

## Determination of stocking density limits for \_\_\_



The first aim of this study was to determine the stocking density limits for Pacific oyster Crassostrea gigas larvae reared in flow-through system (FTS) and recirculating aquaculture systems (RAS). The second aim was to examine biofilm formation on the larval tank wall and its interaction with larvae growth. Three larvae concentrations were tested: 50, 150, and 300 mL–1. Chemical parameters and larvae performance were measured. The biofilm was observed by scanning electron microscopy, and its bacterial composition was investigated by pyrosequencing analysis of part of the 16S rRNA gene. The highest growth (13 μm day–1), survival (87%) and metamorphosis (50%) rates were observed in FTS at 50 larvae mL–1, while lower and similar performances occurred at 150 larvae mL–1 in both systems. At 300 larvae mL–1, performances dropped with occurrence of mortality. Biofilm thickness increased with larval density. The pioneer bacteria were coccobacilli followed by filamentous bacteria. The latter constituted abundant braids at the end of rearing at high larval concentrations. The first colonizers were mainly Rhodobacteraceae (α-Proteobacteria). The

filamentous bacteria were Saprospirae (Bacteroidetes) and Anaerolineae (Chloroflexi). The biofilm was also made up of other minor groups, including Actinobacteria, Planctomycetes,  $\delta$ -,  $\gamma$ -Proteobacteria, and Flavobacteriales. The biofilm's composition was more similar to that found in a sewage reactor than in open-sea collectors, which might negatively influence larval rearing due to potential metabolites. This first study on biofilms provides insights into the interaction between rearing density and larvae performance.

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