Simulation study on the long term utilization of QTL for a disease trait in fish breeding programs

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Abstract

The aim of this study was to investigate, through stochastic simulation, the potential of using genome information and more particularly, information on the identified IPN resistance QTL in salmon breeding program in Norway. The breeding goal of the simulation was composed of two traits. The first trait was measured on the selection candidates as a growth rate whereas the second was measured only on full-sibs of the breeding candidates. The IPN resistance QTL had a very strong effect and was responsible for 83% of the genetic variation. Thus, the potential of using GAS was also tested on a QTL with a small effect only responsible for 20% of the genetic variation. Different values for genetic correlation between these two traits have been tested, 0 and -0.36. The genetic model assumed for the second trait was composed of a QTL segregating together with polygenes. Thus, two schemes were implemented, Gene-Assisted Selection (GAS) - which takes into account QTL information - and Standard Phenotypic Selection (PHE). The genetic gain from GAS and PHE obtained by combining BLUP EBVs and optimum contribution were compared at the same rate of inbreeding. The results showed that GAS led to a faster fixation of the favourable allele and achieved more gain for the second trait in short-term than the PHE. This increased gain is due to the utilization of the optimum contribution procedure. However, after the fixation time, the genetic gain was not maintained and resulted in a long-term loss compare to PHE. In previous publications, it has been showed that using optimization of the weight given to the QTL in Optimized Gene-Assisted selection (GAO) had an effect on avoiding long-term loss. Therefore, it could be interesting to implement GAO in salmon breeding program for the IPN resistance QTL.

Key Words: Gene-Assisted Selection, Genetic Gain, IPN Resistance QTL, Salmon Breeding Program.

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Introduction

In 2009, aquaculture production represented 38% of the global production of fish, crustaceans and molluscs. Indeed, during the last ten years, aquaculture has strongly been developed worldwide and production has almost doubled from 34 million tons to 56 million tons (www.fao.org). This trend will continue in the future since fisheries have reached a maximum. Norway, Spain, Denmark, Italy and France are the main producers in Europe. Atlantic salmon is the main produced species in Norway, representing 88% of the total production. In 2007, Norwegian aquaculture production represented a value of US$ 2.9 billion.

Presently, aquaculture faces issues such as disease outbreak. Despite strict sanitary rules and costly biosecurity procedures (McLoughlin & Weigall, 2002), the spread of disease is responsible for major losses in fish farming and thus represents a high economic cost. In Canada, the United States, and Europe, Infectious Pancreatic Necreosis (IPN) is considered one of major diseases within salmon farming. Brun (2003) observes that an IPN outbreak is associated with high fish losses, of 10-20% on average, reaching up to 100%. Fish which survive the infection become life-long virus carriers and participate both in horizontal transmission (between fish of the same cohort) and in vertical transmission (from parent to offspring via sperm ovarian fluids or eggs) (Bootland *et al.*, 1991). This disease affects both fry in freshwater and post-smolt, just after transfer in seawater. There are currently no treatments for IPN but vaccines have been available since 1995. However, these vaccines with IPN virus antigens do not efficiently prevent IPN outbreaks in post-smolts (Brun, 2003; Ramstad & Midtlyng 2008). Despite considerable effort, the disease has proved to be difficult to control either by vaccination or biosecurity controls. In fish as well as in vertebrates, the first antiviral defence is processed by the innate immune system, using interferons. These interferons activate the expression of many antiviral genes, which are involved in building individual resistance to infection (Verrier *et al.*, 2011). Midtlyng *et al.*, in 2002, showed that family resistances in mortality among Atlantic salmon fry can vary as much as 80% between families with the greatest and least incidence, suggesting the presence of a genetic component in IPN susceptibility. Moreover, Wetten *et al.*, (2007) and Guy et *al.*, (2006) have shown a moderate estimated heritability of the trait: 0.31 and 0.43 respectively. These results indicate that resistance to virus is at least partially based on genetic. Thus, breeding resistant

individuals as parents for the next generation to increase resistance to IPN is a good opportunity to provide effective and sustainable control of the disease.

In 1971, Norway was the first country to implement breeding programs to improve performance and adaptation to Atlantic salmon farming. Since this period, breeding programs have evolved and now include traits such as growth performance, sexual maturation age, disease resistance and quality traits such as flesh colour and fat content (Gjøen, 1997). For growth rate and sexual maturation age, selection is directly applied on breeding candidates. However, for disease-resistant family selection, selection is based on challenge-tested fish for which survival rates of sib-groups are recorded. Those fish which cannot be used as breeding candidates because of vertical transmission risks. Thus, within-family selection cannot be applied and only half a genetic variance for IPN resistance is exploited. Moreover, this method to measure disease-resistance is not only difficult but expensive and disconcerting for animal welfare.

On the other hand, progress in genomics has started to be included in animal breeding programs in recent years. Indeed, for many species, genetic markers have been located on the genome and organized in genetic maps. Genetic markers are DNA sequences that exist in two or more alleles and chose inheritance can be followed. Thus, they are used to detect loci that affect single-gene traits or quantitative traits (QTL). There are three different markers, as denoted by Dekkers (2004); 1) LE markers - loci that are in population-wide linkage equilibrium with the functional mutation; 2) LD markers - loci that are in population linkage disequilibrium with the functional mutation. These markers are also located close (1-5cM) to a QTL; 3) Direct markers which code directly for the functional mutation. They are difficult to find because it is hard to prove that the marker is responsible for a functional mutation. Direct and LD markers possess a great interest in selection plans. They allow selection on genotypes across the population because marked genotypes are associated to particular phenotypes. This information gives us the possibility to increase response to selection using marker-assisted selection (MAS) or gene-assisted selection (GAS). This is particularly interesting for traits that are difficult to improve due to a low heritability and complex phenotypic measurements. This is typically the case for disease resistance (Dekkers 2004).

Houston *et al.* (2008) and Moen *et al.* (2009) present a genetic variance in IPN resistance in Atlantic salmon from Scottish and Norwegian origin respectively. In the

Norwegian study by Moen *et al.* (2009), a genome scan with 136 microsatellite markers was done on post-smolt fish that died or survived after IPN-challenge-test. The study pointed out two significant QTL for IPN resistance from 10 full-sib families of post-smolt. A minor QTL was positioned on chromosome 4 and was responsible of 0.9% of the genetic variation. A major QTL was located on chromosome 21 as it has been found by Houston *et al.* (2008) in the Scottish population. After linkage-based fine-mapping of this major QTL, the position of the QTL has been estimated to range between 23-26 cM on the chromosome. The QTL was responsible for 29% of the phenotypic variation and 83% of the genetic variation. Thus, this QTL explains most of the genetic variation for IPN resistance. Moreover, the major effect QTL on chromosome 21 had a strong effect also for fry resistance. Fry and post-smolt are two life stages taking place in different environments, in fresh and salt water, respectively. During the transition between these two environments, the metabolism undergoes many modifications. Thus, these results imply that the gene underlying IPN resistance is part of innate immune system and that the genetic component of this trait is mainly under control of one major QTL and probably one or two linked genes (Moen *et al.*, 2009). Linkage analysis at the population level showed that there was significant linkage disequilibrium between markers in the QTL region with probable strong linkage disequilibrium between markers and the polymorphism underlying the QTL. A haplotype composed of four markers has proved to be the best predictor of alleles at the underlying polymorphism (Moen *et al.*, 2009). Finding markers linked to QTL or gene(s) controlling IPN resistance in Atlantic salmon is a major issue. Indeed, genotypes at the linked marker(s) can be used to select the best genotypes among breeding candidates. In 2007, Aqua Gen started to use the major QTL found by Moen *et al*. (2009) in the breeding program. Within-family marker-assisted selection (MAS) was implemented to select the most IPN-resistant fish as parents for the next generation. However, MAS can be only carried out within family and within offspring of parents of known QTL genotype. In addition, the four-marker haplotype used is distributed on 10 cM and its stability over generation is not proven (Moen *et al*., 2009). More information about the position of the QTL is needed in order to improve MAS for IPN resistance in salmon. The development of genomic tools will allow map generation with higher density of markers of a type's single nucleotide polymorphisms (SNP) which are a variation in a single nucleotide on the genome and are very frequent. A 16 000 SNPs chip has been developed at the Center of Integrative Genetics (CIGENE).

Many studies have attempted to predict the potential extra rates of genetic gain from marker-assisted selection in a mixed inheritance model where a QTL is segregating with polygenes. Simulations studies from Gibson (1994), Larzul *et al.* (1997) and Pong-Wong & Woolliams (1998) used standard truncation selection where selected parents contributed equally to the next generation. Moreover, equal emphasis was set up on estimated breeding value (EBV) for the QTL and polygenes. Pong-Wong & Woolliams (1998) found that using MAS on an additive QTL (explaining 5% of the phenotypic and 20% of the genetic variance) can improve the rate of genetic gain in a short term but this gain was not maintained in the long-term compared to phenotypic selection. Villanueva et al. (1999) used BLUP and optimum contribution (OC) to estimate the benefits of using QTL information in the estimation of breeding value for increasing short and long-term selection gain. Optimum contribution constrains inbreeding by reducing the increase of the average relationship while optimizing genetic gain (Meuwissen, 1997). Moreover, Dekkers & van Arendonk (1998) have optimized weight on the QTL to maximize response over multiple generations. In 2004, Villanueva *et al,* combined both these methods, from Villanueva *et al*. (1999) and Dekkers & van Arendonk (1998), to increase the rate of genetic gain by optimizing the contributions of selected candidates and by optimizing the emphasis given to the QTL. In this study, the trait selected was controlled by an infinite number of additive loci with an infinitesimal effect, plus a single biallelic QTL with alleles B and b. They compared - using stochastic simulation - three different methods to estimate breeding value:

1) Standard phenotypic selection: EBV was calculated using the phenotypic values of the candidates without taking into account the QTL genotype.

2) Standard gene-assisted selection (GAS): EBV for polygene was calculated using information on the QTL to calculate the breeding value due to the QTL for each individual.

3) Optimized gene-assisted selection (GAO) (Villanueava *et al.,* 2004): EBV for the polygene and BV for the QTL were calculated as in GAS. However, BV due to the QTL was optimized and λ denoted the optimal weight given to BV according to Dekkers & van Arendonk (1998) and Dekkers & Chakratory (2001).

For each of the three selection methods, two selection procedures were compared. The first one was based on linear index (standard truncation selection) and the second one on a quadratic index (optimum contribution) that optimizes the numbers of parents and their contribution to the next generation in order to maximize genetic gain while constraining the rate of inbreeding (Villanueva *et al.,* 1999). For dominant and additive QTL, results of this

study showed that using optimum contribution improved the rate of genetic gain and the sum of genetic gain over generations in comparison to standard truncation selection. But optimum contribution in GAS did not prevented loss of genetic gain in long-term. However, when the weight of the QTL was optimized as in GAO, the loss in long-term was less important than when GAS was applied. It has also been shown that using optimum contribution led to a faster increase in the frequency of the favourable allele and its accelerated fixation. After 15 generations, the highest value for the sum of genetic gain over generations was obtained with GAO for a quadratic index selection strategy. For additive QTL, it was 2.5 and 2.8% higher than conventional phenotypic selection and GAS, respectively. This study showed that a significant extra genetic gain can be obtained and maintained over multiple generations using optimum contribution and optimization of the emphasis given to the QTL. These results have been obtained without increasing of inbreeding because of the optimization of the contribution of the selective candidates.

Thus it is interesting to study - through stochastic simulation – the possible impacts of implementing GAS selection in Norwegian salmon breeding programs using genotypic information for IPN-resistance. A simulation program already exists and allows calculating and predicting genetic gain over multiple generations for two traits (Skaarud *et al.*, 2012). One can be directly measured on breeding candidates as growth rate, and the second trait can be measured on dead fish as quality trait or disease resistance. For this second trait, records are available only on sib-group. Thus, only family breeding values will be attributed to live candidates and all candidates from a same full-sib family will obtain the same breeding value. This means all individuals from the same family will have a similar selection index. If the truncation selection is not restricted, this leads to the selection of individuals of the same family which will increase the rates of inbreeding. In aquaculture, the number of individuals selected from each family is limited to avoid this problem. But this static criterion is not the best way to restrict inbreeding. In recent years, dynamic selection tools have been developed to optimize the genetic contribution from breeding candidates while constraining inbreeding. Optimum contribution (OC) is now recommended in most livestock breeding programs. In aquaculture, simulation studies looked at the benefits of using OC (Sonesson, 2005). However, some difficulties have been reported when applying optimum contribution to fish breeding programs because the number of families and the size of full-sib group are preset due to facilities limitations and tagging costs. Thus, Skaarud *et al.* (2010) have tested four different ways of applying optimum contribution. Among them, OC individual (OCI) seems

to be the most efficient application of OC in fish breeding program. It yielded 6% more genetic gain than others OC methods when h^2=0.25 and 200 families which is a typical scheme in fish breeding program. Therefore, OCI has been used in the simulation program to optimize the contribution of selected individuals to the next generation.

The aim of our study is to evaluate, through stochastic simulation, the potential extra genetic gain obtained from gene-assisted selection (GAS) for IPN resistance in Norwegian salmon breeding programs by using BLUP estimated breeding values and optimum contribution of selected individuals. The selection program will simulate a first quantitative trait with underlying polygenic variation as growth rate and a second trait where a single quantitative trait is segregating with polygenes as IPN resistance. EBVs for the first trait will be estimated using BLUP procedure and two different methods will be used to calculate EBVs for IPN resistance:

1) Conventional BLUP selection, where the total EBV was calculated without correction for QTL genotype.

2) Standard gene-assisted selection (GAS), where information on the QTL is used to estimate the breeding value of individuals.

1. Material and methods

Our simulation study is based on the program developed and used by Skaarud *et al.* (2012) for "Optimizing resources and management of variation in fish breeding schemes with multiple traits". The aim of this paper was to investigate, using stochastic simulation, the effect on the genetic gain of increasing the number of families in a breeding program with selection on two traits. The first trait was measured on all breeding candidates while the second only on full-sibs. In this study, two methods of implementing optimum contribution have been tested: one based on optimizing the contribution from families and the other one from individuals.

1.1 Generating simulated populations

Stochastic simulation is used to generate populations with discrete generations. Each generation is composed of n_{fam} families obtained by random mating. Each family had n_o of candidates for selection (with equal number of males and females) and n_{inf} informative full-sib. These informative animals are the fish on which the second trait is measured. Indeed, to measure and to provide information for this trait, the informative fish need to be killed and thus cannot be selected themselves. The selection was then applied for two traits, growth rate (trait 1) and IPN resistance (trait 2). A polygenic infinitesimal model was assumed *ie* the trait 1 was assumed to be determined by an infinite number of unlinked loci, each with an infinitesimal effect (polygenes). It was measured on breeding candidates and on both sexes. The trait 2 was also composed of polygenes plus a biallelic (B and b) QTL. Therefore, the genetic value of an individual is composed of the genotypic value due to the QTL and the polygenic effect. This polygenic effect was measured on full sibs. Furthermore, according to Skaarud *et al.* (2012), genetic value for trait 1 and the polygenic effect of trait 2, G_i, of unrelated base population animals (generation 0) were sampled from the distribution $\begin{bmatrix} G_1 \\ G_2 \end{bmatrix} = L \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix}$

where $L = \begin{bmatrix} \sigma_{A1} & 0 \\ \dfrac{\rho}{\sigma_{A1}} & \sqrt{\sigma_{A2}^2 - \dfrac{\rho^2}{\sigma_{A1}^2}} \end{bmatrix}$ is a lower triangle matrix from the Cholesky decomposition of the variance/covariance matrix for the two traits, and εi are independent samples from a

standard normal distribution. σ_{A1} and σ_{A2} are respectively genetic standard variation for trait 1 and trait 2 and ρ is the genetic correlation between the two traits. Moreover, a genetic value due to the QTL was adding to the genetic value of trait 2. This value corresponded to *a* for individuals with genotype BB, *d* for individuals with genotype Bb or bB and *–a* for individuals with genotype bb. In the base population, the additive genetic variance explained by the QTL was $\sigma^2_q = 2p(1-p)\alpha^2$ where *p* is the frequency of the favourable allele and α is the average effect of gene substitution equal to a+(1-2p)d. Alleles at the QTL were chosen at random according to the probabilities given by the frequency of the favourable allele. 1, 2, 3 and 4 were the values attributing to genotype bb, Bb, bB and BB respectively where the first letter indicated the allele received from the sire. It was assumed that the QTL and polygenes were in linkage phase equilibrium.

When we combined the parental genotypes to construct genotypes for the offspring, we first corrected the genotypes of the parents for the effect of their QTL alleles. Only the polygenic parts of the genotypes for the parents were added together including a random factor accounting for mendelian sampling as follows:

$$\begin{bmatrix} G_1 \\ G_2 \end{bmatrix} = \frac{1}{2}\begin{bmatrix} G_{1s} \\ G_{2s} \end{bmatrix} + \frac{1}{2}\begin{bmatrix} G_{1d} \\ G_{2d} \end{bmatrix} + \frac{1}{2}\sqrt{1-F_s}L\begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix} + \frac{1}{2}\sqrt{1-F_d}L\begin{bmatrix} \varepsilon_3 \\ \varepsilon_4 \end{bmatrix}$$

G_s, G_d and F_s, F_d are genotypes and inbreeding coefficients of the sire and dam, respectively. The environmental covariance between the traits was assumed to be equal to zero because they are measured on different individuals. Phenotypic values (Pi) were obtained by adding to the total genetic value, a normally distributed environmental component with mean zero and variance σ_e^2.

$$\begin{bmatrix} P_1 \\ P_2 \end{bmatrix} = \begin{bmatrix} G_1 \\ G_2 \end{bmatrix} + \begin{bmatrix} E_1 \\ E_2 \end{bmatrix}, \text{ where } E_{i\sim}N(0,1-\sigma_{Ai}^2).$$

Finally, based on the QTL of the offspring, the value of the QTL genotype, *a*, *d* or *-a*, is added to the polygenic genotype to give the total genotype.

1.2 Estimation of Breeding Values

BLUP procedure was used to estimate breeding value (EBVs) both for trait 1 and the polygenic effect of trait 2. The candidate EBVs varied among individuals for the first trait, because it was based on observations on both the breeding candidates and the informative ones. On the contrary, for the polygenic breeding value of trait 2, only family breeding values were available on the live candidates since all candidates from a given full-sib family received the same breeding values from informants. An index has been constructed where equal weight was put on each trait. For the PHE the index is calculated as I=1/2(EBV1+EBV2), and for GAS I=1/2(EBV1) + 1/2(EBV2+BV) where EBV1 and EBV2 are estimated breeding value for trait 1 and 2 respectively. This index was used in Optimum Contribution (OC) procedure developed by Meuwissen (1997) to maximize the genetic gain while constraining increase in inbreeding, ΔF, to a pre-defined level. Moreover, candidates were genotyped for the QTL and two different schemes were compared to the QTL estimate breeding value: Conventional Phenotypic Selection with no information on the QTL (PHE) and standard Gene-Assisted Selection with utilization of QTL information (GAS) (Villanueva *et al.*, 2004).

Conventional Phenotypic Selection - PHE:

In this scheme, information on the QTL was ignored when BLUP selection was used to calculate EBV. The total initial genetic additive variance ($\sigma^2_q+\sigma^2_u$) and phenotypic values uncorrected for the QTL effect were used to obtain the total EBV.

Standard Gene-Assisted Selection - GAS:

In this scheme, information on the QTL was used. The total estimated breeding value is composed of the estimated polygenic breeding value (EBV) plus the breeding value linked to the QTL (BV). The EBV was obtained from the standard BLUP using the polygenic variance (σ^2_u) and the phenotypic values corrected for the QTL effect (Pi-*a*). The breeding value (BV) for the QTL was $2(1-p)\alpha$ for genotype BB, $(1-2p)\alpha$ for Bb and $-p\alpha$ for bb. p corresponds to the frequency of the favourable allele and was updated every generation. EBV and BV were equally weighted to calculate the total estimated breeding value for trait 2.

1.3 Selection and Mating

For the two different schemes, the selection strategy used was based on optimum contribution of individual (OCI). Optimum Contribution (OC) procedure developed by Meuwissen (1997) maximizes the genetic gain while constraining ΔF to a pre-defined level: 1% per generation. The genetic level at generation t+1 can be defined by $G_{t+1}=c_t'EBV_t$, where c_t is a vector of genetic contributions, and EBV_t is a vector of estimated breeding values for the selection candidates in generation t. In our study, optimum contribution individuals have been implemented according to Skaarud *et al.* (2012), who compared this method with OC Amer (OCA). They found that in fish breeding programs when the number of families increase, OCI always increased the genetic gain while OCA reaches a maximum around 200 families. In OCI, the estimation of contribution is based on the individual's breeding value of best males and females from each family. To accelerate the simulation, only n*sel* (number of selected individual) best males and n*sel* best females within each family received an estimated contribution coefficient, c_m and c_f. Therefore, these selection candidates can contribute as sires or dams to a number of families proportional to their own individual contribution coefficient. Individuals with high contribution coefficient will be used to create several families while individuals with low contribution coefficient will not contribute to any families. Note that with OCI, each sire and dam may now be used in more than one mating depending on their individual quota. The selected sires and dams were mated at random.

1.4 Parameters studied

One of the first parameters to consider when setting up a breeding program is the number of families used as this factor will decide the size and costs of facilities needed. In fish breeding, family selection is used. The larger is the number of families, the higher is the pressure of selection. This results in a larger genetic gain (Woolliams *et al.,* 1999). Skaarud *et al*. (2012) demonstrated that an adequate size for fish breeding programs is between 200 and 300 families when using optimum contribution individuals. Thus, the number of families was set up at 250 with the number of offspring n_o = 50 in each family, and number of informative fish n_{inf} =15. The first trait of this study has a heritability set up at 0.3. The second trait was controlled by a polygene and a QTL where the initial allelic frequency of the favourable allele was 0.15. Moreover, when implementing marker-assisted selection, it is also crucial to know the part of the genetic variation explained by the QTL. Two different values for QTL

effect are then tested. The first objective is to simulate a breeding program based on two traits: growth rate and IPN resistance. Thus, the genetic variation explain by the QTL is set up at 83% for genetic variance and 29% for phenotypic variation (σ^2_q). These are values found by Moen *et al*. (2009) for the QTL for IPN resistance in Atlantic salmon of Norwegian origin. The environmental and polygenic variance was set up to $\sigma^2_e = 0.65$ and $\sigma^2_u = 0.06$ respectively. Thus, the heritability for this trait in these conditions is 0.35. The average effect of gene substitution α is then equal to 1.07 under the condition of an additive QTL $a = 1.07$ and $d = 0$. The effect of this QTL is very large compared to other effects QTL has found in past years. A second value for the QTL effect is then set up at 20% of the genetic variation and 5% for phenotypic variation (σ^2_q). The environmental and polygenic variance was set up to $\sigma^2_e = 0.75$ and $\sigma^2_u = 0.2$ respectively. Thus, the heritability for this trait in these conditions is 0.25. The average effect of gene substitution α is equal to 0.443 under the case of the additive QTL $a = 0.443$ (Pong-Wong & Woolliams, 1998). Moreover, a lack of information about genetic correlation between IPN resistance and other commercial traits have been reported in literature. However, Drangsholt *et al.*, (2011) found a negative correlation between the growth rate and the resistance to the viral disease furonculosis in vaccinated fish whereas no correlation was observed in unvaccinated fish. These results supposed that resistant fish used more energy in immune system than in growth. Thus, both a negative, $\rho = -0.36$, and a null $\rho = 0$ genetic correlation between the two traits were investigated for both values of QTL effect considered.

In summary, two breeding value estimation schemes, PHE and GAS, were run for two different QTL effects (83% and 20%) and two genetic correlations between traits (0 and -0.36) for a total of 12 different schemes tested (Table 1).

Table 1. Different schemes and conditions tested in this study and the abbreviations used. PHE is the standard phenotypic selection and GAS is the gene-assisted selection. L refers to the large QTL effect (83% of the genetic variation) and S refers to a small QTL effect (20% of the genetic variation). 0 and -0.36 refer to the genetic correlation between trait 1 and 2.

		Schemes	
QTL effect	*Genetic correlation between trait1 and 2*	**PHE**	**GAS**
83% (large)	**0**	PHE-L0	GAS-L0
	-0,36	PHE-L-0,36	GAS-L-0.36
20% (small)	**0**	PHE-S0	GAS-S0
	-0,36	PHE-S-0,36	GAS-S-0,36

Each round of simulation was done for 15 generations, i.e. 14 generations of selection repeated 50 times. The first generation was obtained by random mating of individuals of base population without selection. The average genetic gain and the change in frequency of the favourable allele were used to compare the two different schemes. It is also interesting to evaluate the changes in allele frequencies over time and quantify how fast the favourable allele can be led to fixation in gene-assisted selection compared to conventional BLUP selection in a salmon breeding program. Moreover, Pong-Wong & Woolliams (1998) showed that standard gene-assisted selection led to a long-term loss of genetic gain. Thus we compare in each scheme; 1) the number of generations needed to fix the favourable allele in the population and; 2) the genetic gain over generations.

2. Results

2.1 Rate of inbreeding

First, the optimum contribution procedure succeeded in restricting the rate of inbreeding to 1% per generation in both the PHE and the GAS scheme, for different values of the QTL effect and for different genetic correlations between traits 1 and 2 (Table 2).

Table 2 Average and standard deviation of the rate of inbreeding per generation for different schemes and parameters tested.

	ΔF	**s.d.**
PHE-L0	0,0098	0,0001
GAS-L0	0,01	0,0001
PHE-L-0,36	0,0099	0,0001
GAS-L-0,36	0,01	0,0001
PHE-S0	0,0099	0,0001
GAS-S0	0,0099	0,0001
PHE-S-0,36	0,01	0,0001
GAS-S-0,36	0,0101	0,0001

2.2 Evolution of the frequency of the favourable allele

Figures 1 and 2 show the changes in the frequency of the favourable allele for the schemes with a QTL effect of 83 and 20%, respectively. As might be expected, using genotype information in the GAS scheme led to a faster fixation of the favourable allele compare to the PHE. This result was observed for both a large and a small QTL effect and regardless of the genetic correlation. Indeed, after five generations, the allele is fixed in the GAS scheme while, in the PHE, a minimum of thirteen generations (obtained for PHE-L0) is needed to fix the allele. However, in the case PHE-L0, the frequency reached 0.98 after eight generations, whereas it took four generations more to fix the allele completely. Moreover, the fixation of the QTL was not reached at all within 14 generations of selection in the case of a small QTL effect with the PHE. It can be noted that in the GAS scheme, the development of the frequency is very similar for both genetic correlations tested 0 and -0.36, whereas there was a slight difference in the progress of the frequency with the PHE scheme when the genetic correlation was 0 compared to -0.36.

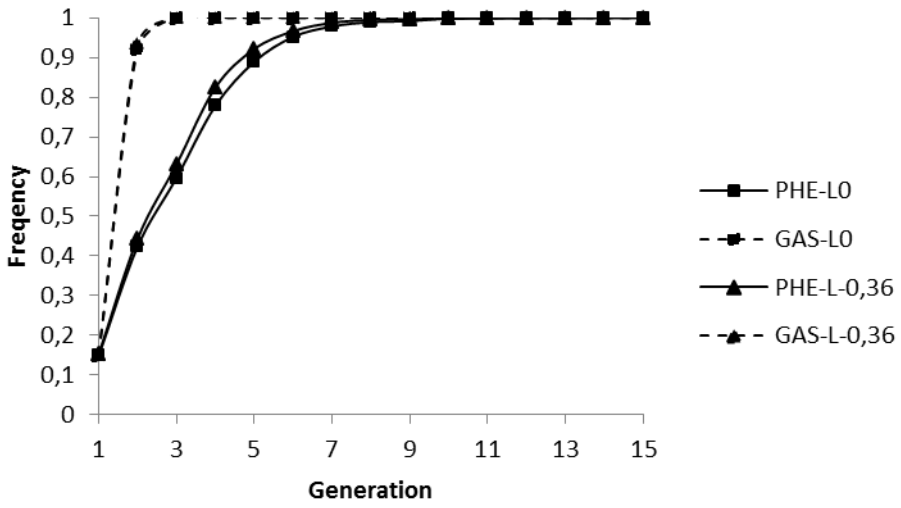

Figure 1 The development of the frequency of the favourable allele during the 14 generations of selection for the PHE and the GAS selection on a large effect QTL (83%) for ρ = 0 and ρ = -0.36.

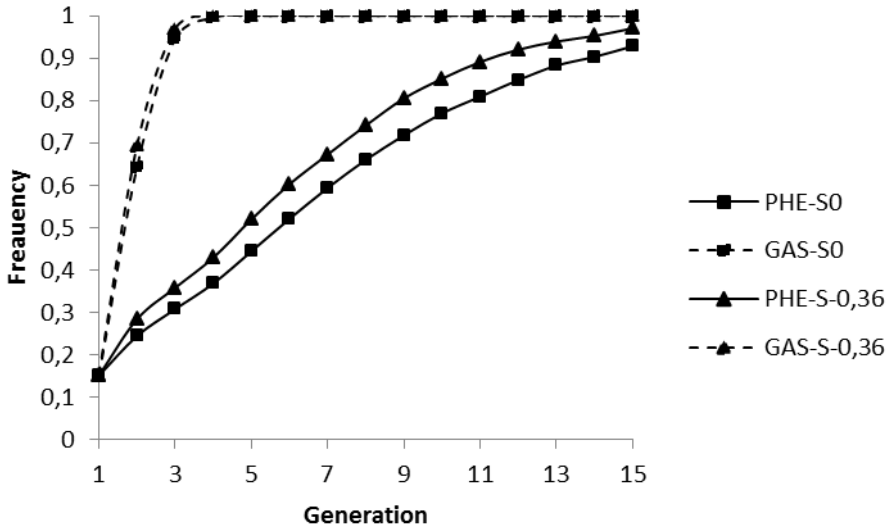

Figure 2 The development of the frequency of the favourable allele during the 14 generations of selection for PHE and GAS selection on a small effect QTL (20%) for ρ = 0 and ρ = -0.36.

2.3 Genetic gain for trait 1

Cumulative genetic gain during the 14 generations of selection for the different schemes and sets of parameters are presented in Figure 3. This figure shows that genetic gain for trait 1 is always higher when the GAS scheme is used, compared to the PHE scheme. For a large QTL effect, GAS resulted in 2.1% and 4.1% greater than the PHE for genetic correlations of 0 and -0.36, respectively. For a small QTL effect, GAS resulted in 0.9% and

1% greater genetic gain than with the PHE. In this second instance, we can see that the highest genetic gain is obtained for a large effect QTL with no genetic correlation between traits.

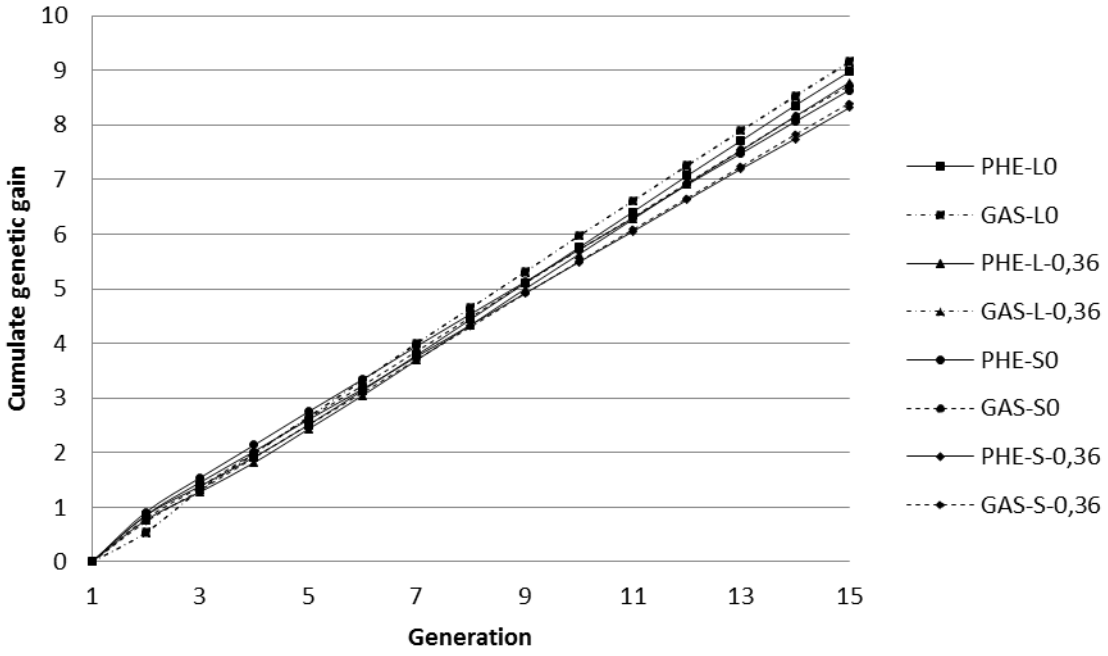

Figure 3 Cumulative genetic gain for trait 1 during the 14 generations of selection for trait 1 for PHE and GAS selection on large and small effect QTL for ρ = 0 and ρ = -0.36.

2.4 Genetic gain for trait 2

When investigating the cumulative genetic gain for trait 2, we first needed to adjust the results to start at the same level for both sizes of the QTL effect. Thus, the values of genetic gain are corrected by the expected QTL effect equal to $p^2 \times a + 2p(1-p) \times d - (1-p)^2 \times a$ or $p^2 \times a - (1-p)^2 \times a$ (since $d = 0$) where p is the initial frequency of the favourable allele and a the additive effect. This value is equal to -0.742 and -0.3101 for a large and a small QTL effect, respectively. Figure 4 shows the evolution of the cumulative genetic gain during the 14 generations of selection for different schemes under a set of parameters. For the 4 sets of parameters tested, we can see that the genetic gain quickly increased until the frequency of the favourable allele approached 1 (Figure 4) in the PHE and GAS scheme for a large QTL effect. After that, for ρ = 0, the genetic gain per generation started to be lower, but still positive (Table 3). This resulted in a slight increase of the cumulative genetic gain. However, in the case of ρ = -0.36, when the allele frequency approaches 1, the genetic gain became

negative (Table 3) and the cumulative genetic gain started decreasing (Figure 4). Moreover, after fixation, the cumulative genetic gain in the PHE scheme is greater than in the GAS scheme (Figure 4). Therefore, there is a long-term loss of genetic gain with GAS. When looking at the results for the small QTL effect, we can also observe a peak in the cumulative genetic gain with GAS when the allele is fixed, while the cumulate genetic gain was more constant for the PHE scheme, for which the favourable allele is not fixed. It is also important to note that the cumulative genetic gain for trait 2 is greater when the QTL underlying this trait has a small effect than when the QTL has a large effect (Figure 4).

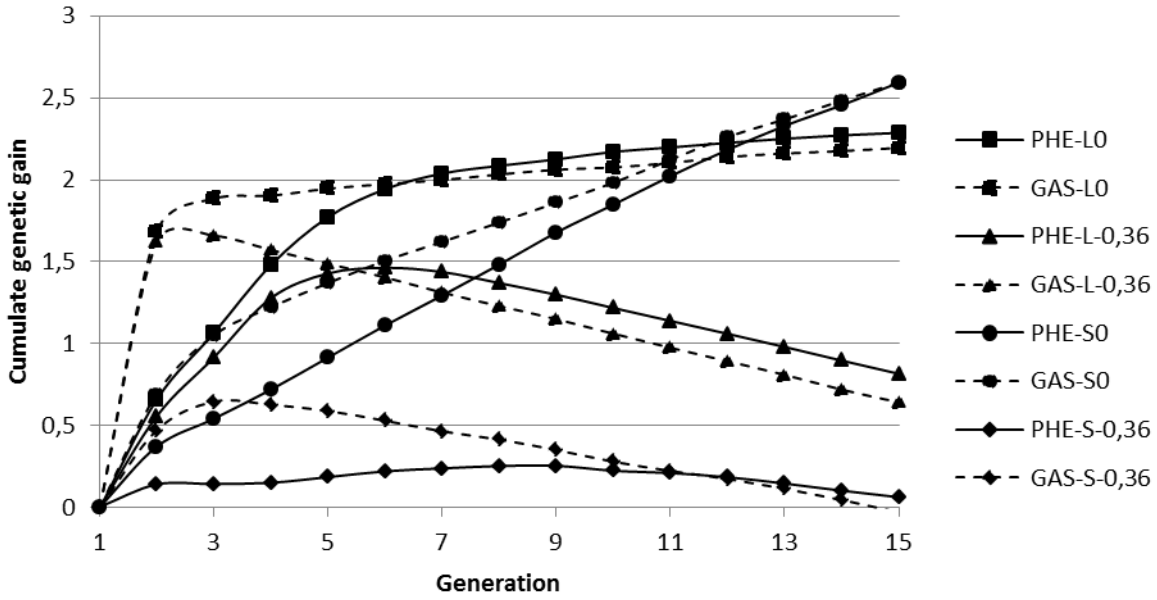

Figure 4 Cumulative genetic gain for trait 2 during the 15 generations of selection for trait 2 for PHE and GAS selection on large and small effect QTL for ρ = 0 and ρ = -0.36.

Table 3 Genetic gain per generation for trait 2 for PHE and GAS selection on large and small effect QTL for ρ = 0 and ρ = -0.36.

Generation	PHE-L0	GAS-L0	PHE-L-0,36	GAS-L-0,36	PHE-S0	GAS-S0	PHE-S-0,36	GAS-S-0,36
1	0	0	0	0	0	0	0	0
2	0,6654	1,6875	0,5597	1,6235	0,3702	0,6954	0,1377	0,4751
3	0,4056	0,2018	0,3579	0,0403	0,1706	0,3581	0,0023	0,1746
4	0,4149	0,0194	0,3624	-0,0866	0,1775	0,1716	0,0076	-0,0185
5	0,2921	0,043	0,1512	-0,0839	0,1948	0,1471	0,0346	-0,0377
6	0,1712	0,0277	0,0339	-0,0833	0,1961	0,1369	0,0356	-0,0595
7	0,0954	0,0246	-0,0251	-0,092	0,1827	0,1151	0,0176	-0,065
8	0,0474	0,0344	-0,0677	-0,0826	0,1889	0,1174	0,0144	-0,0503
9	0,039	0,0286	-0,0708	-0,0799	0,1954	0,1211	0,0016	-0,0633
10	0,0465	0,0144	-0,0809	-0,0889	0,1701	0,1215	-0,0306	-0,0714
11	0,0265	0,0271	-0,0786	-0,0855	0,1742	0,1398	-0,0136	-0,0565
12	0,0285	0,0358	-0,0789	-0,0826	0,1604	0,1393	-0,0252	-0,0486
13	0,0249	0,0219	-0,0801	-0,0828	0,1457	0,1073	-0,0387	-0,0597
14	0,0205	0,0146	-0,0831	-0,0902	0,1262	0,1144	-0,0436	-0,0687
15	0,015	0,0194	-0,0809	-0,0775	0,1378	0,1105	-0,0398	-0,0755

2.5 Average genetic gain per generation for trait 1 and 2

The results summarized in Figure 5 represent the average genetic gain per generation for the two schemes and for all parameters tested. Notable is the negative impact of a negative genetic correlation on the genetic gain in trait 2. For a large QTL in the GAS scheme, the negative genetic correlation led to 29% lower genetic gain than the scheme with no genetic correlation. Moreover, the average genetic gain per generation for trait 2 is close to zero for the PHE while this average is negative in the GAS schemes when the QTL effect is small and the genetic correlation is negative.

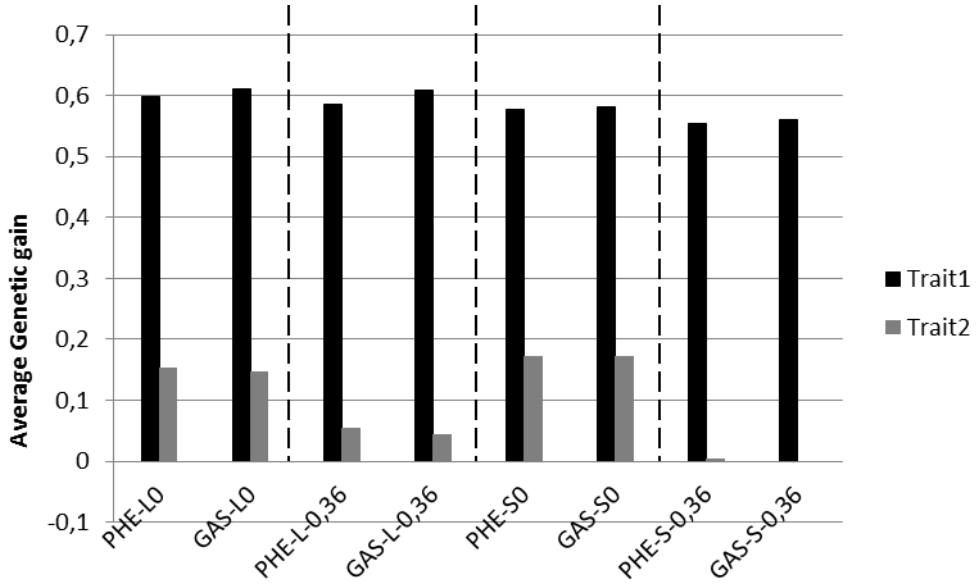

Figure 5 Average genetic gain for trait 1 and trait 2 for PHE and GAS selection scheme, with small and large QTL effect and for ρ = 0 and ρ = -0.36.

3. Discussion

Today, the development of molecular genomics provides more and more information about QTLs and genes with larger or smaller effects on economical important traits. Previous papers such as Villanueva *et al.* (2004), have studied the potential extra genetic gain of introducing genome information and more particularly information on an identified QTL in a breeding program. Our study investigates more specifically the impact of using information on the IPN resistance QTL in breeding programs for Atlantic salmon for the first time. Moreover, this study gives the possibility to analyse the effect of the GAS on a two traits breeding program; a trait controlled by polygenes and measurable on breeding candidates and a second trait controlled by polygenes plus a QTL. Both the GAS and the PHE has been implemented with the use of BLUP EBVs and optimum contribution procedures. The GAS led to a faster fixation of the favourable allele and the OC procedure has fully succeeded in restricting inbreeding to a predefine rate of 1% per generation. From this point, we were able to compare these schemes against each other.

First, the cumulate genetic gain for trait 1 is constantly increasing during the 14 generations of selection for the PHE and the GAS scheme for small and large QTL effects and both genetic correlations tested. The difference observed between schemes after 14 generations of selection is very small and the genetic variation for trait 2 is very similar and is slightly decreasing (Figure 6). These results can be explained by the fact that the selection procedure for trait 1 is the same between PHE and GAS.

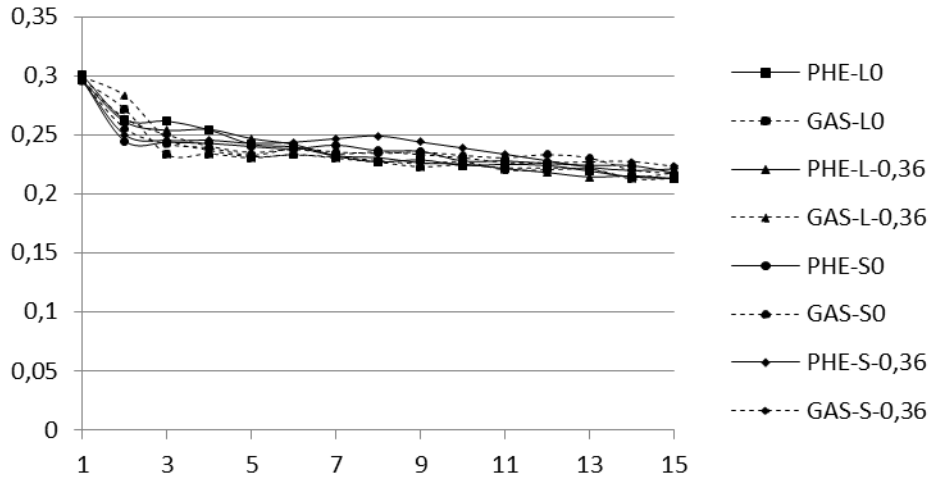

Figure 6 Evolution of the genetic variation for trait 1 during the 15 generation of selection for PHE and GAS selection on small and large QTL effect for ρ = 0 and ρ = -0.36.

With the PHE, selection is based on individual performance of full-sibs without any information on the QTL. The difference in genetic gain among these PHE schemes can be explained by the fact that the large QTL effect is responsible for 29% of the phenotypic variation while the small QTL effect is only responsible for 5%. Thus, individuals who carry the favourable allele for the QTL have a higher chance to be selected in the case of a large QTL effect, resulting in a quicker response to selection compared to a selection on a small QTL effect.

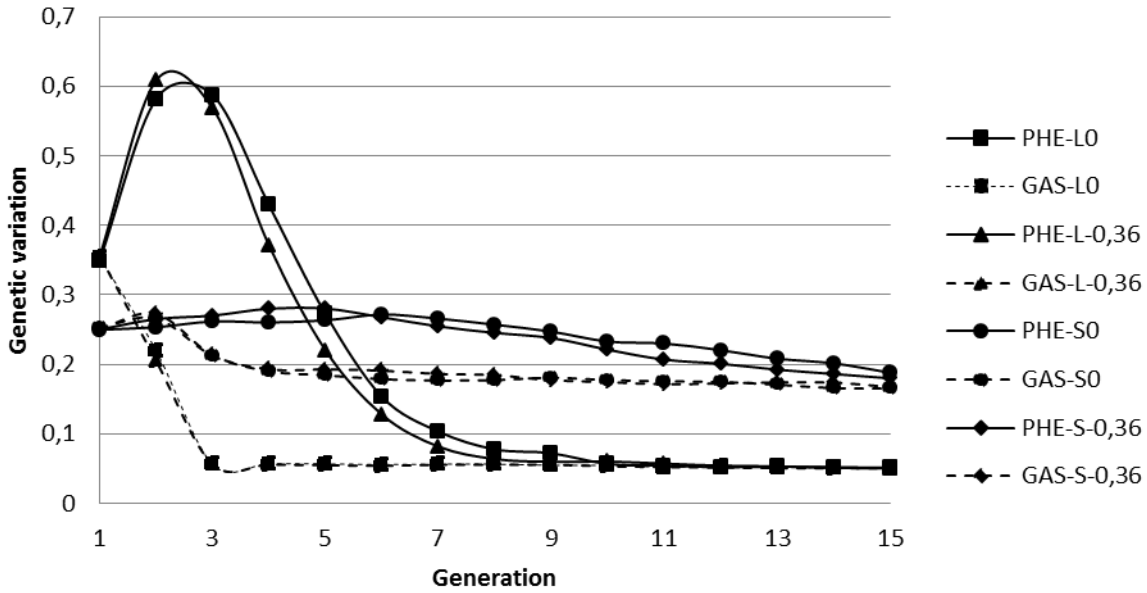

Figure 7 Evolution of the genetic variation for trait 2 during the 15 generation of selection for PHE and GAS selection on small and large QTL effect for ρ = 0 and ρ = -0.36.

Thirdly, when comparing large and small QTL effects, the highest cumulative genetic gain is obtained by the PHE and the GAS schemes in the condition of a small QTL effect for a null genetic correlation, 2.5926 ± 0.0368 and 2.593 ± 0.0374 phenotypic standard deviation, respectively. In contrast, the PHE and the GAS schemes applied on a large QTL effect with no genetic correlation between traits have a cumulate genetic gain of 2.2862 and 2.1944. Thus, the genetic gain for the PHE and the GAS scheme on a small QTL effect was respectively, 11.8% and 15.4% higher than the PHE and the GAS scheme on a large QTL effect. The most likely explanation for this result is that the polygenic variance is much larger in small QTL effect than in large QTL effect conditions (Figure 7). Indeed, after the first few generations, when the QTL is not fixed, the cumulate gain is larger for selection schemes on large QTL effects because the polygenic variance is higher (Figure 7). But for the later generations, when the allele is fixed, the scheme with the small QTL effect has more polygenic variation to select from (Figure 7). Thus, the cumulative genetic gain of the small QTL effect is catching up on the genetic gain of the large QTL effect. In contrast, when the genetic correlation is set up to -0.36, the cumulative genetic gain is higher for selection on a large QTL effect than on a small QTL effect. The genetic gain becomes negative when the frequency of the favourable allele becomes or approaches 1 and results in decreasing the cumulative genetic gain. Indeed, in this condition, the positive selection for trait 1 results in a negative selection for trait 2.

When comparing scheme with (GAS) and without (PHE) information on the QTL, we can differentiate three different periods (Villanueva *et al.*, 1999). 1) When the QTL is segregating in both the PHE and the GAS. 2) When the favourable allele has been fixed in the GAS but not in the PHE. 3) When the favourable allele is fixed in both schemes. In the first period, the GAS gives higher genetic gain due to higher increase in the frequency of the favourable allele but the genetic variation is decreasing (Figure 6). In the second period, the GAS reaches the highest genetic gain and the lowest genetic variation whereas the PHE is still giving gain due to the presence major gene and a higher genetic variation (Figure 6). In the last period, the genetic gain in the PHE scheme is higher than the gain obtained with the GAS. The cumulative genetic gain realized after 14 generations of selection becomes higher with the PHE and results in a long-term loss for cumulative genetic gain for the GAS scheme. The results of the study are consistent with those where the benefit obtained in genetic gain in early generations could not be maintained in long-term when using the GAS scheme (Pong-Wong & Woolliams, 1998; Villanueva *et al.*, 2004). In the four different conditions, the PHE

selection always results in a higher cumulative genetic gain after 14 generations of selection. Villanueva *et al.*, (1999) also suggest that this loss was due to the reduction of selection intensity applied on the polygene underlying the trait 2. This reduction of selection intensity is due to the increased differences in the selective advantage between genetic groups. Therefore, using BLUP EBVs and optimum contribution was not enough to keep a positive genetic gain in a long-term. Moreover, the emphasis given to the QTL was fixed and then not optimal.

Different methods in the prevention of this long term loss by optimizing the allele trajectory have been described. Several of these studies used a continuous time model of the process of fixing an allele based on discrete generation models such as Liu & Woolliams (2010) and others. For the latter, different approaches have been studied within a predefined time horizon (Dekkers & van Arendonk, 1998), constrain to a constant rate of inbreeding (Villanueva *et al*., 2002) or through maximizing progress over the long-term (Pong-Wong & Woolliams, 1998; Villanueva *et al*., 2004). Villanueva *et al.*, (2004) implemented BLUP EBV, optimum contribution and optimized weight from Dekkers & van Arendonk (1998) in order to maximizing the genetic gain over multiple generations with quantitative trait locus selection and control of inbreeding. In this study, stochastic simulation has been used to evaluate the extra genetic gain obtained from Optimized Gene-Assisted selection (GAO) which implements both optimum contribution of selected candidates and optimum weight given to the QTL. In GAO, the selection criterion was EBV + λ BV where EBV and BV were obtained from a simple GAS scheme and λ corresponds to the optimized weight given to the breeding value of the QTL. This optimal weight was obtained by using the deterministic model published by Dekkers & Chakraborty (2001). The aim of their study was to find optimal solutions that maximize the sum of mean total genetic values by generation over the planning horizon of T generation:

$$R = \sum_{t=1}^{T} wtGt$$

Where G_t is the mean total genetic value at the generation t and T is the total number of generations of selection. Truncation selection is applied on an index, I, composed of the QTL and the polygenic breeding value:

$$I_{ijmt} = b_{jmt} \times g_{mt} + (\hat{u}_{ijmt} - \bar{\hat{u}}_{mt}) \quad (1)$$

I_{ijmt} is the index for individual i of sex j and genotype m at generation t. g_{mt} correspond to the mean total breeding value for individuals of genotype m at generation t, deviated from the mean total breeding value of individuals with genotype Bb ($\bar{u}_{2,t}$):

$$g_{mt} = n_m [a + (1 - p_{st} - p_{dt})d] + (\bar{u}_{mt} - \bar{u}_{2,t}) = n_m \alpha + (\bar{u}_{mt} - \bar{u}_{2,t})$$

n_m is an indicator variable equal to 1 for genotype BB, 0 for Bb and bB and -1 for genotype bb. p_{st} and p_{dt} are the frequencies of the favorable allele B in selected sires and dams at generation t. $\alpha_t = a + (1 - p_{st} - p_{dt})d$ and then $g_{1t} = t + \bar{\hat{u}}_{1t} - \bar{\hat{u}}_{2t}$; $g_{2t} = 0$; $g_{3t} = \bar{\hat{u}}3t - \bar{\hat{u}}_{2t}$ and $g_{4t} = -\alpha_t + \bar{\hat{u}}_{4t} - \bar{\hat{u}}_{2t}$. Thus, in this index, the differences between different QTL genotypes are due to the QTL and also due to the linkage disequilibrium generated between the QTL and the polygenes. $\bar{u}_{mt}$ is the mean polygenic breeding value by genotype at generation t and it assumes that $\bar{u}_{mt}$ can be estimated as the average estimated polygenic breeding value by genotype, $\bar{u}_{mt} = \bar{\hat{u}}_{mt}$. In equation 1, $\hat{u}_{ijmt}$ is the estimated polygenic breeding value for individual i and $\bar{\hat{u}}_{mt}$ is the average estimated polygenic breeding value of individuals with genotype m. This value is obtained from BLUP using the polygenic variance and the phenotypic values corrected for the QTL effect.

Selection on the index I imposes truncation selection and maximizing Gt can be formulated as an optimal control problem. Then, optimal fractions selected, $f_{jmt,}$ is used as decision variables instead of the index weight. Dekkers and van Arendonk (1998) demonstrated how the truncation points x_{jmt} , corresponding to the fraction of individuals selected from each genotype at generation t, can be transformed to weights for index I on the standard breeding value for the QTL:

$$b_{jmt} = \sigma_j (x_{jmt} - x_{j2t}) / g_{mt}$$

b_{jmt} is then the optimized weight of the QTL breeding value for individuals of sex j and genotype m at generation t corresponding to λ. In standard gene assisted selection, this weight is fixed while in optimized gene-assisted selection, b_{jmt} is the value that must be optimized in order to maximize the objective function R. This weight differs by sex, genotype and generation. Thus, six different weights are calculated per generation. x_{jmt} is the truncation point corresponding to the fraction of individuals selected, f_{jmt}, from sex j and genotype m at

generation t. σ_j is the standard deviation of polygenic EBV within each genotype equal to $r_j \times \sigma_{pol}$ where σ_{pol} is the polygenic standard deviation and r_j is the accuracy of polygenic EBV for sex j. Optimal fractions selected, f_{jmt}, were derived using optimal control procedures (Dekkers & van Arendonk, 1998).

This procedures implied truncation selection. Therefore, weights could not be derived directly because selection was based on optimum contribution that changed the fraction selected every generation. Thus, optimal weights at generation t were derived assuming truncation selection of the number of parents selected with optimum contribution in the GAS scheme at the same generation t. GAO was run in two steps, 1) first, the GAS scheme was run to obtain, through optimum contribution, the optimized number of males and females selected, 2) in a second time, this number was used as input for the optimal control procedure and the optimized the weights given to the QTL in the GAO scheme.

In 2004, Villanueva *et al.,* compare the genetic gain obtain from selection on an identify QTL with optimized gene-assisted selection (GAO), standard gene-assisted selection (GAS) and standard phenotypic selection (PHE). In their study, the method described by Dekkers & van Arendonk (1998) have been modified to include unequal selection for both sex, nonadditive QTL, multiple QTL and discounted response. Moreover, Villanueva *et al.*, (2004) took into account the reduction of the polygenic genetic variance which was not the case in the model by Dekkers & van Arendonk (1998). They have shown that extra genetic gain can be obtained when QTL information is in use with the optimum contribution of selection candidates and the optimum weight given to the QTL in GAO. Moreover, GAO prevented the long-term loss usually observed in the GAS scheme. When the GAO scheme with optimum contribution was applied on additive QTL, the genetic gain was the same than in the PHE and produced 4% higher gain than the GAS. This study showed that most of the increase of gain was produced by optimization of the selection candidates' contributions. However, the optimization of the weight given to the QTL had a greater effect on avoiding the long-term genetic loss. Therefore, the conflict usually observed between short and long-term genetic gain have been avoided when both procedures, optimum contribution and optimized weight, was implemented.

4. Conclusion

The result of our study is important because the effect of using QTL information in salmon breeding program is now known. It has been demonstrated that using GAS instead of PHE on two traits does not give higher benefits in term of genetic gain for the second trait *i.e.* IPN resistance. Utilization of the GAS led to a faster fixation of the favourable allele and a decrease in the genetic variation. Therefore, it is essential to optimize the weight given to the QTL and then to optimize the allele trajectory. The aim is to keep a high genetic variation to increase the response to selection. Therefore, it would be interesting to investigate the benefits that could bring the utilization of Optimized Gene-Assisted selection in this case. It has already been showed that higher benefits are realized when optimum contribution of selection candidates and optimum weight given to the QTL are combined (Villanueva *et al.,* 2004). It is very important today to investigate the benefits of using QTL information since genomic tools are developed. Indeed, it is expected that more information on the QTL affecting commercial trait will be available in the future. However, implementing GAS in breeding programs has to be seen from an integrative point of view and has to take into account business goals and market needs. Indeed, GAS requires development and integration of procedure for genotyping, for DNA collection and storage and for data analysis that have a certain cost (Dekkers, 2004).

Moreover, the International Collaboration to Sequence the Atlantic Salmon Genome expresses the objective of sequencing the Salmon genome (Davidson *et al.,* 2010). The aim is to produce a genome sequence that identifies and physically maps all of the genes in the Salmon genome. Once the sequence is established, a SNP-chip can be developed. This technology allows to have genotype individuals for a large number of markers mapped on the genome, upon which breeding values can be estimated from these data. This is genomic selection. The relationship matrix among the animals is estimated from the markers instead of the pedigree and the accuracy of EBVs approach 1 (Goddard, 2009). Genomic selection could be implemented in a close future in salmon breeding programs.

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