SPOT LIGHT

Innate immunity in Atlantic salmon as climate change increases risk of disease

LEISHA MCGRATH, PHD

A microfluidics-based gene expression assay to monitor fish health

Atlantic salmon grown in marine farms are increasingly plagued by a parasitic amoeba that attaches to their gills, causing amoebic gill disease. While the condition poses no threat to humans, the salmon have reduced gill function, lower growth rates and can die if left untreated.

In the past the disease would appear seasonally and thus could be somewhat managed on a cyclic basis. Thanks to climate change, amoebic gill disease is now a consistent threat to salmon aquaculture, costing the fish industry an estimated $162 million per year. The ability to regularly monitor fish stocks for the disease before it becomes symptomatic would be a significant contribution to fish health and to farms around the world.

Scientists at the Marine and Freshwater Research Centre in Ireland are looking for a way to assist in the control and management of the disease.

Leisha McGrath, PhD student at the center, focuses her graduate research on the presence of antimicrobial peptides in Atlantic salmon. Her background in biomedical science and the drive to learn more about antimicrobial resistance led her to explore approaches that could minimize the risk of amoebic gill disease in salmon by studying when and where their innate immunity is triggered upon infection.

She designed a study to look at active gene expression in relation to amoebic gill disease, produce those proteins recombinantly in the lab and then test their functionality for antimicrobial effects. Knowledge of the initial immune response in infected fish could potentially offer a method to detect disease onset.

Testing innate immunity

McGrath's research included first characterizing organ-specific expression of antimicrobial peptide (AMP) genes β-defensin-1, -3 and -4 and cathelicidin 2 in healthy Atlantic salmon, and second comparing the expression of these genes in healthy versus asymptomatic Atlantic salmon seven days post challenge with *Neoparamoeba perurans*, the amoeba that causes the disease.

The study design included testing three organs in the fish. McGrath hypothesized that these organs would exhibit a change in innate immune expression upon infection. The gill is the primary site of infection. The gut is an immunologically active tissue in teleost fish. The swim bladder is used for buoyancy in the fish via gaseous exchange, but it contains an epithelial cell component similar to the gill, with evidence of known expressors of innate immune mediators.

An introduction to microfluidics

The team sampled a set of fish prior to infecting them with the amoeba as parasite-free control groups to use for comparison. Then they infected the fish with the parasite and sampled tissues seven days later.

McGrath used Biomark™ HD for gene expression analysis. "We were just in the right place at the right time," she says about using the microfluidics-based system. "We actually started setting up an overwhelming number of qPCR reactions manually in tubes, working out the number of tissues we needed to test, how many replicates would be ideal for this type of experiment and what the cost would be in running so many reactions."

The center had just installed a Biomark HD system, and the team was told they would be able to do all their reactions on one microfluidic array instead of multiples of tubes. “This was very appealing. We thought that would be a much better and simpler approach, so we chose to go with it.”

> **… they were easily able to fit all samples, assays, controls and replicates on the same 48.48 IFC.**

During assay design, they were easily able to fit all samples, assays, controls and replicates on the same 48.48 IFC. This enabled them to add their preferred number of replicates, helped save on cost and enhanced their experimental design. McGrath emphasized that the microfluidics-based approach cut the cost of consumables and reagents, in addition to enabling multiple runs to be completed in a day for a quick turnaround time. “Plus, we didn’t have to go back and do multiple rounds of qPCR, which in itself would introduce more variation and more issues,” she explained.

“The system was very beneficial for us, and the setup was ideal—very straightforward, very easy to follow up and much faster than we had been expecting. So, with all of this, Biomark improved our ability to do this study.”

Upregulation upon initial infection

McGrath found that for three of the four AMP genes, there was a significant upregulation in expression compared with housekeeping genes and controls. Cathelicidin 2 upregulation has been observed in bacterial infections, and the β-defensin are known to be upregulated in other organisms in association with innate immunity. Taking this into consideration, the team’s results suggest that because the fish were fighting a known infection, their innate immune response was activated and working to fight off the parasite.

The findings present the potential to use AMP gene expression to monitor for infection before symptoms begin. McGrath sees this helping with fish health as well because early parasite detection can eliminate stress from treatment.

Stability of data

Given the comprehensive study design, the team was pleased with data output and reproducibility between replicates. “This is great for us because it meant we could rely on the results without having to spend the time to go back and test again.” Data visualization by heat map was also useful to see the data in a different, more holistic context.

A microfluidics-based assay also conserves sample volume. For gene expression studies, it is common to isolate just enough RNA for analysis. The capability to stretch each sample to more assays improves the statistical significance of each result and empowers researchers to ask bigger questions and collect more information.

“The system was very beneficial for us, and the setup was ideal—very straightforward, very easy to follow up and much faster than we had been expecting. So, with all of this, Biomark improved our ability to do this study.”

The system enables you to effectively scale up quickly compared with a traditional method.

“I suppose it’s difficult to visualize it before you see the system work for yourself,” McGrath says. “But once you perform the experiment and get the readout, you understand that compared to a standard reaction volume or standard tube, it is such an advantage to be able to stretch everything out a bit further.”

Moving forward

The study demonstrated that AMP gene expression could be detected seven days after infection, providing preclinical evidence of disease onset before visible symptoms appear. For any innate immune response there is a peak and a trough. McGrath questions whether they caught the peak at seven days, or if seven days is just the start and innate immunity continues for longer.

The team also collected samples from two and three weeks post infection when the disease begins to show in the gills. A follow-up study exploring gene expression activity in this time period could help them better understand disease progression. Additionally, the team would like to explore expression of these genes in other organs.

McGrath notes that their microfluidics workflow could also be well-suited to larger sample cohorts, such as when monitoring fish health or performing detailed gene expression or RNA sequencing. The system enables you to effectively scale up quickly compared with a traditional method. “I think what we’re showing with this study is that the Biomark system enables simple setup across a range of experiments, empowering you to screen bigger populations, monitor pathogen detection, look at different infections or even support trialing vaccines or treatments for response. You could do it all.”

Read the **publication**.

“... the Biomark system enables simple setup across a range of experiments, empowering you to screen bigger populations, monitor pathogen detection, look at different infections or even support trialing vaccines or treatments for response. You could do it all.

Standard BioTools™ Support Steps Up

“I definitely appreciate all the support we received during the actual process and study design. It was easy to ask questions, and the training was very helpful. Once we built confidence that our design was solid, it was easy to continue independently.”

MICRO FLUIDICS MEGA INSIGHTS.

Explore more applications using microfluidics:

standardbiotools.com

STANDARD BIOTOOLS™

Unleashing tools to accelerate breakthroughs in human health™