



Popular science summary of the PhD thesis

PhD student	Giulia Zarantonello
Title of the PhD thesis	Application of integrative molecular approaches to the study of fish pathogens in aquaculture: Microbial community dynamics, pathogen detection and identification using environmental nucleic acids and sequencing: case studies on IHNV, VHSV and MLO.
PhD school/Department	DTU Aqua

Science summary

Aquaculture has grown steadily over the past decades, but its sustainable expansion is challenged by infectious diseases. Traditionally, research has focused on how single pathogens interact with their fish hosts, improving diagnostics and disease management. However, the role of the microbiome, which includes the communities of microorganisms living in and around the fish, is increasingly recognized as a key factor in disease development. Infection can trigger changes in the microbiome, known as dysbiosis, which can influence disease progression and outcomes. This has shifted the perspective from a “one pathogen, one disease” model to the concept of the “pathobiome,” which considers interactions among pathogens, other microbes, the host, and the surrounding environment.

Understanding these interactions can also improve disease detection. The application of methods detecting multiple microorganisms at a time can reveal the presence of pathogens, opportunistic microbes, or microbial biomarkers indicating declining fish health. Moreover, extending the focus also to environmental nucleic acids, which include DNA and RNA found into water, can improve early detection before the appearance of symptoms in the animals. This thesis applied these approaches to selected viral and bacterial infections in rainbow trout (*Oncorhynchus mykiss*).

In the first part, we focused on Novirhabdoviruses, by improving the detection of their RNA in water and by exploring correlations between viral load in water and fish, disease progression, and changes in microbial communities, to identify potential microbial indicators of infection.

The second part is focused on a bacterial infection called red mark syndrome (RMS), a skin disease whose causative agent is uncertain. By combining targeted detection of the suspected pathogen *Midichloria*-like organism (MLO) with a broad survey of the skin microbiome over disease progression and healing, the study provided strong evidence that MLO is most likely the primary agent of RMS, while also showing that opportunistic bacteria can contribute to more complex disease outcomes. Finally, metagenomic sequencing allowed the recovery of the full MLO genome without the need for culture, revealing that the bacterium is part not only a new species but of a previously unknown genus. Analysis of its genome provided insights into its intracellular lifestyle and pathogenic mechanisms.

Altogether, this work highlights how integrating host, pathogen, and environmental microbiomes can deepen our understanding of infectious disease in aquaculture and offer new tools for monitoring and managing fish health.