# PRELIMINARY RESULTS OF EXPERIMENTS TESTING THE FEASIBILITY OF CO-CULTURE OF SHRIMP AND ALGAE IN RAS SYSTEM

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## Introduction

Algae have recently come into focus for their application potential in RAS systems due to the high levels of nutrient uptake, high photosynthesis level, and high growth rate.
In order to demonstrate that algae can be used in RAS cultures as biofilters to purify the culture water of *Paneus vannamei* (whiteleg shrimp) 2 experiments were conducted. In the first experiment the idea of "algae scrubber" on canvas as trickling filter was tested. In the second experiment, native filamentous green algae of the genus *Chaetomorpha* were used.

## Materials and methods

Experiments were carried out between May and September 2021 in two RAS-500 systems designed by AquaMedic Poland located at the Institute of Oceanography, University of Gdansk. Both RAS are identical and have 500 $dm^{3}$ cultivation pools and system operation is controlled and monitored by a dedicated computer. For algae cultivation, additional flow-through tanks (refugia) with a maximum volume of 125 $dm^{3}$ equipped with Aqua Illumination Prime HD lamps were connected to the original systems (Fig. 1-2). In both refugia the same light exposure time was set (10h light and 14h dark cycle), and the same default light parameters were used: 60% blue light, 60% green light, red light, 60% warm white light, 60% cold white light. The values of the main water parameters (temperature, pH, salinity, redox potential) were continuously monitored during the experiments. Additionally during the first 2 weeks and then once a week, the concentration of nitrogen (NH4+, NO3- and NO2-) and phosphorus compounds (PO43-) as well as Fe ions were measured every 2 days with a Hach Lange DR5000 spectrophotometer using sachet tests.
For the first experiment testing the development of algae biofilm on canvas, in the refugium, three 50 x 35 cm nets were mounted on the water supply pipes in such a way that water flowed down them continuously along their entire length.
In the second experiment, filaments of green algae *Chaetomorpha linum* collected locally from the coastal waters of the Baltic Sea were used in variants with nitrogen gas and distilled water pre-treatment, with and without acclimatization.
In both experiments, intensive observations of biofilm and *Ch. linum* filaments were carried out. Additionally, *Ch. linum* biomass was measured on a SBS-LW Balance Scale with a measurement accuracy of 0.001 g. As in the case of water parameters, during the first 2 weeks analyses were performed every 2 days and then once a week.

## Experiment 1 – „Algae scrubber" on canvas

Initially, no biofilm was formed to act as a biofilter, confirming the initial assumption that in the case of a RAS system meeting the high purity requirements no algal colonization of the system would occur (Fig. 3-4). Only after the introduction of *Ch. linum* filaments into the algae tank a biofilm has formed but on the walls of the refugium, consisting mainly of diatoms and green algae (Fig. 5-6). No biofilm formed on the specially prepared canvas, probably because the water flow was too high.

## Experiment 2 – native filamentous green algae *Chaetomorpha* sp.

Native algae without and after acclimatization process showed growth only for a period of several weeks (6-9 depending on the variant of the experiment) (Fig. 5). It was also observed that irrespective of the method used to purify *Ch. linum* filaments obtained from the environment, microalgae were always introduced into the system along the host algae. Hence, only the culture of specially prepared and purified strains e.g. from unialgal cultures can ensure the purity of the RAS system. Furthermore, the decline of the growth of *Ch. linum* after a couple of weeks indicates suboptimal conditions for its development. This is probably due to inadequate light conditions or lack of key micronutrients, which were not measured.

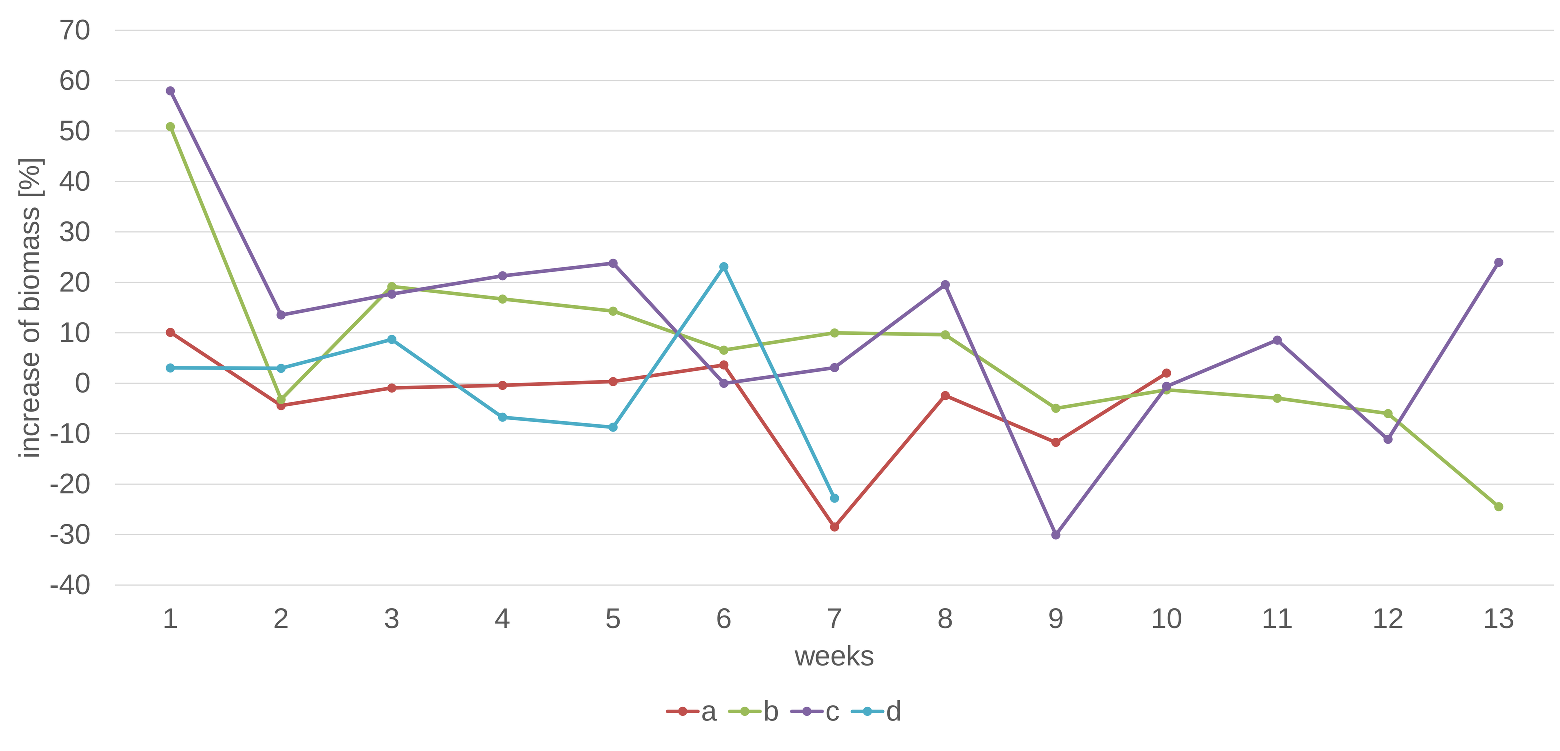


Fig. 5. Diagram showing the percentage increase of biomass of *Chaetomorpha linum*: a. cleaned filaments no acclimatization, b. cleaned filaments acclimatization to 27 PSU, c. cleaned filaments acclimatization to 27 PSU, d. uncleaned filaments without acclimatization.

Fig. 1-2. The example of refugium for algae growth experiments.

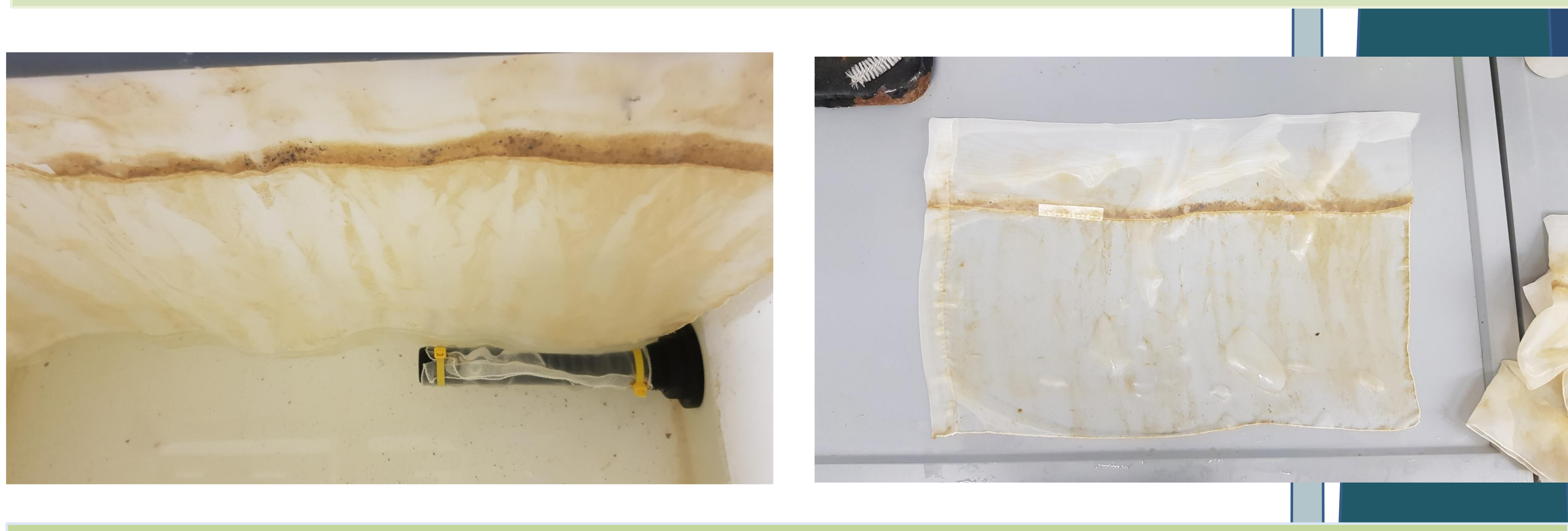

Fig. 3-4. Examples of a film consisting of organic matter developed on a canva after 2 weeks of experiment.

Fig. 5-6. Example of a biofilm consisting of diatoms and green algae developed on refugium walls after introduction of native green algae *Chaetomorpha linum*.

## Effects on water quality and shrimps *P. vannamei*

Based on the experiments, no significant effect of algal co-culture on the reduction of nutrients in RAS systems was recorded, but parameters stabilized and even system malfunctions (e.g. pumps, feeder, heater) did not significantly affect the water quality (Fig. 6).
It was also not observed that the presence of algae in refugia had a negative effect on the growth rate of *P. vannamei*, as their growth was 10% higher than in previous experiments in the same RAS systems.

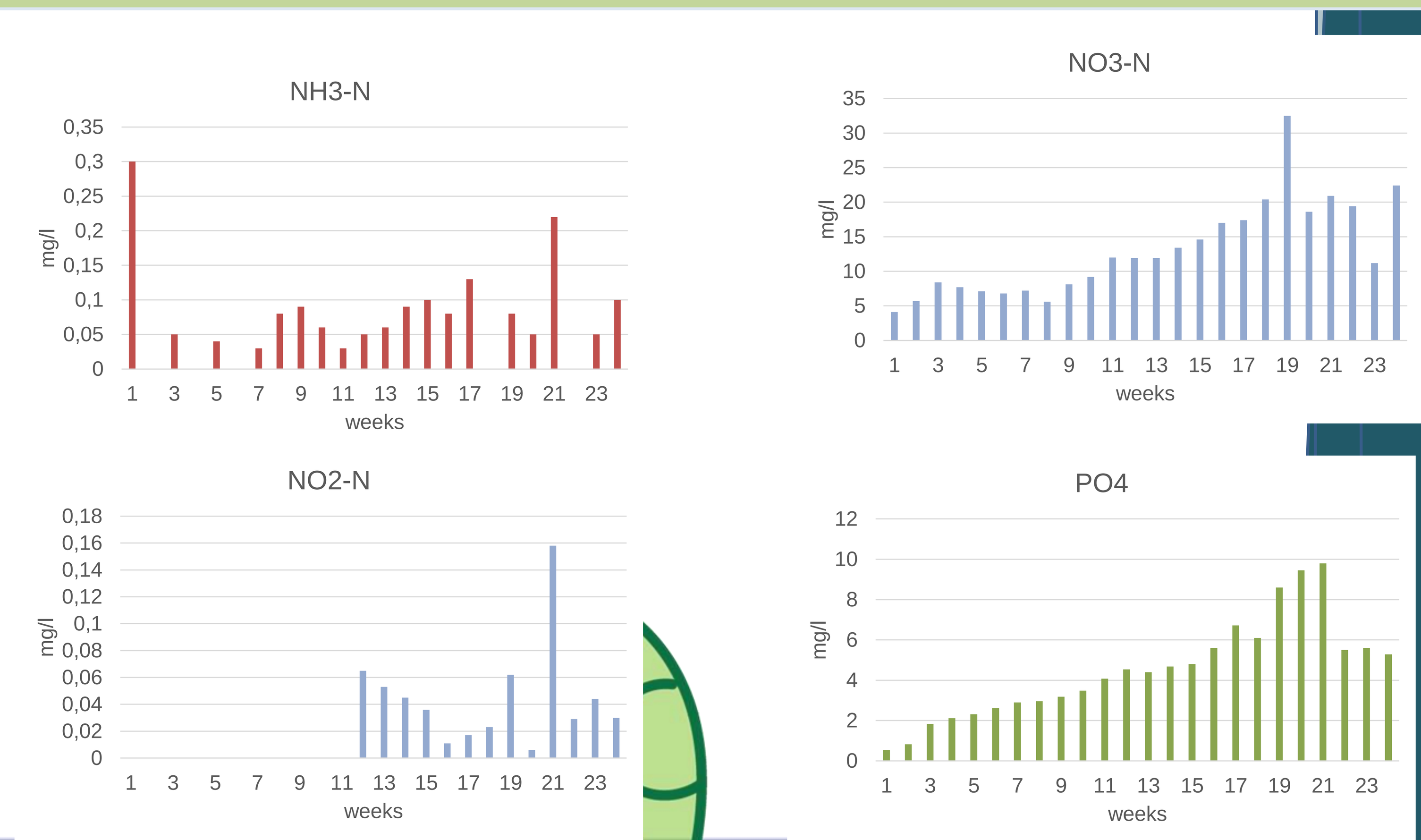


Fig. 6. Diagrams presenting values of measured water parameters for RAS 2 with a set of experiments with *Chaetomoroha linum*.

## Conclusions

Both microalgae and macroalgae can be cultured in RAS with shrimps. The presence of algae in the RAS refugia did not have a negative effect on the growth rate of *P. vannamei*, but further observations are needed to confirm the positive effect.
In order to achieve satisfactory levels of water purification and algal biomass growth, the introduction of selected and pre-adapted algal species is required along with the provision of suitable growth conditions.

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