Aquaculture 511 (2019) 734265

Contents lists available at ScienceDirect

Aquaculture

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

Estimates of genetic correlations between susceptibility of Atlantic salmon to amoebic gill disease in a bath challenge test and a field test

Bjarne Gjerde[a,*], Solomon Antwi Boison[a], Muhammed Luqman Aslam[a], Marie Løvoll[b], Håvard Bakke[c], Simon Rey[d], Marie Lillehammer[a]

[a] Department of Breeding and Genetics, Nofima AS, P.O. Box 210, N-1431 Ås, Norway
[b] VESO Vikan, Beisvågveien 107, Vikan, N-7810 Namsos, Norway
[c] SalmoBreed AS, Sandviksboder 3A, N-5035 Bergen, Norway
[d] FishVet Group, P.O.Box 1012, 0218 Oslo, Norway

ARTICLE INFO

Keywords:
Atlantic salmon
Amoebic gill disease
Bath challenge test
Field test
Growth
Heritabilities
Genetic correlations

ABSTRACT

Estimates of genetic parameters of susceptibility of Atlantic salmon to amoebic gill disease (AGD) were obtained from a bath challenge test with two repeated infections (1612 and 1582 fish, the offspring of 50 sires and 100 dams) and from a field test of their sibs (1156 fish) that were naturally infected and scored once for AGD. A third sibling group were reared in an AGD-free environment and their body weights recorded at harvest. In both challenge tests, susceptibility to AGD were measured using an adapted Taylor gill-score (0–5) where gill-score 3 was divided into three sub-classes 3A, 3B and 3C. In the field test, one gill arch of each animal was swabbed to quantify the amount of *Paramoeba perurans* by RT-qPCR, and a random sample of 126 of the fish were also analysed by RT-qPCR for *Paranucleospora theridion* and *Branchiomonas cysticola*. In the field test, body weights of the fish were recorded at time of gill-scoring and seven months later. In both tests, the distributions of gill-score was quite narrow (large proportion with gill-score 2 and 3A, and none with gill-score 4 and 5). In the field test, average body weight of fish with gill-score 1, 2, 3A and 3B was 17.6, 9.4, 17.9 and 22.2% lower, respectively than fish with gill-score 0. The genetic correlation between gill-score in the bath and the field test was close to zero. Therefore, the present bath challenge test for susceptibility to AGD cannot replace a field test in a selective breeding program. In the AGD-affected environment, the genetic correlation of gill-score with CT was −0.81 ± 0.16 and with body weight − 0.88 ± 0.09. These high genetic correlations indicate that CT and growth may be used as indirect trait measures of susceptibility to AGD. The high genetic correlation between body weights in the AGD-affected and the AGD-free environment (0.86 ± 0.05) indicate a true favourable genetic correlation between susceptibility to AGD and growth in Atlantic salmon. Consequently, selection for increased growth rate will result in a favourable genetic correlated response in susceptibility to AGD. The magnitude of these correlations need to be verified, in particular as the negative effect of decreasing CT-values of *P. theridion* on body weight was found to be larger than that of *P. perurans* and that growth of the fish in the AGD-free environment may be affected by other gill pathogens with negative effect of growth.

1. Introduction

Amoebic gill disease (AGD) is caused by the amoeba *Paramoeba perurans*, which colonizes and induces damage on gills of several fish species. In farming of Atlantic salmon, AGD has been a major problem in Tasmania for decades, and is currently an emerging issue in Northern Europe. In Norway, AGD was first detected in 2006, but has since 2012 caused significant losses in the southern part of the country and especially at locations with high salinity and high seawater temperature during August to November (Fish Health Report 2016, ISSN no. 1893–1480). In Tasmania, selective breeding for lower gill-score from a field test has successfully increased the interval between the freshwater treatments (Brad Evans, pers. comm.). This field-test strategy is dependent on more regular and predictable outbreaks than current situation in Northern Europe, and Norway in particular, where the AGD season is relatively short which leads to incomplete pathogenicity/cycle of the amoebae. Under such circumstances, it would be beneficial to use a challenge test, given that susceptibility to AGD in a challenge test is a good predictor of susceptibility in a field test environment.

Heritability for susceptibility to AGD was reported to be moderate in

* Corresponding author.
E-mail address: bjarne.gjerde@nofima.no (B. Gjerde).

https://doi.org/10.1016/j.aquaculture.2019.734265
Received 12 December 2018; Received in revised form 25 June 2019; Accepted 28 June 2019
Available online 29 June 2019

the Tasmania Atlantic salmon populations (Taylor et al., 2009; Kube et al., 2012). Recently, Lillehammer et al. (2019) found significant genetic variation for susceptibility to AGD both during 1st and 2nd infection both in the challenge test (h^2 = 0.08–0.12) and the field test (h^2 = 0.13–0.20) showing that the susceptibility to AGD in Norwegian Atlantic salmon populations can be decreased through selective breeding. However, the low estimated genetic correlation between susceptibility to AGD in the challenge test and field test (0.07 to 0.38, Lillehammer et al., 2019) indicates that susceptibility to AGD in the previous challenge test was not a good predictor of susceptibility in the field test, and that further development must be done for a challenge test to replace a field test in a selective breeding program. The low genetic correlations could be due to the low observed variation in gill score (0 to 5) in the challenge test, possible due to the high amoebic concentration in the challenge test.

The main objective of the project was to investigate if adjustments of the challenge test (lower amoebic concentration) and the gill-scoring procedure (divide gill-score 3 in three classes 3A, 3B and 3C) could result in both a larger phenotypic and genetic variation in gill-score as well as to a higher genetic correlation between gill-score in the challenge test and the field test. In addition, we investigated whether the concentration of *P. perurans* on the gills, measured by non-destructive RT-qPCR, can replace the subjective gill-scoring procedure.

2. Material and methods

2.1. Fish material

The fish were the offspring of 50 sires and 100 dams from SalmoBreed year-class 2015 (startfed 12. January to 25. February 2015). The fullsib families were reared in separate tanks at Nofima Sunndalsøra, until a body size (average weight ~15 g) suitable for individual tagging (22–28 July) with PIT-tags. From each family, a random sample of 20 fish were tagged for the bath challenge test group, while a random sample of 15 fish were tagged for the field test group.

Sibs of the above-mentioned fish, as well as fish from an additional 182 families (total of 3413 fish from 282 families with an average of 12 fish per family) of the same year-class, were PIT-tagged from 26th May to 17th June 2015, and were grown in a net-cage at LetSea, Dønna in Nordland county (http://letsea.no) from 13th October 2015 until harvest in 28th February 2017.

2.2. Gill-scoring

Gill-scoring of the bath challenge test and the field test was performed using an adapted version of the categorical field evaluation method of Taylor et al. (2009), where gill-score 3 was divided into the three sub-classes 3A, 3B and 3C (Table 1). The method describes the extent of visible white patches on a non-linear scale from 'clear' to 'heavy' to schedule proactive freshwater bath treatments. At advanced infections, this presumptive scoring method is known to have a moderate to good agreement with histopathological diagnosis (Adams et al., 2004), but less reliable for less severe cases (Clark and Nowak, 1999). The degree of lesions is known to be in direct proportion to the infective parasite concentration and progression of the infection (Morrison et al., 2004a,b). A quite recent study reported a high correlation (0.84) between gill score and histology scores (Downes et al., 2018).

2.3. Challenge test

In 2nd week of January 2016, the bath challenge test group (average body weight 110 g) was transported from Nofima, Sunndalsøra to VESO Vikan, Nord-Trøndelag where they were kept in a single 12.5 m^3 tank at a stocking density of < 40 kg/m^3. The fish were acclimatized to 15 °C seawater and kept at this temperature until first bath challenge on 25. January, and thereafter until 1st gill score of 1612 fish on 11.-12. February. After the 1st gill-score was completed the fish were treated with freshwater three times. A 2nd bath challenge was performed on 14. March followed with a 2nd gill score of all 1582 surviving fish on 7. - 8. April.

In both bath challenges the fish were challenged with *P. perurans* using amoebae from VESO Vikan (ref. no. 2014.10.15NO) that were cultivated at VESO Vikan on MYA plates. The amoebic concentration in the water was 500 amoebae/L, which was 1/5 of the concentration used in the two previous challenge tests in Lillehammer et al. (2019).

From 1st bath challenge until 1st score mean water temperature was 15.0 (SD = 0.13) °C and mean salinity 33.1 (SD = 0.18) ‰. After 1st gill score water temperature was reduced to 12.0 (SD = 0.55) °C and mean salinity to 25.0 (SD = 1.1) ‰ until 10. March, after which the temperature was increased to 15 °C prior to the 2nd bath challenge on 14. March. From 2nd bath challenge until 2nd gill score mean water temperature was 14.8 (SD = 0.18) °C, and mean salinity 33.3 (SD = 0.41) ‰.

Prior to the gill-score each fish was sedated with AQUI-S (Isoeugenol; Scan Aqua, Norway) and further anesthetised with benzocaine according to procedure S-1012 at VESO Vikan. During the entire challenge test the fish were given a commercial feed at an amount of 1–2% of their body weight per day.

2.4. Field test

The field-test group was put into a net-cage at Bolaks locality Mjånes, Hordaland on 30th March 2016 at an average body weight of about 190 g. AGD-infection was monitored from early July with regular gill-scoring and gill swabbing of a random sample of the fish. The swab samples were used to determine the severity of AGD from the CT (cycle threshold) values of *P. perurans* obtained from RT-qPCR. The amount of amoeba nucleic acids increases with decreasing CT value. High CT values (e.g. > 40) indicate minimum amount of nucleic acids or environmental contamination of the samples. The mean seawater temperature at 5 m depth at Mjånes was 10.5 (SD = 1.2) ^{0}C in May, 14.6 (SD = 1.2) ^{0}C in June, 15.1 (SD = 0.5) ^{0}C in July, 15.2 (SD = 0.4) ^{0}C in August, 15.9 (SD = 0.5) ^{0}C in September and 14.6 (SD = 1.3) ^{0}C in October until the gill scoring on 8th, 10th and 11th November 2016. At 10 m depth these mean monthly temperatures in May to September were from 0.1 to 0.7 °C higher, and in October until the gill scoring in

Table 1
The gross gill-score used both in the challenge test and the field test. An adaption after Taylor et al. (2009).

Infection level	Gill-score	Gross gill-score description
Clear	0	No sign of infection on any side of the 8 (=2 × 4) gill-arches
Very light	1	In total only 1 white spot on all 16 sides of the gill-arches
Light	2	In total 2–3 white spots on all 16 sides of the gill-arches
Moderate A	3A	In total 4–10 white spots on all 16 sides
Moderate B	3B	In total > 10 white spots up to 15% cover of the total area of all 16 sides
Moderate C	3C	15–20% cover of white spots of the total area of all 16 sides
Advanced	4	20–50% cover of the total area on all 16 sides
Heavy	5	The white spots cover most of the area on all 16 sides

early November 0.5 °C lower.

The gill-scorings of all fish were performed by two experienced persons from VESO Vikan. Prior to gill-scoring each fish was anesthetised with benzocaine. It was not possible to obtain a second gill-score since this first gill-scoring took place so late in the year, and at a decreasing seawater temperature. The field score can, however, not be compared to a first score in a challenge test, since the fish have had (lower) exposure to the amoebae over at least several weeks.

After the above recordings, the fish were put together with the SalmoBreeds' breeding candidates at Mjånes and were not treated with freshwater. Individual body weights of all fish were recorded both at gill-scoring in November 2016 and at preselection of breeding candidates 19th to 28th June 2017.

The body weight and sex (based on inspection of the gonads) of the fish at LetSea (see 2.1) were recorded at slaughter from 28th February to 3rd March 2017. AGD has not yet been identified in Nordland or further north based on screening at exposed sites with high salinity (Fish Health Report 2016, ISSN no. 1893–1480).

During the AGD field test we observed slow development of the gill-scores during the summer and autumn. As a low average gill-score could result in an unreliable estimate of the genetic correlation between susceptibility to AGD in the field test and the bath challenge test, we decided to obtain RT-qPCR CT values for *P. perurans* as a more objective and sensitive measure of the severity of the AGD-infection. The swab samples were obtained from the 2nd anterior gill-arch on the left side of each fish.

A random sample of 126 of these swab samples were also analysed by RT-qPCR for two other microorganisms, the spore-forming unicellular parasite *Paranucleospora theridion* and the intracellular bacteria *Branchiomonas cysticola* that both can cause pathology on gills of Atlantic salmon in seawater (Gunnarsson et al., 2017; Wiik-Nielsen et al., 2017).

2.5. RT-qPCR methods

The semi-quantitative RT-qPCR methods used are validated in-house Fish Vet Group, Oslo, Norway methods based on primers and probes from the following papers; Nylund et al. (2010); Fringuelli et al. (2012) and Mitchell et al. (2013). Validation of the assays included a comparison of RT-qPCR results from gill swabs to RT-qPCR results from gill tissue conserved in RNAlater. The results showed that a higher sensitivity was achieved for *P. perurans* when using gill swabs, similar sensitivity was achieved for *B. cysticola*, and a lower sensitivity was achieved for *P. theridion*.

The reported CT-values are the $\log_2$(cycle threshold value), and thus represent a doubling of the amoebae concentration per unit decrease in the CT-value.

2.6. Housekeeping gene

For *P. perurans*, the mean CT value of the housekeeping gene for the 1141 analysed gill samples was 18.9, and with a much smaller standard deviation (0.82) as compared to the standard deviation (4.17) of the observed CT values (see Table 2).

For *P. theridion*, the mean and standard deviation of the housekeeping gene for the 126 gill samples was 17.5 and 0.60, respectively, as compared to 25.7 and 2.19 for the observed CT values for the same samples. For *B. cysticola*, the mean and standard deviation of the housekeeping gene for the 126 gill samples was 16.6 and 0.84, respectively, as compared to 25.0 and 2.30 for the observed CT values for the same samples.

The standard deviation of the difference in CT value between the observed values and the housekeeping gene values was marginally lower than for the observed CT value; i.e. 4.01 vs. 4.18 for *P. perurans*; 2.10 vs. 2.19 for *P. theridion*, and 2.13 vs. 2.30 for *B. cisticola*.

For *P. perurans*, the correlation coefficient between the observed CT values and the difference in CT values between the observed and the housekeeping gene values was very high (0.98), as was the corresponding correlations for *P. theridion* (0.98) and *B. cisticola* (0.97). Consequently, for each of the three microorganisms we decided to use the observed CT values rather than the difference between the observed values and the housekeeping gene values.

Table 2
Means and standard deviations for Taylor AGD gill-score (score 0–5), modified Taylor AGD gill-score, body weight and RT-qPCR CT in the bath challenge test and the field test.

Type of test and trait	N	Mean	SD
Bath challenge test			
1st gill-score			
- Taylor et al. (2009)	1554	2.26	0.58
- Taylor et al. (2009), modified	1554	3.33	0.94
2nd gill-score	1554	2.70	0.58
- Taylor et al. (2009)	1554	2.70	0.58
- Taylor et al. (2009), modified	1554	3.81	0.72
Field test, AGD environment			
Gill-score			
- Taylor et al. (2009)	1156	2.68	0.58
- Taylor et al. (2009), modified	1156	2.89	0.80
Body weight Nov. 2016, kg	1154	2.18	0.64
Body weight June 2017, kg	861	4.87	1.22
RT-qPCR CT	1141	23.4	4.17
Field test, AGD free environment			
Body weight, g	3392	4.85	1.24

2.7. Statistical analyses

Estimates of (co)variance components for the studied traits were obtained from the following multitrait linear mixed animal model using the ASReml software (Gilmour et al., 2009):

$$\begin{bmatrix} Gs1\_ct \\ Gs2\_ct \\ Gs\_ft \\ CT\_ft \\ Bw1\_ft \\ Bw2\_ft \\ Bw\_ft \end{bmatrix} = Xb + Z_1u + Z_2f + e$$

where the traits Gs1_ct and Gs2_ct is the 1st and 2nd gills-score in the challenge test, respectively; Gs_ft, CT_ft, Bw1_ft and Bw2_ft is gill-score, CT_ft-qPCR, the body weight in November 2016 and the body weight in June 2017 in the AGD field test environment, respectively; Bw_ft is the harvest body weight in the non-AGD field environment; $\boldsymbol{X}$ is the incidence matrix that assign the trait record for each animal to the appropriate level of the fixed effect(s) for the trait, $\boldsymbol{b}$ is a vector of the fixed effects for each trait; $\boldsymbol{Z_1}$ is the incidence matrix that assign the trait record to each animal and $\boldsymbol{u}$ is the vector of additive genetic values for each animal with $u \sim N(0, A\sigma_u^2)$,where σ_u^2 is the additive genetic variance and $\boldsymbol{A}$ is the additive genetic numerator relationship matrix; $\boldsymbol{Z_2}$ is the incidence matrix that assign the observation of each animal to its full-sib family and $\boldsymbol{f}$ is the vector of the effect common to fullsibs other than additive genetics; and $\boldsymbol{e}$ is the vector of random residual effects with $e \sim N(0, I\sigma_e^2)$, σ_e^2 is the environmental residual variance. For the traits Gs1_ct, Gs2_ct and Gs_ft, $\boldsymbol{b}$ is the fixed effect of gill-scoring person each with two levels; for CT $\boldsymbol{b}$ is the overall cage mean; for Bw1_ft and Bw2_ft $\boldsymbol{b}$ is the effect of sex; while for Bw_ft $\boldsymbol{b}$ is the combined effect of sex and sexual maturity. The sex of each fish was determined by using markers located within the *sd*Y gene (Houston et al., 2014). The traits recorded in the challenge test environment, the traits recorded in the AGD field test environment and the harvest body weights recorded in the non-AGD test environment were recorded on different animals from the same families. Therefore, the residual

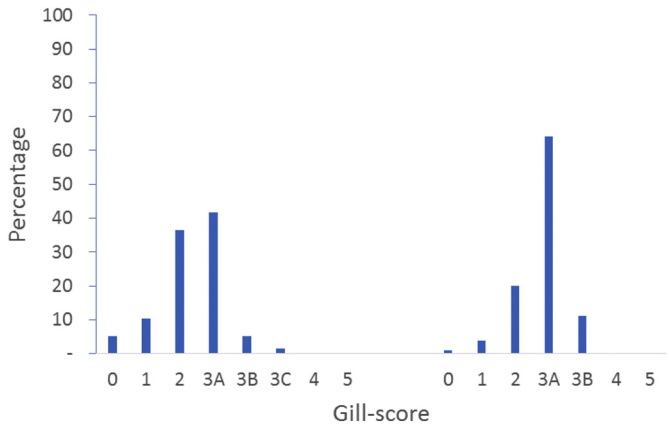

Fig. 1. Distributions of 1st and 2nd gill-score in the challenge test at VESO Vikan.

Table 3
Estimates of heritabilities (on diagonal) and genetic (below diagonal) and residual/phenotypic correlations (above diagonal) for 1st and 2nd gill-score in the challenge test and the gill-score in the field test at the affected AGD environment at Mjånes.

Type of test	Trait	Challenge test		Field test
		1st gill-score	2nd gill-score	Gill-score
Challenge test	1st gill-score	0.20 ± 0.09	0.03/−0.00	–
	2nd gill-score	−0.25 ± 0.26	0.06 ± 0.03	–
Field test	Gill-score	−0.11 ± 0.22	0.14 ± 0.28	0.17 ± 0.08

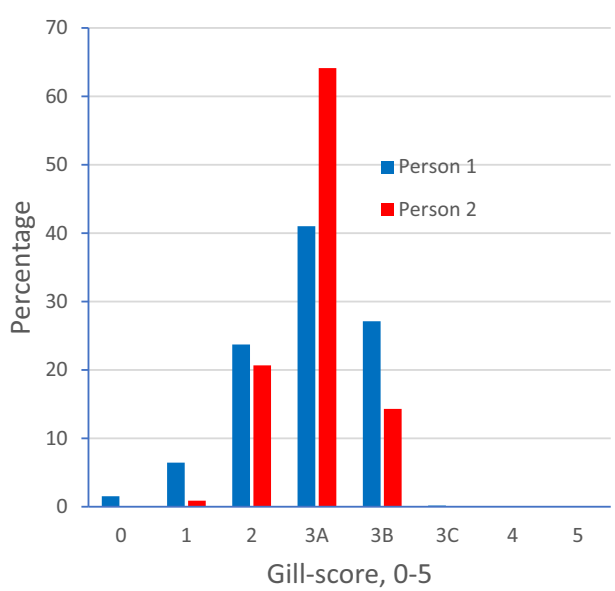

Fig. 2. Distribution of gill-score in the field test at Mjånes.

correlation between these three groups of traits were set equal to zero.

3. Results

3.1. Challenge test

Descriptive statistics for the gill-scores recorded at the two challenge tests are given in Table 2 and Fig. 1. As expected the modified gill-score resulted in both a higher average and standard deviation for gill-score. However, a large proportion of the fish had gill-score 2 and 3A, and none of the fish got gill-score 4 or 5. The estimated genetic parameters are given in Table 3. The heritability obtained for the 1st gill-score was moderate (0.20 ± 0.09) and low for 2nd score (0.06 ± 0.03). The use of the modified gill-score scale had marginal effect on the magnitude of the heritability estimates. The genetic correlation between 1st and 2nd gill-score was negative (−0.25 ± 0.27), but not significantly different from zero.

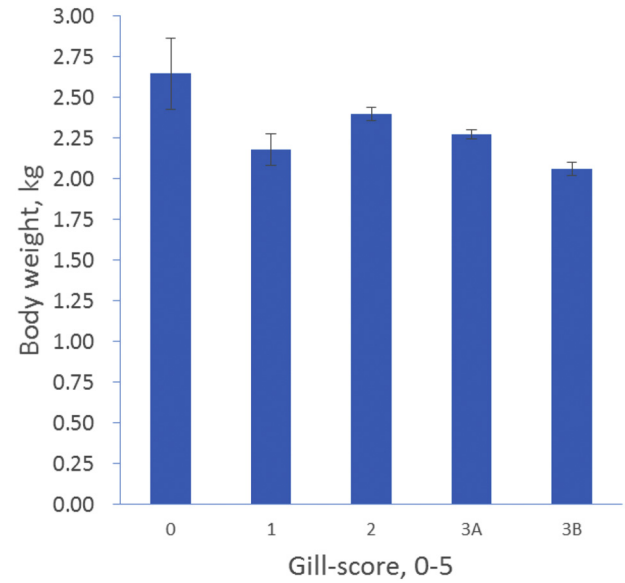

Fig. 3. Mean body weight (± standard error) of fish with different gill-score.

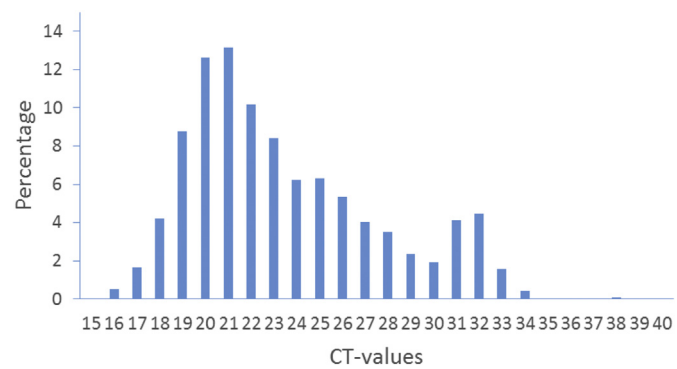

Fig. 4. Distributions of qRT-PCR CT-values for *Paramoeba perurans* in the gill swab samples of 1141 Atlantic salmon in the field test at Mjånes.

3.2. Field test

3.2.1. Monitoring of P. perurans

On 30th June 2016, *P. perurans* was not detected in gills of any of the 20 sampled fish at the Mjånes locality. First sign of an infection of *P. perurans* at this locality was observed on 27th September 2016 when six of the 20 sampled fish had a positive CT value (average 31.2). On 4th and 12th October 2016 four of the 10 fish sampled were positive (average 34.1 and 31.3), and on 18th, 25th and 31st of October 2016, all the 10 sampled fish were positive (average 31.0, 28.8 and 21.6, respectively).

3.2.2. Average gill-score

At the gill-scoring in November 2016, the average gill-score on the adapted gill score (0–7) was 2.89 (Table 2). The distribution of the gill-score was narrow with very few fish with score 0 (8) and 1 (41), only 1 fish with score 3C and none with score 4 or 5 (Fig. 2). Without the extended gill-score (3A, 3B and 3C) most of the fish would have got gill-

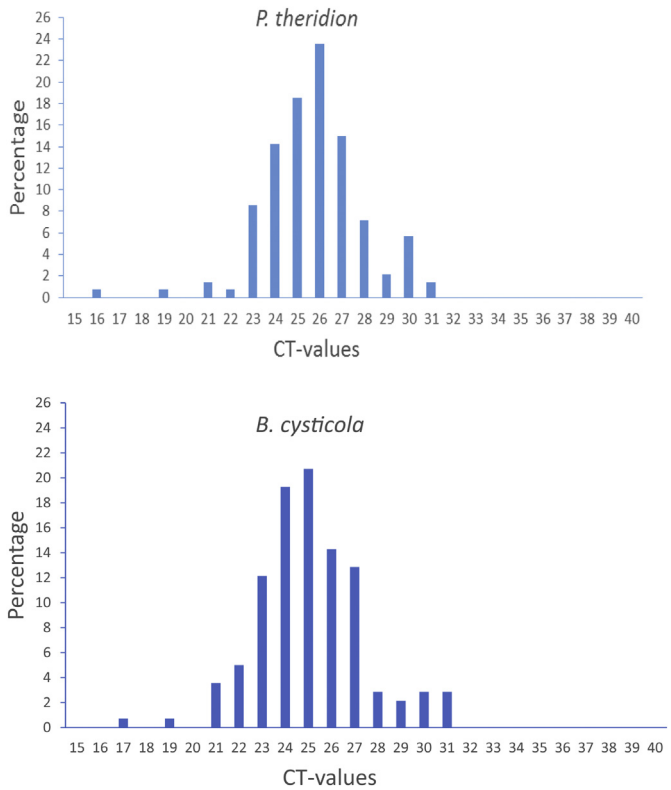

Fig. 5. Distributions of RT-qPCR CT-values due to *Paranucleuspora theridion* and *Branchiomonas cysticola* in the gill swab samples from a random sample of 140 of the 1141 Atlantic salmon in the field test at Mjånes.

score 2 and 3.

3.2.3. Distribution of CT values

The distribution of the CT values due to *P. perurans* are shown in Fig. 4. The average CT (23.4, Table 2) was slightly lower than the average CT of three water samples obtained from one of the anesthetizing bath by a cup (26.9) or by a swab (25.9), and much lower than the average CT of three water samples (33.2) obtained from the hose used to fill the anesthetizing baths. Consequently, the average concentration of amoeba was much higher on the gills of the fish and in the water in the anesthetizing bath, as compared to that in the seawater at the Mjånes location. However, this implies also that any CT value from the swabs above or around 26 possibly comes from contaminated bath water, and that any CT value above or around 33 comes from amoeba in the water itself.

The distribution of the CT values due *P. theridion* and *B. cisticola* are shown in Fig. 5. For this random sample of 126 fish the average CT value of *P. theridion* (mean 25.7, SD = 2.2) and *B. cysticola* (mean 25.0, SD = 2.3) were very similar to that of *P. perudans* (24.5, SD = 4.6), but not directly comparable as different primers and probes (see 2.4) were used for the three microorganisms.

The correlation coefficients of the CT values of *P. perudans* with *P. theridion* and *B. cysticola* was 0.26 and 0.11, respectively; and 0.20 between the two latter. The correlation of gill score with the CT values with *P. perudans, P. theridion* and *B. cysticola* was −0.29 ($P < .0001$), 0.02 ($P > .05$) and 0.13 ($P > .05$), respectively.

3.2.4. Effect of water replacement in the anesthetizing bath on CT-values due to P. perurans

Over the 2½ days recording period the seawater in the anesthetizing bath (a separate bath for each of the two persons that performed the gill-scoring and obtained the gill-samples with the swabs) was replaced with new seawater in total 31 times. The regression of CT on the time within each person by water replacement combination was not significantly different from zero (b = 0.0107 ± 0.0093), and thus no need to account for this effect when estimating genetic parameters for CT.

3.2.5. Effect on body weight

The average body weight of the 1141 fish recorded at Mjånes was 2.18 kg (Table 2). Average body weight of fish with gill-score 1, 2, 3A and 3B was 17.6, 9.4, 17.9 and 22.2% lower, respectively than fish with gill-score 0 (Fig. 3). The regression plots of body weight (g) on CT

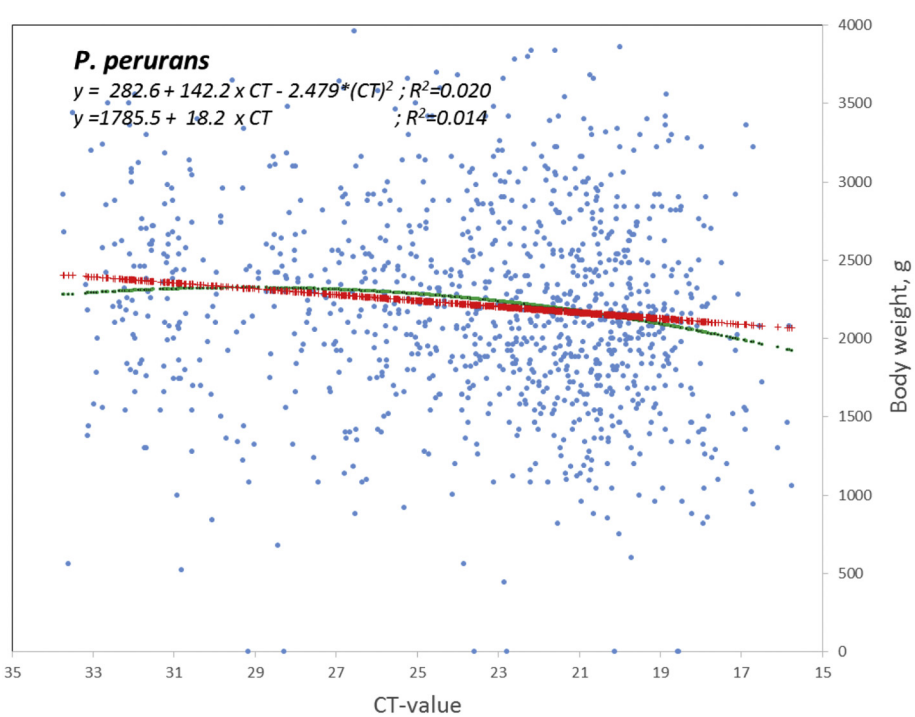

Fig. 6. Regression plots of body weight on RT-qPCR CT-values due to *Paramoeba perurans* in the field test at Mjånes.

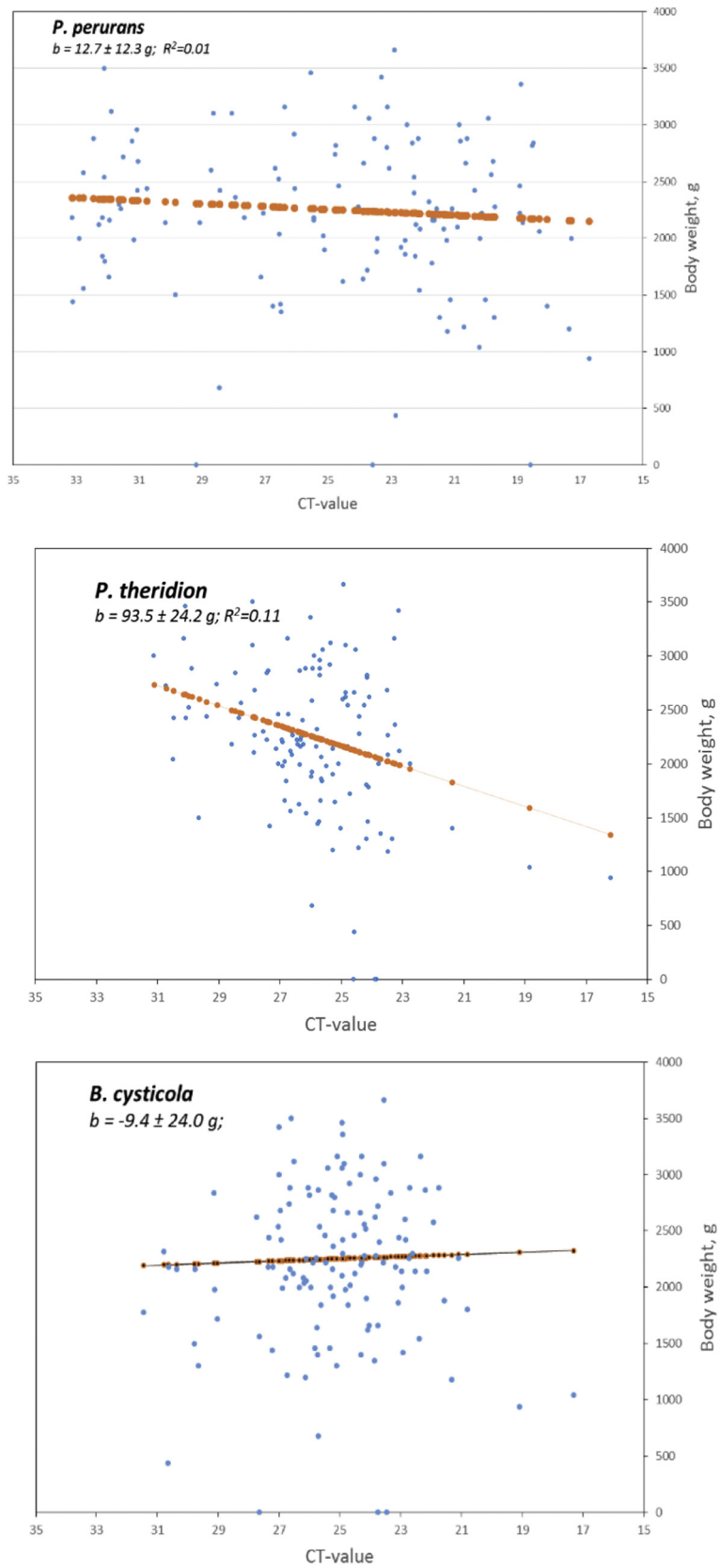

Fig. 7. Regression plots of body weight on RT-qPCR CT-values due to *Paramoeba perurans*, *Paranucleospora theridion and Branchiomonas cysticola* for the sample of 126 fish analysed for each of these three microrganisms.

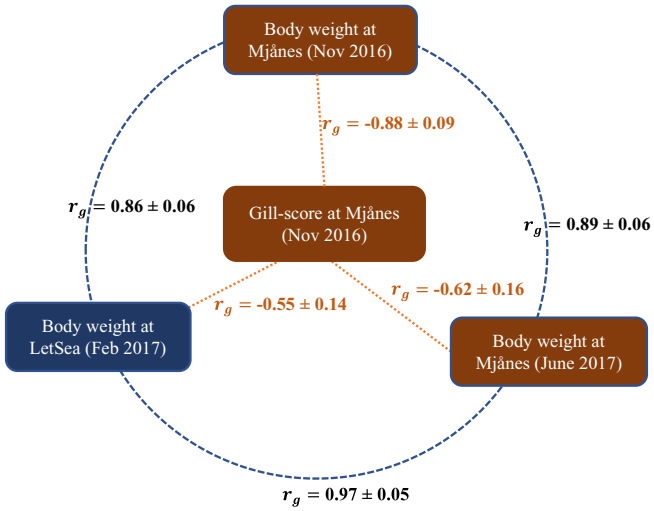

Fig. 8. Genetic correlations of AGD gill-score with the three body weight traits.

(Fig. 6) shows a very low association between the two traits (y = 282.6 + 142.2 x CT – 2.480 x $(CT)^2$; R^2 = 0.020; $P < .001$). For a first degree polynomial only, the regression coefficient was 18.2 ± 4.6 g (R^2 = 0.015; $P < .001$), or 18.2 g reduced body weight per unit decrease (corresponding to a doubling of the amoeba concentration) of the CT value, and similar to the regression coefficient for the sample of 126 fish (Fig. 7).

This negative effect on body weight was also observed with decreasing *P. theridion* CT-values with 93.5 ± 24.2 g (R^2 = 0.11; $P < .001$) reduced body weight per unit decrease in the CT value (Fig. 7); an effect that was reduced to 75 ± 28 g (R^2 = 0.06; $P < .001$) when omitting the two observations in Fig. 7 with CT below 20. For the same sample of 126 fish the effect of *B. cysticola* CT-values was not significantly different from zero ($P > .05$) (Fig. 7), as was the regression coefficient due *P. perurans* for this sample of 126 fish. In a simultaneously analyses of the effect of the CT values of all of the three above mentioned microorganisms on body weight, only the effect of *P. theridion was* significantly different from zero with 96 ± 25 g ($P < .001$) reduced body weight per unit decrease of the CT value.

3.2.6. Estimates of genetic parameters in the AGD field test environment

Estimates of heritabilities for the traits recorded in the field test, and the genetic, residual and phenotypic correlations between the traits are shown in Table 4. Heritability for gill-score was of medium magnitude (0.19 ± 0.05) while that for CT was lower (0.11 ± 0.04). Higher heritability estimates were found for body weight recorded in November 2016 (0.50 ± 0.08) and in June 2017 (0.43 ± 0.07).

The genetic correlation of gill-scores in November 2016 with body weight in November 2016 (−0.88 ± 0.09) and June 2017 (−0.62 ± 0.14) were negative. The residual correlations of body weight with gill-score and CT were low. The genetic correlation of gill-score with concentration of *P. perurans* on the gill (−0.81 ± 0.16) and body weight (−0.88 ± 0.09) were high negative. The genetic correlation between body weights in November 2016 and June 2017 was high (0.89 ± 0.06).

3.3. Challenge test vs. field test

The genetic correlations of gill-score in the field test with 1st (−0.11 ± 0.22) and 2nd (0.14 ± 0.28) gill-score in the bath challenge test were low (Table 3) and not different from zero.

3.4. Correlations between traits in the AGD and non-AGD field test environments

The genetic correlation of gill-score at Mjånes in November 2016 and harvest body weight at LetSea was negative (−0.55 ± 0.14), while the genetic correlations of body weight at LetSea in February 2017 with body weight at Mjånes in November 2016 (0.86 ± 0.05) and June 2017 (0.97 ± 0.05) were high (Table 4).

4. Discussion

In Tasmania the successful selection for increased resistance to AGD in Atlantic salmon has been based on gill-scores from field tests (Taylor et al., 2009; Kube et al., 2012). However, since field-test is dependent on more regular and predictable outbreaks than present in Northern Europe, it would be beneficial to obtain the gill-score data from a controlled challenge test, given that resistance to AGD in a challenge test is a good predictor of resistance in a field test environment. In a recently published study we found that the genetic correlation between gill-score in a bath challenge test and a field test was low (0.07–0.38) and not significantly different from zero (Lillehammer et al., 2019), and thus a poor predictor of resistance to AGD in a field test. Another explanation could be that in the field the fish and their gills are exposed to many other microorganisms (e.g. *P. theridion* and *B. cysticola* as documented in this study), and that the field results reflect a combined effect of several agents, not only *P. perurans*. In the study of Lillehammer et al. (2019), the amoeba concentration was high, and might have influenced the low genetic correlation we observed between the bath challenge test and the field test. Therefore, we reduced the amoebic concentration in the challenge test and used an extended Taylor et al. (2009) gill-scoring scale (gill-score 3 was divided into three classes 3A, 3B and 3C) in both the bath challenge test and the field test. However, the distribution of both the 1st and 2nd gill-score in the bath challenge test were similar to those reported by Lillehammer et al. (2019), in spite of the much lower amoeba concentration. In both tests the heritability for gill-score (0.1–0.2) was of the same magnitude as reported by Lillehammer et al. (2019), but in general lower than the heritability estimates for gill-score from field tests in Tasmania (Taylor et al., 2009; Kube et al., 2012).

In the bath challenge test the genetic correlation between 1st and 2nd gill-score was close to zero and thus in close agreement with the

Table 4
Estimates of heritabilities (on diagonal) and genetic (below diagonal) and residual/phenotypic correlations (above diagonal) for traits recorded at in the AGD field test (Nov. 2016 and June 2017) and in the AGD free environment (Feb. 2017).

Time recorded	Trait	November 2016			June 2017	Feb. 2017[1]
		Gill-score	CT	Body weight	Body weight	–
Nov. 2016	Gill-score	**0.19 ± 0.05**	−0.22/−0.29	0.15/−0.16	0.04/−0.13	–
	CT	−0.81 ± 0.16	**0.11 ± 0.04**	−0.03/0.11	−0.02/0.13	–
	Body weight	−0.88 ± 0.09	0.53 ± 0.18	**0.50 ± 0.08**	0.50/0.67	–
June 2017	Body weight	−0.62 ± 0.14	0.45 ± 0.19	0.89 ± 0.06	**0.43 ± 0.07**	–
Feb. 2017[1]	Body weight	−0.55 ± 0.14	0.33 ± 0.19	0.86 ± 0.05	0.97 ± 0.05	**0.57 ± 0.05**

[1] AGD free environment at LetSea.

estimate reported by Lillehammer et al. (2019). In field tests in Tasmania moderate genetic correlation between 1st and 2nd gill-scores have been reported, and higher genetic correlations among 2nd and later gill-scores (Kube et al., 2012), implying that gill-score at 1st gill-score and later re-infections are different traits, probably due to an interplay of innate and acquired immune responses. Whether 1st and/or 2nd gill-score from a challenge test should be used as selection criterion for increased resistance to AGD depends on their genetic correlation to the breeding objective trait gill-score in a field test.

The close to zero genetic correlation of gill-score in the field test with 1st and 2nd gill-scores in the bath challenge test is in agreement with the estimates reported by Lillehammer et al. (2019). Therefore, as stated earlier gill-scores from a bath challenge test cannot easily replace gill-scores from a field test in a breeding program. However, as a field test is dependent on more regular and predictable AGD outbreaks than yet present in Norway, efforts should be taken to develop a challenge test more similar to that the fish experience in a field test, but will always be restricted by the number of parameters that can be controlled. A first step could be to try a challenge test with an even lower concentration of amoebae than in this study as inoculation with from 500 to down to 10 amoebae cells/L is reported to give both gross and histological evidence of AGD (Morrison et al., 2004a,b). These lower concentrations are similar to the concentration of *Paramoeba* sp. observed in the water column within and among Atlantic salmon cages (approximately 10–50 cells/L) in south-eastern Tasmania (Douglas-Helders et al., 2003). A drawback of this would most likely be a longer lasting and thus more costly challenge test.

The genetic correlation between gill-scores and harvest body weights of the field test was negative (−0.62), but of lower magnitude than between gill score and body weight at scoring (−0.88) (Table 4; Fig. 8). This indicates that the fish during the seven months from November 2016 to June 2017 have not managed to compensate for the loss in growth prior to the gill-scoring in November 2016. If the gill-scored fish had been treated with freshwater after the gill-scoring in November 2016 this correlation (−0.62) may have been lower.

Fish with gill-score greater than zero had reduced body weight as compared to fish with gill-scorer 0 (Fig. 3), indicating a negative effect of AGD and possible also other gill microorganism on growth as shown for *P. theridion* but not *B. cysticola* in this study. This may be explained by a reduction in the oxygen uptake as demonstrated in AGD infected Atlantic salmon in a swim tunnel respirometer (Hvas et al., 2017).

The genetic correlations of body weight with both gill-score (−0.88) and CT (0.53) are much higher than the residual correlations (0.15 and − 0.03) which indicate a true negative and thus favourable genetic correlation between AGD gill-score and growth rate in Atlantic salmon. This is supported by the high genetic correlation (0.86) between body weight at the AGD affected and the AGD-free environment. Therefore, selection for increased growth rate in an AGD-free environment should result in a favourable genetic correlated response in resistance to AGD. However, as 23% ($1–0.88^2$) of the observed genetic variation in AGD gill-scores cannot be explained by growth in the AGD environment, a smaller response to selection is to be expected by selecting for growth only.

The larger negative effect of decreasing *P. theridion* CT-values than of decreasing *P. perurans* CT-values on body weight (Fig. 7) may indicate that the magnitude of the above genetic correlations may also be affected by other pathogens with negative effect on gill health and growth as gills of Atlantic salmon are found to be co-infected with several other pathogens associated with gill health (Gunnarsson et al., 2017; Downes et al., 2017). Therefore, it cannot be ruled out that the fish in the AGD free environment in this study may be affected by other pathogens than *P. perurans* with a negative effect on gill health and growth. The magnitude of the genetic correlations of gill-score at the AGD-environment with growth at both the AGD affected and the AGD free environment, as well as magnitude of the genetic correlation between growth in the two environments, should be verified in a similar experiment where the growth in both environments is measured from the early onset of the pathogen infection until gill-scoring, and thus will require closely monitoring of putative pathogens from the time the smolt are stocked into the net-cages in the sea. At the time of gill scoring in both environments, CT values of several putative gill pathogens e.g. *P. theridion*, should be obtained from which the genetic and residual correlations of growth with CT values of different pathogens can be obtained, as well as genetic and residual correlations among the CT values of the different pathogens. This will provide novel insight into the genetic relationship between the host susceptibility of different gill pathogens.

The high favourable genetic correlation of gill-score with CT values of *P. perurans* strongly indicates that CT-values may be used as an indirect, but more objective and less laborious measure of resistance to AGD than gill-score. However, the cost of gill-scoring may be lower than the cost of RT-qPCR analysis.

Acknowledgements

This project was funded by the Norwegian Seafood Research Fund-FHF grant no. 901147 and by the project partners SalmoBreed, Marine Harvest, VESO Vikan and Nofima. The RT-qPCR analyses of the swab samples were obtained from Fish Vet Group, Oslo, Norway. The fish material was obtained from SalmoBreed AS. Akvaforsk Genetics edited data and build pedigree files.

References

Adams, M.B., Ellard, K., Nowak, B.F., 2004. Gross pathology and its relationship with histopathology of amoebic gill disease (AGD) in farmed Atlantic salmon, *Salmo salar* L. J. Fish Dis. 27, 151–161.

Clark, A., Nowak, B.F., 1999. Field investigations of amoebic gill disease in Atlantic salmon, *Salmo salar* L., in Tasmania. J. Fish Dis. 22, 433–443.

Douglas-Helders, G.M., O'Brien, D.P., McCorkell, B.E., Zilberg, D., Gross, A., Carson, J., Nowak, B.F., 2003. Temporal and spatial distribution of *Paramoeba* sp. in the water column – a pilot study. J. Fish Dis. 26, 231–240.

Downes, J.K., Rigby, M.L., Taylor, R.S., Maynard, B.T., MacCarthy, E., O'Connor, I., Marcos-Lopez, M., Rodger, H.D., Collins, E., Ruane, N.M., Cook, M.T., 2017. Evaluation of non-destructive molecular diagnostics for the detection of *Neoparamoeba perurans*. Front. Mar. Sci. 4, 61. https://doi.org/10.3389/fmars.2017.00061.

Downes, J.K., Yatabe, T., Marcos-Lopez, M., Rodger, H.D., MacCarthy, E., O'Connor, I., Collins, E., Ruane, N.M., 2018. Investigation of co-infections with pathogens associated with gill disease in Atlantic salmon during an amoebic gill disease outbreak. J. Fish Dis. 1–11. https://doi.org/10.1111/jfd.12814.

Fringuelli, E., Gordon, A.W., Rodger, H., Welsh, M.D., Graham, D.A., 2012. Detection of *Neoparamoeba perurans* by duplex quantitative Taqman real-time PCR in formalin-fixed, paraffin-embedded Atlantic salmonid gill tissues. J. Fish Dis. 35 (10), 711–724. https://doi.org/10.1111/j.1365-2761.2012.01395. xFish Health Report., 2016. The fish health situation in Norwegian aquaculture. Veterinærinstituttet. Rapport 4-2017.

Gilmour, A., Gogel, B.J., Cullis, B.R., Thompson, R., 2009. ASReml User Guide Release 3.0. VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Gunnarsson, G.S., Karlsbakk, E., Blindheim, S., Plarre, H., Imsland, A.K., Handeland, S., Sveier, H., Nylund, A., 2017. Temporal changes in infections with some pathogens associated with gill disease in farmed Atlantic salmon (*Salmo salar* L). Aquaculture 408, 126–134.

Houston, R.D., Taggart, J.B., Cézard, T., Bekaert, M., Lowe, N.R., Downing, A., Talbot, R., Bishop, S.C., Archibald, A.L., Bron, J.E., Penman, D.J., Davassi, A., Brew, F., Tinch, A.E., Gharbi, K., Hamilton, A., 2014. Development and validation of a high density SNP genotyping array for Atlantic salmon (*Salmo salar*). BMC Genomics 15, 90 (http://www.biomedcentral.com/1471-2164/15/9).

Hvas, M., Karlsbakk, E., Mæhle, S., Wright, D.W., Oppedal, F., 2017. The gill parasite *Paramoeba perurans* compromises aerobic scope, swimming capacity and ion balance in Atlantic salmon. Conservation Physiol. 5, 1–11.

Kube, P.D., Taylor, R.S., Elliott, N.G., 2012. Genetic variation in parasite resistance of Atlantic salmon to amoebic gill disease over multiple infections. Aquaculture 364, 165–172.

Lillehammer, M., Boison, S.A., Norris, A., Løvoll, M., Gjerde, B., 2019. Genetic parameters for resistance to amoebic gill disease in Atlantic salmon. Aquaculture, submitted.

Mitchell, S.O., Steinum, T.M., Toenshoff, E.R., Kvellestad, A., Falk, K., Horn, M., Colquhoun, D.J., 2013. '*Candidatus* Branchiomonas cysticola' is a common agent of epitheliocysts in seawater-farmed Atlantic salmon *Salmo salar* in Norway and Ireland. Dis. Aquat. Org. 103 (1), 35–43. https://doi.org/10.3354/dao02563.

Morrison, R.N., Crosbie, P.B.B., Nowak, B.F., 2004a. The induction of laboratory-based amoebic gill disease revisited. J. Fish Dis. 27, 445–449.

Morrison, R.N., Crosbie, P.B.B., Nowak, B.F., 2004b. The induction of laboratory-based amoebic gill disease revisited. J. Fish Dis. 35 (10), 711–724. https://doi.org/10.

1111/j.1365-2761.2004.00561.x.
Nylund, S., Nylund, A., Watanabe, K., Arnesen, C.E., Karlsbakk, E., 2010. *Paranucleospora theridion* n. gen., n. sp. (Microsporidia, Enterocytozoonidae) with a Life Cycle in the Salmon Louse (*Lepeophtheirus salmonis*, Copepoda) and Atlantic Salmon (*Salmo salar*). J. Eukaryot. Microbiol. 57 (2), 95–114. https://doi.org/10.1111/j.1550-7408.2009.00451.x.
Taylor, R.S., Muller, W.J., Cook, M.T., Kube, P., Elliott, N.G., 2009. Gill observations in Atlantic salmon (*Salmo salar*, L.) during repeated amoebic gill disease (AGD) field exposure and survival challenge. Aquaculture 290, 1–8.
Wiik-Nielsen, J., Gjessing, M., Solheim, H.T., Litlabø, A., Gjevre, A.-G., Kristoffersen, A.B., Powell, M.D., Colquhoun, D.J., 2017. Ca. *Branchiomonas cysticola*, Ca. *Piscichlamydia salmonis* and Salmon gill pox virus transmit horizontally in Atlantic salmon held in fresh water. J. Fish Dis. 40 (10), 1387–1394.