COMPLETED PROJECT CASE STUDY

MOBILE BIOMARKER DETECTION FOR SEAWATER READINESS MONITORING

PARTNERS

Europharma Scotland | PrimerDesign | University of Stirling's Institute of Aquaculture

PROJECT LEADS

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BACKGROUND

The UK is the third largest producer of Atlantic salmon globally, with overall fish health and welfare the primary challenge to sectoral growth. One issue that can hamper efficient production is the ability for producers to identify the correct time to transfer fish from freshwater production units to seawater net pen sites. Atlantic salmon in the wild spend their first year in freshwater rivers and streams before migrating to the sea. During downstream migration, wild salmon undergo a physiological process called smoltification which adapts their bodies to life in seawater. Unlike their wild counterparts, in farmed salmon the smoltification process needs to be triggered via the use of specialised diets or tailored lighting systems, before the fish is transferred from freshwater production facilities to seawater net pen sites.

Smoltification is a highly managed process and a crucial period in the salmon's lifecycle: transferring to seawater too early can cause non-adapted fish to be more vulnerable to disease and mortality, particularly from infectious diseases such as Infectious Pancreatic Necrosis virus (IPNV). On the other hand, transferring too late can cause 'de-smoltification', i.e. regression to freshwater form. Smoltification is a physiological transformation within individual fish, and therefore it is crucial to understand its development across an entire population.

To assess a population of smolts for seawater readiness, one diagnostic tool producers use is an enzymatic assay that measures the activity of a salt transport protein (Na+ /K+ -ATPase, NKA) within a gill sample. If the activity is above a certain threshold, the smolts are deemed ready for transfer. However, this method is time-consuming with varying results, and is restricted to off-site laboratory analysis. Therefore, a rapid and transportable method of determining seawater readiness would be valuable to increase the rate of data provision and improve the farmers' ability to identify the best time for seawater transfer.

Project lead Europharma worked in collaboration with the University of Stirling and PrimerDesign to develop a rapid biomarker detection assay and mobile laboratory to assess seawater readiness in the field, i.e. on site at smolt hatcheries, to allow testing and direct delivery of results to hatchery managers for timely management decisions.

AIMS

The main aim of this project was to develop a rapid assay and mobile laboratory for assessment of smoltification, followed by assay validation against NKA activity assays along with saltwater challenge and chloride results. To achieve this, project partners established the following objectives:

1. Development of NKA qPCR assay for the Gensig Q16 instrument.
2. Testing the Gensig Q16 platform and mobile laboratory at smolt production facilities in Scotland.

DEVELOPMENT OF NKA QPCR ASSAY FOR THE GENSIG Q16 INSTRUMENT

Advances in technology and genetic analyses have allowed the development of portable real-time polymerase chain reaction (PCR) instruments. These instruments can be used to quantify the genetic material present in a DNA sample.

WORK DONE

Using protocols previously developed at the University of Stirling's Institute of Aquaculture, project partners formulated their own kit for use on the Gensig Q16 device, as well as establishing qPCR primers against NKA for the mobile qPCR instrument.

Furthermore, a mobile laboratory was constructed to validate the assay.

OUTCOMES
Laboratory-based q-PCR assays for NKA were transferred to the mobile qPCR system.
A mobile laboratory was assembled to carry out RNA extraction, reverse transcription and q-PCR on-site (Figure 1):

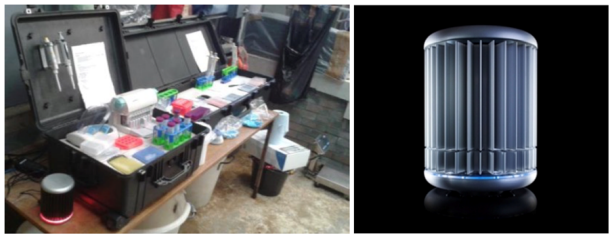

Figure 1. Mobile laboratory developed for on-site sample processing and analysis by the Gensig Q16 instrument

TESTING THE GENSIG Q16 PLATFORM AND MOBILE LABORATORY AT SMOLT PRODUCTION FACILITIES IN SCOTLAND

WORK DONE
In autumn 2017, three hatcheries located in Kintyre, Applecross and South Uist were visited twice each to test early and late points of smoltification using the mobile laboratory and assay.

In all three cases, biomarker detection was within the expected ranges, including consistent readings of positive controls. The samples from the field trips were retested in the laboratory and results indicated the same spread of cycle threshold values (CTs) for initial and final points, as well as for the positive control.

During the field trips, the mobile laboratory and its procedures were adapted to reduce the amount of materials needed per field trip, increasing its portability.

OUTCOMES
A mobile quantitative real-time assay for the detection of a biomarker indicative for smoltification was developed and validated on farms in the 2017 autumn smoltification season.

The Gensig Q16 platform showed that sites could be sampled and data provided to the farmer on the same day as sampling, offering an assay that is rapid and mobile.

The mobile laboratory was transported by plane to Uist, and on two occasions it was transported and operated solely by one person.

The mobile cycler Q16 was operated at temperatures close to freezing (0 – 1°C) without any loss of efficiency or loss of quality in its results. The Q16 was also operated in a car (on the move), indicating the potential to reduce the time on the farm from 5–6h down to 3–3.5h, theoretically making it feasible to attend two sites on the same day.

IMPACT

This project resulted in the successful development and validation of a rapid, portable and low-cost method for assessing seawater readiness using the Gensig Q16 instrument. This tool has the potential to provide farmers with a more accurate and timely assessment of their freshwater fish for seawater readiness. Moreover, project partners have sought to address a common bottleneck within the salmon production cycle by applying innovative technology to reduce the heath and welfare implications of seawater transfer, allowing UK salmon producers to remain competitive and to facilitate overall sectoral growth.

For various commercial reasons, Europharma and PrimerDesign did not continue to develop the mobile assay. Therefore, although significant knowledge was gained, further development and commercialisation of this technology has not continued. Europharma has expressed an interest in revisiting this work with alternative collaborators and technology providers in future.

ADDITIONAL INFORMATION

This work formed a chapter of a PhD thesis that studied molecular diagnostics for use in Atlantic salmon production:

PhD thesis: Michael McGowan (2018): *Molecular regulators of smoltification and viral infection management tools for salmon aquaculture.*

McGowan, M.J. (2018): *Transfer Technology: A new approach to assessing smoltification in salmon.* Fish Farmer Magazine: Institute of Aquaculture Special.

McGowan, M.J., MacKenzie, S., Steiropoulos, N. and Weidmann, M. (2021): *Testing of NKA activity by mobile real time PCR is an efficient indicator of smoltification status. (Submitted to Aquaculture).*