# Improving competitiveness and sustainability—salmon farming industry

Dr. Alastair Hamilton of Landcatch Natural Selection (Landcatch), now part of Hendrix Genetics Aquaculture, UK, is a University College London graduate with a PhD from the Institute of Aquaculture, University of Stirling, UK.

## Introduction

Salmon farming depends on selective breeding to maintain competitiveness and to combat threats to sustainability. One of the biggest threats is sea lice infection, which can have a major impact on the health of farmed salmon and the fish-farming economy.[1]

To develop single-nucleotide polymorphism (SNP) genotyping tools and genomic methods that could be used to breed sea lice resistance in farmed salmon, Landcatch led a collaborative project involving scientists at The Roslin Institute, Edinburgh, UK (including ARK-Genomics and GenePool); the Institute of Aquaculture at the University of Stirling, UK; The University of Glasgow, UK; and Thermo Fisher Scientific. Funding was provided by the UK Government's Technology Strategy Board and Landcatch Natural Selection.

We interviewed Dr. Alastair Hamilton to discuss why the collaboration members chose to develop a high-density SNP-chip for genome-wide studies in salmon to find quantitative trait loci (QTL) and to develop genomic selection for sea lice resistance in order to improve aquaculture breeding programs.

## Customer profile

Dr. Hamilton has worked on the genetics of nematodes, sponges, oysters, and several fish species, focusing primarily on Atlantic salmon and more recently rainbow trout. Since 2002, Dr. Hamilton has led the Molecular Biology department at Landcatch Natural Selection, now part of Hendrix Genetics Aquaculture, in support of its Atlantic salmon breeding program, focusing particularly on breeding for disease resistance. Research priorities include resistance to infectious pancreatic necrosis (IPN), pancreas disease, amoebic gill disease, *Lepeophtherirus salmonis*, *Caligus rogercresseyi,* and salmonid rickettsial septicemia in salmon; and bacterial cold water disease in rainbow trout.

**Thermo Fisher Scientific:** What were the aims of your project, and why did you choose this focus?

**Hamilton:** Sea lice are the single largest economic and welfare problem for the salmon aquaculture industry worldwide, with annual losses estimated at €305M globally, and €33.6M in the UK alone. Prior to the start of the project, Landcatch had demonstrated significant genetic variation in susceptibility to sea lice amongst their stocks. Although very little was known about the genetic architecture, there was some evidence indicating that it was a highly polygenic trait. However, data on sea lice susceptibility cannot be collected from candidate broodstock, so selective breeding was confined to family-based selection. For these two reasons, sea lice resistance was felt to be an exemplary trait for demonstration of the power of this technology. Making a high-density SNP-chip for genome-wide association studies to develop genomic selection was appealing. There was clearly a need to develop a high-density salmon chip as lack of access to such tools was a major barrier to aquaculture breeding.

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The genomic selection approach was attractive, particularly as it is a difficult disease to study. Collecting data is very labor intensive. It is challenging work looking at populations of 2,000 fish, and individually counting sea lice on each fish. It is difficult to do accurately—adult sea lice are mobile, and so can jump on and off fish. Therefore the settlement of juveniles on the fish is used as a proxy for susceptibility. They can only be counted during a short window of 10–15 days post-infection, when an equilibrium settlement level has been reached. At this point, the lice are big enough to be seen physically using a dissecting microscope, but have not yet become mobile. It can take approximately 25 minutes to count the lice on each fish, and it is skilled work.

" … we felt that by moving over to individual selection, we would be able to accelerate progress."

Landcatch had been gathering data for several years to identify susceptible families for breeding programs. They saw clear family differences in susceptibility to sea lice, and this was unevenly distributed, with a long tail of highly susceptible families. This meant that with a conventional family-based breeding program, fairly good progress was possible, as deselecting the most susceptible families could make a significant difference to the overall sea lice population load.

Nevertheless, the very large variation in susceptibility observed within families, along with the inherent difficulty in measuring the trait, meant we were only capturing a fraction of the total genetic variation. While we had been gathering the data for a conventional family-based selection program for some years, the attraction of moving over to the selection of individual candidate broodstock based on the genomic breeding value was very appealing. It is not uncommon to find resistant individuals, even within relatively susceptible families.

**Thermo Fisher:** How did you set up the collaboration with the other parties on the project?

**Hamilton:** The grant application was submitted under the Genomes UK call from the Technology Strategy Board (TSB). The TSB is a UK government–funded initiative to support industrial–academic innovation partnerships. With help from another government partnership initiative, the Knowledge Transfer Network (KTN), we pulled together the finances. We then approached Thermo Fisher Scientific to be a partner in the project. The application also drew on input from several academics at the Roslin Institute, who had been key to projects developing high-density SNP-chips for other farmed species. In particular, Edinburgh Genomics had worked closely with Thermo Fisher Scientific in the past.

We have previously collaborated with Stirling and Edinburgh on a number of projects including one on infectious pancreatic necrosis (IPN) resistance that found a QTL accounting for most of the genetic variation for IPN susceptibility. This was a spectacular success, being the most powerful genetic animal disease marker identified in animal breeding. We had also been collaborating with the University of Glasgow and Stirling on family-based sea lice resistance, leading up to the project.

**Thermo Fisher:** What were the factors that you considered in making your choice of a genotyping technology platform?

**Hamilton:** The decision was influenced by technical considerations, and by the costs of development and genotyping. We were able to work with Thermo Fisher Scientific to fit the costs into the required structures for the grant proposal.

Applied Biosystems™ Axiom™ technology had previously worked well in the hands of our collaborators; ARK-Genomics at the Roslin Institute had considerable experience using it. We felt it fit well with the competition call and had confidence that the technology would work. In our hands it has proven to be very robust.

The project specified development of a high-density SNP-chip, in the first instance. A planned output from one of the later work packages was lower-density panels of SNPs to create a tool suitable for routine genomic selection across traits. This had been envisaged as a very low-density, sea lice–focused panel of a few hundred markers at most, but during the lifetime of the project, the 36–50K Axiom™ arrays became a reality, so that enabled us to come out of the project with a tool for routine use across traits.

**Thermo Fisher:** How did you discover and select the SNPs to use on the array?

**Hamilton:** A significant component of the funding was for SNP discovery, so we did quite a bit of sequencing to discover new SNPs. We included a reduced representation library prepared from a number of different fish populations, with a heavy bias toward Landcatch populations, but including other populations as well. We also had access to an RNA-Seq database from another project. We discovered in excess of a million SNPs and progressively reduced the list, filtering it down *in silico*. We ended up with more than 130,000 SNPs on the high-density chip.

We included “must-have” SNPs from areas that previous research had suggested might be of interest. We wanted all of our SNPs to be mapped, but the vast majority of our SNP list consisted of previously unknown SNPs. However, the genome sequence was in preparation, and an early version had been released, so we were able to select SNPs that were reasonably equally distributed across contigs.

**Thermo Fisher:** What specific challenges did the salmon genome raise and how did you handle those?

**Hamilton:** There are a lot of repeat sequences in salmonid genomes, so SNPs in these areas were filtered out during selection. A particular challenge working with salmonid genomes is a relatively recent whole-genome duplication event, which means that SNP discovery identifies a high proportion of paralogous variants. To help identify these, the SNP discovery phase also included one library prepared from haploid fish, and any SNPs that cropped up in the haploids were filtered out.

Once we started genotyping with the high-density chip, we also saw SNPs that produced multiple clusters, most of which came from the RNA-Seq data. We had been well aware of this risk with the RNA-Seq data, but obviously having SNPs in coding regions was appealing. We didn’t make any use of the multiple cluster SNPs during the sea lice project, nor did we include any of them on the medium-density chip. However, Thermo Fisher Scientific has since developed tools that might enable us to interpret these. We have a significant research project underway looking at another parasitic disease, amoebic gill disease, and I’m looking forward to working closely with Thermo Fisher bioinformaticians to explore how we can include these SNPs in the analysis.

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**Thermo Fisher:** How are the opportunities and challenges different for genomic breeding in aquaculture compared with land animals?

**Hamilton:** Family sizes in aquaculture are obviously different. A single cross can produce tens of thousands of eggs. In terms of the selection differential that you can apply, there are opportunities for making fast progress in aquaculture.

A key difference between aquaculture species and most land-based, domesticated farm animals is that fish are just a few generations away from the wild, so there is a tremendous amount of genetic variation there. Farming at sea means biosecurity is trickier than it is in land-based breeding systems. For that reason, and because aquaculture is a relatively new science, disease has been a big problem, and breeding for disease resistance has been one of the tremendous successes that has been possible in aquaculture.

The models around deploying genomic selection in aquaculture are still being developed. It is clear that there is a great deal that aquaculture can learn from the experiences in the pig, chicken, and dairy industries, but there is a lot that is different about aquaculture.

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**Thermo Fisher:** What role do you think genomic technologies and microarrays can play in aquaculture in the future?

**Hamilton:** Environmental adaptation, feeding regimes, and epigenetics—these will have an impact on aquaculture in the future, particularly if we expand the range of aquaculture production to new geographic areas and new species. There will be focus on feed conversion ratios, adaptability to different diets, etc. For now, disease resistance remains the big one, but we will certainly be looking at other traits.

It is fair to say that the introduction of genomic selection has been a huge success for us, but there is further work to do. SNP-chips are now routinely employed when making livestock breeding decisions, but at the start of this project, salmon aquaculture had no such tools.

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[1] https://phys.org/news/2015-08-salmon-resistant-sea-lice.html