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# Patterns of pair formation and mating in an ectoparasitic caligid copepod *Lepeophtheirus salmonis* (Krøyer 1837): implications for its sensory and mating biology

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Non-invasive observations of single cohort and manipulated populations of the sealouse on laboratory-maintained salmon established the sequence of reproductive events and mating. Protandry occurs with adult male emergence synchronized with pre-adult II female emergence, the stage at which most pair formation and pre-copular guarding takes place. Male competence for pair formation and mating was achieved within 24 h of the final moult and pairing occurred according to the preference hierarchy, virgin adult females > pre-adult II females >> pre-adult I females. This hierarchy broke down when the adult male to unmated female ratio increased rapidly. Males mated successfully not only with newly moulted adult females, but also with older virgin females in which enlargement of the genital complex and premature extrusion of egg strings had occurred. Multiple mating by adult males was demonstrated and may be widespread. Significant inter-host transfer was also demonstrated. Adult males were more mobile than adult females and showed significantly more inter-host transfer. Ablation of the distal tip of the antennules significantly reduced the success of host-finding, pair formation and males' mating.

**Keywords:** sealice; salmon; behaviour; reproduction; chemical ecology

## 1. INTRODUCTION

Sealice, *Lepeophtheirus salmonis* in particular, have attracted interest because of their potential for causing severe pathological effects on salmonids in aquaculture (Wootten *et al*. 1982; Pike 1989). The impact of sealice on marine aquaculture is well documented (Boxshall & Defaye 1993). Recent failures of existing chemotherapeutic methods to control infections (Jones *et al*. 1992) have focused research on alternative methods of control including new chemotherapeutic (Roth *et al*. 1993) treatments, the use of hydrogen peroxide (Thomassen 1993), and biological control using wrasse (Costello 1996) both applied in conjunction with management practices (Bron *et al*. 1993) designed to reduce the opportunities for louse transmission.

Aspects of the biology of sealice, such as structure of the reproductive system and spermatophore transfer (Ritchie *et al*. 1996*a*), mating and reproduction (Anstensrud 1990*a–d*, 1992; Ritchie 1993), development (Johnson & Albright 1991*a*,*b*), pre-infective larval distribution and behaviour (Heuch 1995; Heuch *et al*. 1995; Heuch & Karlsen 1997), sensory and behavioural ecology of the infective larvae and reproductive adults (Bron *et al*. 1993; Pike *et al*. 1993), and sensory ultrastructure (Gresty *et al*. 1993; Hull 1997) may be of value in the development of novel strategies for control.

The potential for semiochemical-based control, as used for control of some insect species (Pickett *et al*. 1997) is investigated here. Little is known about the role of chemosensory perception in mating sealice. This study, using on-host recording of lice of known chronological age and reproductive status, was designed to gather data non-invasively from controlled experimental populations. The main aims of the study were: (i) to follow populations in the laboratory to pinpoint the sequence of reproductive events in a single cohort; (ii) to identify mating strategies; and (iii) to investigate the role of inter-host transfers. The role of the antennules in host and mate location was also examined with reference to mating success.

## 2. MATERIALS AND METHODS

### (a) *Specimen collection and cultivation*

A consistent method of *Lepeophtheirus salmonis* production was used throughout.

#### (i) *Egg collection and copepodid rearing*

Egg strings from mature females on Atlantic salmon (*Salmo salar*) were collected at Scottish West Coast commercial fish-farm cage sites. Adult lice, with egg strings attached, were collected from 2–6 kg salmon during fish harvesting. Lice were removed from newly slaughtered fish, washed twice in seawater, maintained at constant temperature in a cool-box, and transported to Marine Harvest McConnell, Fort William. There, the egg strings were removed by snipping the cases between the last extruded egg of the string and the genital complex, washed in sterile seawater and sorted for maturity on the

 

basis of pigmentation (Ritchie 1993). Mature egg strings were transferred to beakers of aerated seawater. Immature egg strings were matured in a similar way, but with a higher level of aeration to create constant agitation of the strings and thus avoid contamination with micro-organisms. Nauplii emerging from egg strings were allowed to develop in aerated beakers kept in a chamber where temperature and photoperiod were regulated to that of the collection site. Populations of larvae were kept separate to ensure that each beaker contained single age cohorts that had hatched within a known 24-h period. After approximately 50% of the larvae had moulted to the infective copepodid they were cooled and transported to Aberdeen.

(ii) *Infection and rearing to adult*

Copepodids were maintained in Aberdeen, in aerated beakers, in an aquarium for a further 48 h and then allowed to establish on salmon at a rate of 120–200 lice per fish, achieved by placing copepodids in the water column of holding tanks and allowing them to attach naturally. Salmon smolts were kept in either 1 m diameter by 0.6 m deep or 3 m diameter by 1 m deep cylindrical tanks (depending on the number of fish being used) housing 2–500 g fish at a stocking density of 0.8–1 kg $m^{-3}$. The water supply and outlets were closed before exposing fish to infection and the water level was lowered to create a temporary stocking density in the region of 6–8 kg $m^{-3}$. Copepodids were added to the water column and left for 2 h. Over 12 h the water supply was gradually restored to the normal volume, and the outlets reopened. This technique produced single cohorts of lice on the host of known, synchronous chronological age without damage to the host. Stock, infected fish, were maintained in similar aquarium tanks where lice developed to the required age for experimentation.

### (b) *Non-invasive monitoring of single cohort populations*

Neither fish nor lice were handled after infection. The lice were monitored visually *in situ* on the fish in the tanks. The tanks had modified lids to allow direct observation from above and access to the water column. Lice on the dorsal regions of the host were recorded by developmental stage and sex, and their position was logged on drawings of the salmon. The lice on the ventral surfaces were similarly recorded by means of an inverted inspection mirror. Reproductive status and lice interactions were noted. Successful matings (demonstrated by the transfer and correct placement of paired spermatophores), males in pre- or post-copular pairs with females and the developmental stage of any female found in pre-copula were recorded.

All stock fish were conditioned to limit the effects of human presence during sampling. This was achieved by hand-feeding the animals regularly, three times daily, and by frequently but irregularly, approaching tanks and treating them as during an observation.

### (c) *Population manipulation*

(i) *Multiple mating*

Fish, with single cohorts of aquarium reared lice that had been monitored during development, were isolated in experimental tanks on day 19 post-infection, as most of the females were being mated. After 24 h, 40 additional pre-adult II females were introduced into each tank and allowed to settle on the fish. These pre-adults were removed from live stock-hosts without the use of anaesthetic, in a manner similar to the collection of gravid females. The lice were poured gently onto 15 $cm^2$ sections of double twill filter mesh which was then placed, louse-side down, on the surface of the water in the tank. The lice were allowed to naturally attach to salmon and the population was monitored as in § 2*b*.

(ii) *Postponed mating*

A total of three replicates of five aquarium reared cohorts of lice were isolated on day 15 post-infection, at the time of first pre-copular pair formation, to examine the timing requirements for mating with the female. All male and early stage female lice were removed to produce single stage, single sex populations of pre-adult II females and again transferred to experimental tanks. Lice were at a mean density of 36.3 (±1 s.d. of 7.83) per fish, and these were isolated until at least 10 d after the final moult of all individuals. A clear excess (ideal mein of 50 per fish) of adult males 24–72 h post-final moult, removed from other stock fish, was introduced into the tanks containing the experimental fish as described above. The ensuing mixed sex populations were monitored for the next 11 d. It was difficult to accurately record successful mating because the presence of egg strings often prevented examination of the ventral surface of the female genital complex. All lice were therefore collected at the end of the experiment and the females examined for successful mating.

(iii) *Inter-host transfers*

Long-term monitoring of standard cohorts in the laboratory, described above, suggested significant levels of host transfer for all mobile stages (M. Q. Hull, unpublished observation). A manipulation was done to directly demonstrate inter-host transfer and to quantify the level at which it occurs under conditions of minimal experimental handling of hosts and lice.

A total of nine infected fish were kept together and their louse populations monitored until day 21, when nearly 100% of females had moulted to adult and were mated. At this point, six fish were removed and anaesthetized with benzocaine (ethyl p-aminobenzoate). We selected three fish and removed their lice populations then placed them in three separate new tanks. Lice on the remaining three fish were also removed, transferred to aerated beakers containing seawater and neutral red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) at 0.015 g $l^{-1}$ (adapted from Anstensrud 1989) for 1 h to produce dark red-stained lice. These lice were isolated in tanks and allowed to re-establish on their original hosts over a period of 8 h. The three fish with re-established stained lice were placed individually into each of the three tanks, with clean fish, together with one each of the three remaining infected fish, so that each experimental tank finally contained a fish cleared of parasites, a fish with an original population of parasites and a fish whose original population of parasites had been removed, stained, and allowed to re-establish.

Lice populations in each tank of three fish were monitored. The number and colour of the lice on each fish was noted as were those that were observed off the hosts.




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### (d) *Behavioural manipulation: distal ablation of the antennules*

The sensory system of individual lice was altered in an attempt to modify behaviour. Adult male lice were reared in single cohorts, removed to beakers without the use of anaesthetic and then processed. Experimental lice were examined microscopically to select only those that possessed full sets of antennulary setae. Selected males received one of two treatments: half were restrained under the microscope whilst the distal tips of both antennules were removed with a scalpel and then returned to aerated beakers of sea water; the others were similarly manipulated, but cut on the posterior tip of the caudal rami as a wound control. The distal ablated and wound control treatment lice were used immediately. The alternative treatments will be referred to as ablated and control respectively.

After ablation the lice were returned to aerated holding beakers and treated as for intact lice. A total of three replicate populations of 150 ablated and 150 control animals were placed in the water column of 1 m diameter tanks containing five salmon smolts and monitored. Approximately 24 h before introduction of males, populations of 250 aquarium-reared pre-adult II females were removed from pre-copular pairs on stock fish and allowed to re-establish on the fish in each replicate experimental tank. The re-settlement and survival of males, and the number and developmental stage of females were monitored, as was the interaction of the males with these resident females. Data were also collected on the age of the adult females.

Simultaneously, three further replicates of 150 control males were placed in tanks each containing five salmon previously not infected with pre-adult females, as were replicates of 150 unmanipulated aquarium reared males that had been removed from the previous host at the same time as the control and ablated treatments. These latter two treatments were monitored for the first 50 h of the experiment to observe re-settlement rates. All surviving lice at the end of the experiment were removed and fixed for examination.

## 3. RESULTS

### (a) *Non-invasive long-term monitoring*

The mean survival rate of *L. salmonis* declined throughout development. Over two-thirds of lice survived filament attachment and chalimus development to day 14 (the first sample date), but 66% of those present were lost over the next 7 days (figure 1*a*). This mortality coincided with the period of pre-adult development, pair formation and mating. On day 14 the mean lice count was 68.5 pre-adult I females and 64.7 pre-adult II males (figure 1*b*).

Lice moulted to the next stage between days 14 and 17 with males being one developmental stage ahead of females. Both pre-adult I and II females and the first adult males were present on day 15. By day 16 all three mobile stages of the female were present plus pre-adult II and adult males. Between days 17 and 21 males completed their final moult and females were either pre-adult II or adult.

On day 15 approximately 66% of males were adult, both pre-adult I and II females were found, but there were no pre-copular pairs. By day 16 over 80% males were adult and the first pre-copular pairs were observed with all three female stages present. Most pre-copular pairs were adult males with pre-adult II females, some with adults and a few with pre-adult I females. All adult

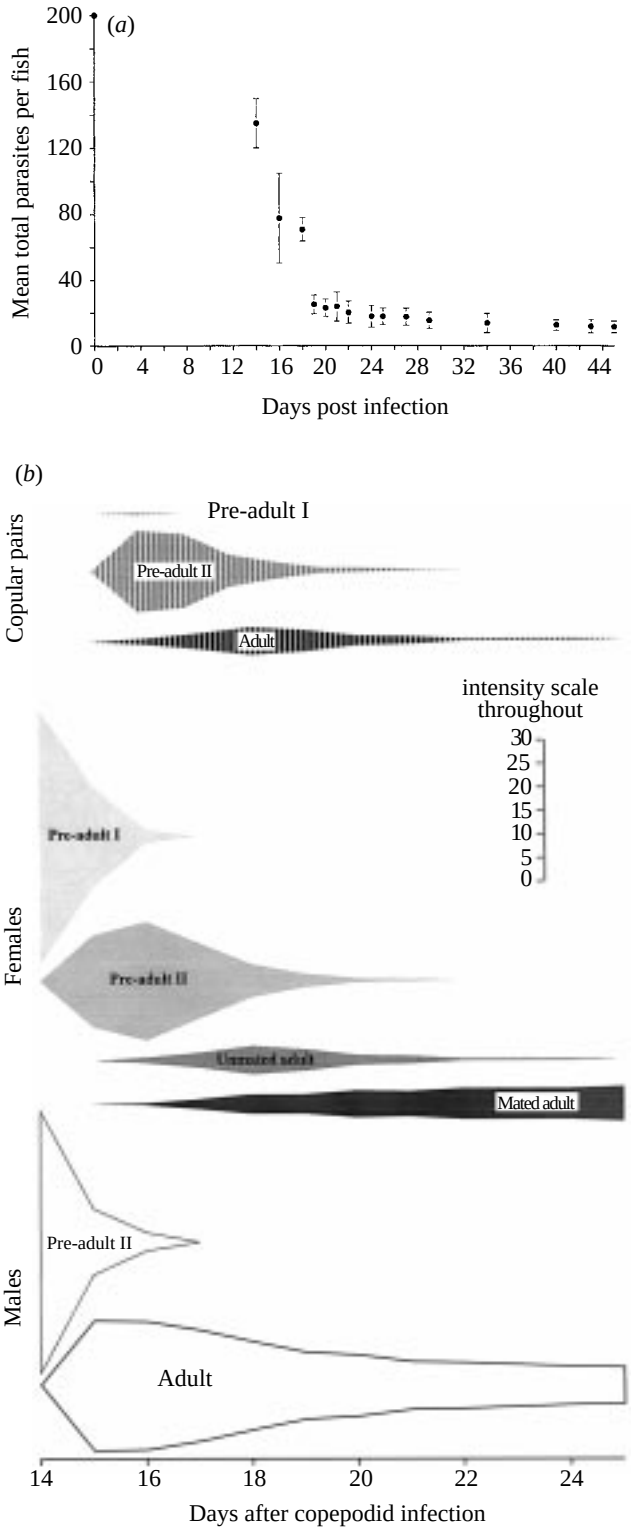


Figure 1. (*a*) Mean (±s.d.) total survival of single lice cohorts after infection onto five salmon smolts per tank ($n=3$). (*b*) Succession and relative mean numbers of individuals during the period of pre-adult and adult stages of development on five salmon smolts expressed as comparative kite diagrams ($n=3$).

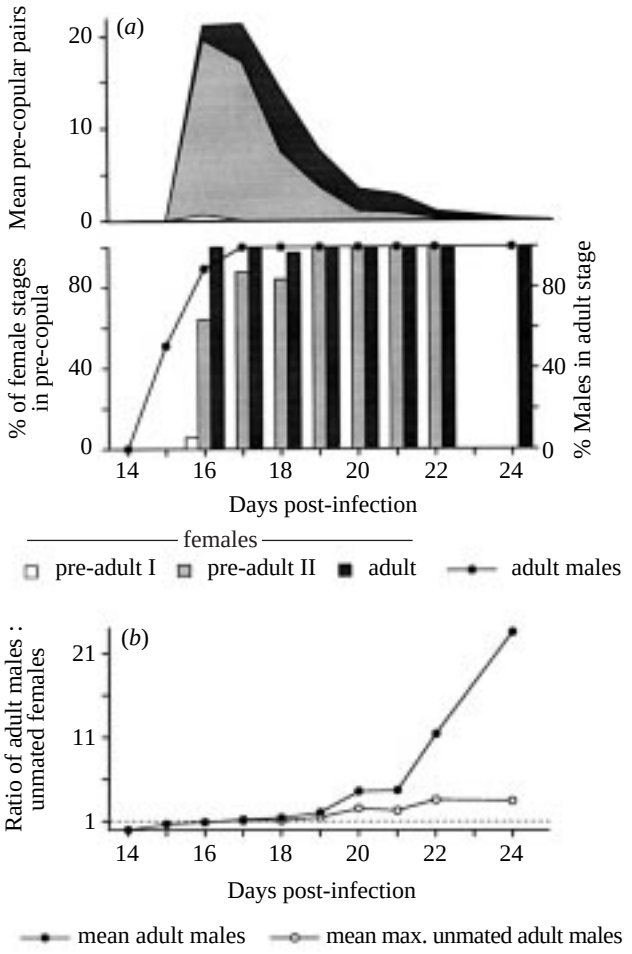


Figure 2. (*a*) Comparison of relative mean numbers and proportion of pre-copular pairs against a line plot of the proportion of males reaching adult stages for the period of pre-adult and adult development on five salmon smolts ($n=3$). (*b*) Comparison of the mean ratio of adult males to unmated females with mean maximum number of adult males not successfully mated with females over same period ($n=3$).

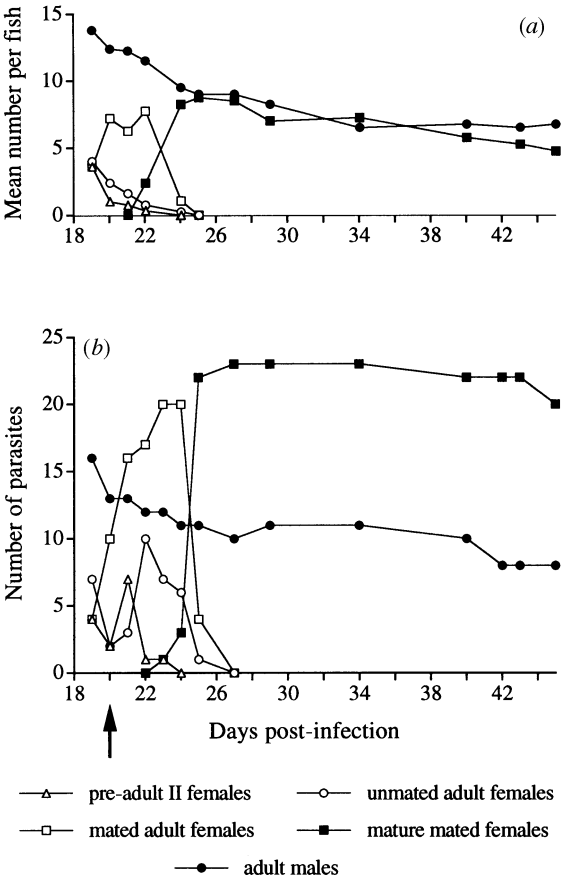


Figure 3. (*a*) Mean numbers of different stages, found between days 19 and 45 post-infection, on five salmon smolts kept communally, demonstrating the developmental progression, mating and maturation pattern for females ($n=3$). (*b*) Numbers of the different stages of lice on a single fish isolated on day 19 post-infection and on which additional pre-adult II were allowed to settle after 24 h (indicated by arrow).

females and over 60% of pre-adult II females were in pre-copula, whereas less than 10% of pre-adult I females were paired.

Adult females first appeared on day 16, as did the first mated female, a minimum of 24 h after the first appearance of adult males. There was a similar lag between the increase in moulted unmated females and the increase in mated lice. At days 24 and 25 post-infection the lag was greater: some moulted females required a minimum of 3 days to be successfully mated.

The first pre-copular pairs occurred on day 16, a minimum of 24 h after emergence of the first adult males. Males appeared to show a preference between the three female developmental stages (figure 2*a*), biased toward later stages and contrasting with the relative abundance of stages present at the time. This preference hierarchy persisted for days 17 and 18 but by day 19, all females present were in copular pairs. By day 25 no unmated females remained.

Between days 15 and 17 post-infection, not all males had become adult, as reflected in the ratio of less than 1:1 for adult males to unmated females (figures 1*b*, 2*a*). The ratio rose above unity by day 17 and increased from then on at either a rapid rate, if all adult males were considered, or more slowly if only the mean maximum number of males that had not successfully mated were counted (figure 2*b*).

### (b) *Population manipulations*

(i) *Multiple mating*

In a single cohort infection numbers of males declined most rapidly over the first 5 days of the observation period (figure 3*a*). Mean numbers rose twice indicating net re-settlement of males from the water column. Females were represented equally by pre-adult II, unmated adult and immature mated adult stages at day 19 (figure 3*a*). Pre-adult II females moulted to adults over the next 5 days. Mating of these new adults was complete 24 h later. By day 22 a proportion of mated adult females was mature, as indicated by expansion of the genital complex and egg string extrusion.

The addition of pre-adult II females on day 20 changed the population structure, as seen by examining a single isolated population (cf. figure 3*a*,*b*). The cumulative mortality of males was very similar to the controls and, although there were more unmated adult females in the altered population, the initial pattern for females was similar. The addition of pre-adult II females created a

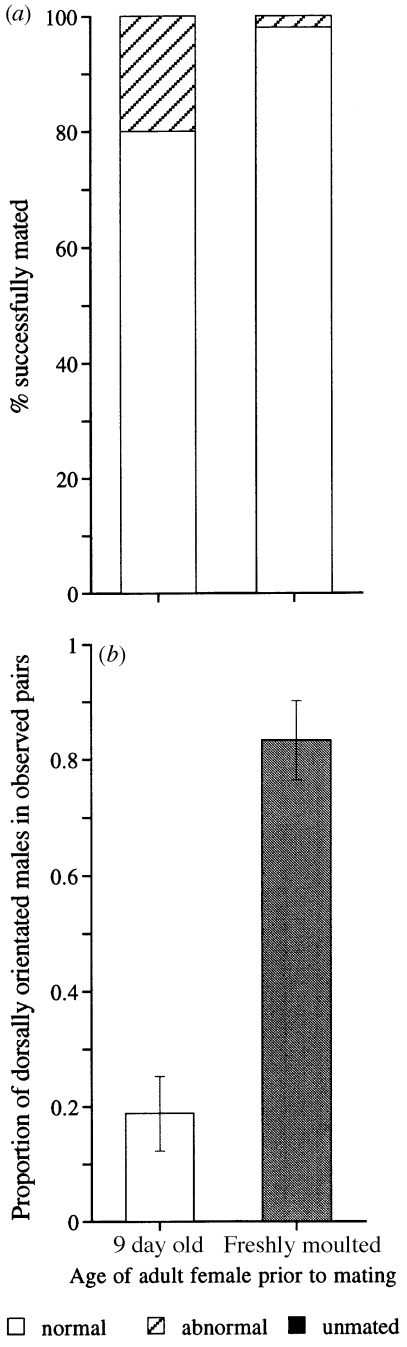


Figure 4. (*a*) Comparison of apparent successful mating between five populations of isolated females and control females. Isolated females were separated from males before maturation, for at least 10 days after terminal moult of female. Control females were allowed to mate soon after their terminal moult ($n=3$). (*b*) Comparison of the mean ($\pm$ s.d.) proportion of dorsally orientated males in copular pairs ($n=3$).

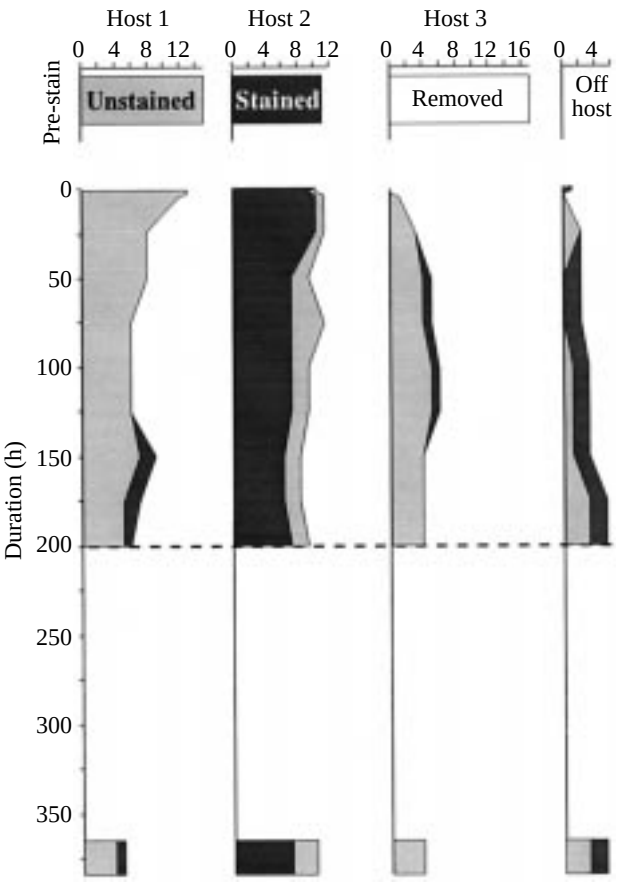


Figure 5. Representative plots of numbers of stained and unstained adult males found on and off individual hosts in a single tank of three fish; before manipulation, over a regularly sampled period of 200 h and an endpoint sample at the termination of the experiment. Each host either had lice left on the host unstained (host 1), lice removed, stained and allowed to re-settle on the host (host 2) or had all lice removed (host 3) before transfer.

secondary peak of unmated adults on day 22. This translated into higher numbers of mated and mature mated adult females. More than twice as many mated females were produced than in the controls and they were twice as numerous as surviving males.

As a minimum of 26 successfully mated females survived to day 25 and a maximum of only four of these were mated before isolation, there were at least 22 successful matings in total. Only 16 males were present on isolation, so at least six matings resulted from multiple mating by a male between days 19 and 25. Further analysis demonstrated that males can mate twice within 4 days. Between days 19 and 21 a minimum of 12 successful matings occurred from the maximum of 16 males. A total of 13 of these males survived, therefore at least nine of them had mated within the previous 48 h. Between days 21 and 23 at least five more matings occurred, at least two of which resulted from multiple mating.

(ii) *Postponed mating*

All surviving females were examined for successful mating (figure 4*a*). Over 80% displayed signs of mating with correct placement of spermatophores and sealing cement in their genital orifices; the remainder had one or both spermatophores attached to the genital complex, but at least one was incorrectly positioned in the genital orifice. No females remained unmated, as in the controls where females typically mated within 24 h of the terminal moult (figures 1*b* and 3*a*). There was an increase in the proportion of abnormally mated females from 1.9 to 19.7%. Hatching studies of the eggs collected showed no significant difference from control females (mean 92.6 per 100, cf. 94.5 per 100 for controls (*t*-test, $p>0.05$)). No

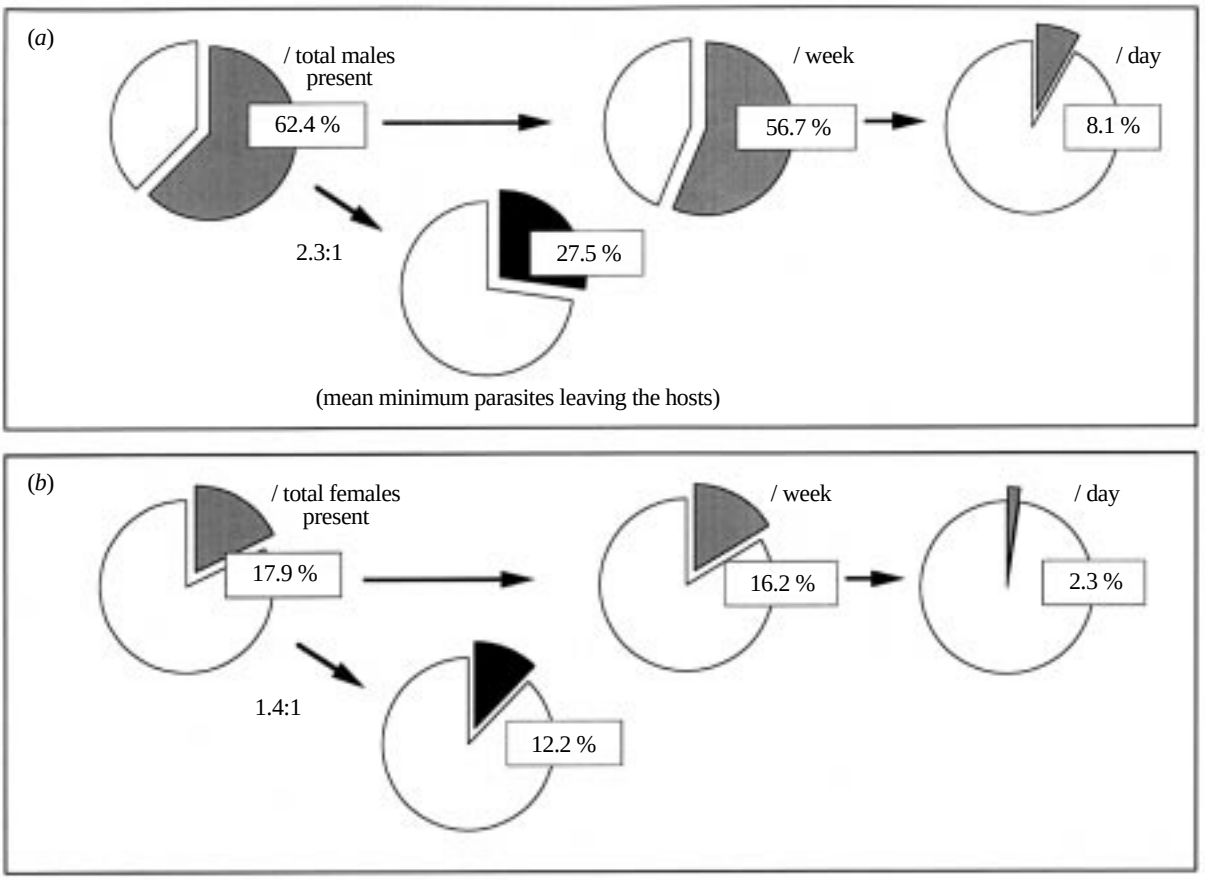


Figure 6. Mean numbers of (*a*) adult male and (*b*) female host transfers observed as a percentage of the total numbers of the parasite stage present at any one time per week and per day. The first of these for each sex is also compared with the mean minimum number of parasites known to have left the host during the experiment (ratio given over arrow) to provide an estimate of the extent of multiple transfers of both sexes.

prolonged mate guarding was observed for pairing with experimental females. Males associated with 9-day-old or older adult females showed a significantly lower proportion of dorsally attached males ($p < 0.01$), an orientation that indicates mate guarding rather than copulation (figure 4*b*).

All unmated adult females isolated before emergence of reproductively competent males showed secondary growth of the genital complex after the terminal moult, a maturation change previously thought to be triggered by mating. A proportion (23.9%) of females produced apparently normal egg strings in the absence of males.

(iii) *Inter-host transfers*

Inter-host transfer was demonstrated by examination of a single replicate of three fish (figure 5). A large proportion of unmanipulated males from host 1 left the host. Some entered the water column, others transferred onto the two other available hosts within the first few hours. The continued fluctuations in numbers on the different hosts and in the water column confirms that transfers continued throughout the experiment. Stained lice from host 2 showed evidence of host transfer only after 50 h. There was no obvious bias toward re-settlement or persistence on particular hosts.

The mean number of successful transfers observed during the first 200 h sampling, was used to calculate the rate of transfer (figure 6). The mean number of adult male transfers was approximately 3.5 times greater than in mated adult females, with a mean transfer rate of 62.4% compared with 17.9% for females. This is equivalent to a mean of 8.1% per day for males and 2.3% for females. To estimate the proportion of this transfer rate derived from multiple transfers of the same lice the mean minimum number of lice leaving hosts was calculated for both sexes from the known maxima that had not been observed to leave salmon during the experiment (figure 6, shown in black). The ratio of mean successful transfers and lice leaving hosts is higher in males (2.3:1) than females (1.4:1), suggesting that not only were males more likely to transfer, but that transferring individuals tend to transfer more often.

### (c) *Behavioural manipulation: distal ablation of the antennules*

(i) *Re-settlement on the host*

Re-settlement was compared in control males on fish pre-infected with pre-adult II females, control males on uninfected fish and intact males that had been off the host for a similar duration and then presented to uninfected fish (see figure 7). There was no significant difference between settlement rates of control and intact males on uninfected fish. Settlement rates of control males on hosts infected with females were slightly lower but not significantly ($p > 0.05$).

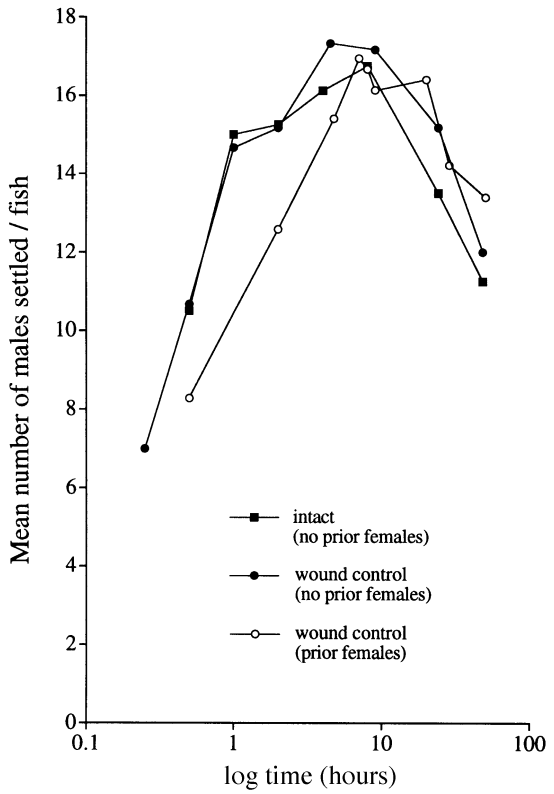


Figure 7. Comparative plots of the mean rate of re-settlement for intact and wound control male *L. salmonis* on five salmon smolts not previously infected with pre-adult II females, and further comparison with the settlement of wound control males on the smolts infected with pre-adult II females 24 h before introduction of males ($n=3$). Data are plotted against time on a $\log_{10}$ scale to permit comparison of early sample points.

Distal ablation significantly altered male behaviour (figure 8). Re-settlement of ablated lice occurred but at significantly reduced levels than in the controls ($p<0.001$ for sample points 0.5–28 h). Significantly higher mean numbers of control males were maintained to the end of the experiment ($p<0.05$ throughout). Long-term survival of those ablated animals that re-settled did not appear to be affected, indeed, ablated lice survived significantly longer than controls (nonlinear repeated measure (RM) ANCOVA, $p=0.023$).

(ii) *Copular pair formation*

*The presence of females on the hosts*

Female lice were initially a mixture of the re-settled pre-adult II females and those that had moulted to adults over the previous 24 h (figure 9*a*). An equal mean number of pre-adult and adult females was present in each population at 20 h. By 100 h over 90% of females had been adult longer than 24 h and all females were adult in both treatments by 150 h. There were fewer females in the ablated population, which became more marked as lice moulted to adult, but the difference was not significant (*t*-test, $p>0.05$ throughout; nonlinear RM ANCOVA 8–225 h, $p=0.092$). The female populations in the two treatments appeared therefore to present comparable environments for re-settlement of either control or ablated males.

The first female to show any morphological change was observed at 9 h (figure 9*b*). All females were pre-adult II 33 h previously and the last females to moult through were between 125 and 150 h. It was assumed therefore that transformation began less than 24 h after the moult.

*Pair formation*

During the first 28 h there were significantly higher numbers of pairs in the controls than in the ablated lice ($p<0.001$ for 4, 6, 7, 8, and 20 h, $p=0.041$, 0.028 and 0.033 for 0.5, 2 and 28 h, respectively). Although not significant, this difference remained until approximately 150 h.

Results for male settlement described above demonstrated significantly higher numbers of controls on hosts. To counteract any effect of this on numbers of females paired, data were analysed as the mean fraction of control and ablated males found in copular pairs (figure 10). With this conversion, mean ratios of controls in copular pairs were still significantly higher for the first 20 h of the experiment, but differences were reduced ($p=0.038$–$0.021$ over the samples). The difference at 28 h was not significant, and there was no significant difference between treatments thereafter.

(iii) *Mating*

Comparison of the mean number of adult females per host with the mean number of mated females shows that distal ablation affected the time taken by males to settle on the host and to copulate (figure 11). The first mated female was observed 8 h after introduction of control males. No mated females were observed with ablated males until 75 h. The mean number of females mated by control males was significantly higher than in ablated lice ($p<0.001$). After approximately 100 h (only 25 h after the first mating was recorded for ablated lice) rates of mating only slightly differed (nonlinear RM ANCOVA, $p=0.965$).

Analysis to counteract the effect of reduced ablated male re-settlement (figure 12) involved the calculation of the male presence index (MPI). This reflects the time available for males to contact and mate with females once on the host, irrespective of the numbers present. It is derived from a running total of the number of males present on each host, when sampled, multiplied by the time those males were present. To obtain the best approximation when data were collected at discrete points, the means of the individual numbers of males present for every two consecutive points were multiplied by the time elapsed between the two points to give the MPI figure, i.e.:

$$T = 0.5, \mathrm{MPI}_{0.5} = ((N_{t_{0.5}} + N_{t_1})/2) \times (t_1 - t_{0.5}),$$

$$T = 1, \mathrm{MPI}_1 = ((N_{t_{0.5}} + N_{t_1})/2) \times (t_1 - t_{0.5}) + ((N_{t_1} + N_{t_2})/2) \times (t_2 - t_1),$$

$$T = 225, \mathrm{MPI}_{225} = ((N_{t_{0.5}} + N_{t_1})/2) \times (t_1 - t_{0.5}) + \ldots ((N_{t_{225}} + N_{t_{150}})/2) \times (t_{225} - t_{150}),$$

where $T$ is time in hours after introduction of males, $N$ is number of males present when sampled.

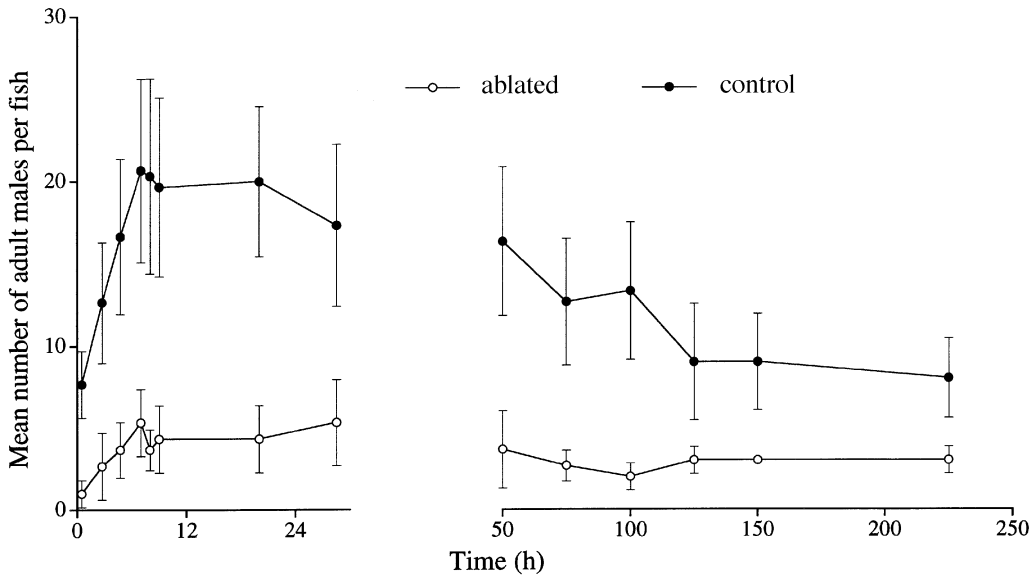


Figure 8. Mean (±s.d.) re-settlement and subsequent persistence of control and ablated adult male *L. salmonis* on populations of five salmon smolts infected with pre-adult II females 24 h before introduction of males ($n=3$).

When the rate of mating between treatments was compared (figure 12) using the MPI, it clearly indicated that time taken for the first female to be mated was significantly longer in ablated lice (mean for control treatment 106.71, mean for ablation treatment 293.46; $p<0.01$). The difference was significant over the duration of the experiment, but this difference mainly reflected the rate of mating in the initial 100 h ($p<0.001$ for 0–340 $MPI_{DA}$, $p=0.874$ for 340–712 $MPI_{DA}$ (nonlinear RM ANCOVA)).

## 4. DISCUSSION

### (a) *Non-invasive long-term monitoring*

This is the first controlled, longitudinal study of a population of a parasitic copepod. Previously, Johnson (1993) followed the survival and development of *L. salmonis* on different body regions of Atlantic and chinook salmon using sacrificial sampling of populations. Anstensrud (1990*b*) also provided detailed information about age of adult emergence in *L. pectoralis*.

Johnson's (1993) host body-region infection intensity data suggest a similar overall pattern of parasite mortality with relatively high first settlement and survival through the attached stages, and substantial loss through development of mobile stages. The population structure of Johnson's (1993) infections at 25 days (234 degree days) post-infection correlates approximately with the first sample in the present study: 14 days (221.4 degree days) post-infection. Anstensrud's (1990*b*) data for age at maturity in *L. pectoralis*, suggest that 50% of males complete the terminal moult *ca.* 25 days (275 degree days) post-infection, and 50% of the females at *ca.* 31 days (341 degree days) post-infection. This is slower than in *L. salmonis*, with an equivalent estimate of *ca.* 14.5 days (229.1 degree days) for males and *ca.* 17 days (268.6 degree days) for females. But both species have a similar relative ratio of rate of development between sexes: *ca.* 1:1.17 and 1:1.24, respectively, between males and females.

Johnson (1993) found a high proportion of precocious adult males, with nearly a third of them completing the terminal moult before emergence of the first pre-adult II female. Occasional highly precocious males were observed (M. Q. Hull, unpublished observations), but this has not been a general feature of laboratory-reared cohorts. The observed synchrony of adult male and pre-adult II female emergence correlates with observations of pair formation. Anstensrud (1992) found male *L. pectoralis* preferred unmated adult females but did not select between pre-adult stages.

Although pre-adult females were available to males from before 14 days no pre-adult males were observed pre-copular guarding, and adult males were not observed paired with females until at least 24 h after the terminal moult of the first male. This strongly suggests that onset of pair formation is determined by the development of and attainment of sexual competence by the male. After onset of pairing the relative numbers of pairs formed with each female stage broadly reflect the developmental composition and stage duration of the female population presented to adult males. On days 16, 17 and 18 the relatively higher proportion of the unmated adult females found in pairs in comparison to pre-adults, and the higher proportion of pre-adult II stages compared with pre-adult I stages, on day 16, indicate that males actively select the more mature female stages, even when the stage structure of the female population is strongly biased against this. This suggests male discrimination of female developmental stage, and possible active rejection of less appropriate females encountered. However, the relative distribution of different mobile stages on the host and the behaviour of females in response to male pair-formation attempts, should also be taken into account (M. Q. Hull, unpublished observations).

The loss of this preference hierarchy occurs after day 19 when two female stages are still present, but the ratio of adult males to unmated females is greater than 1:1, and all unmated females are pre-copular. The hierarchy appears to break down with the increased male competition for available females. As Anstensrud (1990*b*) found in *L. pectoralis*, males of *L. salmonis* can mate more than once. Males will also pair and mate with older genitally 'matured' adult females, so males that fail to mate are not

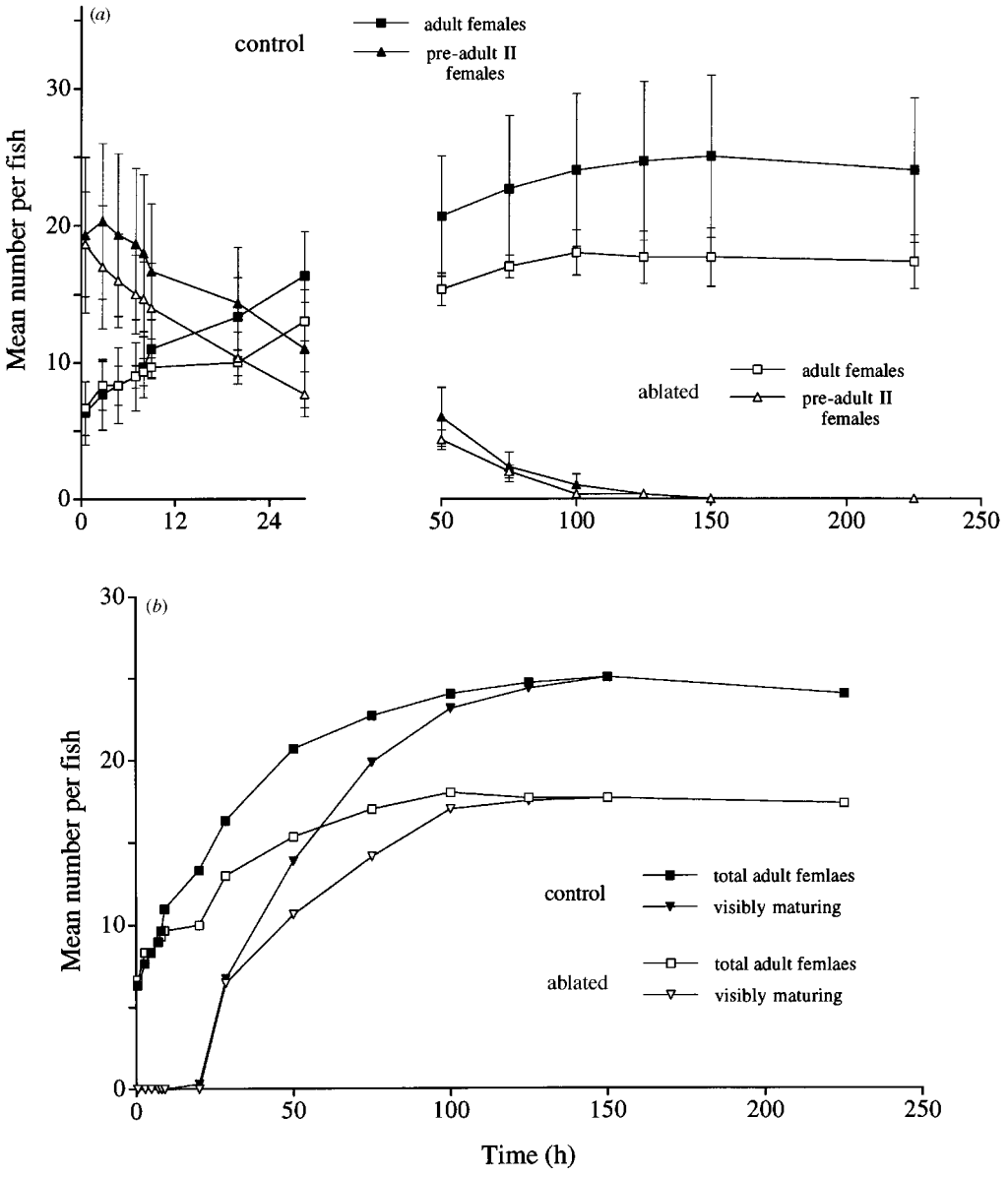


Figure 9. (*a*) Mean (±s.d.) numbers of pre-adult II and adult females on populations of five salmon smolts ($n=3$). (*b*) Mean numbers of freshly moulted and visibly maturing adult females on populations of five salmon smolts ($n=3$).

mechanically prevented from mating by enlargement of the female's genital complex.

### (b) *Population manipulation*

The addition of extra unmated females after all females on the host are either mated or in pre-copula demonstrates multiple mating by males. The presence of more than double the number of mated females only 5 days after introduction of the pre-adult II females, suggested that multiple mating occurred at high levels, and soon after the males finished mating the females of the original cohort.

Temporary isolation of immature females from competent males before the female can be mated and subsequent reintroduction of males at least 9 days after the terminal moult of the females shows that mating of the female in *L. salmonis* is not limited to a specific 'window' after moulting. Anstensrud (1990*b*) demonstrated that female *L. pectoralis* are usually mated immediately after the terminal moult, and that further insemination is precluded by the concurrent application of blocking 'cement' until the attached spermatophores were spent. Ritchie *et al.* (1996*b*) confirmed that this process is typical for female *L. salmonis*. Anstensrud (1990*b*) also demonstrated that once spent spermatophores were lost from the genital complex of female *L. pectoralis*, males were able to re-inseminate them. Loss of spent spermatophores was not noted in *L. salmonis*, but these results show that females age post-moult and the associated morphological changes in the genital complex do not prevent copulation and do not affect the viability of progeny resulting from postponed mating. This study also showed that genital expansion and extrusion of eggs occurred in isolated virgin females. It had been assumed previously that these processes occur only in mated females. It seems unlikely that such unfertilized eggs are viable, but this needs to be tested.

Pre-copular mate guarding is severely reduced when mating has been postponed and there is little advantage in delaying mating with a female that is receptive on

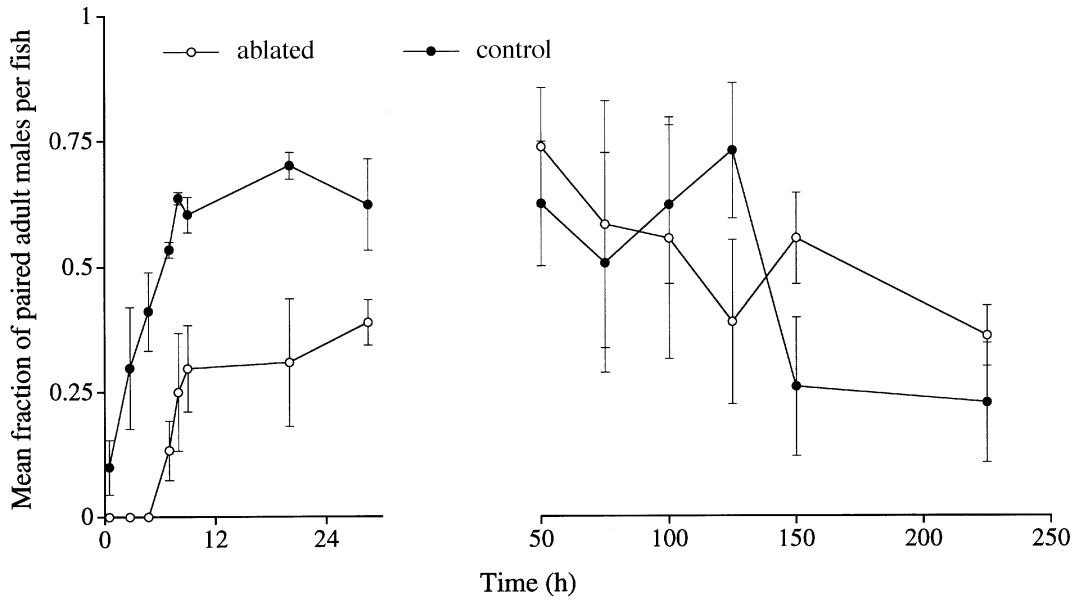


Figure 10. Mean (±s.d.) number of copular pairs expressed as raction of total number of adult males found on populations of five salmon smolts ($n=3$).

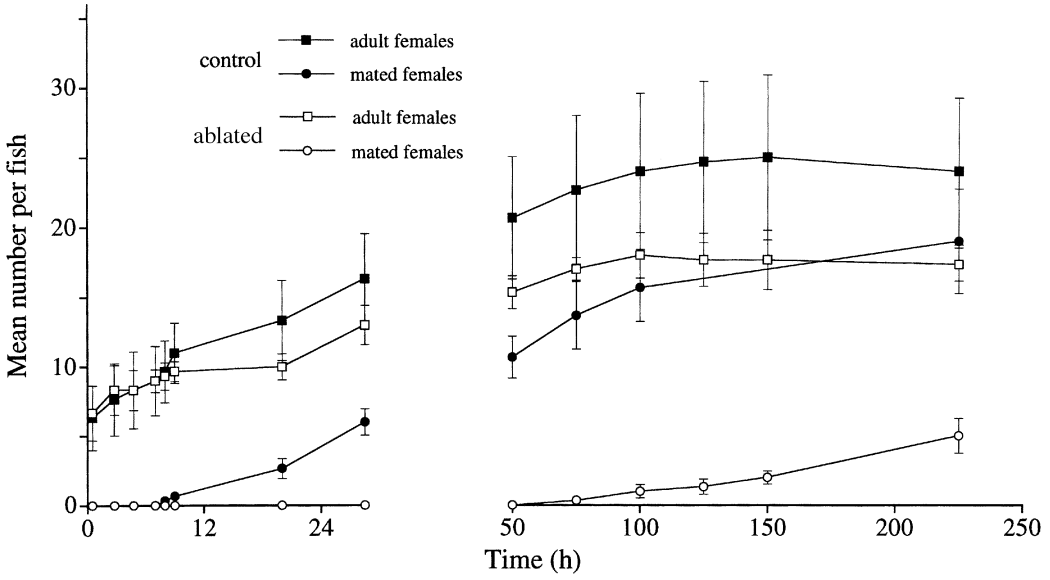


Figure 11. Mean (±s.d.) numbers of confirmed mated females and total adult females on populations of five salmon smolts ($n=3$).

first contact. Some advantage might be gained if a period of time was required by the male to generate new spermatophores.

There has been considerable debate whether active host transfer is a natural behaviour of lice or an artefact. Preliminary work by Bruno & Stone (1990) and Ritchie (1993) demonstrated host transfer under laboratory conditions and that lice may resettle on Atlantic salmon when placed on different fish species. Ritchie (1993) showed high levels of intraspecific inter-host transfer with a minimum of 40% of parasites transferring over an 8-day period. Both these studies used anaesthetic on hosts and parasites, and involved manual transfer of *L. salmonis*. These factors may affect the parasite–host interaction.

The relatively non-invasive technique used here to examine inter-host transfer of adults helped to quantify the rate of transfer of males and females, under relatively undisturbed conditions, after the post-terminal moult mating process is complete. The results represent a reasonable estimation of the rate of host transfer under laboratory conditions. However, because of the method employed, we cannot rule out the possibility of adults leaving their host and then re-settling on the same host. The level of transfer found is lower than that reported in the preliminary studies of Bruno & Stone (1990) and Ritchie (1993). More male transfers were observed, and individual males appear to transfer more often than females. After mating the fecund female must maximize production of viable offspring and would appear to gain little from inter-host transfer as females can produce at least six pairs of egg sacs after one mating (Ritchie 1993).

Adult males would have more to gain from inter-host transfer. Males of *L. salmonis* may mate many times and can mate opportunistically with unmated adult females that were not mated directly after reaching sexual maturity. After mating the male makes no further investment in the fertilized progeny, and might increase the viability of offspring by its departure from that host and




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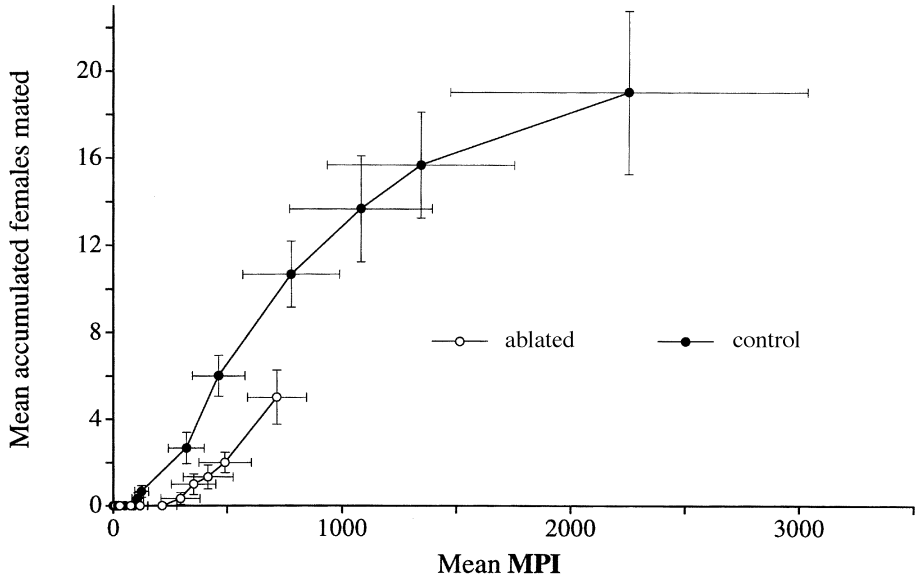


Figure 12. Mean (±s.d.) accumulated females mated against respective mean (±s.d.) male presence index for the two treatments ($n=3$).

the concurrent reduction of its parasite burden. However, reproductive gain would depend on the balance of probability between subsequent settlement of unmated females and encounter with a new host carrying unmated females.

### (c) *Behavioural manipulation: distal ablation of the antennules*

Ablation of the antennule tip profoundly affected lice behaviour. The three key behaviours of host re-settlement, pair formation, and mating were all affected, supporting previous studies (Hull 1997) in which the *in vitro* kinesis response of lice to host odours was lost after ablation.

This effect may not derive purely from disruption of the chemosensory modality of *L. salmonis* as ablation removed putative chemoreceptive setae and four of the putative mechanoreceptors found posteriorly on the apex. These latter receptors in lice may be involved in the control of normal swimming and could contribute to behavioural changes. Accepting that the effects were a result of ablation of this small group of setae in total, caution must be exercised in interpreting the results.

The reduction of host re-settlement in ablated lice suggests an involvement of these sensory setae in the process. Ablation appears to interfere with settlement itself, rather than having an effect after initial contact with the host. The marked reduction in numbers of *L. salmonis* resettling suggests this, and further support comes from the persistence levels of ablated lice that successfully settle on the host.

Ablation affected the rate of the two key reproductive processes after settlement, but the mechanism of action and the implications for the sensory role of the setae are unclear.

The effect of ablation on pair formation was an initial reduction in number of copular pairs, but this difference diminishes over time. Ablation may reduce the ability of the male to recognize and locate appropriate females for pairing, but ablated males still formed pairs, and alternative interpretations are possible. For example, ablation may have affected the capability of males to orientate and move over the host's surface, in which case the initial rate of pair formation would be slow, but the ratio of males in pairs would grow until it was comparable with the controls. As with pair formation, mating is delayed by ablation. In the control the first pre-copular pair was present in the first sample and the first mated female only 8.5 h later. In the ablation treatment, the first pair was observed at 7 h, but the first mating was nearly 70 h later.

Pair formation is affected by ablation during the initial period, but disruption of the early part of mate location and pair formation cannot completely account for the results. Significant numbers of pairs were formed by ablated animals, none of which led to successful mating. This suggests a behavioural deficiency occurring in ablated males during transition from mate-guarding behaviour to active mating.

The sensory implications of this are unclear as contact with the female is common to both sustained mate guarding and all mating. Ablating setae does not appear to affect the late mating of matured females but prevents any mating at the usual post-moult receptive period. A sensory cue may be necessary in the latter situation but not in the former, perhaps involved in the transition from a dorsal guarding behaviour to the ventral mating position.

The mating behaviour of *L. salmonis* was described in detail by Ritchie *et al.* (1996*a*) and resembles that of other parasitic copepods such as *L. pectoralis* (Anstensrud 1990*a–d*; 1992). Preliminary observations of mate searching and testing behaviour by males in the presence of pre-adult and adult females and the clustering of males around moulting females of *L. salmonis* on salmon strongly suggest that long-range, short-range and contact chemical stimuli may be involved, either alone or in combination during pair formation and mate recognition (Ritchie *et al.* 1996*a*). Increased activity of males in the presence of pre-adult II females has been demonstrated (Pike *et al.* 1993) and males exhibit a behavioural response to water-borne chemical cues from salmon-conditioned water introduced to *in vitro* bioassays (Pike *et al.* 1993). Loss of this *in vitro* response after ablation of the distal tips of male antennules was also demonstrated (Hull 1997).

The present results provide further evidence of high parasite mobility both on and between salmon. We emphasize the importance of the chemical ecology of sealice in host and mate finding. Future work is necessary to establish the components of the chemical cues involved in these behaviours and to investigate if these can be used in the control of this main ectoparasitic pest of farmed salmonids.

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## REFERENCES

Anstensrud, M. 1989 A vital stain for studies of behaviour and ecology of the parasitic copepod *Lernaeocera branchialis* (L.) (Pennellidae). *Mar. Ecol. Prog. Ser.* **53**, 47–50.

Anstensrud, M. 1990*a* Moulting and mating in *Lepeophtheirus pectoralis* (Copepoda, Caligidae). *J. Mar. Biol. Ass. UK* **70**, 269–281.

Anstensrud, M. 1990*b* Mating strategies of two parasitic copepods (*Lernaeocera branchialis* (L.)) (Pennellidae) and *Lepeophtheirus pectoralis* (Müller) (Caligidae) on flounder: polygamy, sex-specific age at maturity and sex ratio. *J. Exp. Mar. Biol. Ecol.* **136**, 141–158.

Anstensrud, M. 1990*c* Effects of mating on female behaviour and allometric growth in the two parasitic copepods (*Lernaeocera branchialis* (L. 1767)) (Pennellidae) and *Lepeophtheirus pectoralis* (Müller 1776) (Caligidae). *Crustaceana* **59**, 245–258.

Anstensrud, M. 1990*d* Male reproductive characteristics of two parasitic copepods, *Lernaeocera branchialis* (L.) (Pennellidae) and *Lepeophtheirus pectoralis* (Müller) (Caligidae). *J. Crust. Biol.* **10**, 627–638.

Anstensrud, M. 1992 Mate guarding and mate choice in two copepods, *Lernaeocera branchialis* (L.) (Pennellidae) and *Lepeophtheirus pectoralis* (Müller) (Caligidae), parasitic on flounder. *J. Crust. Biol.* **12**, 31–40.

Boxshall, G. A. & Defaye, D. 1993 *Pathogens of wild and farmed fish: sea lice*. London: Ellis Horwood.

Bron, J. E., Sommerville, C., Wootten, R. & Rae, G. H. 1993 Fallowing of marine Atlantic salmon, *Salmo salar* (L.), farms as a method for the control of sea lice, *Lepeophtheirus salmonis* (Krøyer 1837). *J. Fish Dis.* **16**, 487–493.

Bruno, D. W. & Stone, J. 1991 The role of saithe: *Pollachius virens* (L.), as a host for the sea lice *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* (Nordmann). *Aquaculture* **89**, 201–207.

Costello, M. J. 1996 Development and future of cleaner-fish technology and other biological control techniques in fish farming. In *Wrasse biology and use in aquaculture* (ed. M. D. J. Sayer, J. W. Treasurer & M. J. Costello), pp. 171–184. Oxford: Fishing News Books.

Gresty, K. A., Boxshall, G. A. & Nagasawa, K. 1993 Antennulary sensors of the infective larva of the salmon louse, *Lepeophtheirus salmonis* (Copepoda, Caligidae). In *Pathogens of wild and farmed fish: sea lice* (ed. G. A. Boxshall & D. Defaye), pp. 83–99. London: Ellis Horwood.

Heuch, P. A. 1995 Experimental evidence for aggregation of salmon louse copepodids (*Lepeophtheirus salmonis*) in step salinity gradients. *J. Mar. Biol. Ass. UK* **75**, 927–939.

Heuch, P. A. & Karlsen, H. E. 1997 Detection of infrasonic water oscillations by copepodids of *Lepeophtheirus salmonis* (Copepoda, Caligidae). *J. Plankton Res.* **19**, 735–747.

Heuch, P. A., Parsons, A. & Boxaspen, K. 1995 Diel vertical migration—a possible host-finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids. *Can. J. Fish. Aquat. Sci.* **52**, 681–689.

Hull, M. Q. 1997 The role of semiochemicals in the behaviour and biology of *Lepeophtheirus salmonis* (Krøyer 1837): potential for control. PhD thesis, University of Aberdeen, UK.

Jones, M. W., Sommerville, C. & Wootten, R. 1992 Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. *J. Fish Dis.* **15**, 197–202.

Johnson, S. C. 1993 A comparison of development and growth rates of *Lepeophtheirus salmonis* (Copepoda, Caligidae) on naive Atlantic (*Salmo salar*) and chinook (*Oncorhynchus tshawytscha*) salmon. In *Pathogens of wild and farmed fish: sea lice* (ed. G. A. Boxshall & D. Defaye), pp. 68–80. London: Ellis Horwood.

Johnson, S. C. & Albright, L. J. 1991*a* Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda, Caligidae) under laboratory conditions. *J. Mar. Biol. Ass. UK* **71**, 425–436.

Johnson, S. C. & Albright, L. J. 1991*b* The developmental stages of *Lepeophtheirus salmonis* (Krøyer 1837) (Copepoda, Caligidae). *Can. J. Zool.* **69**, 929–950.

Pike, A. W. 1989 Sea lice—major pathogens of farmed and wild Atlantic salmon. *Parasitol. Today* **5**, 291–297.

Pike, A. W., Mordue, A. J. & Hull, M. Q. 1993 *The potential role of semiochemicals in sea lice control*. Internal report to the Scottish Salmon Growers Association.

Pickett, J. A., Wadhams, L. J. & Woodcock, C. M. 1997 Developing sustainable pest control from chemical ecology. *Agric. Ecosyst. Environ.* **64**, 149–156.

Ritchie, G. 1993 Studies on the reproductive biology of *Lepeophtheirus salmonis* (Krøyer 1838) on Atlantic salmon (*Salmo salar* L.). PhD thesis, University of Aberdeen, UK.

Ritchie, G., Mordue, A. J., Pike, A. W. & Rae, G. H. 1996*a* Observations on the mating and reproductive behaviour of *Lepeophtheirus salmonis* Krøyer (Copepoda, Caligidae). *J. Exp. Mar. Biol. Ecol.* **201**, 285–298.

Ritchie, G., Mordue, A. J., Pike, A. W. & Rae, G. H. 1996*b* Morphology and ultrastructure of the reproductive system of *Lepeophtheirus salmonis* (Krøyer 1837) (Copepoda: Caligidae). *J. Crust. Biol.* **16**, 330–346.

Roth, M., Richards, R. H. & Sommerville, C. 1993 Current practices in the chemotherapeutic control of sea lice infestations in aquaculture—a review. *J. Fish Dis.* **16**, 1–26.

Thomassen, J. M. 1993 Hydrogen peroxide as a delousing agent for Atlantic salmon. In *Pathogens of wild and farmed fish: sea lice* (ed. G. A. Boxshall & D. Defaye), pp. 290–295. London: Ellis Horwood.

Wootten, R., Smith, J. W. & Needham, E. A. 1982 Aspects of the biology of parasitic copepods, *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids and their treatment. *Proc. R. Soc. Edinb.* B **81**, 185–197.