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Growth of the Macroalgae *Ulva lactuca* Cultivated at Different Depths in a Biofloc Integrated System with Shrimp and Fish

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Abstract: The constant production of solids in intensive shrimp and tilapia culture can affect the performance of macroalgae when cultivated in an integrated system, and little is known about culture structures that enhance the performance of macroalgae in biofloc systems. The objective of this work was to evaluate different depths of culture structure for the macroalgae *Ulva lactuca* in an integrated system with *Litopenaeus vannamei* and *Oreochromis niloticus* in a biofloc system. The experiment lasted 70 days, with six systems composed of: a 16 m^3 shrimp tank, a 3 m^3 tilapia tank, and a 3 m^3 macroalgae tank, with water recirculation between tanks. Two treatments were carried out, shallow float, with a structural depth of 10 cm, and bottom float, where the depth was kept at 30 cm from the surface. The shallow float resulted in a growth rate of up to 0.95 ± 0.54% day^{-1}, with biomass loss only at the end of the culture due to the high density of macroalgae, decreasing temperature, and increasing solids concentration. The bottom float had biomass loss throughout the culture cycle. The integrated culture of shrimp, fish, and macroalgae is feasible with the use of shallow floats within 10 cm from the surface.

Keywords: total suspended solids; cultivation structure; temperature; density

Citation: Carvalho, A.; Costa, L.C.d.O.; Holanda, M.; Gonçalves, M.; Santos, J.; Costa, C.S.B.; Turan, G.; Poersch, L.H. Growth of the Macroalgae *Ulva lactuca* Cultivated at Different Depths in a Biofloc Integrated System with Shrimp and Fish. *Phycology* **2023**, *3*, 280–293. https://doi.org/10.3390/phycology3020018

Academic Editors: Marcin Dębowski and Peer Schenk

Received: 28 February 2023
Revised: 1 May 2023
Accepted: 5 May 2023
Published: 10 May 2023

1. Introduction

The shrimp *Litopenaeus vannamei* (Boone, 1931) is the most cultivated crustacean in the world, with current production of approximately 5.8 million tons [1]. Its advancement in production is due to the consequence of better adaptation in the area, the knowledge established about its reproductive cycle, and the simplicity in management practices [2], thus being the main species in an integrated culture system. The tilapia, *Oreochromis niloticus*, also represents an enhancement in integrated culture due to the possibility of a low feed supply (1% of biomass) to induce the intake behavior of the solids, increasing the productivity of the culture with lower costs, as demonstrated by Poli et al. [3] with the integrated culture of shrimp, tilapia, and halophytes. The growth of tilapia production is due to the easiness in obtaining juveniles, the rapid growth, and the possibility of cultivation in several places, being a highly produced species in Brazil and with great economic interest.

In shrimp farming, only 25 to 30% of nitrogen and phosphorus from feed and fertilizers are used by the shrimp, and most of it is leached and lost in the water [4]. Choosing species that can reuse such waste and maximize production is essential for more sustainable systems. The use of organic and inorganic consuming species to take advantage of system waste characterizes Integrated Multi-Trophic Aquaculture (IMTA), which has the advantage of greater sustainability and final productivity [5]. A target species of higher trophic level is

chosen, such as fish or shrimp, and organic and inorganic consuming species are integrated into the system with the objective to, respectively, reuse the organic matter and nutrients available in the water for the growth of new biomass with added economic value [6].

The cultivation of macroalgae associated with other aquatic organisms in aquaculture has gained momentum due to the increasing focus on sustainable systems with nutrient recycling. As inorganic consumers, macroalgae use nitrogen and phosphate compounds in the water for their growth [7]. Several studies have shown a better performance of macroalgae when cultivated in shrimp farm effluent and/or in integrated systems. Nardelli et al. [8] showed that macroalgae growth, oxygen production, and nutrient uptake were directly proportional to increasing trophic levels and species insertions in a system. The production of macroalgae is currently approximately 35 million tons in 2020 compared to 10 million in the 2000s [1]. The rise in economic interest in macroalgae cultivation is attributed to the extraction of by-products such as agar and carrageenan for the food and pharmaceutical industries [2] and the consumption of fresh macroalgae [9].

The use of the integrated system in conjunction with biofloc technology (BFT) has recently advanced to reuse waste and minimal water exchange through the control of nitrogenous compounds performed by a diversity of organisms present in the system [10]. This control in water quality occurs by heterotrophic bacteria, which transform ammonia in the presence of carbon and intense aeration into microbial biomass, and chemoautotrophic bacteria that convert ammonia to nitrite and subsequently to nitrate [11]. The transformation of ammonia to nitrate allows a low-toxicity nitrogen compound to accumulate without reducing water quality [12]. The presence of high concentrations of nutrients in the system can be advantageous for inorganic consuming species that reuse the waste from another species for biomass growth.

The control of total suspended solids in the integrated production of organisms in biofloc is advantageous to maintain water quality and complement the diet of cultured organisms. However, according to Gaona et al. [13], levels of 100 to 300 mg L^{-1} are optimal for the culture system. Organic consumers can also benefit from this production of solids and use them as complementary food sources. In an integrated system of *Litopenaeus vannamei* shrimp and *Mugil liza* mullet, Holanda et al. [14] found reduced solids concentrations in the integrated system compared to shrimp monoculture. Azim and Little [15] also showed that the protein level of tilapia diets can be reduced when tilapia is grown in biofloc due to the supplementation of the diet by microbial flocs. However, high concentrations of solids can negatively impact the performance of cultured organisms, such as shrimp and fish, for which gill obstruction may occur [13].

For macroalgae, the characteristics of the biofloc system impose many challenges to their use and active optimal culture conditions. High concentrations of solids can affect macroalgae growth due to the deposition of total suspended solids in the photosynthesizing tissue [16]. In addition to this deposition, the low transparency of the water can reduce light uptake by the macroalgae in the tanks. Reis et al. [17] show that with increasing depth, the light intensity decreases in a biofloc system due to the bacterial aggregates. Therefore, cultures of lower depth may limit macroalgae to the surface where there is greater light availability.

A shallow depth can limit the movement of macroalgae. The biofloc system has as a characteristic for its maintenance the use of intense aeration for the movement of particles in the water column [10]. This intense aeration can favor the movement of macroalgae in deeper structures, causing the algae to reach the bottom of the structure and then return to the surface, so there is no overlap between them. The overlapping of macroalgae by having a high density in a limited space can cause biomass loss during culture procedures [18]. Better management procedures are needed for the suitability of different organisms in biofloc-integrated cultures.

Adding different species into the culture can also alter the characteristics of the system. As previously mentioned, the insertion of a sessile organism can influence the movement of water and cause the removal of solids that should be suspended [16]. An inorganic con-

sumer can also decrease the concentration of nutrients in the culture, being a bioremediator. Studies by Alencar et al. [18] and Copertino et al. [19] showed macroalgae of the genus *Ulva* with growth rates above 8.0% day^{-1} using shrimp culture effluent. The feasibility of using water from biofloc-rearing macroalgae cultivation still needs to be addressed.

Therefore, the aim of this work was to evaluate the cultivation of the macroalgae *Ulva lactuca* in different water depths in a biofloc-integrated system with shrimp and fish and to determine how macroalgae influence total suspended solids.

2. Materials and Methods

2.1. Experimental Design and Facilities

The experiment was conducted in a greenhouse at the Marine Aquaculture Station (Estação Marinha de Aquicultura—EMA), Institute of Oceanography, Federal University of Rio Grande (FURG), located in Cassino Bach, Rio Grande, Rio Grande do Sul. The macroalgae species used was *U. lactuca*, collected in a natural environment at Cassino Beach (32°17′52.30″ S–52°15′59.80″ W), Rio Grande, RS, Brazil. The macroalgae were taken to the macroalgae laboratory, the epiphytes removed, and then taken to a greenhouse. The algae were kept in a 1 m^3 circular culture structure inside the greenhouse, with 10% biofloc inoculum for adaptation, for 15 days. Mean culture concentrations were 27.7 ± 2.45 mg L^{-1} nitrate and 1.02 ± 0.48 mg L^{-1} phosphate. The identification of the macroalgae was performed using a microscope, observing quadratic cells characteristic of this species and a bilayer of cells, as also identified by Alencar et al. (2010) [18]. The shrimp came from a grow-out culture in the Carcinoculture laboratory at EMA. The juveniles of *O. niloticus* were obtained from a commercial fish farm. The experiment was approved by the Ethics and Animal Welfare Committee of FURG (Case number 23116.005895/2016-42).

To conduct the experiment (70 days of culture), six production systems were used, with constant aeration supplied by a 4CV blower, and daily light intensity of 28.68 ± 8.53 µmol m^{-2} s^{-1}. Each system consisted of three tanks. The first tank had 16 m^3 of usable volume, where 400 shrimp m^{-2} [20] with an initial weight of 4.6 ± 0.01 g were stocked, and water was circulated to a second tank of 3 m^3 of usable volume with the aid of a Boyu submersible pump 75 w (SPA 4000 L/h, BOYU©, Guangdong, China) where 35 fish m^{-3} were stocked, with an initial weight of 177.67 ± 32.06 g. The water passed to the third tank by gravity, where macroalgae were stocked at a density of 2.4 kg per 3 m^3 (or 0.8 kg per m^3), equivalent to 0.1 kg m^{-3} (considering 24 m^3 of the entire system), and the water returned to the shrimp tank by gravity (Figure 1).

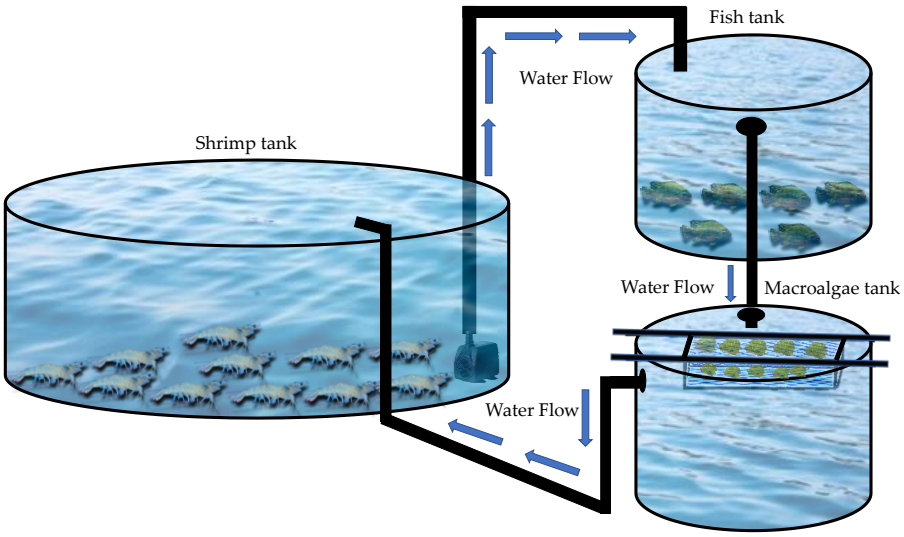

Figure 1. Scheme with a recirculation system, composed of a tank with shrimp of 16 m^3 useful volume, a tank with fish of 3 m^3 useful volume, and a tank with macroalgae of 3 m^3 useful volume, with a float used for the accommodation of the macroalgae.

At the beginning of the experiment, a biofloc inoculum from a grow-out shrimp culture was used with concentrations of 0.1, 0.2, 90.0, 4.7, and 600.0 mg L^{-1} of total ammoniacal nitrogen, nitrite, nitrate, phosphate, and total suspended solids, respectively. When excess solids occurred in the system, a clarification system was used in the tank. The clarifier consisted of a conical tank, with a usable volume of 150 L, made of fiberglass. The water from the shrimp tank was pumped to the top of the clarifier, and by decanting, the denser particles of solids were deposited at the bottom of the clarifier, and the lighter ones on the surface; water with lower solid concentration in the middle of the clarifier returned to the shrimp tank. When the value of total suspended solids exceeded the limit of 300 mg L^{-1} [21], the clarifier was added to the system.

The macroalgae were placed in floats made of PVC pipe and a 5 mm polyethylene mesh, forming a rectangular structure with length, width, and depth of 120 cm, 60 cm, and 40 cm, respectively. Each float with macroalgae was kept in the third tank, with a volume of 3 m^3 and 2.20 m diameter (Figure 1). There was one float for each tank, with macroalgae thallus varying from 10 to 20 cm in length. The experimental design was defined by two treatments (with three repetitions): shallow float, where the depth of the structure was placed within 10 cm from the surface; bottom float, where the depth was kept at 30 cm from the surface. The macroalgae were stirred inside the float, and solids decanted on their blades shaken off twice per day.

The shrimp and fish were fed twice per day (9 a.m. and 5 p.m.). The feed for the shrimp was supplied according to the daily feeding rate proposed by Jory et al. [22]. The fish were fed at a rate of 1% of the biomass to induce them to consume the biofloc and the supplied commercial feed of 36% protein (Guabi Aqua QS 2–3 mm, Guabi Nutrition and Animal Health S.A., Campinas, São Paulo, Brazil).

2.2. Physical and Chemical Parameters

For the routine water quality analyses, due to the recirculation of water between the tanks, the water was collected only from the shrimp tank. For water quality monitoring, temperature (°C), dissolved oxygen (DO, mg L^{-1}), and pH were measured daily in the shrimp tanks using a multiparameter probe (model Pro-20, YSI Inc., Yellow Springs, OH, USA) and a benchtop pH meter (Seven2Go S7 Básico, Mettler Toledo, São Paulo, Brazil). Salinity (‰) was measured twice per week using a multiparameter probe (model Pro-20, YSI Inc., OH, USA). Light intensity was measured using an underwater luxmeter (PROTOMATIC Model 0824861, PCE Instruments, Júpiter, FL, USA). For the water quality analyses, samples were collected from the shrimp tanks in plastic containers and taken immediately for analysis. Total alkalinity (mg $CaCO_3$ L^{-1}) was monitored according to the methodology presented by APHA [23] and was measured twice per week. Calcium hydroxide was used to maintain total alkalinity above 150 mg L^{-1} [24]. Total ammoniacal nitrogen (or TAN, mg L^{-1}) and nitrite (mg L^{-1}) were analyzed according to the methods of UNESCO [25] and Bendschneider and Robinson [26] twice per week. When the concentration of total ammoniacal nitrogen (TAN) was higher than 1 mg L^{-1}, molasses was applied for water quality control [10]. Nitrate (mg L^{-1}) and phosphate (mg L^{-1}) were analyzed using the methodology described by Aminot and Chaussepied [27] and monitored twice per week. Solids analyses in the shrimp tanks were performed twice per week. Turbidity (NTU) was measured by a portable turbidimeter (2100P, Hach®, Loveland, CO, USA), and total suspended solids (or TSS, mg L^{-1}) were quantified by filtration and gravimetry according to the methodology described by Baumgarten et al. [28]. Settleable solids (or SS, ml L^{-1}) were measured using Imhoff cone according to the method proposed by APHA [23].

The solids that were decanted on the macroalgae were quantified every 15 days during the daily stirring process of shaking off solids decanted on macroalgae blades. For that quantification, an additional water collection was performed. There were two situations in each treatment, with macroalgae and without macroalgae. This collection was performed in the 3 m^3 tank where the macroalgae were located (Figure 1) for better quantification of

the solids that were decanted. Following the procedure, first, the water was collected with the macroalgae in the tank. For the second collection, we stirred the macroalgae in the tank so that the solids would again become suspended in the system, and then we removed only the structure with the macroalgae. There was a 10 min waiting time to homogenize the water, and then another water sample was collected in the same tank. Total suspended solids and settleable solids analyses were performed every 15 days.

2.3. Performance of Macroalgae

The biomass yield of the macroalgae was measured biweekly by weighing the fresh biomass, where the macroalgae were removed from the water and left outdoors for 20 min to reduce the humidity. The following equation was used to calculate the Relative Growth Rate (RGR) [29]:

$$\text{RGR (\% d}^{-1}\text{): } 100 \times [\ln(\text{final weight (g)}/\text{initial weight (g)})/(\text{final time} - \text{initial time})] \quad (1)$$

2.4. Shrimp and Fish Performance

Weekly and biweekly biometric measurements were performed for shrimp and fish, respectively, with the aid of a digital scale (BL3200H, MARTE®, Santa Rita do Sapucaí, Minas Gerais, Brazil). For the shrimp, 50 shrimp were collected and weighed. For fish, sampling was performed with 25 tilapia; the animals were anesthetized in clear water with 50 mg L^{-1} with benzocaine hydrochloride [30], and individual weighing was performed. The fish were then taken to a recovery tank and then to their tanks. The performance was analyzed with the following equations:

1. Final average weight (g): final biomass of live animals (g)/total number of animals;
2. Specific growth rate (g $week^{-1}$): weight gain (g)/number of weeks;
3. Final biomass yield (g): sum of final weight of all live animals (g);
4. Feed conversion rate (FCR) = feed offered (g)/(final biomass (g) − initial biomass (g));
5. Productivity (kg m^{-3}): [(final biomass (kg) − initial biomass (kg)) × 100]/tank volume (m^{-3}).
6. Survival (%) = (final number of animals/initial number of animals) × 100;

2.5. Statistical Analysis

The normality and homoscedasticity of the data were verified by the Shapiro–Wilk and Levene tests, respectively. Once the assumptions were met, the *t*-test was performed. The non-parametric Kruskall–Wallis test was used otherwise. A minimum significance level of 5% ($p \leq 0.05$) was applied in all analyses.

3. Results

3.1. Water Quality

The water quality results indicate that there was no significant difference between the two treatments throughout the 70 days of the experiment (Table 1). Figure 2 shows the temperature variation in the tanks during the days of culture, showing a higher temperature at the beginning and decreasing over the weeks. The experiment started at the end of April and ended in May, being the autumn period, close to winter, affecting the temperature of the tanks throughout the culture cycle.

The accumulation of total suspended solids was constant throughout the production cycle due to the production of bacterial biomass and waste from feed and feces, showing that high concentrations were found on days 40 to 48 (Figure 3). The reduction in solid values after these days is due to the use of clarifiers in the system.

Table 1. Mean values (± standard deviation) of water quality parameters for the treatments shallow float (5 to 10 cm depth) and bottom float (25 to 30 cm depth) during 70 days of experimentation.

	Treatments	
Parameters	**Shallow Float**	**Bottom Float**
Temperature (°C)	22.30 ± 1.59	22.28 ± 1.58
DO (mg L^{-1})	6.70 ± 0.56	6.72 ± 0.59
pH	8.00 ± 0.21	8.06 ± 0.20
Salinity (‰)	19.92 ± 0.98	19.00 ± 1.55
Alkalinity (mg$CaCO_3$ L^{-1})	218.14 ± 20.06	219.90 ± 22.30
TAN (mg L^{-1})	0.18 ± 0.20	0.15 ± 0.19
Nitrite (mg L^{-1})	2.16 ± 2.38	1.29 ± 1.30
Nitrate (mg L^{-1})	64.40 ± 28.39	61.71 ± 24.17
Phosphate (mg L^{-1})	5.99 ± 4.24	5.43 ± 3.78
Turbidity (NTU)	219.49 ± 86.66	196.02 ± 68.72
SS (ml L^{-1})	3.88 ± 2.09	5.01 ± 2.27
TSS (mg L^{-1})	307.92 ± 87.48	303.43 ± 91.60

DO = dissolved oxygen; TAN = total ammoniacal nitrogen; TSS = total suspended solids; SS = settleable solids. Temperature and DO (n = 140); pH (n = 70); salinity, alkalinity, TAN, nitrite, nitrate, phosphate, turbidity, SS, and TSS (n = 35).

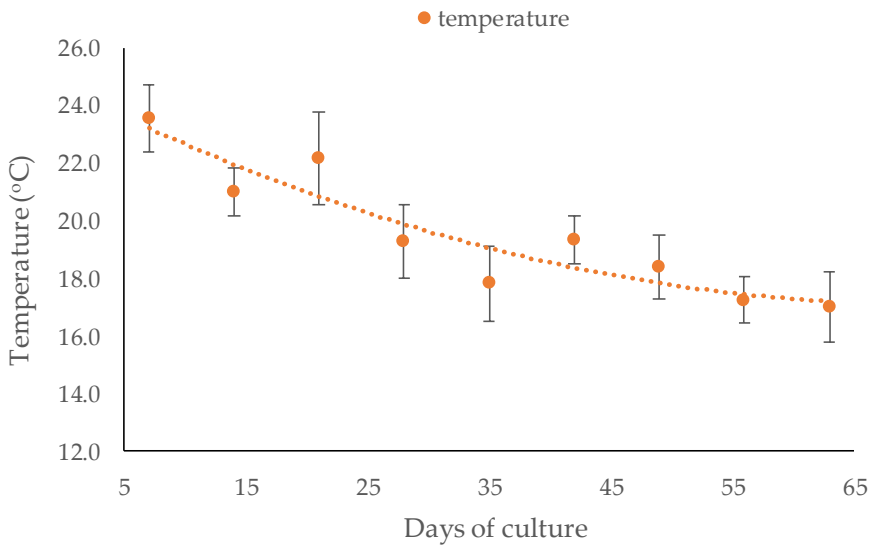

Figure 2. Mean ± standard deviation of the weekly temperature of the shrimp tanks during the 70 days of rearing (n = 6).

The amount of solids decanted on macroalgae laminas, estimated by solid resuspension in the water after being shaken off from macroalgae laminas, significantly ($p < 0.05$) increased the mean concentration of settleable solids (SS) and total suspended solids (TSS) (Table 2). The SS and TSS means were higher when the macroalgae were stirred and removed from the tank and water was collected. There was a difference between tanks with and without macroalgae in total suspended solids of 198.7 ± 113.4 mg L^{-1} for the shallow treatment and 212.5 ± 102.6 mg L^{-1} in the bottom treatment. After removing the macroalgae from the system or moving them in the structures, the solids previously deposited were again suspended in the water, with an overall average increase of 39.4% and 40.1% in total suspended solids in the Shallow and Bottom float treatments, respectively.

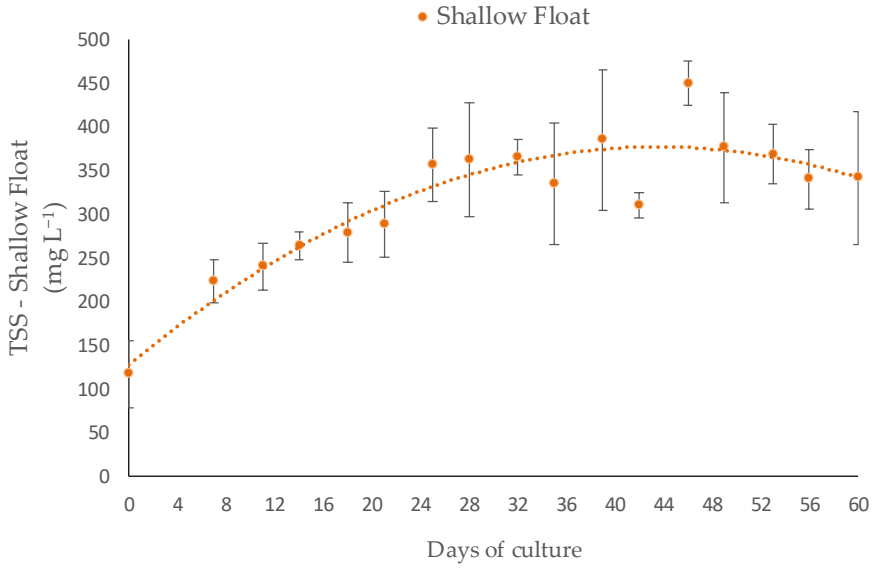

Figure 3. Mean ± standard deviation of total suspended solids (TSS) over the course of the cultivation days (n = 3).

Table 2. Concentrations of settleable solids (SS) and total suspended solids (TSS) (mean ± standard deviation) of the treatments shallow float (5 to 10 cm depth) and bottom float (25 to 30 cm depth), with and without macroalgae in the tank, during 70 days of experiment.

	Treatments			
	Shallow Float		**Bottom Float**	
Parameters	**with Macroalgae**	**without Macroalgae**	**with Macroalgae**	**without Macroalgae**
SS (ml L^{-1})	6.50 ± 3.50 ^{a}	14.50 ± 2.90 ^{b}	8.40 ± 3.50 ^{a}	16.40 ± 1.90 ^{b}
TSS (mg L^{-1})	305.10 ± 84.10 ^{a}	503.80 ± 40.10 ^{b}	317.00 ± 71.30 ^{a}	530.30 ± 40.10 ^{b}

Different letters represent significant differences ($p \leq 0.05$) with and without macroalgae in the same treatment after Student's t-test (n = 12).

In both depth treatments, mean values of SS and TSS were lower in tanks with macroalgae than without macroalgae throughout the experiment. However, differences between TSS values decreased towards the last days of cultivation, when the highest solid concentration in the water was observed (Figure 4).

3.2. Performance of Macroalgae

The biomass of macroalgae in the shallow treatment showed, throughout the experimental period, a significant difference ($p \leq 0.05$) from the bottom treatment. The bottom float treatment showed a decreasing trend in macroalgae biomass throughout the experimental period (Figure 5).

In 70 days of experimentation, the relative growth rate of macroalgae in the shallow float treatment was 0.14 ± 0.14% day^{-1} (Table 3), with an increase in biomass in the first weeks of culture (up to 0.95 ± 0.54% day^{-1}) and a decrease in biomass between sampling on days 41 and 55 of culture. At the end of the experiment, the shallow treatment showed a gain in macroalgae biomass.

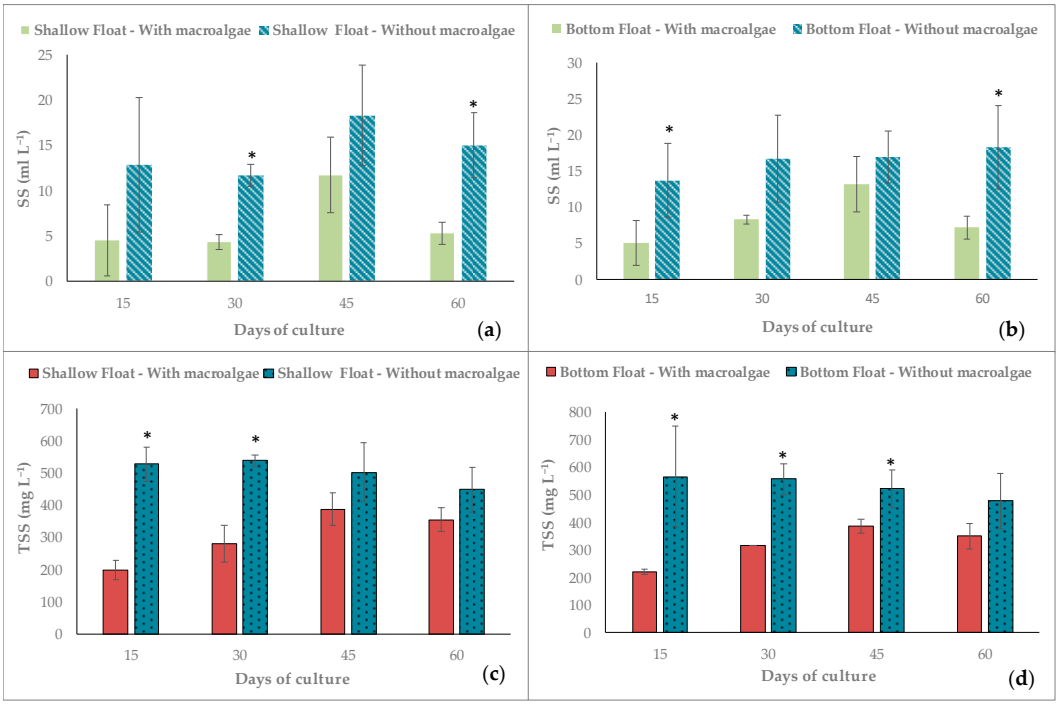

Figure 4. (**a**) Concentration of settleable solids (mean ± standard deviation) in the shallow float treatment (between 5 to 10 cm depth) with and without macroalgae. (**b**) Concentration of settleable solids (mean ± standard deviation) in the bottom float treatment (between 25 to 30 cm depth), with and without macroalgae. (**c**) Concentration of total suspended solids (mean ± standard deviation) in the shallow float treatment (between 5 to 10 cm depth) with and without macroalgae in the pond. (**d**) Total suspended solids concentration (mean ± standard deviation) in the bottom float treatment (between 25 and 30 cm depth) with and without macroalgae. Asterisks (*) represent significant difference ($p \leq 0.05$) with and without macroalgae after Student's *t*-test ($n = 3$).

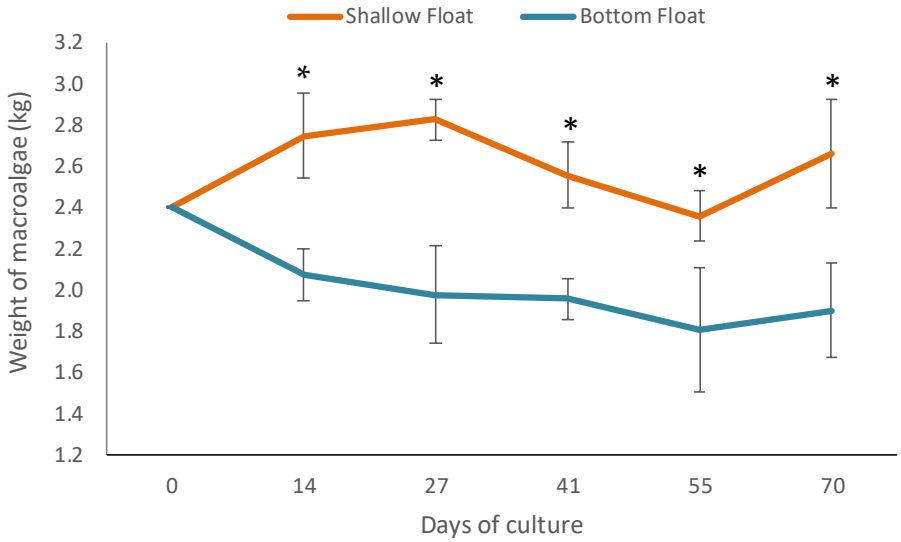

Figure 5. Mean macroalgae weight (kg—fresh weight) of the treatments, shallow float (between 5 to 10 cm depth) and bottom float (between 25 to 30 cm depth) during the 70 days of experiment. Asterisks (*) represent significant differences ($p \leq 0.05$) among treatments after Student's *t*-test ($n = 3$).

Table 3. Macroalgae performance (mean ± standard deviation) of the treatments, shallow float (between 5 to 10 cm depth) and bottom float (between 25 to 30 cm depth) during 70 days of experiment.

	Treatments	
	Shallow Float	**Bottom Float**
SGR (% day^{-1})		
14 day	0.95 ± 0.54 [a]	−1.06 ± 0.43 [b]
27 day	0.23 ± 0.78	−0.39 ± 0.48
41 day	−0.72 ± 0.28	−0.05 ± 0.48
55 day	−0.58 ± 0.19	−0.63 ± 0.84
70 day	0.85 ± 0.58	0.38 ± 0.47
Initial mean weight (kg—FW)	2.40 ± 1.64	2.40 ± 0.88
Final mean weight (kg—FW)	2.63 ± 0.23 [a]	1.94 ± 0.20 [b]
RGR (% day^{-1})	0.14 ± 0.14 [a]	−0.35 ± 0.17 [b]
Biomass gain (kg)	0.26 ± 0.27 [a]	−0.50 ± 0.23 [b]

SGR, specific growth rate; RGR, relative growth rate; FW, fresh weight. Different lower-case letters represent statistical differences between treatments after Student's *t*-test (n = 3).

3.3. Shrimp Performance

There was no difference in the performance of fish and shrimp between the treatments during the 70 days of culture (Table 4). There was growth in the shrimp over the weeks of cultivation. Moreover, there was a weight gain of up to 9.93 ± 1.90 g week^{-1} for the fish, even though they were fed 1% of the biomass.

Table 4. Performance of shrimp and fish (mean ± standard deviation) of the treatments, shallow float (5 to 10 cm depth) and bottom float (25 to 30 cm depth) during 70 days of experiment.

	Treatments	
	Shallow Float	**Bottom Float**
Shrimp		
Final mean weight (g)	8.05 ± 0.52	8.92 ± 0.36
WWG (g week^{-1}) [##]	0.38 ± 0.06	0.48 ± 0.04
Final biomass (kg)	27.90 ± 4.95	27.80 ± 2.94
FCR [#]	2.63 ± 0.47	2.99 ± 0.86
Yield (kg m^{-3})	1.33 ± 0.24	1.32 ± 0.24
Survival (%)	85.64 ± 19.84	76.24 ± 7.53
Fish		
Final mean weight (g)	289.74 ± 30.09	227.15 ± 48.97
WWG (g week^{-1}) [##]	9.93 ± 1.90	8.02 ± 1.75
Final biomass (kg)	26.73 ± 1.73	16.94 ± 8.05
FCR [#]	0.39 ± 0.09	0.49 ± 0.16
Yield (kg m^{-3})	1.27 ± 0.08	0.81 ± 0.38
Survival (%)	88.77 ± 8.04	89.05 ± 6.07

[#]: FCR (food conversion rate); [##]: WWG (weekly weight gain); n = 3.

4. Discussion

Integrated culture requires that water quality conditions meet the optimal levels for the cultivation of all species produced so that stress and poor development do not occur [5]. The use of a mature biofloc inoculum with the establishment of bacteria and the presence of nitrate [31] in this experiment provided high concentrations of total ammoniacal nitrogen and nitrite, which were controlled during culture, and no water quality problems were observed. As there was no control treatment without macroalgae, the uptake of nutrients by macroalgae was not verified. However, since there was no difference in the nitrogen content between the treatments, the loss of biomass in the bottom treatment did not cause nitrogen problems. The biomass of macroalgae used in the experiment was also low compared to the entire volume of the system, probably not causing significant nutrient uptake.

The biofloc system produces high concentrations of solids through heterotrophic bacteria, which use the ammonia produced in the system for their growth. According to Ebeling et al. [11], for each gram of ammonia transformed into bacterial biomass, 4.7 g of dissolved oxygen and 3.5 g of alkalinity are consumed, and 8 g of bacterial biomass is formed given their faster metabolism and establishment compared to chemoautotrophic bacteria. This bacterial biomass produced is part of the total suspended solids, which, in high concentrations, can cause occlusion of the shrimp gills. Thus, a limit of 100 to 300 mg L^{-1} of total suspended solids should be determined [13]. The produced excess solids can be removed using clarifiers [21] and organic consumers in the system, such as tilapia [3].

Unlike shrimp and fish, macroalga is a sessile organism, and despite the movement of solids in the water column caused by vigorous aeration, macroalgae can act as a physical barrier to this movement and cause sedimentation of solids in the system. Brito et al. [16] observed the control of solids and turbidity of the water due to the deposition of particulate material on the macroalgae and increased settleable solids because of the incorporation of macroalgae fragments in the system.

In the present study, solids were deposited on the macroalgae and were not accounted for in the water quality analysis, with an overall average increase of 39.4% and 40.1% in total suspended solids in the shallow and bottom float treatments, respectively. Even when the total suspended solids concentrations were higher than 300 mg L^{-1}, and clarifiers were used, the solids that were decanted on the macroalgae were still there and were not removed from the system. The water going to the clarifier contained only the solids in suspension in the water column. However, the formation of this physical barrier of macroalgae may serve as a substrate for bacteria and aid in water quality; little is known about this relationship or the chemical effects between macroalgae and solids. Manual movement of the macroalgae in the culture structure was necessary at least twice per day to remove the solids from the top of the macroalgae and allow light to enter; however, the deposition of solids quickly occurred again. A sudden increase in the concentration of solids in the water column occurs, which, if for a prolonged time, can cause a drop in tank oxygen due to bacterial respiration [32] and obstruct the gills of cultured animals [13].

Besides the effects on water quality, the deposition of solids on the macroalgae can reduce their performance. The sedimentation of solids can obstruct photosynthetic laminae, reducing light absorption necessary for photosynthesis and hence, the growth of the macroalgae. Carvalho et al. [33] showed that a concentration of up to 400 mg L^{-1} of total suspended solids in culture does not negatively influence the macroalgae, even with deposition. That study was conducted in 3 L transparent carboys, with light entering from the surface and sides of the structure, promoting a wide surface area exposed to light so that the macroalgae had a greater availability of light to enhance performance. In this present experiment, on the other hand, the only light input to the macroalgae came through the surface of the tank. Therefore, it may have affected the growth of the macroalgae during the days of cultivation.

In addition to the deposition, the production of solids from feces, feed residues, and growth of bacterial biomass was continuous throughout the days of culture [13]. High concentrations of solids were found on days 40 to 48, exceeding 400 mg L^{-1} total suspended solids, requiring the permanent use of clarifiers. The accumulation of solids in this period may have influenced the decrease in biomass of the macroalgae between the weighing days 41 and 55. The high organic load may prevent light from entering the water, decreasing the macroalgae's photosynthetic efficiency and overall performance. Our results suggested that better *U. lactuca* performance integrated into a BFT system can be obtained from waters with TSS concentrations lower than 300 mg L^{-1} by using clarifiers.

The increase in depth of the bottom float structures (15 to 25 cm) provides more space for macroalgae movement and greater carrying capacity for the macroalgae biomass. However, according to Luo et al. [34], the biofloc system has great light limitations, with ammonia removal occurring predominantly by bacteria, as they do not need much light and can develop better in the system. Reis et al. [17] worked with different colors and

wavelengths for the culture of shrimp *L. vannamei* and evaluated the penetration of each wavelength at the surface and at 20 and 40 cm of depth. These authors showed that the light penetration decreased with depth due to reflection or absorption by suspended particles in the water. Wavelengths of 79.05 ± 42.00 µmol m^{-2} m^{-1} and of 20.45 ± 23.40 µmol m^{-2} m^{-1} are absorbed, respectively, at the surface and at 20 cm depth, in white light. This decrease of light in the water column may be a determinant in reducing macroalgae growth, causing the loss of biomass seen in the bottom float treatment.

Despite the deposition of solids on the macroalgae in both treatments, the shallow float treatment (5 to 10 cm) provided better conditions for macroalgae growth. The proximity to the surface probably allowed the macroalgae to capture more light for photosynthesis. The performance of the macroalgae was better than that shown by Legarda et al. [35], who observed a decrease in biomass of the macroalgae *U. fasciata* cultivated in an integrated biofloc system. The proximity to the surface and the adaptation of the macroalgae to the biofloc environment before the beginning of the experiment were possibly determining factors for better performance.

Despite the increase in biomass in this experiment in an integrated biofloc system, Resende et al. [36] showed a maximum specific growth rate of 3.91 ± 0.67% d^{-1} of the macroalgae *Ulva* spp. in integrated culture with clear water, during autumn with temperatures ranging from 9.6 to 14.9 °C. This shows that the biofloc system can interfere with the performance of the macroalgae, and that better management procedures can be adopted.

The high specific growth values observed by Resende et al. [36] in an autumn experiment are lower compared to the same experiment conducted in the spring months (specific growth rate of 14.48 ± 3.52% d^{-1}) with temperatures ranging from 19.57 to 28.5 °C and at 26.3 salinity. Culture temperature can be a key factor in macroalgae growth. Sudden changes in temperature can cause stress to the macroalgae, which usually initiate the release of spores for reproduction [19]. The loss of biomass in the last weeks of cultivation in this experiment may be associated with the sudden drop in temperature and consequent reproduction event as the release of spores was verified in the tanks.

In addition to temperature fluctuation, the salinity adopted in the culture also plays an important role in macroalgae physiology. The macroalgae of the genus *Ulva* are adapted to a wide range of salinity; however, Li et al. [37] and Bews et al. [38] point out that the minimum limit of salinity of the culture medium would be 20‰, as this is already a stress factor for the macroalgae and can affect their development. The average salinity in the experiment was 19.46 ± 0.65‰ and may have been a stressor for the macroalgae, causing lower growth rates. Mantri et al. [39] tested the best conditions for induction and spore release and growth of the macroalgae *U. fasciata*, and found that the highest spore release occurred at salinity 15‰, and at salinity 30‰, the highest growth rate (16.1 ± 0.28% day^{-1}) was observed at a mean temperature of 25 °C.

The carrying capacity of the algae culture structure could also have led to the decrease in biomass in the shallow float treatment. The increase in biomass at the beginning of the experiment decreased the free space in the structure, increasing the overlap of the macroalgae, causing shading and decreased light capture. After the biomass decreased and space was released, the macroalgae increased their biomass again in the shallow float treatment. This loss of biomass caused by the increased density was also verified by Alencar et al. [18], who showed that increasing the density of macroalgae in a given space caused decreased growth and loss of biomass throughout the culture cycle, probably due to interspecific competition for light and space.

Thus, better management and water quality parameters still need to be established for maximum macroalgae growth to occur in integrated biofloc culture. The use of clarifiers to remove solids and the maintenance of a concentration of 100 mg L^{-1} of total suspended solids would probably favor greater light input. The shallow float allowed the macroalgae to grow in culture, and the use of aeration within the macroalgae structure would probably be more efficient in moving the macroalgae in the structure, and less settling of solids would occur. An improved management protocol could include partial harvests, which, according

to Fernand et al. [40], can decrease the density, promoting greater light penetration and nutrient availability.

The shrimp, a euryhaline species, can tolerate large variations in salinity [37]. On the other hand, tilapia can be cultured in salinity from 0 to 16‰ with no loss of performance [41]. Therefore, the higher salinity used in this experiment—despite not causing mortality—may have promoted physiological damage to the fish. The low temperature during the weeks of culture influenced the performance of the cultured organisms. For shrimp, according to Ren et al. [42], low temperatures decreases responses to external events and locomotor activities, being a major limiting factor for growth, which might explain the low weight gain and poor apparent feed conversion. According to Nobrega et al. [43], the optimal temperature for tilapia culture would be 28 °C, and at 22 °C, there would be a reduction in food consumption and, consequently, in growth. That may justify the high apparent feed conversion factor. However, weekly weight gain was higher than that of Holanda et al. [44], rearing tilapia in an integrated system with shrimp.

The integrated multi-trophic culture using biofloc technology has shown advances with the objective of generating intensive cultures with a lower amount of waste generation. Brito et al. [16] showed that the integration of the macroalgae *U. lactuca* in shrimp culture resulted in the uptake of nitrogen and phosphate compounds in the system, improving water quality and, consequently, shrimp growth. In the experiment performed, the density of macroalgae used was low due to the large volume of the system, resulting in nutrient uptake. However, the production of biomass in the shallow treatment promoted the reuse of nutrients in the system to form a new product. Another inorganic consumer can be added to the system to maximize nutrient uptake. Poli et al. [3] reported better performance in productivity and nutrient uptake in the integrated system with *Sarcocornia ambigua* compared to monoculture.

Holanda et al. [14] showed a reduction in the organic load of biofloc culture when integrating mullet *Mugil liza* in shrimp culture compared to shrimp monoculture. In this experiment, tilapia were added to both treatments to consume the solids, but there was no quantification of the solids consumption. However, the addition of an organic consumer along with the macroalgae may help to reduce the solids and improve the light incidence in the water, favoring the growth of the macroalgae.

5. Conclusions

The integrated culture in a biofloc system presents distinct characteristics compared to conventional cultures in clear water due to the high load of solids and nutrients. The insertion of the macroalgae in the integrated biofloc system showed deposition of solids on the macroalgae, decreasing the concentrations of total suspended solids and avoiding solids exiting the system by clarification. Even with this result, there was growth of the macroalgae *Ulva lactuca* in an integrated system with the shrimp *Litopenaeus vannamei* and tilapia *Oreochromis niloticus*, proving viability at depths of up to 10 cm in a biofloc system with an average TSS concentration of 300 mg L^{-1}.

Author Contributions: Conceptualization, A.C., L.C.d.O.C., M.G., J.S., L.H.P., G.T. and M.H.; methodology, A.C., L.C.d.O.C., M.G. and M.H.; software, A.C., M.G. and L.C.d.O.C.; validation, A.C., L.C.d.O.C., M.G., J.S., M.H., G.T. and L.H.P.; formal analysis, A.C., M.G., J.S., M.H. and L.C.d.O.C.; investigation, A.C., L.C.d.O.C., M.H., M.G., J.S., G.T. and L.H.P. resources, C.S.B.C., G.T. and L.H.P.; data curation, A.C., L.C.d.O.C. and M.G.; writing—original draft preparation, A.C., L.C.d.O.C. and M.G. writing—review and editing, C.S.B.C., J.S., M.G., G.T., M.H. and L.H.P.; visualization, A.C., L.C.d.O.C., M.H., L.H.P. and G.T.; supervision, C.S.B.C., G.T. and L.H.P.; project administration, L.C.d.O.C., M.H., L.H.P. and G.T.; funding acquisition, C.S.B.C., G.T. and L.H.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ASTRAL Project—H2020 grant Agreement 863034.

Institutional Review Board Statement: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors. The experiment was approved by the Ethics and Animal Welfare Committee of FURG (Case number 23116.005895/2016-42).

Acknowledgments: The authors are grateful for the financial support provided by the European Union (ASTRAL Project—H2020—Grant Agreement 863034). Special thanks to GUABI Nutrition and Animal Health S.A. for donating the commercial diets.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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