

# ADVANCEMENTS IN GILL HEALTH MANAGEMENT FOR ATLANTIC SALMON

## PARTNERS

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## BACKGROUND

The health of gills is a critical topic in salmon farming globally, due to their intimate connection with the aquatic environment in which they are farmed. Hazards such as variable water quality, opportunistic pathogens, and physical injuries from farming practices risks disrupting this balance.

The gills are crucial organ responsible for vital functions like respiration, osmoregulation, and waste excretion, any impairment can affect the fish's growth and overall health. Over the past decade, salmon farmers have been experiencing a significant rise in gill diseases, leading to increased mortality rates and animal welfare concerns. Due to this rise, there is a global demand for better understanding of gill health and practical management tools to address the problem.

This case study explores a comprehensive project aimed at improving diagnostic tools for managing gill diseases in farmed Atlantic salmon. By focussing on the molecular and physiological responses to these conditions, the teams aimed to provide valuable information on the host pathogen interaction. This initiative was a multi-stakeholder collaboration between BioMar, the Institute of Biological and Environmental Sciences at the University of Aberdeen, and Scottish Sea Farms.

## AIMS

The primary aim of this collaboration was to identify diagnostic markers to assess and inform the decision-making process around gill diseases in Atlantic salmon. The work was structured around five key objectives:

1. To establish a repository of gill tissue samples from farmed Atlantic salmon for research purposes
2. To perform detailed examinations of gill tissues to identify disease markers at various stages of the fish's life
3. To validate reliable molecular markers that indicate gill health for use in disease diagnostics

4. To apply the identified markers in developing and testing feeds that could improve gill health
5. To create models that could predict the risk factors associated with gill diseases

## PROJECT OVERVIEW

The project involved extensive sampling and analysis of Atlantic salmon from three marine production sites across Scotland. Sites were chosen because of their geographic location and previously identified as high risk from a gill health perspective. The gill tissues were examined at three levels, each level providing a different perspective and depth of resolution: macroscopic evaluation (gross morphology), gene expression analysis (RNA sequencing), and microscopic examination (histopathology).

Integration of gene expression data with histopathological findings allowed researchers to identify common patterns associated with multifactorial gill diseases, regardless of the sample's origin.

The research activities were organised into six work packages (WPs), each with milestones and deliverables associated with the project objectives:

### WP1: DEVELOPMENT OF A GILL TISSUE BIOBANK

The first work package focused on creating a biobank of gill tissue samples. The milestones included establishing a sampling protocol, collecting tissues, and compiling a database of associated data. The biobank now contains samples from 272 unique fish, collected during ten sampling events across three marine production sites. This repository will support future industrial research on gill diseases.

### WP2: GROSS AND HISTOLOGICAL ASSESSMENT OF GILL DISEASE

In this work package, biological samples from fish gills were analysed for gross morphology and histopathology.

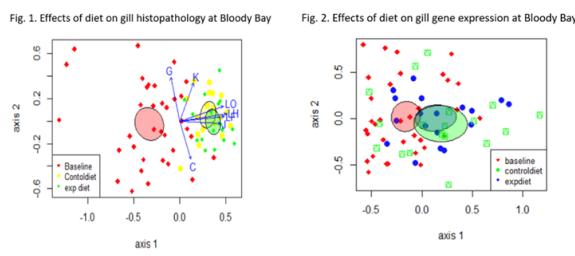
The macroscopic examination used a semi-quantitative scoring system for two key conditions of the salmon gill: proliferative gill disease (PGD) and amoebic gill disease (AGD). Histopathological sections were prepared, digitised and archived, providing a detailed record of gill health at different life stages.

### WP3: IDENTIFICATION OF MOLECULAR BIOMARKERS

The third work package aimed to identify molecular markers that could serve as reliable indicators of gill health. Gene expression analysis was conducted on samples from three sites, representing different environmental and pathological conditions. The results showed that gill tissues with low and high macroscopic scores (PGD scores of 1 and 3, respectively) were indistinguishable at the transcriptomic and histopathological levels, suggesting that gross morphology alone is insufficient for diagnosing gill diseases.

### WP4: APPLICATION OF BIOMARKERS IN FUNCTIONAL FEEDS

This work package focused on applying the identified biomarkers from WP3 to evaluate the effectiveness of functional feeds in improving gill health. A feed trial took place, comparing the effects of functional and control diets on gill histopathology and gene expression. Despite the trial's rigorous design, no significant differences were found between the diets, although changes were observed over time.



*Histopathological examination of the 72 gill samples from the feeding trial demonstrated 1) a significant effect of time (lower histopathology in baseline samples and higher histopathology in final samples) and 2) no effect of functional feed (no significant differences between experimental and control diets) (Fig. 1), as the 95% confidence levels for the two groups completely overlap, indicating no differences between the control and experimental diet.*

### WP5: EPIDEMIOLOGICAL MODELLING

The fifth work package aimed to develop epidemiological models to predict gill disease risks based on collected data. However, due to time constraints and data availability issues, the milestones for this work package were not fully achieved. Future research should address these challenges to develop predictive models that can guide disease management in aquaculture.

### WP6: PROJECT MANAGEMENT

The final work package facilitated the smooth execution of the project through regular meetings and knowledge exchange workshops. These activities facilitated collaboration among researchers, industry partners, and other stakeholders, ensuring that the project remained on track and that the findings were effectively communicated.

## RESULTS

The project archived a comprehensive number of tissue samples within the Biobank. It is believed that further value will be extracted from this, as new techniques become available for researchers. The team identified 462 genes associated with gill inflammation, providing a valuable resource for future research, including 354 protein-coding genes, 35 immunoglobulin gene segments, 15 pseudogenes and 58 non-coding RNAs. Two primary processes dominated the results: immune response driven by pro-inflammatory cytokines and tissue repair mechanisms driven by caspases and angiogenin. Although the study was limited to specific locations and times, the findings offer a foundation for developing more effective diagnostic tools and treatments for gill diseases.

A panel of approximately 40 key markers of interest was developed into PCR assays to distinguish between high and low histopathology gills. These assays were tested in a feed trial, which – although it did not show significant differences between diets – highlighted the potential of gene expression markers in monitoring gill health. Surprisingly, the results indicated that changes in the macroscopic appearance of gills did not correlate with changes in gene expression or histopathology, challenging the effectiveness of current visual scoring systems for gill diseases. This suggests that although traditional methods like gross morphology provide some insights, they are insufficient for understanding the underlying molecular mechanisms of gill diseases.

## IMPACT

This case study highlights the complexity of studying gill diseases in farmed Atlantic salmon and the challenges in diagnosing and managing these conditions. An integrated approach, pulling together histopathology and gene expression analysis, offers a more comprehensive strategy for diagnosing and managing gill health, with the potential to improve fish welfare and reduce economic losses in the aquaculture industry.

The project also had a broader impact on the aquaculture sector. A histopathology workshop organised by one of the project's researchers provided a platform for knowledge exchange on emerging gill diseases, helping to disseminate the findings to industry professionals and academics.

Future research should focus on refining these diagnostic tools and exploring the environmental and biological factors contributing to gill diseases. By continuing to develop and apply molecular biomarkers, the industry can move towards more effective and sustainable methods for managing gill health in farmed salmon.