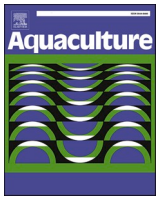
Aquaculture 572 (2023) 739492

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Integrated multitrophic aquaculture system applied to shrimp, tilapia, and seaweed (*Ulva ohnoi*) using biofloc technology

Ana Paula Mariane de Morais [a], Ivanilson Lima Santos [a], Ramon Felipe Siqueira Carneiro [a], Eric Arthur Bastos Routledge [b], Leila Hayashi [a], Marco Antônio de Lorenzo [a], Felipe do Nascimento Vieira [a,*]

[a] *Laboratório de Camarões Marinhos, Departamento de Aquicultura, Universidade Federal de Santa Catarina, Rua dos Coroas 503, CEP 88061-600, Barra da Lagoa, Florianópolis, Santa Catarina, Brazil*

[b] *Pesquisador, Diretoria de Pesquisa e Inovação, Embrapa Sede - PqEB - Av. W3 Norte (Final) S/N - Asa Norte, Brasília, DF 70770-901, Brazil*

A R T I C L E I N F O

Keywords:
Nitrogen
Phosphorus
Biomass
IMTA
Biofloc

A B S T R A C T

Integrated multitrophic aquaculture (IMTA) is a model that integrates organisms from different trophic levels, all sharing the same production system and, consequently, the nutrients. This study adopted shrimp (*Penaeus vannamei*) as the main species, tilapia (*Oreochromis niloticus*) as an organic consumer, and the seaweed (*Ulva ohnoi*) at different densities as an inorganic consumer in a biofloc production system. The study consisted of an experiment with three treatments and four replicates each: 1) No seaweed; 2) Seaweed 1 g L^{-1} (density of 1 g L^{-1} of *U. ohnoi*), and 3) Seaweed 2 g L^{-1} (density of 2 g L^{-1} of *U. ohnoi*). Shrimp (3.82 ± 0.05 g) were stocked in 800 L tanks at a density of 275 shrimp m^{-3}, fish (14.44 ± 0.57 g) in 90 L tanks at a density of 267 tilapia m^{-3}, and 50 and 100 g of seaweed stocked in 50 L tanks, corresponding to treatments with 1 and 2 g L^{-1}, respectively. The integration of *U. ohnoi* at densities of 1 g L^{-1} and 2 g L^{-1} with *P. vannamei* and *O. niloticus* benefited the system through higher nitrogen (13,22 and 13,10% higher in the treatments 1 g L^{-1} and 2 g L^{-1} respectively) and phosphorus recovery (25,57 and 34% respectively) and an increase in total productivity (15,32 and 19,22% respectively), generating an ecological gain. Considering the similar final biomass of seaweed in a multitrophic system with shrimp and tilapia, the use of *U. ohnoi* at a density of 2 g L^{-1} is recommended since nutrient recovery by fish and shrimp was higher at this density.

1. Introduction

The biofloc technology system (BFT) can increase productivity in shrimp farming systems and other aquatic organisms, while reducing environmental impacts. This system serves as a supplementary source of feed for reared organisms. At the same time, it maintains water quality carried out by the microbial community present in the system (Avnimelech, 1999; Wasielesky et al., 2006; Ballester et al., 2010). However, high concentrations of nitrogen in this system can become a problem in aquaculture, accumulating as a result of the excretion of produced organisms, uneaten feed, and organic waste (Timmons and Ebeling, 2010).

The accumulation of these compounds can be controlled by two main pathways, heterotrophic and chemoautotrophic. In the heterotrophic pathway, the addition of an organic carbon source acts on the proliferation of heterotrophic bacteria that helps in the assimilation of ammonia and consequent formation of bacterial biomass (De Schryver et al., 2008; Serra et al., 2015). In the chemoautotrophic pathway, nitrifying bacteria act on the successive oxidation of ammonia to nitrate through the nitrification process, which happens through the absorption of inorganic carbon present in the alkalinity (Ebeling et al., 2006). In addition to nitrogen, phosphorus, total suspended solids, and other nutrients tend to accumulate within the production system in the absence of water exchange.

The increase of inorganic compounds in aquaculture is associated with animal excretion and uneaten feed since shrimp retain 23 to 31% of nitrogen and 10 to 13% of phosphorus (Crab et al., 2007), while the rest of these nutrients remain in the water and must be removed to prevent deterioration and toxicity to animals. The high concentration of total suspended solids is a result of a predominantly heterotrophic system owing to the constant increase of bacterial biomass. During shrimp

* Corresponding author.
E-mail address: felipe.vieira@ufsc.br (F. do Nascimento Vieira).

https://doi.org/10.1016/j.aquaculture.2023.739492
Received 10 October 2022; Received in revised form 12 March 2023; Accepted 18 March 2023
Available online 21 March 2023
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rearing, it is recommended to maintain solids below 600 mg L^{-1} because higher values can affect shrimp performance and must be corrected once exceeded (Schveitzer et al., 2013). Systems with a predominance of chemoautotrophic microorganisms also show an increase in solids; however, in smaller amounts, the addition of these microorganisms results in greater efficiency in the nitrification process (Ferreira et al., 2020; Serra et al., 2021). Thus, the challenge of the BFT system is to control the concentration of solids that tends to increase over time and the use of inorganic nutrients generated during the production cycle. These problems can be solved mechanically using filters or biologically.

In this context, an integrated multitrophic aquaculture (IMTA) system is an alternative to reduce the undesirable surpluses produced in the BFT system since this system integrates organisms from different trophic levels, all sharing the same production system and, consequently, the nutrients. Thus, the organic and inorganic residues generated become inputs (feed or fertilizer) for the lower trophic level species that are part of the system (Chopin et al., 2001).

The application of IMTA can contribute to the reduction of effluents produced and the consequent increase in productivity based on the diversity of species of different trophic levels, thereby allowing better use of the nutrients generated in the BFT system since surplus is consumed by another species, generating, in turn, a better utilization of nutrients present in this system.

The choice of potential candidates for integration in this system must consider the ability to make better use of generated effluents; however, salinity can be a limiting factor. Thus, some species of shrimp, tilapia, and seaweed are suitable candidates to form an IMTA system, as they are euryhaline species able to adapt to a wide range of salinities.

Tilapia is a species already cultured in a biofloc system owing to its adaptability and ability to use flocs as a feed source (Crab et al., 2009; Poli et al., 2019), demonstrating its potential to use the organic surpluses of the system. The genus Ulva stood out by its ability to absorb nitrogen and phosphate compounds within an integrated system in biofloc, improving its composition (Legarda et al., 2021). However, its absorption and growth can be affected by stocking densities as a consequence of seaweed shading that causes a low incidence of light (Martins et al., 2020).

Thus, this study evaluated the IMTA aquaculture system by adopting shrimp (*Penaeus vannamei*) as the main species, tilapia (*Oreochromis niloticus*) as an organic consumer, and the seaweed (*Ulva ohnoi*) at different densities as an inorganic consumer in a biofloc production system.

2. Material and methods

2.1. Study site

The study was carried out for 56 days at the Marine Shrimp Laboratory (LCM), which is part of the Aquaculture Department of the Federal University of Santa Catarina (UFSC), located in Florianópolis, SC, Brazil.

2.2. Biological material

P. vannamei shrimp were obtained from Aquatec®, a commercial laboratory located in Canguaretama, RN, Brazil. Shrimp were maintained in a 50.000 L nursery tank and cultured in a biofloc system until they reached the initial weight of the experiment (3.82 ± 0.05 g).

Nile tilapia fingerlings *O. niloticus* were obtained from the Agricultural Research and Rural Extension Company of Santa Catarina (Epagri) and maintained in 1000 L tanks in a biofloc system until the beginning of the experiment when they reached 14.44 ± 0.57 g. *U. ohnoi* seaweed specimens were collected from the sedimentation pond of the Marine Molluscs Laboratory, Florianópolis, SC, Brazil. They were cleaned with seawater to remove epiphytes and attached animals and then kept at the laboratory for salinity acclimation for one week. This work was approved by the Ethics Committee on Animal Use of the UFSC (Protocol 8,700,240,920).

2.3. Experimental design

The experimental design consisted of three groups with four replicates each, a total of 12 experimental units: 1) No seaweed; 2) Seaweed 1 g L^{-1}; and 3) Seaweed 2 g L^{-1} of *U. ohnoi*.

The experimental units consisted of 800 L and 90 L circular tanks of useful volume to culture the shrimp and the tilapia, respectively, and 50 L rectangular tanks of useful volume to cultivate seaweed. All were placed in a greenhouse. Shrimp production experimental units had an 800 W heater with a thermostat to maintain the temperature at 28 °C and an aeration system with micro-perforated hoses connected to a blower to keep the biofloc in suspension and dissolved oxygen higher than 5 mg L^{-1}. Each shrimp tank also had four rectangular artificial substrates of Needlona® (dimensions of 0.40 × 0.55 m) attached vertically at a proportion of 80% of the surface of the tank, as described by Schveitzer et al. (2013), for the bacterial colonization and increase the available area for shrimp.

The fish and seaweed tanks were equipped with 200 W heaters to maintain the temperature at 28 °C and aeration with an air stone to keep the biofloc in suspension and the oxygen higher than 5 mg L^{-1}. Seaweed tanks had central aeration with perforated pipes to keep the water moving. Both aeration systems were attached to the same blower that aerated the shrimp tanks.

Before the beginning of the experiment, pre-tests were carried out (not published yet) to verify the tolerance of the organisms to salinities. In the first test, shrimp and tilapia were cultured at different salinities, and salinities above 20 g L^{-1} were not recommended. The second test was carried out to determine the lowest salinity the macroalgae *U. ohnoi* can tolerate in a biofloc system, reaching a concentration of 20 g L^{-1}.

At the beginning of the experiment, a quantity of 267 L of mature biofloc from a predominantly chemoautotrophic matrix tank was transferred as inoculum to the shrimp and fish tanks, with the following characteristics: ammonia 0.09 mg L^{-1}; nitrite 0.12 mg L^{-1}; nitrate 15.55 mg L^{-1}; alkalinity 204 mg L^{-1}; pH 7.9; salinity 29 g L^{-1}; orthophosphate 7.0 mg L^{-1}; total suspended solids 553 mg L^{-1}, corresponding to 30% of the volume of each unit and completed with seawater and freshwater to adjust the salinity to 18 g L^{-1}. Throughout the experiment, no water exchange occurred in the experimental units. Freshwater was added only to replace water lost through evaporation.

The system remained in constant recirculation whereby water circulated from the shrimp tank to the fish tank through a Sarlo-Better submerged pump, with a flow rate of 1000 L $hour^{-1}$, and then returned to the shrimp tank by gravity.

Once a week the pump was turned off to transfer water from the seaweed tank to the shrimp tank, followed by filtering 12.5 L of water from the shrimp tank (filter Bag) and returning it to the seaweed tank, which was completed with salt- and/or freshwater to maintain salinity at 20 g L^{-1}. At the end of this procedure, the recirculation system was reconnected according to Legarda et al. (2021).

2.4. Stocking

Shrimp tanks were stocked with 220 animals (275 shrimp m^{-3}) and fish tanks with 24 tilapia (267 tilapia m^{-3}), corresponding to 30% of final shrimp biomass, according to Poli et al. (2019a). Seaweed tanks were stocked with 50 and 100 g of seaweed, corresponding to seaweed 1 g L^{-1} and seaweed 2 g L^{-1} treatments, respectively.

2.5. Feeding

Shrimp were fed with a 40% crude protein commercial feed (Guabitech 1.6 mm Guabi®, 40% of protein and 10% of lipdis) four times a day, according to Jory et al. (2001), with daily amounts adjusted weekly

after biometrics. Tilapia individuals were fed once a day with 38% crude protein commercial feed for omnivorous freshwater fish at 1% of their biomass, considering the imposition of feed restriction to stimulate biofloc consumption, according to Poli et al. (2019).

2.6. Water quality and total suspended solids

Temperature and dissolved oxygen were measured twice a day and salinity once a day with the YSI Pro 2030 m. Ammonia concentration (TAN) (Unesco, 1983), nitrite (N-NO_2) (Strickland and Parsons, 1972), pH (pH-meter Tecnal®), and alkalinity ($CaCO_3$) (APHA/AWWA/WEF. Standard, 2012) were measured twice a week. When pH and alkalinity values were respectively lower than 7.3 and 150 mg L^{-1}, they were corrected with the addition of hydrated lime (Furtado et al., 2011). Nitrate (N-NO_3) (Hach *NitraVer® 5*) and orthophosphate (PO_4) (APHA/AWWA/WEF.Standard, 2012) were measured at the beginning, middle, and end of the experiment. Total suspended solids (TSS) were measured twice a week (APHA/AWWA/WEF.Standard, 2012) and maintained between 400 and 600 mg L^{-1}, levels considered suitable for *P. vannamei* (Gaona et al., 2011), and excess solids were removed using conical settling tanks as recommended by Schveitzer et al. (2013). After this procedure, the sludge volume and its concentration were measured. Then, the volume and concentration of solids were measured. The amount of sludge removed from each experimental unit was quantified according to Poli et al. (2019) as follows: sludge produced (g $tank^{-1}$) = [((TSS$_{final}$ x V1) - (TSS$_{initial}$ x V1) / 1000) + (Σ(TSS$_{sludge}$ x V2))], where V1 is the volume of the experimental unit in liters, TSS$_{final}$ is the TSS concentration at the end of the experiment, TSS$_{initial}$ is the TSS concentration at the beginning of the experiment, TSS$_{sludge}$ is the TSS concentration in the sludge removed, and V2 is the volume of sludge removed.

2.7. Shrimp, tilapia, and seaweed performance

Shrimp performance was evaluated weekly by weighing a sample of 22 shrimp from each experimental unit on a digital scale with a precision of 0.01 g. At the end of the experiment, weekly growth (g $week^{-1}$), survival (%), final mean weight (g), apparent feed conversion ratio (FCR-A), and productivity (kg m^{-3}) were measured.

At the beginning, middle, and end of the experiment, all fish from each tank were weighed. Tilapia performance parameters evaluated were survival (%), final mean weight (g), apparent feed conversion ratio (FCR-A), and productivity (kg m^{-3}).

Seaweed was weighed biweekly to monitor the increase in biomass. At the end of the experiment, the final mean weight (g), final biomass (kg), production (kg m^{-2}), and daily growth rate (% day^{-1}) were evaluated, according to Lignell and Pedersén (1989), as follows: TC = [(Mf / Mi)$^{1/t}$ – 1] x 100, where M_f is the final biomass (g), M_i is the initial biomass (g), and t is the rearing time.

Final biomass and system productivity were evaluated, considering all organisms (shrimp, fish, and seaweed). The total productivity was calculated by dividing the total biomass by the total volume of the system. The total productivity was calculated by dividing the total biomass by the total volume of the system.

2.8. Nitrogen and phosphorus recovery

At the beginning and end of the experiment, samples were collected from each tank, approximately 100 g of shrimp, tilapia, and seaweed. A total of 100 g of each feed used was also collected. The concentration of nitrogen (N) was determined by the Kjeldahl method (NTK), while phosphorus (P) content was determined by the colorimetric method, following the methodology described by AOAC (2005) for whole animals.

The recovery of nitrogen and phosphorus was calculated according to the respective equation recommended by the NRC (National Research Council) (2011): recovery (%) = [(final weight x final nutrient (N or P)) - (initial weight x initial nutrient (N or P) x 100] / nutrient input - feed (N or P).

2.9. Mass balance

The initial biomass of shrimp, tilapia, and seaweed, feed consumed by shrimp and tilapia, and their nitrogen and phosphorus contents were considered sources of nutrients. The final biomass of shrimp, tilapia, seaweed, and effluents was estimated at the end of the experiment. The effluent was calculated considering the initial nitrogen and phosphorus as 100% and the difference of what was removed. For each organism, the biomass from each tank was multiplied by the concentration of nitrogen and phosphorus at the beginning and end of the experiment (Cerozi and Fitzsimmons, 2017).

2.10. Statistical analysis

Data were presented as mean ± standard deviation and subjected to Shapiro-Wilk and Levene tests to prove the prerequisites of normality and homoscedasticity, respectively. Then, an analysis of variance (one-way ANOVA) was applied to verify differences among the treatments, followed by the Tukey test when differences were found (Zar, 2010). All statistical tests were evaluated with a significance level of 5% ($p < 0.05$) and performed using the Statistica® version 7.0 program.

3. Results

3.1. Water quality parameters

Dissolved oxygen remained higher than 5 mg L^{-1}, the temperature was maintained at 28 °C, and the salinity was maintained at 20 g L^{-1} for seaweed tanks and 18 g L^{-1} for shrimp and fish tanks. The pH, alkalinity, nitrogen compounds (ammonia, nitrite, and nitrate), phosphate, and total suspended solids showed no significant difference among treatments throughout the experiment. The total amount of sludge removed during the entire experimental period did not show a significant difference among treatments (Table 1).

3.2. Animal and seaweed performance

The final weight, survival, weekly growth, and feed conversion ratio values for shrimp and tilapia did not show significant differences among treatments. The initial and final biomass of the *U. onhoi* did not show a significant difference among the treatments. Daily growth rate of the

Table 1
Mean and standard deviation of water physical and chemical parameters for 56 days of study in a multi-trophic system with biofloc stocked with 275 shrimp *Penaeus vannamei* m^{-3} (3.81 ± 0.05 g), 267 tilapia *Oreochromis niloticus* m^{-3} (14.24 ± 0.39 g) and different seaweed densities (No seaweed; Seaweed 1 g L^{-1}; and Seaweed 2 g L^{-1} of *Ulva ohnoi*) with four replicates.

Parameter	No seaweed	Seaweed 1 g L^{-1}	Seaweed 2 g L^{-1}	*p*-value
Ammonia (mg L^{-1})	0.33 ± 0.05	0.32 ± 0.04	0.33 ± 0.03	0.97
Nitrite (mg L^{-1})	1.04 ± 0.17	1.00 ± 0.29	1.06 ± 0.14	0.96
Nitrate (mg L^{-1})	13.92 ± 0.50	14.99 ± 1.84	13.72 ± 0.85	0.48
Phosphate (mg L^{-1})	5.32 ± 0.45	5.21 ± 0.59	5.58 ± 0.79	0.77
Alkalinity (mg L^{-1})	172.34 ± 1.02	171.53 ± 1.46	171.44 ± 2.48	0.97
pH	8.13 ± 0.02	8.14 ± 0.05	8.12 ± 0.05	0.37
Total suspended solids (mg L^{-1})	403.52 ± 6.15	404.74 ± 14.34	388.89 ± 29.44	0.82
Sludge produced (kg $tank^1$)	0.35 ± 0.08	0.35 ± 0.06	0.36 ± 0.06	0.99

Different letters in the same line represent a statistical difference with $p < 0.05$.

seaweed showed a significant difference among treatments with different densities of seaweed since higher growth was obtained in seaweed treatment of 1 g L^{-1}. The final biomass and productivity of the system showed a significant difference among treatments, and the lowest values obtained were those in the treatment without seaweed, as shown in Table 2.

3.3. Nitrogen and phosphorus recovery

Nitrogen and phosphorus recovery of the system showed a significant difference among treatments. In both parameters, treatment without seaweed showed lower values than treatments with seaweed at densities of 1 and 2 g L^{-1} for both parameters, as shown in Table 3.

3.4. Mass balance

Nitrogen mass balance showed a significant difference among the experimental groups for fish in the initial phase of the system, in which seaweed treatment of 2 g L^{-1} showed a higher value than the others. In the final phase, the system showed a significant difference with seaweed presence, with higher nitrogen value in both treatments (Seaweed 1 g L^{-1} and Seaweed 2 g L^{-1}), compared to treatment without seaweed (Table 4). At the beginning of the experiment, phosphorus showed a significant difference for fish. At this stage, the no seaweed and seaweed treatment at 1 g L^{-1} were similar and different from seaweed treatment at 2 g L^{-1}, which showed the highest value. At the end of the experiment, treatments in the presence of seaweed (1 and 2 g L^{-1}) were statistically similar and showed a significantly higher phosphorus concentration than treatment without seaweed. For shrimp, at the beginning of the experiment, phosphorus showed no statistical difference among the treatments. At the end of the experiment, seaweed treatment at 2 g L^{-1} showed a significant difference from the other treatments, as shown in Table 5.

Table 2
Mean and standard deviation of growth performance for 56 days of study in a multi-trophic system with biofloc stocked with 275 shrimp *Penaeus vannamei* m^{-3} (3.81 ± 0.05 g), 267 tilapia *Oreochromis niloticus* m^{-3} (14.24 ± 0.39 g) and different seaweed densities (No seaweed; Seaweed 1 g L^{-1}; and Seaweed 2 g L^{-1} of *Ulva ohnoi*) with four replicates.

Parameter	No seaweed	Seaweed 1 g L^{-1}	Seaweed 2 g L^{-1}	*p*-value
Shrimp				
Final weight (g)	12.20 ± 0.19	12.11 ± 0.24	12.42 ± 0.22	0.186816
Survival (%)	88.41 ± 3.61	90.11 ± 3.02	89.09 ± 2.32	0.733517
Feed conversion ratio	2.40 ± 0.16	2.33 ± 0.14	2.39 ± 0.15	0.761413
Weekly growth (g $week^{-1}$)	1.05 ± 0.02	1.04 ± 0.03	1.07 ± 0.03	0.186816
Final biomass (kg)	2.37 ± 107.69	2.40 ± 87.74	2.43 ± 69.57	0.653736
Tilapia				
Final weight (g)	43.98 ± 4.09	45.75 ± 3.71	49.14 ± 2.09	0.149636
Survival (%)	94.74 ± 5.21	97.92 ± 4.17	93.70 ± 4.14	0.421985
Feed conversion ratio	0.52 ± 0.07	0.48 ± 0.05	0.49 ± 0.01	0.585645
Weekly growth (g $week^{-1}$)	3.71 ± 0.51	3.93 ± 0.46	4.36 ± 0.26	0.149636
Final biomass (kg)	0.96 ± 91.42	1.05 ± 78.13	1.09 ± 46.14	0.094444
Ulva onhoi				
Initial biomass (g)	–	50 ± 0.0	100 ± 0.0	–
Final biomass (g)	–	393.01 ± 44.50	458.63 ± 64.05	0.143399
Daily growth rate (% day^{-1})	–	8.00 ± 0.01[a]	5.43 ± 0.01[b]	0.000999
System				
Final biomass (kg)	3.34 ± 0.07[a]	3.85 ± 0.17[b]	3.98 ± 0.14[b]	0.00047
Productivity (kg m^{-3})	3.59 ± 0.07[a]	4.14 ± 0.18[b]	4.28 ± 0.15[b]	0.000151
System conversion	1.31 ± 0.10	1.15 ± 0.08	1.17 ± 0.07	0.053381

Different letters in the same line represent a statistical difference with $p < 0.05$.

Table 3
Mean and standard deviation of nitrogen and phosphorus recovery for 56 days of study in a multi-trophic system with biofloc stocked with 275 shrimp *Penaeus vannamei* m^{-3} (3.81 ± 0.05 g), 267 tilapia *Oreochromis niloticus* m^{-3} (14.24 ± 0.39 g) and different seaweed densities (No seaweed; Seaweed 1 g L^{-1}; and Seaweed 2 g L^{-1} of *Ulva ohnoi*) with four replicates.

Parameter	No seaweed	Seaweed 1 g L^{-1}	Seaweed 2 g L^{-1}	*p*-value
Nitrogen recovery (%)	24.42 ± 0.53[a]	27.65 ± 1.42[b]	27.62 ± 1.35[b]	0.005059
Phosphorus recovery (%)	14.82 ± 8.92[a]	18.61 ± 11.97[b]	19.86 ± 8.94[b]	0.000152

Different letters in the same line represent a statistical difference with $p < 0.05$.

4. Discussion

Inorganic compounds present in the water that will be absorbed by seaweed come mainly from the decomposition of animal feed and excretion (Timmons and Ebeling, 2010) which can cause toxicity in high concentrations with corresponding decreasing growth, or even mortality, of animals. Through the use of IMTA, nitrate and phosphorus that tend to accumulate in the BFT system are consumed by species of lower trophic levels. Given the ability to assimilate nitrogen and phosphorus and convert them into biomass, seaweeds are often used to integrate these systems (Neori et al., 2004).

Maintaining water quality in production systems, especially in those with high stocking densities, is essential for the growth of animals. Seaweed presence in BTF systems is important for the removal of excess nutrients, as verified by Legarda et al. (2020), Martins et al. (2020), and Celino et al. (2010). Additionally, in this system, the presence of a microbial community is responsible for converting ammonia into nitrate, a less toxic compound for organisms. Thus, all water quality parameters remained within the recommended values for *P. vannamei* and *O. niloticus* species throughout the experiment (Van Wyk and Scarpa, 1999a, 1999b; Lin and Chen, 2001, 2003; El-Shafai et al., 2004).

In shrimp production systems, approximately 25% of nitrogen available in feed is incorporated by shrimp, and the surplus is degraded in the water, which, in turn, accumulates and causes toxicity (Crab et al., 2007; Avnimelech, 2015). Using *U. ohnoi* in densities of 1 and 2 g L^{-1} did not influence the bacterial process of nitrogen and phosphorus recovery from the system since ammonia, nitrite, and nitrate remained the same in the treatment without macroalgae.

However, the daily rate of seaweed growth differed as the treatment with lower initial density showed a higher specific growth, which was already expected, owing to the similar amount of initial nutrient in both treatments. Therefore, the treatment with lower density showed better growth conditions. In addition, a higher density of seaweed in a small area could cause competition for light, which limits growth. Despite this, the seaweed from both treatments showed a growth rate higher than 5.0% day^{-1}, which was higher than the 4.3 and 2.7% day^{-1} observed by Martins et al. (2020) with seaweed densities of 2 and 4 g L^{-1}, respectively. Legarda et al. (2020), in turn, could not keep up with the growth rate owing to the mortality of the sea lettuce *Ulva lactuca*, and it was necessary to replace the biomass weekly, which could be attributed to the low availability of ammonia, the first nutrient assimilated by macroalgae. However, in the present work, even with similar water quality parameters, the seaweed *U. ohnoi* managed to grow, which may be an indication of the ability of the genus *Ulva* to convert nitrate into

Table 4
Nitrogen mass balance for 56 days of study in a multi-trophic system with biofloc stocked with 275 shrimp *Penaeus vannamei* m^{-3} (3.81 ± 0.05 g), 267 tilapia *Oreochromis niloticus* m^{-3} (14.24 ± 0.39 g) and different seaweed densities (No seaweed; Seaweed 1 g L^{-1}; and Seaweed 2 g L^{-1} of *Ulva ohnoi*) with four replicates.

N input	Shrimp feeding (g $tank^{-1}$)	Shrimp (g $tank^{-1}$)	Tilapia feeding (g $tank^{-1}$)	Tilapia (g $tank^{-1}$)	Seaweed (g $tank^{-1}$)	Initial total (g $tank^{-1}$)
No seaweed	195.43 ± 3.44	20.58 ± 0.32	16.12 ± 0.68	7.48 ± 0.19[a]	–	239.61 ± 2.97
Seaweed 1 g L^{-1}	193.82 ± 9.41	20.43 ± 0.39	17.27 ± 1.51	7.58 ± 0.12[a]	0.36 ± 0.0	239.46 ± 8.6
Seaweed 2 g L^{-1}	201.44 ± 3.16	20.62 ± 0.08	18.45 ± 1.08	7.96 ± 0.34[b]	0.73 ± 0.0	249.20 ± 3.31
p-value	0.227436	0.660769	0.118517	0.041016	–	0.056506
N output	–	Shrimp (g $tank^{-1}$)	–	Tilapia (g $tank^{-1}$)	Seaweed (g $tank^{-1}$)	Effluent (g $tank^{-1}$)
No seaweed		62.49 ± 2.84		22.97 ± 2.17[a]	–	154.15 ± 2.21
Seaweed 1 g L^{-1}		63.36 ± 2.32		26.79 ± 1.98[b]	3.05 ± 0.40	146.27 ± 7.31
Seaweed 2 g L^{-1}		65.43 ± 1.87		28.05 ± 1.18[b]	3.14 ± 0.56	152.58 ± 4.71
p-value		0.250580		0.008892	–	0.137554

Different letters in the same line represent a statistical difference with $p < 0.05$.

Table 5
Phosphorus mass balance for 56 days of study in a multi-trophic system with biofloc stocked with 275 shrimp *Penaeus vannamei* m^{-3} (3.81 ± 0.05 g), 267 tilapia *Oreochromis niloticus* m^{-3} (14.24 ± 0.39 g) and different seaweed densities (No seaweed; Seaweed 1 g L^{-1}; and Seaweed 2 g L^{-1} of *Ulva ohnoi*) with four replicates.

P input	Shrimp feeding (g $tank^{-1}$)	Shrimp (g $tank^{-1}$)	Tilapia feeding (g $tank^{-1}$)	Tilapia (g $tank^{-1}$)	Seaweed (g $tank^{-1}$)	Initial total (g $tank^{-1}$)
No seaweed	49.85 ± 0.88	1.62 ± 0.02	4.27 ± 0.18	2.19 ± 0.06[a]		57.93 ± 0.74
Seaweed 1 g L^{-1}	49.44 ± 2.40	1.61 ± 0.03	4.57 ± 0.40	2.22 ± 0.03[a]	0.02 ± 0.0	57.86 ± 2.11
Seaweed 2 g L^{-1}	51.38 ± 0.80	1.63 ± 0.01	4.77 ± 0.29	2.33 ± 0.10[b]	0.03 ± 0.0	60.25 ± 0.84
p-value	0.227436	0.660769	0.118517	0.041016		0.074108
P output	–	Shrimp (g $tank^{-1}$)	–	Tilapia (g $tank^{-1}$)	Seaweed (g $tank^{-1}$)	Effluent (g $tank^{-1}$)
No seaweed		4.73 ± 0.21[a]		8.00 ± 0.76[a]	–	45.20 ± 0.50
Seaweed 1 g L^{-1}		4.82 ± 0.18[a]		9.97 ± 0.74[b]	0.22 ± 0.03	42.85 ± 1.91
Seaweed 2 g L^{-1}		5.70 ± 0.16[b]		10.44 ± 0.44[b]	0.24 ± 0.04	43.88 ± 0.90
p-value		0.000071		0.001246		0.074108

Different letters in the same line represent a statistical difference with $p < 0.05$.

ammonia through the nitrate reductase enzyme (Costa Da Costa, 2006).

In the present work, integration of the seaweed in the system did not significantly affect the performance of shrimp or fish. The high survival and weekly growth rate of shrimp observed were above 88% and 1 g $week^{-1}$, respectively, which corresponded to the report by Samocha et al. (2015). Fish showed a survival higher than 93%, a weekly growth rate higher than 3.7 g $week^{-1}$, and a mean increase of 28.5 g, even if fed with only 1% of their biomass, which suggests they used biofloc as a feed source, corroborating the results observed by Poli et al. (2019). However, seaweed presence in the system provided an increase in biomass to the production system compared to the treatment without macroalgae, which demonstrates that seaweed increased the productivity of shrimp and tilapia in addition to removing the inorganic compounds dissolved in the water. The seaweed tested in this work can be used in feed, as tested by Elizondo-González et al. (2018), who successfully evaluated the use of *Ulva lactuca* as an ingredient in shrimp feed, and Legarda et al. (2021), who evaluated the levels of *Ulva fasciata* inclusion in *Seriola dorsalis* feed.

Nitrogen and phosphorus recovery in production systems is important because of the greater amount of these nutrients in feed. In conventional systems, it is common to carry out water exchange when nitrogen compounds are in high concentrations. In addition, feed represents the highest cost of production in aquaculture, and nitrogen is the most expensive ingredient. In the present work, nitrogen recovery was higher in both treatments with seaweed (1 and 2 g L^{-1}), and the same occurred with phosphorus which recovered in greater quantity in the treatments with seaweed. Legarda et al. (2020) obtained similar behavior with sea lettuce presence, observing higher phosphorus incorporation in shrimp and fish, even without direct contact with the seaweed. These results suggest that seaweed release some compound in the water that favors phosphorus recovery. Further research is needed to confirm this behavior. Legarda et al. (2021) used the seaweed *Ulva fasciata* and obtained nitrogen and phosphate recovery of 29.2 and 10.5%, respectively. In an integrated recirculation system, Elizondo-González et al. (2018) used *Ulva lactuca* and obtained nitrogen recovery and phosphorus of 80.0 and 64.0%, respectively, results that demonstrate the efficiency of seaweed in nitrogen and phosphorus recovery. In the present study with *U. ohnoi*, treatment with 1 g L^{-1} showed nitrogen and phosphorus recovery of 27.65 and 18.61%, respectively, and similar results in at a density of 2 g L^{-1}, with 27.62 and 19.86% of nitrogen and phosphorus recovery, respectively.

Even without direct contact, it is possible to attribute the greater incorporation of nitrogen and phosphorus to the presence of seaweed in the system. The low feeding rate of the fish, as an inducement to consume biofloc excess, may have also contributed to the increase of nitrogen and phosphorus in fish. These results show that it is possible to improve system efficiency by increasing nitrogen recovery, diversifying production, and generating the same amount of effluent with a lower concentration of nutrients.

5. Conclusion

The results obtained in the present study prove that the presence of *Ulva ohnoi* in the IMTA system with *P. vannamei* and *O. niloticus* benefited the performance of all species through nitrogen and phosphorus recovery and an increase in total productivity, generating an ecological gain. Considering that *Ulva ohnoi* final biomass was similar, it is recommended to use a density of 2 g L^{-1} in a multitrophic system with shrimp and tilapia since the nutrient recovery by fish and shrimp was higher at this density.

Formatting of funding sources

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, by Fundação de Amparo à Pesquisa e Inovação do Estado de Santa

Catarina (FAPESC project 2020TR728). Felipe do Nascimento Vieira and Leila Hayashi received a productivity research fellowship from CNPq.

CRediT authorship contribution statement

Ana Paula Mariane de Morais: Writing – original draft, Formal analysis, Investigation, Conceptualization, Methodology. **Ivanilson Lima Santos:** Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Ramon Felipe Siqueira Carneiro:** Investigation, Conceptualization, Methodology. **Eric Arthur Bastos Routledge:** Conceptualization, Writing – review & editing. **Leila Hayashi:** Writing – original draft, Writing – review & editing, Conceptualization, Methodology. **Marco Antônio de Lorenzo:** Writing – original draft, Writing – review & editing, Conceptualization, Methodology. **Felipe do Nascimento Vieira:** Writing – original draft, Writing – review & editing, Conceptualization, Methodology.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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