Aquaculture 480 (2017) 83–88

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Cloned and outbred Atlantic salmon display equal parasite dispersion when infected with the salmon louse

K.A. Glover[a,b,*], T. Hansen[c], F. Besnier[a], M.F. Solberg[a], P.G. Fjelldal[c], A.G.S. Eide[a], S. Dalvin[a,b], F. Nilsen[a,b]

[a] Institute of Marine Research, Postboks 1870, Nordnes, N-5817 Bergen, Norway
[b] Sea Lice Research Centre, Dept. of Biology, University of Bergen, N-5020 Bergen, Norway
[c] Institute of Marine Research, Matre Research Station, N-5984 Matre, Norway

ARTICLE INFO

Keywords:
Heritability
Sea lice
Aquaculture
Microsatellite
Breeding
Resistance

ABSTRACT

The salmon louse is an ectoparasitic copepod that infects salmonids in the marine environment. This parasite causes a major economic and biological challenge to the Atlantic salmon farming industry, and efforts to increase the resistance of salmon to this parasite are ongoing. We used a novel approach to investigate the relative importance of genetic and non-genetic factors on individual infection levels with this parasite. A collectively-reared group of 50 cloned (no genetic variation) and 50 outbred (maximum genetic variation) Atlantic salmon were challenged with lice in two replicate tanks. Lice abundance ranged from 19 to 58 and 13 to 50, and fish-weight corrected lice abundance from 16 to 56 and 11 to 50, for the cloned and outbred groups respectively. Thus, the dispersion in infection was identical among individuals within the two experimental groups. We therefore conclude that under the experimental conditions described, the large (> 5-fold) variation in salmon lice abundance observed among individual salmon was primarily caused by random processes, and that host genetic background played no detectable role. While this result does not preclude the possibility of genetic variation for susceptibility, it demonstrates that random and/or non-genetic factors play a dominant role in infection dynamics in tank studies.

Statement of relevance: This is the first study to investigate the distribution of parasite burden on cloned (no genetic variation) and outbred (maximal genetic variation) fish. Our results clearly demonstrate that non-genetic factors represent the primary determinants of *Lepeophtheirus salmonis* infection-levels among individual Atlantic salmon infected in tanks. These novel results are highly relevant to the aquaculture industry where sea lice represent a major challenge to salmon farming, and breeding programs to decrease susceptibility to this parasite are initiated.

1. Introduction

The salmon louse (*Lepeophtheirus salmonis*) is a parasitic copepod infecting salmonids in the marine environment, existing in both the Pacific and the Atlantic as two sub-species respectively (Skern-Mauritzen et al., 2014). Lice attach to the exterior of host fish, and thereafter feed on skin, mucous and blood which ultimately results in open wounds and osmoregulatory failure in heavily infected individuals (Dawson et al., 1998; Wootten et al., 1982). Each year, this parasite causes hundreds of millions of dollars in treatment costs on commercial salmon farms (Costello, 2009), and has been causatively linked with reduced marine survival of Atlantic salmon (*Salmo salar* L.) (Vollset et al., 2016). Consequently, this parasite represents a major challenge to the salmon aquaculture industry (Taranger et al., 2015; Torrissen et al., 2013).

Genetic variation in susceptibility and/or tolerance is well documented in a number of parasite and host systems including fish (Eizaguirre and Lenz, 2010; Klemme and Karvonen, 2017). Some Pacific salmonids exhibit low levels of susceptibility to sea lice and respond fast and efficient to infestations (Braden et al., 2015; Sutherland et al., 2014). In contrast, all of the marine-migrating Atlantic salmonids can all be considered as highly susceptible, and do not mount an immune response against the parasite. Despite this, within Atlantic salmon and sea trout (*Salmo trutta* L.), genetic variation in host susceptibility has been reported among populations (Glover et al., 2001; Glover et al., 2003), and among families within populations (Gharbi et al., 2015; Gjerde et al., 2011; Glover et al., 2005; Holm et al., 2015; Kolstad et al., 2006). Together with studies that have found statistical associations

* Corresponding author at: Institute of Marine Research, Postboks 1870, Nordnes, N-5817 Bergen, Norway.
E-mail address: kevin.glover@imr.no (K.A. Glover).

http://dx.doi.org/10.1016/j.aquaculture.2017.08.008
Received 25 April 2017; Received in revised form 9 August 2017; Accepted 10 August 2017
Available online 12 August 2017

between molecular genetic variation (also transcriptional) and lice abundance or susceptibility (Gharbi et al., 2009; Glover et al., 2007; Holm et al., 2015), these studies collectively suggest that there is additive genetic variation in susceptibility to the salmon louse in Atlantic salmon and sea trout.

Parasite burdens are often aggregated and non-normally distributed among individual hosts (Pierce et al., 2014; Poulin, 2013). The underlying reasons for this are diverse and depend on the type of parasite and experimental-system in which investigations were conducted. Even for parasites that do not proliferate directly on the host, as is the case for the salmon louse that displays planktonic larval stages, distributions are often skewed and a low number of individuals carry a disproportionately high number of parasites. This has been observed within groups of fish when infections are conducted in fish tanks (Gjerde et al., 2011) as well as among fish sampled in the natural environment (Gargan et al., 2016; Jones and Hargreaves, 2007). While the genetic susceptibility (Holm et al., 2015) or physiological status (Krasnov et al., 2015) of the host may play a role in such parasitic distributions, results from experiments where the same fish were infected with salmon lice on multiple occasions (with delousing in-between) display very different ranking of the individuals between infections (Glover and Skaala, 2006; Glover et al., 2004b). Therefore, other non-genetic processes, for example random variation, behavioral differences or differences in individual fish size may also significantly influence the dispersion of salmon lice among individual salmon in both tanks and sea-cages. Understanding the genetic basis of susceptibility to the salmon louse is important in the context of selective breeding, and for identification of potential targets for vaccine development. Furthermore, it would facilitate our understanding of the evolutionary influence this species may elicit on natural populations, especially in farming dense regions where this parasite has been demonstrated to contribute to mortality of salmonids migrating to the sea (Vollset et al., 2016).

In this study, we aimed to investigate the potential influence of host genetic background on the distribution of salmon lice among individual fish using a novel approach. We challenged a cloned (no genetic variation – thus any differences in infection between individuals within this group must be non-genetic) and an outbred (maximum genetic variation – thus any differences in infection between individuals within this group can be both genetic and non-genetic) group of Atlantic salmon with the salmon louse in a common-garden replicated tank experimental design. Our null hypothesis was: There is no difference in the dispersion of infection level between the individual cloned salmon and the individual outbred salmon.

2. Materials and methods

2.1. Overall experimental design

To examine the degree to which host-genetics contributes to the dispersion of *L. salmonis* within a group of fish, we infected two groups of Atlantic salmon. The first group included 50 cloned salmon (no genetic variation among individuals) and the second group included 50 outbred salmon (individuals arising from separate wild and farmed populations and families to maximize genetic variation among individuals). These fish were infected communally, i.e., 25 cloned and 25 outbred fish in each of the two parallel tanks (100 fish in total). Specifically, we wanted to examine if the inter-individual variation in parasite number, upon termination of the experiment, differed between the two groups.

In tank challenge experiments, *L. salmonis* infection level is often positively correlated with fish size, presumably due to the increased surface area available for attachment (Glover et al., 2004a; Glover et al., 2001; Glover et al., 2003). Consequently, fish from both experimental groups (cloned and outbred) were size-selected for this experiment in order to limit both differences in mean size between groups, as well as create as similar as possible size variations within the groups.

2.2. Experimental fish

On 15.02.16, 50 post-smolt salmon originating from a single cloned-family were divided into two replicate ~2.5 m^3 tanks (25 fish in each). These fish originated from a commercial farmed strain (Aqua Gen AS), and were cloned at the Matre field station owned by the Institute of Marine Research using the following approach: Eggs from a doubled haploid female were fertilized with UV treated sperm and subjected to a hydrostatic pressure of 65.500 kPa (TRC-APV, Aqua pressure vessel, TRC Hydraulics Inc., Dieppe, Canada) for 5 min, 37 min and 30 s after fertilization at 8 °C. Genetic validation of the offspring is provided in Supplementary file 1.

On 17.02.2016, 50 outbred post-smolt salmon were divided into the two replicate tanks already containing the cloned salmon. These fish were first sedated and then adipose fin clipped before being added to the experimental tanks. This enabled visual identification of the cloned (not fin clipped) and outbred (adipose fin clipped) fish within the replicate tanks. The 50 outbred salmon selected for this experiment originated from 28 full and half-sibling families from multiple wild and farmed strains of salmon (see validation via genotyping below). This included the domesticated Mowi strain, the wild Figgjo, Etne and Opo populations, and F1 hyrbids between the wild Figgjo population and the farmed Mowi strain. All three wild populations are located on the west of Norway, and two of them, Etne and Opo, have been demonstrated to be partially admixed with farmed escapees (Glover et al., 2012; Glover et al., 2013; Karlsson et al., 2016). The 50 outbred fish were randomly selected from a tank of aged 1 + post-smolt salmon that originated from a mixture of fish from > 100 families from the above-mentioned strains. Therefore, while the 50 outbred fish selected for this study were not from 50 separate families (which would have maximized potential genetic variation among these individuals), they still displayed a very significant amount of genetic diversity due to their multiple origins.

All data for the cloned and outbred fish used in this study, including phenotypic measurements, lice infection levels, genotypic data and family pedigree information are presented (Supplementary file 1).

2.3. Challenge with lice

Cultivation systems for the production of *L. salmonis* are well developed. We used a well-established and standard *L. salmonis* propagation protocol in order to produce the copepodites that were used to infect the fish in this study (Hamre et al., 2009). A total of 7500 copepodites were produced at the wet laboratory facilities at the Institute of Marine Research in Bergen, and transported to the Matre field station where the fish were challenged in two parallel tanks.

On 10.03.2016, the two replicate tanks were infected with 7500 *L. salmonis*. Infection was conducted using a well-established and standard procedure for conducting tank experiments with this parasite (Hamre et al., 2009). In short, this involved reducing the water level in both tanks down to approximately 15 cm deep, and reducing the inflow water to a minimum. This ensured the fish stood evenly in the gentle current and had sufficient oxygen. The copepodites where thereafter added a little at a time to the tanks, and the water flow immediately adjusted so that it took approximately 1 h for the tank to fill up again and start to flush out lice that had not managed to find and attach to a host in that time period. The water temperature during infection was 10 °C (± 0.5) degrees and full salinity 34‰ (± 0.5). These environmental conditions were held throughout the experiment.

The experiment was terminated on 07.04.2016, 28 days post-infection. At this stage the lice had developed to pre-adult I females and pre-adult 2 males. Upon termination, fish were carefully netted with a wet net one at a time from the tanks, given an overdose of the anesthetic metacain (Finquel Vet, ScanVacc, Årnes, Norway), and thereafter measured for weight, length and number of lice. Any lice detaching

from the fish in the net, or in the anesthetic bath were added to the count for that fish. A tissue sample was thereafter taken from each fish to validate experimental group and pedigree via DNA analysis.

2.4. Genotyping of hosts

After the experiment was terminated, all salmon were genotyped with a panel of 18 microsatellite markers. Genotyping served to validate the genetic background of the experimental fish (cloned or outbred), and specifically for the outbred group, to identify all 50 individuals back to their families of origin (Supplementary file 1). It is important to note that identification to family was not conducted to estimate family infection rates in the outbred group of fish (nor compute heritability etc.), but to validate that the initial random selection of individuals from a tank of mixed genetic background, included fish of diverse backgrounds (i.e., multiple families and populations were represented in the selection). The genotyping also served to demonstrate that the cloned fish contained no genetic variation (all cloned fish displayed an identical single allele for each of the 18 markers which are known to be highly or very highly polymorphic in both wild and farmed Atlantic salmon from Norway – Supplementary file 1 (Glover et al., 2009; Glover et al., 2012; Glover et al., 2016; Quintela et al., 2016)).

Microsatellite genotyping was performed in the laboratory using standard isolation and amplification protocols previously described (Glover et al., 2015). Parentage identification was performed with an exclusion based approach implemented in the program FAP (Taggart, 2007), using an identical procedure to previous studies from this laboratory (Solberg et al., 2013a; Solberg et al., 2013b; Solberg et al., 2016).

2.5. Statistical analysis

All statistical analyses were performed in the R programming language and environment (R core team 2016). First, a series of linear models were fitted to test for *fish weight*, *tank* (replicate), and *fish type* as predictors to the number of lice on each fish. As fish weight was the only significant co-factor, we subsequently used the weight-corrected lice count to test for a possible difference in lice infection variability between the two fish types. To do so, we fitted a F test to compare the variance of two samples as in the *var.test* function in R.

2.6. Animal welfare conditions

The experiment was conducted in accordance with International guidelines, and was certified using Norwegian research permit number 8516.

3. Results

Upon termination, three of the cloned salmon (two in tank 1, one in tank 2) were dead (reason undetermined). These individuals were excluded from the analyses, leaving a total of 47 cloned and 50 outbred salmon for the comparisons. None of the experimental parameters (mean fish weight, lice abundance, lice density) showed differences between replicate tanks (all $P = 0.18$–0.96) (Table 1).

The average weight of the fish belonging to the two experimental groups was significantly different, with the cloned fish being slightly larger than the outbred fish ($P = 0.005$) (Table 1). Furthermore, despite efforts to select fish from both experimental groups with similar size distributions, there was a significant difference in distribution of fish size between groups, with the outbred fish showing significantly greater variation in fish size ($P < 0.001$) (Table 1, Fig. 1).

A significant difference in abundance of lice was observed between the two experimental groups, with the cloned fish displaying a slightly higher average number of lice than the outbred fish ($P = 0.03$) (Table 1). Lice abundance was positively correlated with fish size ($P = 0.004$) (Fig. 1). Fish belonging to the cloned group were both larger and displayed a higher abundance of lice than fish belonging to the outbred strain (Table 1). However, once individual fish size was accounted for (see methods), there was no difference in the weight-corrected lice abundance between the cloned and outbred groups, demonstrating equal susceptibility to infection with *L. salmonis* ($P = 0.17$).

A large variation in individual infection level was observed for both groups of fish, ranging from 19 to 58 and 1 to 50 for the cloned and outbred groups respectively (Table 1, Fig. 1). Using the F test to compare the variance within the two experimental groups, no difference for either abundance ($P = 0.7$) nor weight-corrected abundance of lice ($P = 0.81$) was observed between the two groups (Table 1, Fig. 2). Thus, the large variation observed in infection level among individual salmon, was similar between the cloned and outbred groups of salmon. This is despite a significantly greater variation in individual fish size in the outbred as opposed to the cloned group ($P < 0.001$) (Fig. 1), which could have potentially contributed to an increase in parasite dispersion in the outbred group. Alternatively, to remove any possible bias linked to fish weight, we also used a truncated version of the data where all outbred fish smaller than 200 g were removed. This produced two groups of fish of very similar weight (261 ± 37 and 264 ± 23) with outbred fish still being slightly more variable in weight than cloned. Despite this, the variation in lice infection between the two size-sorted groups were not significantly different ($P = 0.39$).

4. Discussion

This study provides an insight into the dynamics of parasite burdens among individual fish where skewed, aggregated and/or highly-variable infection levels are often reported. Specifically, we observed no difference in the dispersion of infection with the salmon louse among a group of cloned (no genetic variation among hosts) and outbred (maximum genetic variation among hosts) salmon (Table 1, Fig. 2). We therefore conclude that under the experimental conditions described, the large (> 5-fold) variation in lice abundance observed among individual salmon was primarily caused by random and/or unidentified processes, and that host genetic background played no detectable role.

Several studies have reported positive heritability estimates for susceptibility to the salmon louse in domesticated Atlantic salmon (Gharbi et al., 2015; Gjerde et al., 2011; Glover et al., 2005; Kolstad et al., 2005; Tsai et al., 2016), while other studies have reported statistical associations between molecular genetic (and genomic) variation and lice abundance (Gharbi et al., 2009; Glover et al., 2007; Holm et al.,

Table 1
Fish size and infection parameters for the cloned and outbred salmon groups when the experiment was terminated.

Strain	Tank	N	Weight (g) ± SD	Range weight	Lice count ± SD	Range lice count	Weight corrected lice count ± SD	Range weight corrected lice count
Cloned	1	24	259.4 ± 25.4	212–305	34.5 ± 10.3	19–58	33.8 ± 9.8	16–58
Outbred	1	25	231.2 ± 51.0	136–325	32.6 ± 9.7	13–50	33.8 ± 8.8	11–48
Cloned	2	23	266.1 ± 22.4	212–326	35.7 ± 8.5	19–49	34.8 ± 8.2	17–46
Outbred	2	25	247.2 ± 54.2	130–344	29.6 ± 7.7	14–43	29.9 ± 8.2	16–50
Cloned	1 + 2	47	262.2 ± 23.0	212–326	35.1 ± 9.3	19–58	34.3 ± 8.9	16–56
Outbred	1 + 2	50	239.9 ± 52.7	130–344	31.1 ± 8.8	13–50	31.9 ± 8.7	11–50

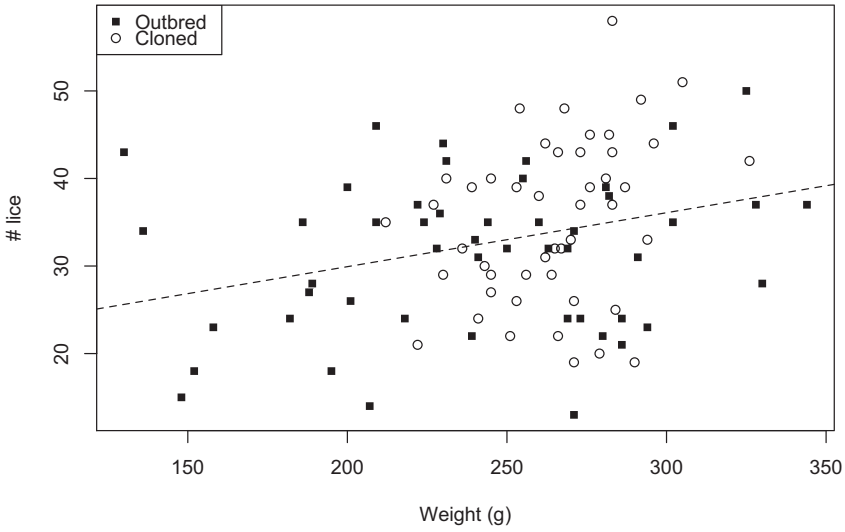

Fig. 1. Fish weight (g) plotted against lice count (n) for 50 outbred (black squares) and 47 cloned salmon (open circles) co-infected with *L. salmonis* in two replicate tanks. Data are shown pooled across replicate tanks. The regression line for # lice predicted by weight for both groups combined is given in dashed line.

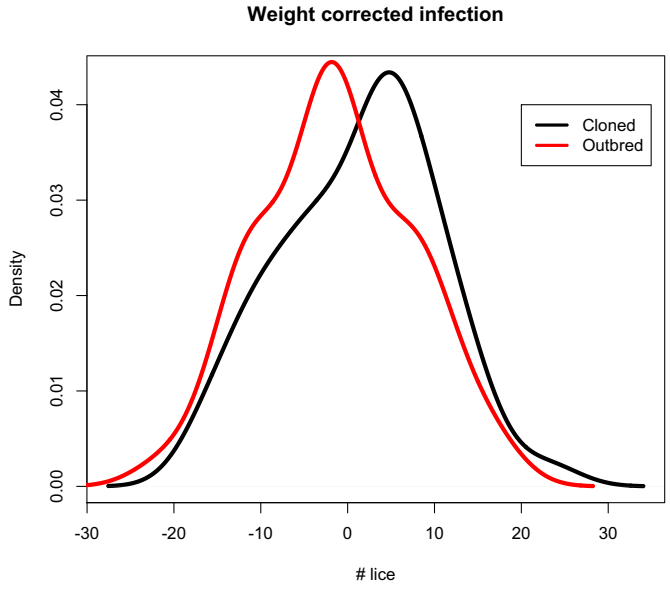

Fig. 2. Distribution of the number of lice, corrected for fish weight, in the outbred (black line) and cloned (red line) groups of salmon. Distributions are not significantly different. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2015; Tsai et al., 2016). The conclusion from the present study, that host-genetics played no detectable role in the observed large variation in the abundance of salmon lice under the given experimental conditions, may appear at first to conflict with the above-mentioned studies. However, our results do not preclude the possibility that Atlantic salmon displays genetic variation in susceptibility. There may be several potential explanations for this apparent discrepancy, however, two stand-out. First, although it would seem unlikely, it is not possible to exclude the possibility that our outbred material, which originated from multiple families from several wild populations and farmed strains, displayed very little or no genetic variation in susceptibility. Second, the homogeneous system in which the fish were infected (i.e., tanks) may potentially mask a genetic influence, and/or disrupt genetic-based behavioral traits that could contribute to heritable differences in infection levels. However, positive heritability estimates have been detected in tank challenges using very similar approaches to that used here (Gharbi et al., 2015; Gjerde et al., 2011), and there is a genetic correlation between results from tank and sea-cage trails (Kolstad et al., 2005).

From the studies cited above, estimates of heritability for susceptibility to salmon lice infection have ranged from 2 to 33%, with some showing confidence intervals overlapping with 0 (i.e., the heritability estimate was not significantly different from 0). Therefore, some pedigree-based trials specifically designed to quantify heritability have failed to detect additive genetic variation (in both tanks and sea-cages). It is therefore possible that the same factor(s) potentially masking additive genetic variation in the current study are also prevalent, to varying degrees, in some of the studies investigating heritability. Identification of the experimental or environmental factors which influence the ability to detect genetic variation in this trait are therefore needed if accurate estimates of heritability are to be obtained, and thereafter fully utilized in breeding programs. While the pedigree-selection approach for high and low-responding salmon families has given a response (Holm et al., 2015), and development of genetic or genomic markers associated with infection levels will assist selection (Gharbi et al., 2009; Holm et al., 2015; Tsai et al., 2016), individual infection levels still strongly overlap even among selected low and high responding families (Holm et al., 2015). Clearly, unidentified and non-genetic parameters also play a major (sometimes dominant) role in the infection levels observed in challenge experiments with this parasite. This is also true for those experiments terminated at a stage where the lice were still attached to the host (i.e. non-motile) (Holm et al., 2015), and therefore could not have been affected by host transfer as has been reported in this species (Ritchie, 1997).

Some studies have infected the same group(s) of salmon with lice on two or more occasions in order to investigate whether results from the first challenge are consistent with the results from subsequent challenges (Glover and Skaala, 2006; Glover et al., 2004b; Holm et al., 2015; Kolstad et al., 2005). These studies have reported that at the group level (i.e., family or strain), there is some degree of congruency in relative susceptibility between challenges (but non-significantly in (Kolstad et al., 2005)). This indicates that the observed differences between experimental groups could not have been caused entirely by random processes, and that average differences in host genetics between these groups were at least partially responsible. However, when the results from the first and subsequent challenges are compared for individual-fish, either in a tank challenge (Glover et al., 2004b) or a sea-cage when infected naturally (Glover and Skaala, 2006), the relative abundance of sea lice observed on an individual fish was completely random between infections. I.e., once fish size was accounted for, there was no relationship between an individual fish's ranking in infection level between the first and subsequent challenges. While this could theoretically be related to individual differences in immune

system learning and response between infections, the Atlantic salmon displays no ability to acquire immunity against this parasite. Therefore, the results of these repeat-challenge studies suggest that the infection level attained on an individual fish is strongly influenced by random processes, and is not primarily the result of its genetic susceptibility. These observations are consistent with the results of the present study, that under certain circumstances, host-genetics plays no detectable, or only a very minor role in an individual host infection level (i.e., where it exists it is overshadowed by other factors). The practical implications of this is that while family-based selection for decreased susceptibility of Atlantic salmon to *L. salmonis* may work (Holm et al., 2015), selection of high or low-responding individual fish within families will be very challenging.

While homozygous clones display no genetic variation, they still exhibit phenotypic variation of varying magnitude (Komen and Thorgaard, 2007). As expected, in many studies the phenotypic variation among clones is reduced compared to outbred controls. For example, in body weight and length in Amago salmon (*Oncohynchus rhodurus*) (Kobayashi et al., 1994), Aya (*Plecoglossus altivelis*) (Taniguchi et al., 1994) and Nile tilapia (*Oreochromis niloticus*) (Muller-Belecke and Horstgen-Schwark, 2000). However, there are also examples of increased variation in weight and length in clonal lines of carp (*Cyprinus carpio*) (Komen and Thorgaard, 2007) and for fluctuating asymmetry and meristic characters in rainbow trout (*Onchorhynchus mykiss*) (Young et al., 1995). It is logical that the relationship between phenotypic variation in cloned and outbred groups of fish will depend on the phenotypic trait in question, the system in which is it measured, and the degree to which the trait has an environmental influence.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.aquaculture.2017.08.008.

Acknowledgements

We would like to acknowledge the help of the staff at the Matre field station for production of fish groups, and Bjørnar Skjold for production of lice. This study was jointly financed with resources from the Norwegian Ministry for Trade and Fisheries, the Norwegian research council (INTERACT - grant number 200510; SFI-Sea Lice Research Centre grant number 203513/03), and the EU Seventh Framework Programme via the AQUAEXCEL project No. 262336. The views expressed in this work are the sole responsibility of the authors and do not necessarily reflect the views of any of the above-mentioned funding bodies.

References

Braden, L.M., Barker, D.E., Koop, B.F., Jones, S.R.M., 2015. Differential modulation of resistance biomarkers in skin of juvenile and mature pink salmon, *Oncorhynchus gorbuscha* by the salmon louse, *Lepeophtheirus salmonis*. Fish Shellfish Immunol. 47, 7–14.

Costello, M.J., 2009. The global economic cost of sea lice to the salmonid farming industry. J. Fish Dis. 32, 115–118.

Dawson, L.H.J., Pike, A.W., Houlihan, D.F., McVicar, A.H., 1998. Effects of salmon lice *Lepeophtheirus salmonis* on sea trout *Salmo trutta* at different times after seawater transfer. Dis. Aquat. Org. 33, 179–186.

Eizaguirre, C., Lenz, T.L., 2010. Major histocompatibility complex polymorphism: dynamics and consequences of parasite-mediated local adaptation in fishes. J. Fish Biol. 77, 2023–2047.

Gargan, P., Karlsbakk, E., Coyne, J., Davies, C., Roche, W., 2016. Sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) infestation levels on sea trout (*Salmo trutta* L.) around the Irish Sea, an area without salmon aquaculture. ICES J. Mar. Sci. 73, 2395–2407.

Gharbi, K., Glover, K.A., Stone, L.C., MacDonald, E.S., Matthews, L., Grimholt, U., Stear, M.J., 2009. Genetic dissection of MHC-associated susceptibility to *Lepeophtheirus salmonis* in Atlantic salmon. BMC Genet. 10.

Gharbi, K., Matthews, L., Bron, J., Roberts, R., Tinch, A., Stear, M., 2015. The control of sea lice in Atlantic salmon by selective breeding. J. R. Soc. Interface 12, 8.

Gjerde, B., Odegard, J., Thorland, I., 2011. Estimates of genetic variation in the susceptibility of Atlantic salmon (*Salmo salar*) to the salmon louse *Lepeophtheirus salmonis*. Aquaculture 314, 66–72.

Glover, K.A., Skaala, O., 2006. Temporal stability of sea louse *Lepeophtheirus salmonis* Kroyer populations on Atlantic salmon *Salmo salar* L. of wild, farm and hybrid parentage. J. Fish Biol. 68, 1795–1807.

Glover, K.A., Nilsen, F., Skaala, O., Taggart, J.B., Teale, A.J., 2001. Differences in susceptibility to sea lice infection between a sea run and a freshwater resident population of brown trout. J. Fish Biol. 59, 1512–1519.

Glover, K.A., Skaala, O., Nilsen, F., Olsen, R., Teale, A.J., Taggart, J.B., 2003. Differing susceptibility of anadromous brown trout (*Salmo trutta* L.) populations to salmon louse (*Lepeophtheirus salmonis* (Kroyer, 1837)) infection. ICES J. Mar. Sci. 60, 1139–1148.

Glover, K.A., Hamre, L.A., Skaala, O., Nilsen, F., 2004a. A comparison of sea louse (*Lepeophtheirus salmonis*) infection levels in farmed and wild Atlantic salmon (*Salmo salar* L.) stocks. Aquaculture 232, 41–52.

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

Glover, K.A., 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.

Glover, K.A., Grimholt, U., Bakke, H.G., Nilsen, F., Storset, A., Skaala, O., 2007. Major histocompatibility complex (MHC) variation and susceptibilty to the sea louse *Lepeophtheirus salmonis* in Altantic salmon *Salmo salar*. Dis. Aquat. Org. 76, 57–65.

Glover, K.A., Hansen, M.M., Skaala, O., 2009. Identifying the source of farmed escaped Atlantic salmon (*Salmo salar*): Bayesian clustering analysis increases accuracy of assignment. Aquaculture 290, 37–46.

Glover, K.A., Quintela, M., Wennevik, V., Besnier, F., Sørvik, A.G.E., Skaala, O., 2012. Three decades of farmed escapees in the wild: a spatio-temporal analysis of population genetic structure throughout Norway. PLoS One 7 (8), e43129.

Glover, K.A., Pertoldi, C., Besnier, F., Wennevik, V., Kent, M., Skaala, Ø., 2013. Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs. BMC Genet. 14, 4.

Glover, K.A., Madhun, A.S., Dahle, G., Sorvik, A.G.E., Wennevik, V., Skaala, O., Morton, H.C., Hansen, T.J., Fjelldal, P.G., 2015. The frequency of spontaneous triploidy in farmed Atlantic salmon produced in Norway during the period 2007–2014. BMC Genet. 16, 37.

Glover, K.A., Bos, J.B., Urdal, K., Madhun, A.S., Sørvik, A.G.E., Unneland, L., Seliussen, B.B., Skaala, Ø., Skilbrei, O.T., Tang, Y., Wennevik, W., 2016. Genetic screening of farmed Atlantic salmon escapees demonstrates that triploid fish display reduced migration to freshwater. Biol. Invasions 18, 1287–1294.

Hamre, L.A., Glover, K.A., Nilsen, F., 2009. Establishment and characterisation of salmon louse (*Lepeophtheirus salmonis* (Kroyer 1837)) laboratory strains. Parasitol. Int. 58, 451–460.

Holm, H., Santi, N., Kjoglum, S., Perisic, N., Skugor, S., Evensen, O., 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 Shellfish Immunol. 42, 384–394.

Jones, S.R.M., Hargreaves, N.B., 2007. The abundance and distribution of *Lepeophtheirus salmonis* (Copepoda: caligidae) on pink (*Oncorhynchus gorbuscha*) and chum (*O-keta*) salmon in coastal British Columbia. J. Parasitol. 93, 1324–1331.

Karlsson, S., Diserud, O.H., Fiske, P., Hindar, K., 2016. Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. ICES J. Mar. Sci. 73, 2488–2498.

Klemme, I., Karvonen, A., 2017. Vertebrate defense against parasites: interactions between avoidance, resistance, and tolerance. Ecol. Evol. 7, 561–571.

Kobayashi, T., Ide, A., Hiasa, T., Fushiki, S., Ueno, K., 1994. Study of biological characters and the genetics of some traits of triploid and gynogenetic diploid on salmonid fishes. 3. Production of cloned Amago salmon *Oncohynchus rhodurus*. Fish. Sci. 60, 275–281.

Kolstad, K., Heuch, P.A., 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.

Kolstad, K., Thorland, I., Refstie, T., Gjerde, B., 2006. Genetic variation and genotype by location interaction in body weight, spinal deformity and sexual maturity in Atlantic cod (*Gadus morhua*) reared at different locations off Norway. Aquaculture 259, 66–73.

Komen, H., Thorgaard, G.H., 2007. Androgenesis, gynogenesis and the production of clones in fishes: a review. Aquaculture 269, 150–173.

Krasnov, A., Breiland, M.S.W., Hatlen, B., Afanasyev, S., Skugor, S., 2015. Sexual maturation and administration of 17 beta-estradiol and testosterone induce complex gene expression changes in skin and increase resistance of Atlantic salmon to ectoparasite salmon louse. Gen. Comp. Endocrinol. 212, 34–43.

Muller-Belecke, A., Horstgen-Schwark, G., 2000. Performance testing of clonal *Oreochromis niloticus* lines. Aquaculture 184, 67–76.

Pierce, A.A., de Roode, J.C., Altizer, S., Bartel, R.A., 2014. Extreme heterogeneity in parasitism despite low population genetic structure among monarch butterflies inhabiting the Hawaiian islands. PLoS One 9, 10.

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

Quintela, M., Wennevik, V., Sørvik, A.G.E., Skaala, Ø., Skilbrei, O.T., Urdal, K., Barlaup, B.T., Glover, K.A., 2016. Siblingship tests connect two seemingly independent farmed Atlantic salmon escape events. Aquac. Environ. Interact. 8, 497–509.

Ritchie, G., 1997. The host transfer ability of *Lepeophtheirus salmonis* (Copepoda: Caligidae) from farmed Atlantic salmon, *Salmo salar* L. J. Fish Dis. 20, 153–157.

Skern-Mauritzen, R., Torrissen, O., Glover, K.A., 2014. Pacific and Atlantic *Lepeoptheirus salmonis* (Krøyer, 1838) are allopatric subspecies: *Lepeoptheirus salmonis salmonis* and *Lepeoptheirus s. oncorhynchi* subspecies novo. BMC Genet. 15, 32.

Solberg, M.F., Glover, K.A., Nilsen, F., Skaala, Ø., 2013a. Does domestication cause changes in growth reaction norms? A study of farmed, wild and hybrid Atlantic salmon families exposed to environmental stress. PLoS One 8 (1), e54469.

Solberg, M.F., Zhang, Z.W., Nilsen, F., Glover, K.A., 2013b. Growth reaction norms of

domesticated, wild and hybrid Atlantic salmon families in response to differing social and physical environments. BMC Evol. Biol. 13, 234.
Solberg, M.F., Dyrhovden, L., Matre, I.H., Glover, K.A., 2016. Thermal plasticity in farmed, wild and hybrid Atlantic salmon during early development: has domestication caused divergence in low temperature tolerance? BMC Evol. Biol. 16, 38.
Sutherland, B.J.G., Koczka, K.W., Yasuike, M., Jantzen, S.G., Yazawa, R., Koop, B.F., Jones, S.R.M., 2014. Comparative transcriptomics of Atlantic *Salmo salar*, chum *Oncorhynchus keta* and pink salmon *O. gorbuscha* during infections with salmon lice *Lepeophtheirus salmonis*. BMC Genomics 15, 17.
Taggart, J.B., 2007. FAP: an exclusion-based parental assignment program with enhanced predictive functions. Mol. Ecol. Notes 7, 412–415.
Taniguchi, N., Han, H.S., Tsujimura, A., 1994. Variation in some quantitative traits of clones produced by chromosome manipulation in Ayu, *Plecoglossus altivelis*. Aquaculture 120, 53–60.
Taranger, G.L., Karlsen, O., Bannister, R.J., Glover, K.A., Husa, V., Karlsbakk, E., Kvamme, B.O., Boxaspen, K.K., Bjorn, P.A., Finstad, B., Madhun, A.S., Morton, H.C., Svasand, T., 2015. Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. ICES J. Mar. Sci. 72, 997–1021.
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. J. Fish Dis. 36, 171–194.
Tsai, H.Y., Hamilton, A., Tinch, A.E., Guy, D.R., Bron, J.E., Taggart, J.B., Gharbi, K., Stear, M., Matika, O., Pong-Wong, R., Bishop, S.C., Houston, R.D., 2016. Genomic prediction of host resistance to sea lice in farmed Atlantic salmon populations. Genet. Sel. Evol. 48, 11.
Vollset, K.W., Krontveit, R.I., Jansen, P.A., Finstad, B., Barlaup, B.T., Skilbrei, O.T., Krkosek, M., Romunstad, P., Aunsmo, A., Jensen, A.J., Dohoo, I., 2016. Impacts of parasites on marine survival of Atlantic salmon: a meta-analysis. Fish Fish. 17, 714–730.
Wootten, R., Smith, J.W., Needham, E.A., 1982. Aspects of the biology of the parasitic copepods *Lepeohtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. Proc. Roy. Soc. B Biol. Sci. 81, 185–197.
Young, W.P., Wheeler, P.A., Thorgaard, G.H., 1995. Asymmetry and variability of meristic characters and spotting in isogenic lines of rainbow trout. Aquaculture 137, 67–76.