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Influence of the frequency of addition of *Navicula* sp. in a biofloc system on the growth of *Penaeus vannamei* post-larvae and the planktonic community

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Abstract

Aim of study: The objective of this study was to evaluate the effect of different addition frequencies of the microalga *Navicula* sp. in a biofloc system on the growth performance of *Penaeus vannamei* post-larvae and the planktonic community.

Area of study: Laboratory of Sustainable Mariculture, Department of Fisheries and Aquaculture, Federal Rural University of Pernambuco, Brazil.

Material and Methods: Four treatments were tested in triplicate over 35 days: a control treatment (BFT - without *Navicula* sp.) and three treatments with *Navicula* sp. additions every 5, 10, and 15 days (BFT 5D, BFT 10D, and BFT 15D). The microalga was added at a density of 10×10^4 cells mL^{-1}, and *P. vannamei* post-larvae (PL10, ~5 mg) were stocked at 3,000 PL m^{-3}.

Main Results: The frequency of *Navicula* sp. addition did not significantly affect water quality parameters, except for settleable solids. Shrimp performance improved in treatments with more frequent additions (BFT 5D and BFT 10D), which showed higher mean final weights (0.44 ± 0.02 g and 0.51 ± 0.01 g) and yields (1.22 ± 0.05 kg m^{-3} and 1.41 ± 0.02 kg m^{-3}) compared to the control. Although the treatments did not significantly affect the composition of the planktonic community, temporal variations were observed, with Cyanophyta reaching up to 95.2% relative abundance. Within the Cyanophyta and protozooplankton groups, *Aphanocapsa* sp. and *Paramecium* sp. were the predominant taxa, respectively.

Research highlights: Adding *Navicula* sp. every 5 or 10 days enhances the growth performance of *Penaeus vannamei* in biofloc systems. Over time, the planktonic composition changed, with a dominance of Cyanophyta. Considering the costs associated with microalgae production, adding *Navicula* sp. every 10 days is recommended for shrimp nurseries in biofloc systems.

Keywords: Diatoms; live feed; microalgae; sustainability.

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La influencia de la frecuencia de adición de *Navicula* sp. en un sistema de biofloc sobre el crecimiento de postlarvas de *Penaeus vannamei* y la comunidad planctónica

Resumen

Objetivo del estudio: El objetivo de este estudio fue evaluar el efecto de diferentes frecuencias de adición de la microalga *Navicula* sp. en un sistema de bioflóculos sobre el rendimiento de crecimiento de las postlarvas de *Penaeus vannamei* y la comunidad planctónica.

Área de estudio: Laboratorio de Maricultura Sostenible, Departamento de Pesca y Acuicultura, Universidad Federal Rural de Pernambuco, Brasil.

Material y Métodos: Se evaluaron cuatro tratamientos por triplicado durante 35 días: un tratamiento control (BFT - sin *Navicula* sp.) y tres tratamientos con adiciones de *Navicula* sp. cada 5, 10 y 15 días (BFT 5D, BFT 10D y BFT 15D). La microalga se agregó a una densidad de 10×10^4 células mL^{-1}, y las postlarvas de *Penaeus vannamei* (PL10, ~5 mg) se cultivaron a una densidad de 3.000 PL m^{-3}.

Resultados principales: La frecuencia de adición de *Navicula* sp. no afectó significativamente a los parámetros de calidad del agua, excepto los sólidos sedimentables. El desempeño del camarón mejoró en los tratamientos con adiciones más frecuentes (BFT 5D y BFT 10D), los cuales presentaron mayores pesos finales medios (0,44 ± 0,02 g y 0,51 ± 0,01 g) y rendimientos (1,22 ± 0,05 kg m^{-3} y 1,41 ± 0,02 kg m^{-3}) en comparación con el control. Aunque los tratamientos no afectaron significativamente la composición de la comunidad planctónica, se observaron variaciones temporales, con una abundancia relativa de Cianofitas de hasta el 95,2%. Dentro de los grupos de Cianofitas y protozooplancton, *Aphanocapsa* sp. y *Paramecium* sp. fueron los taxones predominantes, respectivamente.

Conclusiones: La adición de *Navicula* sp. cada 5 o 10 días mejora el rendimiento de crecimiento de *Penaeus vannamei* en sistemas de bioflóculos. Con el tiempo, la composición planctónica cambió, presentando un dominio de Cyanophyta. Considerando los costos asociados a la producción de microalgas, se recomienda la adición de *Navicula* sp. cada 10 días en criaderos de camarón en sistemas de bioflóculos.

Palabras clave: Diatomeas; alimento vivo; microalgas; sostenibilidad

Introduction

Biofloc technology (BFT) is a culture system that enhances water quality management by assimilating toxic nitrogen compounds through the addition of organic carbon (Avnimelech, 2015; Samocha, 2019). The use of this system provides increased biosecurity due to greater environmental control, higher productivity, and reduced need for large areas for production (Avnimelech, 2015; Samocha et al., 2017).

The biofloc is composed of bacteria, protozoa, microalgae, nematodes, zooplankton, as well as food remains and feces of cultivated animals (Hargreaves, 2013; Samocha et al., 2017). According to Cardona et al. (2015), the bioflocs formed in the system can serve as a food supplement and contribute with approximately 37 to 40% of the food supply (food source) in shrimp culture. In addition, they stimulate the activities of digestive enzymes, which can improve the growth of shrimp (Anand et al., 2013).

Previous studies indicate that shrimp growth in mixed biofloc systems, where autotrophic algae and heterotrophic bacteria coexist, is higher compared to systems dominated solely by bacteria (Marinho et al., 2014; Brito et al., 2016; Xu et al., 2016; Marinho et al., 2017; Abreu et al., 2019). Nutritionally, microalgae have a lipid content varying between 10 to 70% of dry weight. The lipid fraction of microalgae can be rich in long-chain polyunsaturated fatty acids, such as docosahexaenoic (DHA) and eicosapentaenoic acids (EPA) that may exceed 26% of total fatty acid content (Cuellar-Bermudez et al., 2015; Adarme-Vega et al., 2012; Maltsev & Maltseva, 2021; Oliveira et al., 2022). Among the groups of microalgae, diatoms stand out for having a high production of lipids when compared to other groups (Alexander et al., 2015). The presence of these microalgae in the microbial floc could improve the floc nutritional quality by minimizing fatty acids (EPA and DHA) deficiency (Ekasari et al., 2014; Shah et al., 2018; Magaña-Gallegos et al., 2018; Abreu et al., 2019).

The microalgae addition has already shown good results on improving zootechnical of *Penaeus vannamei* performance and reducing pathogenic bacteria (e.g., *Vibrio* spp.) both on laboratory and commercial scale (Molina-Cardenas and Sanchez-Saavedra, 2017; Emerenciano et al., 2022; Oliveira et al., 2023). However, factors such as nutritional quality and/or growth characteristics must be carefully analysed in microalgae species selection for better performance in aquaculture (Shah et al., 2018).

The microalga *Navicula* sp. is a benthic diatom widely used in aquaculture. Khatoon et al. (2009) noted that, when cultivated in Conway medium, the species showed high levels of crude protein, lipids and carbohydrates. This microalga, when added

to the nursery in BFT system, contributed to the shrimp performance and to the composition of polyunsaturated fatty acids in the microbial floc (Brito et al., 2016; Abreu et al. 2019; Silva et al. 2021). In fact, Abreu et al. (2019) found that the addition of *Navicula* sp. (at 10^5 cells mL^{-1}) during 45 days of culture provided a higher productivity (2.42 Kg m^{-3}) and an enrichment of EPA (20:5 n-3) and DHA (22:6 n-3) in shrimp whole body.

In intensive systems characterized by high stocking densities, excessive total suspended solids, and dissolved nutrient accumulation (notably nitrogen and phosphorus), conditions become favorable for the growth of potentially harmful microalgae, especially cyanobacteria and dinoflagellates (Marinho et al., 2014; Samocha, 2019). These algae can harm the water quality and farmed animals, as they are able to form foam, produce toxins and cause oxygen depletion in the growing environment (Anderson et al., 1993; Chorus and Bartram, 1999; Paerl and Huisman, 2009: Guzman-Guillen et al., 2013; Ye et al., 2009, Qiao et al., 2013).

Despite the benefits of *Navicula* sp. addition in the shrimp culture, the best frequency of this diatom addition is still unknown. Given the above, the objective of the present study was to evaluate the best frequency of addition of *Navicula* sp. to *P. vannamei* reared in a nursery biofloc system. Furthermore, this study sought to evaluate the ecological influence of *Navicula* sp. addition at different frequencies in the planktonic community.

Material and methods

Experimental conditions

The experiment was conducted at the Laboratory of Sustainable Mariculture (LAMARSU) of the Department of Fisheries and Aquaculture (DEPAq) of the Federal Rural University of Pernambuco (UFRPE), Recife, Brazil.

The maturation of the biofloc used as initial inoculum was performed 40 days before the beginning of the experiment. Maturation tank (1.4 m³ capacity) was filled with previously chlorinated seawater (35 g L^{-1}) (65% active chlorine) in the proportion of 13 mg L^{-1} active chlorine. After three days under constant aeration, inorganic fertilization with urea (2 g m^{-3} N), triple superphosphate (0.1 g m^{-3} P) and sodium metasilicate (3 g m^{-3} Si) was performed. Subsequently, organic fertilizations with sugarcane molasses and pulverized feed (40% crude protein) were performed to form bacterial biomass.

Before the beginning of the experiment, the water matured in the maturation tank (TAN 0.24 mg L^{-1}, N-NO_2 0.83 mg L^{-1}, N-NO_3 49.7 mg L^{-1}, alkalinity 165 mg L^{-1} $CaCO_3$, pH 8.14, salinity 32 g L^{-1}, PO_4 9.2 mg L^{-1} and settleable solids 4.63 mL L^{-1}) was homogenized and used to supply the experimental units up to 50% of the volume (25 L). The remaining volume (25 L) was filled with seawater (salinity of 35 g L^{-1}) previously chlorinated. Twelve black polyethylene tanks (60 L, 50×35×23 cm) were used as cultivation units, which were constantly aerated by three airstones (2.4 cm in diameter and 2.6 cm in height). To prevent the shrimp from escaping, all experimental units were covered with Nylon screens.

Molasses (30% organic carbon) was added once a day as a carbohydrate source to maintain the C:N ratio at 12:1, according to Ebeling et al. (2006). In addition, calcium hydroxide (Ca $(OH)_2$) (81% Relative Total Neutralizing Power PRNT) was added to 10% (by weight) of the daily diet throughout the study to maintain alkalinity > 100 mg L^{-1} and pH > 7.5. During the experimental period, no water changes were performed, except for the addition of dechlorinated fresh water to compensate for evaporation losses.

To evaluate the effect of the frequency of addition of *Navicula* sp. in the biofloc nursery system, four treatments were tested in a completely randomized design for 35 days. The control treatment remained without the addition of *Navicula* sp. (BFT), while the other three treatments were supplemented with the addition of microalgae every 5 (BFT 5D), 10 (BFT 10D) and 15 days (BFT 15D), with three replicates for each treatment. Post-larvae (PL10) of *P. vannamei* (5 ± 0.03 mg) were obtained from Aquatec LTDA, RN, Brazil and stocked at a density of 3,000 post-larvae m^{-3} (150 shrimp per experimental unit).

Navicula sp. addition

Navicula sp. was obtained from the Live Food Production Laboratory (LAPAVI) and cultured in Conway medium (1.0 mL L^{-1}; Walne, 1966), with salinity of 30 g L^{-1}, pH 8.0, temperature of 25 ± 1 °C, and 37 µmol photons m^{-2} s^{-1} irradiance under 12 h of photoperiod. The microalgae were grown in a 2 L Erlenmeyer flask with constant aeration, and inoculation was done in the exponential phase of the growth curve. The microalgae *Navicula* sp. was added at a concentration of 10^5 cells mL^{-1} in the BFT 5D, BFT 10D and BFT 15D treatments according to the frequency for each treatment.

Water quality

Water quality parameters including temperature (°C), salinity (g L^{-1}), dissolved oxygen (mg L^{-1}) and pH were measured twice a day with the aid of a multiparameter (YSI model 556, Yellow Springs, Ohio, USA). Total ammonia nitrogen (TAN) (APHA, 2012), nitrite-N (NO_2-N) (Fries, 1971) and alkalinity (APHA, 2012) were weekly analysed, while orthophosphate (PO_4) (APHA, 2012) and nitrate-N (NO_3-N) (APHA, 2012) were measured every other week. Settleable solids were analyzed

with the aid of an Imhoff cone (Avnimelech, 2015), twice a week, when levels were greater than 14 mL L^{-1} a settling chamber was used.

Storage, feeding and monitoring of shrimp

During the experiment, the shrimp were fed commercial feed (0.4-1 mm in diameter) composed of 45% crude protein and 8% lipids (Invivo Animal Nutrition and Health), at a feeding frequency of four times per day (8 am, 11 am, 2 pm and 5 pm). The daily feeding rate of 45% of the biomass used at the beginning of the culture was gradually reduced to 15% after 35 days, based on the table of Van Wyk (1999) and adjusted weekly according to the estimated shrimp consumption and the rate of mortality.

Weekly, from the 15th day of culture, biometrics were performed to monitor shrimp growth and to determine the zootechnical performance at the end of the experiment including biomass gain [final biomass (g) - initial biomass (g)]; average final weight [final biomass (g) / number of individuals at the end of culture]; feed conversion ratio (FCR) [total feed offered / biomass gain]; Survival rate (number of individuals at the end of culture / initial number of individuals stocking x 100); SGR [specific growth rate (% day^{-1}) = 100 × (ln final weight (g) − ln initial weight (g))/time (days)]; and yield [(final biomass (kg) / volume of the experimental unit (m^3)].

Plankton composition and structure

Weekly, throughout the experimental period, water samples (500 mL) were collected from the experimental units and filtered through a 250, 125 and 70 µm cylindrical-conical mesh to reduce suspended solids in the sample. Then, the samples were filtered through a 50 µm mesh to retain zooplankton and a 15 µm mesh to retain cyanobacteria, and each sample was concentrated to 25 mL (CETESB, 2011). Subsequently, 2.5 mL aliquots were fixed in 4% formalin and stored in plastic containers for later analysis. For identification and quantification, a Sedgewick-Rafter chamber and a binocular optical microscope (400x magnification) were used. Identification at the genus level was done with the help of identification keys for phytoplankton (Hoek et al., 1995; Bicudo and Menezes, 2006) and zooplankton (Bradford-Grieve et al., 1999; Foissner et al., 1999). Phytoplankton was expressed in cells per millilitre (cells mL^{-1}) following the methodology described by Hötzel and Croome (1999), and zooplankton was expressed in individuals per millilitre (ind mL^{-1}) following the methodology described in APHA (2012). The percentage frequency of occurrence (F) (%) of the species was also calculated according to Rosini et al. (2013) methodology using the following formula: F = [number of sample in which the genus was registered / total collections performed] x 100. The species were considered very frequent ($F \geq 80\%$), frequent ($50\% \geq F > 80\%$), uncommon ($20\% \geq F > 50\%$) or rare ($F < 20\%$).

Statistical analysis

For statistical analysis, data were tested for homogeneity of variances using the Cochran test ($p < 0.05$) and normality using the Shapiro-Wilk test ($p < 0.05$). Then, analysis of variance (ANOVA) was used to analyse the shrimp performance parameters, and when significant differences were observed, the Tukey test was performed ($p < 0.05$) to compare and classify the means between the treatments. Water quality parameters (dissolved oxygen, pH, temperature, salinity, alkalinity and orthophosphate) were analysed by performing ANOVA for repeated measures, followed by Tukey's mean comparison test ($p \leq 0.05$) for normal and homogeneous data. For non-parametric statistical data (dissolved oxygen, TAN, nitrite-N and orthophosphate), Friedman's test ($p \leq 0.05$) with Conover's multiple comparison test with Holm-Bonferroni correction.

The phytoplankton and zooplankton communities were analysed using the PRIMER 6.0 program. Data were previously transformed [log (x +1)] and the Bray-Curtis index was used for similarity and multivariate cluster analysis (Cluster). A bi-factorial ANOSIM similarity analysis with replicates (Factors: Time x Treatments) was performed, with 999 permutations, to identify the difference between groups in relation to species abundance (Clarke, 1993). In addition, the percentage similarity (SIMPER) was used to identify the main typifying species of the groups.

A redundancy analysis (RDA) was performed to explore the relationship between water quality variables and phytoplankton composition. The data were log-transformed prior to analysis. The significance of RDA was evaluated through an ANOVA function with 999 permutations.

Results

The water quality data are summarized in Table 1. It was observed that dissolved oxygen, pH, temperature, salinity, alkalinity and orthophosphate did not differ significantly between treatments ($p \geq 0.05$), with an average variation of 4.07 to 4.54 mg L^{-1}, 7.81 to 8.18, 26.80 to 31.05 ºC, 29.57 to 36.12 g L^{-1}, 125 to 200 mg $CaCO_3$ L^{-1} and 0.22 to 4.10 mg L^{-1}, respectively. Nitrogen compounds as TAN, nitrite-N and nitrate-N also did not differ significantly between treatments ($p \geq 0.05$), with an average of 0.55 ± 0.03 mg L^{-1} (TAN), 0.97 ± 0.06 mg L^{-1} (nitrite-N) and 125.08 ± 76.51 mg L^{-1} (nitrite-N). For settleable solids, treatments with addition of *Navicula* sp. presented the significantly lower values, (BFT 5D: 2.64 mL L^{-1}, BFT 10D: 1.76 mL L^{-1} and BFT 15D: 1.80 mL L^{-1}) than control (7.12 mL L^{-1}).

Table 1. Water quality variables during cultivation (35 days) of post-larvae of *Penaeus vannamei* cultured in a biofloc system with different addition frequencies of *Navicula* sp.

Variables	Treaments			
	BFT	**BFT 5D**	**BFT 10D**	**BFT 15D**
Salinity (g L^{-1})	32.66 ± 0.94	33.97 ± 1.07	32.75 ± 0.78	32.74 ± 1.16
Temperature (ºC)	28.70 ± 0.86	28.79 ± 1.01	28.80 ± 1.14	28.61 ± 1.09
Dissolved oxygen (mg L^{-1})	4.36 ± 0.10	4.33 ± 0.11	4.73 ± 1.38	4.42 ± 0.11
pH	7.98 ± 0.11	7.98 ± 0.10	7.98 ± 0.11	7.98 ±0.09
TAN* (mg L^{-1})	0.47 ± 0.16	0.68 ± 0.20	0.53 ± 0.12	0.50 ± 0.10
Nitrite-N (mg L^{-1})	1.03 ± 0.11	0.87 ± 0.17	1.05 ± 0.28	0.94 ± 0.16
Nitrate-N (mg L^{-1})	118.51 ± 21.61	108.59 ± 26.59	142.12 ± 29.91	131.08 ± 26.38
Alkalinity (mg $CaCO_3$ L^{-1})	159.0 ± 17.95	157.33 ± 16.99	160.0 ± 10.52	160.33 ± 8.06
Orthophosphate (mg L^{-1})	3.74 ± 1.38	3.75 ± 1.36	3.45 ± 1.44	3.62 ± 1.40
Sedimentable solids (mL L^{-1})	7.12 ± 1.75^{b}	2.64 ± 0.62^{a}	1.76 ± 0.38^{a}	1.80 ± 0.39^{a}

The data correspond to the mean ± standard deviation; Mean values in the same row with different superscript letters differ significantly ($p \leq 0.05$); BFT (biofloc system without addition of *Navicula* sp.); BFT 5D (biofloc system with addition of *Navicula* sp. every 5 days); BFT 10D (biofloc system with addition of *Navicula* sp. every 10 days) and BFT 15D (biofloc system with addition of *Navicula* sp. every 15 days).

*TAN - Total ammonia nitrogen.

As for the shrimp performance, no significant differences ($p \geq 0.05$) were observed regarding survival rate and FCR. However, the average final weight, yield and specific growth rate in BFT 5D (0.44 g, 1.22 Kg m^{-3} and 12.82 % day^{-1}, respectively) and BFT 10D (0.51 g, 1.41 Kg m^{-3} and 13.17 % day, respectively) were significantly higher than those in the control treatment (without *Navicula* sp. addition) (0.33 g, 0.92 Kg m^{-3} and 12.14 % day-1) (Table 2).

Table 2. Zootechnical performance of *Penaeus vannamei* reared for 35 days in a biofloc system with different addition frequencies of the microalga *Navicula* sp.

Parameters	BFT	BFT-5D	BFT-10D	BFT-15D
Final weight (g)	0.33 ± 0.04^{b}	0.44 ± 0.02^{a}	0.51 ± 0.00^{a}	0.40 ± 0.01^{ab}
Survival (%)	92.00 ± 1.15^{a}	93.33 ± 0.38^{a}	92.83 ± 0.44^{a}	92.83 ± 0.44^{a}
Feed conversion ratio	1.57 ± 0.07^{a}	1.33 ± 0.18^{a}	1.13 ± 0.12^{a}	1.40 ± 0.15^{a}
SGR* (% dia^{-1})	12.14 ± 0.27^{a}	12.82 ± 0.10^{a}	13.17 ± 0.02^{a}	12.62 ± 0.07^{a}
Yield (Kg m^{-3})	0.92 ± 0.12^{a}	1.22 ± 0.05^{a}	1.41 ± 0.01^{a}	1.12 ± 0.03^{b}

The data correspond to the mean ± standard deviation. Mean values in the same row with different superscript letters differ significantly ($p < 0.05$). BFT (biofloc system without addition of *Navicula* sp.); BFT 5D (biofloc system with addition of *Navicula* sp. every 5 days); BFT 10D (biofloc system with addition of *Navicula* sp. every 10 days) and BFT 15D (biofloc system with addition of *Navicula* sp. every 15 days).

*SGR- specific growth rate.

Regarding the relative abundance and density of the phytoplankton community, there were no significant differences between treatments ($p \geq 0.05$), except the last week, in which treatments BFT-5 and BFT-10 showed lower phytoplankton density (Fig. 1). It was observed that from the third week onwards there was an increase in the total phytoplanktonic density, starting from 45,629 cells mL^{-1} and reaching 99,526 cells mL^{-1}. There was a dominance of Cyanophyta, especially *Aphanocapsa* sp. (from the beginning of culture time), with abundance ranging from 1,304 to 103,775 cells mL^{-1} and relative abundance between 30.8% (beginning) and 94.7% (35th day) (Fig. 2). A total of 6 genera in the initial period and 7 genera in the final period were identified, distributed in the groups Heterokontophyta (*Cyclotella* sp. and *Diatoma* sp.), Chlorophyta (*Closteriopsis* sp., *Mychonastes* sp., *Chlamydomonas* sp.), Cyanophyta (*Aphanocapsa* sp., *Oscillatoria* sp. and *Geitlerinema* sp.) and Dinophyta (*Pyrophacus* sp.).

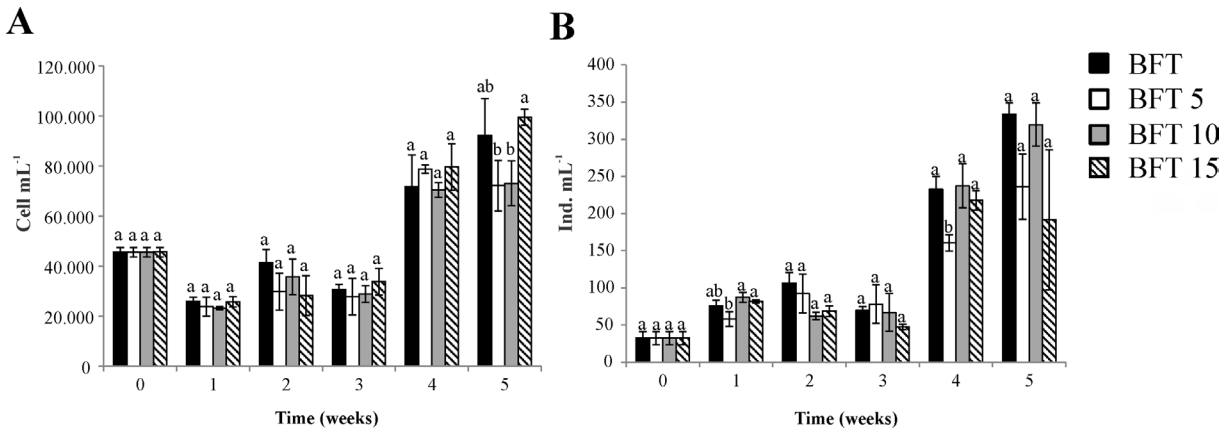
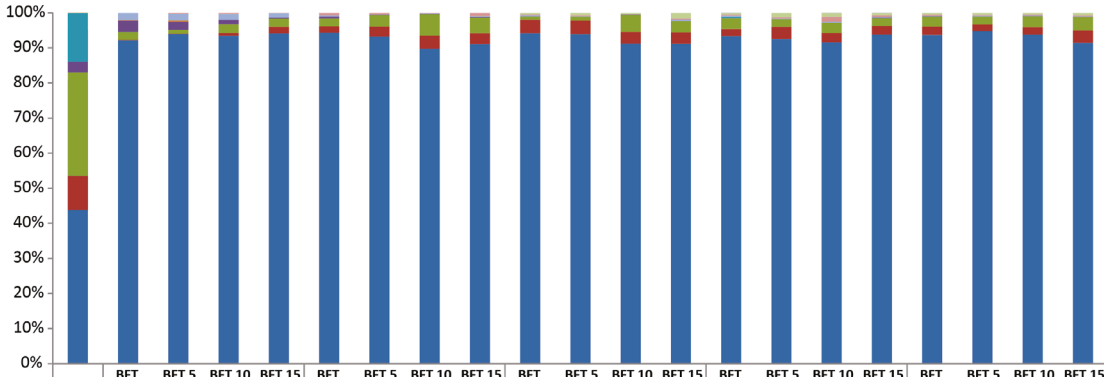


Figure 1. Density of phytoplankton (A) and protozooplankton (B) during the nursery of *Penaeus vannamei* in a biofloc system with the addition of *Navicula* sp. for 35 days.
BFT (biofloc system without addition of *Navicula* sp.); BFT 5D (biofloc system with addition of *Navicula* sp. every 5 days); BFT 10D (biofloc system with addition of *Navicula* sp. every 10 days) and BFT 15D (biofloc system with addition of *Navicula* sp. every 15 days).

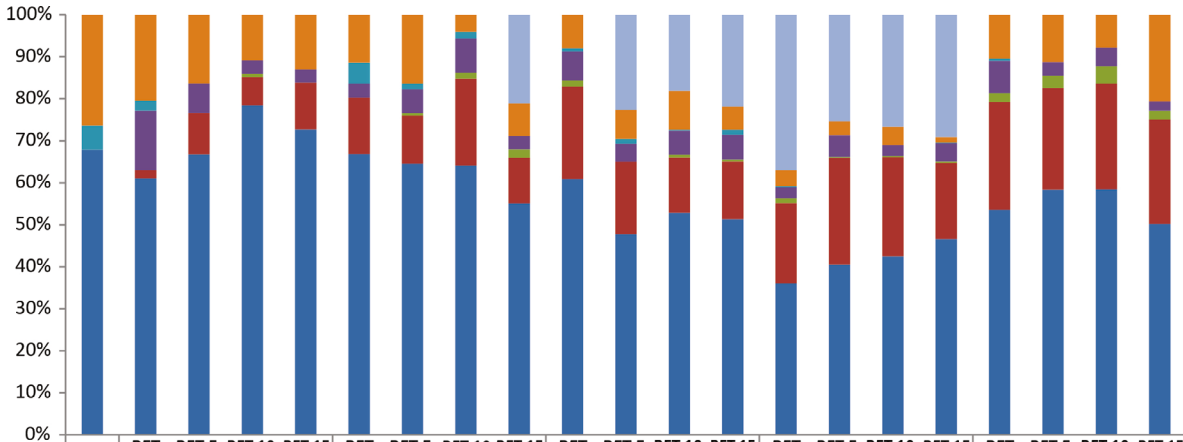
Similarity analysis (ANOSIM) did not show significant differences in the phytoplankton community between treatments (overall R = 0.021, p = 0.36), but it differed over the weeks of culture (overall R = 0.768, p = 0.01), with the initial samples presenting a complete difference from the other weeks (global R = 1, p < 0.01). This difference was also confirmed by the grouping analysis (Cluster), in which the formation of two groups at a cutoff of 70% of similarity was observed. Regarding the frequency of occurrence of taxa, only *Anabaenopsis* sp., *Diatoma* sp. and *Mychonastes* sp. were infrequent (present only in one experimental period), while the others were classified as frequent or very frequent.

In the protozooplanktonic community, only 3 genera were identified at the beginning (*Diffugia lobostoma*, *Euglypha rotunda*, *Paramecium* sp.) and 6 genera at the end (*Hartmannellidae* sp., *Didinium nasutum*, *Frontonia* sp., *Euglypha rotunda*, *Diffugia lobostoma*, *Paramecium* sp.), without any significant difference between treatments over the experimental period (p = 0.23; Fig. 2. The total protozooplankton density ranged from 32.6 ind. mL^{-1} to 633.7 ind. mL^{-1}, and, according to SIMPER, *Paramecium* sp. was the most frequent gender and with the greatest contribution in all treatments, with values from 44.8% to 78.7% (Fig. 3). The similarity analysis (ANOSIM) showed significant differences only between the weeks of cultivation (overall R = 0.730, p = 0.01), with the initial samples differing from the other weeks (overall R between 0.928 and 1, p < 0.01). For frequency of occurrence of taxa, all were present during most of the experimental period, being classified as frequent or very frequent, except for *Difflugia* sp., which was found only in the period between 15 -35 days (infrequent).



	Initial	BFT	BFT 5	BFT 10	BFT 15	BFT	BFT 5	BFT 10	BFT 15	BFT	BFT 5	BFT 10	BFT 15	BFT	BFT 5	BFT 10	BFT 15	BFT	BFT 5	BFT 10	BFT 15	Frequency
		1st week				2nd week				3rd week				4th week				5th week				
Anabaenopsis sp. (Cyanophyceae)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.8	0.0	2.3	2.1	0.0	0.0	0.0	0.0	R - 9.5%
Chlamydomonas sp. (Chlorophyceae)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	143.8	188.8	117.0	486.4	364.0	931.9	875.1	543.4	410.2	485.0	427.0	667.1	F - 57.1%
Geitlerinema sp. (Cyanophyceae)	0.0	0.0	51.2	74.1	14.5	232.7	163.7	42.9	292.2	75.2	45.0	36.9	73.6	212.6	285.3	1.1 x 10^3	336.1	311.9	207.1	216.8	161.1	VF - 90.5%
Oscillatoria sp. (Cyanophyceae)	0.0	527.2	410.7	386.4	380.9	68.8	23.1	35.3	83.3	87.3	40.9	16.4	125.6	287.9	131.1	161.8	154.4	74.3	160.7	108.3	99.5	VF - 95.2%
Diatoma sp. (Bacillariophyceae)	9.9	37.4	78.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	R - 14.3%
Mychonastes sp. (Chlorophyceae)	2.2 x 10^3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	R - 9.5%
Pyrophacus sp. (Dinophyceae)	443.7	842.6	479.5	290.6	81.9	172.4	46.4	55.2	66.0	7.6	2.3	25.8	2.3	231.3	8.5	15.0	103.2	90.9	4.6	18.9	66.5	VF - 100%
Cyclotella sp. (Bacillariophyceae)	4.6 x 10^3	582.2	243.3	576.9	656.3	647.5	1.1 x 10^3	2.3 x 10^3	1445.7	289.9	288.1	1.7 x 10^3	954.99	2.2 x 10^3	1.6 x 10^3	2.3 x 10^3	1.6 x 10^3	2.3 x 10^3	1.9 x 10^3	2.7 x 10^3	3.5 x 10^3	VF - 100%
Closteriopsis sp. (Chlorophyceae)	1.4 x 10^3	45.2	17.5	172.6	535.8	532.8	1.0 x 10^3	1.4 x 10^3	1.0 x 10^3	1.2 x 10^3	988.2	1187.67	968.4	1535.3	2607.3	2018.7	1.9 x 10^3	1.9 x 10^3	1.6 x 10^3	1.9 x 10^3	3.1 x 10^3	VF - 100%
Aphanocapsa sp. (Cyanophyceae)	3.6 x 10^3	2.4 x 10^4	2.0 x 10^4	2.2 x 10^4	2.7 x 10^4	2.8 x 10^4	3.3 x 10^4	3.4 x 10^4	2.9 x 10^4	2.9 x 10^4	2.4 x 10^4	3.2 x 10^4	2.7 x 10^4	6.7 x10^4	6.9 x 10^4	7.1 x 10^4	7.0 x 10^4	7.6 x 10^4	7.7 x 10^4	7.9 x 10^4	82 x 10^4	VF - 100%

Figure 2. Phytoplankton community relative abundance (%) and density (Cell mL^{-1}) in the nursery of *Penaeus vannamei* production with the addition of *Navicula* sp. in a biofloc system. BFT (biofloc system without addition of *Navicula* sp.); BFT 5D (biofloc system with addition of *Navicula* sp. every 5 days); BFT 10D (biofloc system with addition of *Navicula* sp. every 10 days) and BFT 15D (biofloc system with addition of *Navicula* sp. every 15 days).



	Initial	BFT	BFT 5	BFT 10	BFT 15	BFT	BFT 5	BFT 10	BFT 15	BFT	BFT 5	BFT 10	BFT 15	BFT	BFT 5	BFT 10	BFT 15	BFT	BFT 5	BFT 10	BFT 15	Frequency
		1st week				2nd week				3rd week				4th week				5th week				
Difflugia sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.8	0.0	18.7	8.3	12.5	80.5	49.2	59.3	62.6	0.0	0.0	0.0	0.0	R - 38.1%
Diffugia lobostoma	8.5	16.5	10.6	9.2	9.5	8.1	10.4	3.1	9.0	6.2	5.6	4.2	3.1	8.2	6.3	9.8	2.8	32.6	33.6	14.3	59.9	VF - 100%
Euglypha rotunda	1.8	1.9	0.0	0.0	0.0	3.6	0.8	1,2	0.0	0.68	1.0	0.1	0.7	0.4	0.0	0.0	0.4	1.4	0.0	0.0	0.0	F - 71.4%
Frontonia sp.	0.0	11.4	4.6	2.8	2.3	2.3	3.6	6.4	3.7	5.4	3.5	2.6	3.4	5.8	10.0	5.8	9.3	24.1	9.4	8.2	6.5	VF - 95.2%
Didinium nasutum	0.0	0.0	0.0	0.7	0.0	0.0	0.3	1.1	2.4	1.1	0.0	0.3	0.2	2.5	0.5	0.7	0.9	6.4	8.9	7.6	6.0	F - 71.4%
Hartmannellidae sp.	0.0	1.5	6.4	5.7	8.1	9.6	7.2	16.2	12.8	17.1	14.2	6.0	7.8	41.4	49.1	52.7	38.9	79.7	71.9	45.9	72.1	VF - 95.2%
Paramecium sp.	21.9	49.2	43.5	66.7	52.9	47.7	40.8	49.9	64.5	47.0	39.5	24.2	29.3	78.3	78.6	94.7	100.2	166.4	173.7	106.8	145.8	VF - 100%

Figure 3. Relative abundance (%) and density (Ind. mL^{-1}) in *Penaeus vannamei* nursery with the addition of *Navicula* sp. in a biofloc system.

BFT (biofloc system without addition of *Navicula* sp.); BFT 5D (biofloc system with addition of *Navicula* sp. every 5 days); BFT 10D (biofloc system with addition of *Navicula* sp. every 10 days) and BFT 15D (biofloc system with addition of *Navicula* sp. every 15 days).

The RDA was significant (Perm p = 0.001, n = 999) and the first and second canonical axes explained 24.17% and 11.27%, respectively. The R^2 and R^2-adjusted were 0.368 and 0.330, respectively (Fig. 4). Higher abundance of Dinophyta was associated with higher pH and orthophosphate levels, while Cyanophyta, Chlorophyra and Heterokontophyta were associated with TAN.

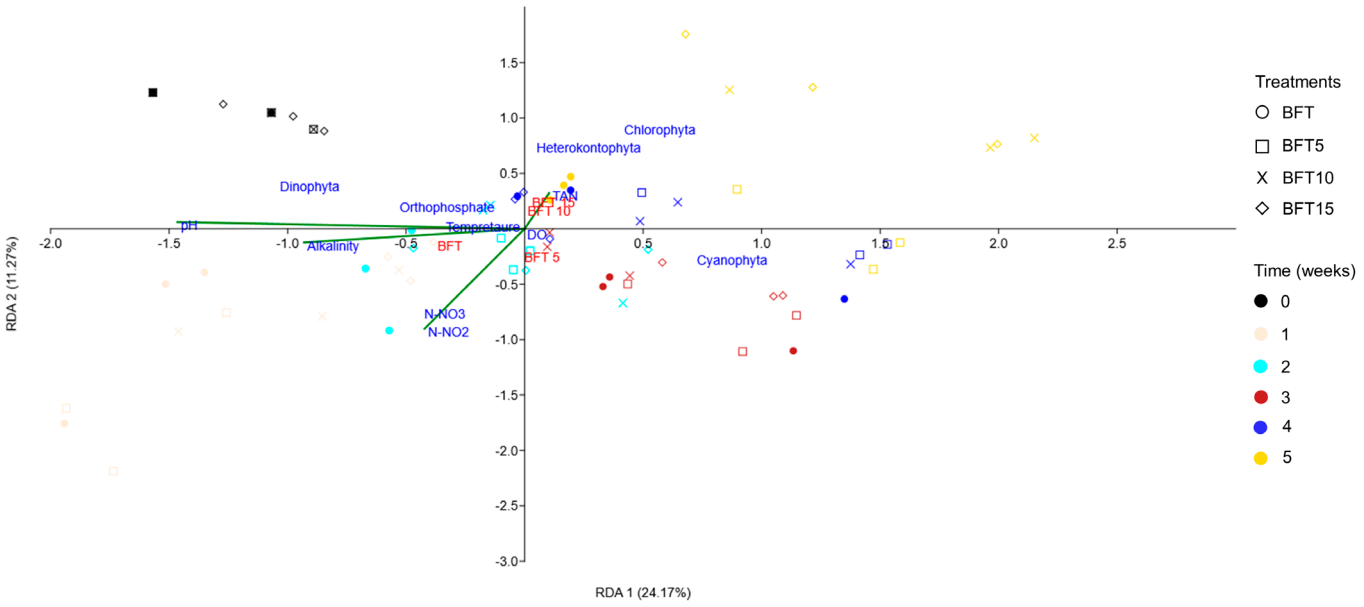


Figure 4. Redundancy analysis (RDA) of phytoplankton community and water quality variables in the nursery of *Penaeus vannamei* with addiction of *Navicula* sp. in a biofloc system. BFT (biofloc system without addition of *Navicula* sp.); BFT 5D (biofloc system with addition of *Navicula* sp. every 5 days); BFT 10D (biofloc system with addition of *Navicula* sp. every 10 days) and BFT 15D (biofloc system with addition of *Navicula* sp. every 15 days).

Discussion

The salinity, temperature, dissolved oxygen and pH of the water remained within the range for *P. vannamei* throughout the culture period (Ponce-Palafox et al., 2013; Samocha, 2019). During the experiment, TAN and N-nitrite values remained within the range for the shrimp, 0.90 and 2.57 mg L^{-1}, respectively (Lin and Chen, 2003; Melo et al., 2016; Samocha, 2019). The low nitrite levels are an indication that the use of 50% of the inoculum from the maturation tank was efficient for the development of heterotrophic and autotrophic bacteria in the system, and that these contributed to the control of the levels of these compounds in the water (De Schryver et al., 2008; Avnimelech, 2015).

The addition of *Navicula* sp. at different frequencies did not cause significant changes in this bacterial community in relation to nitrogenous control, results similar to those observed by Marinho et al. (2017), Brito et al. (2016) and Abreu et al. (2019). According to Avnimelech (2012) and Castillo-Soriano et al. (2013), in intensive systems, bacteria are mainly responsible for the transformation of ammonia. However, photoautotrophic organisms are also able to convert ammonia into algal biomass (Ebeling et al., 2006), but the contribution of organic carbon and the accumulation of particles can reduce the luminosity of the place. This process can reduce the rates of nutrient absorption by algae and, consequently, the conversion of nitrogenous compounds presents in the system.

The biofloc system, with reduced water exchange, can cause nitrate accumulation in the system, especially if the water is reused for several cycles (Kuhn et al., 2010). In the present study, some values above 200 mg L^{-1} were observed in the third week of culture, but these were still lower than the maximum limit recommended by Samocha et al. (2017), of 400 mg L^{-1}. These high levels of nitrate in the system compared to TAN and N-nitrite indicate a good nitrification process in the culture environment (Ebeling et al., 2006). According to Ji et al. (2014), nitrate is the most used source of nitrogen in the physiology of microalgae, however, in the present study, no significant differences were observed with the addition of *Navicula* sp. at different frequencies. This fact may also be linked to the increase in solids levels throughout the culture cycle, which resulted in low availability of light through water column (Table 1).

In order to contribute to the processes of transformation of TAN into microbial biomass and to aid in the oxidation of ammonia into nitrite and nitrate, alkalinity was maintained above 100 mg of $CaCO_3$ L^{-1}, with inorganic carbon (calcium hydroxide) being replaced when necessary. It is important to maintain adequate values for intensive systems, since inorganic carbon is consumed by heterotrophic and nitrifying bacteria, which form the microbial floc, for microbial processes (Ebeling et al., 2006). Therefore, no reductions in pH levels were observed during the experimental period, even with higher frequencies of microalgae addition.

These solids suspended must be kept between 5 and 14 mL L^{-1}, so that the microorganisms present in the floc act positively in maintaining the water quality of the system, and so that a high biological demand for oxygen does not occur (Emerenciano et al., 2017; Samocha, 2019). In the present study, the levels of settleable solids remained within the range for shrimp culture recommended by the afore mentioned authors. However, treatments with the addition of *Navicula* sp. showed lower values when compared to the control, since there was a dilution of solids due to the volume of microalgae added to the system, as reported by Silva et al. (2021).

When analysing the shrimp performance at the end of experimental period, a relation was observed between the frequency of addition of *Navicula* sp. and some of the evaluated parameters, probably due to the benefits of enrichment in the nutritional quality of the food source. Yield and average final weight differed significantly between treatments, and higher values were obtained in those with the lowest microalgae addition frequencies (BFT5D and BFT10D). Similar results were reported by Brito et al. (2016) when inoculating 5 x 10^4 cell mL^{-1} of *Navicula* sp., every 5 days, in the intensive nursery of *P. vannamei* over 35 days (1.76 kg m^{-3}, 0.81 g, 9.41 % day^{-1} and FCR 1.37), as well as by other authors (Marinho et al., 2014; Marinho et al., 2017; Abreu et al., 2019; Silva et al., 2021).

The good shrimp performance results observed in treatments with microalga addition may be related to the ease of shrimps in digesting them, since they have low fiber content (Moss, 2002). In addition, they may also be related to the increased digestibility of the ingredients offered as a result of the addition of plankton, which contributes to increased activity of digestive enzymes (Shah et al., 2018). Furthermore, the addition of this microalga to the culture system is a way to nutritionally improve microbial flocs, mainly in fatty acids. This is because the species has high concentrations in dry weight of protein (494 g Kg^{-1}), lipids (259 g Kg^{-1}), PUFA (110 g Kg^{-1}), DHA (22 g Kg^{-1}) (Khatoon et al., 2009), as well as essential amino acids (Ju et al., 2008). According to Abreu et al. (2019), the addition of *Navicula* sp. in the biofloc system provides higher concentrations of fatty acids (DHA and EPA) in the floc, and all these factors, when combined, provide better shrimp performance.

The composition of the planktonic community reflects water conditions and responds to changes in environmental variables over culture time, which is why it is considered an important biological indicator of water quality (Macedo and Sipaúba-Tavares, 2010). In the present study, an increase in the abundance of the phytoplankton and prozooplanktonic population was observed from the beginning to the end of the experiment, as already reported by other studies in intensive systems (Lima et el., 2022a; Marinho et al., 2017), without any significant influence of *Navicula* sp. in these communities. Still, according to Avnimelech (2007) and Monroy-Dosta et al. (2013), environmental changes and biotic (eg, predation) and abiotic (food availability and carbon sources) factors can affect the diversity and abundance of planktonic communities.

The accumulation of solids, common in intensive systems, can influence the phytoplankton community due to eutrophication (Boyd, 2015; Samocha, 2019), resulting in an increased microalgae biomass and a reduction in the diversity of these organisms.

Moreover, it may also result in the predominance of harmful algae (Hu et al., 2017), as observed in the present study and also reported by Silva et al. (2021) and Lima et al. (2022a; 2022b).

In the phytoplanktonic community, probably due to the high abundance of *Aphanocapsa* sp., a low genera richness was observed. Throughout the culture, Cyanophyta was the most abundant group, mainly represented by *Aphanocapsa* sp., with no significant differences between treatments. In intensive systems, favored by high concentrations of nitrogen and phosphorus, this group can be dominant (Llario et al., 2019), as already reported by other authors (Qiao et al., 2020; Lima et al., 2021). Cyanophyta are the main group of undesirable phytoplankton in shrimp farming, due to their low nutritional value and their potential to deteriorate water quality (blooms and production of toxins), which can negatively affect shrimp quality and even lead to death (Sipaúba-Tavares, 2010; Schrader et al., 2011; Boyd, 2020). Zimba et al. (2006), while investigating the mortality of *P. vannamei* on a commercial farm, reported that dead shrimp had traces of microcystin in their hepatopancreas, caused by the presence of *Microcystis aeruginosa* and *Anabaena* sp.

Several species of zooplankton occur naturally in intensive systems, where they can act in the recycling of nutrients, in the maintenance of water quality and in the nutrition of the cultivated species (Emerenciano et al., 2013). In the present study, *Paramecium* (*Ciliophora*) was the most abundant genus in the protozooplanktonic community throughout the culture period, being present in all samples. Similar results have already been found by other authors for culture in an intensive system (bioflocs and synbiotic) (Widanarni et al., 2012; Lima et al., 2022a). The greater abundance of *Ciliophora* in culture is related to the adaptation of these organisms to high levels of nutrients and solids, which are characteristic of intensive culture systems (Marinho et al., 2014). It is noteworthy that ciliates are able to fix carbon through photosynthesis (Tundisi & Matsumura-Tundisi, 2008), which allows high densities (39–169 per mL) of these organisms to be achieved in intensive shrimp culture (Ballester et al., 2010). In addition, these organisms feed on algae, bacteria and fungi (Nagano and Decamp, 2004), being a rich source of free amino acids, thus providing polyunsaturated fatty acids to shrimp (Decamp et al., 2001).

Conclusion

In conclusion, the addition of *Navicula* sp. did not significantly influence the water quality and planktonic community in relation to the treatments, but improved the shrimp performance, suggesting a potential application in the shrimp nursery in biofloc systems.

Ethical approval: All pertinent institutional and national guidelines for the use of the laboratory animals employed were followed.

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