ABSTRACTS
GROWTH AND NUTRIENT UPTAKE OF THE SEAWEED Ulva ohnoi INTEGRATED IN A Solea senegalensis RECIRCULATING SYSTEM: INFLUENCE OF LIGHTING, STOCKING DENSITY AND CO₂

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The integrated production of fish and seaweeds can improve the sustainability of the marine aquaculture industry. Seaweeds remove the nutrients dissolved in fish-farm effluents and can become a good source of proteins, carbohydrates and bioactive compounds of commercial interest.

In a flow-through fish tank, most of the N dissolved in water occurs as ammonium, and its concentration is constrained by the limits of fish toxicity, giving usually values lower than 1 mg/L of N. In contrast, in a recirculating system, ammonium is oxidized to nitrate, much less toxic for fish, and the concentrations of N can be until 100 times higher. The integration of seaweed production with Recirculating Aquaculture Systems (RAS) can contribute to reduce even more the make-up water needs in RAS, promoting higher versatility in terms of fish farm location.

Ulva ohnoi has been identified as ideal candidate for filtering fish effluents due to their capacity to rapidly absorb and metabolize nitrogen, high growth rates and worldwide distribution. Nevertheless, their highly variable rates of productivity, makes it harder the management routines and jeopardize the continuous and stable supply of biomass.

This work analyzes the influence of lighting, stocking density and CO₂ concentration in the growth and in the phosphorous and nitrogen uptake of Ulva ohnoi, fed with the effluents coming from recirculating tanks of Solea senegalensis, with high nitrate and phosphorous concentrations.

Influence of irradiance and stocking density on growth
Ulva ohnoi was cultivated in three circular indoor tanks (diameter 64 cm and capacity 90 L) with three Photosynthetic Photon Flow Densities (PPFD) (0.163, 0.280 and 0.886 mmol m⁻² s⁻¹), three initial stocking densities (SD) (0.8, 1.6, and 2.4 kg fresh weight (FW) per square meter) and identical photoperiod. For each stocking density, biomass yield (fresh weight) was measured two times per week along three weeks and the amount of biomass was adjusted to the initial stocking density after each measurement. The harvested biomass was dried, yield of dry weight per square meter calculated and nitrogen and phosphorous contents determined.

Nutrients were supplied by two tanks of sole (Solea senegalensis) working in a recirculating system with a nitrifying biofilter. Concentrations of N and P in the recirculating system ranged from 20 to 40 mg N/L and 0.6 to 1.2 mg P/L respectively, being the ammonium concentration negligible in front the nitrate. A high N flow was maintained in all the tanks to avoid N becoming a limiting factor for Ulva growth.

Temperatures and pH were monitored in seaweed tanks and CO₂ concentration was controlled by providing sodium bicarbonate to maintain alkalinity over 100 mg/L CaCO₃ and adding HCl along the light period to maintain a pH 8.24±0.35 at the end of the dark period

Biomass yields obtained, expressed as grams of dry weight (DW) per square meter and day, showed significant differences between PPFD and between stocking densities (Figure 1).

With the lowest PPFD (0.163 mmol m⁻² s⁻¹) the biomass yield decreased when the initial stocking density increased, being maximum and minimum yields ±sd 10.3±1.1 and 4.3±1.7 gDW m⁻² d⁻¹.

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With PPFD 0.280 mmol m\(^{-2}\) s\(^{-1}\) the biomass yield still decreased when initial stocking density increased, but relative differences were lower, with yields ±sd ranging from 15.8±1.6 to 10.6±3.3 gDW m\(^{-2}\) d\(^{-1}\).

PPFD 0.886 mmol m\(^{-2}\) s\(^{-1}\) with the intermediate initial stocking density (1.6 kgFW m\(^{-2}\)d\(^{-1}\)) produced the highest biomass yield (42.8±3.4 gDW m\(^{-2}\) d\(^{-1}\)). That is more than twice the yield obtained with the lower density and around 30% more than using the higher density. When the highest PPFD was combined with the lower initial stocking densities, not only the biomass yield decreased, but also the appearance of the seaweeds worsened; taking a yellowish color, reducing the thallus area and increasing its thickness.

The photosynthetic photon flux per gram of biomass fresh weight (PPF/FW in mmol s\(^{-1}\) gFW\(^{-1}\)) can be calculated from the ratio between PPFD and the average stocking density. The relationship between this value and the Specific Growth Ratio was analyzed and a linear relationship was found for PPF/FW values lower than 0.42mmol s\(^{-1}\) gFW\(^{-1}\), in the ranges of PPFD and stocking densities evaluated. PPF/FW values higher than 0.42mmol s\(^{-1}\) gFW\(^{-1}\) corresponded to the trials with the highest PPFD and the lowest stocking densities, which resulted in the abovementioned problems.

**Influence of inorganic carbon (Ci) on biomass yield**

Photosynthesis and growth of seaweeds can be limited by the availability of Ci. The influence of this factor in our seaweed tanks integrated in a RAS, was evaluated by comparing 3 treatments: In all of them, algae tanks were maintained at PPFD 0.163mmol m\(^{-2}\) s\(^{-1}\), the stocking density 0.8kg m\(^{-2}\) and alkalinity higher than 100 mg/L CaCO\(_3\). In the first treatment a continuous flow rate of 7L/h of water, coming from the recirculation fish growth system (with high CO\(_2\) concentration as a result of fish respiration) was maintained along the light period and hydrochloric acid (HCl) was added to maintain suitable Ci concentrations in the algae tank. In the second treatment, water inlet flow rate was reduced to 4 L/h without HCl addition. Finally, in the third treatment, the water inlet flow rate was reduced to only 2L/h without HCl addition. Even in the second and third treatment the concentration of inorganic N and P in water was high enough to exclude the possibility of these nutrients becoming a constraining factor on seaweed growth (average concentrations for N±sd 14.1±1.2 and 11.9±2.2mg/L; and for P±sd 0.94±0.29 and 0.82±0.25 in the second and third treatment respectively). The average yields of biomass in second and third treatment were, respectively, 27% and 54% lower than those obtained in the first treatment. These results suggest that, when using water from a RAS, despite the very low water inlet flow rates required to provide enough flow of nutrients to the algae tanks, a control of pH and alkalinity is required to guarantee the availability of Ci by the algae.

**Nutrient uptake**

Nitrogen and Phosphorous uptakes were estimated in a specific experiment conducted along 4 weeks with PPFD 0.280 mmol m\(^{-2}\) s\(^{-1}\) and within a range of stocking densities from 0.8 to 1.2 kgFW m\(^{-2}\). Not significant differences in yield were observed between stocking densities, due to the relatively narrow range considered and the dispersion of yield values for each stocking density. Nevertheless, the N and P uptake values, calculated as the difference between incoming and outgoing fluxes of N and P, showed a much lower dispersion, and N uptake showed significant differences, with a slight increase in N uptake when the stocking density increased. This matched with an increase in organic N concentrations measured in seaweed tissue.

Overall N and P uptake ratios obtained with PPFD 0.280 mmol m\(^{-2}\) s\(^{-1}\) were 643 and 57 mg m\(^{-2}\) d\(^{-1}\). Nevertheless, biomass yield multiplied by the N and P content of the seaweed tissue was only 68 and 64% of the mentioned values. N and P uptakes per gram of DW yield were respectively 54 and 4.8 mg gDW\(^{-1}\).

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