

## **Combining metagenomics, water quality analyses, and mathematical modelling to investigate RAS biofilter composition and performance.**

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### **ABSTRACT**

Use of shotgun metagenomics is a powerful way of assessing microbial community structure and functioning in microbial biofilms. Yet, this technique is rarely applied in analyses of microbial communities in biofilters of recirculating aquaculture systems (RAS). Here we demonstrate how shallow sequencing metagenomics combined with a co-assembly strategy can be used to effectively monitor how biofilter dynamics change over time and respond to changes in water quality. Our metagenomic approach was tested in a six months long pilot-scale RAS experiment rearing Atlantic salmon (*Salmo salar*). The experiment involved repeated controlled changes in pH and alkalinity. From our metagenomic data we were able to reconstruct more than 200 near full-length genomes (MAGs) in the biofilters. This included genomes of the most dominating ammonia oxidizing and nitrite oxidizing bacteria. Metagenomic analyses, combined with chemical rate measurements and mathematical modelling of water quality dynamics, revealed that the composition of the biofilter was rather stable over time, but that the biofilter activity was significantly affected by fluctuations in pH and alkalinity occurring over weeks. Our study demonstrates how the combination of metagenomics, chemical rate measurements, and mathematical modelling can provide a deeper understanding of the complex chemical and biological dynamics occurring in RAS systems. Ultimately this can help us move forward when it comes to optimizing RAS in terms of operation and design.