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BRIEF COMMUNICATION

The chemical cues of male sea lice *Lepeophtheirus salmonis* encourage others to move between host Atlantic salmon *Salmo salar*

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Adult male sea lice *Lepeophtheirus salmonis* were more likely to leave host fish Atlantic salmon *Salmo salar* if they detected the chemical cues of other adult male lice than if they detect cues of female lice. The detection of both male and female chemical cues yielded an intermediate response. These results suggest that males use chemical cues to balance competition for resources and mate acquisition, and they highlight the need for further studies of the chemical ecology of this important parasite.

Key words: aggregation; aquaculture; ectoparasite; host selection; parasite behaviour; semiochemical.

The sea louse *Lepeophtheirus salmonis*, is a major pest of farmed salmonids (Costello, 2009), and may contribute to declines in wild salmonid stocks in areas of intense sea cage aquaculture (Krkošek *et al*., 2007). Despite the economic importance of this parasite, little is known of its ecology. Aggregation of parasites on a small proportion of the available hosts is one aspect of parasitology about which there are many hypotheses (Shaw & Dobson, 1995); in sea lice, behaviour could be a contributory factor. Because sea lice reproduce sexually, the presence of potential mates on a host fish is a criterion for host selection and is commonly cited as an explanation for the observed movement of pre-adult and adult (motile) lice between host fish. Hull *et al*. (1998) found that males had a transfer rate of 62·4%, compared to 17·9% in females. In a sea cage not only were motile lice found to move among experimentally infected hosts within a cage, but there was a high level of immigration of lice from the surrounding cages as well (Ritchie, 1997). In an experiment using juvenile pink salmon *Oncorhynchus gorbuscha* (Walbaum 1792), nearly 45% of adult male lice moved among host fish (Connors *et al*., 2011). Males of the freshwater louse *Argulus coregoni* are more likely to move between rainbow trout *Oncorhynchus mykiss*

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(Walbaum 1792) if there is no female present on their original host (Bandilla *et al*., 2008). Similarly, motile *L. salmonis* on *O. gorbuscha* appear to disperse according to the availability of mates (Connors *et al*., 2011). None of these studies attempted to characterize the method by which lice perceive the number or sex of lice on their host.

Securing mating opportunities may often be balanced by the avoidance of competition for resources. This trade-off is well studied in habitat selection by free-living animals (Clobert *et al*., 2001), but data are lacking from equivalent studies of host selection by facultatively dispersing parasites. There is indirect evidence that motile sea lice may distribute themselves among hosts according to this trade-off, including the observation that at low infection intensities, and low resource competition, lice are aggregated among the host population with a few fish carrying the majority of the louse population and most fish carrying few or no lice. As infection intensity and resource competition increase, louse burden in the host population becomes more normally distributed (C. Revie, pers. comm.).

How lice discern the presence or absence of mating partners, and the density of lice on their host fish, is unknown. Improving the understanding of the factors determining louse movement and aggregation could usefully influence sea cage monitoring regimes. Here, a behavioural experiment tested the hypothesis that male lice assessed the infection load and the presence of potential mates on their host fish through the detection of chemical signals, and that this informed their movement between host fish. A 'focal' fish and a 'stimulus' fish were held in a small volume tank with a low flow rate. The number and sex of lice on the stimulus fish were manipulated between treatments, that of the lice on the focal fish held constant, and the amount of movement between fish was recorded. Because of the small volume and low flow, the concentration of chemical cues in the test tanks could be considered equivalent to those experienced by lice on a single fish in an open water setting. This design ensured that there was no chance of tactile communication between the lice on different fish, and provided a destination for lice that moved from the focal fish. The results suggested that lice are more likely to move from a host fish when in receipt of chemical cues from a large number of males than when they can detect the chemical cues from females.

The experiment was conducted at the Marine Environment Research Laboratory (MERL), Machrihanish, Argyll, Scotland. Lice were cultivated in single cohort batches throughout the experiment. Fifteen *Salmo salar* L. 1758 (mean ± S.E. fork length, L_F, 331·36 ± 3·95 mm) were held in each of five tanks of 1 m diameter, with *c*. 600 l of fresh, aerated sea water in a flow-through system. Sea lice copepodids (produced at MERL) were used to infect the fish at a density of 60 lice per fish 19–20 days before the lice were required. The flow of water into the tank to be infected was turned off and the water level was dropped, while aeration was increased. The copepodids were added to the tank and after 2·5 h normal tank conditions were restored. To collect lice, the fish were anaesthetized using 2-phenoxyethanol (P1126; Sigma-Aldrich; www.sigmaaldrich.com) and the lice removed with curved forceps.

Preliminary observations found that maintenance in 0·015 g l^{-1} neutral red solution for 30–40 min gave lice a sufficiently red colour without affecting mortality rates compared with controls (Hull *et al*., 1998). The red colour was still detectable, under bright light, after 7 days on a fish. Dyed and undyed lice spent the same amount of time off fish.

The experiment was conducted in a system comprising 10 opaque white plastic 0·5 m diameter cylindrical tanks. Each tank had a central drain with a standpipe (diameter 1·75 cm) and standpipe cover (diameter 5 cm) to ensure that the water layers mixed. The volume of water in each tank was 33 l. Water was pumped ashore from Machrihanish Bay and passed through a fine mesh net into a reservoir tank; the water temperature was mean ± S.E. $14{\cdot}30 \pm 0{\cdot}02^\circ$ C. Each experimental tank was fed with water pumped from the reservoir tank. Flow rate was the same in each tank (mean ± S.E. l s^{-1}: $2{\cdot}60 \pm 0{\cdot}02$). Two fluorescent bulbs lit the system from above. Each tank had a netting cover, on top of which was laid a strip of black plastic, placing half of each tank in shade (mean ± S.E. shaded: $5{\cdot}8 \pm 1{\cdot}2$ lx; unshaded: $227{\cdot}3 \pm 10{\cdot}5$ lx).

The experimental fish were netted from their home tank and lightly anaesthetized. Each fish was placed in a separate white 10 l bucket of fresh sea water along with lice corresponding to one treatment. In each treatment, the focal fish was infected with 10 adult male lice. The infection burden of the stimulus fish varied between treatments (1, no lice; 2, 10 pre-adult II females; 3, 10 adult males; 4, five pre-adult II females, five adult males). Either the focal or stimulus fish was marked with a distinctive cut in the adipose fin; this was pseudo-randomized such that by the end of the experiment equal numbers of focal and stimulus fish had been marked. Fish were left in the buckets for 1 h, at which point the attachment success of the lice was recorded by counting the number still loose in the bucket. Extra lice were added when it seemed unlikely that the lice remaining in the bucket would attach to the fish. This ensured that all lice that attached to the test fish were healthy and had not been damaged during removal from their rearing host. Once the target number of lice had attached, the fish was transferred to the tank.

During the 7 days of the experiment, fish were checked daily and then were killed with an overdose of anaesthetic, weighed and measured and the number and sex of the lice and whether or not they were dyed was recorded. For tanks in which a fish died before the end of the experiment ($n = 5$ across the whole experiment; 160 fish used in total) these data were taken as well as the number of completed days. No difference between these replicates and the 7 day ones was found. Between replicates each tank was emptied, scrubbed with 70% ethanol and rinsed thoroughly with sea water.

Across the experiment, fish had an L_F of $218{\cdot}33 \pm 21{\cdot}83$ mm and weighed $108{\cdot}54 \pm 10{\cdot}85$ g (mean ± S.E.). Within each tank the two fish used during any replicate were not of different L_F (t-test, $t = 0{\cdot}650$, d.f. = 98, two-tailed $P > 0{\cdot}05$) or masses (t-test: $t = 0{\cdot}564$, d.f. = 98, two-tailed $P > 0{\cdot}05$). As replicates were conducted sequentially there was a significant increase in the size of the fish used in the trials across the 44 day experimental period (regression: L_F: mean change = 46·4 mm; $r^2 = 0{\cdot}59$, $F_{1,98} = 142{\cdot}20$, $P < 0{\cdot}001$; mass: mean change = 83·45 g; $r^2 = 0{\cdot}57$, $F_{1,98} = 130{\cdot}54$, $P < 0{\cdot}001$).

To test the hypothesis that the rate at which male lice left the focal fish depended on the sex and number of lice on the stimulus fish, the proportion of males leaving the focal fish was used as a response variable in a two-way ANOVA in R (version 2.10.1, GUI 1.31; S. Urbanek & S. M. Iacus, R Foundation for Statistical Computing, 2009) in which both treatment and block were used as class variables. That data conformed to the assumptions of this test was confirmed using Bartlett's and Fligner–Killeen

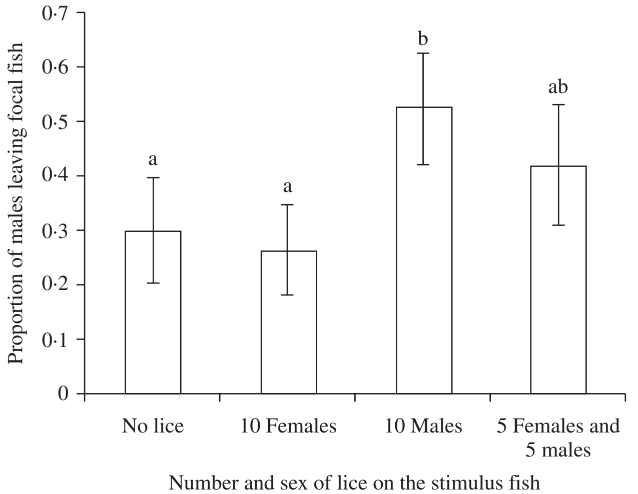

FIG. 1. Proportion of adult male lice *Lepeophtheirus salmonis* leaving the 'focal' fish under each of four different treatments. At the beginning of a 7 day trial, there were 10 adult male lice on the focal fish. The number and sex of lice on the 'stimulus' fish was manipulated as shown: 'females' were pre-adult II lice. Focal and stimulus fish were held in pairs in small tanks with low water flow; pheromone concentration can be considered similar to that experienced by lice on a single fish in a more natural setting. The lower case letters a, b and ab denote treatments between which there was a significant difference ($P < 0{\cdot}05$) in the proportion of males that left the focal fish.

tests. Tukey's honestly significant difference test was run as a *post hoc* test on significant results.

The proportion of males leaving the focal fish varied significantly across the treatments ($F_{2,18} = 8{\cdot}42$, $P = 0{\cdot}001$) and there was no block effect ($F_{9,18} = 1{\cdot}05$, $P > 0{\cdot}05$) (Fig. 1). The proportion of males leaving the focal fish was significantly higher when there were males on the stimulus fish than when the stimulus fish was uninfected ($P < 0{\cdot}01$). Males were also more likely to leave the focal fish when the stimulus fish was infected with male lice than when it was infected with female lice ($P < 0{\cdot}01$). When the stimulus fish was infected with males and females, the proportion of males leaving the focal fish was not significantly different from any other treatment. Of the males leaving the focal fish, $64{\cdot}2 \pm 0{\cdot}03\%$ (mean ± S.E.) were counted on the stimulus fish across all treatments. Of the lice that had left the focal fish and were not counted on the stimulus fish, 15·7% were found loose in the tank and the others were assumed to have been flushed out of the tank or eaten by the fish.

The results suggested that adult male lice were more likely to leave a host fish if they detected the chemical signals of a number of other males than if they detect the chemical signals of females. The proportion of males leaving the focal fish in treatment four, with five females and five males on the stimulus fish, was at an intermediate level. These results supported the hypothesis that lice used chemical cues to assess their host's louse burden and move between hosts based on this information.

The stimulus fish in this experiment were used to present lice on the focal fish with chemical cues of lice attached to a fish, rather than free-swimming lice, and to provide a destination to facilitate counting those that had left the focal fish. It was also important that the lice were able to detect the chemical cues of lice not on their own host fish to eliminate the possibility of tactile cues which are important in this species (Ritchie *et al.*, 1996). One potential disadvantage of this experimental design was that lice from the destination host were also able to move between hosts, subtly changing the treatment. As they would still release chemical cues, however, this need not alter the interpretation of the results.

Chemical communication may be as important to Crustacea, parasitic and free-living, as to terrestrial arthropods (Snell & Morris, 1993; Derby & Sorensen, 2008), and evidence suggests that *L. salmonis* is no exception to this. For animals in aquatic environments with a body size $>0{\cdot}2-5{\cdot}0$ mm, pheromone production dramatically increases mate location (Dusenbery & Snell, 1995); pre-adult and adult *L. salmonis* are usually larger than this. In addition, *L. salmonis* are sensitive to the odour of potential host fish at a concentration of 10^{-4}, tested electrophysiologically (Fields *et al.*, 2007). Louse behavioural responses suggest that males at least are similarly sensitive to conspecific odours, although this has not been tested electrophysiologically (Ingvarsdóttir *et al.*, 2002). Adult male *L. salmonis* show directional responses to water conditioned with the chemical cues of pre-adult II female lice, but not to adult females or adult males, although they do show activation responses to both of these (Ingvarsdóttir *et al.*, 2002). It is, therefore, likely that the male lice used during this experiment were able to detect the chemical cues from the other lice, and that the difference in behaviour between treatments was due to the different cues present in each treatment.

These results indicate a potentially important mechanism by which lice distribute themselves among host fish, but do require further investigation. The experimental set-up is likely to have provided lice with concentrations of cues analogous to those they would experience while on a single fish in an open water setting. The clearest interpretation of the results is that when in receipt of female chemical cues male lice stay on their current host. The attraction of the presence of females is, however, balanced by the resource competition male lice are likely to encounter. If this too is assessed using chemical cues, it explains why a high proportion of male lice moved between fish in the male-only treatment. It is likely that these two effects are density dependent, as is suggested by the intermediate result of treatment four.

The results of this study add to evidence indicating that lice may choose a host based on factors other than those inherent to the fish. Although selectively breeding fish with some resistance has had some success (Glover *et al.*, 2005), genetically homogenous farmed stocks still show variation in susceptibility to infection which seems to be related to their existing louse burden. For example, when individually tagged fish in a sea cage were deloused between infection events, their initial louse burden was a poor predictor of their subsequent burdens. When fish were not deloused, those with high louse burdens in the first sampling event again had the highest, increased, louse burdens in the second sampling event (Glover & Skaala, 2006). Because of the speed of development of resistance to, and the environmental impact of, pharmaceutical control and the limited efficacy of other control methods, more integrated pest management (IPM) is being called for (Denholm *et al.*, 2002). Greater understanding of louse biology and behaviour is needed to effectively plan these IPM strategies.

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