Int Aquat Res
https://doi.org/10.22034/IAR.2022.1936674.1176

ORIGINAL RESEARCH

Effect of stocking density on water quality, plankton community structure, and growth performance of *Litopenaeus vannamei* post-larvae cultured in low-salinity biofloc system

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Received: 04 January 2022 / Accepted: 06 April 2022 / Published online: 16 April 2022
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Abstract This study aimed to investigate the effects of stocking density on *Litopenaeus vannamei* at the nursery phase in low-salinity biofloc system. Post-larvae of *L. vannmei* were cultured for 28 days at 2000, 4000 and 6000 shrimp m^{-3}, and the water quality, plankton composition and growth performance were evaluated. The water quality variables remained within the ideal range for the shrimp culture, and only total suspended solids showed a significant difference between treatments – the highest stocking density has increased the levels of total suspended solids. In regarding to zooplankton structure, the Protozoa group had the greatest diversity regardless of treatment, and also the greatest abundance (> 66%). For phytoplankton structure, Bacillariophyta (*Navicula* sp. and *Cyclotella* sp.) was the dominant class in phytoplankton structure throughout the culture, although a Cyanobacteria bloom has reported at the end of the cultivation at 2000 shrimp m^{-3}. At the end of cultivation, the shrimp yield (0.133 ± 0.028 kg m^{-3}) was significantly higher in the 6,000 shrimp m^{-3}. In contrast, the shrimp survival was significantly higher in the lowest stocking density (71.66 ± 10.36%). Our findings suggest that a density of 6,000 shrimp m^{-3} should be used under these conditions, but an economic feasibility study should be considered in the future.

Keywords Aquaculture . Brackish water . Live food . Semiarid . Shrimp farming

Introduction

Aquaculture is one of the fastest-growing food production sectors. It produces 73.8 million tons, surpassing fishing production. In addition, shrimp farming has grown steadily in recent years, reaching 4.8 million tons in 2015 (FAO 2018). Outstandingly, the Pacific white shrimp *Litopenaeus vannamei* is the main species of shrimp produced in the world. It is a tropical species that when subjected to low temperatures, zootechnical performance may be compromised (Wurmann et al. 2004). This species can be bred in a wide range of salinity levels due to its high osmotic potential (Ponce-Palafox et al. 1997). Interestingly, the production of *L.*

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vannamei in waters with low salinity has become a promising alternative to reduce coastal environmental degradation, contributing to development of sustainable aquaculture. Furthermore, the use of low salinity water has been suggested as a possible strategy to reduce the incidence of diseases in crops (Fonseca et al. 2009; Santos et al. 2009). On the other hand, the nitrogen compounds (i.e., ammonia, nitrite, nitrate) toxicity increases with a decrease in salinity. and this represent an important problem associated with low rates of food intake, reduced growth, increase consumption of oxygen, and even moderate or high mortality (Schuler et al. 2010; Valencia-Castañeda et al. 2018).

Biofloc technology (BFT) is a relatively recent method for shrimp farming. It has contributed to the intensification of this production (El-Sayed 2021) and is more less aggressive to the environment due to minimum/low water exchange. It has a great potential for using the area and produce satisfactory productive results (Krummenauer et al. 2011). In addition, BFT has a greater efficiency in water demand, making it an environmentally friendly system because it reduces environmental impacts and manages to have greater productivity compared to other traditional systems (Krummenauer et al. 2011). Moreover, BFT can considerably reduce production costs and make production feasible in arid and semiarid regions (Sandifer and Hopkins 1996). Therefore, shrimp farming using brackish water from arid and semiarid regions uses water and land that is unsuitable for agriculture, and thus, not compete with (or affect) traditional agriculture.

In addition, BFT is considered a good alternative due to the possibility of establishing crops in continental areas using brackish waters, generating income for arid region populations (Barros et al. 2004). Many studies have been carried out aiming to establish the potential of *L. vannamei* both in fresh and oligohaline waters by taking advantage of natural characteristics of this species, which grows well in freshwater (<0.5-salinity). Yet, the use of oligohaline and/or brackish waters (0.5-5.0-salinity) can reduce energy expenditure of animals due to the presence of certain ions that may facilitate osmoregulation (Samocha et al. 2004; Bezerra et al. 2007). However, there is not an abundant literature showing the effects of *L. vannamei* stocking density in low salinity BFT systems on nitrogen compounds levels, growth performance, and plankton composition.

By using BFT during the nursery phase, good indices of water quality may be maintained, even without changing the water (Xu et al. 2012). Some studies have demonstrated the important role of the BFT system at the shrimp post-larvae, providing a safer environment for animal growth (Krummenauer et al. 2010; Wasielesky et al. 2013). However, there are no studies evaluating plankton composition and growth performance of *L. vannamei* in low-salinity biofloc system. For this reason, this study aimed to evaluate water quality, variation in plankton composition, and the zootechnical performance of *Litopenaeus vannamei* post-larvae cultured in different stocking densities in a low-salinity biofloc system.

Materials and methods

Biological material

This study was conducted at the Laboratório de Experimentação de Organismos Aquáticos of the Universidade Federal Rural de Pernambuco, Brazil. *Litopenaeus vannamei* post-larvae were obtained from a commercial laboratory. The mean weight of the post-larvae was 5.05 ± 0.01 mg and they were cultured for 28 days.

Biofloc preparation

For BFT preparation, oligohaline water (~1.5 salinity) from an artesian well was stored in a matrix tank with 1,000 L and constant aeration. For the formation of microbial aggregates, organic carbon (molasses) was added daily at a carbon: nitrogen (C: N) ratio of 15:1 to favor the development of heterotrophic bacteria, according to Ebeling et al. (2006).

The quantities of molasses added were calculated based on the C:N ratios required taking into account the amount of nitrogen required in feed conversion to ammonia (ΔN) and carbon content in molasses (%C) according to the equations:

$$\Delta Molasses = [\Delta N \times (C:N)] \times \%C^{-1} \quad (1)$$

$$\Delta N = QFeed \times \%NFeed \times \%NExcretion \quad (2)$$

where, QFeed is the amount of feed offered daily, %NFeed is the amount of nitrogen added to the system (%Crude protein / 6.25), and % NExcretrion is the ammonia flow into water either directly from excretion or indirectly from microbial degradation of organic nitrogen residues.

The amount of molasses added to each experimental unit to meet the required C:N ratio in the treatments was calculated using the equations (3)

$$\Delta Molasses = [(QFeed \times \%Nfeed \times \%NExcretion) \text{ x } (C:N)] \times \%C^{-1} \quad (3)$$

The molasses used had about 30% carbon on the dry matter. Thus, using commercial feed containing 40% crude protein (6.4% N) and 50% of nitrogen, the %NExcretion from the feed is calculated according to Avnimelech (1999):

$$\Delta Molasses = [(QFeed \times 0.064 \times 0.5) \times (C:N)] \times 0.30^{-1} = QFeed \times 0.1067 \times (C:N) \quad (4)$$

The equations described were adapted from Avnimelech (1999) and Samocha et al. (2007).

The animals were acclimated in a matrix tank. After 15 days, they were transferred to the experimental units. 15 L were added until ~ 90% of volume, and the rest completed with water 3-salinity.

Experimental design

The experiment was completely randomized with three different densities: 2000, 4000 and 6000 post-larvae m^{-3} and each treatment with four replicates. The post-larvae were cultured in 15 L polyethylene tanks with a porous stone and a blower aeration system to keep the biofloc in suspension maintaining oxygen above 5 mg L^{-1}. No water exchange occurred during this study. Freshwater was only used to replace the water lost by evaporation.

Shrimp post-larvae were fed with commercial feed containing 40% crude protein. Feed was provided six times a day and adjusted according to Wasielesky et al. (2013).

Water quality analysis

Water temperature (ºC), pH, dissolved oxygen (mg L^{-1}), and salinity (PSU) parameters were measured twice a day using a multi-parameter probe (YSI model 55, Yellow Springs, Ohio, USA). Total ammoniacal nitrogen (TAN) (Koroleff 1976), nitrite ($N\text{-}NO_2$) (Golterman et al. 1978), nitrate ($N\text{-}NO_3$) (Mackereth et al. 1978), orthophosphate (P-ortho) (APHA 2005), turbidity (NTU), total suspended solids (TSS) (APHA 2005), and alkalinity (mg L^{-1} $CaCO_3$) (Felföldy et al. 1987) were measured once a week.

Plankton composition

Weekly, two liters of water from each tank were collected from the bottom to surface in each experimental unit. Subsequently, the collected volume was filtered with a 20-µm net, concentrated to 250 mL, and fixed with formaldehyde (4%) for later identification.

Identifying phytoplankton, a taxonomic classification was performed according to Bicudo and Menezes (2006) following keys and specific bibliography of each algae group, including González (1996), Popovský and Pfiester (1990) and Oliveira et al. (2018). For zooplankton, the taxonomic classification was performed according to Newell and Newell (1963).

Quantitative analyses of plankton were conducted in triplicate using a binocular optical microscope (BA300, Motic®, China) and a Sedgwick-Rafter (ind. mL^{-1}). Individuals were counted at 400 or 1,000× of magnification. Colonies, single cells, filaments, and cenobites were considered a single individual (Oliveira et al. 2019). To estimate individual density, the filtered volume was considered.

Table 1 Mean values (± standard deviation) of water quality (physical and chemical variables) of the environment *L. vannamei* post-larvae grew under different stocking densities with biofloc technology for 28 days.

Parameter	Treatment (post-larvae m^{-3})		
	2000	4000	6000
Dissolved oxygen (mg L^{-1})	9.21 ± 0.62	9.34 ± 0.87	9.86 ± 0.76
Temperature (°C)	22.16 ± 1.13	22.16 ± 1.15	22.18 ± 1.1
Alkalinity (mg L^{-1})	255.83 ± 20.01	281.66 ± 16.31	277.5 ± 43.37
pH	8.13 ± 0.12	8.09 ± 0.08	8.06 ± 0.13
Salinity (PSU)	2.48 ± 0.04	2.47 ± 0.04	2.46 ± 0.04
Total suspended solids (mg L^{-1})	150 ± 0.09^{a}	230 ± 0.15^{b}	250 ± 0.01^{b}
Total ammonia nitrogen (mg L^{-1})	0.29 ± 0.29	0.66 ± 0.31	0.35 ± 0.39
Nitrite-N (mg L^{-1})	0.72 ± 0.89	0.84 ± 0.78	1.01 ± 0.80
Nitrate-N (mg L^{-1})	13.31 ± 9.68	20.94 ± 3.08	19.50 ± 5.88
Phosphate-P (mg L^{-1})	51.20 ± 10.22	46.42 ± 9.21	53.10 ± 6.54

Mean values on a same row with different superscript letters differ significantly ($P < 0.05$) by Tukey test.

Shrimp performance

L. vannamei post-larvae performance was evaluated by a weekly biometry. At the end of rearing, survival (%), final mean weight (g), weekly growth rate (WGR, g $week^{-1}$), feed conversion ratio (FCR), and yield (kg m^{-3}) were calculated according to de Moura et al. (2021).

Statistical analysis

Water quality and growth performance data were presented as mean ± standard deviation (n = 4). Normality and homoscedasticity were determined by Shapiro-Wilk and Levene tests, respectively. One-way ANOVA was applied, followed by a Tukey test whenever necessary to verify differences between stocking densities ($P < 0.05$). All analyses were performed using the software Statistica, version 10.0 (StatSoft).

Results and discussion

Water quality

Water quality parameters were summarized in Table 1. Temperature, salinity, dissolved oxygen, pH, $N\text{-}NO_2$, $N\text{-}NO_3$, TAN, $P\text{-}PO_4$, and alkalinity did not show significant differences ($P < 0.05$) among treatments. Only TSS presented significant difference between the treatments, with higher values in the density of 6,000 post-larvae m^{-3} (Table 1).

The abiotic parameters are determining factors for the zootechnical performance of a cultivated species. Salinity, dissolved oxygen, pH, $N\text{-}NO_2$, $N\text{-}NO_3$, TAN, P-, and alkalinity samples remained in ideal conditions for the cultivation of *L. vannamei* (Gaona et al. 2011; Van Wyk and Scarpa 1999). TSS increased with the increase of stocking density, for this reason the higher stocking density (i.e., 6,000 shrimp m^{-3}) had higher value compared to the other treatments. However, it remained within appropriate ranges for the *L. vannamei* culture (Samocha et al. 2007; Gaona et al. 2011).

Temperature remained below of optimal levels for high growth of *L. vannamei* throughout the experimental period. Optimal temperature range for the cultivation of this species range from 26 to 33 ºC. Temperatures above 35 ºC and below 25 ºC can cause damage to zootechnical performance of this species (Van Wyk and Scarpa 1999). In the present study, the temperature was close to 22 ºC, due to the period in which the experiment was conducted (winter), and the non-use of a thermostat. This parameter directly affects food consumption, leaving animals susceptible to diseases and stress (Bardera et al. 2019). According to Ponce-Palafox et al. (1997), temperature directly affects the performance of shrimp, reducing food

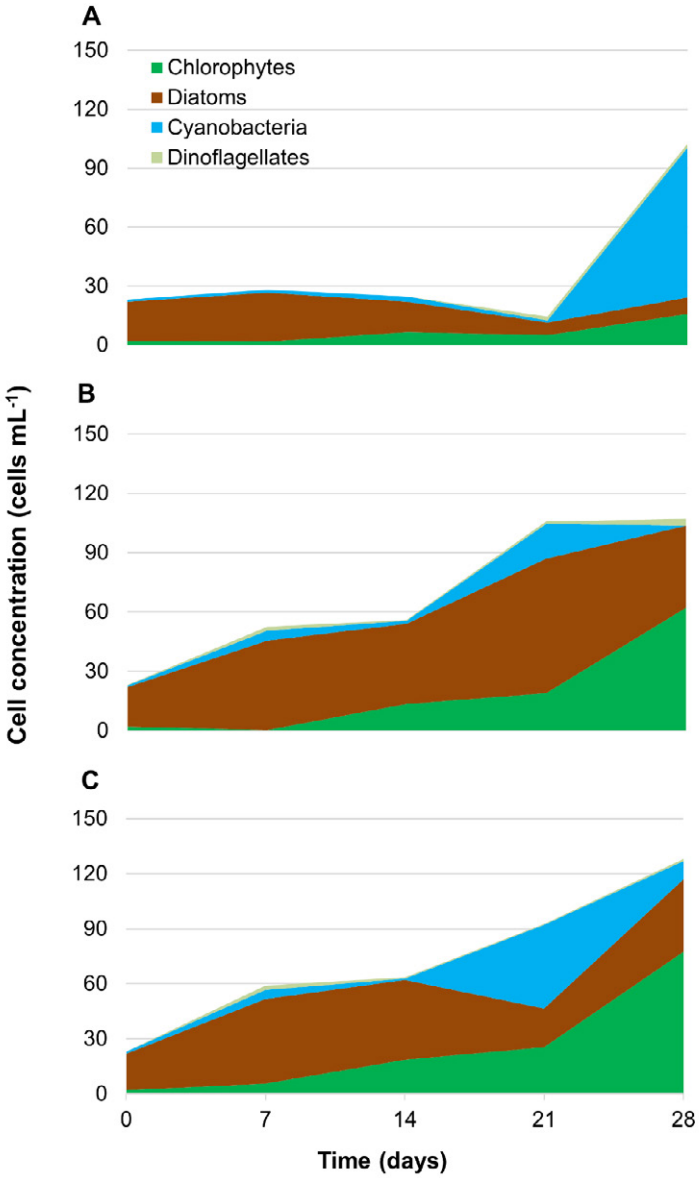


Fig. 1 Mean contribution of phytoplankton groups in temporal evolution during the rearing of *Litopenaeus vannamei* post-larvae in a low-salinity biofloc system. A: 2,000 post-larvae m^{-3}, B: 4,000 post-larvae m^{-3}, and C: 6,000 post-larvae m^{-3}.

consumption and consequently growth.

The nitrification process was efficient during the cultivation, since nitrate-N levels were higher when compared to TAN and nitrite-N, and these two nitrogen compounds remained at the levels recommended by Samocha (2019) for *L. vannamei* cultivation. On the other hand, a relatively high level of phosphorus was found in the present study. The accumulation of phosphorus in BFT culture still is an obstacle, due to the lower absorption capacity of this compound, compared to nitrogen sources, by bacteria and microalgae (Oliveira et al. 2021).

Plankton composition

The evolution of phytoplankton groups in the different stocking densities was shown in Fig. 1. During the cultivation, 16 taxa from four groups were found: six diatoms (Bacillariophyceae): *Navicula* spp., *Cyclotella* sp., *Fragilariforma* sp., *Aulacoseira* sp., *Fragilaria* sp. and *Valva* sp.; four chlorophytes (Chlorophyceae): *Chlorococcum* spp., *Asterococcus* sp., *Stigeoclonium* sp. and *Tetraspora* sp.; two cyanobacteria: *Planktolyngbya* sp. and *Aphanothece* sp.; and one dinoflagellate (Dinophyceae): *Ceratium* sp. On one hand,

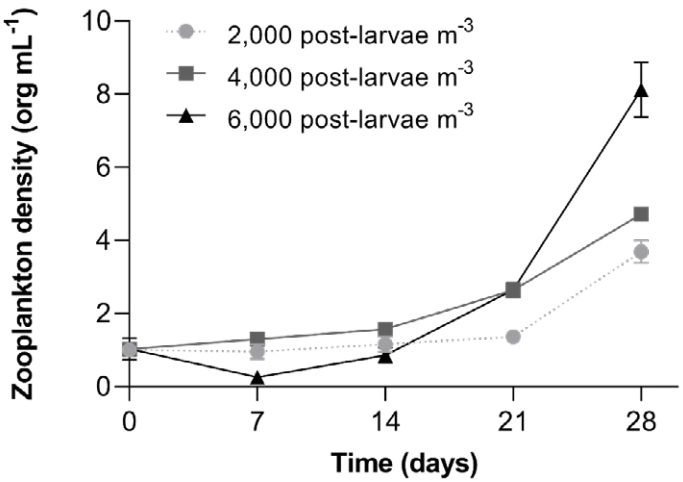


Fig. 2 Zooplankton density (organisms mL^{-1}) during the rearing of *Litopenaeus vannamei* post-larvae in a low-salinity biofloc system

diatoms prevailed in all treatments during the first 14 days of cultivation. On the other hand, between 14th and 21st days, cyanobacteria concentration increased considerably in the two treatments with high stocking densities (4000 and 6000 post-larvae m^{-3}). At the end of cultivation, the 2,000 post-larvae m^{-3} treatment had an exponential growth of cyanobacteria. In all samplings and treatments, dinoflagellates were the less representative group.

Intensification of *L. vannamei* requires establishment of a well-developed planktonic community because it may be used as a food complement (Paiva-Maia et al. 2003). Marinho et al. (2017) reported a reduction in Cyanobacteria concentration throughout cultivation of *L. vannamei* in a biofloc system. These authors reported a gradual reduction in the cyanobacteria densities between the beginning (3,790 cells mL^{-1}) and the end (ranging from 190 to 1,674 cells mL^{-1}) of the cultivation. Herein, oppose findings for Cyanobacteria density were observed, probably, the gradual increase in phytoplankton density reported in the present study was due to the greater acceptance of inert food throughout rearing.

Cyanobacteria peak on the last week of rearing of the 2,000 post-larvae m^{-3} treatment can be justified by the resistance of this group and by the consequent fragility of the community of diatoms and chlorophytes (Oliveira et al. 2019; Ariadi et al. 2019). Although it has not been reported in high densities, the presence of *Ceratium* sp. in all treatments also must be highlighted. The most likely cause for it to be found in a biofloc system is its colonization in reservoirs near the region the present study was conducted (Oliveira et al. 2021; Nascimento Filho et al. 2019). Thus, it is likely that phytoplankton composition in low-salinity biofloc systems will be targeted according to the aquatic body used for maturation of the system. Moreover, the addition of *Navicula* sp. promoted benefits to zootechnical performance of *L. vannamei* post-larvae also reared in a biofloc system and improved the nutritional quality of flocs by increasing fatty acids content (Abreu et al. 2019). In fact, microalgae are recognized for their high nutritional contents of essential amino acids (Ahmad et al. 2020), polyunsaturated fatty acids (Oliveira et al. 2021), carotenoids (Dantas et al. 2019), among others. These compounds, in turn, besides contributing to animal growth, can modulate the intestinal microbiota (Pilotto et al. 2019).

The evolution in the zooplankton concentration and the relative abundance of taxa found in the present study were shown in Fig. 2 and Table 2, respectively. Zooplankton community presented 23 genera from five different groups (Table 2). The Protozoa group presented the greatest diversification and abundance regardless of the treatment and dominated the system throughout cultivation. Rotifers accounted for approximately 30% of total zooplankton regardless of treatment. *Lecane* spp. and *Lepadella* spp. were the most abundant taxa. In all treatments, up to the day 14 of cultivation, the zooplankton community had a density of 4 individuals mL^{-1}, increasing to 8 and 14 individuals mL^{-1} on the days 21 and 28, respectively.

Zooplankton community is the second link in the aquatic trophic web, superior only to microalgae and bacteria (Arruda et al. 2017). Corroborating Castro-Mejía et al. (2017), the protozoa predominance during the cultivation of Nile tilapia (*Oreochromis niloticus*) using the biofloc technology was also ob-

Table 2 Relative abundance (%) of zooplankton during the rearing of *L. vannamei* post-larvae in a low-salinity biofloc system over a 28-day study

Taxa	Treatment (post-larvae m^{-3})		
	2000	4000	6000
Rotifers	32.94	27.64	33.06
Lecane spp.	23.10	15.03	4.95
Lepadella spp.	9.15	11.54	26.62
Euchlanis sp.	0.27	0.14	0.11
Colurella sp.	0.19	0.54	0.45
Encentrum spp.	0.03	0.06	0.01
Brachionus sp.	0.00	0.01	0.00
Keratella sp.	0.00	0.00	0.00
N.I.	0.20	0.34	0.93
Protozoa	66.79	72.15	66.83
Aspidisca sp.	27.97	37.16	30.80
Trinema spp.	13.72	20.58	10.46
Euplotes sp.	12.53	9.99	16.76
Vorticella spp.	5.95	2.38	6.80
Quadrulella sp.	4.49	1.42	1.29
Cothurnia sp.	1.18	0.11	0.28
Paramecium spp.	0.62	0.25	0.15
Dindinium spp.	0.04	0.12	0.16
Centropyxis sp.	0.12	0.08	0.00
Stylonychia sp.	0.04	0.01	0.00
N.I.	0.10	0.06	0.13
Nematode	0.06	0.01	0.01
N.I.	0.06	0.01	0.01
Platyhelminthes	0.15	0.05	0.03
N.I.	0.15	0.05	0.03
Cladocera	0.06	0.15	0.08
N.I.	0.06	0.15	0.08

N.I. Not identified genus

served. Conversely, only the genus *Lecane* was reported. A possible competition between the genera *Lecane* and *Lepadella* has been reported for both genera are related to water eutrophication (Loureiro et al. 2011).

Growth performance

Zootechnical performance results were summarized in Table 3. The final weight and weight gain of shrimp did not present significant differences between treatments ($P > 0.05$) during all 28 experimental days. However, survival was significantly and negatively ($P < 0.05$) affected by the different stocking densities. Moreover, yield was significantly lower by using a lower density ($P < 0.05$) (Table 3).

Productivity was directly affected by stocking density. The high-density treatments presented a better performance than the 2,000 shrimp m^{-3} treatment. A possible explanation for this is the stocking density assigned to each treatment. 2,000 shrimp m^{-3} had a lower number of individuals compared to the other treatments. A possible lower yield would be obtained independent of survival compared to the other treatments.

Table 3 Shrimp performance for 28 days of rearing of *L. vannamei* post-larvae in a low-salinity biofloc system under different stocking densities

Parameters	Treatments (post-larvae m^{-3})		
	2000	4000	6000
Final weight (mg)	52.05 ± 9.13	50.52 ± 1.57	47.61 ± 7.44
Weekly growth rate (mg $week^{-1}$)	11.74 ± 2.28	11.52 ± 1.15	10.64 ± 1.86
Survival (%)	71.66 ± 10.36^{a}	61.67 ± 14.33^{a}	49.72 ± 9.22^{b}
Feed conversion ratio	2.08 ± 0.36	2.11 ± 0.58	2.68 ± 0.89
Yield (kg m^{-3})	0.073 ± 0.007^{b}	0.114 ± 0.068^{ab}	0.133 ± 0.028^{a}

Mean values on a same row with different superscript letters differ significantly ($P < 0.05$) by Tukey test.

This was also reported by Krummenauer et al. (2011), where higher yields were related to higher stocking densities (Ariadi et al. 2019).

In addition to stocking density, the increase in the concentration of potentially toxic cyanobacteria (both *Planktolyngbya* sp. and *Aphanothece* sp.) may have negatively influenced shrimp survival. Lima et al. (2021) reported that the survival of *L. vannamei* cultured in bioflocs was negatively affected by the dominance of cyanobacteria in the system. However, for a better elucidation, a toxin analysis would be necessary to directly infer on this topic.

Successful shrimp nurseries in BFT systems in low-salinity systems has been report (Zacarias et al. 2018; Moura et al. 2021). According to Moura et al. (2021), an inoculation at 3% of seawater (~1,75 g L^{-1} of salinity) mitigated negative effects of the ionic imbalance, improving significantly the growth performance. Although in the present study the salinity was still similar to that reported by these authors, it is worth noting that the ionic composition of brackish water is different from that of seawater (Haris et al. 1972). Moreover, it is probably that lower growth performance (i.e., survival and weekly growth rate) was also negatively impacted of higher nitrogen toxicity at low salinity plus ionic stress on the shrimp (Muqsith et al. 2021; Wafi et al. 2021).

Higher feed conversion factor can be attributed to higher energy expenditure for osmoregulation in shrimp (Camacho-Jiménez et al. 2017), as well as the increase in the load of filamentous cyanobacteria in the composition of flocs (Llario et al. 2019). But considering that brackish waters in semi-arid regions are unsuitable for consumption and agriculture, the use for the culture of *L. vannamei* can still be considered an interesting alternative (Ariadi et al. 2019). Thus, an economic feasibility study can be useful to investigate this issue.

Conclusion

In conclusion, our results reinforce the possibility of the use of low-salinity waters, that are unsuitable for agriculture and human consumption, for production of Pacific white shrimp *L. vannamei*. The stocking density of 6,000 shrimp m-3 was significantly higher than other densities, even with a lower survival. Moreover, stocking density affect the phytoplankton composition throughout the cultivation. Thus, the findings suggest that a density of 6,000 shrimp m^{-3} should be used under these conditions. However, due to the relatively low survival at the end of the nursery phase, it is extremely important that an economic feasibility study be considered in the future.

Data Availability Statement The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval It is not required to get an "Approval of Animal use Protocol" when using invertebrates as experimental animal in Brazil.

Declaration of competing interests The authors declare that they have no conflict of interest.

Authors' contributions: **WAS** Investigation, Formal analysis, Data curation, Writing - Original Draft. JLS: Investigation, Formal analysis, Data curation, Writing - Review & Editing. CYBO: Formal analysis, Data curation, Writing - Original Draft. APMM: Data

curation, Writing - Review & Editing. RASM: Data Curation, Software, Writing - Review & Editing. ULS: Supervision, Project administration, Writing - Review & Editing.

Funding The study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Brazil (Finance Code 001).

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