Seafood CRC Research Travel Grant: Study Tour to Norway

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Project No. 2010/762

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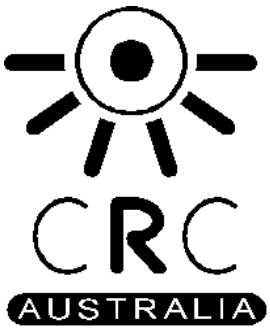

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NON-TECHNICAL SUMMARY

PROJECT NO: 2010/762

TITLE STUDY Tour of Norwegian Fish Breeding

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(PROJECT) OBJECTIVES OF RESEARCH TRAVEL GRANT/ INDUSTRY BURSARY

(i) Discuss strategies for measurement of genetic resistance to parasitic diseases
(ii) Discuss statistical analysis of genetic parameters of (censored) survival traits
(iii) Broaden knowledge on assessment of „robustness" traits including heart morphology and cardiovascular performance.
(iv) Understand aspects of salmon gut health related to high temperature and protein:energy balance in commercial feeds
(v) Attendance at the „Fish Breeders Round Table" meeting in Stavanger, Norway

NON TECHNICAL SUMMARY:

The primary reason for this travel was to attend the Fish Breeders" Round Table in Stavanger, Norway. This is an international forum, where knowledge and experience is exchanged between fish breeding researchers and those involved in applied genetic improvement work on a commercial basis. The forum included over 30 presentations on genomics, genetic models and commercial application of fish breeding. Following the meeting I visited research institutions and Atlantic salmon breeding installations.

The main aim of this study was to examine attempts to select for „robustness" in the Norwegian industry. A key concern is the increasing prevalence of production-related deformities in farmed salmonids which may have serious impact upon production. The fish welfare implication of malformations is a major ethical concern and has serious implications for consumer perceptions of the aquaculture industry. One key finding has been the increased level of heart deformities, which appear to be caused by the rearing environment (exercise and nutrition) but may also be influenced by genetics. Heart abnormalities have serious implications for the robustness of farmed fish. This study tour therefore aimed to understand current efforts to measure genetic contribution to robustness and heart health and the strategies that are being considered to ameliorate this issue. The concept of robustness or resilience has particular application in the Tasmanian salmon industry because fish are frequently handled for amoebic gill disease (AGD) treatment and regularly suffer a level of handling losses.

One issue encountered by the Tasmanian salmon industry is „Summer Gut Syndrome", a condition that causes some fish to produce yellow „casts" and become emaciated during summer. This study tour allowed me to meet with members of the Aquaculture Protein Centre in Oslo and Sunndalsøra. The techniques discussed for examining changes in gut function related to diet and environment and can be directly applied to the SGS condition.

OUTCOMES ACHIEVED TO DATE

- Understanding of how salmon breeding programs in Norway have moved on from the early supported R+D phase to become commercially driven and vertically integrated
- Strengthened links with researchers and commercial breeding programs attempting to develop more „robust" fish and improved cardiovascular function
- Visited research cages and recirculation aquaculture systems (RAS) that are run on a semi-commercial footing.
- Strengthened research linkages in understanding salmonid gut health related to environment and nutrition.
- Following discussions with Dr Ingrid Olesen of Nofima Marin, CSIRO are named as collaborators on a proposed project („HeartBeat") which was submitted to the Norwegian Research Council on 12/10/2011.

OUTPUTS DEVELOPED AS RESULT OF TRAVEL GRANT/ INDUSTRY BURSARY:

Taylor RS, 2011, Selection for AGD resistance in the Saltas SBP (Measurement methods for AGD resistance and genetic ranking). Presentation to the management of AquaGen AS, 5th September 2011, Trondheim, Norway.

Taylor RS, 2011, Summer Gut Syndrome in Tasmania. Presentation at Norwegian School of veterinary Science (Aquaculture protein Centre), 15th September 2011, Oslo, Norway.

ABOUT THE PROJECT/ACTIVITY

BACKGROUND AND NEED

Every four years a small international gathering of commercial aquaculture fish breeders occurs in Norway. This trip provided me with the opportunity to attend the meeting and to meet researchers. This provided the springboard for viewing a number of research and breeding program facilities across Norway.

Of major concern to fish welfare and production is the increasing prevalence of production-related deformities in farmed fish. One of the most serious, but least understood is heart deformities. Research indicates that cardiac malformation is linked to the fishes ability to withstand stressors. Therefore, breeding programs are exploring ways to simply incorporate robustness traits into their overall breeding goal to ensure that future progeny are „well rounded; animals. In Tasmania, the need to frequently crowd and handle fish for freshwater bathing to combat AGD can cause significant losses. The Tasmanian SBP already includes „AGD resistance" in its breeding goal but there are currently no robustness traits in the program. The main aim of this trip was therefore to understand how robustness can be simply measured and how this could be incorporated into the Tasmanian SBP. The calculation of breeding values for susceptibility (susceptible / not susceptible) and longevity (time until failure for susceptible animals) is a developing area in quantitative genetics, these methods are applicable to robustness testing (where fish „fail" at a test time point or may „survive" to the end of the test). Therefore a key aim of this travel was to optimise the measurement and analysis of „AGD resilience" traits.

Part of this tour coincided with Brad Evans Seafood-CRC funded visit to Norway (Project #2011/709). Due to CSIRO"s role in providing R+D and supporting the development of the Saltas Selective Breeding Program (SBP), we were able to gain mutual benefit in sharing a common itinerary for the first week. This also minimised disturbance to our Norwegian hosts.

RESULTS

Travel itinerary

31/08/2011 Dr Morten Rye, Akvaforsk Genetics Centre (AFGC)

Dr Rye explained that in 1969 the Norwegian government funded collection of wild salmon from native rivers in order to ensure a diverse range of genotypes as a basis for selection. In 1971 the Norwegian College of Agriculture built a new research station at Sunndalsøra, followed in 1973 by the marine research station for salmonids on the island of Averøy. In 1975 Akvaforsk began the first family-based breeding scheme for aquaculture. Fish breeding grew in scale with the industry and the needs of research and core breeding production began to diverge, therefore AFGC was established in 1999 to run commercial breeding, the profits from which were put into research. Despite a number of ownership changes, AFGC has grown through money earned in service to the public sector and is involved in 24 aquaculture breeding programs around the world (15 species, 13 countries). AFGC offer technical genetic services (program design, protocols, recording schemes, data systems, breeding estimation, breeding decisions). They do little „hands-on" work and rely on their commercial partners having quality people to handle stock and collect data.

The business model for AFGC was initially to “be a genetic consultancy company” selling a service which was renumerated by (i) fixed annual payment (ii) royalty on use of animals. The royalty varied between clients but was typically 3% to 7%. In the second phase, AFGC became involved in „owned genetic material" through partnerships or AFGC only – for example, they own 10% of SalmoBreed (Salmon) and 60% of MarineBreed (Cod). They are building a Nile Tilapia business in the USA. Althogh “owned material” is building, 80% of AFGC"s business is “technical genetic services”.

Despite the scale of AFGC"s business, it is important to note that they are still inherently linked with research (mainly through NOFIMA). Their ability to remain at the cutting edge of breeding

developments through close association with research allows AFGC to remain a preferred supplier of genetic services.

1/09/2011 to 3/09/2011 Fish Breeders' Round table Meeting, Stavanger, Norway (Appendix 1)

Fish Breeders" Round Table is an international forum where knowledge and experience is exchanged between fish breeding researchers and those involved in applied genetic improvement work on a commercial basis. The conference was small (~50 people) but was an excellent forum to make contacts, being held in a small countryside hotel over four days. The presentations were quite informal, allowing participants plenty of opportunity to discuss the topics. Although there were 35 talks, I will limit my notes to some of the more relevant presentations:

Sven Arild Korsvoll (AquaGen AS): Breeding program for A. salmon in Chile

Chile and Norway have similar coastlines for salmon production but the density of salmon leases is lower in Norway. The growth potential in Chile is higher (40 t/km^2) than in Norway (10 t/km^2). Much of the selective breeding in the Chilean industry is done by Norwegian companies. In the past, the genetic nucleus was kept in Norway and eggs were taken to Chile. However, since 2009 the need for increased biosecurity has prevented the importation of eggs. Therefore, in 2008 AquaGen set up a nucleus (420 sibling families to the Norwegian nucleus) and is now running an independent program. The genetic links between the two programs allows for comparison of genetic and environmental effects (GxE).

The author reports a high genetic correlation (r_g) of growth between Norwegian and Chilean stocks. The AquaGen Chile breeding goal is (i) growth freshwater and seawater (ii) IPN resistance (iii) SRS resistance (iv) ISA resistance (v) Sea lice resistance [h^2 = 0.17 for Caligus test] (vi) fillet yield (vii) swimming performance. The program also uses SNP selection. Currently the regulation is that broodstock must be in freshwater for 12 months prior to spawning, this allows a certain amount of marine performance testing to be carried out at sea. The industry is currently debating the pros and cons of holding broodstock solely in freshwater and performance testing siblings at sea (biosecurity measure as practiced in the Tasmanian SBP). The Chileans see this option as expensive given that the breeding program is only a small part of the value chain. Direct growth measures from freshwater held broodstock have a moderate r_g (~0.5) with marine growth.

Jørgen Ødegård (NOFIMA): Genetic Models and modelling fish diseases

In aquaculture breeding programs, resistance to infectious diseases is tested by injection, bath challenge, cohabitation or natural challenge. Data can be treated as cross sectional (one record per animal, binary data) or longitudinal (time to failure with censored values, risk expressed as a function of time).

Using a linear model for cross sectional data can give reasonable results when survival is close to 50% because the residuals are normally distributed; at the extremes this model is inaccurate. The linear model underestimates heritability, the heritability estimate will drop at extreme survivals. This can be overcome by using a threshold model (Probit, Logit) but there may be extreme category problems when fixed effects are included.

Longitudinal survival models such as the proportional hazards model have been developed to account for censoring. These models cannot be run on standard software (such as AsReml), but algorhythms have been developed to run in DMU.

In analysis of time-to-event data, classical survival models ignore the presence of potential nonsusceptible (cured) individuals, which, if present, will invalidate the inference procedures. Time to death and survival may be different traits, if a trial is stopped early (say at 50% survival) the wrong EBV"s for survival would be calculated. In Fig. 1, Family 2 appear to be worse than Family 1 early on, whereas eventually Family 2 is superior.

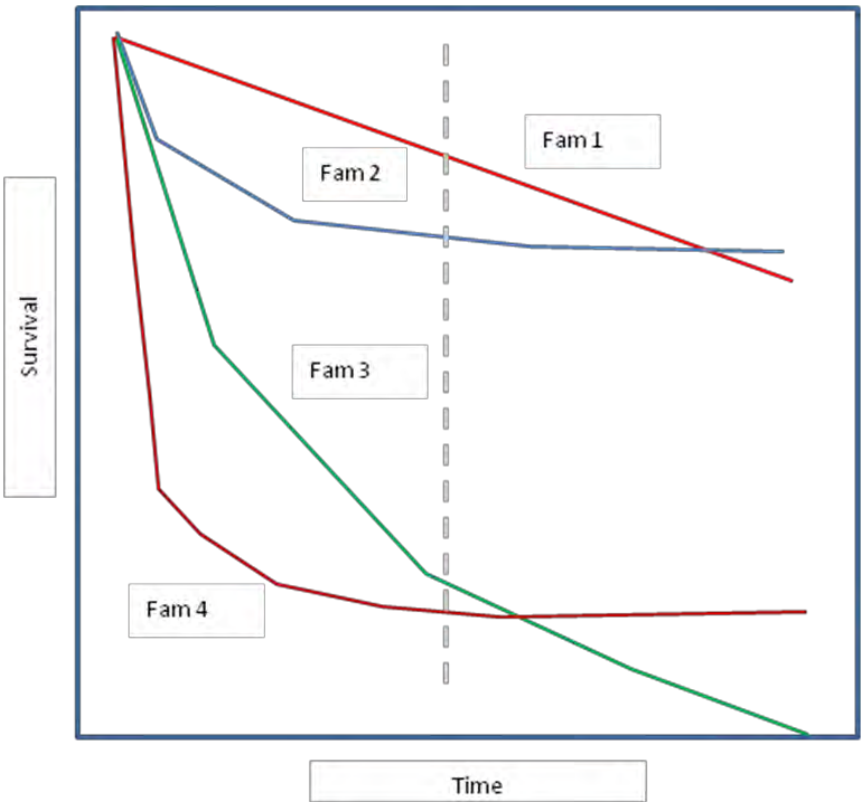


Fig. 1: Example of survival curves for four families

In a study of Taura syndrome in shrimp „Ødegård 2011" found a low rg (0.22 ± 0.25) between susceptibility (h^2 = 0.41 ± 0.07) and endurance (h^2 = 0.07 ± 0.03), indicating that selection on „time to death" would be suboptimal. In non-lethal (chronic) diseases survival is an inaccurate measure of resistance, it is better to look for a correlated indicator trait (e.g. growth)

(Reference: Ødegård, J., Gitterle, T., Madsen, P., Meuwissen, T.H.R., Yazdi, M.H., Gjerde, B., Pulgarin, C., Rye, M. (2011) Quantitative genetics of Taura syndrome resistance in Pacific white shrimp (Penaeus vannamei): A cure model approach. Genetics Selection Evolution, 43:14).

Roberto Neira (University of Chile, Aquainnovo S.A.): resistance to Caligus rogercresseyi and Piscirickettsia salmonis in Atlantic salmon: genetic parameters.

Aquainnovo have over 10 years experience in applied aquaculture species, working on Coho salmon, Atlantic salmon, Rainbow trout and Tilapia. The diseases SRS (Salmon Rickettsia Syndrome) and Sea lice (*Caligus*) have been a problem since the early days of the Chilean Atlantic salmon industry. Resistance to *Caligus* and SRS have not previously been in the breeding goal, so Aquainnovo have developed testing for the interaction between the two problems (i.e. SRS resistance in the presence of *Caligus*). The test has been done on four levels by controlled tank challenge with replicates: (i) Resistance to SRS (ii) Resistance to *Caligus* (iii) Combined resistance (iv) GxE interactions.

There is sufficient genetic variation for *Caligus* resistance to include it in the breeding goal, heritability was measured at 0.34 (sessile stages) and 0.06 (mobiles). High genetic correlation (rg = 0.77 to 0.99) with sessile lice counts on „fins only" means that fin counts can be used as a rapid assessment method. Co-infection (SRS and *Caligus*) produced 42% mortality (SRS only) and 100% mortality (SRS + *Caligus*), r_g = 0.19 to 0.54. Resistance to the two diseases is not antagonistic. Overall resistance to *Caligus* is not a high priority for the breeding program because it is treated and does not cause mortality. However, the interaction between *Caligus* and SRS is very important.

Although *Caligus* are not an issue in the Tasmanian industry, this study illustrates that the interaction of pathogens needs to be considered in breeding programs. Therefore, if new diseases become established in Tasmania, interactions with AGD should be considered.

Ashie Norris (Marine Harvest): Heritability of spinal deformities in Atlantic salmon.

When fish from the breeding nucleus or nucleus backup were moved from a production site (Inver Bay) to the broodstock site (Mulroy Bay), 30-50% deformities was reported. Some of this stock had been subjected to elevated hatchery incubation temperature to synchronise first feeding (spawning had been spread across 12 days). Deformities „humpback", „lordosis", „scoliosis", „jaw deform" and „short tail (kyphosis)" were assessed by dissection. Spinal deformities were described by severity and position (cranial, mid-cranial, middle, mid-caudal, caudal) and some fish were sent for x-ray to look for fusions of two or more vertebrae. The severity of spinal fusion showed little relationship to the severity of external phenotype, external examination was likely to miss some spinal deformity. The main deformity seen was humpback (86% of deformities, mostly in the cranial to middle positions). The results indicated that deformity was actually higher in the „normal" incubation batch (5-7°C) than in the batch that were held at 8-10°C between 90 degree days and first feeding. Deformity was found to be non-heritable and confounded with the size of the dam. Further examination found that the problem was confined to families held at Mulroy only and that there was a high r_g between family weight and deformity. The authors raised the hypothesis that rapid growth following transfer to Mulroy had been nutrient limited, farm staff confirmed that normal grower feeds had been used rather than broodstock-specific diet. Testing of phosphorus levels in bones would have been useful.

This study is important because it illustrates that deformities can occur at any stage in the salmon's life cycle. The critical issue is that fast growing fish (whether in the hatchery or at sea) can quickly become nutrient limited. Any genetic tendencies for deformity will only be an issue if the rearing environment/nutrition is inadequate.

Harvard Bakke (SalmoBreed): Improving lice resistance through selecvtive breeding in Atlantic salmon.

The Salmobreed multiplier produces 280 million eggs across three hatcheries. Broodstock are spread across 4 sea sites for production of multiplier lines and nucleus. Average generation time is 3.5 years (mixture of 3 and 4 year old broodstock). Resistance to salmon lice (*Lepeophtheirus salmonis*) is tested at NOFIMA's Averoy test station using controlled natural infection (known number of copepodids released into a small tarpaulin surrounded pen). Approximately 14 days after infection the sessile stages are counted. Heritability of $h^2 = 0.26 \pm 0.07$ is highly correlated with controlled tank challenge ($r_g = 0.87$ to 0.99). There is a positive phenotypic relationship between lice count and body size (weight). There is an inconsistent low positive genetic correlation between sealice resistance and ISA resistance. The program has only tested resistance to first infection so far, this differs from Tasmanian work to measure genetic variation of AGD resistance, where first infection and resistance to reinfection are different traits. Although SalmoBreed broodstock are held at sea, there has been no measurement upon potential brood animals, family breeding values are obtained from sibling tests. Harvard estimates a gain of 37% in sealice resistance between 2009 and 2012.

Derrick Guy (Landcatch Natural Selection): Field and experimental challenege testing for IPNv resistance in Atlantic salmon.

IPNv can cause significant losses at freshwater fry stage (22 – 60%) and following marine stocking (0 – 30%). The discovery of a single locus QTL that explains most of the variation in IPNv resistance has major impact upon the breeding program. Resistance is normally measured by controlled tank challenge. Therefore, data from field testing was examined, showing rg = 0.89 between year groups and 0.68 between marine and freshwater. IPN is a special case amongst aquatic diseases because it is linked to a single QTL. Most disease resistances are polygenic/continuous traits.

Ingrid Olesen (NOFIMA): Why is only <10% of aquaculture production based on genetically improved seed?

There are 101 breeding programs for finfish around the world (13 Atlantic salmon, 13 Rainbow trout), but these are mainly temperate species. There are 166 species of farmed fish and 76 of shellfish, yet only 8.5% of production is based on genetic improvement. In comparison, temperate farming is more established and has very few species.

The benefits of selective breeding are easy to illustrate, for instance 8 generations of salmon breeding have improved feed conversion efficiency by >30%. The benefit to cost ratio for salmon breeding is around 15, while Tilapia could be up to 60. The question is who receives the benefit of decreased risk and improved efficiency and fish welfare? Based on a value of genetic gain (~10c per kg of fish/year) and proportion of turnover (breeding nucleus 0.4%, eggs 0.7%, smolt 12.4% and growout 86.5%), the value goes to growout and the customer. Therefore, to take advantage of breeding it is better to be vertically integrated. Breeding programs need clear royalty structures to remain viable, this is in the long-term interest of the grower. A major issue is „resource copying" where farms incorporate improved stock into their own breeding schemes. Breeding programs need tools to control the use of their biological material (i) biological – sterile production (ii) legal – branding, patent, transfer agreements (iii) „Sui generis" – mandatory certificate of origin verified by DNA markers.

Renate Kvingedal (Ewos Innovation): Mapping gut microflora in Atlantic salmon using molecular methodologies

Although this paper was not related to breeding programs, it outlined a molecular technique to investigate gut microflora assemblages. This work is of particular interest in Tasmania because "Summer Gut Syndrome" (SGS) can cause significant production losses. One feature of SGS is that tthere is a marked shift in hind-gut bacteria abundance and assemblage. It was also interesting to learn that feed companies have their own fish challenge tank facilities which are used for nutrition and disease work.

Renata described the development of a qPCR designed to reflect the main species clusters found in 400 gastrointestinal tracts from Norway and Scotland. A trial looking at Soya bean meal (SBM) and prebiotics identified increased bacterial load with higher levels of SBM. The test was also run across a variety of fishmeal inclusion levels. As fishmeal was reduced, there was an overgrowth of *Corynebacterium glutamicum* (an aerobic bacterium that is used industrially to produce lysine in animal feeds). A low fishmeal diet plus prebiotic gave 10% growth improvement.

Harri Venvilainen (MTT Agrifood Research): The use of microsatellites in data recording and breeding value estimates

Genotyping of individuals in test populations is very expensive. Therefore, this paper reports a simulation study whereby the measured trait was divided into classes (body weight – small, medium, large) and the genetic material from each class was pooled to estimate the genetic contribution from each parent to that class. Instead of genotyping all fish in the trial, costs can be limited to only three or four microsatellite runs. EBV"s were compared to true (parental) EBV"s, r = 0.76. This method is not suggested for use on the nucleus, but could be useful for validation populations.

The method has potential for use within the Tasmanian SBP if performance of commercial populations (derived from the SBP) needs to be assessed cost-effectively.

Victor Martinez (University of Chile): Genetics of *Piscirickettsia salmonis* resistance.

The Tasmanian strain of *Piscirickettsia salmoni*s is of low pathogenicity but it is an „emerging" disease that needs to be monitored. This talk was useful because it described results for assessment of genetic variation of Rickettsia resistance in Chile.

Rickettsia was first reported in Chile in 1989, it causes significant economic loss to the industry. It is an intracellular bacterium that responds poorly to antibiotics. A challenge test was designed using 58 full-sib families (85 sibs/family), infection was by i.p. injection across two tanks. Some families showed different mortality curves, achieving the same overall loss but having markedly different curves. Victor discussed the implications in relation to the onset of losses (endurance vs survival). There was also evidence of tolerance (growth despite pathogen load) and resistance (ability to resist the pathogen). This concept has direct relevance to AGD in Tasmania, one of the aims of my study was to explore tolerance („resilience") to acute (handling) stress, but the ability to continue growing despite a level of AGD pathology may be an important consideration for the breeding program.

Genome-wide association shows 8 SNP"s related to resistance in 2 genomic regions.

Anna Sonesson (NOFIMA): Genetic parameters for obesity traits in Atlantic salmon

Increased adiposity leads to decreased heart health (arteriosclerosis and inflammation). There is little known about the genetics of lipid and lipid related health in Atlantic salmon. Using a group of large salmon, heritabilities (animal model, sex as fixed effect) were: weight 0.34, visceral fat weight 0.27, heart weight 0.41, cardiosomatic index 0.31, heartfat 0.28. Genetic correlation visceral fat and CSI = -0.42; heart fat and CSI = -0.60, suggesting developmental issues in the heart with high fat. It was noted (Ashie Norris) that fish held on high energy/offshore farms have fewer heart fat and deformity issues.

4/09/2011 Fish Breeders Round Table farm tour

Following the fish breeders meeting, a day-tour of farm establishments was organised. This provided the opportunity to visit the Centre for Aquaculture Competance AS (CAC) and Sterling White Halibut. CAC was set up in 2003 by Marine harvest and is a joint venture with Skretting, Akva and the Veterinary Institute. It is a marine cage farm with a unique funding model (funded by Marine Harvest, research is paid by fee, farmed product is sold commercially). Situated on a 300 m deep lease, the facilities include a feed store/office/laboratory housed on a large barge (Fig. 2), 12 steel cages (20x20 m) on conjoined walkway and a 160 m circumference Polar Cirkel pen (which holds 1000 t of fish). One million fish are grown on the site. The main research aim is to reduce fish meal/oil usage to 1 kg for each 1 kg salmon produced. Feeding is via Akva centralised feed system, this farm is the commercial scale test-bed for new Akva and Skretting products. Mortalities are removed by „lift-up" and nets inspected monthly by dive/video record. Gill nets are set around the farm and any capture of escaped fish (of representative size) is immediately followed by a full dive inspection.

To control disease (especially sea lice) growing areas are rotated so that each year one fjord holds smolt, one holds growers and one is to fallow. Once smolt are stocked to a site they are taken through to harvest. Sealice are managed by subsampling and counting 20 fish per pen per week. Once levels reach 1 adult or 5 lice per fish the farm has to treat. Due to issues with parasite resistance, lice are regularly sent to the laboratory and tested for susceptibility; the most effective treatment is then used by all farmers in a region. Lice are also managed by holding wrasse in the pens (numbers equivalent to 3% of the salmon population).

The farm has tested 1000 t of triploids, which were produced using the Tasmanian hatchery protocol as part of EuroTrip. The European salmon strains do not suffer from jaw deformity when triploided. The manager reported excellent growth and high fillet yield, though the main interest in triploids is in minimising potential impact upon wild salmon.

CAC do not use supplementary oxygen or aeration. Temperatures peak at 18°C and oxygen is always between 60 and 90% saturation. Growth from 200 g to 5 kg in 14 to 16 months is standard. The farm does a lot of work on schooling behaviour using a camera system and report that fish often crowd in the warmest water or lowest oxygen bands, localised stocking densities may reach 225 kg/m^3. The farm has developed a mixing unit that attaches to the centralised feeder system to release feed at depth so that schooling behaviour can be influenced.

Fig. 2: Centre for Aquaculture Competance, Langavika

Sterling White halibut is also Marine Harvest owned. The farm consists of twenty 20x20 m cages of 30 m depth. In each cage there are four „apartments" for the halibut (*Hippoglossus hippoglossus*). Each apartment consists of a stack of fifteen 6 m diameter shelves. The fish are fed on a high fish meal diet. The main production issue is maturation, with males maturing at 2.5 kg and females at 7.5 kg the main technical aim is to develop all-female production. There are also issues with external pigmentation and flesh fat (once fat levels exceed 10% there is a problem with fat spotting in the flesh). There are no issues with jaw or eye development. Although there are reported to be few diseases associated with halibut farming, atypical furunculosis can be an issue. The production cycle employed by SWH is long, involving one year in the hatchery to 5 g, the fish are then moved to another landbased facility for another year (now 500 g) before transferring to seacages for another 2.5 years.

5/09/2011 to 7/09/2011 AquaGen AS, Trondheim

Arne Storset (Senior Scientist) organised for Brad Evans and I to visit the AquaGen head office in Trondheim. This was an opportunity to formally present aspects of the Tasmanian SBP and salmon farming whilst receiving presentations from key members of the AquaGen management team.

AquaGen are co-owned by EW Group GmbH (52.5%), Skretting, Marine Harvest and Cermaq. The AquaGen strains of Atlantic salmon and Rainbow trout represent 60% of Norway"s production and are the result of over 10 generations of selection. The company had considerable egg exports to Chile but these ceased after new biosecurity laws in 2008-9. The company backed up the breeding nucleus in Chile and is now running a separate (but genetically linked) program there. A technical challenge being faced in Chile is that broodstock will no longer be sent to seawater, therefore AquaGen are interested in the experiences of the Tasmanian SBP.

AquaGen in Norway maintains a breeding nucleus of 800 core families. Until 2005 the program was based upon three separate yearclass lines, but these were amalgamated and genetic links are maintained to the multipliers through the use of cryopreserved Elite milt, which is produced every third year. There are currently 22 traits in the breeding goal (http://aquagen.no/En/Breeding+Genetics/Broad+Selective+Breeding+Goals.9UFRrS3Q.ips). The company runs two multiplier lines of elite fish (labelled „Robust"[R] and „Effective"[E]) that are different products produced by varying the breeding goal (R are weighted for disease resistance and swim performance, E for growth and commercial traits). The use of two elite lines ensures that a cross between the lines will use unrelated parents. Eggs which are IPN QTL selected achieve approximately 5 c price advantage over untested eggs. Production of QTL tested eggs has risen from 35 million in 2009 to 150 million in 2011. The advantage of these eggs has already been realised by hatcheries that have experienced natural IPN

challenge, results from across AquaGen"s customers indicate 47.5% survival in susceptible lines, 76.6% in RxE and 97.7% in RxE QTL.

On 6th and 7th September we visited Aquagen"s freshwater facilities at Hemne and Tingvoll. Biosecurity rules in Norway permit brood fish to be held at sea and then moved back to freshwater for conditioning and spawning, though fish must remain within disease control zones. Because the hatcheries are built at sealevel on deep fjords, the broods can simply be well-boated and pumped into the freshwater tanks. Unlike Tasmania, the spawning method is lethal, so fish are not reconditioned and reused. This allows spawned animals to be tested for pathogens and egg batches from suspect parents to be disposed of. The broodstock are separated to three production regimes to extend the spawning season: „Early" are lit at sea from spring and not lit in the hatchery for mid-September stripping; „Natural" are held at ambient light throughout and are stripped in November; „Late" are lit from late May to delay spawning until January. Once in freshwater, all broodstock are held at 17°C until 30 days pre-spawning, before dropping to 7°C. Freshwater photoperiod is 8D:16N. Eyed egg production is further extended by temperature and chilling regimes. Natural pH at Tingvoll is 5.27 which is raised to 6.6 for spawning.

Eggs for the nucleus are incubated in a system of 10 L Chilean bulk bins (the eggs of two females per bulk). Commercial production is in 180 L bulk bins.

A unique and recent development of the AquaGen system is that 25,000 smolt from the nucleus are swim-trialled as a robustness measure. The company has built a large „aquadrome" test unit (Fig. 3) which is designed to give a linear water flow in the upper test chamber. The test lasts for three hours and water speed is stepped through 0.4 m/sec increases until all fish have failed, the failed fish falling back onto an inclined screen and PIT tag ID registered. Because water is recirculated it is necessary to oxygenate the water to 110% saturation prior to testing, fish are allowed to familiarise themselves for 15 minutes pre-test with the current at low speed. Each test run holds 200 kg of fish (~1000 fish). The ability to test every fish to exhaustion gives a number of options for genetic analysis of the data because fish can be scored at „time to failure" or „failure speed". Heritability of swimming is around 0.25. A similar trial that I have previously developed to test swimming resilience of AGD affected fish in the Tasmanian SBP is limited to a binary classification of failed/not failed due to limitations with pump speed and test vessel design. Further research is being conducted on Aquagen „winners" and „losers" by Harald Takle (Nofima).

Tingvoll is a multiplier stripping station, 6000 salmon are taken through the station to produce 70 to 100 million commercial eggs. Broodstock tanks are 10mx1.5m or 8mx2m. The process is simply to condition brood fish, strip eggs and despatch them to commercial hatcheries. There is no egg incubation on-site.

On 7/09/2011 we visited NOFIMA"s research station at Sunndalsøra. A detailed introduction was given by Per Brunsvik (Station Manager): The hatchery was established in 1971 to collect wild salmon and establish the breeding program prior to commercialisation of AquaGen. The station is now purely for research, much of which is performed on contract and „in confidence" for commercial companies. The station enjoys ready access to seawater and freshwater which has been used for cooling at the nearby powerplant (thus at 9 to 16°C) and boasts the newly opened Nofima Centre for Recirculation in Aquaculture (http://www.nofima.no/marin/en/artikkel/4869733954035201089). Research is centred upon fish nutrition, animal welfare and breed technologies. All salmonids are brought in as production eggs, there are no fish returned from saltwater.

Greta Baeverfjord gave an overview of research into fish deformities that have been undertaken as part of the European FineFish project. The number one factor causing malformation to heart, internal organs and spinal development is temperature. If this is too high there are changes to heart shape and malformations such as septum traversum and situs inversus. Excess cooling to <1°C prior to 100 °day age also causes issues. The second issue is mineral availability, especially phosphorus and zinc. This is especially important during periods of fast growth (low FCR). A breakpoint of 16°C is suggested for marine deformities. Spinal deformity is best seen by X-ray, mammogram x-ray has the best settings for fish work (dental x-ray would be unsuitable). There is genetic variation of spinal deformity (linked to mineral utilization ability), but selective breeding is not an efficient way to fix the problem – it is much better to control rearing environment and nutrition.

Fig. 3: 'Aquadrome' swimming performance test unit at AquaGen Hemne.

Bendik Fyhn Terjesen gave us an overview of Nofima Centre for Recirculation in Aquaculture. This facility is unique in Norway as the only recirculation research facility of approximate commercial size (1750 m²). The centre features six experimental sections and has a total volume of 1100 m³. The centre has access to both freshwater and seawater. The system is run on a semi-commercial basis, with commercial production at 500,000 smolt per annum.

11/09/2011 to 16/09/2011 NOFIMA Marin

Nofima Marin is a business oriented research institute engaged in R & D, innovation and knowledge transfer for the national and international fisheries and aquaculture industry, concentrating upon breeding and genetics, feed and nutrition, fish health, efficient and sustainable production, seafood processing and product development. This stage of my tour was hosted by Bjarne Gjerde (Senior Scientist) and involved time at the Marine Research facility at Averøy and the Research Laboratory at Ås.

The Averøy station (Fig. 4) was established in 1973 and consists of sixty-four 5x5x5 m (125 m^3) certified experimental cages and twenty 7x7x7 m (350 $m^{3)}$ with additional storage units for experimental fish (2400 m³ each). The facility has laboratories and a small processing area and is run on a semi-commercial basis, selling research capacity and producing approximately 300 t of product. Most work is nutrition based, so each pen is fitted with electronic feeders and „lift-up" feed traps to measure feed waste amounts. The Farm Manager (Michel Sarmiento Guajardo) gave me a full tour of the facility. Because of my interest in breeding for resistance to parasitic diseases, the sealice work carried out at Averøy (as previously described by Harvard Bakke at Fish Breeders) was of particular interest. This technique has been applied since 2008 and is included in the SalmoBreed breeding goal. This year, AquaGen are also purchasing research time to have their animals assessed. The test involves removing lice egg strings (available from naïve *Lepeophtheirus* bred in Tromsø), enumerating the eggs and sorting copepodids by hatching date. Initial work involved tank infections,

but this tends to produce an unnatural settlement because of the behaviour of the fish, settlement of 80% of copepodids can be achieved in tank. The cage test involves releasing 100 copepodids per fish into a liner enclosed cage and oxygenating for 2-3 hours to achieve 15% settlement success. The number of lice per fish is counted approximately 2-3 weeks after infection, this process requires 4 people (plus two handling fish) and achieves approximately 100 fish/hour, with fish held in individual buckets that also need to be checked for motile lice. There is a strong operator effect in the data. The level of light can also affect counts, so in winter these are performed indoors using a standard „daylight" spectrum lamp. Based upon CSIRO"s work linking gill score to bathing frequency, Nofima plan to look at the consistency of reinfection and the „time to treatment threshold".

Bjarne explained that SalmoBreed produces its breeding nucleus of 300 families as three distinct lines, with a 1x2 breeding design. Linkages are maintained between the lines through use of frozen milt, allowing males to be used more intensively. Genetic services are provided by Nofima. Most of the activity is in the four multipliers, each of which are self-controlled. The program benchmarks its progress by estimating improvement from breeding values and comparing with actual performance. Bjarne noted that siblings of sealice tested fish (10 best and 10 worst families) had been assessed following six months of natural challenge with a 20% difference in lice number recorded. It was noted that selection for lice resistance would be more powerful if within-family variation could be utilised, but tested animals from Averøy cannot be taken back to freshwater. Therefore, whole genome selection is the logical choice for future development – this could be cost-effective if applied on a categorical scale of phenotypic trait grouping.

Fig. 4: Averøy Research Station – 5x5 m pens showing feed units and feed waste „lift-up" system.

SalmoBreed are now able to utilise the IPN QTL (the availability has been controlled intellectual property for two years). The company have developed a polygenic QTL for Pancreas Disease (PD) which shows some promise and they hope to develop QTL"s for lice resistance. They are planning to

test for swimming performance as a robustness trait and will measure how this trait links to long-term performance traits (growth, survival).

At Ås I met with a Jørgen Ødegård and discussed genetic analysis of survival traits that can be applied to disease resistance and robustness testing, he is familiar with this issue as he is responsible for the analysis of AquaGen"s swim test. Because the proposed „AGD Resilience" test will include data on „time to failure" with censored results, the use of a „test day survival score" divided to 10-30 categories seems to be the best option for our analysis. Data will have to be corrected phenotypically for gill score. Dr Ødegård suggested that DMU software would be more flexible. Potential collaboration between Nofima and CSIRO was discussed to assist in developing survival models.

I also spoke to Ingrid Olesen about Nofima"s proposed study on heart deformities and robustness. The aim of the study is to measure swimming ability and commercial performance of a test population of SalmoBreed progeny in relation to cardiac malformation. The effects of exercise and rearing environment will be included to understand the interaction of genetics and environment in robustness traits. From this discussion a project ("HeartBeat") has been developed and submitted to the Norwegian Research Council with CSIRO as a contributor.

On 15th September I met with Prof. Åshild Krogdahl at the Norwegian School of Veterinary Sciences (APC Aquaculture Protein Centre) and presented an overview of SGS in Tasmania. There have been a number of summer issues explored in Norway include "floating shit" and fat belching. The issue of Rainbow Trout Gut Enteritis (RTGE) was also discussed. Gut microbiota plays a key role in disease resistance (e.g. increased susceptibility to IPN). The APC researches replacement of fish protein, including the use of Soy meal (causes cell proliferation and flattened mucosa), issues can be worse if fed with Lupin meals. Lupins are also implicated in stomach ulcers (maybe a diet hardness issue).The APC studies gut gene expression and gut flora assemblages in relation to diet, it was noted that bacteria in casts can differ from adherent bacteria in the mucosa and lumen.

I also had opportunity to meet with Prof. Trygve Poppe at the Veterinary Institute. He has documented many changes in heart morphometry in aquacultured fish. This was an opportunity for me to learn a little more about image analysis techniques that have been employed. Trygve also discussed the usefulness of colour Doppler in non-destructively measuring heart shape and volume.

INDUSTRY IMPACT

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

The focus of this study was the assessment of robustness traits in breeding programs and the impact of cardiac morphology upon robustness. I currently have an „AGD Resilience" funding application with the FRDC, if this project is approved the concepts discussed in Norway may be incorporated into the project. Although „Robustness" is assessed at AquaGen (and will be incorporated by SalmoBreed), the true value of this trait remains untested in terms of its impact upon growth and survival in the field. With AGD in mind, it is possible that growth between measures (i.e. the ability to deal with chronic stress) could be assessed in relation to the „acute" stress of the swimming test.

The main outcome was that CSIRO are linked to Nofima on their „HeartBeat" project in conjunction with SalmoBreed. This project was submitted to the Norwegian Research Council on 12/10/2011. Further cooperation between Nofima and CSIRO is likely to develop, particularly involving the genetic analysis of survival data.

Updated information on the cause of deformities, in particular the increased mineral requirement during periods of high growth, is of particular importance to the Tasmanian industry.

SUMMARY OF CHANGE IN INDUSTRY

The main focus of this study was the assessment of robustness traits in breeding programs. The SBP needs to ensure that selection for commercial traits (disease resistance, growth, etc.) does not negatively impact upon cardiac health and robustness. Although no immediate impact is envisaged, the long-term benefit is that resilience may be incorporated into the breeding goal. Non-destructive testing (swim performance, doppler heart imaging) will allow resilience to be assessed cost-effectively and routinely. Improved animal welfare (through breeding and husbandry) is a positive for long-term customer perception of the industry.

WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?

If research indicates that there is genetic variation in resilience traits, these may be incorporated into the breeding goal of the SBP if it is cost-effective. It is also likely that husbandry techniques can be improved to reduce stressors during marine growout.

WHAT BARRIERS ARE THERE FOR CHANGES TO OCCUR?

Before a selection trait is incorporated into a breeding program, it is necessary to establish that there is adequate genetic variation in the trait and that there is cost benefit in selection that cannot be achieved through improved rearing environment. The selection trait should be that closely correlated to the objective trait (in this case, robustness). It is preferable that selection traits are simple and cost effective to measure, as the difficulty and cost of measurement and selection may outweigh the benefits.

IF NOT ALREADY HAPPENING, WHEN WILL THE CHANGES OCCUR?

The uptake of robustness traits into the SBP is dependent upon the results and recommendations of research projects and recommendations to the saltas SBP Technical Committee. On the current proposed research schedule, we hope to recommend a strategy in 2013. Some aspects of the work may be adopted sooner, depending upon results from Norwegian breeding programs.

WHAT IS THE LIKELIHOOD THAT THESE CHANGES WILL OCCUR?

There is a high chance that robustness traits will be adopted by the Saltas SBP. The project addresses priority areas of the Tasmanian Salmonid Growers Association 2011 R&D Strategy, including „AGD – Selective breeding program" and „AGD – determination of what is actually killing the fish". Industry has reiterated it"s need to minimise AGD bath handling losses, so is likely to back efforts to produce „well rounded" animals from the SBP.

WHAT BARRIERS ARE THERE TO DOPTION OF THESE CHANGES AND WHAT ACTION COULD BE TAKEN TO OVERCOME THESE?

It is likely that the swim-test will have to be improved so that it can be assessed as a „continuous" trait. However, there is value in a simple binary trait („failed" „did not fail" as currently proposed. The cost of altering the test will be assessed.

The need to adopt robustness traits into the SBP will be gauged upon the cost of assessment and the estimated benefit of improved welfare and commercial improvement. Any addition of objective traits to the breeding goal may lower selection pressure upon other commercial traits. Therefore, the genetic correlation between „robustness" and commercial traits needs to be considered by the SBP Technical Committee before implementation..

COMMUNICATION OF PROJECT/EXTENSION ACTIVITIES

WHAT IS THE OUTPUT THAT NEEDS TO BE COMMUNICATED?

Selective breeding allows us to select for important commercial traits (such as growth and disease resistance). It is also necessary to ensure that animal welfare is addressed by producing animals that are able to deal with the stress of handling operations, changes in nutrition and adverse environmental conditions. The inclusion of robustness traits in selection programs will ensure that aquaculture production remains sustainable and profitable.

WHO IS/ARE THE TARGET AUDIENCE/S?

The Tasmanian Salmonid Industry and Saltas SBP.

WHAT ARE THE KEY MESSAGES?

That „Robustness" traits are being incorporated into Norwegian salmonid breeding programs.

That cardiac health is closely linked to robustness. There is benefit in collecting data on variation in cardiovascular traits even if selection pressure is not applied. This gives the industry a benchmark to assess whether cardiac health is changing as a correlated trait.

That the main cause of deformities is rapid growth. The primary cause is warm water in the hatchery rearing phase, but it is still possible to have profound effects at sea. Low FCR during periods of fast growth causes minerals to become less available, this affecting skeletal and organ development.

COMMUNICATION CHANNELS

Channel	*Who by*	*When*
Saltas Technical Commitee	Richard Taylor	Ongoing
Salmon Industry (TSGA)	Richard Taylor	Ongoing

LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS

WHAT IS YOUR FEEDBACK?

This bursary assisted me in attending the Fish Breeders Round table and visiting a number of research and fish breeding establishments in Norway. The main value of the trip was in developing relationships with Researchers working on similar issues. The ability to develop knowledge and networks is essential for my career development and will benefit the Tasmanian salmon aquaculture industry. It is likely that robustness and cardiac traits will be important in other aquacultured species in Australia.

ACKNOWLEDGEMENTS

I would like to thank the CRC for supporting my trip to Norway. I am particularly grateful to Dr Nick Robinson for his assistance with the CRC application and in arranging contacts in Norway.

- I would like to thank Dr Arne Storset for spending three days to show Brad Evans and I around the AquaGen facilities and for his open and helpful explanations of the large scale operation of a commercial breeding company. The professionalism of the people we met was reflected in the excellent quality of the facilities and fish that we witnessed.

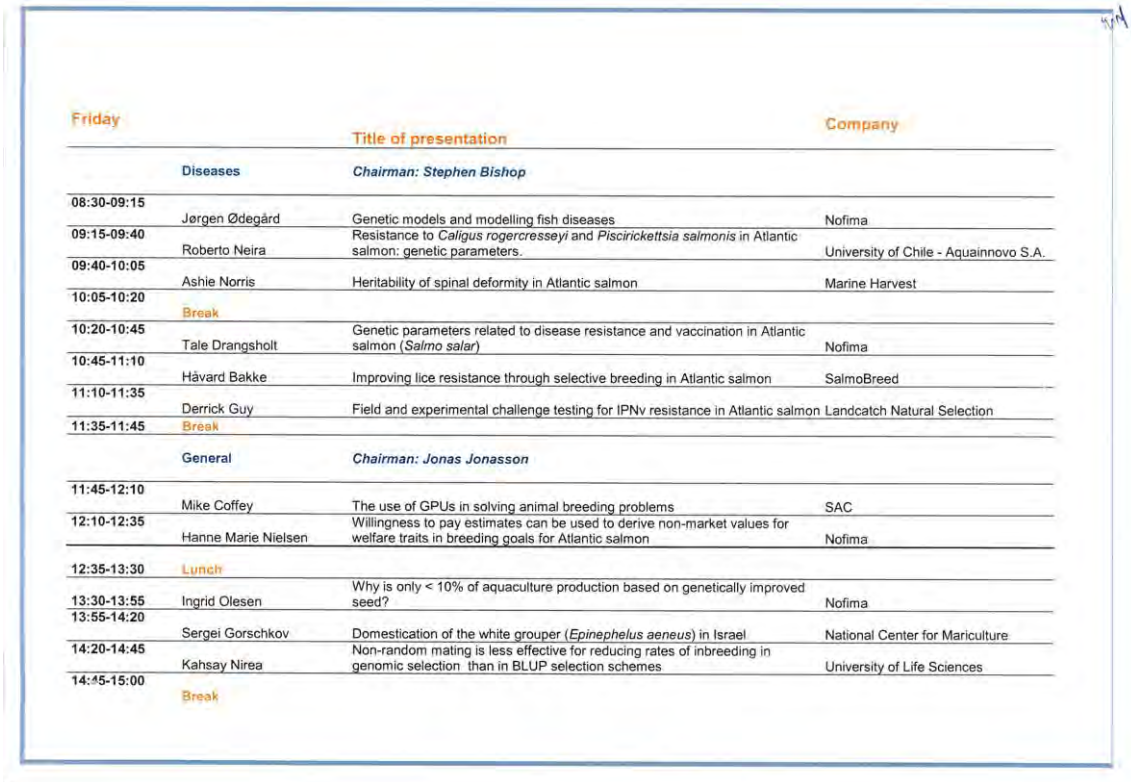
- Dr Bjarne Gjerde from Nofima for taking me to Averøy and Sunndalsøra and then driving to Oslo. I particularly enjoyed the opportunity to discuss aspects of fish breeding research and to witness the Norwegian scenery.
- Dr Kari Kolstad (Director of Research) for supporting my visit to Nofima"s office at Ås, I appreciated the opportunity to network with Nofima scientists.

APPENDIX

Appendix 1 : Fish Breeders Round Table presentations and list of participants

Program Fish Breeders' Round Table 2011
September 1-3, Sola Strand Hotel, Stavanger, Norway

Thursday		Title of presentation	Company
13:30-13:35	Welcome		Nofima
	Design of breeding programs	***Chairman: Nick Elliott***	
13:35-14:00	Peter Amer	Simulation of breeding scheme designs for new aquaculture species	AbacusBio Ltd
14:00-14:25	Robbert Blonk	Small scale breeding programs for natural mating species	WUR/IMARES
14:25-14:50	Hein van der Steen	Multiple versus single line development in shrimp	Stonebridge breeding
14:50-15:05	Break		
15:05-15:30	Nick Robinson	Breeding for "unconventional" situations- How feasible is walkback selection for barramundi?	Nofima
15:30-15:55	Hans Komen	Low cost breeding programs	WUR
15:55-16:20	Sven Arild Korsvoll	Breeding program for A. salmon in Chile	AquaGen AS
16:20-16:35	Break		
16:35-17:00	Brad Evans	Atlantic salmon selective breeding: the Tasmanian Approach	Salmon Enterprises of Tasmania (SALTAS)
17:00-17:25	Panteleon Kakie	The challenges of breeding fish in Africa	Kachuks Agro Farms Co.
17:25-17:50	Alex Safari	Fish breeding and genetics in WorldFish: where to now?	WorldFish
18:10	Pick up from the hotel reception for dinner at Måltidets Hus		



Friday		Title of presentation	Company
	Diseases	***Chairman: Stephen Bishop***	
08:30-09:15	Jørgen Ødegård	Genetic models and modelling fish diseases	Nofima
09:15-09:40	Roberto Neira	Resistance to *Caligus rogercresseyi* and *Piscirickettsia salmonis* in Atlantic salmon: genetic parameters.	University of Chile - Aquainnovo S.A.
09:40-10:05	Ashie Norris	Heritability of spinal deformity in Atlantic salmon	Marine Harvest
10:05-10:20	Break		
10:20-10:45	Tale Drangsholt	Genetic parameters related to disease resistance and vaccination in Atlantic salmon (*Salmo salar*)	Nofima
10:45-11:10	Håvard Bakke	Improving lice resistance through selective breeding in Atlantic salmon	SalmoBreed
11:10-11:35	Derrick Guy	Field and experimental challenge testing for IPNv resistance in Atlantic salmon	Landcatch Natural Selection
11:35-11:45	Break		
	General	***Chairman: Jonas Jonasson***	
11:45-12:10	Mike Coffey	The use of GPUs in solving animal breeding problems	SAC
12:10-12:35	Hanne Marie Nielsen	Willingness to pay estimates can be used to derive non-market values for welfare traits in breeding goals for Atlantic salmon	Nofima
12:35-13:30	Lunch		
13:30-13:55	Ingrid Olesen	Why is only < 10% of aquaculture production based on genetically improved seed?	Nofima
13:55-14:20	Sergei Gorschkov	Domestication of the white grouper (*Epinephelus aeneus*) in Israel	National Center for Mariculture
14:20-14:45	Kahsay Nirea	Non-random mating is less effective for reducing rates of inbreeding in genomic selection than in BLUP selection schemes	University of Life Sciences
14:45-15:00	Break		

15:00-15:25	Bjarne Gjerde	The effect of early culling with respect to body weight at tagging on genetic gain and biases of genetic gain for harvest body weight in fish breeding programs	Nofima
15:25-15:50	Jan Sunde	Cryopreservation of milt	BioKapital AS/ Cryogenetics AS
15:50-16:15	Marc Vandeputte	Parentage asssignment in an octoploid species, the Siberian sturgeon *Ascipenser baeri*	INRA
16:15-16:30	Break		
16:30-16:55	Morten Rye	Response to selection for increased growth rate in Nile tilapia in China	Akvaforsk Genetics Center
16:55-17:20	Renate Kvingedal	Mapping gut microflora in Atlantic salmon using molecular methodologies	EWOS Innovation AS
19:00		Dinner at Sola Strand Hotel	

Saturday		Title of presentation	Company
	Genomics	***Chairman: Anna Sonesson***	
08:30-09:15	Mike Goddard	Genomics in aquaculture breeding	DPI, University of Melbourne
09:15-09:40	Ross Houston	Genome-wide and trait-linked SNP discovery using RAD sequencing in Atlantic salmon	The Roslin Institute
09:40-10:05	Matthew Baranski	QTL mapping for PD resistance in Atlantic salmon	Nofima
10:05-10:20	Break		
10:20-10:45	Hossein Yazdi	Quantitative Trait Loci (QTL) analysis using Bayesian approach for resistance to Pancreas disease in salmon	Akvaforsk Genetics Center
10:45-11:10	Matthew Cleveland	Imputation in sparsely-genotyped pedigrees	Genus plc
11:10-11:35	Harri Vehviläinen	The use of microsatellites in data recording and breeding value estimation	MTT Agrifood Research
11:35-12:00	Victor Martinez	Genetics of *Piscirickettsia salmonis* resistance	University of Chile
12:00-13:00	Lunch		
13:00-13:25	Thomas Moen	Industry-level performance evaluation of IPN-resistant salmon eggs selected by means of MAS	Aqua Gen AS
13:25-13:50	Mark Henryon	Breeding fish for resistance to disease is difficult even with genomic selection	Århus University/ Danish Agriculture and Food Council
13:50-14.15	Woo-Jai Lee	A new approach to improve genetic gains by using genomics and quantitative genetics tools	GenoMar AS
14:15-14:30	Break		

	Other traits	*Chair: Morten Rye*	
14:30-14:55	Anna Sonesson	Genetic parameters for obesity traits in Atlantic salmon	Nofima
14:55-15:20	Pierrick Haffray	Does fish need head? Genetic parameters of production trait and bone development in trout	SYSAAF
15:20-15:45	Theodor Kristianson	Genetic correlation between growth and maturation in Atlantic cod	Stofnfiskur
15:45-16:10	Olafur Kristjansson	How to obtain appropriate genetic parameters for growth and carcass quality traits in Atlantic salmon	Stofnfiskur
16:10-16:30	Summing up		

Nofima

AKVAFORSK Genetics Center AS

AquaGen

SalmoBreed

The Research Council of Norway

Nofima

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