

## GEOMAR Helmholtz Centre for Ocean Research

### Incubation Experiments of Assemble+ Translates

This research activity received funding from the European Union's Horizon 2020 research and innovation program through the project Assemble Plus TRANSLATES (Trace Gases in Selected Aquaculture Sites in Southern Portugal) under grant agreement No. 730984.

The research activity aims to observe the impact of aquaculture on the nitrogen cycle, on deoxygenation, and the production or consumption of climate relevant trace gases. We aim to understand the processes involved in the production/consumption pathways of trace gases such as nitrous oxide, nitric oxide, methane, and carbon monoxide.

Part of this project is an incubation experiment in an aquaculture effluent site in the Algarve Region in Portugal.

#### Study Site and Set-up

On 18 May 2021, we collected effluent waters in 3.5L glass bottles from the aquaculture facility of Estação Piloto de Piscicultura em Olhão (EPPO) in Portugal. The collected samples were then transferred to an area exposed to ambient light conditions with constant flow of water (to minimize temperature fluctuations) at the CCMAR in the Universidade do Algarve.

Bottles were incubated in incubation vessels under conditions of light and dark to interrogate the impact of UV on photochemical pathways of NO and CO. A further set of incubations were poisoned with sodium azide to prevent microbial production of nitric oxide.



Figure 1. Collection of water sample from the aquaculture facility of Estação Piloto de Piscicultura em Olhão (EPPO) in Olhão, Portugal

Aside from trace gases concentration, ancillary parameters such as nutrient, O<sub>2</sub>, pH, marker pigments, CDOM, flow cytometry were determined at the start and end of the incubation.

As part of this research project, we measured NO and CO production in an incubation experiment to identify potential production and consumption processes. We used the lab bench pH, dissolved oxygen, and temperature sensors with the FireSting®-Pro multi-analyte meter with high sensitivity and accuracy.



Figure 1. Collection of water sample from the aquaculture facility of Estação Piloto de Piscicultura em Olhão (EPPO) in Olhão, Portugal

#### Results and Discussion

Shown below are the initial results we obtained from our incubation experiment. Using the FireSting®-Pro based sensor system, we observed an 89% increase of oxygen concentration ( $398.96 \pm 1.03 \mu\text{mol/L}$  from  $210.43 \pm 1.88 \mu\text{mol/L}$ ) after 48 hours of incubation in light incubation.

Using a Micromac 1000 Portable Analyzer System, we also noted that ammonium, nitrate, and nitrite were consumed (below detection limit) while phosphate concentration decreased by about 86% in light incubations after 48 hours. This may probably be attributed to photosynthetic activity of phytoplanktons (Chemical Equation:  $6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$ ) which consumes nutrients and produces oxygen.

Meanwhile, we observed that after 24- and 48-hours incubation, pH decreased by 0.20 to 0.50 pH unit from initial pH 8.00.

This experiment showed the capability of the lab-bench FireSting®-Pro multi-analyte meter in measuring dissolved oxygen, pH, and temperature for biogeochemistry incubation experiments.

Due to its high accuracy and precision, it may serve as alternative to conventional methods such as the Winkler

method for dissolved oxygen and pH titration for pH determination.

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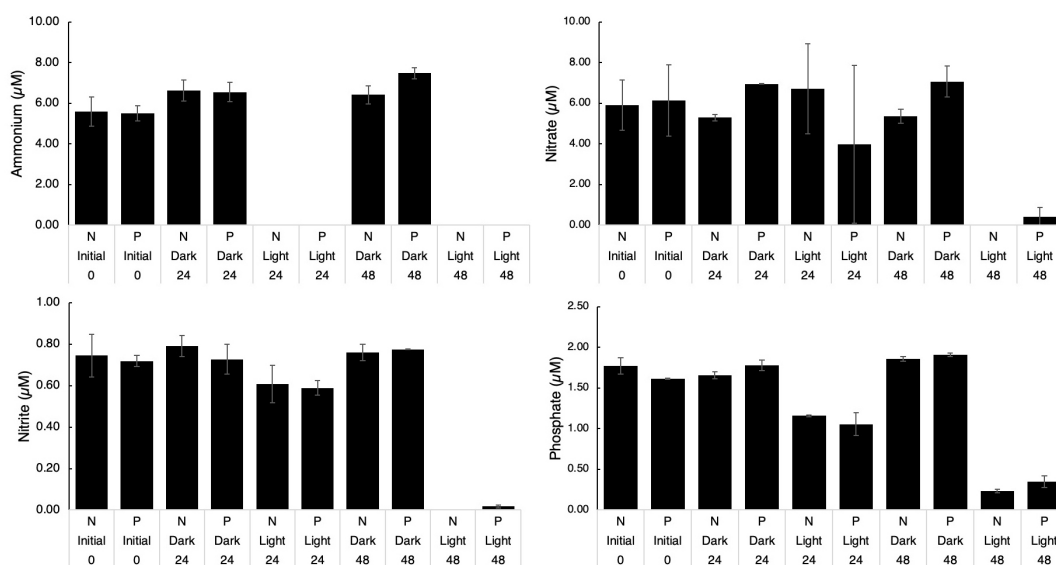


Figure 3. Dissolved ammonium, nitrite, nitrate, and phosphate concentrations of aquaculture effluent samples incubated for 48 hours (P-Poisoned, N-Not Poisoned)

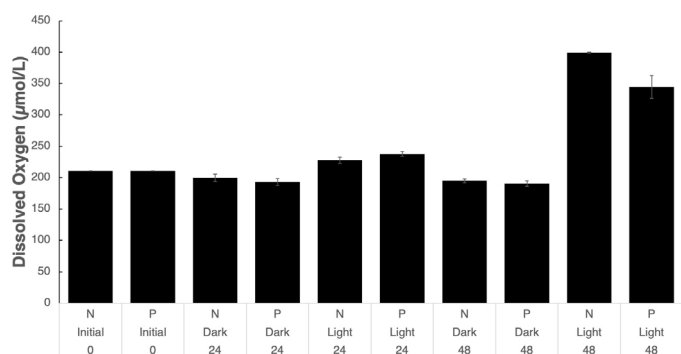


Figure 4. Dissolved oxygen concentration of aquaculture effluent samples incubated for 48 hours (P-Poisoned, N-not poisoned)

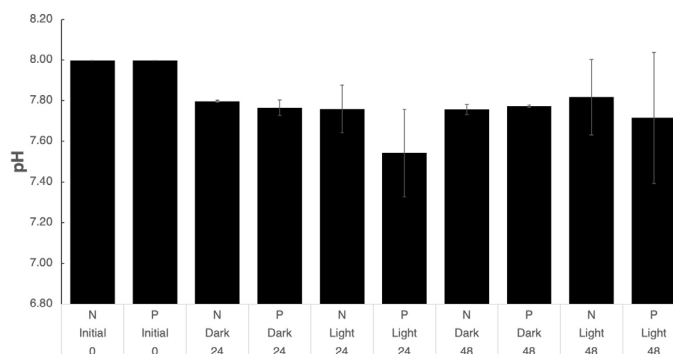


Figure 5. pH of aquaculture effluent samples incubated for 48 hours (P-Poisoned, N-not poisoned)

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