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ARTICLE

Sea lice, sockeye salmon, and foraging competition: lousy fish are lousy competitors

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Abstract: Pathogens threaten wildlife globally, but these impacts are not restricted to direct mortality from disease. For fish, which experience periods of extremely high mortality during their early life history, infections may primarily influence population dynamics and conservation through indirect effects on ecological processes such as competition and predation. We conducted a competitive foraging experiment using outmigrating juvenile Fraser River sockeye salmon (*Oncorhynchus nerka*) to determine whether fish with high abundances of parasitic sea lice (*Caligus clemensi* and *Lepeophtheirus salmonis*) have reduced competitive abilities when foraging. Highly infected sockeye were 20% less successful at consuming food, on average, than lightly infected fish. Competitive ability also increased with fish body size. Our results provide the first evidence that parasite exposure may have negative indirect effects on the fitness of juvenile sockeye salmon and suggest that indirect effects of pathogens may be of key importance for the conservation of marine fish.

Résumé : Les pathogènes sont une menace pour les espèces sauvages à l'échelle planétaire, mais leurs impacts ne se limitent pas à la mortalité directe découlant de maladies. Pour les poissons, caractérisés par une période de mortalité extrêmement élevée au début de leur cycle de vie, les infections pourraient influencer principalement la dynamique des populations et la conservation par le biais d'effets indirects sur des processus écologiques comme la concurrence et la prédation. Nous avons mené une expérience d'approvisionnement concurrentielle en utilisant des saumons rouges (*Oncorhynchus nerka*) juvéniles du fleuve Fraser dans leur migration vers la mer, afin de déterminer si les poissons présentant une forte abondance de poux du poisson (*Caligus clemensi* et *Lepeophtheirus salmonis*) parasitiques présentaient également une capacité concurrentielle réduite durant l'approvisionnement. Les saumons rouges très infectés avaient un taux de succès de consommation de nourriture 20 % plus faible, en moyenne, que les poissons légèrement infectés. La capacité concurrentielle augmentait également avec la taille du corps du poisson. Nos résultats sont les premiers à indiquer que l'exposition aux parasites pourrait avoir des effets négatifs indirects sur l'aptitude des saumons rouges juvéniles et donnent à penser que les effets indirects des pathogènes pourraient être d'importance capitale pour la conservation des poissons de mer. [Traduit par la Rédaction]

Introduction

Pathogens are a major threat to wildlife around the world (Dobson and Foufopoulos 2001; Smith et al. 2009). From outbreaks of protozoan parasites in wild bumble bees (Meeus et al. 2011) to viral epidemics in wild carnivores and African apes (Hofmeyr et al. 2000; Leroy et al. 2004; Origgi et al. 2012), new cases of pathogen-induced mortality and population declines continue to be identified, with few management attempts showing clear benefits of intervention (Woodroffe 1999). The emergence of diseases in many wildlife populations is attributable to anthropogenic influences that promote pathogen transmission and virulence, such as climate change, habitat loss, introduced species, pollution, and domesticated animals (Brearley et al. 2013; Daszak et al. 2000; Harvell et al. 2002). For extinction risk, pathogens are primarily a threat when a reservoir host population maintains high pathogen prevalence in a sympatric threatened host species as it declines towards extinction (De Castro and Bolker 2005; Krkošek et al. 2013).

Impacts of pathogens on their hosts go beyond direct mortality from disease. Host–parasite systems provide particularly noteworthy examples of behavioural (Carney 1969), physiological (Kristan and Hammond 2000), and morphological (Johnson et al. 1999) changes to hosts that can indirectly reduce their survival. Pathogens can modulate crucial ecological processes affecting the host, such as competition or predation (Hatcher et al. 2012), and this may be particularly important for fishes whose life histories typically include extreme mortality from these interactions (Groot and Margolis 1991; Hixon and Jones 2005). These high mortality rates of fish in the absence of pathogens make it difficult to understand the implications of pathogen infection on fish populations. Consequently, studies restricted to direct effects on survival or physiology (e.g., Jakob et al. 2013) are only tangentially relevant for fish populations because they ignore how pathogens interact with other processes that can lead to high mortality, such as competition and predation.

Salmon (*Oncorhynchus* spp. and *Salmo salar*) exemplify the complexity of fish disease ecology. Epidemics in wild fish created by parasite spill-back from farmed fish that act as reservoir hosts for ectoparasitic sea lice (*Lepeophtheirus salmonis*) can cause mortality that exceeds previous fisheries catch and may threaten persistence for some species (Krkošek et al. 2007). In other systems, impacts of parasitism do not scale up to the population level, potentially due to modulation of predation pressure (Peacock et al. 2014). Theoretically, parasitism can act synergistically or antagonistically with predation depending on where the predator–prey system lies along a type II functional response, whereby predator intake rate increases with prey density toward an asymptote

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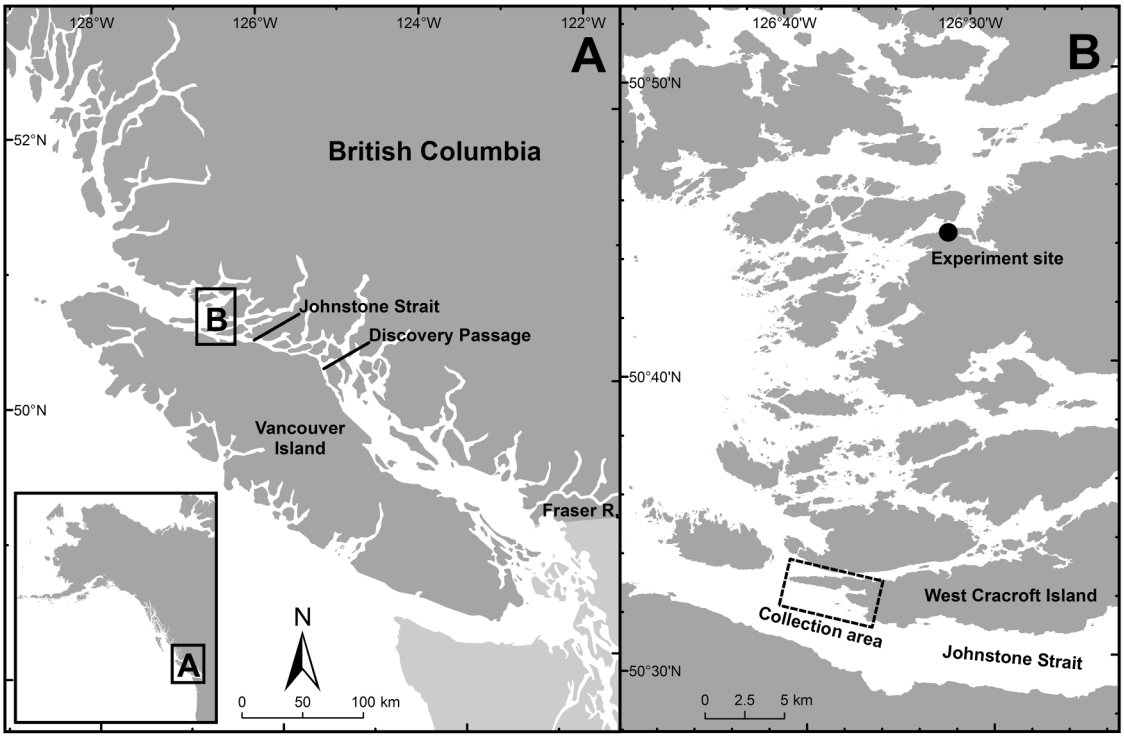
Fig. 1. Locations of the collection area and the competitive foraging experiment. Outmigrating juvenile sockeye salmon were captured in the region defined by the dashed rectangle in May and June 2013.



(Krkošek et al. 2011). Sockeye salmon (*Oncorhynchus nerka*) are of great ecological, cultural, and economic importance to the west coast of North America (Cooke et al. 2004; Eliason et al. 2011) and are therefore a strong candidate species for conservation research. Recently, the decline of Canada's iconic Fraser River sockeye stocks triggered a $26 million dollar federal judicial inquiry into the causes of the decline (Cohen 2012*a*, 2012*b*, 2012*c*), which identified disease interactions of sockeye with salmon aquaculture operations as a major management uncertainty and research priority.

Populations of domesticated salmon represent a reservoir host population of *Caligus clemensi* sea lice, which can infect wild juvenile sockeye (Price et al. 2011). Infection risk for juvenile sockeye is exacerbated by the generalist nature of the *C. clemensi*; Pacific herring (*Clupea pallasii*) can also be infected by *C. clemensi* at high abundances and are likely a second sea louse reservoir to sockeye (Beamish et al. 2009; Morton et al. 2008). However, it is not known how or whether parasite exposure fits into the long-term decline of sockeye productivity. Direct mortality of juvenile sockeye from *C. clemensi* infection has not been estimated, but it is likely very low; in a laboratory setting, *L. salmonis* infection does not cause direct mortality except at extreme abundances not seen in wild fish (Jakob et al. 2013). Because Pacific salmon experience high mortality during their early marine life (Bax 1983; Parker 1968) and early marine growth is crucial for survival (Beamish et al. 2004; Farley et al. 2007; Moss et al. 2005), any impact of *C. clemensi* on sockeye population dynamics is probably indirect, by modulating competition or predation. Here, we performed a foraging experiment to test whether the competitive ability of juvenile Fraser River sockeye differs with sea louse abundance. Competitive ability, in this case, was defined as intake during a food pulse of limited quantity and duration between hungry fish. We found that infection is associated with reduced competitive ability, whereas large body size is associated with greater competitive ability. Collectively, these results indicate that elevated parasitism, potentially due to infection from farmed salmon, may have indirect effects on survival and conservation of an iconic fish.

Materials and methods

Sea louse life cycle

Sockeye salmon on the south coast of British Columbia are infected by two species of sea louse: *L. salmonis*, a specialist on salmonids, and *C. clemensi*, a generalist on many fish species. The life cycle of the sea louse has an early free-living phase (nauplius stages), a phase in which the louse is attached to its host (copepodid and chalimus stages), and a mobile phase during which the louse is able to move around on the surface of its host (pre-adult and adult stages). The number of chalimus stages differs among louse genera. Here, we use the term "large chalimus" to describe the final two chalimus stages for *C. clemensi*, which has four chalimus stages, and the final chalimus stage for *L. salmonis*, which has two chalimus stages (Hamre et al. 2013; Kabata 1972). We use "motile" to encompass all pre-adult and adult stages of louse development. Adult lice reproduce sexually, with attached females extruding pairs of egg strings whose eggs hatch into nauplii. The generation time for *L. salmonis* is 40 to 52 days at 10 °C and shorter at warmer temperatures (Johnson and Albright 1991), and the generation time for *C. clemensi* is probably similar based on other *Caligus* species (Hogans and Trudeau 1989; Piasecki and MacKinnon 1995).

Study area and sampling design

We collected juvenile sockeye salmon in northern Johnstone Strait, British Columbia, every 2 to 9 days from 29 May to 13 June 2013 (Fig. 1) during the three and a half weeks that sockeye were observed in this region. Our collection area is a migration bottleneck for juvenile sockeye, and individual collections were made after visual confirmation of sockeye presence, usually through surfacing behaviour. Fish were captured by a purse seine (bunt: 27 m × 9 m with 13 mm mesh; tow: 46 m × 9 m with 76 mm mesh) designed for hand retrieval from a small (6 m) motorized vessel. Capture typically occurred between 5 and 40 m from shore.

Table 1. Infection abundances of sea lice before and after the competitive foraging experiment with juvenile sockeye salmon.

	Pre-experiment criteria				Post-experiment means			
	Lightly infected		Heavily infected		Lightly infected		Heavily infected	
Trial	Large chalimus	Motile	Large chalimus	Motile	Large chalimus	Motile	Large chalimus	Motile
1	≤2	0	≥5	≥2	1.1±0.2	1.4±0.4	4.4±0.5	2.3±0.5
2	≤2	0	≥5	≥2	0.6±0.3	0.8±0.3	3.3±0.2	3.2±0.4
3	0	0	≥4	≥2	0.4±0.2	0.8±0.2	2.3±0.2	2.7±0.5
4	≤2	0	≥5	≥1	0.8±0.1	0.4±0.2	4.3±0.7	1.6±0.3
5	≤2	0	≥5	≥2	0.3±0.2	0.7±0.2	3.1±0.4	2.1±0.4
Overall					0.3±0.1	0.8±0.1	3.5±0.2	2.4±0.2

Note: Louse means are shown with ± standard error (SE). "Large chalimus" includes chalimus III and IV stage *C.clemensi* and chalimus II stage *L. salmonis*. "Motile" encompasses the pre-adult and adult stages of both louse species.

Captured fish were initially held alongside the boat in a pocket of the bunt end of the net that was carefully set with sufficient depth and width to allow the fish to swim with minimal apparent stress and to prevent contact with the netting. The maximum time that fish were held in the net was approximately 0.5 h, during which time sockeye were removed from the net. The vast majority of bycatch was other juvenile Pacific salmon, but Pacific sand lance (*Ammodytes hexapterus*), threespine stickleback (*Gasterosteus aculeatus*), rockfish (*Sebastes* spp.), and Pacific herring were also captured in low numbers (fewer than 10 non-salmon bycatch individuals in each set). Fish were transferred from the net by allowing them to swim into a seawater-filled 3.79 L milk jug with the base cut off and then transferred into one of three seawater-filled insulated fish totes (0.58 m deep and 0.97 m × 0.55 m across) by submerging the milk jug and allowing the fish to swim out. All subsequent transfers of fish were performed using this technique. During initial collections, fish were observed in 13.2 L transparent plastic aquaria to check carefully for sea louse detachment resulting from transfer. No sea lice were seen detaching from their host during these or subsequent collections. Collection ceased when we had reached a density of approximately 150 fish per tote. We did not always capture enough fish in our first set to reach the desired density in the three totes; in these cases we set the net again and continued adding fish to the totes. When the first fish had been in a tote for 2 h, we ended the collection and no more fish were added. No species other than sockeye salmon were kept or assessed for sea louse infection.

Fish transportation and holding

The juvenile sockeye were transported for approximately 1 h by boat to the experimental facility in Cramer Passage, in the Broughton Archipelago, British Columbia. We used ice packs and battery-powered aquarium bubblers to ensure adequate temperature and aeration during transportation. Approximately 1600 fish were captured and transported over the course of the sampling period. All fish survived transportation except during one collection due to poor weather during the transportation process. Fish from this collection were released and not used in the experiment. Apart from the fish in this collection, transported sockeye did not exhibit behaviours indicative of stress such as gasping, clamped fins, or unusual movement other than being easily startled into the corners of the tote.

The experimental facility consisted of several floating wooden docks that supported four ocean net pens in a location chosen for shelter from wave action. Upon arrival at the facility, sockeye were transferred immediately to one of three net pens. One net pen was 2.1 m deep and 2.8 m × 2.8 m across, another was 2.3 m deep and 4.4 m × 3.2 m across, and the third was 2.8 m deep and 6.1 m × 6.1 m across; each had 4 mm mesh walls and floors. Each collection of approximately 450 fish was stored in a separate net pen.

The fish were fed frozen adult brine shrimp (Brine Shrimp Direct, Ogden, Utah, USA) in the holding pens. The shrimp had minimal size variation and were added into the middle of the net pen after being thawed in an equal volume of seawater. Feeding occurred every 2 h during daylight hours such that an average of 3 g of brine shrimp was fed to each fish over the course of the day.

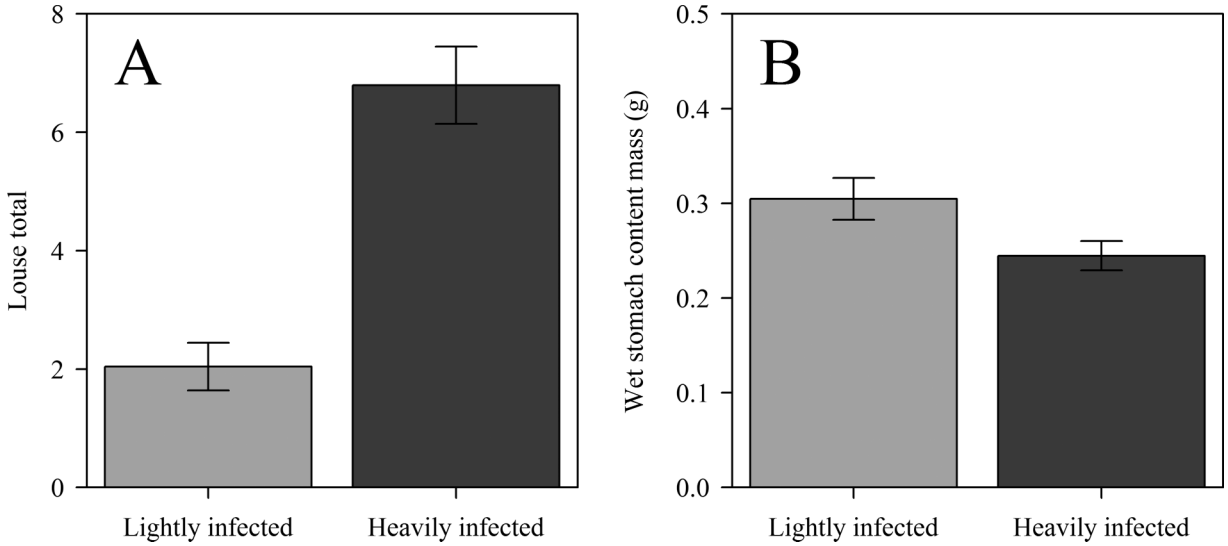
Competitive foraging experiment

After 3 to 10 days in the holding pen, sockeye were selected for the foraging experiment. Each trial used fish from only one collection (i.e., all fish caught in a single day). Individual fish were transferred from the holding pen to 13.2 L transparent plastic aquaria and visually assessed for infection of large chalimus and motile stage lice. A more detailed assessment of louse infection could not be performed without handling the fish, which would have increased the likelihood of louse detachment. Because of the high prevalence of infection (>99%), it was not possible to obtain enough uninfected fish to form infected and uninfected groups. Instead, we selected 10 fish in each of the upper and lower extremes of the distribution in louse load from among approximately 300 fish, on average. Our selection criteria for the infection categories changed among trials depending on the abundance of lice on the fish, but for all trials the minimum difference in louse load between the lightly infected and heavily infected groups was three large chalimus stage lice and one motile louse (Table 1). Fish with scars, open wounds, hemorrhaging fins, or other external signs of poor health were not used in the experiment. Five trials of 20 fish (10 in each infection category) were initiated, though one fish escaped during a trial.

After fish were assigned to an infection category, they were transferred to the experimental net pen (2.3 m deep and 4.4 m × 3.2 m across). Once there, fish were fed an excess quantity of brine shrimp every hour during daylight hours to ensure that all had the opportunity to feed. We began depriving the fish of food approximately 24 h after they were placed into the experimental net pen. The foraging experiment was initiated 30 min after sunrise 2 days later, which corresponded to 36 h after the beginning of food deprivation.

To initiate the experiment, 20 g of frozen brine shrimp was fed to the fish in the same manner as in the holding pens. After 2.5 min of foraging, the fish were startled by a deliberate sudden movement by the experimenter so that they would not feed on any remaining brine shrimp. In a test run, most of the brine shrimp were consumed at the end of 2.5 min, and the most successful foragers stopped feeding after this point. Following the experiment, the holding pen was pulled up to form a shallow pool and the fish were captured using milk jugs. They were then transferred to individual sterile sample bags (Whirl-Pak Write-On Bags; Nasco, Fort Atkinson, Wisconsin, USA) and euthanized with a lethal dose of MS-222 (240 mg·L^{-1}). We assessed each fish for sea

Fig. 2. Louse total and wet stomach content mass recorded post-experiment for both infection categories of juvenile sockeye. Error bars show the 95% confidence intervals for the mean value.



louse infection by hand lens (Krkošek et al. 2005), including smaller louse life stages, and placed them on ice. Temperature and salinity were measured inside the net pens at the surface and 1 m depth immediately following the experiment (see online supplementary data, Table S1[1]).

Dissection

We recorded the fork length, body depth, and wet body mass of each fish in the laboratory and noted any tissue damage or evidence of poor health. Stomachs were excised between the lower oesophagus and the pyloric sphincter and their wet contents weighed. For each fish, half of the heart and two gill arches with filaments were removed and stored in an RNA preservative (RNAlater; Life Technologies, Burlington, Ontario, Canada) at 4 °C overnight and –20 °C thereafter.

To further assess the health status of our experimental fish, we screened the tissue samples from all the fish in the first trial and half the fish in trials 2–5 for salmonid alphavirus (SAV), infectious salmon anemia virus (ISAV), and piscine reovirus (PRV) (see Appendix A for methods). The samples tested from trials 2–5 were from the five least successful foragers in the heavily infected group and the five most successful foragers in the lightly infected group, as determined by their stomach content masses after the experiment. These fish were chosen to provide the largest difference in competitive abilities while maintaining even ratios of lightly infected and heavily infected fish.

Statistical analyses

To test for differences in wet stomach content masses and post-experiment louse loads between infection categories, we used Welch's *t* test for two samples of unequal variance. We used linear regression to determine whether wet stomach content masses increased with fish body size. This body size term was the result of a principal component analysis (PCA) used to convert our three measures of body size (fork length, body depth, and wet body mass discounted by stomach content mass) into one linearly uncorrelated body size variable. The data from the first principal component explained 93% of the original variation in body size data.

To evaluate which parameters best predicted the wet stomach content masses and therefore the competitive abilities of our experimental fish, we performed model selection using Akaike's information criterion corrected for small sample sizes (AIC_c) (Hurvich and Tsai 1989). We fit eight mixed-effects models to the data using trial number as a random effect on the intercept in each. Our models were constructed a priori according to our hypotheses and included the biologically relevant combinations and pairwise interactions of three fixed effects: the fish's infection category (i.e., highly or lightly infected), the total number of lice on the fish after the experiment, and the body size variable from the PCA. We included the post-experiment louse total term in our candidate model set to assess whether louse dispersal among fish during the experiment and development from chalimus to motile stages influenced our ability to predict competitive ability from the initial conditions of the fish. All analyses were performed in R 3.0.1 (R Core Team 2013) using the nlme and MuMIn packages.

Results

Fish that had been assigned to the lower infection category before the experiment had less than one-third as many sea lice after the experiment as those in the higher infection category (Fig. 2A). Sockeye in the lower infection category had no motile sea lice before entering the experimental pen, but they averaged 0.8 motile lice per fish after the experiment (Table 1), which equated to 58% of the fish having motile lice. Despite the increase in motile lice and decrease in large chalimus lice over the course of the experiment, we were able to identify the original infection categories of the fish after the experiment because of the criteria that distinguished them initially (see Fig. S1[1]). Each trial initially had a minimum difference of three large chalimus between the two infection categories; after each trial, the two groups of fish still had different numbers of large chalimus, even though the overall chalimus abundances decreased. Although 96% of motile lice infecting the experimental fish were *C. clemensi*, a second species of louse, *L. salmonis*, accounted for 4% of motile lice. The mean (±SD) length of our experimental fish was 11.2 ± 1.1 cm, their mean depth was 2.0 ± 0.2 cm, and their mean body mass discounted by stomach content mass was 12.2 ± 4.0 g. Mean fish body size did not differ between infection categories (two-sample *t* test, $t = 0.34$, df = 97, $p = 0.74$).

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2014-0284.

Table 2. Model selection statistics for the mixed-effects models fit to the stomach content mass data from the competitive foraging experiment.

Rank	Model*	ΔAIC_c†	w_i‡
1	Pre-infection + size	0	0.977
2	Pre-infection	8.21	0.016
3	Pre-infection × size	10.33	0.006
4	Size	13.93	0.001
5	Post-infection + size	15.09	0.001
6	Intercept only	20.66	0
7	Post-infection	23.84	0
8	Post-infection × size	28.68	0

*All models include a random effect on the intercept for trial number. Terms included were the infection category of the fish before the experiment (Pre-infection), the number of lice on the fish after the experiment (Post-infection), and the body size variable from the principal component analysis (Size). Parameter estimates (±SE) for the top model were 0.304 0.014 for the intercept, −0.058 ± 0.011 for the highly infected group, and 0.020 ± 0.004 for the body size variable derived from the principal component analysis (n = 99). Models with interactions (e.g., "Pre-infection + size") include lower-order main effects.

†Difference from the top model AIC_c (ΔAIC_c).

‡Akaike model weight (w_i).

Adding brine shrimp to the experimental net pen caused a feeding frenzy, suggesting that the experiment conditions were highly competitive. All fish were observed feeding in each trial, and brine shrimp were the only organisms found in the digestive tracts of the fish after the experiment.

Of the 60 experimental sockeye screened for viruses, none were positive for ISAV or SAV. Two fish tested positive for PRV, one from each infection category.

Juvenile sockeye competitive ability was related to both sea louse infection and body size. The top model of eight models fit to the stomach content mass data included terms for both of these predictors. This model accounted for 97.7% of model support using Akaike weights and was 8.21 AIC_c units lower than the second top model (Table 2), indicating that it had substantially more empirical support than any other model in our model set (Burnham and Anderson 2002). The standard deviation of the random intercept was 0.0245. The top three models accounted for 99.8% of model support, and all included a term for the infection category of the fish.

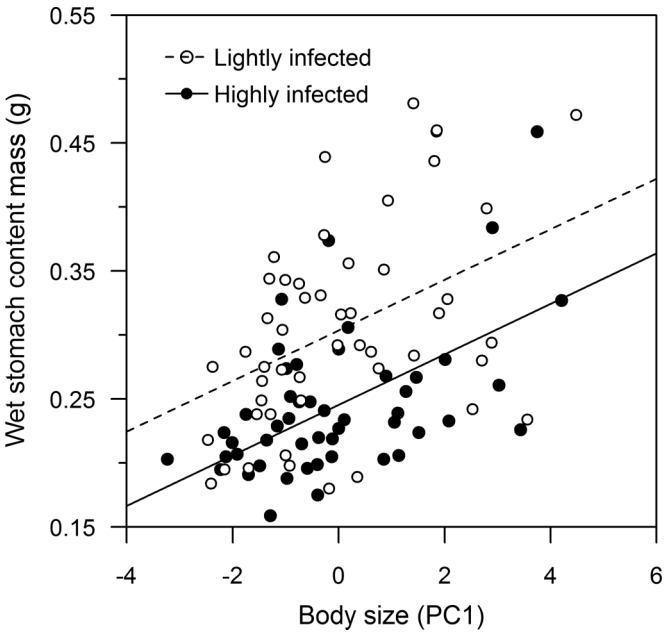
As predicted, highly infected sockeye had lower competitive abilities in the experiment relative to lightly infected fish. Highly infected fish were 20% less successful at consuming food than lightly infected fish, on average (Fig. 2B); the mean (±SE) stomach content mass of the fish was 0.305 ± 0.011 g for the lightly infected sockeye (n = 50) and 0.245 ± 0.008 g for the highly infected sockeye (n = 49). Also as predicted, wet stomach content masses increased with body size for each infection category (Fig. 3), and this relationship was consistent among all trials (see Fig. S2[1]).

Discussion

Theory predicts that complex host–pathogen dynamics may arise in multihost systems, particularly when pathogens modulate other ecological processes such as competition and predation (Hatcher et al. 2012). Our results suggest that parasitic sea lice may modulate intraspecific competition among wild juvenile sockeye salmon, where higher sea louse abundances produce lower competitive abilities in hosts. Our results also indicate higher competitive abilities in larger fish, but that there was little evidence that larger fish were less impaired by infections than smaller fish.

The relevance of our results to wild-feeding fish depends on how well the experimental environment represented the natural conditions encountered by outmigrating juvenile sockeye. Our

Fig. 3. Wet stomach content masses as a function of body size for fish of both infection categories. Each point represents an individual fish. The unitless body size variable was derived from a principal component analysis using three correlated measures of body size (PC1 = the first principal component). Regression lines use the coefficients from the main effects of the top mixed-effects model, which includes terms for infection category and body size (Table 2).



experiment simulated a limited patch of a single prey type. The physical characteristics of northern Johnstone Strait cause biological production in this body of water to be extremely low (McKinnell et al. 2014). Juvenile sockeye prey availability is thought to be so low in this region that McKinnell et al. (2014) put forth a "trophic gauntlet hypothesis", in which sockeye suffer an energy deficit while travelling the 150 km through Johnstone Strait, and the individuals that survive this journey are only those with sufficient energy reserves. Although the patchiness and diversity of sockeye prey in Johnstone Strait are not yet known, the prey limitation in this region is such that feeding opportunities for wild juvenile sockeye may be limited and individuals must therefore compete.

There are several potential mechanisms by which sea lice might reduce the competitive ability of juvenile sockeye. These include, but are not limited to, visual impairment, swimming impairment, stamina reduction, and antagonistic behaviour from larger or more dominant fish. Further research is needed to determine which mechanisms apply; only antagonistic behaviour from larger fish can be discounted because there was no difference in size between the infection categories of our experiment. Our objective was to determine whether highly infected fish have reduced competitive abilities because of the implications of such an outcome. In equal-opportunity, prey-limited environments, lower competitive abilities should reduce foraging success, which determines the energy that can be allocated to growth. For fish, growth can be used as a surrogate component of fitness (Schluter 1995) and can determine the outcome of competition and predation (Sogard 1997). Indeed, as shown in our experiment, size also influences competitive foraging success, suggesting a further delayed effect of louse infection on competitive ability.

Despite having no motile sea lice upon entering the experimental net pen, 58% of the fish in the lower infection category had motile lice afterwards (Table 1). This indicates either the development of chalimus stage lice into motile lice, the transfer of motile lice from individuals in the higher infection category, or the at-

tachment of motile lice from the environment during the 60 h that the fish were held in the experimental pen. We believe louse development was the primary driver of this result for four reasons: (*i*) no sea lice were observed to detach from their hosts during the handling process, (*ii*) the mesh size of the net pens was small enough to hinder motile louse influx, (*iii*) abundances of large chalimus stage lice were lower after the experiment than they were before (Table 1), and (*iv*) although no study has clarified the timing of the life cycle for *C. clemensi*, the dominant species in our experiment (96%), work on two other *Caligus* species suggests that a realistic estimate of development time between the final *C. clemensi* chalimus stage and the adult stage could be between 21 and 71 degree-days, which would result in a considerable portion of large chalimus stage lice being able to molt into motile lice between our infection assessments (on average, 29 degree-days at 1 m depth; González and Carvajal 2003; Piasecki and MacKinnon 1995).

Since the treatment groups were not created experimentally, it is possible that fish with different parasite loads differed in some aspect of condition that may have predisposed them to louse infection. One such possibility is pre-existing viral disease. For a separate investigation, we screened a subset of our fish for three viruses of potential concern: SAV, ISAV, and PRV. None of the 60 experimental fish tested positive for ISAV or SAV, and only two were positive for PRV, one from low and one from high louse infection categories. In addition to viral screening following the experiment, we assessed the external conditions of the fish before the experiment and their internal conditions immediately after. We chose fish for the experiment that did not have substantial scarring, open wounds, or hemorrhaging fins. During dissection, we confirmed that the fish did not have swollen kidneys, pale gills, discoloured livers, internal bleeding, or any other obvious internal sign of poor health. Though not conclusive evidence, the absence of the three viruses tested in all but two fish and the apparently healthy internal and external conditions of the fish help argue against the hypothesis that the driver of the effect seen in our competitive foraging experiment was an underlying health difference rather than sea louse infection.

Juvenile sockeye salmon were primarily infected with *C. clemensi*, although *L. salmonis* accounted for 4% of motile lice on experimental fish. This result is consistent with the relative abundances of louse species reported by Price et al. (2011) on juvenile Fraser River sockeye earlier along their migration route. However, the highest infection prevalences reported by Price et al. were considerably lower (84% in 2007 and 62% in 2008 for *C. clemensi*) than the prevalence we observed immediately after capture (>99%), which prevented us from challenging groups of uninfected fish in the experiment. This discrepancy could be a result of year-to-year variation in environmental conditions, increased transmission from salmon farms in the Discovery Passage, British Columbia (Fig. 1A) (Price et al. 2011), or transfer from another louse source further along the migration route, such as Pacific herring. Regardless, the high infection prevalence observed is of concern given the potential fitness consequence of sea louse exposure demonstrated by our experiment.

A potential alternative explanation for our results could be that highly infected fish have reduced appetites. Farmed Atlantic salmon (*Salmo salar*) adults with high numbers of *L. salmonis* can have reduced appetite, but there has been no published evidence of such an effect in wild or juvenile fish. In fact, previous work indicates that infected wild juvenile pink salmon (*Oncorhynchus gorbuscha*) are willing to accept higher predation risk to access food (Krkošek et al. 2011). Additionally, all of our experimental fish joined the feeding frenzy upon food introduction. One other potential alternative explanation for our results is that highly infected fish have increased metabolic rates and therefore faster digestion. Should this effect occur, it is unlikely to have confounded our data because the fish were immediately euthanized after the experiment ended, and therefore differences in stomach content mass are unlikely to be the result of differences in digestive rates. Differential competitive ability between infection categories of fish therefore remains the most plausible mechanism behind our results.

Although we were not able to confirm the origin of our fish genetically, there is a very high probability that they were from the Fraser River, given the timing and the large pulse of fish moving through the region, rather than from the few small local systems (Groot and Cooke 1987; Price et al. 2013). We ensured that we collected juvenile sockeye during the peak of the Fraser River sockeye outmigration by monitoring the progress of the run through observers in the Discovery Islands.

Fraser River sockeye are of substantial economic and ecological importance. After a two-decade decline in Fraser River sockeye productivity, record-low adult returns in 2009 prompted intense public and scientific scrutiny of their management. The resulting federal judicial inquiry by the Cohen Commission provided 75 recommendations to the Canadian government on the management and conservation of Fraser River sockeye. The final report postulated that the causes of the decline may originate in the nearshore marine waters where the fish grow (Cohen 2012*c*, p. 59), and one of the recommendations explicitly stated the need for research on determining "what pathogens are encountered by Fraser River sockeye salmon along their entire migratory route, and the cumulative effects of these pathogens" (Cohen 2012*c*, p. 61). By showing that juvenile Fraser River sockeye with high abundances of sea lice have reduced competitive abilities, our work provides the first evidence for a fitness consequence of parasite exposure in Fraser River sockeye.

Sea louse infestations of domesticated salmon in British Columbia are currently managed using in-feed treatment with a parasiticide (emamectin benzoate ("SLICE"); Intervet/Schering-Plough Animal Health, Boxmeer, The Netherlands) (Saksida et al. 2010). In the Broughton Archipelago, treatments preceding juvenile pink (*O. gorbuscha*) and chum salmon (*Oncorhynchus keta*) outmigrations have resulted in dramatic reductions in louse levels of wild fish and positive conservation outcomes for local wild populations (Peacock et al. 2013). Louse loads of outmigrating sockeye are still very high in Johnstone Strait, as we observed, and elevated sea louse abundances have been linked to salmon farms earlier on the sockeye migration route in the Discovery Passage (Price et al. 2011). Under the conditions of their Licence for Finfish Aquaculture, farms are only required to initiate treatment when the abundance of lice exceeds three motile *L. salmonis* per fish. No policy exists for reducing *C. clemensi* levels, despite this being the primary louse species infecting juvenile sockeye migrating through this region. A treatment regime aimed to minimize *C. clemensi* infestations on farmed fish in the Discovery Passage prior to and during the migration of juvenile sockeye in this region could reduce one potentially major stressor on one of the most important salmon populations in the world.

Our results are important within the context of marine conservation for species like sockeye salmon that are susceptible to infection by pathogens with complex multihost dynamics. Both fish farms and Pacific herring act as reservoir hosts from which *C. clemensi* can be maintained in the environment even when there are few or no sockeye present. Other species of salmon for which sea lice have been implicated as causes of concern are primarily infected with the salmonid specialist *L. salmonis* (e.g., Gargan et al. 2012; Krkošek et al. 2007), so the potential for highly abundant reservoirs of other fish species is considerably reduced. Fraser River sockeye, however, are mainly parasitized by the generalist *C. clemensi*, which infect other species such as Pacific herring. This additional reservoir host exacerbates any threat to sockeye from sea louse infection, such as reduced ability to forage successfully, because the potential exists for a stronger Allee effect and greater extinction risk if the sockeye population becomes sufficiently low

(Krkošek et al. 2013). This complexity is compounded by indirect effects of parasites on fish populations. For salmon, early marine survival largely depends on growth (Beamish et al. 2004; Farley et al. 2007), so the results of resource competition among individuals likely govern their survival. The implications of a pathogen-induced reduction in competitive ability could therefore determine the survival of infected individuals. Understanding how pathogens like sea lice interact with fundamental ecological processes that determine fish survival is essential for effective marine conservation of populations vulnerable to pathogen infection.

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References

Bax, N.J. 1983. Early marine mortality of marked juvenile chum salmon (*Oncorhynchus keta*) released into Hood Canal, Puget Sound, Washington, in 1980. Can. J. Fish. Aquat. Sci. **40**(4): 426–435. doi:10.1139/f83-061.

Beamish, R., Mahnken, C., and Neville, C. 2004. Evidence that reduced early marine growth is associated with lower marine survival of coho salmon. Trans. Am. Fish. Soc. **133**(1): 26–33. doi:10.1577/T03-028.

Beamish, R., Wade, J., Pennell, W., Gordon, E., Jones, S., Neville, C., Lange, K., and Sweeting, R. 2009. A large, natural infection of sea lice on juvenile Pacific salmon in the Gulf Islands area of British Columbia, Canada. Aquaculture, **297**(1–4): 31–37. doi:10.1016/j.aquaculture.2009.09.001.

Brearley, G., Rhodes, J., Bradley, A., Baxter, G., Seabrook, L., Lunney, D., Liu, Y., and McAlpine, C. 2013. Wildlife disease prevalence in human-modified landscapes. Biol. Rev. **88**(2): 427–442. doi:10.1111/brv.12009.

Burnham, K.P., and Anderson, D.R. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York.

Carney, W.P. 1969. Behavioral and morphological changes in carpenter ants harboring dicrocoeliid metacercariae. Am. Midl. Nat. **82**(2): 605–611. doi:10.2307/2423801.

Cohen, B.I. 2012*a*. Cohen Commission of inquiry into the decline of sockeye salmon in the Fraser River — final report. The uncertain future of Fraser River sockeye. Vol. 1: The sockeye fishery. Minister of Public Works and Government Services Canada, Ottawa, Ont.

Cohen, B.I. 2012*b*. Cohen Commission of inquiry into the decline of sockeye salmon in the Fraser River — final report. The uncertain future of Fraser River sockeye. Vol. 2: Causes of the decline. Minister of Public Works and Government Services Canada, Ottawa, Ont.

Cohen, B.I. 2012*c*. Cohen Commission of inquiry into the decline of sockeye salmon in the Fraser River — final report. The uncertain future of Fraser River sockeye. Vol. 3: Recommendations, summary, process. Minister of Public Works and Government Services Canada, Ottawa, Ont.

Cooke, S.J., Hinch, S.G., Farrell, A.P., Lapointe, M.F., Jones, S.R., Macdonald, J.S., Patterson, D.A., Healey, M.C., Van, Der, and Kraak, G. 2004. Abnormal migration timing and high en route mortality of sockeye salmon in the Fraser River, British Columbia. Fisheries, **29**(2): 22–33. doi:10.1577/1548-8446(2004)29[22:AMTAHE]2.0.CO;2.

Daszak, P., Cunningham, A.A., and Hyatt, A.D. 2000. Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science, **287**(5452): 443–449. doi:10.1126/science.287.5452.443.

De Castro, F., and Bolker, B. 2005. Mechanisms of disease-induced extinction. Ecol. Lett. **8**(1): 117–126. doi:10.1111/j.1461-0248.2004.00693.x.

Dobson, A., and Foufopoulos, J. 2001. Emerging infectious pathogens of wildlife. Philos. Trans. R. Soc. B Biol. Sci. **356**(1411): 1001–1012. doi:10.1098/rstb.2001.0900.

Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale, M.K., Patterson, D.A., Hinch, S.G., and Farrell, A.P. 2011. Differences in thermal tolerance among sockeye salmon populations. Science, **332**(6025): 109–112. doi:10.1126/science.1199158.

Farley, E.V., Murphy, J.M., Adkison, M.D., Eisner, L.B., Helle, J.H., Moss, J.H., and Nielsen, J. 2007. Early marine growth in relation to marine-stage survival rates for Alaska sockeye salmon (*Oncorhynchus nerka*). Fish. Bull. **105**(1): 121–130.

Gargan, P.G., Forde, G., Hazon, N., Russell, D.J.F., and Todd, C.D. 2012. Evidence for sea lice-induced marine mortality of Atlantic salmon (*Salmo salar*) in western Ireland from experimental releases of ranched smolts treated with emamectin benzoate. Can. J. Fish. Aquat. Sci. **69**(2): 343–353. doi:10.1139/f2011-155.

González, L., and Carvajal, J. 2003. Life cycle of *Caligus rogercresseyi*, (Copepoda: Caligidae) parasite of Chilean reared salmonids. Aquaculture, **220**(1–4): 101–117. doi:10.1016/S0044-8486(02)00512-4.

Groot, C., and Cooke, K. 1987. Are the migrations of juvenile and adult Fraser River sockeye salmon (*Oncorhynchus nerka*) in near-shore waters related. *In* Sockeye salmon (*O. nerka*) population biology and future management. *Edited by* H.D. Smith, L. Margolis and C.C. Wood. Canadian Special Publication of Fisheries and Aquatic Sciences, No. 96. pp. 53–60.

Groot, C., and Margolis, L. 1991. Pacific salmon life histories. UBC Press, Vancouver, B.C., Canada.

Hamre, L.A., Eichner, C., Caipang, C.M.A., Dalvin, S.T., Bron, J.E., Nilsen, F., Boxshall, G., and Skern-Mauritzen, R. 2013. The salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) life cycle has only two chalimus stages. PLoS ONE, **8**(9): e73539. doi:10.1371/journal.pone.0073539.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., and Samuel, M.D. 2002. Climate warming and disease risks for terrestrial and marine biota. Science, **296**(5576): 2158–2162. doi:10.1126/science.1063699.

Hatcher, M.J., Dick, J.T., and Dunn, A.M. 2012. Diverse effects of parasites in ecosystems: linking interdependent processes. Front. Ecol. Environ. **10**(4): 186–194. doi:10.1890/110016.

Hixon, M.A., and Jones, G.P. 2005. Competition, predation, and density-dependent mortality in demersal marine fishes. Ecology, **86**(11): 2847–2859. doi:10.1890/04-1455.

Hofmeyr, M., Bingham, J., Lane, E., Ide, A., and Nel, L. 2000. Rabies in African wild dogs (*Lycaon pictus*) in the Madikwe Game Reserve, South Africa. Vet. Rec. **146**(2): 50–52. doi:10.1136/vr.146.2.50.

Hogans, W., and Trudeau, D. 1989. Preliminary studies on the biology of sea lice, *Caligus elongatus*, *Caligus curtus* and *Lepeophtheirus salmonis* (Copepoda: Caligoida) parasitic on cage-cultured salmonids in the lower Bay of Fundy. Can. Tech. Rep. Fish. Aquat. Sci. 1715.

Hurvich, C.M., and Tsai, C.-L. 1989. Regression and time series model selection in small samples. Biometrika, **76**(2): 297–307. doi:10.1093/biomet/76.2.297.

Jakob, E., Sweeten, T., Bennett, W., and Jones, S. 2013. Development of the salmon louse *Lepeophtheirus salmonis* and its effects on juvenile sockeye salmon *Oncorhynchus nerka*. Dis. Aquat. Org. **106**(3): 217–227. doi:10.3354/dao02642.

Johnson, P.T., Lunde, K.B., Ritchie, E.G., and Launer, A.E. 1999. The effect of trematode infection on amphibian limb development and survivorship. Science, **284**(5415): 802–804. doi:10.1126/science.284.5415.802.

Johnson, S., and Albright, L. 1991. Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. J. Mar. Biol. Assoc. U.K. **71**(02): 425–436. doi:10.1017/S0025315400051687.

Kabata, Z. 1972. Developmental stages of *Caligus clemensi* (Copepoda: Caligidae). J. Fish. Board Can. **29**(11): 1571–1593. doi:10.1139/f72-245.

Kristan, D.M., and Hammond, K.A. 2000. Combined effects of cold exposure and sub-lethal intestinal parasites on host morphology and physiology. J. Exp. Biol. **203**(22): 3495–3504.

Krkošek, M., Morton, A., and Volpe, J.P. 2005. Nonlethal assessment of juvenile pink and chum salmon for parasitic sea lice infections and fish health. Trans. Am. Fish. Soc. **134**(3): 711–716. doi:10.1577/T04-133.1.

Krkošek, M., Ford, J.S., Morton, A., Lele, S., Myers, R.A., and Lewis, M.A. 2007. Declining wild salmon populations in relation to parasites from farm salmon. Science, **318**(5857): 1772–1775. doi:10.1126/science.1148744.

Krkošek, M., Connors, B.M., Ford, H., Peacock, S., Mages, P., Ford, J.S., Morton, A., Volpe, J.P., Hilborn, R., Dill, L.M., and Lewis, M.A. 2011. Fish farms, parasites, and predators: implications for salmon population dynamics. Ecol. Appl. **21**(3): 897–914. doi:10.1890/09-1861.1.

Krkošek, M., Ashander, J., Frazer, L.N., and Lewis, M.A. 2013. Allee effect from parasite spill-back. Am. Nat. **182**(5): 640–652. doi:10.1086/673238.

Leroy, E.M., Rouquet, P., Formenty, P., Souquiere, S., Kilbourne, A., Froment, J.-M., Bermejo, M., Smit, S., Karesh, W., and Swanepoel, R. 2004. Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science, **303**(5656): 387–390. doi:10.1126/science.1092528.

McKinnell, S., Curchitser, E., Groot, K., Kaeriyama, M., and Trudel, M. 2014. Oceanic and atmospheric extremes motivate a new hypothesis for variable marine survival of Fraser River sockeye salmon. Fish. Oceanogr. **23**(4): 322–341. doi:10.1111/fog.12063.

Meeus, I., Brown, M.J.F., De, Graaf, D.C., and Smagghe, G.U.Y. 2011. Effects of invasive parasites on bumble bee declines. Conserv. Biol. **25**(4): 662–671. doi:10.1111/j.1523-1739.2011.01707.x.

Morton, A., Routledge, R., and Krkošek, M. 2008. Sea louse infestation in wild juvenile salmon and Pacific herring associated with fish farms off the east-central coast of Vancouver Island, British Columbia. N. Am. J. Fish. Manage. **28**(2): 523–532. doi:10.1577/M07-042.1.

Moss, J.H., Beauchamp, D.A., Cross, A.D., Myers, K.W., Farley, Jr, E.V.,

Murphy, J.M., and Helle, J.H. 2005. Evidence for size-selective mortality after the first summer of ocean growth by pink salmon. Trans. Am. Fish. Soc. **134**(5): 1313–1322. doi:10.1577/T05-054.1.

Origgi, F.C., Plattet, P., Sattler, U., Robert, N., Casaubon, J., Mavrot, F., Pewsner, M., Wu, N., Giovannini, S., Oevermann, A., Stoffel, M.H., Gaschen, V., Segner, H., and Ryser-Degiorgis, M.-P. 2012. Emergence of canine distemper virus strains with modified molecular signature and enhanced neuronal tropism leading to high mortality in wild carnivores. Veterinary Pathology Online, **49**(6): 913–929. doi:10.1177/0300985812436743.

Parker, R.R. 1968. Marine mortality schedules of pink salmon of the Bella Coola River, central British Columbia. J. Fish. Board Can. **25**(4): 757–794. doi:10.1139/f68-068.

Peacock, S.J., Krkošek, M., Proboszcz, S., Orr, C., and Lewis, M.A. 2013. Cessation of a salmon decline with control of parasites. Ecol. Appl. **23**(3): 606–620. doi:10.1890/12-0519.1.

Peacock, S.J., Connors, B.M., Krkošek, M., Irvine, J.R., and Lewis, M.A. 2014. Can reduced predation offset negative effects of sea louse parasites on chum salmon? Proc. R. Soc. B Biol. Sci. **281**(1776): 20132913. doi:10.1098/rspb.2013.2913. PMID:24352951.

Piasecki, W., and MacKinnon, B.M. 1995. Life cycle of a sea louse, *Caligus elongatus* von Nordmann, 1832 (Copepoda, Siphonostomatoida, Caligidae). Can. J. Zool. **73**(1): 74–82. doi:10.1139/z95-009.

Price, M.H.H., Proboszcz, S.L., Routledge, R.D., Gottesfeld, A.S., Orr, C., and Reynolds, J.D. 2011. Sea louse infection of juvenile sockeye salmon in relation to marine salmon farms on Canada's west coast. PLoS ONE, **6**(2): e16851. doi:10.1371/journal.pone.0016851.

Price, M.H.H., Glickman, B.W., and Reynolds, J.D. 2013. Prey selectivity of Fraser River sockeye salmon during early marine migration in British Columbia. Trans. Am. Fish. Soc. **142**(4): 1126–1133. doi:10.1080/00028487.2013.799517.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Saksida, S.M., Morrison, D., and Revie, C.W. 2010. The efficacy of emamectin benzoate against infestations of sea lice, *Lepeophtheirus salmonis*, on farmed Atlantic salmon, *Salmo salar* L., in British Columbia. J. Fish Dis. **33**(11): 913–917. doi:10.1111/j.1365-2761.2010.01192.x.

Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. Ecology, **76**(1): 82–90. doi:10.2307/1940633.

Smith, K.F., Acevedo-Whitehouse, K., and Pedersen, A.B. 2009. The role of infectious diseases in biological conservation. Anim. Conserv. **12**(1): 1–12. doi:10.1111/j.1469-1795.2008.00228.x.

Sogard, S.M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bull. Mar. Sci. **60**(3): 1129–1157.

Woodroffe, R. 1999. Managing disease threats to wild mammals. Anim. Conserv. **2**(3): 185–193. doi:10.1111/j.1469-1795.1999.tb00064.x.

Appendix A. Description of methods for viral analysis

Virus screening was performed at the Atlantic Veterinary College. Salmonid alphavirus (SAV) was tested using TaqMan primers and probe sequences for the Q_nsP1 real-time PCR assay, a broad-spectrum assay for all SAV subtypes (Hodneland and Endresen 2006). Real-time reverse transcription (RT)-PCR was also performed to detect infectious salmon anemia virus (ISAV) and piscine reovirus (PRV) using TaqMan probes for ISAV segment 8 (Snow et al. 2006) and PRV segment L1 (Haugland et al. 2011). Samples were considered positive at C_t values less than or equal to 37.5 for SAV, 35.25 for ISAV, and 39.9 for PRV. Verification of ISAV results was accomplished with conventional RT-PCR using segment 8 (Devold et al. 2000) and segment 6 HPR primers (Kibenge et al. 2009).

References

Devold, M., Krossøy, B., Aspehaug, V., and Nylund, A. 2000. Use of RT-PCR for diagnosis of infectious salmon anaemia virus (ISAV) in carrier sea trout *Salmo trutta* after experimental infection. Dis. Aquat. Organ. **40**(1): 9–18. doi:10.3354/dao040009. PMID:10785858.

Haugland, Ø., Mikalsen, A.B., Nilsen, P., Lindmo, K., Thu, B.J., Eliassen, T.M., Roos, N., Rode, M., and Evensen, Ø. 2011. Cardiomyopathy syndrome of atlantic salmon (*Salmo salar* L.) is caused by a double-stranded RNA virus of the Totiviridae family. J. Virol. **85**(11): 5275–5286. doi:10.1128/JVI.02154-10. PMID:21411528.

Hodneland, K., and Endresen, C. 2006. Sensitive and specific detection of Salmonid alphavirus using real-time PCR (TaqMan®). J. Virol. Methods, **131**(2): 184–192. doi:10.1016/j.jviromet.2005.08.012.

Kibenge, F., Godoy, M.G., Wang, Y., Kibenge, M., Gherardelli, V., Mansilla, S., Lisperger, A., Jarpa, M., Larroquete, G., Avendaño, F., Lara, M., and Gallardo, A. 2009. Infectious salmon anaemia virus (ISAV) isolated from the ISA disease outbreaks in Chile diverged from ISAV isolates from Norway around 1996 and was disseminated around 2005, based on surface glycoprotein gene sequences. Virol. J. **6**(1): 88. doi:10.1186/1743-422X-6-88. PMID:19558648.

Snow, M., McKay, P., McBeath, A., Black, J., Doig, F., Kerr, R., Cunningham, C., Nylund, A., Devold, M., and Vannier, P. 2006. Development, application and validation of a Taqman® real-time RT-PCR assay for the detection of infectious salmon anaemia virus (ISAV) in Atlantic salmon (*Salmo salar*). Dev. Biol. **126**: 133–145. PMID:17058489.