Sea lice infection of juvenile pink salmon (*Oncorhynchus gorbuscha*): effects on swimming performance and postexercise ion balance

L. Nendick, M. Sackville, S. Tang, C.J. Brauner, and A.P. Farrell

Abstract: Sea lice (*Lepeophtheirus salmonis*) infection negatively affected swimming performance and postswim body ion concentrations of juvenile pink salmon (*Oncorhynchus gorbuscha*) at a 0.34 g average body mass but not at 1.1 g. Maximum swimming velocity (U_{max}) was measured on over 350 individual pink salmon (0.2–3.0 g), two-thirds of which had a sea lice infection varying in intensity (one to three sea lice per fish) and life stage (chalimus 1 to preadult). For fish averaging 0.34 g (caught in a nearby river free of sea lice and transferred to seawater before being experimentally infected), the significant reduction in U_{max} was dependent on sea lice life stage, not intensity, and U_{max} decreased only after the chalimus 2 life stage. Experimental infections also significantly elevated postswim whole body concentrations of sodium (by 23%–28%) and chloride (by 22%–32%), but independent of sea lice developmental stage or infection intensity. For fish averaging 1.1 g (captured in seawater with existing sea lice), the presence of sea lice had no significant effect on either U_{max} or postswim whole body ions. Thus, a single *L. salmonis* impacted swimming performance and postswim whole body ions of only the smallest pink salmon and with a sea louse stage of chalimus 3 or greater.

Résumé : Les infections à poux de mer *Lepeophtheirus salmonis* affectent négativement la performance de nage et la concentration corporelle d'ions après la nage chez les jeunes saumons roses (*Oncorhynchus gorbuscha*) à une masse corporelle moyenne de 0,34 g, mais non à 1,1 g. Nous avons mesuré la vitesse maximale de nage (U_{max}) chez 350 saumons roses individuels (0,2–3,0 g) dont les deux-tiers portaient des infections à poux de mer d'intensité variable (un à trois poux par poisson) et de stades différents du cycle biologique (larve chalimus 1 à pré-adulte). Chez les poissons de masse moyenne de 0,34 g (prélevés dans une rivière adjacente, libres de poux de mer et transférés en eau de mer avant d'être infectés expérimentalement), la réduction significative de U_{max} dépend du stade du cycle biologique des poux de mer et non de l'intensité de l'infection; elle commence à diminuer seulement après le stade chalimus 2. Les infections expérimentales accroissent aussi significativement les concentrations de sodium (de 23–28 %) et de chlorures (de 22–32 %) du corps entier après la nage, mais indépendamment du stade de développement des poux de mer et de l'intensité de l'infection. Chez les poissons de masse moyenne de 1,1 g (capturés en mer déjà infectés de poux de mer), la présence de poux de mer n'a aucun effet significatif sur $U_{max,}$ ni sur les ions corporels totaux après la nage. Ainsi, un seul *L. salmonis* affecte la performance de nage et les ions corporels totaux après la nage de seulement les plus petits saumons roses et seulement si le pou est au stade chalimus 3 ou un stade plus avancé.

[Traduit par la Rédaction]

Introduction

Juvenile pink salmon (*Oncorhynchus gorbuscha*) move into seawater (SW) soon after emergence from gravel and begin their ocean migration at an especially small body size (~0.2 g) (Takagi et al. 1981; Weatherley and Gill 1995). Thus, unlike most other salmonids, pink salmon have little time to physiologically prepare for the ionoregulatory requirements of life in SW. Possibly as a result, they double their whole body ion concentrations with first entry into SW, but then progressively reduce whole body ion concentrations during the next 1–3 months in association with a tripling of gill Na^+,K^+-ATPase enzyme activity (Grant et al. 2009), a life history pattern very different from most other Pacific salmon.

An additional ionic challenge for juvenile pink salmon may occur when they are infected with sea lice *Lepeophtheirus salmonis*, which feed on the mucus, skin, and blood of their host (Jones et al. 2006; Jones and Hargreaves 2007) and disrupts ionic balance in other salmonid hosts (Bjørn and Finstad 1997; Wagner et al. 2003; Wells et al. 2006). It has been suggested that juvenile wild pink salmon become

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infected with *L. salmonis* at an intensity and prevalence previously unseen when they migrate past aquaculture sites containing infected Atlantic salmon (Morton et al. 2004). In fact, the concern over this parasite is such that sea lice infections to juvenile wild pink salmon populations have been regularly monitored for nearly a decade in the Broughton Archipelago, British Columbia, Canada, an area of active aquaculture (Krkošek et al. 2006; Jones and Hargreaves 2007). From a reported high of 90% prevalence in 2002, sea lice prevalence on juvenile pink salmon has declined to around 10% (Jones and Hargreaves 2009), an infection level that is still likely higher than background (Gottesfeld et al. 2009).

At a high intensity (the number of sea lice per fish), *L. salmonis* can kill its host. For example, two motile sea lice reportedly kill juvenile pink salmon (body mass not given but likely <3 g; Krkošek et al. 2006). Moreover, low sea lice intensities cause sublethal behavioural, immunological, and physiological impacts (Johnson and Fast 2004; Wagner et al. 2008). Sea lice intensity, the developmental stage of the sea louse, and the fish size all factor into such impacts. For example, when pink salmon have grown to 0.7 g, they become more tolerant of sea lice (Jones et al. 2008). Therefore, any assessment of sea lice impact on fish, such as the one reported here, must necessarily consider combinations of fish size, as well as sea lice intensity and developmental stage.

In terms of sublethal effects of *L. salmonis*, impairment to swimming performance (Wagner et al. 2003) and ionic homeostasis (Wootten et al. 1982; Grimnes and Jakobsen 1996; Bjørn and Finstad 1997) stand out as being likely to reduce salmonid fitness in nature. Yet, we know nothing concerning such sublethal effects on juvenile pink salmon when they are at their smallest in SW and possibly most sensitive to additional stressors. Therefore, the purpose of the present study was to test the hypothesis that sea lice, by damaging the skin of small pink salmon, disrupt ionic homeostasis and impair maximum swimming speed. To this end, swimming ability and ionic homeostasis were determined for the first time in 0.2–3.0 g pink salmon infected with *L. salmonis* at different levels of lice intensity (one to three lice per fish) and with different developmental life stages of sea lice (chalimus 1 through to preadult). Maximum swimming velocity (U_{max}) was individually assessed with two constant acceleration tests separated by a 30 min recovery period. Ionic homeostasis was assessed by measuring postswim whole body concentrations of sodium ($[Na^+]$) and chloride ($[Cl^-]$).

Materials and methods

River-caught (RC) fish

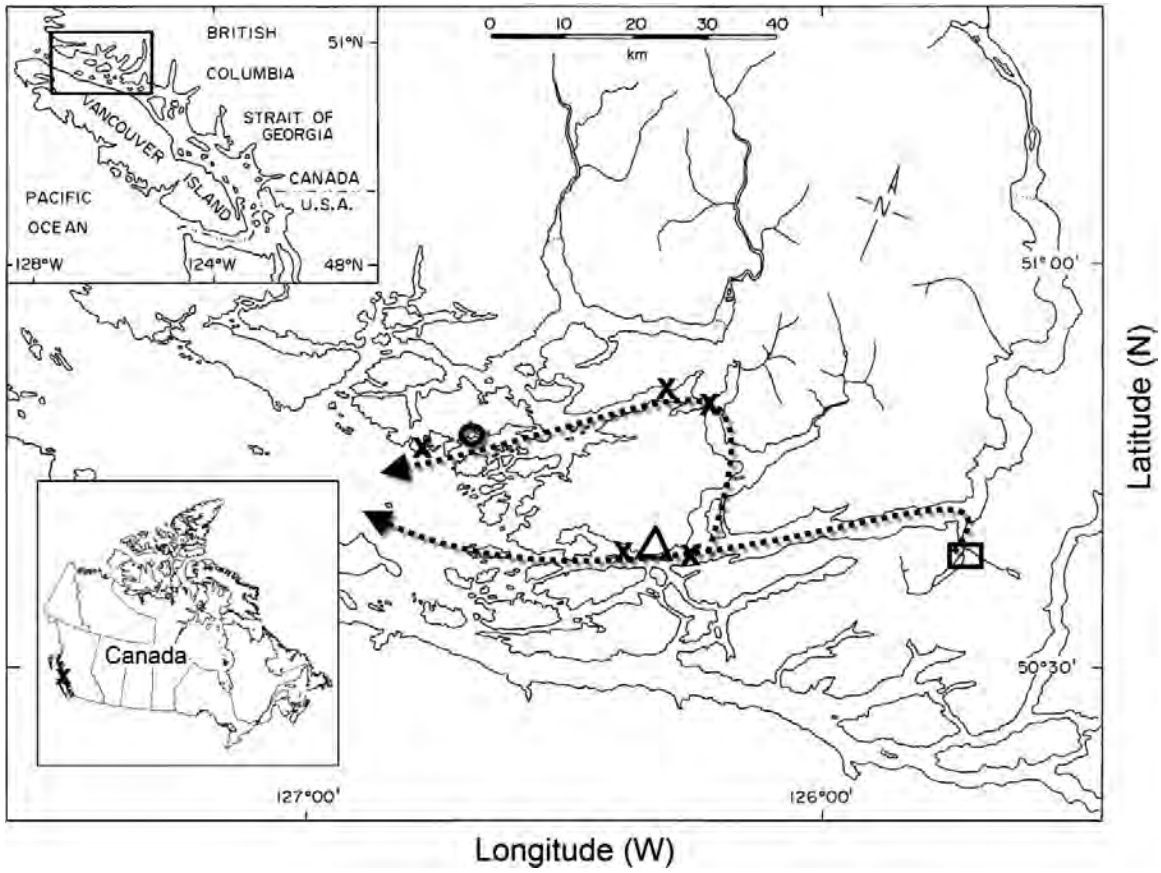
The experiments were conducted at a field laboratory constructed on a float located at Dr. Islets, Knight Inlet, a major migratory corridor for pink salmon in the Broughton Archipelago (Fig. 1). Approximately 2000 river-caught (RC) juvenile pink salmon were obtained locally on 27 March from an enumeration screw trap while actively migrating down the Glendale River, which drains into Knight Inlet (Fig. 1). Many of these fish were used in a companion study. The Glendale stock of pink salmon is the largest contributor to the Broughton Archipelago pink salmon population, contributing 89% of the odd-year cycle and 39% of the even-year cycle (Brooks and Jones 2008). Collection from a freshwater (FW) site ensured the fish would be free of *L. salmonis* (White 1940) and the controlled experimental infection with *L. salmonis* would be a novel challenge. Fish were transported in FW by boat to the field laboratory where they were introduced to SW over a 12 h period as salinity was gradually increased at 7.0 °C. Fish were then fed up to twice daily with a commercial feed (Bio-Vita starter feed; Bio-Oregon, Longview, Washington) in 60 L tanks, receiving flow-through, aerated, SW (32–34 ppt, 7.0 to 8.5 °C) pumped from 30 m below the surface.

Gravid sea lice collection and experimental infection of pink salmon

Gravid adult *L. salmonis* were collected during a harvest of adult Atlantic salmon (*Salmo salar* L.) at Wicklow Point fish farm on 24 March 2008 (Fig. 1) and transported in aerated SW to Dr. Islets, where egg strings were removed. Groups of eggs were established in 4 L closed vessels containing aerated SW according to the egg maturity, and the progression from hatching, through the naupliar stage to the infectious copepodid stage, was monitored daily using a dissecting scope. Once the infectious copepodid stage became dominant, experimental infections were carried out (3 and 13 April) by exposing RC fish for 4 h to ~23 copepodids·fish^{-1} in a static infection bath containing 2 L of SW. Of the 581 fish exposed to sea lice, 511 were successfully infected (88% prevalence). They were then allowed to recover for 24 h before being separated according to sea lice intensity (one, two, three, and four or more) into holding aquaria outfitted with flow-through SW (as described above). Some of these fish were used in a companion study. Sea lice intensity and development were monitored daily so that swim tests could be performed at predetermined sea lice development stages (day postinfection, DPI). Only fish with an attached sea lice intensity of one to three were tested. However, because the fish shed some or all their sea lice, for example 159 fish had lost all their sea lice by 14 DPI, fish were reassigned according to sea lice intensity at 14 DPI and some fish that previously had had four or more sea lice were moved into the appropriate container for fish with one to three sea lice. The ability of juvenile salmon to readily shed *L. salmonis* in a laboratory environment is well established (Connors et al. 2008; Jones et al. 2008; Morton et al. 2008). Here we report sea lice intensity (from one to three) and development stage (chalimus 1 (the first attached lice stage) to preadult 1 (the first motile lice stage)) recorded at the time of the swim test. Given that most RC fish started with a higher infection intensity than that reported, which was especially true for tests with more advanced stages of sea lice where fish had a longer period to shed the sea lice, the impacts reported here consistently under-represent the historic sea lice intensity of the individual.

Sham-treated and untreated RC fish were similarly maintained and tested. Sham-treated fish (n = 150) were subject to the same infection protocol as described above, but the infection bath contained no sea lice (SW filtered through 60 μm nitex mesh). Untreated fish (n = 30) were not exposed to any kind of protocol.

Fig. 1. Broughton Archipelago showing potential outward migration routes of juvenile pink salmon (dashed line). Dr. Islets field station (triangle), lice harvest site (Wicklow Point fish farm, open circle), freshwater fish collection site (Glendale River, square), and seawater fish collection sites (crosses).



Swimming test

A total of 147 RC fish (mean mass = 0.34 ± 0.02 g) were subjected to a repeated maximum swimming (U_{max}) performance test (Nendick et al. 2009). These tests were conducted using duplicate Blazka-type (Blazka 1960) swim tunnels (26.4 mm in diameter and 100.0 mm in length; mini-swim tunnel, Loligo Systems, Denmark), which allowed up to six fish to be individually tested daily, consisting of at least one control fish, and a fish at each of the three sea lice intensities. After light anaesthesia (0.05 g·L^{-1} MS222 (tricaine methane sulphonate), Sigma Aldrich, Sigmaaldrich.com), fish were visually assessed for lice intensity and developmental stage using a dissecting scope as necessary and transferred to the swim tunnel. They recovered within 1 min and were left for a further 5–10 min before the water velocity was increased from 0 to 1.7 cm·s^{-1}. At this water velocity, fish oriented themselves into the current, swam with minimal effort, and were left for a further 60 min. The repeated U_{max} test involved accelerating the water at a rate of 0.05 cm·s^{-2} in 1.4 cm·s^{-1} increments every 30 s until the fish fatigued. Fatigue was defined as the time when fish rested with its caudal fin on the posterior grid and did not move when lightly prodded. U_{max} (BL·s^{-1}) was calculated according to Nendick et al. (2009). After fatigue, fish were allowed to recover at a velocity of 1.7 cm·s^{-1} for just 30 min before the swim test was repeated using an identical protocol. The recovery ratio (Jain et al. 1998; Tierney and Farrell 2004) was determined by expressing the second U_{max} value as a ratio of the first U_{max} value. Water velocity (cm·s^{-1}) was calibrated using frame-by-frame video analysis (30 frames·s^{-1}) of neutrally buoyant particles moving through the swim chamber. The cross-sectional area of the fish was <10% of the cross-sectional area of the swim tunnel, and consequently the solid blocking effect of the fish was not calculated (Bell and Terhune 1970). Average water temperature was controlled to within 1.0 °C of the ambient water temperature.

Swimming ability was tested over a period of 36 days on a total of 147 RC fish, of which 83 fish were infected with sea lice that had developed up to the preadult (DPI 26–28). It was not possible to test more advanced sea lice maturity because shedding made it impossible to maintain a sufficient number of infected fish. Furthermore, to increase statistical power, two fish infected with four motile sea lice were added to the group of fish infected with three sea lice. There was no significant difference between sham-treated and untreated RC fish for either U_{max} (5.38 ± 0.13 and 5.05 ± 0.11 BL·s^{-1}, respectively) or recovery ratio (0.96 ± 0.02 and 1.00 ± 0.03, respectively). Therefore, the results for these fish were pooled to create a larger control group and increase the statistical power of comparisons with infected fish.

Body ion analyses

After the second U_{max} test, fish were removed and euthanized in 0.2 g·L^{-1} MS222 before sea lice were removed to as-

sess their developmental stage according to Johnson and Albright (1991). Fish were weighed, rinsed in FW, patted dry, and fork length (BL) was measured. The body was wrapped in tinfoil and frozen in liquid nitrogen until later analysis, at which time the fish was thawed, the head was removed, and the remaining tissue was processed according to Grant et al. (2009). Whole body [Cl^-] was measured using a digital chloridometer (Haake Buchler Instruments Inc., Saddlebrook, New Jersey, USA), and whole body [Na^+] was measured using a flame atomic absorption spectrometer (Spectra AA; Varian, Victoria, Australia).

Ocean-caught (OC) fish

Experiments with larger fish (up to 3.0 g) were necessarily performed with wild, ocean-caught (OC) juvenile pink salmon with or without a pre-existing *L. salmonis* infection. These fish were collected, as needed, from the nearby shoreline by beach or purse seine throughout April, May, and June (Fig. 1). Their sea lice infection history prior to capture was unknown. Controlled sea lice infections with RC fish had to be abandoned in the absence of a reliable source for gravid sea lice, in part because of pink salmon shedding so many sea lice and OC fish having such a low sea lice prevalence and intensity. Sea lice prevalence averaged ~10% among >10 000 OC fish, with a sea lice intensity of ~1. Therefore, OC fish were graded for sea lice immediately after capture and excess uninfected fish were released. OC fish were transported in aerated SW to the field laboratory, where they were held for at least 16 h prior to further handling and sorting for sea lice intensity and development stage but used within 7 days for experiments. Sea lice infection intensity decreased during holding, and consequently sea lice intensity at the time of the swimming test is reported. The repeated U_{max} swimming tests were performed over a 49-day period using 214 OC fish (mean mass = 1.14 ± 0.05 g), of which 165 were infected with one to tree sea lice. Six fish infected with four motile sea lice were pooled with those infected with three motile sea lice. To further increase statistical power, louse developmental stages were grouped. Chalimus 1 and 2 were grouped and are referred to as ''early chalimus'', chalimus 3 and 4 were grouped and are referred to as ''late chalimus'', and preadult and adult lice were grouped and are referred to as ''motile''.

Statistical analysis

Values are reported as mean values ± standard error of the mean (SEM). Data for OC fish and RC fish were analysed separately even though their range of body mass overlapped. Body length and mass, whole body ion concentrations, and U_{max} were analyzed for statistically significant differences resulting from sea lice infections using a one-way analysis of variance (ANOVA) with Holm–Sidak post hoc test ($P < 0.05$). Recovery ratio was analysed for statistical significance using a *t* test. Because there were no significant differences between the first and second U_{max} values (i.e., a recovery ratio not different compared with 1.0; $P > 0.05$), quantitative comparisons used the second U_{max}. A linear model was designed using R software to examine the relative contributions of measured variables to U_{max}. Because time, fish growth, DPI (RC fish only), days in SW (RC fish only), and sea lice development were related variables, the RC fish data were presented according to sea lice development stage (i.e., chalimus 1–4 and preadult) with control fish matched according to a similar number of days in SW. The data for OC fish were grouped as a function of fish length because of their unknown infection history and time in SW.

Results

Control fish

U_{max} of control RC fish was unchanged during the month of testing ($P > 0.05$). Even so, relative swimming speed (U_{max} in $BL \cdot s^{-1}$) was dependent on body length for lice-free RC fish and OC fish (Fig. 2), as expected (Brett 1965; Brett and Glass 1973). Therefore, U_{max} for control RC fish was significantly greater than U_{max} for control OC fish ($P < 0.05$) because they were smaller in length ($P < 0.05$). Recovery ratios for control RC fish (0.98 ± 0.02) and OC fish (0.99 ± 0.02) were not significantly different from each other or from unity ($P < 0.05$), again as expected (Jain et al. 1998).

Controlled sea lice infections

RC fish grew in length from 34 to 41 mm ($P < 0.05$, Table 1) while the sea lice on them developed from chalimus 1 to preadult 1. Among the 511 fish successfully infected with sea lice, 329 fish initially had one to three sea lice per fish, and the remaining 182 fish had four or more sea lice. During the first 14 DPI and among the fish with one to three sea lice, 120 fish were used for experiments, 159 fish lost their sea lice, and 5 fish died (two fish each with one sea louse and three fish each with three sea lice). At 14 DPI, the remaining 35 infected fish were supplemented with 99 fish from the 182 fish initially with four or more sea lice (some had had up to 30 copepodids, and there had been two fish mortalities by 14 DPI). Among these fish transfers were the following: 10 fish each with one sea louse, 20 fish each with two sea lice, and 15 fish each with three sea lice). Between 14 DPI and 28 DPI, 95 fish were tested, 22 fish lost their sea lice, and 17 fish died.

Swimming performance of RC fish

RC fish infected with one chalimus 1 or 2 (at 3–13 DPI) had the same ($P > 0.05$) U_{max} as control fish (Fig. 3). However, U_{max} was significantly reduced (–20.4%) after the sea louse had developed to chalimus 3 (at 14 DPI) when compared with control fish (Fig. 3). U_{max} was significantly reduced ($P < 0.05$) by a similar amount (–26.5% and –37.9%, respectively) with subsequent life stages (one chalimus 4 or one preadult 1) when compared with controls. Therefore, the impact of one sea louse on U_{max} did not change significantly ($P > 0.05$) between chalimus 3 and preadult 1 sea lice (Fig. 3). In addition to U_{max} not decreasing significantly with each successive developmental stage, sea lice intensity had no significant effect on U_{max} beyond the effect seen with one louse per fish for all developmental stages, except for three chalimus 4 sea lice, which reduced U_{max} beyond that observed for one chalimus 4 (Fig. 3). Thus, the impact of *L. salmonis* on swimming performance of the smallest of juvenile pink salmon in SW was limited to sea louse stage

Fig. 2. Maximum swimming performance (U_{max}) of river-caught fish (open circles) and ocean-caught fish (closed circles) not infected with sea lice (control) plotted relative to fork length (L). The slope of the regression fit to both groups is $U_{max} = 0.036L + 6.55$ ($r^2 = 0.26$, $P < 0.0001$).

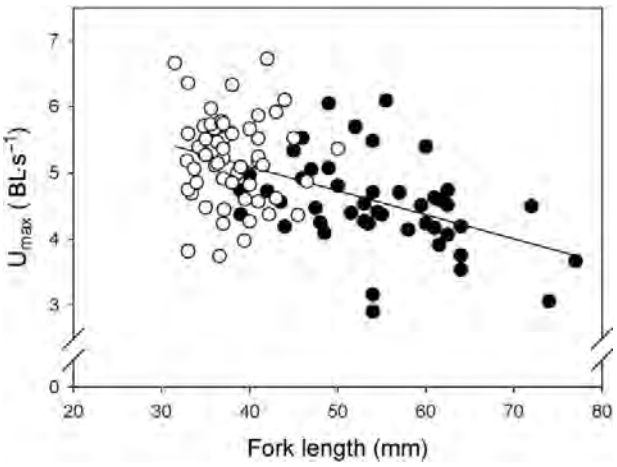


Table 1. Growth of river-caught fish following experimental infection with sea lice.

Lice development stage	DPI stage observed*	*n*	Fork length (mm ± SEM)
Chalimus 1	3	20	34.2±0.6a
Chalimus 2	7	32	35.0±0.4ab
Chalimus 3	14	29	36.4±0.7b
Chalimus 4	19	36	38.4±0.6c
Preadult 1	26	20	40.8±0.5d

Note: Dissimilar letters represent a significant difference in fork length ($P < 0.05$). SEM, standard error of the mean.

*Sea lice development stage reported as days postinfection (DPI).

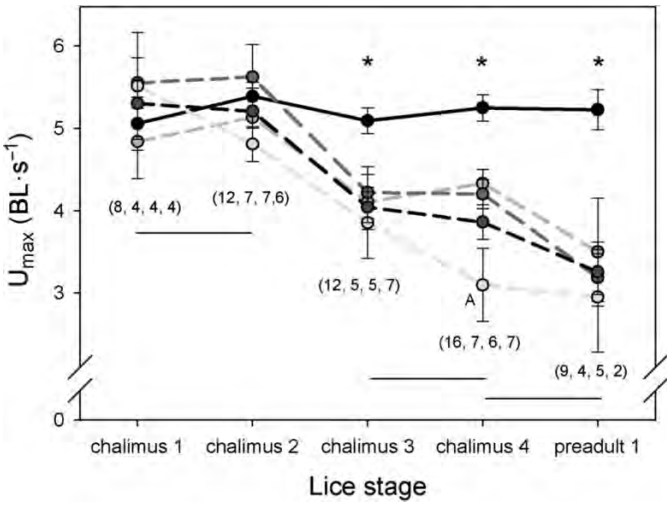
of chalimus 3 and greater and independent of sea lice intensity between one and three.

Ionic regulation of RC fish

Following the second U_{max} test, whole body [Na^+] and [Cl^-] were significantly elevated in infected RC fish compared with control ($P < 0.05$; Fig. 4). Even so, the percent disruption to whole body ions was essentially independent of the developmental stage of sea louse (23%–28% for [Na^+] and 22%–32% for [Cl^-]). The only exception was chalimus 2, which increased [Na^+] by 36%. Thus, the postswim disruption of ionic homeostasis caused by up to three sea lice was largely unrelated to the developmental stage of the sea louse.

Low statistical power prevented reliable ANOVA statistics on the [Na^+] and [Cl^-] data according to sea lice intensity and developmental stage. Instead, a linear model considered fish mass, fish length, and postswim body [Na^+] and [Cl^-], along with sea lice intensity and developmental stage as factors influencing U_{max}. Only sea lice intensity and developmental stage had statistically significant effects on U_{max}. Preadult sea lice was the only development stage that significantly ($P < 0.05$) decreased U_{max} independent of lice load, whereas chalimus 1 was the only developmental stage that had no effect on U_{max} independent of sea lice intensity ($P > 0.05$). Chalimus 2, 3, and 4 stages all significantly decreased U_{max}, depending on sea lice intensity ($P < 0.05$) (significance was reached with three sea lice for chalimus 2, and two or more sea lice for chalimus 3 and 4).

Fig. 3. Maximum swimming performance (U_{max}) of river-caught fish in the presence and absence of sea lice infection. Control fish (black solid line) and fish with sea lice infection intensity of one (light grey, dashed), two (grey), three (dark grey), and all infection intensities pooled (black dashed line). An asterisk (*) denotes significant differences between control and infected (pooled or individual) values (analysis of variance (ANOVA), $P < 0.05$), while discontinuous horizontal lines denote a difference among the lice development stages (independent of lice intensity) and an uppercase letter indicates the single difference among lice intensity for a given lice development stage. Numbers within parentheses are the number of fish used (reading left to right) for control, one louse, two lice, and three lice per fish, respectively. Values are mean ± standard error of the mean (SEM).



A potential confounding factor in the analysis of whole body [Na^+] and [Cl^-] was the previous finding that whole body [Na^+] and [Cl^-] of uninfected juvenile pink salmon naturally decreases with growth in SW (Grant et al. 2009). In the present study, this tendency was not statistically significant ($P > 0.05$) for either control or infected RC fish, perhaps because of the shorter duration of the present experiments or because samples were postswim rather than resting.

OC fish

Individual OC fish never spent more than a week at the field laboratory, but U_{max} was assessed over a 49-day period, during which the fish being captured and tested progressively increased in length. As expected, U_{max} decreased with fish length for both control and infected OC fish ($P < 0.05$; Fig. 5). Furthermore, there was no significant ($P > 0.05$) difference between U_{max} for control and infected fish (all developmental stages combined; Fig. 5). Similarly, while postswim [Na^+] and [Cl^-] also declined with fish length for both control and infected OC fish, there was no significant effect of sea lice infection when compared with control fish ($P > 0.05$; Fig. 6).

When a linear model examined the effect on U_{max} of sea lice intensity, sea lice developmental stage, fish mass, fish

Fig. 4. Postswim body [Na^+] (triangle, left *y* axis) and [Cl^-] (circle, right *y* axis) of control (black) and infected (grey) river-caught fish, plotted as a function of lice development stage. An asterisk (*) denotes significant differences between control and infected fish (analysis of variance (ANOVA), $P < 0.05$). There were no significant differences in body [Na^+] or [Cl^-] among different lice development stages ($P > 0.05$). Values are mean ± standard error of the mean (SEM).

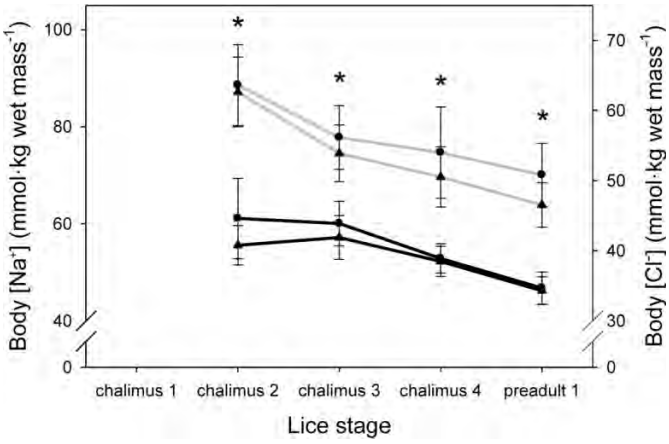
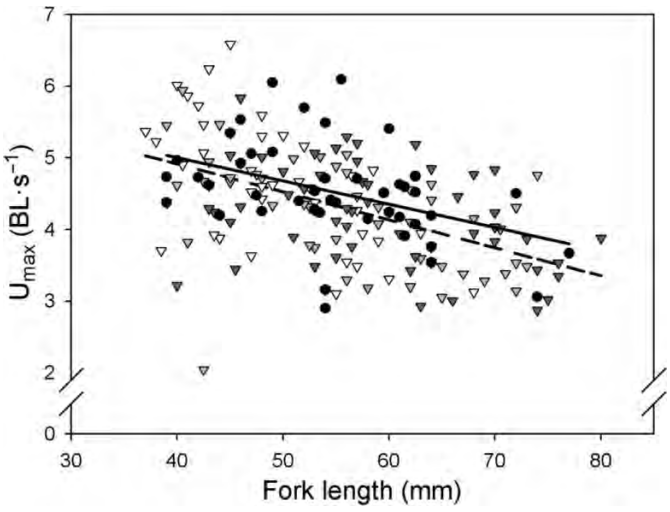


Fig. 5. Maximum swimming performance (U_{max}) of individual control ($n = 45$, black circles) and sea lice infected ($n = 165$, inverted triangles) ocean-caught fish plotted as a function of fork length (L). Sea lice infected fish are grouped by development stage: early chalimus (open), late chalimus (grey), and motile (dark grey). Regression lines of control (solid line, $U_{max} = -0.03L + 6.3$, $r^2 = 0.178$, $P < 0.001$) and infected (dashed line, $U_{max} = -0.04L + 6.5$, $r^2 = 0.261$, $P < 0.001$) ocean-caught fish are not significantly different from one another.



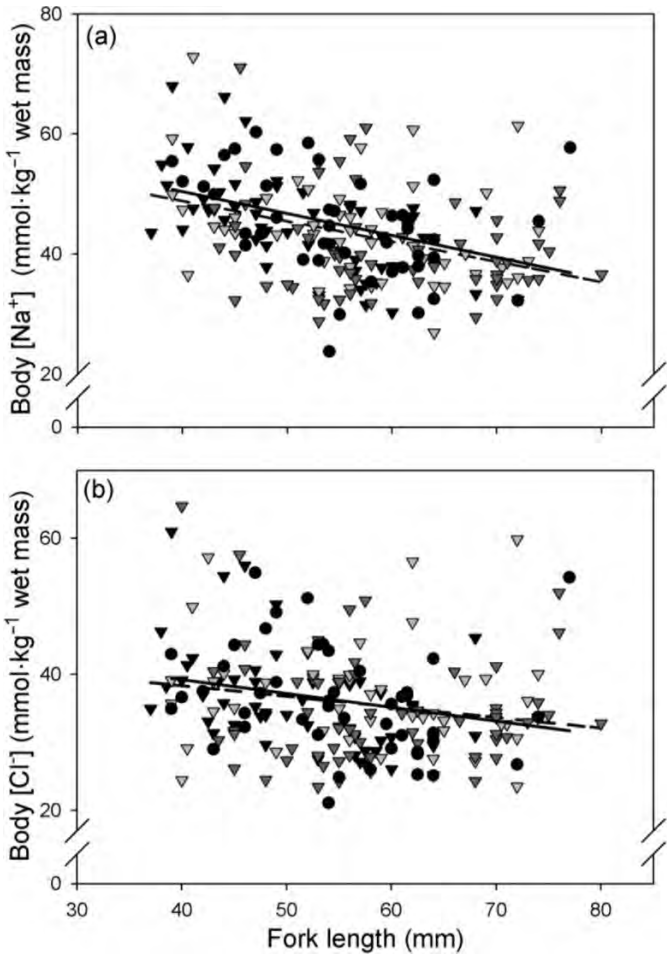
length, whole body [Na^+], and whole body [Cl^-], the only factor significantly decreasing U_{max} was three motile lice ($P < 0.05$). Curiously, fish length did not reach statistical significance ($P = 0.09$) as a contributor to U_{max} in this model, unlike the linear regression analysis.

Discussion

A single *L. salmonis* had a negative impact on swimming performance and postswim whole body ions, but this was limited to pink salmon averaging 0.34 g with a developmental stage of chalimus 3 or greater. However, no sublethal impact of sea lice was detected on pink salmon averaging 1.1 g. Furthermore, the sublethal impacts on the smaller pink salmon were not accentuated by tripling sea lice intensity, except for the chalimus 4 stage. Although mortality of infected fish in this study was limited to about 1%, there are clearly sublethal effects of infection in fish of 0.34 g.

The present results are important in the context of the controversial debate surrounding the impact of salmon aquaculture on wild salmon populations. It has been suggested that juvenile wild pink salmon in the Broughton Archipelago become infected with *L. salmonis* at an intensity and prevalence previously unseen when they migrate past aquaculture sites containing infected Atlantic salmon (Morton et al. 2004). While this suggestion has been challenged (Brooks

Fig. 6. Postswim body (*a*) [Na^+] and (*b*) [Cl^-] of control ($n = 44$, black circles) and infected ($n = 158$, inverted triangles) ocean-caught fish, plotted as a function of fish fork length (L). Sea lice infected fish are grouped by development stage: early chalimus (open), late chalimus (grey), and motile (dark grey). Regression lines of control (solid line, [Na^+] = $-0.36L + 65.0$, $r^2 = 0.138$, $P < 0.05$; [Cl^-] = $-0.202L + 47.3$, $r^2 = 0.053$, $P = 0.128$) and infected (dashed line, [Na^+] = $-0.582L + 75.2$, $r^2 = 0.34$, $P < 0.05$; [Cl^-] = $-0.394L + 56.8$, $r^2 = 0.185$, $P < 0.05$) ocean-caught fish are not significantly different from one another.



2005), mathematical modelling has further suggested that wild pink salmon populations in this region will collapse as a result of such sea lice infection pressure (Krkošek et al. 2007). But even this model output has not been without controversy, being challenged (Brooks and Jones 2008; Riddell et al. 2008) and the challenge being rebutted (Krkošek et al. 2008). Still missing in this entire debate is reliable information on sublethal effects of *L. salmonis* for the smallest, and likely the most sensitive, life stage of juvenile pink salmon. Our results suggest that if a pink salmon averaging 0.34 g carries just one chalimus 3 sea louse, then U_{max} and ionic homeostasis after exhaustive swimming will be impaired. Such an impairment could apply to about 10% of the population of juvenile pink salmon in the Broughton Archipelago, given that the current prevalence of *L. salmonis* in the region is around 10% and sea lice intensity is around 1 (Jones and Hargreaves 2009). We might expect such impacts could affect the fish's ability to swim within a school, acquire food, avoid predation, and successfully migrate to the open ocean.

We did not observe extensive mortality as a result of the controlled sea lice infections, but then infections were only monitored through to the motile stage with up to three sea lice per fish. Mortality over 14 DPI was 1.1% for fish infected with one to three sea lice and 1.0% for fish infected with four or more sea lice. Given the report that two motile sea lice kill juvenile pink salmon (Krkošek et al. 2006), we were surprised at our low fish mortality rate, especially since some of the initial sea lice infection intensities were very high (>10 sea lice for a 0.3 g fish). Other contributing factors to this low mortality rate could include the ability of pink salmon to grow rapidly and readily shed sea lice. By growing rapidly, a pink salmon entering SW could double its body mass each month (Grant et al. 2009), potentially reaching 1 g in 2 months, becoming better adapted to SW (Grant et al. 2009) and becoming far more tolerant of *L. salmonis* (Jones et al. 2006; present study). It takes about 1 month at 15 °C for *L. salmonis* to grow from an infectious copepodid to a mature adult (Johnson and Fast 2004), during which time the salmon is also growing. Shedding of sea lice by pink salmon was recently recognized in mathematical models by Krkošek et al. (2009), who reported chalimus mortality rates on salmon of between 0.0002 and 0.0257 day^{-1}. In the present study, 147 of 329 fish lost all sea lice over 14 days, and thus loss of sea lice averaged 0.07 day^{-1} assuming each fish was originally infected with an average of two sea lice; a value over two orders of magnitude higher than that reported by Krkošek et al. (2009). In nature, there is also the possibility of reinfection with sea lice, something that has only been modelled (Krkošek et al. 2009) rather than experimentally tested to our knowledge. Discovery of the smallest of pink salmon infected with a gravid sea louse in the wild would likely reflect such reinfection or stunted salmon growth.

We made various attempts not to under-report sea lice impacts in the present study. For RC fish, we knew that their initial infection intensity was typically greater than when tested, especially for the stages beyond 14 DPI when the majority of fish tested had started with four or more sea lice. Furthermore, chalimus 3 and preadult results for three sea lice per fish actually had four sea lice to increase statistical power. Also, we cannot exclude the possibility that OC fish lost sea lice during capture and transport, since neither possibility was monitored. Furthermore, fish with sea lice infections were given two swim tests to fatigue and only a short recovery period in between. While there was never any statistical difference between the two levels of swimming performance, we still reported U_{max} for the second swim test, the one more likely to be impaired by sea lice. Lastly, the expected effect of body size on both U_{max} and whole body $[Na^+]$ and $[Cl^-]$ was considered when evaluating sea lice impacts. This consideration proved to be extremely important, since we did not detect impacts on pink salmon averaging 1.1 g.

There were some unexpected findings. A priori, it was anticipated that the disruption to ionoregulation and swimming performance would be directly dependent on lice stage and on lice intensity and inversely related to fish size. However, tripling sea lice intensity up to chalimus 4 stage had no consistent additive effect on U_{max} compared with one sea louse. This result suggests that the additional drag imposed by a sea louse was not the principal reason for reducing U_{max}, otherwise each additional louse would have slowed U_{max} further, perhaps in an exponential fashion. While the potential drag effects of sea lice would necessarily be reduced on the larger (mean 1.1 g) OC fish, juvenile pink salmon also develop scales on their skin when they reach 0.7 g (Jones et al. 2008), reducing the impact to ionoregulation and swimming of parasitic feeding on the skin.

Likely then, sea lice impair U_{max} through disruption of the skin epithelial barrier and ionoregulatory homeostasis, as proposed. However, a study of whole body ions in resting fish is still needed to test this idea further. Here we measured an elevation in postswim whole body $[Na^+]$ and $[Cl^-]$, but previous studies linked an elevation of resting plasma ion levels with impaired swimming performance for juvenile coho salmon (*Oncorhynchus kisutch*) parr (Brauner et al. 1992) and smolts (Brauner et al. 1994). The concern is that when swimming in SW and without any disruption to the skin's integrity by sea lice, salmon are expected to take on ions and lose water as a result of the osmo-respiratory compromise (Sardella and Brauner 2007). Since the level of ionic disturbance for both $[Na^+]$ and $[Cl^-]$ did not change for the most part with sea lice intensity or development, it is possible that U_{max} decreases because whole body ions have reached an upper threshold level.

Other studies have previously documented effects of sea lice on both ion regulation and swimming performance in much larger salmonids. For example, artificially infected brown trout (*Salmo trutta*) smolts (90 g) had elevated plasma Cl^- levels and decreased hematocrit after the first appearance of preadult *L. salmonis* (Bjørn and Finstad 1997). Again in brown trout (19–70 g), sea lice infection significantly increased plasma Cl^-, osmolality, glucose, lactate, and cortisol while significantly reducing haematocrit (Wells et al. 2006). Likewise, adult Atlantic salmon (600 g) infected with *L. salmonis* had elevated plasma chloride level and a 20% reduction in critical swimming speed (Wagner et al. 2003). Even so, there is a danger extrapolating among fish of a different size and species. In the present study, impacts were evident in the smallest of pink salmon at a sea lice density of 3 sea lice·g^{-1}, whereas for brown trout the

threshold was 13 sea lice·g^{-1} (Wells et al. 2006) and 0.1 sea lice·g^{-1} for Atlantic salmon (Wagner et al. 2003).

In summary, in association with a disruption to postswim ionic homeostasis, U_{max} of nearly the smallest pink salmon found in SW was significantly reduced after artificial infection with a single chalimus 3 through to preadult *L. salmonis*. However, these impacts were unaffected by tripling sea lice intensity on fish averaging 0.34 g and were not present at all for fish averaging 1.1 g. It appears more likely that the impact of sea lice on U_{max} was a result of an ionoregulatory imbalance rather than a consequence of increased surface drag. Although there was very limited mortality associated with sea lice infection in this study, there were large and significant sublethal disturbances in the smallest pink salmon shortly following SW entry, indicating that this is a very sensitive stage to sea lice parasitism.

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