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Salmon lice evasion, susceptibility, retention, and development differ amongst host salmonid species

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With different ecological characteristics amongst salmonid species, their response to parasitic infestation is likely to vary according to their spatial and temporal overlap with the parasite. This study investigated the host–parasite interactions amongst three species of salmonids and the ectoparasitic salmon louse, *Lepeophtheirus salmonis*. To determine any variation in infestation parameters amongst salmonids, single population groups of Atlantic salmon (*Salmo salar*), chinook salmon (*Onchorhynchus tshawytscha*), and previously-infested and naïve sea trout (*Salmo trutta*) were exposed to a controlled infestation challenge. We found that chinook salmon and both sea trout groups were more susceptible to acquiring lice than Atlantic salmon. Behavioural responses during infestation were more pronounced in Atlantic and chinook salmon. Parasite development was similar in lice attached to Atlantic salmon and sea trout, but hindered on chinook salmon. At 16 days post-infestation, chinook salmon had reduced lice loads to the same level as Atlantic salmon, whilst sea trout retained their lice. These results demonstrate differences in interactions with *L. salmonis* amongst these species, and highlight the vulnerability of sea trout to infestation.

Keywords: anti-parasite behaviour, *Lepeophtheirus salmonis*, *Onchorhynchus tshawytscha*, *Salmo salar*, *Salmo trutta*.

Introduction

Host defenses against parasites transpire over ranging levels, from large-scale avoidance behaviours to species-specific immunological responses (Boots and Bowers, 1999; Boots and Haraguchi, 1999; Moore, 2002). Behaviour influences the success rate of infection by the parasite through avoidance or deflective actions (Hart, 1992; Moore, 2002; Ezenwa *et al.*, 2016). Behaviours can represent an adaptive trade-off, with the other pillars of defense representing an energetic cost that induces a selection for the most efficient form (Boots and Bowers, 1999). The alternative lines of defense are investment in physiological or immunological resistance, or mitigating the fitness cost of infections through tolerance mechanisms (Råberg *et al.*, 2009; Adelman and Hawley, 2017). These approaches are not mutually exclusive in their efficacy; for instance, behaviours can influence susceptibility (inextricably linked to resistance; Daly and Johnson, 2011) or increase

the subsequent tolerance to infection (Sears *et al.*, 2013). Most often, hosts spread their risk and will have some combination of defense behaviours and physiological mechanisms that increase their resistance to infection (Karvonen *et al.*, 2004). These factors can shift in terms of cost investment, even within the same species, or species that occupy the same environment. Populations may face different environmental pressures or ecological factors that influence the need for parasite defense (Johnson *et al.*, 2012; Sears *et al.*, 2015).

Salmonids represent an ideal model to study potential differences amongst species or populations, as they coexist in the same environments under various circumstances. Through potentially increasing infection pressures driven by the production of Atlantic salmon (*Salmo salar*), wild salmonids that share the waters with farms are likely experiencing more intense and frequent epizootics, particularly with parasites (e.g. Bjørn *et al.*,

2011; Serra-Llinares *et al.*, 2014; Shephard *et al.*, 2016). The magnitude of effect is likely to vary amongst salmonid species, depending on their biology and ecology (Thorstad *et al.*, 2015; Vollset *et al.*, 2016). This difference would manifest through variation in life-history strategies, in that investment into resistance or tolerance would be linked to their risk of encounter and duration of exposure to the pathogen (e.g. Sears *et al.*, 2015; Klemme and Karvonen, 2017). For example, both Atlantic salmon and chinook salmon (*Oncorhynchus tshawytscha*) species leave the river synchronically, and migrate to oceanic waters for one to several years before returning for spawning (Healy, 1991; Klemetsen *et al.*, 2003). Sea trout (*Salmo trutta*), on the other hand, remain in coastal waters to feed, but the migration period can be short-lived and highly unpredictable, interspersed with forays into freshwater habitats (Thorstad *et al.*, 2016). As aquaculture facilities are mostly situated near the coast, the exposure to aquaculture-promoted diseases and parasites is likely to vary amongst these species, driving host–parasite interactions.

There is indeed documented variation amongst species and populations in relation to susceptibility to salmon lice (*Lepeophtheirus salmonis*), with ranging conclusions. When comparing Atlantic salmon and sea trout, Dawson *et al.* (1997) found that sea trout were more susceptible and retained more lice than salmon. On the other hand, Glover (2003) showed an opposite pattern with higher susceptibility reported in Atlantic salmon. Little is known about the interactions between salmon lice and chinook salmon, but a seminal tank experiment showed that Atlantic salmon were more susceptible to infestation than chinook (Johnson and Albright, 1992). However, early surveys of wild-caught salmonids reported the highest infestation levels of lice on chinook salmon, compared with other Pacific salmonid species (Nagasawa, 1987). Essentially, existing knowledge on host–parasite interactions amongst these species can be inconclusive when comparing between studies. The current level of salmon lice abundance is allegedly causing declines in wild salmonid populations (Krkosek *et al.*, 2007; Miller *et al.*, 2014; Shephard and Gargan, 2017), and therefore we require updated experimental knowledge on the louse susceptibility and tolerance in affected salmonid species.

In this study, we compared salmon lice avoidance, susceptibility, retention, and development amongst three different salmonid species, including two species of salmon (Atlantic and chinook), and two groups of sea trout (experienced and naïve to infestation). Unlike the previous studies from the field in variable environments, we tested these factors in an experimental setting with a high degree of control and standardization. We conducted an infestation challenge in a tank environment, using a single stock of infective lice for all groups. Specifically, we aimed to determine whether these groups differed in their susceptibility to salmon lice infestation, and whether lice stay on their host and have the same level of developmental success across salmonid species.

Material and methods
Experimental fish
Four groups of salmonids were tested in this study: hatchery-reared chinook salmon, domesticated Atlantic salmon, and wild sea trout, previously experienced with and naïve to infestation. Chinook salmon were sourced from the US National Marine Fisheries Service, Little Port Walter Marine Research Station (LPW) located on lower Baranof Island in south-east Alaska.

Chinook eggs were fertilized (August 2015) and incubated in MariSource vertical incubators, then collected as pre-eyed (314 Temperature Unit) eggs (September 2015). The eggs were transported on ice to Bergen, Norway, by chartered and commercial air service, and subsequently transferred to the Matre Research Facility in western Norway. They were raised with a standard grow-out regime applied to domesticated Atlantic salmon. Atlantic salmon (AquaGen strain) were used as the base comparison as they represent the most commonly available host for salmon lice. They were sourced from a standard stock at the research facility, and comparable in size to the chinook salmon. Sea trout post-smolts were caught in the Matre river estuary with a bag-net (June 2016) during a period of natural emigration and were therefore unlikely to have yet experienced infestation. The sea trout were held in the same tank facilities, and almost half of the sea trout ($n = 57$) were subjected to infestation (approx. parasite load of 15 lice per fish; August 2016), whilst the latter half remained naïve to infestation ($n = 42$). No lice were still attached to the experienced sea trout after 4 weeks. At the initiation of the experimental period, groups were similar in weight, with Atlantic salmon the largest (258 ± 50 g; mean $\pm$ SD) and chinook salmon the smallest (209 ± 88 g). Sea trout were in between, and almost identical in size to each other (experienced: 225 ± 106 g; naïve: 225 ± 107 g).

Experimental setup
In February 2017, the experimental fish were transferred to their experimental tanks 5 days prior to the infestation challenge. Each experimental group had four replicate tanks (0.35 m^{-3}), each with ~15 fish ($N_{\text{group}} \approx 60$; Table 1). Because of the limited catch of wild sea trout, the number of individuals in these groups were fewer (see Table 1). Keeping sea trout and Atlantic salmon in the same tank during an infestation challenge has been shown to have an effect on subsequent infestation levels (Dawson *et al.*, 1997). Hence, species were not mixed in the experimental tanks. All groups were provided with the same feed (Spirit Supreme 3 mm, Skretting) according to a standard feeding regime for their size, maintained with a natural lighting schedule, and held at $12 °C$.

Infestation challenge
The copepodids used in the infestation challenge were produced in the laboratory in the Matre Research Station, and were third generation wild lice, sourced from a farm in Masfjorden. Eggstrings collected from females were incubated for 7 days at $12 °C$. Approximately 7200 copepodids were collected after incubation, which were then aliquoted into containers for each tank, to provide an infestation pressure of 30 copepodids fish^{-1}. The method of incubation and abundance estimation is described in Hamre *et al.* (2009).

Immediately prior to the infestation period, water level was lowered to 20 cm (0.17 m^{-3}) and flow reduced to ~4 l min^{-2}. When fish had acclimatized to these conditions (<1 min), infective copepodids were added into the water adjacent to the water outlet, with little disturbance to the fish. Over time, the tank slowly refilled, and after the 50-min infestation period, the original flow rate was reinstated. Fish were left in this state until the first lice assessment.

Table 1. Lice retention within the four species of salmonids tested, between 3–13, and 3–16 days post-infestation (dpi).

Time comparison	Fish group	n (x dpi–y dpi)	Difference in lice abundance (%)	Wilcoxon signed-rank test	
				W	P
3–13 dpi	Chinook salmon	59–27	−55	1 442	<0.001
	Atlantic salmon	60–30	+11	915	0.110
	Sea trout, experienced	57–19	−12	1 057	0.128
	Sea trout, naïve	42–16	−3	545	0.402
3–16 dpi	Chinook salmon	59–30	−68	1 224	<0.001
	Atlantic salmon	60–25	−4	762	0.159
	Sea trout, experienced	57–31	−18	554	0.876
	Sea trout, naïve	42–23	−16	314	0.993

Behavioural observation

During the infestation period, the frequency of behaviours was recorded from visual observation for 5 min prior to, and 40 min during the infestation challenge. Observations were made for 1 min every 5th minute, and the behaviours quantified included burst swimming, jumping/rolling, and muscle twitches (see full description in Bui *et al.*, 2017b). Burst swimming behaviours were counted when a marked increase in swimming speed was observed, resulting in displacement of the fish from their position in the shoal. A jump was recorded if the fish accelerated and broke the surface of the water, either with their whole body airborne, or with a slower "roll" at the surface (Furevik *et al.*, 1993). Twitching behaviour was a muscular reaction, whereby the body of the fish contorted along the midline in an "S" shape.

Lice assessment

At 3 days post-infestation (dpi), when lice were expected to still be at the copepodid stage, fish were lightly sedated in the tank (medomidate hydrochloride: 0.1 g/100 l) and collected by hand to transfer into full sedation (medomidate hydrochloride: 1 g/100 l). Body weight and lice abundance were recorded for each individual, and they were returned to their experimental tank and monitored for full recovery. At 13 dpi, lice were expected to be at the mobile stage, however lice are known to develop slower on chinook salmon (Johnson and Albright, 1992), therefore a subsample of fish were collected at 13 dpi (4–10 fish per tank; fewer from the sea trout tanks as they had fewer to begin with). The remaining fish in the tank were assessed at 16 dpi, in order to capture the development rate of lice within this moulting period. Weight and length of fish were recorded at 13 and 16 dpi.

Statistical analyses

Lice avoidance behaviours

Frequency of individual behaviours (bursts, jumps, and muscle twitches, totalled across the sample intervals) were added together to create a value for total anti-parasite behaviours, which was compared amongst groups using a generalized linear model with a Gaussian distribution. Model assumptions were checked by assessing residual plots. Two-way ANOVAs were used in post-hoc analyses.

Initial lice density

Lice abundance was converted to lice density (lice cm^{-2}) to standardize infestation levels amongst varying body sizes. This approach removes the correlation between infestation intensity and host size (Glover *et al.*, 2004a). Density was calculated as a function of lice abundance relative to body surface area, where body surface area was calculated as $13.9W^{0.61}$ ($W =$ weight in grams; Frederick *et al.*, 2017). The distribution of lice density diverged from a normal distribution for each fish group (Shapiro–Wilk test: $p < 0.01$ for chinook salmon, Atlantic salmon, and experienced sea trout), except for the naïve sea trout ($p = 0.091$). As such, the nonparametric Kruskal–Wallis rank sum test was used to compare densities amongst groups at 3 dpi.

Lice development rate

With the known development rate of *L. salmonis* on Atlantic salmon at 12 °C (Johnson and Albright, 1991), we expected no difference in lice stages at 3 dpi. Thus, we compared proportions of each lice stage amongst the fish groups for 13 and 16 dpi. Within each lice stage, abundance was weighted with total abundance per individual, and compared amongst groups with a generalized linear model. The model used a quasibinomial distribution and included tank as a random effect.

In generalized linear models, observations with zero weight are not used for calculating dispersion. Because of the absence of particular stages of lice on chinook salmon, analyses were reduced and excluded the chinook group for pre-adult I females at 13 dpi, and pre-adult II males and females at 16 dpi. No analysis was conducted for adult male lice at 16 dpi as so few observations were made.

Lice retention

Differences in lice density at 13 and 16 dpi was tested with the Kruskal/Wallis rank sum test. Change in lice density over time (between 3 and 13 dpi, and 3 and 16 dpi) was compared amongst groups with the Wilcoxon signed-rank test.

All analyses were conducted in the R environment (version 3.1.0; R Development Core Team, 2015) using the inbuilt packages, as well as the mvnormtest (mshapiro.test function for test of normality) package.

Ethical note

This experiment was conducted according to the regulations set by the Norwegian Regulation on Animal Experimentation (application ID: 8228).
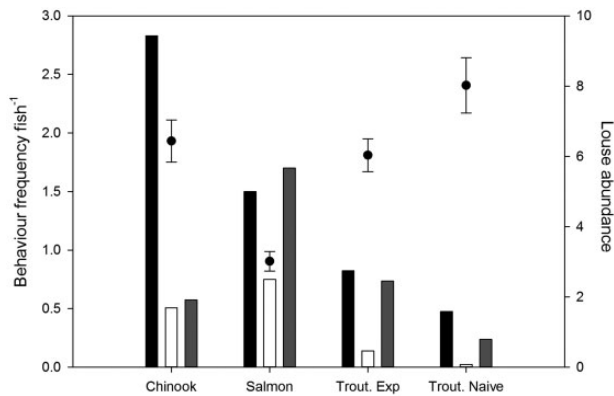
Figure 1. Frequency of anti-parasite behaviours within the salmonid species tested (chinook salmon, Atlantic salmon, and sea trout experienced and naïve to infestation), within a 40 min infestation period. Behaviours assessed were burst swimming (black bars), jumping (unfilled bars), and muscle twitches (grey bars). To characterize the relationship between behaviour and its efficacy in reducing parasite attachment, the subsequent lice levels acquired after the infestation challenge is also shown (black markers with SE bars), as assessed 3 days post-infestation.

Results

Lice avoidance behaviour

Fish behaviour during infestation was pronounced compared with their normal profile. The frequency of burst swimming, jumps, and muscle twitches was negligible prior to the infestation challenge ($<$2 of any behaviour observed in each tank) but escalated immediately with the introduction of infective lice. Burst swimming was most prominent in chinook salmon (Figure 1), whilst the other three groups displayed frequencies of bursts and muscle twitches at similar levels. Jumping behaviour was not common in sea trout but most pronounced in Atlantic salmon (Figure 1). Overall, the frequency of behaviours was not different between chinook and Atlantic salmon (GLM: $z = 1.42$, $p = 0.157$), with means of 3.9 and 4.0 total behaviours per fish, respectively. However, experienced sea trout exhibited less behaviours (mean $= 1.7$, $z = -5.8$, $p < 0.001$), and naïve sea trout exhibited even fewer (mean $= 0.7$, $z = -9.7$, $p < 0.001$). Groups were compared within each displayed behaviour, showing that sea trout generally exhibited lower frequencies in jumps and muscle twitch behaviours compared with both chinook and Atlantic salmon (post-hoc ANOVAS: Supplementary Table S1). Experienced and naïve sea trout behaved similarly, with no significant differences found for any behaviour (Figure 1, Supplementary Table S1).

Initial lice density

Three days after infestation, naïve sea trout had the most lice (average eight lice per fish, Figures 1 and 2) with chinook salmon levels at a similar level (average six lice per fish). Atlantic salmon had acquired the least parasites, with an average of three lice per fish (Figures 1 and 2). Lice density was strongly different amongst the fish groups (Kruskal–Wallis rank-sum test: $H = 54.22$, df $= 3$, $p < 0.001$; Figure 2). Atlantic salmon (mean rank score $= 60$) had significantly lower lice densities than all the other groups (chinook salmon, naïve and experienced sea trout, mean rank scores $= 123$, 123, and 143, respectively).
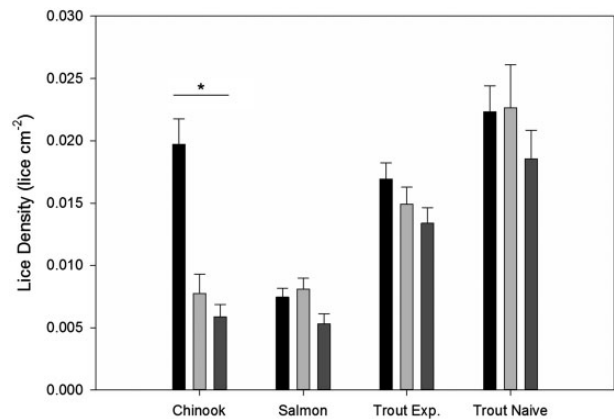
Figure 2. Levels of salmon lice attachment over time amongst salmonid species (chinook salmon, Atlantic salmon, and sea trout experienced and naïve to infestation), as measured by abundance relative to host surface area. Lice levels were assessed at 3 (black bars), 13 (light grey bars), and 16 (dark grey bars) days post-infestation (dpi). Error bars indicated the standard error of the mean, whilst the asterisk indicates statistical difference in lice density over time within a group (Wilcoxon signed-rank test).

Lice development rate

At 3 dpi, 100% of attached lice were at the copepodid stage on all fish groups (Figure 3), but from 13 dpi onwards, there was a marked difference between the four species in the development rate of salmon lice.

The development rate of lice was slowest on chinook salmon, as mainly sessile stages (chalimus I and II) were found over the 16-day period (Figure 3). Both at 13 and 16 dpi, chinook salmon had a significantly larger proportion of sessile stages (chalimus I and II means: 81 and 16% at 13 dpi, $p_{13dpi} < 0.037$ for both; 70 and 26% at 16 dpi, $p_{16dpi} < 0.001$; Supplementary Table S2), and reduced presence of the later stages (all $p < 0.001$; Figure 3).

The development rate was comparable amongst the other three tested groups, with slight differences both at 13 and 16 dpi. On Atlantic salmon, at 13 dpi 63% of lice were mobile (pre-adult I males), increasing to 84% at 16 dpi (pre-adult I females and pre-adult II males, Figure 3). The proportion of chalimus II ($p_{13dpi} = 0.032$; 16 dpi non-significant), pre-adult I male ($p_{13dpi} < 0.001$; $p_{16dpi} = 0.021$), and pre-adult I females ($p_{13dpi} < 0.001$; $p_{16dpi} < 0.001$) differed in Atlantic salmon compared with the other groups at 13 and 16 dpi, as did pre-adult II males ($p_{16dpi} < 0.001$) and females ($p_{16dpi} < 0.001$) at 16 dpi (Figure 3).

For sea trout, lice were equally distributed between sessile (chalimus II) and mobile (pre-adult I male) stages at 13 dpi, whereas at 16 dpi, mobile stages (pre-adult II males and females) were most prevalent (Figure 3). Lice developed at similar rates amongst the two sea trout groups, with statistical differences in proportions present with chalimus II ($p_{13dpi} < 0.001$ and 0.006 for experienced and naïve sea trout, respectively) and pre-adult I males (both $p_{13dpi} < 0.001$ for experienced and naïve sea trout) at 13 dpi, and pre-adult I females (both $p_{16dpi} < 0.001$ for experienced and naïve sea trout) at 16 dpi (Figure 3). When compared with Atlantic salmon, development rate appeared slower at 13 dpi but accelerated to become slightly faster than salmon at 16 dpi (Figure 3), where pre-adult II males were more frequent in sea trout than salmon (mean $= 50$ and 52, $p_{16dpi} = 0.056$ and 0.029
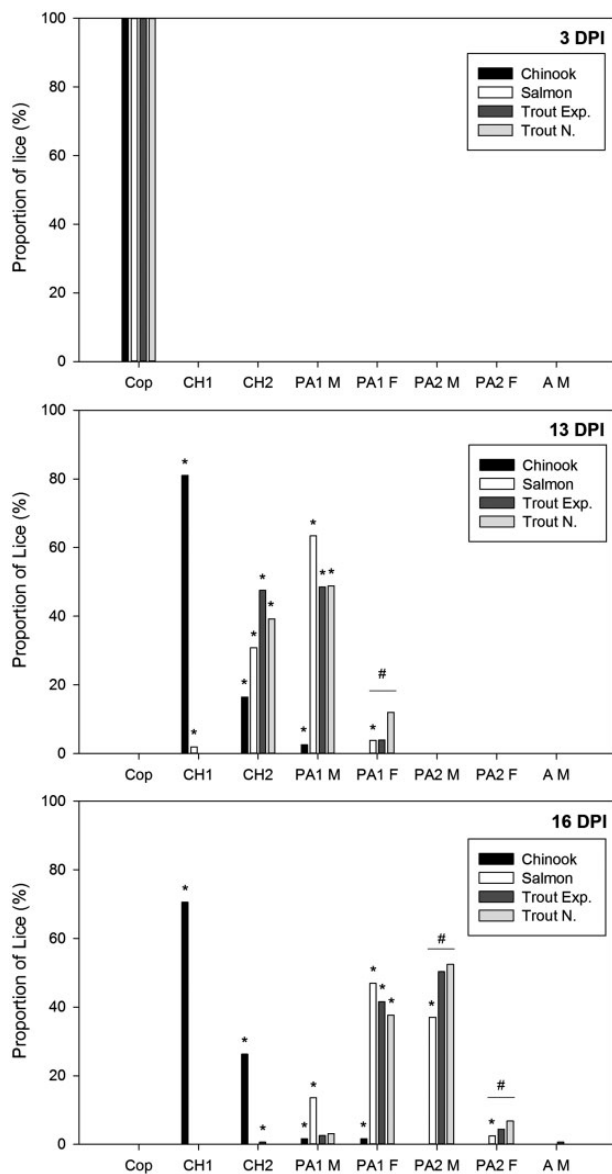
Figure 3. Distributions of development stages of *L. salmonis* on the tested salmonid species (chinook salmon, Atlantic salmon, and sea trout experienced and naïve to infestation) at 3, 13, and 16 days post-infestation (dpi), represented as the mean proportion of stages of lice attached to a host. Lice were categorized into: copepodids (Cop), chalimus I (CH1), chalimus II (CH2), pre-adult I males and females (PA1 M and PA1 F, respectively), pre-adult II males and females (PA2 M and PA2 F, respectively), and adult males (AM). Asterisks indicate significant differences in the group (generalized linear models), and hashes indicate when chinook salmon were removed from the analysis.

for experienced and naïve sea trout compared with mean = 37 for salmon).

Lice retention

The average percentage loss of attached lice was much higher in chinook salmon at 68%, compared with experienced and naïve sea trout with similar losses of 18 and 16%, respectively (Figure 2, Table 1). Atlantic salmon retained their parasites the most

amongst the groups, with only 4% loss over the 16 days. At 13 dpi, the lice density level amongst groups remained significantly different ($H = 23.88$, df = 3, $p < 0.001$; Figure 2), but shifted from the pattern observed at 3 dpi. Densities in chinook salmon and Atlantic salmon (mean rank scores = 33 and 39, respectively) were lower than amongst naïve and experienced trout (mean rank scores = 63 and 62, respectively). The pattern in difference amongst the groups remained the same from 13 dpi to the termination of the trial at 16 dpi ($H = 40.36$, df = 3, $p < 0.001$; rank mean scores = 37, 35, 70, and 79 for chinook salmon, Atlantic salmon, experienced and naïve sea trout, respectively).

When comparing lice density with time, within each fish type, only the chinook salmon significantly reduced their infestation status by 16 dpi (Wilcoxon signed-rank test: $W = 1224$, $p < 0.001$) whereas the other groups remained similar to their initial levels (Table 1). The same pattern was evident at 13 dpi (Table 1).

Discussion

We found clear differences in evasive behaviour, lice susceptibility, and retention amongst the species. Atlantic salmon and chinook salmon showed a more pronounced behavioural response to infestation compared with sea trout. Whilst Atlantic salmon had the lowest susceptibility to lice, Chinook salmon demonstrated low retention of attached lice, with initial infestation densities reducing to the same level as Atlantic salmon after 10 days. Negligible difference was found in infestation parameters and behaviour between sea trout that were naïve or experienced to lice. When attached, lice on chinook salmon developed at a substantially slower rate compared with those on the other salmonids, whereas the rate was quite similar amongst sea trout and Atlantic salmon. Sea trout and Atlantic salmon also retained a similar level of lice throughout the experimental period, indicating a low capability for rejection.

Avoidance behaviour and infestation parameters

Both chinook and Atlantic salmon showed more prominent behaviours. Sea trout exhibit a diverged life-history strategy that differs from that of Atlantic salmon and chinook salmon. Instead of obligatory migration to the open ocean, sea trout remain in coastal waters and fjords (Thorstad *et al.*, 2016). This near-shore migration behaviour potentially subjects them to constant infestation pressure by salmon lice, in the areas where the highest concentrations of lice are generally found. This longer-term exposure and interaction with salmon lice, combined with the proximity of fresh-water refuges, should result in alternate selective pressures in host defense mechanisms against parasites compared with off-shore-migrating salmonid species. In particular, the continuous accessibility of brackish and fresh-water allows for habitat selection that can influence infestation status: highly infested individuals can essentially "delouse" themselves by staying close to river outlets or re-entering the river (Birkeland and Jakobsen, 1997; Wells *et al.*, 2006; Gjelland *et al.*, 2014). Thus, sea trout investment in broad-scale anti-parasite behaviours fits their ecology, whereby the energetic expenditure of spatial relocation is more efficient than the development of physiological defenses. Forays into rivers are shown to be highly efficient at removing young stages of salmon lice (through their intolerance to freshwater, Wright *et al.*, 2016).

We observed elevated frequencies of jumping, burst swimming, and muscle twitches in the two obligatory open-ocean

migrating species compared with sea trout. In contrast to sea trout, Atlantic and chinook salmon have a short window where they are subject to risk of infestation during their out-migration, as they pass through coastal waters with potentially high concentrations of sea lice. With no option in the open ocean for freshwater removal of attached lice, these salmonid species must moderate infestations either during exposure or once infested. The expected defense mechanism against lice is thus likely to lean towards fine-scale behaviours at the point of infestation, or physiological resistance. Fine-scale behaviours could include evasive actions as quantified in this study, or changes in depth preference. Out-migrating Atlantic salmon often swim in low-salinity surface waters as they leave the rivers. This is partly due to osmotic and orientation needs and to benefit from the outflowing currents, but this behaviour also has the potential added benefit of avoidance of parasite-risky depths (Davidsen *et al.*, 2008; Plantalech Manel-La *et al.*, 2009; Thorstad *et al.*, 2012).

Although we observed higher occurrence of evasive behaviours amongst the salmon species, the relationship between anti-parasite behaviours and subsequent parasite loads is not eminently clear, and thus there is likely to be an interaction between the behaviours exhibited and the genetic background of each species. Behavioural defenses are not mutually exclusive in the repertoire of initial host–parasite interactions, but are coupled with numerous other host- and parasite-centric factors (MacKinnon, 1998; Tucker *et al.*, 2000). One such element that is relevant in this study is the genetic differences amongst these salmonid species that innately influences susceptibility. Variation in susceptibility to salmon lice exists at the species level (Johnson and Albright, 1992; Fast *et al.*, 2002), population level within species (Glover, 2003; Glover *et al.*, 2001, 2004a, 2005; Bui *et al.*, 2017a), and at the individual level (Glover *et al.*, 2004b) due to inter-individual variation, such as personalities (Klemme and Karvonen, 2016) or other characteristics (Fevolden *et al.*, 1993; Kittilsen *et al.*, 2012). Here, we found early evidence of differential susceptibility to salmon lice at the species level, whereby chinook salmon and sea trout acquired a higher parasite load compared with Atlantic salmon.

Chinook and Atlantic salmon have previously been shown to initially acquire the same infestation load (Johnson and Albright, 1992); the differences we observed could be through the use of a domesticated strain of Atlantic salmon, however the origin of those experimental fish in Johnson and Albright (1992) is unknown. Sea trout were more susceptible to infestation compared with Atlantic salmon in this study, which follows Dawson *et al.* (1997). Where our results showed a ∼2.3–3 times greater lice level in the trout groups compared with Atlantic salmon, the effect size found by Dawson *et al.* (1997) was only 1.1 times more lice on trout. In contrast, Glover (2003) showed higher susceptibility in farmed salmon compared with three populations of trout, with effect sizes of 1.09–1.48 times greater lice density on salmon. Amongst these studies and ours, differences exist in the sample time point (and therefore post-settlement factors) and the experimental setup (including fish size, tank size, source population, etc.), and consequently, comparisons are loosely made here. The mechanisms for susceptibility or initial resistance is still not well understood, however evidence shows that there is little initial resistance or immune response in the salmon species (Johnson and Albright, 1992; Wagner *et al.*, 2008). To our knowledge, no studies have yet definitely addressed the physiological response of sea trout to salmon lice infestation.

Lice development rate and retention

The development and retention of parasites varied amongst the salmonid species tested, likely through a combination of natural lice mortality and physiological or immune defense. Chinook salmon were found to retard the rate of development for attached lice in comparison to Atlantic salmon and sea trout. This finding is in accordance to Johnson (2006), who reported slower development rates for chinook than Atlantic salmon (adult stage reached in 400–450 vs. 250–300 day degrees, respectively). This divergence in development rate was evident in Johnson's study (1993) after 5 dpi (50 day degrees); in this study, the difference became apparent at 13 dpi (156 day degrees) due to the timing of sampling.

Johnson (1993) considered chinook salmon as more resistance to infestation, although species-specific defense mechanisms in the hosts were not found. Instead, there is a possibility of broad responses such as the excretion of growth inhibitors or substances that obstruct feeding activity could have altered the nutritional status of the host. Although the underlying defense mechanisms could be species-specific to *L. salmonis* or a generalized response, the low survival of lice attached to chinook salmon indicates a level of resistance to infestation. Thus, from the results of the present and prior studies, initial infestation in chinook salmon is not indicative of how many lice will progress to the adult stage. Mortality rates of attached lice will lead to short-term effects of infestation in chinook, whilst their slower rate of development insinuates decreased virulence and lice propensity for reproduction.

Resistance to disease or parasites is often a trade-off between risk of infection and energy investment for defense mechanisms, sometimes dictated by the interaction between host life-history traits and associated fitness strategies (Lee, 2006). For example, within the same genus, species of tadpoles with shorter life-spans invested more into short-term defense mechanisms against a parasite, such as avoidance and resistance, whilst longer-living species exhibited higher tolerance and almost no avoidance behaviours (Sears *et al.*, 2015). The difference in retention between Atlantic salmon and sea trout was found to be marginal in this study. This contrasts to their response to a freshwater nematode, whereby sea trout exhibited higher resistance to infection than Atlantic salmon (Klemme and Karvonen, 2017). Even though closely related, they have developed different trade-offs in parasite defense likely through selective pressures of their environment.

The effect of previous exposure: experienced vs. Naïve sea trout

We found no significant differences in evasion behaviour, number of acquired lice, lice retention, or developmental rate between experience and naïve trout. These results suggest that even though sea trout have the potential to be repeatedly infested during their coastal marine feeding migrations, previous exposure to lice does not preclude an increased immune response. The similarity in their evasive behavioural profile and lice retention is likely due to the broad-scale nature of their avoidance; detours into fresh water habitats for de-lousing are potentially energetically cheaper than acquiring physiological defences. Birkeland (1996) reported return to freshwater in previously unexposed, heavily-infected sea trout within 4 days of release, and sea return of the same individual after 20 h of successful delousing. However, longer stays in freshwater can have a higher energetic cost both in form of lost

feed and growth opportunities, and even negative growth; sea trout body mass can decrease by 23.5% in 37 days when recovering in freshwater from severe infestation levels (Birkeland and Jakobsen, 1997). As salmon lice get harder to shed with advancing stages (up to 8 days for the chalimus stages and older; Wright *et al.*, 2016), and motile stages cause considerably more damage and osmoregulatory issues to their host (Jónsdóttir *et al.*, 1992), selection would lean towards frequent freshwater forays to rapidly remove attached lice, before fitness is reduced from the severity of infestation.

Conclusions

Although these results stem from infestation in an artificial environment and single population samples, they provide evidence for a pattern of differences amongst these species. The variation in behaviour and susceptibility suggests that the length and intensity of parasite infection pressure potentially drives the species' investment choices in defense traits (see Miller *et al.*, 2007; Johnson *et al.*, 2012). It appears that sea trout are more susceptible hosts than Atlantic salmon, which may have negative consequences for trout populations that exist in environments with high infestation pressure. Although sea trout may rely on freshwater forays to control heavy infestation levels, this could affect their growth potential at sea. Further, infected sea trout may function as a reservoir for lice, increasing the infestation potential both for wild and domesticated salmon in the sea.

The lice developed quickly on Atlantic salmon, suggesting that long-term contributions of infective stages of lice will be higher for those populations compared with others attached to chinook salmon, depending on the temperature and salinity. The implications of more rapid development are further amplified through the increased reproductive output of female lice attached to Atlantic salmon, whereby egg production is approximately double that of lice attached to chinook salmon (Johnson, 1993). Thus, evidence suggests that lice populations from Atlantic salmon hosts will contribute more to the proliferation and success of the parasite. Less is known about the virulence of lice on sea trout hosts, however with the negligible loss of attached lice and similar development rate as in Atlantic salmon observed in this study, the effect of the sea trout pool of lice populations is expected to be analogous.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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References

Adelman, J. S., and Hawley, D. M. 2017. Tolerance of infection: a role for animal behavior, potential immune mechanisms, and consequences for parasite transmission. Hormones and Behavior, 88: 79–86.

Birkeland, K. 1996. Consequences of premature return by sea trout (*Salmo trutta*) infested with the salmon louse (*Lepeophtheirus salmonis* Krøyer): migration, growth, and mortality. Canadian Journal of Fisheries and Aquatic Sciences, 53: 2808–2813.

Birkeland, K., and Jakobsen, P. J. 1997. Salmon lice, *Lepeophtheirus salmonis*, infestation as a causal agent of premature return to rivers and estuaries by sea trout, *Salmo trutta*, juveniles. Environmental Biology of Fishes, 49: 129–137.

Bjørn, P. A., Sivertsgård, R., Finstad, B., Nilsen, R., Serra-Llinares, R. M., and Kristoffersen, R. 2011. Area protection may reduce salmon louse infection risk to wild salmonids. Aquaculture Environment Interactions, 1: 233–244.

Boots, M., and Bowers, R. G. 1999. Three mechanisms of host resistance to microparasites-avoidance, recovery and tolerance-show different evolutionary dynamics. Journal of Theoretical Biology, 201: 13–23.

Boots, M., and Haraguchi, Y. 1999. The evolution of costly resistance in host–parasite systems. The American Naturalist, 153: 359–370.

Bui, S., Dalvin, S., Dempster, T., Skulstad, O. F., Edvardsen, R. B., Wargelius, A., and Oppedal, F. 2017a. Origin matters: susceptibility, behaviour, and parasite retainment of Atlantic salmon to the salmon louse (*Lepeophtheirus salmonis*). Journal of Fish Diseases, in press.

Bui, S., Oppedal, F., Samsing, F., and Dempster, T. 2017b. Behaviour in Atlantic salmon confers protection against an ectoparasite. Journal of Zoology, in press.

Daly, E. W., and Johnson, P. T. 2011. Beyond immunity: quantifying the effects of host anti-parasite behavior on parasite transmission. Oecologia, 165: 1043–1050.

Davidsen, J. G., Plantalech Manel-la, N., Økland, F., Diserud, O. H., Thorstad, E. B., Finstad, B., Sivertsgård, R., *et al.* 2008. Changes in swimming depths of Atlantic salmon *Salmo salar* post-smolts relative to light intensity. Journal of Fish Biology, 73: 1065–1074.

Dawson, L. H. J., Pike, A. W., Houlihan, D. F., and McVicar, A. H. 1997. Comparison of the susceptibility of sea trout (*Salmo trutta* L.) and Atlantic salmon (*Salmo salar* L.) to sea lice (*Lepeophtheirus salmonis* (Krøyer, 1837)) infections. ICES Journal of Marine Science, 54: 1129–1139.

Ezenwa, V. O., Archie, E. A., Craft, M. E., Hawley, D. M., Martin, L. B., Moore, J., and White, L. 2016. Host behaviour-parasite feedback: an essential link between animal behaviour and disease ecology. Proceedings. Biological Sciences, 283: 20153078.

Fast, M. D., Ross, N. W., Mustafa, A., Sims, D. E., Johnson, S. C., Conboy, G. A., Speare, D. J., *et al.* 2002. Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice. Diseases of Aquatic Organisms, 52: 57–68.

Fevolden, S. E., Nordmo, R., Refstie, T., and Røed, K. H. 1993. Disease resistance in Atlantic salmon (*Salmo salar*) selected for high or low responses to stress. Aquaculture, 109: 215–224.

Frederick, C., Brady, D. C., and Bricknell, I. 2017. Landing strips: model development for estimating body surface area of farmed Atlantic salmon (*Salmo salar*). Aquaculture, 473: 299–302.

Furevik, D. M., Bjordal, Å., Huse, I., and Fernö, A. 1993. Surface activity of Atlantic salmon (*Salmo salar* L.) in net pens. Aquaculture, 110: 119–128.

Gjelland, K. Ø., Serra-Llinares, R. M., Hedger, R. D., Arechavala-Lopez, P., Nilsen, R., Finstad, B., Uglem, I., *et al.* 2014. Effects of salmon lice infection on the behaviour of sea trout in the marine phase. Aquaculture Environment Interactions, 5: 221–233.

Glover, K. 2003. Differing susceptibility of anadromous brown trout (*Salmo trutta* L.) populations to salmon louse (*Lepeophtheirus salmonis* (Krøyer, 1837)) infection. ICES Journal of Marine Science, 60: 1139–1148.

Glover, K., Nilsen, F., Skaala, Ø., Taggart, J., and Teale, A. J. 2001. Differences in susceptibility to sea lice infection between a sea run

and a freshwater resident population of brown trout. Journal of Fish Biology, 59: 1512–1519.

Glover, K. A., Aasmundstad, T., Nilsen, F., Storset, A., and Skaala, Ø. 2005. Variation of Atlantic salmon families (*Salmo salar* L.) in susceptibility to the sea lice *Lepeophtheirus salmonis* and *Caligus elongatus*. Aquaculture, 245: 19–30.

Glover, K. A., Hamre, L. A., Skaala, Ø., and Nilsen, F. 2004a. A comparison of sea louse (*Lepeophtheirus salmonis*) infection levels in farmed and wild Atlantic salmon (*Salmo salar* L.) stocks. Aquaculture, 232: 41–52.

Glover, K. A., Nilsen, F., and Skaala, Ø. 2004b. Individual variation in sea lice (*Lepeophtheirus salmonis*) infection on Atlantic salmon (*Salmo salar*). Aquaculture, 241: 701–709.

Hamre, L. A., Glover, K. A., and Nilsen, F. 2009. Establishment and characterisation of salmon louse (*Lepeophtheirus salmonis* (Kroyer 1837)) laboratory strains. Parasitology International, 58: 451–460.

Hart, B. L. 1992. Behavioral adaptations to parasitism: an ethological approach. The Journal of Parasitology, 78: 256–265.

Healy, M. C. 1991. Life history of chinook salmon (*Oncorhynchus tshawytscha*). *In* Pacific Salmon Life Histories, pp. 311–394. Ed. by C. Groot, and L. Margolis. UBC Press, Vancouver, Canada.

Johnson, P. T., Rohr, J. R., Hoverman, J. T., Kellermanns, E., Bowerman, J., and Lunde, K. B. 2012. Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. Ecology Letters, 15: 235–242.

Johnson, S. C. 1993. A comparison of development and growth rates of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on naive Atlantic (*Salmo salar*) and chinook (*Oncorhynchus tshawytscha*) salmon. *In* Pathogens of Wild and Farmed Fish: Sea Lice, pp. 68–82. Ed. by G. Boxshall, and D. Defaye. Ellis Horwood, London.

Johnson, S. C. 2006. A comparison of development and growth rates of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on naive Atlantic (*Salmo salar*) and chinook (*Oncorhynchus tshawytscha*) salmon. *In* Pathogens of Wild and Farmed Fish: Sea Lice. Ed. by G. Boxshall, and D. Defaye. CRC Press.

Johnson, S. C., and Albright, L. J. 1991. Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. Journal of the Marine Biological Association of the United Kingdom, 71: 425–436.

Johnson, S. C., and Albright, L. J. 1992. Comparative susceptibility and histopathology of the response of naïve Atlantic, Chinook and coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). Diseases of Aquatic Organisms, 14: 179–193.

Jónsdóttir, H., Bron, J. E., Wootten, R., and Turnbull, J. F. 1992. The histopathology associated with the pre-adult and adult stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases, 15: 521–527.

Karvonen, A., Seppälvä, O., and Valtonen, E. T. 2004. Parasite resistance and avoidance behaviour in preventing eye fluke infections in fish. Parasitology, 129: 159–164.

Kittilsen, S., Johansen, I. B., Braastad, B. O., and Overli, O. 2012. Pigments, parasites and personality: towards a unifying role for steroid hormones? PLoS ONE, 7: e34281.

Klemetsen, A., Amundsen, P-A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F., and Mortensen, E. 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. Ecology of Freshwater Fish, 12: 1–59.

Klemme, I., and Karvonen, A. 2016. Learned parasite avoidance is driven by host personality and resistance to infection in a fish-trematode interaction. Proceedings. Biological Sciences, 283: 20161148.

Klemme, I., and Karvonen, A. 2017. Vertebrate defense against parasites: interactions between avoidance, resistance, and tolerance. Ecology and Evolution, 7: 561–571.

Krkosek, 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: 1772–1775.

Lee, K. A. 2006. Linking immune defenses and life history at the levels of the individual and the species. Integrative and Comparative Biology, 46: 1000–1015.

MacKinnon, B. M. 1998. Host factors important in sea lice infections. ICES Journal of Marine Science, 55: 188–192.

Miller, K. M., Teffer, A., Tucker, S., Li, S., Schulze, A. D., Trudel, M., Juanes, F., *et al.* 2014. Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evolutionary Applications, 7: 812–855.

Miller, M. R., White, A., and Boots, M. 2007. Host life span and the evolution of resistance characteristics. Evolution, 61: 2–14.

Moore, J. 2002. Parasites and the Behavior of Animals, Oxford University Press, New York, NY.

Nagasawa, K. 1987. Prevalence and abundance of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on high-seas salmon and trout in the North Pacific Ocean. Nippon Suisan Gakk, 53: 2151–2156.

Plantalech Manel-La, N., Thorstad, E. B., Davidsen, J. G., Økland, F., Sivertsgård, R., McKinley, R. S., and Finstad, B. 2009. Vertical movements of Atlantic salmon post-smolts relative to measures of salinity and water temperature during the first phase of the marine migration. Fisheries Management and Ecology, 16: 147–154.

R Development Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Råberg, L., Graham, A. L., and Read, A. F. 2009. Decomposing health: tolerance and resistance to parasites in animals. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 364: 37–49.

Sears, B. F., Snyder, P. W., and Rohr, J. R. 2013. Infection deflection: hosts control parasite location with behaviour to improve tolerance. Proceedings of the Royal Society of London B: Biological Sciences, 280: 20130759.

Sears, B. F., Snyder, P. W., and Rohr, J. R. 2015. Host life history and host–parasite syntopy predict behavioural resistance and tolerance of parasites. Journal of Animal Ecology, 84: 625–636.

Serra-Llinares, R. M., Bjørn, P. A., Finstad, B., Nilsen, R., Harbitz, A., Berg, M., and Asplin, L. 2014. Salmon lice infection on wild salmonids in marine protected areas: an evaluation of the Norwegian 'National Salmon Fjords'. Aquaculture Environment Interactions, 5: 1–16.

Shephard, S., and Gargan, P. 2017. Quantifying the contribution of sea lice from aquaculture to declining annual returns in a wild Atlantic salmon population. Aquaculture Environment Interactions, 9: 181–192.

Shephard, S., MacIntyre, C., and Gargan, P. 2016. Aquaculture and environmental drivers of salmon lice infestation and body condition in sea trout. Aquaculture Environment Interactions, 8: 597–610.

Thorstad, E. B., Todd, C. D., Uglem, I., Bjørn, P. A., Gargan, P. G., Vollset, K. W., and Halttunen, E. 2015. Effects of salmon lice *Lepeophtheirus salmonis* on wild sea trout *Salmo trutta*—a literature review. Aquaculture Environment Interactions, 7: 91–113.

Thorstad, E. B., Todd, C. D., Uglem, I., Bjørn, P. A., Gargan, P. G., Vollset, K. W., Halttunen, E., *et al.* 2016. Marine life of the sea trout. Marine Biology, 163: 47.

Thorstad, E. B., Whoriskey, F., Uglem, I., Moore, A., Rikardsen, A. H., and Finstad, B. 2012. A critical life stage of the Atlantic salmon *Salmo salar*: behaviour and survival during the smolt and initial post-smolt migration. Journal of Fish Biology, 81: 500–542.

Tucker, C. S., Sommerville, C., and Wootten, R. 2000. The effect of temperature and salinity on the settlement and survival of

copepodids of *Lepeophtheirus salmonis* (Krøyer, 1837) on Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases, 23: 309–320.

Vollset, K. W., Krontveit, R. I., Jansen, P. A., Finstad, B., Barlaup, B. T., Skilbrei, O. T., Krkošek, M., *et al.* 2016. Impacts of parasites on marine survival of Atlantic salmon: a meta-analysis. Fish and Fisheries, 17: 714–730.

Wagner, G. N., Fast, M. D., and Johnson, S. C. 2008. Physiology and immunology of *Lepeophtheirus salmonis* infections of salmonids. Trends in Parasitology, 24: 176–183.

Wells, A., Grierson, C. E., MacKenzie, M., Russon, I. J., Reinardy, H., Middlemiss, C., Bjørn, P. A., *et al.* 2006. Physiological effects of simultaneous, abrupt seawater entry and sea lice (*Lepeophtheirus salmonis*) infestation of wild, sea-run brown trout (*Salmo trutta*) smolts. Canadian Journal of Fisheries and Aquatic Sciences, 63: 2809–2821.

Wright, D. W., Oppedal, F., and Dempster, T. 2016. Early-stage sea lice recruits on Atlantic salmon are freshwater sensitive. Journal of Fish Diseases, 39: 1179–1186.

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