#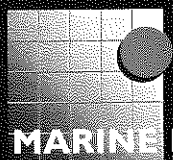 Development of a Management Strategy for the Reduction/Elimination of Sea Lice Larvae, *Lepeophtheirus salmonis,* parasites of salmon and trout
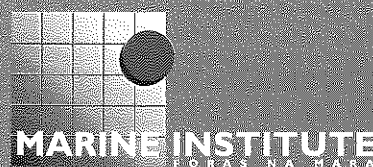
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The Marine Institute

DEVELOPMENT OF A MANAGEMENT STRATEGY FOR THE REDUCTION/ELIMINATION OF SEA LICE LARVAE,
Lepeophtheirus salmonis,
PARASITES OF FARMED SALMON AND TROUT

by
G. O'Donoghue, M. Costelloe, and J. Costelloe

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Abridged Report

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ABSTRACT

Sea lice are copepod ectoparasites of fish, belonging to the family Caligidae. Their importance to marine salmonid culture stems from the extensive damage they may inflict on hosts through feeding and contact abrasion. The principal species associated with cultured salmonids is *Lepeophtheirus salmonis* (Kroyer, 1838), a large salmonid-specific species reported as a problem for aquaculture in a number of countries. The objectives of the present study were: (a) to examine the production and distribution of larval stages of *Lepeophtheirus salmonis* within a cage containing *Salmo salar* in order to identify specific spawning cues and larval frequencies and intensities; (b) to identify precisely the behavioural patterns of sea lice larvae over a variety of tidal and diurnal cycles; (c) to monitor environmental parameters and (d) having identified the specifics of spawning and larval behaviour, to identify potential management strategies for the elimination of a high percentage of sea lice larvae produced on fish farms. Larval plankton samples along with mobile lice samples were taken during two growing cycles on a fish farm on the west coast of Ireland. Highest densities of larvae were recovered during neap tides following synchronous spawning episodes within the female population. Gravid females were recorded during the winter months; however, spawning intensity remained low until late Spring. Sea lice larvae migrated vertically within the water column with highest densities recorded during slack water normally associated with high tide. The results of this study increases our knowledge of the complex behaviour and life cycle of the louse. The occurrence and the location of high densities of larvae within salmon cages have been identified. This information provides a sound basis from which management strategies can be developed in order to reduce lice intensities on the farm.

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1. INTRODUCTION

1.1. General Introduction

Sea lice are copepod ectoparasites of fish, belonging to the family Caligidae. Their importance to marine salmonid culture stems from the extensive damage they may inflict on hosts through feeding and contact abrasion (Jones *et al.,* 1990; Jonsdottir *et al.,* 1992). Sea lice are at present considered the most serious parasite pathogen of marine farmed salmonids and are estimated to cause losses totalling up to 15% of production (Roth *et al.,* 1993).

The principal species associated with cultured salmonids in Ireland is *Lepeophtheirus salmonis* (Krøyer, 1838). The caligid life cycle is direct (i.e., single host) and is normally considered to consist of ten stages, separated by moults and divisible into five identifiable phases (Schram, 1993). After hatching from paired egg-strings, two free-living 'nauplius' stages allow dispersion in the plankton and are followed by an infective 'copepodid' stage which re-establishes contact with a host. Following settlement, the copepodid moults through four chalimus stages. The subsequent 'pre-adult' phase is sexually differentiated and comprises two stages. The 'adult' phase is fully mature and the adult female produces a number of batches of paired egg-strings which hatch into the water column to continue the cycle (Kabata 1979, Johnson & Albright 1991a, Schram, 1993).

The majority of studies have concentrated mainly on the diagnostic features of the adult louse and its larval stages, survival and behaviour of the louse in various environmental conditions and infestations on farmed or wild fish (Boxshall *et al.,* 1993). There has been little information available on the dispersion and distribution of the larval stages in the wild.

1.2. Population dynamics

In general, there is a paucity of information on the reproductive output of *L. salmonis* and the timing and intensity of spawning is virtually unknown. The number of eggs produced within an egg-string has been shown to vary considerably (Wootten *et al.,* 1982; Gravil, 1996), confirming that there are wide variations in reproductive output. Other studies have suggested that the intensity and timing of infestation events of caligid copepods in wild or cultured fish populations may be predicted from previous local production of nauplius I of the parasite (Tully, 1992). However, this relationship is not well established. A history of parasitic intensity and population structure of lice on farmed fish from a particular site will aid in the reduction of parasitic impact on host fish using control decisions based on predictions of future levels of infestations and impacts.

1.3. Vertical migration

Heuch *et al.* (1995) contend that sea lice larvae exhibit a diel vertical migratory pattern with highest numbers of larvae occurring predominantly in the surface waters during daylight hours. Aqua-Fact (1994, 1995), however, reported a tidally induced migration with the larvae

occurring in the surface water during flooding tides. Gravil (1996) could find no evidence of a vertical migration although she states that this may be due to the sampling procedure (samples were taken every three hours at 5m intervals from the surface to the benthos). However, she reports that live naupliar stages of *L. salmonis* (no copepodids recovered) with ample lipid reserves were concentrated in the upper surface layers of the water column over the sampling period. Diel vertical migration in plankton has been recognised for more than a century (Longhurst, 1976), but it is only relatively recently that plankton vertical migration over tidal timescale has been reported (Hough & Naylor, 1991; Zeng & Naylor, 1996).

1.4.   Ecology of the louse

In previous Aqua Fact studies (1994, 1995) it was found that sea lice larvae exhibited a vertical migration to the surface water in the three to four hour period prior to high water in both light and dark conditions. Given that the range of hosts of *L. salmonis* is limited on the west coast of Ireland, it would be most advantageous for the parasite to have evolved a strategy where highest densities of the infective stage occur when salmonids are also at their most numerous, i.e. near the river mouths during smolt migrations. In general, results from the 1995 study enhanced and strengthened the original hypothesis first put forward in 1994, that in any given bay which contains both a fish farm and a river system there is more than one source of lice infection, the impact of each being determined to a large extent on the oceanographic conditions within the bay, the number of ovigerous lice present at any one time within the population source and the behaviour of the larvae.

It is a requirement of a parasite to find a host, in this case a salmonid. Therefore, the behaviour and life cycle of the host will have a strong influence on the parasite. It is imperative for the louse to have its infective stage at a maximum density when hosts are at their maximum density. Free-living infective stages of parasites usually have developed behaviour that augments the frequency of host encounters (Combes, 1991). Combes (*loc. cit.*) states that any mutation or group of mutations that increases the probability of parasite infective stages coming into contact with hosts will be selected for. Behavioural responses by copepodids to locate suitable hosts have evolved in many parasitic copepods, particularly when it is selective in its choice of host. Copepodids of *Lernaernicus sprattae* migrate towards the sea surface at night where their host, *Sprattus sprattus*, congregates (Schram & Anstensrud, 1985) while *Haemobaphes intermedius*, a copepod gill parasite of intertidal fish, use environmental cues to synchronise egg hatching with times when hosts are concentrated in a small volume of water (Roth, 1988).

1.5.   Summary of findings to date

In general, results of plankton studies carried out in selected bays returned very low levels of *L. salmonis* larvae while studies close to fish farms in the same bays gave higher levels with highest densities of larvae being recorded within the cages. During the course of work, initiated in 1994, a number of findings were made which contribute to understanding the biology of sea lice:

- On 03.08.94, samples were taken from within and either side of an isolated Bridgestone® cage containing salmon with a lice load of *c.* 50,000 gravid females. Samples taken from either side of the cage were in the tidal current flowing into and out of the cage respectively. The fouling on the net was also examined for the presence of larvae. A control cage without fish was sampled to establish background levels of larvae on the farm. Highest levels of larvae (66.1 m^{-3}) were recorded from the surface tows within the cage. Densities increased to a peak at high water. There was a rapid decrease in numbers of larvae in the surface tows taken immediately after this peak. Relatively few larvae were recovered from samples taken 10m either side of the cage indicating that the rise and fall in larval numbers was not due to larvae being washed into and out of the cage. The majority of larvae remained above 8m throughout a full tidal cycle.

- In general, results from the single cage studies suggested that larvae were most likely to be found in the surface tows in the three hour period before high water in the days on or after neap tides.

- Surface tows showed a significant relationship between larval numbers and distance from the farm. Highest numbers of larvae were recorded 10m from the farm with a 90% reduction found at 1km.

- Trial cage experiments, in which salmon smolts were positioned at various distances between a salmon farm and wild fishery, revealed higher infestation rates of *L. salmonis* in the inner estuarine cages located furthest from the farm. Analysis of the population structure of the lice on these smolts suggest that infestation occurs in pulses rather than as a continuous event.

- In 1995, surface tows taken twice weekly at various locations along the length of Killary Harbour from April to May, recovered 18 nauplii and 121 copepodids. Larvae were only found sporadically, the majority (70.5%) being recovered in the first week of sampling at the stations located in the inner harbour near the mouth of the Erriff.

- Distribution of larvae within a cage was investigated at Killary Salmon Farm over a 24 hour period. Larval densities exhibited a cyclic trend corresponding to the state of the tide. Highest densities were recovered from surface samples taken in the 2 - 3 hour period prior to predicted high water during both day and night tides.

Results from 1995 indicated that sea lice larvae occurred sporadically in the inner estuarine area of Killary Harbour. Given that the range of hosts of *L. salmonis* is limited, it would be most advantageous for the parasite to have evolved a strategy where highest densities of the infective stage occur when salmonids are also at their most numerous, i.e. near the river mouths during smolt migrations. The results of the 1995 programme can be summarised as follows:

- Little variation was observed between temperature at surface and bottom waters.

- Salinity values at the inner stations exhibited a wide range of values reflecting the freshwater influence in these areas. Salinity was more stable at the farm stations.

- The majority of larvae recovered at the farm were at the nauplius stage.

- The maximum density found in a single tow was located in a shallow area of the inner estuary corresponding to an area where sea trout would feed. These larvae were presumed to have originated from lice on wild fish resident or migrating through the area. Alternatively, the larvae found in the estuary may have hatched from egg-strings which either fell off lice on salmon during the previous season or from adult lice which remained dormant on vegetation and rocks or in the sediment until conditions were more favourable in the Spring. In order to test this hypothesis it was decided to take samples of bottom sediments in Killary Harbour and in Golam Harbour for comparison. The study was carried out to coincide with the rise in temperature naturally associated with a Spring bloom.

- No cysts/larval egg-strings of *L. salmonis* were recovered in the sediments of the inner Killary site or in those from Golam Harbour.

1.6. Objectives and targets of the current project as part of the Operational Programme for Fisheries (1994-1999)

Following the Aqua-Fact studies (1994-1995) there was a need for further specific research into the behaviour and biology of sea lice in order to develop a management strategy to control *L. salmonis* on farmed salmonids. The control of sea lice without recourse to chemical treatment is highly desirable as the different stages of sea lice exhibit a differential susceptibility to chemical 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).

The objectives of the study, as part of the Operational Programme for Fisheries (1994-1999) were:
- to examine the production and distribution of the larval stages of *L. salmonis* within a cage containing *Salmo salar* in order to identify specific spawning cues, frequencies and intensities.
- to identify precisely the behavioural patterns of sea lice larvae over a variety of tidal and diurnal cycles,
- to monitor environmental parameters such as water temperature, salinity, lux and secchi depth to ascertain their influence during the above cycles,
- to examine the population dynamics of that population of parasites producing the larvae in question,
- to examine the morphometrics of females that were found on farmed fish and compare them to females parasitising wild fish,
- to examine the phenomenon of vertical migration of sea lice larvae both in cage systems and in experimental mesocosms and finally,
- having assessed the specifics of spawning and larval behaviour, to identify potential management strategies for the elimination of a high percentage of sea lice larvae in a fish farm environment.

2. STUDY AREAS

Each of the sampling sites were located on the west coast of Ireland. Initially, Killary Harbour was chosen as the main sampling site. However, lice levels on the farmed fish located within the harbour were kept extremely low with the result that few larvae were recovered within the cages. Consequently, the sampling programme was changed to Golam Harbour where relatively high numbers of gravid *L. salmonis* were present on the farmed fish. Killary Harbour and Ardmore Bay were the locations of vertical migration studies.

Golam Harbour (Fig. 2.1.) is positioned at the outer point of Kilkieran Bay where it connects with the North Sound of Galway Bay. The harbour is formed by a configuration of islands giving the appearance of a semi-enclosed bay with a western opening. A previous study (Ottway, 1986) found that soundings within the harbour were in good agreement with the Admiralty chart with a maximum depth between 7-8m. Ottway (*loc. cit.*) further found that there is thorough water mixing within the harbour, and good exchange with the oceanic water outside. The Golam Teóranta production site is located in the Harbour and accommodates one set of four Turmec cages. A separate block of three polarCirkel® cages used for smolt input is located *c.* 270m to the west of the Turmec® cages. The site has been in production since 1988 and present output consists of 130 tonnes of fish per annum produced over a two year cycle.
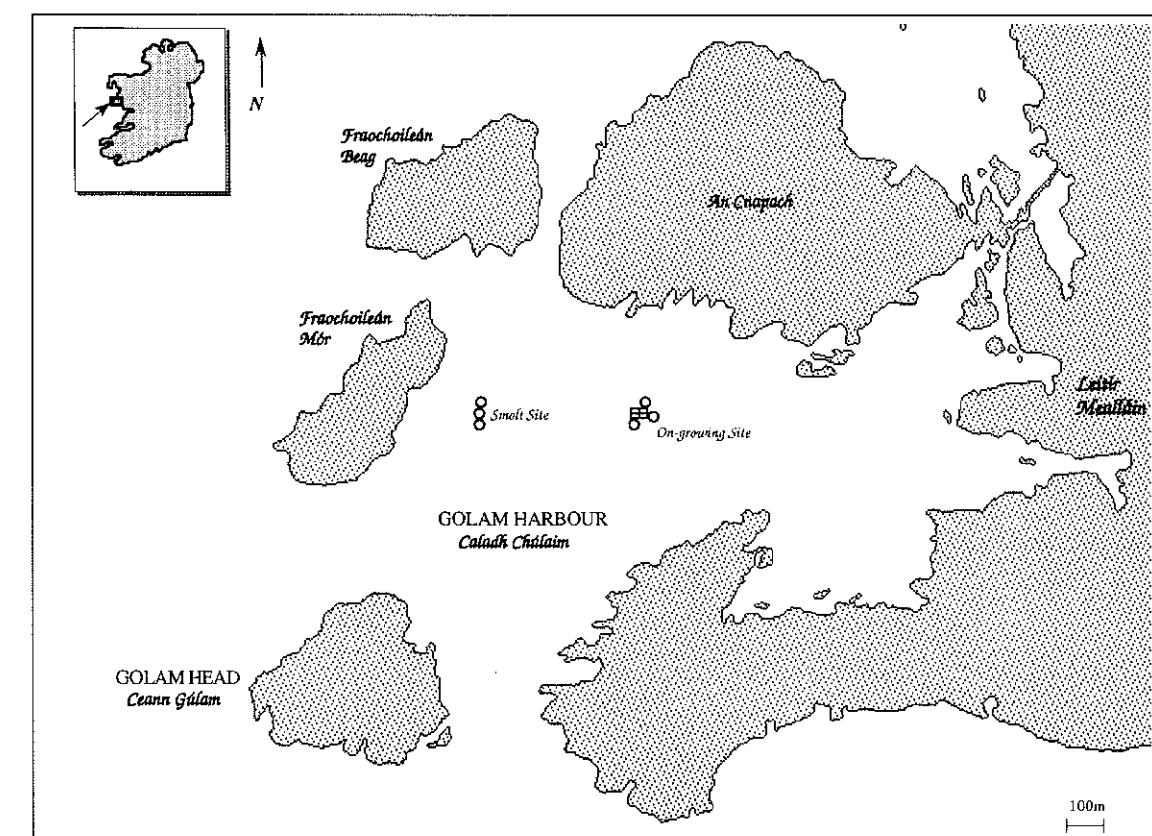

Figure 2.1:
Position of the Turmec® and PolarCirkel® cages which make up the Golam Teóranta production site.

Killary Harbour is a large fjord-like inlet opening to the south-east of the Inishdeigil Islands and forms most of the seaward boundary between Mayo and Galway. The fjord, which is approximately 14 km long and 700m wide, runs initially in a south-easterly direction until reaching the elbow or turn of the harbour, positioned half way along its length, after which it runs in an easterly direction. In terms of water structure, Killary Harbour can be described as a partially mixed estuary.

Ardmore Bay is situated in the south west end of Kilkieran bay due north of Golam Harbour. Vertical migration studies were carried out at Emerald fishfarm which is located within the bay. Unlike Golam Harbour, the bay is open and forms part of the Kilkieran Bay water mass. Currents in the vicinity of the fishfarm are highly variable in direction with mixed gyres and eddies depending on the stage of the tide.

3. MATERIALS & METHODS

3.1. Environmental parameters

Temperature and salinity profiles were obtained using a WTW Conduktometer equipped with the necessary probes. Light intensity was measured using an LX-101 digital lux meter and the relative water turbidity was estimated using a Secchi disk.

Current measurements were recorded using a Surface Readout Directional Current meter. Measurements were taken inside and outside the cage with the current meter suspended either from the centre or the side of the cage unit at the required depth. Speed and direction records were logged periodically as impeller revolutions per 50 seconds and degrees, respectively. The revolutions were later transformed into metres per second in the laboratory.

3.2. Larval distribution

3.2.1. Field sampling procedure

Sampling commenced in Killary Harbour on 02.04.96. However, lice levels within the cages at Killary Salmon Farm were extremely low and were predicted to remain low for some time. Consequently, the sampling programme was changed to Golam Harbour. Sampling began at this site on 10.06.96.

Larval densities in the surface waters of the sampled cage were monitored by taking three replicate plankton samples twice per week from 10.06.96 to 14.10.96 and once per week from 24.10.96 to 17.07.97. The newly hatched naupliar stage is 0.54±0.04mm in length (Johnson & Albright, 1991b), hence, the surface water was sampled by slowly pulling a 1.5m long, conical plankton net, with a mesh of 150µm, by hand across the diagonal/diameter of the cage/polarCirkel®. An estimation of the volume of water sampled was evaluated by multiplying the mouth area of the net by the standard distance towed. The efficiency of the net was not measured and larval densities are taken to be minimum values for the area being sampled.

In general, tows were taken on the flooding tide within one hour prior to high water. After the tow, the plankton net was washed down with sea water and the residue was stored in plankton jars and preserved with 4% formalin, buffered by sea water, for later analyses.

3.2.2. Laboratory procedure and identification

Samples were not left for more than three days in preservative before sorting as the distinctive pigmentation in the larvae was found to fade if left over an extended period. On analysis, the samples were washed on the 150µm sieve and then sorted under a binocular microscope and larval lice stages removed. Larval stages were identified as naupliar and copepodid stages according to Johnson & Albright (1991b) and Schram (1993).

3.3. Sea lice population structure and dynamics

3.3.1. Field sampling procedure

In order to examine the intensity and population structure of sea lice on the host fish, on each sampling occasion twelve fish were randomly removed from the experimental cage using a hand net. A maximum of three to four fish were taken at a time and anaesthetised by being placed in a bin containing a benzocaine/acetone mixture in sea water. The fish were then examined individually for the presence of the mobile stages of lice. These were removed using a forceps and placed in 100ml sample bottles (one bottle per fish) containing 4% formalin buffered in sea water. The salmon were returned to the cage after examination. On completion of sampling the bin water in which the fish were anaesthetised was strained through a 200µm sieve and the sieve and bin checked for any residual lice.

3.3.2. Laboratory procedure and identification of mobile stages.

In the laboratory, samples were not left for more than three days in preservative before identification. Each sample was sorted under a binocular microscope (x10) and individuals were identified according to sex, life-cycle stage and species using descriptions by Johnson and Albright (1991b), Schram (1993) and Ritchie *et al.* (1993). Female lice were sorted into 5 different categories, pre-adult I and II, virgin, non-gravid and ovigerous. Virgin females were not inseminated and had a reduced genital complex. Non-gravid females lacked egg-strings while ovigerous females carried egg-strings (Figure 3.1.). Male lice were sorted into three different categories, Pre-adult I and II and adult (Figure 3.1.). After identification, the lice were transferred to alcohol for storage.

3.4. Vertical Migration

Studies on vertical migration of sea lice larvae in cage systems were undertaken on a total of five occasions throughout the study. The details of each trial, i.e. dates, duration and tidal status are outlined in Table 3.1.

Trials 1 and 2 were undertaken in Golam Harbour in the cage which was being sampled for larval distribution at that time. Three replicate samples were taken as described in Section 3.2.1., at sub-surface and 3m depths, every hour for the duration of the experiments. Those samples taken at depth in the cage, i.e. at 3m for Trials 1 - 2, and at 5m for Trials 3 - 5, were taken by lowering the weighted plankton net to the required depth and then towing the net across the cage. The weighted net was attached to a surface buoy using ropes of the respective lengths. The net was lowered to, and raised from, these depths using this rope to keep the mouth of the net in a vertical position in order to reduce contamination by larvae from the surface waters.

Additionally one sub-surface tow was taken every hour 20m west of the cage in order to examine the movement of larvae into the cage on the flooding tide and out of the cage on the ebbing tide. These samples were taken by slowly towing the plankton net behind a boat over a distance of *c.* 25m Samples were processed according to methods outlined in Section 3.2.2.

Trials 3 - 5 were undertaken in Ardmore Bay in outer Kilkieran Bay in the second year of the study. Three replicate samples were taken in a polarCirkel® containing *c.* 50,000 salmon, at sub-surface and 5m depths every hour for the duration of the experiments, as described in Section 3.3.1. Sub-surface tows were also taken every hour 20m tidally upstream and tidally downstream of the cage in order to examine the movement of larvae into and out of the cage. These samples were taken by slowly towing the plankton net behind a boat over a distance of *c.* 25m Samples were processed according to methods outlined in Section 3.2.2.

Pre adult and adult categories of female *L. salmonis* :
(A) pre adult I; (B) pre adult II; (C) virgin; (D) non gravid; (E) ovigerous; (F) aged.

Pre adult and adult stages of male *L. salmonis:*
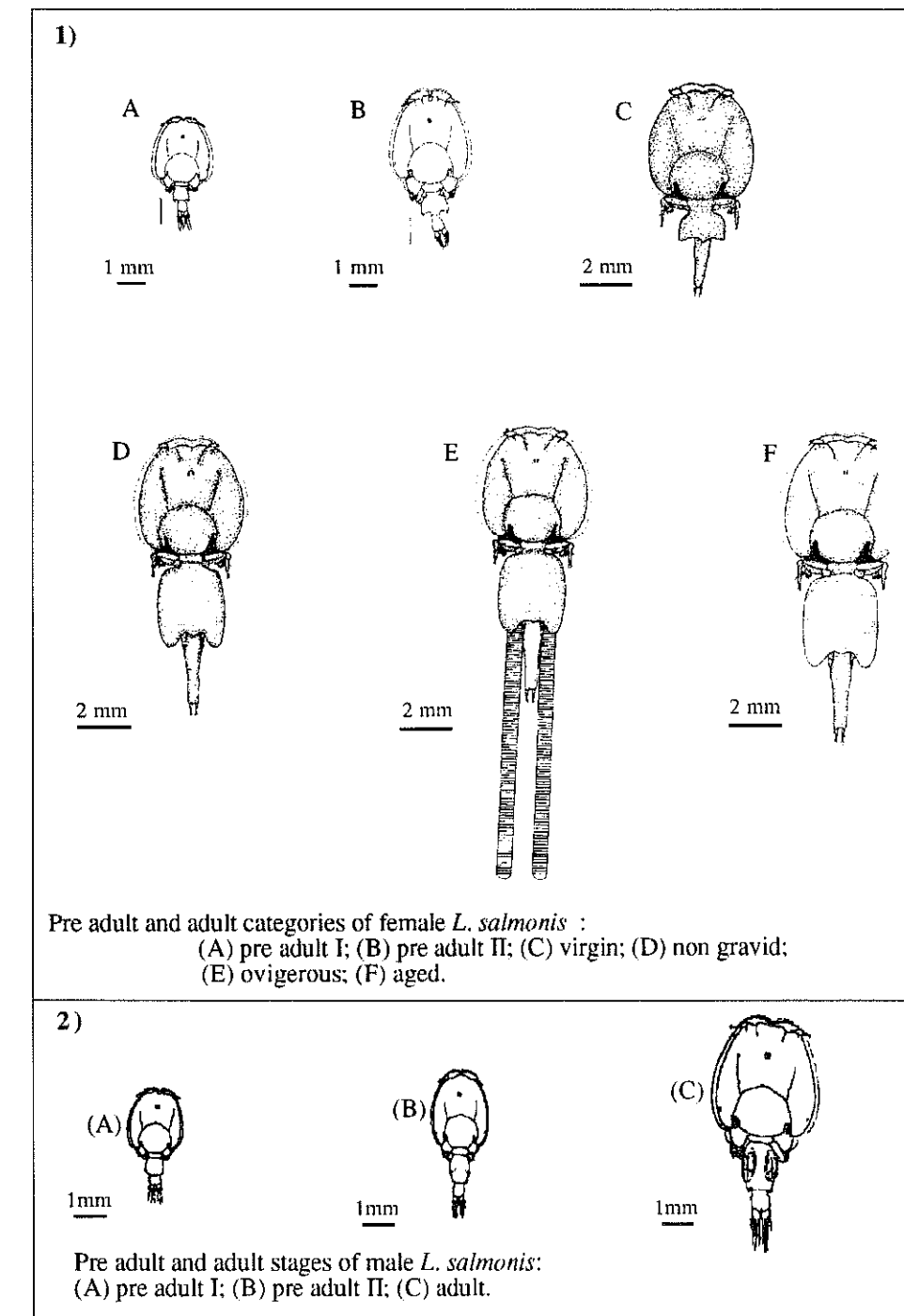(A) pre adult I; (B) pre adult II; (C) adult.

Figure 3.1:
Pre adult and adult categories of *L. salmonis:* 1) Female, 2) Male

During Trial 1, currents were measured inside the cage at the surface and at five metres. During Trials 4 and 5, current movements were monitored inside the cage at a depth of three metres and outside, at the surface and at a depth of 5m.

Table 3.1. Dates, times and tidal status for each vertical migration study				
Trial:	**1**	**2**	**3**	**4**	**5**
Location:	Golam (12h)	Golam (24h)	Ardmore (12h)	Ardmore (12h)	Ardmore (12h)
Date:	18.06.96	20-21.06.96	29.05.97	16.07.97	22.07.97
Time:	12:00 - 24:00	11:00 - 10:00	09:00 - 21:00	10:00 - 21:00	09:00 - 20:00
Tide Times:	LW13:09 1.1m	LW14:42 1.3m	HW11:26 4.5m	HW15:04 4.5m	LW13:20 1.1m
	HW19:44 4.7m	HW21:01 4.5m	LW17:34 1.2m	LW21:14 1.2m	HW19:41 4.7m
		LW02:58 1.2m			
		HW09:31 4.3m			
Tidal Status:	Spring	Spring	Neap	Neap	Spring

3.5. Data analysis

Larval intensity and the mobile lice data were initially analysed using a Microsoft Excel spread sheet. The mean abundance, mean intensity and % prevalence of lice infestation on the fish examined were calculated. The term mean abundance, mean intensity and prevalence follow the definitions of Margolis *et al.* (1982):

1) mean abundance can be defined as the mean number of parasites per fish examined (including the bin residue),

2) mean intensity, the mean number of parasites per infected fish,

3) prevalence, the percentage of infected fish

The term infection level is also used to collectively incorporate the concepts abundance, mean intensity and prevalence (Nagasawa *et. al.*, 1993). Larval densities recorded in the surface plankton samples taken during neap and spring were compared using the Wilcoxon signed ranks test as were current measurements recorded inside and from outside a cage.

4. RESULTS

4.1. Introduction

The sampling programme to investigate the production and distribution of larval stages of *L. salmonis* within a cage was changed from Killary Harbour to Golam Harbour after three months sampling. Given the low larval recovery from Killary Harbour (see Figure 4.1), little information could be interpreted from these results. However, it was later found that few larvae are produced in the early part of the year, even if ovigerous lice are present on the fish (see Section 4.3).

Naupliar and copepodid stages of both *Lepeoptheirus salmonis* and *Caligus elongatus* were recovered in the plankton tows taken over both years of the programme. However, the numbers of *C. elongatus* recorded were low and sporadic and the results presented concentrate entirely on *L. salmonis*. No differentiation was made between nauplius I and nauplius II stages of development. Furthermore, the numbers of copepodids recovered were low and the results present the densities of naupliar stages only.
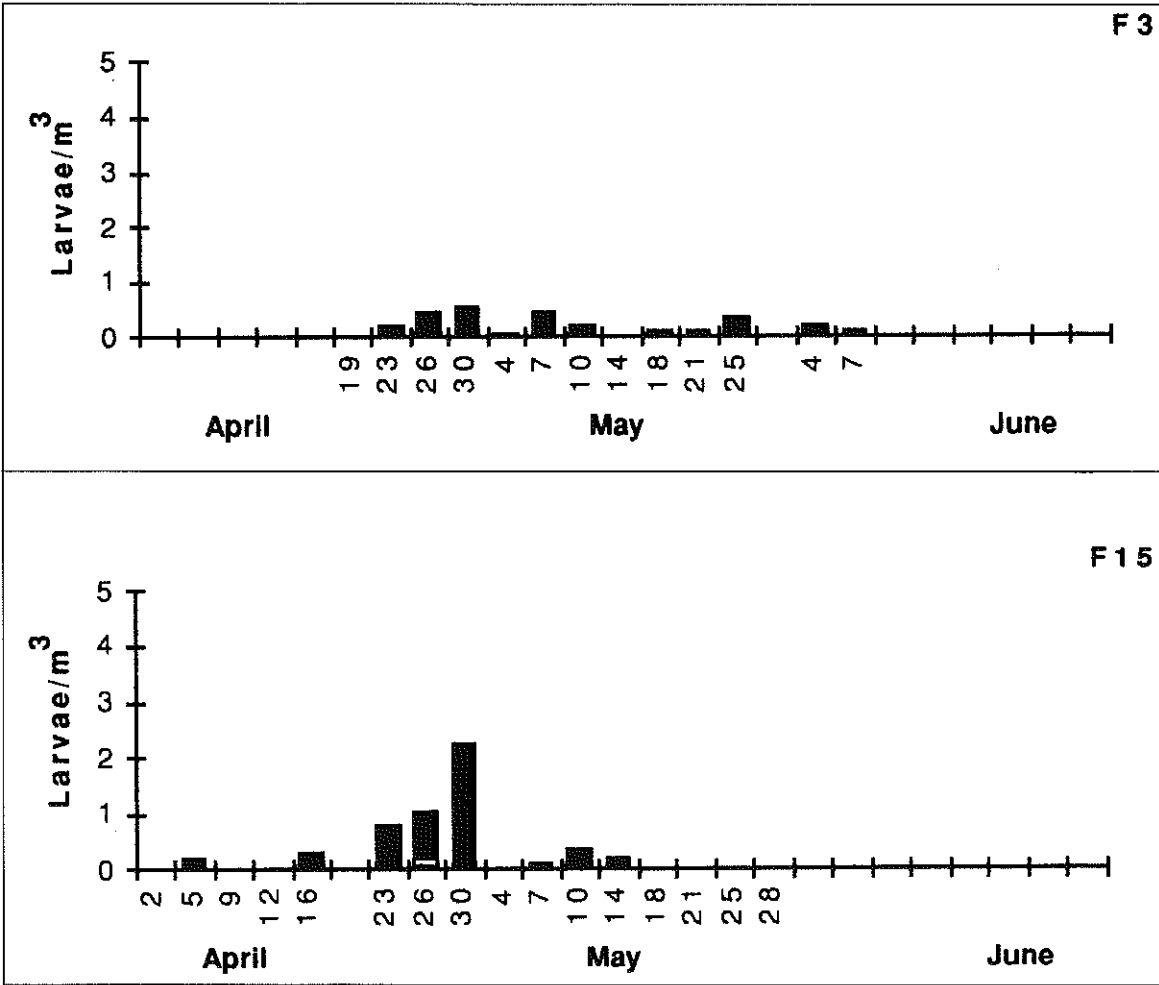

Figure 4.1
Larval returns from surface tows taken in two cages in Killary Harbour, April-June, 1996

4.2. Environmental Data

During the course of the project, water temperature and salinity were measured from sub-surface to off-bottom, the depth depending on the stage of the tide. Daily results were averaged to give a mean temperature and salinity on the days of sampling. Mean water temperatures followed the pattern typical of inshore waters ranging from a high of 16°C in the summer months of 1996 to a mean low of 5.7°C recorded in January 1997. Mean water temperatures began to rise again slowly over the following months until a maximum mean of 14.5°C was reached in mid-June, 1997. Mean salinity values, in general, ranged between 30 - 35S.

Light intensity and water transparency were measured simultaneously with plankton samples on most sampling dates and the recorded values are presented in Figure 4.2. Zero light intensity values are from days on which sampling occurred at dawn or dusk. Given that measurements were taken relative to high water which occurred at different times of the day, there are no apparent trends in either light intensity or water transparency values. It is interesting to note that even though light intensity was much reduced at the beginning of 1997, water clarity was greater than that measured in the following summer months when light intensity increased.

4.3. Larval densities over time

Larval sampling in the cages commenced on 10.06.96 and continued until mid-October when the '95 generation fish were completely harvested from the site. Sampling recommenced on 24.10.96 with the introduction of the '96 year fish to the on-growing site and continued until 17.07.97. Given that the Golam Teóranta production site is a commercial farm, the number of fish within the sampled cage was continuously changing due to harvesting, grading, mortalities, *etc*. Although difficult to quantify exactly, the approximate number of fish within the sampled cage at any one time is given in Table 4.1 along with the treatment history.

Larval densities recorded over both year cycles are presented in Figure 4.3. As the number of fish, together with their associated ovigerous lice load, changed over time within the sampled cage, the numbers of larvae per cubic metre recovered in each plankton tow were standardised to the numbers of larvae per cubic metre per 1,000 fish. This standardisation facilitates a comparison between sampling dates.

Larvae were recorded on all sampling dates during 1996 (Figure 4.3a). However, there was a general fluctuation in larval densities with relatively high densities (maximum 10.6 m^{-3} on 24.06.96) occurring for a number of days periodically within the cage. Larval densities were generally less than 4 m^{-3} on all other dates. The densities of larvae after the 03.09.96 were ≤1 m^{-3} until the end of sampling when all the remaining fish were harvested by 14.10.96.
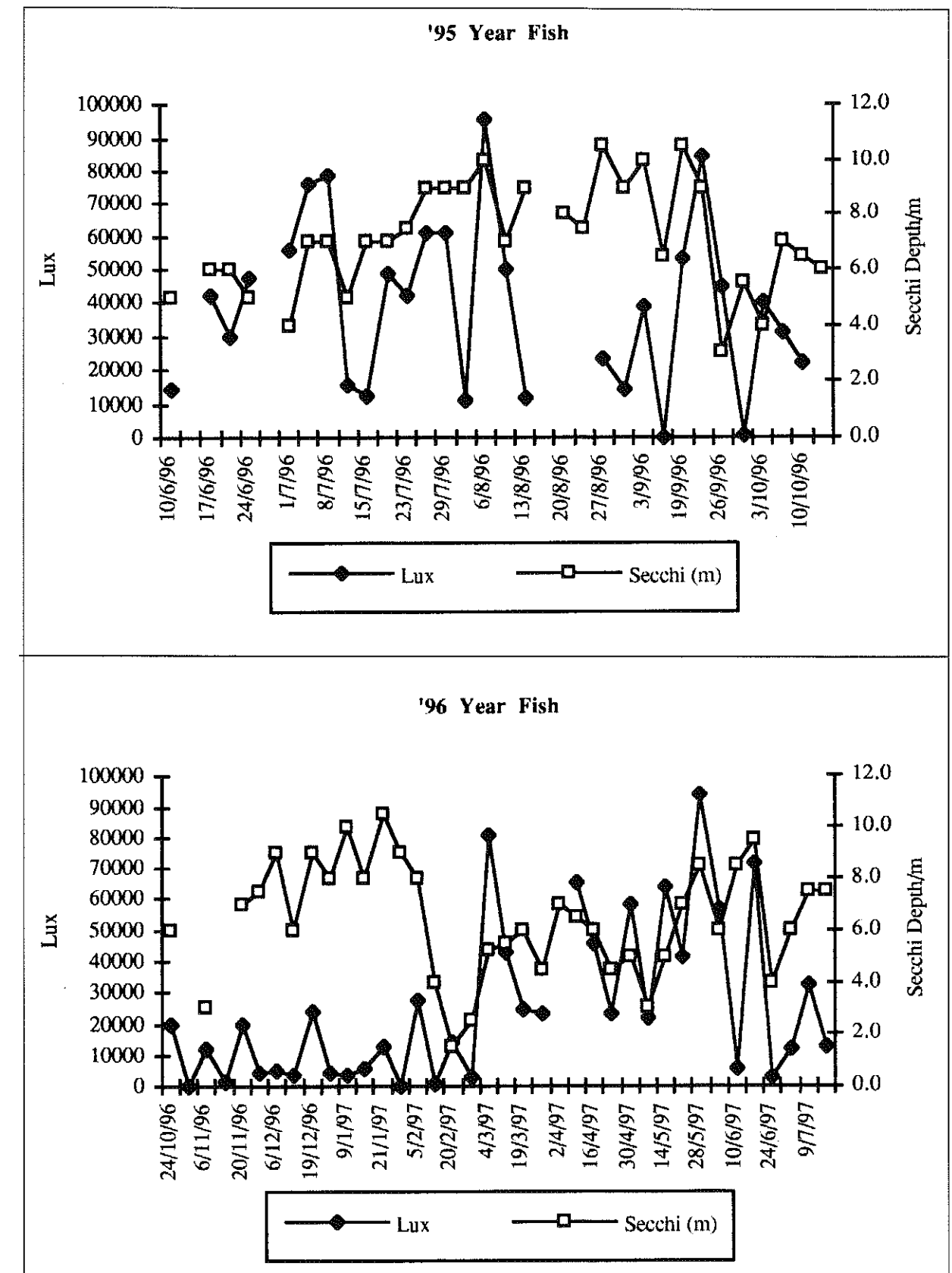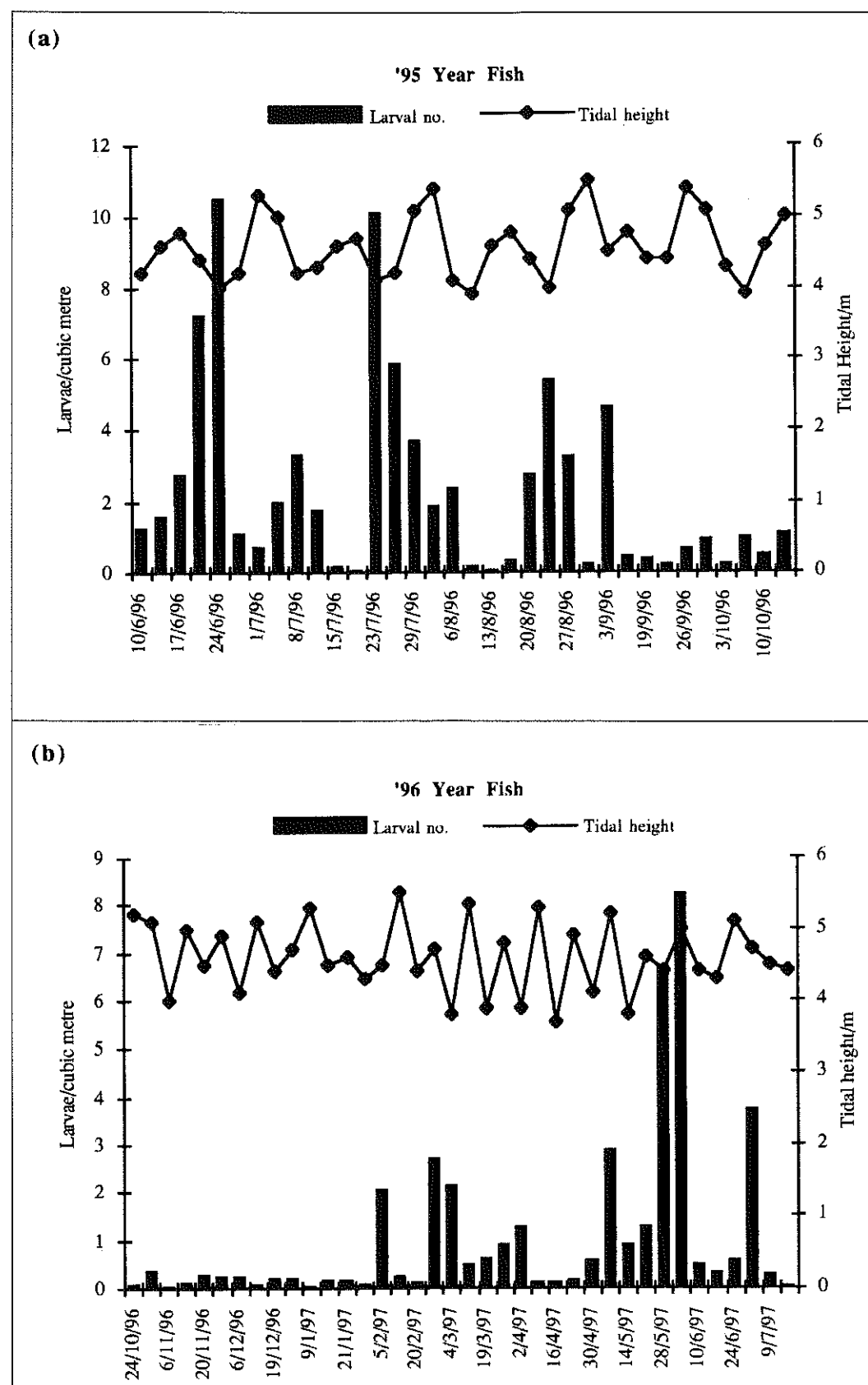

Figure 4.2:
Lux measurement and Secchi depth recorded in Golam Harbour during 1996 and 1997

(a)

'95 Year Fish

(b)

'96 Year Fish

Figure 4.3:
Mean numbers of L. salmonis larvae per cubic metre per 1,000 fish with tidal height for (a) the '95 fish cycle and (b) the '96 fish cycle.

Table 4.1:
Approximate number of fish and treatment history within the cages sampled in Golam Harbour between 10.06.96 and 17.07.97.

| Date	Number of Fish	Fish History
10-6-96	6000 ('95 generation)	downgraded fish
15.7.96	5800	moved to new cage
24.7.96	5800	lice treatment
6.9.96	4800	lice treatment
15.10.96	4000	fully harvested
21.10.96	18000 ('96 generation)	moved to site
26.11.96	6540	graded
7.12.96	8000	graded
9.1.97	7900	weight analysis
1-3.2.97	7900	storms/very windy
10-3-97	7800	lice treatment
18/19.3.97	7700	lice treatment
24.4.97 - 5.6.97	–	harvesting continuously
25.6.97	1000	net change
17.7.97	1000	last larval sampling

The results of larval density in the sampling cage containing the '96 year fish are presented in Figure 4.3b. The number of larvae recovered in tows over the winter months were low, remaining <1 m-3 until the end of January. Densities were variable over the following months but were <3 m-3 until the end of May when there was a significant increase with recorded densities of 6.7 m-3 on 28.5.97 and 8.22 m-3 on 4.6.97. Apart from one date (02.07.97; 3.7 m-3) larval densities recorded after 04.06.97 were <1 m-3.

It is notable that the highest densities occurred on dates coinciding with neap tides during the first quarter of the moon when sampling the '95 generation fish. The data were statistically tested using a Wilcoxon signed ranks test to examine the null hypothesis that densities were not higher on those dates on which neap tides occur. Data for dates on which neap tides occurred were compared with those of both the previous and following spring tide with the result that the null hypothesis was rejected each time, i.e., there were significantly higher numbers of larvae recovered during neap tides than those recovered during spring tides (P<0.001).

4.4. Sea lice population dynamics

In general there were high numbers of parasites on both year classes during the summer/autumn months. The prevalence of *L. salmonis* on the fish was 100% throughout the sampling period. Apart from minor fluctuations, there was little change in the population structure throughout the sampling period.

The mean intensity of male and female lice are presented separately in Figures 4.4. and 4.5. for the '95 and '96 year generation fish, respectively. In general, the adult lice have a higher mean intensity compared to the pre-adult stages. The population had already been established in the first year of sampling ('95 year class). The mean intensities of both the gravid females and adult males show large fluctuations during the summer months. While the pre-adult stages also show some variation in numbers, this is not to the same extent as the adults. The mean intensity of both pre-adult and adult stages are lower in the winter months as seen from the second year of sampling ('96 year class). Mean intensities of the male and ovigerous female adults increase rapidly in the second half of April. Pre-adult male and female levels also increase but not to the same extent as the adults. However, on one occasion (14.05.97) the mean intensity of pre-adult females is higher than the adult level.
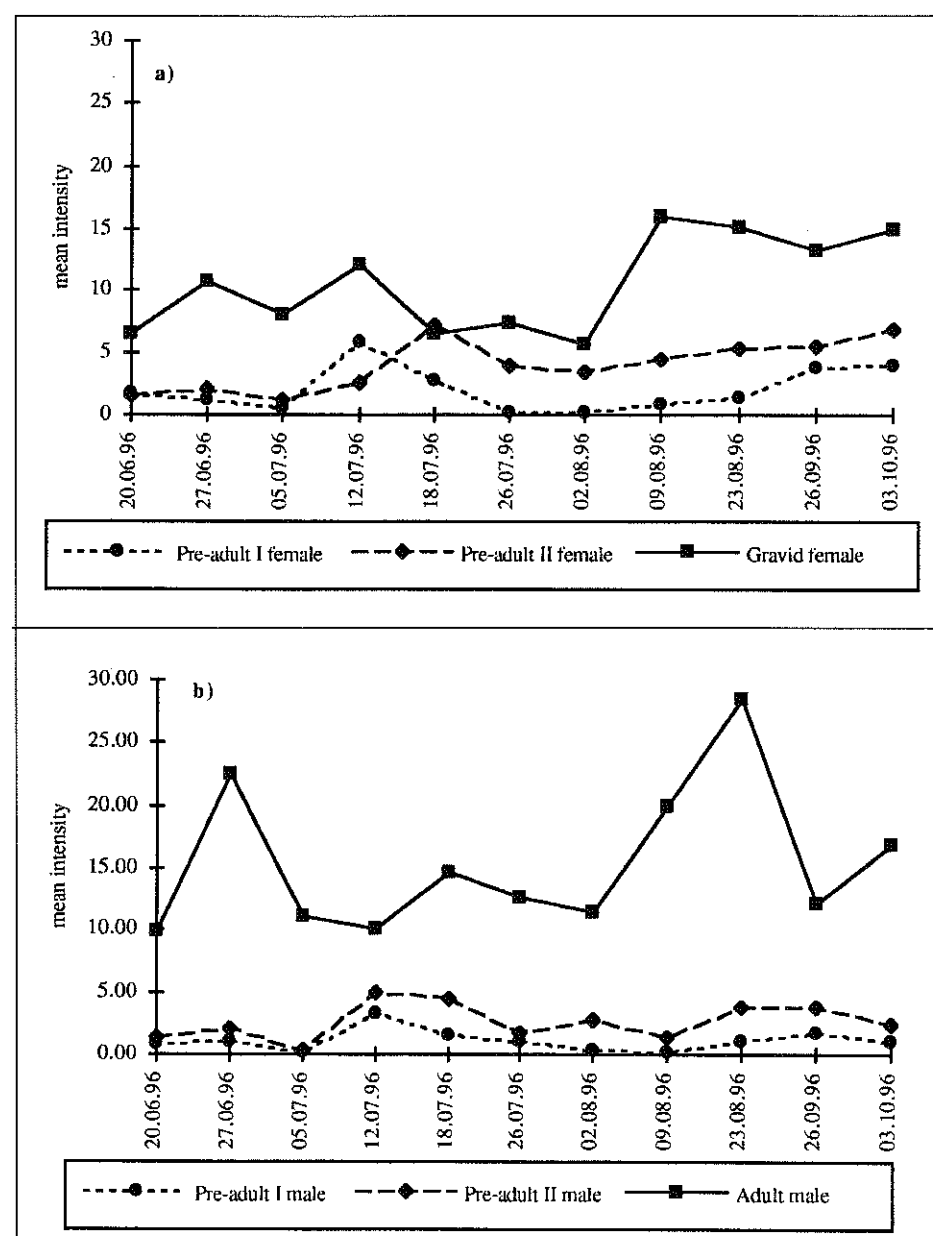
Figure 4.4:
Mean intensity of *L. salmonis* collected from the '95 fish
(a) pre-adult and adult females, (b) makes. (No. of fish = 12 per sampling date.
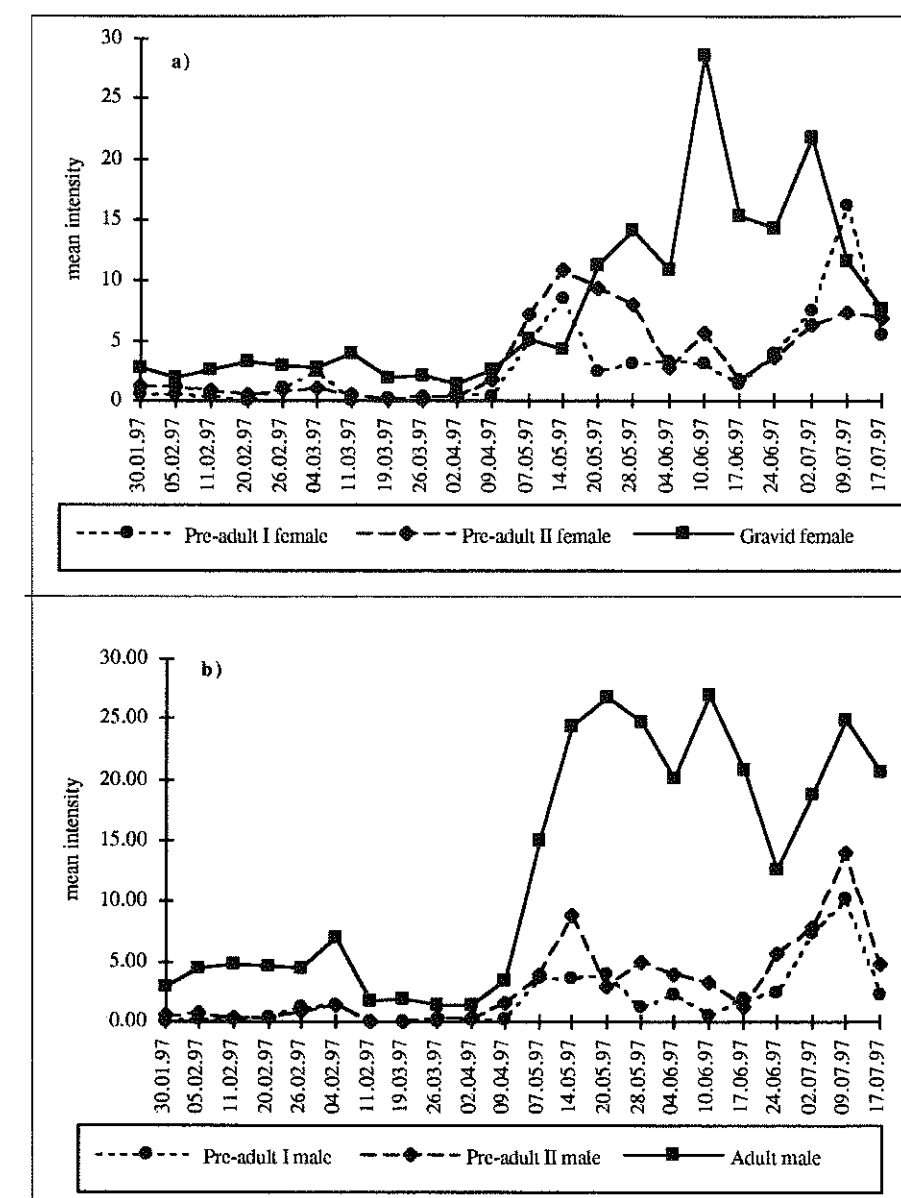
Figure 4.5:
Mean intensity of *L. salmonis* collected from the '96 fish
(a) pre-adult and gravid females, (b) makes. (No. of fish = 12 per sampling date.

4.5. Vertical migration

Throughout the vertical migration studies the majority of larvae recovered were at the nauplius stage of development. Few copepodid stages were found. Consequently, only nauplii are dealt with in the data and these are referred to as larvae per cubic metre.

The first two trials were undertaken in July, 1996 in Golam Harbour during the spring tide period. During Trial 1, mean water temperatures ranged from 15.65°C - 16.15°C. Light intensity was greatest at the beginning of the experiment and decreased gradually until dusk, as would be expected for a mid-summers day. The Secchi depth remained between 8.5 and 9m until it could no longer be read after dusk. Current movement inside the cage during this trial was negligible and below the precision level of the meter.

The mean density of larvae recovered in the surface waters and at 3m are presented in Figure 4.6. Highest densities of larvae occurred around the low water period between 13:00 and 14:00 and remained high up until 15:00. Densities decreased over the following three hours until high water when densities increased again and remained relatively high during the following four hours. The densities of larvae at 3m exhibited a similar pattern over the sampling period.
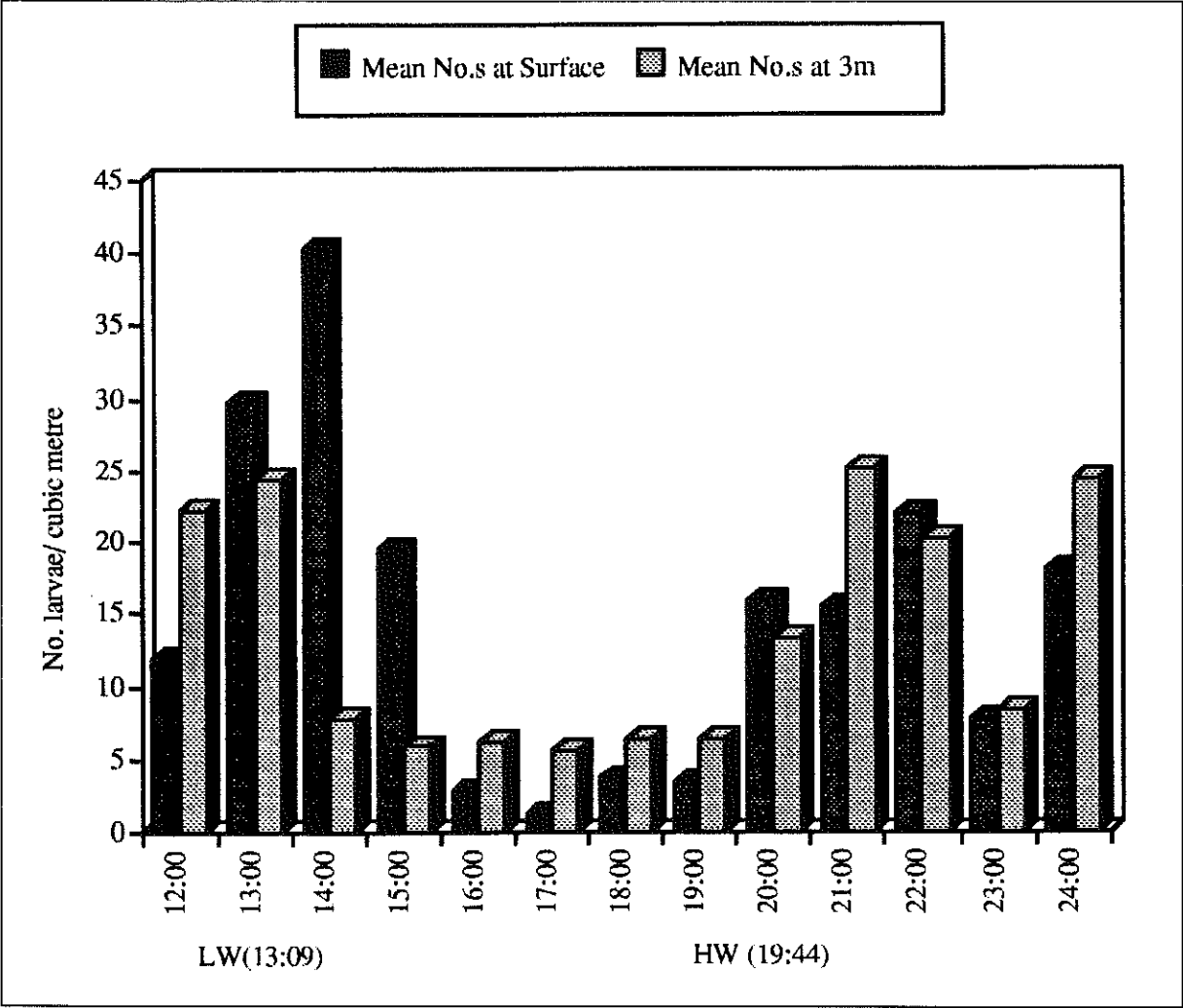
Figure 4.6:
Mean numbers of larvae per cubic metre recovered at the surface and at 3m during the 12hr vertical migration study on 18.06.96.

The densities of larvae recovered in plankton tows taken 20m from the cages show a similar pattern with highest densities recorded before low water and after high water, with few or no larvae found during the flooding tide (Figure 4.7). These tows were taken on the seaward side of the cage block.
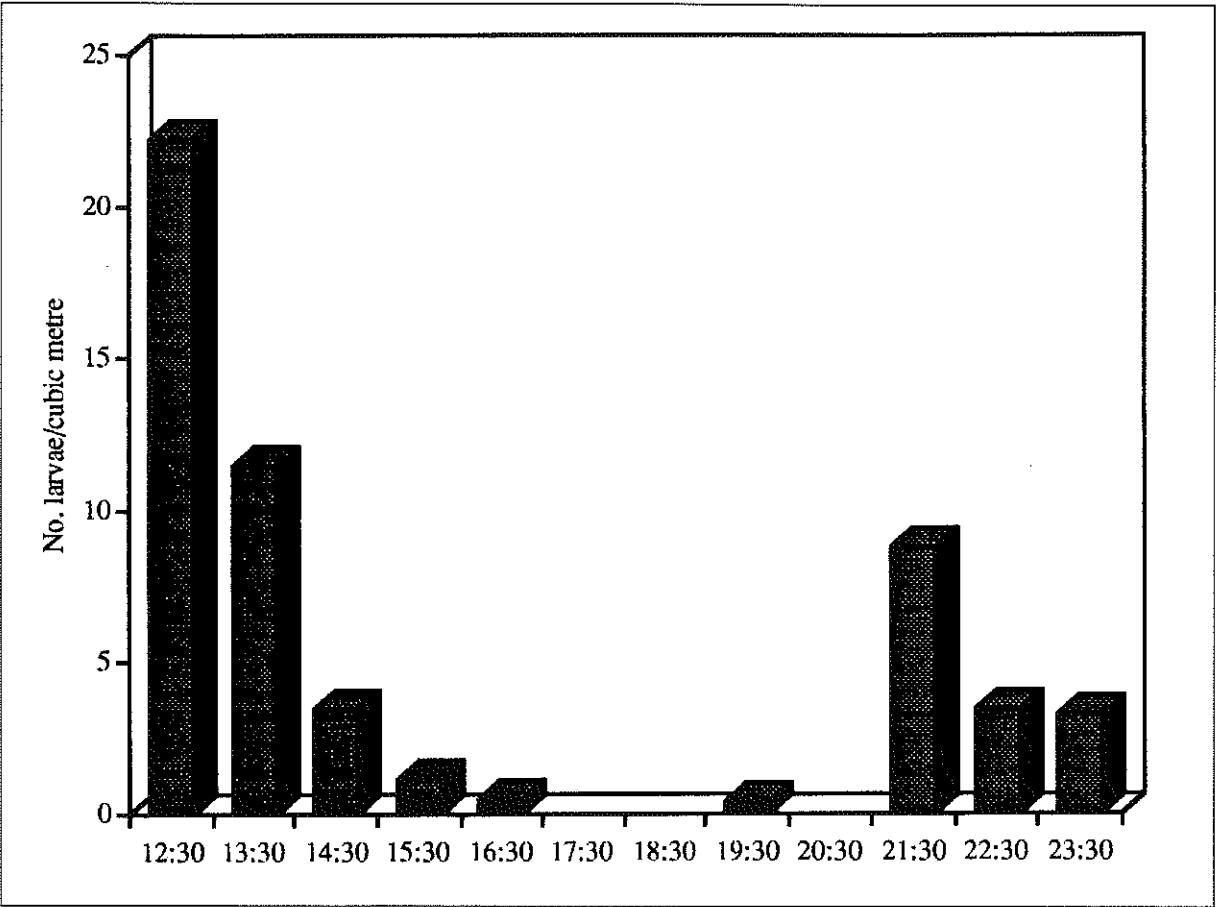
Figure 4.7:
Mean numbers of larvae per cubic metre recovered in the surface tow taken 20m away from the cages during the 12hr vertical migration study on 18.06.96.

The second trial took place two days later on the 20.06.96, running for a twenty four hour period. The mean water temperature ranged between 15.5–16.5°C during the sampling period. Lux and Secchi measurements gave highest readings during the brightest daylight hours falling off to zero during the night. Current movement inside the cage during this trial was negligible and below the precision level of the meter.

Larval densities recorded during this trial are presented in Figure 4.8. Apart from one ebbing tide period, larval densities at the surface and 3m were similar. During the ebbing tide following high water at 21:00, surface densities were generally higher than at 3m. During both flooding tides sampled, larval densities were lower at both depths compared with densities at any other stage of the tide.

The results of surface plankton tows taken 20m west of the cage units showed a similar pattern to inside the cage (Figure 4.9.). Highest larval densities were found during the ebbing tide and slack water periods at high and low water. Relatively few larvae are recorded during the flooding tide.
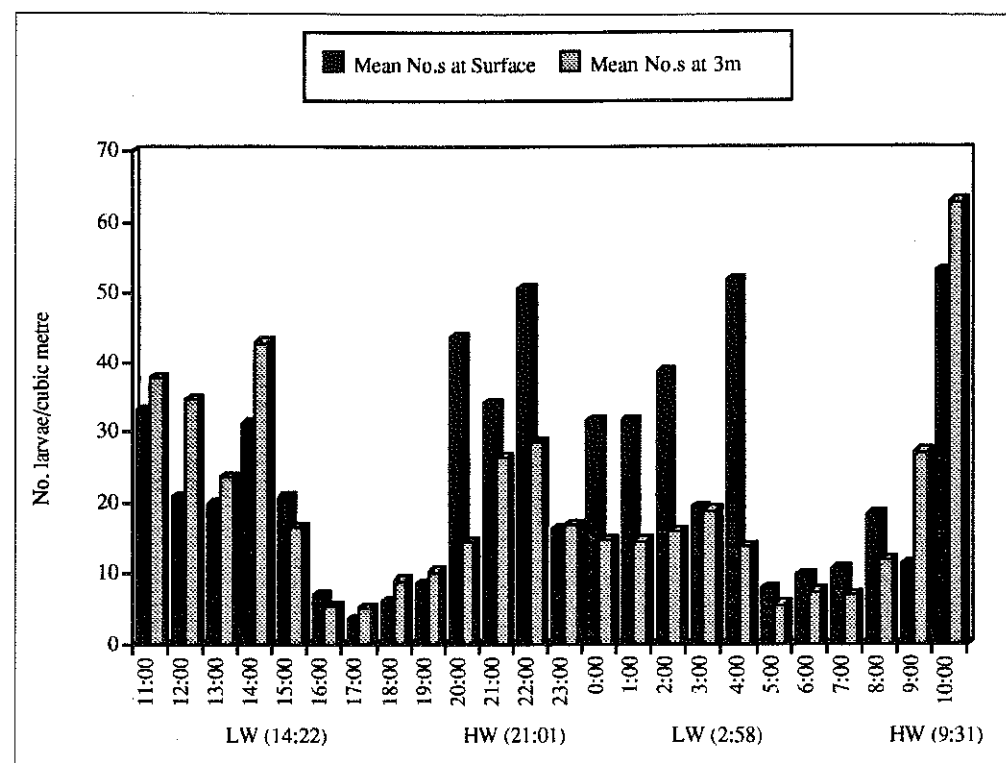
Figure 4.8:
Mean numbers of larvae pre cubic metre recovered at the surface and at 3m during the 24hr vertical migration study on 20-21.06.96, in Golam Harbour.
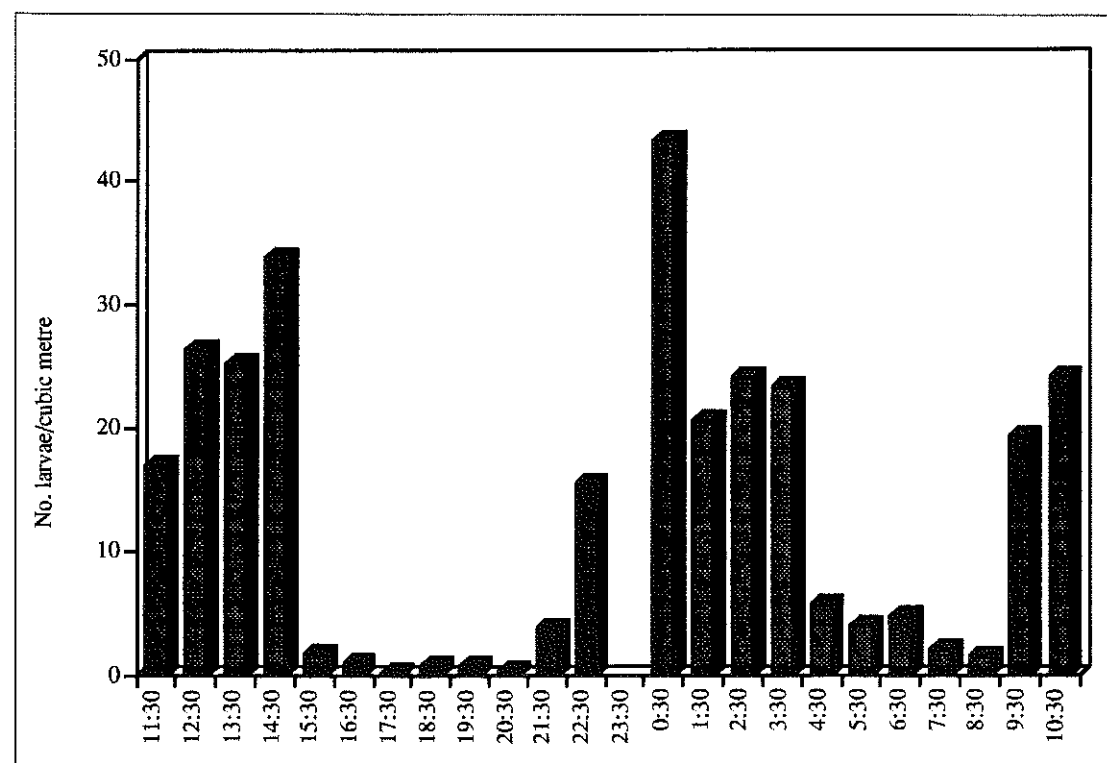

Figure 4.9:
Mean numbers of larvae pre cubic metre recovered in the surface tow taken 20m away from the cages during the 24hr vertical migration study on 20-21.06.96, in Golam Harbour.

The subsequent three vertical migration experiments took place in a Bridgestone® cage in Ardmore Bay in the summer of 1997. Throughout the studies, current speeds inside the cage, were <0.1 ms^{-1} when above the meters precision limit while comparable currents outside were in the region of 0.1 ms^{-1}.

Trial 3 was carried out on the last quarter neap tides on the 29.05.97. Mean water temperature ranged between 14.0 - 14.7°C with little fluctuation in salinity. Light intensity showed a typical regime, with the brightest part of the day at around 13:00 and then falling gradually until dusk.

The numbers of larvae per cubic metre recovered in the surface waters were low at the start of the experiment, remaining at ≤20 until 14:00 when a ten-fold increase occurred (Figure 4.10.). This increase peaked at 15:00 and subsequently dropped to 40.m^{-3} and then back to below 20.m^{-3}. There was one smaller peak at 19:00 and another subsequent drop in levels thereafter. Those samples taken at 5m show a similar pattern, however, the numbers of lice were initially high and then dropped off with a later peak at 16:00. There was a small rise in numbers at 19:00 and like those at the surface, was followed by a subsequent drop in densities. Densities of larvae recorded tidally upstream and downstream were generally low when compared to the densities recovered within the cage (Figure 4.11).

Figure 4.10:
Mean numbers of larvae per cubic metre recovered at the surface and at 5m during the 12hr vertical migration study on 29.05.97, in Ardmore Bay.

Trial 4 was undertaken on the 16.07.97, during the first quarter neap tides. The densities of larvae recorded inside and outside the cage were generally low when compared to the previous trial. Larval densities from the surface samples and 5m were similar and densities at both depths were generally higher on the flooding tide compared to ebbing (Figure 4.12.). Densities recorded outside the cage were variable and showed no trend throughout the experiment (Figure 4.13).

Figure 4.11:
Mean numbers of larvae per cubic metre recovered during the 12hr vertical migration study in Ardmore on 29.05.97, (a) upstream of the cage, (b) downstream.

Figure 4.12:
Mean numbers of larvae per cubic metre recovered at the surface and at 5m during the 12hr vertical migration study on 16.07.97, in Ardmore Bay.

Figure 4.13:
Mean numbers of larvae per cubic metre recovered during the 12hr vertical migration study in Ardmore Bay on 16.07.97, (a) upstream of the cage, (b) downstream.

The final experiment was carried out during the full moon spring tide on the 22.07.97. Larval densities recorded at 5m and the surface were again similar throughout the study (Figure 4.14.). Larval densities before and after low water were low c. 2 m^{-3} but gradually increased with the flooding tide to peak before high water at c. 10 m^{-3}. Larval densities recorded 20m either side were similar to those recorded within the cage and exhibited the same pattern (Figure 4.15.).

In each of the above studies, larval densities in the top 5m of the water column were variable over time. However, when comparing each of the experiments, little pattern could be detected in larval densities relative to the stage of the tide other than that highest densities were generally found during slack water at high water.
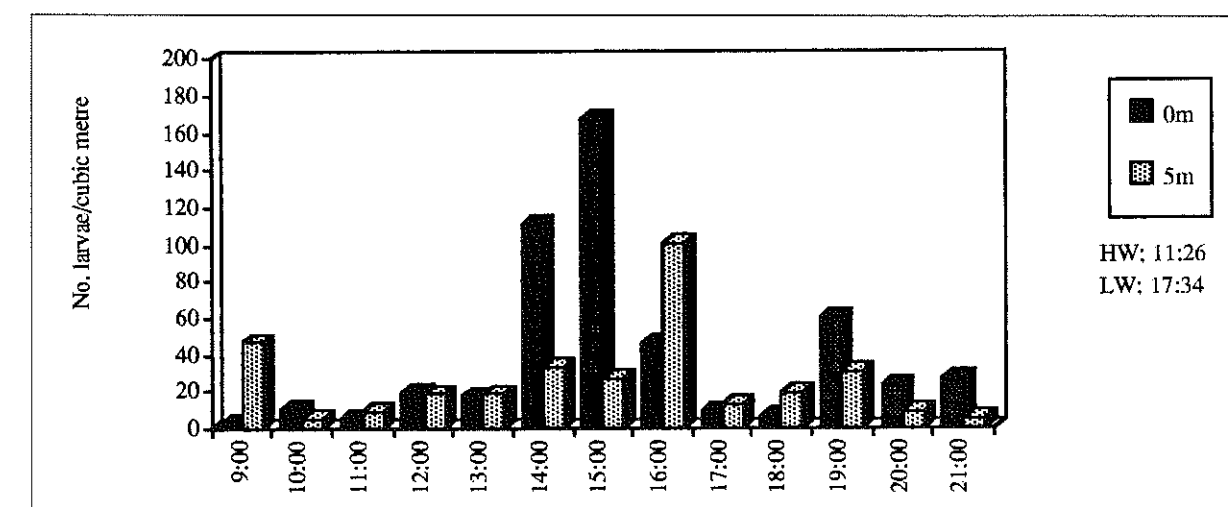
Figure 4.14:
Mean numbers of larvae per cubic metre recovered at the surface and at 5m during the 12hr vertical migration study on 22.07.97, in Ardmore Bay
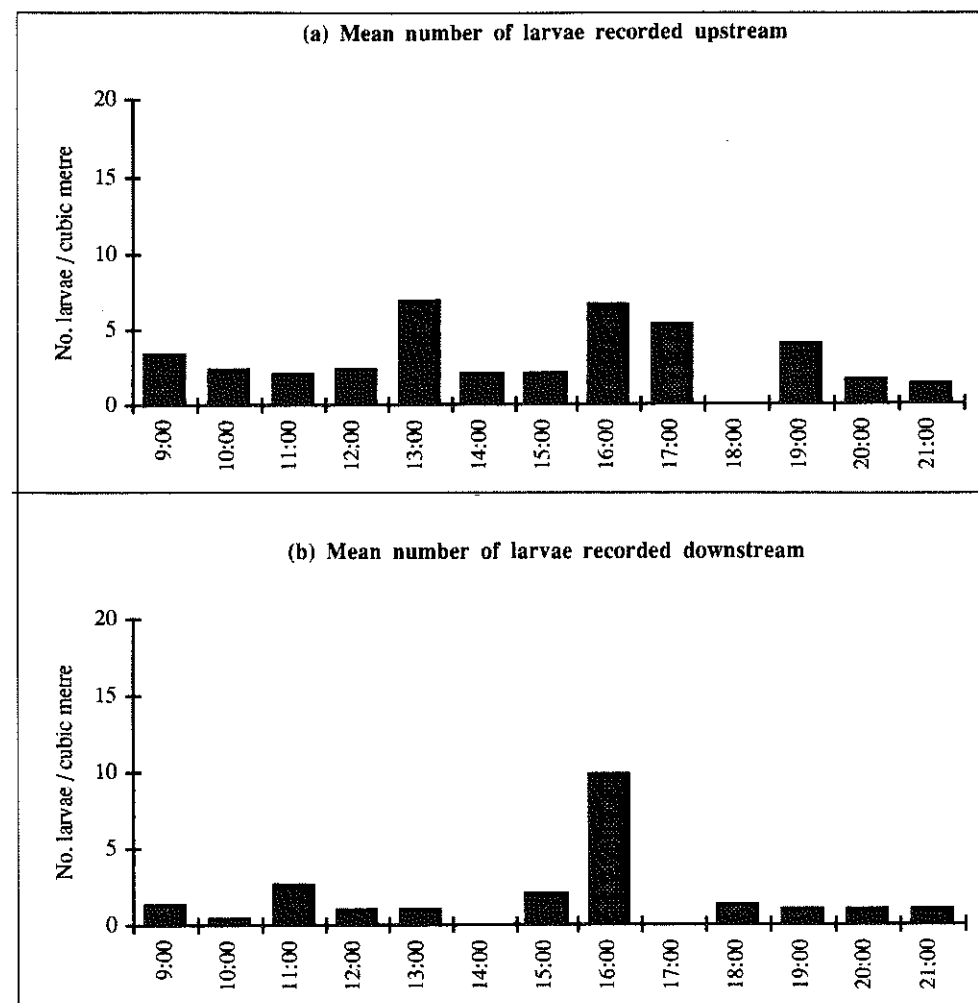

Figure 4.15:
Mean numbers of larvae per cubic metre recovered during the 12hr vertical migration study in Ardmore Bay on 22.07.97, (a) upstream of the cage, (b) downstream
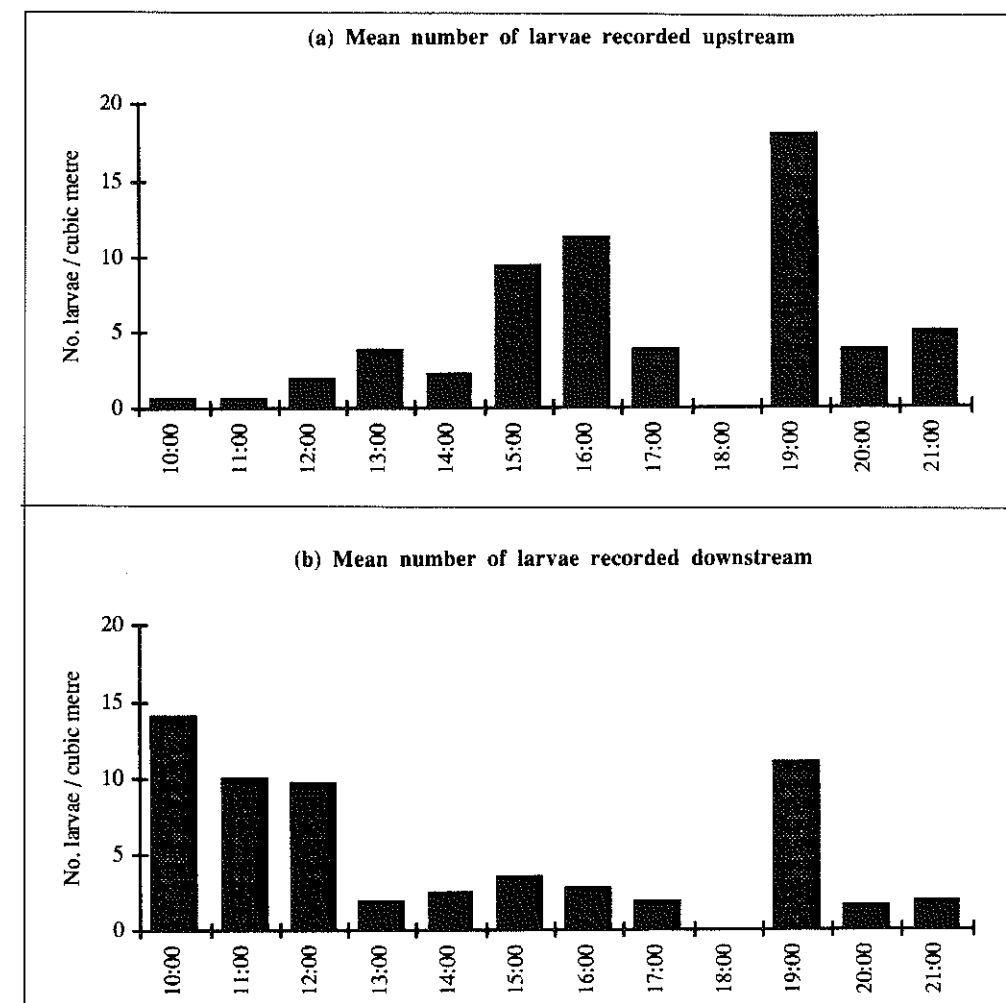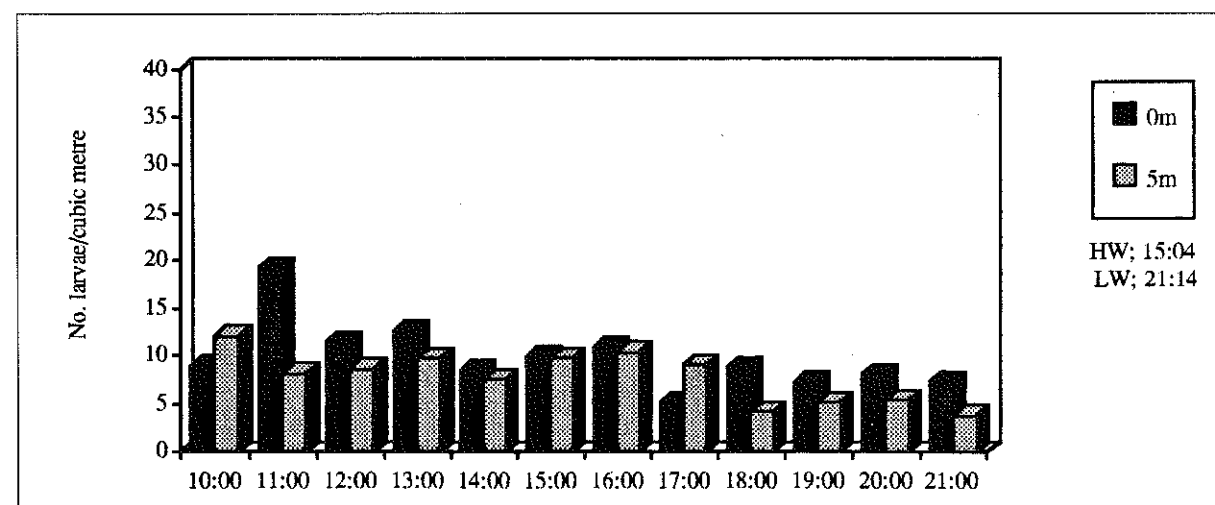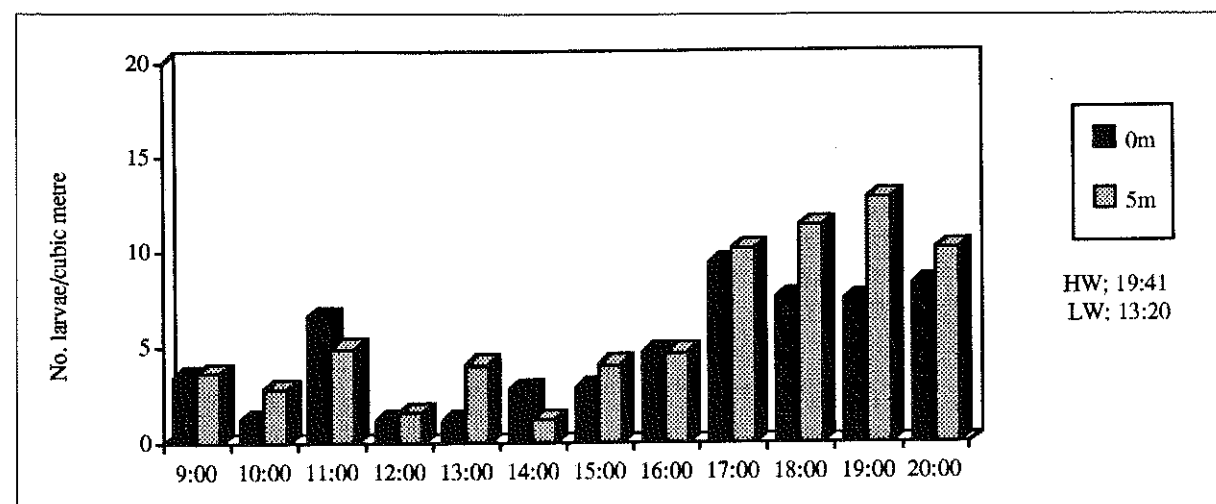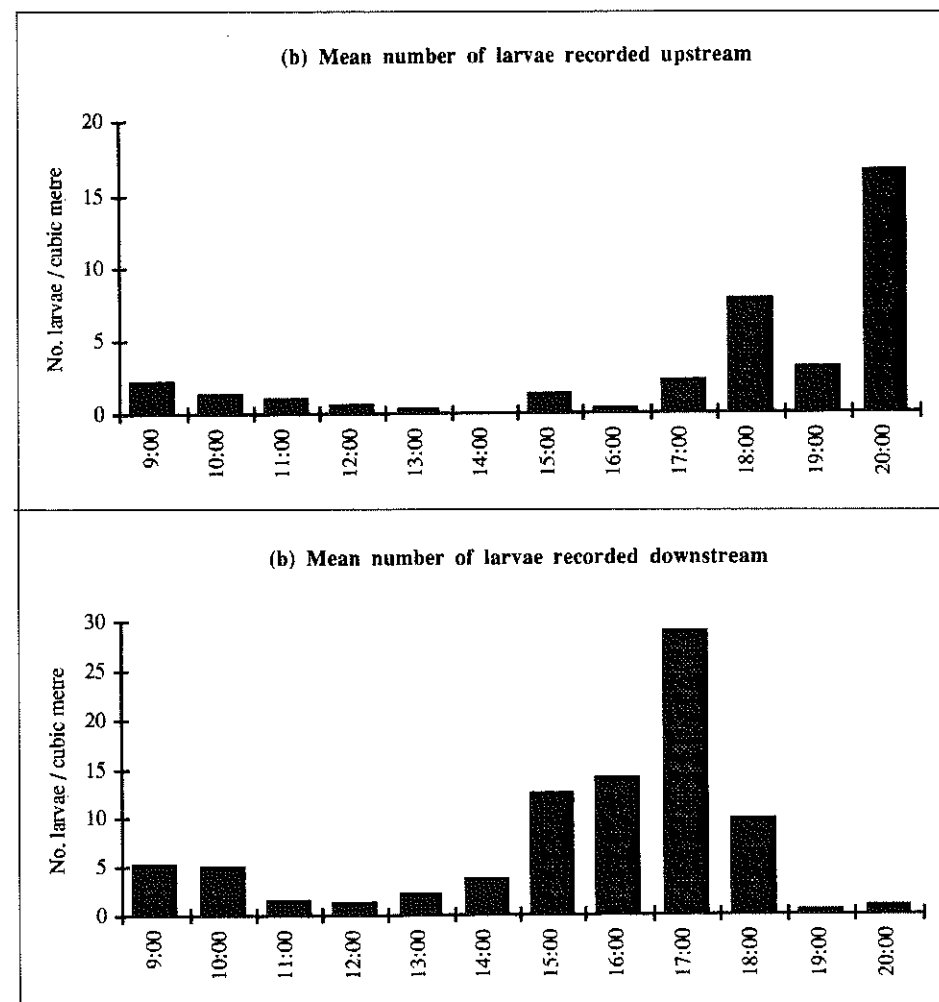
5. Discussion

5.1. Larval sea lice density

For infection to occur, the infective stages of L. salmonis and a suitable host have to meet. However, the extent and mechanism of the dispersion of the early lice stages is largely unknown (McVicar, 1997). During early studies on the distribution of the larval stages of sea lice, few planktonic stages of L. salmonis were recovered from selected bays on the west coast of Ireland (Aqua-Fact, 1994), Scotland (Gravil, 1996) or Norway (Boxaspen, 1997) even though adult lice were abundant on salmon held in cages in these areas. However, when sampling methodology was refined and concentrated close to and in salmon cages, substantial larval densities were recorded (Aqua-Fact, 1995, Costelloe et al., 1996). Similarly, high densities of larvae were recorded within salmon cages during the current study (see Section 4.3.) and it was found that these densities were higher in the summer months with a reduction in numbers in late autumn of 1996 and the beginning of 1997. This reduction corresponds to a reduction in parasitic intensity which is related to the decrease in water temperature during these times. Hogans & Trodeau (1989) reported a similar reduction of parasitic intensity over the winter period. Additionally, there have been reports concerning the viability of eggs produced during winter. Richie et al. (1993) stated that there appeared to be more areas of disorganised or discoloured eggs present in L. salmonis egg-strings during winter, suggesting a seasonal variation in the condition of the eggs. However, Gravil (1996) observed no seasonal variation in egg viability in her study but found a seasonal variation in fecundity with maximum number of eggs in the egg-strings occurring in March and minimum numbers in October which was directly correlated with both temperature and female cephalothorax size.

Few infective copepodid stages were recovered during the sampling period during this study and the studies mentioned above i.e., Aqua-Fact, 1995, Costelloe et al., 1996. A similar finding was recorded by Gravil (1996) when sampling within Scottish salmon cages. The number of copepodids will be reduced by dilution, predation and natural mortality of the larvae as they develop and therefore, there is a natural reduction of copepodids in the water column compared to the earlier free swimming stages. Johnson et al. (1993) reported an average of 16% of L. salmonis eggs attaining infective status, whilst Johnson & Albright (1991a) found this figure to be 27% and Gravil (1996) reported a figure of 18%. In addition, it is highly probable that as the larvae develop to the copepodid stage, they immediately attach to the available reservoir of hosts present within the cage. When this reservoir of hosts are not available i.e., outside the cage, it should be possible to locate copepodids as was the case in studies carried out by Costelloe et al. (1996) and Costelloe et al. (in press), both studies being carried out in inner estuarine areas.

There was a temporal variation in larval densities during the summer months with distinct time periods in which larval densities were relatively high compared to the overall mean. These peaks in larval density are particularly obvious during the first year of sampling (June - October 1996 on '95 generation fish) and correlated to neap tide periods (see Figure 4.3a.). A similar pattern is not obvious from the second year of sampling which may be

related to an increase in handling and treatment of these fish compared to the first year of sampling (see Table 4.1). Grading, harvesting and other physical disturbances of fish such as crowding when reducing the depth of the net during treatments together with the actual chemicals used in treatment can cause a loss of lice or induce spawning in gravid females. Such occurrences will interfere with parasitic intensity on the fish, mask natural spawning patterns and generally interfere with the natural development of the lice population. During the period of highest larval densities in 1997 (May - June), the fish in the sampled cage and the surrounding Turmec® cages were being harvested continuously. Lateral transfer of lice have been noted on fish during periods of harvest (Jackson et al., 1997) and physical and temperature shocks will cause hatching of larvae which may not have occurred if left undisturbed. Following harvesting, additional fish were added to the Turmec® cages from the surrounding polarCirkel® cages. Due to this fish movement and general disturbance, any patterns in larval lice densities, which may have occurred naturally, will not be obvious from the results. In contrast, the harvest period during 1996 had more or less finished when sampling commenced, and consequently, the lice population on these fish were left undisturbed.

The periodicity in larval densities during the first year of sampling is due to one of two reasons or a combination of both; the larvae were present in equal densities throughout the sampling period (i.e., continuous supply of larvae) but there was a variability in the larval recovery rate for a number of possible reasons (e.g. flushed from the cage, wrong sampling method etc.) or the larvae were only present in the cage periodically .

There are a number of factors that must be taken into consideration when investigating larval densities within a cage. During previous studies (Aqua-Fact, 1994 and 1995) it was shown that larval densities in the surface water of the cage varied depending on the stage of the tide e.g. mid-flood, high water etc. Consequently, during the current study it was decided to take samples at the same stage of the tide (i.e., within one hour of high water) on each sampling date. Additionally, as the number of larvae produced within a cage will depend on the ovigerous lice load and fish numbers were not constant over the sampling period, all density data were standardised to the number of larvae per cubic metre per 1,000 fish. Temporal comparisons could then be made between samples.

During studies in Ardmore Bay, Costelloe et al. (1996) concluded that retention of larvae within the cage sampled was the main factor responsible for higher numbers of larvae being recorded inside a cage compared to densities outside. Gravil (1996) also found a higher concentration of nauplii within cages compared to immediately outside the net and reported that the most plausible explanation for this was a combination of the retentive nature of the nets and the dispersal that will occur immediately outside the cages. There are many factors which may cause the retention of larvae within a cage. For example, a structure such as a net will alter the flow field when placed in a current: owing to the resistance of the net, part of the flow will be forced under and to the outside of the net while the remainder will flow through the net at a lower speed (Løland, 1993). The mesh size and the degree of fouling on the net, e.g., tunicates, hydrozoans and algae, will further affect current movement into and out of the net, with fouling acting as a baffle to decrease the penetration of currents through the net. The very presence of the fish themselves may

act as an additional barrier to the movement of current through the cage. In addition, the position of the cage within the fish farm system will determine the strength of the current acting on it in relation to the prevailing currents within the general area. A cage located 'downstream' in a configuration of cages will experience a reduction in current strength due to its position in the wake of the other cages. This would be more significant in a block of cages with a combined mooring system than single cages such as polarCirkel® and Bridgestone® cages which may be moored independently of each other. 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. Iwama (1991) noted a drop in current velocity relative to the outside current inside a 20x20x6m net cage with a 5cm mesh and stocked at 1.6kg fish/m^3 of 65%, and a decline in flow rate in successive cages aligned parallel to the current.

While the net in a cage system may act as a barrier to prevailing currents it does not completely hinder the movement of sea lice larvae to and from the cage. Larvae may be flushed out of the cage by water currents or by passive dispersion. Once away from the point source, there will be a dilution of numbers with distance from the cage due to the large receiving body of water. It is notable that the cyclical pattern observed in the present study was synchronised with the lunar tide, in that highest densities were recorded on dates coinciding with the neap tide. It is possible, therefore, that differences in currents and tidal range between spring and neap tide periods caused the variance in the observed larval densities within the cage with a greater number of larvae being washed from the cage during spring tides. Higher densities of larvae were recorded outside the cages during spring tide periods compared to neaps during the vertical migration studies (see section 4.4). However, these studies were carried out at different locations, two of the three spring tide studies being carried out in Golam Harbour, while Ardmore Bay was the location during two neap tides and one spring tide. The difference between larval densities during spring and neap tides outside the cages may reflect the oceanographic difference between the two sites and not current or tidal differences affecting the cage due solely to tidal state as might be assumed. Additionally, although the studies were carried out during spring tide periods in Golam Harbour, current measurements made inside the net were below the precision level of the meter over the tidal range indicating negligible currents. However, no measurements were recorded outside the cage on this occasion and the effect of the net on current strengths within the cage cannot be assessed. Current measurements recorded inside the cage in the trials in Ardmore Bay were significantly lower (Wilcoxon signed ranks test; P<0.05) than those recorded outside.

Larval densities within the cage will be related to the number of spawning individuals producing those larvae. Figure 5.1. presents the larval densities recorded in 1996 with the total number of ovigerous lice present within the cage sampled. Prior to the occurrence of peaks in larval densities there were decreases in the total number of ovigerous lice, presumably brought about by synchronous spawning rather than a physical loss of lice. It is unclear what impulse(s) may have caused these spawnings. The hatching and development of the early stages of L. salmonis is highly variable, even under constant environmental conditions, and relatively small changes in temperature and salinity will have a further influence which highlights the complex nature of this parasite (Gravil, 1996). Gravil (loc. cit.)
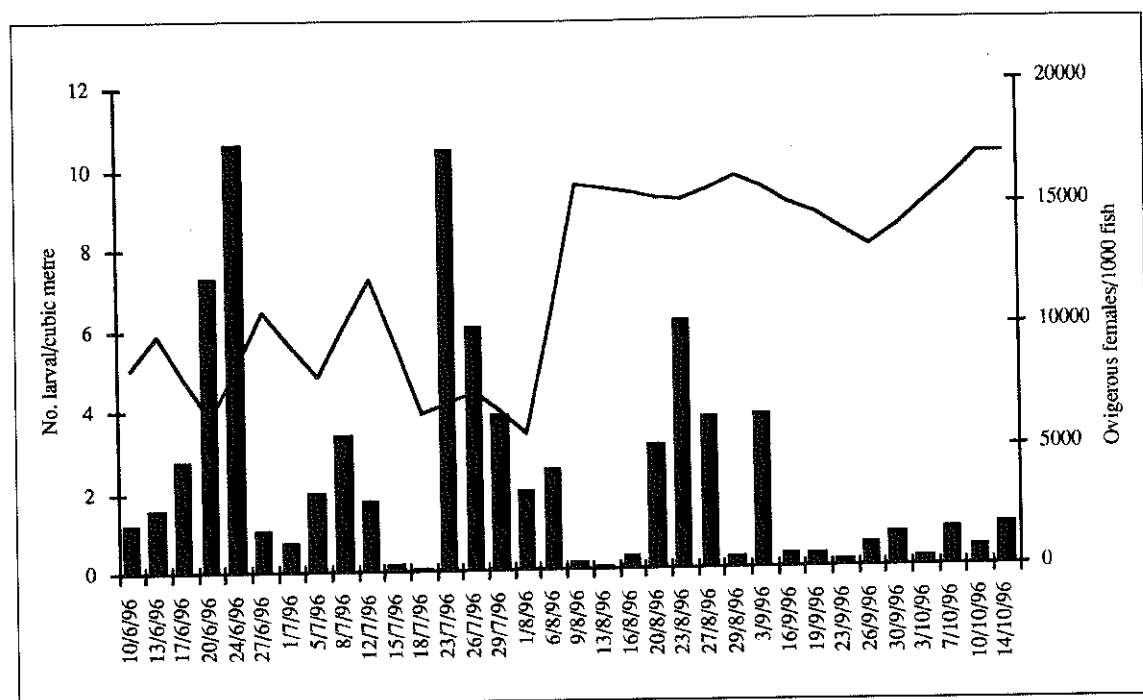
Figure 5.1:
Density of larvae in the sampled cage relative to the number of ovigerous lice per 1,000 fish. (Larval densities are standardized to larvae per cubic metre per 1,000 fish).

suggested that nauplii within the egg excrete solutes to increase the concentration gradient across the egg membrane, causing an influx of water by osmosis resulting in hatching. Given that the nauplii can detect changes in environmental conditions *e.g.* pressure and temperature (Bron *et al.*, 1993; Gravil, 1996), it is possible that it can use these or other cues to initiate hatching during favourable conditions.

Synchronous spawning by crustaceans is not unknown and has been reported previously by other authors. Christy (1978), reports data on reproductive cycles in individually marked male and female fiddler crabs, *Uca pugilator*. The data reported suggest that the phase relationship between female reproductive cycles and the semilunar cycle of tidal amplitude and current velocity is an adaptation to maximise the probability that the final larval stage will settle on substrates suitable for adults. Hatching in a variety of other crustaceans, including other non-parasitic copepods, is regulated by abiotic factors such as illumination, tides and temperature (DeCoursey, 1983, Sastry, 1983). In some species of parasitic copepods it has been suggested that environmental cues trigger egg hatching (Poulin *et al.*, 1990b). Roth (1988) reported that egg hatching in *Haemobaphes intermedius*, a copepod gill parasite of intertidal fish, may be under the influence of tides and other environmental stresses. *H. intermedius* may use these cues to hatch at times when several hosts are concentrated in a small volume of water, (i.e., tidal pools), thus facilitating the transmission of the parasite. However, in general, there is little published data on synchronous spawning in planktonic copepods. 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. Gravil (1996) found that hatching was not observed to be directly influenced by environmental stimuli such as photoperiod or exposure to light or darkness. She suggests that by having two non-infective naupliar stages, there is little point in environmental cues initiating hatching and would seem highly unlikely that the naupliar stage will demonstrate host-finding capabilities because of their inability to infect fish. However, behaviour that would bring or keep the nauplii in areas inhabited by hosts would increase the chances of a future host-parasite encounter by the infectious copepodid stage due to the spatial overlap of each other. It would be a waste of the available energy reserve of the larval stages should the nauplii stages disperse into areas not frequented by salmonids. Additionally, non-dispersal of the infective larvae would result in pulses of infection on fish as they encounter these larvae. Peaks or pulses in hatching of parasitic copepodids have been reported previously (Roth, 1988; Tully, 1989; Poulin *et al.*, 1990b, Costelloe *et al.*, 1995) while Costelloe *et al.* (in press) recorded high densities of larvae in localised areas in an inner estuary.

The durations of peak numbers of larvae were approximately 4 - 5 days on each occasion in which high densities of larvae were recorded within the cages. Gravil (1996) noted that the mean duration of hatching of *L. salmonis* larvae was 9 days at 10°C, although in the last 5 days, only 0.7% of eggs hatched while the majority (78.1%) hatched within the first 48 hours. Combining these figures with a cumulative average life span of the naupliar I and II stages of approximately 2 days at 15°C (Johnson & Albright, 1991a), the observed levels of larval densities suggest that a large number of ovigerous lice spawned during the same time period indicating a synchronicity in spawning presumably initiated by an environmental cue. It is highly unlikely that the observed level of hatching, over such a relatively short time span, would occur if larvae were simply to hatch as they developed without such a cue.

Although there may be a number of factors affecting larval densities within the cage as discussed above, it is probable that the variance in larval densities is mainly due to a periodicity in spawning output with changes in current strengths due to tidal stage also playing a part. Further detailed studies, incorporating histological examinations of egg development, need to be carried out to ascertain the exact nature and degree of the spawning pattern in *L. salmonis*.

5.2. Population dynamics

L. salmonis was present on the salmon throughout the year with each life stage occurring on each sampling occasion. This observation was also made on commercial marine fish farms in Scotland where *L. salmonis* was recorded on Atlantic salmon throughout the year (Wootten *et al.*, 1982; Gravil, 1996). However, it is difficult to assess the natural progression of the population during the current study as interference caused by fish movement, treatments, general handling and harvesting caused disruptions in the lice population structure either through physical removal of various life stages or reduced/increased spawnings triggered by physical disturbance. Additionally, the copepodid and chalimus stages were not enumerated and it is therefore impossible to follow development and infection success from free-swimming larvae to adult parasite on the fish. When sampling

began in 1996, the lice population on the salmon was well established with adult male and female *L. salmonis* the dominant stages present. The natural progression from male pre-adult to adult stages is unclear and there is no evidence of a succession of generations. Little change occurred in the pre-adult mean intensity although there were significant increases in the adult mean intensity (see Figure 4.5). Similarly, pre-adult II male intensity values were higher than pre-adult I values. Given that the duration of pre-adult male I and II is 8 days and 9 days at 10°C, respectively, and will be reduced at higher temperatures, it is possible that peaks in the pre-adult population were missed with the sampling regime employed, i.e. sampling every 7 - 10 days. Additionally, males have been observed to migrate from fish to fish, particularly the adult stage (Jaworski and Holm, 1992). A succession in generations was noticeable in the female population on these fish (95 generation). Following a reduction in the ovigerous mean intensity at the end of June 1996, the pre-adult I female intensity value peaked on 12.07.96 and pre-adult II on 18.07.96 (Figure 4.5.). The fish were treated on the 24.07.96 and a subsequent increase in adult females was not observed. Following this cohort from a larval peak on 24.06.96 (Figure 4.3a.) to a pre-adult I peak on 12.07.96 and pre-adult II on 18.07.96, the time between the occurrence of larvae and pre-adult I was 17 days with a further 6 days to pre-adult II at a mean water temperature of *c.* 15°C. Although caution must be exercised in taken these times due to the periodicity of sampling frequency, they agree with times put forward by Johnson and Albright (1991a) of 25 - 32 days between larvae and pre-adult I females and a further 8 - 10 days to pre-adult II at the lower temperature of 10°C.

The population structure on the '96 generation fish was difficult to interpret as infestation did not follow the expected natural pattern of pre-adult I to adult. This is possibly due to sampling frequency and the handling and treatment of fish as discussed above. However, relatively low infestation levels occurred until early May 1997 after which there is a dramatic increase in mean infestation levels. In a paper discussing infestation parameters, Tully (1992) states that reproductive output of *L. salmonis* is expected to increase in spring and reach a maximum in early summer in Ireland as is the case here. Tully (*loc. cit.*) goes on to suggest that the intensity and timing of this peak in output may also vary between years depending on the rate of change in temperature. It is interesting to note that the high level of intensity of adult males occurred prior to pre-adult females (see Figure 4.5.) which is in keeping with the life cycle of the louse whereby adult males will copulate with pre-adult females and deposit a pair of spermatophores over the females genital apertures after the females final moult into adult (Costello, 1993).

Regular monitoring of mobile lice levels on all salmonid farms in Ireland has been ongoing since 1991, with sampling of each fish year class at each site twice per month between March and May and monthly for the remainder of the year. The general trend observed from this programme was similar to the data presented in this report, with higher numbers in the summer months and lower levels during the winter period (Dept. of Marine, pers. comm.). Boxaspen (1997) reported a marked increase in lice abundance (4 - 5 fold) between May and June on untreated sentinel salmon while Hogans & Trodeau (1989) found a similar pattern in their studies on *Caligus elongatus* and *Lepeophtheirus salmonis* in the Bay of Fundy, Canada. The results of this study showed that there was a greater proportion of females in the population particularly in late autumn and early winter. Eggs extruded in late

autumn remain dormant and unharmed in the winter months, being eventually released in the spring. Johannessen (1978) observed that the duration of the egg bearing period increases at decreasing temperatures. Laboratory tests showed that at 9°C the egg bearing period lasted about three times as long as at 11.5°C. This constitutes a difference of about nine days in the summer months to about 40 days in the winter months.

In addition to temperature considerations, fish size would have contributed to the lower intensities recorded on the fish in the early part of 1997 during the current study. Having been introduced into Golam Harbour the previous April, the '96 generation fish sampled at the beginning of 1997 were relatively small compared to the '95 generation fish sampled during 1996. Jaworski & Holm (1992) suggested that an increase in the number of lice with fish size results from the obvious fact that bigger fish have larger body surface areas potentially available to parasites.

Information on the life span of the adult louse is sparce, in particular the adult female. Boxshall (1974) estimated the total life span of *L. pectoralis* (Müller, 1776) to be 10 months on place (*Pleuronectes platessa* L.) with an overwintering population of adult lice ready to shed their eggs in early spring when the sea temperatures increases. Longterm cycles and longevity estimates were not possible during the present study due to harvesting of the fish being sampled. However, the ectoparasitic platyhelminth, *Udonella caligorum* (Johnson, 1835), and attached filamentous green and red algal mats were located on the female lice recovered on several occasions indicating that these lice were present on the fish for at least a number of months without moulting. Jacobsen & Gaard (1997) found that adult female lice survive on salmon during the winter period in the sea. These authors further found that the observation of the higher abundance of adult lice compared with the number of the younger stages on the hosts indicated an accumulation of adult stages. The less positively skewed frequency distribution of lice on two sea winter salmon, as compared with one sea winter fish, also indicated a stabilising tendency which they suggested indicated a regulatory mechanism of infestations.

In general, the population dynamics of the sea lice on the fish sampled in the present study were controlled to a large extent by the temperature of the ambient sea water and handling of the fish on the farm. Low levels in parasite intensity were observed between January and April when temperatures were low. The parasite intensity subsequently increased with rising temperatures. Although the natural development of the lice population on the fish is interfered with by fish husbandry and management practices, the movement of generations could be observed within the population as discussed above. Movement of generations or cohorts of *L. salmonis* within the population have been reported by other authors, most notably on studies on untreated fish (*e.g.* Tully, 1989, Costelloe *et al.*, 1996).

5.3. Vertical migration

During previous studies by Aqua-Fact, *L. salmonis* larval densities were found to be variable in the surface water in a cage environment over 12 and 24 hour periods. This variability appeared to be associated with the tide as larval densities increased with the

flooding tide, peaked at high water and dropped again as the tide turned in the majority of the studies (see Aqua-Fact 1994, 1995 and 1996). On one sampling occasion, larval numbers increased from 0.1 m^{-3} at low tide, to a peak of 66.1 m^{-3} in the surface waters at high water and immediately dropped again as the tide turned. Retention of larvae within the cage sampled was always high as few larvae were found immediately outside the net and it was postulated that the larvae migrated to and from the surface water, the main driving force being the state of the tide possibly coupled with light intensity. During the present study, larval densities were also variable over the tidal cycle. However, this variability was not correlated in the same manner as had previously been observed. On a number of occasions, high densities were found at low water as well as high water, while on one study (16.07.97) there was little variability in densities over the 12 hour period in which samples were taken. Relatively high densities of larvae were recovered outside the cages in those studies carried out in Golam Harbour indicating that either retention within the cage is not as high at this site compared to the other sites studied or that there is a general retention within the harbour without a diluting effect outside the cages. Given that highest densities occurred inside and outside more or less at the same time, the latter would seem to be the main factor involved. Although these larvae had to originate originally from inside the cage, there should be a time lag between high densities recorded either side of the net if they were all being washed directly out of the cage. It is more probable that the high densities recorded outside were a result of an accumulation of larvae washed from all of the cages at the site over an extended time period.

Vertical migrations of plankton species are well documented (e.g. Hardy & Paton, 1947, Bainbridge, 1961, Raymont, 1983, Heuch , 1995 and Heuch et al., 1995). Although transportation and maintenance of pelagic larvae in any given body of water are largely controlled by oceanographic factors, larval behaviour can influence their eventual destination. This may occur by larvae regulating their position in the pelagic environment (Chia, 1989). 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. The observed vertical movement of L. salmonis larvae is possibly a behavioural adaptation related to larval transport or retention within a particular area leading eventually to host location. Costello (1993) suggests that nauplii and copepodids of L. salmonis may control their vertical distribution in the water column, perhaps using diurnal and tidal activity patterns, such that they lie in the path of salmonids migrating to sea. Recently in Norway, Heuch et al. (1995) found copepodids of L. salmonis exhibited a diel vertical migration in closed mesocosms.

It is unclear what apparent cues might initiate vertical migration of L. salmonis larvae. Naylor (1992) suggests that many crustaceans maintain characteristically zoned patterns of distribution by physiologically controlled patterns of locomotor activity which persist in constant conditions in the laboratory. That is, they possess biological clocks which permit behavioural anticipation of unfavourable states of tidal, diel and neap/spring cycles of environmental change. In addition, precise zonal recovery is often attained by varying responses to environmental cues at different stages of daily or tidal cycles. Light intensity may be an important factor in the case of L. salmonis. A strong phototropic response has

been reported for copepodids of L. salmonis by Johannessen (1978) and Wootten et al. (1982) and has also been reported for copepodids of L. dissimulatus (Lewis, 1963) and L. pectoralis (Boxshall, 1976). Gravil (1996)reported that both nauplii and copepodid stages of L. salmonis were observed to react photopositively to light although, for both the nauplius stages, a correlation was found to exist between increasing light intensity and increasing photo response while no such relationship was found to exist between light intensity and the response of the copepodid stage. This suggests that the copepodid 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 increased sensitivity to higher intensities. The findings of the studies conducted by Heuch et al. (1995) strongly suggest that light intensity is of major importance for the vertical distribution of L. salmonis copepodids. However, these experiments were carried out in an artificial environment where the main environmental stimulus was light intensity. The involvement of light in vertical migration in zooplankton is not an unusual phenomenon and it is well documented in the literature (see Raymont, 1983). Experiments conducted by Bron et al. (1993) with copepodids of L. salmonis agree with previous reports that there is a positive phototactic reaction. At the highest light intensities, the larvae swam almost directly towards the light source while at lower intensities the movement was more meandering (see Bron et al., 1993).

Although light may have an influence on the observed behaviour, it is not the only stimulus having an impact on the movement of the larvae. Larval densities also increased at the surface on high water when sampling was carried out during darkness. Larvae will experience pressure increases from the flooding tide. This increase in pressure may be a causative factor in the movement of larvae to the surface. Other studies have shown that pressure may have some influence upon plankton migration. Certain inshore surf plankton species are sensitive to pressure changes and wave actions which later impose a diurnal rhythm, causing them to swim upwards from the sand on a rising tide (Raymont, 1983). Results obtained with zoea and megolopa stages of Portunus and Carcinus showed a high proportion of these swim for periods of up to three hours when subjected to pressures equivalent to 5, 10, 15 and 20m depths. DeVries et al. (1994) cite zoeae of crabs as being extremely sensitive to rates of changes in pressure, salinity, and temperature and respond to rates of change they may encounter through vertical movement. Often, increases in abundance occur during the second half of the rising tide corresponding to times of maxima in the environmental variables (DeVries et al., 1994). The response of L. salmonis copepodids to pressure under experimental conditions has shown that increased and decreased pressure resulted in active upward swimming and passive sinking responses, respectively, although no pressure organ was detected (Bron et al., 1993). Similarly, Gravil (1996) reported a negative geotaxis in both the first and second nauplius stage which she suggests, combined with the positive phototactic nature of the free-swimming larvae, will help maximise the spatial overlap between host and parasite. These results were further highlighted when it was found that an inverse correlation existed between depth and numbers of naupliar stages obtained in plankton samples taken in salmon cages (Gravil, loc. cit.).

6. SUMMARY & CONCLUSIONS

The main results of the study can be summarised as follows:

- highest densities of larvae were recovered during neap tides,
- larval densities were low during the winter months,
- while overall parasite intensity was reduced, gravid females were recorded over the winter period. However, spawning intensity remained low until late spring,
- spawning occurred throughout the study but distinct peaks were observed prior to larval density peaks which suggests an element of synchronicity in spawning behaviour,
- sea lice larvae migrated up and down through the water column with highest densities occurring in the surface water during slack current periods normally associated with high tide.

One of the main aims of the project was to examine the possibility of developing a parasite control mechanism. Tully (1992) outlined the need for studies on the intensity and timing of infestation events of caligid copepods. He went on to state that the best strategy to control sea lice populations is to reduce peaks in parasite intensity. Peaks in parasite intensity can be predicted by quantifying site characteristics such as temperature and larval densities within the cages. The majority of research to date has 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 farm industry (Gravil, 1996). At present lice on fish farms are controlled using a combination of husbandry techniques, single bay management, monitoring and treatment. Costello (1993) outlines in detail the present and possible future methods of control: important considerations in the control method include its efficiency, stress caused to the fish, financial cost, handling hazards, environmental effects, marketing implications, availability and ease of application. Obviously a cost effective, environmentally safe but efficient control is desirable. However, a single method encompassing these requirements is currently not available.

The results of this study increases our knowledge of the complex behaviour and life cycle of the louse. Information regarding parameters that control and influence the reproductive output of sea lice together with detailed information on the occurrence and specific location of high densities of larvae within salmon cages have been identified. This information provides a sound basis from which management strategies can be developed in order to reduce lice intensities on the farm. In view of these data, it may be possible to introduce an integrated physical/chemical control method which would combine husbandry techniques along with monitoring and chemotherapy to create an efficient yet environmentally safe approach to lice control.

Although lice were present on the fish over the winter months, larval densities in the cage were low and consequently infestation pressure was reduced during this period. Females did

not begin to spawn in significant numbers until water temperatures began to rise in the spring. In order to reduce the intensity of this first major spawn, it is advisable to treat the fish prior to any rise in water temperature. However, the efficacy of current chemical treatments, during the early part of the year in particular, is low due to water turbidity, low temperatures, differential susceptibility *etc.* which makes treatment of the lice at this time difficult. Given that infection levels are low during the winter, a lice treatment towards the end of the year when water temperatures are dropping, would significantly reduce the intensity of spawning the following spring. Additionally, chemical treatments should be effective on all stages of the parasite including the free living naupliar and copepodid larvae. During the present study, adult lice numbers were reduced following lice treatments. However, high densities of larvae were subsequently recorded in the cages, presumably due to eggs hatching from the physical/chemical shock of the treatment, which developed into the next generation on the fish. Current treatments are not effective on all stages of the parasite although a number of chemicals are currently under trial which may overcome this problem.

However, even if internal transfer on the farm is kept at a minimum, a natural source of lice infection can originate from lice on wild fish (Costelloe et al., 1995, in press, Jackson et al., 1997). Although less severe in estuaries which do not have a salmonid fishery, this source cannot be mitigated against and some form of lice treatment will be required throughout the year. The current practice on most farms is to treat the fish once lice numbers reach a certain infection level. This level will depend on the management strategy of the individual farm although all fish farmers are instructed by the Department of the Marine to maintain an ovigerous lice abundance of as close to zero as is practical particularily during critical periods. It is postulated that, given the knowledge obtained during the present study on temporal and spatial occurrence of larval lice, it will be possible to remove up to 80% of the larvae produced at a farm location by a simple physical, environmentally friendly, removal method. The benefits of such a technique to the environment and also to the final product image will be significant in that:

- the lice larvae are targeted; the fish are left undisturbed,
- the need for chemical lice treatments will be reduced or eliminated and thereby reducing both the chemical input into the environment and the effect on the fish.
- it will be cost effective; current lice treatments are very expensive and numerous treatments can put severe financial pressure on the farm owners, particularly in relatively small family owned operations.

It is unknown what the infestation pressure resulting from the remaining 20% of the larvae produced within a cage will be. It may turn out that this number of larvae is all that is required for full infection to occur. However, it is more likely that infection levels will drop significantly as the larvae are diluted similar to the situation occurring in offshore sites. Jackson *et al.* (1997) recorded low transmission rates within an offshore site where there was no chemical or biological lice control and incomplete separation of fish generations. This was attributed to dispersion and dilution of the infectious larval stages.

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

control methods. Although the present study has provided invaluable information to the development of a management strategy to control sea lice, field trials are required to assess the effectiveness of larval removal in relation to infection levels in a farm situation before the actual benefits of this proposed method are known.

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