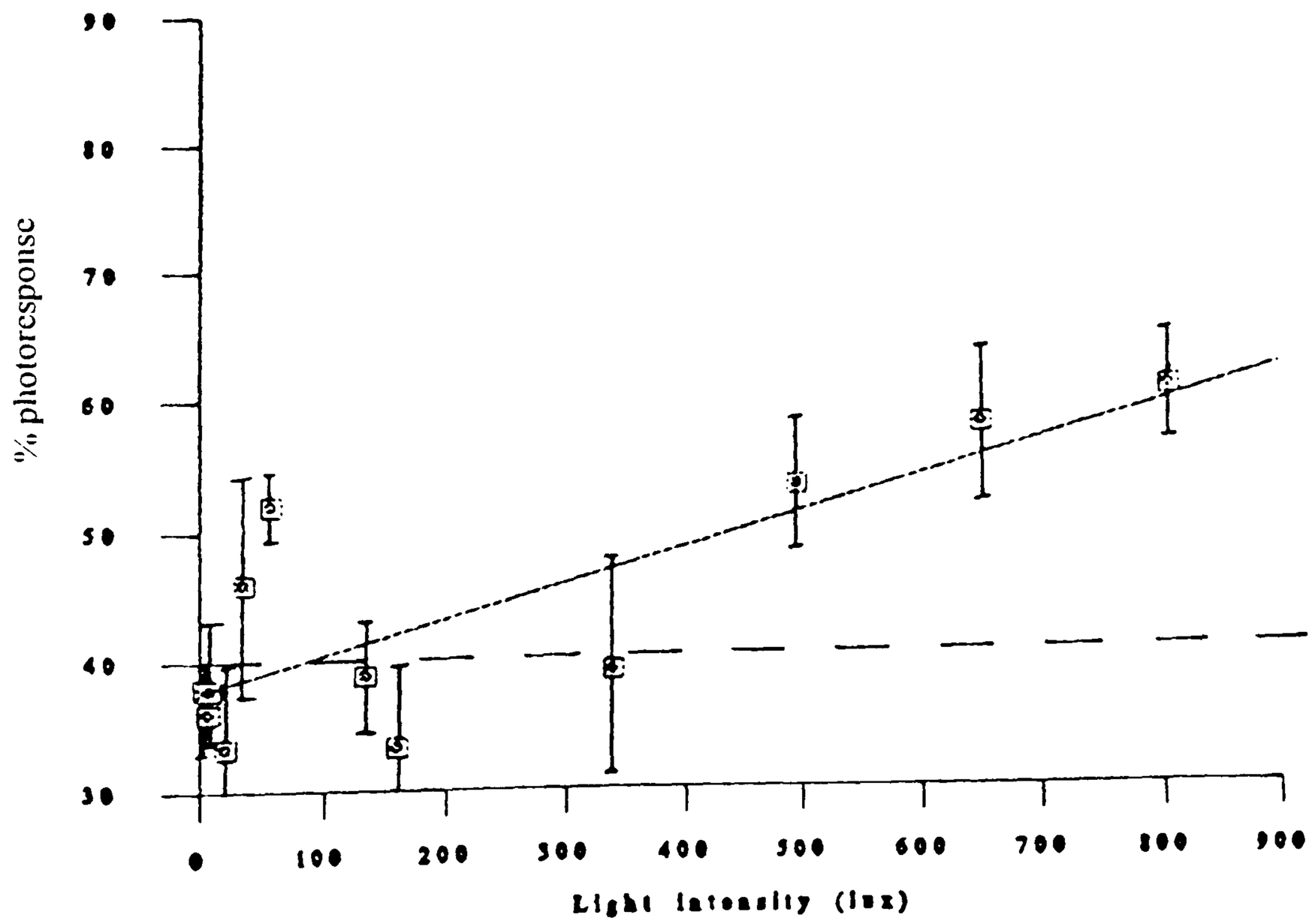


Fig 5.5. Photoresponse of *L. salmonis* nauplius II stage to varying light intensity: below dashed line indicates no directed response.



positive photoresponse at different intensities, and a correlation was found to exist, which is shown graphically in fig 5.4, between the percentage of larvae demonstrating such a response and increasing intensity ($r^2 = 0.794$, $p < 0.001$). This indicates that the photoresponse of *L. salmonis* nauplius I stages is greater at higher intensities, and there is no response below 200 lux.

ii. Nauplius II stage

The photoresponse of *L. salmonis* nauplius II stages is shown in fig 5.5., and it can be seen that a positive photoresponse is first observed to occur at intensities greater than 85 lux. This appears to indicate that this stage either has a stronger response or is more sensitive to light than the previous nauplius I stage. However, at some intensities above 85 lux, and up to 340 lux, a positive photoresponse was not seen. Significant variations were observed to exist however, between the numbers of larvae recorded from each compartment of the test chamber at different intensities (ANOVA, $p < 0.005$) and a relationship was found to exist between numbers demonstrating a positive photoresponse and increasing intensity ($r^2 = 0.620$, $p < 0.005$). This is shown graphically in fig. 5.5.

iii. Copepodid stage

The copepodid stage of *L. salmonis* was seen to demonstrate a positive photoresponse at all light intensities tested (fig. 5.6.), even down to levels as low as 3 lux. It would therefore appear to be more sensitive to light than the previous naupliar stages. Analysis of variance, however, showed that there did not exist a significant variation ($p = 0.06$) in the numbers exhibiting this photoresponse over the intensity range tested (3 to 850 lux), suggesting that there does not exist a relationship between

Fig 5.6. Photoresponse of *L. salmonis* copepodids to varying light intensity: below dashed line indicates no directed response.

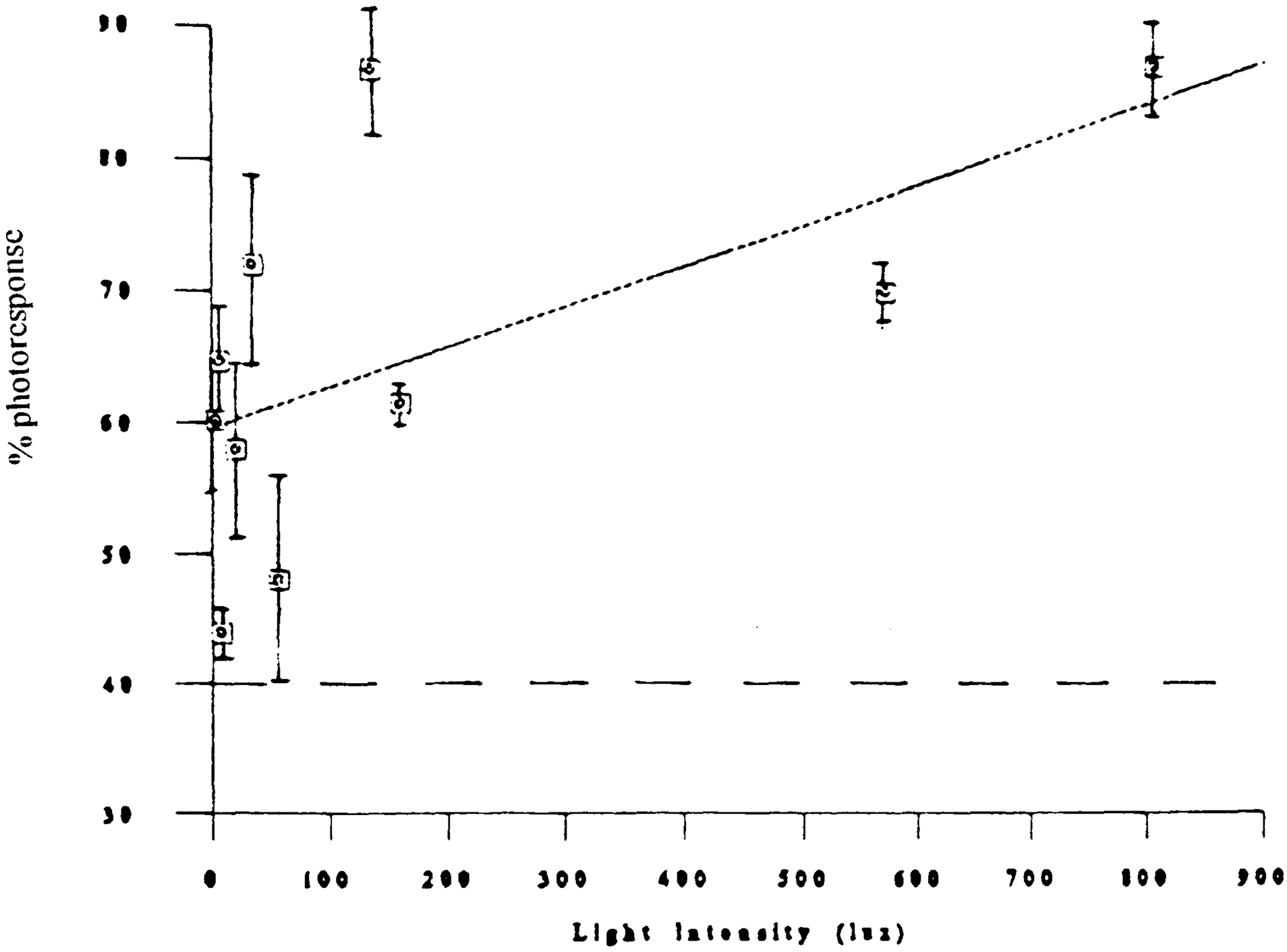
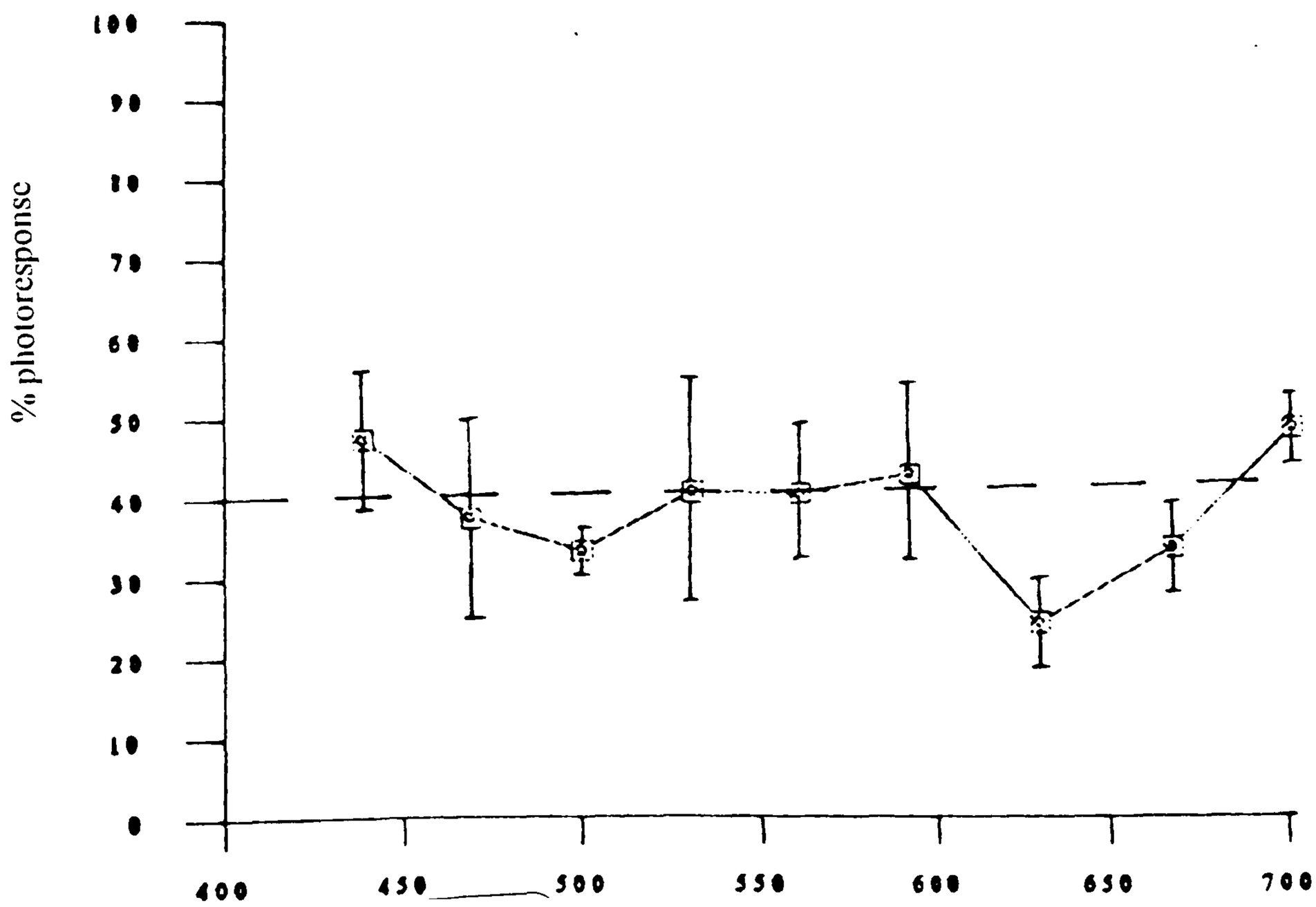


Fig 5.7. Photoresponse of *L. salmonis* nauplius I stage to varying wavelengths: below dashed line indicates no directed response.



increasing intensity and increasing response. Indeed, a plotted regression confirmed this ($r^2 = 0.366$, $p = 0.064$) and is shown graphically in fig 5.6.

5.3.1.2. Wavelength

No significant peaks of phototaxis were seen at any of the wavelengths tested for the first nauplius stage of *L. salmonis* (fig 5.7.). However in contrast, the second nauplius stage was observed to exhibit a significant positive phototaxis at a wavelength of 500nm (Dunn's test, $p < 0.05$), as can be seen in fig. 5.8. The photoresponse of the infective copepodid stage to light of different wavelengths is shown in fig. 5.9., and it can easily be seen that this stage demonstrates two peaks of positive phototaxis at 500 and 561nm, with both of these peaks being significantly different from the response of the infective stage to the other wavelengths tested (Dunn's test, $p < 0.05$).

5.3.2. Photoresponses to simulated "natural" light

The aim of the angular light distribution experiments was to observe if the positive photoresponses of the first three stages of *L. salmonis* seen under an artificial light regime were reversed under a natural light simulation. Due to the differences in the experimental apparatus (i.e. no compartmentalised chamber for recording final numbers), it is not possible to directly compare results. Instead, the numbers of larvae present in the top section of the marked test chamber in the ALD chamber were recorded at all intensities tested and during control experiments. If a photonegative response is demonstrated, then it can be expected that there will be fewer larvae present in the top section of the test chamber than during the control experiments when larvae were not exposed to light during the 180 second test period.

Fig 5.8. Photoresponse of *L. salmonis* nauplius II stage to varying wavelengths: below dashed line indicates no directed response.

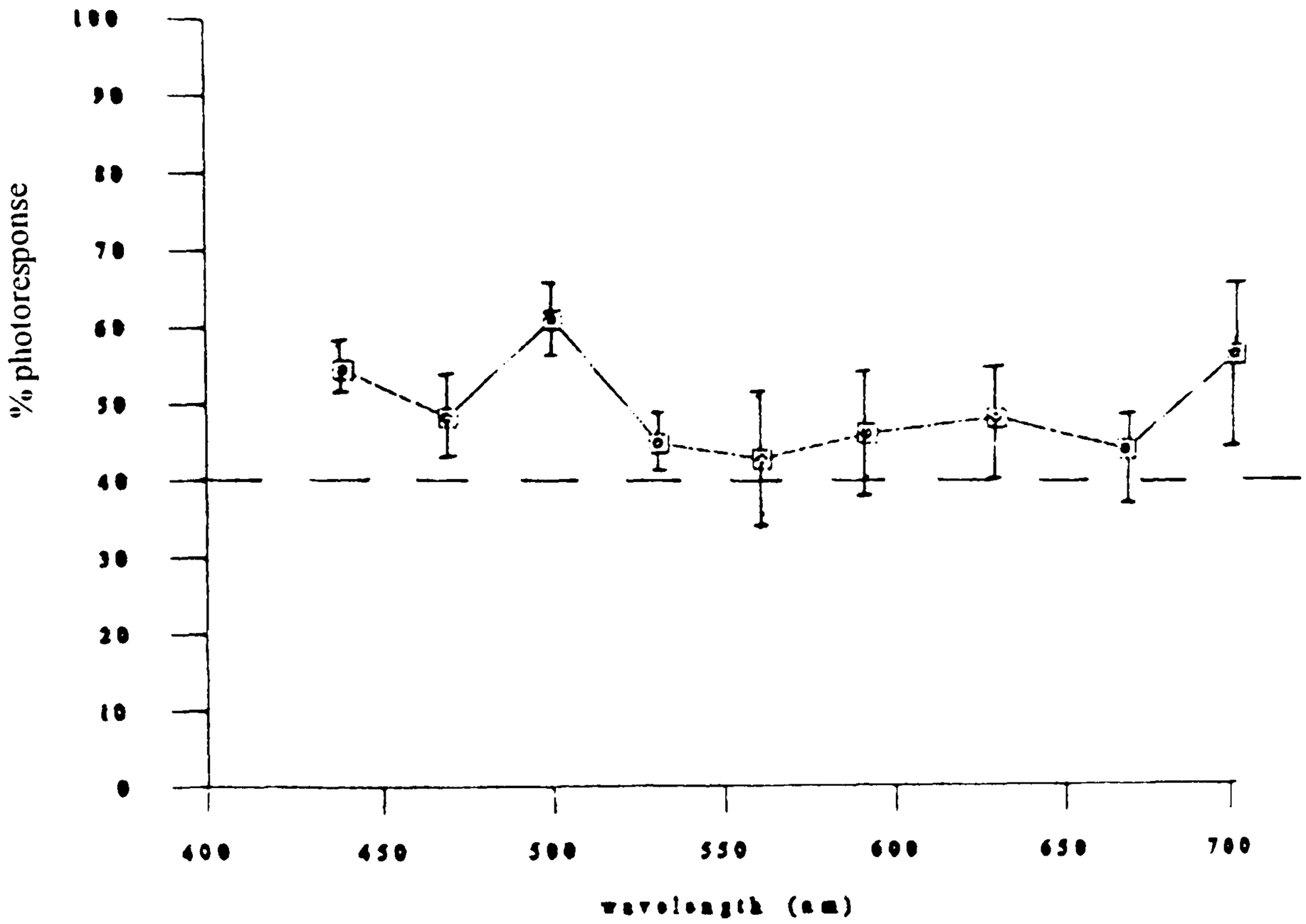
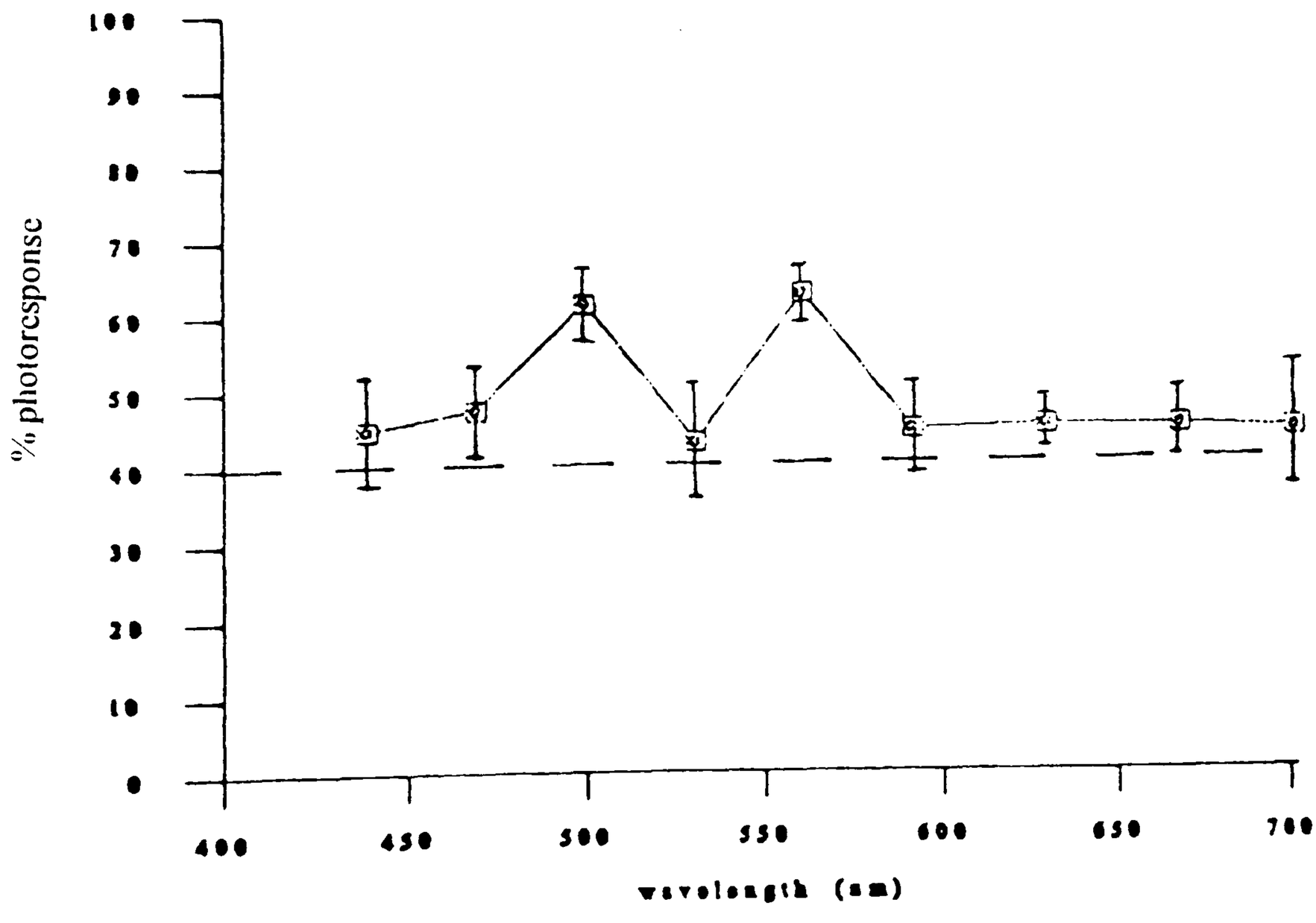


Fig 5.9. Photoresponse of *L. salmonis* copepodids to varying wavelengths: below dashed line indicates no directed response.



i. Control experiments

Table 5.2. gives the percentage of *L. salmonis* nauplius I, nauplius II and copepodid stages present in the top portion of the test chamber. As can be seen, the proportions present remained constant for the three different stages.

ii. Nauplius I stage

The percentage of nauplius I stages present in the top section of the test chamber at different light intensities is given in fig. 5.10. As can be seen from the graph, at intensities of greater than 85 lux, the proportions of larvae present in the upper chamber were higher than the controls. However a Mann-Whitney test performed on each intensity demonstrated that this photopositive response was only significant at intensities of 134 lux or greater ($p<0.01$ for all intensities above and including 160 lux) with the exception of 340 lux, where there was no significant difference between the controls and the experimental sample ($p=0.08$).

iii. Nauplius II stage

Like the nauplius I stage, nauplius II larvae also did not appear to demonstrate a negative phototaxis under an angular light distribution. Again at intensities of over 85 lux, the proportions of larvae present in the upper chamber were higher than the controls (fig 5.11). A Mann Whitney test performed on each intensity demonstrated that this photopositive response was significant at intensities of over 134 lux ($p<0.01$ for all intensities above and including 134 lux) with the exception of 490 lux where there was no significant difference between the experimental and control samples ($p=0.06$).

iv. Copepodid stage

Fig 5.10. Photoresponse of *L. salmonis* nauplius I stage to an angular light distribution: below dashed line indicates no directed response.

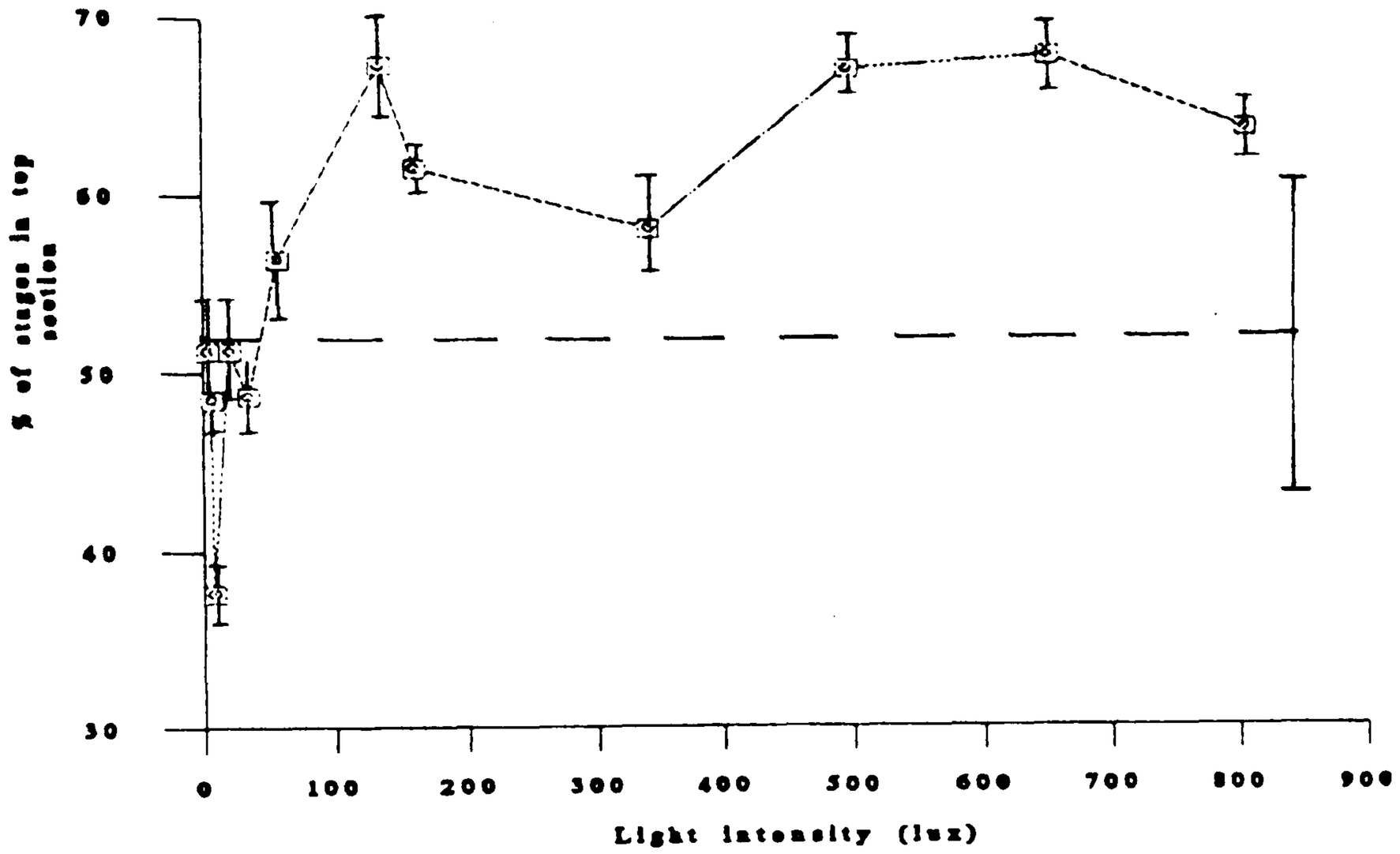
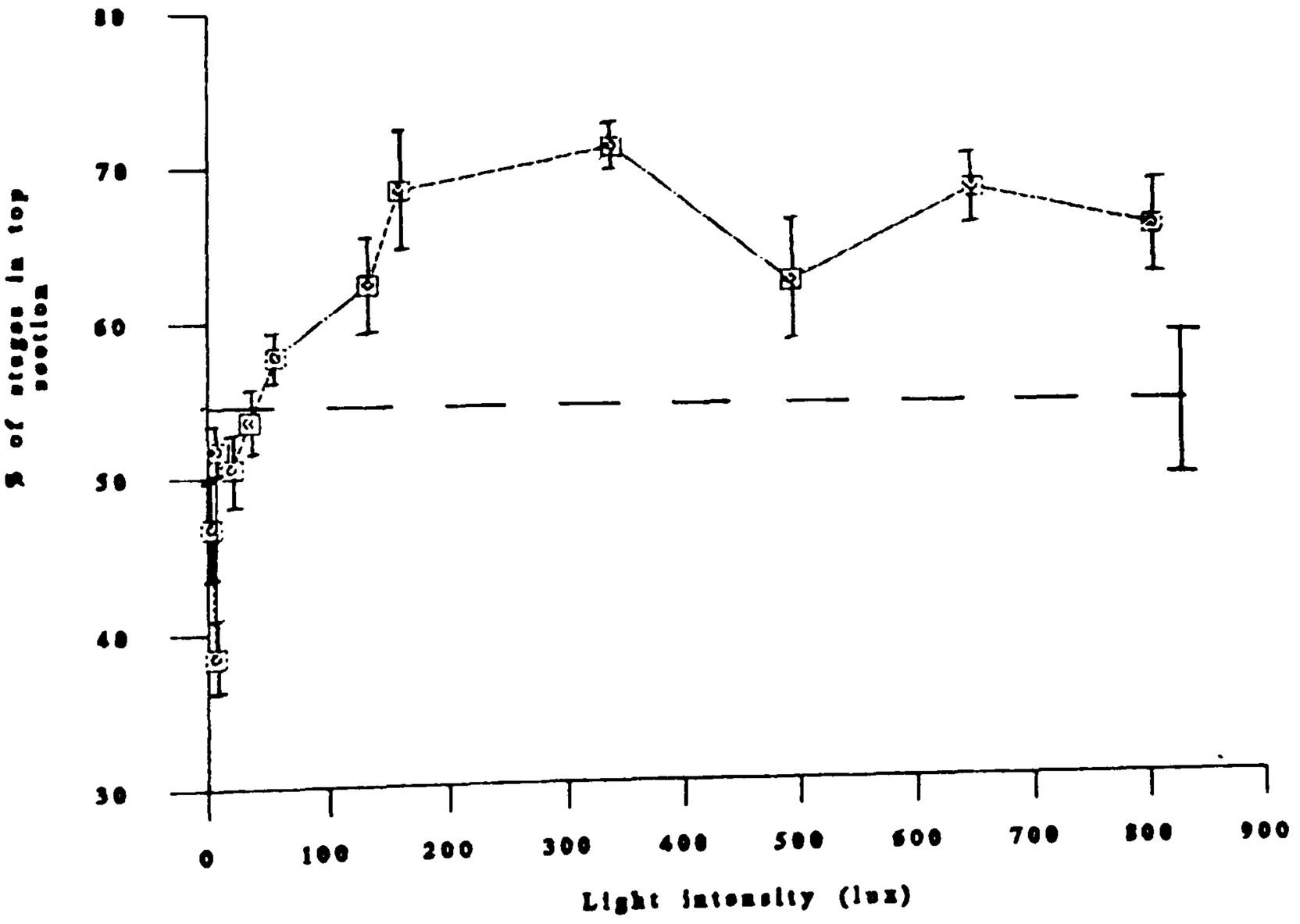


Fig. 5.11. Photoresponse of *L. salmonis* nauplius II stage to an angular light distribution: below dashed line indicates no directed response.



The proportion of copepodids present in the top section of the test chamber was observed to be higher at all intensities tested when compared to the control (fig. 5.12.). However, a Mann Whitney test demonstrated that this photopositive response was only significant at intensities above and including 134 lux ($p<0.01$ for all intensities).

5.3.3. Behavioural responses to gravity

Observations on the responses of the naupliar stages were taken by watching the direction and speed of the stages against a white background. This allowed the larvae to be seen as black "specks" moving up and down the column of water. Both nauplii I and II were observed to swim upwards in response to increasing pressure generated by the manometer. Movement appeared to be by directed upward swimming with interspersed periods of passive sinking. The distance gained during the directed swimming appeared to exceed that "lost" when the nauplii descended during their passive sinking periods. This directed swimming appeared to be most pronounced when the pressure was at its greatest level but again there were always periods of passive sinking. However nauplii did appear to spend more time actually swimming compared to those under a lower pressure (i.e. 1m head of water). When pressure was decreased, nauplii continued to "sink and swim," though the period of time spent actively swimming seemed to be reduced.

5.3.4. Swimming patterns

5.3.4.1. Microscope observations

The swimming patterns of both the naupliar and copepodid stages of *L. salmonis* were observed to consist of two parts, causing a characteristic "hop and rest" motion to be seen. All three stages were observed to either sink passively when present in a container with sufficient water to allow suspension in the water column or remain

Fig. 5.12. Photoresponse of *L. salmonis* copepodids to an angular light distribution: below dashed line indicates no directed response.

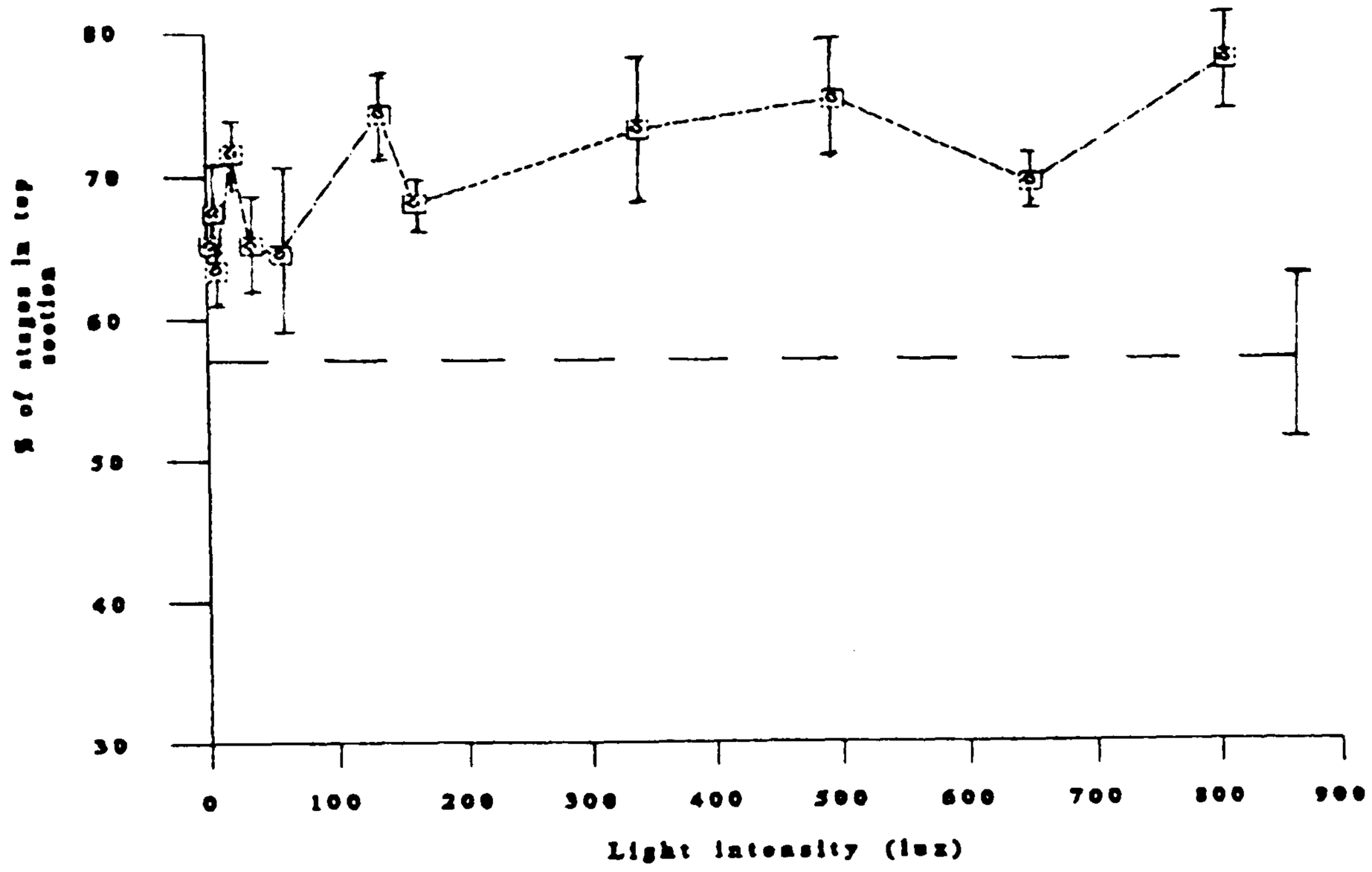
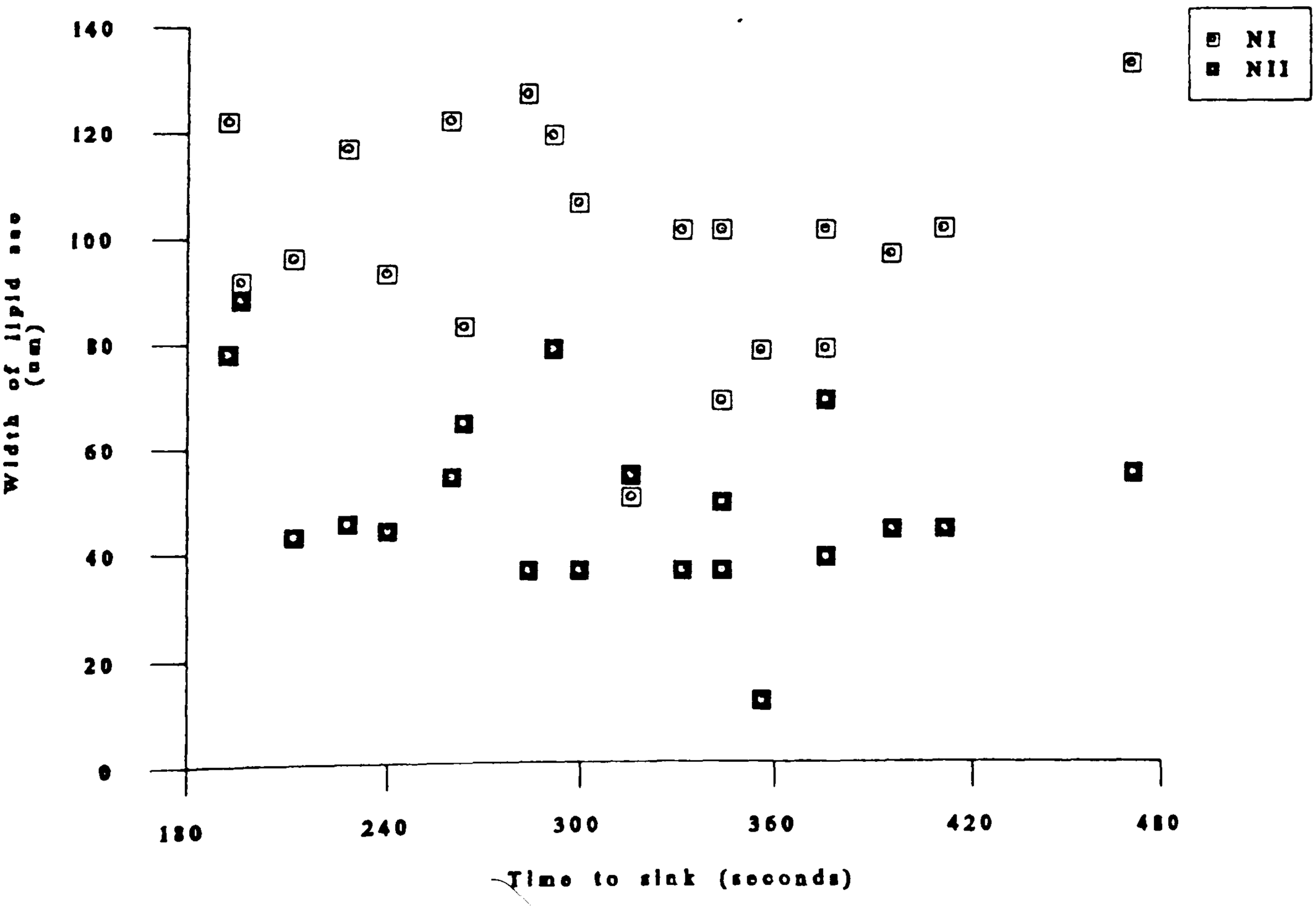


Fig. 5.13. Relationship between *L. salmonis* naupliar lipid reserves and passive sinking rates.



resting on the bottom of a petri-dish containing a shallow amount of water. During any passive sinking, the nauplii remained upright, whilst the copepodids were observed to become inverted and sink anterior end first. After a period of sinking or equivalent resting, upward or horizontal movement (in the case of larvae observed in petri-dishes) movement occurred in the form of a "hop" which was either followed again by a period of passive sinking or another "hop." The period spent actively moving during a "hop" was always less than that spent passively sinking in the water column or resting in a petri-dish. This enabled a net advance forward. Although this pattern was the same for both the naupliar stages and the copepodids, it was observed that copepodids generally exhibited a greater frequency of "hops" than the earlier stages.

5.3.4.2. Image analysis observations

Both *L. salmonis* nauplii and copepodids were recorded by video for their vertical swimming movements. Again, the characteristic swimming movements of all three stages were observed. From the video observations it was noted that the first antennae of the naupliar stages were held completely upright above the anterior region during passive sinking, whilst the copepodids were observed to turn through 180 degrees to sink "head" first. Using the number of frames per second and the marked grid on the test chamber, it was possible to calculate both the passive sinking speeds and the directed swimming speeds of the nauplii and the copepodids.

i. Velocity

No significant differences were observed between the swimming speeds of the first and second naupliar stages (paired t-test, $p=1.00$) and so they were not treated separately. The mean sinking speed of the naupliar stages ($n=60$) was calculated to be 0.09cm ($\pm$ 0.01) per second or 5.43cm per minute, whilst the mean speed of each short

upward hop was 1.25cm (± 0.16) per second, with this being equivalent to 75.00cm per minute. The copepodid stage (n=30) was found to sink at a rate of 0.10cm (± 0.03) per second or 5.94cm per minute, whilst the velocity attained during a spontaneous "hop" was calculated to be 2.14cm (± 0.24) per second or 128.24cm per minute. If the copepodids were mechanically stimulated during observations by tapping the top of the test chamber, the active swimming movements exhibited were observed to be of a greater magnitude. The mean velocity (n=30) attained during these stimulated swimming motions was observed to be 6.48cm (± 2.56) per second or 410.55cm per minute, approximately 3.2 times the speed of an unstimulated copepodid. The maximum speed recorded was 10.23cm per second, this being equivalent to 614.2cm per minute.

ii. Duration

It was observed during microscope observations and from the results of Chapter 4 that both nauplii and copepodids spend a greater proportion of time passively sinking compared to active swimming. By varying the wait cycle of the image analyser, it was possible to dictate the number of frames taken per second, and thus the amount of time spent both actively swimming and sinking could be estimated. However, it must be remembered that all recordings took place at room temperature, with the chamber also being illuminated by strong light. Thus, the actual duration may be invalid for transposing to the natural environment, due to temperature differences, but it is the proportion of time spent either swimming or sinking that is being investigated.

It was estimated that the time taken for each individual "hop" by the naupliar stages (n=30) was a maximum of 0.04 seconds. This was recorded with no wait cycle inserted, with the camera recording 25 frames per second, its maximum capability.

Unfortunately, as the camera could not record frames any faster, it was impossible to estimate the minimum and mean duration's. The mean time spent passively sinking between two active "hops" by the naupliar stages was calculated to be 0.45 seconds (n=30), though this was highly variable (S.D.± 0.50), ranging from 0.20 seconds to 1.16 seconds. By multiplying the mean velocity with the mean duration, it can be estimated that the distance travelled during each "hop" would be 0.05cm, whilst that covered during each individual sinking motion is 0.04cm, thus allowing a net upward movement of *L. salmonis* nauplii in the water column of 0.01cm.

The duration of each hop for *L. salmonis* copepodids (n=30) was found to be greater than that of the naupliar stages, 0.67 seconds (± 0.33) compared to 0.04 seconds. However, the duration of passive sinking between hops was found to be similar, 0.47 seconds compared to 0.45 seconds for copepodids and nauplii respectively, though again this was found to be highly variable (S.D. ± 0.29). Again, a net upward movement of 1.38cm was calculated to occur, as the distance travelled downwards will be less than that achieved during a hop, 0.05cm compared to 1.43cm.

5.3.4.3. Buoyancy and lipid reserves

The relationship between sinking time and lipid reserves from 40 nauplii (NI, n=20; nauplius II n=20) can be seen in fig 5.13. Analysis of variance revealed that there was no significant difference between the time taken to descend the water column (30cm in distance) and the width of the lipid reserves in *L. salmonis* naupliar stages (p = 0.77 for nauplius I, 0.12 for nauplius II). Confirmation that nauplii with less lipid do not sink faster than those with greater supplies was given by the fact that a fitted regression curve was also not significant ($r^2 = 0.001$, $p = 0.77$ for nauplius I, $r^2 = 0.130$, $p = 0.12$).

5.3.5. Behavioural responses to temperature

5.3.5.1. Laboratory observations

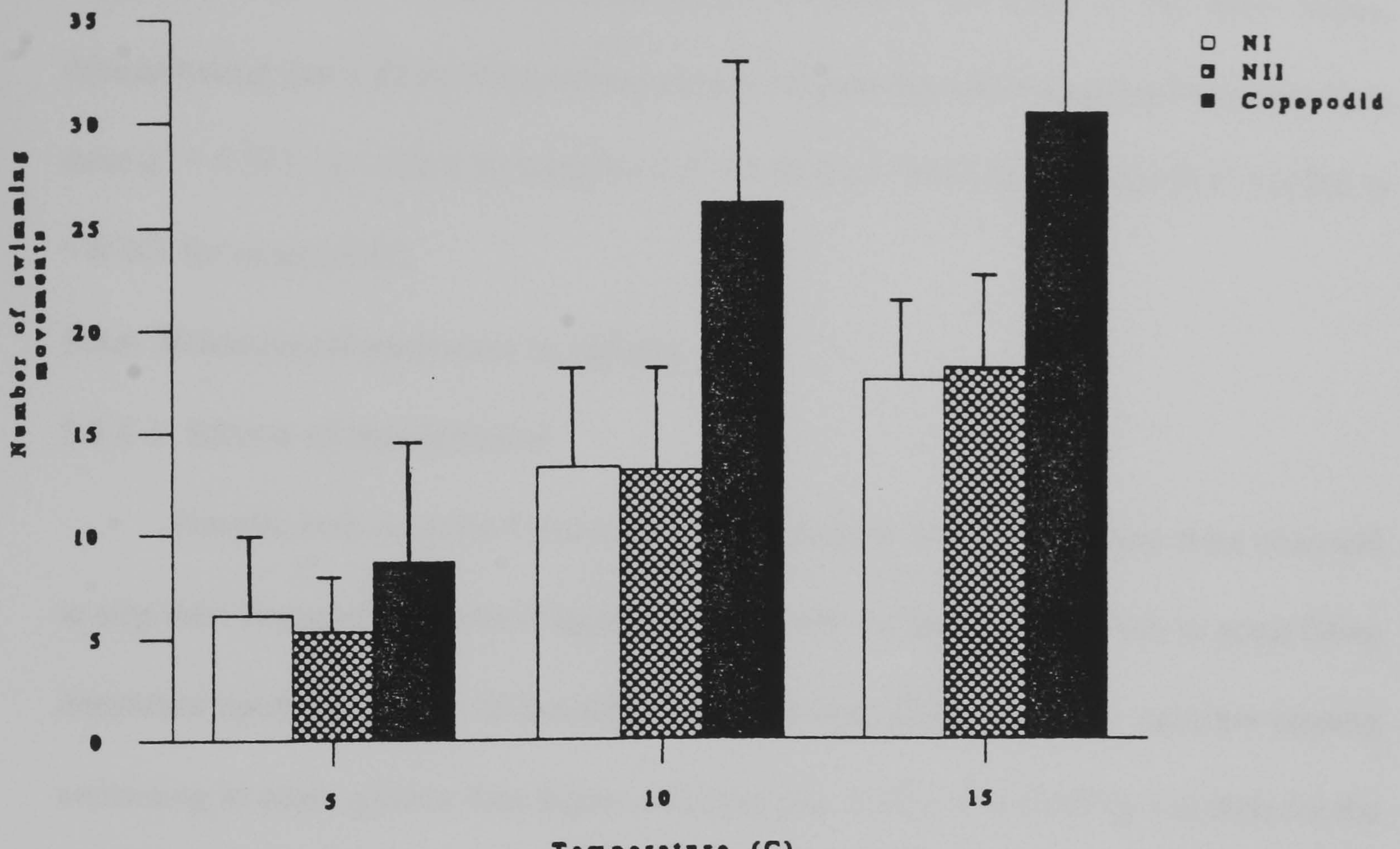
Both nauplius I, nauplius II and copepodid stages of *L. salmonis* were observed to be less active when kept at low temperatures. At 5°C, larvae kept in glass beakers were not observed to accumulate at the upper surface layer of water in the beaker, in contrast to those kept at 10 and 15°C. Instead, they showed reduced swimming movements, which caused them to tend to aggregate at the bottom of containers, occasionally showing short bursts of activity. This would elevate them a little in the water column, but observations with a microscope showed that this short period of activity was then followed by a longer passive period, where the larvae sunk back down. In 10 and 15°C, larvae of all stages were observed to spend more time actively swimming, than passively sinking.

5.3.5.2. Quantitative analysis

The mean number of spontaneous swimming movements demonstrated by *L. salmonis* nauplius *I*, nauplius II and copepodid larvae at different temperatures over 120 seconds are shown in figs. 5.14. No significant differences were observed between the two naupliar stages (unpaired t-test, $p = 0.89$) and so they were pooled for comparison against the copepodid stage. As can be seen graphically, the copepodid stage is much more active than the first two naupliar stages at 10 and 15°C, exhibiting a greater number of swimming movements or "hops" (unpaired t-test, $p < 0.001$) when compared to these earlier stages at these temperatures. However, at 5°C, there was no significant difference between the number of swimming movements between the naupliar stages and the copepodid stage (unpaired t-test, $p = 0.13$).

Analysis of variance demonstrated that there was a significant difference in the number of spontaneous swimming movements for each of the three stages at different

Fig. 5.14. Relationship between the frequency of swimming movements of *L. salmonis* naupliar and copepodid stages and temperature.



temperatures ($p < 0.001$ for nauplius I, $p < 0.001$ for nauplius II, $p < 0.001$ for copepodids), indicating that temperature does have an effect upon the duration of active swimming by the free-swimming larval stages. This was confirmed by the fitting of correlation's between temperature and the number of swimming movements for each of the three stages, demonstrating that a direct relationship between temperature and swimming frequency does exist ($r^2 = 0.527$, $p < 0.001$ for nauplius I, $r^2 = 0.561$, $p < 0.001$ for nauplius II, $r^2 = 0.566$, $p < 0.001$ for copepodids).

5.3.6. Behavioural responses to salinity

5.3.6.1. Effects of concentration

Nauplii, both nauplius I and nauplius II, placed in low salinity water were observed to stop their characteristic swimming movements, with the time taken for this to occur being dependent upon the salinity of the water they had been placed in; lower salinities causing swimming to cease quicker than higher salinities (fig. 5.15.), $r^2 = 0.697$ ($p < 0.005$) for the nauplius I stage and $r^2 = 0.748$ ($p < 0.001$) for the nauplius II stage. The cessation of swimming was observed to be due to the appendages becoming dysfunctional, and no longer being held in their normal position. The first antennae of the naupliar stages, after being exposed to low salinity water appear to no longer be able to be held outstretched in front of the nauplius at the anterior end, but instead are seen to "fall" to the side of the body, being held perpendicular to the body. The second antennae and the mandibles also do not remain in their normal outstretched position, but again "fall" with these pairs lying by the side of the body.

The copepodid stage was also observed to lose swimming ability when placed into low salinity surroundings, with the time taken for this to occur again decreasing with decreasing salinity (fig. 5.16), $r^2 = 0.981$ ($p < 0.001$). However, as well as this function

Fig. 5.15. Effect of lowered salinity upon the swimming ability of *L. salmonis* nauplius I and nauplius II.

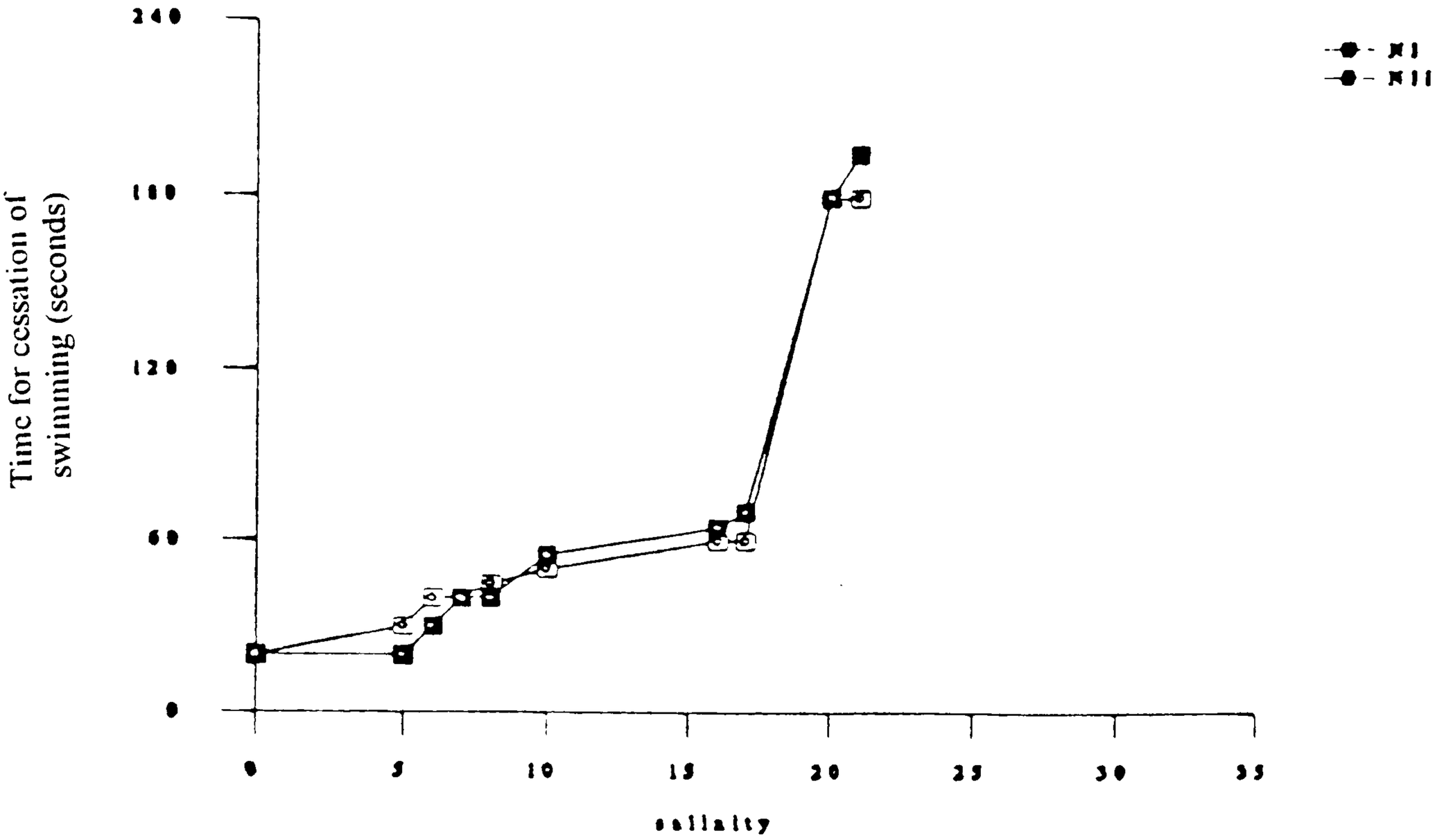
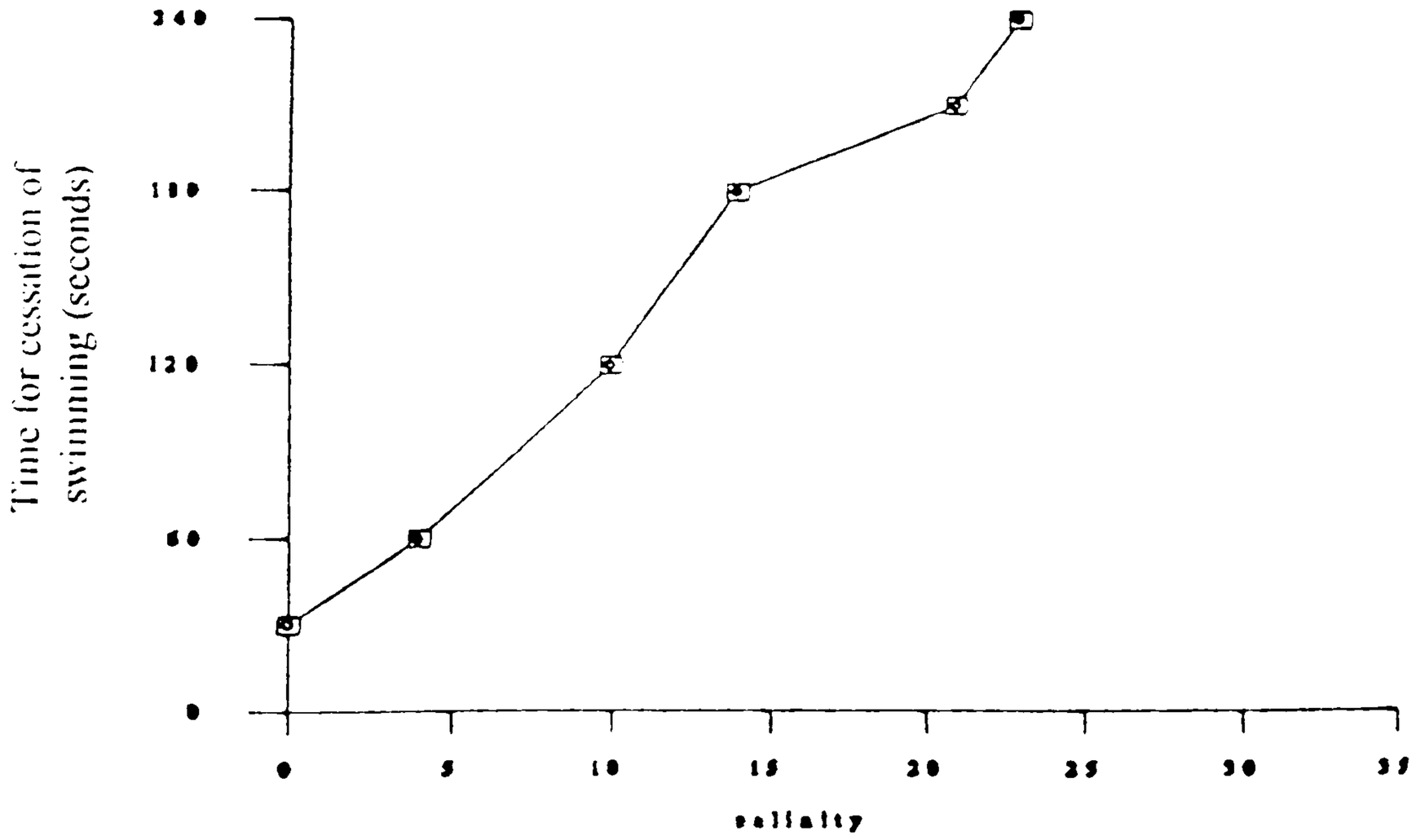


Fig. 5.16. Effect of lowered salinity upon the swimming ability of *L. salmonis* copepodids.



being compromised in the copepodid by low salinities, the peristaltic activity of the gut of the copepodid was also seen to be affected. The regular peristaltic movements of the gut were observed to reduce in frequency when exposed to a decrease in salinity. The magnitude of reduction was dependent upon the salinity, with lower salinities causing a much more reduced movement (table 5.3.). Some natural recovery however, was seen to occur. In salinities of 21‰ or greater, although swimming ability and gut movements were initially compromised by the drop in salinity, after a period of time, the activity of the copepodid was seen to increase. Although the activity levels of the appendages (and the gut) were not observed to return to their original levels, they did reach such a level that allowed active swimming, and suspension in the water column when placed in 400ml of the test water in a 500ml glass beaker was seen to occur.

5.3.6.2. Ability to recover

Both nauplius I and nauplius II stages were observed to stop all swimming activity in salinities of less than or equal to 25‰, in both 60 and 120 second exposures. Copepodids did not appear as sensitive to environmental change as the naupliar stages, firstly taking longer to lose their ability to swim in very low salinities, and secondly, not losing their ability at all in some medium salinities (table 5.4.).

Both the naupliar stages behaved in a similar manner, with recovery occurring in approximately the same time when placed in the same strength seawater (table 5.5.). The time to recover was dependent upon the salinity the nauplii were exposed to and the duration of exposure. The pattern of recovery was also the same in both the naupliar stages. The first indication of recovery was the return of the ability of the appendages to move, twitching being observed. At this point the appendages were still lying by the side of the body. This twitching was then followed by a return of the appendages to their normal

Table 5.5. Time for recovery to occur in *L. salmonis* nauplii exposed to low salinity water and then transferred to full strength seawater (time recorded in seconds).

Salinity (‰)	Movement of appendages seen	Appendage position regained	Response to stimulation	Suspended in water column
120 second exposure to test salinity				
0	180	600	840	900
5	180	600	720	900
10	120	240	360	480
15	120	180	240	300
20	60	240	240	300
25	0	120	180	240
240 second exposure to test salinity				
0	never	never	never	never
5	300	600	960	1260
10	120	360	480	600
15	60	300	360	480
20	60	120	180	300
25	0	60	60	180

Table 5.6. Mean time (± 1 S.D.) taken for the moult to occur between *L. salmonis* first and second naupliar stages and the second naupliar stage and the copepodid.

Stage	Mean time (minutes)	S.D.
Nauplius I to Nauplius II	10.53	4.34
Nauplius II to Copepodid	12.21	3.87

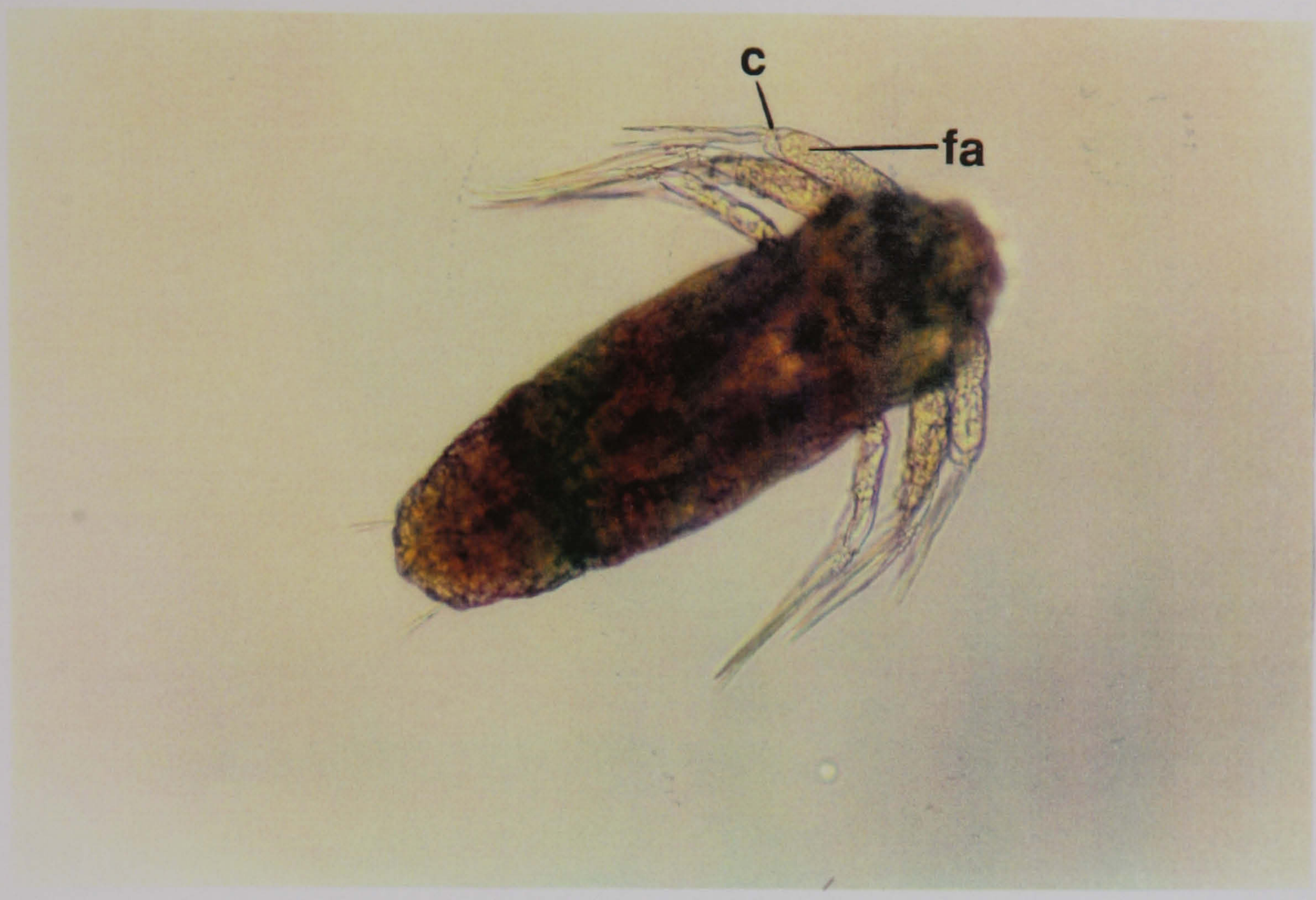
position, moving away from the body into a more "upright" position, still twitching. At this point, if both naupliar stages were stimulated by a jet of water emitted from a pipette, they showed some sporadic swimming, whereas prior to this, stimulation by this method caused no reaction in the nauplii. Once the appendages had returned to their normal position, spontaneous swimming was seen to occur, the larvae becoming suspended in the water column. Even with very low salinities, this total recovery was seen to occur, although the duration to recovery was much longer in those exposed to lower salinities for a longer amount of time.

5.3.7. Moulting times

5.3.7.1. Moult from nauplius I to nauplius II

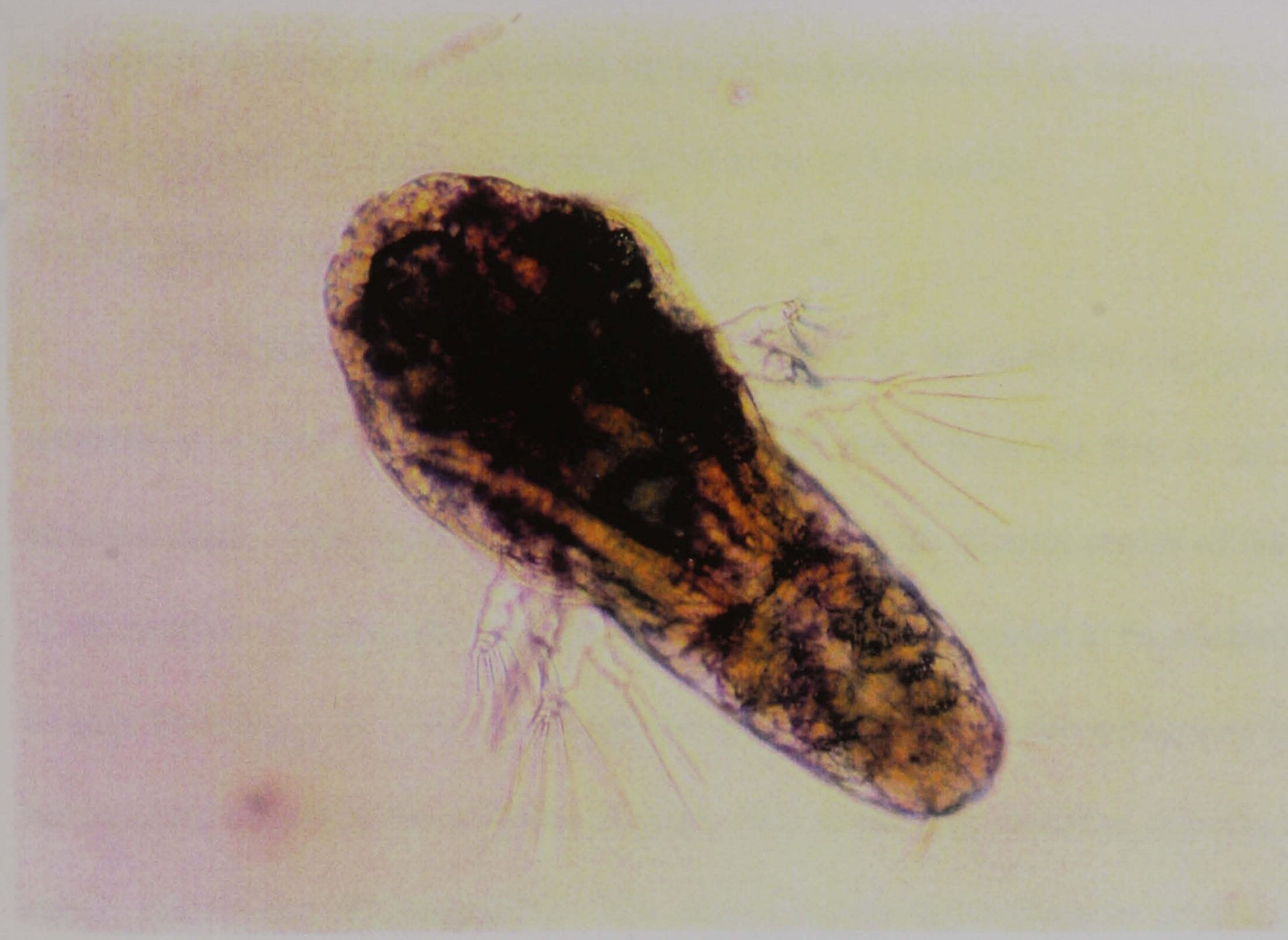
Fig 5.17 shows an *L. salmonis* nauplius I stage ecdysing to reveal the second nauplius stage, and table 5.6. gives the mean time taken for the cuticle to be shed and the second nauplius stage to be released (n=20). It was observed from observations during moulting and from the appearance of the shed cuticle post-ecdysis, that the cuticle splits in a latitudinal manner across the dorsal surface of the head region. The anterior end of the nauplius is then forced out of this opening. Due to this, swimming was observed to cease as the appendages were withdrawn from the old cuticle surrounding them, though twitching of all three pairs was seen to continue, this movement perhaps promoting the shedding of the cuticle. The first antennae are the first pair of appendages to show this "withdrawing," followed shortly by the second antennae and lastly be the mandibles. When the second antennae withdraw, this is when the enlarged distal spine on the endopodite can first be observed, this being a characteristic morphological difference between the first and second nauplius stages (Johannessen, 1978). Once all three pairs of appendages have been withdrawn, the old

Fig 5.17. (a) *Lepeophtheirus salmonis* nauplius I stage commencing moulting to release a nauplius II stage. Note the withdrawing of the first antennae (fa) from the surrounding cuticle (c) (x100 magnification.)



(b) *Lepeophtheirus salmonis* nauplius II stage emerging anteriorly from the shed cuticle that surrounded the nauplius I stage (x100 magnification).

Fig. 5.18. (a) *Lepeophtheirus salmonis* copepodid emerging anteriorly from the nauplius II cuticle (x100 magnification).



(b) *Lepeophtheirus salmonis* copepodid completing the moult from the nauplius II stage. The copepodid emerges through a split in the anterior region of the old cuticle (x100 magnification).

cuticle is then observed to be shed posteriorly (fig. 5.17) by the twitching of the body of the nauplius. Once this was observed to occur, the resultant second nauplius stage was able to actively swim and retain its position suspended in the water column. The mean time taken for this to occur was found to be 10.53 minutes.

5.3.7.2. Moult from nauplius II to copepodid

The moult from the second nauplius stage to the infective copepodid stage occurred in a very similar manner to that of the moult from the first to the second nauplius stage. Again a split was observed to occur in the anterior region of the cuticle of the nauplius II stage, allowing the head region of the copepodid to be released as can be seen from fig 5.18. The appendages were again observed to be withdrawn from the surrounding cuticle of the nauplius II stage, with swimming therefore ceasing, and the cuticle finally cast off posteriorly. The mean time for this ecdysis to occur (n=20) is given in table 5.6, and as can be seen, the time is very similar to that taken for the moult from the first to the second naupliar stage, 12.21 minutes compared to 10.53 minutes respectively.

5.4. Discussion

This study has attempted to elucidate the factors that control or influence the behavioural responses of the free-swimming larval stages of *L. salmonis*. Increased information on the behaviour of sea lice will allow a better insight into the ecology of such species whilst free-swimming in the natural environment. This in turn may help to understand the infection patterns observed on farms and the dynamics of the larval stages.

5.4.1. Swimming behaviour

According to Kabata (1981), the most important aspect of the locomotory activities of parasitic copepods is the swimming of the dispersal stages, since they are directly responsible for the location of the host and the survival of the parasite. It is therefore surprising that there is little information available in the published literature regarding the swimming abilities of naupliar and copepodid stages of such parasites, and the factors which control, modify and influence such patterns.

5.4.1.1. Description of swimming patterns

From the light experiments it was shown that swimming occurred in both light and in darkness by *L. salmonis* nauplii or copepodids. Movement was caused by short "hops" of the parasite, almost always in an upwards direction followed by a period when the larva stopped such activity, and sank passively. The duration of the passive sinking was often longer than that of the directed swimming motion. However, the distance gained in the active swimming stage was always greater than the distance covered when descending. From image analysis observations, it was possible to estimate the speed of both naupliar and copepodid stages of *L. salmonis* during active swimming and also passive sinking. For the naupliar stages, the sinking velocity was

calculated to be approximately 5.43cm per minute, whilst directed movement was found to average 75cm per minute. The copepodid stage was found to sink at a very similar rate to that of the nauplii, this being 5.94cm per minute. However, the velocity it attains during either spontaneous swimming or stimulated swimming was observed to be greater that that exhibited by the nauplii. The average speed of a spontaneous "hop" was 128.24 cm per minute, whilst that of a stimulated movement was 410.55cm per minute. It therefore appears that a copepodid will exhibit a faster rate of movement in response to a possible host-originated cue compared to the speed attained during spontaneous swimming in order to maintain its position in the water column.

Because of the considerable difference in swimming and sinking speeds and the time spent doing each activity, there will be an overall upward movement in the water column. This is especially evident for the infective copepodid stage, with approximately 0.05cm being covered during one individual passive sinking period, whilst during an active spontaneous movement 1.43cm can be covered. There are few reports on the swimming speeds of free-living copepods and none at all of their larval stages or parasitic copepods. However, a report by Pavlova (1981) estimated that the highest velocity recorded in a single "jump" by a female free-living copepod, *Rhincalanus nasutus* Giesbrecht, was 208.5cm per second, which is equivalent to approximately 1251cm per minute, almost 17 times faster than *L. salmonis* nauplii and 3 times the speed of stimulated copepodids. However, the author stated that the average swimming velocity of *R. nastus* as well as three other free-living copepod females, *Euchirella curticauda, Scolecithrix danae* (Lubbock) and *Euchaeta marina* varied between 1.6 and 3.0cm per second (96 to 180cm per minute), a much closer

speed to that attained by *L. salmonis* nauplii and unstimulated copepodids, 1.25 and 2.14cm per second respectively (75.00 and 128.24 per minute).

This "hop and sink" behaviour was reported for *L. salmonis* by Wootten *et al.* (1982) and by Bron *et al.* (1993a). Such behaviour, according to Wootten *et al.* (1982), would appear to have value in seeking out a free-swimming host that is likely to be in the upper layers of the water column. Of course, it may be argued, that since it is only the copepodid stage that can infect a host, such behaviour would be of little value to the naupliar stages, and that such swimming to enter or remain in the surface layers would be a waste of their finite energy supplies. However, it is due to these supplies being limited that the nauplii may exhibit such swimming, and possess their positive photo- and geotactic natures. If the first two naupliar stages did not enter and/or remain in the upper region of the water column, then once the moult to the infective copepodid stage had occurred, this stage would be much lower down in the water column. Although, in such a case, it would no doubt have more energy reserves remaining, the expenditure that would be necessary to reach the surface layers of the water and spatially overlapping potential hosts by constant directed swimming may be too much to then allow successful infection and establishment. Indeed, Poulin *et al.* (1990a) noted spontaneous activity by the copepodid stage of *Salmincola edwardsii*, and stated that such behaviour probably helps in bringing the parasite into the range of hosts.

No differences were observed between the swimming patterns of the two naupliar stages with no significant variations occurring in the amount of time spent actively swimming or sinking. At 10°C, the numbers of active "hops" observed per minute for the first and second nauplius stages were 6.8 and 6.6 respectively. The copepodid stage, however, was observed to be much more active than these earlier

stages, exhibiting approximately 13.10 "hops" per minute at the same temperature. This difference in activity may be explained by the copepodid stage not only having to remain in spatial overlap with their hosts, but also react to their presence in order to infect them. There would be little point in nauplii responding to stimuli indicative of a host when they do not have the ability to infect.

Swimming patterns of the larval stages of other parasitic copepods have been briefly described in the literature, but all are confined to the patterns exhibited by the copepodid stage (Wilkes, 1966; Anderson & Rossiter, 1969b; Kabata, 1981). Kabata (1981) stated that such descriptions tended to be uninformative, and indeed most are short observations that are an aside to the main topic of research.

In contrast, swimming patterns in free-living copepods have been more extensively studied, though these tend to concentrate on the behaviour and abilities of the adult stages. However, it is worth considering such descriptions and results, as it can be argued that the first three stages of *L. salmonis*, and in particular the two naupliar stages, are free-swimming and may possess many of the characteristics of their free-living counterparts.

Hwang, Turner, Costello, Coughlin & Strickler (1993) reported that females of the calanoid copepod *Centropages hamatus* Lilljeborg exhibited four modes of behaviour - a slow-swim (movement of feeding appendages only), break (no appendages moving), fast-swim and grooming (brushing of antennae through feeding appendages). Due to the lecithotrophic nature of the free-swimming stages of *L. salmonis* the slow-swimming and grooming behaviour may not be observed, but the "hop and sink" pattern seen in the present study appears to be similar to that of the break and fast-swim behaviour of *C. hamatus*. Tiselius & Jonsson (1990) also observed

such behaviour in *Acartia clausi*, with the copepod either sinking motionless or exhibiting short jumps. Wong, Ramcharan & Sprules (1986) stated that the swimming behaviour of the calanoid copepod *Diaptomus minutus* consisted of periods of rest interrupted by short jumps of approximately one to two body lengths and, although this was again for adult stages, Buskey, Coulter & Strom (1993) summarised the swimming patterns of copepod nauplii describing them either as "jerky" or "stop and go," as was seen for *L. salmonis* nauplii and copepodids.

Alexander (1990) considered this hop and sink technique used by some copepods to be an adaptation for the prevention of sinking and, according to the author, is more economical than constant "hovering." Animals are denser than either fresh- or seawater and will therefore tend to sink, unless they have adaptations that give buoyancy. Alexander (1990) states that since very small organisms sink slowly and can be kept suspended by natural turbulence, they therefore do not have to evolve buoyancy aids, though this is only effective for organisms of less than 150μm in diameter. *L. salmonis* nauplii and copepodids are larger than this critical size given by Alexander (1990), and therefore fall into the category of animals that have to develop adaptations against sinking and hence the "hop and sink" swimming behaviour. It was thought that the presence of lipids, as well as acting as an energy supply might also act as a buoyancy device that would reduce the energy needed by *L. salmonis* nauplii and copepodids to remain in the upper part of the water column. Indeed, Yayanos, Benson and Nevenzel (1978) investigated the lipids from *Calanus plumchrus* Marukawa and looked at their contribution towards the buoyant properties of the organism. The authors stated that the buoyancy gained by lipids could influence the vertical location of an animal in the water column. However, in the present study, no relationship was

found between the amount of lipid remaining in the naupliar stages and the sinking rate of *L. salmonis*. As was claimed by Yayanos *et al.* (1978) the presence of lipids can only positively contribute to the overall buoyancy of an organism, but it may be the case that such a small amount is present within the body of *L. salmonis* free-swimming stages, that changes in its volume due to its utilisation will not have a noticeable effect upon the sinking rate.

5.4.1.2. Effect of temperature on swimming

Temperature was seen to significantly affect the number of periods spent by *L. salmonis* nauplii and copepodids actively swimming, with the number of "hop" movements increasing with temperature, confirming the observations on activity described in Chapter 4. Such an increase in activity was also observed for the free-living copepod *Temora longicornis* by Gill & Crisp (1985), with the frequency of the limb beat of the adult female and the consequent swimming rate increasing with increasing temperature.

Prah & James (1977) studying the digenean parasite *Schistosoma mansoni* also noted that, at increasing temperatures, the cercarial stage was more active and its increased infective ability proved. Since activity and infectivity are inextricably linked, it may therefore be expected that as temperatures increase seasonally at fish farms, the combination of a decreased generation time and a more active infective stage will increase the potential for hosts to have increased parasite burdens. Also, the effect of temperature upon the activity and the subsequent infectivity of the parasite must be taken into account when performing on-going infection experiments.

5.4.1.3. Effect of salinity on swimming

Salinity was observed to have a profound effect on the swimming abilities of both the naupliar and copepodid stages of *L. salmonis*, and again the two naupliar stages behaved in a similar manner. Swimming in the naupliar and copepodid stages was found to halt in salinities of 21‰ or 23‰ or below respectively. Naupliar stages were never observed to recover this ability in lowered salinities, though the copepodid stage did appear to possess a greater tolerance and acclimation with the ability to swim returning after a few minutes in salinities of greater than 20‰. Nauplii were seen to recover, however, when exposed to low salinity water for only a short period of time (either 120 or 240 seconds) and then returned to full strength seawater, with those exposed for the shortest duration recovering the quickest. The pattern of recovery was consistent with that observed in copepodids placed in low salinity water, with the first signs of recovery being appendage movement followed by their return to the characteristic position, the ability to swim in reaction to stimulation and finally by spontaneous "hop and sink" swimming.

According to Lance (1962), the swimming activity of free-living copepods is depressed in water columns of reduced salinity, with the resulting activity being dependent both on the degree of dilution and on the period of immersion. The ability of free-living crustacean larvae to react to changing salinities has been documented. Latz & Forward (1977) found that the larva of the estuarine crab *Rhithropanopeus harrisii* reversed its normal positive phototactic response in response to reduced salinity water, with only a 1 to 2% drop being necessary for this reversal. As well as this directed swimming, downward movement in the form of passive sinking has also been observed for free-living copepod species (Lance, 1962; Grindley, 1964; Harder, 1968) in relation to reduced salinity. This appears to be the case for *L. salmonis* nauplii. Latz

& Forward (1977) stated that directed or passive downward movement by pelagic larvae in order to remove them from areas of low salinity may be a general characteristic. From the results of Chapter 3 it was observed that *L. salmonis* nauplii are very sensitive to low salinities, and cessation of swimming may well be an adaptation to remove them from areas of low salinity. Freshwater or water of low salinity will tend to be found higher up in the water column due to the relative densities of the different types of water, these being approximately 1,000kg/m^3 and 1,026kg/m^3 for freshwater and seawater respectively (Alexander, 1990). Sinking in response to low salinity will thereby hopefully carry *L. salmonis* nauplii into water of higher salinities. *L. salmonis* copepodids were, however, observed to acclimate to salinities of between 21 and 25‰ (after which swimming did not cease) after a period of time. For this stage of the parasite, it may also be the case that avoidance of low salinities occurs by vertical sinking due to the copepodids losing the ability to swim, but, that after a certain point in time at these "medium-strength" salinities, the copepodid commences its swimming again, since it still has to locate and successfully infect a host. By not swimming, its chances, as already discussed, will be reduced. *L. salmonis* copepodids were observed to have a higher tolerance of low salinities compared to the naupliar stages as seen in Chapter 3 and at such medium salinities, the longevity of this stage was seen not to be significantly reduced. At lower salinities however, longevity did decrease, and so this may be why at low salinities, like the naupliar stages, the copepodid loses all ability to swim, in an attempt to reach deeper water that may be more saline.

Heuch (1995) described experimental evidence for the aggregation of *L. salmonis* copepodids in step salinity gradients. The author suggested that copepodids in the natural environment aggregate in these gradients in order to exploit the aquatic

odour trails of hosts, the trails being found in such density gradients. Whether one regards the use of chemosensory response as a likely host-finding mechanism or not, there is no evidence at present to suggest that *L. salmonis* copepodids use such odour trails. Bron (1994) did not find any chemosensory response in *L. salmonis* copepodids. It may well be the case that, as suggested in the present study, the larvae are merely showing an avoidance of potentially damaging low salinity water.

5.4.2. Responses to light

5.4.2.1. Intensity

Both naupliar and copepodid stages of *L. salmonis* were observed to react photopositively to light in a narrow horizontal beam. By using a horizontal beam, any responses to gravity that may exist will be negated as the larvae will not be travelling vertically to a stimulus. The copepodid stage possessed the strongest response, reacting positively to all intensities tested, even down to as low as 3 lux. The naupliar stages showed a comparatively weaker response, with the NI stage first demonstrating a positive taxis at intensities of greater than 200 lux, and the NII stage being slightly more photopositive, first reacting at intensities of 85 lux. It may be argued that due to the copepodid stage having a greater swimming ability than the two naupliar stages, the nauplius stages would not have long enough to traverse the test chamber towards the light source. However, the exposure time for the experiments was 180 seconds, which, from the swimming observations, was considered long enough for any response to be shown.

For both the nauplius stages, a correlation was found to exist between increasing light intensity and increasing photoresponse. However, no such relationship was found to exist between light intensity and the responses of the copepodid stage, suggesting

that the copepodid stage is very sensitive to light at all intensity levels, whereas the earlier nauplius stages have a threshold level at which they first react followed by an increasing sensitivity to higher intensities. Such behaviour in these free-swimming larval stages will no doubt enhance the chances of an infection encounter by the copepodid stage by keeping the parasite in spatial overlap with potential hosts during daylight hours. Such behaviour has also been reported by Bron *et al.* (1993a) for the *L. salmonis* copepodid stage, but these authors did not look at the responses of the nauplius stages. Wootten *et al.* (1982) and Johannessen (1978) did describe a positive photoresponse in all three stages of *L. salmonis* but their results are restricted to laboratory observations without any quantitative measurements. MacKinnon (1993) described a similar response of the copepodid stage of *Caligus elongatus* to horizontal light beams as that in the present study, and by Bron *et al.* (1993a), but again ignored the nauplius stages.

The study also attempted to examine whether the responses to directional light, such as the horizontal light beam show the true photobehaviour of copepods, or whether this induces laboratory artefacts. The optical arrangement does not simulate the angular light distribution (ALD) of light underwater from daylight or moonlight, and Forward (1988) states that normal photic orientation is only possible when the experimental illumination conditions approximate to a normal ALD. For example, Stearns & Forward (1984a) demonstrated that the free-living copepod *Acartia tonsa* displayed a positive phototaxis at all intensities using a horizontal light beam, but the same authors (Stearns & Forward, 1984b) then proceeded to show, using an ALD system that this was an apparent artefact, with copepods showing a positive phototaxis only in response to decreasing light intensities, and a photonegative response at high

intensities. If the first study was to be accepted, then one would expect *A. tonsa* to show what is termed a "reverse migration" (Hutchinson, 1967), with the copepod ascending to a minimum depth during the day and descending to a maximum at night. However, the ALD experiments revealed that a "nocturnal migration" (Hutchinson, 1967) occurs, with *A. tonsa* reaching a minimum depth between sunset and sunrise and a maximum depth during the day.

For this reason, the ALD experiments were performed in the present study to ascertain whether the photobehaviour observed by *L. salmonis* is different under "natural" light distribution and, if so, what effect this would have on the distribution of the larvae in the water column? In contrast to Stearns & Forward (1984a, 1984b), the results for *L. salmonis* nauplii and copepodids using ALD did not show any change in their photobehaviour. Their responses still indicated a "reverse" migration pattern when only phototaxis is taken into account. *L. salmonis* copepodids reacted positively to intensities of 134 lux or greater. Although this is a higher threshold than that observed for artificial light, it should be noted that the actual intensities within the water will be lower using the angular distribution of light than the measured intensities outside (measuring light intensity within the water was not possible due to the meter not being waterproof) due to total internal reflection (Forward *et al.*, 1984). Nauplii of both stages were also seen to react positively to intensities of over 134 lux (outside of the water column).

It might have been expected that the free-swimming larval stages of *L. salmonis* would show photobehaviour similar to that exhibited by free-living copepods under ALD. However, it must be remembered that such behaviour will be disadvantageous to species of parasitic copepods whose hosts inhabit the upper surface layers of the water

column. Free-living copepods tend to show nocturnal migrations to avoid predation by visual predators during the day. Although *L. salmonis* larvae are obviously at risk from such predators, avoidance would be detrimental to their chances of locating potential hosts.

5.4.2.2. Wavelength

Since *L. salmonis* larval stages react strongly to light and this is influencing their distribution in the natural environment, it is logical to expect that their visual spectral sensitivity should be adapted to the spectrum of light underwater. The first nauplius stage of *L. salmonis* was not observed to show any peaks of phototaxis in the wavelengths tested (430 to 700nm), but the second nauplius and the copepodid stages were seen to react to light at 500nm and 500 and 561nm respectively. In the open ocean, the available spectrum compresses to wavelengths in the blue region around 470nm whilst in coastal regions the available spectrum shifts to longer wavelengths in the region of about 500 to 550nm (Dartnall, 1975; Jerlov, 1976). Therefore, as stated by Forward (1988), if zooplankton are sensitive to spectral light during the day, there should be a regular shift in spectral sensitivity to the blue-green regions, as was observed in the present study for *L. salmonis* NII and copepodid stages. It is surprising that the NI stage did not show any spectral sensitivity but it may be the case that the bands of wavelength tested were too broad or that the NI's response was just not strong enough. By maximising spectral sensitivity in the blue-green range, larvae will also maximise their positive photoresponse to light, as light underwater will be of this wavelength.

However, as well as showing these peaks, the NII and the copepodid stage of *L. salmonis* also show a broad range of sensitivity from 430 to 700nm. Stearns &

Forward (1984a) also found this for *Acartia tonsa* and reported that this was unusual since, according to Forward (1976), most members of the zooplankton show narrow ranges or peaks. However, Stearns & Forward (1984a) suggest that a functional advantage of a broad range is a broad sensitivity in aquatic environments from clear water to turbid estuaries. Since *L. salmonis* is not restricted to either the open ocean or coastal waters, the physical conditions of these two environments being very different, it may be the case that the parasite has developed this broad sensitivity as well as its maximal peaks as an adaptation to life in different water conditions.

5.4.3. Responses to gravity

Bron *et al.* (1993a) has already demonstrated a pressure response in *L. salmonis* copepodids confirming Johannessen's (1975) earlier suggestion that such a response was possible in the parasite. However, Bron *et al.* (1993a) did not study the geotactic nature of the earlier naupliar stages. In the present study, a negative geotaxis like that previously reported for the copepodid stage by Bron *et al.* (1993a) was observed in both the first and second nauplius stage. The positively phototactic nature of the free-swimming larvae combined with their geotactic responses will help maximise the spatial overlap between host and parasite. If larvae only responded to light, then during the hours of darkness, *L. salmonis* would no longer be attracted towards the surface layers of the water column, where salmon tend to inhabit according to Hogans & Trudeau (1989) and Huse & Holm (1993). However, by showing spontaneous swimming without light stimulation and possessing a negative geotactic response, larvae will be able to remain within the surface layers. Bron *et al.* (1993a) suggested that such behaviour would bring *L. salmonis* larvae into the surface waters. Such behaviour will only be advantageous for parasites whose hosts inhabit the surface

layers, but for those species that parasitise benthic fish, for example *Lepeophtheirus pectoralis*, such behaviour will not promote infection. Species such as these will have had to develop alternative responses in order to maximise their infection chances such as that reported by Boxshall (1976) for *L. pectoralis* copepodids. After an initial, active phase, he found that they became photonegative. This would increase the chances of an infection encounter occurring between *L. pectoralis* and its potential demersal hosts such as *Platichthys flesus* L. and *Pleuronectes platessa* L. However it is not known whether *L. pectoralis* copepodids exhibit a positive geotaxis or whether this negative phototaxis overrides any pressure response the parasite may possess.

A negative geotactic pressure response is common in free-living invertebrates (Morgan, 1984) and according to Bron *et al.* (1993a) perhaps should not be unexpected in the larvae of parasitic copepods. If this is the case, then it may be expected that *L. salmonis* and *L. pectoralis* may not possess an adaptive pressure response in order to spatially overlap with either a pelagic or demersal host respectively, but instead may share a similar response which is a common phenomenon throughout marine invertebrates. However Morgan (1984) does state that behavioural responses to hydrostatic pressure are presumably of an adaptive nature and will vary in accordance with the life cycle of the species concerned. Until more is information is available about the pressure responses of all marine invertebrates, it may not prove possible to determine or separate such specific gravitational responses from other taxes or responses exhibited by individual species of parasitic copepods. Due to this, the magnitude of the role of geotaxis in the distribution of *L. salmonis* in the natural environment will remain speculative.

5.4.4. Moulting and its implications for distribution

5.4.4.1. Duration of moulting

The time taken for the moult to occur from both the NI stage to the NII stage, and from the NII stage to the copepodid stage was found to be very similar, taking approximately 10 and 12 minutes respectively. During this time, no swimming was observed to occur due to the withdrawal of the first and second antennae and the mandibles from within the old cuticle. Because of this, larvae were unable to remain suspended in the water column, and were, in all cases, found to be lying on the bottom of petri-dishes. It was originally thought that such inability to maintain position would have strong implications upon the distribution of these stages in the water column in the natural environment, with the position of both the NI stage and the NII stage being lost upon moulting to the NII and the copepodid stage respectively, passive sinking taking them away from the surface layers of water. However, due to the relatively short moulting times and the sinking velocity of the naupliar stages of *L. salmonis*, the loss of position in the water column may not prove to be such a major factor as at first anticipated. By multiplying the time taken for the moult with the average sinking speed of the nauplii, the downward distance covered can be estimated. Thus the distance travelled downwards is estimated to be 57.18cm and 66.30cm during the NII/NII moult and the NII/copepodid moult respectively. With the upward swimming speeds that can be exhibited by the resultant NII and copepodid stages of 75 and 128.24cm per minute respectively, it can be expected that this loss of position may be overcome due to the positive phototaxis and negative geotaxis exhibited by the larvae. Therefore it is highly likely that these taxes not only keep larvae in the upper surface layers in between moults but also return them to this area following the sinking that occurs during ecdysis. The relatively short moult times and the behavioural responses of the larvae

should combine to counteract any detrimental effect on their ability to remain within the upper surface layers.

From the present study, it was observed that both the naupliar and copepodid stages exhibit a range of behavioural responses that will aid them in remaining in the upper layers of the water column, helping them overcome such problems as their negative buoyancy and the passive sinking that occurs during the two moults between the hatching of the first larval stage and the infective copepodid stage. Such responses are of vital importance since, in order to possess any chance of infection occurring, a parasite and host must be in spatial overlap at some point in the parasite's life cycle. Although some of the behavioural characteristics observed were similar, if not identical, to those exhibited by free-living copepods such as swimming patterns, responses to gravity and spectral wavelength, others such as responses to light intensity were in contrast, indicating, that although the first three stages of *L. salmonis* are "free-living" whilst in the water column, they have adapted some of their behavioural responses to account for their parasitic life cycle.

CHAPTER 6

DISTRIBUTION IN THE NATURAL ENVIRONMENT

6.1. Introduction

At the time this study was performed there were only two published reports of attempts to obtain free-swimming stages of sea lice from the natural environment. Johannessen (1978) obtained a single wild first nauplius stage of *Lepeophtheirus salmonis* in a plankton haul at a depth of 2m, but no specimens were found in the surface waters, and no samples were taken at deeper depths. Heegaard (1947) reported that no nauplii of *Caligus curtus* were found above 40m in plankton hauls.

Consequently, there was no information available regarding the ecology of the free-swimming larval stages of *L. salmonis* or indeed any other species of sea lice with respect to their distribution and dispersal in the water column in and around commercial fish farms. Although information is available on the biology and behaviour of the first three stages of sea lice, this has been restricted to laboratory based studies. It is possible to extrapolate these laboratory findings in order to hypothesise what will occur in the natural environment but, unless sampling programmes are undertaken, the ecology of the naupliar and copepodid stages will remain speculative. As already discussed in Chapter 3, research in Ireland has already attempted to link the estimated production of the first nauplius stage of *L. salmonis* from gravid females infecting wild and farmed salmon with future infection levels on wild sea trout, *Salmo truttae* (Tully & Whelan, 1993). The difficulties in this with respect to the lack of information available on the hatching and development of *L. salmonis* were also discussed in Chapter 3 but, even if and when the factors controlling and modifying such parameters are understood, there still remains a significant gap in data concerning the distribution and dispersal of the free-swimming larval stages in the natural environment

that should be filled in order to clarify hypothetical correlations made between sea lice infections.

Despite this lack of information, there have been a few reports on the vertical distribution of Atlantic salmon in a caged environment, that may shed some light on the ecology of the free-swimming larval stages of sea lice. Huse & Holm (1993), in a study on the vertical distribution of Atlantic salmon within cages of different depths found that infection of fish during the summer by *L. salmonis* was significantly higher in shallow netpens (6m in depth) compared with deep netpens (20m in depth). This they considered to be due to the fish in 20m pens remaining at a deeper position in the water column during high light levels and maximum solar elevation. This suggests that the infective stage of *L. salmonis*, at least, is present in higher numbers in the upper surface layers of the water column than compared to lower layers. Indeed, the authors report that the occurrence of parasites was at a maximum in the surface layer during the summer, though gave no details of what they meant by this - free-swimming larvae in the water column or the numbers of pre-adult and adult lice recorded on the fish? This seems to suggest that the distribution of *L. salmonis* larvae is governed by the light, though no details were given as to the infection levels or distribution of the parasite in times other than summer. Huse, Bjordal, Fernö & Furevik (1990) citing Johanessen (1975), reported that the pelagic larvae of *L. salmonis* were phototactic, and that a preliminary study indicated that *L. salmonis* infections were reduced in a group of Atlantic salmon kept in a pen covered by a light-proof roof compared to a control group in an uncovered pen. However, they do not state whether this is due to the fish occupying a deeper depth or whether this is due to the infective copepodid stage no longer being concentrated in the surface layers of the water column due to their

phototactic nature. In a more detailed experiment, Huse *et al.* (1990) found that fish held in some uncovered pens had significantly higher *L. salmonis* infection levels compared to covered pens, indicating that shading does have an effect on the levels of louse infection. However, in other covered and uncovered pens, there were no differences in the infection levels, and the authors concluded that shading reduced the infection rate of *L. salmonis* in marine netpen rearing of Atlantic salmon only to a marginal degree. They also noted that the behaviour of the fish did not change in covered or uncovered pens with respect to vertical distribution indicating that it is the effect of light that concentrates the infective copepodid stage at least in the surface layers causing the increased infection levels seen in uncovered pens, and that shaded pens would disperse this concentration due to the decreased light levels.

These two studies, plus the report of Johanessen (1978) constitute the only information available upon the distribution of the free-swimming larval stages of *L. salmonis* indicating that, under the influence of light, larval stages, or at least the infective stage, concentrate in the upper layers of the water column. However this contradicts the distribution of *C. curtus* reported by Heegaard (1947), who found nauplii only below 40m depth in day samples, though the author did state that the number of hauls performed was small and "so give no absolute certainty."

There has, however, been a study on the distribution of the larvae of *Lernaeenicus sprattae* which may help to understand the ecology of the pelagic free-swimming stages of parasitic copepods. Schram & Anstensrud (1985) sampled the Oslofjord for the larval stages of *L. sprattae* and found both nauplii and copepodid stages, though the copepodid stage tended to dominate. They concluded that copepodids most probably undertake diurnal vertical migrations, with larvae being most

frequently recorded in neuston tows taken at night at between 0 and 90cm depth. This appears to be in contrast to the data available for *L. salmonis*, but Schram & Anstensrud (1985) state that laboratory studies show that the copepodid stage of *L. sprattae* exhibits a photonegative response at high light levels with a photopositive response being seen only in dull illumination, and hence the diurnal migration observed. In the light of the laboratory studies with *L. salmonis* copepodids and the first two naupliar stages, which showed the positively phototactic nature of the larvae, the results of Schram & Anstensrud (1985) concerning the distribution of *L. sprattae* may not be applicable to the ecology of *L. salmonis*.

As well as the vertical distribution of the free-swimming larvae of *L. salmonis* and other species of sea lice in the natural environment being unknown, data on the effect of dispersal on the horizontal distribution of larvae are also lacking.

Schram & Anstensrud (1985) found that the vast majority of *L. sprattae* larvae obtained were from neuston tows taken in the inner part of the Oslofjord with lower numbers obtained in the outer and middle fjord. The authors attributed this to the lack of horizontal water flow in the inner fjord, with the larvae subsequently not being dispersed, but remaining relatively concentrated. The effect of currents on the dispersal of such pelagic larval stages of parasitic copepods must indeed be large due to their small size and weak swimming ability. Whitfield *et al.* (1988) considered that the dispersal of *Lernaeocera branchialis* would be compounded by current-induced movements of nauplii and copepodids. Research attempting to link production of nauplius I stages of *L. salmonis* from farmed and wild salmon to future infection levels on wild sea trout (Tully & Whelan, 1993) therefore appears to be rather premature, since, until the dispersal patterns of such pelagic stages is known, and their subsequent

effects upon the infection dynamics understood, it surely cannot be possible to safely correlate infection levels from one farmed host species with the future effects on another, wild, host species.

The collection and collation of data on the distribution and dispersal of free-swimming larval stages of species of sea lice such as *L. salmonis* will not only provide data upon the infection dynamics of the parasite and thus essential information for the fish farming industry, but may also help in the search for alternative biological or managerial methods of control for sea lice rather than remaining totally dependent upon chemical treatments, the use of which may lead to the development of drug resistance (Jones *et al.*, 1992) in populations of *L. salmonis* along with the environmental concerns that accompany the use of such chemicals. Information upon the ecology of the first three free-swimming stages of *L. salmonis* may also help to clarify issues that exist in the controversy that has developed after the collapse of wild sea trout stocks. Such a situation has occurred on the west coasts of Ireland and Scotland and the fish farming industry has been prematurely blamed in the public press (Clover, 1994: Wilkie, 1995). Such blame has been apportioned without comprehensive scientific evidence that the wild sea trout becoming infected with *L. salmonis*. The origin of the lice has been assigned to the presence of fish farms.

The following study was therefore undertaken, in order to attempt to elucidate the natural vertical and horizontal distribution of the naupliar and copepodid stages of *L. salmonis* and *C. elongatus* in the water column in and around a commercial fish farm, and to observe if there exist any spatial or temporal patterns in such distributions and what effect these may have on the population dynamics of the species. The use of a light lure was also employed to determine if the free-swimming larval stages are

distributed according to their phototactic nature as has been observed in the laboratory, and if this could be exploited as a future alternative biological control method.

6.2 Materials & Methods

6.2.1. Plankton sampling

6.2.1.1. Sampling site

Plankton samples were taken at an Atlantic salmon farm on the west coast of Scotland. A site was chosen that had been established for a number of years and that had a persistent sea lice problem, fish being infected in large enough numbers throughout the year to necessitate treatment.

6.2.1.2. Sampling equipment

A sampling device was constructed using a 12V D.C pump (Jabsco) powered by a heavy duty 12V battery, to which a weighted inlet hose and an outlet hose were attached, with both these tubes having an internal diameter of 25mm. When the pump was turned on, water from the outlet hose was directed and filtered through a 60μm plankton net, which had been fitted with a removable 30ml plastic pot at the end secured by a jubilee clip. Once a sample had been taken, this pot could be removed and sealed with a screw-top lid, wrapped with Nesco film and labelled. A fresh pot was used for each sample.

6.2.1.3. Sampling method

Samples were taken vertically in the water column at 5m intervals, from the surface (0m) to the benthos. This profile was sampled for plankton at two locations outside the cages and one inside the cages, these being:

i. Between the nets and the walkway, immediately adjacent to the cages,

ii. On the other side of the walkway adjacent to open water approximately 2m from the nets,

iii. At the surface and at 5m inside the 6m deep cages.

Samples were collected over a period of 24 hours and for each 24 hour period sampled, six samples were taken at 0800, 1100, 1400, 1700, 2000 and 0200 hours at each depth. At each depth, 100 litres of water was pumped, and filtered through the plankton net. The net was then rinsed with filtered seawater and the plastic pot removed and sealed, and another one put in its place. The pot was labelled with the time and date when the sample was taken, and its depth and position. On return to the laboratory, the samples were centrifuged with a plankton centrifuge and the resulting plankton was fixed using a 5% concentration of buffered formalin, the final volume of each sample being 15ml. This was achieved by resuspending the centrifuged plankton in 7.5ml of the filtered seawater and adding the same amount of 10% buffered formalin. The water spun off in the centrifuge was checked for the presence of any plankton, and none was found. The samples were then analysed for the presence of the free-swimming stages of *L. salmonis* and *Caligus elongatus*, as well as for free-living zooplankton. As each sample contained 15ml, for each 15x1ml aliquots were scanned at x40 magnification on a compound microscope using a Sedgewick Rafter Chamber.

Any larval stages that were obtained from the plankton samples were removed from the Sedgewick Rafter Chamber and stained with Sudan Black B to look at their lipid reserves, using the method described in Chapter 4.

The time taken to pump 100 litres of water was initially timed and the water from the pump was filtered for this amount of time in all subsequent samples. After changing the depth and the position for the next sample, the pump was run for a few seconds prior to filtering in order to expel any remaining water in the tubes from the previous depth.

6.2.1.4. Sampling programme

Ten 24 hour period depth profiles were sampled from the same cage and from the same location outside the cage. The sampling programme took place between November 1994 and January 1995 over eleven weeks, one sample being taken every week with the exception of one week in December when there was a two week gap instead of the usual 7 days. On each occasion the surface water temperature and salinities at each depth were recorded.

The numbers of gravid adult females of both species of sea lice present on 5 fish were also noted to see if there existed any correlation between these and the levels of larvae in the water column. Fish were individually hand-netted out of the cage and killed by a blow to the head, and the numbers of gravid adult females recorded.

6.2.1.5. Extended sampling

Initial samples gave low yields of larvae and it was decided to extend the sampling time to filter larger amounts of water. These samples were only taken within the cages at 0 and 5m depth and at one point in time (June). Increased volumes of water were filtered in the same manner as described above, these volumes being 200, 400, 800 and 1600 litres of water. The samples were then centrifuged and analysed for the presence of the free-swimming stages of *L. salmonis* and *C. elongatus*.

6.2.2. Light lure

6.2.2.1. Sampling equipment

A prototype light lure designed and manufactured by Terecos was used to sample for the free-swimming stages of *L. salmonis* and *C. elongatus* taking advantage of their phototaxis. The lure was attached to an inlet hose (internal diameter 50mm) approximately 8m long which was attached to a diesel pump usually employed for grading fish. The outlet

hose, also 50mm in diameter was then directed into the 60μm plankton net with the pot attached. The lure was connected to a 12V heavy duty battery which powered the light.

6.2.2.2. Sampling method

The lure was placed in the cages at either 0 or 5m depth and the light switched on for a set amount of time. After this period of time, the pump was then switched on and 100 litres of water was pumped and filtered and subsequently fixed and analysed for the presence of free-swimming stages of *L. salmonis*, *C. elongatus* and zooplankton in the same manner as was described in section 6.2.1.3.

6.2.2.3. Sampling programme

Before the light was switched on at each occasion, a control sample was taken so as to compare the plankton profiles gained with and without the light switched on. The light was the switched on for 1, 2, 4, 8, 18 and 24 hours on separate days at both 0 and 5m within the cages, making a total of 12 sampling occasions, and 24 samples. Two samples were taken on separate days per week over a total of 6 weeks during the months of June and July. Again the temperature and salinity levels were recorded at the site, as well as the number of gravid adult females of both species present on 5 fish in adjacent cages.

6.3. Results

6.3.1. Site description

The site chosen was on the West coast of mainland Scotland. Although not within a sea loch system, the site was comparatively sheltered, present on the edge of a small bay. The site was accessible from the land by a walkway out to the cages, of which there were approximately 20 pens. The site was flushed by a tidal current running down the sound where the cages were, between the mainland and a small island. Two rivers fed into the sound via the bay. The site had been established for 3 years and its current practice for the control of sea lice was the use of hydrogen peroxide.

6.3.2. Pump sampling

6.3.2.1. Sampling on different days

6.3.2.1.1. Total numbers of larvae

i. *Lepeophtheirus salmonis*

Larvae of *L. salmonis* were obtained in each of the ten 24 hour sample periods at location (ii). The total numbers of larvae of *L. salmonis* found within each 24 hour period immediately outside the cage are shown in Table 6.1. No larvae were found at location (iii). The larvae obtained at location (i) were only used in horizontal distribution analysis, as only 2 depths were tested (0 and 5m) compared to the 5 depths sampled at location (ii) and (iii). The mean number of larvae found during each 24 hour period at location (ii) was 34.8 and analysis of variance demonstrated that there was no significant differences between the numbers obtained in the ten 24 hour periods sampled ($p = 1.00$), and due to this the sample periods were pooled for further analysis.

ii. *Caligus elongatus*

Table 6.1. Total numbers of *L. salmonis* and *C. elongatus* free-swimming larval stages obtained in plankton samples

Sample number	0m		5m		10m		15m		20m		Total number
	L.s.	*C.e.*	*L.s.*	*C.e.*	*L.s.*	*C.e.*	*L.s.*	*C.e.*	*L.s.*	*C.e.*	*L.s.*
1	9	4	21	8	3	0	2	0	0	0	35
2	11	3	21	2	2	0	2	0	0	0	36
3	10	0	14	0	5	0	3	0	0	0	32
4	17	0	10	0	3	0	4	0	0	0	34
5	12	0	15	0	2	0	5	0	0	0	34
6	12	0	11	0	1	0	3	0	0	0	27
7	15	0	17	0	4	0	2	0	0	0	38
8*	10	0	14	0	4	0	7	0	0	0	35
9*	10	0	20	0	3	0	3	0	0	0	36
10	14	0	22	0	1	0	4	0	0	0	41
Mean	12.00	0.70	16.50	1.00	2.80	0	3.50	0	0	0	34.80
S.D.	2.58	1.47	4.35	2.54	1.32	0	1.58	0	0	0	3.68

L.s. = *L. salmonis*
C.e. = *C. elongatus*
* denotes two week interval between samples instead of the usual week

C. elongatus was only found in the first two 24 hour sample periods, and hence subsequent statistical analysis was not possible. The total number of *C. elongatus* larvae found on each sampling period is also shown in Table 6.1.

6.3.2.1.2. Numbers of gravid adult females on Atlantic salmon

i. *Lepeophtheirus salmonis*

The mean number of gravid *L. salmonis* females present on each of the sample days is given in Table 6.2. Although there was a significant difference in the numbers of gravid females present over the sampling period (ANOVA, $p < 0.001$), there was no correlation between the total number of larvae obtained within each 24 hour period and the number of ovigerous females present on the fish sampled ($r^2 = 0.013$, $p = 0.758$).

ii. *Caligus elongatus*

No gravid adult *C. elongatus* females were recorded from the fish sampled over the course of the programme.

6.3.2.1.3. Temperature and salinity levels

The temperature and mean salinity levels on each of the sampling days are given in Table 6.3. The highest temperatures were recorded in the first samples, and then gradually declined. There was no correlation between temperature and the numbers of adult or larval *L. salmonis* obtained ($r^2 = 0.34$, $p= 0.07$; $r^2= 0.04$, $p=0.58$). There was also no correlation between salinity and the numbers of adult or larval *L. salmonis* stages obtained ($r^2= 0.48$, $p=0.54$; $r^2= 0.02$, $p=0.73$).

6.3.2.2. Circadian rhythm

i. *Lepeophtheirus salmonis*

The distribution of the larval stages of *L. salmonis* over a 24 hour period (the ten 24 hour sample periods having been pooled) is shown in fig. 6.1. Statistical

Table 6.2. Mean (± 1 S.D.) numbers of *L. salmonis* and *C. elongatus* gravid adult females present on *S. salar* during the pump-sampling programme

Sample number	Number of fish examined	Mean (± 1 S.D.) number of gravid females present	
		L. salmonis	*C. elongatus*
1	5	6.0 ± 2.89	0
2	5	4.8 ± 2.49	0
3	5	8.4 ± 2.88	0
4	5	5.2 ± 2.28	0
5	5	1.4 ± 1.14	0
6	5	1.8 ± 3.03	0
7	5	0.4 ± 0.55	0
8*	5	2.6 ± 1.95	0
9*	5	3.4 ± 2.19	0
10	5	3.4 ± 0.89	0

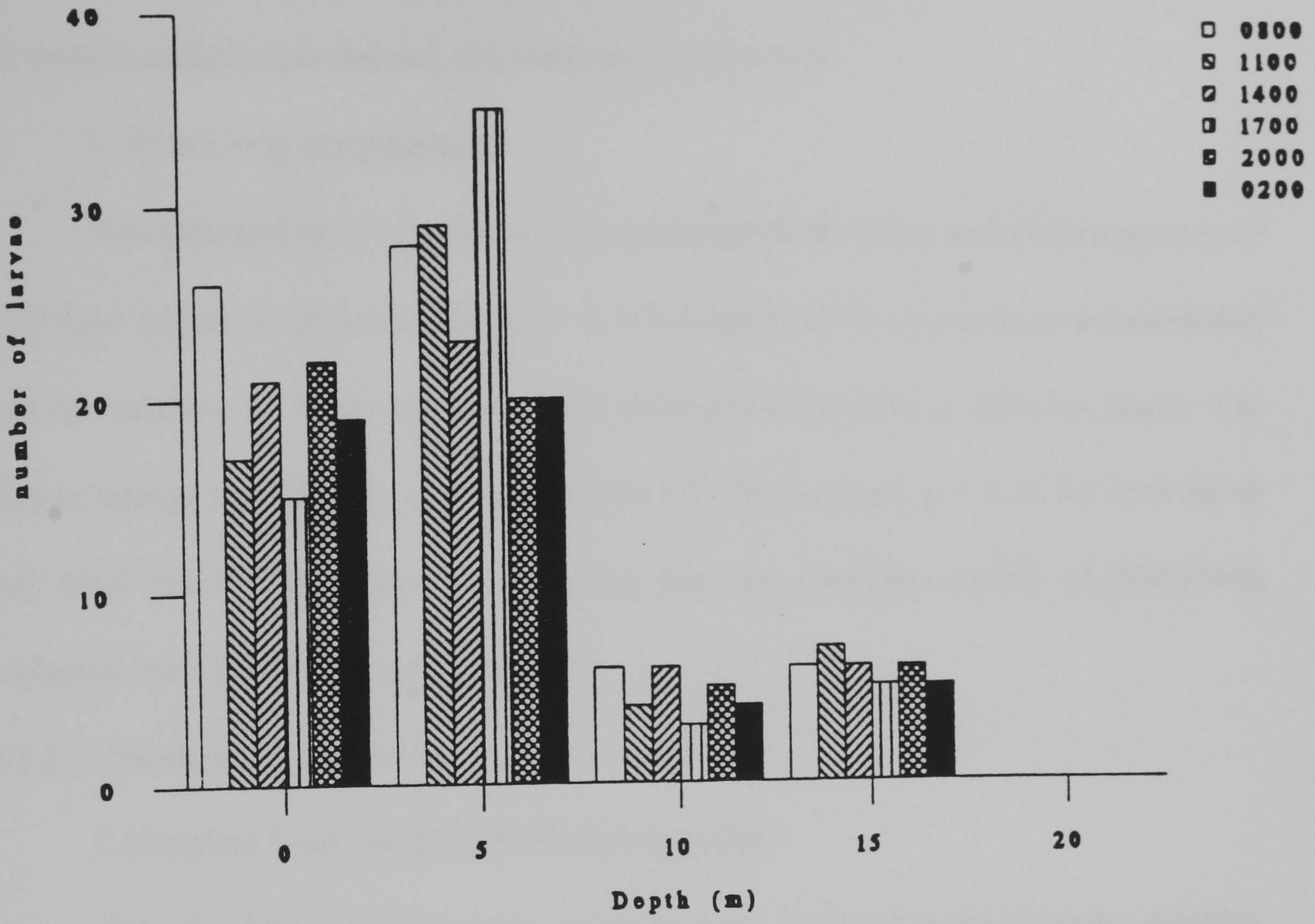
* denotes two week interval between samples instead of the usual week

Table 6.3. Temperature and mean salinity levels over the sampling programme

Sample number	Surface water temperature (°C)	Mean (±1 S.D.) salinity level (‰)
1	8.5	28.67 ± 1.03
2	7.0	28.80 ± 1.10
3	8.0	26.40 ± 1.14
4	8.5	22.20 ± 1.30
5	7.5	25.20 ± 1.92
6	7.0	25.51 ± 1.22
7	5.5	23.20 ± 0.45
8*	6.0	29.83 ± 1.17
9*	5.0	28.17 ± 0.41
10	5.5	26.57 ± 0.53

* denotes two week interval between samples instead of the usual week

Fig 6.1 Total depth profile of *L. salmonis* larvae sampled over ten 24 hour sampling periods.



analysis demonstrated that there was no significant variation in the numbers of larvae found at different depths at different times of the day (ANOVA, $p = 0.998$), and hence for further analysis (see below), the numbers were pooled.

ii. Free-living zooplankton

The distribution of total free-living copepods (both larval and adult stages) over a 24 hour period is shown in fig. 6.2. A two-way analysis of variance demonstrated that the numbers of these members of the zooplankton present at different depths was seen to change significantly over time within a 24 hour period, $p < 0.05$ for both larval and adult free-living copepods, indicating that the plankton profile of free-living copepods has a diurnal/circadian nature.

6.3.2.3. Depth profile of larvae

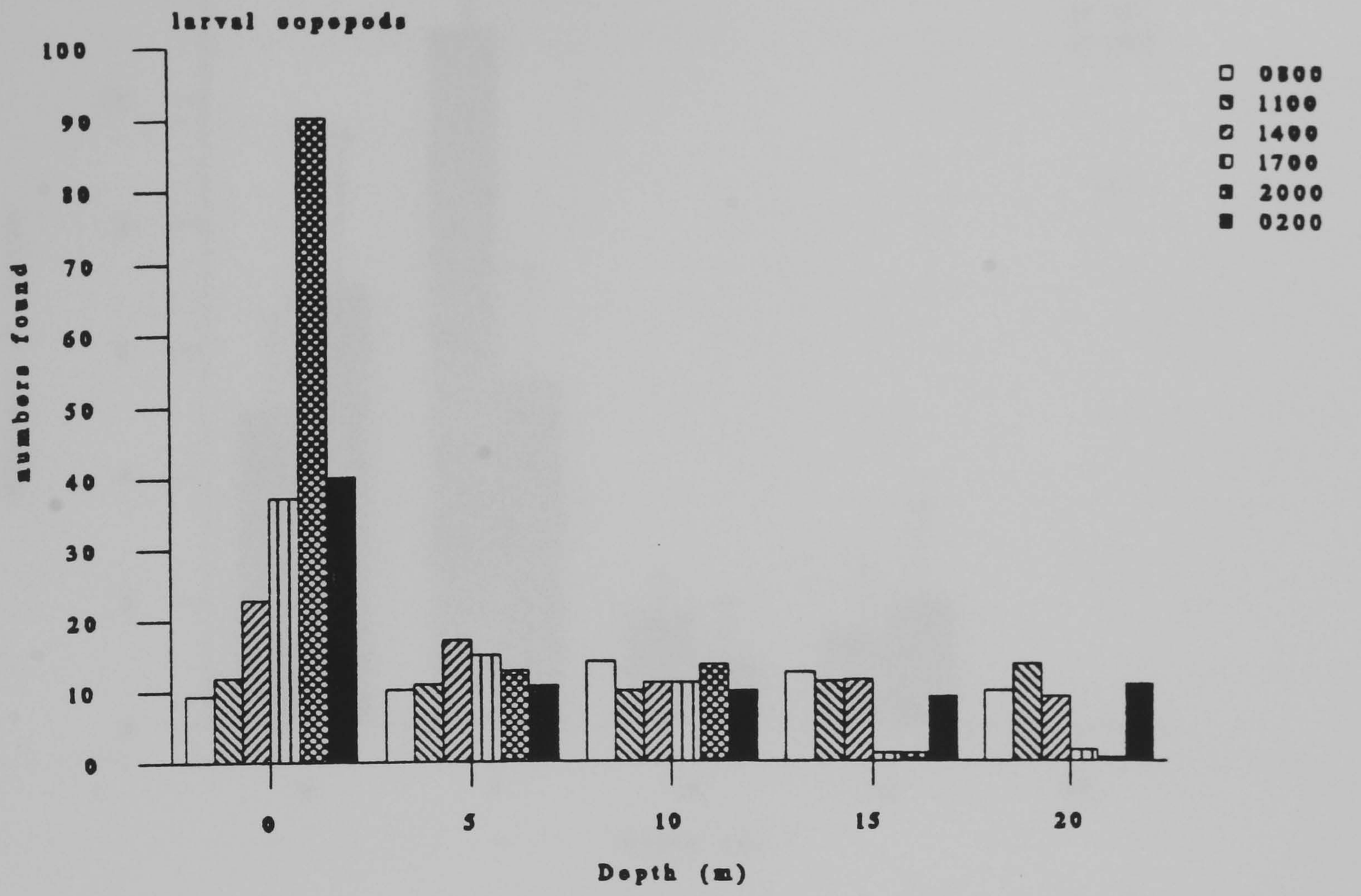
i. Nauplius I and Nauplius II treated separately

Only the first two stages of *L. salmonis* were obtained in the plankton samples, the infective copepodid stage never being found. The distribution of the first and second naupliar stages of the parasite is given in fig. 6.3, which shows graphically the mean numbers found. A t-test on the distribution of both these stages in the water column demonstrated that there was no significant variation between the proportions of each naupliar stage at each depth ($p = 0.91$), and hence the two stages were no longer treated separately.

ii. Naupliar stages combined

The combined depth profile of the two naupliar stages of *L. salmonis* is shown in fig. 6.4. Analysis of variance revealed that there was a significant difference between the numbers found at each of the 5 depths (0, 5, 10, 15 and 20m) sampled ($p < 0.001$), and it was demonstrated that a correlation existed between depth and the

Fig 6.2 Total depth profile for free-living larval and adult copepods sampled over ten 24 hour sampling periods.



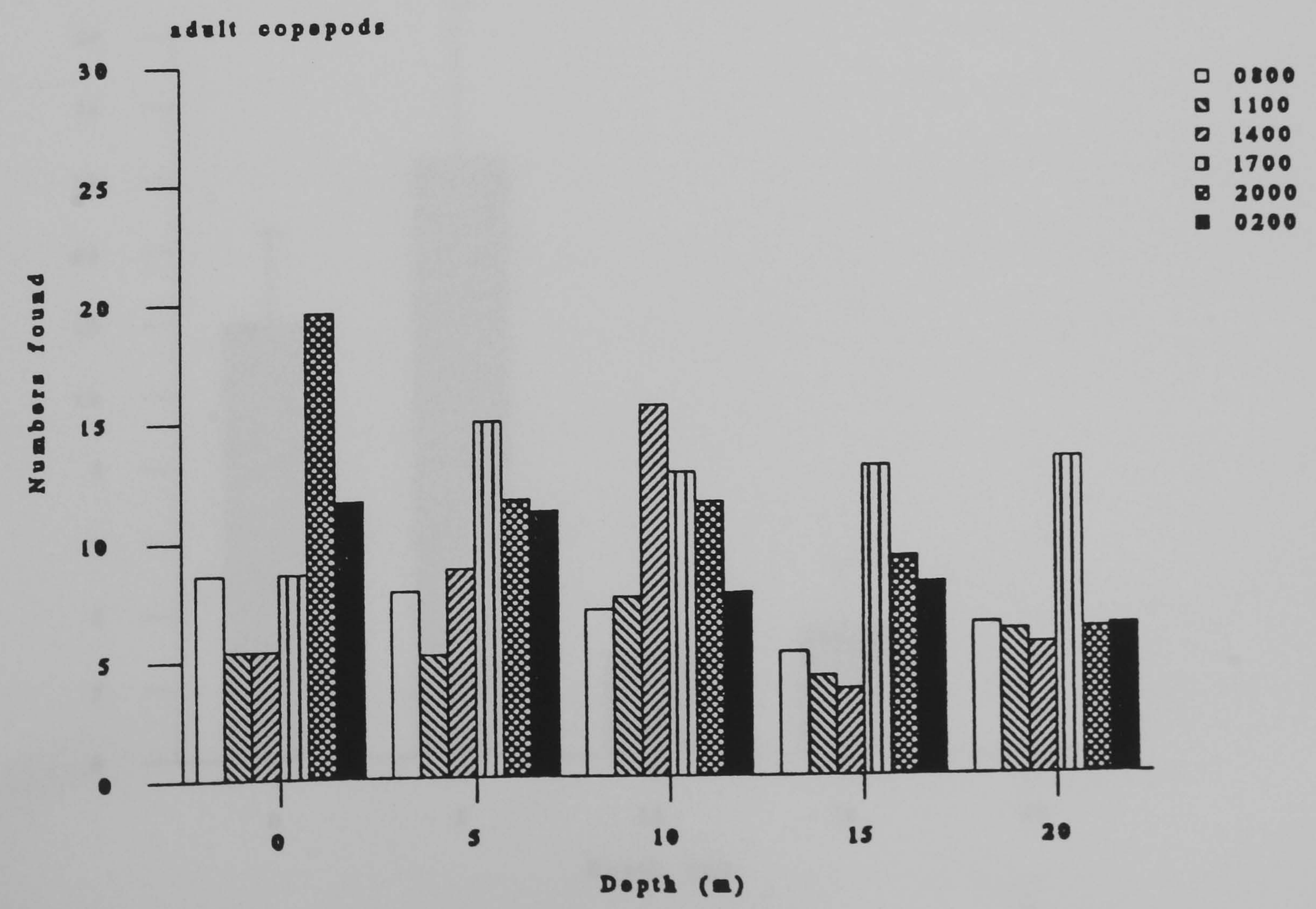


Fig 6.3 Depth profile of the first and second free-swimming naupliar stages of *L. salmonis*

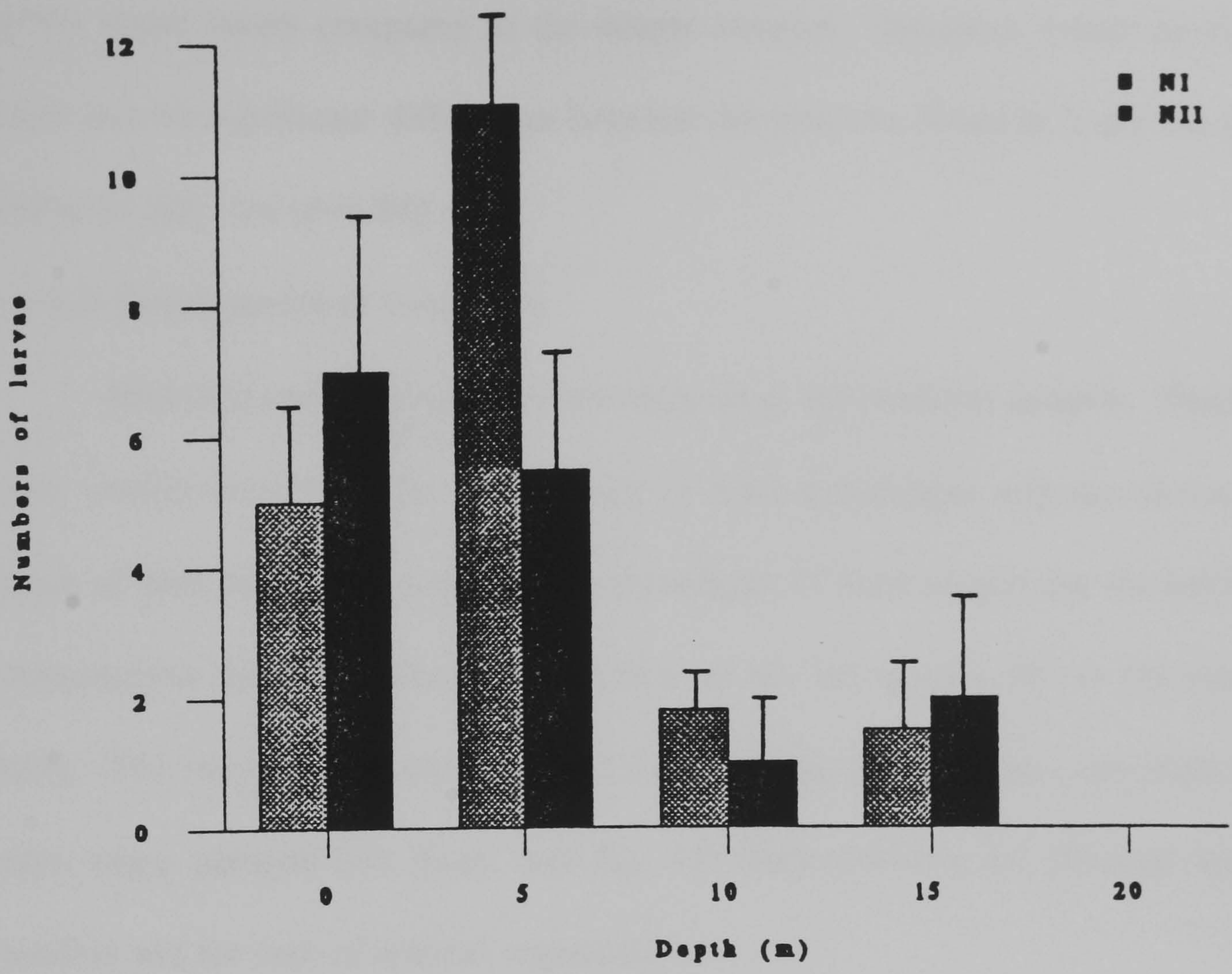
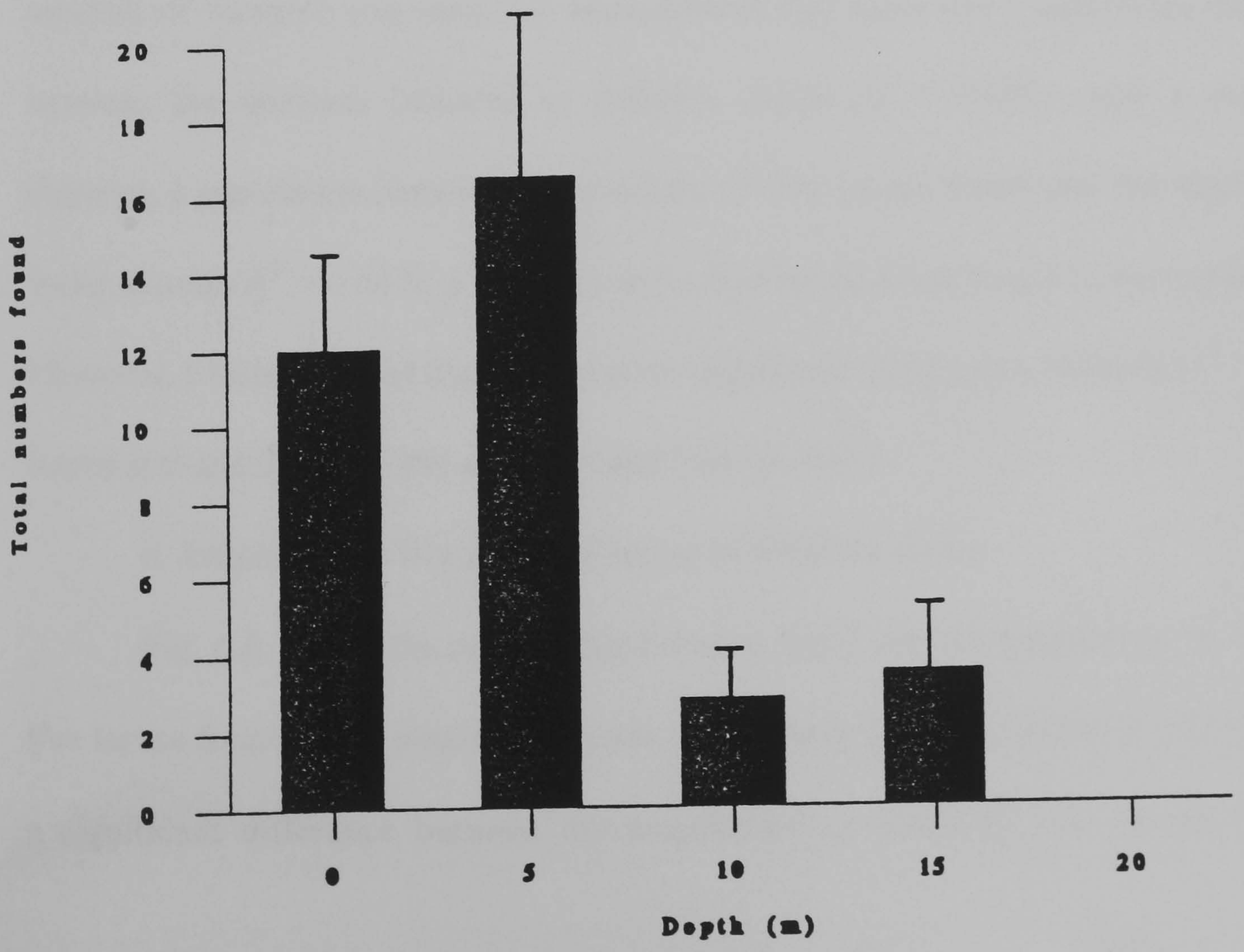


Fig 6.4 Overall depth profile of the first two free-swimming naupliar stages of *L. salmonis*



numbers of naupliar stages found ($r^2 = 0.634$, $p < 0.001$), with more larvae being found in the upper layers compared to the deeper samples. However, t-tests revealed that there was no significant differences between the numbers found at 0 and 5m ($p=0.33$) and at 10 and 15m ($p=0.88$).

6.3.2.4. Depth profile of live larvae

Both live and dead nauplii were obtained in the plankton samples. Dead nauplii were readily recognised by the position of their appendages and the obvious break down of their internal structure. The appendages of dead nauplii are not held in their characteristic upright position seen in live larvae, but quickly fall to the side of the body. This can easily be seen by fig. 6.5 and 6.6, fig. 6.5 showing a specimen that was alive when pumped and fixed, and fig. 6.6. demonstrating the changed appendage position and the lack of internal organisation.

i. Total numbers of live larvae

The distribution of live nauplii in the water column is given in fig. 6.7. Again analysis of variance was used and demonstrated that there was a significant difference between the numbers obtained at different depths ($p < 0.001$), with a regression showing a correlation between the numbers of live larvae found and the depth in the water column ($r^2 = 0.58.6$, $p < 0.001$), more live larvae being found in the upper layers. However, t-tests revealed that there was no significant differences between the numbers found at 0 and 5m ($p=0.48$) and at 10 and 15m ($p=0.29$).

ii. Proportion of live and dead larvae at different depths

Fig. 6.8. shows the relationship between depth and the proportions of dead and live larvae found in the plankton samples. Analysis of variance indicated that there was a significant difference between the proportions of dead and live nauplii found at

Fig 6.5. Live *Lepeophtheirus salmonis* nauplius stage obtained in a plankton sample. The appendages are held in their usual characteristic position (x100 magnification)

Fig. 6.6. Dead *Lepeophtheirus salmonis* nauplius stage obtained in a plankton sample. The internal structure of the larva has broken down (x100 magnification).

Fig 6.7 **Distribution of live *L. salmonis* naupliar stages found in the water column.**

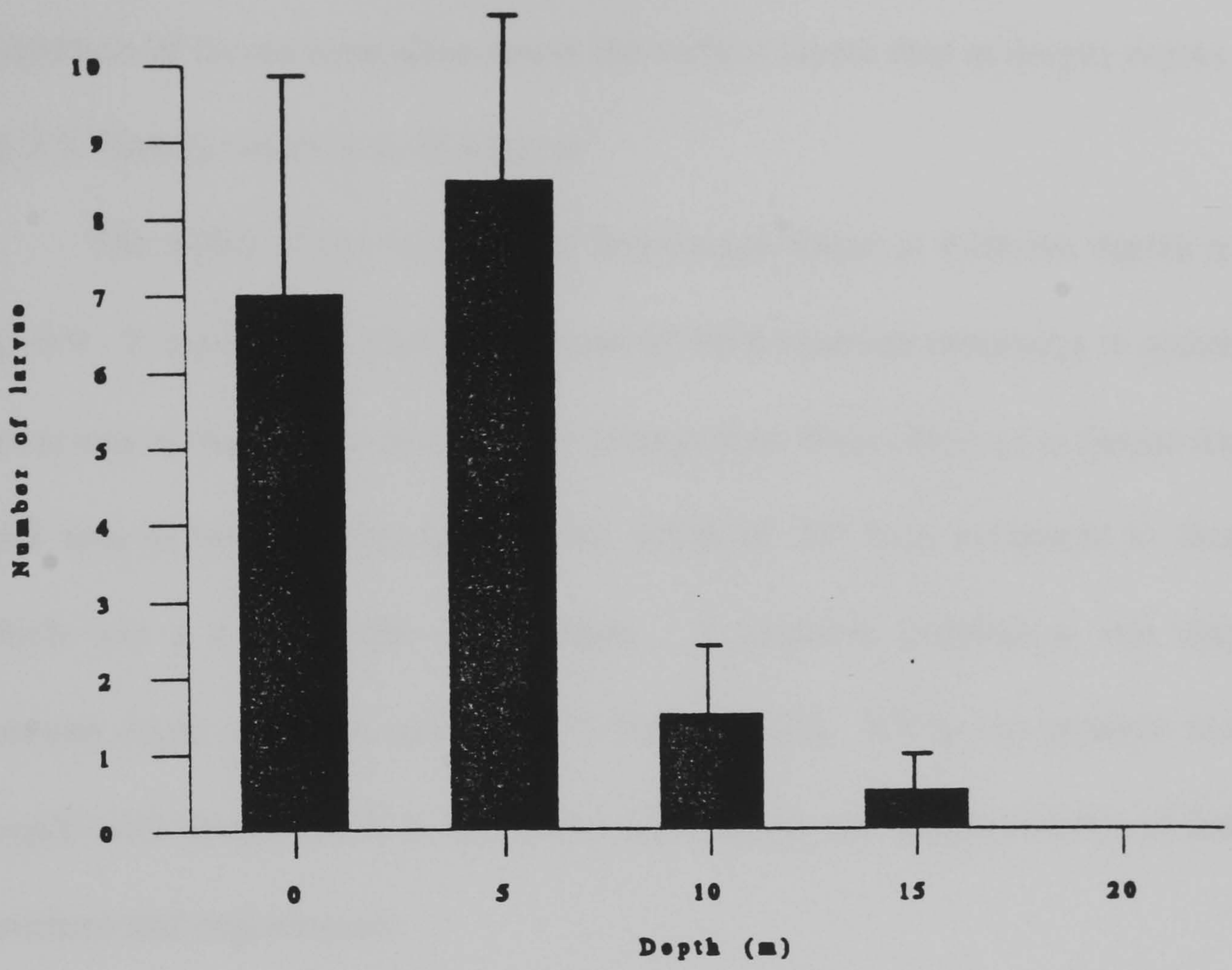
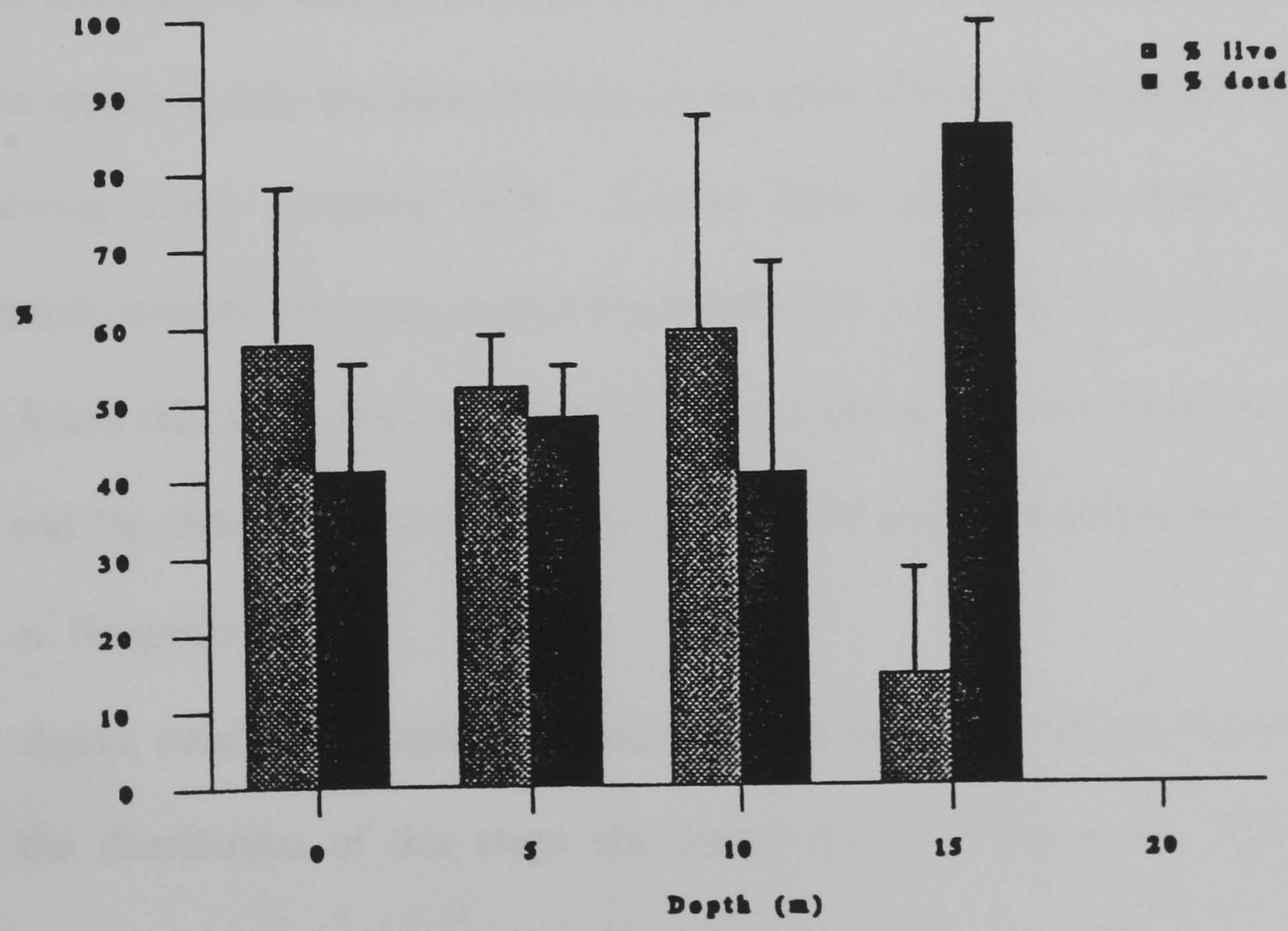


Fig 6.8 Percentage of live and dead *L. salmonis* naupliar stages at different depths in the water column.



different depths ($p < 0.005$), with a correlation being found between depth and the percentage of live and dead stages obtained ($r^2 = 0.277$, $p < 0.005$). Thus a greater proportion of larvae were alive nearer the surface layers than at deeper depths.

6.3.2.5. Energy reserves in live larvae

The width of the lipid area of live nauplii found at different depths is shown in fig. 6.9. It can be seen that the amount of lipid reserves remaining is greater in those larvae nearer the surface of the water column than those obtained at deeper depths. The lipid area in larvae at 0m had a mean width of 101.3μm compared to those at 15m which had a mean width of 60.78μm. A negative correlation was demonstrated between depth and lipid width ($r^2 = 0.70$, $p < 0.05$). It was not possible to stain dead nauplii with Sudan Black B due to the degradation and decomposition of their internal structure and organisation.

6.3.2.6. Numbers of larvae found inside and outside cages

i. Nauplius I

Fig. 6.10 shows the distribution of the first naupliar stage both inside (location (i)) and immediately outside the cages (between the nets and the walkway) (location (ii)) and approximately 2m from the cage on the other side of the walkway adjacent to free-flowing water (location (iii)). Larvae were only found either inside or immediately outside the cages, none being found after a distance of 2m from the nets. It was found that there were significantly more nauplius I larvae inside the cages at both 0 and 5m, than outside the cages (t-test, $p < 0.001$ and 0.005 respectively).

ii. Nauplius II

Again, nauplius II stages were only found inside or immediately adjacent to the cages, the distribution of this stage also being shown in fig. 6.10. There was a

Fig 6.9 Mean width of the lipid reserves of live naupliar stages of *L. salmonis* obtained in the plankton samples and stained with Sudan Black B.

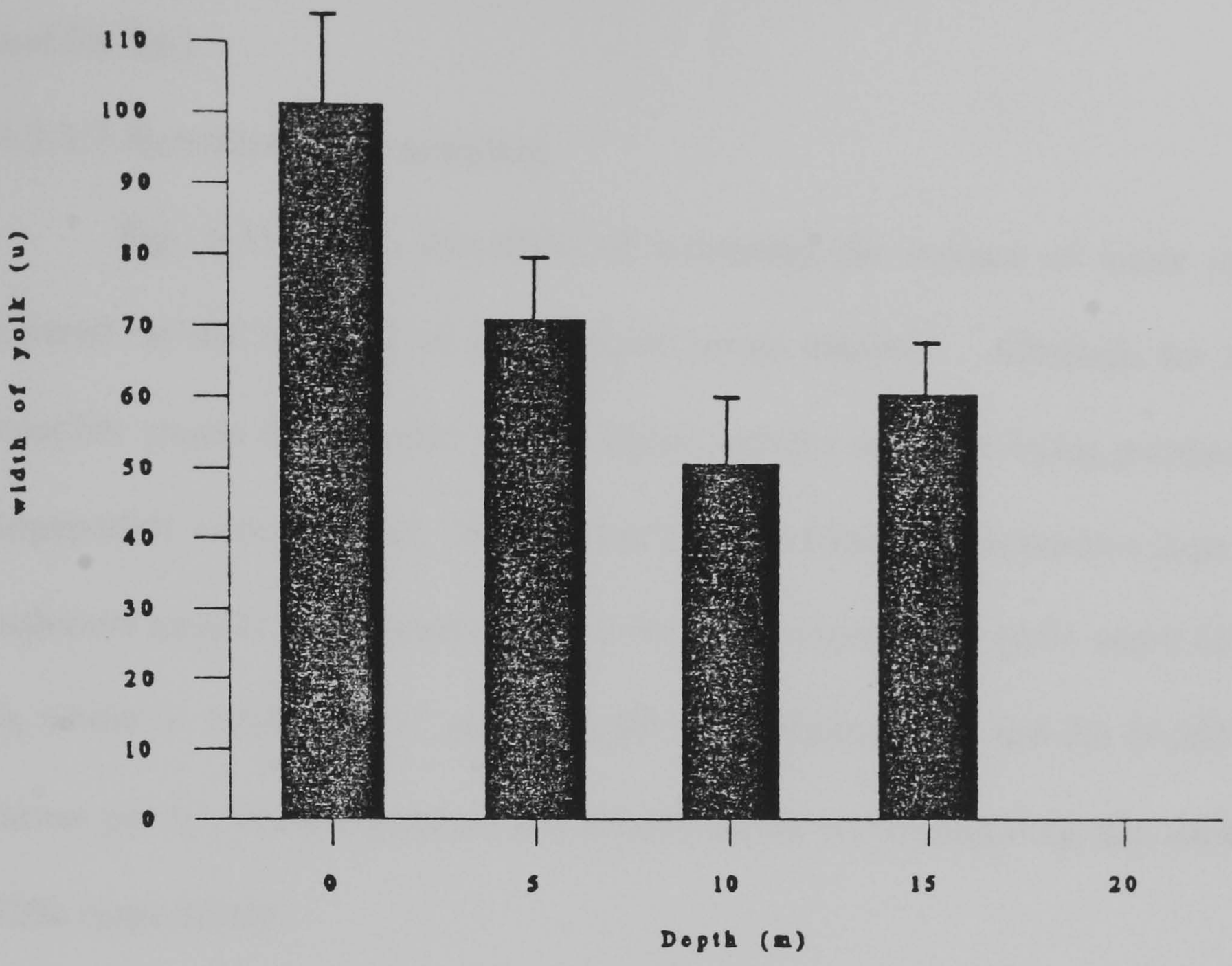
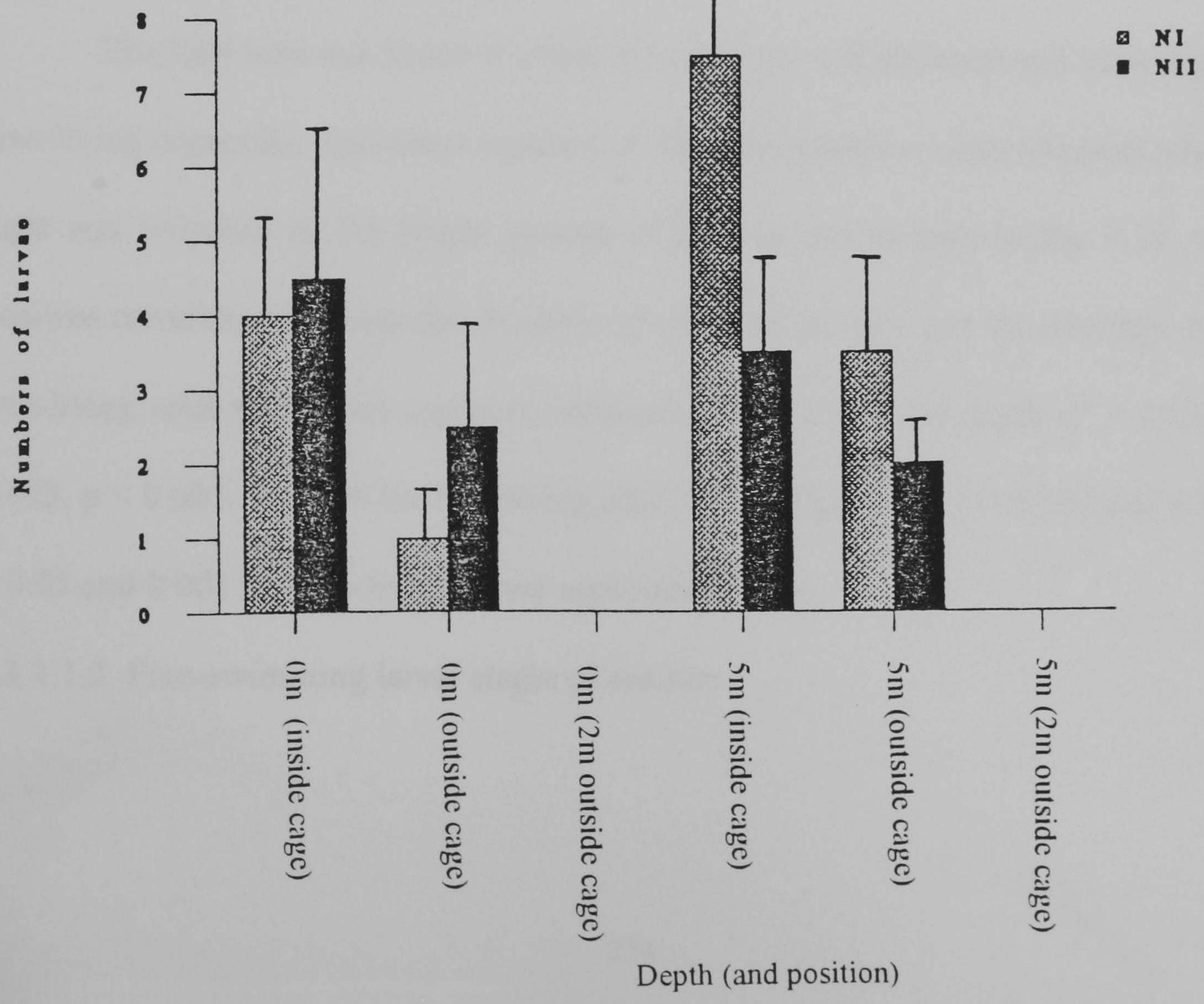


Fig 6.10 Numbers of the first and second naupliar stage of *L. salmonis* obtained at 0 and 5m depth at three different locations: inside the cage, immediately outside the cage, and approximately 2m away from the cage.



concentration of the second nauplius stage inside the cages at 0 and 5m compared to outside, with the differences in these numbers being significant (t-test, $p < 0.05$ for 0m and for 5m).

6.3.2.7. Extended pump sampling

Fig. 6.11 shows the effect of increasing the volume of water pumped and filtered on the numbers of *L. salmonis* larvae obtained. Although the numbers of naupliar stages did increase with a higher volume of water being pumped, again no copepodids were obtained. In 100 litres of water filtered from inside a cage, 1 and 2 *L. salmonis* nauplii were found at 0 and 5m depth respectively (0.01 and 0.02 larvae per l), whilst in 1600 litres 11 and 8 nauplii were obtained at 0 and 5m (0.007 and 0.005 larvae per l). The temperature and salinity levels on the sampling day were 13°C and 32‰ respectively.

6.3.3. Light lure sampling

6.3.3.1. Effect of exposure to a light stimulus

6.3.3.1.1. Free-living plankton

The light lure was found to affect the numbers of both larval and adult stages of free-living copepods. Increased numbers of these zooplankton were obtained when the light was switched on for longer periods of time as can be seen in fig. 6.12, with a positive correlation between the duration of exposure to light and the numbers of both free-living adult and larval copepods obtained at both 0 and 5m depth ($r^2 = 0.910$ and 0.693, $p < 0.001$ and 0.05 for free-living adult copepods at 0m; $r^2 = 0.566$ and 0.889, $p < 0.05$ and 0.001 for free-living larval copepods at 5m).

6.3.3.1.2. Free-swimming larval stages of sea lice

Fig 6.11 **Relationship between the volume of water pumped and filtered and the number of *L. salmonis* naupliar stages obtained.**

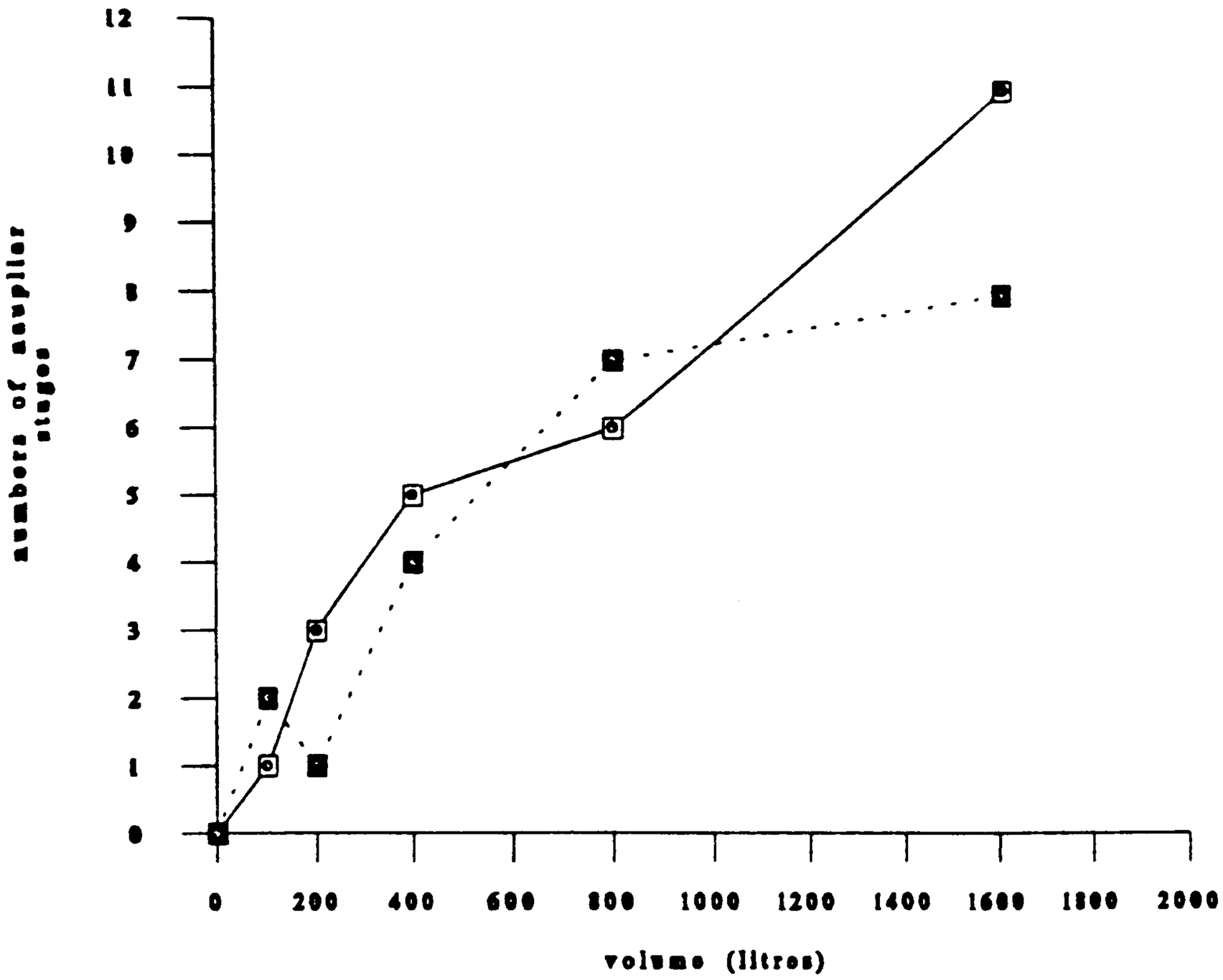
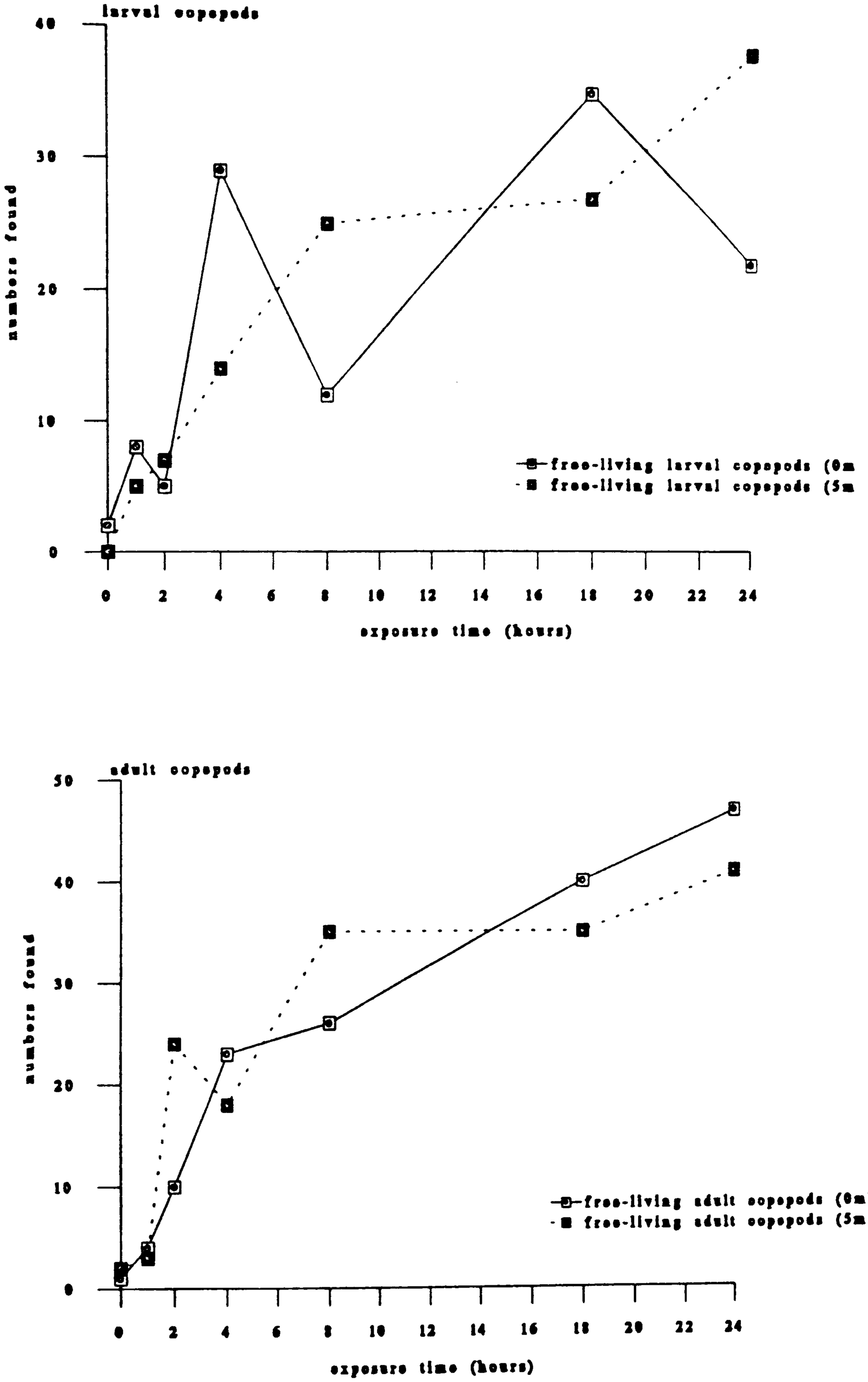


Fig 6.12 **Relationship between time of exposure to a light lure and the number of free-living larval and adult copepods obtained in the plankton samples.**



In contrast to the positive relationship between duration of exposure to light and numbers of free-living copepods obtained, it was found that no correlation existed between the time of exposure to the light lure and the subsequent numbers of sea lice larvae obtained (r^2 = 0.038 and 0.264, p = 0.677 and 0.238 at 0 and 5m respectively), with the levels remaining relatively constant as can be seen in fig. 6.13. Again, only *L. salmonis* naupliar stages were obtained, with the infective copepodid again being absent in the samples. No adult or larval stages of *C. elongatus* were obtained in the light lure samples.

6.3.3.2. Numbers of gravid *L. salmonis* adult females

The mean number of gravid *L. salmonis* females present on each of the light lure sample days is given in Table 6.4. No significant difference existed in the numbers of gravid females present over the light lure sampling period (ANOVA, p = 0.806), and therefore it was not possible to test a correlation between the total number of larvae obtained within each light lure control sample (light switched off) and the number of ovigerous females present on the fish sampled.

6.3.3.3. Temperature and salinity levels

The temperature and mean salinity levels on each of the light lure sampling days are given in Table 6.5. It can be seen that the levels of these two parameters remained relatively constant over the 12 sampling periods ranging from 12.0 to 14.0°C and 29 to 34‰ respectively.

Fig 6.13 Relationship between time of exposure to a light lure and the number of free-swimming naupliar stages of *L. salmonis* obtained in the plankton samples.

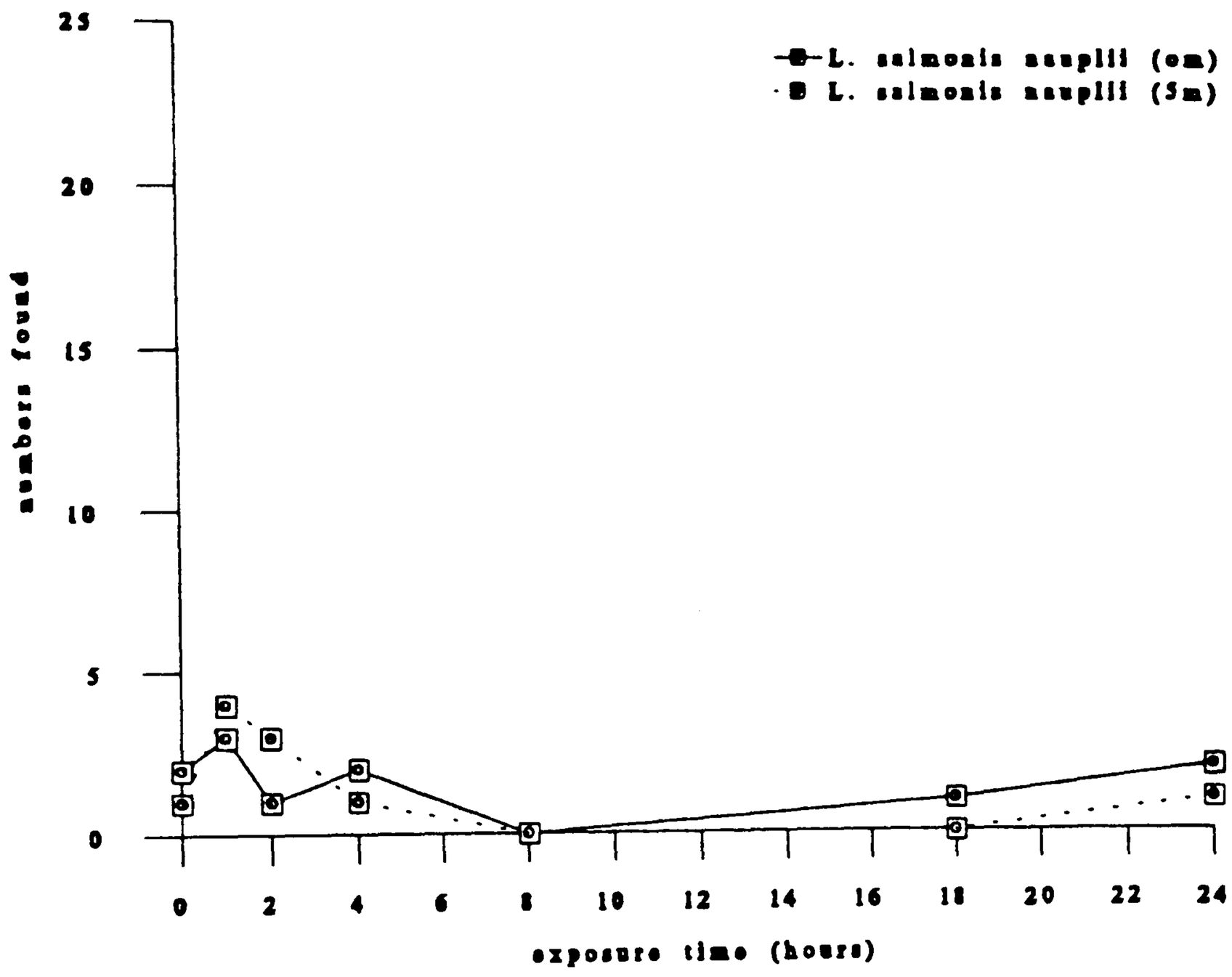


Table 6.4. Mean (± 1 S.D.) numbers of *L. salmonis* and *C. elongatus* gravid adult females present on *S. salar* during the light lure sampling programme

Depth and time	Number of fish examined	Mean (± 1 S.D.) number of gravid females present	
		L. salmonis	*C. elongatus*
0m 1 hour	5	0.8 ± 0.84	0
5m 1 hour	5	1.6 ± 1.67	0
0m 2 hours	5	1.4 ± 1.14	0
5m 2 hours	5	0.2 ± 0.05	0
0m 4 hours	5	0.4 ± 0.55	0
5m 4 hours	5	1.0 ± 1.00	0
0m 8 hours	5	2.0 ± 1.00	0
5m 8 hours	5	2.8 ± 1.92	0
0m 18 hours	5	1.4 ± 0.55	0
5m 18 hours	5	0.4 ± 0.55	0
0m 24 hours	5	0.8 ± 0.84	0
5m 24 hours	5	1.2 ± 1.30	0

Table 6.5. Temperature and mean salinity levels over the light lure sampling programme

Sample number	Surface water temperature (°C)	Salinity level (‰)
0m 1 hour	12.0	32
5m 1 hour	12.5	33
0m 2 hours	12.5	32
5m 2 hours	12.5	34
0m 4 hours	13.0	31
5m 4 hours	12.5	32
0m 8 hours	13.5	33
5m 8 hours	13.0	32
0m 18 hours	12.5	29
5m 18 hours	13.5	31
0m 24 hours	14.0	33
5m 24 hours	13.0	32

6.4. Discussion

This part of the study was undertaken in order to describe the distribution of *L. salmonis* and *C. elongatus* in the water column in the vicinity of a fish farm. From previous chapters, survival, environmental tolerance, activity, infectivity and behavioural characteristics have been elucidated from laboratory based studies. By combining such information with data gained through the sampling programme in the natural environment, it should be possible to gain a broader understanding of the biology and ecology of the species.

6.4.1. Distribution of sea lice in the water column

The naupliar stages of both *L. salmonis* and *C. elongatus* were found in the plankton samples, though the infective copepodid stage was not found. Johannessen (1978) reported the finding of a single wild first nauplius stage of *L. salmonis* in a plankton haul, but apart from this passing comment, at the time of the present study there were no other published reports of the free-swimming larval stages of *L. salmonis* being found in the plankton, and none at all available for *C. elongatus*. Heegaard (1947) did state that the nauplii of *Caligus curtus* were obtained in plankton hauls, but gave no further details on the stages or numbers found.

Since the study, two reports have become available on the occurrence of free-swimming larval stages of *L. salmonis* in the natural environment (Anon., 1996; Costelloe, Costelloe & Roche, 1996). Both are concerned with the horizontal distribution of the larvae in the water column on wein areas off the west coast of Ireland.

No free-swimming stages of the mobile pre-adult or adult stages of *L. salmonis* or *C. elongatus* were found in the present study, although the presence of caligid

parasites has been reported previously. Hardy (1970) stated that although parasitic copepods spend only a limited time in the plankton, *C. elongatus* may often be found swimming freely when in transit from one host to another, and the presence of the adults of this species has also been reported by Scott (1901), Pearson (1904), Neilson, Perry, Scott & Valerio (1987) and Anon. (1996). Adults of other *Caligus* species have also been obtained in plankton samples, with Heegaard (1972) reporting the finding of *Caligus coryphaenae* Steenstrup & Luetken and *Caligus productus* Dana and Ruangpan, & Kabata (1984) reporting *Caligus epidemicus.*

No pre-adult or adult stages of *Lepeophtheirus salmonis* were found in the plankton samples. There has only been one report of pre-adult and adult stages of *L. salmonis* being found in the plankton (Bron, 1994), but this was in samples taken using a light trap, and none was found in samples taken using a pump technique similar to the one used in the present study (Bron, pers. comm.). The lack of reports describing the finding of mobile stages of *L. salmonis* in the plankton compared to those concerned with *Caligus* species appears to indicate that the pre-adult and adult stages of *L. salmonis* are less likely to leave a host and be present in the water column in an attempt to infect another host, than other caligid species. It is not known why such established parasites leave hosts, or indeed whether it is a chosen strategy or if the parasite has simply become detached, but personal observations during the present study indicated that *Caligus elongatus* pre-adult and adult stages were much more active and mobile both over the surface of salmon as well as when held in beakers containing only seawater when compared to their *L. salmonis* counterparts. Pike (1989) also stated that despite both species being able to move freely over the skin of the host, *C. elongatus* is generally the most active of the two and may leave the surface of the

host's body more readily to become temporarily free-swimming. However, according to Bruno & Stone (1990), both *C. elongatus* and *L. salmonis* mobile stages were found to transfer from saithe, *Pollachius virens* L., to Atlantic salmon under experimental conditions by free-swimming in horizontal and vertical planes. However, the percentage of *L. salmonis* pre-adult and adult stages transferring from Atlantic salmon to saithe was lower than that transferring from saithe to salmon perhaps demonstrating that *P. virens* is not a natural host for *L. salmonis*, which is recognised as a parasite largely restricted to members of the Salmonidae of the genera *Salmo, Salvelinus* and *Oncorhynchus* (White, 1942a; Boxshall, 1974b), with parasites perhaps leaving the saithe in order to locate a suitable host. Indeed, Bruno & Stone (1990) reported that no adult *L. salmonis* stages were observed on wild saithe, and suggest that *L. salmonis* cannot mature on a non-salmonid "host." No transfer "rates" were given by the authors for either *L. salmonis* or *C. elongatus* transferring between Atlantic salmon, and therefore the natural tendency of both species to leave the host and/or transfer to another salmon is not known, and cannot be compared to determine if *C. elongatus* is the more mobile species of the two.

Only 7 nauplii of *C. elongatus* were obtained over the initial plankton sampling programme, with these being found in the first two 24 hour samples. No gravid adult *C. elongatus* females were present on the salmon sampled at the site. This is thought to be due to the time period over which the sampling programme took place being between November and January since *C. elongatus* is considered to be a warm water species (Kabata, 1979). The size of the population of *C. elongatus* at the fish farm sampled can be considered to be very small as indicated by the lack of gravid adult females present. This lack may explain the absence of naupliar stages and pre-adult

and adult stages found in the plankton. Due to the decreasing temperatures experienced prior to and over the 11 weeks owing to the onset of winter, it is not surprising that nauplii were only obtained in the first two weeks. The temperature recorded during the first week was 8.5°C and since there were no gravid adult females infecting the fish sampled (no treatment had occurred in the cages sampled), it may be the case that temperatures had fallen to a low enough level to halt development of the species. If this did occur, then hatched nauplii would not complete the life cycle and so fish would not become reinfected. This would then subsequently halt the production of the next generation of nauplii due to no larvae from the previous one completing development.

According to Kabata (1979), *L. salmonis* has a northern circumpolar distribution, and it might therefore be expected that the parasite will be present throughout the winter (Bron *et al.*, 1993c). In the present study, both gravid adult females and nauplii were recorded from fish and in the plankton throughout the sampling programme between November and January. It has been shown (Wootten *et al.*, 1982) that by sampling one site over a period in time, a wave of infection and the subsequent progression of development can be observed. Due to this domination of particular stages at different points in time, it was thought that there may exist a correlation between the numbers of gravid adult females present on the fish and the numbers of larvae found in the water column on the same sampling day, but this did not prove to be the case. This may seem surprising, as it would be expected that when the mean numbers of gravid adult females per fish are at their peak, the number of larvae being produced would be greatest. However, this may have been obscured by the fact that such low numbers of larvae were obtained. For example, during the first sampling period (sample number 1), a total of 35 *L. salmonis* nauplii were obtained immediately

ouside the cages (location (ii)), but it must be remembered that this is an amalgamation of 5 sampling depths and 6 sampling times, making a total of 30 individual samples. This represents a mean of 1.17 larvae per individual sample. For all 300 samples at location (ii), a total of 348 nauplii were obtained. This is a mean of 1.16 per sample and is equivalent to 11.6 larvae m^{-3}. Due to these low numbers, no significant variation was observed in the numbers of larvae obtained between different individual samples, or over the sampling programme. Low numbers of *L. salmonis* larvae were also reported by Anon. (1996). In the main sampling area, Killary Harbour, off the west coast of Ireland, 12 sites were sampled on a varying number of occasions. The six sites where the most larvae (nauplii and copepodids) were obtained from surface plankton tows were pooled, and totalled 135 larvae. Each surface tow (27 at each of the 6 sites) filtered approximately 24,630 litres of water, a much greater volume compared to the 100 litres pumped per sample in the present study. The number of larvae per m^3 in Anon's (1996) study therefore calculates out to be a much smaller figure than the present study, 0.03 compared to $11.6m^{-3}$. However, the six sites sampled by Anon. (1996) are not in the close vicinity of a fish farm, and hence probably explain their low figures.

Another factor affecting the lack of a correlation between gravid females and larvae obtained is that of egg development. The time from egg string production to hatching is not a matter of hours at such temperatures, but more a matter of days. Johnson & Albright (1991) determined *L. salmonis* egg development duration (i.e. time spent from production of the ovisac to hatching) as being 8.6 days at 10°C, whilst Johanessen's (1978) determination of "time to hatch" for the same species was 10 to 14, 25 and 33 to 39 days at 11.5, 9.5 and 9°C respectively. Counting gravid adult

females infecting salmon on a commercial fish farm in order to estimate the mean numbers per fish obviously does not allow the calculation of the time left until hatching begins, as one does not know how long it has been since the egg strings were extruded. Therefore, it may not be feasible to expect a correlation between the number of ovigerous females and the numbers of larvae in the water column at the same point in time, since hatching may not occur for many hours. Also, since ectoparasites have been reported as having an overdispersed distribution on their hosts (Fryer, 1966; Crofton, 1971, Boxshall 1974c; Poulin *et al.* 1991), by only sampling 5 fish, the true infection pattern may not have been observed. Epidemiological studies at the Institute of Aquaculture have however demonstrated that a sample of 5 fish does give a relative abundance and is a suitable sample size (Sommerville pers. comm.).

6.4.1.1. Vertical distribution

6.4.1.1.1. Nauplius I and II profiles

No significant differences were found between the vertical distributions of the first and second nauplius stages of *L. salmonis* and, due to this, these two stages were not further analysed separately. The similar distribution patterns of the NI and NII stages may not seem surprising due to their similar swimming abilities and behavioural patterns seen in Chapter 5. However, it was thought that due to the existence of the moult between these first two stages and the inability of the larvae to swim during this, that the second nauplius stage may have generally been found at lower depths than the first naupliar stage, and it was interesting to note that although only 3 larval stages were obtained that were undergoing the ecdysis from NI to NII, they were found at the deeper depths of 10 and 15m. Also, the second nauplius stage may be expected to be less buoyant due to the depletion of the finite energy reserves in the form of adding to

the hypothesis that the first larval stage would be found nearer the surface than NII larvae. However, as already stated this did not prove to be the case confirming the earlier laboratory results (present study, Chapter 5) which also found no relationship between lipid width and buoyancy. It has been demonstrated that *L. salmonis* nauplii not only exhibit a photopositive response but also a negative geotaxis (present study, Chapter 5), the latter originally described by Bron *et al.* (1993) for the infective copepodid stage. The combination of these two may help to override the effects of the ecdysis giving the similar vertical distributions of the first two free-swimming stages of *L. salmonis* observed in the present study.

6.4.1.1.2. Diurnal rhythm

From the plankton samples taken in the sampling programme, it was possible to analyse the numbers of free-living copepods present at different depths in the water column at different times of the day. Significant variations were observed to exist between numbers of both adult and larval free-living copepods found at different depths over a 24 hour period, with the numbers obtained at the surface (0m) being greatest between the hours of 1700 and 0200 hours, and falling to a minimum between 0800 and 1400 hours. This illustrates a nocturnal vertical migration of both larvae and adult free-living copepods, which is characterised according to Hutchinson (1967), by a single daily ascent, with a minimum depth reached between sunset and sunrise and maximum depth attained during the day. Most zooplankton species undergo daily patterns of vertical migration with the most common type being either nocturnal or twilight migrations (Forward, 1988), and a lesser type being a reverse migration. Adult copepod species have been previously reported as undertaking nocturnal migrations, as appears to be the case in the present study, with both *Acartia tonsa* Dana (Stearns,

1983) and *Mesocyclops edax* S.A. Forbes (Woodmansee & Grantham, 1961) demonstrating this vertical pattern. Forward (1988) attributed the behaviour of crustaceans exhibiting nocturnal vertical migrations to their photoresponses and photobehaviour being adapted to lighting conditions at times of migration at sunrise and sunset. This study has shown that the photoresponses and behaviour of *L. salmonis* nauplii and copepodids are dissimilar to those of free-living zooplankton with larvae showing a photopositive response to both artificial and simulated natural angular light, in contrast to zooplankton, which tend to demonstrate a "false positive" response to simulated light, but a reverse negative geotaxis under angular conditions (Forward, 1988).

It may therefore be expected as a result of photoresponses, that *L. salmonis* larvae would undertake a daily reverse migration, which consists of an ascent to a minimum depth during the day and descent to a maximum depth at night (Hutchinson, 1967). However, the results from the present study indicated that there were no significant differences in the depth profiles of *L. salmonis* nauplii at different times of the day, indicating that no vertical migrations of any kind occur. This seems surprising, since a study by Huse *et al.* (1990) appears to indicate that light concentrates the infective copepodid stage at least of *L. salmonis* in the surface layers. The authors stated that Atlantic salmon kept in shaded pens had reduced infection rates of the parasite compared to those fish held in uncovered pens, with fish not showing any difference in behaviour with respect to their vertical distribution in the two types of cages. This suggests that shading disperses any concentration of larval stages, explaining the decreased infection levels observed by the authors. However, significant variations in infection rates were not observed for all of the cages, with a number

showing no differences. Therefore the results of Huse *et al.* (1990) require substantiation before it can be concluded from their study that *L. salmonis* larval stages are concentrated near the surface due to just their photoresponses. Of course, this may be due to the low numbers of larvae obtained in the present study masking any pattern that may exist, but the negative geotaxis exhibited by the naupliar stages (present study, Chapter 5) and the presence of buoyant lipid reserves may, on the other hand, combine to keep these first two free-swimming stages in the surface layers throughout the night without the aid of a photoresponse. The report by Anon (1996) concluded that a migration of *L. salmonis* larvae (they did not distinguish between the stages) did occur with greater numbers of larvae being obtained in the surface (0m) just before highwater compared to 5m. However, there was no statistical evidence for this, and the conclusions appear to be unsubstantiated. Indeed, the authors themselves state that they drew this conclusion from one site only, a second site tested showing no distribution differences. Heuch, Parsons & Boxaspen (1995) studied the distribution patterns of *L. salmonis* nauplii and copepodids held in enclosures in the sea at depths between 0 and 12m. They reported that nauplii only showed small differences in their position in the water column between day and night. However, they did not statistically test their results for these stages, and hence their findings require corroboration. Heuch *et al.* (1995) described a diel vertical migration in copepodids held in these enclosures, with the larvae decending at night in the absence of light. They reported that, on average, the copepodid showed a downwards migration at night of between 1.3 and 1.6m, from a depth of around 2.0m to approximately 3.5m. If such a migration does occur, then even if copepodids had been obtained in the present study, such a pattern would have probably not been observed as sampling took place only at 5m intervals. However only

2 out of the 4 experiments performed by Heuch *et al.* (1995) on the copepodid's distribution were statistically significant at the $p < 0.05$ level, and this therefore makes their observations tenuous.

Since the infective copepodid stage was not obtained during the course of the sampling programme, its distribution in the water column can only be speculative. Like the naupliar stages, it shows a positive photoresponse and a negative geotaxis (Bron *et al.*, 1993; present study, Chapter 5), which may combine to keep it in the surface layers, both during the day and the night, and thus having a similar vertical distribution pattern to the naupliar stages. There would only be an advantage of possessing a vertical migration pattern if the host also exhibited one, so maximising spatial overlap between host and parasite. Poulin, Curtis & Rau (1991) suggested that due to the behaviour of the brook trout, *Salvelinus fontinalis*, the fish being quiescent at night and spending more time near the bottom of rivers, the copepodid stage of the ectoparasitic copepod *Salmincola edwardsii* may maximise its chances of infection by being distributed at the bottom of the water column, a feature which was seen in laboratory observations. However, the authors do not suggest that the infective copepodid stage changes its distribution during the day (i.e. exhibiting a reverse migration) by ascending to where the fish are distributed during daylight hours. If stages such as the copepodids of *L. salmonis* and *S. edwardsii* were to perform vertical migrations, it would no doubt cause a faster depletion of their finite energy reserves due to the traversing of the water column once daily (assuming a descent is performed by passive sinking and not active swimming), which may provide another reason as to why such migrations are not observed for *L. salmonis*. Schram & Anstensrud (1985), however, found that the copepodid stage of *Lernaeenicus sprattae* was most frequently

recorded in the plankton at 0 to 90cm depth during the night, suggesting a nocturnal vertical migration, in contrast to the findings of the present study with respect to *L. salmonis* nauplii. However, laboratory studies by the authors demonstrated that the infective stages of *L. sprattae* exhibit a photonegative response at high light levels, with a positive response only being observed at low levels of illumination, in similarity to many free-living zooplankton (Forward, 1988), but in contrast to the free-swimming larval stages of *L. salmonis*. The authors attribute this behaviour of *L. sprattae* to the behaviour of its host, the sprat *Sprattus sprattus* L. The sprat has been found to undertake diurnal vertical migrations during the summer, coming close to the surface during the night (Baaken, 1973), and it therefore appears that the parasite mimics this behaviour to increase its spatial overlap and infection chances. However, during the winter, according to Schram & Anstensrud (1985), the sprat avoids upper water, spending most of its time in deeper water. It is not known though as to whether the *L. sprattae* changes its distribution also, remaining deeper and not undergoing vertical migrations though this seems unlikely. Huse & Holm (1993) found that the vertical distribution of farmed Atlantic salmon kept in deep pens changes seasonally, with the fish remaining at around 5m during winter and spring, but preferring deeper waters when not feeding during the summer. It may be the case that *L. salmonis* larvae remain in the upper layers over the whole year, as seen in the present sampling programme, maximising their chances of infection from autumn through to spring, but that their chances of infecting wild salmon in the summer are reduced, due to the changed behaviour of the fish. However there is little information on the vertical distribution of wild Atlantic salmonids in coastal waters (Heuch *et al.*, 1995) and it may not be

possible to extrapolate the behaviour of wild fish from the results of Huse & Holm (1993).

6.4.1.1.3. Distribution of live larvae

It was found in the present study that an inverse correlation existed between depth and numbers of *L. salmonis* naupliar stages obtained in the plankton samples, indicating that greater numbers of larvae are present in the surface layers of the water column than at deeper depths. Huse & Holm (1993) found that Atlantic salmon preferred to be deeper in the water column during the summer avoiding high light levels and maximum solar elevation, when light penetration in the sea reaches its highest level. During these times it was observed that salmon held in deep pens (20m in depth) had significantly lower lice burdens in the form of *L. salmonis* compared to those kept in shallow pens (6m in depth). This suggests that salmon that are able to reach deeper depths (i.e. those held in 20m cages) have a decreased chance of becoming infected and thus the spatial overlap between host and parasite must be greatly reduced. Results from the present study appear to confirm this, since greater numbers of nauplii were found at depths of 0 to 5m compared to numbers obtained between 10 and 20m. Obviously it may be argued that it is the distribution of the copepodid stage that is critical, since this is the infective stage. However, since this stage was not obtained in the sampling programme, its distribution can only be hypothesised. Due to its positive phototactic and negative geotactic nature, as already stated, it can be expected that the distribution of the copepodid stage will be similar to that of the naupliar stages, and will be present in greater numbers nearer the surface.

Within the numbers of nauplii found, it was also demonstrated that a relationship existed between the proportion of live and dead nauplii obtained and depth,

with a greater proportion of dead nauplii found at deeper depths. This is not surprising, as dead nauplii will continue to passively sink in the water column, and so will not remain in the surface layers like their live counterparts. If this pattern of distribution is extended to the copepodid stage, this would mean that fish near the surface layers are not only exposed to a greater number of infective stages, but also a greater proportion of live stages, and therefore it is not surprising that Huse & Holm (1993) reported that fish in shallow pens had greater infection rates of *L. salmonis* than those allowed the freedom to go deeper in the water column. Heuch *et al.* (1995) also concluded that the presence of copepodids "gathering" in surface layers causes the high lice loads on farmed salmon .

As well as increased numbers and higher proportions of live *L. salmonis* nauplii being obtained nearer the surface, it was found in the present study that the amount of energy remaining in the live naupliar stages (measured as the width of the lipid "sac") was greater in those at 0 and 5m compared to those at 10 or 15m. Again this is not unexpected since, to remain in the upper layers of the water column requires energy expenditure in the form of active swimming. As shown in Chapter 4, copepodids of *L. salmonis* switch to a non-swimming strategy if they have not located or successfully infected a host after a certain point in time in order to conserve their remaining energy. Such a strategy may also occur in the naupliar stages, allowing them to still enter and complete moults and continue their development. However, it may be the case that they carry on swimming until there is not enough energy remaining to allow active swimming to continue. Either way, this would result in nauplii with low amounts of energy reserves to continue to passively sink in the water column and thus they would be found at deeper depths compared to those nauplii with relatively plentiful supplies.

Presumably such a distribution will also occur for the infective copepodid stage due to the activity patterns demonstrated in Chapter 4. This would mean that fish present near the surface layers, would not only be exposed to greater numbers of live larvae, but also that any copepodids present would have greater energy reserves, allowing them to be more active and therefore more infective (present study, Chapter 4), explaining the observations of Huse & Holm (1993).

6.4.1.2. Horizontal distribution

Three lateral points at 0 and 5m depth were sampled in the present study to determine the effect of the nets on the horizontal distribution of the free-swimming larval stages of *L. salmonis*. These points were (i) inside the cages, (ii) immediately outside the cages between the nets and the walkway and (iii) on the other side of the walkway immediately adjacent to free-flowing water. No larvae were found in the third location adjacent to free-flowing water with this point being approximately 2m from the nets. Larvae were, however, obtained immediately outside the cages, though lower numbers were recorded for both the first and second nauplius stage at this location compared to the numbers obtained inside the cages. This difference in numbers inside the cages compared to outside was more pronounced for the NI stage. This is probably due to the constant seeding of NI larvae within a cage from gravid adult females present on the host population, and the dispersal that will occur during the time taken for development to the second stage, which at 10°C takes approximately 36 hours (present study, Chapter 3). However, as already stated, a higher concentration of both stages was found within the cages. It is highly unlikely that these first two stages possess any host finding capabilities, and this concentration is most probably due to a retentive characteristic of the nets. Braaten & Stigebrant (1991) found that current

speeds in Atlantic salmon pens in Norway were reduced by one to two thirds when compared to undisturbed currents outside the farms. Huse *et al.* (1990) considered that fouling of net pens by algae and invertebrates represented an important problem in net pen operations due to decreased water exchange. In support of this, personal observations of the nets at the fish farm sampled revealed that many were covered with fine filamentous algae which would no doubt have reduced the flushing of the cages and cause the observed concentrations of *L. salmonis* larvae observed.

The amount of larval dispersal that occurs for *L. salmonis* is at present, of prime importance considering the controversy concerning the collapse of wild sea trout stocks on the west coasts of Ireland and Scotland. The two opposing arguments are that this collapse is caused by sea lice infections originating from farmed salmon, a view that has been much publicised by the national press (Clover, 1994; Wilkie, 1995), and the other that fish farming has in no way contributed to such collapses. Already there has been a published study attempting to establish a link between the production of *L. salmonis* NI stages from farmed and wild salmon with infection rates seen at a later date on wild sea trout on the west coast of Ireland present in the same embayments (Tully & Whelan, 1993). Without knowing the dispersal dynamics of the larvae this seems a large and indeed dangerous step to take, as such correlation's may influence governmental decisions over the emotive debate regarding the future of fish farming without constituting solid scientific evidence.

Results from the present study appear to indicate that either a large dispersal of larval stages occurs almost immediately outside the confines of fish farm cages, with no naupliar stages being found 2m away from the nets, or that larvae remain within the nets. A study performed in Ireland (Anon., 1995) found *L. salmonis* larvae 200m from

a fish farm, but stated that numbers obtained had dropped by 97.7%. The report by Anon. (1996) also found decreased numbers outside cages (up to 100m away) compared to tows within the cages. Costelloe *et al.* (1996) described an inverse correlation between the distance from a fishfarm and the numbers of *L. salmonis* larvae (nauplii and copepodids) obtained in plankton tows. The most plausible explanation of the low numbers of larvae obtained outside the cages in the present study is a combination of the retentive nature of the nets (also suggested by Costelloe *et al.*, 1996) and the dispersal that will occur immediately outwith the cages. Due to the extremely low numbers of larvae obtained in the present study in each individual sample, it is not surprising that following dispersion, no nauplii were found almost immediately adjacent to the cages next to undisturbed flowing water. No details are available concerning the sampling programme performed in Ireland by Anon. (1995), and it may be the case that much larger and more numerous samples were taken, in which case greater numbers of larvae would be expected to be obtained both around the site and at distances away from the site. Anon. (1996) did find *L. salmonis* larvae 100m away from the cages in surface plankton tows. The volume of water filtered by in the study was far in excess of that pumped in the present studt, 24,630 litres compared to 100 litres, and the number of larvae found were on average less than 1.00 m^{-3}. At 1 km from a fish farm, Costelloe *et al.* (1996) found a maximum concentration of 0.4 larvae m^{-3}, also after filtering almost 25,000 litres. These reports clearly show that a massive volume of water needs to be filtered in order to find the larvae once they are outside the cages of a fish farm.

From these studies, it may therefore be assumed that a massive amount of dispersion must occur. Schram & Anstensrud (1985) found that the majority of *L.*

sprattae larvae were found in the inner part of the Oslofjord, with much lower numbers being obtained in the middle and outer fjord. The lack of numbers found in these parts was attributed by the authors to current flow, with the inner fjord demonstrating a lack of horizontal water flow, and thus not dispersing the larvae, but allowing them to remain relatively concentrated. Whitfield *et al.* (1988) reported that the dispersal of *Lernaeocera branchialis* would be affected by the current-induced movements of the nauplii and copepodid stages. It is therefore clear that until the effects and patterns of dispersion of *L. salmonis* are understood, it will not be possible to determine the actual value and contribution of fish farms as a source of sea lice infection for wild fish.

6.4.1.3. Absence of the copepodid stage

No infective copepodid stages of either *L. salmonis* or *C. elongatus* were obtained during the sampling programme. Although this can be explained for *C. elongatus* in terms of the season over which the sampling took place, with this parasite probably no longer constituting a reproducing population on the farm, as observed by the lack of gravid adult females present, *L. salmonis* was found to be present over the whole sampling period. Both ovigerous females and naupliar stages were recorded infecting fish and in the water column respectively, and the lack of copepodid stages obtained is frustrating. Anon. (1996) did find 128 *L. salmonis* copepodids in plankton tows in a number of bays off the west coast of Ireland, a mean of 0.57 per sample. However no details on either the volume of water filtered or on the vertical or horizontal distribution is available. The distribution and ecology of this important stage is therefore still unknown and will consequently be purely speculative.

However, there are a number of potential reasons that may explain the lack of *L. salmonis* infective stages in the present study. Firstly, over the sampling programme,

low temperature and salinity levels were experienced at the site sampled due to the prevalent weather conditions. Temperature was found to range between 5 and 8.5°C, and salinity, 22 to 29‰. The low salinities were primarily caused by heavy rainfall and the subsequent run-off from the surrounding hills and increased river flows into the small bay where the farm was sited. It has been demonstrated in the present study (Chapter 3) and in previous published reports (Johannessen, 1978; Johnson & Albright, 1991) that both low temperature and salinity can reduce or even halt the development of *L. salmonis*.

In the present study (Chapter 3), it was demonstrated that egg strings hatched in salinities of 20‰ or below either did not hatch, or hatched, but development to the infective copepodid stage was not reached. Even at salinities of 25‰, developmental success to the copepodid stage was found to be very low, with only 0.03% of eggs successfully reaching this stage. Johnson & Albright (1991) also demonstrated that *L. salmonis* egg strings held in low salinity water failed to successfully develop, with only 0.01% of eggs becoming active copepodids at 25‰. Although it was shown in Chapter 3 that copepodids are more tolerant of environmental extremes such as salinity compared to the earlier more sensitive naupliar stages, copepodids were able to tolerate salinities down to 20‰ without their longevity being significantly effected. If the naupliar stages are exposed to such concentrations, development will not occur to this stage. Schram & Anstensrud (1985) also demonstrated that nauplii of *L. sprattae* were more sensitive to extreme environmental conditions than the copepodid stage, and that since development did not occur below 15‰, the parasite would be absent in certain hydrographic areas such as the Baltic Sea due to its low salinity barring development. Johnson & Albright (1991) suggested that *L. salmonis*, like *L. sprattae* may be

excluded from low salinity areas due to the reduced hatching success and survival of the early stages, and since low salinities were experienced during the sampling programme, it may be the case that development of the free-swimming larval stages of the parasite was compromised, and hence the lack of copepodids obtained.

It has also been suggested that the level of infection of Atlantic salmon with *L. salmonis* is inversely related to rainfall and river discharge (White, 1942b) with this usually being assumed to be due to the reduced developmental success described above. However, another factor that may help to explain the lack of copepodids obtained in the present study may be the fact that increased rainfall and associated run-off which may increase the flushing and dispersal of larvae from the fish farm site. Such an increased run-off and river flow was observed over the sampling period due to heavy rainfall and Gaines & Bertness (1992) demonstrated a correlation between settlement rates of the larvae of the barnacle *Semibalanus balanoides* L. and flushing times indicating that, with increased flushing, there was an associated decrease in settlement in a specific area, no doubt caused by the dispersal of the larvae.

Temperature has also been shown to have a significant effect on the development of the larval stages of parasitic copepods. In the present study (Chapter 3), it was found that development to the copepodid stage of *L. salmonis* was reduced at 7.5°C and halted at 5°C. Pike *et al.* (1993) showed reduced developmental success for *C. elongatus* at 5°C, with higher mortalities of naupliar stages causing a subsequent decrease in the numbers of copepodids obtained. Indeed Kinne (1970) stated that at low temperatures, shedding of the cuticle in some crustaceans may be blocked. Temperatures ranging between 5 and 8.5°C were experienced over the sampling

programme, and these combined with the effects of salinity already discussed may have caused the lack of *L. salmonis* copepodid stages obtained.

However, later repeated samples in high salinities and optimum temperatures yielded no copepodids. An alternative explanation therefore may be that there are normally reduced numbers of copepodids present in the water column compared to the earlier stages. Johnson *et al.* (1993) reported an average of 16% of *L. salmonis* eggs within egg strings attaining infective status, whilst Johnson & Albright (1991) found this figure to be 26.8% in 30‰ in flowing water. In the present study (Chapter 3), 18.33% of eggs were found to successfully complete development to the copepodid stage, and due to constant mortalities of the early stages, it is clear that the proportion of each subsequent stage will be much less than the first naupliar stage. From the plankton sampling programme the number of NII stages obtained was less than the number of NI stages, 145 compared to 193 respectively, and it can be assumed that the number of copepodids in the water column will be an even lower figure, especially as the time taken to reach this stage from hatching (96 hours at 10°C) would allow more time for dispersal. Indeeed, Costelloe *et al.* (1996) reported that the ratio of copepodids to nauplii increased in surface tow samples taken further away from the fish farm and that this was due to development (of nauplii to copepodids) and to natural mortality. They also reported that only low numbers, 0.28 copepodids m^{-3}, were obtained The mean number of nauplii obtained in the present study immediately outside the cages at 0m depth was 20 larvae m^{-3}. It may be the case that greater volumes of water needed to be sampled in the present study in order to obtain the copepodid stage in the plankton, taking into account their reduced numbers and density due to mortality and dispersal, and for this reason the extended sampling programme

took place. The temperature and salinity levels during this remained high, 13°C and 32‰ respectively, so negating the possibility of reduced developmental success already discussed. However, again no copepodids were obtained, despite a direct relationship being found to exist between numbers of nauplii obtained and the volume of water pumped. In the light of the recently available studies by Anon. (1996) and Costelloe *et al.* (1996), where an even greater volume of water pumped resulted in very low numbers of larvae being obtained, it is now clear that a massive amount of water needs to be sampled in order to obtain the larvae. In the present study it was difficult to pump a greater volume of water than 1600 litres due to the drain on the battery (the power source had to be portable).

Dispersal and environmental conditions may not be the only explanations as to why no copepodids were obtained in the present study and only low numbers were obtained by Anon. (1996) and Costelloe *et al.* (1996). One potential explanation may be the simplest one of all. Once the moult has occurred to the copepodid stage, the parasite is immediately ready to infect, and it can be assumed that it will do this at the earliest available opportunity. *L. salmonis* copepodids developed from eggs produced by females present on a host population within a fish farm will have a massive number of hosts available in high densities. Since there occurs a concentration of naupliar stages within the cages due to the retentive effect of the nets, this is probably also the case for the copepodid, and so once moulted, it will almost immediately infect one of the thousands of hosts available, explaining perhaps why no copepodids are obtained in the plankton. If this is the case, then it will always prove difficult to elucidate the distribution of the copepodid stage in the natural environment, and that its ecology will remain speculative.

6.4.2. Efficacy of the light lure

Due to the environmental problems associated with the available chemical treatments (Egidius & Møster, 1987), the differential susceptibility by different stages of sea lice to them (Walday & Fonnum (1989) for Aquaguard, Thomassen (1993) for hydrogen peroxide) and the potential problems regarding possible development of resistance against such compounds (Jones *et al.*, 1992), the need for alternative management controls exists. For this reason, the light lure was developed, with the lure attempting to exploit the phototactic nature of the lice (Nuttall, 1995). As already stated, it has been seen in laboratory studies (present study, Chapter 5) that the free-swimming larval stages of *L. salmonis* are photopositive under all light conditions, with the infective copepodid stage showing the strongest response. Initial tank-based studies by the developers of the light lure appeared to demonstrate that the *L. salmonis* copepodid was strongly attracted to the light emitted by the lure (Binnie, pers. comm.), and therefore the lure was tested out in the present study in order to test the efficacy of it as an alternative control method in the natural environment. To possess this potential, the lure would have to be seen to attract the free-swimming stages of *L. salmonis* in the water column around a commercial fish farm. By removing such stages from the water column by pumping at frequent intervals, it can be assumed that salmon would be exposed to fewer copepodids, and consequently stand a lower chance of becoming infected.

6.4.2.1. Effect of the lure on parasitic copepods

The results from the light lure sampling programme strongly indicated that the device would not be able to act as an alternative method for the control of sea lice populations. No copepodids of either *L. salmonis* or *C. elongatus* were obtained from

plankton samples taken from the water surrounding the light lure at either 0 or 5m depth. Although *L. salmonis* naupliar stages were obtained in the plankton samples from the lure, the numbers did not increase with increased exposure to the lure, indicating that, despite their positive photoresponses, increased light from the lure did not have an effect upon their distribution. This may seem to contradict earlier results, but it should be noted that nauplii were found in the surface layers (0 to 5m) during the night indicating that perhaps there are other responses that influence their vertical profile, such as a geotaxis and buoyancy aids in the form of stored lipids. It may be argued that, due to the positive phototaxis during the day in the presence of natural light, combined with a negative geotaxis, an increase in larval numbers attracted to the lure would not be expected. However, because of the lack of natural light at night, it may have been expected that the lure emitting out light, would have attracted an elevated number of larvae. However, in samples taken overnight, and collected early in the morning (18 and 24 hour samples), no increase in numbers was observed. It therefore seems to be the case that although the free-swimming naupliar stages of *L. salmonis* possess a photopositive response, there are other factors that either act alone or combine to influence their vertical distribution more.

L. salmonis copepodids showed a stronger positive phototactic response than their naupliar counterparts (present study, Chapter 5), and it would therefore be expected to have found more of them in the light lure samples than nauplii. None was found, and Bron (1994), also using a light trap, stated that such a device seemed ineffective in attracting additional *L. salmonis* copepodids. Anon (1996) noted that no *L. salmonis* larvae were recovered during a light trap experiment off Ireland. Since the copepodid has the ability to infect a host after the completion of the NII-copepodid

moult, at the first available opportunity it seems unlikely that it will choose not to infect one of the many hosts present but instead be attracted towards light emitted from the lure.

Although it is recognised that just one lure would not be sufficient to control sea lice, farms apparently needing between 10 and 20 lures (Nuttall, 1995), the light lure as a device to reduce lice infection rates on farms would appear, from these results, to be of little or no value.

6.4.2.2. Effect of the lure on free-living zooplankton

Despite its lack of success with respect to attracting the free-swimming stages of sea lice, the light lure was found to attract both adult and larval copepods, with greater numbers being obtained with increased exposure to the light. Bron (1994) also found that light traps increased the numbers of zooplankton obtained in plankton samples, and Jones (1971) found that copepods such as *Temora longicornis, Acartia clausi, Centropages hamatus* and *Paracalanus* and *Pseudocalanus* species were obtained in light traps. Both Sheard (1941) and Hale (1953) show the use of light to be a method that improves the collection of marine organisms, but for zooplankton this may seem contradictory, since the majority of species demonstrate either nocturnal or twilight vertical migrations (Forward, 1998). However, it must be remembered that these migrations observed in the water column are due to the angular distribution of light in the natural environment, this distribution being caused by light penetrating the surface of the water. A light lure or trap placed below the surface will emit light that will not have an angular distribution, but instead will mimic light used in those experiments performed in the laboratory using a narrow light field. Frequently, in such experiments, zooplankton are observed to be positively phototactic to high light

intensities, with for example, the copepod *Acartia tonsa* being found by Stearns & Forward (1984a) to be positively phototactic to all light intensities in the laboratory. Hence, it can be expected that if free-living copepods are exposed to a light lure in the natural environment, they will demonstrate this "artificial" photoresponse and so be attracted towards it, explaining the higher numbers of copepods obtained in the present study.

It can be concluded from the sampling programme in the natural environment in the present study that there still exists gaps in data available concerning the distribution and ecology of the free-swimming stages of *L. salmonis* and *C. elongatus*, especially with respect to the infective copepodid stage of both species. However, it can be concluded that live naupliar stages of *L. salmonis* with "ample" lipid reserves appear to concentrate in the upper surface layers of the water column, with numbers being higher within cages compared to outside, a large dispersal occurring immediately outwith the nets. If this distribution is extended to the copepodid stage, it appears that the only feasible method of reducing infections by the reduction of exposure of hosts to the infective stage may be to hold fish in deeper cages, when during the summer, they prefer deeper waters only coming to the surface to feed (Huse & Holm, 1993). This, however would only reduce infections during the summer at the most, since salmon remain at around 5m in depth during the winter and spring (Huse & Holm, 1993). Research into other biological and/or managerial methods for control must therefore continue, otherwise the industry will continue to rely on the use of chemical compounds.

CHAPTER 7

GENERAL SUMMARY & CONCLUSIONS

7. General Summary and Conclusions

The present study has investigated a wide variety of aspects of the biology and ecology of the free-swimming stages of *Lepeophtheirus salmonis* and to a lesser extent *Caligus elongatus*. The majority of earlier research had been devoted largely to the parasitic stages, with little time being spent on the free-swimming naupliar and copepodid stages. This was no doubt due to the need for the development of managerial and chemical control methods for sea lice when it first became apparent that such parasites were becoming a major disease problem in the fish farming industry. However due to the need for both alternative control measures and for information regarding the possible interactions between infections on farmed and wild salmonids, the present study was undertaken in order to attempt to elucidate data on the first three free-swimming larval stages of *L. salmonis* and *C. elongatus*.

Information regarding parameters that control and influence the reproductive output of sea lice as well as the subsequent hatching and development of the free-swimming stages were elucidated, with particular attention paid to the effects of the energy reserves on the activity and infectivity of the copepodid stage. These combined with laboratory behavioural studies allowed hypotheses to be drawn regarding the distribution of the naupliar and copepodid stages in the natural environment, which were then subsequently tested by an extensive sampling programme around a commercial fish farm.

At present, infection levels of *L. salmonis* on fish farms throughout the year are very variable, and the parameters that control these patterns are not clearly understood. From the present study it was observed that such levels may be partly due to both the

seasonal and infrapopulational variation in reproductive output of *L. salmonis*. Temperature was observed to have a significant influence on the number of eggs produced over the year, with fewer numbers being observed to be produced in the summer when compared to winter. Although Ritchie *et al.* (1993) had also studied the fecundity of *L. salmonis* previously, the authors did not report on whether any seasonal change actually occurred in the total number of eggs. It was therefore unclear as to whether the seasonality in fecundity reported by Ritchie *et al.* (1993) was due to a change in the total number, a change in the viable number of eggs or a combination of the two. The present study demonstrated that a change occurs in the total number of eggs seasonally, whilst the proportion of normal to abnormal eggs remains relatively constant.

The phenomenon of a greater number of eggs being produced in the summer was also seen from the literature to be common in the majority of free-living and other parasitic copepods, with only the reports of Evans & Diaz (1978) for *Microstella norvegica* and Johnston & Dykeman (1987) for *Salmincola salmoneus* being contrary to this. At first this may seem to be an adaptation to overcoming high mortality in perhaps less optimal conditions by producing more offspring or, for free-living copepods in particular, to maximise offspring production in time for the spring bloom of phytoplankton. However, both from the available literature and from the present study, it was observed that body size also has a controlling effect on the number of eggs produced, there being a direct relationship between body size of adult females and their fecundity. Body size was observed to have an inverse relationship with temperature. This was attributed to an increase in generation time and decreased moult frequency (Kurata, 1962) at lower

temperatures causing a larger size increment to occur between moults. Thus, although this relationship between temperature and reproductive output may serve to increase the numbers of larvae produced, it may not be an adaptation that has evolved in response to environmental conditions, but instead is as a consequence of the relationship between temperature and body size attained in organisms that possess a life cycle interspersed with moults.

Unlike egg number variation, *L. salmonis* was not observed to conform to the accepted pattern of seasonal egg size variation as is seen in free-living crustaceans. Although a seasonal variation was observed in the present study and by Ritchie *et al.* (1993), it was the opposite to what is observed in free-living species. In such species, it appears that eggs produced during the winter tend to be of a larger size than those produced during the summer months (Steele & Steele, 1975a; Sheader, 1983; Crawford & Daborn, 1986), though the factors that control the size of the eggs do not appear to be fully understood. The factors that control the size of *L. salmonis* eggs were also not elucidated, but from the seasonal studies, and from those by Ritchie *et al.* (1993), it is clear that *L. salmonis* eggs are larger in the summer, and smaller in the winter.

The naupliar stages of sea lice and the copepodid stage whilst present in the water column are lecithotrophic in nature, being dependent upon energy reserves laid down during egg development. From studies on the utilisation of such reserves in the form of lipids, depletion was reported for the first time, and it was also seen that it was dependent upon temperature, a faster depletion occurring at higher temperatures. It was concluded that this was due to a combination of both an increased intrinsic metabolic rate, and also a

higher activity level. It can therefore be expected that if eggs of the same size were produced throughout the year, containing the same amount of lipid, then larvae produced during the summer would have a shorter time in which to locate and infect a host, assuming that they had enough energy to successfully develop to the infective copepodid stage. The larger eggs produced by *L. salmonis* during the summer were demonstrated to have a greater lipid reserve as the width of the lipid area was directly related to the size of the egg. Thus the production of larger eggs with increased energy reserves over the summer may be an adaptation to accommodate the faster depletion of such stores at higher temperatures, allowing the larvae to have an adequate amount of time in which to infect a host.

The proportion of viable and non-viable eggs was not seen to vary significantly seasonally and was also not related to the number of eggs produced per brood in any way. The controlling and regulating influences over the development of eggs are therefore worthy of further study. Further work needs to be undertaken in order to elucidate whether the abnormal/ non-developing eggs observed in both *L. salmonis* and *C. elongatus* ovisacs are either unfertilised, or fertilised but have aborted. If they have not been fertilised, due to the depletion of sperm from the spermatophore sealing the females genital opening, then it might be expected that, if remating does not occur, later broods would have a lower proportion of eggs showing normal development. If remating does occur however, then it may be expected that the proportions of viable and non-viable eggs will remain relatively constant over the number of broods produced.

It has been observed for free-living copepods that there is a decline in egg production with increasing age (Smyly, 1970; Hopkins, 1977; Parrish & Wilson, 1978), and Anstensrud (1990) stated that older, virgin *Lepeophtheirus pectoralis* females produced a fewer number of eggs once mated when compared to younger virgin females. Thus, there appears to be other factors aside from those parameters elucidated in this study that may control the level of reproductive output and the subsequent development of sea lice. At present, it may still not be possible to produce a standardised figure for the fecundity of *L. salmonis* or indeed other species of sea lice due to such unknown factors. However, the present study has assisted the progression of such a model by the provision of information on how a number of factors affect reproductive output and highlighting areas which should subsequently be investigated. As well as seasonal variations controlled by external abiotic factors, infrapopulational differences were evident from the present study, with perhaps the age of the female, the number of broods she has produced and the time of mating all contributing to the final fecundity of each individual female.

It has been suggested that *L. salmonis* is adapted to an estuarine or coastal environment, rather than a strictly marine/ high salinity environment (Bass & Murphy, 1995). Although some results from the present study seem to confirm this hypothesis, such as the behavioural responses to particular wavelengths of light that are common in coastal areas, other data obtained appears to contradict this. The main evidence from the present study for *L. salmonis* not being specifically a coastal or estuarine species is that development of the early free-swimming larval stages is halted in low salinity water. The egg and the naupliar stages appear to be more sensitive than the later infective copepodid

stage, but even this stage is sensitive to low salinities. Hatching of live nauplius I stages was not seen to occur in salinities of less than 15‰ and even at this level, less than 5% of *L. salmonis* nauplius I stages released 24 hours after hatching had begun were showing any signs of activity. Development to the copepodid stage only occurred in salinities of 25‰ or greater, though at 25‰, only very low levels, 0.03% of the total number of eggs within an ovisac, attained infective status. This appeared to be due to development to the second nauplius stage initially being compromised by lowered salinity levels. At 20‰ salinity, only approximately 2% of hatched nauplius I stages moulted successfully to the second naupliar stage, with this number subsequently being seen to increase as salinity levels were raised. With increased naupliar success, so the proportion of larvae developing to the infective copepodid stage increased. Thus, it seems highly unlikely that *L. salmonis* has specifically adapted to a coastal or estuarine life as, in such areas, the salinity of the water bodies are not constant but instead fluctuate greatly, with such changes being detrimental to the development of the egg and naupliar stages.

L. salmonis copepodids were observed to be more tolerant of lowered salinities than these earlier naupliar stages, both in their ability to survive and in their behavioural responses. Copepodids were observed to be able to tolerate a relatively wide range of salinities from 15 to 35‰, with their longevity within this range not being significantly different. However, the lifespan of the infective stage was significantly reduced at salinities of 10‰ and below, but this "critical" level is at a much lower salinity than could be tolerated by the earlier naupliar stages. The swimming abilities of the naupliar and copepodid stages were also seen to differ when exposed to lowered salinities. Copepodids

could remain actively swimming for approximately twice as long in lowered salinity water when compared to the naupliar stages, and natural recovery in some lowered salinities was also seen to occur in the infective stage whereas this was only observed in the naupliar stages when they were transferred back to full strength seawater.

These differences in environmental tolerances of the naupliar and the copepodid stages may signify a marked difference in the strategies of the two types of larvae. The naupliar stages have no infection capability and, due to this, are also highly unlikely to utilise any host-locating mechanisms. However, in order to increase the chances of survival of the parasite by the location and infection of a host by the copepodid stage, the naupliar stages must maximise the chances of such a host encounter occurring, and thus be present in an environment in which the host is likely to inhabit. From the behavioural studies it was observed that all three of the free-swimming larval stages of *L. salmonis* were photopositive and geonegative in nature, bringing them in spatial overlap with salmonid hosts which, according to Hogans & Trudeau (1989) and Huse & Holm (1993) tend to aggregate in the upper surface layers. However, when exposed to water of lowered salinity, the naupliar stages, were seen to cease swimming and instead passively sink in the water column. This, as has been documented for free-living copepods (Latz & Forward, 1977), appears to be a behavioural adaptation in order to take such larval stages out of an unfavourable environment. By staying in an area of lowered salinity, *L. salmonis* nauplii would be compromising their individual chances of survival and thus the continued development of the parasite to the stage where it is able to infect a host. *L. salmonis* copepodids on the other hand are the infective stage and, as such, their principal adaptation

is the location and infection of a suitable host as soon as possible. It is unlikely that an infective stage which is non-active would have a high chance of infecting a host. Therefore it is not surprising that this stage can both survive and swim in lowered salinity water for longer than the naupliar stages and also show a rapid recovery of their ability to swim after a certain period of time. If this stage is suddenly exposed to a flush of low saline water, then it appears to first of all employ an avoidance strategy of non-swimming, like that seen in the naupliar stages, in order to reach deeper and therefore more dense and more saline waters. However, although this sinking behaviour will increase its chances of survival in the water column, it will also concomitantly decrease its chances of infection. Infection encounters are promoted by an increase in the activity of the infective stage. If this stage is not exhibiting any activity then it will not be maximising its chances of remaining in spatial overlap with the host or its likelihood of an infection encounter, nor will it be responding to host-originated stimuli and/or cues.

The copepodid stage of *L. salmonis* was also seen to adopt other behavioural strategies over its lifespan, again in order to maximise the chances of host location and subsequent parasite survival. The copepodid stage appears to have evolved two host-locating strategies which are employed at different times over its lifespan in order to maximise its chances of host infection given that it has limited energy reserves. Early on in the limited lifespan of *L. salmonis* copepodids it was observed that, as well as swimming in response to cues such as water currents that may mimic the stimuli produced by potential hosts, the infective stage also swam spontaneously even when there was no stimulation present. This behaviour was also observed by the random distribution of *L.*

salmonis copepodids in the dark during the experiments to test their phototactic responses. However, after a certain point in time, this behaviour changed, and swimming was only observed in response to external stimulation. It was concluded that this was a switch from one strategy to a second by the parasite in order to conserve its remaining finite energy reserves. If the energy reserves had depleted to such an extent that swimming could no longer occur, this period of swimming in response to stimulation would not have been seen. Instead no swimming would have occurred, a situation which does happen, but only after the period of swimming only in response to stimulation. Considering the decline in energy reserves, the finding that older copepodids are less infective than their younger counterparts is hardly surprising. Added to this "equation" is the effect of temperature upon the rate of lipid depletion due to increased metabolic rates and activity at higher temperatures. Such phenomena will play an important role in the success of infection. This may be influenced in part by both the role of temperature and its effect upon activity and subsequent infectivity of *L. salmonis* copepodids, and by the age-dependent infectivity status of these stages.

Hatching of *L. salmonis* nauplii was not observed to be influenced by environmental stimuli such as photoperiod or exposure to light or darkness. Hatching in response to such factors is probably only an advantage to those parasitic species that hatch to release a stage which is immediately infective, such as *Salmincola* spp. and *Haemobaphes* spp. (Poulin *et al.*, 1990b; Roth, 1988). By having two non-infective naupliar stages in the life cycle of *L. salmonis*, there would be little point in such factors initiating hatching since there exists a relatively long period of time (e.g. 96 hours at

10°C) before infection can occur. During this time, the distribution of the free-swimming larval stages will be affected and dispersed by water currents, and hence "synchronised hatching" would be of little value in maximising the chances of infection. Indeed, during the plankton sampling programme, no "waves" of newly hatched nauplii I stages were observed, with the numbers of larvae being found in individual samples over the entire sampling programme remaining relatively constant.

The sampling programme carried out in the present study confirmed many of the hypotheses that had been developed from laboratory based work, such as the vertical distribution of the larvae in the water column. However, although some of the ecology of the first two naupliar stages was elucidated, the distribution of the infective copepodid stage of both *L. salmonis* and *C. elongatus* remains unknown and only speculative due to the absence of copepodids obtained in the plankton samples. It was concluded that the reason for this absence was most likely to be the interaction of a number of factors, including temperature, salinity, dispersal and mortality rates. Temperature was seen to have a significant effect on the development of *L. salmonis* in the present study, with low temperatures either reducing developmental success or halting it altogether. It was found that it was the moult from the nauplius II stage to the infective copepodid stage that was affected the most, with either nauplii not entering the moult at all, or entering it but not successfully completing it. This, combined with low salinity levels that were experienced during the sampling programme may have combined to reduce the numbers of copepodids of either *L. salmonis* or *C. elongatus* present in the water column. Also, from the developmental studies, it was observed that a large mortality occurred before the second

nauplius stage was reached, indicating that there was likely to be much lower numbers of infective stages present. Whether this mortality is due to the first nauplius stage being the most sensitive, or whether individuals that are less "fit" are removed from the system is still unclear, though further work in this area would solve this problem. In the natural environment, the horizontal distribution of the free-swimming larval stages of sea lice is controlled by the dispersive nature of the water body. This was very evident from the plankton sampling programme where a massive dispersal effect appeared to exist once the nauplii were outside the cages.

In the light of the results of the present study, the model of Tully and Whelan (1993) discussed earlier should be revisited. The model had attempted to correlate *L. salmonis* nauplius I production with future infection rates on salmonids. Criticisms of the model were that it would carry many inaccuracies due to the number of assumptions included. Attempting to relate production with future infection levels from an estimate of the number of ovigerous females present on a salmonids on a fish farm without comprehensive knowledge either factors that effect the fecundity or the biology and ecology of the free-swimming stages will mean that the models "end result" will always be treated with a certain amount of scepticism. Information gained from the present study, if fed into such a model (or similar) would not only allow a more accurate prediction of the number of nauplius I stages actually produced, but also an estimate of the number of stages that will develop successfully to the infective stage. Although by bringing such factors into a model, an increasing amount of complexity will certainly be caused, it will help to greatly ameliorate the degree of inaccuracy carried.

Due to the economic importance of the fish farming industry and the cost and problems associated with the treatment of sea lice, there is an ongoing need for the development of novel control methods. One such method was tested in the present study, this being the Terecos light lure, the equipment intending to take advantage of the positive phototactic behaviour of *L. salmonis* and other species of sea lice. However, in the trials undertaken, no positive results were obtained, despite the obvious success in attracting free-living larval and adult copepods in the natural environment. Such results serve to highlight the complex nature and biology of the free-swimming larval stages of parasitic copepods. Although such stages may exhibit various behavioural and developmental responses in relation to abiotic factors as shown in the present study, only one response at a time was tested. It may be necessary to test a combination of factors but then there are difficulties associated with the subsequent interpretation of results and their implications. Therefore, although sea lice nauplii and copepodids possess a phototactic nature, this alone will not control their ecology and distribution, but will interact with the multitude of other responses to abiotic and biotic factors. Due to this, it may not prove possible to exploit just one individual behavioural characteristic as the light lure attempted to do, but instead all such characteristics may have to be taken into account.

The results of this study have therefore provided increased knowledge of an area of sea lice biology that has previously been poorly understood. This provides a sound basis from which further studies, such as those outlined here, can attempt to increase our understanding of the biology and ecology of the free-swimming larval stages of sea lice, and help to make more accurate predictions of their population dynamics.

CHAPTER 8

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