The effect of sea lice infestation on the salinity preference and energetic expenditure of juvenile pink salmon (*Oncorhynchus gorbuscha*)

Sandra J. Webster, Lawrence M. Dill, and Kevin Butterworth

Abstract: Ocean-going juvenile salmonids heavily infected with salmon louse, *Lepeophtheirus salmonis*, have been observed prematurely returning to freshwater. This change in salinity preference may be an attempt either to regain osmotic balance or to remove the lice. For either hypothesis to be true, freshwater habitats must provide infected fish with a higher net fitness than saltwater habitats. The objectives of this study were to use behavioural titration to quantify the energetic cost of different salinities to infected and uninfected pink salmon (*Oncorhynchus gorbuscha*) and to determine if infection alters salinity preference. Results demonstrate that infection changes the salinity preference of fish from saltwater to freshwater. The cost paid by these freshwater-preferring infected fish foraging in saltwater increased with lice density during trials conducted between 13–33 days after infection. Other infection-induced behavioural changes include a 14-fold increase in the jumping frequency of infected versus control fish and a decrease in foraging between 13 and 33 days after infection.

Résumé : On a observé que certains jeunes salmonidés fortement infestés de poux du saumon, *Lepeophtheirus salmonis*, en route pour l'océan retournent prématurément en eau douce. Ce changement de préférence de salinité peut être une tentative pour retrouver un équilibre osmotique ou pour se débarrasser des poux. Pour que l'une ou l'autre de ces hypothèses se vérifie, il faut que l'habitat d'eau douce procure aux poissons infectés une fitness nette plus grande que les habitats d'eau salée. Les objectifs de notre étude sont d'utiliser le dosage comportemental pour mesurer le coût énergétique des diverses salinités chez des saumons roses (*Oncorhynchus gorbuscha*) infectés et sains et pour déterminer si l'infection modifie la préférence de salinité. Nos résultats démontrent que l'infection change la préférence de salinité des poissons, de l'eau salée vers l'eau douce. Lors d'essais menés entre 13 et 33 jours après l'infection, le coût payé par ces poissons qui préfèrent l'eau douce, mais qui se nourrissent en eau salée, augmente en fonction de la densité des poux. Parmi les autres changements comportementaux induits par l'infection, on note une augmentation de l'ordre de 14 fois de la fréquence des sauts chez les poissons infectés par comparaison aux poissons témoins et une diminution de la recherche de nourriture entre les jours 13 et 33 après l'infection.

[Traduit par la Rédaction]

Introduction

Parasites can induce behavioural changes in their hosts (Poulin 1994). These behavioural changes occur either because the parasite will enhance its fitness by increasing its probability of transmission to future hosts or because the host is compensating for the cost of the parasite (Poulin 1995). Host-mediated behavioural changes include avoiding or reducing the probability of becoming infected (Downes et al. 1986; Rogowski and Stockwell 2006) or ridding themselves of infection once it has occurred through behavioural fever (Kluger et al. 1975; Covert and Reynolds 1977; Elliot et al. 2002), self-medication (Clark and Mason 1988; Clayton and Wolfe 1993), or physical removal (e.g., allogrooming (Hutchins and Barash 1976; Barton 1985; Hart and Hart 1992) and self-grooming (Murray 1987)).

These behavioural changes may come at a cost. For example, pupfish (*Cyprinodon tularosa*) have higher body condition and larger body size when in moderately saline habitats. However, when the parasitic pupfish trematode, *Ascocoytl* sp., is present in these habitats, pupfish prefer highly saline habitats where their growth and condition are reduced but the trematode is less abundant (Rogowski and Stockwell 2006).

The salmon louse, *Lepeophtheirus salmonis*, is a common parasite of salmonid fishes. At moderate loads, it has a mini-

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mal effect on the host (Wootten and Smith 1982). However, heavily infected ocean-going juvenile sea trout (*Salmo trutta*), pink salmon (*Oncorhynchus gorbuscha*), and coho salmon (*Oncorhynchus kisutch*) have been observed returning to freshwater during unusually early stages of their life history (Birkeland 1996; Bjorn et al. 2001; A. Morton, Raincoast Research, Simoon Sound, BC V0P 1S0, personal communication). Sea trout postsmolts, which typically return to freshwater in the autumn to overwinter, have been observed ascending rivers within a few weeks of their downstream smolt migration, a phenomenon known as premature return (Tully et al. 1993; Birkeland 1996; Bjorn et al. 2001). Unlike sea trout, Pacific salmon are anadromous and semelparous and therefore normally only return to freshwater once, to spawn as adults. However, they have been observed returning to freshwater within weeks of entering the ocean (A. Morton, personal communication). Returning to freshwater from saltwater is not only osmotically costly (McKeown 1984; Wendelaar Bonga 1997), but as growth is generally better in the ocean than in freshwater (Randall et al. 1987; Groot and Margolis 1991), decreased time in saltwater may result in reduced growth rates (Birkeland 1996; Mortensen et al. 2000) and thus reduced marine survival, owing to size-selective predation risk (Holtby et al. 1990; Sogard 1997), and reduced fecundity as an adult (Dickerson et al. 2002).

It is unlikely that freshwater return in juvenile salmonids is parasite-mediated (i.e., beneficial to the parasite and thus parasite-driven) because sea lice typically do not survive longer than 1–2 weeks in freshwater (Hahnenkamp and Fyhn 1985; Finstad et al. 1995), and they are not capable of reproducing or transferring to a new host in this environment (Wootten and Smith 1982). Hence, freshwater return is thought to be beneficial to the host and thus host-mediated.

There are two commonly presented hypotheses as to why salmonids infected with sea lice return to freshwater. Sea lice do not survive long in freshwater, hence salmonids may be returning to freshwater to kill the lice. Alternatively, salmonids may be returning to freshwater to regain osmotic and ionic balance. Juvenile salmonids heavily infected with sea lice exhibit high gill Na^+/K^+-ATPase activity and osmoregulatory breakdown (Grimnes and Jakobsen 1996; Nolan et al. 1999), both of which result in increased energetic costs. If these costs become sufficiently high, infected fish may be unable to maintain osmotic balance in saltwater. As a result, infected fish may return to freshwater because of the benefits associated with lower ionic gradients.

Sea lice infestation may modify salmonid behaviour in ways other than habitat choice. For instance, fish leaping behaviour is believed to occur in part because of infection by ectoparasites and may be a means of dislodging the parasite (Gudger 1944). This is supported by the observation that fish in net pens exhibit increased leaping behaviour during increased levels of louse infestation and decreased leaping after delousing (Furevik et al. 1993).

The objectives of this study were to advance the current understanding of how sea lice infestation affects juvenile pink salmon behaviour and to determine potential effects of infection on the salmon's physiological state and energetic expenditure. We conducted a series of laboratory tests to (*i*) determine if risk of infection is related to fork length and condition factor, (*ii*) determine if infection alters salinity preference, foraging rate, frequency of leaping and rolling, and (or) Na^+/K^+-ATPase activity of juvenile pink salmon, and (*iii*) quantify and compare the energetic cost of different salinities to infected and uninfected salmon and determine if costs change with sea lice load.

Materials and methods

Fish and fish maintenance

Experiments were conducted on hatchery-reared, pink salmon parr from the Seymour River Fish Hatchery in British Columbia, Canada. Fish were collected from the hatchery on 29 March 2004 after they had volitionally emigrated from the incubator. Fish were then transferred to the Fisheries and Oceans Canada Laboratory in West Vancouver where they were kept indoors in one of three identical 250 L flow-through tanks at natural photoperiod. Tanks were initially supplied with a continuous flow of air-equilibrated well water (salinity 0‰, temperature 9.8 ± 0.2 °C, and dissolved oxygen 97.8 ± 17.0% air saturation; mean ± standard deviation, SD). Tank salinity was increased 2 days after the fish arrived to 10.0‰ ± 0.6‰ and was gradually increased 10 days later to a final salinity of 28.2‰ ± 0.9‰. Trials began 5 weeks after arrival to the laboratory. Fish were fed a ration (2% biomass·day^{-1}) of commercial salmon pellets (EWOS Canada Ltd.) twice daily.

Salmon louse culture and artificial infection

On three separate occasions, ovigerous sea lice were collected from sea-farmed Atlantic salmon (*Salmo salar*) near Egmont, British Columbia, Canada. On the day after collection, egg strings were removed from the lice and cultured in 4 L chambers. The egg strings hatched within 2 days, and the nauplii were cultured through to the copepodid stage before infecting the salmon. The free-living stages were monitored daily until 90% of the sea lice had reached copepodid stage.

Three separate infections were conducted, each consisting of 100 fish (see Table 1 for data on fish fork length and mass). Fish were lightly sedated with 30 ppm clove oil for 5 min until loss of orientation and significant reduction in gill cover movement were observed. The fish were anaesthetised to reduce the stress of the procedure and to limit the flow of water across the gills, which reduces the number of copepodids passing over them and thus the potential for the sea lice to attach to the gill filaments. Fish were then placed in a 4 L bath containing approximately 4000 sea lice copepodid larvae. After 15 min had lapsed, the contents of the sea lice bath, including the fish, were transferred into a 150 L flow-through tank continuously supplied with air-equilibrated seawater (see Table 1 for data on water quality). Following attachment to the salmon, the development of the lice through the different life stages was as follows: copepodid at 6 days after infection (DPI), chalimus stage between 8 and 14 DPI, and pre-adult stage between 16 and 33 DPI (temperature ranged between 9.5 and 12.5 °C). Control groups were anaesthetised with 30 ppm clove oil for 5 min before a sham infection.

Table 1. Water quality and fish body sizes (± standard error) during sea lice infection procedures.

Experiment	Condition	Mass[a] (g)	Length[a] (mm)	Dissolved O_2 (%)	Temperature (°C)	Salinity (‰)
1. Risk of infection trial	Infected	2.64±0.20	69.1±1.6	93.8±4.9	11.4±0.8	28.7±1.0
2. Energetic cost trial 1	Infected	0.63±0.05	43.2±0.9	90.6±7.1	9.7±0.5	28.5±0.9
	Control			91.4±6.6	9.8±0.6	28.4±0.9
3. Energetic cost trial 2 and leaping and rolling trial	Infected	4.23±0.36	81.5±2.4	92.7±5.0	12.2±1.2	28.8±1.1
	Control			92.1±5.1	12.2±1.1	28.8±1.0

[a]Averages are taken from samples of 20 fish before the experiment and division into treatment groups.

Experimental procedures

Risk of infection

A group of 100 salmon from the infected group were placed in a 150 L flow-through tank continuously supplied with seawater. Fifty fish were removed 2 days after infection and were individually anaesthetized in a dilute solution of clove oil and killed by a blow to the head. Immediately following death, lice load (number of lice per fish) and lice density (number of lice per gram of fish), as well as any skin lesions (holes in the epidermis; Bjorn and Finstad 1998) and damage to the fish's fins, were recorded along with fish fork length (mm) and mass (g). After 14 DPI, the remaining 47 fish (three fish died before data collection) were removed and subjected to the same protocol.

Leaping and rolling behaviour

Immediately following infection, two groups of 100 fish each, one control and one infected, were placed in side-by-side 150 L fibreglass tanks. For 1 h each day, starting at 1 DPI and continuing until 14 DPI, the two groups of fish were observed simultaneously for leaping and rolling behaviour. Leaping occurred when the fish's body cleared the water surface, and rolling occurred when the fish's body partly broke the water surface (Grimnes and Jakobsen 1996). Every 4 days 10–15 fish were randomly removed from each tank, anaesthetized, and sacrificed with a blow to the head. The mass and fork length of these fish were recorded, as was their lice load. To account for the decrease in the total number of fish throughout the trial, the frequency of leaping and rolling behaviour was expressed as leaps or rolls per fish.

Salinity preference and energetic cost

We used behavioural titration to quantify the energetic cost for infected and control fish to reside in habitats of differing salinity. Behavioural titration is based on the assumption that an animal will leave a habitat (A) when the net benefit of foraging there decreases below the net benefit of foraging in an alternate habitat (B) (Brown 1988). The animal's intake rate at the time it leaves habitat A for habitat B is referred to as the quitting harvest rate (QHR) (Pyke 1978, 1980; Hodges 1981). As costs in habitat A become greater relative to those in habitat B, QHR should increase. Therefore, the value of QHR represents the greater energetic demand in habitat A relative to habitat B. This method of measuring cost is well established in the literature (Brown 1988; Kotler and Blaustein 1995; Webster and Dill 2007).

Because it can be logistically difficult to measure the QHR of a forager when it leaves a patch, it is often assumed that the density of food remaining in the patch at the time of departure (giving-up density or GUD) is an acceptable surrogate for QHR (Brown 1988, 1992). However, because a change in abiotic condition can alter intake rate (Webster and Dill 2007), it was important to measure QHR directly. This was logistically possible in this study because we could record how long a fish was in a patch before giving up and how much food was consumed in that time.

The behavioural titration was conducted as a two-part experiment. In part 1, individual salmon were given a choice between one of two habitats that differed only in salinity. Salmon should choose the habitat that provides the highest net benefit (in this case the lowest cost). If infection with sea lice increases the cost of residing in saltwater relative to freshwater, infected fish should require a higher feeding rate, compared with control fish, to remain there.

In part 2 of the experiment, 100 capelin (*Mallotus villosus*) eggs were added to the vacant and presumably more costly habitat (hereafter referred to as the alternate habitat) to increase its net benefit above that of the preferred habitat. As the eggs were consumed in the alternate habitat, the fish's net energy intake rate decreased owing to depletion (Webster and Dill 2007). The fish should return to the preferred habitat when its net energy intake rate in the alternate habitat decreases to a point just below the cost of residing in the preferred habitat. The fish's intake rate at the time it leaves the alternate habitat is the QHR (Pyke 1980; Brown 1988). By calculating the difference in the QHRs of control and infected fish, the effect of sea lice infection on the energetic cost of residing in habitats of different salinities can be measured.

Part 1: salinity preference

Two sets of trials were conducted; the first from 15 May to 7 June (hereafter referred to as trial 1) and the second from 7 to 21 August (hereafter referred to as trial 2). Experiments on fish in trial 1 ran between 13 and 33 DPI, whereas experiments on fish in trial 2 ran between 6 and 22 DPI. All trials began with an acclimation period during which fish were held, in groups of eight, in 60 L aquaria that were divided into two identical habitats that fish could easily swim between. The aquaria were maintained under a cycle of 16 h day – 8 h night and were supplied with seawater (trial 1, 28.5‰ ± 0.9‰, 14.1 ± 1.0 °C; trial 2, 28.8‰ ± 0.9‰, 10.1 ± 0.4 °C). Fish were fed capelin eggs daily to satiation.

Following acclimation, individual fish were placed into 20 L experimental aquaria (41 cm × 20 cm × 25 cm) that were divided into two habitats by 10 cm high, clear Plexiglas™ dividers. The two habitats were identical except in salinity: one habitat contained freshwater (0‰) and the other saltwater (28.6‰ ± 0.9‰). The side of the aquarium that

contained saltwater was alternated between trials to reduce the possibility that a side preference would affect habitat choice. A layer of freshwater over the denser saltwater allowed the fish to move between the two habitats. Daily salinity monitoring revealed that the movement of fish during trial 1 resulted in a significant amount of mixing of the two habitats. This mixing resulted in an increase in the salinity of the freshwater (to 6.2‰ ± 1.7‰) and a decrease in the salinity of the saltwater (to 18.5‰ ± 4.2‰) compared with initial condictions. To minimize mixing during trial 2, the two habitats were kept at different salinities using a duplex flow-through system (freshwater, 1.0‰ ± 1.2‰; saltwater, 28.8‰ ± 0.8‰). This system maintained the salinity differential by continuously replacing the freshwater and saltwater throughout the experiment (see Webster and Dill 2007).

After the fish were introduced to the experimental aquaria, they were left overnight to explore both habitats. On the following morning, 50 capelin eggs were placed in the vacant side of the aquaria. This provided the fish with food and ensured that the fish experienced both habitats. Three hours later, 50 eggs were placed in the opposite habitat (originally the preferred side) to ensure that the fish perceived equal food availability in both habitats. Most fish (70%) had returned to their preferred habitat before the addition of eggs and, therefore, were not required to switch a second time. This reduces the probability that fish learned to switch habitats following the addition of eggs. At the end of the day, all remaining eggs were removed. To camouflage the white capelin eggs during part 2 of the trial, 50 g of brown and white mottled aquarium gravel was scattered across each side of the aquaria. This required the fish to search for eggs, ensuring that the fish experienced a decreasing intake rate over time (an assumption of QHR tests; Brown 1988, 1992). On the following morning (15 h later), salinity preference was determined by observing fish habitat preference every 10 min for 1.5 h. Fish were observed by peering through slits in light occlusion blinds that provided a front view of the aquaria. The habitat in which fish spent ≥80% of their time was considered the "preferred" habitat. Fifteen infected fish and 11 control fish were excluded from the data set because preference was unknown after 1.5 h, resulting in a final sample size of 32 and 34 control fish and 37 and 36 infected fish in trials 1 and 2, respectively.

Part 2: quantifying energetic cost

Part 2 of the experiment followed immediately after the completion of part 1. As soon as habitat preference was determined, we dispersed 100 capelin eggs over the gravel in the alternate habitat and left the fish to forage. Once the fish returned to the preferred habitat, the remaining eggs (R) were removed and counted and the total time spent foraging (T) was recorded. If a fish did not cross the divider to forage during the experiment, it was excluded from part 2, resulting in a final sample size of 25 and 19 control fish and 18 and 13 infected fish in trials 1 and 2, respectively.

To test whether foragers were experiencing a steadily diminishing rate of return, we conducted a series of intake-rate trials. These trials followed the same format as parts 1 and 2 above with one exception. In part 2, fish were allowed only to forage for a predetermined amount of time: 5 min (control, N = 6; infected, N = 5), 15 min (control, N = 7; infected, N = 7), 30 min (control, N = 5; infected, N = 9), or 60 min (control, N = 7; infected, N = 6).

At the end of every trial, fish fork length (FL; to the nearest 0.1 cm) and mass (M; to the nearest 0.1 g) were recorded. We calculated condition factor (CF) using the equation $CF = (M/FL^3)\cdot 100$ (McCormick and Naiman 1984).

We also measured gill Na^+/K^+-ATPase activity on crude gill homogenates as described by McCormick (1993). Following death of the fish by a blow to the head, gills were dissected, immediately frozen in liquid nitrogen, and subsequently stored at –70 °C. ATPase activity was normalized to total homogenate (protein) (measured using the bicinchoninic acid method with bovine serum albumin standards; Sigma-Aldrich). All samples were run in triplicate. Ouabain-sensitive ATPase activity is expressed as micromoles of ADP per milligram of protein per hour.

Statistical analyses

We calculated the instantaneous intake rate of individual fish at the time of departure from the foraging habitat (QHR) following the same procedure as that of Webster and Dill (2007). This procedure involved plotting the number of eggs eaten (Y) against time spent foraging (t) during intake-rate trials and fitting a curve to the data. The curve that best described the data was of the form

$$(1) \qquad Y = a(1 - e^{-bt})$$

where a represents the maximum number of eggs available to be eaten (in this case 100), and b is the shape constant. Because intake rate differed between experimental fish, individual values of b needed to be determined to calculate QHR. This was done by fitting eq. 1 to each fish's data on time in the alternate habitat and eggs eaten (T, R). The QHR of each individual was then calculated by evaluating the first derivative (dY/dt) of eq. 1 for that fish at the time of departure from the alternate habitat.

Multiple regression was used to determine if lice density or lice load was correlated with fork length or condition factor. Because of the high correlation between body mass and fork length (R^2 = 0.948), body mass was excluded from all analyses. A χ^2 test was used to determine if sea lice infection affected salinity preferences, and a nonparametric Kruskal–Wallis test was used to determine if the probability of foraging during a trial differed as a result of infection. Lastly, a general linear model (GLM) was used to determine if the frequency of leaping and rolling behaviour was affected by infection state (control versus infected) and (or) DPI and to determine if QHR was affected by salinity preference, fork length, condition factor, Na^+/K^+-ATPase activity, and (or) lice density.

Results

Risk of infection

At 2 DPI, all exposed salmon were infected with an average of 8.4 ± 0.6 standard error (SE) lice per fish, but none had skin lesions. Sea lice density decreased with fork length (R^2 = 0.244, $F_{[2,47]}$ = 7.567, P = 0.001) but was not correlated with condition factor (P = 0.317). There was no rela-

tionship between lice load and fork length or condition factor ($F_{[2,47]}$ = 1.117, P = 0.336).

At 14 DPI, only 12 fish remained infected (N = 47), resulting in an average of 0.6 ± 0.2 SE lice per fish. However, nine of the fish that still hosted sea lice had skin lesions, and 13 of the fish that no longer hosted sea lice had skin lesions, with a group average of 0.8 ± 0.2 SE lesions per fish. Lice load (R^2 = 0.348, $F_{[2,44]}$ = 11.734, P < 0.001) and density (R^2 = 0.410, $F_{[2,44]}$ = 15.279, P < 0.001) were both negatively correlated with fork length. There was no relationship between condition factor and lice load or density (P = 0.768 and P = 0.843, respectively).

Leaping and rolling

Lice were present on all exposed fish (mean 12.2 lice per fish) at the start of leaping and rolling trials. By 14 DPI, prevalence (proportion of fish infested with sea lice) had declined to 40%, with an average of 2.2 lice per fish. Consistent with the results from the "risk of infection" trials, density of sea lice was negatively correlated with fork length (R^2 = 0.292, $F_{[2,33]}$ = 6.798, P = 0.003). There was no relationship between lice load and fork length (R^2 = 0.142, $F_{[2,33]}$ = 2.735, P = 0.080).

The frequency of rolling behaviour (rolls per fish per hour) was greater for infected fish than for control fish ($F_{[1,24]}$ = 5.293, P = 0.030) and decreased significantly with increasing DPI ($F_{[1,24]}$ = 5.486, P = 0.028). However, there was no interaction between infection state and DPI ($F_{[1,24]}$ = 3.531, P = 0.072). Leaping behaviour was also significantly more common in infected fish than in control fish ($F_{[1,24]}$ = 46.802, P < 0.001; Fig. 1). The frequency of leaping behaviour (leaps per fish per hour) decreased with increasing DPI ($F_{[1,24]}$ = 27.183, P < 0.001; Fig. 1) but decreased at a greater rate among infected fish (F value for DPI interaction is $F_{[1,24]}$ = 18.968, P < 0.001;).

Energetic costs and salinity preference

The assumption that foragers experience a steadily diminishing intake rate was met. The curve that best fit the data was an exponential rise to an asymptote with the constant b equal to 0.036 ± 0.007 SE (R^2 = 0.757) for control fish and 0.034 ± 0.006 SE (R^2 = 0.647) for infected fish; b did not differ significantly between control and infected fish ($F_{[1,50]}$ = 0.337, P = 564).

In trial 1, control fish were significantly more likely to forage during a trial than were infected fish (X^2 = 5.629, P = 0.022); however, there was no difference in trial 2 (X^2 = 0.149, P = 0.699).

When given a choice between two habitats of different salinity, infected fish preferred the freshwater habitat (trial 1, χ^2 = 4.568, P = 0.033; trial 2, χ^2 = 4.000, P = 0.046; Fig. 2), whereas control fish preferred the saltwater habitat (trial 1, χ^2 = 4.5, P = 0.034; trial 2, χ^2 = 9.529, P = 0.002; Fig. 2). The energetic cost (QHR) to forage in the alternate habitat differed between trials ($F_{[7,68]}$ = 8.533, P < 0.001), but there was no effect of infection (Fig. 3). The cost for saltwater-preferring fish to forage in freshwater was 78% greater for fish in trial 2 than for fish in trial 1.

In trial 1, the GLM analysis showed that the cost for freshwater-preferring fish to forage in saltwater increased with lice density and the cost for saltwater-preferring fish to forage in freshwater decreased with lice density ($F_{[1,16]}$ = 5.429, P = 0.034; Fig. 4*a*). In trial 2, QHR was significantly greater for saltwater-preferring fish foraging in freshwater than for freshwater-preferring fish foraging in saltwater ($F_{[1,11]}$ = 8.969, P = 0.012; Fig. 4*b*), but there was no relationship between lice density and QHR ($F_{[1,11]}$ = 1.698, P = 0.427).

The analysis of variance (ANOVA) on gill Na^+/K^+-ATPase activity revealed no significant variation within trials (trial 1, $F_{[3,59]}$ = 0.583, P = 0.629; trial 2, $F_{[3,42]}$ = 1.012, P = 0.397; Fig. 5). However, fish in trial 1 had lower Na^+/K^+-ATPase activity than fish in trial 2 ($F_{[1,108]}$ = 33.656, P < 0.001).

Fig. 1. Leaping frequency of sea lice infected (□) and control (▲) pink salmon (*Oncorhynchus gorbuscha*) fry as a function of days after infection. Lines give expected values estimated from a general linear model, total R^2 = 0.841.

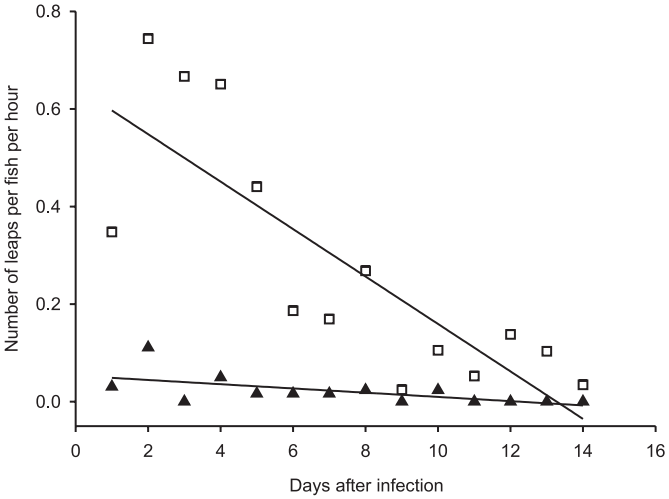
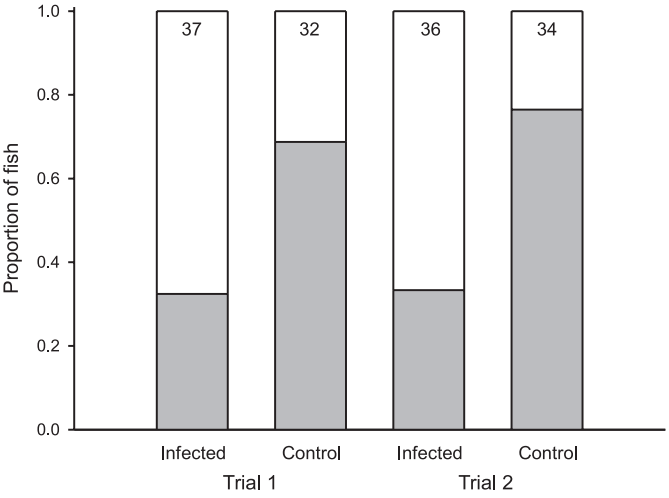


Fig. 2. Proportion of sea lice infected and noninfected pink salmon (*Oncorhynchus gorbuscha*) selecting freshwater (open bars) versus saltwater (shaded bars). Numbers give sample size.



Discussion

In recent years there have been a number of reports regarding the potential negative impact of sea lice infestation

Fig. 3. Variation in quitting harvest rate (QHR) in the alternate, less preferred habitat as a function of trial, salinity preference, and sea lice infection. Shaded bars represent saltwater-preferring fish; open bars represent freshwater-preferring fish. Different letters denote significant differences ($P < 0.020$); 95% confidence intervals are shown.

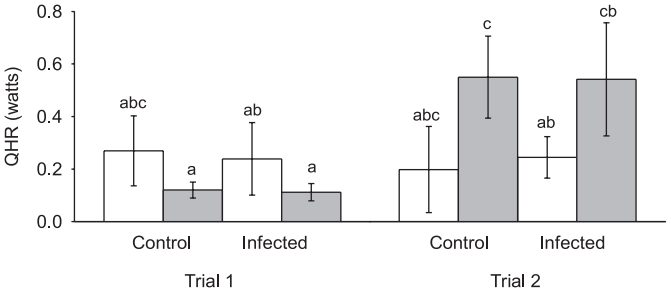
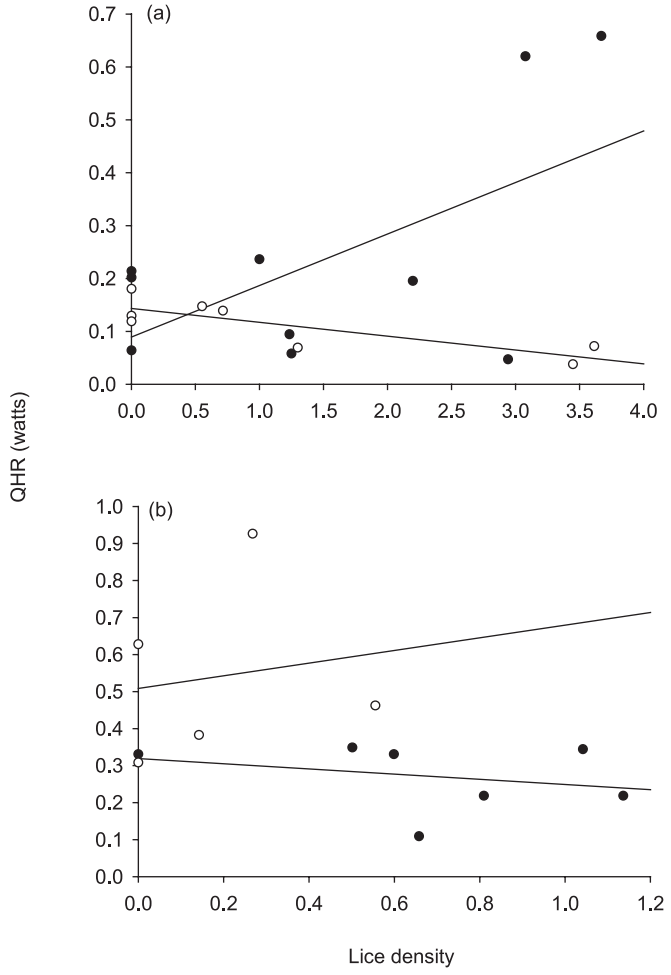
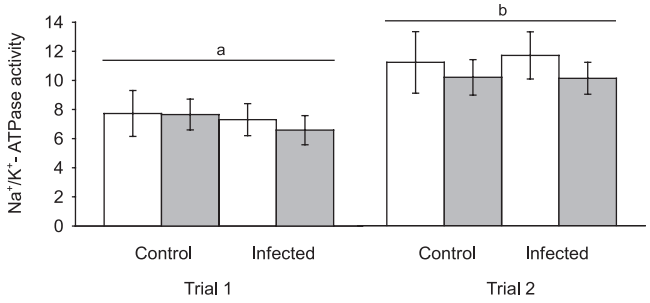


Fig. 4. Relationship between quitting harvest rate (QHR) and lice density for saltwater-preferring fish foraging in freshwater (○) and freshwater-preferring fish foraging in saltwater (●) during (*a*) trial 1 (13–33 days after infection) and (*b*) trial 2 (6–22 days after infection). Lines are best-fit lines for ○ and ● as predicted from a general linear model.



on juvenile salmonids (Pike and Wadsworth 2000; Morton and Williams 2003; Morton et al. 2004). However, few studies have directly assessed the effect of sea lice infestation on

Fig. 5. Mean (±95% confidence interval) gill Na^+/K^+-ATPase activity as a function of salinity preference and sea lice infection. Shaded bars represent saltwater-preferring fish; open bars represent freshwater-preferring fish. Within-trial variation in Na^+/K^+-ATPase activity was not significant, but activity was greater overall in trial 2 than in trial 1.



juvenile salmonid behaviour and those that have, have used Atlantic salmon (Grimnes and Jakobsen 1996) and sea trout (Birkeland and Jakobsen 1997). Using controlled laboratory experiments, we have demonstrated that sea lice infestation alters the behaviour of juvenile pink salmon in a number of ways.

Infestation changes the salinity preference of juvenile pink salmon from saltwater to freshwater. In addition, in trials run 13–33 DPI (trial 1), density of sea lice was positively related to the cost for these freshwater-preferring fish to forage in saltwater. There was no relationship between lice density and energetic costs in trials run 6–22 DPI (trial 2), possibly because few lice had reached the pre-adult stage at which they are known to affect osmotic balance (Grimnes and Jakobsen 1996). Pre-adult lice were present in trial 1.

The freshwater preference of infected fish is consistent with field (Tully et al. 1993; Birkeland 1996; Bjorn et al. 2001) and experimental (Birkeland and Jakobsen 1997) observations of a premature return of infected sea trout to rivers and estuaries. Premature freshwater return is hypothesised to be an attempt to regain osmotic and ionic balance and (or) to remove sea lice. A number of studies have demonstrated a relationship between infection by pre-adult stage sea lice and osmotic costs resulting from increased Na^+/K^+-ATPase activity and eventually osmotic breakdown (Grimnes and Jakobsen 1996; Bjorn and Finstad 1997; Finstad et al. 2000). In the present study we found no difference in Na^+/K^+-ATPase activity between control and infected fish, possibly because infection levels during our trials were not sufficiently high. Nolan et al. (1999) found that Atlantic salmon had to be infected with a minimum of 10 pre-adult and adult lice for a change in Na^+/K^+-ATPase activity to be observed 10 DPI. However, Nolan et al. (1999) used significantly larger fish than we did in the study. The fact that we found no difference in Na^+/K^+-ATPase activity indicates that the role of this enzyme in maintaining homeostasis remained relatively intact and, therefore, was not associated with the freshwater preference we observed in infected fish.

The freshwater preference that was observed, however, may still be related to ion regulation. Early stages of sea lice infestation have been reported to result in a primary stress response (Grimnes and Jakobsen 1996), which has been linked to an increase in catecholamines (Wendelaar Bonga

1997). An increase in catecholamines can cause increased permeability of tight junctions in the gills and thus an increase in ion diffusion rates (Tully and Nolan 2002). A relationship between stress and avoidance of saltwater has been observed in salmon following exposure to toxins (Kruzynski and Birtwell 1994), handling (Price and Schreck 2003*a*), and pathogens (Price and Schreck 2003*b*). In addition, the affinity for saltwater decreases proportionally with the persistence or severity of the stressor (Price and Schreck 2003*a*, 2003*b*). This is consistent with our observation that the energetic cost paid by freshwater-preferring fish foraging in saltwater 13–33 DPI increased with lice density.

The freshwater preference of infected fish may be an attempt by the fish to remove the lice; however, it is unlikely that this is the only reason. Infected fish with lice and without (fish whose lice had already fallen off) exhibited a preference for freshwater, providing evidence against the hypothesis that the shift to freshwater is made in an attempt to remove lice. However, it is unknown whether or not the fish that were no longer infected at the end of the trials were infected at the time of entry into the freshwater habitat. Therefore, it is possible that the fish entered freshwater to remove sea lice and remained in freshwater because of osmotic and ionic benefits.

Fish may also attempt to remove lice by leaping. We found that the frequency of leaping was 14 times greater in infected fish than in control fish. This observation is consistent with the observation in net pens that fish infested with sea lice demonstrate increased levels of leaping behaviour relative to uninfected fish (Furevik et al. 1993). This behaviour is likely energetically costly and may result in increased risk of predation by aerial predators. Therefore, to be occurring with such high frequency, it must also be providing infected fish with a significant benefit. Determining if this benefit is related to lice removal requires further study.

Though Na^+/K^+-ATPase activity did not differ between infected and uninfected fish, fish in trial 2 did have higher Na^+/K^+-ATPase activity than fish in trial 1. This is not surprising as Na^+/K^+-ATPase activity increases as a function of saltwater residency (Hoar 1988; McCormick et al. 1989), and before starting trial 2, fish had been in saltwater for 45 days longer than fish in trial 1. This difference in Na^+/K^+-ATPase is likely the reason why the energetic cost paid by saltwater-preferring fish foraging in freshwater was significantly higher (78%) for fish in trial 2 than for fish in trial 1 (Webster and Dill 2006).

The abiotic conditions and the prevalence and density of sea lice per fish used in this study were representative of what has been observed in the wild (Morton et al. 2005). Under these conditions, we found that at 2 DPI, there was no relationship between lice load and body size, but at 14 DPI, smaller fish hosted a greater lice load than did larger fish. This finding suggests that smaller fish may be less able than larger fish to rid themselves of lice. We also observed that during trials in which fish were infected with mobile chalimus- and pre-adult-stage lice (trial 1), they were significantly less likely to forage than uninfected fish, but this pattern was not observed in fish infected with copepodid and chalimus-stage lice (trial 2). This finding is consistent with a previous study reporting a decrease in feeding by Atlantic salmon when the louse was in its motile, premating stage (Dawson et al. 1999) and with numerous other studies of the effects of parasites on host foraging behaviour (e.g., Smith and Smilowitz 1976; Schreck et al. 1997; Danyk et al. 2005).

The long-term effect of sea lice infestation on salmon populations is unknown. However, the behavioural changes reported in this study suggest that even relatively low levels of sea lice infestation have the potential to impact a fish's fitness. Infection may result in an increase in time spent at the water surface owing to both increased jumping behaviour and freshwater-preferring fish staying closer to the surface in stratified habitats. This increase in time at the surface may increase predation risk (Kramer 1983; Collis et al. 2001). In addition, an increase in the time spent in freshwater habitats reduces time available to forage in the more productive saltwater habitats, possibly reducing growth rates (Mortensen et al. 2000). A reduced growth rate of juveniles may result in an increase in the time that they are vulnerable to size-selective predators (Holtby et al. 1990; Sogard 1997). Also, if fish cannot make up this lost growth, their body size at the time of spawning will be smaller and their fitness may be still further reduced (Beacham and Murray 1993; Dickerson et al. 2002).

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