Salmon lice as models for understanding life history evolution of parasites under intensive farming

Mathias Stølen Ugelvik

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Abstract

Human activities are drastically changing ecological conditions for living organisms and this leads to evolutionary changes. One example is the appearance and spread of resistance against drugs and antibiotics, but we also have evidence that new environmental conditions select for changes in how living organisms allocate resources between growth, reproduction and longevity. In parasites, such changes in life history traits could have several consequences, since life history traits are often closely connected to transmission rates, epidemiology and virulence.

Intensive farming of Atlantic salmon (*Salmo salar*) represents one such case of a severe change in ecological conditions, both to salmon and its parasites. This industry has increased rapidly during the last decades, and has caused an increase in the abundance of salmon lice (*Lepeophtheirus salmonis*). Salmon farms represent new ecological conditions for salmon lice, with year-round presence of a high number of susceptible hosts and reduced life expectancy due regular exposure to anti-parasitic drugs and slaughtering of hosts. From life history theory, we predicted that conditions in farms should select for higher investment into early reproduction, because future prospects of survival and reproduction are reduced. An increase in early reproductive investment could be manifested in several ways, for example by faster development, higher fecundity or by investing more into each offspring. If an organism increases its investment in reproduction it will need more resources and, for parasites, these resources must be taken from the host. We therefore predicted that an increase in early reproduction would also be associated with more virulent parasites.

The rapid increase of salmon farming in the last decades makes this a good model-system to test how human activities might affect life history and virulence of parasites. In this study we compared in the laboratory the life history traits and virulence of salmon lice sampled from locations with salmon farms (Frøya and Bergen) to those of lice sampled from locations without farms (Oslo and Eastern Scotland).

Salmon lice life history traits suggest that they are adapted to low and unpredictable host availability. Under natural conditions the sexually reproducing louse may therefore be selected to increase aggregation (Paper I) in order to maximize the chance of finding a mating partner. In accordance with this, we found that individual fish already infected with lice were more susceptible to new infections (Paper I). However, in salmon farms the number and density of susceptible hosts is much higher, which has increased lice intensities. Under such

conditions aggregation could result in too high parasite loads. High loads of salmon lice on infected fish results in a reduction in lice fecundity, probably caused by increased resource competition on the host (Paper II). There was no effect of parasite load on lice survival (Paper II).

We found differences in life history traits between lice from locations with farms (Frøya and Bergen) compared to lice from locations without farms (Eastern Scotland and Oslo), suggesting that conditions in farms select for higher investment into early reproduction (Paper III). In our study lice from farms had lower overall fecundity and survival after infection, but higher fecundity at the first reproductive event (Paper III). Lice from farms also had lower adult survival, but their offspring had higher infection success, suggesting that lice in farms invest more in offspring quality (Paper III). Variation in these life history traits is likely caused by ecological conditions in salmon farms selecting for accelerated life history strategies, at the cost of a reduction in future survival and reproduction (Paper III). Lastly, lice from locations with farms caused more harm to their hosts. We suggest that this could be caused by conditions in farms such as reduced lifespan of hosts, the use of drugs to reduce lice abundances and the high density of susceptible hosts available (Paper IV).

List of papers

Paper I: Mathias Stølen Ugelvik, Thor Mo, Adele Mennerat and Arne Skorping (2017). Atlantic salmon infected with salmon lice are more susceptible to new lice infections. *Journal of Fish Diseases* **40**: 311-317.

Paper II: Mathias Stølen Ugelvik, Arne Skorping and Adele Mennerat (2017). Parasite fecundity decreases with increasing parasite load in the salmon louse *Lepeophtheirus salmonis* infecting Atlantic salmon *Salmo salar*. *Journal of Fish Diseases* **40**: 671-678.

Paper III: Adele Mennerat, Mathias Stølen Ugelvik, Camilla H. Jensen and Arne Skorping (2017). Invest more and die faster: the life history of a parasite on intensive farms. *Evolutionary Applications* (in press): 10.1111/eva.12488.

Paper IV: Mathias Stølen Ugelvik, Arne Skorping, Olav Moberg and Adele Mennerat (2017). Evolution of virulence under intensive farming: Salmon lice increase skin lesions and reduce host growth in salmon farms. *Journal of Evolutionary Biology* (in press): 10.1111/jeb.13082.

Contents

1. Introduction

1.1 Life history theory

Organisms are selected based on their fitness (*i.e.* relative genetic contribution to the next generation) which depends on underlying life history traits *i.e.* those characterizing the pace at which organisms reproduce and die (Poulin, 1996; Stearns, 2000; Roff, 2002; Poulin, 2007; Mennerat *et al.*, 2012). Everything else being kept equal, an increase in each particular life history trait (*e.g.* maturation rate, fecundity, lifespan) results in higher fitness. Given how closely life history traits are related to fitness, one could expect them to display little variation in natural populations, but on the contrary they vary both within and between species. Variation in life history traits could be maintained by spatial or temporal environmental heterogeneity, because selection pressure might vary over time and from location to location thus maintaining variation in these traits.

Moreover, not all fitness related traits can be maximized simultaneously, as organisms have access to limited time and energy, and the number of possible combinations can be further limited by physiological, phylogenetical or ecological constraints (Stearns, 1992; Poulin, 1996; 2007). A change in one trait that is beneficial for the organism's fitness might therefore result in a change in another trait that is associated with reduced fitness, *i.e.* these traits are involved in an evolutionary trade-off (Stearns, 1989; Roff, 2002). Organisms are therefore under selection for the optimal strategy, which is the combination of traits that results in highest fitness in a given environment.

Since an individual has limited amount of time and energy available, iteroparous organisms that allocate more resources into current reproduction either by producing more offspring or by investing more into each offspring, have less resources available for survival and future reproduction (Minchella and Loverde, 1981; Stearns, 1989; Stearns, 1992; Roff, 2002). This prediction was confirmed in an experimental study on blue tits (*Parus caeruleus*) infected with fleas, where increased investment into current reproduction to compensate for the effect of the parasites reduced survival and future reproduction (Richner and Tripet, 1999). Another study on the common eider (*Somateria mollissima)* also supports this expectation; birds with experimentally increased clutch size (increasing the cost of incubation) had lower fecundity the subsequent year (Hanssen *et al.*, 2005). This therefore represents an evolutionary trade-off between current and future reproduction and the optimal strategy between these traits depends on life expectancy; a reduction in future life expectancy

should select for higher early reproductive effort, because prospects for future survival and reproduction are reduced (Stearns, 2000; Roff, 2002)

1.2 Parasites, life-histories, and virulence

Parasites show a wide range of different life history strategies and are probably affected by similar trade-offs as free-living organisms (Poulin, 1996; Trouvé et al., 1998). However, they live on a host and therefore their life history is also affected by host traits such as longevity, immune status and body size (Morand *et al.*, 1996; Poulin, 1996; Sorci *et al.*, 1997; Morand and Sorci, 1998; Gandon *et al.*, 2001; Viney and Cable, 2011). Parasites are expected to rapidly respond to changes in host life history and due to larger population sizes, often higher mutation rates and shorter generation times parasites evolve faster than most hosts (Anderson and May, 1982; Crossan et al., 2007; Lebarbenchon et al., 2008; Cressler et al., 2016).

By relying on resources from their host, parasites cause some degree of reduction in host fitness and this is termed virulence (Read, 1994; Kennedy et al., 2016). Virulence theory is based on the presence of a virulence-transmission trade-off, with virulence having both fitness costs (reduced longevity) and benefits (current production of transmission stages) for parasites (Anderson and May, 1982; Alizon *et al.*, 2009; Alizon and Michalakis, 2015). Parasites are under selection for the virulence level that results in highest parasite fitness (Ebert, 1994; Alizon et al., 2009; Cressler et al., 2016; Kennedy et al., 2016), which is suggested to be at intermediate levels of virulence (Jensen *et al.*, 2006; Fraser *et al.*, 2007; de Roode *et al.*, 2008). The level of virulence is affected by ecological conditions, for instance an increase in the density of susceptible hosts is predicted to select for parasites with accelerated life histories and thus higher virulence (Bull, 1994; André and Hochberg, 2005; Mennerat *et al.*, 2010; Berngruber *et al.*, 2013; Borovkov *et al.*, 2013; Cressler *et al.*, 2016; Kennedy *et al.*, 2016). Factors reducing host and parasite life expectancy are expected to select for higher current reproduction and thereby increased virulence because the prospects for future transmission and survival are reduced (Anderson and May, 1982; Gandon *et al.*, 2001; Ebert and Bull, 2008; Cressler *et al.*, 2016; Kennedy *et al.*, 2016; Rozins and Day, 2017). Furthermore, if the host is co-infected with other parasite genotypes or other parasite species, increased within host competition reduce future life expectancy and select for increased virulence (Nowak and May, 1994; May and Nowak, 1995; Ebert and Bull, 2008). According to this theory, changes in one or several of these factors, for instance due to anthropogenic activities, might select for more harmful parasites.

1.3 Man-made evolution and intensive farming

Human activities are drastically changing ecological and environmental conditions through the effects of global warming, introduction of exotic species, harvesting of natural populations and habitat destruction (Palumbi, 2001; Ashley *et al.*, 2003; Stockwell *et al.*, 2003; Renaud *et al.*, 2005; Hendry *et al.*, 2011; Thrall *et al.*, 2011; Budria and Candolin, 2014; Cable *et al.*, 2017). This alters the selection pressure on both free-living and parasitic organisms and might lead to evolutionary changes (Palumbi, 2001; Carroll et al., 2007; Lebarbenchon et al., 2008; Rogalski et al., 2017). Rapid evolutionary responses have been shown with the appearance and spread of antibiotic resistance in bacteria (Hamilton-Miller, 2004), resistance against commonly used drugs in agriculture (Thrall *et al.*, 2011; Aaen *et al.*, 2015) and changes in life history traits (Leignel and Cabaret, 2001; Jørgensen et al., 2007; Heino et al., 2015).

Intensive farming has increased globally and especially fish farming has expanded rapidly during the last decades (FAO, 2016). Salmon farming in Norway has for instance increased from 160 thousand metric tons in 1994 to more than 1300 thousand metric tons in 2015 (The Norwegian Directorate of Fisheries, 2016). Intensive food production systems are characterized by a high density of hosts with little genetic variability that have been under selection for increased growth rates (Nowak, 2007; Mennerat *et al.*, 2010; Peeler and Feist, 2011; Kennedy *et al.*, 2016). These conditions might select for changes in parasite life history traits, for instance high host availability increases parasite transmission, leads to higher parasite abundance and is expected to select for individuals that rapidly utilize these resources by increasing early reproduction (Mennerat *et al.*, 2010; Kennedy *et al.*, 2016). Hosts in farms are also under artificial selection for increased growth rates and are regularly slaughtered. Together with the use of anti-parasitic drugs this reduces the prospects for parasite survival. This is expected to select for parasites that invest more into early reproduction and as parasites rely on resources from their hosts this should increase virulence (Skorping and Read, 1998; Mennerat *et al.*, 2010; Atkins *et al.*, 2012; Kennedy *et al.*, 2016).

In this thesis we explored the following hypotheses:

I: We predicted that the high density of susceptible hosts that are present throughout the year, together with shorter life expectancy due to regular slaughtering of hosts and the use of anti-louse drugs in farms should select for lice that utilize this large resource either by an increase in early fecundity or by producing offspring of higher quality (or both). However, organisms have limited amounts of resources available, higher allocation to one fitness trait (early

reproduction), should therefore come at a fitness cost. We therefore expected that higher investment into early reproduction should result in a reduction in future reproduction and survival.

II: Salmon lice rely on resources from their host to grow and reproduce, so changes in ecological conditions in farms could also affect virulence. We expected that high density of susceptible hosts and shorter life expectancy in farms should increase the fitness benefit of virulence (*i.e.* increased transmission), while the cost of virulence is unaffected or reduced. Thus conditions in salmon farms should increase fitness of lice that exploit their host more intensively and this is predicted to lead to increased virulence in farms.

2. Study system

We investigated these predictions by infecting salmon smolts with lice originating from locations either with or without farms. We compared infection success, fecundity, adult survival and virulence of lice sampled from "wild" and "farmed" locations under the same ecological conditions in the laboratory.

The host, the Atlantic salmon, is naturally found throughout the northern Atlantic and is anadromous with genetically distinct populations in different rivers (Glover et al., 2012). Salmon are experiencing altered conditions after the introduction of salmon farms, where high densities of salmon are kept stationary for the duration of the production cycle (about 18 months). Under natural conditions salmon are migratory with smolts migrating out of the river to the sea in the spring or early summer, while mature adults return to their natal river to spawn in the autumn after spending one to four years at sea (Forseth *et al.*, 2017).

The expansion of salmon farming has also drastically changed ecological conditions for salmon lice and has been associated with increasing lice abundances (Morton *et al.*, 2004; Krkošek *et al.*, 2006; Torrissen *et al.*, 2013). The salmon louse (*Lepeophtheirus salmonis*) is an ectoparasitic copepod (family Caligidae) browsing on the skin of salmonids and thereby causing skin damage, osmoregulatory stress and increasing the host`s susceptibility to secondary infections (Grimnes and Jakobsen, 1996; Pike and Wadsworth, 2000; Costello, 2006). Furthermore, this parasite seems able to suppress the host's immune response (Fast et al., 2007; Skugor et al., 2008; Holm et al., 2015), might affect the age of returning salmon (Vollset *et al.*, 2014) and is even suggested to affect host behavior (Øverli *et al.*, 2014; Bui *et al.*, 2016). Salmon lice are naturally occurring in the Northern Atlantic mostly infecting wild

Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*) (Johnson and Albright, 1991; Pike and Wadsworth, 2000). They are iteroparous and can produce up to 11 clutches (*i.e.* egg strings) (Heuch *et al.*, 2000; Whelan, 2010; Mennerat *et al.*, 2012). They have a direct life-cycle and developmental time is temperature dependent (Hamre et al., 2013). The life cycle consists of eight development stages separated by molting and of these, three stages are free-living, with larvae drifting in the water relying on nutrients stored in the yolk sac. The life cycle starts when planktonic nauplius larvae are released and spread with water currents. A few days later, these larvae further develop into infectious copepodites. The copepodites that find and infect a suitable host later develop through two chalimus stages while remaining attached to the fish (especially on the fins), before molting into the first of two motile pre-adult stages (Bjørn and Finstad, 1998; Pike and Wadsworth, 2000; Costello, 2006). The life cycle is completed with the final molt to the adult stage, when males and females mate to produce the next generation of eggs (Pike and Wadsworth, 2000).

3. Summary of papers

Paper I

In order to better understand how intensive farming practices affect salmon lice life history and virulence, we first needed to know more about their general ecology and how lice life history traits might have evolved under natural conditions. Parasites have been shown to affect host susceptibility to new infections, either by increasing or reducing susceptibility to subsequent infections. Surprisingly, considering the negative impact salmon lice have in farms, no study has so far explored this. We therefore experimentally tested whether previous exposure to salmon lice affects susceptibility to new lice infections, and found that fish already infected with adult salmon lice are more susceptible to lice than naïve fish. Making infected hosts more susceptible to new infections might be a mechanism to increase aggregation for the lice. Under natural conditions wild hosts are often found in low densities over vast areas at sea, thereby making it difficult for the small, short lived salmon lice copepodites to find and infect a host. Higher aggregation might be adaptive for the parasite, as it increases the probability that individuals of both sexes infect the same host, and hence the likelihood of reproduction for this dioecious parasite.

Paper II

Salmon farming has drastically increased the number of available hosts for salmon lice and this has led to higher lice abundances in coastal waters. We therefore needed to know how these increased infra-population densities might affect fitness-related traits of the louse. We studied the effect of parasite load (*i.e.* number of adult female lice on the host) on lice fecundity and survival for the five first clutches using lice from four different localities (Frøya, Bergen, Eastern Scotland and Oslo). We found that the fecundity of salmon lice decreased significantly with increasing parasite loads for the first three clutches, while the correlation disappeared later in the infection. There was no effect of parasite load on lice survival in our study. We interpret this as an indication that the negative effect of density on fecundity found in our study may be due to higher on-host competition with increasing parasite loads.

Paper III

The life of a salmon louse in a farm is very different from natural conditions. Here, the parasite has access to an abundance of susceptible hosts, but the adult stage has a higher mortality due to shorter host lifespan combined with the use of anti-parasitic drugs. We predicted that lice from locations with farms should invest more into early reproduction, at the expense of future reproduction and survival. To explore this we compared fecundity, infection success, and adult survival of lice collected from locations with salmon farms (Frøya and Bergen) to that of lice from locations without farms (Eastern Scotland and Oslo). We found that lice from farms have both lower overall fecundity and higher infection success than those from locations with no farms. Lice from unfarmed areas had lower fecundity at the first clutch and had higher adult survival. In agreement with our prediction there seems to be a shift over to investment in early reproduction on lice from farmed locations, at the cost of a reduction in future reproduction and survival. This suggests that conditions in salmon farms select for changes in salmon lice life history.

Paper IV

According to virulence theory conditions in farms are expected to select for increased virulence. To investigate this we therefore infected Atlantic salmon smolts with lice either from locations with farms (Frøya or Bergen) or from locations without farms (Eastern Scotland or Oslo), and compared how virulent they were on their hosts. We used two proxies

for virulence; skin damage as short-term effect and growth (weight and length gain) as long-term effect on host fitness. We found that lice sampled from locations with salmon farms (Frøya and Bergen) created bigger lesions and had a more negative impact on host growth than those from locations without farms. This suggests that higher virulence in lice from locations with farms might be caused by ecological conditions in salmon farms.

4. Discussion

If salmon lice have responded to changes in selection regimes associated with salmon farming, we predicted that by comparing the life history of lice from locations that have been farmed for > 40 years (*i.e.* about 240 lice generations) to that of lice from locations without farms, we should be able to determine how, and to what extent, their life history traits have evolved. In addition, because salmon lice are parasites where life history seems to correlate with virulence (Mennerat *et al.*, 2012), we predicted that these changes should also be reflected in higher levels of virulence.

We found that lice from farms started to reproduce slightly later and produced more eggs in their first clutch; they had lower fecundity later in the infections than those from locations without farms (Paper III). They also displayed higher infection success (*i.e.* ability to infect hosts and develop until maturity) and higher adult mortality (Paper III). Lastly lice from farms inflicted more skin damage to their hosts and reduced host weight gain more (Paper IV), suggesting that ecological conditions in farms selects for consistent changes in both life history traits and virulence.

In our study lice from both locations with and without farms were raised in the laboratory for at least three generations prior to the start of the study, which makes it unlikely that the observed differences reflect environmental variation at the sites of origin. This is further supported by the fact the distances between “farmed” and “wild” locations are about the same, so differences in traits between locations with and without farms could not be explained by geographical distance alone. For locations without salmon farms distance to the nearest farm was at least 200 km at the time of sampling and they were located outwards in relation to water currents. This should make them sufficiently separated from locations with farms for life history traits to reflect selection pressure in that area. Moreover, farmed locations used in our study have been intensively farmed for more than four decades and in these areas the number of farmed hosts far outweighs the number of wild conspecifics. The

selection pressure in these areas should therefore mostly be driven by ecological conditions in farms.

There has been a discussion on the degree of gene flow in salmon lice using neutral genetic markers, with some studies report no genetic differentiation (Todd *et al.*, 2004), while other found a weak, but significant differentiation between sites (Glover *et al.*, 2011). Nevertheless, small or no differentiation measured from neutral genetic markers does not necessarily prevent traits under selection from varying between locations (Kirk and Freeland, 2011). We therefore interpret these differences as genetically-based, adaptive responses of lice to farming conditions (Paper III and IV). However, our data are only correlative, and would now require experimental confirmation (*e.g.* selection experiments) to better understand how lice life history and virulence respond to changes in ecological conditions. Additionally, our study only involved two locations with farms and two without farms; since salmon is now also farmed on other continents where salmon lice also are present (North America), it would be interesting to explore how general our findings are worldwide, and how these differences are expressed in countries where salmon farming has more recently been established.

4.1 Short-term responses to farming

Salmon lice are extensively studied due to the negative impact on production in salmon farms, but much about the ecology of this marine parasite remains unknown. To be better able to predict how farming practices might affect life history and virulence we needed more information on how short-term ecological changes in farms affects this parasite *(i.e.* how high parasite loads affects fitness and aggregation in this host-parasite system). We therefore infected salmon with different infection histories in the same infection tank and found that already infected salmon are more susceptible to new lice infections than naïve fish (Paper I), which could be caused by the ability of salmon lice to modulate the host's immune response (Fast *et al.*, 2007; Skugor *et al.*, 2008; Tadiso *et al.*, 2011). This may increase the probability of both sexes infecting the same host and thereby ensure reproduction for the sexually reproducing salmon lice (Paper I). Modulating the host's immune response might be a mechanism used by salmon lice to increase aggregation, which under natural conditions with low host and parasite densities probably is adaptive for the parasite (Paper I). However, aggregation likely has both fitness costs and benefits for parasites, as it may not only increase the probability of reproduction, but also result in negative density-dependent fitness effects

(Shaw and Dobson, 1995). Salmon farms has increased louse densities and lice from farms also have higher infection success (Paper III), resulting in higher parasite loads. Under these conditions mechanisms increasing aggregation might no longer be adaptive for the lice as it could result in too high parasites loads. There seems to be a cost of high infra-population densities in salmon louse as we found negative density-dependence in fecundity with increasing parasite loads for the first three clutches (Paper II). However, we found no effect of parasite load on the survival of adult lice (Paper II). We suggest that decreasing fecundity with higher parasite loads is caused by increased resource competition on the host (Paper II). Furthermore the lack of an efficient host immune response, together with the tendency of lice to make infected hosts more susceptible to new lice infections and negative density-dependence in fecundity suggests that neither hosts nor lice are adapted to high lice densities (Paper I and II).

4.2 Life history in farms

Organisms have limited amount of time and resources available for reproduction. Life history theory therefore predicts an evolutionary trade-off between current and future reproduction for iteroparous organisms (Stearns, 1992; Roff, 2002). The optimal strategy between these two traits that results in highest fitness will depend on ecological conditions such as the probability of future survival.

Under natural conditions adult salmon lice have good prospects for future survival on the host, but the potential for transmission at a given time is low, because there is a low density of susceptible hosts available. The iteroparous reproductive strategy and long reproductive period of salmon lice could therefore be an adaptation to increase the probability of some offspring coming into contact with a host under conditions of low host densities. Moreover, due to the migratory behavior of salmon, host densities also varies throughout the year, the long reproductive period of salmon lice could be a seen as a bet-hedging strategy towards and unpredictable resource (Stearns, 1992; Beaumont *et al.*, 2009). However, in salmon farms large numbers of susceptible hosts are available throughout the year and the expected lifespan is lower. We therefore predicted that conditions in farms should select for increased investment into early reproduction in salmon lice.

We found that lice from locations with farms produced more eggs in their first clutch than lice from locations without farms (Paper III). This suggests that lice from farms invest more into early reproduction and this in agreement with our prediction (Paper III). Investment into increased early reproduction is probably adaptive in farms, as large numbers of

susceptible hosts are kept at high densities. These conditions should select for lice that rapidly utilize this large available resource, a resource that has been constantly expanding the last decades, to increase transmission and thus infect many new hosts. Additionally, reduced prospects for future reproduction and survival should select for higher early reproduction. There seem to be a fitness cost of high early reproduction in salmon lice, as we found that lice from farms had both lower future reproduction and also lower adult survival (Paper III). However, the cost of high intrinsic mortality is likely to be low in farms, because the extrinsic mortality due to slaughtering of hosts and the use of drugs is higher than under natural conditions. Higher investment into current reproduction should then increase fitness even at the cost of reduced survival and future reproduction.

Furthermore, lice from locations with farms had higher infection success (Paper III), suggesting that lice from farms have a higher investment into each offspring than those from "wild" locations. In farms the probability of a copepodite coming into contact with a host is much higher than under natural conditions; fitness should thus increase more by investing more resources into each offspring, than producing a high number of low quality offspring. In addition, farming has increased lice abundances, resulting in higher lice loads on the fish, and therefore competition for resources is likely to be harder in farms. This could select for lice that invest more into each offspring, hence increasing the offspring's ability to compete against conspecifics for resources on the host (Paper II).

We expected that salmon lice from locations with farms should compensate for higher adult mortality by maturing faster and starting reproducing earlier than those from locations without farms, but this prediction was not supported by our data (Paper III). On the contrary, those from farms started to reproduce slightly later than lice from unfarmed locations (Paper III). This might seem surprising, however even though the difference was significant, the effect was small; in addition, the fact that copepodites from farmed lice had higher ability to infect a host (Paper III), suggests that the differences in the onset of reproduction may be driven by a trade-off between the start of reproduction and the offspring's ability to infect hosts. If such a trade-off exists it would prevent lice from combining high ability to infect a host with short maturation time.

All in all our findings are in agreement with a trade-off between early and future reproduction in salmon lice and suggests that farming have selected for accelerated life history of salmon lice and this might also have implications for virulence.

4.3 Virulence in farms

Increased density of susceptible hosts, shorter lifespan, and higher rates of multiple infections in farms due to higher lice abundance have probably selected for increased virulence in salmon farms, as suggested by our observations that salmon lice from farmed locations both reduced growth more and inflicted larger wounds on their hosts than "wild" lice (Paper IV). Unfortunately, the effect of each of these factors on the evolution of virulence in salmon lice cannot be assessed, because our findings are correlative. Furthermore, higher lice abundances in farms (Morton et al., 2004; Krkošek et al., 2006; Torrissen et al., 2013) and higher infection success of lice from farms (Paper III) together with the ability of salmon lice to increase aggregation (Paper I) leads to higher parasite loads. This further increases the harm done to infected hosts by such macroparasites, for which the pathogenic effect is related to the number of parasites (May and Anderson, 1990). Our findings therefore suggest that salmon farming not only have increased lice abundances, but also selected for lice with higher intrinsic virulence, which both might cause more harm to both wild and farmed salmonids (Paper IV).

Our findings of increased virulence associated with intensive farming are in agreement with studies on other host-parasite systems. The bacterial pathogen *Flavobacterium columnare* infecting salmonids have increased in virulence during the last decades and highly virulent strains are more common within farming tanks than in the environment, suggesting that conditions in farms select for higher virulence (Pulkkinen et al., 2010; Sundberg et al., 2016). The increased virulence of the salmon anemia virus has also been associated with ecological conditions in fish farms (Nylund *et al.*, 2003; Murray and Peeler, 2005). Moreover, Marek`s disease virus infecting poultry has increased in virulence following intensification of production and it has been suggested that farming practices such as high host numbers, short generation time, reduced host genetic variation and the use of vaccines could have driven the selection for increased virulence (Nair, 2005; Atkins et al., 2012; Read et al., 2015; Rozins and Day, 2017). Together with our study this suggests that intensive farming practices can have a selective effect for increased virulence in several host-parasite systems.

Wild Atlantic salmon populations are highly structured genetically due to the fact that salmon tend to migrate to their natal river to spawn (Forseth *et al.*, 2017). The genetic structure in salmon might be disrupted by the introduction of large number of genetically similar farmed hosts originating from a few farmed genotypes (Norris *et al.*, 1999; Forseth *et*

al., 2017). Furthermore, farmed escapees might go up rivers and hybridize with wild Atlantic salmon, reducing genetic variability between populations and also reducing the degree of local adaptation (Glover et al., 2012). Reduction in genetic variation is suggested to select for increased virulence as parasites adapt to the most common host genotype, at the cost of becoming less adapted to other genotypes (Ebert, 1998; Kennedy *et al.*, 2016). Genetic variation amongst hosts might therefore limit the spread and severity of infectious diseases (Ebert, 1998; Altermatt and Ebert, 2008). This expectation was confirmed in a study on the microsporidia *Pleistophora intestinalis* infecting *Daphnia magna* were the parasite had higher ability to infect and higher virulence on hosts from the same location, suggesting that parasites adapt to local host genotypes (Ebert, 1994). In our experiment we only infected farmed hosts with lice, so we cannot rule out the possibility that lice from locations with farms are better adapted to farmed hosts than lice from locations with no farms. Future studies should explore potential ongoing local adaptation processes, *e.g.* by infecting both farmed and wild hosts with lice from both locations with and without farms.

5. Conclusions and future research

We found that lice from farms had accelerated life history strategies compared to those from locations without farms, as they have increased investment into early reproduction, but reduced future reproduction. Additionally, they have higher ability to infect and develop on the host, but lower adult survival than lice from locations without farming. Lice from locations with farms also caused more harm to their host (*i.e.* virulence). All in all our findings support predictions from evolutionary theory, that current farming practice have selected for faster life history strategies and increased virulence of salmon lice.

Much research is conducted on salmon lice, due to its negative impact on production and fish welfare in salmon farms. However, these studies mostly focus on genetics and drug resistance and not on ecological and evolutionary mechanisms addressed in this thesis. We have shown that much about the ecology of salmon lice is still unknown, so more research should be conducted on this parasite. This could hopefully shed light on both ecology and evolution of parasites in general, but could also be important to find novel ways to reduce salmon lice abundances in farms.

Findings in this study are correlative, so to be certain that variation in life history and virulence between locations with and without farms are adaptive for the parasite and associated with ecological conditions in farms, we should now conduct experimental

approaches such as selection experiments, where we experimentally select either for fast or slow reproduction or manipulate host density to see how this affects lice life history and virulence. Furthermore, we have only collected lice from four locations (two with and two without salmon farms). To see how general our findings are, more comparisons should be conducted using lice collected from several locations. Lastly, to test whether salmon lice show local adaptation to common host genotypes, we should also conduct infection experiments on both wild and farmed hosts using lice from locations with and without farms. Based on our findings we cannot rule out the possibility that lice from locations with farms are better adapted to farmed host genotypes, which were used in this study. This is something that should be addressed in future studies in this host-parasite system.

Intensive farming is often relying on the use of drugs to reduce parasite abundances and this is often highly efficient in the short term. However, the extensive use of drugs and shorter life expectancy under farming are likely to result in adaptive responses in parasites, which might unintentionally select for more harmful parasites in the long run. Predicting how changes in living conditions for parasites might affect specific life history traits may not always be straightforward, because of the presence of trade-offs, and the observed effects could appear counterintuitive from what one might expect when considering traits separately.

Intensification of farming practices is likely to continue in the future as human populations are growing. We therefore need to find new methods to control parasites that are not likely to result in more harmful parasites due to changes in life history and virulence, and carefully consider the ecological and evolutionary consequences of new drugs and management strategies on parasites. To achieve this, more research on how intensive farming practices affect parasite evolution in economically important host-parasite systems is urgently needed.

6. References

Aaen, S. M., Helgesen, K. O., Bakke, M. J., Kaur, K. & Horsberg, T. E. (2015). Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends in Parasitology* **31**, 72-81.

Alizon, S., Hurford, A., Mideo, N. & Van Baalen, M. (2009). Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *Journal of Evolutionary Biology* **22**, 245-259.

Alizon, S. & Michalakis, Y. (2015). Adaptive virulence evolution: the good old fitness-based approach. *Trends in Ecology & Evolution* **30**, 248-254.

Altermatt, F. & Ebert, D. (2008). Genetic diversity of *Daphnia magna* populations enhances resistance to parasites. *Ecology Letters* **11**, 918-928.

Anderson, R. M. & May, R. M. (1982). Coevolution of hosts and parasites. *Parasitology* **85**, 411-426.

André, J. B. & Hochberg, M. E. (2005). Virulence evolution in emerging infectious diseases. *Evolution* **59**, 1406-1412.

Ashley, M. V., Wilson, M. F., Pergams, O. R. W., O'Dowd, D. J., Gende, S. M. & Brown, J. S. (2003). Evolutionarily enlightened management. *Biological Conservation* **111**, 115-123.

Atkins, K. E., Read, A. F., Savill, N. J., Renz, K. G., Islam, A. F., Walkden-Brown, S. W. & Woolhouse, M. E. (2012). Vaccination and reduced cohort duration can drive virulence evolution: Marek's disease virus and industrialized agriculture. *Evolution* **67**, 851-860.

Beaumont, H. J. E., Gallie, J., Kost, C., Ferguson, G. C. & Rainey, P. B. (2009). Experimental evolution of bet hedging. *Nature* **462**, 90-93.

Berngruber, T. W., Froissart, R., Choisy, M. & Gandon, S. (2013). Evolution of virulence in emerging epidemics. *PLoS Pathogens* **9**, e1003209.

Bjørn, P. A. & Finstad, B. (1998). The development of salmon lice (*Lepeophtheirus salmonis*) on artificially infected post smolts of sea trout (*Salmo trutta*). *Canadian Journal of Zoology* **76**, 970-977.

Borovkov, K., Day, R. & Rice, T. (2013). High host density favors greater virulence: a model of parasite-host dynamics based on multi-type branching processes. *Journal of Mathematical Biology* **66**, 1123-1153.

Budria, A. & Candolin, U. (2014). How does human-induced environmental change influence host-parasite interactions? *Parasitology* **141**, 462-474.

Bui, S., Oppedal, F., Stien, L. & Dempster, T. (2016). Sea lice infestation level alters salmon swimming depth in sea-cages. *Aquaculture Environment Interactions* **8**, 429-435.

Bull, J. J. (1994). Perspective: virulence. *Evolution* **48**, 1423-1437.

Cable, J., Barber, I., Boag, B., Ellison, A. R., Morgan, E. R., Murray, K., Pascoe, E. L., Sait, S. M., Wilson, A. J. & Booth, M. (2017). Global change, parasite transmission and disease control: lessons from ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**.

Carroll, S. P., Hendry, A. P., Reznick, D. N. & Fox, C. W. (2007). Evolution on ecological time-scales. *Functional Ecology* **21**, 387-393.

Costello, M. J. (2006). Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology* **22**, 475-483.

Cressler, C. E., McLeod, D. V., Rozins, C., Van Den Hoogen, J. & Day, T. (2016). The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. *Parasitology* **143**, 915-930.

Crossan, J., Paterson, S. & Fenton, A. (2007). Host availability and the evolution of parasites life-history strategies. *Evolution* **61**, 675-684.

de Roode, J. C., Yates, A. J. & Altizer, S. (2008). Virulence-transmission trade-offs and population divergence in virulence in a naturally occurring butterfly parasite. *Proceedings of the National Academy of Sciences* **105**, 7489-7494.

Ebert, D. (1994). Virulence and local adaptation of a horizontally transmitted parasite. *Science* **265**, 1084-1086.

Ebert, D. (1998). Experimental evolution of parasites. *Science* **282**, 1432-1436.

Ebert, D. & Bull, J. J. (2008). The evolution and expression of virulence. In *Evolution in health and disease* (Stearns, S. C. & Koella, J. C., eds.), pp. 153-167. Oxford: Oxford Univeristy Press.
FAO (2016). The state of world fisheries and aquaculture 2016.
Fast, M. D., Johnson, S. C., Eddy, T. D., Pinto, D. & Ross, N. W. (2007). *Lepeophtheirus salmonis* secretory/excretory products and their effects on Atlantic salmon immune gene regulation. *Parasite Immunology* **29**, 179-189.
Forseth, T., Barlaup, B. T., Finstad, B., Fiske, P., Gjøsæter, H., Falkegård, M., Hindar, A., Mo, T. A., Rikardsen, A. H., Thorstad, E. B., Vøllestad, L. A. & Wennevik, V. (2017). The major threats to Atlantic salmon in Norway. *ICES Journal of Marine Science*.
Fraser, C., Hollingsworth, T. D., Chapman, R., de Wolf, F. & Hanage, W. P. (2007). Variation in HIV-1 set-point viral load: epidemiological analysis and an evolutionary hypothesis. *Proceedings of the National Academy of Sciences of the USA* **104**, 17441-17446.
Gandon, S., Jansen, V. A. A. & van Baalen, M. (2001). Host life history and the evolution of parasite virulence. *Evolution* **55**, 1056-1062.
Glover, K., Quintela, M., Wennevik, V., Besnier, F., Sorvik, A. & Skaala, O. (2012). Three decades of farmed escapees in the wild: a spatio-temporal analysis of Atlantic salmon population genetic structure throughout Norway. *PLoS ONE* **7**, e43129.
Glover, K. A., Stølen Å, B., Messmer, A., Koop, B. F., Torrissen, O. & Nilsen, F. (2011). Population genetic structure of the parasitic copepod *Lepeophtheirus salmonis* throughout the Atlantic. *Marine Ecology Progress Series* **427**, 161-172.
Grimnes, A. & Jakobsen, P. J. (1996). The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. *Journal of Fish Biology* **48**, 1179-1194.
Hamilton-Miller, J. M. (2004). Antibiotic resistance from two perspectives: man and microbe. *International Journal of Antimicrobial Agents* **23**, 209-212.
Hamre, L., Eichner, C., Caipang, C., Dalvin, S., Bron, J., Nilsen, F., Boxshall, G. & Skern-Mauritzen, R. (2013). The Salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) life cycle has only two chalimus stages. *PLoS ONE* **8**, e73539.
Hanssen, S. A., Hasselquist, D., Folstad, I. & Erikstad, K. E. (2005). Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. *Proceedings of the Royal Society B: Biological Sciences* **272**, 1039-1046.
Heino, M., Pauli, B. D. & Dieckmann, U. (2015). Fisheries-induced evolution. *Annual Review of Ecology, Evolution, and Systematics* **46**, 461-480.
Hendry, A. P., Kinnison, M. T., Heino, M., Day, T., Smith, T. B., Fitt, G., Bergstrom, C. T., Oakeshott, J., Jørgensen, P. S., Zalucki, M. P., Gilchrist, G., Southerton, S., Sih, A., Strauss, S., Denison, R. F. & Carroll, S. P. (2011). Evolutionary principles and their practical application. *Evolutionary Applications* **4**, 159-183.
Heuch, P., Nordhagen, J. & Schram, T. (2000). Egg production in the salmon louse *Lepeophtheirus salmonis* (Kroyer) in relation to origin and water temperature. *Aquaculture Research* **31**, 805 - 814.
Holm, H., Santi, N., Kjøglum, S., Perisic, N., Skugor, S. & Evensen, Ø. (2015). Difference in skin immune responses to infection with salmon louse (*Lepeophtheirus salmonis)* in Atlantic salmon (*Salmo salar* L.) of families selected for resistance and susceptibility. *Fish and Shellfish Immunology* **42**, 384-394.
Jensen, K. H., Little, T., Skorping, A. & Ebert, D. (2006). Empirical support for optimal virulence in a castrating parasite. *PLoS Biology* **4**, e197.
Johnson, S. & Albright, L. (1991). The developmental stages of *Lepeophtheirus salmonis* (Kroyer, 1837) (Copepoda, Caligidae). *Canadian Journal of Zoology* **69**, 929 - 950.
Jørgensen, C., Enberg, K., Dunlop, E. S., Arlinghaus, R., Boukal, D. S., Brander, K., Ernande, B., Gårdmark, A., Johnston, F., Matsumura, S., Pardoe, H., Raab, K., Silva, A., Vainikka, A., Dieckmann, U., Heino, M. & Rijnsdorp, A. D. (2007). Ecology: managing evolving fish stocks. *Science* **318**, 1247-1248.
Kennedy, D. A., Kurath, G., Brito, I. L., Purcell, M. K., Read, A. F., Winton, J. R. & Wargo, A. R. (2016). Potential drivers of virulence evolution in aquaculture. *Evolutionary Applications* **9**, 344-354.
Kirk, H. & Freeland, J. R. (2011). Applications and implications of neutral versus non-neutral markers in molecular ecology. *International Journal of Molecular Sciences* **12**, 3966-3988.

Krkošek, M., Lewis, M. A., Morton, A., Frazer, L. N. & Volpe, J. P. (2006). Epizootics of wild fish induced by farm fish. *Proceedings of the National Academy of Sciences of the USA* **103**, 15506-15510.
Lebarbenchon, C., Brown, S., Poulin, R., Gauthier-Clerc, M. & Thomas, F. (2008). Evolution of pathogens in a man-made world. *Molecular Ecology* **17**, 475 - 484.
Leignel, V. & Cabaret, J. (2001). Massive use of chemotherapy influences life traits of parasitic nematodes in domestic ruminants. *Functional Ecology* **15**, 569-574.
May, R. M. & Anderson, R. M. (1990). Parasite-host coevolution. *Parasitology* **100**, 89-101.
May, R. M. & Nowak, M. A. (1995). Coinfection and the evolution of parasite virulence. *Proceedings of the Royal Society of London B* **261**, 209-215.
Mennerat, A., Hamre, L., Ebert, D., Nilsen, F., Davidova, M. & Skorping, A. (2012). Life history and virulence are linked in the ectoparasitic salmon louse *Lepeophtheirus salmonis*. *Journal of Evolutionary Biology* **25**, 856-861.
Mennerat, A., Nilsen, F., Ebert, D. & Skorping, A. (2010). Intensive farming: evolutionary implications for parasites and pathogens. *Evolutionary Biology* **37**, 59-67.
Minchella, D. J. & Loverde, P. T. (1981). A cost of increased early reproductive effort in the snail *Biomphalaria glabrata*. *American Naturalist* **118**, 876-881.
Morand, S., Legendre, P., Gardner, S. L. & Hugot, J.-P. (1996). Body size evolution of oxyurid (Nematoda) parasites: the role of hosts. *Oecologia* **107**, 274-282.
Morand, S. & Sorci, G. (1998). Determinants of life-history evolution in nematodes. *Parasitology Today* **14**, 193-196.
Morton, A., Routledge, R., Peet, C. & Ladwig, A. (2004). Sea lice (*Lepeophtheirus salmonis*) infection rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the nearshore marine environment of British Columbia, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 147-157.
Murray, A. G. & Peeler, E. J. (2005). A framework for understanding the potential for emerging diseases in aquaculture. *Preventive Veterinary Medicine* **67**, 223-235.
Nair, V. (2005). Evolution of Marek's disease – A paradigm for incessant race between the pathogen and the host. *The Veterinary Journal* **170**, 175-183.
Norris, A. T., Bradley, D. G. & Cunningham, E. P. (1999). Microsatellite genetic variation between and within farmed and wild Atlantic salmon (Salmo salar) populations. *Aquaculture* **180**, 247-264.
Nowak, B. F. (2007). Parasitic diseases in marine cage culture – An example of experimental evolution of parasites? *International Journal for Parasitology* **37**, 581-588.
Nowak, M. A. & May, R. M. (1994). Superinfection and the evolution of virulence. *Proceedings of the Royal Society of London B* **255**, 81-89.
Nylund, A., Devold, M., Plarre, H., Isdal, E. & Aarseth, M. (2003). Emergence and maintenance of infectious salmon anaemia virus (ISAV) in Europe: a new hypothesis. *Diseases of Aquatic Organisms* **56**, 11-24.
Palumbi, S. R. (2001). Evolution - Humans as the world's greatest evolutionary force. *Science* **293**, 1786-1790.
Peeler, E. J. & Feist, S. W. (2011). Human intervention in freshwater ecosystems drives disease emergence. *Freshwater Biology* **56**, 705-716.
Pike, A. W. & Wadsworth, S. L. (2000). Sealice on salmonids: their biology and control. *Advances in Parasitology* **44**, 233 - 337.
Poulin, R. (1996). The evolution of life history strategies in parasitic animals. *Advances in Parasitology* **37**, 107-134.
Poulin, R. (2007). *Evolutionary Ecology of Parasites*: Princeton University Press, Oxfordshire, 2. edn.
Pulkkinen, K., Suomalainen, L. R., Read, A. F., Ebert, D., Rintamaki, P. & Valtonen, E. T. (2010). Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. *Proceedings of the Royal Society B: Biological Sciences* **277**, 593-600.
Read, A. F. (1994). The evolution of virulence. *Trends in Microbiology* **2**, 73-76.

Read, A. F., Baigent, S. J., Powers, C., Kgosana, L. B., Blackwell, L., Smith, L. P., Kennedy, D. A., Walkden-Brown, S. W. & Nair, V. K. (2015). Imperfect vaccination can enhance the transmission of highly virulent pathogens. *PLoS Biology* **13**, e1002198.
Renaud, F., De Meeüs, T. & Read, A. F. (2005). Parasitism in man-made ecosystems. In *Parasitism and ecosystems* (Thomas, F., Renaud, F. & Guégan, J.-F., eds.), pp. 155-170. Oxford, UK.: Oxford university press.
Richner, H. & Tripet, F. (1999). Ectoparasitism and the trade-off between current and future reproduction. *Oikos* **86**, 535-538.
Roff, A. D. (2002). *Life history evolution*. Sunderland, Massachusetts: Sinauer Assoiates, Inc.
Rogalski, M. A., Gowler, C. D., Shaw, C. L., Hufbauer, R. A. & Duffy, M. A. (2017). Human drivers of ecological and evolutionary dynamics in emerging and disappearing infectious disease systems. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**.
Rozins, C. & Day, T. (2017). The industrialization of farming may be driving virulence evolution. *Evolutionary Applications* **10**, 189-198.
Shaw, D. J. & Dobson, A. P. (1995). Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* **111**, 111-133.
Skorping, A. & Read, A. F. (1998). Drugs and parasites: global experiments in life history evolution? *Ecology Letters* **1**, 10-12.
Skugor, S., Glover, K., Nilsen, F. & Krasnov, A. (2008). Local and systemic gene expression responses of Atlantic salmon (*Salmo salar* L.) to infection with the salmon louse (*Lepeophtheirus salmonis*). *BMC Genomics* **9**, 498.
Sorci, G., Morand, S. & Hugot, J. P. (1997). Host-parasite coevolution: comparative evidence for covariation of life history traits in primates and oxyurid parasites. *Proceedings of the Royal Society B: Biological Sciences* **264**, 285-289.
Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional Ecology* **3**, 259-268.
Stearns, S. C. (1992). *The evolution of life histories*. Oxford: Oxford University Press.
Stearns, S. C. (2000). Life history evolution: successes, limitations, and prospects. *Naturwissenschaften* **87**, 476-486.
Stockwell, C. A., Hendry, A. P. & Kinnison, M. T. (2003). Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution* **18**, 94-101.
Sundberg, L.-R., Ketola, T., Laanto, E., Kinnula, H., Bamford, J. K. H., Penttinen, R. & Mappes, J. (2016). Intensive aquaculture selects for increased virulence and interference competition in bacteria. *Proceedings of the Royal Society B: Biological Sciences* **283**, 10.1098/rspb.2015.3069.
Tadiso, T., Krasnov, A., Skugor, S., Afanasyev, S., Hordvik, I. & Nilsen, F. (2011). Gene expression analyses of immune responses in Atlantic salmon during early stages of infection by salmon louse (*Lepeophtheirus salmonis*) revealed bi-phasic responses coinciding with the copepod-chalimus transition. *BMC Genomics* **12**, 141.
Thrall, P. H., Oakeshott, J. G., Fitt, G., Southerton, S., Burdon, J. J., Sheppard, A., Russell, R. J., Zalucki, M., Heino, M. & Ford Denison, R. (2011). Evolution in agriculture: the application of evolutionary approaches to the management of biotic interactions in agro-ecosystems. *Evolutionary Applications* **4**, 200-215.
Todd, C., Walker, A., Ritchie, M., Graves, J. & Walker, A. (2004). Population genetic differentiation of sea lice (*Lepeophtheirus salmonis*) parasitic on Atlantic and Pacific salmonids: analyses of microsatellite DNA variation among wild and farmed hosts. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 1176 - 1190.
Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O. T., Nilsen, F., Horsberg, T. E. & Jackson, D. (2013). Salmon lice – impact on wild salmonids and salmon aquaculture. *Journal of Fish Diseases* **36**, 171-194.
Trouvé, S., Sasal, P., Jourdane, J., Renaud, F. & Morand, S. (1998). The evolution of life-history traits in parasitic and free-living platyhelminthes: a new perspective. *Oecologia* **115**, 370-378.
Viney, M. & Cable, J. (2011). Macroparasite life histories. *Current Biology* **21**, R767-R774.

Vollset, K. W., Barlaup, B. T., Skoglund, H., Normann, E. S. & Skilbrei, O. T. (2014). Salmon lice increase the age of returning Atlantic salmon. *Biology Letters* **10**.
Whelan, K. (2010). A review of the impacts of the salmon Louse, *Lepeophtheirus salmonis* (Krøyer, 1837) on wild salmonids. *The Atlantic Salmon Trust, Perth*.
Øverli, Ø., Nordgreen, J., Mejdell, C. M., Janczak, A. M., Kittilsen, S., Johansen, I. B. & Horsberg, T. E. (2014). Ectoparasitic sea lice (*Lepeophtheirus salmonis*) affect behavior and brain serotonergic activity in Atlantic salmon (*Salmo salar L*.): perspectives on animal welfare. *Physiology and Behavior* **132**, 44-50.

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

M S Ugelvik[1], T Mo[1], A Mennerat[1,2] and A Skorping[1]

1 Department of Biology, University of Bergen, Bergen, Norway
2 Ecologie et Dynamique des Systèmes Anthropisés (FRE 3498), CNRS/Université de Picardie Jules Verne, Amiens, France

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 (Pennycuick 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).

Correspondence *M S Ugelvik, Universitetet i Bergen, Institutt for biologi, Postboks 7803, N-5020 Bergen, Norway (e-mail: mathias.ugelvik@uib.no)*

Journal of Fish Diseases

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 form 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 programing 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).

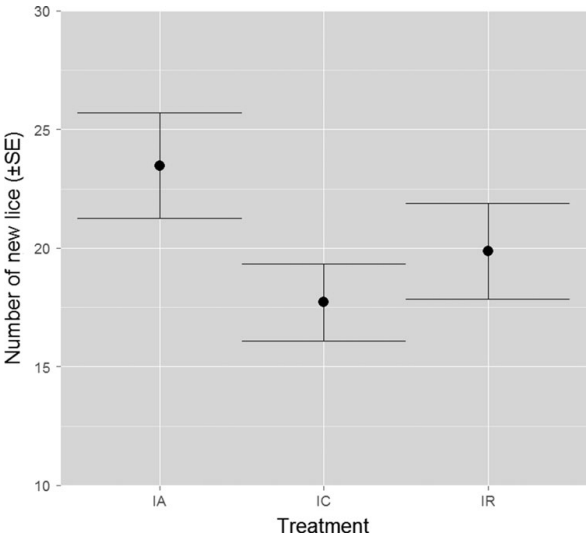

Figure 1 Mean number of new lice (±SE) for the three treatment groups for both replicates: IA, infected twice with adult lice form the first infection remaining; IR, Infected twice, but adult lice from the first infection removed prior to the second infection; and IC, control group of naive fish not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

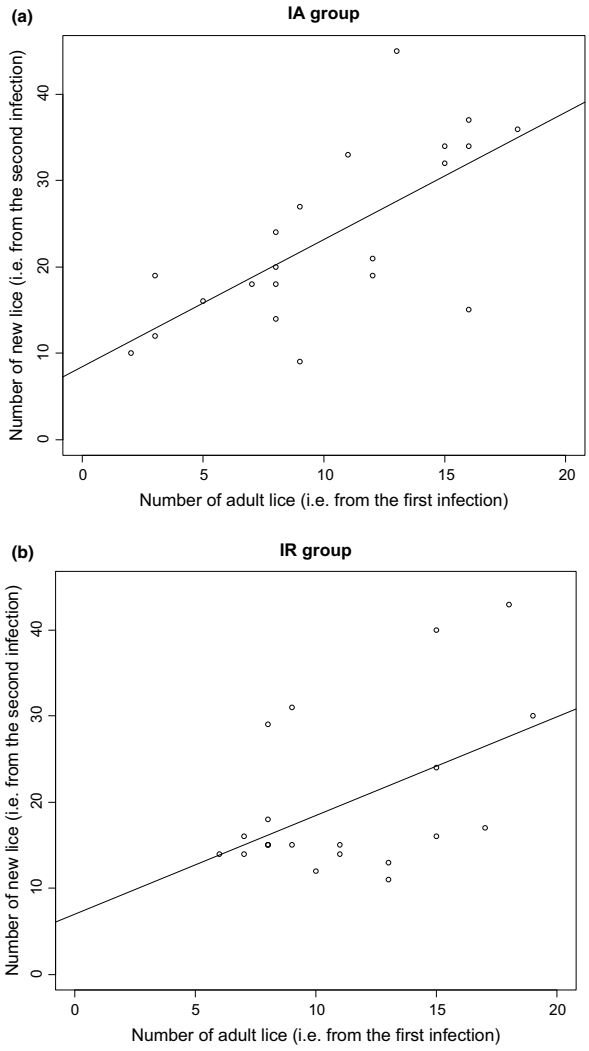

Figure 2 Linear regressions showing the relationship between the number of lice in the first and second infection, (a) for the currently infected group (IA) (Spearman's rank, S = 497.88, $P = 0.0007$, rho = 0.68, z = 2.96, Slope = 1.5), (b) for the group from which adult lice where removed before the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

References

Albon S.D., Stien A., Irvine R.J., Langvatn R., Ropstad E. & Halvorsen O. (2002) The role of parasites in the dynamics of a reindeer population. *Proceedings of the Royal Society B-Biological Sciences* **269**, 1625–1632.

Alizon S., Hurford A., Mideo N. & Van Baalen M. (2009) Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *Journal of Evolutionary Biology* **22**, 245–259.

Anderson R.M. & Gordon D.M. (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373–398.

Anderson R.M. & May R.M. (1978) Regulation and stability of host-parasite population interactions. I. Regulatory processes. *Journal of Animal Ecology* **47**, 219–247.

Barnard C.J., Behnke J.M., Gage A.R., Brown H. & Smithurst P.R. (1998) The role of parasite-induced immunodepression, rank and social environment in the modulation of behaviour and hormone concentration in male laboratory mice (*Mus musculus*). *Proceedings of the Royal Society of London B: Biological Sciences* **265**, 693–701.

Ben-Ami F., Mouton L. & Ebert D. (2008) The effects of multiple infections on the expression and evolution of virulence in a Daphnia-endoparasite system. *Evolution* **62**, 1700–1711.

Costello M.J. (2006) Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology* **22**, 475–483.

Cox F.E. (2001) Concomitant infections, parasites and immune responses. *Parasitology*, **122**(Suppl.), 23–38.

Crofton D. (1971) A model of host–parasite relationships. *Parasitology* **63**, 343–364.

Cross D.A. & Klesius P.H. (1989) Soluble extracts from larval *Ostertagia ostertagi* modulating immune function. *International Journal for Parasitology* **19**, 57–61.

Ebert D., Lipsitch M. & Mangin K.L. (2000) The effect of parasites on host population density and extinction: experimental epidemiology with *Daphnia* and six microparasites. *The American Naturalist* **156**, 459–477.

Fast M.D., Johnson S.C., Eddy T.D., Pinto D. & Ross N.W. (2007) *Lepeophtheirus salmonis* secretory/excretory products

and their effects on Atlantic salmon immune gene regulation. *Parasite Immunology* **29**, 179–189.

Galvani A.P. (2003) Immunity, antigenic heterogeneity, and aggregation of helminth parasites. *Journal of Parasitology* **89**, 232–241.

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

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

Goodwin L.G., Green D.G., Guy M.W. & Voller A. (1972) Immunosuppression during trypanosomiasis. *British Journal of Experimental Pathology* **53**, 40–43.

Greenwood B.M., Bradley-Moore A.M., Bryceson A.D. & Palit A. (1972) Immunosuppression in children with malaria. *Lancet* **1**, 169–172.

Heuch P., Nordhagen J. & Schram T. (2000) Egg production in the salmon louse *Lepeophtheirus salmonis* (Kroyer) in relation to origin and water temperature. *Aquaculture Research* **31**, 805–814.

Holm H., Santi N., Kjøglum S., Perisic N., Skugor S. & Evensen Ø. (2015) Difference in skin immune responses to infection with salmon louse (*Lepeophtheirus salmonis)* in Atlantic salmon (*Salmo salar* L.) of families selected for resistance and susceptibility. *Fish and Shellfish Immunology* **42**, 384–394.

Hudson P.J., Dobson A.P. & Newborn D. (1998) Prevention of population cycles by parasite removal. *Science* **282**, 2256–2258.

Kolstad K., Heuch P., Gjerde B., Gjedrem T. & Salte R. (2005) Genetic variation in resistance of Atlantic salmon (*Salmo salar*) to the salmon louse *Lepeophtheirus salmonis. Aquaculture* **247**, 145–151.

Lightowlers M.W. (2010) Fact or hypothesis: concomitant immunity in taeniid cestode infections. *Parasite Immunology* **32**, 582–589.

May R.M. & Nowak M.A. (1995) Coinfection and the evolution of parasite virulence. *Proceedings of the Royal Society of London B: Biological Sciences* **261**, 209–215.

Mennerat A., Nilsen F., Ebert D. & Skorping A. (2010) Intensive farming: evolutionary implications for parasites and pathogens. *Evolutionary Biology* **37**, 59–67.

Mennerat A., Hamre L., Ebert D., Nilsen F., Davidova M. & Skorping A. (2012) Life history and virulence are linked in the ectoparasitic salmon louse *Lepeophtheirus salmonis. Journal of Evolutionary Biology* **25**, 856–861.

Nowak M.A. & May R.M. (1994) Superinfection and the evolution of virulence. *Proceedings of the Royal Society of London B: Biological Sciences* **255**, 81–89.

Øverli Ø., Nordgreen J., Mejdell C.M., Janczak A.M., Kittilsen S., Johansen I.B. & Horsberg T.E. (2014) Ectoparasitic sea lice (*Lepeophtheirus salmonis*) affect behavior and brain serotonergic activity in Atlantic salmon (*Salmo salar L.*): perspectives on animal welfare. *Physiology and Behavior* **132**, 44–50.

Pennycuick L. (1971) Frequency distributions of parasites in a population of three-spined sticlebacks, *Gasterosteus aculeatus* L., with particular reference to the negative binomial distribution. *Parasitology* **63**, 389–406.

Pike A. & Wadsworth S. (2000) Sealice on salmonids: their biology and control. *Advances in Parasitology* **44**, 233–337.

Poulin R. (2010) Parasite manipulation of host behavior: an update and frequently asked questions. In: *Advances in the Study of Behavior* (ed. by H.J. Brockmann), pp. 151–186. Academic Press, Burlington.

Poulin R. (2013) Explaining variability in parasite aggregation levels among host samples. *Parasitology* **140**, 541–546.

Rajakumar S., Bleiss W., Hartmann S., Schierack P., Marko A. & Lucius R. (2006) Concomitant immunity in a rodent model of filariasis: the infection of *Meriones unguiculatus* with *Acanthocheilonema vitae. Journal of Parasitology* **92**, 41–45.

Shaw D.J. & Dobson A.P. (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* **111**, S111–S133.

Skugor S., Glover K., Nilsen F. & Krasnov A. (2008) Local and systemic gene expression responses of Atlantic salmon (*Salmo salar* L.) to infection with the salmon louse (*Lepeophtheirus salmonis*). *BMC Genomics* **9**, 498.

Tadiso T., Krasnov A., Skugor S., Afanasyev S., Hordvik I. & Nilsen F. (2011) Gene expression analyses of immune responses in Atlantic salmon during early stages of infection by salmon louse (*Lepeophtheirus salmonis*) revealed bi-phasic responses coinciding with the copepod-chalimus transition. *BMC Genomics* **12**, 141.

Wilson K., Bjørnstad O.N., Dobson A.P., Merler S., Poglayen G., Randolph S.E., Read A.F. & Skorping A. (2002) Heterogeneities in macroparasite infections: patterns and processes. In: *The Ecology of Wildlife Diseases* (ed. by P.J. Hudson, A. Rizzoli, B.T. Grenfell, H. Heesterbeek & A.P. Dobson), pp. 6–44. Oxford University Press, Oxford.

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

M S Ugelvik[1], A Skorping[1] and A Mennerat[1,2]

1 Department of Biology, University of Bergen, Bergen, Norway
2 Ecologie et Dynamique des Systèmes Anthropisés (FRE 3498), CNRS/Université de Picardie Jules Verne, Amiens, France

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

Correspondence *M S Ugelvik, Universitetet i Bergen, Institutt for biologi, Postboks 7803, N-5020 Bergen, Norway (e-mail: mathias.ugelvik@uib.no)*

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

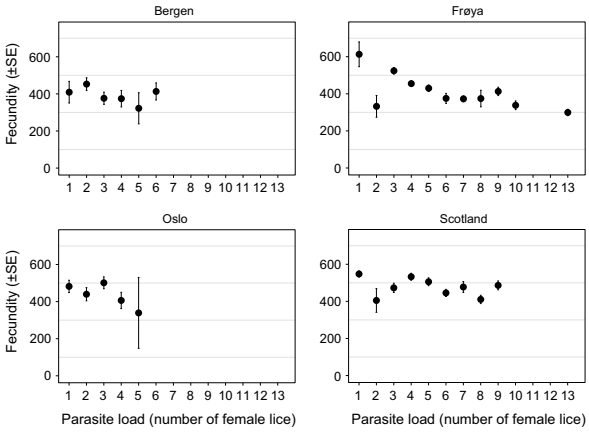

Figure 1 Relation between parasite load and salmon lice fecundity, in lice of the four origins: Bergen (n = 13), Frøya (n = 15), Oslo (n = 14) and Scotland (n = 13).

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

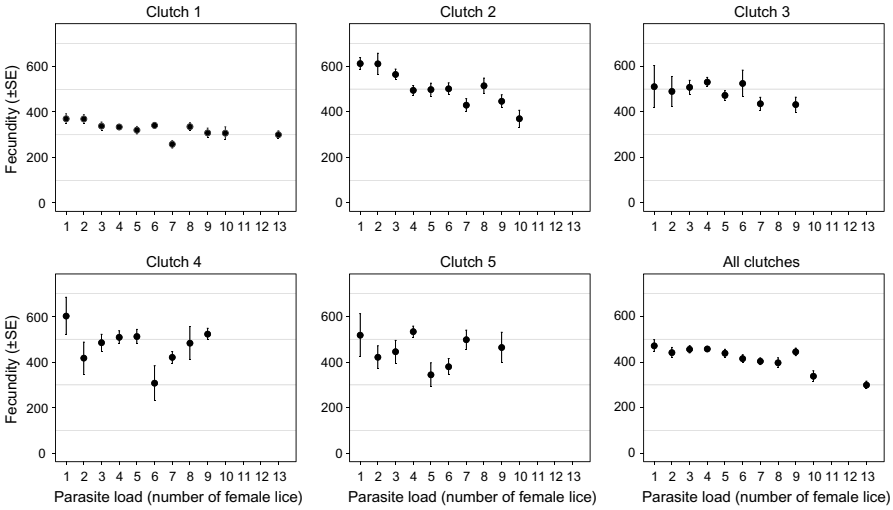

Figure 2 Relationship between parasite load and salmon lice fecundity in the five-first clutches for all hosts from all four origins. The overall relation for all successive clutches is shown in the bottom right panel.

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

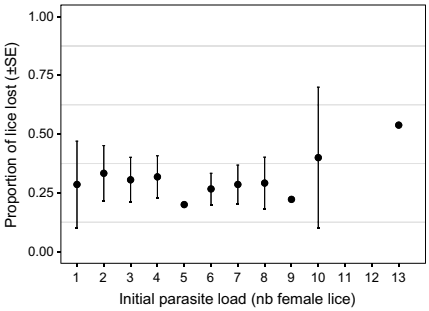

Figure 3 Relationship between initial parasite load (at first reproduction) for all hosts from all four origins and the proportion of female salmon lice lost (i.e. a proxy for lice mortality) during the course of the experiment.

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.

References

Anderson R.M. & Gordon D.M. (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373–398.

Bleay C., Wilkes C.P., Paterson S. & Viney M.E. (2007) Density-dependent immune responses against the gastrointestinal nematode *Strongyloides ratti*. *International Journal for Parasitology* **37**, 1501–1509.

Churcher T.S., Filipe J.A. & Basanez M.G. (2006) Density dependence and the control of helminth parasites. *Journal of Animal Ecology* **75**, 1313–1320.

Costello M.J. (2006) Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology* **22**, 475–483.

Costello M. (2009) How sea lice from salmon farms may cause wild salmonid declines in Europe and North America and be a threat to fishes elsewhere. *Proceedings of the Royal Society B: Biological Sciences* **276**, 3385–3394.

Ebert D., Zschokke-Rohringer C.D. & Carius H.J. (2000) Dose effects and density-dependent regulation of two microparasites of *Daphnia magna*. *Oecologia* **122**, 200–209.

Fast M., Ross N., Mustafa A., Sims D., Johnson S., Conboy G., Speare D., Johnson G. & Burka J. (2002) Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. *Diseases of Aquatic Organisms* **52**, 57–68.

Fast M.D., Johnson S.C., Eddy T.D., Pinto D. & Ross N.W. (2007) *Lepeophtheirus salmonis* secretory/excretory products and their effects on Atlantic salmon immune gene regulation. *Parasite Immunology* **29**, 179–189.

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

Heuch P., Nordhagen J. & Schram T. (2000) Egg production in the salmon louse *Lepeophtheirus salmonis* (Kroyer) in relation to origin and water temperature. *Aquaculture Research* **31**, 805–814.

Holm H., Santi N., Kjøglum S., Perisic N., Skugor S. & Evensen Ø. (2015) Difference in skin immune responses to infection with salmon louse (*Lepeophtheirus salmonis)* in Atlantic salmon (*Salmo salar* L.) of families selected for resistance and susceptibility. *Fish and Shellfish Immunology* **42**, 384–394.

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

Krkošek M., Lewis M.A., Morton A., Frazer L.N. & Volpe J.P. (2006) Epizootics of wild fish induced by farm fish. *Proceedings of the National Academy of Sciences of the United States of Amercia* **103**, 15506–15510.

Lagrue C., Poulin R. & Cohen J.E. (2015) Parasitism alters three power laws of scaling in a metazoan community: Taylor's law, density-mass allometry, and variance-mass allometry. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 1791–1796.

Lebarbenchon C., Brown S., Poulin R., Gauthier-Clerc M. & Thomas F. (2008) Evolution of pathogens in a man-made world. *Molecular Ecology* **17**, 475–484.

Luong L.T., Vigliotti B.A. & Hudson P.J. (2011) Strong density-dependent competition and acquired immunity constrain parasite establishment: implications for parasite aggregation. *International Journal for Parasitology* **41**, 505–511.

Mennerat A., Nilsen F., Ebert D. & Skorping A. (2010) Intensive farming: evolutionary implications for parasites and pathogens. *Evolutionary Biology* **37**, 59–67.

Mennerat A., Hamre L., Ebert D., Nilsen F., Davidova M. & Skorping A. (2012) Life history and virulence are linked in

the ectoparasitic salmon louse *Lepeophtheirus salmonis*. *Journal of Evolutionary Biology* **25**, 856–861.

Morton A., Routledge R., Peet C. & Ladwig A. (2004) Sea lice (*Lepeophtheirus salmonis*) infection rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the nearshore marine environment of British Columbia, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 147–157.

Øverli Ø., Nordgreen J., Mejdell C.M., Janczak A.M., Kittilsen S., Johansen I.B. & Horsberg T.E. (2014) Ectoparasitic sea lice (*Lepeophtheirus salmonis*) affect behavior and brain serotonergic activity in Atlantic salmon (*Salmo salar L.*): perspectives on animal welfare. *Physiology and Behavior* **132**, 44–50.

Paterson S. & Viney M.E. (2002) Host immune responses are necessary for density dependence in nematode infections. *Parasitology* **125**, 283–292.

Pike A.W. & Wadsworth S.L. (2000) Sealice on salmonids: their biology and control. *Advances in Parasitology* **44**, 233–337.

Pittman K., Pittman A., Karlson S., Cieplinska T., Sourd P., Redmond K., Ravnøy B. & Sweetman E. (2013) Body site matters: an evaluation and application of a novel histological methodology on the quantification of mucous cells in the skin of Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **36**, 115–127.

Poulin R. (2007) Are there general laws in parasite ecology? *Parasitology* **134**, 763–776.

Pulkkinen K., Suomalainen L.R., Read A.F., Ebert D., Rintamaki P. & Valtonen E.T. (2010) Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. *Proceedings of the Royal Society B: Biological Sciences* **277**, 593–600.

Selvan S., Campbell J.F. & Gaugler R. (1993) Density-dependent effects on entomopathogenic nematodes (*Heterorhabditidae* and *Steinernematidae*) within an insect host. *Journal of Invertebrate Pathology* **62**, 278–284.

Shaw D.J. & Dobson A.P. (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* **111**, S111–S133.

Skorping A. & Read A.F. (1998) Drugs and parasites: global experiments in life history evolution? *Ecology Letters* **1**, 10–12.

Skugor S., Glover K., Nilsen F. & Krasnov A. (2008) Local and systemic gene expression responses of Atlantic salmon (*Salmo salar* L.) to infection with the salmon louse (*Lepeophtheirus salmonis*). *BMC Genomics* **9**, 498.

Tadiso T., Krasnov A., Skugor S., Afanasyev S., Hordvik I. & Nilsen F. (2011) Gene expression analyses of immune responses in Atlantic salmon during early stages of infection by salmon louse (*Lepeophtheirus salmonis*) revealed bi-phasic responses coinciding with the copepod-chalimus transition. *BMC Genomics* **12**, 141.

Todd C.D., Walker A.M., Hoyle J.E., Northcott S.J., Walker A.F. & Ritchie M.G. (2000) Infestations of wild adult Atlantic salmon (*Salmo salar* L.) by the ectoparasitic copepod sea louse *Lepeophtheirus salmonis* Krøyer: prevalence, intensity and the spatial distribution of males and and females on the host fish. *Hydrobiologia* **429**, 181–196.

Tully O. & Whelan K. (1993) Production of nauplii of *Lepeophtheirus salmonis* (Kroyer) (Copepoda, Caligidae) from farmed and wild salmon and its relation to the infestation of wild sea trout (*Salmo trutta L*) off the west coast of Ireland in 1991. *Fisheries Research* **17**, 187–200.

Ugelvik M.S., Mo T., Mennerat A. & Skorping A. (2016) Atlantic salmon infected with salmon lice are more susceptible to new lice infections. *Journal of Fish Diseases* doi: 10.1111/jfd.12514.

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III

DR. ADELE MENNERAT (Orcid ID : 0000-0003-0368-7197)

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Invest more and die faster: the life history of a parasite on intensive farms

Adèle Mennerat[1,2], Mathias Stølen Ugelvik[1], Camilla Håkonsrud Jensen[1] & Arne Skorping[1]

[1] Department of Biology, University of Bergen, Bergen, Norway

[2] Ecologie et Dynamique des Systèmes Anthropisés (FRE 3498), CNRS / Université de Picardie Jules Verne, Amiens, France

Running head: Intensive farming and parasite life histories

Corresponding author:

Adèle Mennerat

Tel: +47 55584214

Fax +47 55584450

email: adele.mennerat@uib.no

Abstract

Organisms are expected to respond to alterations in their survival by evolutionary changes in their life history traits. As agriculture and aquaculture have become increasingly intensive in the past decades, there has been growing interest in their evolutionary effects on the life histories of agri- and aquacultural pests, parasites and pathogens. In this study we used salmon lice (*Lepeophtheirus salmonis*) to explore how modern farming might have affected

life history evolution in parasites. We infected salmon hosts with lice from either farmed or unfarmed locations, and monitored life history traits of those parasites in laboratory conditions. Our results show that compared to salmon lice from areas unaffected by salmon farming, those from farmed areas produced more eggs in their first clutch, and less eggs later on; they achieved higher infestation intensities in early adulthood, but suffered higher adult mortality. These results suggest that salmon lice on farms may have been selected for increased investment in early reproduction, at the expense of later fecundity and survival. This calls for further empirical studies of the extent to which farming practices may alter the virulence of agricultural parasites.

Keywords: human-induced evolution, life history trade-offs, *Lepeophtheirus salmonis*, *Salmo salar*, intensive aquaculture

Introduction

Agri- and aquacultural practices have become increasingly intensive in the past decades, which represents a change in the ecology of domesticated species (Turcotte, Araki, Karp, Poveda, & Whitehead, 2016). In parallel there has been growing concern that this may also cause evolutionary changes in the life histories of agricultural pests and diseases (Kennedy et al., 2015; Lebarbenchon, Brown, Poulin, Gauthier-Clerc, & Thomas, 2008; Mennerat, Nilsen, Ebert, & Skorping, 2010; Rogalski, Gowler, Shaw, Hufbauer, & Duffy, 2016; Rozins & Day, 2016). High local densities of animals or plants, reduced genetic variation, and breeding of stocks for a high output usually characterize intensive agricultural systems. While farming-induced evolution of parasite virulence is being explored in a number of reviews and theoretical studies (e.g. Mennerat et al. 2010, Kennedy et al. 2015, Rozins and Day 2016), empirical studies so far have mainly focused on short-term issues like drug resistance,

producing new treatments, and reducing the economic losses caused by parasites (but see Leignel and Cabaret 2001, Pulkkinen et al. 2010, Sundberg et al. 2016).

For iteroparous organisms, a well-established cost of current reproduction is a decrease in future (or residual) reproductive value, which is a combination of future fecundity and survival (Minchella & Loverde, 1981; Roff, 2002; Stearns, 1992). Therefore, an individual that restrains its current reproduction will have more resources available to invest in growth and survival, and thereby benefit from higher future fecundity. A second trade-off is that between the number of offspring and the amount of resources invested into each of them (e.g. Smith and Fretwell 1974). Since the amount of resources available for reproduction is limited, organisms have the option of either making few, high quality offspring or many offspring of a lower quality. How these trade-offs are resolved depends on the shape of the survival curve (Stearns, 1992); both free-living and parasitic species are therefore expected to respond to alterations in their survival (including human-induced) by evolutionary changes in their life history traits (Skorping, Jensen, Mennerat, & Högstedt, 2016).

One of the most rapidly growing forms of food production in recent years is intensive aquaculture. The Norwegian Atlantic salmon (*Salmo salar*) mariculture stocks alone have increased from about 160 thousand to more than 720 thousand metric tonnes from 1994 to 2015, while the number of licenced farms has only increased from 811 to 974, which means that both density and turnover rate of fish on each farm have increased dramatically (Norwegian Directorate of Fisheries, 2015). As with other forms of intensive food production, fish in intensive aquaculture facilities are usually densely stocked and kept in monocultures. These unnaturally high population sizes do not only translate into more potential hosts for the parasites, but can also be viewed as a highly predictable year-round resource as fish with a migratory behavior are kept in cages (Kennedy et al., 2015; Mennerat

et al., 2010). In addition, compared with other farming practices (e.g. swine or chicken domestication) salmon mariculture is relatively recent, and there still exist areas both untouched by these food production practices and relatively isolated from farmed areas. This makes salmon farming an especially good system for studying parasite evolutionary responses to human-induced changes in host ecology.

The ectoparasitic sea lice (Caligidae) that feed on the skin, mucus and blood of salmonid fish are amongst the most widespread marine parasites in salmon aquaculture (Costello, 2006). Within this family it is the salmon louse (*Lepeophtheirus salmonis*) that has received most attention because of the problems it causes for both the industry and wild salmonid populations. Its life cycle consists of eight developmental stages (Hamre et al., 2013), and calculations indicate that about seven generations can be produced in one year (Whelan, 2010). The salmon louse has an iteroparous life cycle, and up to 11 successive pairs of egg strings (*i.e.* clutches) have been reported (Heuch, Nordhagen, & Schram, 2000; Mennerat et al., 2012).

Before mariculture started and in areas still unaffected by aquaculture, the salmon louse was depending on migrating salmon that sporadically came into the fjords and on their offspring swimming out, as well as on the resident sea trout populations in coastal areas. The salmon louse's iteroparous life cyle can therefore be viewed as a bet-hedging strategy to an unpredictable host resource (e.g. Beaumont et al. 2009). Salmon lice epidemics started to be reported soon after cage culture began, in the 1960's in Norway, the 1970's in Scotland and in the 1980's in North America, and were attributed to the significantly increased host resource in the sea (Mennerat et al., 2010; Pike & Wadsworth, 2000). In addition to a high host density "farmed" lice are also exposed to frequent host culling and chemical treatments (Denholm et al., 2002), both of which lead to shorter life expectancies of adults on the host (Mennerat et al., 2010).

The differences between the environments of "farmed" and "wild" salmon lice may select for different strategies: infective stages of "farmed" lice have a high probability of finding a host, but due to treatments and culling of hosts the prospect of a long adult life once infection has been achieved is rather poor. For "wild" lice chances of infecting a host are much lower, but life expectancy after infection may be better. This shift in selection from wild to farmed host populations may have favored increased investment in current reproduction (Mennerat et al., 2010), which can be achieved by increasing early fecundity and/or by producing eggs of a better quality.

In this paper we focus on the double hypothesis that (1) "farmed" lice have been selected to achieve higher reproductive output soon after maturity, and invest more than "wild" lice in offspring quality, and (2) such a shift towards increased current reproduction comes at the cost of decreased fitness later in life. In salmon lice the quality of offspring (*i.e.* larvae) is expressed in their ability to find a suitable host, infect it, and develop on it until adulthood. We investigated these predictions by comparing infection success, fecundity, and adult survival of salmon lice sampled from relatively isolated areas where there has never been any salmon farming, to lice coming from areas where salmon have been intensively farmed for several decades.

Materials and methods

L. salmonis sample

In this study we monitored the life history of salmon lice from four different groups, infecting salmon hosts maintained in individual tanks. Two groups (hereafter referred to as "wild lice") were sampled from areas where there had never been any salmon farming at the time of sampling. These areas are relatively isolated from farmed areas, both geographically (by a

radius of at least 200 km) and due to the outwards direction of marine currents (Oslofjord in Norway and Angus in Scotland, Heuch et al. 2000). Hence these two groups may be assumed to be the closest possible representatives of salmon lice as they were before salmon farming started. The two other groups of lice ("farmed lice") were sampled from salmon farms located approximately 450 km apart on the western coast of Norway (Austevoll and Frøya) where salmon farming has been taking place for about four decades, *i.e.* approximately 280 lice generations (Figure 1). Egg strings were collected from 38 - 50 female lice from at least 15 hosts per group, hatched in the laboratory (see e.g. Lars A. Hamre, Glover, & Nilsen, 2009), and pooled together. Before the experiments all four groups of lice were bred for at least three generations in 500 L tanks containing 15-20 naive fish in each (Industrilaboratoriet, Bergen, Norway), to reduce differences between groups of lice due to different environmental conditions at their site of origin.

Infection procedure

We used Atlantic salmon smolts (80-120 g) originating from the same cohort (Industrilaboratoriet, Bergen, Norway) and kept in single aquaria supplied with UV-treated seawater with a flow rate at about 2-6 L/min, and 12 h daylight. 15 fish were infected with lice from Austevoll, Frøya and Scotland. Due to the accidental death of one fish that could not be replaced (it died the day before infection), only 14 fish were infected with lice from Oslo. Because of space limitations (laboratory rooms could not contain more than 30 individual fish tanks), we carried out this study in two different rooms, each containing one farmed and one wild group of lice (Room 1: Austevoll & Oslofjord; Room 2: Frøya & Angus). 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 one hour,

during which the water flow was stopped, the water level lowered and air was supplied directly into the tanks (as described e.g. in Mennerat et al. 2012). Due to differences in the dimensions of the tanks, water volume during infection differed (either 10L or 20L) between the two experimental rooms. We adjusted the number of copepodites accordingly (*i.e.* added 40 copepodites in the smaller and 80 in the bigger tanks) so that all fish were exposed to a similar density of copepodites (4 copepodites per liter). Copepodites were counted using a broad-end sterile pipette.

Handling of fish and lice

All fish were hand-fed twice a day with 0.5 g of 3 mm standard industrial food pellets, following the manufacturer's recommendation. From day 40 post-infection, they were inspected daily; the number of adult females on each fish was recorded, as well as the date at which individual female lice extruded their egg strings. When all female lice on a fish had egg strings, the fish were anesthetized with 1.5 g metacaine (MS-222) per 20 L of seawater, and all adult lice were carefully removed from the fish and placed in a cool box with seawater. 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. The fish were gently lifted by hand so that the top of their back emerged above the surface. After placing the lice back onto the host skin, the fish were observed for a few minutes to make sure re-attachment was successful. For each pair of egg strings a picture of whole egg strings was taken with low magnification (3.5 x) to measure total egg string length. In addition, pictures were taken with higher magnification (20 x) 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 euthanized with an overdose of 3.0 g of metacaine per 15 L of seawater.

Statistical analysis

All analyses were performed using the *lme4* package in the statistical programming environment R 3.2.2 (http://r-project.org), All models presented here were validated by visual inspection of the normality and heteroscedasticity of residuals, and for all four of them including random effects resulted in lower AIC values than models with only fixed effects, indicating a better fit.

Timing of reproduction

After producing their first pair of eggstrings, adult female lice kept producing new pairs of eggstrings at an interval of approximately 12 days (see Table 1). In order to test whether the timing of reproduction (*i.e.* the dates at which female lice produce eggs, measured in days post-infection) differed between farmed and wild lice, we used a generalised linear mixed-effects model fitted with a Poisson distribution (g*lmer*) including status (farmed vs wild) and reproductive event (from 1 to 5) as factors. Room (1 vs 2) and Tank (nested within Room) were used as random effect factors.

Fecundity

The total number of eggs contained in each egg string was estimated by dividing total egg string length by average egg length, and fecundity was calculated as the sum for each pair of egg strings (Mennerat et al., 2012; Ugelvik, Skorping, & Mennerat, 2016). Fecundity of wild and farmed salmon lice was compared using a linear mixed-effect model (*lmer*) with status (farmed vs wild) and reproductive event (from 1 to 5) as factors. Because parasite load varied

across fish hosts and is known to negatively affect salmon lice fecundity (Ugelvik et al., 2016), we also included the number of female lice on each fish as a covariate. Room (1 vs 2) and Tank (nested within Room) were used as random effect factors.

Infection success

As a measure of infection success, we used the number of lice that reached maturity on each fish, as this variable summarizes both successful attachment and development on the host. Both male and female lice were recorded; however male salmon lice displayed a clear tendency to jump off the fish (pers. obs.; Hamre and Nilsen 2011). This resulted in male lice being washed out of the tanks at variable rates on different hosts, and we could not always ascertain whether those males actively jumped off or simply got detached as a result of death. Consequently we only compared the infection success of females between farmed and wild lice. We used a generalised linear mixed-effects model fitted with a binomial distribution (g*lmer*) including status (farmed vs wild) as a factor, Room (1 vs 2) as a random effect factor. The number of trials was defined as the number of copepodites used at infection.

Adult mortality

To test whether adult mortality differed between farmed and wild salmon lice, we compared the number of female lice remaining on the fish after the fifth reproductive event relative to the initial number (*i.e.* at the first reproductive event). We used a generalised linear mixed-effects model fitted with a binomial distribution (g*lmer*) including status (farmed vs wild) as a factor, and Room (1 vs 2) as a random effect factor. The number of trials was defined as the initial number of adult females on the fish.

Results

Farmed lice reproduced slightly (2.6 / 63.1 days, *i.e.* 4%) but significantly later than wild lice ($P = 0.04$), and this difference did not vary over time (status * reproductive event, $P = 0.22$, Table 2, Figure 2). Overall fecundity was lower in farmed than in wild lice ($P = 0.03$), differed among reproductive events ($P < 10^{-4}$) and was negatively affected by parasite load ($P = 0.03$, Table 3). However, fecundity was higher for farmed than for wild lice at the first reproductive event ($P = 0.004$), and lower at the second ($P < 10^{-4}$) and third ($P < 10^{-3}$) reproductive events (Table 4, Figure 3; see also Figure S1). Farmed lice also had a higher infection success than wild lice (farmed: 0.16 ± 0.01, $N = 27$ hosts; wild: 0.12 ± 0.01, $N = 29$ hosts; d.f. = 1, Chisq = 5.28, $P = 0.02$). Finally, adult mortality was higher in farmed than in wild lice (farmed: 0.38 ± 0.06, $N = 29$; wild: 0.23 ± 0.05, $N = 27$; d.f. = 1, Chisq = 3.67, $P = 0.055$, Figure 4; see also Figure S2).

Discussion

Compared to salmon lice sampled from unfarmed areas, those from farmed areas did not develop faster, but produced more eggs in their first clutch, and fewer eggs afterwards. They achieved higher infestation intensities at maturity, but displayed higher adult mortality and hence their numbers declined more rapidly afterwards. All in all, our results indicate that lice sampled from farmed areas invested more in early reproduction than lice from unfarmed areas, at the expense of later fecundity and survival.

A study by Todd *et al.* (2004) found no genetic differentiation between Scottish, East-Canadian and North-Norwegian lice based on neutral genetic markers, and concluded that the salmon lice in the North Atlantic consists of one single panmictic population. Noticeably though, the study sample consisted almost exclusively of lice coming from areas that were either farmed or located downstream from farmed areas. The Scottish sample consisted of 11

locations on the Western coast, which has been farmed for decades, pooled together with only one location on the Eastern, non-farmed coast. A later, more extensive survey including locations from Canada, Ireland, Shetland, Faroe Islands, and Norway found a weak, but significant genetic differentiation as well as some degree of isolation by distance (Glover et al., 2011). Neutral genetic differentiation throughout the North Atlantic is nevertheless likely weak at most, due to larval interchange between farmed and wild stocks combined with oceanic migration of wild hosts, which is assumed sufficient to prevent large, neutral genetic divergence in salmon lice in the North Atlantic. However the story may well differ for those loci that are under selection, as even weak selection may lead to local increases in the selected alleles, as long as the populations are large enough to be free from genetic drift. Recombination would prevent such selection-driven differentiation from being detected by microsatellite studies, unless the microsatellites used are closely linked to the genes under selection (Todd et al., 2004). Between-population differences in characters under selection in salmon lice are largely understudied, but one recent study showed that positively selected traits such as drug resistance can rapidly spread across farms of the North Atlantic (Besnier et al., 2014). In our study we found significant differences in life history traits (*i.e.* traits tightly linked to fitness) between farmed and wild groups of lice that had previously been raised in the lab for at least three generations, indicating that those differences likely have a genetic basis. Hence our current interpretation is that these traits may have started to differentiate due to selective changes caused by intensive salmon farming. The correlative nature of this study does however not allow us to determine the causes of such differentiation. Given that farmed salmon vastly outnumber wild salmon and represent a distinct genetic pool, ongoing local host adaptation might also partially explain our observations.

It is somehow unclear at this stage what consequences such adaptive changes may have in epidemiological terms, and knowledge about the life history of salmon lice is still expanding (e.g. (Ugelvik, Mo, Mennerat, & Skorping, 2016; Ugelvik et al., 2016). From earlier studies it seems that faster life histories correlate with higher levels of virulence (Mennerat et al., 2012), which is consistent with virulence evolution theory and more specifically the existence of a virulence – transmission tradeoff (Alizon, Hurford, Mideo, & Van Baalen, 2009; Cressler, Rozins & Day, 2015). One may therefore expect lice from farmed areas to display higher levels of virulence than those from unfarmed areas, and this seems to be the case (Ugelvik, Skorping, Moberg, & Mennerat, 2017). However, these results remain correlative, and experimental approaches (e.g. artificial selection and / or experimental evolution) would be very useful in determining whether such apparently adaptive changes in the life history and virulence of salmon lice as well as other agricultural parasites are being caused by intensive farming.

Seen from the parasite´s point of view, many of the ecological conditions that we see in farmed versus wild salmon, are also recognized in other intensive farming systems. For example, both poultry and pig farms are characterized by rapid host turnover rates, low genetic variability of hosts and frequent use of antiparasitic drugs. While low host genetic variability may increase the speed of parasite adaptation (e.g. Altermatt and Ebert 2008), shorter host lifespan, as well as frequent medication, reduce parasite life expectancy and thereby the prospects of future reproduction. Our main finding, of a shift to a higher investment in current reproduction, might therefore be relevant to intensive farming in general, and not just salmon farms.

Authors' contributions

AS provided funding; AM and AS designed the study; AM, MSU and CHJ carried out the study and collected data; AM did the statistical analysis and wrote the draft, partly based on an earlier draft written by CHJ; AS and MSU provided comments on the manuscript.

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Data archiving statement

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4db01

References

Alizon, S., Hurford, a, Mideo, N., & Van Baalen, M. (2009). Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *Journal of Evolutionary Biology*, *22*(2), 245–59. https://doi.org/10.1111/j.1420-9101.2008.01658.x

Altermatt, F., & Ebert, D. (2008). Genetic diversity of Daphnia magna populations enhances

resistance to parasites. *Ecology Letters*, *11*(9), 918–28. https://doi.org/10.1111/j.1461-0248.2008.01203.x

Beaumont, H. J., Gallie, J., Kost, C., Ferguson, G. C., & Rainey, P. B. (2009). Experimental evolution of bet hedging. *Nature*, *462*(1476–4687 (Electronic)), 90–93. https://doi.org/10.1038/nature08504

Besnier, F., Kent, M., Skern-Mauritzen, R., Lien, S., Malde, K., Edvardsen, R. B., … Glover, K. a. (2014). Human-induced evolution caught in action: SNP-array reveals rapid amphi-atlantic spread of pesticide resistance in the salmon ectoparasite Lepeophtheirus salmonis. *BMC Genomics*, *15*(1), 937. https://doi.org/10.1186/1471-2164-15-937

Costello, M. J. (2006). Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology*, *22*(10), 475–83. https://doi.org/10.1016/j.pt.2006.08.006

Cressler, C. E., Mc, L. D., Rozins, C., J, V. D. H., & Day, T. (2015). The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. *Parasitology*, 1–16. https://doi.org/10.1017/S003118201500092X

Denholm, I., Devine, G. J., Horsberg, T. E., Sevatdal, S., Fallang, A., Nolan, D. V, & Powell, R. (2002). Analysis and management of resistance to chemotherapeutants in salmon lice, Lepeophtheirus salmonis (Copepoda: Caligidae). *Pest Management Science*, *58*(6), 528–36. https://doi.org/10.1002/ps.482

Glover, K. A., Stølen, Å. B., Messmer, A., Koop, B. F., Torrissen, O., & Nilsen, F. (2011). Population genetic structure of the parasitic copepod Lepeophtheirus salmonis throughout the Atlantic. *Marine Ecology Progress Series*, *427*, 161–172. https://doi.org/10.3354/meps09045

Hamre, L. A., Eichner, C., Caipang, C. M. A., Dalvin, S. T., Bron, J. E., Nilsen, F., … Skern-Mauritzen, R. (2013). The Salmon Louse Lepeophtheirus salmonis (Copepoda: Caligidae) Life Cycle Has Only Two Chalimus Stages. *PLoS ONE*, *8*(9), 1–9.

https://doi.org/10.1371/journal.pone.0073539

Hamre, L. A., Glover, K. A., & Nilsen, F. (2009). Establishment and characterisation of salmon louse (Lepeophtheirus salmonis (Krøyer 1837)) laboratory strains. *Parasitology International*, *58*(4), 451–460. https://doi.org/10.1016/j.parint.2009.08.009

Hamre, L. A., & Nilsen, F. (2011). Individual fish tank arrays in studies of Lepeophtheirus salmonis and lice loss variability. *Diseases of Aquatic Organisms*, *97*(1), 47–56. https://doi.org/10.3354/dao02397

Heuch, P. A., Nordhagen, J. R., & Schram, T. A. (2000). Egg production in the salmon louse [Lepeophtheirus salmonis (Krøyer)] in relation to origin and water temperature. *Aquaculture Research*, 805–814.

Kennedy, D. A., Kurath, G., Brito, I. L., Purcell, M. K., Read, A. F., Winton, J. R., & Wargo, A. R. (2015). Potential drivers of virulence evolution in aquaculture. *Evolutionary Applications*, 344–354. https://doi.org/10.1111/eva.12342

Lebarbenchon, C., Brown, S. P., Poulin, R., Gauthier-Clerc, M., & Thomas, F. (2008). Evolution of pathogens in a man-made world. *Molecular Ecology*, *17*(1), 475–84. https://doi.org/10.1111/j.1365-294X.2007.03375.x

Leignel, V., & Cabaret, J. (2001). Massive use of chemotherapy influences life traits of parasitic nematodes in domestic ruminants. *Functional Ecology*, (15), 569–574.

Mennerat, A., Hamre, L., Nilsen, F., Ebert, D., Davidova, M., & Skorping, A. (2012). Life history and virulence are linked in the ectoparasitic salmon louse Lepeophtheirus salmonis. *Journal of Evolutionary Biology*, *25*, 856–861.

Mennerat, A., Nilsen, F., Ebert, D., & Skorping, A. (2010). Intensive farming: evolutionary implications for parasites and pathogens. *Evolutionary Biology*, *37*(2–3), 59–67.

Minchella, D. J., & Loverde, P. T. (1981). A Cost of Increased Early Reproductive Effort in the Snail Biomphalaria glabrata. *The American Naturalist*, *118*(6), 876–881.

https://doi.org/10.1086/283879

Pike, A., & Wadsworth, S. (2000). Sealice on salmonids: their biology and control. *Advances in Parasitology*, *44*, 233–337. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10563397

Pulkkinen, K., Suomalainen, L.-R., Read, A. F., Ebert, D., Rintamäki, P., & Valtonen, E. T. (2010). Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. *Proceedings. Biological Sciences / The Royal Society*, *277*(1681), 593–600. https://doi.org/10.1098/rspb.2009.1659

Roff, D. A. (2002). *Life history evolution*. (Sinauers, Ed.). Massachusetts, U.S.A.: Sunderland.

Rogalski, M. A., Gowler, C. D., Shaw, C. L., Hufbauer, R. A., & Duffy, M. A. (2016). Human drivers of ecological and evolutionary dynamics in emerging and disappearing infectious disease systems. *Philosophical Transactions of the Royal Society B*, (box 1).

Rozins, C., & Day, T. (2016). The industrialization of farming may be driving virulence evolution. *Evolutionary Applications*, 189–198. https://doi.org/10.1111/eva.12442

Skorping, A., Jensen, K. H., Mennerat, A., & Högstedt, G. (2016). When to Reproduce? A New Answer to an Old Question. *The American Naturalist*, *187*(4), 540–546. https://doi.org/10.1086/685423

Smith, C. C., & Fretwell, S. D. (1974). The Optimal Balance between Size and Number of Offspring. *The American Naturalist*, *108*(962), 499–506. https://doi.org/10.1086/282929

Stearns, S. C. (1992). *The evolution of life histories*. (Oxford University Press, Ed.). Oxford.

Sundberg, L., Ketola, T., Laanto, E., Kinnula, H., Bamford, J. K. H., Penttinen, R., & Mappes, J. (2016). Intensive aquaculture selects for increased virulence and interference competition in bacteria. *Proceedings of the Royal Society B: Biological Sciences*, *283*(20153069). https://doi.org/10.1098/rspb.2015.3069

Todd, C. D., Walker, a M., Ritchie, M. G., Graves, J. a, & Walker, a F. (2004). Population genetic differentiation of sea lice (Lepeophtheirue salmonis) parasitic on Atlantic and Pacific salmonids: analyses of microsatellite DNA variation among wild and farmed hosts. *Canadian Journal of Fisheries and Aquatic Sciences*, *61*(7), 1176–1190. https://doi.org/10.1139/f04-069

Turcotte, M., Araki, H., Karp, D., Poveda, K., & Whitehead, S. (2016). The eco-evolutionary impacts of domestication and agricultural practices on wild species. *Philosophical Transactions of the Royal Society B*.

Ugelvik, M., Mo, T., Mennerat, A., & Skorping, A. (2016). Atlantic salmon infected with salmon lice are more susceptible to new lice infections. *Journal of Fish Diseases*, *in press*(1971), 1–7. https://doi.org/10.1111/jfd.12514

Ugelvik, M. S., Skorping, A., & Mennerat, A. (2016). Parasite fecundity decreases with increasing parasite load in the salmon louse Lepeophtheirus salmonis infecting Atlantic salmon Salmo salar. *Journal of Fish Diseases*, 1–8. https://doi.org/10.1111/jfd.12547

Ugelvik, M. S., Skorping, A., Moberg, O., & Mennerat, A. (in press). Evolution of virulence under intensive farming: Salmon lice increase skin lesions and reduce host growth in salmon farms. *Journal of Evolutionary Biology*, in press. https://doi.org/10.1111/jeb.13082

Whelan, K. (2010). A Review of the Impacts of the Salmon Louse , Lepeophtheirus salmonis (Krøyer , 1837) on Wild Salmonids. *Atlantic Salmon Trust*, (August), 27.

	N		Mean date ± SE (days P.I.)		Mean residual fecundity ± SE (corrected for parasite load)	
Reproductive event	Wild	Farmed	Wild	Farmed	Wild	Farmed
First	83	121	63.1 ± 0.52	65.7 ± 0.63	-147.7 ± 6.1	-118.2 ± 8.1
Second	74	112	76.4 ± 0.98	77.3 ± 0.76	58.4 ± 10.6	30.9 ± 11.0
Third	66	88	87.8 ± 0.83	89.8 ± 0.89	67.5 ± 14.4	30.6 ± 12.9
Fourth	59	73	99.1 ± 1.17	103.1 ± 0.93	58.3 ± 15.4	57.1 ± 12.5
Fifth	53	65	111.7 ± 1.20	114.6 ± 1.10	24.7 ± 18.7	41.8 ± 15.8

Table 1. Timing and fecundity of the first five reproductive events, for farmed and wild salmon lice. N = number of individual females, SE = standard error.

	Estimate	SE	z	p
Intercept	3.98	0.07	54.58	-
Status	-0.04	0.02	-2.06	0.04
Rep. event	0.14	0.003	39.87	$< 10^{-4}$
Status x Rep. event	0.006	0.005	1.22	0.22

Table 2. Effects of status (wild vs farmed), reproductive event (from 1 to 5), and their interaction on the timing of reproduction of salmon lice.

	Estimate	SE	t	p
Intercept	383.34	37.30	10.28	-
Status	33.01	14.08	2.35	0.03
Rep. event	48.91	3.40	14.39	$<10^{-4}$
Parasite load	-6.39	3.13	-2.04	0.03

Table 3. Effects of status (wild vs farmed), reproductive event (from 1 to 5), and parasite load (number of female lice per host) on salmon lice fecundity.

Reproductive event	Estimate	SE	t	p
First	-32.85	10.74	8.32	0.004
Second	83.34	16.50	17.56	$< 10^{-4}$
Third	83.30	20.57	12.64	$< 10^{-3}$
Fourth	22.52	25.90	0.68	0.41
Fifth	25.93	29.38	0.57	0.45

Table 4. Effect of status (wild vs farmed) on salmon lice fecundity, for separate reproductive events. A mixed-effects model was used, including the number of female lice on each fish as a covariate, and Room (1 vs 2) and Tank (nested within Room) as random effect factors. Only the effects of status are reported here.

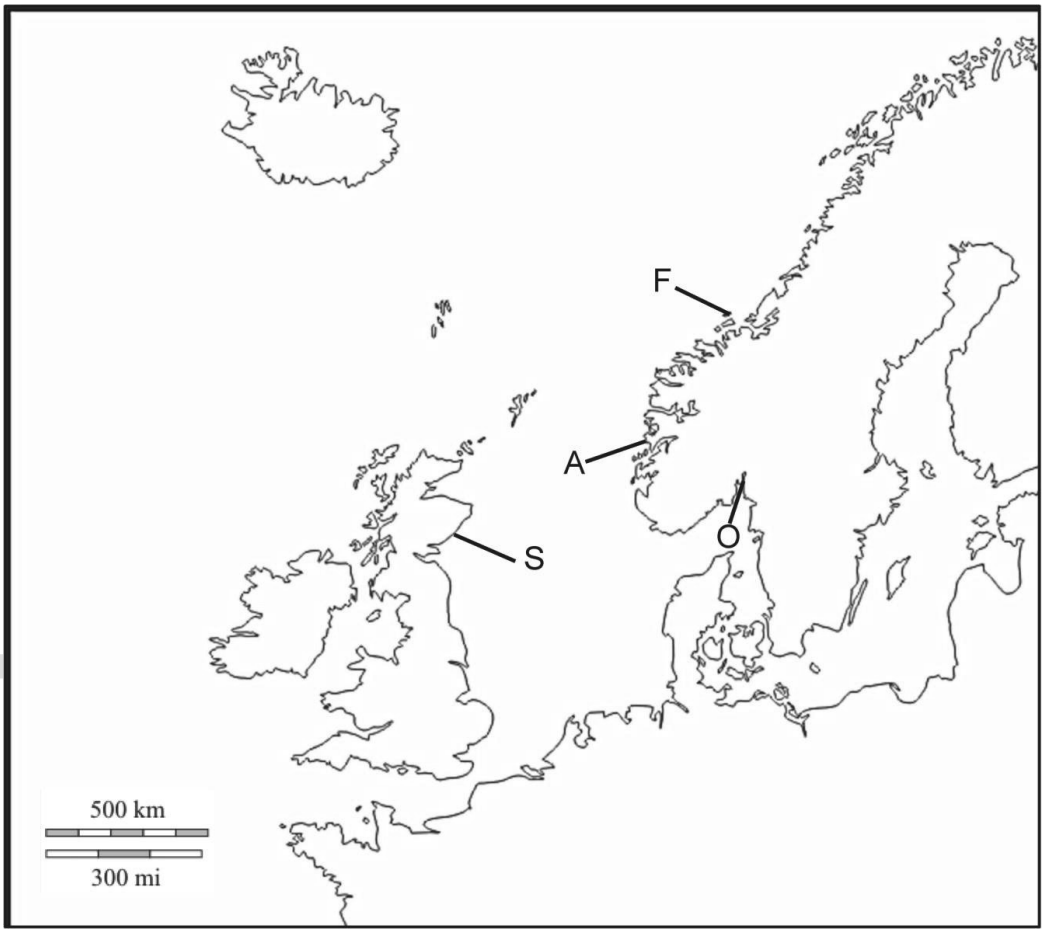
Figure 1. Map of Northern Europe showing the sampling locations of the four study groups of salmon lice. A: Austevoll (farmed); F: Frøya (farmed); O: Oslofjord (unfarmed); S: Scotland (unfarmed).

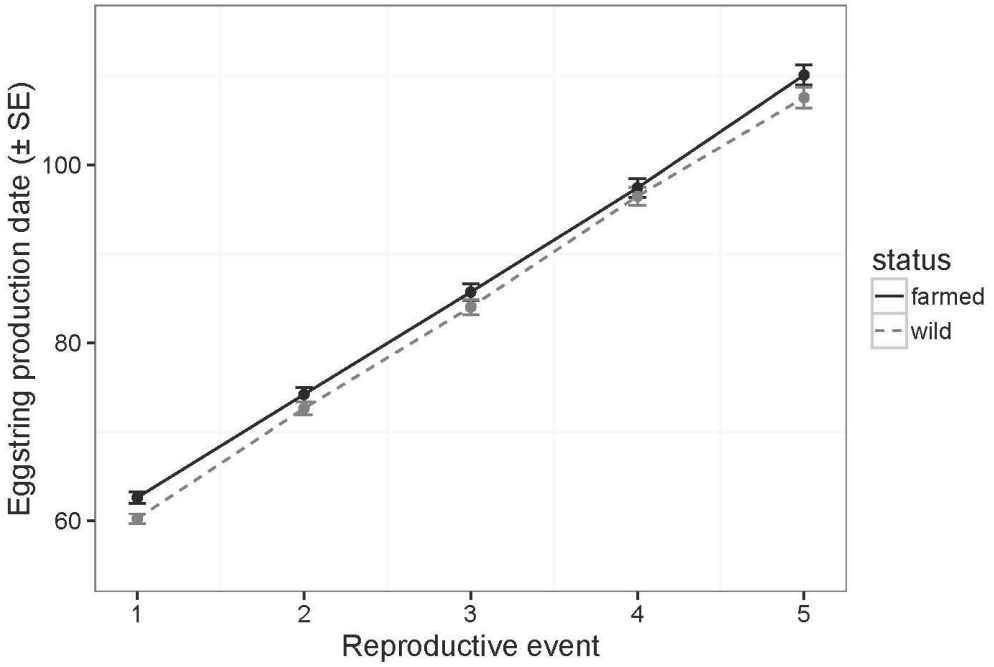
Figure 2. Timing of eggstring production (in days post-infection) of female lice originating from either Atlantic salmon farms ("farmed") or from unfarmed areas ("wild"), for the first five reproductive events.

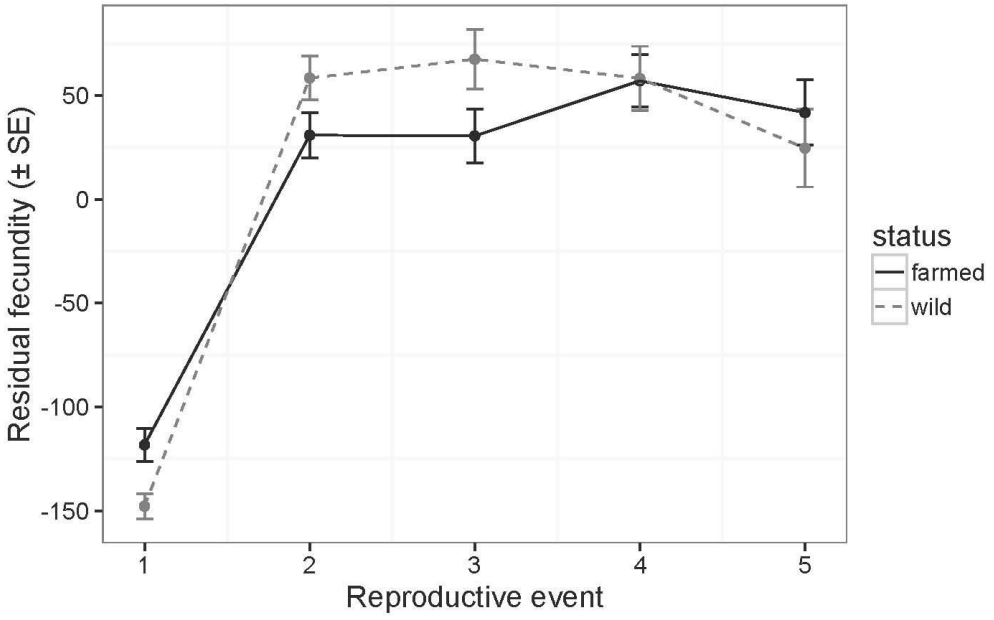
Figure 3. Fecundity (number of eggs produced) of female lice originating from either Atlantic salmon farms ("farmed") or from unfarmed areas ("wild"), for the first five reproductive

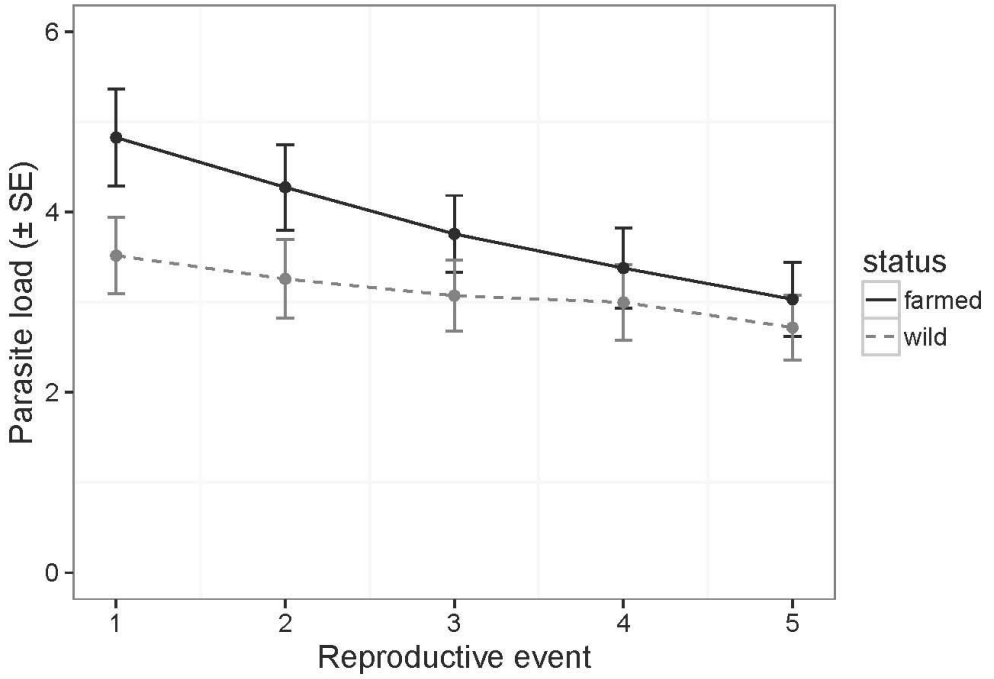
events. This figure represents residual fecundity after controlling for the effect of parasite load on lice fecundity (see Methods).

Figure 4. Decrease in parasite load (due to adult mortality) of female salmon lice originating from either Atlantic salmon farms ("farmed") or from unfarmed areas ("wild").









IV

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Evolution of virulence under intensive farming: salmon lice increase skin lesions and reduce host growth in salmon farms

M. S. UGELVIK*, A. SKORPING*, O. MOBERG* & A. MENNERAT*†

*Department of Biology, University of Bergen, Bergen, Norway
†Ecologie et Dynamique des Systèmes Anthropisés (FRE 3498), CNRS/Université de Picardie Jules Verne, Amiens, France

Keywords:

host–parasite interaction;
human-induced evolution;
intensive aquaculture;
Lepeophtheirus salmonis;
Salmo salar.

Abstract

Parasites rely on resources from a host and are selected to achieve an optimal combination of transmission and virulence. Human-induced changes in parasite ecology, such as intensive farming of hosts, might not only favour increased parasite abundances, but also alter the selection acting on parasites and lead to life-history evolution. The trade-off between transmission and virulence could be affected by intensive farming practices such as high host density and the use of antiparasitic drugs, which might lead to increased virulence in some host–parasite systems. To test this, we therefore infected Atlantic salmon (*Salmo salar*) smolts with salmon lice (*Lepeophtheirus salmonis*) sampled either from wild or farmed hosts in a laboratory experiment. We compared growth and skin damage (i.e. proxies for virulence) of hosts infected with either wild or farmed lice and found that, compared to lice sampled from wild hosts in unfarmed areas, those originating from farmed fish were more harmful; they inflicted more skin damage to their hosts and reduced relative host weight gain to a greater extent. We advocate that more evolutionary studies should be carried out using farmed animals as study species, given the current increase in intensive food production practices that might be compared to a global experiment in parasite evolution.

Introduction

Parasites (including pathogens) rely on resources drawn from a host to survive, grow and reproduce, and therefore, they reduce host fitness. This obligatory effect of parasites is termed virulence (Read, 1994). A parasite that exploits the host at a higher rate will be able to invest more in current reproductive output, but will also likely cause increased virulence. Such a strategy can shorten the lifespan of both the host and the parasite and hence reduce future reproduction of the parasite (Anderson & May, 1982; Frank & Schmid-Hempel, 2008; Alizon & Michalakis, 2015; Kennedy *et al.*, 2016). We would therefore expect a trade-off between the fitness costs (reduced parasite survival) and benefits (increased current reproductive output) of virulence (Anderson & May, 1982; Frank & Schmid-Hempel, 2008; Alizon & Michalakis, 2015; Kennedy *et al.*, 2016). Empirical studies from a number of host–parasite systems have shown that intermediate levels of virulence are optimal, that is result in the highest fitness for the parasite (Jensen *et al.*, 2006; Fraser *et al.*, 2007; de Roode *et al.*, 2008; Doumayrou *et al.*, 2013).

Theory predicts that this optimal level of virulence can be modulated by ecological conditions such as increasing host density, shorter host lifespan or increased competition between different parasite genotypes (Ewald, 1995; Ebert & Bull, 2008; Alizon *et al.*, 2009; Borovkov *et al.*, 2013). For example, the fitness cost for parasites overexploiting their host will be smaller when host density is increasing, because transmission stages will have a higher chance of finding new hosts (Ewald, 1995; Mennerat *et al.*, 2010; Kennedy *et al.*, 2016). Furthermore, as parasite abundance tends to increase with host density (Arneberg *et al.*, 1998), we should expect higher rates of multiple infections and increased within-host competition. Higher levels of virulence are therefore expected to be selected for with increasing host densities (Alizon *et al.*, 2009; Mennerat

Correspondence: Mathias Stølen Ugelvik, Institutt for biologi, Universitetet i Bergen, Postboks 7803, N-5020 Bergen, Norway. Tel.: (+47)55584214; fax: (+47) 55584450; e-mail: mathias.ugelvik@uib.no

et al., 2010; Cressler *et al.*, 2016; Kennedy *et al.*, 2016). Moreover, shorter parasite lifespan, due either to higher host mortality rates or lower parasite survival, is also expected to select for faster reproducing parasites and hence higher levels of virulence (Skorping & Read, 1998; Mennerat *et al.*, 2010; Kennedy *et al.*, 2016).

During the last decades, there has been an increasing concern that human practices, and in particular increasingly intensive farming and aquaculture, might affect the evolution of parasites and pathogens (Skorping & Read, 1998; Leignel & Cabaret, 2001; Palumbi, 2001; Murray & Peeler, 2005; Nowak, 2007; Lebarbenchon *et al.*, 2008; Lynch *et al.*, 2008; Mennerat *et al.*, 2010; Kurath & Winton, 2011), and a handful of studies have explored this empirically (Pulkkinen *et al.*, 2010; Sundberg *et al.*, 2016). Arguably, intensive farming could be considered as a natural, global experiment in parasite life-history evolution (Skorping & Read, 1998). Compared to natural host–parasite populations, intensive food production systems are characterized by high densities of hosts and shorter parasite life expectancies, due to the regular use of antiparasitic drugs combined with selection for fast-growing hosts that are regularly slaughtered (Nowak, 2007; Mennerat *et al.*, 2010; Kennedy *et al.*, 2016).

Intensive salmon farming has expanded rapidly during the last decades. Among the many parasites and pathogens that may infect farmed salmon, the ectoparasitic salmon louse (*Lepeophtheirus salmonis*) is among those that represent the biggest challenge to the industry (Torrissen *et al.*, 2013; Murray *et al.*, 2016). This marine, sexually reproducing copepod browses on the skin of salmonids, thereby causing skin damage and osmoregulatory stress, as well as increasing the risk of secondary infections (Pike & Wadsworth, 2000; Costello, 2006). Salmon lice have a direct life cycle consisting of eight development stages separated by moulting (Hamre *et al.*, 2013), and development time is about 60 days, but is temperature dependent. Under natural conditions, salmon lice rely on migratory Atlantic salmon and small residential populations of sea trout (*Salmo trutta*). However, the introduction of salmon farms has significantly increased both the number and density of available susceptible hosts in coastal waters. Moreover, these hosts are present throughout the year. Previous studies indicate a virulence-transmission trade-off for salmon lice, with earlier reproduction being associated with higher fecundity and reduced host growth (Mennerat *et al.*, 2012). The potential for rapid response to selection in salmon lice has both been studied theoretically (McEwan *et al.*, 2015) and exemplified by the appearance and rapid spread of drug resistance in those parasites (Besnier *et al.*, 2014; Aaen *et al.*, 2015).

Salmon lice and their hosts therefore do not only represent an excellent study system in virulence evolution; due to their huge economic and environmental impact, understanding how farming practices might alter their evolutionary optimal level of virulence is also highly relevant. To investigate this, we compared virulence levels of salmon lice from either farmed or unfarmed areas, using replicate infections of salmon hosts maintained individually in laboratory conditions. As proxies for virulence, we used two different measures – degree of skin damage and host growth rate.

Materials and methods

Experimental set-up

Salmon lice were collected from four locations: two samples from farmed hosts originating from Frøya ('F'), Norway, and Bergen ('B'), Norway, and two samples from wild hosts originating from unfarmed areas, namely the Angus coast in Scotland ('S'), and from Oslofjorden in Norway ('O'). The locations where we collected lice from wild hosts are located upstream from farms, and at the time of sampling, no salmon farms existed within a radius of at least 200 km. At each location, egg strings were collected from 38–50 female lice from at least 15 hosts, hatched in the laboratory and pooled together. Prior to the experiment, lice from all four origins were reared for at least three generations in the laboratory on naive Atlantic salmon (Industry laboratory, Bergen, Norway). To test for differences in virulence, we performed 15 replicate infections for each of the four origins, using a total of 60 Atlantic salmon smolts originating from the same cohort (Industry laboratory, Bergen, Norway; weight: 80–175 g; length: 20–26.5 cm). For these comparisons, the fish were individually kept in tanks with constant flow of UV-treated and filtered normal seawater (flow rate 2–6 L min^{-1}; temperature 7.6–8.6 °C; salinity 35 ppm; and 12-h daylight) and fed 500 mg of 3-mm commercial pellets twice a day. Each room had a capacity of 30 individual tanks; comparison one (O and B, Room 1) was conducted in one room, whereas comparison two (S and F, Room 2) was conducted in another.

The initial experimental design consisted of 15 fish (i.e. replicate infections) per lice origin; however, one fish infected with O lice died prior to the infection; in addition, two fish infected with the S lice and two fish infected with the B lice did not have any adult female lice and were therefore excluded from the statistical analysis. The final sample was therefore S (n = 13), F (n = 15), O (n = 14) and B (n = 13).

Infection

Prior to the infection, all fish were anesthetized with MS-222 (75 mg L^{-1}) and their initial weight and length were recorded. Later the same day, each fish was submitted to the same infection procedure, as described in Mennerat *et al.* (2012): water level was

lowered, water flow stopped, and oxygen provided directly into the tank for 1 h, during which each fish was exposed to infectious salmon lice copepodites (4 copepodites L^{-1}, i.e. 40 copepodites in 10 L of water in Room 1 and 80 copepodites per fish in 20 L of water in Room 2).

Data collection

From day 40 until day 130 post-infection, the number of gravid and nongravid female lice on each host was recorded daily by visually inspecting the fish in their tanks. When all lice on a fish had produced a pair of egg strings (i.e. a clutch), the fish was anesthetized with MS-222 (75 mg L^{-1}) and all lice were gently removed from the host. The number of male and female lice and the total fish length and weight were recorded. Fish were then covered in a transparent plastic film (Toppits®), and the area of skin damage caused by lice was drawn onto the plastic film using a permanent marker. These drawings were later scanned, and the area(s) with skin damage was measured in mm^2 using ImageJ. v. 1.43 for Windows (http://rsweb.nih.gov/ij). Lice were later the same day returned to their original host. This procedure was followed until all lice had completed their fifth clutch (130 days post-infection), after which the fish were euthanized one by one with an overdose of MS-222 (200 mg L^{-1}).

Statistical analysis

We compared three different variables between farmed and wild salmon lice: the area of skin damage caused by the parasites and relative weight and length gain of their salmon hosts. For skin damage, we used a linear mixed-effects model (lme) with status (wild vs. farmed), number of female lice on the fish and days post-infection (i.e. time) as explanatory variables. Lice were clustered among individual hosts that were spread in two different laboratory rooms. The model therefore also included tank (i.e. host) nested within room as random effects. First-order autocorrelation was used to account for repeated measurements. This significantly improved the model and was therefore kept in the final model. For relative weight and length gain, we also used linear mixed-effects models (lme) with status (wild vs. farmed), number of female lice on the fish and days post-infection (i.e. time) as explanatory variables. The hosts were in two different laboratory rooms, so the models had room as random effect. All models were validated by visually inspecting that residuals were normally distributed, and the data for skin damage were square-root-transformed to fit this expectation (uploaded as supplementary material).

All analyses were performed using the nlme package in the statistical program environment R 3.2.2 (http://r-project.org).

Results

This study involved a total number of 204 adult female lice infecting 55 salmon hosts. Both length and weight of the hosts increased with time (days PI), but skin damage was more variable (Tables 1 and 2). This was probably caused by the combined effects of browsing of the lice and continuous healing of the host skin. Furthermore, relative length and weight gain of the host and the area of skin damage were significantly affected by the number of female lice on the fish ($P < 0.02$, Tables 1 and 2). Lice from farmed areas inflicted larger skin damage to their host than lice from unfarmed areas ($P = 0.0001$, Tables 1 and 2A, Fig. 1) and reduced relative host weight gain more than those from unfarmed areas ($P = 0.03$ Fig. 2, Tables 1 and 2B). Relative host length gain did not significantly differ according to lice status (wild vs farmed), although it tended to be reduced in hosts carrying lice from farmed areas (Fig. 3, Tables 1 and 2C).

Discussion

In this study, we found that, compared to lice sampled from unfarmed areas, those collected from farmed

Table 1 Summary statistics of the variables used in the mixed-effects models.

	Min	Max	Mean	SE
Oslo (n = 14)				
Area of skin damage (mm^2)	0	439.96	112.18	10.55
Initial fish weight (g)	80	136	113.86	4.84
Final fish weight (g)	161	223	208.17	4.63
Initial fish length (cm)	21	25	23.71	0.31
Final fish length (cm)	25.5	29	27.92	0.25
Number of female lice on fish	1	3	2	0.25
Scotland (n = 13)				
Area of skin damage (mm^2)	0	219.21	68.25	54.75
Initial fish weight (g)	97	175	142.53	4.46
Final fish weight (g)	172	233	198.69	5.51
Initial fish length (cm)	20.5	25	23.10	0.25
Final fish length (cm)	25.5	28	26.48	0.23
Number of female lice on fish	0	9	5	0.59
Bergen (n = 13)				
Area of skin damage (mm^2)	0	670.98	214.01	20.46
Initial fish weight (g)	106	170	132.57	5.09
Final fish weight (g)	208	276	230.9	6.96
Initial fish length (cm)	22.5	26.5	24.54	0.29
Final fish length (cm)	28	30.5	28.85	0.28
Number of female lice on fish	0	6	3	0.38
Frøya (n = 15)				
Area of skin damage (mm^2)	0	295.06	85.84	6.47
Initial fish weight (g)	122	170	143.8	3.29
Final fish weight (g)	148	225	193.47	6.09
Initial fish length (cm)	22	24	23.17	0.19
Final fish length (cm)	24.5	27.7	26.32	0.25
Number of female lice on fish	1	10	6	0.56

Table 2 Results from the linear mixed-effects models (lme) for weight, length and skin damage.

	numDF	denDF	Value (±SE)	F	*P*
(A) Skin damage					
Intercept	1	259	0.879 ± 3.3	6.99	0.0087
Status (wild vs. farmed)	1	259	−0.29 ± 0.89	16.11	0.0001
Number of female lice	1	259	0.84 ± 0.15	420.05	< 0.0001
Days PI	1	259	0.089 ± 0.01	86.11	< 0.0001
Status × female lice	1	259	0.48 ± 0.22	0.57	0.45
Status × days PI	1	259	−0.03 ± 0.01	5.49	0.019
(B) Weight					
Intercept	1	43	−0.19 ± 0.6	5.60	0.0226
Status (wild vs. farmed)	1	43	−0.79 ± 0.5	4.82	0.0335
Number of female lice	1	43	−0.008 ± 0.02	6.88	0.012
Days PI	1	43	0.007 ± 0.005	12.96	0.0008
Status × female lice	1	43	−0.056 ± 0.02	1.86	0.18
Status × days PI	1	43	0.009 ± 0.005	3.45	0.07
(C) Length					
Intercept	1	43	0.11 ± 0.1	108.34	< 0.0001
Status (wild vs. farmed)	1	43	−0.18 ± 0.1	1.80	0.187
Number of female lice	1	43	−0.005 ± 0.003	11.99	0.0012
Days PI	1	43	0.0005 ± 0.001	6.07	0.0178
Status × female lice	1	43	−0.01 ± 0.005	1.00	0.32
Status × days PI	1	43	0.002 ± 0.001	3.49	0.068

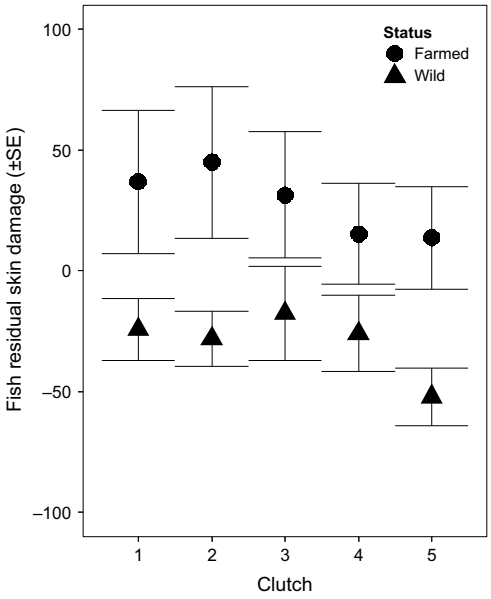

Fig. 1 Fish residual skin damage (±SE) corrected for number of female lice depending on clutch number (egg string) and status of lice (wild vs. farmed).

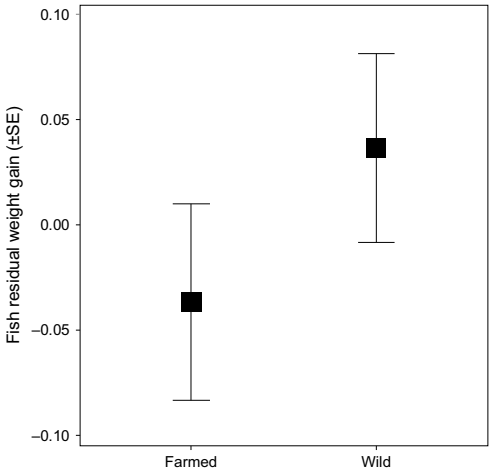

Fig. 2 Relative fish residual weight gain (±SE) from start of the experiment to the fifth egg string corrected for number of female lice depending on lice status (wild vs. farmed).

areas both inflicted larger skin damage and caused a greater reduction in the relative weight gain of their hosts.

Two different proxies for virulence were used in this study, namely the effects of lice on skin damage and those on host growth (weight and length). Wounds caused by salmon lice do not only open the skin barrier and create osmotic imbalance, but the associated bleeding may also lead to reduced haematocrit (Grimnes & Jakobsen, 1996) and hence might impair aerobic capacity and locomotion. In addition, the immune response

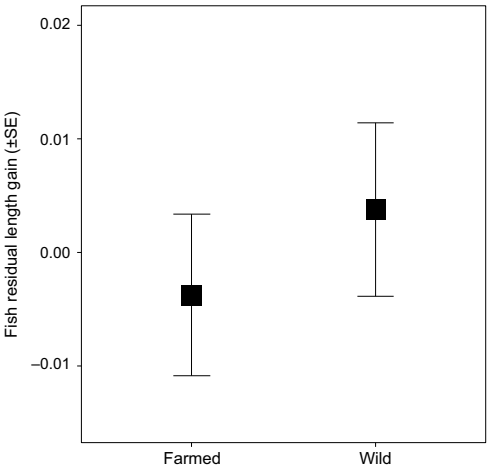

Fig. 3 Relative fish residual length gain (±SE) from start of the experiment to the fifth egg string corrected for number of female lice depending on lice status (wild vs. farmed).

is down-regulated in the skin of infected Atlantic salmon, either due to increased stress (e.g. cortisol) levels or to direct effects of lice on gene expression (Krasnov *et al.*, 2012). Skin damage caused by salmon lice can therefore reduce the fitness of their hosts in several ways, for example via an increased risk of secondary infections or by reducing the amount of energy available for growth, foraging and escaping predators. Juvenile growth in salmon is positively correlated with adult (post-smolt) survival and recruitment (Friedland *et al.*, 2000, 2005; Peyronnet *et al.*, 2007). Our combined measures of skin damage and body growth therefore appear as appropriate proxies for virulence in this host–parasite system (Mennerat *et al.*, 2012). Moreover, these measures should be highly relevant to the aquaculture industry, given the substantial costs of subclinical effects of salmon lice on host growth, including in locations where lice abundances seem to be under control.

In our study, all lice were reared in the laboratory under the same conditions for at least three generations prior to the experiment. It thus appears unlikely that the differences observed here between lice from farmed and unfarmed areas are due to environmental differences at the sites of origin. Variation that might be due to different infestation intensities and different laboratory rooms was controlled for in our analyses. In addition, the fish hosts used here were from the same cohort, so differences in virulence between groups should reflect differences in the lice more than in the hosts. Taken together, our results therefore suggest that there is an intrinsic (i.e. genetically based) difference between farmed and wild lice in the propensity to cause skin damage and reduce growth. Assuming that lice sampled from areas without any salmon farming are closer to ancestral 'wild' lice than those sampled from farmed areas, our results suggest that increased virulence of salmon lice may have arisen as a result of altered selection related to farming conditions. Although our results remain correlative and are based on lice collected at only four marine sites (two with farms and two without farms), they are intriguing and call for more experimental research and broader sampling of both hosts and parasites associated with fish farms.

We found a reduction in relative fish weight gain, but not in relative fish length gain, which suggests that farmed lice reduced host body condition more than wild lice. Moreover, our experiment was carried out in UV-treated seawater, which protected hosts with skin damage from secondary infections. In natural conditions, co-infections are common (Kotob *et al.*, 2016), especially in farms where hosts might also be affected by other waterborne diseases (e.g. infectious salmon anaemia ISA, salmon pancreas disease SPD, vibriosis). The significantly larger skin damage caused by lice from farmed areas is therefore likely to affect host growth more than our laboratory results show.

Under natural conditions, salmon lice rely on relatively small populations of migratory Atlantic salmon and residential populations of sea trout, with additional variation in host density with season. The low number of available hosts, as well as variation in host number and density over time, might restrain selection for high virulence in parasites. Intensive salmon farming has not only increased the number and density of hosts, but has also resulted in a continuous presence of hosts in coastal waters throughout the production cycle (around 18 months). In addition, farmed salmon probably have shorter lifespan than wild fish due to regular slaughtering; this could also reduce the expected lifespan of the parasites and thereby select for higher virulence.

Our findings are consistent with evidence from previous studies indicating changes in parasite virulence related to fish farming. The fish bacterial pathogen *Flavobacterium columnare* affecting salmonids seems to have caused increased host mortality in the last decades, and environmental monitoring shows that the switches from low-virulence to high-virulence strains are happening within farming tanks (Pulkkinen *et al.*, 2010; Sundberg *et al.*, 2016). The infectious salmon anaemia virus has increased in virulence, and the conditions within salmon farms were proposed as a possible explanation (Nylund *et al.*, 2003; Murray & Peeler, 2005). The same trend seems to emerge in other types of animal farms; for example, it was suggested that higher virulence of Marek's disease virus infecting poultry may be due to farming practices such as vaccination and selection for shorter host lifespan (Atkins *et al.*, 2012).

All in all, it seems that the well-acknowledged increase in salmon abundance may not be the only

aspect that should raise concern when discussing salmon farming practices (Tully & Whelan, 1993; Morton *et al.*, 2004; Krkošek *et al.*, 2005; Torrissen *et al.*, 2013). Evolutionary theory predicts that the conditions typical of intensive farming may select for life histories resulting in higher transmission rates and, in case of a transmission-virulence trade-off, also higher virulence. As the repeated appearance of drug resistance clearly demonstrates, parasites of farmed animals can respond quickly to selective changes. Our comparison of two areas with farms against two areas without farms suggests that virulence levels already might have changed in salmon lice. Comparisons based on samples from a wider area, as well as experimental tests, should now be conducted to better assess how general our findings are. The underlying life-history changes are currently under study, but a bigger research effort is now urgently needed to better understand the selective effects of intensive farming on parasites and pathogens. Agri- and aquaculture are likely to become more and more intensive worldwide as a result of a fast-growing human population, and their sustainability cannot be achieved without a better knowledge of how parasite life histories are likely to respond to such human-altered selection.

Author's contribution

AS and AM designed the study; AM and OM collected the original lice; MSU and AM did the experiments and analysed the data; MSU wrote the first draft; AM, OM and AS provided critical revisions and comments to the manuscript.

Ethics statement

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

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Conflict of interest

The authors declare that they have no conflict of interests.

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References

Aaen, S.M., Helgesen, K.O., Bakke, M.J., Kaur, K. & Horsberg, T.E. 2015. Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends Parasitol.* **31**: 72–81.

Alizon, S. & Michalakis, Y. 2015. Adaptive virulence evolution: the good old fitness-based approach. *Trends Ecol. Evol.* **30**: 248–254.

Alizon, S., Hurford, A., Mideo, N. & Van Baalen, M. 2009. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *J. Evol. Biol.* **22**: 245–259.

Anderson, R.M. & May, R.M. 1982. Coevolution of hosts and parasites. *Parasitology* **85**: 411–426.

Arneberg, P., Skorping, A., Grenfell, B. & Read, A.F. 1998. Host densities as determinants of abundance in parasite communities. *Proc. R. Soc. B: Biol. Sci.* **265**: 1283–1289.

Atkins, K.E., Read, A.F., Savill, N.J., Renz, K.G., Islam, A.F., Walkden-Brown, S.W. *et al.* 2012. Vaccination and reduced cohort duration can drive virulence evolution: Marek's disease virus and industrialized agriculture. *Evolution* **67**: 851–860.

Besnier, F., Kent, M., Skern-Mauritzen, R., Lien, S., Malde, K., Edvardsen, R. *et al.* 2014. Human-induced evolution caught in action: SNP-array reveals rapid amphi-atlantic spread of pesticide resistance in the salmon ectoparasite *Lepeophtheirus salmonis*. *BMC Genom.* **15**: 937.

Borovkov, K., Day, R. & Rice, T. 2013. High host density favors greater virulence: a model of parasite-host dynamics based on multi-type branching processes. *J. Math. Biol.* **66**: 1123–1153.

Costello, M.J. 2006. Ecology of sea lice parasitic on farmed and wild fish. *Trends Parasitol.* **22**: 475–483.

Cressler, C.E., McLeod, D.V., Rozins, C., Van Den Hoogen, J. & Day, T. 2016. The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. *Parasitology* **143**: 915–930.

Doumayrou, J., Avellan, A., Froissart, R. & Michalakis, Y. 2013. An experimental test of the transmission-virulence trade-off hypothesis in a plant virus. *Evolution* **67**: 477–486.

Ebert, D. & Bull, J.J. 2008. The evolution and expression of virulence. In: *Evolution in Health and Disease* (S.C. Stearns, J.C. Koella, eds), pp. 153–167. Oxford University Press, Oxford.

Ewald, P.W. 1995. The evolution of virulence: a unifying link between parasitology and ecology. *J. Parasitol.* **81**: 659–669.

Frank, S.A. & Schmid-Hempel, P. 2008. Mechanisms of pathogenesis and the evolution of parasite virulence. *J. Evol. Biol.* **21**: 396–404.

Fraser, C., Hollingsworth, T.D., Chapman, R., de Wolf, F. & Hanage, W.P. 2007. Variation in HIV-1 set-point viral load: epidemiological analysis and an evolutionary hypothesis. *Proc. Natl Acad. Sci. USA* **104**: 17441–17446.

Friedland, K.D., Hansen, L.P., Dunkley, D.A. & MacLean, J.C. 2000. Linkage between ocean climate, post-smolt growth, and survival of Atlantic salmon (*Salmo salar* L.) in the North Sea area. *ICES J. Mar. Sci.* **57**: 419–429.

Friedland, K.D., Chaput, G. & MacLean, J.C. 2005. The emerging role of climate in post-smolt growth of Atlantic salmon. *ICES J. Mar. Sci.* **62**: 1338–1349.

Grimnes, A. & Jakobsen, P.J. 1996. The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. *J. Fish Biol.* **48**: 1179–1194.

Hamre, L., Eichner, C., Caipang, C., Dalvin, S., Bron, J., Nilsen, F. *et al.* 2013. The Salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) life cycle has only two chalimus stages. *PLoS ONE* **8**: e73539.

Jensen, K.H., Little, T.J., Skorping, A. & Ebert, D. 2006. Empirical support for optimal virulence in a castrating parasite. *PLoS Biol.* **4**: e197.

Kennedy, D.A., Kurath, G., Brito, I.L., Purcell, M.K., Read, A.F., Winton, J.R. *et al.* 2016. Potential drivers of virulence evolution in aquaculture. *Evol. Appl.* **9**: 344–354.

Kotob, M.H., Menanteau-Ledouble, S., Kumar, G., Abdelzaher, M. & El-Matbouli, M. 2016. The impact of co-infections on fish: a review. *Vet. Res.* **47**: 98.

Krasnov, A., Skugor, S., Todorcevic, M., Glover, K.A. & Nilsen, F. 2012. Gene expression in Atlantic salmon skin in response to infection with the parasitic copepod *Lepeophtheirus salmonis*, cortisol implant, and their combination. *BMC Genom.* **13**: 130.

Krkošek, M., Lewis, M.A. & Volpe, J.P. 2005. Transmission dynamics of parasitic sea lice from farm to wild salmon. *Proc. R. Soc. B Biol. Sci.* **272**: 689–696.

Kurath, G. & Winton, J. 2011. Complex dynamics at the interface between wild and domestic viruses of finfish. *Curr. Opin. Virol.* **1**: 73–80.

Lebarbenchon, C., Brown, S., Poulin, R., Gauthier-Clerc, M. & Thomas, F. 2008. Evolution of pathogens in a man-made world. *Mol. Ecol.* **17**: 475–484.

Leignel, V. & Cabaret, J. 2001. Massive use of chemotherapy influences life traits of parasitic nematodes in domestic ruminants. *Funct. Ecol.* **15**: 569–574.

Lynch, P.A., Grimm, U. & Read, A.F. 2008. How will public and animal health interventions drive life-history evolution in parasitic nematodes? *Parasitology* **135**: 1599–1611.

McEwan, G.F., Groner, M.L., Fast, M.D., Gettinby, G. & Revie, C.W. 2015. Using agent-based modelling to predict the role of wild refugia in the evolution of resistance of sea lice to chemotherapeutants. *PLoS ONE* **10**: e0139128.

Mennerat, A., Nilsen, F., Ebert, D. & Skorping, A. 2010. Intensive farming: evolutionary implications for parasites and pathogens. *Evol. Biol.* **37**: 59–67.

Mennerat, A., Hamre, L., Ebert, D., Nilsen, F., Davidova, M. & Skorping, A. 2012. Life history and virulence are linked in the ectoparasitic salmon louse *Lepeophtheirus salmonis*. *J. Evol. Biol.* **25**: 856–861.

Morton, A., Routledge, R., Peet, C. & Ladwig, A. 2004. Sea lice (*Lepeophtheirus salmonis*) infection rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the nearshore marine environment of British Columbia, Canada. *Can. J. Fish Aquat. Sci.* **61**: 147–157.

Murray, A.G. & Peeler, E.J. 2005. A framework for understanding the potential for emerging diseases in aquaculture. *Prev. Vet. Med.* **67**: 223–235.

Murray, A.G., Wardeh, M. & McIntyre, K.M. 2016. Using the H-index to assess disease priorities for salmon aquaculture. *Prev. Vet. Med.* **126**: 199–207.

Nowak, B.F. 2007. Parasitic diseases in marine cage culture – An example of experimental evolution of parasites? *Int. J. Parasitol.* **37**: 581–588.

Nylund, A., Devold, M., Plarre, H., Isdal, E. & Aarseth, M. 2003. Emergence and maintenance of infectious salmon anaemia virus (ISAV) in Europe: a new hypothesis. *Dis. Aquat. Organ.* **56**: 11–24.

Palumbi, S. 2001. Evolution - Humans as the world's greatest evolutionary force. *Science* **293**: 1786–1790.

Peyronnet, A., Friedland, K.D., Maoileidigh, N.Ó., Manning, M. & Poole, W.R. 2007. Links between patterns of marine growth and survival of Atlantic salmon *Salmo salar*, L.. *J. Fish Biol.* **71**: 684–700.

Pike, A.W. & Wadsworth, S.L. 2000. Sealice on salmonids: their biology and control. *Adv. Parasitol.* **44**: 233–337.

Pulkkinen, K., Suomalainen, L.R., Read, A.F., Ebert, D., Rintamaki, P. & Valtonen, E.T. 2010. Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. *Proc. R. Soc. B Biol. Sci.* **277**: 593–600.

Read, A.F. 1994. The evolution of virulence. *Trends Microbiol.* **2**: 73–76.

de Roode, J.C., Yates, A.J. & Altizer, S. 2008. Virulence-transmission trade-offs and population divergence in virulence in a naturally occurring butterfly parasite. *Proc. Natl Acad. Sci. USA* **105**: 7489–7494.

Skorping, A. & Read, A.F. 1998. Drugs and parasites: global experiments in life history evolution? *Ecol. Lett.* **1**: 10–12.

Sundberg, L.-R., Ketola, T., Laanto, E., Kinnula, H., Bamford, J.K.H., Penttinen, R. *et al.* 2016. Intensive aquaculture selects for increased virulence and interference competition in bacteria. *Proc. R. Soc. Lond. B Biol. Sci.* **283**, doi: 10.1098/rspb.2015.3069.

Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O.T., Nilsen, F. *et al.* 2013. Salmon lice – impact on wild salmonids and salmon aquaculture. *J. Fish Dis.* **36**: 171–194.

Tully, O. & Whelan, K.F. 1993. Production of nauplii of *Lepeophtheirus salmonis* (Krøyer) (Copepoda: Caligidae) from farmed and wild salmon and its relation to the infestation of wild sea trout (Salmo trutta L.) off the west coast of Ireland in 1991. *Fish. Res.* **17**: 187–200.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Model for skin damage: model validation for skin damage, to check that data are normally distributed (using the qqnorm function in R).

Figure S2 Model for length gain: model validation for length gain, to check that data are normally distributed (using the qqnorm function in R).

Figure S3 Model for weight gain: model validation for weight gain, to check that data are normally distributed (using the qqnorm function in R).

Data deposited at Dryad: https://doi.org/10.5061/dryad.68r7s

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