FINAL REPORT

AWARD RECIPIENT: Torin Philpott - Brood stock to Fry hatchery manager

ADDRESS: Petuna Aquaculture, 134 Tarleton Street, East Devonport, TAS 7310

HOST ORGANISATION: Petuna Aquaculture

DATE: 23 July 2019

ACTIVITY UNDERTAKEN

Participation in the University of Stirling's two-week professional development course in Aquatic Animal Reproduction and Genetics. This course covers broodstock management, spawning, egg quality and gender control. Learning outcomes of this course include understanding the importance of genetic management for the long-term maintenance and improvement of hatchery produced finfish species, as well as understanding the importance of combining reproductive and genetic technologies to ensure the sustainable and cost-effective production of high-quality seed for the Tasmanian salmonid aquaculture industry.

OUTCOMES ACHIEVED TO DATE

The Reproductive and Genetics course covered a range of topics that will be useful to transition into current practices in Tasmania. Topics included broodstock management through the manipulation of photoreceptors to advance or delay spawning, breeding program strategies, spawning practices, the time of ovulation in broodstock and its detrimental effect on fertilisation and offspring.

Genetic management using a breeding program could be one of the major improvements in the salmonid industry. With the industry still in its infancy in comparison to terrestrial farming (pigs, chickens and cows), there is potential to maximise genetic gain through targeted performance traits, shortening salt water growth times, and target the genetics of the salmon who have adapted to the current Tasmanian farming environment.

This can be achieved by creating a population of fish with the known parent's genetics. With equal egg number contributed to the population from the parents, multiple groups are created dependent on the company or industry setup. A small group created to become future broodstock is called the Nucleus. The number of the other groups created is dependent on the number of saltwater sites. The Nucleus Broodstock are kept at the hatchery, while the other groups are transferred to the saltwater sites and are evaluated from transfer to harvest for the traits in which the company are wanting to follow. This information is then used to select the future offspring, using the siblings from the Nucleus of the same genetics that perform well to the traits the company are selecting against.

Background and Need

The farming of Atlantic salmon in Tasmania takes place in one of the warmest salmon farming regions of the world, and with this comes fast growth rates. The warm waters mean Atlantic salmon can be grown to a marketable size within 16-18 months, however, with this faster growth rate there are some negative challenges such as early maturation with consequent downgrade in flesh quality and reduced market value. There is also an increase in susceptibility to disease such as Amoebic Gill Disease (AGD), Pilchard Orthomyxovirus (POMV) and *Yersinia ruckeri* to name a few in both seawater and freshwater sites.

The Atlantic salmon industry in Tasmania is a closed population. The industry is not permitted to import more stock or different strains from other countries, nor source new stock from wild caught, nor, for biosecurity reasons, able to move broodstock from a seawater site back to a freshwater hatchery. The companies growing Atlantic Salmon in Tasmania need to be proactive in ensuring the genetic diversity and success of the population moving forward.

This training opportunity will lead to improving knowledge in genetics and reproduction strategies for Atlantic salmon farmed in Tasmania.

Objectives

The aim of this project is to improve the overall fish health and survival of larvae and eggs at Petuna Aquaculture's Cressy hatchery in Tasmania by upskilling current staff and then to pass on this new knowledge to other team members. The University of Stirling, Scotland, offer a two-week professional development course in Aquatic Animal Reproduction and Genetics. This course covers broodstock management, spawning, egg quality and gender control. Learning outcomes of this course include understanding the importance of genetic management for the long-term maintenance and improvement of hatchery produced finfish species; and understanding the importance of combining reproductive and genetic technologies to ensure the sustainable and cost-effective production of high-quality seed for the industry.

The Course

The module was part of a 6-month postgraduate course with each topic taking 2 weeks to be covered. The module covered the genetic and reproductive management of captive fish populations and was held between 30th October to 10th November 2017. The reproductive biology of important finfish species was described and how this can require different management approaches. The principles of sexual maturation and its control and manipulation using environmental cues was described in several important species. The genetic principles of broodstock management and selective improvement was presented, as were the consequences of genetic degradation caused by poor practice. The emphasis was placed on efficient hatchery production and the sustainable management and improvement of aquaculture stocks, with a focus on finfish. A broad range of species were used to illustrate the module.

Students completing the module were expected to:

- have a basic understanding of the reproductive biology and its control in major farmed freshwater and marine finfish;
- understand the importance of genetic management for the long-term maintenance and improvement of hatchery produced finfish species;
- understand the importance of combining reproductive and genetic technologies to ensure the sustainable and cost effect production of high-quality seed for the industry.

The module was taught as a series of lectures (as listed in Appendix I), demonstrations and practicals, including visits to a salmonid and a tropical research facility. Staff from the Genetics and Reproduction Research Group and the Sustainable Aquaculture Research Group at the University of Stirling contributed to the module.

Learning Outcomes

The Reproductive and Genetics course covered a range of topics that will be useful to transition into current practises in Tasmania. Topics included broodstock management through the manipulation of fish sensory inputs to advance or delay spawning, breeding program strategies, spawning practices, the time of ovulation in broodstock and its detrimental effect on fertilisation and offspring.

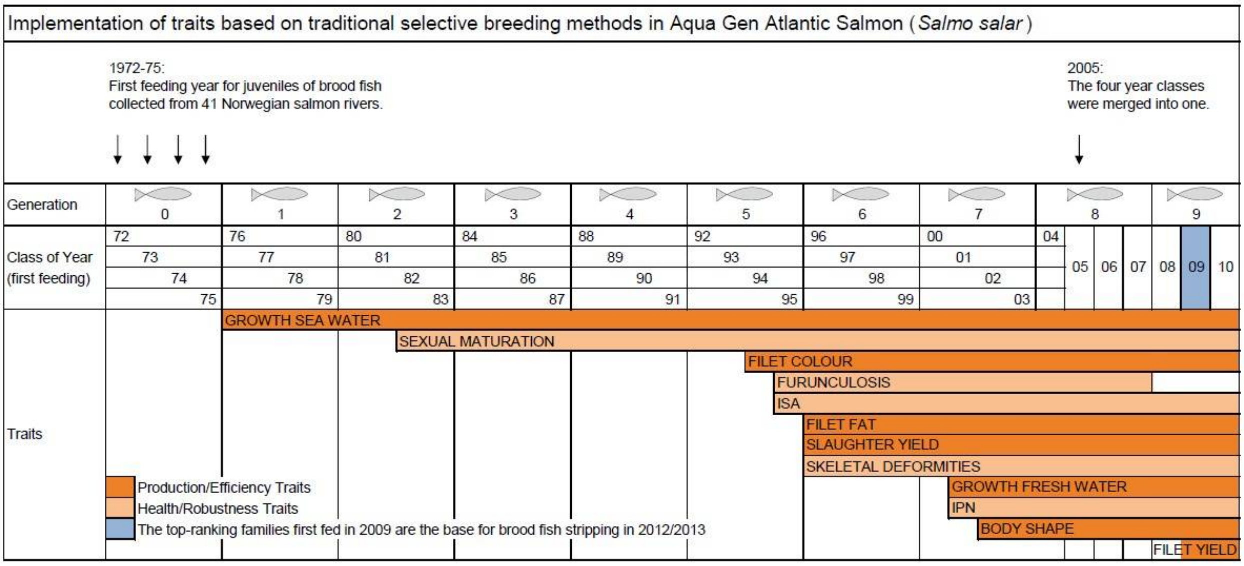
<u>Breeding Programs</u> - Genetic management through the use of a breeding program could be one of the major improvements in the salmonid industry. With the industry still in its infancy in comparison to terrestrial farming (pigs, chickens and cows), there is potential to maximise genetic gain through targeted performance traits, shortening SW growth times, producing robust population, and overall reduce the production cost at sea and enhance the final product.

This can be achieved by creating a population of fish with the known parent's genetics. With equal egg number contributed to the population from the parents, multiple groups are created dependent on the company or industry setup. A small group created to become future broodstock is called the Nucleus. The number of the other groups created is dependent on the number of saltwater sites. The Nucleus Broodstock are kept at the hatchery, while the other groups are transferred to the saltwater sites and are evaluated from transfer to harvest for the traits in which the company are wanting to follow. This information is then used to select the future offspring, using the siblings with the same genetics from the nucleus that performs well to the traits the company are selecting against. In the past couple of years Petuna has completed this with one grow out site at Rowella. The data and performance for the sea site will be analysed and the siblings with the same genetics with be used in the spawning in 2020. In 2019, Petuna had 2 groups of "Evaluation pen's", with one group sent to Rowella in July and another to Macquarie harbour in August. These fish will be harvested and analysed ready for the spawning in 2021.

<u>Year-round Egg Production</u> - Common practice for salmon production in Scotland and Norway is for the year-round production of the "best quality" eggs, with most of the hatcheries now opting for egg suppliers such as AquaGen to supply these eggs with up to four inputs throughout the year. These eggs are the progeny of parents from breeding programs which select for a range of traits and heritability

performance factors which date back to the 1970's as shown in Figure 1. Companies such as these opt for out of season broodstock to ensure they can maintain quality eggs for the demand.

Figure 1: AquaGen traits selections history.



Salmonid species grown in Tasmania only spawn once a year. The transition to manipulate small populations of broodstock to either advance or delay spawning around the natural spawning period in May is desired. This will maximise production, minimise risk to the industry and provide high quality eggs all year around.

In recent years, Tasmanian salmon producers have started to manipulate small populations of broodstock to advance spawn in March-April. However, due to our current climate the further advancing manipulation for broodstock would require a large financial investment if a broodstock facility hasn't been established; the use of production tanks could be used but will impact on the number of smolts a hatchery can produce. A broodstock facility may be required to ensure the broodstock can be in their peak condition and ensure their gametes will be in optimum condition at fertilisation. These gametes with the correct incubation conditions will result in a stronger and heathier embryo, which when hatch will have a high-quality food source from its yolk sac created from the female broodstock to transform into a larger, stronger and more robust fry when starting to feed.

Controlling maturation and spawning can be obtained through the use of either hormonal induction or environmental control. A problem that can occur with the reliance on natural spawning is the female broodstock are ready spawning and the male's gametes aren't ready for action. Another problem that can occur naturally is the combination of cues aren't there, for example the natural lighting should have the broodstock ready for spawning however the water temperature is unseasonably higher than normal. The use of hormonal induction is primarily to induce the spawning and to synchronise the timing of spawning, best used on the males to ensure good sperm movement and synchronise ovulation with the female broodstock. Environmental control of maturation is achieved through the use of photo period and temperature. This practise is best for controlling the female broodstock. However, by further advancing maturation away from their natural spawning time, fewer fish achieve the energetic status for gonad development, or a size to complete maturation, and so this is arrested or inhibited. This can provide a positive tool with which to prevent early maturation in female production batches and possibly reduce the reliance on triploid production batches. This outcome can be done through the manipulation of the decision window by shortening it, as shown in the following graphs (Figures 2-4).

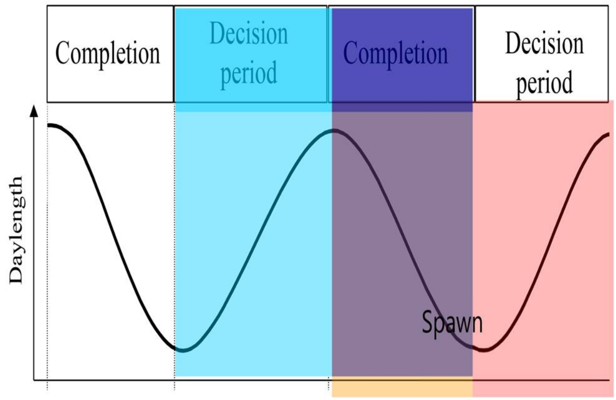


Figure 2: Shows the decision period, where broodstock decide if they are going to produce eggs and the number of eggs to produce. The quality of eggs is all dependent upon the health of the broodstock, and environmental conditions. Completion time where the broodstock puts energy into the eggs ready to spawn.

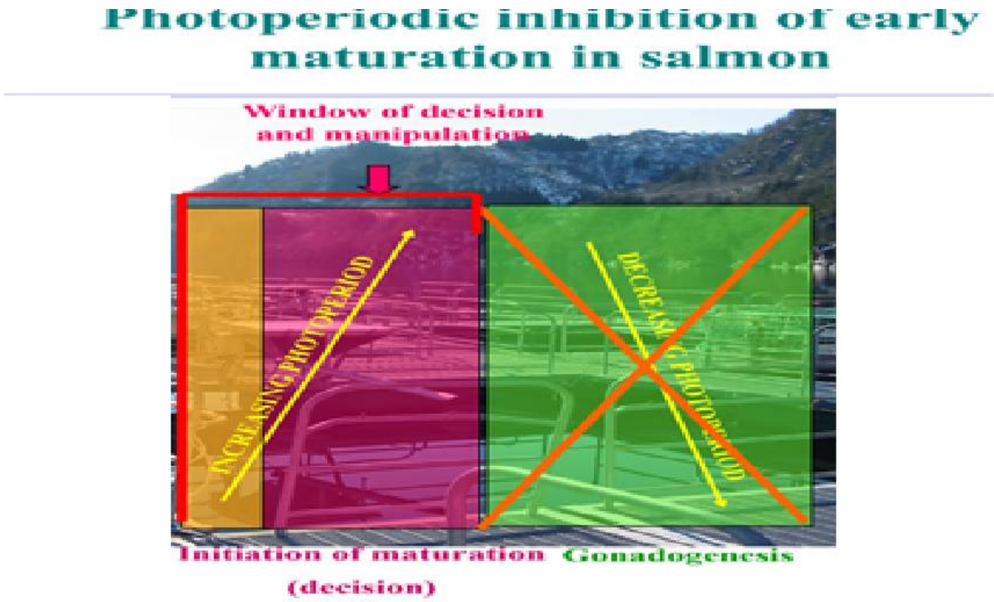


Figure 3: Demonsration of how the manipulation photoperiod (if shortened) can effect the decision window and inhibit the maturation of stock. Usually benificial for production stocks. The other option is to have a continual summer to not give the fish the decreasing photoperiod and the trigger to put energy into gametes (eggs and sperm).

The key factors effecting fertilised egg quality include the nutritional status of hens, environmental conditions, hormonal induction, the timing and process of stripping, milt quality, fertilisation and genetic manipulation. Monitoring these points during their critical timing throughout the broodstock decision and completion windows and spawning process will increase the success of a hatchery's egg ferlilsation rate and fry health.

Egg Stripping - Some noticeable differences from Tasmanian practices were apparent in the handling of broodstock leading into spawning and during the stripping process. For egg quality, stripping needs to occur during the optimum time of ovulation. The process involves checking the females regularly during the spawning period. Any females that are ready are moved to holding pens/tanks for 2-3 days, until stripping, as it takes 3-5 days to reach optimum fertility after ovulation. While I was there, lecturer Dr John Taylor, who specialises in salmonids at the Stirling University, was involved in a study trying to determine the optimum time of ovulation through the use of ovarian fluid; hopefully this can create an indicator tool to pinpoint the optimum time of ovulation. If this was possible, it would make the selection of ovulatiing female broodstock easier, instead of requiring highly skill staff to assess the broodstock.

The graph in Figure 4 illustrates the importance of stripping broodstock at the optimum time, as removing eggs prematurely or too late when eggs over-ripen will seriously reduce the survival of the eggs and even fry post-hatch.

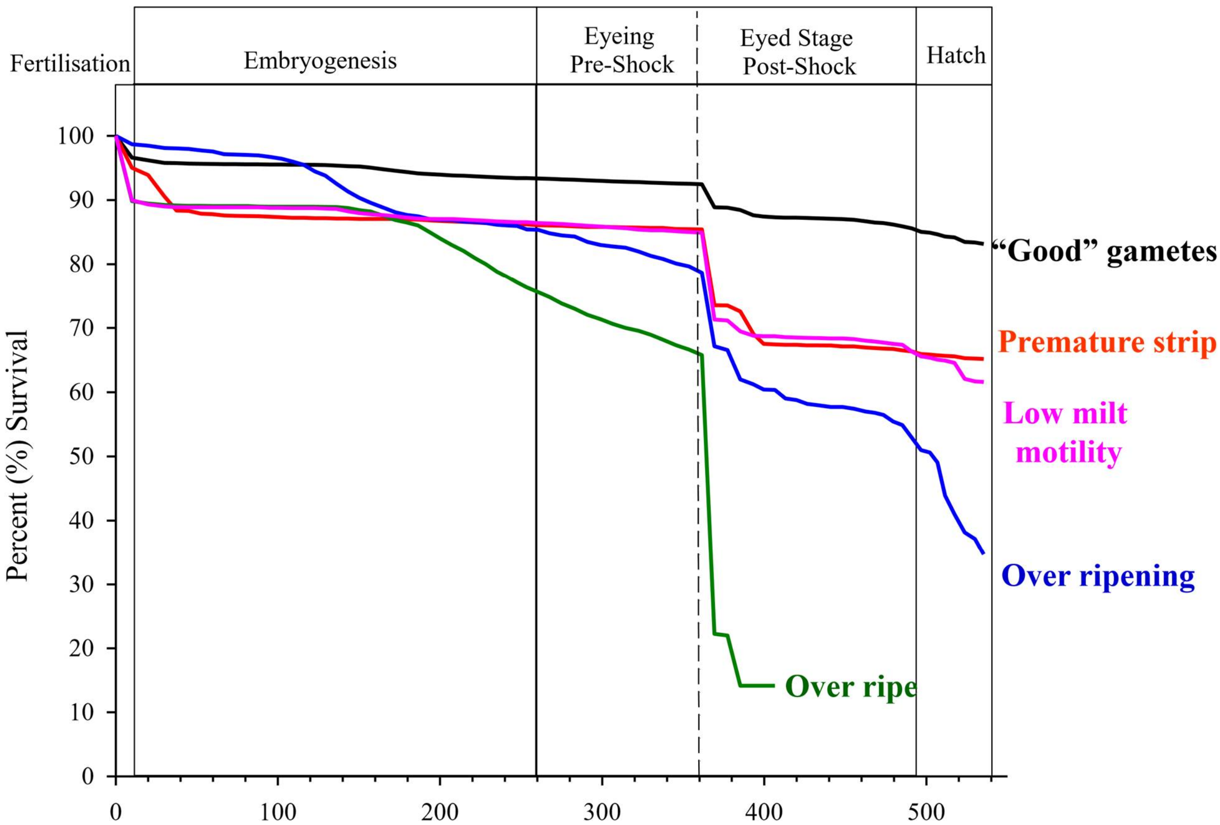


Figure 4: Comparison of egg survival based on stripping timing of hens.

A common practice for the stripping of eggs from the hens is to euthanise them with an overdose of anaesthetic and inject air into the female's abdomen to push the eggs out, as shown in Images 1 and 2. This process reduces the chances of damage to the eggs compared to manual stripping of eggs from the abdomen. Manual stripping can result in forcing eggs from the egg sac that may not have reached ovulation stage and been released into the abdomen. Once all eggs have been stripped from each hen, they are kept separated and fertilized with a predetermined cross with milt from a male (image 3).

In Scotland, as part of disease management program, all females are checked for Infectious Pancreatic Necrosis Virus, using ovarian fluid and kidney samples taken for testing. Eggs are kept separate until the results are available from the laboratory. Eggs were also disinfected prior to the water hardening with an isotonic saline and Buffodine® solution (buffered iodine for egg disinfection) to prevent bacteria entering the egg.

Image 1: Female broodstock euthanased and ready to be stripped	
Image 2: Female placed into cradle and stripped using air injection	

Image 3: Buckets of individual eggs with specifically selected males. Males were checked and milt frozen 2-3 weeks prior to spawning time.

Benefits and Adoption

Since completing this course in late 2017, Petuna has made some modifications to how the broodstock are handled and conditioned prior to stripping. Previously broodstock were housed in either flow-through or recirculation tanks for the duration of summer (warmer months). Then leading into the final phase prior to spawning broodstock were transferred to flow-through tanks or ponds. Petuna are now utilising the first feeding system, in this system we can control the environmental conditions with lighting and temperature. Doing this we can either advance or delay the spawning window on our broodstock. In 2019, Petuna were able to spawn the flowthrough stock (natural) in May, and 2 batches of delayed spawning in June and late July. All the broodstock are held in a tank environment and not in pond for both the flowthrough (natural and recirculation environments). This gives Petuna staff the ability to handle all broodstock to check for ovulation prior to spawning then transfer ovulating females to holding tanks for stripping in 3-5 days' time. This insures all broodstock are checked and no fish are missed compared with when checking fish in the ponds.

Other major changes were to the stripping and fertilization process previously carried out at Petuna. Changing from the manual task of hand stripping the eggs from the females (requiring 3-4 skilled staff) to using air injection method (1-2 staff) to remove the eggs from the females. Another major change to the spawning process is the fertilisation process. Previously once stripped and quality checked the eggs were pooled together prior to fertilisation. A problem with this is sometimes an individual egg can look good prior to fertilisation but post fertilisation and once in contact with water would turn white. These eggs can then potentially become substrate for fungus during incubation stage which can potentially kill the viable eggs if not managed through treatments. The practice at Petuna now is for eggs to be left in their individual buckets and fertilized. After fertilisation if the eggs start turning white due to having a weak egg shell (water in contact with protein inside the egg turns white) the eggs from one female can be discarded. Once eggs have passed their quality check post fertilisation they are pooled together in the designated incubator.

Petuna has also modified its broodstock selections, by creating a Petuna breeding program, with evaluation pens of both Atlantic salmon and Rainbow trout at Macquarie harbour (Strahan) and an Atlantic Salmon pen at Rowella (Tamar river). These grow out sites are very different growing environments. Using DNA sequencing we can analyse the families with higher mortality over the warmer more challenging months (summer), as well as fillet colour and growth from these fish and use the information to select future broodstock for their siblings back at the hatchery when they mature in their 3rd year. It was also decided to not utilise the SALTAS breeding program due to the evaluation pen in the south of Tasmania with the focus on AGD resistance as well as growth and fillet colour.

Conclusion

The unique environment in which Tasmanian salmon farming takes place is challenging. The educational experience at Stirling University and the sites that I visited in Scotland have allowed me to recognise where improvements could be made in our industry and at Petuna Aquaculture.

Acknowledgements

I'd like to thank all those involved in organising this further development course, Phillipa Sims, Petuna Aquaculture, staff at the Stirling University and finally the FRDC for allowing me this opportunity.

Appendix I

Lecture 1 – Introduction	- Prof. Brendan McAndrew
Lecture 2 – Genetic marker slides	- Prof. Brendan McAndrew
Lecture 3 – Reproductive Strategies	– Herve Migaud
Lecture 4 – Endocrinology of reproduction	– Herve Migaud
Lecture 5 – Gonadal development	– Herve Migaud
Lecture 6 – Environmental reproductive management	– Herve Migaud
Lecture 7&8 – Genetics of Broodstock management	- Prof. Brendan McAndrew
Lecture 9 – Light perception and lighting set ups	– Herve Migaud
Lecture 10 – Hormonal Induction	– Herve Migaud
Lecture 11&12 – Quantitative Genetics	- David Penman
Lecture 13 – Selective Breeding	- Professor Brendan McAndrew
Lecture 14 – Manipulation of sex ratio and ploidy	- David Penman
Lecture 15 – GM and Aquaculture	- David Penman
Lecture 16 – Genomics and Bioinformatics	- Dr Michaël Bekaert
Lecture 17 – Potential Genetics Impact	- David Penman
Lecture 18 – Tilapia	- David C. Little
Lecture 19 – Marine Hatcheries	- Andrew Davie
Lecture 20 – Hatchery Production of Carps	- Dave Penman
Lecture 21 – Salmonid Hatcheries	- Dr John Taylor
Lecture 22 – Hatchery Production of Catfish	- Dave Penman