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Integrated multitrophic aquaculture in biobloc technology with different fertilization approaches

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ABSTRACT

The aim of this study was to evaluate the performance of *Penaeus vannamei* and *Oreochromis niloticus* in an integrated multi-trophic system with biofloc technology using different strategies. Two treatments were developed: TC (chemoautotrophic) and TH (heterotrophic). The TC treatment started 46 days before stocking the animals, with inorganic fertilization. In the TH treatment, organic fertilization was carried out after stocking the animals. Four hundred shrimp·m^{-2} (1.00 ± 0.04 g) and 45 tilapia fry·m^{-3} (25 ± 0.50 g) were stocked for 86 days. Water quality parameters such as temperature, oxygen, and pH were monitored daily. Total ammonia, nitrite, nitrate, phosphate, total suspended solids, and settleable solids were monitored twice a week. Prior fertilization in the chemoautotrophic treatment avoided high concentrations of nitrogen compounds in the system, due to the prior establishment of bacteria, compared to the heterotrophic treatment. The increase in nitrogen compounds in heterotrophic treatment led to higher water use, reduction in feed and consequently lower shrimp growth performance compared to the chemoautotrophic system. Tilapia's growth was not affected. Therefore, the use of prior fertilization in chemoautotrophic treatment allowed for a higher final weight, yield of shrimp, and greater environmental sustainability.

Keywords: Chemoautotrophic; Heterotrophic; Integrated multi-trophic system; Water quality; Inputs; Pilot production project.

Aquicultura multitrófica integrada em tecnologia de bioblocos com diferentes abordagens de fertilização

RESUMO

O objetivo deste estudo foi avaliar o desempenho de *Penaeus vannamei* e *Oreochromis niloticus* num sistema multitrófico integrado com a tecnologia de bioflocos, utilizando diferentes estratégias. Foram desenvolvidos dois tratamentos: TC (quimioautotrófico) e TH (heterotrófico). O tratamento TC começou 46 dias antes do repovoamento dos animais, com fertilização inorgânica. No tratamento TH, a fertilização orgânica foi realizada após o povoamento dos animais. Quatrocentos camarões·m^{-2} (1,00 ± 0,04 g) e 45 alevinos de tilápia·m^{-3} (25,00 ± 0,50 g) foram estocados e cultivados por 86 dias. Os parâmetros de qualidade da água, como temperatura, oxigênio e pH, foram monitorados diariamente. O nitrogênio amoniacal total, nitrito, nitrato, fosfato, sólidos suspensos totais e sólidos sedimentáveis foram monitorados duas vezes por semana. A fertilização prévia no tratamento quimioautotrófico evitou altas concentrações de compostos nitrogenados no sistema, por causa do estabelecimento prévio de bactérias, em comparação com o tratamento TH. O aumento dos compostos nitrogenados no tratamento TH levou a maior uso de água, redução da alimentação e, consequentemente, menor desempenho de crescimento do camarão em comparação com o sistema quimioautotrófico. O crescimento da tilápia não foi afetado. Portanto, o uso de fertilização prévia no tratamento TC permitiu maior peso final, produtividade do camarão e maior sustentabilidade ambiental.

Palavras-chave: Quimioautotrófico; Sistema multitrófico integrado; Qualidade de água; Insumos; Projeto de produção piloto.

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INTRODUCTION

The production of Pacific white shrimp (*Penaeus vannamei*) is one of the most important in the aquaculture world. The species was the most produced in 2022, with 6.8 million tons (FAO, 2024), and has potential for integrated systems due to its economic interest and easier management, since it can be produced under temperature variations of 23 to 30°C, salinity of 10 to 30 ppm, and a wide protein requirement in the diet ranging from 20 to 40% without affecting its growth (Araneda et al., 2020; Gao et al., 2016). In fish farming, tilapia (*Oreochromis niloticus*) is one of the most produced fish species in Brazil and worldwide (Roriz et al., 2017), it also has higher resistance to temperature and salinity variations, as well as rapid growth (FAO, 2024).

Both species can be cultivated in intensive systems, characterized by increased animal density in a smaller volume of water, resulting in high yields at the end of cultivation (Holanda et al., 2022). However, the growth of aquaculture in intensive systems has aggravated some problems, such as the disposal of residual effluent from production without adequate treatment (Naylor et al., 2009). Effluents from aquaculture production have high loads of dissolved nutrients such as ammonia, nitrite, nitrate, and phosphate, as well as suspended organic matter (Queiroz et al., 2020). Therefore, for aquaculture to continue to grow, studies must be carried out to develop sustainable systems that have less negative impact on the environment.

The adoption of the biofloc system (BFT) aims to improve the use of water and increase yields with the minimum amount of water renewal required (Wasielesky et al., 2006). In this production system, the ammonia and nitrite present in the water are converted into nitrate by heterotrophic and chemoautotrophic bacteria. This allows the water to be used in more than one cultivation cycle (García-Ríos et al., 2019; Robles-Porchas et al., 2020). The development of heterotrophic bacteria occurs through the manipulation of the carbon (C) and nitrogen (N) ratio, in which organic carbon from sources such as sugar cane molasses, glucose, wheat bran, and cellulose is an energy source for the growth of heterotrophic bacteria (Brandão et al., 2021). This bacterial group appears quickly in the system and is efficient at removing ammonia, transforming it into bacterial biomass and giving rise to bioflocs (Khanjani et al., 2016). Throughout the production process, there is an increase in the concentration of bioflocs, which can be measured as total suspended solids, and must be removed from the system through filters or decanters when it reaches concentrations of between 300 and 500 $mg \cdot L^{-1}$ (Gaona et al., 2017). At the same time, chemoautotrophic bacteria develop and colonize, which is slower, but highly efficient at oxidizing ammonia and nitrite into nitrate. This group of bacteria can be manipulated in the system by adding inorganic carbon, present in salts such as ammonium chloride and sodium nitrite (Ebeling et al., 2006). It is important to note that the formation of microbial flocs is lower in environments dominated by chemoautotrophic bacteria.

Therefore, the dominant group of bacteria in the BFT system can be induced according to the type of fertilization adopted, resulting in the accumulation of total suspended solids and inorganic compounds such as nitrate and phosphate (Silva et al., 2013). To minimize these problems and maximize the use of water, integrated cultivation systems (IMTA) have been associated with BFT, providing a bioremediation system (Holanda et al., 2022). The main objective of the IMTA system is to optimize the use of organic and inorganic waste, by producing different species with different trophic levels. The system can be composed of a fed target species (such as shrimp or fish), filter species (such as molluscs and omnivorous fish), as well as primary producer (such as macroalgae, microalgae and/or halophytes) (Carvalho et al., 2023; Chopin, 2015). The adoption of the IMTA system can contribute to improving water quality, since there is better use of the nutrients generated in the BFT system by the cultivation of species of different trophic levels, and a consequent reduction in feed conversion (Holanda et al., 2022; Morais et al., 2023).

The aim of this study was to evaluate the production of *P. vannamei* shrimp and *O. niloticus* tilapia in IMTA with BFT using different fertilization approaches during the cultivation period.

MATERIAL AND METHODS

Origin of the animals

The experimental protocol for this work was submitted to and approved by the Ethics Committee for the Use of Animals of the Universidade Federal do Rio Grande, protocol no. 23116.005895/2016-42.

The shrimp (*P. vannamei*) post-larvae were acquired from a commercial laboratory (Aquatec, Canguaretama, RN, Brazil) and transported to the Marine Station of Aquaculture, where they were kept in a raceway tank in an agricultural greenhouse with BFT technology until the start of the experiment. The tilapia fry (*O. niloticus*) was obtained from a commercial fish farm (Camaquã, RS, Brazil) and also in an agricultural greenhouse in two 4-m^3 tanks with clear water and constant aeration. Before starting the experiment, the tilapia fry was acclimatized into salinity 20 for 10 days, with 10% of the fresh water being replaced with seawater every two days.

Experimental design

The experiment lasted 86 days and was conducted at the Marine Aquaculture Station from the Universidade Federal do Rio Grande, Rio Grande do Sul, Brazil. Six experimental systems were used, with each system consisting of a 16-m^3 tank, in which 400 shrimp/m^2 (1.00 ± 0.04g) were stocked, and a 4-m^3 tank, in which 45 tilapia fry/m^3 (25.00 ± 0.50 g) were stocked. All the tanks had aeration provided by a 4HP blower and air distribution by diffusion hoses (Aero Tube). The water in the tanks circulated with the help of a submerged pump (BOYU 4,000 $L \cdot h^{-1}$), installed in the shrimp tank, which pumped the water into the tilapia tank 24 hours a day, and by gravity returned it to the shrimp tank (Fig. 1). All treatments were exposed to a natural photoperiod, with 13:10 hours of light/dark at the end of January and February, changing to 12:12 hours of light/dark in March and April, according to the weather in Southern Brazil.

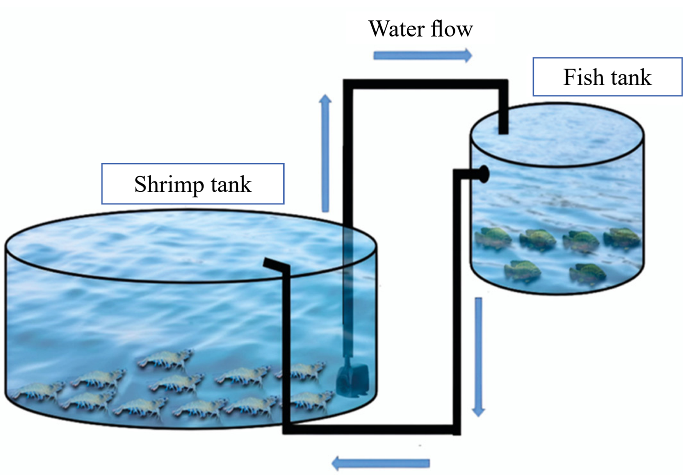


Figure 1. Experimental design of the shrimp and fish cultivation units.

The experiment included two treatments: chemoautotrophic treatment (TC), and heterotrophic treatment (TH), with three replicates each. To develop the chemoautotrophic system, three 16-m^3 tanks were randomly selected and individually fertilized daily for 46 days before the start of the experiment, with 1 $mg \cdot L^{-1}$ of ammonium chloride (NH_4Cl) (Neon Comercial, São Paulo, SP, Brazil) and 1 $mg \cdot L^{-1}$ of sodium nitrite ($NaNO_2$) (Neon Comercial, São Paulo, SP, Brazil) without exposure to light, to stimulate the growth of nitrifying bacteria, as described by Ferreira et al. (2020).

For the heterotrophic system, the animals were stocked in clear water, and the concentration of total ammonia nitrogen was monitored daily until it reached 1 $mg \cdot L^{-1}$. When ammonia exceeded 1 $mg \cdot L^{-1}$, sugar cane molasses was added, following the carbon (C) and nitrogen (N) ratio of 15C:1N, until the total ammonia nitrogen concentrations stabilized (Avnimelech, 1999; Ebeling et al., 2006). The initial concentrations of water quality parameters in each treatment are presented in Table 1.

Table 1. Mean ± standard deviation of water quality parameters in the chemoautotrophic and heterotrophic treatment at the start of the experiment.

Parameters	Treatments	
	Chemoautotrophic	**Heterotrophic**
Temperature (°C)	30.08 ± 1.56	30.52 ± 1.53
Dissolved oxygen ($mg \cdot L^{-1}$)	6.0 ± 0.27	5.82 ± 0.35
pH	8.06 ± 0.03	7.77 ± 0.10
Alkalinity ($CaCO_3 \cdot L^{-1}$)	203.3 ± 2.9	100.0 ± 5.0
Total ammoniacal nitrogen ($mg \cdot L^{-1}$)	2.37 ± 0.46	0.50 ± 0.10
Nitrite ($mg \cdot L^{-1}$)	1.61 ± 0.83	0.02 ± 0.00
Nitrate ($mg \cdot L^{-1}$)	22.70 ± 3.02	0.00 ± 0.00
Total suspended solids ($mg \cdot L^{-1}$)	157.33 ± 54.42	35.00 ± 15.00

Water quality

Dissolved oxygen and temperature were checked twice a day using a digital oximeter (YSI model Pro-20, United States of America). The pH (bench pH meter, Mettler Toledo, FEP20, Brazil) was measured once a day in the morning. Salinity was measured once a week using a multiparameter (HANNA HI9829). To control salinity, fresh water was added to the system to correct the loss due to evaporation. The pH and alkalinity were corrected by applying calcium hydroxide when the values were lower than 7.5 and 150 mg $CaCO_3 \cdot L^{-1}$, respectively (Furtado et al., 2014). Total ammoniacal nitrogen and nitrite were analyzed daily (Bendschneider & Robinson, 1952; UNESCO, 1983) in both treatments, until stabilization and the appearance of nitrate in the system. When necessary, applications of molasses were made to control spikes in total ammoniacal nitrogen. In the case of nitrite peaks, the water was renewed together with a reduction in the amount of feed offered. Nitrate concentrations (Amionot & Chaussepied, 1983) and alkalinity (APHA, 1989) were measured twice a week. Total suspended solids (TSS) (Strickland & Parsons, 1972) and settleable solids (SS) were measured twice a week according to the methodology described by the American Public Health Association (APHA, 2005). To control and reduce excess TSS, 250-L conical fiberglass clarifiers were used whenever TSS reached 350 $mg \cdot L^{-1}$.

Productive performance and feed management

To determine zootechnical performance, weekly biometrics were carried out for the shrimp and biweekly biometrics for the fish. A digital scale accurate to 0.01 g (Marte Cientifica, AD 2000, Brazil) was used to weigh the animals. The following calculations were made to determine growth performance (Eqs. 1–6):

$$\text{Average final weight (g)} = \sum \text{final weight of live animals (g)} / \text{total number of animals} \quad (1)$$

$$\text{Weekly weight gain } (g \cdot week^{-1}) = \text{(final weight - initial weight) (g)} / \text{number of weeks} \quad (2)$$

$$\text{Feed conversion rate (FCR)} = \text{feed offered (g)} / \text{(final biomass (g) - initial biomass (g)} \quad (3)$$

$$\text{Survival (\%)} = \text{(final number of animals} / \text{initial number of animals)} \times 100 \quad (4)$$

$$\text{Final biomass (g)} = \sum \text{final weight of all live animals (g)} \quad (5)$$

$$\text{Yield } (kg\ m^{-3}) = [\text{(final biomass (kg) - initial biomass (kg)} \times 1000] / \text{useful tank volume (L)} \quad (6)$$

The shrimp and tilapia were fed twice a day (8 a.m. and 4 p.m.) with commercial feed specific to their species and size. The feed used for the shrimp was Guabi Active 40, 1.6 mm (40% crude protein), and for the tilapia Guabi Mirim 2 mm (36% crude protein). The shrimp's feeding rate was monitored using the methodology by Jory et al. (2001), while the tilapia fed 1% of the tank's biomass throughout the experimental period to stimulate consumption of the biofloc.

Statistical analysis

All results were expressed as mean ± standard deviation. The homogeneity and normality of the data were previously tested using the Levene's and Shapiro-Wilk's tests, respectively, and when they did not show homogeneity and normality, the data were transformed. Once the assumptions were met, the data was submitted to Student's t-test. The analyses were carried out at a minimum significance level of 5% or $p < 0.05$ (Zar, 2010).

RESULTS

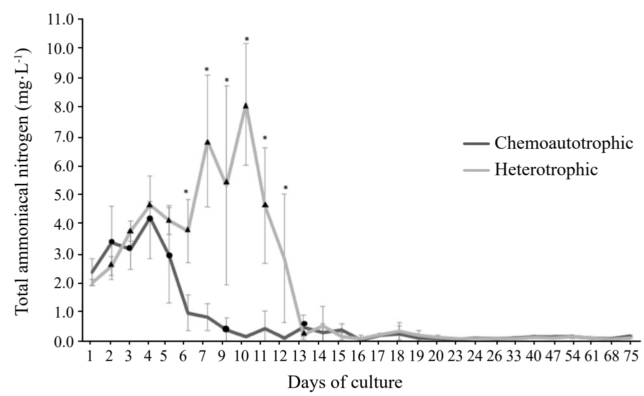
Water quality

Parameters including temperature, dissolved oxygen, pH, alkalinity, and salinity did not differ between treatments during the experimental period (Table 2).

Table 2. Water quality parameters (mean ± standard deviation) in the chemoautotrophic and heterotrophic treatments over 86 experimental days.

Parameters	Treatments	
	Chemoautotrophic	Heterotrophic
Temperature (°C)	27.39 ± 1.97	27.27 ± 2.31
Dissolved oxygen ($mg \cdot L^{-1}$)	5.57 ± 0.49	5.76 ± 0.49
pH	7.79 ± 0.09	7.83 ± 0.13
Alkalinity ($CaCO_3 \cdot L^{-1}$)	169.5 ± 18.8	162.2 ± 18.5
Salinity	20.99 ± 0.79	24.65 ± 1.03

The concentration of total ammoniacal nitrogen was significantly higher in the TH treatment in the first 13 days, and then this compound stabilized in the treatment (Fig. 2).



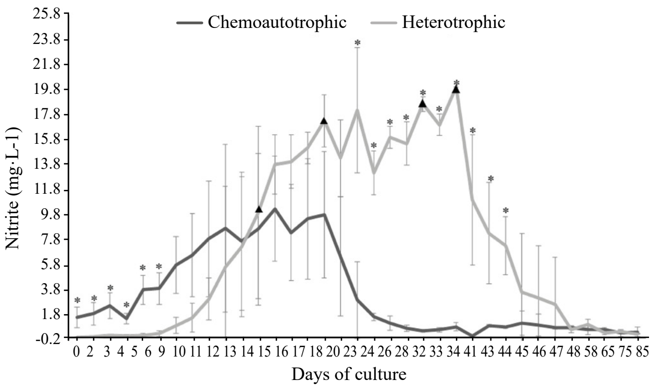
*A significant difference between the treatments on the same day, after Student's T-test; ball: the application of molasses in the chemoautotrophic treatment; triangle: the application of molasses in the heterotrophic treatment.

Figure 2. Average levels of total ammoniacal nitrogen in chemoautotrophic and heterotrophic treatments throughout the experimental period.

Nitrite was significantly higher ($p < 0.05$) in the TH treatment during a long period and only showed a reduction after the 41st day of the experiment, unlike the TC system, which showed lower nitrite concentrations from the 20th day of the experiment (Fig. 3).

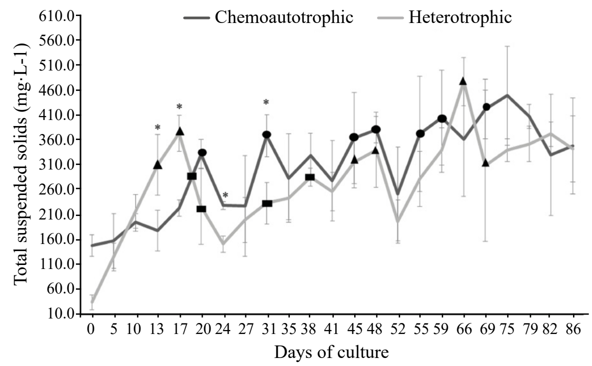
Regarding nitrate, the TC treatment showed significantly higher values ($p < 0.05$) compared to the TH treatment throughout the experimental period (Fig. 4).

The concentration of TSS was significantly higher ($p < 0.05$) in the TH treatment in the first two weeks, after which the



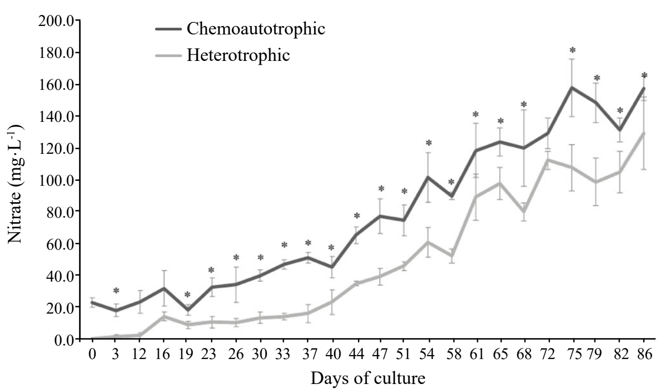
*A significant difference between the treatments on the same day, following Student's t-test; triangle: water renewals in the heterotrophic treatment.

Figure 3. Average nitrite levels in chemoautotrophic and heterotrophic treatments throughout the experimental period.



*A significant difference between the treatments on the same day, after Student's t-test; ball: the days of clarification in the chemoautotrophic system; triangle: the days of clarification in the heterotrophic system; square: the days of water renewal in the heterotrophic treatment.

Figure 5. Average levels of total suspended solids in chemoautotrophic and heterotrophic treatments throughout the experimental period.



*A significant difference between treatments on the same day, following Student's t-test.

Figure 4. Average nitrate levels in the chemoautotrophic and heterotrophic treatments throughout the experimental period.

TC system showed a significant difference compared to the TH treatment. Both treatments showed clarification, but only the TH treatment had its water renewed on days 18, 20, 31, and 38 due to high nitrite concentrations, keeping TSS concentrations at acceptable levels until day 59 (Fig. 5).

Animal performance

The shrimp performance was significantly more efficient ($p < 0.05$) in the TC treatment than in the TH treatment, except for the feed conversion rate, which showed no significant difference ($p > 0.05$) between the treatments (Table 3). For the fish, there was no significant difference ($p > 0.05$) between the treatments for any of the parameters evaluated (Table 3).

Table 3. Growth performance and survival results of shrimp (*Penaeus vannamei*) and tilapia (*Oreochromis niloticus*) produced in a multitrophic system and chemoautotrophic and heterotrophic bioflocs over a period of 86 days*.

Shrimp	**Treatments**	
	Chemoautotrophic	**Heterotrophic**
Average initial weight (g)	1.00 ± 0.04	1.00 ± 0.04
Final average weight (g)	11.30 ± 0.36[a]	8.25 ± 0.34[b]
Weekly weight gain (g week^{-1})	0.81 ± 0.03[a]	0.57 ± 0.03[b]
Feed conversion rate	1.86 ± 0.12	1.98 ± 0.19
Survival (%)	90.34 ± 5.38	85.08 ± 5.97
Gain biomass (kg)	73.26 ± 5.55[a]	48.18 ± 4.58[b]
Yield (kg·m^{-2})	4.06 ± 0.28[a]	2.81 ± 0.23[b]
Fish	**Chemoautotrophic**	**Heterotrophic**
Average initial weight (g)	25.00 ± 0.50	25.00 ± 0.50
Final average weight (g)	171.62 ± 17.81	180.25 ± 9.23
Weekly weight gain (g·week^{-1})	11.53 ± 1.40	12.25 ± 0.73
Feed conversion rate	0.91 ± 0.35	0.75 ± 0.10
Survival (%)	76.19 ± 25.43	83.81 ± 8.93
Gain biomass (kg)	18.61 ± 8.53	22.21 ± 3.88
Yield (kg·m^{-2})	1.15 ± 0.43	1.33 ± 0.19
Shrimp and fish	**Chemoautotrophic**	**Heterotrophic**
Gain biomass (kg)	104.24 ± 12.11 [a]	82.77 ± 1.40 [b]
Yield (kg·m^{-2})	5.21 ± 0.61 [a]	4.14 ± 0.07 [b]

*Data are expressed as mean ± standard deviation. Different letters on the same line indicate significant differences ($p < 0.05$) between treatments, after Student's t-test.

Inputs

The amount of feed offered was significantly higher ($p < 0.05$) in the TC treatment compared to TH. The use of molasses was significantly higher ($p < 0.05$) in the TH treatment than in the TC treatment. There was no significant difference ($p > 0.05$) between the treatments for calcium hydroxide (Table 4).

Table 4. Quantities of inputs used in the chemoautotrophic and heterotrophic treatments during the 86 experimental days*.

Inputs	Treatments	
	Chemoautotrophic	Heterotrophic
Shrimp feed (kg)	407.52 ± 1.84[a]	284.78 ± 1.42[b]
Fish feed (kg)	89.28 ± 1.21	89.28 ± 1.21
Calcium hydroxide (kg)	8.25 ± 1.98	9.53 ± 0.32
Molasses (kg)	5.15 ± 4.33[b]	13.26 ± 1.82[a]
Ammonia chloride (kg)	2.82 ± 0.1[#]	-
Sodium nitrite (kg)	2.82 ± 0.1[#]	-
Total water use (m^3)	20.00 ± 1.00[b]	44.00 ± 1.00[a]
Use of water/shrimp ($L\cdot kg^{-1}$)	278.92 ± 28.03 [a]	918.74 ± 86.93 [b]
Use of water/fish ($L\cdot kg^{-1}$)	1307.39 ± 713.45	2026.16 ± 389.48
Use of water/organism ($L\cdot kg^{-1}$)	223.91± 29.73 [a]	625.22 ± 12.48 [b]

*Data are expressed as mean ± standard deviation. Different letters on the same line indicate significant differences ($p < 0.05$) between the treatments after Studen's t-test; #salts used previously only in the chemoautotrophic treatment.

DISCUSSION

Different strategies for preparing the water in the BFT system can have significant impacts on both the water quality parameters and the performance of the animals cultivated. In terms of yield, the maintenance of nitrogen compounds is essential for positive results in cultivation. The concentration of total ammoniacal nitrogen in production systems is influenced by a number of factors, such as high stocking density, excretion, the amount of feed supplied, and decomposing organic matter (Stewart et al., 2006). Despite being toxic to aquatic organisms, this compound is controlled in the BFT system by adding a source of organic carbon as a way of favoring the growth of bacteria in the system. The heterotrophic system showed maximum concentrations of 8 $mg\cdot L^{-1}$ of total ammoniacal nitrogen at the beginning of the experiment. This is probably because the animals were stocked in clear water, in which there were no bacteria established in the medium to carry out the cycling of nitrogen compounds, requiring a greater supply of molasses to encourage the growth and stabilization of heterotrophic bacteria in the medium (Avnimelech & Kochba, 2009).

In the chemoautotrophic system, prior fertilization using ammonium chloride and sodium nitrite promoted the growth of ammonia- and nitrite-oxidizing bacterial groups capable of cycling these compounds in the system. The same was observed by Ferreira et al. (2020), when they analyzed the influence of three different water preparation strategies in a *P. vannamei* nursery: heterotrophic, mature, and chemoautotrophic. In this experiment, despite the pre-colonization of these bacteria in the chemoautotrophic system, after stocking the shrimp and tilapia, there were total ammoniacal nitrogen peaks of up to 5 $mg\cdot L^{-1}$. This is probably due to the amount of ammonia produced by the organisms and the feed offered being higher than the pre-established bacteria's ability to oxidize this compound, necessitating the supply of molasses, even if in a lower quantity compared to the heterotrophic system.

During the experiment, both treatments showed nitrite peaks, but this event was higher in the heterotrophic system, reaching 18 $mg\cdot L^{-1}$, taking 32 days to return to acceptable levels. Ferreira et al. (2020) carried out organic fertilization with feed and molasses when starting a heterotrophic system, three days before stocking the animals, with a (C:N) ratio of 15:1, and found maximum nitrite values of 4.23 $mg\cdot L^{-1}$, which is lower than the value found in this study. This result may also be related to the fact that this experiment used an integrated system with pilot-scale production of shrimp and tilapia, which generated more waste and feed inputs. The use of more precise calculations for initial fertilization with molasses, considering feed intake and animal excrement, as carried out by Brandão et al. (2021), can prove to be more effective in controlling nitrite in animal stocking in a heterotrophic system.

Although previous studies have reported that *P. vannamei* is tolerant to high nitrite concentrations (26.4–29 $mg\cdot NO_2\text{-}N\cdot L^{-1}$) in salinities between 15 and 35 ppm (Cohen et al., 2005; Handy et al., 2004; Lin & Chen, 2003), the results obtained in the present study suggested that there was a negative influence between the higher nitrite concentrations and the performance of the shrimp, since at the salinity of 20 ppm, in the heterotrophic treatment, the zootechnical indices of the shrimp were significantly lower than those of the animals in the chemoautotrophic treatment. These results were corroborated by Vinatea et al. (2010), who observed that *P. vannamei* showed a reduced growth rate when exposed to concentrations between 0.72 and 9.49 $mg\cdot NO_2\text{-}N\cdot L^{-1}$ in a super-intensive raceway system without water exchange. In addition, in this experiment, 30% of the water in the heterotrophic system was renewed as a way of controlling nitrite, along with a 30% reduction in the shrimp's feed throughout the period when nitrite was high (30 days), being factors that affected shrimp performance.

For the chemoautotrophic system, the nitrite peak reached 11 $mg \cdot L^{-1}$ and lasted 22 days, indicating that fertilization with ammonium chloride and sodium nitrite for 46 days before stocking the animals favoured the colonization of nitrifying bacteria that were able to remove this compound from the system in a shorter period of time, which is confirmed by the appearance of nitrate in the system, indicating that the nitrogen cycle was being carried out. In this sense, the high nitrite concentrations found in the chemoautotrophic treatment may be related to the lack of substrate in the tanks, as nitrifying bacteria have adherence characteristics to proliferate, so the use of substrates increases the contact area in the system and facilitates the colonization of this group, accelerating their proliferation and avoiding peaks of toxic compounds in the cultivation medium for long periods (Ferreira et al., 2015; Lara et al., 2021).

Nitrate is one of the elements that accumulates in closed systems without water renewal, as it is the case with the BFT system (Burford & Williams, 2001). However, the concentration of these compounds can be affected by the type of fertilization chosen. Nitrate is the end product of the nitrification process, which occurs with the oxidation of ammonia to nitrite and then to nitrate by nitrifying bacteria and, of these three compounds, nitrate is the least toxic to organisms (Burford & Williams, 2001). The high value of nitrate in the chemoautotrophic treatment can be explained by the fact that fertilization with ammonium chloride and sodium nitrite salts favoured the growth and predominance of nitrifying bacteria, which oxidize ammonia and accumulate it. For the heterotrophic system, the high nitrate concentrations found differ from the concentrations presented in Brandão et al. (2021) and Ferreira et al. (2020) and may be related to the type of fertilization carried out, this provided sufficient nitrogen for nitrifying bacteria to be able to establish over the time and reduce this available ammonia to nitrate.

In addition to nutrients, the production of solids and organic matter is constant in the biofloc system. One of the advantages of the chemoautotrophic BFT system is the lower production of TSS, given that the predominant bacteria in this system consume approximately 1 g of ammonia and produces 0.20 g of TSS. In contrast, heterotrophic bacteria, when consuming the same amount of ammonia, produce 8.07 g of TSS (Ebeling et al., 2006). However, there was no difference in the concentration of TSS between the treatments in the present study, which may be related to the use of clarifiers to maintain this parameter at acceptable levels in the production of 350 $mg \cdot L^{-1}$, according to Gaona et al. (2017), as well as due to water renewals in the heterotrophic treatment, carried out in order to control the high concentrations of nitrite, which led to a reduction in the organic matter present in this system.

In this study, using an integrated system with BFT technology with different fertilizations, better performance of shrimp was observed in chemoautotrophic treatment when compared to the heterotrophic treatment, which may be related to water quality, since the concentrations of ammonia and nitrite were lower in the chemoautotrophic treatment. The zootechnical performance values found in this treatment were similar to those found by other authors such as Holanda et al. (2022) using the IMTA system with BFT technology in shrimp production and Ferreira et al. (2021), with mullet in the grow-out of shrimp monoculture.

The use of prior fertilization in chemoautotrophic treatment promoted a lower concentration of toxic nitrogen compounds due to the previously established bacterial biomass, demonstrating that maintaining water quality and providing the correct feed promote better conditions for animal growth. In the heterotrophic treatment, which began in clear water without established bacterial communities, higher peaks of nitrogen compounds occurred, which remained in the system for longer, which required changes in feeding management, such as reducing the feed supply, to reduce nitrite levels and prevent animal mortality. However, chronic exposure to nitrite may have caused stress, impairing the zootechnical performance of the animals, since studies report that *P. vannamei* presents reduced growth when exposed to high chronic concentrations of nitrite (2 to 20 $mg \cdot L^{-1}$) at salinity 30, which may cause an imbalance in the intestinal microbiota (Huang et al., 2020). Despite the mortality that occurred, the high levels of ammonia and nitrite did not significantly interfere with the survival of the animals in either treatment, demonstrating that water renewal and feed reduction in the heterotrophic treatment were efficient. Similar results were observed by Valencia-Castañeda et al. (2017), who tested the influence of water renewal and feed reduction and proved that these methods are effective for animal survival.

In addition to maintaining nitrogen compounds in chemoautotrophic treatment, another important factor such as water composition may have contributed to better shrimp performance in terms of nutrition. According to Khanjani et al. (2023), biofloc has high nutritional value and, in some cases, can contribute to the diet and performance of the cultivated organism. In our study, a longer preparation time for chemoautotrophic treatment and not requiring water changes enabled higher growth of the bacterial community and increased food availability for the shrimp.

Integrated production allows the use of organic consumers with filtering characteristics to feed on the waste present in the system, which ends up generating a better feed conversion factor. In the present study, tilapia was fed 1% of the total biomass, forcing the fish to consume the bioflocs. Holanda et al. (2022) also obtained low feed conversion values (0.60 ± 0.0) in an IMTA system with feed supply at 1% of the total fish biomass, but with weekly growth slightly lower than that found in our study, which was 12 $g \cdot week^{-1}$, which may be related to the high density used by the authors, which was 100 $fish \cdot m^{-3}$, while in the present study it was 45 $fish \cdot m^{-3}$. The integrated multitrophic system provides the diversification of production systems, enabling the cultivation of different species in the same environment, as long as the water quality requirements for each species are met (Chopin, 2015).

CONCLUSION

Multitrophic cultivation with prior chemical fertilization, in chemoautotrophic treatment, showed better control of nitrogen compounds such as ammonia and nitrite, in addition to less water use, consequently influencing better weight gain for the shrimp and greater productivity. The use of waste produced by the organisms in the system provides higher sustainability, increasing production, which is one of the advantages of the multitrophic system.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data will be provided upon request.

AUTHORS’CONTRIBUTION

Conceptualization: Santos, J., Carvalho, A., Holanda, M., Poersch, L.H.; **Formal Analysis:** Santos, J., Carvalho, A., Gonçalves, M.; **Investigation:** Santos, J., Carvalho, A., Gonçalves, M.; **Methodology:** Santos, J., Holanda, M., Poersch, L.H., Gonçalves, M.; **Data curation:** Santos, J., Carvalho, A., Gonçalves, M.; **Writing – original draft:** Santos, J., Carvalho, A.; **Project Administration:** Holanda, M., Wasielesky Júnior, W., Poersch, L.H.; **Writing – review & editing:** Holanda, M., Gonçalves, M., Wasielesky Júnior, W., Poersch, L.H.; **Resources:** Wasielesky Júnior, W., Poersch, L.H.; **Final approval:** Carvalho, A.

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DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE TOOLS

No artificial intelligence tools were used.

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REFERENCES

American Public Health Association (APHA) (1989). *Standard Methods for the Examination of Water and Waste Water* (16th ed.). APHA, AWWA, WPFC.

American Public Health Association (APHA). (2005). *Standard methods for the examination of water and wastewater* (21st ed.). APHA, AWWA, WPFC.

Amionot, A., & Chaussepied, M. (1983). *Manuel des analyses chimiques en milieu marin*. Centre National pour l’exploitation des oceans.

Araneda, M., Gasca-Leyva, E., Vela, M. A., & Domínguez-May, R. (2020). Effects of temperature and stocking density on intensive culture of Pacific white shrimp in freshwater. *Journal of Thermal Biology*, 94, 102756. https://doi.org/10.1016/j.jtherbio.2020.102756

Avnimelech, Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, 176(3-4), 227-235. https://doi.org/10.1016/S0044-8486(99)00085-X

Avnimelech, Y., & Kochba, M. (2009). Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks, using N-15 tracing. *Aquaculture*, 287(1-2), 163-168. https://doi.org/10.1016/j.aquaculture.2008.10.009

Bendschneider, K., & Robinson, R. J. (1952). New spectrophotometric method for the determination of nitrite in water. *Fresenius Environmental Bulletin and Advances in Food Sciences*.

Brandão, H., Xavier, Í. V., Santana, G. K. K., Santana, H. J. K., Krummenauer, D., & Wasielesky, W. (2021). Heterotrophic versus mixed BFT system: Impacts on the water use, suspended solids production and growth performance of *Litopenaeus vannamei*. *Aquacultural Engineering*, 95, 102194. https://doi.org/10.1016/j.aquaeng.2021.102194

Burford, M. A., & Williams, K. C. (2001). The fate of nitrogenous waste from shrimp feeding. *Aquaculture*, 198(1-2), 79-93. https://doi.org/10.1016/S0044-8486(00)00589-5

Carvalho, A., Costa, L. C. O., Holanda, M., Poersch, L. H., & Turan, G. (2023). Influence of total suspended solids on the growth of the sea lettuce *Ulva lactuca* integrated with the pacific white shrimp *Litopenaeus vannamei* in a biofloc system. *Fishes*, 8(3), 163. https://doi.org/10.3390/fishes8030163

Chopin, T. (2015). Marine aquaculture in Canada: Well-established monocultures of finfish and shellfish and an emerging Integrated Multi-trophic Aquaculture (IMTA) approach including seaweeds, other invertebrates, and microbial communities. *Fisheries*, 40(1), 28-31. https://doi.org/10.1080/03632415.2014.986571

Cohen, J. M., Samocha, T. M., Fox, J. M., Gandy, R. L., & Lawrence, A. L. (2005). Characterization of water quality factors during intensive raceway production of juvenile *Litopenaeus vannamei* using limited discharge and biosecure management tools. *Aquacultural Engineering*, 32(3-4), 425-442. https://doi.org/10.1016/j.aquaeng.2004.09.005

Ebeling, J. M., Timmons, M. B., & Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. *Aquaculture*, 257(1-4), 346-358. https://doi.org/10.1016/j.aquaculture.2006.03.019

Ferreira, G. S., Silva, V. F., Martins, M. A., Silva, A. C. C. P., Machado, C., Seiffert, W. Q., & Vieira, F. N. (2020). Strategies for ammonium and nitrite control in *Litopenaeus vannamei* nursery systems with bioflocs. *Aquacultural Engineering*, 88, 102040. https://doi.org/10.1016/j.aquaeng.2019.102040

Ferreira, G. S., Santos, D., Schmachtl, F., Machado, C., Fernandes, V., Bögner, M., Schleder, D. D., Seiffert, W. Q., & Vieira, F. N. (2021). Heterotrophic, chemoautotrofic and mature approaches in bioflocs system for pacific white shrimp. *Aquaculture*, 533, 736099. https://doi.org/10.1016/j.aquaculture.2020.736099

Ferreira, L. M. H., Lara, G., Wasielesky Jr., W., & Abreu, P. C. (2015). Biofilm versus biofloc: Are artificial substrates for biofilm production necessary in the BFT system. *Aquaculture International*, 24, 921-930. https://doi.org/10.1007/s10499-015-9961-0

Food and Agriculture Organization (FAO) (2024). *The state of world fisheries and aquaculture: blue transformation in action*. Rome: Food and Agriculture Organization of the United Nations. FAO.

Furtado, P. S., Poersch, L. H., & Wasielesky, W. (2014). The effect of different alkalinity levels on *Litopenaeus vannamei* reared with biofloc technology (BFT). *Aquaculture International*, 23(1), 345-358. https://doi.org/10.1007/s10499-014-9819-x

Gao, W., Tian, L., Huang, T., Yao, M., Hu, W., & Xu, Q. (2016). Effect of salinity on the growth performance, osmolarity and metabolism-related gene expression in white shrimp *Litopenaeus vannamei*. *Aquaculture Report*, 4, 125-129. https://doi.org/10.1016/j.aqrep.2016.09.001

Gaona, C. A. P., Almeida, M. S., Viau, V., Poersch, L. H., & Wasielesky Jr., W. (2017). Effect of different total suspended solids levels on a *Litopenaeus vannamei* (Boone, 1931) BFT culture system during biofloc formation. *Aquaculture Research*, 48, 1070-1079. https://doi.org/10.1111/are.12949

García-Ríos, L., Miranda-Baeza, A., Coelho-Emerenciano, M. G., Huerta-Rábago, J. A., & Osuna-Amarillas, P. (2019). Biofloc technology (BFT) applied to tilapia fingerlings production using different carbono sources: Emphasis on comercial applications. *Aquaculture*, 502, 26-31. https://doi.org/10.1016/j.aquaculture.2018.11.057

Handy, M., Samocha, T., Patnaik, S., Gandy, R., & McKee, D. (2004). Nursery trial compares filtration system performance in intensive raceways. *Global Seafood Alliance*.

Holanda, M., Wasielesky Jr., W., Lara, G. R., & Poersch, L. H. (2022). Production of marine shrimp integrated with tilapia at high densities and in a biofloc system: choosing the best spatial configuration. *Fishes*, 7(5), 283. https://doi.org/10.3390/fishes7050283

Huang, M., Xie, J., Yu, Q., Xu, C., Zhou, L., Qin, J. G., Chen, L., & Li, E. (2020). Toxic effect of chronic nitrite exposure on growth and health in Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*, 529, 735664. https://doi.org/10.1016/j.aquaculture.2020.735664

Jory, D. E., Cabrera, T. R., Dugger, D. M., Fegan, D., Lee, P. G., Lawrence, A. L., Jackson, C. J., McIntosh, R. P., & Castañeda, J. (2001). A global review of shrimp feed management: status and perspectives. In C. L. Browdy & D. E. Jory (Eds.), *The new wave: proceedings of the special session on sustainable shrimp culture* (pp. 104-152). The World Aquaculture Society.

Khanjani, M. H., Mozanzadeh, M. T., Sharifinia, M., & Emerenciano, M. G. C. (2023). Biofloc: A sustainable dietary supplement, nutritional value and functional properties. *Aquaculture*, 562, 738757. https://doi.org/10.1016/j.aquaculture.2022.738757

Khanjani, M. H., Sajjadi, M. M., Alizadeh, M., & Sourinejad, I. (2016). Nursey performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a biofloc system: the effect of adding different carbon sources. *Aquaculture Research*, 1-11. https://doi.org/10.1111/are.12985

Lara, G. R., Poersch, L. H., & Wasielesky Jr., W. (2021). The quantity of artificial substrates influences the nitrogen cycle in the biofloc culture system of *Litopenaeus vannamei*. *Aquacultural Engineering*, 94, 102171. https://doi.org/10.1016/j.aquaeng.2021.102171

Lin, Y.-C., & Chen, J.-C. (2003). Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. *Aquaculture*, 224(1-4), 193-201. https://doi.org/10.1016/S0044-8486(03)00220-5

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, e739492. https://doi.org/10.1016/j.aquaculture.2023.739492

Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliot, M., Farrell, A. P., Forster, I., Gatlin, D. M., Goldburg, R. J., Hua, K., & Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 15103-15110. https://doi.org/10.1073/pnas.0905235106

Queiroz, H. M., Ferreira, T. O., Taniguchi, C. A. K., Barcellos, D., Nascimento, J. C., Nóbrega, G. N., Otero, X. L., & Artur, A. G. (2020). Nitrogen mineralization and eutrophication risks in mangroves receiving shrimp farming effluents. *Environmental Science and Pollution Research*, 27, 34941-34950. https://doi.org/10.1007/s11356-020-09720-1

Robles-Porchas, G. R., Gollas-Galván, T., Martínez-Porchas, M., Martínez-Cordova, L. R., Miranda-Baeza, A., & Vargas-Albores, F. (2020). The nitrification process for nitrogen removal in biofloc system aquaculture. *Reviews in Aquaculture*, 12(4), 2228-2249. https://doi.org/10.1111/raq.12431

Roriz, G. D., Delphino, M. K. V. C., Gardner, I. A., & Gonçalves, V. S. P. (2017). Characterization of tilapia farming in net cages at a tropical reservoir in Brazil. *Aquaculture Reports*, 6, 43-48. https://doi.org/10.1016/j.aqrep.2017.03.002

Silva, K. R., Wasieleski Jr., 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

Stewart, N. T., Boardman, G. D., & Helfrich, L. A. (2006). Characterization of nutrient leaching rates from settled rainbow trout (*Oncorhynchus mykiss*) sludge. *Aquaculture Engineering*, 35(2), 191-198. https://doi.org/10.1016/j.aquaeng.2006.01.004

Strickland, J. D. H., & Parsons, T. R. (1972). *A pratical handbook of seawater analysis* (2nd ed.). Fisheries Research Board of Canada.

United Nations Educational, Scientific and Cultural Organization (UNESCO) (1983). *Chemical methods for use in marine environmental monitoring. Manual and Guides 12*. Intergovernamental Oceanographic Commissiony.

Valencia-Castañeda, G., Vanegas-Pérez, R. C., Frías-Espericueta, M. G., Chávez-Sánchez, M. C., Ramírez-Rochin, J., & Páez-Osuna, F. (2017). Comparison of four treatments to evaluate acute toxicity of nitrite in shrimp *Litopenaeus vannamei* postlarvae: Influence of feeding and the renewal water. *Aquaculture*, 491, 375-380. https://doi.org/10.1016/j.aquaculture.2017.12.037

Vinatea, L., Gálvez, A. O., Browdy, C. L., Stokes, A., Venero, J., Haveman, J., Lewis, B. L., Lawson, A., Shuler, A., & Leffler, J. W. (2010). Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super-intensive raceway culture with zero water exchange: interaction of water quality variables. *Aquacultural Engineering*, 42(1), 17-24. https://doi.org/10.1016/j.aquaeng.2009.09.001

Wasielesky, W., Atwood, H., Stokes, A., & Browdy, C. L. (2006). Effect of natural production in a zero-exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture*, 258(1-4), 396-403. https://doi.org/10.1016/j.aquaculture.2006.04.030

Zar, J. H. (2010). *Biostatical analysis* (5th ed.). Pearson Prentice Hall.