JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.13082

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

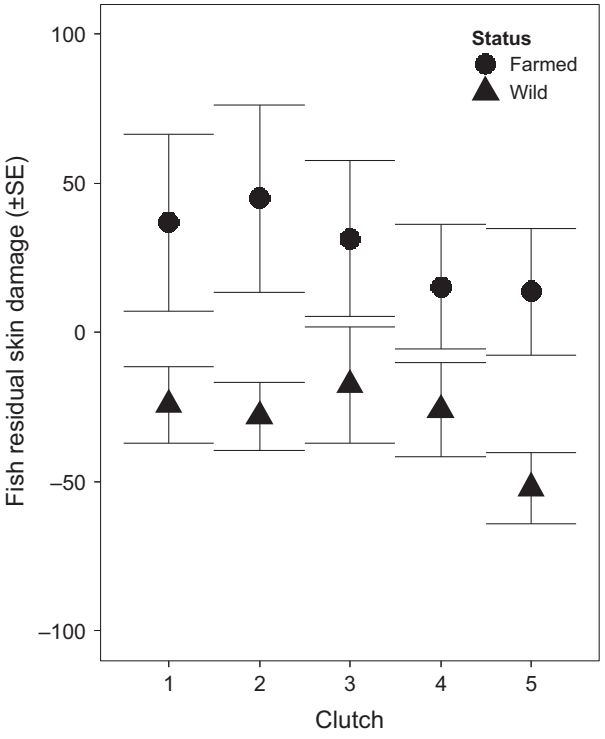

Fig. 1 Fish residual skin damage (±SE) corrected for number of female lice depending on clutch number (egg string) and status of lice (wild vs. farmed).

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

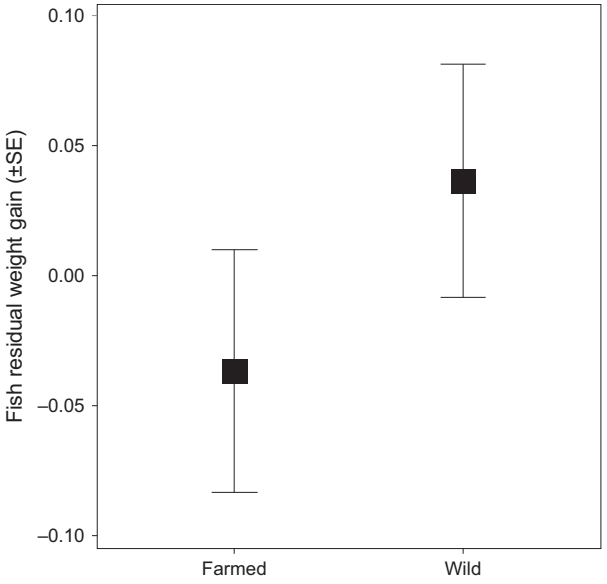

Fig. 2 Relative fish residual weight gain (±SE) from start of the experiment to the fifth egg string corrected for number of female lice depending on lice status (wild vs. farmed).

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

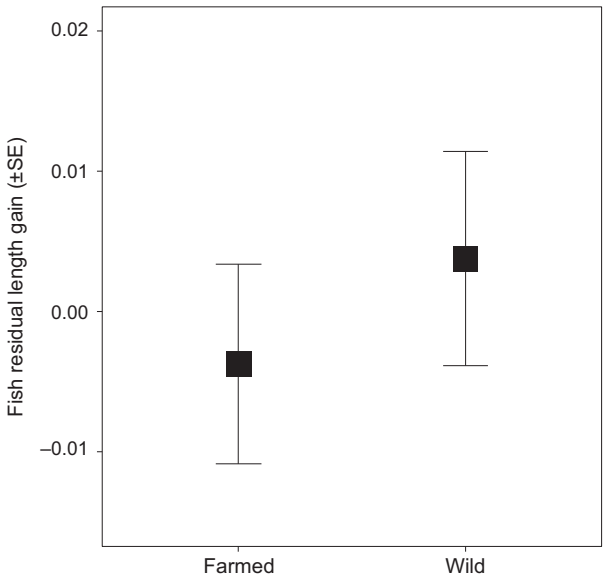

Fig. 3 Relative fish residual length gain (±SE) from start of the experiment to the fifth egg string corrected for number of female lice depending on lice status (wild vs. farmed).

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).

Acknowledgments

We are grateful to Camilla H. Jensen, Lars Hamre and Per Gunnar Espedal for help in the laboratory, and to Frank Nilsen for guidance in the starting phase of the study.

Conflict of interest

The authors declare that they have no conflict of interests.

Funding

This research was funded by the University of Bergen and a grant from the Norwegian Research Council to A. Skorping (grant no 186140).

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

Received 20 January 2017; revised 28 March 2017; accepted 29 March 2017