Gain obtained by marker assisted selection in salmonids

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Summary

In no other animal breeding sector has the use of marker assisted selection (MAS) contributed as much as it has in breeding of salmonids. MAS has been used as a tool in AquaGen for 10 years, mainly in selection for resistance to infectious diseases but also for fillet colour and resistance to sea lice. The effect of the different quantitative trait loci (QTL) has varied, but for all of them implementation and validation has been important. Major QTLs for infectious pancreatic necrosis (IPN) resistance have been found in both Atlantic salmon and rainbow trout. The fraction of fish selected for the IPN QTL in salmon has increased steadily, and relative to the pre-QTL situation a 75% reduction of disease outbreaks has been seen in Norway. In Chile, Salmonid Rickettsial Syndrome (SRS) remains one of the main challenges. Eggs selected for higher resistance to SRS were launched in 2014, and the fish selected for this QTL have performed better than ‘standard’ fish in a field study. During the production in sea, the mortality due to SRS is significantly lower in cages with the SRS QTL than in cages with the standard fish. From data collected on fillet colour from around 6000 fish from 3 year classes of the breeding nucleus, AquaGen detected 3 highly significant QTLs for fillet colour. These QTLs, have been confirmed over generations and populations, and contribute to increasing the level of and reducing variation in colour of the fillet. These QTLs have contributed significantly to improved fish welfare, survival and quality, translating into increased profitability for the whole industry. They have also made the industry more aware of the potential of genetic selection.

Keywords: quantitative trait loci, Atlantic salmon, rainbow trout, infectious pancreatic necrosis, fillet colour; salmonid rickettsial syndrome

Introduction

In farming of salmonids, as in other production species, most of the traits of interest are quantitative traits. It is difficult to think that a single gene could have major influence on performance for specific traits. Nevertheless, with genetic markers came the possibility to look for major genes affecting a quantitative trait, and the detection of a QTL for infectious pancreatic necrosis (IPN) in Atlantic salmon showed that major genes could be found (Houston et al, 2008, Moen et al, 2009, Moen et al., 2015). This QTL was shown to explain ~80% of the genetic variation in various populations, exemplifying the potential and value of genetic markers as tools for selection.

The detection of this specific QTL gave opportunities for application of genomics in aquaculture. For instance, it contributed substantially to the focus on genomic tools in selective breeding, such as development of high-density SNP chips, which have become a key
technology for functional genomics studies as well as for advanced genomic selection. In 2009, AquaGen launched its first product based on MAS, selected for higher resistance to IPN in Atlantic salmon (Salmo salar L.). Since then, the number of target traits for MAS has increased to a total of 10 for Atlantic salmon, rainbow trout (Oncorhynchus mykiss) and coho salmon (Oncorhynchus kisutch). The aim of this study was to inform about some of the QTLs found in salmonids, and investigate what gain has been obtained by applying MAS in the commercial production of salmonid eggs.

**Material and methods**

The material in this study are populations of Atlantic salmon, rainbow trout and coho salmon belonging to the breeding company AquaGen. The populations are based on wild fish, captured 30-50 years ago, thus having been selected for 12 to 16 generations. The breeding stations are located both in Norway and Chile, and provide improved genetics to the salmon and trout farming industries around the world, in the form of fertilized eggs.

MAS was applied by utilising single markers (single nucleotide polymorphisms (SNPs) or microsatellites), or haplotypes of such single markers, on male and/or female candidates. Candidates harbouring the desired combinations of marker genotypes were used as parents in egg production. MAS was also applied in the selection of parents for the breeding nucleus and multiplier populations.

**Results**

QTLs for totally 10 traits have been introduced to the salmon industry by AquaGen (Table 1). Most of the QTLs are found in Atlantic salmon, but two were discovered in rainbow trout and one in coho salmon. Most of these traits associated with QTLs are related to resistance against specific infectious diseases. These diseases have all caused large losses of fish during the history of the salmon farming industry. As far as we know, none of these diseases appear in wild salmon populations to a large extent, which could be part of the explanation for why the QTLs have not yet been fixed by natural selection.

The QTLs have been detected from phenotypes and genotypes, both from controlled experiments and field sources. Following the detection of the QTLs, a major job has been to validate the findings and to implement the results in the eggs that AquaGen produce. High density SNP chips have been used to identify markers in strong linkage disequilibrium (LD) with the QTL, facilitating MAS within the entire candidate populations. Each male can have up to 2 million offspring, so males contribute to more offspring than females. Hence, the selection strategy has focused more on males than on females; those males that carry the good alleles being selected as sires for the commercial production of eggs. In the following sections data from validation of three QTLs will be presented.

**Table 1. List of traits with QTLs in three salmonid species that AquaGen has introduced to the salmon industry.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Trait</th>
<th>Year introduced to the industry</th>
<th>Approximate fraction of genetic variance explained by the QTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic salmon</td>
<td>Infectious pancreatic necrosis (IPN)</td>
<td>2009</td>
<td>80 %</td>
</tr>
<tr>
<td></td>
<td>Pancreas Disease (PD)</td>
<td>2010</td>
<td>10 %</td>
</tr>
<tr>
<td></td>
<td>Fillet colour</td>
<td>2013</td>
<td>18 % + 10 % + 5 %</td>
</tr>
<tr>
<td></td>
<td>Cardiomyopathy</td>
<td>2014</td>
<td>28 %</td>
</tr>
</tbody>
</table>
IPN

For Atlantic salmon, the IPN QTL was found in a controlled post-smolt challenge test, and the results were confirmed, and fine-mapping performed, in data from a challenge test on fry from the same population (Moen et al. 2009). Other research groups also identified this IPN QTL (Houston et al., 2008), and most salmon egg producers have now implemented this QTL in their selection. Similar studies where performed in rainbow trout, also resulting in a major QTL for IPN. The survival within different genotypes in different fry challenge experiments for both Atlantic salmon and rainbow trout can be seen in Figure 1. For both species, the experience from the field is that both homozygous fish (Qq) and homozygous for the resistant allele (QQ) are the good performers.

**Figure 1. Survival in percentage for the genotypes for IPN-QTL in Atlantic salmon and rainbow trout, with standard errors. All experiments are IPN challenge tests performed on fry. The dark blue bars include homozygous IPN sensitive fish (qq), and the light blue include homozygous IPN resistant fish (QQ). For rainbow trout experiments, Sp and Wb are indicating challenge by different IPN virus isolates.**

Since 2009, the fraction of Atlantic salmon selected for this QTL has increased worldwide, resulting in a decrease of IPN outbreaks both in freshwater and in seawater production. As seen in Figure 2, prior to 2010 the number of IPN outbreaks in the Norwegian salmon industry was stable at 200 per year, but after the introduction of the QTL-selected salmon eggs in commercial production, problems associated with IPN have decreased, and after 2014 less than 40 IPN outbreaks per year have been registered (Hjeltnes et al. 2017). In Chile, egg production based on MAS for IPN was initiated in 2010. Although a significant reduction in the number of IPN outbreaks has been observed in the companies that have acquired this product, the implementation of several other biosecurity measures during the same time period preclude a clear discrimination of specific causes. Nevertheless, nowadays almost 100% of the eggs sold by AquaGen Chile are requested to be selected by using the IPN QTL.

The QTL for IPN resistance in rainbow trout was also introduced to the market in 2015 by AquaGen, but because of problems with identifying the broodfish carrying the resistance alleles at the QTL the trout industry did not see any improvement the first year. Improvements in selection were done, and from 2015 to 2016 the number of registered outbreaks of IPN in rainbow trout dropped from 11 to 2. The fish health report of 2016 from the Norwegian Veterinary Institute states that “The reduction in number of IPN outbreaks in
rainbow trout can, in all probability, be credited to use of QTL stocks.” (Hjeltnes et al 2017). The use of this marker in the Chilean breeding program of trout was introduced during 2016. The first eggs produced using this strategy were all given to one company, which has not reported any outbreak of IPN in this group during the freshwater phase.

Figure 2. The number of IPN outbreaks in Norway since 2008, for Atlantic salmon sites in sea and freshwater and rainbow trout in freshwater. Source: Norwegian veterinary institute.

SRS in Chilean salmonids

Salmonid Rickettsial Syndrome (SRS) remains one of the main challenges for the Chilean salmon farming industry. It is the cause of approximately 80% of mortalities due to infectious diseases and it has historically account for up to 3% of the total mortality during a production cycle of Atlantic salmon (SIFA-Sernapesca, 2015). The disease is caused by the intracellular bacteria *Piscirickettsia salmonis* and the main control strategies rely on more than 10 commercial vaccines that have had a limited effect and two types of antibiotics (florfenicol and oxytetracycline). In fact, the disease is responsible for the use of close to 0.53g of antibiotics per harvested kg of salmon (Sernapesca, 2017) and costs associated with it have been estimated to be approximately USD 700 million/year (Intesal, 2015).

In 2011 AquaGen started the search for a QTL for SRS resistance, aiming to contribute to the control of SRS in the Chilean industry. The search was based on a post smolt challenge tests using a cohabitation model developed for Atlantic salmon in a controlled environment. The following genome-wide association study (GWAS), utilising a 220k SNP-chip, revealed a QTL that explained 14% of the genetic variation. During 2014 and the beginning of 2015 the first eggs produced using MAS for SRS-resistance were delivered to seven different salmon companies in Chile. Each company kept these eggs separated from other production batches during the whole freshwater phase. In order to validate the effect of the marker in different commercial conditions, these groups of fish were then transferred to 100 different cages at ten different sea sites. Each sea site has a different percentage of cages containing QTL SRS fish, the remaining cages being stocked with standard AquaGen fish (i.e. without the QTL SRS).

All sites were harvested between May 2016 and September 2017. As expected, a significant increase in mortality was observed at the time when water temperatures rose up to levels known to favour development of the bacteria (spring-summer). Comparison of total and SRS-specific mortality between the two products QTL-IPN and QTL-IPN-SRS, shown that products with the SRS-QTL had a significant lower mortality (P < 0.05) in both analyses (Figure 3). Interestingly, both types of products showed a mortality that was below the industry mean for total mortality, indicating that fish with the SRS-QTL may have a favourable epidemiological effect on all fish held at the same site.
Figure 3. Box-plot of QTL-IPN and QTL-IPN-SRS cumulative mortality. A) Cumulative total mortality and B) Cumulative SRS mortality. Black dots indicate the mean of each group of cages. The red line indicates the industry mean. In both comparisons, there is significant difference (p<0.05) between the two products.

Fillet colour

From data collected on fillet colour from around 6000 fish from 3 year classes of the breeding nucleus, AquaGen detected 3 highly significant QTLs for fillet colour (RED QTL). Fillet colour was measured as astaxanthin (mg/kg) in fillet, estimated from near-infrared spectroscopy (NIR) (Folkestad et al 2008). The effect of the QTLs is mostly additive, hence the fish with zero copies of the good alleles are the fish with the lowest level of astaxanthin in the fillet (4.9-6.5 mg/kg) and fish with six copies of the good allele have the highest level of astaxanthin (>7.5 mg/kg).

The detected QTLs where found in populations of harvest size (4-5 kg), but the effect of the QTLs have been confirmed for fish at various life stages in sea production. In a sample of 200 fish from 224 g up to 7 kg, the average amount of astaxanthin increased by 1.4 mg/kg from the group with 1-2 copies of the good alleles to the group with 5-6 copies of the good allele (Figure 4).

The QTLs have also been confirmed in other commercial stocks. While sampling at a harvest plant, fillet colour was measured with a Roche SalmoFan™. Two of the QTLs were confirmed in this rather small material (240 fish), and the group of fish with zero copies of the good allele had an average score below 27. In the Norwegian industry standard of quality in farmed salmon, the critical level is set at an average of 27 for fish ≤3 kg with minimum score at 24.

Signals from the industry indicate that it is becoming increasingly more difficult to achieve high levels of pigmentation in Atlantic salmon. The documentation of the RED QTL shows that salmon selected for these QTLs will have less variation in fillet colour, and the
elimination of the individuals not carrying the favourable alleles will contribute to an increase in average fillet colour.

Figure 4. Least-squares means with standard error bars for astaxanthin (mg/kg) in groups of fish with increasing number of good alleles (Q). The general linear model includes effect of size and fish group.

Conclusions

QTLs and MAS have been used in farmed salmonids to increase performance for specific traits and to produce differential genetics for 10 years. The QTLs have contributed significantly to both fish welfare and quality, resulting in an increased gain for the whole industry. The salmonid farming industry has with the QTLs, become aware of the powerful tool that genetics is.

List of References


