BOLETIM DO INSTITUTO DE PESCA
Scientific Article

Sea lettuce (*Ulva ohnoi*) cultivation in biofloc technology: growth performance and characterization of bioactive compounds

Jamilly Sousa Rocha[1*], Daniele Santos[1], Mateus Aranha Martins[1], Claúdia Marlene Bauer[2], Marcelo Maraschin[2], Leila Hayashi[1], Felipe do Nascimento Vieira[1]

[1]Universidade Federal de Santa Catarina – Centro de Ciências Agrárias – Departamento de Aquicultura – Florianópolis (SC), Brazil.
[2]Universidade Federal de Santa Catarina – Centro de Ciências Agrárias – Departamento de Fitotecnia – Florianópolis (SC), Brazil.
*Corresponding author: jamillysr@hotmail.com

ABSTRACT

This work evaluated the biofloc technology cultivation of *Ulva ohnoi* on its growth performance and biocompounds contents. *Ulva ohnoi* was cultivated under an initial density of 6 g·L^{-1} for 28 days using water from a biofloc tank which was exchanged daily at a 90% rate. Temperature, salinity, and illuminance were measured daily. Algae growth and their density was adjusted weekly. Results showed an average plant growth of 1.15%·day^{-1} (49.5 g·week^{-1}). A significant difference was observed when comparing the initial (2.64 ± 0.3%) and final (4.62 ± 0.2%) ulvan concentration, in addition to a protein increase of 30.2%. No statistical differences were found for concentrations of phenolics and chlorophylls. An increase in flavonoids was observed on days 7 and 14 (0.41 ± 0.04; and 0.41 ± 0.07 µg·g^{-1} of dry weight), as well as a decrease in carotenoids (41.3%). In conclusion, increases in protein and ulvan were observed after *Ulva ohnoi* was cultivated in bioflocs.

Keywords: BFT; Biocompounds; Macroalgae; Protein; Shrimp effluent; Ulvan.

Cultivo de alface-do-mar (*Ulva ohnoi*) em tecnologia de bioflocos: desempenho de crescimento e caracterização de compostos bioativos

RESUMO

Este trabalho avaliou o cultivo da *Ulva ohnoi* em sistema de bioflocos quanto ao seu desempenho de crescimento e teor de biocompostos. *Ulva ohnoi* foi cultivada sob uma densidade inicial de 6 g·L^{-1} por 28 dias usando água de um tanque de biofloco, trocado diariamente a uma taxa de 90%. Temperatura, salinidade e iluminância foram medidas diariamente. O crescimento das algas e sua densidade foram ajustados semanalmente. Os resultados mostraram um crescimento médio das plantas de 1,15%·dia^{-1} (49,5 g·semana^{-1}). Observou-se diferença significativa quando comparadas as concentrações, inicial (2,64 ± 0,3%) e final (4,62 ± 0,2%), além de um aumento proteico de 30,2%. Não foram encontradas diferenças estatísticas para as concentrações de fenólicos e clorofilas. Um aumento nos flavonoides foi observado nos dias 7 e 14 (0,41 ± 0,04; e 0,41 ± 0,07 µg·g-^{1} de peso seco), bem como uma diminuição nos carotenoides (41,3%). Em conclusão, aumentos na proteína e ulvana foram observados após o cultivo de *U. ohnoi* em bioflocos.

Palavras-chave: BFT; Composição bioquímica; Macroalgas; Proteína; Efluente de camarão; Ulvana.

Received: June 22, 2023 | **Approved:** October 16, 2023.

INTRODUCTION

Ulva ohnoi is a seaweed belonging to the genus *Ulva* (Linnaeus, 1753), which has in its composition proteins, vitamins, minerals, amino acids, polyunsaturated fatty acids, and several bioactive compounds, such as photosynthetic and phenolic pigments (Angell et al., 2015). This seaweed offers an attractive source of nutritionally important metabolites, generating a growing interest in the industrial sector, and can be used as a natural source of high-value-added compounds (Bikker et al., 2016) and low-cost organic molecules, such as polysaccharides (Alves et al., 2013).

Among the polysaccharides extracted from green algae, ulvan is a sulfated polysaccharide (Lahaye and Axelos, 1993) present in their cell wall. Ulvan is strongly associated with proteins and is considered a functional ingredient as it has shown positive effects on the immune system of aquatic organisms (Peso-Eharri et al., 2012). Other important biocompounds from algae include pigments, of which chlorophyll is the primary one, being essential in plant metabolism, due to their role in absorbing light energy in photosynthesis. Carotenoids are the secondary or accessory ones, which are synthesized only by algae, plants, and microorganisms (Goodwin, 1962). These compounds have biological functions in these organisms, acting as antioxidants, membrane stabilizers, and accessory pigments in photosynthesis (Guaratini et al., 2009).

It is essential to understand how environmental factors influence macroalgae growth performance and production of biocompounds, in view of their optimization. Nutrient levels, luminosity and water temperature are some of the key points to successful cultivation. Macroalgae can be cultivated in continental areas, open lagoons, ponds, and laboratories (Sebök et al., 2017), presenting ideal characteristics for cultivation in aquaculture, including in Integrated Multi-trophic Aquaculture (IMTA) systems. IMTA provides nutrient bioremediation capability (Bolton et al., 2009; Hoang et al., 2016), benefits to co-cultivated organisms, and economic diversification through the production of other value-added aquaculture products (Chopin et al., 2001). Species of the genus *Ulva* are promising in this system, since they present considerable rates of assimilation of nutrients that are used for their growth, such as nitrogen in different forms (ammonia, nitrite, nitrate), for example (Troell et al., 2003).

IMTA and biofloc technology (BFT) have been pointed out by researchers as viable alternatives to traditional systems. BFT is characterized by being an intensive or super-intensive system with low water exchange and higher biosecurity than other systems, providing nutritional supplementation for the cultured animals through their consumption of microbial flocs (Avnimelech, 2015). However, despite the positive points presented by the biofloc system, there is an increase in the accumulation of feed residues, excreta, and inorganic compounds in the system, since shrimp retain only a small percentage of nutrients (23-39% of nitrogen and 10-35% of phosphorus) (Silva et al., 2013; Thakur and Lin, 2003). The disposal of this effluent rich in inorganic and organic nutrients is still a challenge, as it can negatively impact the environment (Avnimelech, 2015; Avnimelech and Ritvo, 2003; Crab et al., 2007).

Several studies have been carried out to minimize these negative effects caused by the intensification of fed aquaculture. In this sense, the use of macroalgae has shown great potential, since these organisms take advantage of the residual nutrients available through animal production (Xu et al., 2008a; b). Martins et al. (2020) cultivated two species of macroalgae (*U. ohnoi* and *Ulva fasciata*) using biofloc effluent from shrimp farming as fertilizer and obtained positive results in growth and nutrient uptake in both species. The integration of *Ulva fasciata* in the cultivation of shrimp and mullet in a biofloc system retained phosphorus and nitrogen, in addition to increasing the concentration of chlorophyll and carotenoids without affecting the productivity of the ulvan extract (Legarda et al., 2021). According to Khoi and Fotedar (2011), the macroalgae *Ulva lactuca* increased the rate of conversion of nutrients into total biomass in the integrated systems (shrimps and algae) and removed part of the inorganic nitrogen dissolved in the closed aquaculture recirculation system. This removal is the result of photosynthetic activities that facilitate the absorption of nutrients from effluents, improving the water quality of cropping systems (Nelson et al., 2001).

Cultivating macroalgae using water from biofloc systems introduces distinct environmental conditions compared to natural habitats. These controlled environments offer advantages, such as nutrient enrichment and stable conditions, which may positively influence macroalgae growth and biocompound production (Pedra et al., 2017). However, variations in water quality and light distribution can also present challenges, affecting the quantity and composition of biocompounds in addition to algae biomass production (Revilla-Lovano et al., 2021).

Green macroalgae, besides acting as agents to remove organic and inorganic nitrogen compounds from the aquatic environment, also have a high capacity for absorbing compounds such as ammonia (Cohen and Neori, 1991) and nitrate (Lapointe and Tenore, 1981; Naldi and Viaroli, 2002). In general, macroalgae have shown promising results and aroused economic interest in several sectors, whether in the form of fresh biomass or algal extracts. For example, in a work carried out by Cruz-Suárez et al. (2010), *Ulva clathrata* supplementation in shrimp feed provided

a positive effect on the growth rate of 60% and decreased the feed conversion rate by up to 45%. In addition, green macroalgae supplementation also improves shrimp immunity (Valente et al., 2006). Given all the benefits mentioned above, discoveries and functionalities in the use of macroalgae have emerged, showing the importance of knowing their biochemical and nutritional characteristics. Still, it is worth mentioning that no work was carried out to analyze possible differences in the characteristics of macroalgae cultivated in biofloc. Therefore, this work evaluated the cultivation of the macroalgae *U. ohnoi* on its growth performance and changes in its proximate and biochemical composition after cultivation in a biofloc system.

MATERIAL AND METHODS

Macroalgae

Macroalgae were collected in the sedimentation pond of the Marine Molluscs Laboratory of the Federal University of Santa Catarina (LMM/UFSC), whose water temperature and salinity at the time of collection were 24°C and 30‰ respectively. All biomass was stocked in plastic containers (20 L) with water from the pond and transported to the Macroalgae Section of the Marine Shrimp Laboratory from the Federal University of Santa Catarina (LCM/UFSC). The epiphytes and any material encrusted in the macroalgae were manually removed and were then washed with salt water. After this procedure, the macroalgae were kept for 2 days in a circular polyethylene tank with 500 L, initial temperature of 24°C, and salinity of 32‰. The temperature and salinity were increased gradually until reaching the values that were to be used in the experiment (28°C, 39‰). The tank was equipped with an aeration system with three porous stones.

Experimental design

Three experimental units were used, consisting of rectangular white polypropylene boxes with a capacity of 60 L (55.5x37.7x31 cm) and a useful volume of 40 L, containing constant aeration and an HT-1900 100W heater. The initial density of macroalgae cultivation was 6 $g \cdot L^{-1}$ and the cultivation time was 28 days. The water used for the experiment was collected from the LCM biofloc matrix tank (capacity of 40·m^{-3}), where 5,000 *Litopenaeus vannamei* shrimp with approximately 15 g were cultured in a biofloc system. Shrimps were fed 4 times a day with commercial feed (Guabi Poti Evolution: 35% crude protein, 7.5% lipids, 4% fiber, 14% mineral matter, 10% moisture) at a rate of 3% of their biomass according to a feeding table (Van Wyk and Scarpa, 1999). Weekly, water quality parameters were evaluated in this tank, which remained constant throughout the experiment: total ammonia nitrogen 0.11 ± 0.1 $mg \cdot L^{-1}$, nitrite-N 0.09 ± 0.01 $mg \cdot L^{-1}$, pH 7.69 ± 0.01, dissolved orthophosphate 4.13 ± 1.4 $mg \cdot L^{-1}$, total suspended solids 544 ± 42.8 $mg \cdot L^{-1}$, alkalinity 188 ± 10.5 $mg \cdot L^{-1}$ and nitrate-N 57.1 ± 16.7 $mg \cdot L^{-1}$. All parameters were within the recommended range for the shrimp (Van Wyk and Scarpa, 1999).

The water exchange of the experimental units was performed once a day (morning), throughout the experiment, at a rate of 90% of the useful volume of the box. The water from the matrix tank was taken to the experimental units through a water recirculation system coupled to an aquarium pump and a 25 µm polyester bag filter to remove solids. The pump was turned on only when the experimental units were renewed. Lighting was provided naturally inside an agricultural greenhouse with 70% shading. To evaluate the macroalgae growth, weekly weighing was performed with a digital scale and water excess was removed using a manual centrifuge before weighing. The daily growth rate was calculated according to the equation GR (%·day^{-1}) = [(Fb / Ib) $^{1/t}$ -1] x 100, where Fb (final biomass), Ib (initial biomass), and t (time), proposed by Yong et al. (2013). The density of each experimental unit was then adjusted to an initial density of 6 $g \cdot L^{-1}$ and the remainder of the sample was saved for proximate and biochemical analyses. The proximate composition of the algae was performed according to the methods described by the Association of Official Analytical Chemists - International AOAC (1999).

Ulva ohnoi dry weight

To determine the dry weight, a wet sample of 5 g was put in a beaker and weighed on an analytical balance. The sample was then placed in an oven and dried at 60 °C for 24 hours to remove water.

Extraction and quantification of total phenolic compounds and photosynthetic pigments

Samples of *U. ohnoi* (3 g fresh weight, n = 3) were macerated in a crucible with liquid N2 and 5 mL of 80 % methanol (v/v). The material was incubated for 1 h in the dark and then centrifuged (12000 g, 10 min). Finally, the supernatant was recovered by vacuum filtration. The total contents of phenolic compounds were determined by the Folin-Ciocalteu colorimetric method (λ = 750 ηm), as described by Randhir et al. (2002). The gallic acid standard curve was used to calculate the analyte levels (Sigma-Aldrich, St. Louis, MO, USA – 100 - 1250 µg·mL^{-1}, y = 0.0108x, r2 = 0.999). The results, in triplicate, were expressed in mg of gallic acid equivalent ($C_7H_6O_5$) per g of dry mass.

The extraction and quantification of carotenoids and chlorophylls was performed with samples of fresh seaweed which were stored at −20 °C. Chlorophylls and carotenoids

were extracted with 1.5 mL of dimethyl sulfoxide at 40 °C for 40 min and quantified according to the methodology of Hiscox and Israelstam (1979). The equations used for obtaining the concentrations of chlorophylls and carotenoids are as follows:

$$\text{Chlorophyll a}=12.9*A665-3.45*A649$$

$$\text{Chlorophyll b}=21.99*A649-5.32*A665$$

$$\text{Total carotenoids}=(1000*A480-2.14*\text{chl a}-70.16*\text{chl b})\div 220$$

Where A480, A649 and A665 are the values obtained through reading the samples at the respective wavelengths in the spectrophotometer (480 nm, 649 nm and 665 nm) and chl a and chl b correspond to the chlorophyll a and b values.

Extraction and quantification of total flavonoids

The total flavonoid content was determined by the colorimetric method described by Chang et al. (2002), with adaptations to algal extract carried out by the research group of the Plant Morphogenesis and Biochemistry Laboratory (LMBV). An aliquot of 0.5 mL of extract of each sample was used to determine the total flavonoid content in the presence of 0.5 mL of methanolic aluminum chloride solution (2%) and 2 aliquots of 5 mL of ethanol P.A. The material was then vortexed and the sample remained protected from light for 1 h. Then, the absorbance reading was performed at 420 nm in a UV-Visible spectrophotometer (UV-2000A, Instrutherm). The calculation of the total flavonoid content was based on the quercetin standard curve (10 to 200 $\mu g \cdot mL^{-1}$ – r2 = 0.99; y = 0.010x). The analyzes were performed in triplicate and the results were expressed as mg of quercetin equivalent per g of dry mass of the extract.

Ulvan extraction

The ulvan polysaccharide extract was prepared according to the methodology described by Paulert et al. (2009) with modifications. Fresh macroalgae were autoclaved following the proportion of 50 g of fresh mass to 300 mL of distilled water for two hours at 110 °C. Then, the supernatant was incubated with three volumes of P.A ethanol for 48 h at -20 °C, and then filtered and lyophilized for 24 h at -54 °C and 0.160 mBar. Finally, the ulvan yield was calculated using the following formula: ulvan yield = [[dry ulvan (g)/fresh macroalgae (g)] x 100]/percent dry matter. For this calculation, the dry ulvan obtained from the extraction and the fresh mass used in the process were considered, and the result was divided by the dry matter of the macroalgae.

Protein content

The physical-chemical characterization analyzes were performed in triplicate according to the methodologies described by AOAC (1999): protein (LECO) Dumas method 990.03, conversion factor of 5 (Angell et al., 2016) and ether extract by Soxhlet using the 920.39C method.

Environmental variables

Throughout the experiment, the following environmental variables were monitored: dissolved oxygen and temperature (YSI 55, YSI Inc., Yellow Springs, OH, USA), as well as salinity (refractometer Instrutherm®) and illuminance (digital luxmeter Hikari HLX-881A) were measured twice a day. The illuminance values were measured above the water surface and were later converted to quantum irradiance ($\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$) by multiplying them by 0.018 (Gensler, 1986).

The water quality variables of the matrix biofloc tank were measured with the following methods: salinity (YSI 55, YSI Inc., Yellow Springs, OH, USA), pH (YSI 55, YSI Inc., Yellow Springs, OH, USA), TSS, alkalinity, total ammonia and nitrite-N (APHA, 1995; Strickland and Parsons, 1972), nitrate-N (HACH method 8039, cadmium reduction) and phosphate (APHA, 1995; Strickland and Parsons, 1972).

Statistical analysis

All results were calculated as mean ± standard deviation. The data were submitted to Shapiro-Wilk's and Brown-Forsythe's tests to assess the prerequisites of normality and homogeneity of variances, respectively. Then, an analysis of variance (one-way ANOVA) was used, followed by Dunnett's test of multiple comparisons. For the initial and final quantification of ulvan, Student's test was used. In all cases, the significance level employed was 5% and all analyses and visualizations were performed using the softwares GraphPad Prism version 9.2 and jamovi (jamovi project, 2022).

RESULTS

Water quality

The temperature, salinity, dissolved oxygen and photon irradiance of the laboratory cultivation period are presented in Table 1. Throughout the entire experiment, temperature ranged from 25.5°C to 29.9°C, salinity ranged from 38‰ to 40‰, dissolved oxygen ranged from 6.4 $mg \cdot L^{-1}$ to 8.5 $mg \cdot L^{-1}$ and photon irradiance ranged from 3.4 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$ to 18.4 $\mu mol\ photons\ m^{-2} \cdot s^{-1}$.

Table 1. Temperature, salinity, dissolved oxygen and photon irradiance: mean, minimum (min) and maximum (max) during 28 days of *Ulva ohnoi* cultivation in the laboratory.

Day	Temperature (°C)		Salinity (‰)		Dissolved oxygen (mg·L^{-1})		Photon irradiance (μmol photons·m^{-2}·s^{-1})	
	Mean	Min – Max	Mean	Min – Max	Mean	Min – Max	Mean	Min – Max
7	27.5 ± 0.4	26.3-29.4	40 ± 0.0	39-40	7.2 ± 0.2	6.4-7.9	15.3 ± 10.4	12.8-17.1
14	27.9 ± 1.6	26.0-29.8	39 ± 0.5	39-40	7.7 ± 0.4	7.1-8.5	11.4 ± 12.3	5.5-18.4
21	28.0 ± 1.4	26.8-29.9	39 ± 0.0	38-40	7.6 ± 0.2	7.0-8.3	11.7 ± 16.9	4.5-18.1
28	26.9 ± 0.6	25.5-28.2	40 ± 0.0	38-41	7.6 ± 0.1	7.0-8.2	8.6 ± 74.4	3.4-16.7

Values are represented as mean ± standard deviation (n = 3).

Algal growth performance and characterization of biocompounds

Algae growth performance is presented in Table 2 and Fig. 1. The overall specific growth rate across the experiment was 2.6 ± 0.8 %·day^{-1}, while the accumulated final biomass before the weekly partial harvests was on average 289.3 ± 15.2 g.

No statistical differences were observed in relation to the concentration of phenolic compounds and chlorophylls a and b (Table 3). Regarding flavonoids, a statistical difference was observed on days 7 and 14. It was also observed that carotenoids decreased approximately 41.3% in macroalgae cultivated after 28 days in biofloc (Table 3). In the case of ulvan, a significant difference was observed when comparing the initial (0.54 ± 0.06%) and final (0.92 ± 0.05%) concentrations (Fig. 2).

Values represent the mean ± confidence interval (n = 3) and the lowercase letters represent significant differences on initial and final yield ($p < 0.05$).

Statistical differences were observed in protein concentration throughout the cultivation period, with values of 14.52 ± 0.1% at the beginning and 18.91 ± 0.17% at the end, representing an increase of 30.2 % (Fig. 3).

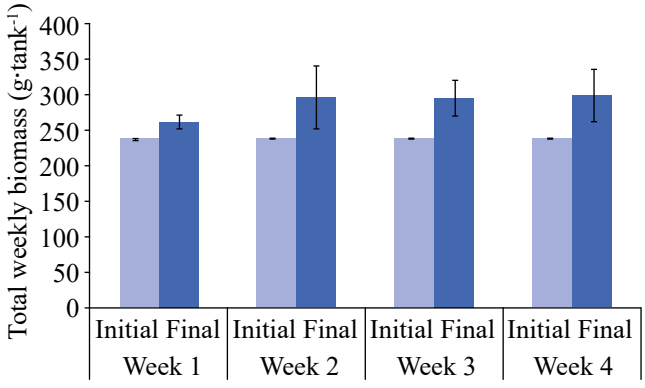

Figure 1. Complete weekly total biomass (g) of *Ulva ohoi* cultivated using water from biofloc technology for 28 days after weekly partial harvests returning the standing biomass to the initial value. No statistical significance was observed across time.

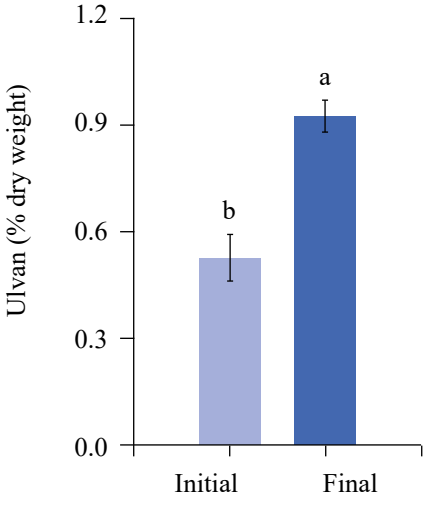

Figure 2. Yield of *Ulva ohnoi* ulvan at the beginning and end (after 28 days) of the culture period in water from a biofloc system.

Table 2. Growth rates of *Ulva ohnoi* cultivated for 28 days in biofloc measured through weekly biomass gain and specific growth rate.

U. ohnoi	Day 7	Day 14	Day 21	Day 28
Weekly biomass gain (g)	23.3 ± 9.3	57.3 ± 43.9	56.7 ± 24.9	60.0 ± 37.0
Specific growth rate (%·day^{-1})	1.3 ± 0.5	3.0 ± 2.2	3.0 ± 1.2	3.2 ± 1.8

Data are presented as arithmetic mean ± standard deviation (n=3).

Table 3. Concentration of phenolic compounds, flavonoids, chlorophylls a and b, and carotenoids from *Ulva ohnoi* cultivated for 28 days using water from a biofloc system.

	Day 0	Day 7	Day 14	Day 21	Day 28
Phenolic compounds (mg EAG·g^{-1} DW)	0.24 ± 0.01	0.33 ± 0.2	0.27 ± 0.1	0.19 ± 0.03	0.19 ± 0.01
Flavonoids (mg EQ·g^{-1} DW)	0.3 ± 0.0 ^{b}	0.4 ± 0.04 ^{a}	0.4 ± 0.07 ^{a}	0.3 ± 0.05 ^{b}	0.3 ± 0.03 ^{b}
Chlorophyll a (µg·g^{-1} DW)	78.5 ± 4.4	63.4 ± 13.6	53.4 ± 36.4	71.8 ± 47.3	80.6 ± 28.2
Chlorophyll b (µg·g^{-1} DW)	70.1 ± 3.6	57.7 ± 14.1	62.8 ± 49.4	80.0 ± 44.4	72.4 ± 25.5
Carotenoids (µg·g^{-1} DW)	32.2 ± 2.8 ^{b}	29.2 ± 3.6 ^{b}	19.5 ± 11.4 ^{b}	24.5 ± 1.4 ^{b}	18.9 ± 1.5 ^{a}

The values represent the mean ± confidence interval (n = 3) and the lowercase letters represent the significant differences among the days of cultivation ($p < 0.05$). DW: dry weight.

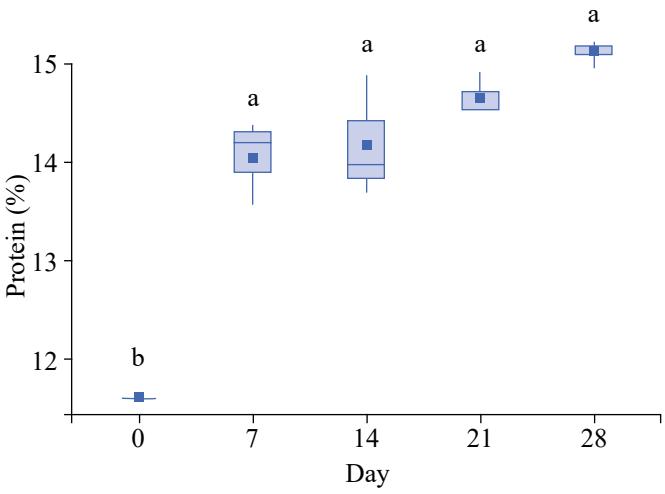

Figure 3. Boxplot graph of protein concentration of *Ulva ohnoi* during the 28-day culture in biofloc.

Squares denote the means (n = 3) and letters represent the significant differences between day 0 compared to days 7, 14, 21 and 28 ($p < 0.05$).

DISCUSSION

Various factors within the cultivation system, employed in this study using water from a biofloc shrimp tank, can affect both the growth performance and biochemical composition of *U. ohnoi*. This dynamic aquatic environment involves factors such as water quality variables, e.g. temperature, pH, and nutrient availability, in addition to light intensity. Moreover, the biofloc system's richness in nutrients, particularly nitrogen and phosphorus, creates favorable conditions for macroalgae growth (Khanjani et al., 2022). Understanding these interactions is essential for comprehending the mechanisms behind our observed results and gaining valuable insights into the nuances of *U. ohnoi* cultivation within the biofloc system.

Water quality parameters are essential to maintain adequate environmental conditions in aquaculture (Boyd and Tucker, 2014). In the present work, the water quality parameters were in the ideal range found for *U. ohnoi* species (Angell et al., 2015; Notoya, 1999; Ohno, 1988). Still, it is a fact that different species have different tolerance ranges to certain conditions, and regarding *U. ohnoi*, it is known that the ideal salinity range for growth fluctuates from 25 to 40‰, according to Angell et al., 2015. The fact that the salinity found in this study was close to the higher optimal limit could have negatively influenced its growth performance and biocompounds composition.

In addition to the water quality parameters, nutrient availability in the cultivation is another important factor for the growth of *Ulva* species (Imchen, 2012). Despite the nutrients available in biofloc, the macroalgae growth was relatively low due to some stressors, such as high stocking density. Martins et al. (2020) observed that *U. ohnoi* cultivated in biofloc under a dilution rate of 25% of biofloc water and 75% of seawater and a density of 2 g·L^{-1} had a significantly higher growth rate when compared to 4 g·L^{-1} density (4.3%·day^{-1} compared with 2.7%·day^{-1}, respectively). In both cases, the growth rate was higher than that observed in this study (2.6±0.8%·day^{-1}), which was possibly a result of the higher stocking density.

Another possibility for the low growth rate might have been the reduced light intensity brought about by the biofloc water

which, although filtered, was not diluted in this experiment, as in other ones employing similar experimental designs, where growth rates ranging from 2.7%·day^{-1} (Martins et al., 2020) up to 8.0 %·day^{-1} (Morais et al., 2023) having been observed, with the filtered biofloc water comprising 25% and 20% of the macroalgae unit volume, respectively. However, due to the differences in stocking densities, the confirmation of this hypothesis escapes the scope of this study, requiring a specific experimental design to answer it.

Having in view the prospect of including macroalgae in aquafeeds, the contents of antioxidant compounds (carotenoids, chlorophylls, phenolics, and flavonoids) are important because of their anti-inflammatory properties, in addition to the possibility of being used as alternative natural antibiotics (Peso-Echarri et al., 2012). These compounds vary according to the macroalgae species, spatial and temporal abiotic interactions within environmental parameters, anthropogenic interventions, and biotic interactions (Stengel et al., 2011).

Eismann et al. (2020) reported a wide range of carotenoids contents, from 0.005 to 900 mg·g^{-1} of fresh weight in *Ulva* sp. Raymundo et al. (2004) quantified carotenoid levels in green algae, obtaining average values ranging from 72.27 to 129.04 mg·kg^{-1}. The lowest content was found in the species *U. fasciata*, while the highest was observed in the macroalgae *Enteromorpha intestinalis*. Similarly, Legarda et al. (2021) found carotenoid concentrations values ranging from 4.99 ± 0.73 µg·g^{-1} to 16.46 ± 3.41 µg·g^{-1} of the fresh weight of *U. fasciata* integrated with shrimp and mullet in a biofloc system. There was a reduction of carotenoids at the end of the cultivation in the present study, but it remained within the values found in literature. According to Chakraborty and Santa (2008), the profile of carotenoids (vitamins) in macroalgae depends on endogenous and exogenous factors, such as the difference in salinity from the place the macroalgae were collected to the place of cultivation. Kakinuma et al. (2004) reported that *Ulva pertusa* showed changes in some physiological and biochemical aspects when cultivated under salinity fluctuations. The authors also reported that, in response to high salinity, a decrease in the total pigment content (carotenoids and chlorophyll a and b) can be observed. These changes cause irreversible damage to the photosynthetic activity of the seaweed, which might explain both the reduction in carotenoid content throughout the experiment and the relatively poor growth rates observed in this study when compared to previous publications.

In the literature, chlorophyll values are diverse and expressed in different units because they can be determined by different extraction methods. Raymundo et al. (2004) reported average chlorophyll values ranging from 151.48 to 411.51 mg·kg^{-1} for the species *Chaetomorpha anteninna* and *Codium decorticatum*, respectively, determined through the use of acetone as the solvent. Legarda et al. (2021) found values of 72.15±12.07 (initial) and 294.66±16.46 µg·g^{-1} (final) of the fresh weight of *U. fasciata* cultivated in biofloc. Hiscox and Israelstam (1979) suggested that DMSO (dimethyl sulfoxide) is a superior method compared to acetone for extracting chlorophyll a and b in green algae. This is attributed to its high diffusion capacity through semi-permeable membranes and its effectiveness as a protein carrier (Ronen and Galun, 1984). In the present work, there were no significant differences in chlorophyll concentrations through the DMSO method. A possible explanation is that the content and presence of pigments may vary according to factors such as reproduction, growth phase, environmental changes, and water salinity, the latter being a limiting factor for the species in this work (Chakraborty et al., 2010; Kakinuma et al., 2004; 2006).

Phenol extraction yield depends on the variety of active compounds with different properties and polarities, which can be affected according to the solubility of the solvent. For the extraction of polyphenols from a plant matrix, polar solvents such as ethanol, methanol, acetone, and ethyl acetate are used (Parekh and Chanda, 2007). Legarda et al. (2021) quantified, using methanol as a solvent, the phenolic compounds of *U. fasciata* cultivated in biofloc and obtained values of 0.32±0.05 (initial) and 0.19±0.03 µg·g^{-1} (final) of dry weight. In the present work, methanol was also used as a solvent for the analysis of green macroalgae cultivated in biofloc, and results within the standards found in the literature were found (Dimova et al., 2019; Legarda et al., 2021). These concentrations may be the result of differences in the chemical composition among the phenolic compounds of the macroalgae, the solvents used in the protocols, or the involvement in the antioxidant activity of other compounds, such as chlorophylls and carotenoids (Raymundo et al., 2004). The same authors reported that for the species *E. intestinalis*, a higher percentage of inhibition (75%) was obtained with the methanolic extract, which had total phenolic values of 610.31 mg 100·g^{-1} of biomass. This was in comparison to *U. fasciata*, which had a similar statistically equivalent amount of phenolic compounds (635.53 mg 100·g^{-1}). However, despite the similar levels of phenolic compounds, there were significant differences in the antioxidant effectiveness between the methanolic extracts of the two species, supporting the theory mentioned above.

Flavonoids represent one of the most important groups of phenolic compounds. The taxonomic classification and

species distribution influence the metabolism and production of these compounds, which result in different concentrations (Machado et al., 2008). Al-Malki et al. (2018) evaluated the impact of several solvents on the flavonoid yield of *U. lactuca* collected in the Red Sea in Saudi Arabia, and found different values according to each solvent used in the methodology (34.5±4.8 ethanol, 60 ± 7.0 ethyl acetate, 31.2±3.3 chloroform, in mg QE/g extract). Seal et al. (2015) reported values of 9397.0 mg EQ·100 g^{-1} and 9894.0 mg EQ·100 g^{-1} of dried algae for extracts from the macroalgae *Nitella flagelliformis* using acetone and methanol as solvents, respectively. In the present study, the solvent used in the methodology was ethanol, and we obtained a statistical difference in the second and third weeks of cultivation. However, the biofloc system, in general, did not interfere with the flavonoid yields according to the values found in the literature about macroalgae collected in a natural environment (Al-Malki et al., 2018; Farasat et al., 2014).

Ulvan is the main water-soluble carbohydrate of members of the order Ulvales (Ray and Lahaye, 1995). In general, this polysaccharide has been gaining prominence due to its biological properties, such as anti-carcinogenic, anti-proliferative, antiviral, antioxidant, antihypertensive, anti-inflammatory, anticoagulant, among others (Collén et al., 2011; Costa et al., 2010; Glasson et al., 2017; Karnjanapratum and You, 2011; Qi et al., 2012). In aquaculture, ulvan has potential to be applied to aquafeeds due to possible improvements in feed efficiency, immunostimulant action, and enhancement of fish health (Peso-Echarri et al., 2012). Pitta et al. (2022) evaluated the performance of *U. fasciata* cultivated in IMTA at six different densities ranging from 1 to 6 kg m^{-3}. They found values of ulvan in the algae biomass ranging from 14.2% to 18.4% of its dry weight using the methodology of Paulert et al. (2009). Similarly, Castelar et al. (2014), who also employed the same extraction method, reported ulvan productivity values of 15.6±5.2% for *Ulva flexuosa* cultivated in the sea, which were comparable to those cultivated in tanks at 20.2±3.9%. In the present work, where the same method of ulvan extraction was used, the cultivation of *U. ohnoi* in biofloc increased the concentration of this polysaccharide in the macroalgae by 75%. This increase is explained by the fact that ulvan is a heteropolysaccharide, which is present in the *Ulva* cell wall and is strongly associated with proteins (Collén et al., 2011; Karnjanapratum and You, 2011; Qi et al., 2012), which also increased in the macroalgae when comparing the initial sampling time with the final one.

In the biofloc system, nutrients such as nitrogen and phosphorus accumulate, and macroalgae can absorb and transform this nitrogen into protein (Duke et al., 1989). Our results clearly show this, since the macroalgae had 30 % more protein in their final composition, which showed that they can absorb the nitrogenous compounds offered by the biofloc system to increase the protein content. The same was found by Legarda et al. (2021), who cultivated the macroalgae *U. fasciata* in biofloc and obtained an increase of 182 % in the concentration of nitrogen in its composition at the end of the cultivation.

CONCLUSION

The cultivation of *U. ohnoi* in biofloc technology resulted in statistically significant increases in both protein and ulvan contents, which could have positive implications for its application as a feed additive or a feed ingredient in aquafeeds. Room for future studies include possible effects of both salinity and stocking density in the biocompounds characterization of the species, both factors which could have affected the results of this study and would require further elucidation.

ETHICAL APPROVAL

All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the authors.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data will be made available by the authors upon reasonable request.

AUTHOR CONTRIBUTIONS

Conceptualization: Rocha JS, Hayashi L, Vieira FN; **Formal analysis:** Rocha JS, Santos D, Martins MA, Bauer CM; **Acquisition of funding:** Vieira FN; **Research:** Rocha JS, Santos D, Martins MA, Bauer CM; **Methodology:** Rocha JS, Maraschin M, Hayashi L, Vieira FN; **Project administration:** Vieira FN; **Resources:** Maraschin M, Hayashi L, Vieira FN; **Supervision:** Vieira FN; **Visualization:** Rocha JS, Martins MA; **Writing – Preparation of original draft:** Rocha JS, Santos D,

Martins MA, Bauer CM, Maraschin M, Hayashi L, Vieira FN; **Writing - Proofreading and editing:** Rocha JS, Martins MA.

FUNDING

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Finance Code 001.

ACKNOWLEDGMENTS

The authors would like to thank the team at the Laboratório de Camarões Marinhos for helping to carry out the experiment.

REFERENCES

Al-Malki, A.L.; Barbour, E.K.; Al-Zahrani, M.H.; Moselhy, S.S. 2018. Impact of Various Solvents on Yield and Activity of Phenolics and Flavonoids of *Ulva lactuca* (Chlorophyta) Algae. *Journal of Pharmaceutical Research International*, 24(3): 1-7. https://doi.org/10.9734/JPRI/2018/44875

Alves, A.; Sousa, R.A.; Reis, R.L. 2013. A practical perspective on ulvan extracted from green algae. *Journal of Applied Phycology*, 25: 407-424. https://doi.org/10.1007/s10811-012-9875-4

Angell, A.R.; Mata, L.; Nys, R.; Paul, N.A. 2015 Indirect and direct effects of salinity on the quantity and quality of total amino acids in *Ulva ohnoi* (Chlorophyta). *Journal of Phycology*, 51(3): 536-545. https://doi.org/10.1111/jpy.12300

Angell, A.R.; Mata, L.; Nys, R.; Paul, N.A. 2016. The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. *Journal of Applied Phycology*, 28: 511-524. https://doi.org/10.1007/s10811-015-0650-1

Association of Official Analytical Chemists (AOAC). 1999. Official Methods of Analysis. Arlington: OMA.

American Public Health Association (APHA). 1995 Standard method for the examination of water and waste water. Washington: American Public Health Association.

Avnimelech, Y. 2015. *Biofloc Technology*: a practical hand book. Baton Rouge: World Aquaculture Society.

Avnimelech, Y.; Ritvo, G. 2003. Shrimp and fish pond soils: Processes and management. *Aquaculture*, 220(1-4): 549-567. https://doi.org/10.1016/S0044-8486(02)00641-5

Bikker, P.; Krimpen, M.M.V.; Wikselaar, P.V.; Tan-Houweling, B.; Scaccia, N.; Hal, J.W.V.; Huijgen, W.J.J.; Cone, J.W.; Contreras-López, A.M. 2016 Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals and biofuels. *Journal of Applied Phycology*, 28: 3511-3525. https://doi.org/10.1007/s10811-016-0842-3

Bolton, J.J.; Robertson-Andersson, D.V.; Shuuluka, D.; Kandjengo, L. 2009. Growing *Ulva* (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a swot analysis. *Journal of Applied Phycology*, 21: 575-583. https://doi.org/10.1007/s10811-008-9385-6

Boyd, C.E.; Tucker, C.S. 2014. *Handbook for aquaculture water quality*. Auburn: Craftmaster Printers.

Castelar, B.; Reis, R.P.; Calheiros, A.C.S. 2014. *Ulva lactuca* and *U. flexuosa* (Chlorophyta, Ulvophyceae) cultivation in Brazilian tropical waters: recruitment, growth, and ulvan yield. *Journal of Applied Phycology*, 26: 1989-1999. https://doi.org/10.1007/s10811-014-0329-z

Chakraborty, S.; Santa, S.C. 2008. Biochemical composition of eight algae collected from Suberdan. *Indian Journal of Marine Science*, 37(3): 329-332.

Chakraborty, S.; Santra, S.C.; Bhattacharya, T. 2010 Seasonal variation of enzyme activity and stress metabolites in eight benthic macro algae with fluctuations in salinity of Sunderban estuary, India. *Indian Journal of Marine Sciences*, 39(3): 429-433.

Chang, C.-C.; Yang, M.-H.; Wen, H.-M.; Chern, J.-C. 2002 Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food Drug Analysis* 10(3): 178-182. https://doi.org/10.38212/2224-6614.2748

Chopin, T.; Buschmann, C.H.; Troell, M.; Kautsky, N.; Neori, A.; Kraemer, G.P.; González-Zertuche, J.A.; Yarish, C.; Neefus, C. 2001. Integrating seaweeds into marine aquaculture systems: a key toward sustainability. *Journal of Phycology*, 37(6): 975-986. https://doi.org/10.1046/j.1529-8817.2001.01137.x

Cohen, I.; Neori, A. 1991. *Ulva lactuca* Biofilters for Marine Fishpond Effluent. I. Ammonium Uptake Kinetics and Nitrogen Content. *Botanic Marine*, 34: 475-482. https://doi.org/10.1515/botm.1991.34.6.475

Collén, P.N.; Sassi, J.-F.; Rogniaux, H.; Marfaing, H.; Helbert, W. 2011. Ulvan Lyases Isolated from the Flavobacterium Persicivirga ulvanivorans Are the first Members of a New Polysaccharide Lyase Family. *Journal of Biological Chemistry*, 286(49): 42063-42071. https://doi.org/10.1074/jbc.M111.271825

Costa, L.; Fidelis, G.P.; Cordeiro, S.L.; Oliveira, R.M.; Sabry, D.A.; Câmara, R.B.G.; Nobre, L.T.D.B.; Costa, M.S.S.P.; Almeida-Lima, J.; Farias, E.H.C.; Leite, E.L.; Rocha, H.A.O. 2010. Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomedicine & Pharmacotherapy*, 64: 21-28. https://doi.org/10.1016/j.biopha.2009.03.005

Crab, R.; Avnimelech, Y.; Defoirdt, T.; Bossier, P.; Verstraete, W. 2007 Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*, 270(1-4): 1-14. https://doi.org/10.1016/j.aquaculture.2007.05.006

Cruz-Suárez, L.E.; León, A.; Peña-Rodríguez, A.; Rodríguez-Peña, G.; Moll, B.; Ricque-Marie, D. 2010. Shrimp: *Ulva* co-culture: A sustainable alternative to diminish the need for artificial feed and improve shrimp quality. *Aquaculture*, 301(1-4): 64-68. https://doi.org/10.1016/j.aquaculture.2010.01.021

Dimova, D.; Dobreva, D.; Panayotova, V.; Makedonski, L. 2019. Dpph antiradical activity and total phenolic content of methanol and ethanol extracts from macroalgae (*Ulva rigida*) and microalgae (*Chlorella*). *Scripta Scientifica Pharmaceutica*, 6(2): 37-41. https://doi.org/10.14748/ssp.v7i2.7369

Duke, C.S.; Litaker, W.; Ramus, J. 1989. Effects of temperature, nitrogen supply, and tissue nitrogen on ammonium uptake rates of the Chlorophyte seaweeds *Ulva curvata* and *Codium decorticatum. Journal of Phycology*, 25(1): 113-120. https://doi.org/10.1111/j.0022-3646.1989.00113.x

Eismann, A.I.; Reis, R.P.; Silva, A.F.; Cavalcanti, D.N. 2020 *Ulva* spp. Carotenoids: Responses to environmental conditions. *Algal Research*, 48: 101916. https://doi.org/10.1016/j.algal.2020.101916

Farasat, M.; Khavari-Nejad, R.A.; Nabavi, S.M.B.; Namjooyan, F. 2014. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. *Iranian Journal of Pharmaceutical Research*, 13(1): 163-170.

Gensler, W.G. 1986. *Advanced agricultural instrumentation*: design and use. Dordrecht: Springer. https://doi.org/10.1007/978-94-009-4404-6

Glasson, C.R.K.; Sims, I.M.; Carnachan, S.M.; Nys, R.; Magnusson, M. 2017. Cascading biorefinery process targeting sulfated polysaccharides (ulvan) from *Ulva ohnoi. Algal Research*, 27: 83-391. https://doi.org/10.1016/j.algal.2017.07.001

Goodwin, T.W. 1962. *Carotenoids, their comparative biochemistry*. New York: Chemical Pub. Co.

Guaratini, T.; Cardozo, K.H.M.; Pinto, E.; Colepicolo, P. 2009. Comparison of Diode Array and Electrochemical Detection in the C30 Reverse Phase HPLC Analysis of Algae Carotenoids. *Journal of Brazilian Chemistry Society*, 20(9): 1609-1616. https://doi.org/10.1590/S0103-50532009000900007

Hiscox, J.D.; Israelstam, G.F. 1979. A Method for Extraction of Chlorophyll from Leaf Tissue without Maceration. *Journal of Botanic*, 57(12): 1332-1334. https://doi.org/10.1139/b79-163

Hoang, T.H.; Qin, J.G.; Stone, D.A.J.; Harris, J.O.; Duong, D.N.; Bansemer, M.S. 2016. Colour changes of greenlip abalone (*Haliotis laevigata* Donovan) fed fresh macroalgae and dried algal supplement. *Aquaculture*, 456: 16-23. https://doi.org/10.1016/j.aquaculture.2016.01.022

Imchen, T. 2012. Recruitment Potential of a Green Alga *Ulva flexuosa* Wulfen Dark Preserved Zoospore and Its Development. *PLoS ONE*, 7(3): e32651. https://doi.org/10.1371/journal.pone.0032651

Jamovi project. 2022. jamovi. (Version 2.3) [Computer Software]. Available at: https://www.jamovi.org. Accessed on: Oct. 24, 1995.

Kakinuma, M.; Coury, D.A.; Kuno, Y.; Kozawa, Y.; Inagaki, E.; Yoshiura, Y.; Amano, H. 2006. Physiological and biochemical responses to thermal and salinity stresses in a sterile mutant of *Ulva pertusa* (Ulvales, Chlorophyta). *Marine Biology*, 149(1): 97-106. https://doi.org/10.1007/s00227-005-0215-y

Kakinuma, M.; Kuno, Y.; Amano, H. 2004. Salinity stress responses of a sterile mutant of *Ulva pertusa* (Ulvales, Chlorophyta. *Fisheries Science*, 70(6): 1177-1179. https://doi.org/10.1111/j.1444-2906.2004.00921.x

Karnjanapratum, S.; You, S.G. 2011. Molecular characteristics of sulfated polysaccharides from Monostroma nitidum and their in vitro anticancer and immunomodulatory activities. *International Journal of Biological Macromolecules*, 48(2): 311-318. https://doi.org/10.1016/j.ijbiomac.2010.12.002

Khanjani, M.H.; Zahedi, S.; Mohammadi, A. 2022. Integrated multitrophic aquaculture (IMTA) as an environmentally friendly system for sustainable aquaculture: functionality, species, and application of biofloc technology (BFT). *Environmental Science Pollution Research*, 29(45): 67513-67531. https://doi.org/10.1007/s11356-022-22371-8

Khoi, L.V.; Fotedar, R. 2011. Integration of western king prawn (*Penaeus latisulcatus* kishinouye, 1896) and green seaweed (*Ulva lactuca* Linnaeus, 1753) in a closed recirculating aquaculture system. *Aquaculture*, 322-323: 201-209. https://doi.org/10.1016/j.aquaculture.2011.09.030

Lahaye, M.; Axelos, M.A.V. 1993. Gelling properties of water-soluble polysaccharides fromproliferating marine green seaweed (*Ulva* spp.). *Carbohydrates Polymers*, 22(4): 261-265. https://doi.org/10.1016/0144-8617(93)90129-R

Lapointe, B.E.; Tenore, K.R. 1981. Experimental outdoor studies with *Ulva fasciata* Delile. I. Interaction of light and nitrogen on nutrient uptake, growth, and biochemical composition. *Journal of Experimental Marine Biology and Ecology*, 53(2-3): 135-152. https://doi.org/10.1016/0022-0981(81)90015-0

Legarda, E.C.; Silva, D.; Miranda, C.S.; Pereira, P.K.M.; Martins, M.A.; Machado, C.; Lorenzo, M.A.; Hayashi, L.; Vieira, F.N. 2021. Sea lettuce integrated with Pacific white shrimp and mullet cultivation in biofloc impact system performance and the sea lettuce nutritional composition. *Aquaculture*, 534: 736265. https://doi.org/10.1016/j.aquaculture.2020.736265

Machado, H.; Nagem, T.J.; Peter, V.M.; Fonseca, C.S.; Oliveira, T.T. 2008. Flavonoids and potential therapeutic. *Boletim do Centro de Biologia da Reprodução*, 27(1-2): 33-39.

Martins, M.A.; Silva, V.F.; Tarapuez, P.R.; Hayashi, L.; Vieira, F.N. 2020 Cultivation of the seaweed *Ulva* spp. with effluent from a shrimp biofloc rearing system: Different species and stocking density. *Boletim do Instituto de Pesca*, 46(3): e602. https://doi.org/10.20950/1678-2305.2020.46.3.602

Morais, A.P.M.; Santos, I.L.; Carneiro, R.F.S.; Routledge, E.A.B.; Hayashi, L.; Lorenzo, M.A.; Vieira, F.N. 2023. Integrated multitrophic aquaculture system applied to shrimp, tilapia, and seaweed (*Ulva ohnoi*) using biofloc technology. *Aquaculture*, 572: 739492. https://doi.org/10.1016/j.aquaculture.2023.739492

Naldi, M.; Viaroli, P. 2002. Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agardh in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Sacca di Goro, Po River Delta, Italy). *Journal of Experimental Marine Biology and Ecology*, 269(1): 65-83. https://doi.org/10.1016/S0022-0981(01)00387-2

Nelson, S.G.; Glenn, E.P.; Conn, J.; Moore, D.; Walsh, T.; Akutagawa, M. 2001. Cultivation of *Gracilaria parvispora* (Rhodophyta) in shrimp-farm effluent ditches and floating cages in Hawaii: a two-phase polyculture system. *Aquaculture*, 193(3-4): 239-248. https://doi.org/10.1016/S0044-8486(00)00491-9

Notoya, M. 1999. *Utilization of* Ulva *spp. and environmental restoration*. Tokyo: Seizandou.

Ohno, M. 1988. Seasonal Changes of the Growth of Green Algae, *Ulva* sp. in Tosa Bay, Southern Japan. *Marine fouling*, 7(1-2): 13-17. https://doi.org/10.4282/sosj1979.7.13

Parekh, J.; Chanda, S.V. 2007. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish Journal of Biology*, 31(1): 53-58.

Paulert, R.; Talamini, V.; Cassolato, J.E.F.; Duarte, M.E.R.; Noseda, M.D.; Smania Junior, A.; Stadnik, M.J. 2009. Effects of sulfated polysaccharide and alcoholic extracts from green seaweed *Ulva fasciata* on anthracnose severity and growth of common bean (*Phaseolus vulgaris L.*). *Journal of Plant Diseases and Protection*, 116: 263-270. https://doi.org/10.1007/BF03356321

Pedra, A.G.M.; Ramlov, F.; Maraschin, M.; Hayashi, L. 2017. Cultivation of the red seaweed *Kappaphycus alvarezii* with effluents from shrimp cultivation and brown seaweed extract: Effects on growth and secondary metabolism. *Aquaculture*, 479: 297-303. https://doi.org/10.1016/j.aquaculture.2017.06.005

Peso-Echarri, P.; Frontela-Saseta, C.; González-Bermúdez, C.A.; Ros-Berruezo, G.F.; Martinez-Graciá, C. 2012. Polisacáridos de algas como ingredientes funcionales en acuicultura marina: alginato, carragenato y ulvano. *Revista de Biología Marina y Oceagrafía*, 47(3): 373-381. https://doi.org/10.4067/S0718-19572012000300001

Pitta, J.P.M.P.; Pontes, M.D.; Castelar, B.; Hamacher, C. 2022. Desempenho de curto prazo de *Ulva fasciata* produzida em diferentes densidades em aquicultura multitrófica integrada. *Conjecturas*, 22(9): 1-17. https://doi.org/10.53660/CONJ-1385-AG05

Qi, H.; Liu, X.; Zhang, J.; Duan, Y.; Wang, X.; Zhang, Q. 2012. Synthesis and antihyperlipidemic activity of acetylated derivative of ulvan from *Ulva pertusa. International Journal of Biological Macromolecules*, 50(1): 270-272. https://doi.org/10.1016/j.ijbiomac.2011.11.006

Ray, B.; Lahaye, M. 1995. Cell-wall polysaccharides from the marine green alga *Ulva* "*rigida*" (ulvales, chlorophyta). Extraction and chemical composition. *Carbohydrate Research*, 274: 252-261. https://doi.org/10.1016/0008-6215(95)00138-J

Raymundo, M.S.; Horta, P.; Fett, R. 2004. Atividade antioxidante in vitro de extratos de algumas algas verdes (Chlorophyta) do litoral catarinense (Brasil). *Revista Brasileira Ciências Farmaceutica*, 40(4): 495-503. https://doi.org/10.1590/S1516-93322004000400007

Revilla-Lovano, S.; Sandoval-Gil, J.M.; Zertuche-González, J.A.; Belando-Torrentes, M.D.; Bernardeau-Esteller, J.; Rangel-Mendoza, L.K.; Ferreira-Arrieta, A.; Guzmán-Calderón, J.M.; Camacho-Ibar, V.F.; Muñiz-Salazar, R.; Ávila-López, M.C. 2021. Physiological responses and productivity of the seaweed *Ulva ohnoi* (Chlorophyta) under changing cultivation conditions in pilot large land-based ponds. *Algal Research*, 56: 102316. https://doi.org/10.1016/j.algal.2021.102316

Randhir, R.; Shetty, P.; Shetty, K. 2002. L-Dopa and total phenolic stimulation in dark germinated fava bean in response to peptide and phytochemical elicitors. *Process Biochemistry*, 37(11): 1247-1256. https://doi.org/10.1016/S0032-9592(02)00006-7

Ronen, R.; Galun, M. 1984. Pigment extraction from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyll degradation. *Environmental and Experimental Botany*, 24(3): 239-245. https://doi.org/10.1016/0098-8472(84)90004-2

Seal, T.; Halder, N.; Chaudhuri, K.; Sinha, S.N. 2015. Evaluation of antioxidant activities of algae and effect of solvent extraction system. *International Journal of Pharmaceutical Sciences and Research*, 6(3): 1273. https://doi.org/10.13040/IJPSR.0975-8232.6(3).1273-78

Sebök, S.; Herppich, W.B.; Hanelt, D. 2017. Development of an innovative ring-shaped cultivation system for a land-based cultivation of marine macroalgae. *Aquacultural Engineering*, 77: 33-41. https://doi.org/10.1016/j.aquaeng.2017.01.005

Silva, K.R.; Wasielesky Junior, W.; Abreu, P.C. 2013. Nitrogen and phosphorus dynamics in the biofloc production of the Pacific white shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society*, 44(1): 30-41. https://doi.org/10.1111/jwas.12009

Stengel, D.B.; Connan, S.; Popper, Z.A. 2011. Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. *Biotechnology Advances*, 29(5): 483-501. https://doi.org/10.1016/j.biotechadv.2011.05.016

Strickland, J.D.H.; Parsons, T.R. 1972. *A practical handbook of seawater analysis*. Ottawa: Fisheries Research Board of Canada.

Thakur, D.P.; Lin, C.K. 2003. Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems. *Aquacultural Engineering*, 27(3): 159-176. https://doi.org/10.1016/S0144-8609(02)00055-9

Troell, M.; Halling, C.; Neori, A.; Choppin, T.; Buschmann, A.H.; Kautsky, N.; Yarish, C. 2003. Integrated mariculture: asking the right questions. *Aquaculture*, 226(1-4): 69-90. https://doi.org/10.1016/S0044-8486(03)00469-1

Valente, L.M.P.; Rema, P.; Gouveia, A.; Matos, J. 2006 Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, 252(1): 85-91. https://doi.org/10.1016/j.aquaculture.2005.11.052

Van Wyk, P.; Scarpa, J. 1999. *Water Quality and Management*. In: Van Wyk, P.; Davis-Hodgkins, M.; Laramore, R.; Main, K.L.; Mountain, J.; Scarpa, J. (eds.). Farming Marine Shrimp in Recirculating Freshwater Systems. Tallahassee: Division of Aquaculture. pp. 128-138.

Xu, Y.; Fang, J.; Tang, Q.; Lin, J.; Le, G.; Liao, L.V. 2008a. Improvement of water quality by the macroalga, *Gracilaria lemaneiformis* (Rhodophyta), near aquaculture effluent outlets. *Journal World Aquaculture Society*, 39(4): 441-571. https://doi.org/10.1111/j.1749-7345.2008.00180.x

Xu, Y.; Fang, J.; Wei, W. 2008b. Application of *Gracilaria lichenoides* (Rhodophyta) for alleviating excess nutrients in aquaculture. *Journal of Applied Phycology*, 20(2): 199-203. https://doi.org/10.1007/s10811-007-9219-y

Yong, Y.S.; Yong, W.T.L.; Anton, A. 2013. Analysis of formulae for determination of seaweed growth rate. *Journal of Applied Phycology*, 25: 1831-1834. https://doi.org/10.1007/s10811-013-0022-7