COMPLETED PROJECT CASE STUDY

DEVELOPMENT OF DIAGNOSTIC TECHNIQUES TO ASSESS FISH HEALTH AND IMPACT OF SEA LICE TREATMENT

PARTNERS

University of the West of Scotland | Kames Fish Farm Ltd | Mowi | Randox Diagnostics, Europharma

PROJECT LEADS

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BACKGROUND

Controlling sea lice is a significant challenge in salmonid aquaculture. Various treatments are available, but their chronic impacts on fish remain poorly understood. Anecdotal evidence suggests that sea lice treatment can contribute to mortality and behavioural changes in Scottish trout and salmon farming. Current assessment methods rely on time-consuming observations and histology, lacking a non-lethal, cost-effective and rapid alternative. Developing such a method could help tailor effective treatments, reducing mortality and recovery time, increasing feeding rate, and ultimately improving overall productivity in fish farming and reducing feed wastage.

Commercially, a standard vet visit for histological analysis costs approximately £650-850, with a waiting time of around seven days for a diagnosis. With rapid diagnosis, avoiding this seven-day waiting period could yield significant savings. Also, early detection allows timely implementation of changes, mitigating complications and preventing further losses.

This project innovates by repurposing automated medical/veterinary diagnostic technologies to assess fish health; a novel approach in aquaculture. The non-lethal testing of endpoints and biomarkers on a large scale offers advantages in reducing variation and establishing biomarker background levels. Correlating biomarkers with sea lice treatment methods and post-treatment infection onset will inform the development of efficient treatments. This faster, cost-effective, and validated technique contrasts with the destructive, labour-intensive histopathology techniques currently used. Commercially, it could lead to a more affordable, rapid, and on-site method for assessing fish health, facilitating swift implementation of measures to address issues, and reducing mortality, food wastage, treatment time, and productivity loss.

ORIGINAL AIM

This project aimed to repurpose medical diagnostic techniques for assessing salmonid fish health in aquaculture. Objectives included establishing baseline clinical chemistry levels over a year, evaluating chronic impacts of sea lice treatments on Rainbow trout and Atlantic salmon, and optimising treatments based on correlations with behavioural and immunodeficiency endpoints.

> *The ultimate goal is to use these biomarkers for early detection of health issues, such as diseases, before organ or tissue injury occurs.*

WORK DONE

Re-purposing and validation of existing medical diagnostic techniques to assess the health of salmonid fish in aquaculture

Originally, the project proposed 15 clinical chemistry endpoints. Adjustments were made upon discovering that some biomarkers were unsuitable for fish. Following discussions with sector partners (Kames and Mowi), the biomarkers were increased to 22, as seen in the table below. Sector partners selected these biomarkers and the additional cost was covered by partners, allowing for the project extension from 12 to 16 months.

Randox Biomarkers	Diagnostic Significance
Alanine Aminotransferase (ALT)	Liver (hepatocyte injury) & Muscle
Alkaline Phosphatase (ALP)	Liver (Hepatobiliary disease and toxic response)
Total Protein (TP)	Protein profile
Albumin (ALB)	Protein profile & Liver
Total Bilirubin (TBIL)	Liver function
Creatinine (CREAT)	Kidney function
Amylase (AMY)	Pancreas function
Creatine Kinase (CK-NAC)	Muscle damage
Lactate Dehydrogenase Pyruvate (LDP)	Muscle damage & Haemolytic Anaemia
Urea (UR)	Liver function & Gill function
Ammonia (AMMON)	Liver function & Gill function
Copper (Cu)	Minerals
Iron (Fe)	Minerals
Zinc	Minerals
Magnesium (Mg)	Electrolytes
Calcium (Ca2+)	Electrolytes
Phosphorus (PHOS)	Electrolytes
Sodium (Na^+)	Electrolytes & Osmoregulation
Potassium (K^+)	Electrolytes
Chloride (Cl^-)	Electrolytes & Osmoregulation
Haemolysis	Haemoglobin concentration
Lipaemia	Lipids in blood

The project faced technical challenges using a human blood and serum instrument. Issues included reagent dilutions, serum concentration adjustments, and machine clogging affecting results. Despite challenges, Dr Josip Barisic, supported by Randox, overcame these obstacles through trial and error. Eventually, the Randox Daytona clinical chemistry instrument successfully measured 22 endpoints in both Atlantic salmon and Rainbow trout throughout the project.

Extensive experimental work was conducted to standardise methodologies and minimise experimental variability in biomarker expression. This involved establishing protocols for fish blood collection, serum processing, sample transportation, and lab storage. A comparison between fresh and frozen serum samples, as well as investigations into the effects of storage temperature, transportation conditions, and repeated freeze/thaw cycles, were performed. The study also explored the impact of haemolysis on biomarker expression, including the use of compounds like HemoBind for its clearance. The developed protocols aimed to generate more consistent samples for biochemical analysis.

Establishing background levels of chosen clinical chemistry endpoints in Rainbow trout and Atlantic salmon

Due to technical issues during the initial two months, biomarker data collection for trout began in February, and in March for salmon. Trout background serum samples were collected every 2-4 weeks from February to November. For salmon, samples were collected from March to December. Despite challenges in scheduled sampling due to the dynamic nature of fish farming, the collaboration with on-site staff from both companies was crucial. Their efforts provided the necessary serum samples for this ambitious study. The data obtained establishes, for the first time, a background level of the chosen 22 biomarkers, offering insights into the normal range of expression over an extensive 10–11-month period.

The collected values were used to calculate reference ranges for each biomarker over the period, additionally, the study explored the impact of fish size on biomarker expression in Rainbow trout, revealing significant differences in seven out of the 18 investigated biomarkers.

Measuring the chronic impact of various sea lice treatments on Rainbow trout and Atlantic salmon

Our aquaculture partners acknowledged the stress induced by sea lice treatment on fish, but lacked precise information on the actual impact. Understanding this impact is crucial for determining optimal timing and methods, and minimum intervals between sea lice treatments, with the goal of minimising the impact on fish health.

The investigation into sea lice treatment impact surpassed the original proposal scope, driven by increased budget, project duration and additional contributions from aquaculture partners in sample provision. Information on treatment types and frequencies was gathered, and serum sampling was conducted immediately before, after and at various intervals post-treatment. Practical limitations occasionally altered this strategy. Histology samples were collected, when possible, along with data on physical and behavioural fish characteristics.

Samples were collected post-treatment using Alphamax (deltamethrin), Salmosan (azamethiphos), freshwater, Hydrolicer, and Thermolicer for both salmon and trout. The extensive data generated is beyond the scope of this report, but a deeper and more detailed insight into results, as well as a wider discussion, can be found in the published articles listed below.

CONCLUSIONS

The project's objectives were not only achieved but surpassed the initial plan funded by SAIC, thanks to our industry partners' extra contributions. This additional support enabled an expanded investigation with 22 biomarkers, increased testing of fish serum samples, and an extended project post-doc employment by four months. Consequently, all original objectives were exceeded.

This work yielded two main outcomes: Firstly, a database was developed for baseline levels of 22 clinical biomarkers in Atlantic salmon and Rainbow trout over an 11-month period. This data establishes a normal biomarker range, enabling comparison with samples from fish facing health challenges to indicate clinical significance. The ultimate goal is to use these biomarkers for early detection of health issues, such as diseases, before organ or tissue injury occurs. These findings were crucial for integrating the high-throughput biomarker approach into routine health practices and form the foundation for a successful KTP application between UWS and Mowi.

The study's second outcome was a shift in fish husbandry practices based on data highlighting the impact of sea lice treatment on fish health. Our aquaculture partners, previously unaware of the severity of the impact ten days post-treatment, have begun adjusting their approach. They have now increased the recovery time between exposures, providing the fish with more time to recover before the next exposure. This change not only benefits fish welfare but also carries economic advantages, as healthier fish are less prone to infections with related losses.

The success of the project and its innovative diagnostic approach to fish health has led to further collaborations and grant submissions within the consortium. Notable projects included an investigation into failed Rainbow trout in aquaculture with SAIC, a Knowledge Transfer Partnership (KTP) with Innovate UK for the development of rapid fish health assessment, and a BBSRC submission for diagnostic techniques assessing anaemia in aquaculture-reared Atlantic salmon. Additionally, discussions with Scottish Enterprise were stablished for the potential participation in their High Potential Spinout Programme, aimed to establish a commercial spin-out company offering the fish health diagnostic service to the aquaculture sector.

ADDITIONAL INFORMATION

Results from this project were presented at the invitation of SAIC during the Aquaculture UK conference (Aviemore 23 – 24 May, 2018), at the World Aquaculture Society, Aquaculture America 2018 conference (Las Vegas, 19-22 Feb, 2018), and the World Aquaculture Society, Aqua 2018 conference in Montpellier, France 25-29 August 2018. All of these conferences are attended by delegates from both industry and academia.

Mr Stuart Cannon (Kames Fish Farming) was invited to present at the 7th annual world congress of aquaculture and fisheries, Weihai, China, which included some results from this study.

Barisic, J., Cannon, S. & Quinn, B. Cumulative impact of anti-sea lice treatment (azamethiphos) on health status of Rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) in aquaculture. Sci Rep 9, 16217 (2019). https://doi.org/10.1038/s41598-019-52636-1