

Effects of laboratory salmon louse infection on mortality, growth, and sexual maturation in Atlantic salmon

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Elevated salmon louse infection pressure generated by salmon farming represents a major threat for wild Atlantic salmon (*Salmo salar*). This study explored the effect of salmon lice on mortality, and body and gonad growth in F1 wild Atlantic salmon. Mature males (jacks) and immature fish were either infected with two different louse infection intensities (LIIs, 0.08 or 0.35 lice g⁻¹) or kept as uninfected controls. Fish maturation was thereafter environmentally stimulated in seawater, followed by transfer to freshwater for 38 d to simulate river ascendance. No females matured, while 99% of the initially immature males started puberty. Jacks had high, and immature and maturing fish low, seawater mortality, independent of lice. The parasites had an LII-dependent negative effect on growth in length, weight, and condition factor in seawater. In freshwater, after the lice had detached, fish that were previously infected in seawater had reduced growth in length but not weight when compared to the uninfected control. The parasites did not affect relative gonad size in any fish phenotypes. The present results show that Atlantic salmon has a complex, and unexplored, regulation of growth when recovering from lice infection under laboratory settings. Further, the results suggest that possible negative effects of salmon louse on reproductive success in Atlantic salmon is most likely governed by the reduced body size and condition. However, further work on possible effects of salmon louse on semen quality is encouraged.

Keywords: aquaculture, Atlantic salmon, growth, mortality, salmon louse, sexual maturation, wild.

Introduction

There are about 100000 parasite species (Rodhe, 2002) distributed among over 14000 marine fish species globally (Nelson, 1994). These parasites can have different negative effects on their host and host populations, such as increased mortality and reduced growth, condition, and reproductive success (Sindermann, 1987; Lloret *et al.*, 2012; Ryberg *et al.*, 2020; 2021). These negative effects can greatly impact animals in production systems and those that coexist in ecosystems with farming activities.

Increased production of farmed anadromous Atlantic salmon (*Salmo salar*) (ICES, 2020) has caused an elevated infection pressure of the ectoparasitic copepod salmon louse (*Lepeophtheirus salmonis*) on their wild conspecifics (Dempster *et al.*, 2021). In parallel, the abundance of wild Atlantic salmon has declined, partially attributed to the rise of salmon lice due to farming (Parrish *et al.*, 1998; Krkosek *et al.*, 2007; Chaput, 2012). Mature female lice attached to farmed salmon in sea cages produce and release larvae into the surrounding environment continuously throughout the year (Costello, 2009; Taranger *et al.*, 2015; Forseth *et al.*, 2017). These planktonic larval stages of lice drift with the water currents, and are distributed along the Norwegian coast and inside the fjords (Myksvoll *et al.*, 2018; Asplin *et al.*, 2020). The free-swimming larvae progress through two non-infectious nauplii stages before reaching the infective copepodid stage (Hamre *et al.*, 2013). After the infective copepodids attach to the host fish, they are fixed in the same position while moulting through a further two developmental stages to reach the pre-adult stage,

whereby the louse changes morphology so that they can move around the host to seek better resources (Bjørn and Finstad, 1998; Hamre *et al.*, 2013). At this stage, the louse's feeding behaviour causes skin damage in the host, which may eventually result in open wounds and secondary bacterial infections if infection intensity is high (Jónsdóttir *et al.*, 1992; Wagner *et al.*, 2008; Jones and Beamish, 2011). High-infection intensity may also cause high circulating cortisol, ionic imbalance, increased mortality, and reduced growth and condition both with and without creating open wounds, when the lice reach the pre-adult stage (Grimnes and Jakobsen, 1996; Fjelldal *et al.*, 2020). The negative effect of salmon louse on growth rate, condition, and survival is worsened with increasing temperature (Godwin *et al.*, 2020), which also decreases and increases lice-mediated changes in relative liver and heart size, respectively (Medcalf *et al.*, 2021). Some subclinical physiological consequences of louse infection are changes in blood glucose (Bowers *et al.*, 2000) and reduced swimming performance (Wagner *et al.*, 2003; Bui *et al.*, 2016).

Severe louse infections on outward migrating wild Atlantic post-smolts increase the likelihood of seawater mortality (Thorstad *et al.*, 2012; Shephard and Gargan, 2017; Bøhn *et al.*, 2020), while more moderate infections may cause reduced growth and condition without increasing mortality; indeed, a negative correlation between louse infection level and body condition has been reported in river-running wild Atlantic salmon (Susdorf *et al.*, 2018a). Modelling has estimated that louse-mediated changes in body condition can cause population declines in wild Atlantic salmon due to changes in

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marine survival, fecundity, and age at sexual maturation (Sustorf *et al.*, 2018b). Furthermore, Vollset *et al.* (2014) compared the return rate of hatchery-reared Atlantic salmon smolts that were unmanipulated or pre-treated for protection against salmon lice, and concluded that infection increased the age at maturity of returning adult Atlantic salmon, either by affecting age at puberty or through disproportionately increased mortality in early maturing fish. However, modelling showed that although delayed maturity may partially compensate for reduced survival, the impact of delayed maturity on population growth is negligible compared to effects of direct mortality (Vollset and Krkosek, 2021).

Salmon lice are naturally occurring parasites and are prevalent on ~100% of the wild homing Atlantic salmon (Torrissen *et al.*, 2013). This level of prevalence was evident even before the beginning of salmon aquaculture (Johannessen, 1975); however, abundances on wild homing salmon have now increased due to the elevated infection pressure from intensive farming (Dempster *et al.*, 2021). Salmon lice can survive on Atlantic salmon for over 1 year in laboratory settings (Hamre *et al.*, 2009); thus, adult lice on homing fish may have attached as copepodids during outward post-smolt migration, particularly in one-sea-winter fish (grilse). The presence of chalimus and pre-adult stage lice on wild Atlantic salmon caught by floating long-lines in the open ocean, with higher numbers in two-sea-winter compared to one-sea-winter fish, indicates that infection also occurs in the ocean (Jacobsen and Gaard, 1997). Further, Fjørtoft *et al.*, (2019) investigated the role of wild salmonids as vectors of pyrethroid-resistant salmon lice, and found that homing wild Atlantic salmon carried less resistant lice than wild sea trout and farmed salmon, which may also indicate some infection in the ocean. However, salmon lice on wild adult Atlantic salmon caught offshore are predominately adults, while chalimus stages are more frequently observed on wild adult Atlantic salmon captured inshore (reviewed in Torrissen *et al.*, 2013), suggesting re-infection of homing fish once they enter coastal waters or fjords. In a study conducted in 1973 in western Norway, before the negative effects of salmon farming became apparent, homing wild salmon caught along the coast had an average of 10 lice per fish, predominantly made up of adult females and pre-adults (Johannessen, 1975). Salmon that migrate to rivers located in fjords may use several days during the fjord migration alone (Hansen *et al.*, 1993). Jonsson *et al.* (1990) studied the homing of Atlantic salmon to River Imsa in southwestern Norway, and found that one-sea-winter fish were first registered in June both at the coast and ascending River Imsa, while older fish were first registered in April at the coast and in July when ascending the river. With these periods of occupying areas where infection risk can be high, re-infection of homing salmon in coastal waters and the development of pre-adult lice before river ascendance (Hamre *et al.*, 2019) is a possible scenario. To our knowledge, there are no experimental studies on salmon louse effects on sexual maturation in Atlantic salmon, and there is no evidence for possible effects of louse re-infection on homing maturing Atlantic salmon.

This study investigates this potential effect in an experimental setting, using sexually mature male (jacks, i.e. Fjellidal *et al.*, 2007) and immature male and female F1 wild Atlantic salmon infected with salmon louse copepodids, to determine whether low or high infection levels influenced maturation status, growth, condition factor, or gonad size.

Material and methods

In brief, this experiment used sexually mature males, and immature male and female Atlantic salmon (of wild origin) infected at two different infection intensities (0.08 and 0.35 lice g^{-1}). The onset of puberty was stimulated in infected immature fish by introducing a maturation-inducing environmental regime (16°C and LD24:0; Fjellidal *et al.*, 2011) when the lice had reached the chalimus 1 stage. When the lice reached the pre-adult stage, water salinity was changed from salt- to freshwater to simulate river ascendance. Maturation status was inspected after 38 d in freshwater. The louse infection was applied in November; following a normal maturation cycle, Atlantic salmon are either fully mature or immature, without the presence of maturing fish, at this time of the year. Hence, it is an ideal timepoint to study immature and mature fish, or artificially stimulate puberty in immature fish. The study consisted of quadruplicated groups of Atlantic salmon post-smolts, either infected with two different louse infection intensities or kept as uninfected controls. Response parameters were mortality, growth in length and weight, condition factor, and gonad size.

Fish stock

The Atlantic salmon used in the present experiment were first-generation wild origin fish brought to the Institute of Marine Research as eyed eggs in January 2016, originating from wild caught Atlantic salmon (male and female) from River Etne, Vestland county, western Norway. First feeding was in April 2017. The fish were reared in a flow-through system under continuous light and constant 12°C until the summer solstice, when the temperature was changed to ambient based on the local river source. The oxygen saturation of the outlet water was always >80% and water salinity was steady at 0.8 ppt. Photoperiod was changed from continuous light to simulated natural light (western Norway, 60°N 5°E) in October 2017. In May 2018, 500 fish were PIT-tagged (Glass tag 2, 12 mm, TrackID AS, Stavanger, Norway) and transferred to eight seawater tanks (1 × 1 m, n fish = 63 per tank) (9°C) as yearling (1+) smolts under a simulated natural photoperiod.

Ethical statement

All experiments were performed at the Institute of Marine Research (IMR), Matre Research Station (60°N 5°E, western Norway), which is authorized for animal experimentation (Norwegian Food Safety Authority, facility 110) in accordance with international guidelines, certified using Norwegian research permit number 14982.

Experimental setup

Salmon lice copepodids that were used for the infection challenge were produced according to standard methods (Hamre *et al.*, 2009) at ~8°C, sourced from IMR's in-house production stock. Lice were transferred to the experimental facilities in cooler boxes, and infection challenges occurred on the same day as lice were transferred.

On 6 November 2018 (day 0), 480 salmon post-smolts (~280 g) were sedated (0.1 g l^{-1} tricaine methanesulfonate; Finquel MS-222), measured for fork length and body weight, checked for maturity status based on external examination, and randomly distributed between twelve 1 × 1 m seawater

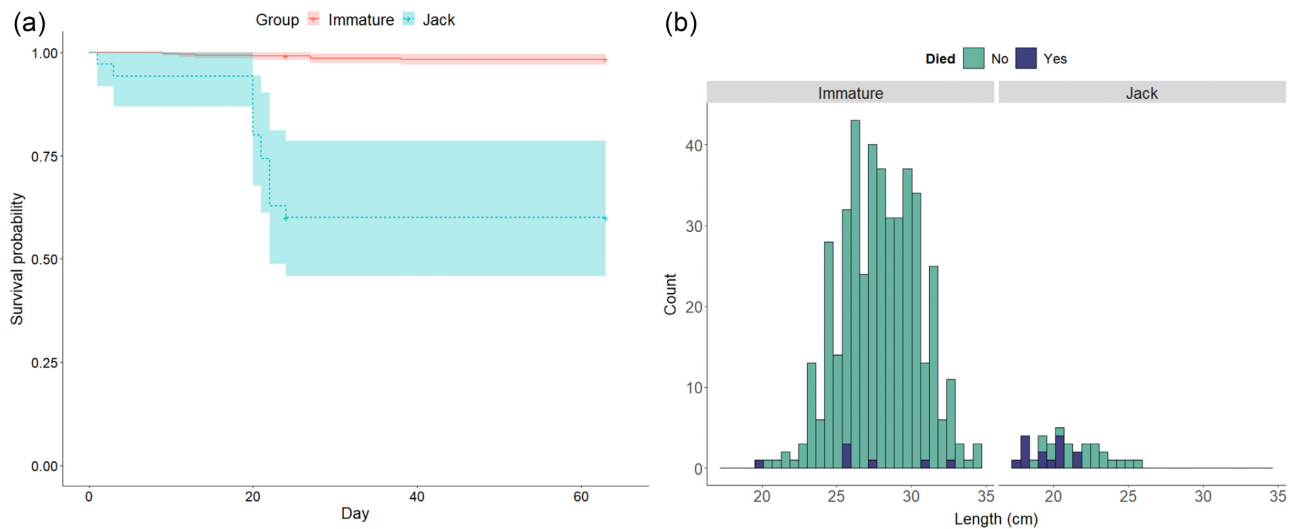


Figure 1. (a) Survival plot relating to the reproductive phenotype on day 0 in Atlantic salmon initially reared on 9°C seawater and natural photoperiod, then 16°C seawater and constant light from day 15, and finally, 16°C freshwater and constant light from day 24. The data are from a Cox proportional hazards model with a random effect and include the predicted mean \pm 95% CI band. (b) Histogram showing the relationship between size and reproductive phenotype on day 0, and whether the fish died during the duration of the experiment.

tanks ($n = 40$ per tank; 9°C). All tanks had equal water supply, flow rate, temperature, feeding rate, photoperiod, and light intensity. Fish with a mature parr phenotype and/or running milt were categorized as jacks ($n = 18$), while fish with a silvery immature phenotype without running milt were categorized as immature (Fjelldal *et al.*, 2011). Jacks and immature fish were reared in a common garden in the same tanks. On 15 November (day 9), eight of the tanks were infected with salmon lice (*L. salmonis*) copepodids, while four tanks remained as uninfected controls. In all 12 tanks (8 infected, 4 uninfected), the water level was reduced to half, and water flow was stopped before adding copepodids (10 d post-hatch) to the 8 infection tanks. Then, in all tanks, the water flow was turned back to the original level after 20 min. Four tanks received 2600 copepodids per tank (65 lice per fish), while the remaining four tanks received 7700 copepodids per tank (193 lice per fish), with an expected infection success of 50% (Dalvin *et al.*, 2020), creating low- and high-intensity infection groups. The present louse levels were selected based on earlier experimental work, and the level known to occur in wild Atlantic salmon (Fjelldal *et al.*, 2020). Under laboratory settings, infection intensities >0.2 pre-adult lice g^{-1} may cause physiological consequences (Fjelldal *et al.*, 2020). The national surveillance programme of salmon lice on wild salmonids (NALO) catches wild salmonids using traps or nets along the Norwegian coast yearly. Of the 2501 wild Atlantic salmon postsmolts captured in 2019 in the NALO project (Nilsen *et al.*, 2019), 31% were infected with salmon lice. Among the seven regions studied, and infected fish, estimated mean lice infection intensity ranged from 0.06 to 0.67 lice g^{-1} , where two regions had >0.5 lice g^{-1} (0.51 and 0.68 lice g^{-1}), while five regions had estimated infection intensities <0.2 lice g^{-1} (0.06–0.19 lice g^{-1}). On 21 November (day 15), in order to stimulate puberty in immature fish, photoperiod was shifted from simulated natural to continuous light, and water temperature was gradually increased to 16°C over a 5 d period (Figure 1). The maturation-stimulating regime was introduced 6 d post-infection when the lice were at the chalimus 1 stage of development (Hamre *et al.*, 2019), which is before the mo-

bile stage (pre-adult I) when severe adverse physiological effects of lice can arise (Grimnes and Jakobsen, 1996; Bowers *et al.*, 2000; Finstad *et al.*, 2000). The lice were then allowed to develop on the fish until they reached the pre-adult stage, at which point the fish had been under the maturation regime for 9 d (day 24). We did not inspect for maturity status at this point herein, but we have unpublished results from other studies showing that onset of male puberty is synchronous and evident within 9 d, as shown by elevated gonadosomatic index (GSI) and plasma 11-ketotestosterone, under the currently used maturation regime. Lice numbers on fish were counted 15 d post-infection on 30 November (day 24). The number of degree days from infection to sample day was 200. Sampling involved netting one fish at a time from their respective tanks, applying sedation (0.01 g l^{-1} metomidate hydrochloride; Aquacalm vet., Scan Aqua AS, Årnes, Norway), recording the PIT tag, measuring fork length and body weight, checking maturity status, and counting lice. Fish were sedated in individual anaesthetic baths, and therefore any lice that detached in the bath were counted towards the lice count of that fish. By the time of sampling, mobile pre-adult II male, and pre-adult I and II female stages had developed (Hamre *et al.*, 2019). Because of the synchronized infection challenge across groups, only louse abundance and not the stages were quantified. In addition to the lice on the fish, there were also lice attached to the tank walls, so the number of lice per tank was also counted. All fish subjected to lice infection on day 9 had lice on day 24, i.e. there was 100% infection prevalence. The fish were allowed to recover from anaesthesia for 10 min in a separate tank with oxygenated water before transferring back to the rearing tank. After the sampling, the tank water was changed to freshwater, while temperature and photoperiod remained unchanged. Mean values for water salinity were 34.53 (min 33.72, max 34.88) and 0.85 ppt (min 0.56, max 0.89) in sea- and freshwater, respectively. The fish were reared under these conditions until 7 January 2019 (day 62) when the experiment was terminated, and a final freshwater sampling was performed once the fish were euthanized by an overdose with anesthetic (0.5 g l^{-1} tricaine methanesulfonate; Finquel

MS-222). At this point, PIT tag number, maturity phenotype, sex, gonad weight, and body length and weight were recorded. All fish were dissected. Males were categorized as either jacks, maturing or immature based on the external morphology of the fish and gonad, and GSI: (i) jack: parr phenotype and white coloured mature testis with running milt and elevated GSI; (ii) maturing male: immature body phenotype and pink coloured maturing testis with elevated GSI, without running milt; and (iii) immature male: immature phenotype of both body and testis as well as low GSI.

The outline of the experiment and environmental data are presented in Supplementary Figure S1.

Calculations and statistical analysis

Infection intensity (II) was calculated using $II = LnFw^{-1}$, where Ln is the number of lice on infected fish and Fw is the body weight (g) of infected fish at the time of counting lice on day 24.

The condition factor (CF) was calculated using $CF = (FwFl^{-3})100$, where Fw is body weight (g) and Fl is fork length (cm).

The gonadosomatic index (GSI) was calculated using $GSI = (Gw*100)Fw^{-1}$, where Gw is gonad weight and Fw is body weight.

The data were transferred to the R Statistical software (version 4.0.4, R Core Team, 2021) for all analyses. The packages “nlme” (Pinheiro *et al.*, 2019), “MuMIn” (Barton 2019), “coxme” (Therneau, 2019), “survminer” (Kassambara *et al.*, 2021), “emmeans” (Lenth, 2021), and “ggplot2” (Wickham, 2016) were used for analysis and graphical presentation. Throughout, model diagnostics were assessed via q-q plots and standardized vs. predicted residual plots. When comparing models, we considered the model with the lowest Akaike information criterion (AICc) to best describe the data when weighted against complexity (Hurvich and Tsai, 1991). The one immature male was excluded from all analyses.

We fitted ten linear mixed-effects (LME) models to the lice count and density data (Supplementary Tables S1–S4). The most complex model included fixed effects for treatment (two levels, low or high), phenotype (three levels, immature females, maturing males, jacks), weight (from day 0, continuous), and the interaction between treatment and phenotype. All models included tank as a random effect on the intercept and lice count was natural log-transformed to remove heteroskedasticity in the model residuals.

We fitted 18 Cox proportional hazards models to the survival data (Supplementary Tables S5–S6). As the sex of the fish that died was not determined, we classified fish only as immature or jack. The most complex model included fixed effects for treatment (three levels, control, low, or high), phenotype (two levels, immature or jack), and length (from day 0, continuous), all possible interactions, and tank as a random effect.

We fitted ten LME models to the body weight, length, and condition data from day 24 (Supplementary Tables S7–S12). The most complex included fixed effects for treatment (three levels), phenotype (three levels), initial body weight/length/condition (day 0), all possible interactions, and tank as a random effect on the intercept. Model selection was performed in a way that all three fixed effects should be included. The same approach was used for body weight, length, and condition on day 63 (Supplementary Tables S13–S18) while controlling for body weight/length/condition on day 24.

For the body-weight data, we used the $\text{weight} = \text{varPower}()$ function to correct for heteroskedasticity, whereas the length data on day 24 were natural log-transformed to ensure normality, of the residuals.

The residuals from LME models using GSI or gonad weight were heteroskedastic, not normally distributed, and could not be improved by transformation. Therefore, we used the residuals from an LME model that had gonad weight (natural logged) as the dependent variable, the interaction between the fixed effects weight (on day 63, natural logged) and phenotype (three levels), and tank as a random effect. The most complex model included phenotype (three levels) and treatment (three levels) as fixed effects, their interaction, and tank as a random effect on the intercept (Supplementary Table S19).

Results

Infection intensity on day 24

There was 100% prevalence in both treatment groups. For both the number of lice per fish and the infection intensity, the model with the most support included treatment and weight, but not phenotype (Supplementary Tables S1–S4). The number of lice per fish and infection intensity were greater in the high- vs. low-infection groups (*post-hoc*, least-squares means lice count; 95% CI: low, 20.5, 12.9–32.4, and high, 90.5, 56.3–145.5; infection intensity: low, 0.06, 0.04–11, and high, 0.32, 0.20–0.51). For lice count, there was a positive association with weight (LME, $\beta = 0.004$, $df = 253$, $t = 10.4$, $p \leq 0.001$), but a negative association for infection intensity (LME, $\beta = -0.001$, $df = 253$, $t = -2.1$, $p = 0.039$).

At the lice counting on day 24, on average 34 and 359 lice were attached to the tank wall in the low- and high-intensity tanks, respectively (Table 1). Mean infection intensity (lice g^{-1}) in the four low-infection tanks ranged between 0.03 and 0.11, and between 0.33 and 0.36 in the four high-infection tanks (Table 1).

Mortality

Dead fish were categorized as jacks or immature fish based on external phenotype and/or presence of running milt (not dissected). A total of 21 fish (4% of the total population) died during the experiment. Of these, 18 died during the lice challenge, whereas 3 died during the follow-up period in freshwater. The model with the lowest AICc score included the fixed-effects phenotype and length and their interaction, but not treatment (Supplementary Tables S5–S6). In total, 78% of the fish that died during the lice challenge were jacks (Figure 1a), whereas all those that died during the freshwater period were immature. Of those that died, smaller jacks were more likely to die than larger jacks, whereas size had no notable effect on survival in immatures (Figure 1b).

Lice effects on growth

The most appropriate model for length and weight during the infection period included treatment, phenotype, and their interactions with initial size (Supplementary Tables S7–S10). For body condition, the most supported model included the interaction between treatment and phenotype (Supplementary Tables S11–S12). Weight and length gain were reduced by sea lice infestation, with a stronger effect in the more infested treatment irrespective of phenotype (Figure 2a and b). Body con-

Table 1. Lice infection intensity and abundance (mean \pm SE) on day 24.

Tank no.	Infection	Infection intensity (lice g^{-1} fish)	Lice abundance (lice fish $^{-1}$)	Lice unattached (lice tank $^{-1}$)
1	High	0.35 \pm 0.02	96 \pm 6	412
2	High	0.36 \pm 0.02	100 \pm 7	349
3	High	0.35 \pm 0.02	97 \pm 7	350
4	High	0.33 \pm 0.03	105 \pm 11	323
5	Low	0.03 \pm 0.00	11 \pm 1	52
6	Low	0.09 \pm 0.01	29 \pm 2	7
7	Low	0.11 \pm 0.01	31 \pm 3	32
8	Low	0.10 \pm 0.01	30 \pm 2	44

Lice unattached are lice that were attached to the tank walls on day 24. Four tanks served as uninfected controls.

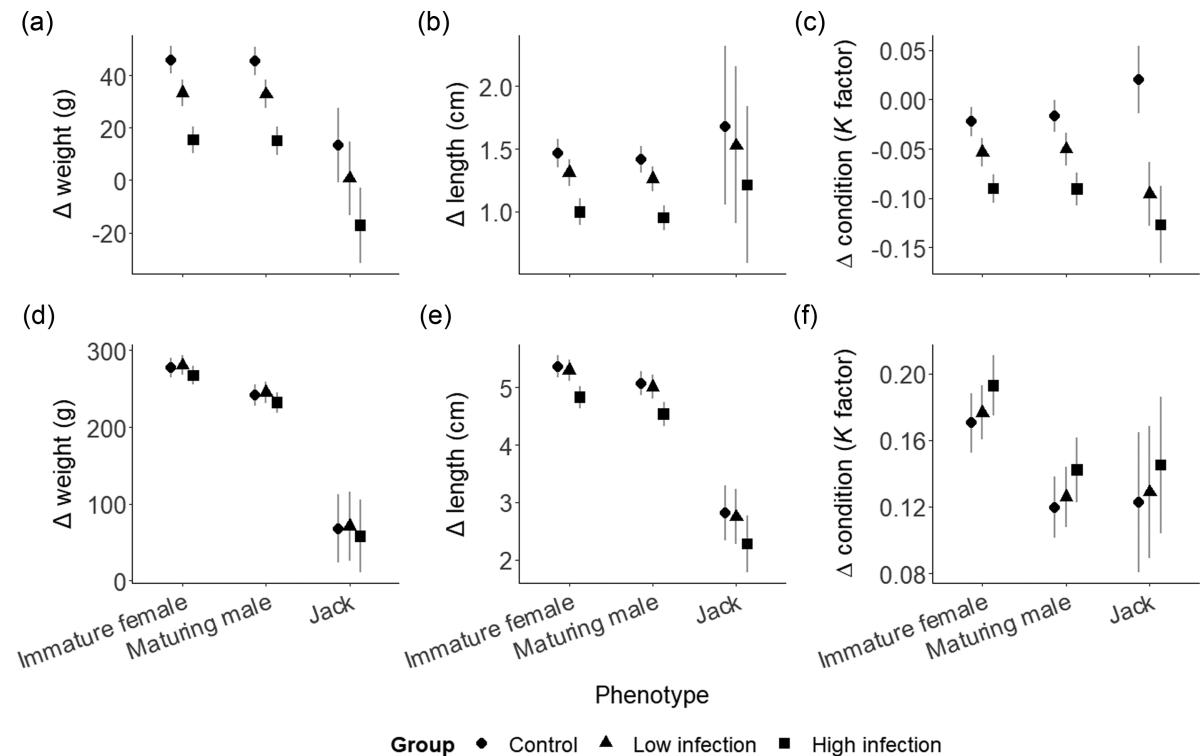


Figure 2. Predicted size and condition changes between days 0–24 (a)–(c) and days 24–63 (d)–(f). The weight (a), length (b), and condition increase (c) is standardized against the population mean (282 g, 27.61 cm, and 1.33 K factor, respectively) on day 0. The weight (d), length (e), and condition (f) increase is standardized against the population mean (318 g, 28.95 cm, and 1.28 K factor, respectively) on day 24.

condition generally dropped in all treatments, and this was magnified by lice infestation and more so in jacks compared to females and maturing males (Figure 2c).

Post infection, the most appropriate models included (i) phenotype and the interaction with initial size, (ii) fixed effects of treatment, phenotype, and initial size, and (iii) treatment and phenotype and their interactions with initial condition for weight, length, and condition, respectively (Supplementary Tables S13–S18). Weight gain was unrelated to treatment, but values were lower in jacks (Figure 2d). The high-infection group showed lower length growth than the control and low-infected fish (Figure 2e). All groups gained condition, but more so in the high-infected than low-infected and control fish (Figure 2f).

Sexual maturation

All females had an immature phenotype in terms of body and ovary morphology/coloration, as well as a low GSI (Figure

3). The percentage of males at the terminal sampling was 47 in the control group, 44 in the low-infection group, and 42 in the high-infection group. Among the sampled males, there were 23 jacks, 149 maturing, and 1 immature (Figure 3). The proportion of jacks and maturing males was equal between groups.

Of the 23 jacks, 18 and 22 of these were also registered as jacks on day 0 and 24, respectively, based on external phenotype and/or presence of running milt. All the 149 maturing males were registered as immature on days 0 and 24 based on external phenotype.

The most supported model suggests phenotype *per se* did not influence lice counts (Supplementary Table S1) or density (Supplementary Table S3). However, jacks were smaller (see above) and there was a positive association between weight and lice number (LME, $\beta = 0.003$, $df = 251$, $t = 9.3$). Therefore, without the size correction, phenotype was included in the most supported models for lice counts and density; jacks

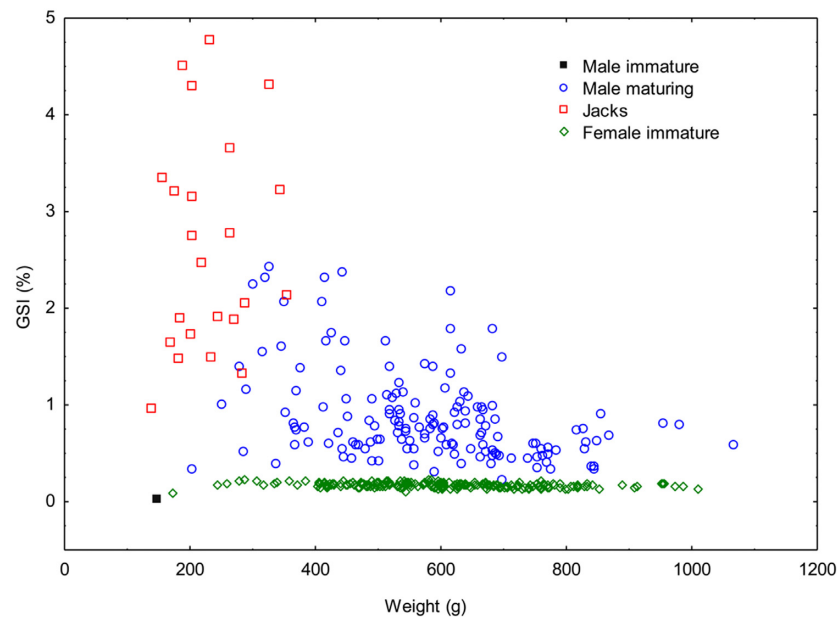


Figure 3. GSI plotted against body weight after 38 d in freshwater on day 62 (7 January). Data from treatment groups (control, low infection, and high infection) are pooled.

had fewer lice and a higher louse density than immature females and maturing males.

Louse effects on sexual maturation

Although there was a tendency for jacks and immature females to have negative trends between louse infection and relative gonad size, model selection provided no support for a treatment effect on relative gonad size (Supplementary Table S19).

Discussion

High seawater mortality in jacks

Jacks, which were already sexually mature at infection, had high mortality in seawater independent of louse infection. The mortality was most likely related to reduced hypoosmoregulatory ability caused by sexual maturation (reviewed in Taranger *et al.*, 2010).

There was no effect of reproductive phenotype on louse counts when correcting for body size in the present study. Johannessen *et al.* (1975) found high lice levels on sexually mature compared to immature farmed salmon in sea cages, while Krasnov *et al.* (2015) found that sexual maturation and administration of both 17β -estradiol and testosterone in immature fish increased the resistance of Atlantic salmon to salmon lice. Why the effect of sexual maturation on infection susceptibility differs between these studies is at present unclear.

Reduced growth but not relative testis size by louse infections in jacks

Jacks that were infected with salmon lice showed reduced growth, while there was no relationship between louse infection and relative testis size. In line with this observation, Skilbrei and Wennevik (2006) concluded that non-lethal infection levels of salmon louse may reduce growth and hence size at spawning in Atlantic salmon populations. Atlantic salmon

males have several strategies to get access to matings (Fleming, 1998) and body size is an important dominance factor, where large males win proximity to the female creating sperm precedence (Fleming *et al.*, 1997; Mjølnerød *et al.*, 1998). Moreover, testis size and volume of ejaculate increase with male size (Fleming, 1996). Hence, salmon louse could potentially have reduced the reproductive success of jacks in the present study, caused by reduced body size.

Salmon louse may also affect semen quality. In northern pike (*Esox lucius* L.), high seminal plasma osmolality reduces sperm motility (Alavi *et al.*, 2009). In Atlantic salmon, infection with salmon louse can increase blood plasma osmolality (Grimnes and Jakobsen, 1996; Fjellidal *et al.*, 2020), but the effect on seminal plasma osmolality is unknown. Wild maturing salmon may stay at the coast—where the louse infection pressure is highest—for a period before river ascendance (Jonsson *et al.*, 1990). Water flow is important for river ascent, and large salmon may wait until the autumn increase in water discharge before they enter the river, especially in smaller rivers (Jonsson, 1991). Thus, in years with dry summers, salmon may nearly complete sexual maturation in seawater, making a scenario with lice-induced increase in seminal plasma osmolality plausible. Further, lice-induced physiological changes may potentially affect earlier stages of testicular development with long-term effect on semen quality. This should be further investigated.

Low seawater mortality in maturing males and immature females—does fish size matter?

Excluding the jacks, total mortality from infection until the lice reached the pre-adult stage was 1.0% among infected fish. Fjellidal *et al.* (2020) conducted a laboratory louse infection trial on fish from the same cohort (not the same individuals) as used in the present study, when the fish had been in seawater for 27 d, and were on average 40 g. In that study, louse infection intensity was 0.38 lice g^{-1} (equal to the current high intensity group) and total mortality among infected fish was 24%

when the lice reached the pre-adult stage. The fish used in the present study were larger at transfer to seawater, had been in seawater for 189 d, and were on average 290 g at time of infection. This may indicate that the time spent in seawater and/or size plays a role in louse-mediated mortalities. Indeed, fish size can be positively correlated to lice loads in infection challenges (Tucker *et al.*, 2002; Glover *et al.*, 2004), although there have been no published reports on whether larger salmon tolerate infections better than smaller conspecifics.

Freshwater-deloused fish showed reduced growth in length but not weight

Louse infection in the current study reduced growth in length and weight, and condition factor in seawater. Fjelldal *et al.* (2020) found a negative relationship between SGR and plasma ions and cortisol in fish from the same cohort that were infected with equal lice levels, but at a smaller body size and shorter stay in seawater. Hence, as in this study, it is possible that at high infection levels, fish experienced physiological consequences of the infection when the lice reached the pre-adult stage. However, we did not euthanize fish on day 24 when the lice reached the pre-adult stage, as the goal of this study was to check for maturity on day 62. Herein, louse infection had a negative long-term effect on growth in length after the fish had shed the lice in freshwater. This was not observed in weight, resulting in re-establishment of a normal condition factor. This result demonstrates that the fish recover normal weight gain after shedding lice, while growth in length—skeletal growth—remains slow. The increase in weight gain is most probably caused by increased appetite but can also have been boosted by increased feed efficiency. The results may indicate that the impact of louse infections on skeletal growth is stronger and longer lasting than on appetite and metabolism, or that the fish have a complex regulation of growth that allows for optimal recovery after infection. Such an interaction between muscular and skeletal growth may work through altered endocrine, autocrine, or paracrine functions, but there is at present no rationale to explain the current louse-mediated effect on skeletal growth in immature females and maturing males. Under natural environmental conditions, maturing adult Atlantic salmon show decreased appetite from early June (Kadri *et al.*, 1997). In some populations, mature Atlantic salmon feed on terrestrial and freshwater invertebrates during their upstream freshwater spawning migration, but the energy gained is probably low (Johansen, 2001). Thus, the present results, generated by a study where the fish were fed *ad libitum* with high-energy diets and did feed and grow in freshwater, are not directly extrapolatable to wild Atlantic salmon. Anadromous brown trout (*Salmo trutta*), sea trout, are known to delouse themselves by entering freshwater and then return to the energy-rich seawater to feed and grow (Birkeland, 1996). Further studies should examine the growth pattern in length and weight in sea trout that have re-entered seawater after freshwater delousing.

Conclusions and further directions

That pre-adult salmon louse had an infection intensity negative effect on growth and condition factor without affecting relative testis size in maturing and mature males suggests that possible negative effects of louse on reproductive success in Atlantic salmon are most likely governed by reduced body size.

Herein, we used stimulatory environmental conditions to induce a rapid onset of sexual maturation. In nature, wild salmon make the decision to mature during the late autumn nearly 1 year before spawning (Thorpe, 1994). Whether the fish decides to mature or not is dependent on its growth rate (e.g. McCormick and Naiman, 1984; Friedland and Haas, 1996), lipid stores (Rowe *et al.*, 1991; Silverstein *et al.*, 1998; Shearer and Swanson, 2000), and genetics (Ayllon *et al.*, 2015; Barson *et al.*, 2015). There is a close relationship between body condition and lipid stores in Atlantic salmon (Herbinger and Friars, 1991). Thus, factors that impact seawater growth and condition negatively in wild salmon may affect age at maturation. One such factor is the salmon louse (Vollset *et al.*, 2014). To make them more extrapolatable to natural conditions, further laboratory studies should be designed to elaborate how reduced growth and body condition imposed by louse infection impacts age at puberty under natural photoperiod and temperature, and study if infection impacts egg and semen quantity and quality. The currently used model with continuous light and high temperature to stimulate maturation could be elaborated further by introducing it to fish that are infected with pre-adult or adult lice, where the lice have already reduced fish growth and condition factor, and to explore possible interactions between fish genetics and louse infections.

Author contributions

PGF, ØK, SB, and TJH conceived and designed the experiment. PGF, TWK, TJH, and SB set up the experiment and collected the experimental data. TWK performed the statistical analysis. PGF, TWK, TJH, ØK, and SB analysed and interpreted the data. PGF, TWK, and SB wrote the first draft of the manuscript. All the authors critically reviewed the intellectual content of the manuscript and gave their approval for the final version to be published.

Supplementary Data

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

Conflict of Interest statement

The authors declare that they have no competing interests.

Data availability statement

The datasets analysed in the current study are available from the corresponding author on request.

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