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Parasite fecundity decreases with increasing parasite load in the salmon louse *Lepeophtheirus salmonis* infecting Atlantic salmon *Salmo salar*

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Abstract

Aggregation is common amongst parasites, where a small number of hosts carry a large proportion of parasites. This could result in density-dependent effects on parasite fitness. In a laboratory study, we explored whether parasite load affected parasite fecundity and survival, using ectoparasitic salmon lice (*Lepeophtheirus salmonis* Krøyer, 1837) infecting Atlantic salmon (*Salmo salar*) hosts. We found a significant reduction in fecundity with higher parasite load, but no significant effect on survival. Together with previous findings, this suggests that stronger competition amongst female lice under high parasite load is a more likely explanation than increased host immune response.

Keywords: density dependence, ectoparasite, fecundity, *Lepeophtheirus salmonis*, survival.

Introduction

Aggregation seems to be one of the very few general laws governing the distribution of most living organisms: the variance in local densities is consistently observed across taxa to be greater than the mean (Lagrue, Poulin & Cohen 2015). This observation is especially marked in parasites, where it is commonly observed, within host populations, that a minor proportion of hosts carries a major proportion of parasites (Shaw & Dobson 1995; Poulin 2007); in other terms, a majority of parasites reproduce at high densities, while the rest experiences very low densities. Heterogeneity in the probabilities of host–parasite encounters and in host susceptibility is often assumed to be the main proximate reasons why infection levels are unevenly distributed within host populations. Additionally, the observed level of aggregation might be modulated by negative and positive density-dependent effects (Anderson & Gordon 1982). On the one hand, since parasites withdraw resources from their hosts to produce offspring, one might expect stronger resource competition combined with higher levels of host immune response in heavily infested hosts, that is negative density-dependent effects. This can in extreme cases counterbalance aggregation (Luong, Vigliotti & Hudson 2011). On the other hand, high densities may dilute the risk of being targeted by the host's immune system or, for dioecious parasites, increase the chances of finding mates, hence selecting for mechanisms that increase aggregation (i.e. whereby the establishment of new parasites is made easier on already infected hosts) (Churcher, Filipe & Basanez 2006; Ugelvik *et al.* 2016).

Production of Atlantic salmon (*Salmo salar*) has expanded rapidly along the Norwegian coast and as predicted by epidemiological theory, parasite and pathogen abundances increased accordingly, to the point that they now represent a major

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Journal of Fish Diseases

challenge to the farming industry as well as to wild salmonid species. It has been suggested that changes in environmental conditions following the introduction of intensive farming have not only increased parasite densities, but may also have selected for changes in their life history (Skorping & Read 1998; Lebarbenchon *et al.* 2008; Mennerat *et al.* 2010; Pulkkinen *et al.* 2010).

The salmon louse (*Lepeophtheirus salmonis* Krøyer, 1837) is a horizontally transmitted ectoparasite feeding on the skin of salmonids (Costello 2006), causing changes in host behaviour (Øverli *et al.* 2014), skin damage, osmoregulatory stress and increased risk of secondary infections (Costello 2006). Salmon lice have a life cycle consisting of eight development stages (Hamre *et al.* 2013), and they reproduce sexually on their host and are iteroparous. At each reproductive event, they typically produce a pair of strings comprising eggs bound together in a matrix (Heuch, Nordhagen & Schram 2000; Mennerat *et al.* 2012).

The introduction of salmon farming has provided salmon lice with a predictable and constant high density of hosts, thereby sustaining much larger lice populations on individual fish than usually observed on wild hosts. One may expect epidemic outbreaks on farms to either be alleviated or aggravated by density-dependent effects, depending on whether these are negative or positive. In order to make realistic models of salmon lice population dynamics, we therefore need to know how increasing infrapopulation densities are affecting fitness-related traits of the parasite.

Here, we report results from a laboratory study where individual salmon hosts were infected with salmon lice originating from four locations in the northern Atlantic. We measured fecundity and survival of lice and investigated the relationship between infestation intensity and parasite fitness over five consecutive reproductive events.

Materials and methods

Experimental setup

In this study, we used 59 Atlantic salmon smolts (weight 80–175 g; length 200–265 mm, Industry Laboratory, Bergen, Norway) that were kept in individual tanks. All tanks had a constant flow of sea water (flow rate 2–6 L min^{-1}; temperature 7.6–8.6 °C; salinity 35 ppm), 12 h daylight, and all fish were fed 500 mg of 3-mm commercial pellets twice a day. For reasons of space in the laboratory, this study took place in two different rooms (30 individual tanks per room). The dimensions of the tanks were identical within each room, but differed between the rooms (30 L vs. 55 L). This was taken into account in both the infection procedure and the statistical analysis (see below).

Lepeophtheirus salmonis sample

To increase the variation in our sample, and since fecundity might vary according to origin, salmon lice from four locations were used: two strains collected from wild hosts originating from the Oslofjord, Norway (thereafter 'O') and the east coast of Scotland ('S'), and two strains collected from farmed hosts originating from Austevoll near Bergen, Norway ('B'), and Frøya, Norway ('F'). Fish carrying O and B lice were maintained in one room, while those carrying F and S lice in the other. To reduce potential environmental effects due to the different origins, lice from all four origins were maintained for at least three generations in bigger tanks prior to the start of this study (20–25 naïve fish per tank). The initial design consisted in infecting 15 fish per lice origin. However, one fish died accidentally the day before the study started and could not be replaced. In addition, two fish infected with the S lice and two fish infected with the B lice carried only male lice and could not be included in the analysis. The final sample therefore involved 14, 13, 13 and 15 fish hosts, respectively, infected with lice from the O, S, B and F origins.

Infection procedure

Prior to infection, the fish were anesthetized with MS-222 (75 mg L^{-1}), measured (initial length and weight) and taken back to their respective tanks for recovery. Later the same day, they were exposed to *L. salmonis* copepodites (i.e. infective stages) for 1 h, during which the water flow was stopped, the water level lowered and air was supplied directly into the tanks (as described in Mennerat *et al.* 2012). Due to differences in the dimensions of the tanks, water volume during infection differed (either 10 L or 20 L) between the two experimental rooms. We adjusted the

number of copepodites accordingly (i.e. added 80 copepodites in the bigger and 40 in the smaller tanks) so that all fish were exposed to a similar density of copepodites (four copepodites per litre). Copepodites were counted using a broad-end sterile pipette.

Handling of fish and lice

All fish underwent the same treatment and from day 40 post-infection the numbers of gravid and non-gravid female lice recorded by visual inspection of the tanks. When all female lice on a fish had become gravid, the fish were anesthetized with MS-222 (75 mg L^{-1}). The lice were carefully picked from the fish and taken to the laboratory in a cooled box. Egg strings were detached from gravid females by gently pulling them with a curved forceps, after which the lice were returned to their original salmon host until the next reproductive event. To do so, the fish hosts were gently lifted by hand so that the top of their back emerged above the surface. After placing the lice back directly on the host skin, the fish were observed for a few minutes to make sure re-attachment of the lice was successful. For each pair of egg strings, a picture of whole egg strings was taken with low magnification (3.5×) to measure total egg string length. In addition, pictures were taken with higher magnification (20×) at five distinct places along the egg string to estimate average egg length. All pictures were taken using Leica Application Suite connected to a Leica Z16APOA microscope (Leica Microsystem). This procedure was followed until day 130 post-infection, when all lice had completed their fifth reproductive event, after which the fish were killed with an overdose of MS-222 (200 mg L^{-1}).

Statistical analysis

Parasite load vs. fecundity. Directly counting the total number of eggs in each egg string was difficult under the microscope, because egg strings were longer than the optical field. To estimate the total number of eggs contained in each egg string, we therefore divided the measured total length of each egg string by the average egg length (estimated from the five detailed pictures taken along each egg string). Fecundity was then obtained for each individual female louse by calculating the total number of eggs comprised in each pair of egg strings (i.e. clutch). Since fecundity is a count data, we fitted the data using a generalized mixed-effects model (glmmPQL) with a quasi-Poisson distribution. As explanatory variables, we included parasite load (number of female lice on the fish) as a covariate, origin of the lice and clutch number (wherever relevant) as a fixed-effect factors, as well as two-way interactions. Because fecundity was measured from lice kept on distinct fish (i.e. in distinct tanks), and because those tanks were set up in two distinct batches (i.e. laboratory rooms), tank and room were included as random factors (tank nested within room). This allows controlling for effects due to slight differences in tank design between the two rooms.

Parasite load vs. survival. Some adult female lice died over the course of the experiment. From daily visual inspections of the fish, it did not seem that lice loss happened more often on the day of handling than during the 8- to 10-day intervals between handling sessions. We calculated the proportion of lice lost (our proxy for louse mortality) during the five-first reproductive events, relative to the initial numbers of adult female lice. We then explored the relation between this proportion and initial parasite load with a generalized mixed-effects model (glmmPQL) fitted with a quasibinomial distribution and including room as random factor, initial density as a covariate, origin as a fixed-effect factor, as well as the interaction between the two.

All analyses were performed using the MASS and NLME packages in the statistical programming environment R 3.2.2 (http://r-project.org). All analyses were repeated while controlling for host size (fish length) by including it as a covariate. Fish length was never significantly related to parasite fecundity (all $P > 0.20$), and controlling for it did not change any of the results. It therefore seems that parasite load is an appropriate proxy for parasite density in this study, and for the sake of clarity, analyses including fish length are not presented here.

Results

Parasite load vs. fecundity

Fecundity overall decreased with parasite load ($P < 10^{-3}$) and differed across origins ($P = 0.05$). There was a significant interaction between parasite load and origin ($P = 0.01$), as well as a

significant interaction between parasite load and clutch number ($P < 10^{-4}$) (Table 1, Fig. 1).

Because the link between parasite load and fecundity differed across clutches, we then ran separate models for each of the five-first egg production events. Fecundity differed across origins (all $P < 0.01$ except clutch 4: $P = 0.10$) and decreased with parasite load in the first ($P = 0.003$), second ($P < 10^{-4}$) and third ($P = 0.05$) clutches. Wherever parasite load was related to fecundity, the interaction with origin was not significant (Table 2, Fig. 2).

Parasite load vs. survival

The proportion of lice lost over the first five reproductive events differed across origins ($P = 0.04$), but was not significantly related to initial parasite load ($P = 0.90$). There was no significant interaction between parasite load and origin ($P = 0.41$) (Table 3, Fig. 3).

Discussion

We found a negative relation between parasite load and fecundity for the first, second and third reproductive events, but no relation between parasite load and lice survival. Our analyses further indicate that parasite load in our study is an appropriate proxy for parasite density. To our knowledge, these data are the first to demonstrate a connection between infrapopulation density and life history traits in salmon lice.

Table 1 Overall effect of parasite load on salmon lice fecundity, measured over repeated clutches (lice from four different origins). Results from a mixed-effects generalized model (glmm fitted with a quasi-Poisson distribution) including fecundity as dependent variable, tank (nested within experiment) as random effect factor, origin and clutch number as fixed-effect factors and density (number of female lice) as covariate

	numDF	denDF	*F*	*P*
Intercept	1	960	64767.72	$<10^{-4}$
Parasite load	1	960	12.53	$<10^{-3}$
Origin	3	51	2.79	0.05
Clutch number	1	960	0.93	0.34
Parasite load × Origin	3	960	3.71	0.01
Parasite load × Clutch number	1	960	20.31	$<10^{-4}$

numDF, numerator degrees of freedom; denDF, denominator degrees of freedom.

The density-dependent decrease in fecundity in our study could in theory be explained either by an upregulation of the host's immune system or by a more intense intraspecific resource competition with higher lice densities, or a combination of the two. The host is providing the parasite with resources to grow, reproduce and survive, and should therefore be seen as a limited resource (Ebert, Zschokke-Rohringer & Carius 2000). In addition to the effects of intra- or interspecific competition, parasites are affected by host immune responses that, depending on the host–parasite system, might increase with higher parasite densities (Paterson & Viney 2002; Bleay *et al.* 2007). Distinguishing between these two mechanisms is often difficult (Paterson *et al.* 2002). However, a density-dependent increase in immune response seems unlikely to explain the observed results for several reasons. Atlantic salmon show high susceptibility to lice compared to other salmonids (Johnson & Albright 1992; Fast *et al.* 2002). In addition, the lice seem able to downregulate the host's immune response (Fast *et al.* 2007; Skugor *et al.* 2008; Tadiso *et al.* 2011). This immunosuppressive effect caused by lice was also found to increase with the number of lice on the host (Holm *et al.* 2015). Altogether, these observations suggest that the host is not upregulating its immune system with higher lice densities, and it therefore appears that host immune response might only play a minor role in the density-dependent reduction in parasite fecundity observed here.

On the other hand, both the reduction in fecundity and the fact that lice survival was not affected by density indicate that resource competition may be a likely explanation, as also suggested in nematodes, where increased resource competition with higher densities may result in reduced fecundity (Selvan, Campbell & Gaugler 1993). In addition, we only observed a negative density-dependent effect on fecundity during the first three reproductive events. This also seems consistent with our interpretation that these density-dependent effects are caused by resource competition, because during the experiment, densities were decreasing due to lice mortality (Table 3, Fig. 3), likely leading to less intense competition for host resources over time.

It might be argued that parasite loads in this study were generally too low in relation to host

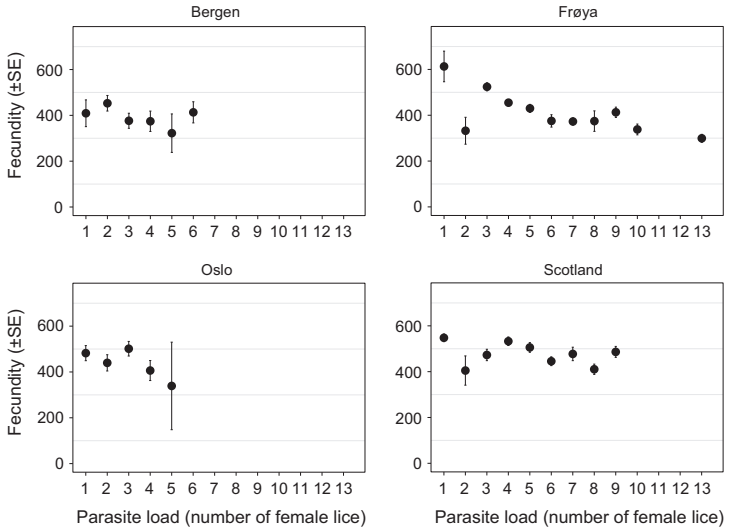

Figure 1 Relation between parasite load and salmon lice fecundity, in lice of the four origins: Bergen (n = 13), Frøya (n = 15), Oslo (n = 14) and Scotland (n = 13).

Table 2 Effects of parasite load on salmon lice fecundity, tested for the five-first clutches separately (lice from four origins). Results from mixed-effects generalized models (glmm fitted with a quasi-Poisson distribution) run for separate clutches and including fecundity as dependent variable, tank (nested within experiment) as a random effect factor, origin as a fixed-effect factor and density (number of female lice) as covariate

	numDF	denDF	*F*	*P*
Clutch 1				
Intercept	1	160	108270.67	$<10^{-4}$
Parasite load	1	47	9.88	0.003
Origin	3	47	9.96	$<10^{-4}$
Parasite load × Origin	3	47	0.74	0.53
Clutch 2				
Intercept	1	147	122091.20	$<10^{-4}$
Parasite load	1	147	44.61	$<10^{-4}$
Origin	3	43	11.47	$<10^{-4}$
Parasite load × Origin	3	43	2.51	0.07
Clutch 3				
Intercept	1	135	60039.05	$<10^{-4}$
Parasite load	1	42	4.02	0.05
Origin	3	42	4.17	0.01
Parasite load × Origin	3	42	1.38	0.26
Clutch 4				
Intercept	1	116	31572.62	$<10^{-4}$
Parasite load	1	116	0.77	0.38
Origin	3	45	2.19	0.10
Parasite load × Origin	–	–	–	–
Clutch 5				
Intercept	1	103	22640.59	$<10^{-4}$
Parasite load	1	103	0.23	0.63
Origin	3	43	4.03	0.01
Parasite load × Origin	3	43	6.06	$<10^{-3}$

numDF, numerator degrees of freedom; denDF, denominator degrees of freedom.

size to result in resource competition amongst lice. This might be true if salmon lice were spread evenly on the host; however, a number of observations suggest that some areas on the fish are preferred over others. First, adult male and female lice typically occupy different areas on the fish, where male lice are mainly found on the head while adult females, who have higher energetic needs, usually are found on the posterior ventral and dorsal midline (Todd *et al.* 2000). This has been suggested to be caused by the larger females competitively displacing the smaller males from more profitable areas (Todd *et al.* 2000). Under conditions of high infrapopulation densities, one might also expect some adult females to be outcompeted from the posterior ventral and dorsal midline by better competitors. Indeed, changes in the distribution of mobile lice stages with density have been reported (Hull, 1996 in Pike & Wadsworth 2000). Furthermore, a review by Costello (2006) argues that the disappearance of *Caligus elongatus* from hosts coinciding with the establishment of salmon lice could be due to interspecific competition. Resource competition therefore appears as a likely important mechanism in the epidemiology of salmon ectoparasites that might have been overlooked so far. It is not clear, however, why some areas on the host would be more profitable than others, but recent studies have shown that mucous cells, for example, are not evenly distributed on the host's body (Pittman *et al.* 2013). Further studies would be required to

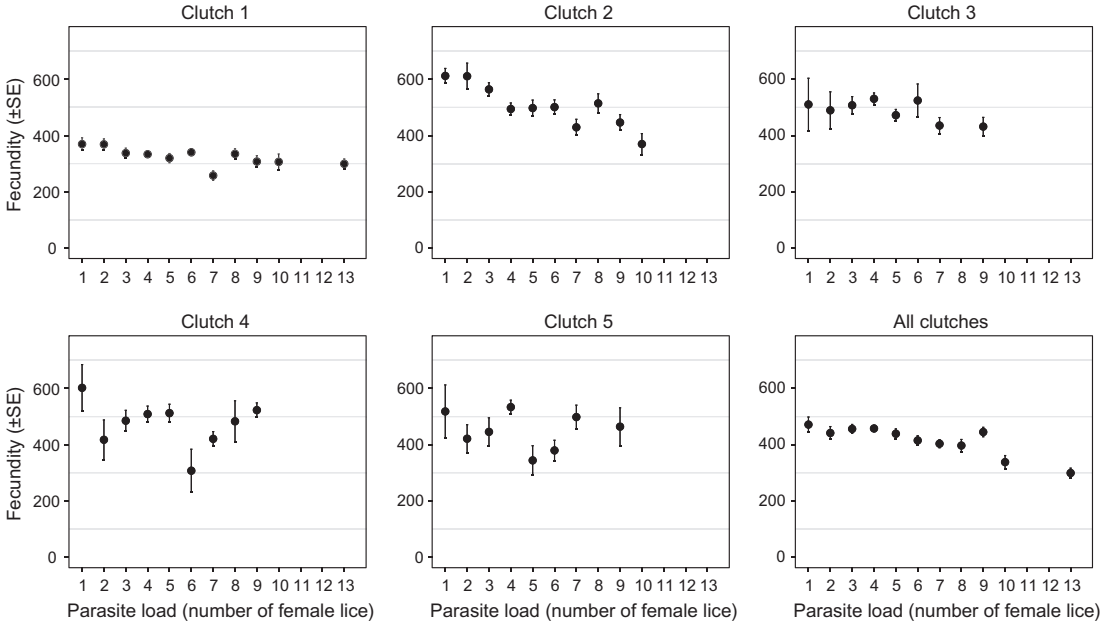

Figure 2 Relationship between parasite load and salmon lice fecundity in the five-first clutches for all hosts from all four origins. The overall relation for all successive clutches is shown in the bottom right panel.

Table 3 Effects of the initial parasite load on salmon lice mortality over the course of the experiment (lice from four origins). Results from a mixed-effects generalized model (glmm fitted with a quasibinomial distribution) including proportion of lice lost (i.e. a proxy for lice mortality) as dependent variable, tank as random effect factor, origin as fixed-effect factor and initial density (number of female lice) as covariate. numDF, numerator degrees of freedom; denDF, denominator degrees of freedom

	numDF	denDF	*F*	*P*
Intercept	1	47	14.77	$<10^{-3}$
Initial parasite load	1	47	0.02	0.90
Origin	3	47	3.06	0.04
Parasite load × Origin	3	47	0.97	0.41

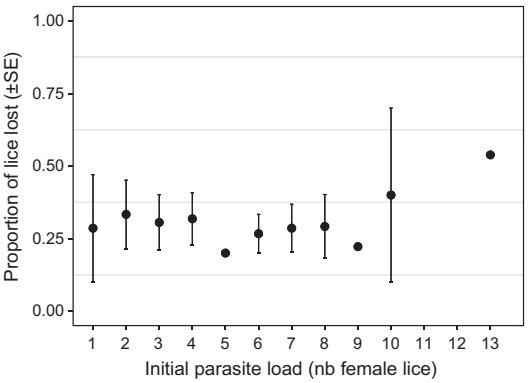

Figure 3 Relationship between initial parasite load (at first reproduction) for all hosts from all four origins and the proportion of female salmon lice lost (i.e. a proxy for lice mortality) during the course of the experiment.

better understand why salmon lice seem to aggregate around – and possibly compete for – some specific areas and discard others.

Finally, the observed reduction in fecundity with higher densities, combined with the lack of reduction in lice survival, indicates that neither salmon lice nor their hosts are adapted to prevent the building up of high lice densities. Recent results indicate that mechanisms that increase aggregation could even be selected for in natural populations, where both host availability and lice intensities are low, because they would improve the mating opportunities of the lice (Ugelvik *et al.* 2016).

Epizootics with lice have been an increasing problem after the introduction of commercial salmon farming (Morton *et al.* 2004; Costello 2009). Several studies have found higher densities of infectious lice larvae surrounding farms than at other localities, suggesting higher infection pressure on both wild and farmed salmonids in those areas (Tully & Whelan 1993; Morton *et al.* 2004; Krkošek *et al.* 2006). The intensive use of antiparasitic treatments on salmon farms seeks to lower

parasite loads in a continuous way over time; however, our results indicate that salmon lice fecundity may increase as a result. How these two combined mechanisms may affect the population dynamics of salmon lice should be further studied both theoretically and empirically.

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Ethics statement

All applicable institutional and national guidelines for the care and use of animals were followed (application ID 5549, Forsøksdyrutvalget).

Conflict of interest

The authors declare that they have no conflict of interest.

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Author's contribution

AS and AM designed the study, MSU and AM did the experiments and analysed the data, MSU wrote the first draft, and AM and AS provided critical revisions and comments to the manuscripts.

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