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Is genetic resistance to AGD from a bath challenge-test a good predictor of genetic resistance from a field-test?

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Report

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Summary/recommendation:

Estimates of genetic parameters for resistance to AGD were obtained based on gill-scores from a bath challenge test and a field test. In the field test, the amount of *P. perurans* on the gills was obtained by CT-qPCR. In the field test, the body weights were recorded at the time of gill-scoring. The body weight of a sib group reared in an AGD-free environment were recorded at harvest. The genetic correlation between gill-score in the bath challenge test and the field was close to zero. A bath challenge test for resistance to AGD can therefore not replace a field test in a selective breeding program. Efforts should be taken to develop a challenge test more similar to that the fish experience in a field test, e.g. a cohabitation test. In the AGD-affected environment the high genetic correlation of gill-score with CT-qPCR for *P. perurans* (r_g=0.81 ± 0.16) and body weight (r_g=-0.88 ± 0.09) indicate that CT-qPCR and growth may be used as indirect trait measures of resistance to AGD. The high genetic correlation between the body weights in the AGD-affected and the AGD-free test environment (r_g=0.86 ± 0.05) indicate a true favourable genetic correlation between resistance to AGD and growth in Atlantic salmon. Consequently, selection for increased growth rate will result in a favourable genetic correlated response in resistance to AGD. These genetic correlation need to be verified in a similar experiment.

Summary/recommendation in Norwegian:

Estimat av genetiske parametrar for motstandskraft mot AGD vart rekn ut basert på gjelle-poeng frå ein badesmitte test og ein felt test. I felt testen vart mengde *P. perurans* på gjellene målt ved CT-qPCR. I felt testen vart kroppsvekt registrert ved registrering av gjelle-poeng. Kroppsvekta av ein gruppe sysken som vart oppdretta på ein AGD-fri lokalitet vart registrert ved slakting. Den genetiske korrelasjonen mellom gjelle-poeng i badesmitte testen og felt testen var nær null. Ein badesmitte test for motstandskraft mot AGD kan difor ikkje erstatte ein felttest i eit avlsprogram. Det bør gjerast forsøk på å utvikle ein smittetest som liknar på i ein felt test, f.eks. ein kohabitant test. I det AGD påverka lokaliteten viser den høge genetiske korrelasjonen mellom gjelle-poeng og CT-qPCR (r_g = 0.81 ± 0.16) og kroppsvekt (r_g = -0.88 ± 0.09) at CT-qPCR og vekst kan brukast som eit indirekte mål for motstandskraft mot AGD. Den høge genetiske korrelasjonen mellom kroppsvekt i eit AGD-påverka og AGD-fritt testmiljø (r_g = 0.86 ± 0.05) indikerer ein sann gunstig genetisk korrelasjon mellom motstandskraft mot AGD og tilvekst hos laks. Difor vil utval for auka tilvekst vil resultere i ein gunstig genetisk korrelert respons i motstandskraft mot AGD. Storleiken på desse genetiske korrelasjonane bør etterprøvast i eit liknande forsøk.

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1 Summary

1.1 Extended English summary

Amoebic gill disease (AGD) in Atlantic salmon is caused by the amoeba *Paramoeba perurans*, which colonizes and performs damage on gill tissue. Estimates of genetic parameters of resistance to AGD were obtained from a bath challenge test at VESO Vikan with two repeated infections (with 1612 and 1582 fish, respectively) and from a field test with natural infection at the Bolaks locality Mjånes, Hordaland (1156 fish). The fish were the offspring of 50 sires and 100 dams from SalmoBreeds breeding nucleus. In both tests, resistance to AGD were measured using an adapted Taylor gill-score (0-5) where gill-score 3 was divided into the three sub-classes 3A, 3B and 3C. In the field test we got gill-score data from one AGD infection only as this infection occurred late in the year in the year (September – October) and with gill-scoring in early November 2016) at decreasing seawater temperature. In the field test, a gill-tissue sample was obtained from a gill-arch of each fish using a swab from which the amount of *P. perurans* was obtained using CT (cycle threshold) values from qRT-PCR. The body weight of the field test fish were recorded at the time of gill-scoring as well as seven months later. A group of their sibs were reared in an AGD-free environment and their harvest body weights were also recorded.

In the bath challenge test, the distributions of gill-score was quite narrow (a large proportion of the fish with gill-score 2 and 3A, and none with gill-score 4 and 5) and similar to the gill-score distribution in a recently finished Research Council of Norway (RCN) project, in spite of the much lower amoeba concentration (500 vs. 2000 per L) in this FHF project. The same narrow distribution of the gill-scores was found in the field test. 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 reduced body weight might be due to a reduced oxygen uptake.

In the bath challenge test heritability for gill-score (0.20 for 1st and 0.06 for 2nd infection) was of the same magnitude as those from two bath challenge-tests in the RCN-project. The genetic correlation between the 1st and the 2nd gill score was negative (r_g=-0.25 ± 0.26) but not significantly different from zero. In the field test, the heritability was 0.19 ± 0.05 for gill-score, 0.11 ± 0.04 for CT-qPCR, and 0.50 ± 0.08 and 0.57 ± 0.05 for body weight in November 2016 and June 2017, respectively. The low genetic correlations of gill-score in the field test with both 1st (r_n=-0.06 ± 0.22) and 2nd (r_g=0.16 ± 0.28) gill-score in the bath challenge test, was also found in the RCN project, and this shows that gill-score from a bath challenge test is a bad measure of resistance to AGD in a field test. A bath challenge test for resistance to AGD can therefore not replace a field test in a selective breeding program. However, as a field-test for resistance to AGD is dependent on more regular and predictable outbreaks than yet present in Norway, efforts should be taken to develop a challenge test with a test environment more similar to that the fish experience in a field test with natural AGD infection, e.g. a cohabitation test.

In the AGD-affected environment the high genetic correlation of gill-score with concentration of *P. perurans* (CT-qPCR) on the gills (r_g=0.81 ± 0.16) and body weight (r_g=-0.88 ± 0.09) indicate that CT-qPCR of *P. perurans* and growth may be used as indirect trait measures of resistance to AGD. The genetic correlation between body weight at Mjånes in November and harvest body weight of their sibs in an AGD-free environment (LetSea, Dønna) was high (r_g=0.86 ± 0.05), but lower than the genetic correlation between harvest body weight at Mjånes in June 2017 and LetSea (r_g=0.97 ± 0.05). However,

the genetic correlation between gill-score at Mjånes and body weight at LetSea was negative (r_g=-0.55 ± 0.14). These results indicate a true favourable genetic correlation between resistance to AGD and growth in Atlantic salmon. Consequently, selection for increased growth rate will result in a favourable genetic correlated response in resistance to AGD, while an additional correlated genetic gain can be obtained by selection for growth in an AGD environment. These genetic correlation need to be verified in a similar experiment.

Accuracy of breeding values increased by 28.9% when using genomic information as compared with pedigree information. Therefore, we can conclude that use of genomic information will increase genetic gain for resistance to AGD substantially, both through higher accuracy of the breeding values and higher selection intensity as use of genomic breeding values makes it possible to select the best breeding candidates within families with no record on gill-score. For e.g. early detection of AGD, CT-qPCR for *P. perurans* obtained from a tissue sample of the gills can replace the subjective gill-scores. However, to obtain a reasonable accurate and precise estimate of the true average gill-score of the fish in e.g. a cage at least 30 fish need to be sampled from the cage.

1.2 Extended Norwegian summary

Amøbe gjelle sjukdom (AGD) hos laks er forårsaka av amøben *Paramoeba perurans* som koloniserer og skader gjellene. Vi rekna ut genetiske parametrar for motstandskraft mot AGD basert på data frå ein badesmitte test ved VESO Vikan og feltdata etter naturleg infeksjon ved Bolaks lokalitet Mjånes, Hordaland. Fisken var avkom frå 50 hannfisk og 100 hofisk frå SalmoBreed sin avlskjerne. I begge testane vart motstandskraft mot AGD målt ved bruk av ein tilpassa Taylor skala (poeng 0-5) der poeng 3 vart delt opp i dei tre underklassane 3A, 3B og 3C. I badesmittetesten vart det gitt gjelle-poeng i kvar av to etterfylgjande smittetestar (1612 og 1582 fisk), medan vi i felt testen berre fekk gjelle-poeng frå ein AGD infeksjon (1156 fisk) fordi denne infeksjonen kom seint på året (september-oktober) og registreringa av gjelle-poeng vart gjort tidleg i november 2016 ved minkande sjøvasstemperatur. I felt testen tok vi ein vevsprøve frå ein gjellebue på kvar fisk ved hjelp av ein vattpinne, og som vart analysert for mengde *P. perurans* som CT (cycle threshold) verdiar ved hjelp av qRT-PCR. I felt testen vart vekta av fisken registrert både ved gjelle-scoring og sju månader seinare. Ei sysken gruppe vart oppdretta på ein AGD fri lokalitet og vekt av desse vart registrert ved slakting.

I badesmitte testen var fordelinga av gjelle-poeng smal (ein stor del av fisken med poeng 2 og 3A, og ingen med poeng 4 og 5), og lik fordelinga av gjelle-poeng i eit nyleg avslutta Norges Forskingsråd (NFR) prosjekt, trass i ein s lågare amøbe konsentrasjon (500 mot 2500 amøbar per liter vatn) i dette FHF prosjektet. Det same smale fordeling av gjelle-poeng vart funne i felt testen. I felt testen var gjennomsnittleg kroppsvekt for fisk med gjelle-poeng 1, 2, 3A og 3B høvesvis 17.6, 9.4, 17.9 og 22.2% lågare enn for fisk med gjelle-poeng 0. Den reduserte vekta kan ha sin årsak i eit redusert oksygen opptak.

I badesmitte testen var arvegrada for gjelle-poeng (0.20 for 1. og 0.06 for 2. infeksjon) av same storleik som i dei to badesmitte testane i NFR prosjektet. Den genetiske korrelasjonen mellom gjelle-poeng registrert ved 1. og 2. infeksjon var negativ (r_g=-0.25 ± 0.26), men ikkje signifikant ulik frå null. I felt testen var arvegrada 0.19 ± 0.05 for gjelle-poeng, 0.11 ± 0.04 for CT-qPCR og høvesvis 0.50 ± 0.08 og 0.57 ± 0.05 for kroppsvekt registrert i november 2016 og juni 2017. Låg genetisk korrelasjon mellom gjelle-poeng i felt testen og gjelle-poeng både i 1. (r_g=-0.06 ± 0.22) og 2. (r_g=0.16 ± 0.28) infeksjonsrunde i badesmitte testen vart også funnet i NFR prosjektet, og viser at gjelle-poeng frå ein badesmitte test er eit dårleg mål for motstandskraft mot AGD i ein felt test. I eit avlsprogram kan ein difor ikkje erstatte ein felt test for AGD med ein badesmitte test. Men ettersom ein felt test mot AGD er avhengig av meir regelmessige AGD utbrot enn det ein har i Norge i dag, bør ein gjere forsøk på å utvikle ein smittetest som liknar på ein felt test, f.eks. ein kohabitant test.

I den AGD-påverka lokaliteten tyder storleiken på den genetiske korrelasjonen mellom gjelle-poeng og konsentrasjonen av *P. perurans* (CT-qPCR) på gjellene (r_g=0.81 ± 0.16) og kroppsvekt (r_g=-0.88 ± 0.09) at CT-qPCR og vekst kan brukast som indirekte mål for motstandskraft mot AGD. Den genetiske korrelasjonen mellom vekt på Mjånes i november 2016 og vekt i eit AGD-fritt miljø (LetSea, Dønna) var høg (r_g=0.86 ± 0.05), men lågare enn den genetiske korrelasjonen mellom vekt på Mjånes i juni 2017 og LetSea (r_g=0.97 ± 0.05). Den genetiske korrelasjonen mellom gjelle-poeng og vekt på LetSea var negativ (r_g=-0.55 ± 0.14). Desse resultata tyder på ein sann gunstig genetisk korrelasjon mellom motstandskrafta mot AGD og vekst hos laks. Difor vil utval for auka tilvekst vil resultere i ein korrelert genetisk framgang i motstandskraft mot AGD, og at ein kan oppnå ein ytterlegare korrelert genetisk framgang ved å gjere utval for tilvekst i eit AGD-miljø. Desse genetiske korrelasjonane må etterprøvast i eit liknande forsøk.

Sikkerheita på avlsverdiane auka med 28.9% ved bruk av genomisk informasjon samanlikna med stamtavleinformasjon. Vi kan difor konkludere med at bruk av genomisk informasjon vil auke den genetisk framgangen for motstandskraft mot AGD vesentlig, både gjennom høgare sikkerheit på avlsverdiane og høgare seleksjonsintensitet ettersom bruk av genomiske avlsverdiar gjer det mogleg å gjere utval av dei beste avlskandidatane i kvar familie utan å registrere gjelle-poeng på desse. For f. eks. tidleg påvising av AGD kan ein bruke CT-qPCR verdiar for *P. perurans* frå ein vevsprøve av gjellene. Men for å få eit rimeleg sikkert og nøyaktig estimat av det sanne gjennomsnittlege gjelle-poeng for fisk i f. eks. ein merd må ein ta ein slik prøve av minst 30 fisk i merden.

2 Introduction

Amoebic gill disease (AGD) is caused by the amoeba *Paramoeba perurans*, which colonizes and performs damage on gill tissue 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 Tasmania, selective breeding for lower gill-score from a field test has successfully increased the interval between the treatments. This field-test strategy is dependent on more regular and predictable outbreaks than yet present in Northern Europe where the AGD season is relatively short. Under such circumstances, it would be beneficial to use a challenge test, given that resistance to AGD in a challenge test is a good predictor of resistance in a field test environment.

In the Research Council of Norway (RCN) project Genetics for amoebic gill disease resistance in Atlantic salmon– 235783/E40 (2014-2016) we found significant genetic variation for resistance to AGD both during 1st and 2nd infection in the two challenge tests performed, as well as in a field test. The estimated heritabilities (0.10 to 0.20) show that resistance to AGD in Norwegian Atlantic salmon can be improved through selective breeding, as documented in Tasmania Atlantic salmon where selective breeding for lower gill-score from a field test has successfully increased the interval between the AGD treatments and thus the treatment cost (Brad Evans, pers. comm.). However, the low estimated genetic correlation between resistance to AGD in the challenge test and field test (0.07 to 0.38) means that resistance in a challenge test is a bad predictor of resistance in the field, and that a challenge test cannot 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 tests, maybe due to a too high concentration of amoebae in the challenge test.

The project was a cooperation between Nofima, VESO Vikan and SalmoBreed. VESO Vikan was responsible for the challenge test and SalmoBreed for the field test, while Nofima was responsible for the experimental design, statistical analyses of the data and for writing all reports and popular and scientific papers. The project was run by a steering group and a project group with the following persons:

Steering group appointed by FHF
Håvard Bakke (SalmoBreed), Petter Arnesen (Marine Harvest), Tor Eirik Homme (Grieg Seafood) and Roy Hjelmeland (Marine Harvest). FHF-responsible for the project: Merete Bjørgan Schrøder, later replaced by Eirik Sigstadstø.

Project group
Bjarne Gjerde, Nofima (leader), Marie Lillehammer (Nofima), Hooman Moghadan (Nofima) and Marie Løvoll (VESO).

3 Problems and objectives

In the recently finished RCN-project (235783/E40) we documented genetic variation in resistance to AGD, both in a bath challenge test and a field test. However, in the RCN project we found a low genetic correlation between resistance to AGD in the bath challenge test and the field test, meaning that a challenge test for AGD cannot replace a field test in a selective breeding program. However, as a field test strategy is dependent on more regular and predictable AGD outbreaks than presently in Norway, selection for increased resistance to AGD depends on an improvement of the present bath challenge test. The main objective of the project was to investigate if an improvement 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 and a higher genetic correlation between gill-score in the challenge and the field test. In addition, we investigated whether the concentration of amoeba on the gills, measured by non-destructive and more sensitive CT-qPCR, could replace the subjective and rougher human gill-scoring procedure.

During the AGD field test we observed slow development of the gill-scores during the summer and autumn. A low average gill-score could result in an unreliable estimate of the genetic correlation between resistance to AGD in the field test and the challenge test. Consequently, prior to the gill-scoring in the field test we looked for a more objective and sensitive measure of AGD-infection in the field test. In recent years, efforts have been made to quantify the severity of the infection by quantifying the amount of amoeba in a tissue sample from the gills obtained by a swab, from which the severity of AGD is determined using CT (cycle threshold) values obtained from qRT-PCR. For example, if the average CT value is < 29 means abundant nucleic acid, between 30-37 moderate amounts of nucleic acid, while CT > 37 indicate minimal amounts of nucleic acid, at infection state or environmental contamination.

As an extension of the project, we received additional fund from FHF (ID 14140) to investigated whether amoeba concentration (1/CT-qPCR) on the gills obtained using swabs could be a better predictor of AGD-infection than the subjective gill-score, and to investigate to what degree amoeba concentration on the gills from swabs can be used as a non-destructive diagnostic for AGD-infection.

4 Material and methods

4.1 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 100 fullsib families were reared in separate tanks at Nofima Sunndalsøra, until a body size (mean 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.

4.2 Gill-scoring

The gill-scoring was performed using an adapted version of the categorical filed evaluation method of Taylor et. al. (2009), and is a gross measure of the degree of host response to the presence of *P. perurans*. The method describes the extent of visible white patches on a scale of 'clear' to 'heavy' (**Table 1**) to schedule proactive freshwater bath treatments. In advanced infections, this presumptive scoring method is known to have a moderate to good agreement with histopathological diagnosis (Adams et al., 2004), but is less reliable for less severe cases (Clark and Nowak, 1999). The degree of lesion development is known to be in direct proportion to the infective parasite concentration and progression of the infection (Morrison et al., 2004).

4.3 Challenge test

In January 2016, the bath challenge test group (average body weight 110 g) was transported from Nofima, Sunndalsøra to VESO Vikan, Nord-Trøndelag. After acclimatization, the fish were bath challenged with *P. perurans* on the 21st of January 2016, using an amoeba from VESO Vikan (ref number 2014.10.15NO), and that were cultivated at VESO Vikan on MYA plates. The amoeba concentration in the water was 500 amoebas/L, which was 1/5 of the concentration used in the two challenge tests in the RCN project (Lillehammer et al., 2017). Just after the 1st gill-score was completed (11. and 12.02.2016), all the gill-scored fish (1612) were treated with freshwater three times, with the last freshwater treatment one week before the second bath challenge. The trial was terminated 3½ weeks post the second challenge with gill scoring of all fish (2nd gill-score, 7. and 8.04.2016, 1582 fish). None of the fish had gill-score 4 or 5. The time needed for the fish to develop from gill-score 3B/3C to gill-score 4 takes longer time then from 2 to 3. In this bath challenge test we probably had to wait for at least two weeks more to get individuals with gill-score 4.

4.4 Field test

The field-test group was put into a net-cage at Bolaks locality, Mjånes, Hordaland on 30th of March 2016. AGD-infection was monitored by gill-scores and gill swabbing (swabbing started from September 2016) a random sample of 10 fish once a week for CT-qPCR analyses for *P. perurans*. The gill-scoring of all fish took place on 8th, 9th and 10th of November 2016, and were performed by two experienced persons from VESO Vikan. A gill-tissue sample from each fish was obtained by a swab from the 2nd anterior gill-arch on the left side of the fish. Since this first gill-scoring took place so late in the year, and at a decreasing seawater temperature, it was not possible to obtain a second gill-score as planned for in the project proposal. After the above recordings, the fish were put together with the

SalmoBreeds' breeding candidates at Mjånes without being treated with freshwater. During the gill scoring in November 2016 and June 2017, individual body weights of were recorded.

Sibs of the fish at Bolaks, Mjånes were also grown at LetSea, Dønna, Nordland.The body weights of these fish, as well as the body weight of the fish from an additional 200 families from the same year-class reared in the same cage, were slaughtered on 28th of February 2017. The sex of the fish (3413) were also recorded. AGD has as yet not been identified north of Nord-Trøndelag with screening at exposed sites with high salinity (Fish Health Report 2016).

4.5 Genome wide association study and genomic predictions

All the gill-scored fish (1145) were genotyped with a 55K NOFSAL02 SNP-chip, but 139 of these fish did not passed the initial quality control during the SNP calling step with the Affymetrix axiom analysis suite. After additional genotype and sample quality checks, three (3) more samples were discarded with 46629 markers remaining. The remaining 1003 fish were used for genome wide association and estimate genomic breeding values. Genome wide association and genomic prediction analysis was performed using linear mixed animal as:

$$y = scoreperson + [M\alpha] + Zu + e$$

where y is a vector of AGD gill-scores, $scoreperson$ is the fixed effect of the gill-scoring person (two scorers); M is the incidence matrix for SNP containing marker genotypes, α is the allele substitution effect of SNP, Z is the incidence matrix of genotyped individuals, u is the vector of genomic breeding values with $u \sim N(0, G\sigma_u^2)$, where σ_u^2 is the additive genetic variance, and e is the vector of random residual effects with $e \sim N(0, I\sigma_e^2)$. The G matrix is a genomic relationship matrix (GRM) which was computed according to VanRaden (2008) as $\frac{MM'}{2*\sum_{i=1}^{Nsnp} p_i(1-p_i)}$; where p_i is the allele frequency of second allele and $Nsnp$ is the total number of SNP markers. SNPs were considered genome or chromosome-wide significant when they crossed the threshold $P \leq 9.28 \times 10^{-7}$ and $P \leq 2.78 \times 10^{-5}$, respectively. Genomic breeding values were obtained without the $M\alpha$ covariate of the above model. Pedigree-based breeding values were computed by replacing the G matrix with the numerator relationship matrix. Accuracy of genomic or pedigree based predictions were assessed by randomly assigning 80% (n=790) of the animals for model training and the 20% (n=213) as validation animals. This procedure was replicated 50 times and for each replicate, accuracy of prediction was computed as the correlation (r_{corr}) of the pre-corrected gill-scoresy_{adj} with the estimated pedigree (PBLUP) and the genomic (GBLUP) breeding values. The r_{corr} value was scaled by the square root of the heritability.

4.6 Number of fish needed to obtain CT-qPCR values by non-lethal gill swabbing that reflect a true AGD gill-score

To confirm the presence of AGD on fish in a cage or tank, a molecular diagnostic test may be used, in addition to gill-score. This diagnostic test checks for the presence of the *N. perurans* on a random sample of the fish. However, treatment of fish only commences when the level of infections is severe. The presence of the amoeba is confirmed with a diagnostic test, while the severity of the AGD infection, which will trigger treatment, is based on a gill-score (e.g. as in **Table 1**). While qPCR traditionally have been performed on tissue sampled from euthanized fish, sampling by swabbing the gills permits sampling of live fish. Euthanasia of fish for sampling can represent a significant yearly economical loss to the unit. In addition, sampling by swabbing is quicker and less technically difficult

and can be carried out by technical staff after training. The idea is to detect the presence of amoeba in a tissue sample from the gills obtained by a swab, from which the concentration of *P. peruans* is determined using CT values obtained from qRT-PCR analysis. While care should be taken when correlating CT values to severity of AGD, lower CT values generally mean high load of amoebae while high CT values mean low levels of amoebae.

In the filed data from Mjånes the phenotypic correlation between gill-scores and CT-qPCR *P. peruans* values was low (r_p =-0.29), showing that the latter is a bad predictor of AGD gill-score. In a previous bath challenge experiment at VESO Vikan this correlation was found to be a little higher (r_p=-0.5). To answer the question: *how many fish needs to be sampled to obtain qPCR values by non-lethal gill swabbing that reflect a true AGD gill-score in a cage/tank*, a simulation study was undertaken.

Input parameters for the simulation was obtained from field data at Mjånes, as well as other studies (**Table 2**). We sampled from a multivariate distribution ($MVN(means, G)$, where $means$ represent the mean of each trait and G is the (co)variance information between the traits (see Table 3). Approximately 6,000 fish were simulated for each replicate. From this generated dataset, we performed the following: (1) Obtain a random sample of e.g. five fish from a cage with 6000 fish; (2) Compute the mean AGD gill-score and the mean CT-qPCR value for the sample and store the results; (3) Repeat this for the desired number of replicates (in this case 1000).

5 Results, discussion and conclusions

5.1 Challenge test

The distribution of the gill-score for both 1st and 2nd gill-score (**Figure 1**) were similar to those observed in the NRC-project in spite of the much lower amoeba concentration (500 vs. 2500 per L), and with a large proportion of the fish with gill-score 2 and 3A, and none with gill-score 4 and 5. The heritability for gill-score (0.20 for 1st and 0.06 for 2nd infection), was of the same magnitude as those from the three bath challenge-tests in the RCN-project. The use of a more detailed gill-score (separate gill-score 3 into 3A, 3B and 3C) had marginal effect on the magnitude of the heritability estimates. The genetic correlation between 1st and 2nd gill-score was negative but low (-0.25 ± 0.27) with a large confidence interval spanning the estimates obtained from the three bath challenge tests in the RCN-project (**Table 3**).

5.2 Field test

On 30th June 2016 none of the 20 fish sampled had a positive CT-qPCR value. First sign of an AGD-infection at the Mjånes locality was observed on 27th September 2016 when six of the 20 fish sampled had a positive CT-value (average value 31.2). On 4th and 12th October 2016 four of the 20 fish sampled were positive (average CT value 34.1 and 31.3), and on 18th, 25th and 31st of October 2016, all the 10 fish sampled were positive with average CT-value 31.0, 28.8 and 21.6.

At the scoring on 8th to 10th November 2016, the average gill-score was 2.5 (CV=27.7 %). 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 (**Figure 2**). Without the extended gill-score (3A, 3B and 3C) most of the fish would have got gill-score 2 and 3.

The average body weight of the 1141 fish recorded at Mjånes was 2.18 kg (SD=0.64; CV=29.5 %). 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 (**Figure 3**). Most likely, this is a negative effect of AGD on the growth of Atlantic salmon, e.g. due to a reduced oxygen uptake.

The distribution of the CT-qPCR values of the gill-tissue samples obtained by the swabs are shown in **Figure 4** (low values are associated with high amoeba concentration and vice versa). The average CT-value was 23.4 (SD=4.17; CV=17.8 %). This average CT-value was slightly lower than the average CT-value of three water samples obtained from one of the anesthetizing bath obtained by a cup (26.9) or by a swab (25.9), but much lower than the average CT value of three water samples (33.2) obtained from the hose used to fill the anesthetizing baths. Therefore, 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.

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-qPCR values on the time within each person by water replacement combination was not significantly different from zero (b=0.0107 ± 0.0093, **Figure 5**), and thus no need to account for this effect when estimating genetic parameters for the qPCR-CT-values.

5.3 Estimates of heritabilities and correlations

Estimates of heritabilities for the traits recorded in the field test, as well as the genetic, residual and phenotypic correlations between the traits are shown in **Table 4**. Heritability for gill-score (0.19 ± 0.05) was similar to that for 1st gill-score in the field test in the RCN-project (0.20 ± 0.03), while that for qPCR-CT was lower (0.11 ± 0.04). Higher heritability estimates were found for body weight in November 2016 (0.50 ± 0.08) and in June 2017 (0.43 ±0.07).

The relatively high genetic correlations of gill-score with concentration of *P. perurans* obtained from gill swabs (0.81 ± 0.16) and body weight (-0.88 ± 0.09 and thus favourable) indicate that CT-qPCR of *P. perurans* and body weight (i.e. growth rate) may be used as indirect trait measures of AGD gill-score. Both growth and CT-qPCR are more objective measures than gill-scores and can be obtained with less work-load (more fish per man-hour) than gill-scores, but the CT-qPCR values at a high cost (~NOK 250/sample).

As expected, the genetic correlation between body weight in June 2017 and gill-score in November 2016 at Mjånes was negative (-0.62), but of lower magnitude than between the same two traits in November 2016 (-0.88) (**Table 4; Figure 6**). This may indicate that the fish during the seven months from November 2016 to June 2017 have not manage to compensate for the loss in growth prior to the gill-scoring in November 2016. If the gill-scored fish in had been treated with freshwater this correlation (-0.62) may have been lower.

5.4 Challenge test vs. field test

The low 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 (**Table 3**), as also found in the RCN-235783/E40 project, show that gill-score from a bath challenge test is a bad predictor of gill-score in a field test. The present AGD challenge test can therefore not replace a field test in a selective breeding program. However, as field-test is dependent on more regular and predictable outbreaks than yet present in Norway, it would be beneficial to develop a challenge test with a test environment more similar to that the fish experience in a field test, e.g. a cohabitation test.

5.5 Growth in an AGD-affected and AGD-free environment

The high negative genetic correlation between gill-score and body weight at Mjånes in November 2016 may be interpreted as a negative effect of AGD on growth. In that case, a low genetic correlation between body weight in an AGD-affected and AGD-free grow-out environment is to be expected. However, the genetic correlation between body weight at Mjånes (AGD affected environment) and harvest body weight at LetSea (AGD-free environment) was high (0.86 ± 0.05), but slightly lower than the genetic correlation between harvest body weight at Mjånes in June 2017 and LetSea (0.97 ± 0.05) (**Table 4; Figure 6**). In addition, the genetic correlation between gill-score at Mjånes and body weight at LetSea was negative (-0.55 ± 0.14) (**Table 4; Figure 6**). These results indicate a true negative and thus favourable genetic correlation between AGD gill-score and growth in Atlantic salmon, and supported by the low residual correlation between the traits (Table 4). Therefore, selection for increased growth rate in an AGD-free environment should result in a favourable genetic correlated response in resistance to AGD. An additional correlated genetic gain in resistance to AGD can be obtained by selection for increased growth in an AGD-affected environment. However, the negative

genetic correlations of gill-score at Mjånes with growth at both Mjånes and LetSea need to be verified in a similar experiment.

5.6 Genome-wide QTL-search

Manhattan plot of GWAS analysis with distribution of –log10 P-values across different chromosomes is given in **Figure 7**. For this population, we did not find any QTL crossing genome or chromosome-wide significant threshold level ($P \leq 9.28 \times 10^{-7}$ and $P \leq 2.78 \times 10^{-5}$ respectively).The result suggests that, AGD resistance is controlled by many infinitesimal gene effect, thus genomic selection should be the preferred approach to improve resistance to AGD.

5.7 Cross validation of conventional and genomic breeding values

Accuracy of breeding values increased by 28.9% when using genomic information as compared with pedigree information (**Table 5**), which is higher than that found in the NRC-project (9-15%). Use of genomic breeding values makes it possible to select within families among the breeding candidates with no recorded gill-score. As genetic gain is proportional to both accuracy and intensity of selection, we therefore can conclude that use of genomic information will increase genetic gain for resistance to AGD substantially as compared with classical selection.

5.8 Use of CT-qPCR values for *P. perurans* as a non-destructive molecular diagnostic for AGD

From **Figure 8 and 9** we can see that when the number of fish sampled from the cage is low (5 and 10, red and gold coloured points, respectively), the mean CT-qPCR values for the replicates ranges from 15.5 to 25.0 and the range in the corresponding mean gill-scores about two gill-score units. For r_p=-0.6 the range in mean gill-scores is only marginally less than for r_p=-0.3. Thus, for 5 and 10 fish sampled it is not possible to draw any conclusion from the CT *P. perurans* values as to the average gill-score of the fish in the cage. For 30 fish sampled, the range in the mean CT values are much less and the range in the corresponding mean gill-score values within one gill-score unit; marginally less for r_p=-0.6 than for r_p=-0.3. Therefore, when using CT-qPCR values from the fish sampled, a minimum of 30 fish are needed to obtain a reasonable accurate and precise estimate of the true average AGD score of the fish in the cage.

5.9 Main results and recommendations

- The project documented genetic variation in resistance to AGD, both in the bath challenge test and the field test.

- The genetic correlation between gill-score in the bath challenge test and the field was close to zero. A bath challenge test for resistance to AGD can therefore not replace a field test in a selective breeding program.

- As 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, e.g. a cohabitation test.

- The high genetic correlation of gill-score with the amount of *P. perurans* on the gills measured by CT-qPCR (r_g=0.81 ± 0.16) and body weight (r_g=-0.88 ± 0.09) indicate that CT-qPCR for *P. perurans* on the gills and growth may be used as indirect trait measures of resistance to AGD.

- The high genetic correlation between the body weights in the AGD-affected and the AGD-free test environment (r_g=0.86 ± 0.05) indicate a true favourable genetic correlation between resistance to AGD and growth in Atlantic salmon. Consequently, selection for increased growth rate will result in a favourable genetic correlated response in resistance to AGD. However, these genetic correlation need to be verified in a similar experiment.

- The use of genomic information will lead to a 29% increased genetic gain per generation compared to using pedigree information only, and therefore helping to reduce both the number of treatment and the severity of AGD infections.

- The amount of *P. perurans* on the gills can be measured by CT-qPCR. At least 30 fish need to be sampled from a population to obtain a reasonable accurate and precise estimate of the severity of an AGD infection.

6 Deliverables

6.1 Oral presentations

- Aslam, L. 2016. Genetics of amoebic gill disease (AGD) resistance in Atlantic salmon. Havbruk 2016, Scandic Havet Bodø, April 18-20.
- Gjerde, B. 2016. Genetic parameters for resistance to amoebic gill disease in Atlantic salmon. FHF dialog meeting on AGD, Værnes, 23. June.
- Aslam, L. 2017. GWAS for resistance to AGD based on gill-score data from the field test at MH, Ireland. Steering group/Project group meeting at Park Inn, Gardermoen 19. January.
- Moghadam, H. 2017. Transcriptomics of AGD infection – initial assessments. Steering group/Project group meeting at Park Inn, Gardermoen 19. January.
- Boison, S. A. 2017. Genomic prediction of resistance to AGD based on gill-score data from the field test at MH, Ireland. Steering group/Project group meeting at Park Inn, Gardermoen 19. January.
- Gjerde, B. 2017. Quantitative genetics of resistance to AGD in Atlantic salmon. Frisk Fisk 2017, Bergen 1. and 2. February.
- Gjerde, B. 2017. Quantitative genetics of resistance to AGD in Atlantic salmon. International Gill Health Initiative, University of Bergen, 27. and 28. April.
- Lillehammer, M. 2017. Quantitative genetics of resistance to AGD in Atlantic salmon. Seminar at Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, 16. June.

6.2 Publications

- Gjerde, B., Boison, S. A., Lillehammer, M., Aslam, L., Løvoll, M. Quantitative genetics and genomics of resistance to AGD from a challenge test and a field test. In manuscript. To be submitted to Aquaculture in November 2017.
- Moghadam, H., Boison, S., Aslam, L., Lillehammer, M. Gjerde, B. Transcriptomic and genomics of resistance to AGD in Atlantic salmon. In manuscript. To be submitted to Frontier Genetics in November 2017.
- Rey, S., Kraugerud M., Lie, K.I., Hellberg, H.H., Boison, S. A., Bakke, H, Gjerde, B. Evaluation of a non-lethal method of diagnostic sampling gill tissue for qPCR in Atlantic salmon. A first draft of a paper in first half of 2018.
- Gjerde, B. Genetisk variasjon i motstandskraft mot AGD hos laks. To be submitted to Norsk fiskeoppdrett in Nov-Dec. 2017.
- Lillehammer, M. and Gjerde, B. A mixture model approach to estimate genetic variation in healthy growth of Atlantic salmon exposed to AGD. A spin off publication that will be written in 2018.

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8 Tables and Figures

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 (=2x4) 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

Table 2 Mean (standard deviation) AGD score and CT values and the phenotypic correlations used in the simulation. The results are shown in Figure 6 and 7

Param. set	Trait	Mean	SD	r_p
1	Gill-score	2.0	0.80	
2		3.0	0.80	
1	CT-qPCR	20.0	3.70	
2		25.0	3.70	
1	Gill-score vs. qPCR-CT			-0.3
2				-0.6

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 gill-score in the field test

Type of test	Trait	Challenge test		Field test
		1st score	2nd 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

Table 4 Estimates of heritabilities (on diagonal) and genetic (below diagonal) and residual/phenotypic correlations (above diagonal)

Time recorded	Trait	November 2016			June 2017	AGD free env.
		Gill-score	1/CT-qPCR	Body weight	Body weight	-
Nov. 2016	Gill-score	**0.19 ± 0.05**	0.22/0.29	0.15/-0.16	0.04/-0.13	-
	1/CT-qPCR	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**	**-**
AGD-free env.	Body weight	-0.55 ± 0.14	-0.33 ± 0.19	0.86 ± 0.05	0.97 ± 0.05	**0.57 ± 0.05**

Table 5 Mean and median accuracy of selection using cross-validation with only genotyped animals

			Accuracy = r_{corr}	
Estimation method	**Training**	**Test**	**Mean (SD)**	**Median**
PBLUP	790	213	0.52 (0.10)	0.52
GBLUP	790	213	0.67 (0.10)	0.67

PBLUP – pedigree based estimation of breeding values

GBLUP – genomic based estimation of breeding values

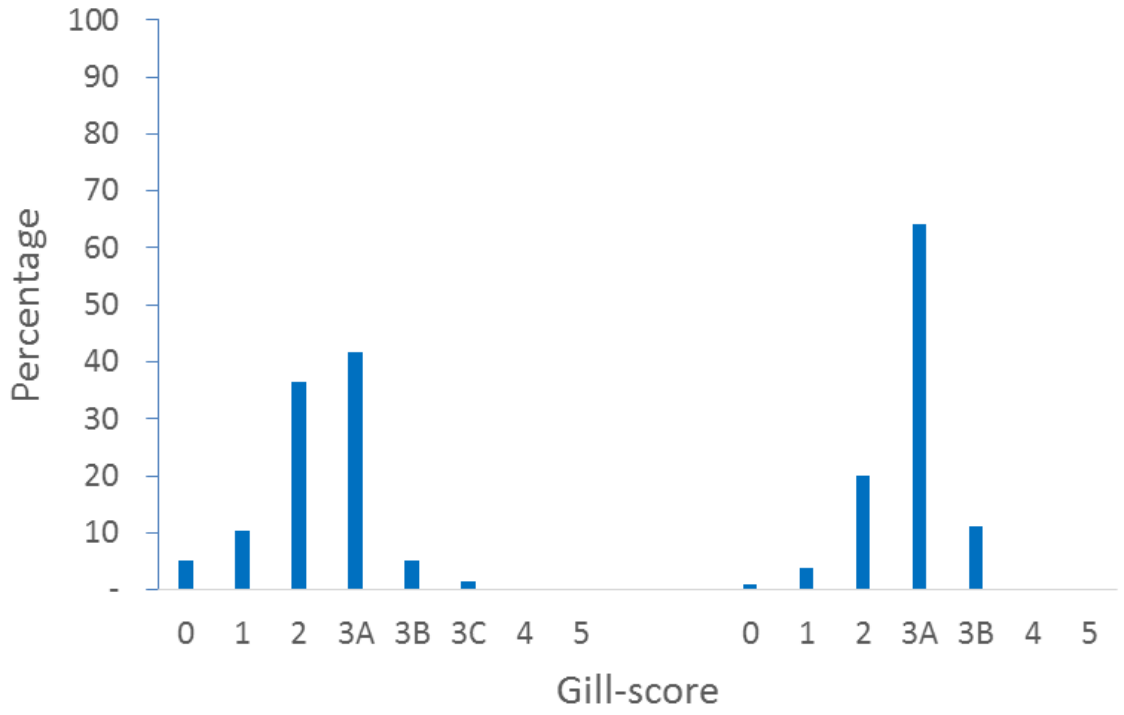

Figure 1 *Distributions of gill-score at the first and second gill-score at VESO Vikan*

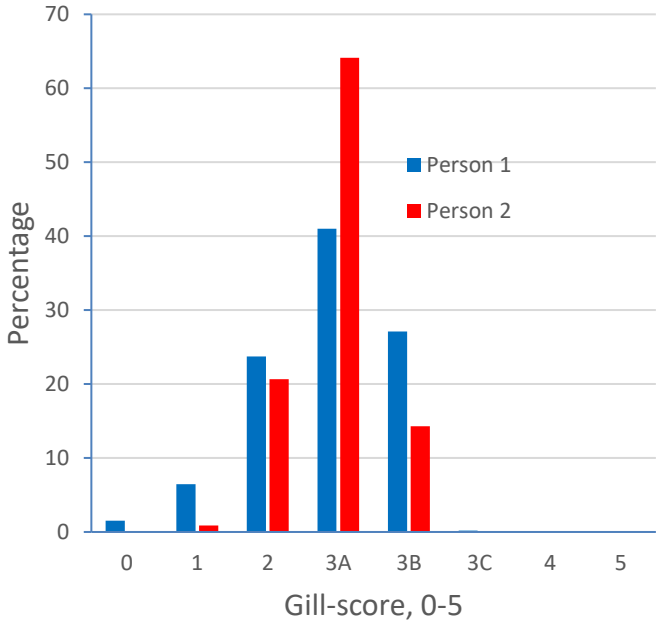

Figure 2 *Distributions of gill-score in the field test at Bolaks, Mjånes*

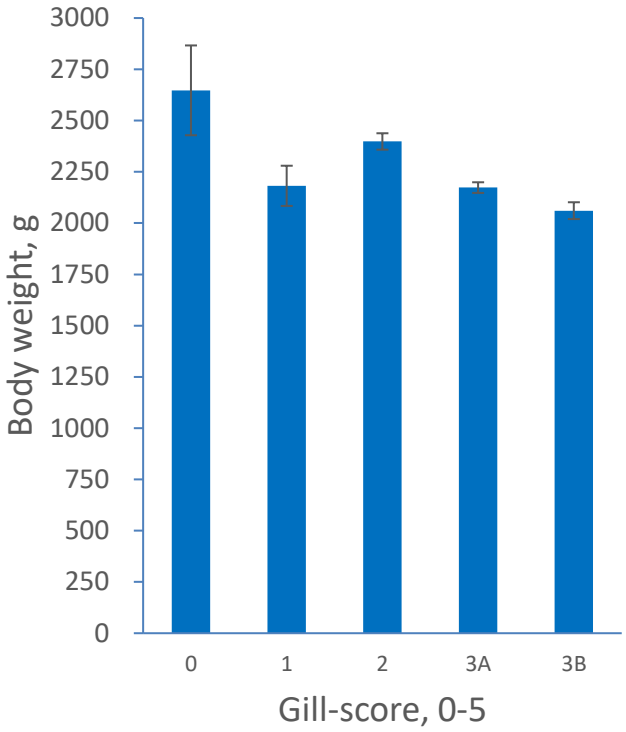

Figure 3 *Mena body weight of fish with gill-score 1, 2, 3A and 3B at Bolaks, Mjånes*

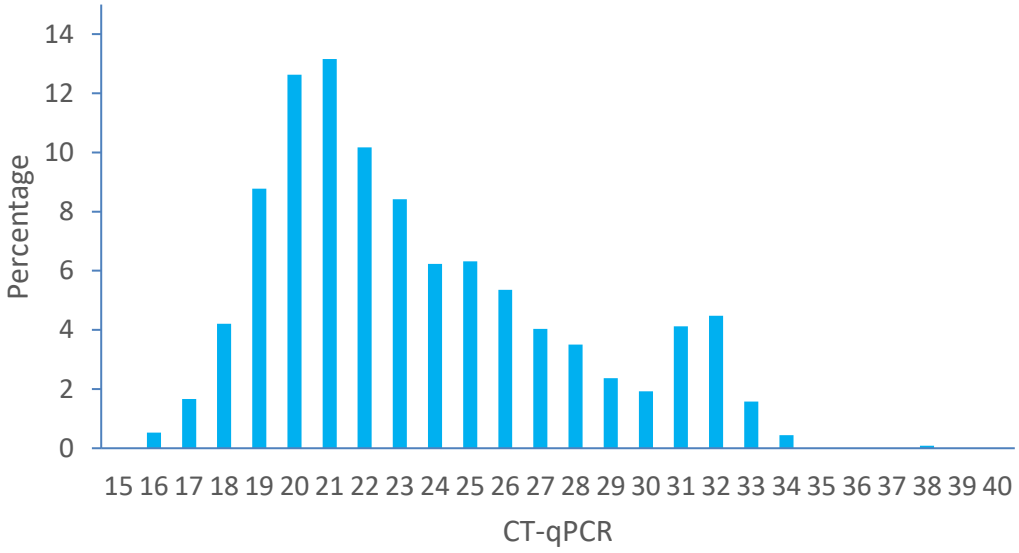

Figure 4 *Distributions of CT-qPCR values in the field test at Bolaks, Mjånes*

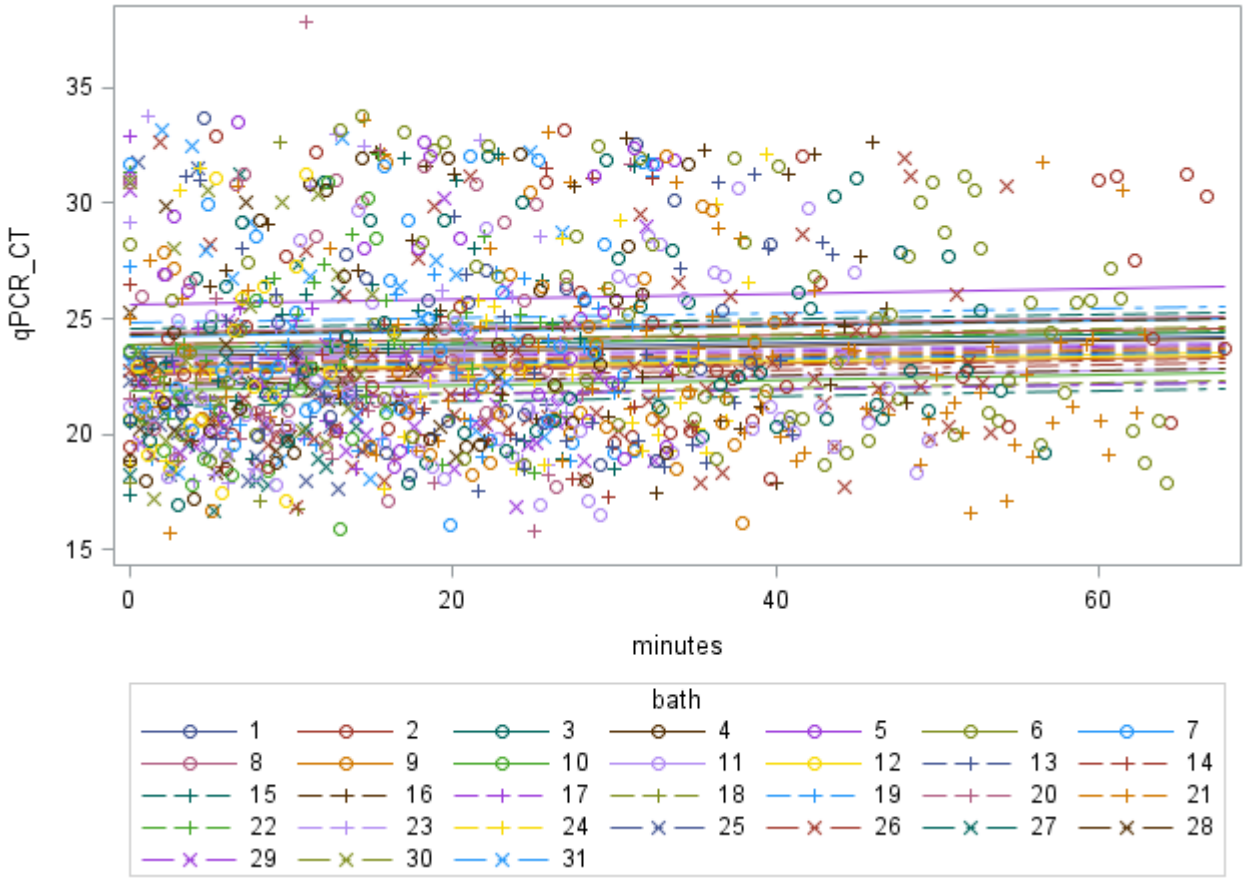

Figure 5 *Regression of CT-qPCR values on time in the anaesthetizing bath*

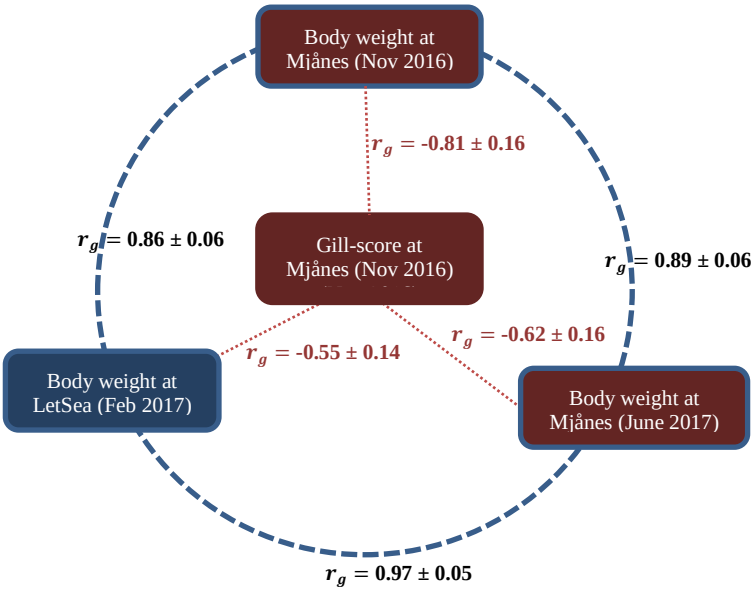

Figure 6 *Genetic correlations of AGD score with the three body weight measures*

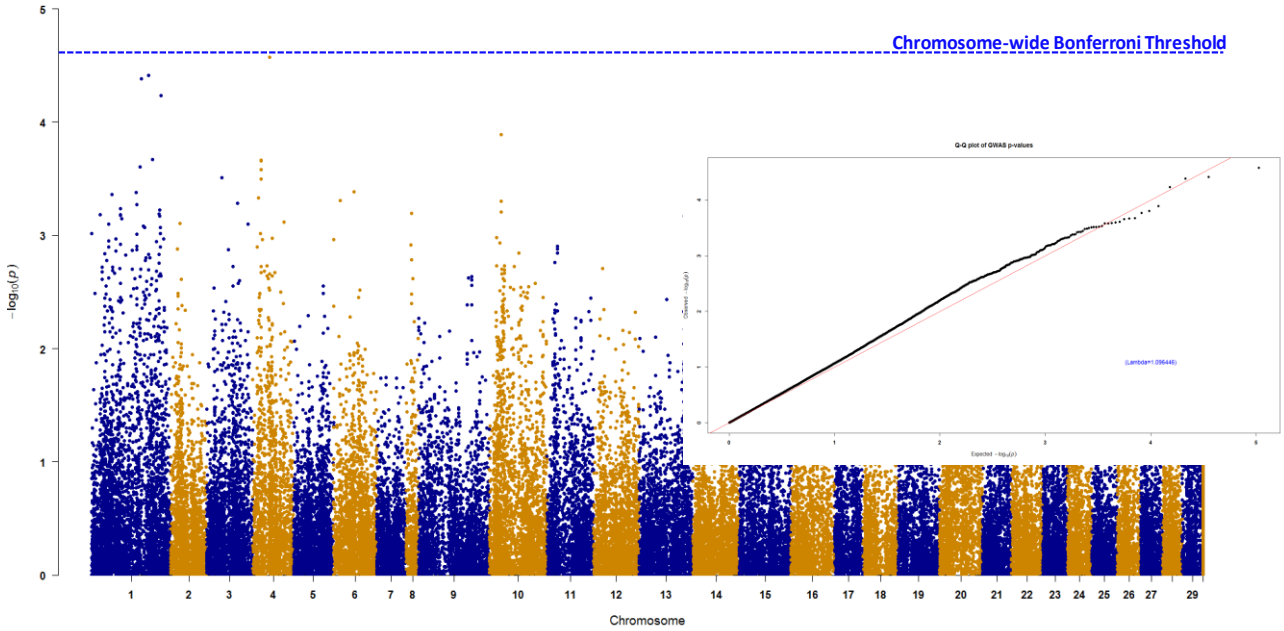

Figure 7 *Manhattan plot for distribution of P-values across different chromosomes. Chromosome 30 represent markers belonging to unknown chromosome(s), quantile-quantile plot with distribution of observed vs. expected p-values is also given with obtained inflation factor (λ = 1.09)*

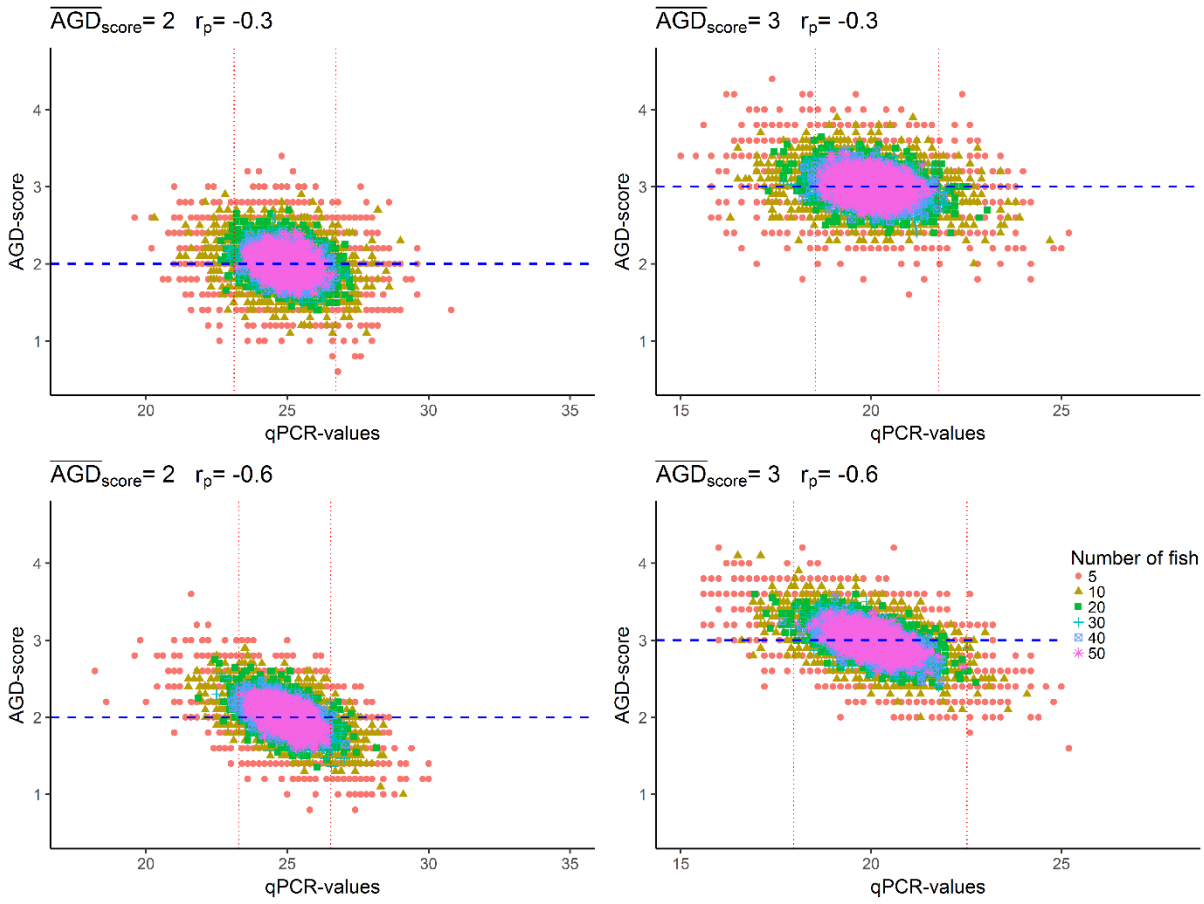

Figure 8 *Plot of mean CT-qPCR values against mean AGD gill-score values for six different number of fish sampled 1000 times from a cage with 6000 fish to infer the severity of AGD infection, for two different true mean gill-scores and two different phenotypic correlation between gill-score and CT-qPCR. The input parameters are given in Table 2*

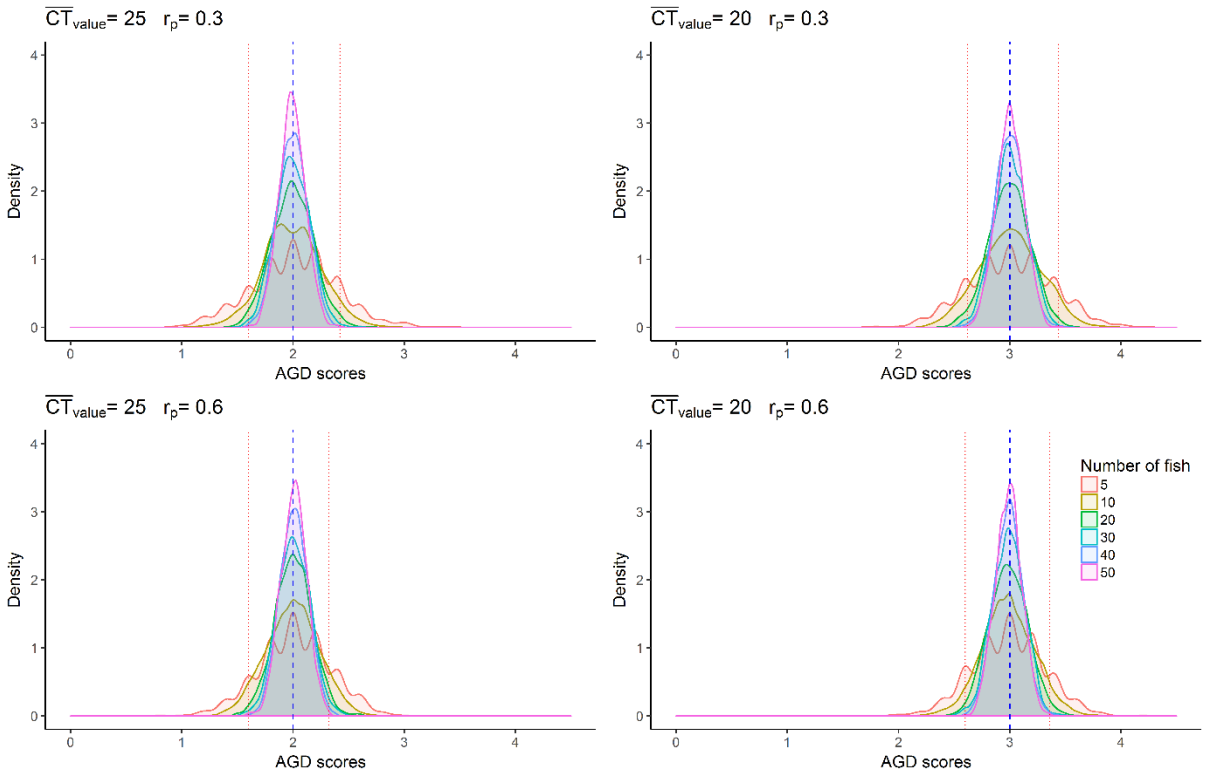

Figure 9 *Distribution of mean gill-scores for six different number of fish sampled 1000 times from a cage to infer the severity of the AGD infection in the cage. The input parameters are given in Table 2*

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