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Comparison between biofloc technology system and aquamimicry in the cultivation of *Litopenaeus vannamei* in lined ponds in Southern Brazil

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ABSTRACT

This work compared biofloc technology and aquamimicry technologies in *Litopenaeus vannamei* lined ponds, using a density of 60 shrimp·m^{-2}. The experiment lasted 120 days, with two treatments, bioflocs (TBio) and aquamimicry (TMi), and three replications for each. In both treatments, the average values of the water quality parameters were as follows: temperature was 24.0 ± 0.32 °C, dissolved oxygen was 8.00 ± 0.45 mg·L^{-1}, pH was 8.40 ± 0.20, and alkalinity was 240.01 ± 37.15 mg·L^{-1}. The control of water quality was effectively maintained in both treatments, indicating the aquamimicry system's capability to efficiently recycle the nutrients found in the lined ponds' water. Furthermore, both treatments demonstrated efficiency in shrimp production, and the shrimp from the TMi treatment reached an average final weight of 11.73 ± 2.21 g, average survival of 53.3 ± 15.2%, and productivity of 3.56 ± 0.15-ton·ha^{-1}. The TBio shrimp reached a final weight of 11.48 ± 1.25 g, survival of 63.3 ± 8.16%, and productivity of 4.08 ± 1.10-ton·ha^{-1}. The present study demonstrated that TMi treatment ponds presented zootechnical performances close to those of TBio treatment ponds. The results achieved can contribute to the improvement of this cultivation system to use it in higher stocking densities.

Keywords: Organic fertilization; Rice bran; Probiotic; Prebiotic; Synbiotics; Biofloc.

Comparação entre o sistema de bioflocos e aquamimicry no cultivo de *Litopenaeus vannamei* em viveiros revestidos no sul do Brasil

RESUMO

Este trabalho comparou as tecnologias sistema de bioflocos e *aquamimicry* em viveiros revestidos de *Litopenaeus vannamei*, usando densidade de 60 camarões·m^{-2}. O experimento teve duração de 120 dias e envolveu dois tratamentos, bioflocos (TBio) e *aquamimicry* (TMi), e três repetições para cada um. Em ambos os tratamentos, os valores médios dos parâmetros de qualidade da água foram os seguintes: temperatura = 24,0 ± 0,32 °C, oxigênio dissolvido = 8,00 ± 0,45 mg·L^{-1}, pH = 8,40 ± 0,20, e alcalinidade = 240,01 ± 37,15 mg·L^{-1}. O controle da qualidade da água foi efetivamente mantido em ambos os tratamentos, indicando a capacidade do sistema *aquamimicry* de reciclar eficientemente os nutrientes encontrados na água dos tanques revestidos. Além disso, ambos os tratamentos demonstraram eficiência na produção de camarões. Os camarões procedentes do tratamento TMi atingiram peso final médio de 11,73 ± 2,21 g, sobrevivência média de 53,3 ± 15,2% e produtividade de 3,56 ± 0,15 ton·ha^{-1}. Os camarões do TBio alcançaram peso final de 11,48 ± 1,25 g, sobrevivência de 63,3 ± 8,16% e produtividade de 4,08 ± 1,10 ton·ha^{-1}. O presente estudo demonstrou que viveiros do tratamento TMi apresentaram desempenhos zootécnicos próximos aos dos viveiros do tratamento TBio. Os resultados alcançados podem contribuir para o aprimoramento desse sistema de cultivo com o objetivo de utilizá-lo em densidades de estocagens mais elevadas.

Palavras-chave: Fertilização orgânica; Farelo de arroz fermentado; Probiótico; Prebiótico; Simbióticos; Bioflocos.

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INTRODUCTION

Aquaculture has shown important technological advances in relation to production systems. In the context of increased productivity, shrimp have been important organisms in this evolution, mainly because they are also highly profitable, especially *Litopenaeus vannamei*. This species can tolerate a wide range of salinities and withstand high stocking densities (Liu et al., 2017; Jaffer et al., 2020).

In addition to increasing productivity, technologies have emerged with the objective of increasing biosafety, also enabling the reduction of production costs (Panigrahi et al., 2020; Valenti et al., 2021; Khanjani et al., 2023). One of these technologies that has been used is the biofloc system (biofloc technology system–BFT), which consists of the formation of aggregates of microorganisms (bacteria, phytoplankton, and zooplankton) in suspension in the water column (Avnimelech, 2007). These organisms remain associated with particles, colloids, organic polymers, and dead cells (Wasielesky Jr. et al., 2006; Khanjani et al., 2023). The development of this microbiota is stimulated by the constant circulation of the water column, constant aeration, and addition of organic carbon to the water in concentrations that maintain a carbon: nitrogen ratio close to 15: 1 (Avnimelech, 1999).

In this system, heterotrophic bacteria play an important role in the formation of biofloc, assimilating organic carbon and ammoniacal nitrogen dissolved in the water into the bacterial biomass, thus reducing the ammonia concentration and improving the quality of water for cultivation (Esparza-Leal et al., 2015; Gaona et al., 2016). Furthermore, the BFT system has other advantages, such as increased biosafety and reduced need for water renewal, besides the fact that bioflocs have high nutritional value, contributing to shrimp nutrition and thus reducing the amount of feed offered (Emerenciano et al., 2012). However, the BFT system has some limitations, such as the need to maintain particulate material suspended in the water column, increasing energy expenditure with the aeration system, the need for technical assistance, high installation cost, among others (Gaona et al., 2016; Romano, 2017).

An approach that can be an intermediary between the use of conventional systems and the BFT system is called the synbiotic or *aquamimicry* system. This system emerged in the 1990s in Thailand from the modification in the fertilization process of shrimp ponds, due to the need to reduce production costs by using low stocking densities to prevent the onset of diseases (early mortality syndrome, for example).

Aquamimicry, as a shrimp culture method, offers a solution to the limitations of traditional BFT system by incorporating elements of conventional methods (Khanjani et al., 2022). This modification has shown promising results, leading to enhanced water quality and improved zootechnical performance in the shrimp farming environment (Li et al., 2009; Khanjani et al., 2022). The underlying principle of this method is the belief that shrimp farming practices can achieve greater sustainability by emulating the natural aquatic environment within aquaculture settings. This approach is characterized by a well-balanced and cost-effective system that replicates natural conditions through the provision of zooplankton for shrimp nourishment and the utilization of beneficial bacteria to enhance water quality (Romano, 2017; Nisar et al., 2022).

The Aquamimicry Aquaculture Alliance defines this system:

> [...] a simulator of the natural conditions of primary and secondary estuarine production, stimulating the growth of beneficial bacteria, phytoplankton blooms, as well as the increase of zooplankton populations, which serve as supplemental nutrition to the cultivated shrimp by helping in maintaining water quality (apud Romano, 2017).

The central aspect of the management approach involves regularly applying fermented carbon/grain sources, including rice bran, soybean meal, and wheat bran, in conjunction with water probiotics like *Bacillus* sp. (Khanjani et al., 2022; Nisar et al., 2022; Santos et al., 2022a, 2022b). Microorganisms or enzymes can be employed to enhance the solubility of the carbon source in water and expedite the breakdown of nutrients. Bacteria (e.g., *Lactobacillus* sp. and *Bacillus* sp.), fungi, and yeast (e.g., *Saccharomyces* sp.) have demonstrated involvement in producing hydrolytic enzymes that decrease fiber and carbohydrate content while boosting the protein solubility of carbon sources (Romano et al., 2017; Khanjani et al., 2022).

Prebiotic can be defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of specific health-promoting bacteria that can improve host health (Gibson & Roberfroid, 1995). The prebiotic source generally consists of oligosaccharides from bran fermentation (rice, wheat or soy), which can stimulate the growth of beneficial bacteria such as Rhodobacteraceae, improving nutrient digestion as well as the immune responses of cultivated organisms. The probiotic source consists of live microorganisms (generally *Bacillus* sp. bacteria, among others) that exert beneficial effects on the health of the host, modulating the intestinal microbiota and increasing resistance against pathogens. For example, Li et al. (2009) kept *L. vannamei* shrimp for 28 days in a symbiotic system composed of *Bacillus* sp. and a mixture of oligosaccharides (IMO

900P) and found that, after a challenge with white spot syndrome virus, individuals present in this system showed greater phagocytic activity by hemocytes and other favorable immune responses, resulting in significantly lower mortality.

A marked distinction between the BFT system and symbiotic technology refers to the management of the C : N ratio. For the BFT system, a certain degree of technical experience is required to obtain the proper ratio (15 : 1), while this proper ratio is less important for the symbiotic system, which can be managed by less experienced aquaculture practitioners (Khanjani et al., 2022). Another difference between these two systems is that the symbiotic technology is normally based on cultivation using large, excavated ponds and low stocking densities, whereas the BFT system manages high animal densities within smaller ponds.

However, there is a gap in knowledge on the comparison between the symbiotic and BFT systems in terms of productivity level. In this sense, studies that aim to increase stocking densities using symbiotic systems can help to improve this technology, increasing productivity and mitigating the possibility of mortality in farmed shrimp. Therefore, this work aimed at the comparison between the BFT and symbiotic systems for the cultivation of marine shrimp *L. vannamei* in lined ponds, in relation to water quality and zootechnical performance indexes.

MATERIALS AND METHODS

Origin of shrimp and management

The nauplii of *L. vannamei* were obtained in a commercial laboratory and transported to the larviculture sector of the Marine Aquaculture Station "Professor Marcos Alberto Marchiori", of the Institute of Oceanography at the Universidade Federal do Rio Grande (EMA/IO-FURG). The nauplii were cultured until they reached the post-larval stage (PL) of 15 days. Subsequently, the post-larvae were placed in nursery tanks inside a greenhouse, where they remained until they reached an average weight of 1.44 g. Finally, they were transferred to lined experimental tanks for the beginning of the experiment.

Experimental design

Juveniles of *L. vannamei* were introduced into six lined ponds, each with an area of 600 m^2, located at the Marine Aquaculture Station (EMA/IO-FURG). These ponds are built with high-density polyethylene (HDPE) lining, ensuring that there is no contact between water and soil. The stocking density was set at 60 $shrimp \cdot m^{-2}$.

The experiment was conducted over a period of 120 days, from December 2018 to March 2019, and consisted of two treatments with three replications each. Three ponds used the BFT (TBio) system, while the other three ones used the aquamimicry (TMi) technology.

Inputs and management

In TBio treatment, three days before shrimp stocking, the ponds were inoculated with 1% water with bioflocs from continuous shrimp farming containing low ammonia and nitrite levels, but increasing nitrate levels. In addition to this inoculum, an initial fertilization was carried out using 30 $mg \cdot L^{-1}$ of a nitrogen source (shrimp feed with 35 %CP) and 30 $mg \cdot L^{-1}$ of a carbon source (sugar cane molasses). The objective of the organic fertilization was to maintain an initial C: N ratio of 15 : 1, which promoted the growth of the heterotrophic microbial community (Avnimelech, 1999). Once the total ammonia nitrogen (TAN) levels reached 1.0 $mg \cdot L^{-1}$ after stocking, a carbon source, specifically cane molasses, was applied at a ratio of 6 : 1 (Xu et al., 2016). This ratio was chosen to account for the feed's ratio of 9 : 1, resulting in an approximate 15 : 1 ratio. This application served to support the growth of heterotrophic bacteria and reduce the concentration of ammonia.

Additionally, in order to regulate the total suspended solids (TSS) concentration, periodic renewals were conducted by introducing seawater or water from wells into the ponds. The TSS levels were consistently maintained below the recommended maximum threshold of 400 $mg \cdot L^{-1}$ (Avnimelech, 2012; Schveitzer et al., 2013; Gaona et al., 2016).

In preparation for the TMi treatment, three days prior to shrimp transfer, the ponds underwent pre-fertilization with 24-hour fermented rice bran as a carbon source. This involved applying 40 $g \cdot L^{-1}$ of fermented rice bran in water, along with the addition of a commercial probiotic (Sanolife PRO W, INVE Aquaculture) at a dosage of 200 $mg \cdot L^{-1}$. This approach was based on an adapted protocol inspired by Kubitza (2018), excluding the use of urea. Rice bran, with a C: N ratio of 20 : 1, was chosen as it promotes the development of heterotrophic bacteria and aids in reducing TAN levels throughout the cultivation cycle. Unlike the BFT system, in which the carbon source application depends on the ammonia concentrations in the water of tanks or ponds, the symbiotic one requires systematic carbon applications, regardless ammonia concentrations. Further fertilization procedures during the experimental period followed the methodology proposed by Kawahigashi (1992, 1998): with a concentration of fermented rice bran of 20 $g \cdot L^{-1}$ and 150 $mg \cdot L^{-1}$ of probiotic pond every day throughout the growing season.

In all treatments, continuous aeration was provided using paddle wheel aerators (Trevisan, Palotina, PR, Brazil) with a power of 33 $HP \cdot ha^{-1}$. Shrimps were fed twice daily with a commercial feed (Guabi) containing 40% crude protein, and feeding trays were utilized to regulate and monitor feed consumption. These feeding trays served as a reference for feeding tables based on Jory et al. (2001)'s work.

Preparation of fermented rice bran

The preparation of the fermented rice bran was performed in a 300-L water tank, in which sieved rice bran (2.0 mm mesh), probiotic and previously chlorinated and dechlorinated seawater were added. After the storage date, the anaerobic fermented compound was added every two days. This fermented compound was prepared with rice bran, commercial probiotic and water chlorinated, then dechlorinated, and kept in an anaerobic environment.

Physicochemical parameters

Dissolved oxygen concentrations and water temperature were assessed twice daily (at 8 a.m. and 4 p.m.) using a digital oximeter (Pro-20 YSI®). pH levels were also measured twice a day using a digital pH meter (Mettler Toledo®). Additionally, water transparency was measured daily using a Secchi disk. To analyze nitrogen compounds, water samples were collected on a daily basis to measure the concentrations of total ammonia-nitrogen (TAN) (UNESCO, 1983), nitrogen-nitrite ($N\text{-}NO_2^-$) (Strickland and Parsons, 1972), and nitrogen-nitrate ($N\text{-}NO_3^-$) (UNESCO, 1983). Weekly, salinity was checked with the aid of an optical refractometer (Atago), and alkalinity was estimated according to American Public Health Association (APHA, 1998). Weekly determination of TSS involved gravimetry, i.e., 20 mL samples of water were filtered through GF 50-A glass fiber filters (Strickland and Parsons, 1972). Water turbidity, on the other hand, was measured once a week using a Hach model 2100P turbidimeter.

Microbial community

For the analysis of the microbial community, three sampling days of the experimental period were chosen for collecting water samples. All water samples were stored in amber glass flasks (50 mL) containing acidic Lugol solution ($1.0 - 2.0\ \% \cdot vv^{-1}$) for further counting of phytoplankton cells, colonial organisms, and cyanobacteria trichomes. Chosen three sampling days corresponded to:

- Initial (October 11, 2018);
- Middle (January 31, 2019);
- Final (March 28, 2019) of the experimental period.

Microorganisms counted were divided into phytoplankton groups (chlorophytes, diatoms and cyanobacteria), protozooplankton (flagellates, ciliates) and zooplankton groups (rotifers).

Cell identification and counting were performed using an Axiovert A1 inverted microscope (Zeiss) and Utermöhl sedimentation chambers with a coupled AxioCam MR (Zeiss) camera for capturing selected images. The entire area of the sedimentation chamber was inspected for counting microorganisms between 20 to 50 µm (100X magnification). At least 30 random fields were considered for counting microorganisms between 5 to 20 µm (200X), and another 30 random fields for counting ones between 2.0 and 10 µm (400X). There were counted 200 units (cells or filaments), whose number threshold gave a margin of error < 20% (Lund et al., 1958). Both taxonomic classification and taxonomic status of all species or genera were checked according to algaebase.org website (Guiry and Guiry, 2019).

Zootechnical performance

On the stocking day, an initial biometric assessment was conducted (N=100 individuals per nursery) to estimate the average weight of the shrimp being stocked. Throughout the experiment, weekly biometric measurements were carried out, involving 60 randomly selected individuals per pond. Each shrimp was individually weighed using a digital scale with a precision of 0.01 g (AS 1000 C, Marte). After being weighed, the shrimps were returned to their respective ponds. Using the collected biometric data, the average weight of the shrimp was calculated, allowing for the adjustment of the weekly feed quantity to be offered. This adjustment was made in accordance with the guidelines provided in a review article by Jory et al. (2001). Thus, at the end of experiments, weight gain of the shrimp for each pond was obtained through Eq. 1:

$$G\ (g/week) = (Wf\ (g) - Wi\ (g)) / NS \qquad (1)$$

Where: Wf: the final weight; Wi: the initial weight; NS: the weeks of end the experiment.

The estimation of the feed conversion rate (FCR) was performed using Eq. 2:

$$FCR = \text{feed offered (g)} / \text{biomass increment (g)} \qquad (2)$$

That is, biomass increment derived from final weight – initial weight (g).

Estimated survival was calculated through Eq. 3:

$$S (\%) = (\text{final number} / \text{initial number}) \times 100 \qquad (3)$$

After harvesting, with the final biomass data, productivity data were obtained.

Statistical analysis

Statistical analysis was conducted to determine significant differences ($p < 0.05$) in zootechnical performance and physical-chemical parameters using Student's t-test. Prior to the analysis, the homoscedasticity of variances was assessed using the Levene's test, and the normality of data distribution was checked using the Kolmogorov-Smirnov test. To meet the assumptions of the t-test, survival data were transformed using the square root arc sine method (Zar, 2010).

RESULTS

Physicochemical variables

Means and standard deviations of the physicochemical variables, as well as total ammonia-nitrogen (TAN), nitrite ($N\text{-}NO_2^-$), nitrate ($N\text{-}NO_3^-$), are shown in Table 1.

The average temperature during the experimental period was 24 °C (Fig. 1). No significant differences ($p < 0.05$) were observed between the two treatments in terms of temperature. However, certain fluctuations in temperature during the study period were closely associated with the passage of cold fronts across the region (observed in weeks 1, 6, 8, 11, 13 and 15). The lowest temperatures were measured in the first and last weeks.

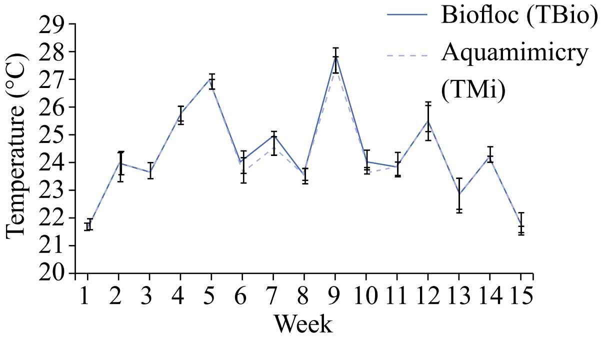

Figure 1. Water temperature (°C) variation for biofloc and aquamimicry systems in lined ponds, during the experimental weeks. The arrows indicate the entry of cold fronts in the region during the experimental period. Mean values and standard deviations presented.

Table 1. Values (mean ± standard deviation) of the physicochemical variables of water quality and nutrients in intensive cultivation ponds of white shrimp *Litopenaeus vannamei*, for the biofloc (TBio) and aquamimicry (TMi) treatments*.

Physicochemical variables	**Treatment**	
	TBio	**TMi**
Temperature (°C)	24.34± 0.36	24.19± 0.28
Dissolved oxygen ($mg \cdot L^{-1}$)	8.08± 0.31	7.85± 0.60
pH	8.47± 0.18	8.44± 0.22
Salinity	11.09± 1.18	11.42± 2.42
Transparency (cm)	17.08 ± 0.84	19.50 ± 2.48
Turbidity (NTU)	112.77± 53.12	116.04± 63.07
Alkalinity ($mg\ CaCO_3 \cdot L^{-1}$)	245.30± 37.95	232.57± 36.36
Total suspended solids (TSS $mg \cdot L^{-1}$)	190.38± 70.81	165.71± 77.32
Total ammonia nitrogen ($mg \cdot L^{-1}$)	0.24± 0.29	0.25± 0.32
Nitrogen nitrite ($mg \cdot L^{-1}$)	0.33 ± 0.47	0.12± 0.16
Nitrogen nitrate ($mg \cdot L^{-1}$)	1.52± 1.00	2.82± 2.21
Water exchange (% $volume \cdot day^{-1}$)	0.92± 0.18^a	3.35± 0.85^b

*Different letters in the same row denote significant statistical differences ($p < 0.05$).

The mean concentrations of dissolved oxygen in the treatments did not exhibit any significant differences ($p > 0.05$) (Fig. 2).

Throughout the experimental period, there were rare instances of decrease in the mean values of dissolved oxygen in both treatments. The minimum and maximum concentrations recorded were 3.83 and 11.38 mg·L^{-1}, respectively. Additionally, there were no significant differences observed in the average pH values during the experiment. The mean salinity values remained relatively stable throughout the study, with a slight decrease noted towards the end of the experimental period. This salinity alteration occurred due to renovations carried out with underground water, whose salinity was 8. That water input was mainly to replace the evaporated water and keep certain environmental parameters in control, such as nitrogen compounds and TSS.

The average values of water transparency had significant differences ($p < 0.05$) between treatments, mainly during the middle of the experimental period, but they remained close and reduced at the end (Fig. 3). Moreover, a reduction in TBio transparency was noted beginning the seventh week and lasted until the 10th week (14.8 cm), whereas for TMi an increase in transparency was observed between weeks 5 and 12, reaching the value of 32 cm.

Contrary to transparency pattern, there was an increase in turbidity (Fig. 4) in TBio between weeks 4 and 12, while for TMi there was an increase in turbidity between weeks 5 and 12, demonstrating an inverse relationship between turbidity and transparency.

There was an increase in alkalinity from the eighth week for TBio, and from the 11th week for TMi (Fig. 5). Maximum alkalinity values were observed in the 15th week (TBio) and 14th week (TMi).

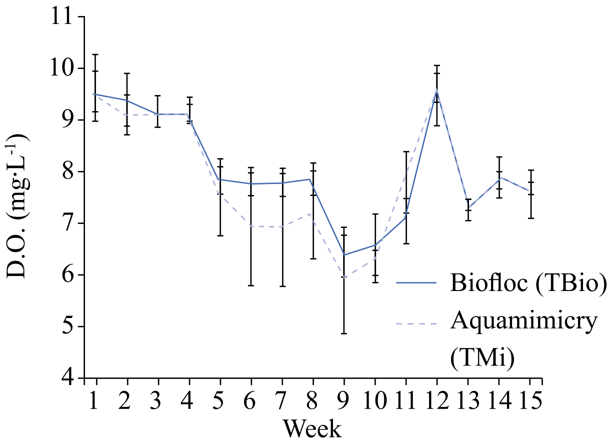

Figure 2. Dissolved oxygen (D.O.) (mg·L^{-1}) variation for biofloc and aquamimicry systems in lined ponds, during the experimental weeks. Mean values and standard deviations presented.

Regarding TSS (Fig. 6), maximum values were obtained in the nineth week (313.90 mg·L^{-1} for TBio) and in the 15th week (360 mg·L^{-1} for TMi),

Mean TAN concentration was less than 1 mg·L^{-1} throughout the cultivation period (Fig. 7). However, there was a peak at the sixth week six for the TMi, with maximum value = 1.12 mg·L^{-1}. The increase in TAN concentrations for both systems was controlled during the experimental period using water renewals.

Nitrite, in turn, remained at low concentrations (< 0.2 mg·L^{-1}) throughout almost the entire experimental period for both

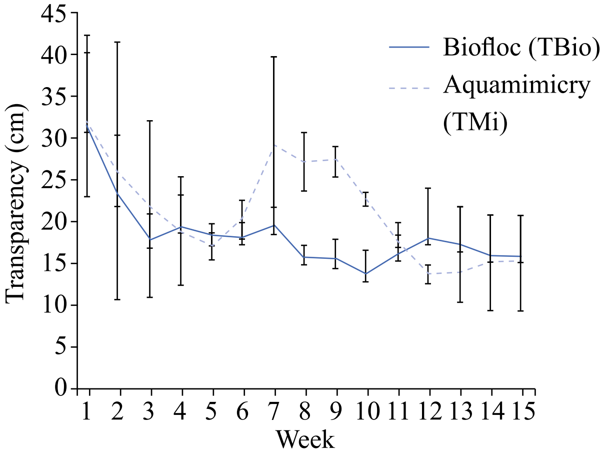

Figure 3. Transparency (Secchi disk, cm) for biofloc and aquamimicry systems in lined ponds, along the experimental weeks. Mean values and standard deviations presented.

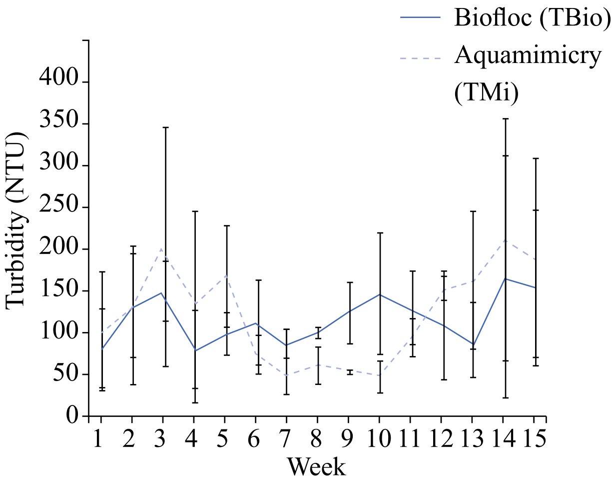

Figure 4. Turbidity (NTU) for biofloc and aquamimicry systems in lined ponds, along the experimental weeks. Mean values and standard deviations presented.

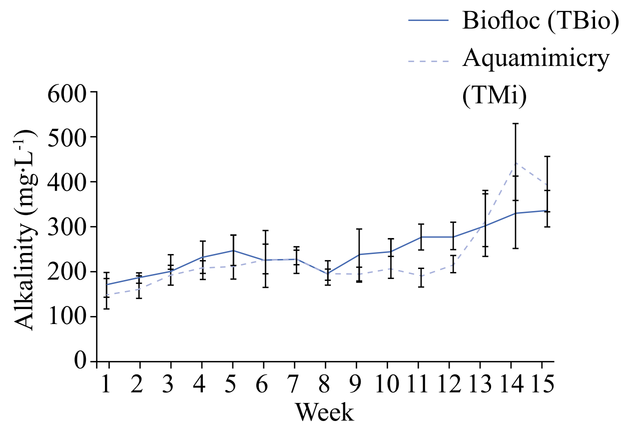

Figure 5. Alkalinity (mg·L^{-1}) for biofloc and aquamimicry systems in lined ponds, along the experimental weeks. Mean values and standard deviations presented.

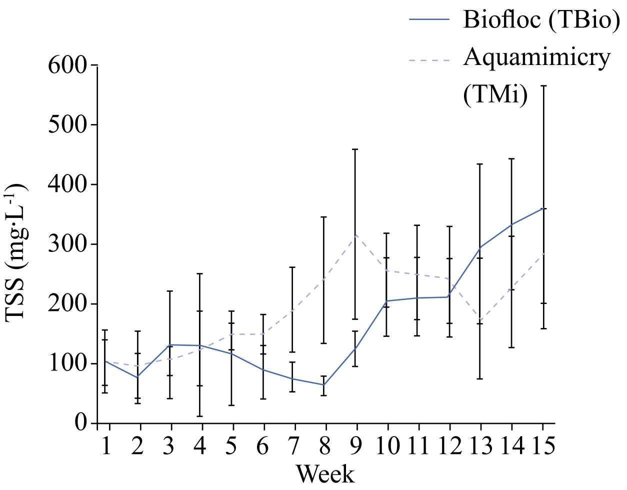

Figure 6. Variation of total suspended solids (mg·L^{-1}) for biofloc and aquamimicry systems in lined ponds, along the experimental weeks. Mean values and standard deviations presented.

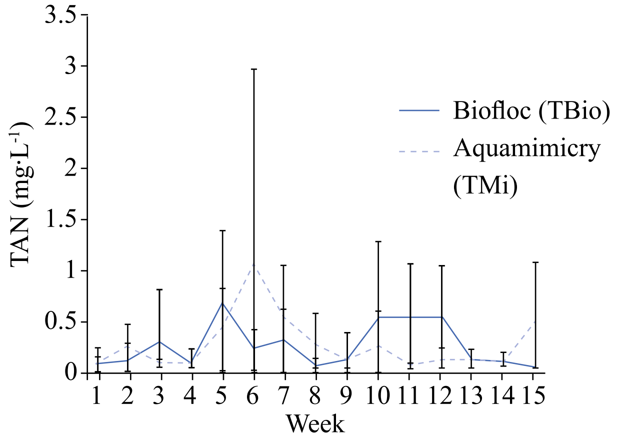

Figure 7. Total ammonia-nitrogen (TAN) concentration (mg·L^{-1}) for biofloc and aquamimicry systems in lined ponds, along the experimental weeks. Mean values and standard deviations presented.

treatments (Fig. 8). There was a considerable increase in the 11th week for TBio (1.09 ± 1.37 mg·L^{-1}) and the 14th week for TMi (0.67 ± 0.93 mg·L^{-1}).

There were no significant differences in nitrate concentration observed between treatments during the entire experimental period (Fig. 9). However, there was a noticeable increase in the 10th week, with values of 3.66 mg·L^{-1} for the TBio treatment and 8.33 mg·L^{-1} for the TMi treatment.

Regarding water changes, it was necessary to perform them more frequently during half of the experimental period, mainly in the TMi treatment ponds due to cyanobacterial blooms, which affected the well-being of the shrimp, reducing food consumption and causing mortality. The average water renewal values are show in Fig. 10.

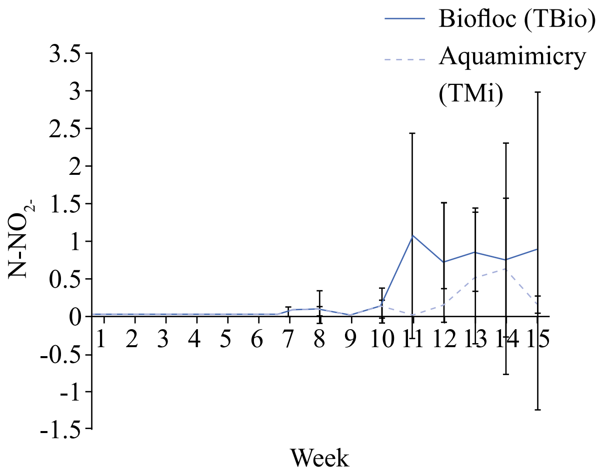

Figure 8. Nitrogen-nitrite (NO_2^-) concentration (mg·L^{-1}) for biofloc and aquamimicry systems in lined ponds, over the experimental weeks. Mean values and standard deviations presented.

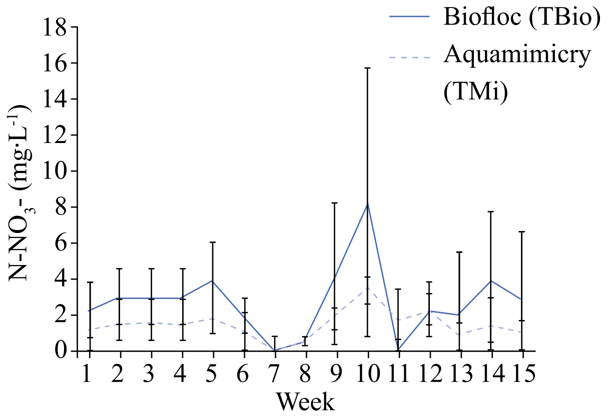

Figure 9. Nitrogen-nitrate (NO_3^-) concentration (mg·L^{-1}) for biofloc and aquamimicry systems in lined ponds, along the experimental weeks. Mean values and standard deviations presented.

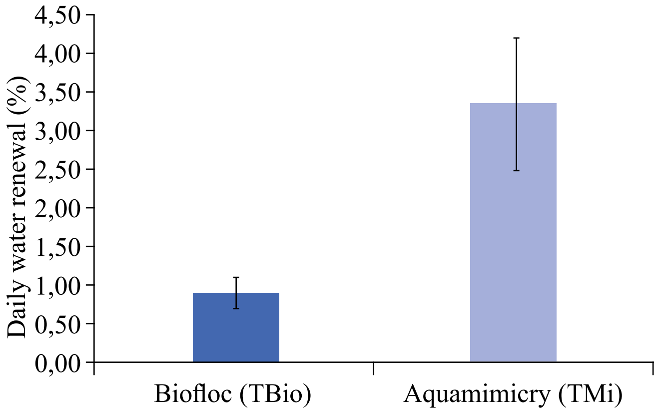

Figure 10. Average daily value for water renewal for the biofloc and aquamimicry systems in lined ponds, over the experimental weeks. Mean values and standard deviations presented.

Microbial community

There was no significant difference ($p < 0.05$) between the two treatments in terms of abundance of planktonic organisms. These abundance values are represented in Table 2.

There were higher concentrations of chlorophytes mainly in the intermediate and final periods of the experiment (1.10×10^9 and 6.64×10^8 cells·L^{-1}, respectively) in the TBio treatment. TMi treatment showed lower abundance of these organisms: 1.46×10^8 cells·L^{-1} (in the intermediate period) and 4.04×10^8 cells·L^{-1} (in the final experimental time). Diatoms were mainly represented by *Chaetoceros muelleri* and *Amphora* sp. in both treatments. The highest concentrations were found in the TBio treatment, with 5.84×10^7 cells·L^{-1} (intermediate period) and 1.29×10^8 cells·L^{-1} (final period), while for TMi 9.80×10^6 cells·L^{-1} and 6.34×10^6 cells·L^{-1} were observed, corresponding to the initial and final periods, respectively.

Cyanobacteria, mainly represented by the species *Nodularia spumigena*, were constant throughout the study period. They can produce harmful toxins (Teikari et al., 2018). There was a substantial increase in these organisms at the end of the experiment, mainly in the TMi one, with maximum value of 2.08×10^8 filaments·L^{-1}. In the TBio treatment, the concentration of cyanobacteria reached 3.86×10^7 filaments·L^{-1}. In both treatments, cyanobacteria blooms were evident in all ponds in the experiment, using water renewal for both treatments (TMi and TBio) for cyanobacteria population control.

During the entire experimental period, flagellates did not show high numbers. In the final period of the experiment, the TMi treatment had a count of only 2.86×10^8 cells·L^{-1}. However, during the initial period, the TMi treatment was more abundant than the TBio treatment (5.27×10^6 and 1.41×10^6 cells·L^{-1}, respectively). Interestingly, in the intermediate period, the highest abundance of flagellates was observed in the TBio treatment (8.91×10^6 cells·L^{-1}). Ciliates were more abundant in the initial and intermediate periods of TMi treatment (1.01×10^6 and 2.09×10^4 L^{-1} cells, respectively). However, there was no record on the final period of the experiment.

On the other hand, TBio treatment presented ciliates abundance of 9.04×10^3 cells·L^{-1} (initial time), 6.48×10^6 cells·L^{-1} (intermediate time) and 9.04×10^4 cells·L^{-1} (final time). Rotifers were abundant, especially in the initial and final experimental periods. In the initial period, TMi presented 4.33×10^4 individuals·L^{-1}, while TBio treatment presented 1.95×10^4 individuals·L^{-1}. At the final time of cultivation, there was greater abundance in TBio (4.28×10^4 individuals·L^{-1}), while no rotifers were observed in TMi. In the intermediate experimental period, the lowest abundance of rotifers was observed with 952 individuals·L^{-1} in TMi and no records in TBio.

Zootechnical performance

Table 3 presents the zootechnical performance parameters. Statistical analysis revealed no significant differences between

Table 2. Abundance values (cells, filaments, and individuals·L^{-1}) ± standard deviations of the main groups of microorganisms found in *Litopenaeus vannamei* cultivation grow out, over 120 days, for the biofloc and aquamimicry treatments.

Organisms	Biofloc	Aquamimicry
Chlorophytes (cells·L^{-1})	$3.80 \times 10^8 \pm 4.88 \times 10^8$	$1.17 \times 10^8 \pm 1.70 \times 10^8$
Diatoms (cells·L^{-1})	$4.99 \times 10^7 \pm 5.06 \times 10^7$	$4.27 \times 10^6 \pm 3.98 \times 10^6$
Cyanobacteria (filaments·L^{-1})	$2.23 \times 10^7 \pm 1.33 \times 10^7$	$2.78 \times 10^7 \pm 1.25 \times 10^7$
Flagellates (cells·L^{-1})	$3.54 \times 10^6 \pm 4.28 \times 10^6$	$5.91 \times 10^7 \pm 1.26 \times 10^8$
Ciliates (cells·L^{-1})	$1.34 \times 10^6 \pm 2.87 \times 10^6$	$2.11 \times 10^5 \pm 4.51 \times 10^5$
Rotifers (individuals·L^{-1})	$1.27 \times 10^4 \pm 1.87 \times 10^4$	$9.23 \times 10^3 \pm 1.90 \times 10^4$

Table 3. Mean (± standard deviation) of zootechnical performance parameters in ponds with intensive culture of white shrimp *Litopenaeus vannamei* for two treatments: biofloc and aquamimicry*.

Parameters / Treatments	Biofloc	Aquamimicry
Initial weight (g)	1.44 ± 1.25	1.44 ± 1.25
Final weight (g)	11.48 ± 1.50	11.73 ± 2.21
Weekly weight gain (g)	0.63 ± 0.09	0.64 ± 0.13
Survival (%)	63.3 ± 8.16	53.3 ± 15.2
Productivity (ton ha^{-1})	4.08 ± 0.11^a	3.56 ± 0.15^b
Feed conversion rate (FCR)	1.70 ± 0.05	1.75 ± 0.07

*Different letters in the same row denote significant statistical differences ($p < 0.05$).

treatments in terms of final shrimp weights at the end of the 120-day experimental period. The weekly weight gain was similar in both treatments, and there were no significant differences observed in shrimp survival rates between the treatments. However, the lowest survival rate (40%) occurred in a pond of the aquamimicry system, while in the TBio system the lowest rate was 50%. The productivity values were significantly different between the treatments, being this difference above 500 $kg \cdot ha^{-1}$ of the shrimps of the TBio treatment and those of the TMI treatment and the TBio.

DISCUSSION

Throughout the experimental period, the TBio and TMi treatments exhibited similar temperature levels, with no significant differences observed. However, it is important to notice that the recorded temperatures remained below the optimal range for shrimp growth (Ponce-Palafox et al., 1997). Also, temperature fluctuations reached down to 10 °C during the daytime. We suggest that lower temperatures may have reflected in low growth of shrimps (Souza et al., 2016).

Previous studies carried out in the same experimental units had reported those kinds of high fluctuations in temperature during daytime (Zemor et al., 2019; Poersch et al. 2021). That decreasing temperature even in the summer across the subtropical region, as at the extreme south of Brazil, tends to happen due to the passage of meteorological cold fronts associated with southern winds (Möller et al., 2001). Regarding salinity, the reduction in ponds over the experimental period was due to cyanobacterial blooming, using a centrifugal pump using well water, which had low salinity (around 8.0). Despite the salinity levels being outside the ideal range, the productive performance of *L. vannamei* was not affected. This species is tolerant of a wide salinity range, from 1.0 to 50 ppt, and it is often used as a model species to investigate mechanisms of osmoregulation and salt tolerance (Jaffer et al., 2020).

Dissolved oxygen concentrations had a slightly decreasing pattern, which was expected, as it is related both to the respiration of the shrimp and the biota present in the ponds and to the nutrient remineralization process. It is worth noting that the concentrations remained within adequate levels for survival and growth (Van Wyk and Scarpa, 1999). Also related to the metabolism of the microbiota (phytoplankton), the average pH values ranged from 8.0 to 8.5. This pH range may be particularly related to cyanobacterial blooms that were intense. Even so, this pH range is considered to be an optimal range for shrimp growth (Van Wyk and Scarpa 1999).

Particularly, the transparency increase noticeable in TMi might also be related to the passage of cold front, because this should have caused temperature decrease and extended cloud cover. Both parameters could lead to phytoplankton crashes (Chen et al., 2015). Other explanation for this pattern in transparency would be the water renewal made to impede the overgrowth of cyanobacteria in the TMi treatment. Contrarily, TBio did not show such transparency increase pattern, that was probably due to the use of sugarcane molasses, which is naturally dark. At the same time, the flake composition was more prone to growth of heterotrophic organisms, i.e., without the need for light incidence. Coupled with the increase in transparency, there was a decrease in turbidity in the TMi, which usually is inversely related to transparency. Despite this relationship between transparency and turbidity, a temperature increase immediately after the passage of cold front was accompanied by an increase in turbidity. We suggest that the establishment of transparency increase, and relatively higher temperature might have promoted the growth of photoautotrophic organisms, as observed by Gaona et al. (2016), whose greater abundance had contributed to the increase in turbidity.

Overall, the levels of alkalinity and TSS were maintained within appropriate ranges (Schveitzer et al., 2013). Alkalinity (around 200 mg $CaCO_3 \cdot L^{-1}$) showed any significant difference between treatments. This level in alkalinity was also associated with the pumping of underground water (250 mg $CaCO_3 \cdot L^{-1}$). For instance, Furtado et al. (2014) suggested that alkalinity should be kept close to the ideal concentration of 150 mg $CaCO_3 \cdot L^{-1}$, which benefits the formation of bioflocs, as well as the establishment of nitrifying bacteria. It was noteworthy a trend in TSS increase for both TBio and TMi treatments. This trend was expected for the TBio; there would be an ideal condition for increased concentrations in bioflocs and TSS within the water column (Gaona et al., 2016). On the other hand, one reduction in TSS for the TBio treatment towards last weeks of the experimental period was due to water renewals. These water renewals were needed for controlling TSS concentration and maintaining the health condition of the shrimps.

Concerning the nutrient concentrations, all nitrogenous forms were measured at low levels in general. At some experimental time periods and ponds, for instance, ammonia concentration reached 3.25 $mg \cdot L^{-1}$ (see Fig. 6). This may be associated with some mortality of shrimp, as well as the decomposition of food offered, but not consumed by shrimps. We suggest even that high cyanobacterial abundance and biomass might have caused partly that shrimp mortality. Also, a passage of cold front may have implicated in reduced numbers of nitrifying bacteria and, therefore, in lowering nitrification process that tended to convert ammonia into less toxic nitrogenous forms.

Similarly, there were measured some increases in nitrite concentration mainly in TBio treatments, whose concentration could be related to the imbalance of numbers of bacteria that oxidized nitrite to nitrate. More specifically, those latter kinds of bacteria should attain slower growth than ammonia oxidizing bacteria, leading to an elevation in nitrite concentration in shrimp culture (Hagopian and Riley, 1998). Nitrate, in turn, showed no difference between the two treatments. Even considering a tendency for nitrate accumulation in all treatments, this nutrient had mean values of 1.52 ± 1.00 $mg \cdot L^{-1}$ for TBio and 2.82 ± 2.21 $mg \cdot L^{-1}$ for TMi. These nitrate levels would be due to the ammonia conversion into its fewer toxic forms by the action of nitrifying bacteria. Furthermore, nitrate must have been assimilated into phytoplankton biomass that kept nitrate levels under some control.

Traditional cultivation systems use water renewal as a way to reduce the concentrations of nitrogenous compounds and phytoplankton concentrations, which, if too high, can cause depletion of dissolved oxygen in the grow-out ponds. In semi-intensive systems, the water renewal rate is around 5% per day, while in intensive systems it can be increased to 10% per day (Boyd et al., 2007). However, the biofloc system offers a more sustainable alternative to these conventional methods. With its minimal water exchange requirements and reduced feed intake, it has emerged as an economical and environmentally friendly technology for advancing aquaculture (De Schryver et al., 2008). Conventional aquaculture systems that depend on regular water changes generally require a high volume of up to 80 m^3 per kilogram of shrimp produced. In contrast, intensive shrimp farming systems with zero water exchange techniques can operate with significantly lower water volumes, ranging from only 1 to 2.26 m^3 per kg of shrimp (Hargreaves, 2006).

In the context of intensive systems, the concept of aquamimicry can also be implemented, offering more benefits and opportunities for sustainable shrimp farming. In this scenario, a centralized drainage system is established to transfer water from the fattening tank to the settling tank. The effluent collected in this drainage system is normally pumped (Vijayan, 2019). During the experiment, blooms of cyanobacteria *Nodularia spumigena* occurred in several experimental units, mainly in the TMi treatment ponds. To combat this problem, water renovations were carried out. Even so, with intensive stocking density, these renewals were lower than those used in semi-intensive ponds.

Microorganisms present in a shrimp farming system generally determine several aspects, including the growth, survival, and productivity of reared species. In relation to phytoplankton, chlorophytes reached high concentrations from the middle experimentation time onwards in both treatments. This abundance of chlorophytes can be considered normal, since light incidence and nutrient availability tended to promote the development of any kind of phytoplankton group, including other diatoms and cyanobacteria (Pereira Neto et al., 2008). For instance, diatoms were abundant in the TBio treatment throughout the whole study period. In nursery, diatoms often predominate under conditions of high concentration of suspended organic matter, which represent very well the conditions in intensive shrimp farming systems.

In contrast, cyanobacteria were observed in all ponds, with a higher prevalence in the TMi treatment during the intermediate phase of the study. It is important to notice that certain cyanobacteria species can produce toxins that have adverse effects on shrimp physiology and may lead to increased mortality rates (Gonçalves-Soares et al., 2012). Ambient with temperatures >20 °C, wide pH range (6 to 9), variable salinity (from 7 to 30), and high light intensity altogether tended to favor the cyanobacteria growth and biomass in cropping systems (Silva, 2005).

Nodularia spumigena was very noticeable cyanobacteria species during the study period. This species produces the nodularin toxin, a potent hepatotoxin that can be bioaccumulated and cause deleterious effects including mortality (Kankaanpaa et al., 2002). We can consider that the conditions found in the ponds were also favorable for the development of that cyanobacteria species as well to other phytoplankton taxa. Probably, *N. spumigena* was less affected by turbidity level and high ammonia concentration. It is well known that an excessive increase in *N. spumigena* could affect zooplankton production and, consequently, shrimp production (Ger et al., 2016).

Moreover, *N. spumigena* produces allelochemicals, which can inhibit competing algae and invertebrate herbivores. Given that, zooplankton would tend to avoid cyanobacteria as a food source (Berry et al., 2008), and rather than they would feed on algae (diatoms, chlorophytes) that competed with those cyanobacteria. In this trophic interaction, zooplankton groups (ciliates, rotifers) would release remineralized nutrients, further favoring the growth of cyanobacteria and causing changes in the structure and dynamics of plankton community. Also, this zooplankton abundance should be preyed by *L. vannamei* individuals, which should release nutrients that stimulated the growth of cyanobacteria, as positive feedback. Due to their ability to form visually prominent blooms and outcompete other photosynthetic organisms, cyanobacteria had a significant impact on the overall structure of the pelagic/crop ecosystem. In this way, we could expect some long-term effects on grazing pressure exerted by zooplankton if these cyanobacterial blooms lasted for a longer period. These changes may have reflected in a decrease in top-down control by zooplankton (ciliates and rotifers over flagellates and phytoplankton, at least) in the experimental ponds, highlighting the importance of the top-down as well bottom-up effects on the cyanobacteria blooms (Karjalainen et al., 2007).

Shrimp growth was monitored weekly in the experimental units, and no significant differences were observed between TBio and TMi in relation to the average final weight. Some studies have shown that symbiotic systems, such as the aquamimicry system used here, should allow for better feed conversion and, consequently, for an increase in growth when compared to conventional systems. Whether growing shrimp at low density (Hai and Fotedar, 2009), in high density within a symbiotic system (Oktaviana et al., 2014), both have already shown good zootechnical performance. Hussain et al. (2021), comparing BFT system and symbiotic type system and using densities of 125 shrimp·m^2, had similar growths and high survival.

Our TBio and TMi indeed showed similar growth, albeit low growth rates mainly for the former treatment. This poor zootechnical performance may be related to adverse temperature conditions throughout the study period, as well as to the presence of cyanobacterial blooms (particularly *N. spumigena*). Costa et al. (2018), performing an experiment in the same facilities and with an average temperature of 24.2 °C, but with a higher density (125 m^{-2}), obtained similar growth values. It was notable that FCR we estimated for both treatments were higher than the expected ones (Hai and Fotedar, 2009). Our FCR should reflect some mortality not visually observed in the routines, with feed offered but not fully consumed, as well as they should imply an eventual mortality rate caused by exposure to Cyanobacteria, which was often in the TBio and TMi treatments.

Regarding shrimp survival, no significant differences were found ($p < 0.05$): 63.3 ± 8.16% (TBio) and 53.3 ± 15.2 % (TMi) in our study. Contrarily, average survival rate > 80% in nursery with BFT treatment had been considered as a good goal in intensive shrimp farming systems (Martinez-Porchas et al., 2020). Our lower shrimp survival rates might be associated with the presence of cyanobacterial blooms, especially within the TMi treatment during the intermediate experimental period. On the other hand, Tbio showed productivity of 4.08-ton·ha^{-1}, whereas TMi attained 3.56-ton·ha^{-1}. This significant difference ($p<0.05$) between the treatments should be a direct reflex of survival rates obtained, being also linked to the cyanobacterial blooms. As these blooms were more intense in the TMi ponds, we suggest that their lower survival and yield rates corroborate the susceptibility of *L. vannamei* cultivation during cyanobacteria blooms in southern Brazil.

CONCLUSIONS

The present study demonstrated that TMi ponds displayed the same overall performance as the TBio ponds. The water quality could be efficiently controlled in both treatments, demonstrating that the TMi system can be capable of nutrient cycling efficiently. Zootechnical performance was, in general, similar between treatments, and differences in survival and productivity rates were associated with high concentrations of cyanobacteria, mainly in the TMi treatment ponds. Water changes had a positive impact on the reduction of blooms, although more studies are needed to prevent their appearance or reduce the negative impacts related to them. Even performing water exchanges, the systems showed efficiency in nutrient cycling, even at higher stocking densities, as renewal were lower than normally performed in traditional systems.

With the refinement and further validation studies of commercial protocols, it is expected that productivity and survival rates will be improved and the symbiotic system (aquamimicry) can provide better results as efficient as the bioflocs in water utilization and shrimp performance. The present study demonstrated that TMi treatment ponds presented zootechnical performances close to those of TBio treatment ponds. The results achieved may contribute to the improvement of this cultivation system for higher stocking densities.

CONFLICT OF INTERESTS

Nothing to declare.

AUTHORS' CONTRIBUTIONS

Conceptualization: Wasielesky, W, Fóes, G; **Investigation:** Catalani K, Zuñiga R, Sousa M S; **Data Curation:** Catalani K; **Formal Analysis:** Catalani K, Zuñiga R; Sousa M S; **Funding Acquisition:** Wasielesky W; **Validation:** Wasielesky W, Fóes G; **Supervision:** Wasielesky W, Fóes G; **Writing — Original Draft:** Catalani K; **Writing — Review & Edition:** Catalani K

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

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