

Water microbiota in a recirculating aquaculture system for environmental sustainability and fish welfare

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Abstract

Recirculating aquaculture systems (RAS) are a promising technology of fish production to reduce aquaculture environmental impact. Water recirculation relies on the stability of physical, chemical and biological processes to increase biosecurity. Although, disruptions in RAS systems can cause fish disease outbreaks by opportunistic pathogenic bacteria with an economic impact. The aim of this study was to characterize the water microbiota across the different sectors of a flatfish (*Solea senegalensis*) RAS unit, and understand its relation with the water quality parameters. Analysis was focused on the beneficial microbial community for a better environmental sustainability, but also on the opportunistic agents that threaten the fish welfare. Water samples were collected from four sectors at different filtration stages. For microbial diversity, DNA was extracted from the water samples, sequenced by Illumina MiSeq® and sequences output analyzed by SilvaNGS. Results show that *Proteobacteria* and *Bacteroidetes* were the most abundant phyla, and potential pathogenic bacteria were detected. They also indicate that salinity shifts can affect the structure of the bacterial community.

Keywords: RAS, microbial community, physico-chemical parameters.

1. Introduction

Recirculating aquaculture systems (RAS) optimize fish production and environmental sustainability by means of reducing water usage, waste management and nutrient recycling (Martins et al., 2010) but disruptions can cause fish disease outbreaks by opportunistic pathogenic bacteria. In a RAS, a beneficial bacterial community is used to treat fish metabolic waste that is released into the water. Apart from other parameters (like pH, salinity, temperature or nutrients), this is a challenging area to monitor. Next Generation Sequencing (NGS) technologies have permitted a deeper understanding of the diversity and abundance of the bacterial community (Martínez-Porchas & Vargas-Albores, 2017). This study aims to characterize the water microbiota of a RAS, and understand its relation with the water quality parameters across an aquaculture production unit.

2. Materials and Methods

Samples were collected from an established aquaculture production unit with four main systems: Open System inlet (OS), Breeding Stock (BS), Weaning (W2) and Pre-Ongrowing (PO). The later three are independent systems; BS and W2 are kept at salinity 35 while PO is kept at salinity 15. Water was filtered into Sterivex™ Filter Units with a 0.22 µm Millipore Express (PES) membrane for DNA extraction. DNA extraction was performed with PowerWater Sterivex™ DNA Isolation Kit, extracted DNA was quantified by Qubit™ 4 fluorometer with a required concentration higher than 5 ng/µL. Samples were prepared for Illumina Sequencing and sequence data was processed at GenoInseq (Cantanhede, Portugal). Sequences were then processed by the NGS analysis pipeline SILVA rRNA gene database project (SILVAngs 1.3) (Quast et al., 2013). In addition, water was characterized in terms of temperature, salinity and pH provided by the production daily monitoring.

3. Results & Discussion

In the RAS unit studied, *Proteobacteria* and *Bacteroidetes* were the most abundant phyla in the different water compartments analysed, with reported abundancies between 34-87 % and 7-55%. They are followed by *Chloroflexi* (0.1-21%), *Patescibacteria* (0.1-15%) and *Planctomycetes* (1-5%). In the different sampling points belonging to the same RAS system, the bacterial community composition was relatively stable (Figure 1). A different observation was found when comparing the three independent systems studied, with PO being dominated by the genera *Leucothrix* and *Pseudoalteromonas*; the BS by *Polaribacter* and an uncultured *Cryomorphaceae*; and W2 by *Tenacibaculum* and an uncultured *Ardenticatenaceae*. The dominating genera in the OS were *Novosphingobium* and *Pseudoalteromonas*. Differences in microbiota composition appear to be related with shifts in salinity, along with other water quality parameters. Current work is focused on the elucidation of the relations between the microbiota community and the environmental parameters, as well as on the relation between different microbiota groups with relevance for water quality and fish welfare.

This work will contribute for the definition of the core microbiome diversity and structure of a healthy, established, community in this particular aquaculture unit,

in order to determine optimum conditions for fish welfare and environmental sustainability of the production.

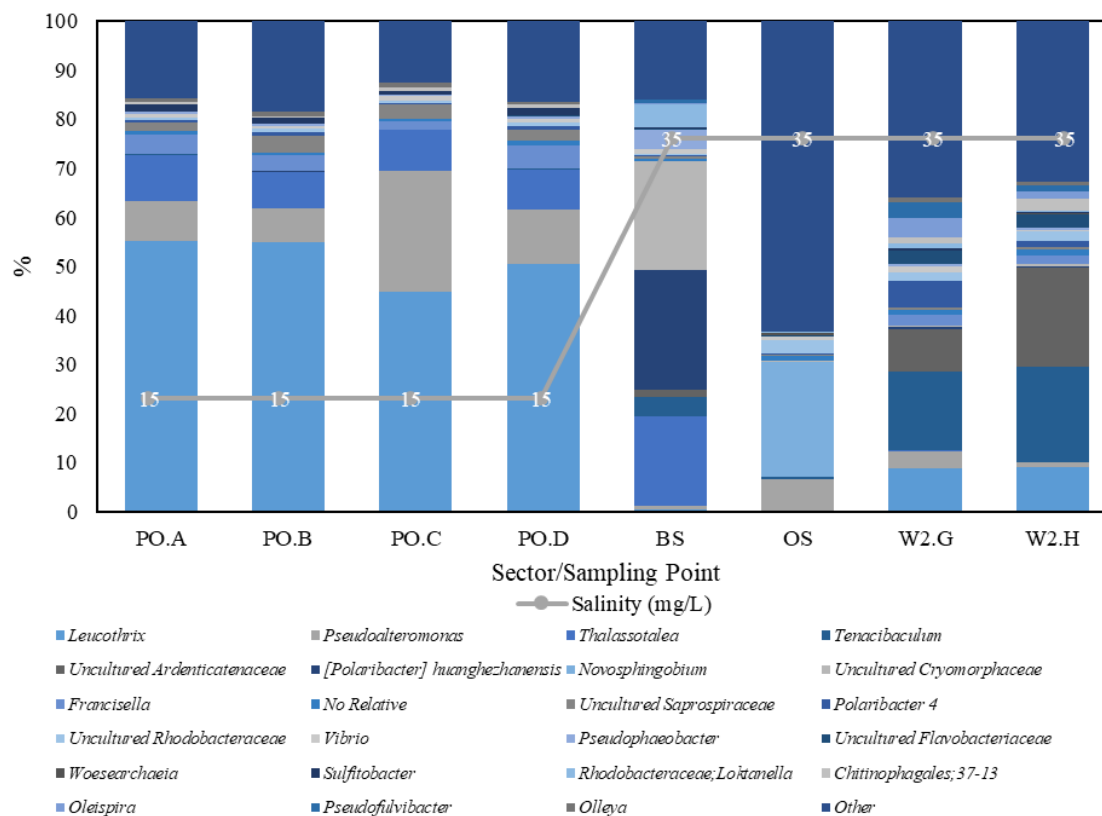


Figure 1. Salinity and bacterial community (with abundances > 5%) at the genera level in eight different points from four independent aquaculture sectors: Pre-Ongrowing (PO, A: before treatment, B: after biological filtration, C: after degasification; D: after complete treatment), Breeding Stock after treatment (BS), Open System inlet (OS), Weaning 2 (W2, A: before treatment; B: after treatment).

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