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Genetic parameters of resistance to amoebic gill disease in two Norwegian Atlantic salmon populations

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ABSTRACT

Amoebic gill disease (AGD) is an increasing problem in Northern Europe and selective breeding has been shown as a good strategy to manage this disease in Tasmania. To explore the possibilities to perform selective breeding for AGD-resistance in Norwegian Atlantic salmon, two controlled challenge tests and one field (on-farm) test were performed with fish from two different breeding companies. For the field test, full-sibs of the same families from one of the controlled challenge tests were exposed to AGD naturally in a net-cage in the sea in Ireland. In all three experiments, two rounds of AGD infection were run, and all fish were assessed when the average gill score had progressed to an advanced threshold. Following assessment, all fish were bathed in freshwater. Heritability for resistance against both first and second AGD infection in the challenge tests and the field test ranged from 0.09 to 0.20. Estimated genetic correlation between gill score in the challenge test and field test resistance ranged from 0.02 to 0.34 and was not significantly different from 0. The genetic correlation between gill score in the first and second infection of the field test was 0.69 and ranged from 0.09 to 0.18 in the two challenge tests. These results indicate that AGD resistance in Norwegian Atlantic salmon can be improved by selective breeding. However, further work to optimize the controlled challenge test to better predict field test resistance is required.

1. Introduction

Amoebic gill disease (AGD) is a parasitic disease of marine salmonid and non-salmonid fish caused by the amoeba *Paramoeba perurans* (Young et al., 2008; Crosbie et al., 2012). Attachment of amoebae to the gill initiates a localised host cellular response, including hyperplasia and hypertrophy of the gill epithelium and lamellar fusion (Adams and Nowak, 2001). Progression of the disease causes inappetance, respiratory distress and cardiovascular compromise (Munday et al., 2001) Nowak, 2012). If left untreated, AGD can cause a high level of mortality, which is directly related to the level of advanced gill pathology in a population (Taylor et al., 2009). Although AGD can occur year-round, it is most prevalent in warmer water and high salinity (Clark and Nowak, 1999). In farmed Atlantic salmon in Tasmania, AGD has been a major problem since the inception of salmon farming in the mid 1980's, and regular freshwater treatments are necessary to control the disease. AGD treatments account for 10–20% of the production cost of Australian farmed salmon (Nowak, 2012). The treatment is also a welfare issue for the fish due to the physiological stress of the freshwater bath.

In Tasmanian Atlantic salmon, AGD-resistance has been shown to be heritable (Taylor et al., 2009; Kube et al., 2012) and selective breeding has increased the bathing interval and reduce the cost of AGD management (Kube et al., 2012). In Ireland, AGD was only reported during periods of record sea temperatures at sites experiencing oceanic salinities (Rodger and McArdle, 1996; Rodger, 2014). Though recent work has confirmed that *P. perurans* has been present on Irish farmed salmon at least since 1995 (Downes et al., 2018). In 2011 and 2012, AGD became the largest infectious health problem for the salmon industry in Ireland, Scotland and France (Rodger, 2013). The first documented occurrence of AGD in Norway was in 2006 (Steinum et al., 2008), and the amoeba has been found regularly on the southwest coast since and reported further north every year (www.vetinst.no).

The success of the Tasmanian selective breeding program for Atlantic salmon indicates that selection could be a good strategy to fight AGD in Atlantic salmon populations in Northern Europe as well. An effective selective breeding program is dependent on phenotypic and

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genetic variation for the trait under selection. For Atlantic salmon populations reared under Northern European farming conditions genetic parameters for resistance to AGD are yet to be estimated. Although a controlled tank challenge was attempted early on (Taylor et al., 2007), the Tasmanian Atlantic salmon breeding program use phenotypes obtained from the field, a strategy that is dependent on regular natural disease development. In Europe, and particularly in Norway, the AGD season is too short to ensure adequate natural AGD expression in challenged populations (www.vetinst.no). Selective breeding is therefore dependent on the use of tank based challenge. Such a test has been developed by VESO (www.veso.no), this study was the first commercial scale use of this challenge for testing genetic resistance to AGD.

Tasmanian research has shown that resistance against first and later infections are different traits and that resistance against later infections are more heritable than against first infection (Kube et al., 2012). The aim of this study was to estimate genetic parameters for AGD resistance both in an artificial challenge test and under field conditions in Europe.

2. Materials and methods

Two controlled challenge tests of approximately 3000 fish were conducted, both with two rounds of infection, where fish were treated with freshwater between the two infections and gill scored some weeks after each infection, where gross gill pathology was regularly assessed and fish were treated in freshwater when this had progressed to an advanced level.. Full and half sibs of the fish from one of the controlled challenge tests were also tested during a natural infection in a field test in Ireland. The fish were placed in a commercial location and gill scored before freshwater treatment after two consecutive infections, to obtain an estimate of the genetic correlation between AGD resistance in the controlled challenge test and the field test.

2.1. Fish material

During this study, three experiments were carried out, with three different batches of fish. One batch of fish (SB-C) originated from the breeding nucleus of SalmoBreed and was used in a controlled challenge test. The other two batches originated from the breeding nucleus of Marine Harvest, and were used in a controlled challenge test (MH-C) or a field test (MH-F). The SB-C batch was created by random mating of 50 males and 100 females, where each male was mated to two females and each female to one male only. Start feeding date varied from January 15th to March 10th 2014. From each full sib family, 30 fish were transported to VESO Vikan for smoltification and entered a controlled tank challenge test (total 3000 fish). The SB-C full-sib families were reared in separate tanks from the eyed egg stage through to startfeeding until tagging. After tagging both the MH-fish and the SB-fish had common rearing of families (within batch). The two batches of fish from Marine Harvest were from 150 full sib families, created from mating 75 males to 150 females, where each male was mated to two females and each female to one male only. The fish was produced in Ireland, and the MH-C batch was transferred to Norway as eyed eggs. They were hatched and reared at the Nofima facilities in Sunndalsøra. After smoltification, 20 fish from each full-sib family (the MH-C batch, total 3000 fish) were transferred to VESO Vikan to be challenged by the amoeba. A minimum of 40 fish from each family (the MH-F batch, total 6100 fish) were placed in a net pen at the South West farm of Marine Harvest in Inishfarnard, Ireland, where AGD is a common problem. The SB-fish were PIT-tagged at an average weight of ~15 g and the MH-fish (both the MH-C and MH-F fish) at ~45 g. During tagging of the two MH-batches, a fin clip sample from each fish was taken for microsatellite genotyping and parental assignment.

2.2. The SB-C controlled challenge test

The SB-C fish were transferred from SalmoBreed facilities and arrived at VESO Vikan on 19th of December 2014 at an average weight of 97 g. They were stocked to a single seawater tank (12,560 L) held at 12 °C and salinity > 31‰. The fish were smoltified and allowed to acclimatize before starting the challenge test on the 13th of February 2015. Temperature during infection and development of disease was kept at 15 °C with > 31‰ salinity. The fish were infected with an amoeba culture isolated from an outbreak in Ireland in 2014, cultivated at VESO Vikan on MYA plates. Mortalities during and before first infection was ~10%. Water volume during the challenge was 3770 L and amoeba concentration was 2400 amoebas/L. Oxygen was added to the tank as long as the water volume was reduced. After 4 h of bathing, water level was increased again to normal level. After the challenge, a sample of 30 fish was gill scored weekly to monitor the development of the disease. Level of disease on single fish was scored using gill scores (0–5), as described by Taylor et al. (2009). All gill surfaces were examined and a score of 1 was given if one white spot was present on one of the gill surfaces; score 2 was given if the total number of spots over all gill surfaces was 2–3; score 3 and 4 was given when < 20% and < 50% of the total gill surface was covered by white spot, respectively; score 5 meant that > 50% of the gill surface was covered by white spot. When the average gill score of the sample reached 2.0 all fish in the tank were individually gill scored prior to treating the population with freshwater. The freshwater treatment was conducted by reducing the water level in the tank as much as possible without compromising the welfare of the fish (subjectively judged) and adding freshwater until salinity dropped below 3‰. Oxygen was added to the tank during the freshwater treatment. The fish were kept on freshwater for 3 h before seawater was added until salinity was > 31‰. After freshwater treatment, 532 fish were removed from the trial to reduce biomass. These were selected randomly within family. After freshwater treatment, temperature was lowered to 12 °C and the fish were allowed to recover for 10 days. Thereafter, temperature was increased to 15 °C and the fish were re-challenged with *P. perurans* using the same bath challenge method as described for the first challenge, but the second infection was performed with a higher amoeba concentration of 9350 amoeba/L. During the freshwater treatment and re-challenge mortality of > 40% occurred. Therefore, for fish welfare reasons, the second major gill-scoring and the termination of the experiment was performed 16 days post re-challenge, even though average score in the tank was still below 2.0. Neither the AGD status of the fish, the treatment procedure or environmental factors could fully explain the mortality and the major cause(s) are unknown. A full overview of the development of estimated gill score in each of the two challenge tests is summarized in Fig. 1, also including time for first and second infections and the freshwater treatments.

2.3. The MH-C controlled challenge test

The MH-C fish arrived at VESO Vikan on 28th of March 2014 at an average weight of 106 g. They were infected with *P. perurans* twice, following the same protocol as the SB-C fish (Section 2.2) with a few exceptions described here. After acclimatization, the fish were bath challenged with *P. perurans* on 29th of April 2014. The amoeba used originated from an outbreak in Rogaland/Hordaland, Norway in 2012 and had been cultivated at VESO Vikan on malt yeast agar (MYA) plates since then. Temperature during the infection was kept at 12 °C, but increased from 12 °C to 15 °C 3 weeks post inoculation since the disease developed more slowly than expected. When the average gill score of the sample reached 2.0 all fish in the tank were individually gill scored prior to treating the population with freshwater. After freshwater treatment, temperature was lowered to 12 °C and the fish were allowed to recover for 10 days. Thereafter, temperature was increased to 15 °C and the fish were re-challenged with *P. perurans* using the same bath challenge method as described for the first challenge, but with a slightly lower amoeba concentration (2160 amoeba/L). A sample of 30 fish were gill scored regularly after the second challenge until the average

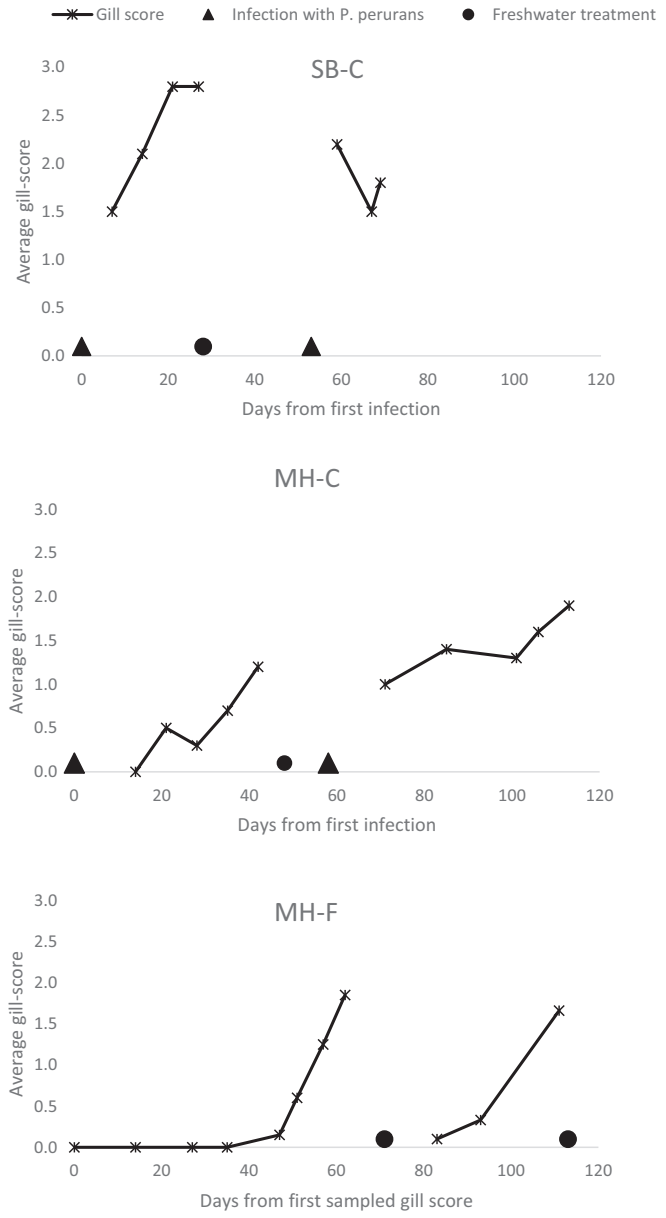

Fig. 1. Development of gill score for first and second infection for both the controlled challenge (SalmoBreed – SB-C and Marine Harvest – MH-C) and field test. (Marine Harvest – MH-F). Each x represents an average of 30 randomly sampled individuals except for the last x in each line series, where all fish were scored to represent the first or second gill scoring events.

score in the tank was around 2.0. Then, a final scoring of all fish was performed before the experiment was terminated. During this trial, total mortality was ~10%.

2.4. The MH-F field test

The fish were pit tagged at an average weight of 61 g and were put into a net-cage in the sea at a commercial farm in Inishfarnard, Ireland on the 24th of April 2014. Amoebic gill disease was first reported in June 2014 at the farm. The monitoring of the development of AGD in the test pen was done by regularly gill-scoring of a sample of 30 fish. The first inspection was 21st of May. Inspection intervals thereafter depended on the stage of infection (more often when scores increased), starting at every second week, but increased to every 4–6 days as gill index increased. When the estimated average score in the pen exceeded 2.0, a major scoring was conducted the 29th of July 2014 by two teams that scored a total of 3663 fish throughout 1 day. The fish to be gill scored were sampled from the pen, until the daylight was no longer appropriate for gill scoring. The fish were treated with freshwater on 31st July. The fish were left at the same location and samples of 30 fish were scored regularly until a new infection had established. A second major scoring was conducted on 12th of September, with records on 3468 fish. The fish were then treated with freshwater. During the first week after this second freshwater treatment a mortality event occurred and killed > 20% of the fish. The cause of death was not known. The IDs of the dead fish were recorded which allow us to estimate the heritability of the mortality and of the genetic correlations between gill score and mortality.

2.5. Statistical analyses

The data was analyzed in ASReml (Gilmour et al., 2009) with a linear animal model to estimate genetic and residual (co)variances of AGD-resistance for the first and second infection, under field and controlled conditions, respectively. The SB-C data were analyzed with a model containing 2 traits (gill score from first and second infection in the challenge test), further referred to as the SB-model. The MH-F and MH-C data were analyzed together with a model containing 5 traits (gill score after first and second infection from the field test, first and second infection from the challenge test and mortality to treatment in the field test) further referred to as the MH-model. Both models can be written as:

$$y = Xb + Zu + e$$

where y is a vector of the 2 or 5 traits, b is a vector of the fixed effects of scoring person, u is a vector of breeding values for each animal and e is a vector of random residual effect. X and Z are design matrices to assign records to the fixed and random effects, respectively. The estimated breeding values were assumed to follow a multivariate normal distribution with variance-covariance matrix G_{SB} (SB-model) or G_{MH} (MH-model), where

$$G_{SB} = \begin{bmatrix} \sigma^2_{gC1} & \\ \sigma_{gC1gC2} & \sigma^2_{gC2} \end{bmatrix}$$

$$G_{MH} = \begin{bmatrix} \sigma^2_{gC1} & & & & \\ \sigma_{gC1gC2} & \sigma^2_{gC2} & & & \\ \sigma_{gC1gF1} & \sigma_{gC2gF1} & \sigma^2_{gF1} & & \\ \sigma_{gC1gF2} & \sigma_{gC2gF2} & \sigma_{gF1gF2} & \sigma^2_{gF2} & \\ \sigma_{gC1gM} & \sigma_{gC2gM} & \sigma_{gF1gM} & \sigma_{gF2gM} & \sigma^2_{gM} \end{bmatrix}$$

where ${\sigma_{gC1}}^2$, ${\sigma_{gC2}}^2$, ${\sigma_{gF1}}^2$, ${\sigma_{gF2}}^2$ and ${\sigma_{gM}}^2$ denote genetic variance for the first and second gill score in the challenge test, the first and second gill score in the field and mortality (after freshwater treatment in the field) respectively. Genetic covariances were estimated between each trait in each model, as indicated in the G-matrices, using the same trait subscripts as for the genetic variances. Because none of the fish had records from both the field test and the challenge test, residual covariance between animals for those tests were assumed to be 0, while the residual covariance between first and second infection within the same test was estimated by the model. A random effect common to full-sibs other than additive genetic effect was also fitted. A significant random effect common to full sibs can be observed when full-sib families are reared separately prior to tagging, since the performance of full-sibs will not only be due to the genes they share but also due to the environment they shared earlier after hatching. A random effect common to full-sibs was more likely to affect the SB batch than the MH batches, since the SB families were reared in separate tanks until tagging while MH families were mixed to a common rearing tank immediately after hatching. In this study non-additive genetic and early environmental effects are confounded. However, when this effect was included in the

Table 1
Number of fish recorded in each dataset for first (1st) and second (2nd) infection with the Amoeba (*Paramoeba perurans*).

Batch	1st infection only	2nd infection only	Both infections
SB-C	1812	–	845
MH-C	139	531	1417
MH-F	1017	852	2616

SB-C: SalmoBreed controlled challenge test. MH-C: Marine Harvest controlled challenge test. MH-F: Marine Harvest field (on-farm) test in Ireland. The same full-sib families were used for the MH-F and MH-C tests.

model, the variance component associated with the effect common to full-sibs converged to a numerically small value. We performed a likelihood ratio test based on the log-likelihoods from a full model and reduced model (effect common to full-sib was excluded) and this effect was non-significant, thus it was excluded from the analysis.

3. Results

3.1. Descriptive statistics

The development of the average gill score of the fish sampled prior to the first and second gill-scoring for each challenge and field test are given in Fig. 1. In the challenge tests, the disease developed quite fast after infection. In the field, the disease developed slowly for the first 6 weeks before a large seemingly exponential development in gill score occurred.

The numbers of fish in each gill score category are summarized in Table 1 for the two tank challenge tests and the field test. Due to technical issues (reading the PIT-tags and connecting PIT tag to gill scores) and mortalities, only 845 fish were recorded during the second gill scoring in the SB-C test, compared to the 2657 fish that were recorded during the first gill scoring. For the MH challenge test, 1556 and 1948 fish were scored for the first and second gill scoring, respectively. The total number of fish that were recorded for the first and second gill scoring of the field test were 3633 and 3468, respectively.

The distributions of gill scores for the six different gill-scoring events are given in Fig. 2. In the tank challenge tests, low phenotypic variation in gill score was a problem in both trials. The variance of gill scores was most variable in the SB-C trial, spanning from 0.19, first scoring event to 0.84 in the second scoring event. The gill scores in the MH-C-trial was 1.20 ± 0.78 and 1.90 ± 0.58 for the first and second scoring event, respectively. In the MH-C trial, score 2 was over-represented (~75% of the records) in the second infection, while in the SB-C trial, score 3 was over-represented (~85% of the records) in the first infection (Fig. 2), explaining the lower phenotypic variance found in these two scoring events. There was a good representation of fish for each score for both the first and second infection in the field test, although the highest categories were not well represented. The average gill score ± standard deviation was 1.85 ± 0.77 and 1.66 ± 0.80 for the first and second gill scoring event, respectively, somewhat lower than the attempted average score of 2.0.

The fixed effects of person on gill score were also significant for several of the gill scorings. The number of people participating in each gill scoring varied from 2 to 7 (Fig. 3). The effect of person was included in the model for all gill score traits, although not significant for all traits.

3.2. Genetic parameters

Analyses of the SB-C data (Table 2) showed no significant genetic correlation between first and second gill scorings, although a positive correlation was indicated with a large standard error. A lower heritability with a marginally larger standard error was found for gill scores after the second as compared to the first infection, possibly due to the reduction in number of samples because of mortalities between the first and second infection. The cause of the mortality in this trial is not known, and may be related to the disease status of the fish, and hence not random.

Heritabilities for gill score estimated from the MH-C and MH-F data ranged from 0.09 to 0.20 (Table 3). The highest heritability was obtained from the first infection in the field test. Genetic correlation between first and second infection was high (0.69) in the field test, but low (0.09) in the challenge test. Genetic correlations between field and challenge tests ranged from 0.02 to 0.34 and were not significantly different from zero. The heritability for mortality under the field test on the observed scale was low (0.06). Analyzing mortality on the underlying scale in a single trait sire-dam model gave a heritability of 0.12 ± 0.03. In addition, relatively low genetic correlations, not significantly different from zero, were observed between mortality and gill scores both in the field test and challenge test. For all trials, the residual correlations between first and second gill-scoring (Table 2 and 3), and between field-test mortality and field gill-scoring (Table 3), were close to 0.

4. Discussion

We obtained moderate heritability for resistance to AGD, both under field (h^2 of 0.12–0.20) and controlled challenge test conditions (h^2 of 0.09–0.13) in two Norwegian Atlantic salmon populations (Marine Harvest and SalmoBreed breeding populations). However, heritability estimates in this study were lower than previously reported by Kube et al. (2012) ($h^2$of 0.14–0.40).

Heritability of gill score during first infection was higher in the field test than in the tank challenge tests, despite the controlled environmental conditions in the tank challenge test. However, the two controlled tank challenge tests had low phenotypic variation in gill scores (Fig. 2) that was probably attributed to the high concentration of amoeba in the challenge test, compared to other challenge tests (Adams et al., 2012) and much higher than what can be expected under field conditions. The effect of parasite concentration on the observed phenotypic distribution is unknown. The challenge test needs to be developed to express larger variation in gill score among the animals, for instance by changing the dosage of amoeba or the infection method. To extend the infection period is not desirable for economic and for fish welfare reasons. Hence, a challenge test model or a phenotypic measure that expresses variation in susceptibility to AGD within the time period tested in this study would be of large value. Currently available continuous measures are more disruptive to the fish and more expensive to analyze (Taylor et al., 2007).

The two tank challenge tests used different strains of amoeba, which seemed to affect the development of the disease. The strain of amoeba used for the MH-C-trial was cultured for several passages on MYA plates prior to this study, while the Irish strain of amoeba, used for SB-C had fewer passages on MYA plates prior to the trial. The culturing may, especially for the MH-C trial, have reduced the virulence of the amoeba (Bridle et al., 2015). Under the MH-C tank trial, the disease developed slower and after 7–8 weeks post challenge the average gill score was lower than the desired average score of 2.5. However, in the SB-C-trial, the disease developed much faster (Fig. 1), indicating higher virulence of the strain of amoebas, although it could also reflect differences between the fish strains used. Despite this difference in disease development, low phenotypic variation for gill score was a problem in both trials and seems to be a problem related to other aspects of the tank challenge than the concentration and the virulence of the amoebas.

The genetic correlation between the first and second infection in the field test was higher (r_g = 0.69 *vs.* 0.45) than previously reported by Kube et al. (2012), indicating that performing a gill score after first infection can be used as a predictor for resistance against later infections. This correlation was however significantly lower than unity indicating that gill score at first and second infection should still be

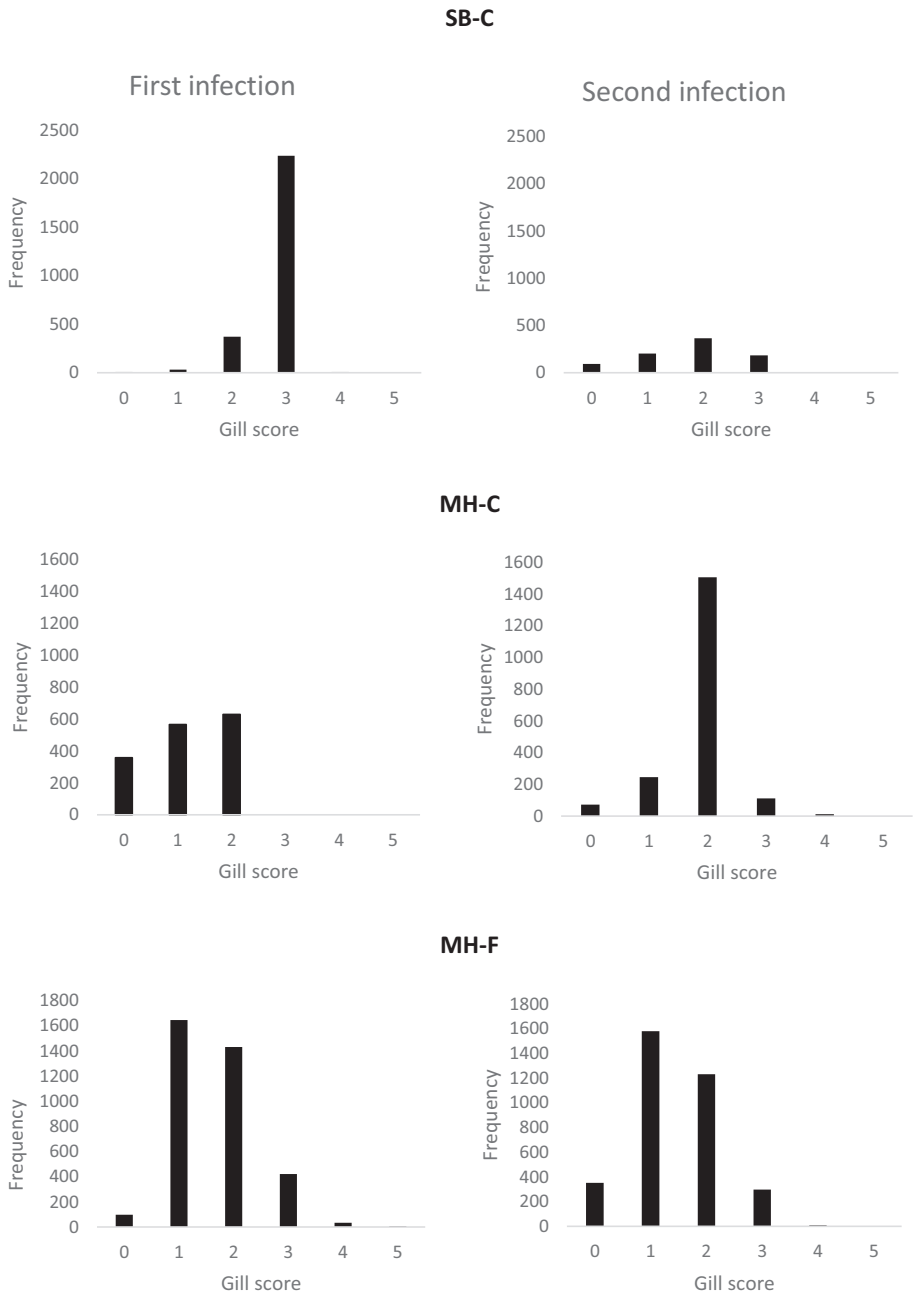

Fig. 2. Phenotypic distribution of gill scores from each of the six-major gill scoring events.

treated as different traits. Both in the field test and in the controlled challenge tests, the residual correlations between gill scores of first and second infections were very close to 0, indicating that the recorded gill scores after second infection were not affected by scar tissues or residue amoeba, i.e. amoeba from the first infection that managed to survive the freshwater treatment. In the controlled challenge test, the genetic correlation between resistance against first and second infection was low and non-significant in both experiments. The non-significant correlation between first and second scoring in the tank challenge, as well as the non-significant correlation between gill scores from the tank challenge and the field test, all indicate that the tank challenge is not fully optimized to serve as a predictor for AGD resistance in the field. The amoeba concentration in the tank challenge test was high, as is common in such controlled challenge tests to ensure a rapid development in the disease, which reduces the costs of the experiment. The fast development of the disease may however reduce the variation between fish in time to develop disease symptoms since they are all infected almost at the same time, while in the field, time of infection may be more variable among individuals and reflect genetic variation in susceptibility to the amoeba. This may affect the magnitude of the heritability if the reduction in genetic variance among fish is not proportional to the reduction in phenotypic variance. Fish density was also considerably higher in the tank challenge test than in the field test, which may also change how the disease spreads in the population. Further work to control environmental variables like salinity and temperature as well as amoeba dosage and fish density is needed to develop a tank challenge model that predicts field resistance with higher accuracy.

Reducing AGD infection might involve selective breeding, vaccine development and other on-farm management control measures. However, selection for increased resistance to AGD seems like the most promising tool and cost effective way to reduce AGD infections in the

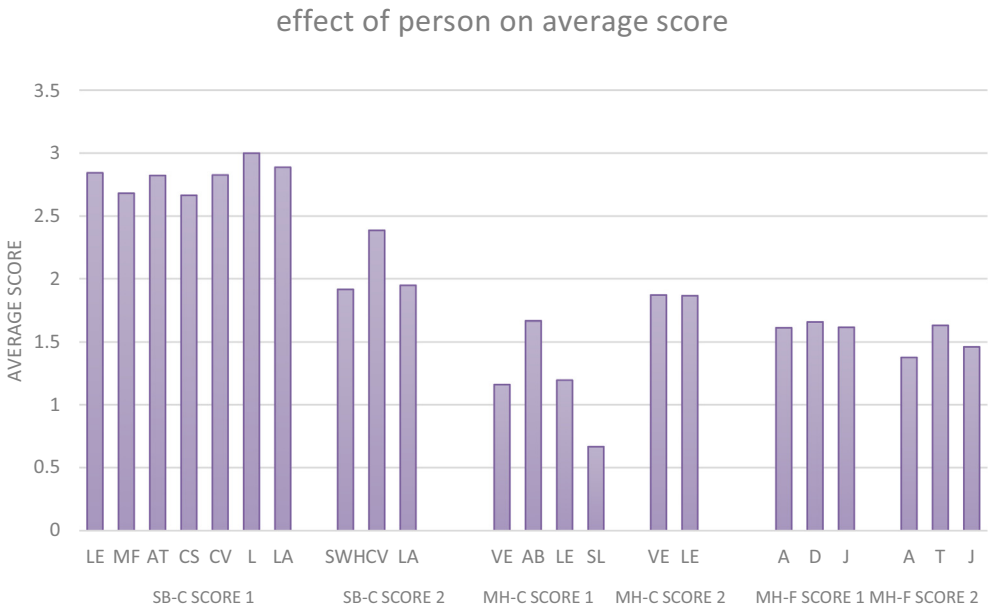

Fig. 3. Average gill score by operator at each of the major gill assessment events. Each person is represented by their initials on the x-axis and the data is sorted by scoring event.

Table 2
Heritabilities (on the diagonal), genetic correlations (above the diagonal) and residual correlations (below the diagonal) obtained from the SalmoBreed (SB-C) challenge test.

	1st infection	2nd infection
1st infection	0.13 ± 0.03	0.18 ± 0.27
2nd infection	−0.01	0.09 ± 0.04

future (Kube et al., 2012), and significant heritable variation was found for all tests in this experiment. Selective breeding for increased resistance to AGD would therefore be a possible strategy also in Norway and other European countries. The largest obstacle now seems to be a reliable test where the genetic variation in resistance is expressed. Among the tests in this study, the field test gave both the highest heritability and the highest genetic variation, and there was a relatively high genetic correlation between the two rounds of infection. Although routine field tests are undertaken in Tasmania, fewer and irregular AGD outbreaks in make this a less reliable testing strategy in Northern Europe. If a successful selective breeding program is being run for several generations, increased resistance will also increase the need for an effective controlled challenge test model that will mimic the development of the disease under field conditions, since field outbreaks will be less common because of higher resistance. In a controlled challenge test, this can be achieved by manipulation of amoeba concentration, salinity, temperature, fish density, infection method or other environmental factors. As stated earlier, further work is needed to optimize the controlled challenge test. A proper challenge test model needs to achieve relatively rapid development of disease but also to express genotypic and phenotypic variation in gill score. As a part of this work, a more detailed gill score scale may be needed.

The relatively low heritability of AGD-resistance will require large number of sibs to obtain reliable breeding values for AGD-resistance. Using sib information for selection will mean that only half of the additive genetic variance (between- families) will be utilized and therefore accuracy of selection is expected to be maximized with large sibling groups. However, selection accuracy could be increased through marker assisted or genomic selection (Meuwissen et al., 2001). Genomic selection would allow for both within- and between- family selection and thus higher selection intensity without gill score records on the selection candidates. Further research on the importance of genomic information over conventional selection methods that rely on pedigree information to increase resistance to AGD is needed.

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Table 3
Heritabilities (on the diagonal), genetic correlations (above the diagonal) ± standard error and residual correlations (below the diagonal) obtained from the Marine Harvest controlled challenge (MH-C) and field (MH-F) tests.

	Challenge test		Field test		
	1st infection	2nd infection	1st infection	2nd infection	Mortality
Challenge test					
1st infection	0.11 ± 0.03	0.09 ± 0.22	0.34 ± 0.18	0.20 ± 0.20	0.48 ± 0.18
2nd infection	0.00	0.13 ± 0.03	0.02 ± 0.17	0.22 ± 0.18	0.10 ± 0.19
Field test					
1st infection	–	–	0.2 ± 0.03	0.69 ± 0.10	0.04 ± 0.15
2nd infection	–	–	0.06	0.12 ± 0.03	0.09 ± 0.17
Mortality	–	–	−0.03	0.03	0.06 ± 0.01

Genetic Centre for their help with editing data and building pedigree files. The statistical analysis was performed using the computer cluster Titan at the University of Oslo.

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