



Comparison of Water Quality Parameters on biofloc and Nursery Culture Systems in *Litopenaeus vannamei* (boone, 1931)

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KEYWORDS

ABSTRACT

Shrimp production is entirely dependent on the physical, chemical, and biological qualities of the water. To adequately manage shrimp production, a basic understanding of water quality is essential. In the present study, bioflocs and a nursery culture system were cultured in an experiment for 30 days to collect data on water quality and its influence on biofloc growth. In addition, molasses was added to the culture water as an inexpensive supply of carbon to help the bioflocs develop. The following physico-chemical parameters of water were periodically analysed: temperature, pH, salinity, dissolved oxygen, total ammonia nitrogen, nitrite-nitrogen, nitrate-nitrogen, phosphorus, chlorophyll a, total suspended solids, floc volume, and turbidity. At the end of the experiment, the shrimp's survival rates were above 95%, with significant differences between the two groups ($P > 0.05$); additionally, the shrimp's growth (in terms of final weight, weight gain, and specific growth rate) was significantly better in biofloc and nursery culture system ($P > 0.05$). As a result, shrimp may be successfully grown in biofloc and nursery culture systems after an examination of physical-chemical parameters identified the ideal range for shrimp survival.

1. INTRODUCTION

Aquaculture is one of the most significant food production sectors nowadays since it produces more proteins for human consumption. Compared to all other industries that provide animal food, aquaculture output has increased more quickly, from 3.9% of total production by weight in 1970 to 27.3% in 2000. One of

the most significant industries worldwide is the production of shrimp and prawns (Bianchi et al, 2014; Reddy and Naik, 2020). Recent years have seen a number of significant and innovative developments in shrimp culture operations, including the introduction of probiotics of various kinds and bioflocs of various carbon sources to improve several and growth rates,

while also enhancing immune capacity and assisting in a significant rise in production rates. Bioflocs are known to improve shrimp health by reducing the presence of infectious pathogens and acting as natural immune enhancer that aid in the development of the shrimp's disease resistance system (Avnimelech, 1999; Kuhn et al., 2008; Fatimah et al., 2019).

Shrimp farming poses a significant ecological issue because to the inputs, particularly feed and its constituents, which are known to accumulate in the culture environment and gradually decrease water quality, impose stress, and ultimately leads to disease outbreaks. Biofloc Technology, which is based on microbial manipulation of the aquaculture system in the form of Carbon: Nitrogen ratio feed, has shown promising results when utilized in the aquaculture of candidate species (Khanjani et al., 2017). The biofloc technology was created to enrichment production by adding additional external carbon sources to the feed. Different carbon sources promote the growth of the indigenous microbiota in diverse ways, which has a significant impact on water quality, the creation of in-situ feed, and the usage of flocs by cultured organisms (Kuhn et al., 2008). In the nursery phase, higher stocking densities are used, which reduces production costs. In temperate climates, this tactic increases crop production. At the end of the growth period, it also supports survival and disease prevention. In addition, when compared to ponds, nurseries provide superior feeding efficacy, more regulated environmental conditions, improved biosecurity, and better facility utilization (Fatimah et al., 2019; Anand et al., 2022). As a result, the creation of high-quality rearing throughout the nursery stage is the most crucial component for effective shrimp growth. Most frequently raised across the world is the white shrimp *Litopenaeus vannamei* (Boone). It is a suitable option for intensive and/or biosecure closed grow-out procedures due to its rapid growth, robust survival in high-density culture, and disease resistance (Cuzon et al. 2004). In the present study water quality metrics in *Litopenaeus vannamei* biofloc and nursery culture systems were compared.

2. MATERIALS AND METHODS

Design of Systems and Experiments

A 30-day study was conducted to compare at Pacific white shrimp production in biofloc and nursery culture

systems in terms of shrimp growth and survival as well as water quality. The study was carried out at the Annamalai University, Center of Advanced Study in Marine Biology in Parangipettai, Tamil Nadu, India. The usable capacity of the cylindrical experimental tanks was 0.22m³. Air stones connected to four blowers (each with a 120m³/hour capacity) provided constant turbulent aeration to the experimental tanks from the bottom of the tanks. By filtering seawater with a 32ppt concentration via a 150-250µm mesh size from the nearby Vellar estuary (Laramore et al., 2001). Calcium hypochlorite (35% chlorine concentration) was used to disinfect the tanks and achieve a residual chlorine concentration of at least 10 ppm for 48 hours to kill disease carriers (International Office of Epizootics, 2009). The residual chlorine level was measured a week later, and sodium thiosulphate was added (7 mg/l for every 1 mg/l residual chlorine) (Van Wyk and Scarpa, 1999). For two to three days, water was pumped into the experimental tanks, which were fertilized with dolomite (10 g/m³, superphosphate (15 g/m³), and urea (15 g/m³) for the two treatment tanks. To encourage the growth of bioflocs, 50 litres of suitable saline water were autoclaved, and the required carbon sources were added to that feed before it was aerated for a day and evenly dispersed to simply the treatment tanks for five days (Panigrahi et al., 2019). Saline water was added to the experimental tanks on a weekly basis to compensate for evaporation losses.

Biofloc formation

Avnimelech (2019) suggested amounts of organic carbon sources were used to begin the formation of biofloc. To promote the growth of heterotrophic bacteria, each ingredient was dissolved in freshwater, incubated for 48 hours, and then added to the culture tanks at a 12:1 C/N ratio. The amount of pellet feed given to shrimp over the course of three days served as the basis for the addition of carbohydrates based on the protein composition of the feed (Serra et al., 2015; Hai et al., 2020).

Assessment of water quality parameters

The parameters were therefore examined at regular intervals for various physio-chemical variables such as temperature, pH, salinity, DO (dissolved oxygen), Total Ammonia Nitrogen (TAN), Nitrite-Nitrogen (NO₂-N), Nitrate-Nitrogen (NO₃-N), Phosphate-Phosphorus (PO₄-P), Chlorophyll a, Total Suspended Solids (TSS), Floc volume (FV) and Turbidity. The water temperature

was determined using a mercury bulb thermometer. Salinity and pH were determined using a hand refractometer and a Scan-Eutech instrument, Singapore, respectively. The dissolved oxygen was measured using a modified Wrinkler's method, according to Strickland and Parson, (1972). The levels of ammonia, nitrites, nitrates and chlorophyll were measured twice a week using standard methods (APHA, 1985). Total suspended solids (TSS) analyses were performed on water samples acquired for suspended material analysis using the Strickland and Parsons method (Strickland and Parsons 1972). Imhoff cones were used to measure the floc volume once a week beginning in Week IV (Eaton et al., 1995).

Performance of Shrimp Growth

The following techniques were used on a weekly basis to calculate the Average Body Weight (ABW), Specific Growth Rate (SGR), and survival rate of *L.vannamei* seeds. Shrimp were harvested at the end of the experiment, and the survival rate was computed (Khanjani et al., 2016).

$$ABW (g) = \frac{\text{Total weight of Shrimp collected (g)}}{\text{Number of Shrimps}}$$

$$SGR = \frac{\text{Final weight} - \text{Initial weight (g)}}{\text{Num. of culture days}}$$

Statistical

The mean (Avg) and standard deviation (SD) of each parameter were determined as descriptive statistics to characterize the variance of each parameter. The variance among the chosen culture waters was examined using a one-way ANOVA and the Kruskal-Wallis (KW) test. Principal component analysis (PCA) was utilized to identify correlations and trends in water quality while simultaneously reducing computing load. Pearson correlation analysis was used to establish the key factors influencing water quality, and the results were shown as corrplots. All of the aforementioned statistical analyses were performed using the R language version 4.0.5., (R Development Core Team, 2018). The Kruskal-Wallis test was performed using the 'dplyr' component of the 'R' programming language (Kassambara, 2019). Principal Component Analysis (PCA) was used to run the "ggfortify" R programme (Tang et al., 2016). The Pearson correlation analysis was plotted using 'R' software's 'ggcorrplot' function (Kassambara and Kassambara, 2019).

3. RESULTS AND DISCUSSION

Water quality is crucial role in aquaculture for the growth and survival of nursery cultured species as well as biofloc. Any changes in physicochemical factors can have an impact on shrimp growth, development, and maturity. As a result, water quality was monitored on a regular basis during the experiment period. The result of Mean with Standard Deviation (SD) of water quality parameters in shrimp *L. vannamei* biofloc and nursery culture are presented in Table 1.

Temperature

Temperature is one of the prominent environmental factors influencing the photosynthesis in water, physiological response of cultured organisms, the decomposition of organic materials, and subsequent biochemical processes. According to Wyban et al., (1995) stated that temperature is a significant factor controlling the shrimp growth. In the present study, water temperature ranged between 28.1 °C and 30.2°C (Figure 1a). The statistical study revealed that water temperature varied significantly between biofloc and nursery culture KW $\chi^2= 1.58$; $df = 1$; $P> 0.05$. Caipang et al., (2022) suggested that, the temperature ranged between 27 and 32°C was quiet suitable for the better development of shrimp larvae. This factor has a direct impact on food consumption, leaving animals vulnerable to diseases and stress (Bardera et al. 2019). The present study's findings closely match the patterns seen in earlier research (Godoy et al., 2012; Tierney and Ray, 2018)

pH

In aquaculture environment, pH plays a vital role and its influences many of physiological functions such as oxidative stress, immunological suppression and disease susceptibility (Han et al., 2018; Duan et al., 2019). The high level of pH causes stress in the shrimp, resulting in soft shell and low survival rate. In the present study, pH maintained ranged from 7.9 to 8.5 with maximum at Biofloc and a minimum at nursery culture (Figure 1b). The results of the statistical analysis also showed significant (KW $\chi^2= 6.44$; $df = 1$; $P<0.05$) differences in the water pH between the biofloc and nursery culture systems. Similar pH values to those observed in this experiment were found in *L. vannamei* by Wasielesky et al. (2006) and Godoy et al. (2012).

Salinity

Salinity of the water may be used to describe the total solids after all carbonates have been converted to oxides.

All organic molecules have been oxidized and chlorides have replaced all of the bromide and iodide components. These values are within the acceptable range for *L. vannamei* (26 to 32ppt) (Mustafa et al., 2022). In the present study, salinity content varied from 31 to 32ppt (Figure 3c). The statistical analysis also revealed that the water salinity was significantly different (KW $\chi^2=1.62$; df = 1; $P>0.05$) between the biofloc and nursery culture systems, with the biofloc system having higher water salinity. According to Emerenciano et al., 2012, salinity levels in biofloc treatments were higher than in water exchange treatments due to increased evaporation in zero - water exchange systems. Similar findings were reported by Godoy et al., 2012 and Mustafa et al., 2022.

Nutrients

Nutrients are molecules in water that may be directly utilized by biota for cell growth in the context of water quality. Aquatic organisms require a variety of nutrients, including ammonia, nitrate, nitrite, and phosphate (Hlordzi et al., 2020). Ammonia concentrations in water can be detected as a result of diminishing ammonia excretion by aquatic organisms, increased ammonia levels in blood, and detrimental effects on membrane integrity and enzyme-catalyzed activities. Ammonia reduces the ability of blood to transport oxygen and increases the usage of oxygen by tissues and gills. Bacteria may create ammonia in water when they break down nitrogenous compounds in an oxygen-depleted environment. In water, both ionised (NH_4) and unionized (NH_3) ammonia exist. Unionized ammonia in aquaculture ponds is a more harmful kind of ammonia due to its ability to quickly infiltrate cell membranes. Temperature, pH, and salinity, to a lesser extent, all influence the amount of NH_3 (Bower and Bidwell, 1978; Venkateswarlu et al., 2019).

In aquatic environments, nitrate is often the crucial nutrient that controls the growth of plankton. In the present study, the highest levels of nitrate and nitrite were found in the nursery culture system and lowest were found in the biofloc culture system and significant differently KW $\chi^2=2.16$; df = 1; $P>0.05$ and KW $\chi^2=1.32$; df = 1; $P>0.05$ respectively (Figure 1e, f). These high concentrations, though, were comparable to those reported by da Silva et al. (2022), who carried out a study in a closed system during the nursery phase, where nitrite and nitrate concentrations exceeded 1.01 (mg L⁻¹) and 20.94 (mg L⁻¹) respectively, in the final week of the

experiment. However, this exposure was only short-term, so it had no impact on the performance of farmed shrimp.

In shrimp farming systems, supplementary feed and direct excretion of cultivated aquatic creatures are the main sources of ammonia compounds. Ammonia is a significant consequence of the protein catabolism in crustaceans. When ammonia levels are high, the blood's ability to carry oxygen is reduced. It is well known that non-ionized ammonia (NH_4) is more dangerous. While the NH_4 levels should be around 0.1 mg/L, the optimal total ammonia concentration shouldn't be more than 1 mg/L. In this study, the concentration of TAN ranged between 0.02 and 0.08ppm and the statistical analysis results show a significant difference (KW $\chi^2=0.18$; df = 1; $P>0.05$) (Figure 3d). According to earlier studies by De Schryver and Verstraete (2009), biofloc heterotrophic bacteria can absorb ammonia 40 times faster than nitrification bacteria. This may help reduce the amount of ammonia in the culture system. These findings were also in agreement with an earlier study conducted by Mustafa et al., (2022) and da Silva et al. (2022).

In aquaculture ponds, phosphate is a crucial nutrient that controls phytoplankton growth and increase the base of the food chain. In the present study, the concentration of phosphate ranged between 0.10 and 0.18ppm and there were significant differences between (KW $\chi^2=0.001$; df = 1; $P>0.05$; Figure 2a). Phosphate concentrations in both treatments increased in a distinct pattern during the culture period. The results of the current investigation are in agreement with earlier studies by Godoy et al., 2012; da Silva et al. (2022).

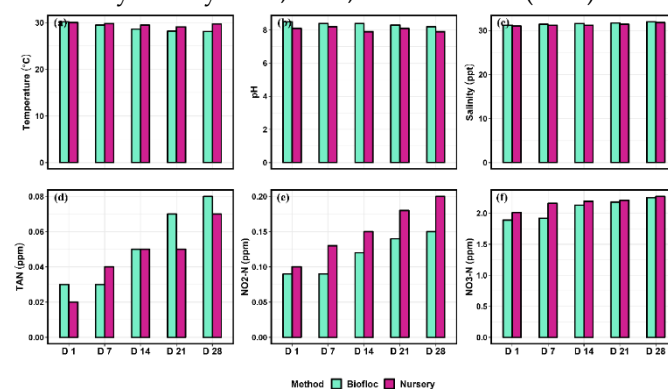


Figure 1. Physicochemical water quality parameters in the biofloc and nursery culture system throughout a 30-day whiteleg shrimp rearing period (a) temperature,

(b) pH, (c) salinity, (d) Total Ammonia Nitrogen (e) Nitrite-Nitrogen and (f) Nitrate-Nitrogen .

Dissolved oxygen

Dissolved oxygen has a direct influence on feed intake and maturation, which has a significant impact on growth and output. Dissolved oxygen influences the solubility and accessibility of several nutrients in culture water system. Dissolved oxygen deficit can directly harmful to shrimp. It can also cause stress, delay development and moulting, and increase the quantity of hepatotoxic metabolic processes in shrimp, all of which can lead to death. Low dissolved oxygen concentrations can potentially impair a substances oxidation state, causing it to transition from an oxidized to a reduced state (Godoy et al., 2012; Lien and Giao, 2020). In the present study, the water dissolved oxygen for biofloc and nursery culture system white-leg shrimp ranges from 6.7 to 7.1mg/l and there were significant differences between (KW $\chi^2= 6.43$; df = 1; $P<0.05$; Figure 2b). These findings were also in agreement with an earlier study conducted by Godoy et al., 2012; Mustafa et al., 2022; Caipang et al., 2022.

Turbidity, Total Suspended Solid and Floc Volume

The amount of suspended matter in the water influences its turbidity, or its ability to impede sunlight. High turbidity reduces sunlight penetration into the water and decreases the ponds ability to produce oxygen (Lien and Giao, 2020). The true indicators of biofloc development in a culture operation are FV and TSS (Ray et al., 2010). In our study, the turbidity concentration ranged from 11.21 to 18.5ppm (Figure 2f) and there were significant differences between (KW $\chi^2= 6.81$; df = 1; $P<0.05$), the main reason could be due to shrimp activities, food left and phytoplankton causing increasing turbidity(Zhao et al., 2016). In the current study, the average TSS and floc volume concentration for the bifloc and nursery culture systems varied from 121.5 to 254.6 ppm and 7.8 to 18.4 ml/l, respectively (Figure 2e, f). The statistical analysis also revealed that the water TSS and floc volume was significantly different (KW $\chi^2= 1.62$; df = 1; $P<0.05$ and KW $\chi^2= 6.81$; df = 1; $P<0.05$ respectively). The quantity of phytoplankton in the pond, soil erosion, feed added to the pond, and mechanical movement in the pond all contribute to an increase in total suspended particles (shrimp movement, waves). The findings of this study agreed with those of

Wang et al. (2015), who found that gradually raising the levels of both BFV and TSS in an *L. vannamei* culture system by adding varying amounts of molasses, maize flour, and wheat bran. According to several researches, controlling the C/N ratio or adding other carbon sources may have an impact on biofloc formation and the management of water quality (Burford et al. 2004; Hari et al. 2004). Additionally, these results was consistent with those of a previous investigation by Godoy et al., 2012; Zhao et al., 2016 and Huang et al., 2021.

Chlorophyll

Chlorophyll a (Chl a), which accounts for 1% to 2% of total dry weight of algae, may be used to accurately estimate the amount of microalgae. According to Ju et al. (2008), suggested carbon sources in the biofloc system favour microbial growth over microalgae and using carbon sources by heterotrophic bacteria to manage ammonia in rearing tanks promotes succession and bacterial dominance over microalgae. In the present study, chlorophyll concentration varied between 112.1 and 121.3 $\mu\text{g/l}$. According to the statistical analysis, chlorophyll differed considerably between biofloc and nursery culture (KW $\chi^2= 6.81$; df = 1; $P<0.05$; Figure 2c). Previous studies have demonstrated that microalgae frequently inhabit biofloc. They could live as free cells in the water column, allowing them to congregate even in situations where heterotrophic bacteria were the dominant microbial species (Xu and Pan, 2012; Lien and Giao, 2020). Furthermore, their findings matched those of a previous study by Godoy et al., 2012; Wasielesky et al., 2013 and Panigrahi et al., 2017.

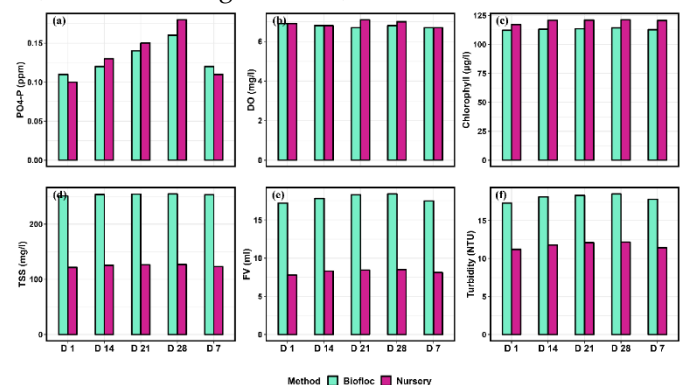


Figure 2. Physicochemical water quality parameters in the biofloc and nursery culture system throughout a 30-day whiteleg shrimp rearing period (a) Phosphate-Phosphorus, (b) Dissolve oxygen, (c) Chlorophyll 'a', (d) Total Suspended Solids (e) Floc volume and (f) Turbidity.

Table 1. Physicochemical parameters (Mean and SD) of the experimental units recorded during the culture period. Different superscript letters in a row indicate significant difference ($p < 0.05$) between treatments.

Parameters	Biofloc (Mean and SD)	Nursery (Mean and SD)
Temperature (°C)	28.92±0.81 ^a	29.62±0.31 ^a
pH	8.36±0.10 ^a	8.04±0.12 ^b
Salinity (ppt)	31.58±0.27 ^a	31.32±0.27 ^a
TAN (ppm)	0.05±0.02 ^a	0.05±0.02 ^a
NO ₂ -N (ppm)	0.12±0.02 ^a	0.15±0.04 ^a
NO ₃ -N (ppm)	2.07±0.14 ^a	2.17±0.09 ^a
PO ₄ -P (ppm)	0.13±0.02 ^a	0.13±0.03 ^a
DO (mg/l)	6.78±0.07 ^b	6.90±0.14 ^a
Chlorophyll a (µg/l)	113.12±0.73 ^b	120.14±1.49 ^a
TSS (mg/l)	253.42±1.22 ^a	124.66±1.99 ^b
FV (ml)	17.84±0.46 ^a	8.23±0.26 ^b
Turbidity (NTU)	18.00±0.42 ^a	11.73±0.37 ^b

Pearson correlation

Pearson correlations in a selected biofloc and nursery culture, the strength and direction of the linear relationship between the water qualities were evaluated. In biofloc culture, the salinity was found to exhibit a positive correlation with chlorophyll, turbidity, ammonia, nitrate, nitrite, phosphate, TSS and FV. Total ammonia showed significant correlation with all the parameters except dissolved oxygen. There is a strong positive relationship of salinity with chlorophyll 'a' and total ammonia with NO₂-N (Figure 3). Among nursery culture system, salinity strong positive relationship of salinity with all the environmental parameters, similarly chlorophyll, turbidity, ammonia, nitrate, nitrite, phosphate, TSS and FV showed significant correlation with all the parameters except temperature and pH (Figure 4). According to statistical studies, nutrients and other chemical components indicated by Mustafa et al. (2022) as having an influence on shrimp growth and survival.

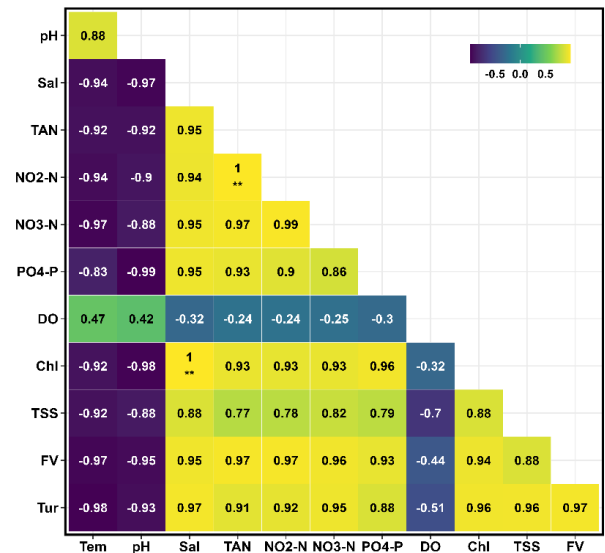


Figure 3. Pearson correlation coefficient between various physicochemical parameters in biofloc

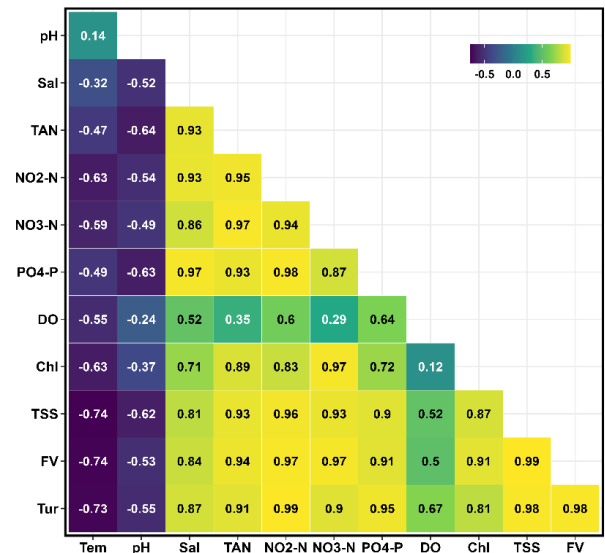


Figure 4. Pearson correlation coefficient between various physicochemical parameters in nursery culture system

Principal Component Analysis

The water quality was assessed using a principal component analysis in comparison to biofloc and nursery culture water. The first two components accounted 87.73% of total variation, with 48.84% and 38.89.15% explained by components 1 and 2, respectively. Temperature, dissolved oxygen, chlorophyll, ammonia, nitrate, nitrite, and phosphate were found to be positively correlated with nursery culture system, whereas other environmental parameters such as pH, salinity, TSS, turbidity and FV were found to be negatively correlated with biofloc culture system. Shrimp production is primarily

influenced by temperature, salinity, pH, and dissolved oxygen, as well as other physical and chemical aspects of water quality (Nguyen et al., 2019). The results in the present study are in accordance with previous investigation by Mustafa et al., (2022).

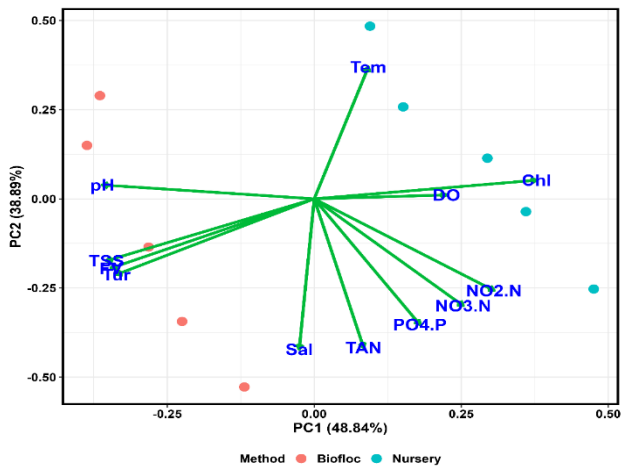


Figure 5. Principal component analysis drawn for the relationship between environmental parameters and culture methods

Growth Performance

There were no significant changes between treatments for any of the shrimp production parameters (Table 2). The survival rates for the biofloc and nursery culture systems were 95.5% and 95%, respectively; the biofloc treatment was quantitatively greater than the nursery culture system. Similarly, in the biofloc treatment, SGR and survival were all quantitatively greater. Many research have shown that biofloc and nursery culture system has a beneficial impact on shrimp culture (Godoy et al., 2012; Panigrahi et al., 2017; Shinn et al., 2018; Hai et al., 2020; Huang et al., 2021; Anand et al., 2022; Caipang et al., 2022). The developed biofloc and nursery in the culture system, according to a number of studies, may enhance growth performance and feed utilization of *L. vannamei* in addition to maintaining good and stable water quality (Xu and Pan, 2012; Luis-Villaseñor et al., 2015; Serra et al., 2015; Khanjani et al., 2016; Tierney and Ray, 2018; Tinh et al., 2021; Chethurajupalli and Tambireddy, 2021).

Table 2. Growth characteristic of *L. vannamei* seeds in the biofloc and nursery culture systems

Growth Parameters	Biofloc	Nursery
Initial weight (g)	0.50±0.1	0.50±0.1
Final body weight (g)	7.5±0.1	7.6±0.2
SGR	0.24±0.01	0.24±0.01
Survival (%)	95.5±0.2	95.0±0.1

4. CONCLUSION

In this study, all treatments produced nearly similar numbers of shrimp. The fact that biofloc systems may require more robust aeration, which might increase cost, is a significant factor for producers to take into account. Overall, while choosing a shrimp nursery culture system, it is important to take into account the potential for quicker shrimp development, the consistency of water quality dynamics, and the prices of the energy and equipment. Due to more thorough filtration, nursery systems had faster bacterial establishment periods and better water quality than biofloc systems. The fact that biofloc systems increased oxygen requirements would necessitate more robust aeration, which could increase costs, is a crucial factor for producers to take into account. Overall, while choosing a system, managers of shrimp nurseries should take into account the possibility for fast shrimp growth, consistency in water quality dynamics, cost of equipment and energy, and other factors. More study is needed to better understand the pathways and processes that govern shrimp feeding physiology, as well as how biofloc and nursery might be regulated to increase shrimp production performance.

Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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