



Atlantic salmon infected with salmon lice are more susceptible to new lice infections

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Abstract

Aggregation is commonly observed for macroparasites, but its adaptive value remains unclear. Heavy infestations intensities may lead to a decrease in some fitness-related traits of parasites (e.g. parasite fecundity or survival). However, to a dioecious parasite, increased aggregation could also increase the chance of finding individuals of the opposite sex. In a laboratory experiment, we tested if previous experience with salmon lice (*Lepeophtheirus salmonis*) affected susceptibility of Atlantic salmon (*Salmo salar*) to later exposure to the same parasite species. We found that currently infected fish got higher intensities of new lice than naive fish. This suggests that hosts already carrying parasites are more susceptible to new lice infections. For this dioecious parasite, such positive density dependence might be adaptive, ensuring successful reproduction under conditions of low lice densities by increasing the probability of both sexes infecting the same host.

Keywords: aggregation, *Lepeophtheirus salmonis*, positive density dependence, *Salmo salar*, susceptibility.

Introduction

To most parasites, the chance of reaching a new host appears to be extremely low. Compared to the host, parasite infection stages are tiny, with limited mobility and longevity. Soon after they

are released they are dispersed by wind or water, some end up in unsuitable hosts, others are predated upon by a multitude of organisms, and the vast majority die without ever making contact with a suitable host. Wherever the parasite in question is a dioecious species, the prospects of transmitting its genes get even worse: at least two infectious stages of different sexes will have to infect the same host in order to succeed. If transmission success was relying on stochastic processes only, we would expect a random distribution of parasites in host populations. However, the opposite is commonly observed, in particular for macroparasites, which are often found highly aggregated in host populations: most hosts have few or no parasites, while a few hosts carry the majority of the parasite population (Pennycuik 1971; Anderson & Gordon 1982; Shaw & Dobson 1995; Galvani 2003; Poulin 2013). Proposed explanations for this phenomenon include spatial or temporal (i.e. seasonal) aggregation of transmission stages prior to infection, variability in host susceptibility and differences in host behaviour (Shaw & Dobson 1995). A number of studies have shown that the degree of aggregation can have crucial effects on both parasite virulence and on the role of parasites as regulators of host populations (Nowak & May 1994; May & Nowak 1995; Hudson, Dobson & Newborn 1998; Ebert, Lipsitch & Mangin 2000; Albon *et al.* 2002; Wilson *et al.* 2002; Ben-Ami, Mouton & Ebert 2008; Alizon *et al.* 2009). Understanding the causes and consequences of such aggregated distributions has therefore been at the forefront of parasite population biology since the seminal works of Crofton (1971) and Anderson & May (1978).

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Negative density-dependent mechanisms, where increased infestation intensity may either reduce the chance of new infections or increase the death rate of already established parasites, will limit the degree of aggregation of the parasite population (Anderson & Gordon 1982). Density-dependent parasite-induced host mortality or host immune responses would have the same effect. Parasites that do not reproduce sexually on their hosts (either because they reproduce asexually or because they have not reached maturity yet) would in these conditions benefit from infesting hosts that are not already carrying large numbers of parasites. However, for dioecious parasites reproducing on their host, the chance of successful mating will increase with parasite density. Shaw *et al.* (1995) suggested a trade-off between aggregation and random distribution in dioecious parasites, where the optimal level of parasite aggregation would depend on the relative costs of reduced host and/or parasite lifespan due to high parasite densities and reduced chances for parasites of finding individuals of the opposite sex due to low infection levels.

The salmon louse is a common ectoparasite on salmonid fish. In its natural environment (i.e. their usual habitat until aquaculture started a few decades ago), this parasite depends on wild salmonids. The female louse disperses its larvae in fjords and in the ocean, first as nauplii before they moult into the infective copepodid stage (Costello 2006). These copepodids are relatively short-lived and depend on energy reserves from the yolk sac (Pike & Wadsworth 2000). Within a time-window of a few weeks, they have to find and successfully infect a host swimming around in a huge volume of water and in relatively low densities. Even if one or more copepodids are able to find and infect a host, this is no guarantee for success. The salmon louse is a dioecious species, so in order to reproduce the parasitic stages that happen to infect a common host must be of separate sexes. Salmon lice, like most other parasites, compensate for this low infection probability by producing a high number of offspring (Heuch, Nordhagen & Schram 2000; Costello 2006), but increased aggregation would probably also be advantageous to these parasites, because this would increase the chance of finding individuals of the opposite sex. One possible mechanism that could lead to higher aggregation would be a higher susceptibility of already infected

hosts— that is a positive density-dependent infection rate.

In this laboratory study, we experimentally tested whether *Salmo salar* hosts that had already been infected once with salmon lice differed from naive hosts in susceptibility to subsequent infections with lice. We also explored how the numbers of lice found on individual fish in the second infection related to the numbers found in the first infection, and whether this relationship was affected by removal of the established lice prior to the second infection.

Material and methods

We used 63 naive Atlantic salmon smolts (200–300 g) originating from the same cohort (Industry laboratory) and salmon lice (*Lepeophtheirus salmonis*) from a laboratory strain originating from Gulen, Norway. Fish were kept in 500-L rearing tanks filled with UV-treated and filtered normal sea water (salinity 35 ppm; temperature 7.5–8.7 °C) with constant water flow (oxygen level > 80%), 12-h daylight and fed with 1 g of 3-mm commercial pellets per day.

To test whether fish that had previously been infected with lice were more likely to acquire new infections than naive fish, we divided the fish into three treatment groups each consisting of 21 fish. In two groups, the fish were infected a first time, and the lice from this first infection were either left (IA) or removed (IR) from the fish before proceeding to a second infection; a third control group was treated and manipulated similarly, but only exposed to lice in the second infection (IC). Two replicate rearing tanks were used for each treatment group (Table 1).

Table 1 Mean, standard error (SE) and minimum and maximum values for the number of new lice for replicate 1 (a) and replicate 2 (b) depending on treatment: IA, infected twice with adult lice from the first infection remaining; IR, Infected twice, but adult lice from the first infection removed prior to the second infection; and IC, control group of naive fish not previously exposed to lice

Treatment	Mean	Min	Max	SE	N
(a)					
IA	27.1	14	37	2.7	10
IR	23.7	14	43	3.2	11
IC	19.5	8	39	2.9	11
(b)					
IA	20.2	9	45	3.2	11
IR	15.6	11	30	1.2	10
IC	15.8	9	21	1.7	10

Estimating the number of copepodids

Copepodids were kept in a 1-L cup filled with filtered and UV-treated normal sea water (35 ppm). After stirring the water to equally distribute the copepodids, a 10-mL sample was taken using a 10-mL serological pipette (Sterilin) rapidly inserted through the entire water column. The water was then poured through a sieve, copepodids were flushed out with sea water into a counting chamber and counted using a microscope. Based on the number of copepodids present in the 10-mL water sample, we estimated the volume of water needed to have approximately 60 copepodids per fish.

First infection

Fish from both replicate tanks of the control group (IC) were moved to an infection tank and got a sham infection (no copepodids added), while fish from the IA and IR groups were infected with about 60 copepodids in a common infection tank (each infection tank contained fish from one IA and one IR replicate). The infection procedure lasted for 1 h during which water level was lowered, water flow stopped and oxygen supplied directly into the tanks. Fish were later returned to their rearing tanks.

Second infection

The second infection took place 47 days after the first infection. Prior to the infection, all fish were anesthetized one by one with MS-222 (75 mg L⁻¹) and tagged with two T-bar extra small anchor tags (Floy[®]), placed in the dorsal fin using a Mark3 fine fabric-tagging gun (Floy[®]). This was carried out to keep track of individual fish, and additionally, each treatment group had a designated tag colour. Adult lice on the IR and IA group were counted and sexed. For the IR group, all lice were carefully removed from the fish with a curved forceps prior to the second infection, while fish in the IA group kept all adult lice from the first infection. Equal numbers of fish (9–11 fish) from the three treatment groups were then moved to common infection tanks. Infection followed the procedure described for the first infection, after which the fish were separated by tag colour and returned to their respective rearing tanks.

The experiment was terminated 83 days after the first infection. The fish were killed one by one with an overdose of MS-222 (200 mg L⁻¹), and all lice from each fish were collected. Pre-adult (i.e. from the second infection) and adult lice (i.e. lice from the first infection, for the IA group only) were then counted and sexed.

Statistical analysis

We first tested whether infestation intensity in the second infection differed across treatment groups using generalized linear mixed-effects model (glmmPQL) fitted with Quasi-Poisson distribution, number of new lice as a dependent variable, treatment as a factor and tank as a random factor. Treatment groups were compared pairwise by setting the relevant treatment level (IC, IR or IA) as reference with the relevel function.

The relationship between the number of lice in the first and second infection was investigated using a Spearman's rank correlation test on the data from the IA and IR group. All analyses were performed using the NLME, PLYR and MASS packages in the statistical programming environment R 3.3.3 (<http://r-project.org>).

Results

The number of new lice per fish was significantly higher for the IA group compared to the control (IC) group (glmmPQL, DF = 59, $T = -2.29$, $P = 0.03$, Fig. 1, Table 1), but not compared to the IR group (glmmPQL, DF = 59, $T = -1.44$, $P = 0.156$, Fig. 1, Table 1). There was no significant difference between the IR and the control (IC) group (glmmPQL, DF = 59, $T = 0.86$, $P = 0.39$).

Within the IA group, we found a positive correlation between the number of adult lice present from the first infection and the number of new lice that established in the second infection, with a slope of 1.5 (Spearman's rank, $S = 497.88$, $P = 0.0007$, $\rho = 0.68$, Fig. 2a). There was also a positive correlation between the number of lice in the first and second infection for the IR group, but in that group the correlation was weaker and the slope closer to 1 (Spearman's rank, $S = 988$, $P = 0.11$, $\rho = 0.36$, Fig. 2b, Slope = 1.1).

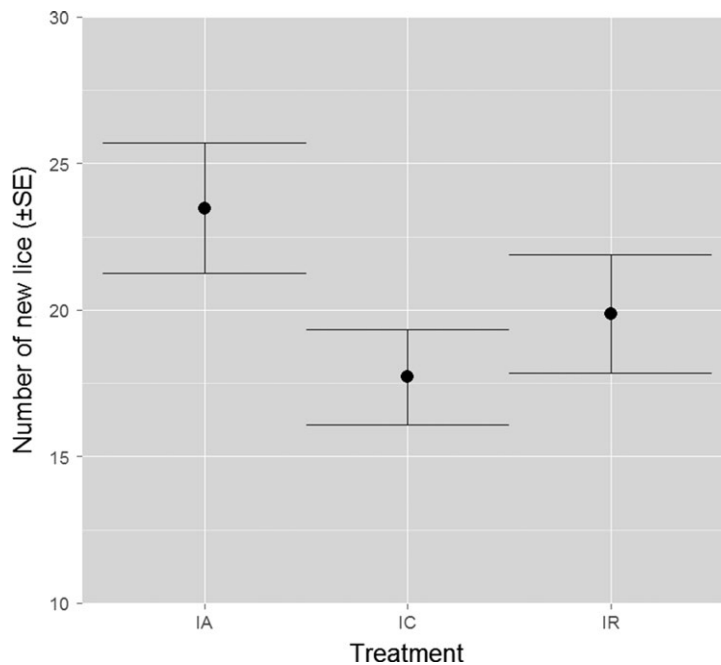


Figure 1 Mean number of new lice (\pm SE) for the three treatment groups for both replicates: IA, infected twice with adult lice from the first infection remaining; IR, Infected twice, but adult lice from the first infection removed prior to the second infection; and IC, control group of naive fish not previously exposed to lice.

Discussion

We found that those salmon that carried lice from an earlier infection got higher intensities of lice in the second infection compared to the control group consisting of naive hosts. This result suggests that hosts currently infected with salmon lice are more susceptible to new infections than naive hosts.

In our study, the second infection took place in common tanks containing fish from the three treatment groups that were exposed together to copepodids for 1 h. Arguably, temporal or spatial heterogeneity in the distribution of copepodids within the infection tank could partially account for the differences in infection levels among treatment groups, if, for example, fish that were previously reared together (i.e. from the same treatment group) tended to stay grouped in the infection tank. We, however, think this is unlikely, as the tank water was stirred upon addition of copepodids, and the number of copepodids added was high. In addition, the high host density within the infection tanks meant that all hosts were in close proximity to each other, and hence, different exposure to copepodids alone is unlikely to explain our results.

In the IR group, where lice from the first infection had been removed prior to the second infection, we found a positive correlation between the

number of lice from the first and the second infection. In other words, some hosts got high numbers of lice, and some got low numbers of lice in both infections. This suggests that susceptibility to salmon lice varies in a consistent way among hosts, likely due to genetic factors. Previous studies found a genetic component in susceptibility, even though the estimated heritability is regarded as low (Glover, Nilsen & Skaala 2004; Glover *et al.* 2005; Kolstad *et al.* 2005). If genetic differences in susceptibility are the only reason for this correlation, we would expect a slope around 1, as we found in the IR group. In the IA group, however, when lice from the first infection were still present during the second infection, the slope was higher, suggesting that both genetics and past experience affected susceptibility in the second infection.

The ability to modulate the host's immune response, which may affect host susceptibility to later parasite exposure, is reported for many parasites (Cox 2001). Several studies have for instance observed concomitant immunity, which occurs when already infected hosts are immune to re-infections while already established parasites are left unharmed (Rajakumar *et al.* 2006; Lightowers 2010). In other cases, some parasites may have immunosuppressive effects, making the host more susceptible to new infections (Goodwin *et al.* 1972; Greenwood *et al.* 1972; Cross & Klesius 1989; Barnard *et al.* 1998). Some evidence

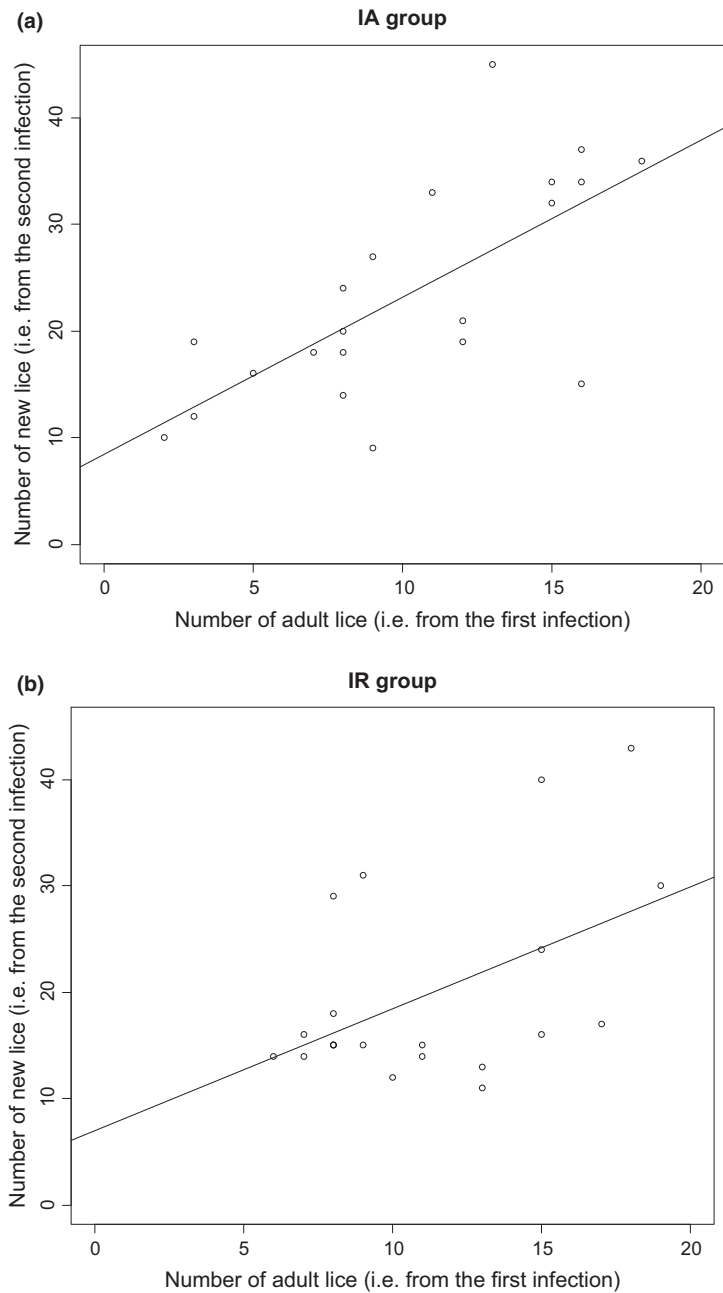


Figure 2 Linear regressions showing the relationship between the number of lice in the first and second infection, (a) for the currently infected group (IA) (Spearman's rank, $S = 497.88$, $P = 0.0007$, $\rho = 0.68$, $z = 2.96$, Slope = 1.5), (b) for the group from which adult lice were removed before the second infection (IR) (Spearman's rank, $S = 988$, $P = 0.11$, $\rho = 0.36$ Slope = 1.1).

was found that salmon lice too are able to modulate the immune response of Atlantic salmon (Fast *et al.* 2007; Skugor *et al.* 2008; Tadiso *et al.* 2011) and that they display density-dependent immunosuppressive effects (Holm *et al.* 2015). In our study, the higher numbers of lice, together with the stronger positive correlation found in the IA group compared to the IR group, further indicate that adult lice present on the fish make their hosts more susceptible to new infections.

It has previously been shown that infected salmon display both reduced locomotor activity and a stronger general stress response (Øverli *et al.* 2014). An alternative explanation for the higher lice intensities found on IA hosts in our study could therefore be that those hosts were less mobile and therefore easier to attach to than uninfected hosts. Even though such a mechanism might apply to infection events occurring under natural conditions, where hosts are swimming in

huge volumes of water, in this study infection was carried out using a high density of copepodids in a relatively small volume of water. It therefore seems unlikely that host behaviour only could suffice to explain our results. Besides, even assuming that reduced mobility of hosts may partly contribute to the differences found here, it does not contradict our findings, as altered behaviour of infected hosts is very often a reflection of an adaptive parasite strategy rather than a mere collateral damage of infection (i.e. host manipulation) (Poulin 2010). More experimental studies would be needed in this area to assess the extent to which salmon lice manipulate the behaviour of their hosts, in addition to regulating down its immune system – both of which would concur in increasing host susceptibility.

For wild populations of salmon lice, mechanisms increasing aggregation appear to be adaptive because they would increase the chances of separate sexes infecting the same host. Selection on hosts for counteracting this has probably been weak, because salmon densities have been too low to result in lice epidemics. However, under the current conditions increasingly favouring high lice densities (e.g. intensive salmon farming), mechanisms that increase aggregation are no longer adaptive, resulting in too high parasite densities, which might not only decrease host fitness (Menerat *et al.* 2010, 2012), but also reduce lice fecundity (Ugelvik *et al.* submitted). Changing ecological conditions following the introduction of intensive salmon farming might therefore select for mechanisms that reduce aggregation, and future studies should take more explicitly into account the adaptive causes and consequences of the many ways salmon lice interact with their hosts.

Ethics statement

Salmon used in the experiment was treated according to Norwegian regulations. Approval (application ID 5549, Forsøksdyrutvalget) and necessary licences were obtained before the experiment was conducted.

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Author's contribution

AS, MSU and TM designed the study. TM and MSU conducted the experiment. TM, MSU and AM analysed the data. AS and MSU wrote the first draft, and AM and TM provided critical revisions and comments to the manuscript.

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