

CULTIVATION OF THE SEAWEED *Ulva* spp. WITH EFFLUENT FROM A SHRIMP BIOFLOC REARING SYSTEM: DIFFERENT SPECIES AND STOCKING DENSITY*

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ABSTRACT

This work evaluated the use of effluent from a marine shrimp biofloc rearing system to cultivate two species of the green seaweed of the genus *Ulva*. First, the growth of two *Ulva* species, *U. ohnoi* and *U. fasciata*, was evaluated. Second, the best-performing species was cultivated under two different stocking densities (2 g L⁻¹ and 4 g L⁻¹) to evaluate both growth and nutrient uptake rates, considering total ammonia nitrogen, nitrate, and orthophosphate. In both cases, environmental variables were monitored, and the cultivation medium, consisting of 25% biofloc water and 75% seawater, was exchanged weekly. *Ulva ohnoi* grew significantly better, considering all variables evaluated ($p < 0.05$). The smaller stocking density produced a higher specific growth rate ($p < 0.05$). Yield, however, was unaffected ($p \geq 0.05$). No significant differences in the nutrient uptake rates were observed ($p \geq 0.05$). Overall, this work highlights the importance of species selection for seaweed destined for aquaculture. Additionally, it also optimizes the cultivation of seaweeds, specifically *U. ohnoi*, using effluent from biofloc systems.

Keywords: BFT; biomitigation; growth performance; *Litopenaeus vannamei*; macroalgae; water quality.

CULTIVO DA MACROALGA *Ulva* spp. COM EFLUENTE DE CRIAÇÃO DE CAMARÃO MARINHO EM BIOFLOCOS: DIFERENTES ESPÉCIES E DENSIDADE DE ESTOCAGEM

RESUMO

Este trabalho avaliou o uso de água de um cultivo de camarão marinho em bioflocos como fertilizante no cultivo de *Ulva*. Inicialmente, o crescimento de duas espécies de *Ulva*, *U. ohnoi* e *U. fasciata*, foram avaliados. Subsequentemente, a alga com melhor desempenho foi cultivada sob duas densidades de estocagem (2 g L⁻¹ e 4 g L⁻¹), e o crescimento e a absorção de nutrientes (nitrogênio amoniacal total, nitrato e ortofosfato) foram avaliados. Em ambos os casos, variáveis ambientais foram monitoradas e o biofoco na concentração de 25% foi trocado semanalmente. *Ulva ohnoi* apresentou um crescimento significativamente melhor para todas as variáveis consideradas ($p < 0,05$). A menor densidade de estocagem produziu uma taxa de crescimento específico significativamente maior ($p < 0,05$), embora a produtividade não tenha sido afetada ($p \geq 0,05$). Diferenças significativas na absorção de nutrientes também não foram observadas ($p \geq 0,05$). No geral, este trabalho destaca a importância da seleção de espécies de macroalgas destinadas à aquicultura. Além disso, otimiza a viabilidade de se cultivar macroalgas utilizando água de sistemas de bioflocos.

Palavras-chave: alga marinha; BFT; biomitigação; desempenho produtivo; *Litopenaeus vannamei*; qualidade da água.

INTRODUCTION

Biofloc technology is an aquaculture technique that has been successfully applied in the rearing of many species, such as the marine shrimp *Litopenaeus vannamei* and the tilapia *Oreochromis niloticus* (Dauda, 2019). This kind of technology shows many advantages, such as reduced water and land area usage (Avnimelech et al., 2008), successful control of toxic nitrogenous wastes under intensive rearing conditions

(Avnimelech, 2015), and recycling of non-assimilated nutrients into microbial biomass that can be used as a supplemental food source (Burford et al., 2004; Avnimelech, 2007). However, particulate matter and dissolved substances still accumulate in the rearing unit and have to be controlled. This fact raises concerns in an age of environmentally conscious consumers, producers, and legislators.

The excess of particulate matter generated in biofloc systems can be harnessed by underfed fish (Poli et al., 2019), while seaweeds could be used to take advantage of the excess dissolved inorganic nutrients (Chopin et al., 2008). Seaweeds already have huge economic relevance, their worldwide production volume being approximately 32.4 million tonnes, of which 97.1% is provided by aquaculture (FAO, 2020). Specimens of the genus *Ulva*, for instance, can be cultivated for human and aquatic animal consumption (Fleurence et al., 2012), or for the extraction of biologically active substances, such as ulvan (Silva et al., 2013). Earlier works evaluating their cultivation in a biofloc system environment, or employing biofloc-rich water as fertilizer, are scarce, and some are not focused on assessing the production performance of the macroalgae *per se* (Brito et al., 2014; Peña-Rodríguez et al., 2016).

Taking into account the potential of seaweeds, in general, and *Ulva*, in particular, to take advantage of excess dissolved inorganic nutrients generated in biofloc systems, as well as their economic relevance, this work aimed to evaluate two aspects of *Ulva* cultivation using water from a shrimp biofloc rearing system as fertilizer. First, we assessed the growth of two different species of *Ulva*, *U. fasciata* and *U. ohnoi*, collected from sea and lagoon, respectively, in the city of Florianópolis, SC, Brazil. Next, the best-performing seaweed was submitted to another experiment to assess its cultivation under two stocking densities (2 g L⁻¹ and 4 g L⁻¹), in which both the productivity and nutrient uptake rates were assessed. In both cases, environmental variables were also monitored.

MATERIAL AND METHODS

The two experiments were conducted at the Marine Shrimp Laboratory (LCM), which is part of the Department of Aquaculture of the Federal University of Santa Catarina (UFSC).

Seaweed from different locations

Ulva fasciata was collected from Prainha da Barra da Lagoa (approx. 27°34'26.2"S, 48°25'15.1"W), while *U. ohnoi* was collected from a set of brackish water lagoons near LCM (approx. 27°34'57.2"S, 48°26'32.4"W). They were rinsed with water of suitable salinities (33 g L⁻¹ seawater in the case of the former and 25 g L⁻¹ brackish water for the latter) to remove associated fauna, flora and detritus, followed by stocking in 800 L tanks (useful volume) filled with the respective salinities for acclimation inside a greenhouse under natural irradiance. Daily, the water was renewed, and the salinity of the brackish water unit was adjusted to 33 g L⁻¹ at the rate of 1 g L⁻¹ per day. The tanks were equipped

with 800 W heaters, and the temperature was adjusted to 25°C at the rate of 1°C per day.

Both species were evaluated in an experiment conducted in a completely randomized design in triplicate, lasting from 4 June 2019 to 25 June 2019. Experimental units consisted of U-shaped tanks with 60 L of useful volume for the macroalgae culture, equipped with 100 W heaters to maintain temperature at 25°C and two air stones connected to a blower aeration system to maintain water circulation and adequate dissolved oxygen concentrations at night. The units were placed inside a greenhouse under natural irradiance. During the experimental period, the approximate average daily sunlight duration was 10.4 ± 0.0 h, based on data obtained through the National Oceanic and Atmospheric Administration (NOAA) Solar Calculator (<https://www.esrl.noaa.gov/gmd/grad/solcalc/calcdetails.html>).

Initially, 15 L of water (salinity 34.5 ± 1.5 g L⁻¹) from a shrimp biofloc rearing unit was filtered through a bag-type filter and mixed with 45 L of seawater (salinity 33 g L⁻¹) in each of the 60 L tanks for a final salinity of approximately 33 g L⁻¹. The seaweeds (199.57 ± 8.30 g) were then stocked and cultivated under a density of 3.3 g L⁻¹. Weekly, tank water was discarded, and the same dilution procedure was performed. During this weekly procedure, biofilm present on the tanks was removed and the algae were also screened for adhered organisms.

Water temperature, dissolved oxygen concentration and illuminance were measured twice daily using an oximeter (YSI Pro20) and a digital luxmeter (Hikari HLX-881A). The values of illuminance (lux) were then converted to quantum irradiance (μmol photons m⁻² s⁻¹) by multiplying them by 0.018 (Gensler, 1986).

Total ammonia nitrogen (TAN) (Grasshoff et al., 1983), nitrite (NO₂⁻) (Strickland and Parsons, 1972), nitrate (NO₃⁻) (APHA, 2005), orthophosphate (PO₄³⁻) (APHA, 2005), alkalinity (APHA, 2005), pH (pHmetro Tecnal®) and salinity (Eco-Sense YSI EC30) were measured once a week. The water samples were collected immediately after the weekly biofloc dilutions.

Macroalgae were weighed weekly until harvest after gently squeezing to remove excess water. Growth performance was assessed through the following variables:

$$\text{Biomass gain (g)} = W_t - W_0 \quad (1)$$

$$\text{Specific growth rate (SGR)} (\% \text{ wet weight day}^{-1}) = \left[\left(\frac{W_t}{W_0} \right)^{\frac{1}{t}} - 1 \right] * 100 \quad (2)$$

$$\text{Yield (g wet weight m}^{-3} \text{ day}^{-1}) = \frac{(W_t - W_0)}{V} \quad (3)$$

where W_t and W_0 are the final and initial weights, respectively, t is the experimental period in days, and V is the unit volume in m³. The specific growth rate was calculated according to Yong et al. (2013).

Different algae densities

Ulva ohnoi specimens were collected again from a set of small lagoons near LCM, rinsed with brackish water to remove associated

fauna, flora and detritus, and stocked in 800 L tanks (useful volume) in an acclimation room under a 12/12 h photoperiod. Daily, the water was renewed, and salinity was gradually raised from 25 g L⁻¹ to 33 g L⁻¹ at the rate of 1 g L⁻¹. The tanks were equipped with 800 W heaters to allow an increase in temperature to 28.5°C at the rate of 1°C per day.

Two densities (2 g L⁻¹ and 4 g L⁻¹) of *U. ohnoi* were evaluated in an experiment conducted in a completely randomized design in quadruplicate, which took place between 26 September 2019 and 16 October 2019 (Figure 1). Experimental units were the same as those employed in the aforementioned experiment. The average sunlight duration during the experimental period was 12.5 ± 0.2 h, based on data obtained through the NOAA Solar Calculator.

Initial seaweed biomass was 120.38 ± 0.24 g and 240.85 ± 0.34 g for the 2 g L⁻¹ and 4 g L⁻¹ treatments, respectively. They were stocked in the 60 L tanks after an initial dilution. The first and then weekly dilutions were performed as in the first experiment.

Environmental variables were monitored as in the first experiment, the difference being the addition of total suspended solids (TSS) (APHA, 2005), which were evaluated weekly. Algae growth was assessed as in the first experiment.

In addition to the water samples collected right after the dilution, water samples were also collected right before the new dilution for the analysis of TAN (Grasshoff et al., 1983), nitrate (APHA, 2005)



Figure 1. Experimental design used for the evaluation of two stocking densities of *Ulva ohnoi* (2 and 4 g L⁻¹) using effluent from a biofloc shrimp rearing unit.

and orthophosphate (APHA, 2005). The results of these analyses were not used in the environmental monitoring tables, as they reflected conditions already affected by the different treatments. Instead, they were used for the result of nutrient uptake rates.

Statistical Analysis

All data were submitted to Shapiro-Wilk and Levene tests to assess normality and homoscedasticity, respectively. Percentage data were arcsine transformed before the normality assessment. When both assumptions were met, the Student's t-test was employed. When the equality of variances assumption was not met, the Welch's t-test was performed. Otherwise, if the normality assumption was not met, the non-parametric Mann-Whitney U-test was used (Zar, 2010). Results were considered statistically significant when $p < 0.05$. Data analysis was performed using *jamovi* software (Version 1.0) (Jamovi, 2019).

RESULTS

Significant differences for the environmental variables evaluated were observed only for pH in the first experiment and temperature for the second one, with the higher values being observed in the *U. ohnoi* and 2 g L⁻¹ groups, respectively (Table 1). Regarding the growth performance, *U. fasciata* exhibited a decrease in biomass, resulting in a significant lower final biomass when compared to *U. ohnoi* (Table 2). In the assessment of the two stocking densities of *U. ohnoi*, statistically significant differences were observed for final biomass and specific growth rate, in which the higher value occurred for algae cultivated in the 4 g L⁻¹ and 2 g L⁻¹, respectively (Table 2). Finally, no significant differences for the nutrient uptake rates were found (Table 3).

DISCUSSION

In the first experiment, both species were subjected to similar environmental conditions, as demonstrated by the lack of statistical significance. The pH value was the exception, albeit the difference was numerically small. For the second experiment, a significant difference was observed, but only for temperature, which was again a numerically small difference. When comparing the two experiments, the rise in temperature observed in the second one was due to higher air temperatures and longer sunlight duration occurring in the months of September and October compared to June. As regards the differences in the concentrations of nutrients, they were a result of variations in the water quality of the biofloc shrimp tanks from which the water was collected. It is difficult to discuss potential differences in the growth performance of *U. ohnoi* when comparing the two experiments due to these variations and to the different stocking densities employed. Possible effects of nutrient concentrations and water temperature on its culture should merit specific studies.

In the literature, water quality values within the range observed in this study for the cultivation of *Ulva* species were reported for dissolved oxygen, temperature, pH and salinity (Khoi and

Table 1. Environmental variables monitored throughout the two three-week experiments evaluating algae species (*Ulva fasciata* and *Ulva ohnoi*) and algae density in the culture of *U. ohnoi* when employing water from a biofloc system as fertilizer.

Variables	Seaweed species		p-value	Densities (<i>U. ohnoi</i>)		p-value
	<i>U. fasciata</i>	<i>U. ohnoi</i>		2 g L ⁻¹	4 g L ⁻¹	
Dissolved oxygen (mg L ⁻¹)	6.71±0.60	6.73±0.62	0.831	5.88±0.48	5.92±0.54	0.559†
Temperature (°C)	23.8±3.0	24.0±3.1	0.539†	30.3±1.4	29.9±1.4*	0.005†
Photon irradiance (μmol photons m ⁻² s ⁻¹)	58.5±44.8	92.0±90.7	0.103†	85.7±73.4	83.7±70.4	0.818†
TAN (mg L ⁻¹)	0.20±0.17	0.22±0.18	0.691†	0.10±0.23	0.13±0.13	0.182†
NO ₂ ⁻ (mg L ⁻¹)	0.12±0.12	0.07±0.05	0.243‡	0.08±0.04	0.06±0.05	0.200†
NO ₃ ⁻ (mg L ⁻¹)	3.4±1.0	2.2±1.2	0.257	7.56±1.83	6.33±1.37	0.148
PO ₄ ³⁻ (mg L ⁻¹)	1.06±0.08	0.87±0.18	0.168	0.27±0.04	0.24±0.03	0.080†
pH	8.20±0.03	8.26±0.05*	0.035	8.64±0.21	8.54±1.46	0.057†
Alkalinity (mg CaCO ₃ L ⁻¹)	159±9	166±18	0.329‡	125±15	127±16	0.620†
Salinity (g L ⁻¹)	34.3±0.5	34.4±0.3	0.267†	34.0±0.2	34.1±0.3	0.341
TSS (mg L ⁻¹)	-	-	-	173±46	155±38	0.194†

Data presented as mean ± standard deviation. *Statistically significant. ‡Welch's t-test. †Mann-Witney U-test. When symbol absent, Student's t-test. TAN: Total ammonia nitrogen. TSS: Total suspended solids.

Table 2. Growth performance variables assessed throughout the two three-week experiments evaluating algae species (*Ulva fasciata* and *Ulva ohnoi*) and algae density in the culture of *Ulva ohnoi* when employing water from a biofloc system as fertilizer.

Variable	Seaweed species		p-value	Densities (<i>U. ohnoi</i>)		p-value
	<i>U. fasciata</i>	<i>U. ohnoi</i>		2 g L ⁻¹	4 g L ⁻¹	
Final biomass (g)	189.0±74.6	379.6±51.7*	0.022	301.9±24.8	378.8±53.0*	0.039
Biomass gain (g)	-†	182.5±50.3	-	181.5±24.9	137.9±53.1	0.188
SGR (%day ⁻¹)	-†	3.0±0.6	-	4.3±0.4	2.7±0.7*	0.006
Yield (g m ⁻³ day ⁻¹)	-†	138.3±38.1	-	137.5±18.8	104.5±40.2	0.188

Data presented as mean ± standard deviation. *Statistically significant. Student's t-test used in all instances. SGR: Specific Growth Rate. †There was no growth, no biomass gain and no yield.

Table 3. Nutrient uptake rates assessed in a three-week experiment evaluating algae density in the culture of *Ulva ohnoi* when employing water from a biofloc system as fertilizer.

Variables	2 g L ⁻¹	4 g L ⁻¹	p-value
TAN uptake efficiency (%)	77.71±26.89	83.79±16.26	0.712
NO ₃ ⁻ uptake efficiency (%)	58.40±10.97	44.64±18.81	0.253
PO ₄ ³⁻ uptake efficiency (%)	96.78±1.64	94.54±2.10	0.145

Data presented as mean ± standard deviation. Student's t-test used in all instances. TAN: Total ammonia nitrogen.

Fotedar, 2011; Mantri et al., 2011; Zou, 2014; Ge et al., 2018), as well as irradiance (Fortes and Lüning, 1980; Sand-Jensen, 1988; Ruangchuay et al., 2012). The concentrations of TAN, nitrite, nitrate, and orthophosphate were also within the range found in other studies (Khoi and Fotedar, 2011; Ge et al., 2018). Alkalinity remained above 100 mg L⁻¹, which is recommended to improve the availability of inorganic carbon for algae (Oca et al., 2019).

When comparing the growth of the two species, *U. ohnoi* showed significantly higher values for all variables evaluated. These results are in agreement with previous assays performed in our laboratory in which *U. fasciata* collected from the sea did

not grow well when cultured using the same methodology as described in the Material and Methods of this work for the first experiment (Unpublished data). In fact, a decrease in algae biomass was observed, similar to the case of the present study. Perhaps seaweeds growing in the lagoon were more adapted to culture conditions. For instance, growing under different environmental conditions in their natural habitats, such as stagnant water, as opposed to the wave-exposed algae collected at the beach, could have been the cause of the impaired growth. In fact, even intraspecific morphological variation is observed for wild algae populations subjected to different environmental conditions, such as wave exposure (Bociąg et al., 2013). Different morphologies can then affect nutrient uptake rates (e.g., Raven and Taylor, 2003). We also noted that, in the experimental units, *U. ohnoi* remained closer to the water surface than *U. fasciata*. Considering that light intensity is attenuated as water depth increases, that fact may have played a role in the differing performances. Another explanation could be related to the water temperatures to which each species was adapted. It is known that this environmental variable affects the growth rate of seaweeds, with different species exhibiting different optimal temperatures for maximum growth (Mantri et al., 2011; Nakamura et al., 2020). *U. ohnoi* collected in the lagoons could have been more adapted to the warmer

water temperatures occurring in the greenhouse, in contrast to *U. fasciata* collected from the sea.

In the second experiment, the final biomass was significantly higher in the stocking density of 4 g L⁻¹ of *U. ohnoi*; however, the specific growth rate was significantly higher in the lower stocking density. This could be explained either by lack of nutrients or by self-shading. However, no significant differences between treatments were observed for the initial concentration of nutrients, or the nutrient uptake rates. Alkalinity, an indicator of inorganic carbon availability, was also statistically equal among treatments. Therefore, a more likely explanation is that of self-shading caused by excessive algae biomass, which could have been caused by the type of aeration system employed, which did not promote an adequate circulation of the algae in the water column. This phenomenon of decreasing growth performance as algae stocking density increases is often observed in the literature. For instance, there was a significant decrease in the growth rate of *Gracilaria tikvahiae* when its stocking density increased from 0.5 g L⁻¹ to 10 g L⁻¹ (Kim and Yarish, 2014). In another study, the algae *Agarophyton vermiculophyllum* exhibited a significant decrease in its specific growth rate when the stocking density increased from 0.2 g L⁻¹ to 2 g L⁻¹ (Shin et al., 2020). In the case of yield, no significant differences were observed. Still, this suggests that a lower stocking density of algal biomass is to be preferred, considering that similar yields are obtained with fewer inputs.

The growth results of both experiments were altogether consistent with data reported for *Ulva* spp., namely *U. fasciata* (Mantri et al., 2011), *U. lactuca* (Khoi and Fotedar, 2011), *U. reticulata* (Msuya et al., 2006; Msuya, 2007) and *U. ohnoi* (Lawton et al., 2013).

Considering that no significant differences were observed in the nutrient uptake rates when comparing the two densities, future studies could assess if higher concentrations of biofloc effluent could produce different results, having in mind that probable higher water turbidities could compromise the growth performance and yield of the macroalgae.

Overall, the results obtained in this work add to previous results showing the feasibility of using water from biofloc rearing units as a fertilizer for the culture of macroalgae, in general (Pedra et al., 2017; Shin et al., 2020), and *Ulva*, in particular (Ge et al., 2018).

CONCLUSION

When comparing the two algae species, we found that *U. ohnoi* performed significantly better than *U. fasciata* when cultured using effluent from a biofloc shrimp tank. Furthermore, in the assessment of different stocking densities of *U. ohnoi* (2 and 4 g L⁻¹), the lower one proved to be more efficient as a significantly higher specific growth rate was achieved. Also, *U. ohnoi* has a high capacity to retain the nutrients from the biofloc water. This work highlights the importance of species selection for wild macroalgae destined for aquaculture, as growth performance can vary greatly from one species to another. In addition, it also optimizes the cultivation of seaweeds and, specifically *U. ohnoi*, using water from biofloc systems.

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