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APPLIED RESEARCH

Water quality management strategies for super-intensive culture of *Penaeus vannamei* with biofloc system: A case study in Virginia, USA

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Abstract

The aim of this case study was to investigate and describe water quality and growth performance of *Penaeus vannamei* produced in a super-intensive biofloc system (BFT) under diverse water conditions (seawater and low-salinity water) in the state of Virginia, USA. Three individual indoor super-intensive grow-out trials were conducted using a stocking density of 500 shrimp m^{-3} at salinities of 23 g L^{-1} for 70 days (TI), 28 g L^{-1} for 69 days (TII), and 2 g L^{-1} for 30 days (TIII). Key water quality management strategies included: (TI) BFT system; (TII) BFT system and probiotic; (TIII) BFT system, probiotic, water reuse, and artificial substrate. TII exhibited the highest mean total ammonia nitrogen (TAN) concentration (2.60 mg L^{-1}), and the greatest variation in TAN levels, which were controlled from day 35 onward. TAN levels remained relatively stable in TIII. TI exhibited the highest mean nitrite concentration (3.66 mg L^{-1}) and the greatest variation, with a spike that was successfully controlled from day 35 onward. In

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contrast, nitrite concentrations in TII displayed a continuous upward trend from day 28 without any indication of stabilization. Nitrite concentrations in TIII remained consistently low, never exceeding 0.5 mg L^{-1} throughout the experiment. Nitrate concentrations exhibited a consistent upward trend across all trials. The final weight of shrimp in TI and TII was 11.03 g and 13.62 g respectively, while in TIII it was 4.86 g. Survival rates were 77.67% in TI, 86.00% in TII, and 87.00% in TIII. Yield values were 4.28, 5.81, and 2.12 kg m^{-3} for TI, TII, and TIII, respectively. Our findings indicated slower nitrification rates in TI and TII compared to TIII. Water reuse, probiotic inoculation, and artificial substrates in TIII resulted in significantly lower and more stable levels of ammonia and nitrite compared to TI and TII, indicating a mature system with improved control for nitrogenous compounds. These findings demonstrate the successful application of integrated BFT strategies, including water reuse, probiotic inoculation, and artificial substrates, in enhancing the super-intensive indoor production of *P. vannamei* using both seawater and low-salinity water. These results have significant implications for the development of shrimp farming in the state of Virginia, USA.

KEYWORDS
artificial substrates, nitrogenous compounds, probiotics, shrimp growth, water reuse

1 | INTRODUCTION

Crustaceans constitute one of the major groups in global aquaculture production, reaching 12.7 million tons in 2022 (FAO, 2024). In the United States, crustacean production corresponded to 76 thousand tons in the same year (FAO, 2024), with seawater shrimp production primarily concentrated in Florida, Hawaii, and Texas (USDA, 2018). Globally, *Penaeus vannamei* dominates crustacean aquaculture production (FAO, 2024), which possess a great adaptability to a wide variation in salinity, temperature, and high stocking densities (Prangnell, Lupatsch, et al., 2019; Roy et al., 2010; Van Wyk et al., 1999). These characteristics, including their adaptability to a wide range of environmental conditions, such as salinity, allow cultivation in low-salinity waters and make *P. vannamei* a suitable candidate for production in closed systems. However, traditional shrimp farming practices have intensified over time, leading to increased organic loading within production systems. This intensification carries significant risks, including heightened susceptibility to disease outbreaks and increased environmental impact due to effluent discharge (Emerenciano et al., 2022; Nguyen et al., 2019). These concerns highlight the need for alternative aquaculture systems. The biofloc system (BFT) represents a promising alternative to traditional systems, offering a more environmentally friendly approach to shrimp production.

BFT is characterized by the fertilization of water with a simple organic carbon source such as molasses or dextrose. This stimulates the growth of heterotrophic bacteria, which in turn assimilate the ammonia released from feed and animal waste (Avnimelech, 2012). Proper BFT system requires maintaining a carbon: nitrogen (C:N) ratio close to 15:1 (Avnimelech, 2012). This management strategy facilitates the growth of microbial aggregates, known as bioflocs, which are composed of bacteria, algae, zooplankton, fungi, and organic matter (Khanjani et al., 2022). This diverse microbial community within the BFT includes chemoautotrophic bacteria, which play a crucial role in nitrification. These bacteria oxidize ammonia and nitrite to nitrate, a less toxic nitrogenous compound for shrimp (Ebeling et al., 2006).

In a scenario where coastal and land prices are steadily increasing (Pimentel et al., 2023), BFT offers several key advantages. These include enhanced biosecurity, reduced land use through high stocking densities, increased production yields, lower water exchange requirements, and the potential for water reuse (Krummenauer et al., 2014; Raza et al., 2024; Silveira, Rosas, Krummenauer, Fróes, et al., 2022). Furthermore, BFT enables indoor shrimp production, providing greater control over environmental variables such as temperature. This allows for year-round production independent of weather fluctuations. The use of BFT, in conjunction with the physiological adaptability of *P. vannamei*, enables shrimp culture in low-salinity water, allowing production in inland regions, closer to consumer markets. However, successful production hinges on effective management practices and market prices consumers are willing to pay.

Key management strategies to enhance nitrogenous compound control and shrimp growth include fertilizing water with organic carbon sources, inoculating with probiotic microorganisms, reusing mature water as inoculum, and using artificial substrates (Bezerra et al., 2024; Krummenauer et al., 2014; Lara et al., 2021; Serra et al., 2015). Probiotic microorganisms such as those of the genus *Bacillus*, in addition to their impact on the health of the host can have significant effects on water quality, as they act to degrade organic matter and remove phosphate from the water (Hlordzi et al., 2020). The use of mature water inoculum provides an initial load of microorganisms that will accelerate nitrogen cycling, avoiding ammonia and nitrite spikes during the culture (Krummenauer et al., 2014). The insertion of artificial substrates into the culture medium provides an area for the establishment of nitrifying bacteria, improving the nitrification process (Lara et al., 2021). It is important to test the effect of these management strategies on seawater and under low-salinity conditions, which could help expand marine shrimp production to regions far from the coast.

Controlling nitrogenous compounds is crucial, particularly in low-salinity culture, as these compounds are toxic to shrimp and can negatively impact culture performance. The establishment of a mature and diverse microbial community within the BFT system facilitates water reuse and effective utilization of artificial substrates over multiple production cycles. Evaluating the effectiveness of these technologies in indoor settings within the United States is essential to promote the adoption of safe and environmentally sustainable BFT-based shrimp farming practices. The aim of this case study was to investigate and describe the water quality dynamics and shrimp growth performance in three independent indoor super-intensive *P. vannamei* grow-out trials, utilizing a BFT with both seawater and low-salinity water, conducted in the state of Virginia, USA.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This study investigated the water quality dynamics and the growth performance of *Penaeus vannamei* in three independent grow-out trials conducted at the Virginia Seafood Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, USA. In each trial, patterns of nitrogenous compounds and growth of *P. vannamei* were investigated in super-intensive systems with BFT. Trial I (TI) was conducted over 70 days with a salinity of 23 g L^{-1}, trial II (TII) lasted 69 days with a salinity of 28 g L^{-1}, and trial III (TIII) was conducted for 30 days using low-salinity water (2 g L^{-1}). Experimental units for TI had a volume of 300 L, while those for TII and TIII had a volume of 100 L. All trials maintained constant aeration (dissolved oxygen >5.00 mg L^{-1}), and temperature (approximately 29°C). A summary of the main characteristics of each trial is presented in Table 1.

2.2 | Experimental conditions

For TI, seawater with a salinity of approximately 23 g L^{-1} was prepared by mixing tap freshwater with artificial sea salt (Instant Ocean, Virginia, USA). This seawater was then treated with 10 g m^{-3} of sodium hypochlorite and subsequently dechlorinated with ClorAm-x (AquaScience Technologies & Research Inc., Missouri, USA). *P. vannamei* postlarvae were obtained from a commercial hatchery (Sea Products Development, Texas, USA) and were reared in a nursery system for 19 days, reaching a mean weight of 0.056 ± 0.003 g (mean ± standard deviation) prior to stocking in experimental tanks. Following a 19-day nursery period, postlarvae were stocked in experimental units at a density of 500 shrimp m^{-3}. The trial started with clear water, and ammonia control was maintained through applications of molasses, following a carbon: nitrogen (C:N) ratio of 15:1 (Avnimelech, 2012; Ebeling et al., 2006). Molasses (Golden Barrel, Pennsylvania, USA) was added only when total ammonia nitrogen (TAN) reached 1 mg L^{-1}. No water exchange was performed throughout the trial.

For TII, seawater with a salinity of 28 g L^{-1} was prepared by mixing tap freshwater with artificial sea salt (Instant Ocean, Virginia, USA). This seawater was subsequently chlorinated with 10 g m^{-3} of sodium hypochlorite and then dechlorinated using ClorAm-x (AquaScience Technologies & Research Inc., Missouri, USA). *P. vannamei* postlarvae were obtained from a commercial hatchery (Homegrown shrimp, Florida, USA) and reared in a nursery system for 30 days, reaching a mean weight of 0.10 ± 0.04 g. Following the nursery phase, postlarvae were stocked in the experimental units at a density of 500 shrimp m^{-3}. The experiment started with clear water, and ammonia control was maintained through the application of dextrose, following a C:N ratio of 15:1 (Avnimelech, 2012; Ebeling et al., 2006). Dextrose (Earthborn elements, USA) was added only when total ammonia (TAN) levels reached 1 mg L^{-1}. Daily probiotic supplementation was implemented throughout the trial. A commercial probiotic powder

TABLE 1 Characteristics of experimental conditions for super-intensive *P. vannamei* grow-out trials with biofloc technology (BFT) system.

Variables	Trials TI	TII	TIII
Key water quality management strategy	BFT system	BFT system and probiotic	BFT system, probiotic, water reuse, and artificial substrate
Phase	Grow-out	Grow-out	Grow-out
Time (days)	70	69	30
Stocking density (shrimp m^{-3})	500	500	500
Initial weight[a]	0.056 ± 0.003	0.10 ± 0.04	1.27 ± 0.06
Number of repetitions	2	2	2
Salinity (g L^{-1})	23	28	2
Carbon source	Molasses	Dextrose	Dextrose
Probiotic (g m^{-3})	N/A	3[b]	0.4[c]
Artificial substrates	N/A	N/A	15 × 15 cm K1 media pillows

Abbreviation: N/A, not available.
[a]Mean values ± standard deviation.
[b]Dosage applied daily.
[c]Dosage applied weekly.

(Sanolife MIC, INVE aquaculture, Dendermonde, Belgium) containing *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus* (5×10^{10} colony forming units (CFU) g^{-1}) was applied daily at a rate of 3 g m^{-3} to both the water column and feed.

For TIII, 100% of the water volume in each experimental unit was reused from a previous *P. vannamei* culture cycle conducted at a salinity of 28 g L^{-1}. This recycled water exhibited the following characteristics of two replicates (mean ± standard deviation): TAN: 1.60 ± 0.42 mg L^{-1}, nitrite nitrogen (NO_2^--N): 37.75 ± 0.99 mg L^{-1}, nitrate nitrogen (NO_3^--N): 180.00 ± 28.28 mg L^{-1}, alkalinity: 160.00 ± 0.00 mg L^{-1}, total suspended solids (TSS): 248.00 ± 22.63 mg L^{-1}, and pH: 7.70 ± 0.00. The salinity of this inoculum was gradually reduced to 2 g L^{-1} over a 13-day period. Each experimental unit was equipped with a 15 × 15 cm pillow containing K1 Kaldnes Biological media, which was used as an artificial substrate to enhance the nitrification process. *P. vannamei* postlarvae were obtained from a commercial hatchery (Homegrown shrimp, Florida, USA) and reared in a nursery system at a salinity of 28 g L^{-1} for 35 days, reaching a mean weight of 1.27 ± 0.06 g. Following the nursery period, shrimp were gradually acclimated to a salinity of 2 g L^{-1} over 7 days and then stocked in the experimental units at a density of 500 shrimp m^{-3}. Dextrose was used as a carbon source to control ammonia levels, applied when TAN concentrations reached 1 mg L^{-1}, following a C:N ratio of 15:1 (Avnimelech, 2012; Ebeling et al., 2006). Water changes of 10% of the tank volume were conducted as needed to maintain TAN levels below 1 mg L^{-1}. Weekly, 0.4 g m^{-3} of Sanolife Mic probiotic (INVE aquaculture, Dendermonde, Belgium), containing *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus*, was applied to the water column.

2.3 | Water quality

In all trials, daily measurements were conducted for temperature (°C), salinity (g L^{-1}), dissolved oxygen (DO, mg L^{-1}measured using a YSI Pro2030 multiparameter probe, Ohio, USA), and pH (measured using a Hanna HI-98107 pH meter, Rhode Island, USA). Weekly measurements were performed for TAN (mg L^{-1}; determined using Hach method 8038), NO_2^--N (mg L^{-1}; determined using Hach method 8507), NO_3^--N (mg L^{-1}; determined using Hach method 8039), TSS (mg L^{-1}; measured using a Hach DR900 portable multiparameter colorimeter), and settleable solids (SS, mL L^{-1}; determined according to Eaton et al., 1995). In TII, due to a sampling error encountered from day 51 onward, NO_2^--N data are presented only up to day 50. Alkalinity (mg L^{-1}; determined according to APHA, 2012), was measured weekly in TI and TII, while in TIII it was analyzed twice a week. When alkalinity values fell below 150 mg L^{-1}, adjustments were made by adding sodium bicarbonate, following Furtado et al. (2011).

2.4 | Feed management and shrimp growth

In TI and II, shrimp were fed three times daily, while in TIII, feeding was administered twice daily. All trials utilized a commercial diet containing 35% crude protein (Zeigler Bros, Pennsylvania, USA). The feed ratio was adjusted weekly according to Jory et al. (2001).

At the conclusion of each trial, shrimp were sampled to determine final weight (g), survival rate (%), feed conversion ratio (FCR), weekly growth rate (WGR, g $week^{-1}$), and yield (Kg m^{-3}).

2.5 | Data analysis

Descriptive statistical analysis was conducted on water quality parameters, including mean, standard deviation, minimum, and maximum values. For shrimp growth data, a descriptive analysis was also performed, and the mean and standard deviation were calculated. The concentration patterns of TAN, NO_2^--N, NO_3^--N, and TSS were presented in graphs, incorporating mean values and standard deviation. Data analysis was performed using R software version 4.3.1 (R core team, 2023) with the following packages: ggplot2 (Wickham, 2016), Rmisc (Hope, 2022), ggbreak (Xu et al., 2021), and ggbump (Sjoberg, 2020).

3 | RESULTS

3.1 | Water quality

Descriptive statistics of water quality variables for trials TI, TII, and TIII are summarized in Table 2. Mean temperature across all trials ranged between 29 and 30°C, and dissolved oxygen (DO) levels consistently exceeded 5 mg L^{-1}. The mean pH in TI was 7.43, while TII and TIII exhibited higher mean pH values exceeding 8.00. Salinity in TI varied between 20 and 27 g L^{-1}, with a mean of 23.56 g L^{-1}. In TIII the mean salinity was 2.20 g L^{-1}.

The mean TAN in TII was 2.60 mg L^{-1}, and in TI was 1.25 mg L^{-1}. In TIII, the mean concentration remained below 1 mg L^{-1} (Table 2). TI exhibited a pronounced TAN spike on day 14, reaching 2.54 mg L^{-1}, which was subsequently controlled within the following weeks (Figure 1a). TII demonstrated the greatest variability in TAN levels, with spike concentrations reaching 4.85 mg L^{-1} on day 14 and 5.13 mg L^{-1} on day 35 (Figure 1a). From day 35 onward, TAN levels in TII began to stabilize, culminating in a concentration of 1.87 mg L^{-1} at the experiment's conclusion (Figure 1a). In contrast, TIII displayed more consistent TAN behavior compared to the other trials. However, two instances of TAN exceeding 1 mg L^{-1} required water exchanges (Figure 1a).

The mean NO_2^--N concentration in TI (3.66 mg L^{-1}) was higher than in the other trials (Table 2). Moreover, TI exhibited greater variability in NO_2^--N levels, culminating in a pronounced spike that reached 11.80 mg L^{-1} on day 35 (Figure 1b). This elevated level was subsequently controlled in the following weeks, with concentrations decreasing to below 0.50 mg L^{-1} between days 48 and 62 (Figure 1b). TII demonstrated a gradual increase in NO_2^--N concentration from day 28, reaching a peak of 4.77 mg L^{-1} on day 50 (Figure 1b). In contrast, TIII maintained consistently low NO_2^--N levels throughout the experimental period, with concentrations remaining below 0.5 mg L^{-1} (Figure 1b).

The mean NO_3^--N concentration was higher in TII (63.33 mg L^{-1}) and TIII (54.80 mg L^{-1}), compared to TI (24.88 mg L^{-1}) (Table 2). In TI, NO_3^--N levels exhibited an increasing trend from day 55 onward (Figure 1c). Similarly, in TII, NO_3^--N concentrations began to rise from day 43. In contrast, TIII demonstrated a consistent upward trend in NO_3^--N levels throughout the experimental period (Figure 1c).

Trial II exhibited the highest mean alkalinity concentration at 198.50 mg L^{-1}, exceeding the values observed in TI and TIII (both above 120 mg L^{-1}) (Table 2). Similarly, TII displayed the highest mean TSS concentration at 214.20 mg L^{-1}, compared to 99.55 mg L in TI and 146.20 mg L^{-1} in TIII (Table 2). In TII, TSS reached peak concentrations of 396.50 mg L^{-1} on day 36 and 366.00 mg L^{-1} on day 43 (Figure 2). In contrast, TI exhibited a maximum TSS concentration of 198.00 mg L^{-1} on day 50 (Figure 2). TIII showed a slight upward trend in TSS, with concentrations increasing from 146.50 mg L^{-1} at the beginning to 167.50 mg L^{-1} at the end of the experiment (Figure 2). Finally, TI demonstrated the highest mean SS concentration at 26.59 mL L^{-1}, while TII and TIII exhibited lower values at 14.67 and 17.70 mL L^{-1}, respectively (Table 2).

3.2 | Shrimp growth

The descriptive statistics of shrimp growth performance in the three trials (TI, TII, and TIII) are presented in Table 3. In TI, the *P. vannamei* final weight was 11.03 g, with a survival rate of 77.67%, WGR of 1.10 g $week^{-1}$, FCR of 1.65,

TABLE 2 Water quality variables during super-intensive grow-out of *P. vannamei* with biofloc technology (BFT) system.

Variables	Trials TI	TII	TIII
Temperature (°C)	29.22 ± 0.97 (28.10–30.80)	30.04 ± 0.94 (26.50–32.80)	29.45 ± 0.27 (29.00–30.00)
DO (mg L^{-1})	6.07 ± 0.28 (5.40–6.70)	5.51 ± 0.27 (4.90–6.20)	6.06 ± 0.27 (5.40–6.60)
pH	7.43 ± 0.45 (6.01–8.44)	8.14 ± 0.21 (7.20–8.40)	8.15 ± 0.22 (7.70–8.60)
Salinity (g L^{-1})	23.56 ± 1.67 (20.80–27.20)	28.00 ± 0.00 (28.00–28.00)	2.20 ± 0.16 (2.10–2.60)
TAN (mg L^{-1})	1.25 ± 0.67 (0.29–3.17)	2.60 ± 1.65 (0.47–5.75)	0.88 ± 0.30 (0.40–1.20)
NO_2^--N (mg L^{-1})	3.66 ± 4.57 (0.01–13.10)	1.12 ± 1.67 (0.02–4.78)	0.20 ± 0.07 (0.12–0.30)
NO_3^--N (mg L^{-1})	24.88 ± 27.39 (0.90–99.10)	63.33 ± 71.15 (0.30–180.00)	54.80 ± 10.25 (45.00–71.00)
Alkalinity (mg L^{-1})	138.80 ± 33.84 (80.00–200.00)	198.50 ± 34.83 (120.00–240.00)	128.90 ± 23.98 (80.00–180.00)
TSS (mg L^{-1})	99.55 ± 55.54 (5.00–204.00)	214.20 ± 117.85 (0.00–464.00)	146.20 ± 30.37 (101.00–185.00)
SS (mL L^{-1})	26.59 ± 29.09 (0.00–101.00)	14.67 ± 16.40 (0.00–60.00)	17.70 ± 9.01 (8.00–30.00)

Note: Data are presented as mean ± standard deviation (minimum–maximum). TI, TII, and TIII represent Trial I, Trial II, and Trial III, respectively.
Abbreviations: DO, dissolved oxygen; NO_2^--N, nitrite nitrogen; NO_3^--N, nitrate nitrogen; SS, settleable solids; TAN, total ammonia nitrogen; TSS, total suspended solids.

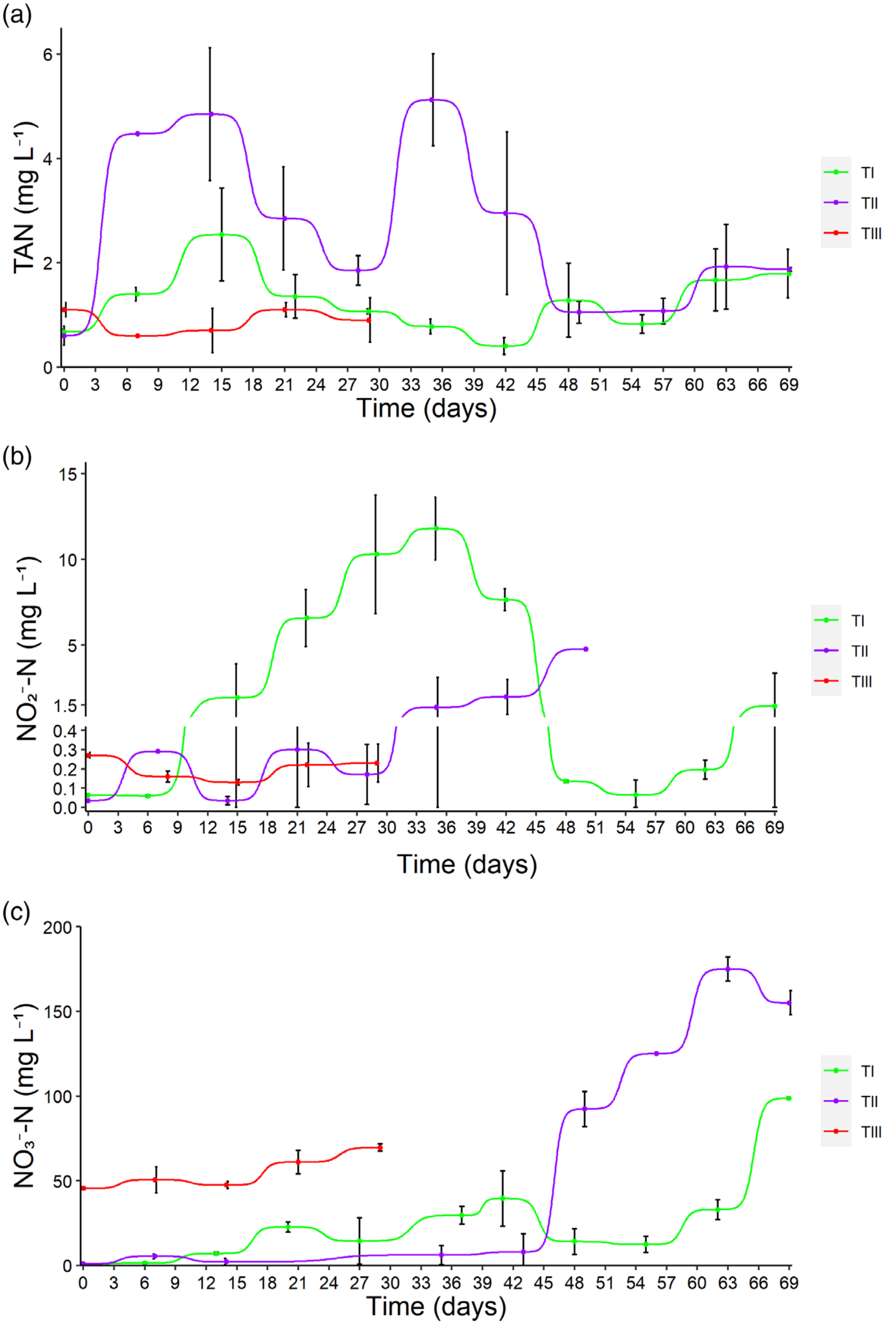

FIGURE 1 Concentration of total ammonia nitrogen (TAN, a), nitrite nitrogen (NO_2^--N, b), and nitrate nitrogen (NO_3^--N, c) during super-intensive grow-out of *P. vannamei* with biofloc technology (BFT) system. TI: Trial I; TII: Trial II; TIII: Trial III.

and yield of 4.28 kg m^{-3}. TII exhibited a final weight of 13.62 g, survival rate of 86.00%, WGR of 1.35 g week^{-1}, FCR of 1.84, and yield of 5.81 kg m^{-3}. In TIII, the final weight was 4.86 g, with a survival rate of 87.00%, WGR of 0.83 g week^{-1}, FCR of 1.62, and a yield of 2.12 kg m^{-3}.

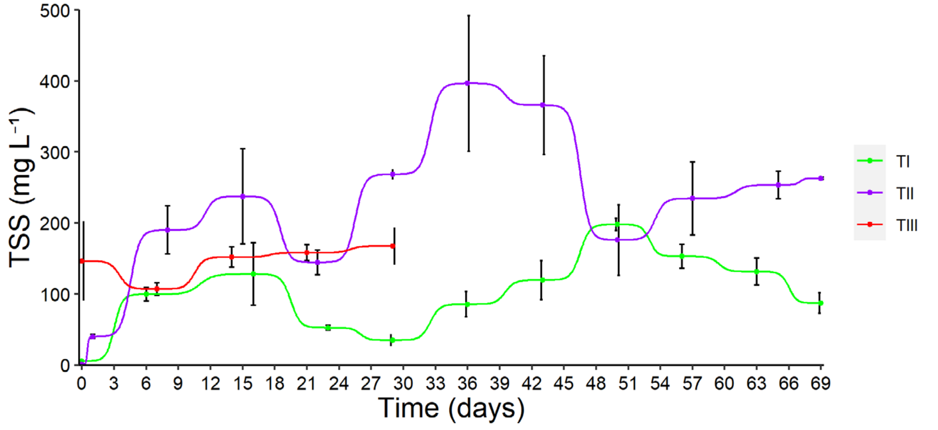

FIGURE 2 Concentration of total suspended solids (TSS) during super-intensive grow-out of *P. vannamei* with biofloc technology (BFT) system. TI: Trial I; TII: Trial II; TIII: Trial III.

TABLE 3 Growth performance of *P. vannamei* at the conclusion of super-intensive grow-out trials with biofloc technology (BFT) system.

	Trials		
Variables	**TI**	**TII**	**TIII**
Initial weight (g)	0.056 ± 0.003	0.10 ± 0.04	1.27 ± 0.06
Final weight (g)	11.03 ± 2.08	13.62 ± 1.72	4.86 ± 0.61
Survival (%)	77.67 ± 1.41	86.00 ± 11.31	87.00 ± 7.07
WGR (g $week^{-1}$)	1.10 ± 0.21	1.35 ± 0.17	0.83 ± 0.14
FCR	1.65 ± 0.28	1.84 ± 0.01	1.62 ± 0.48
Yield (kg m^{-3})	4.28 ± 0.73	5.81 ± 0.03	2.12 ± 0.42

Note: Data are presented as mean ± standard deviation.
Abbreviations: FCR, feed conversion ratio; TI, Trial I; TII, Trial II; TIII, Trial III; WGR, weekly growth rate.

4 | DISCUSSION

The BFT system, characterized by being a closed production system, allows precise control over environmental variables. In the present case study, in all three trials, temperature, dissolved oxygen (DO), and pH remained within the recommended ranges for *P vannamei* culture in BFT systems (Samocha & Prangnell, 2019). Mean alkalinity values across the trials were maintained within the recommended ranges for shrimp farming, exceeding the minimum threshold of 100 mg L^{-1} (Van Wyk et al., 1999). However, fluctuations in alkalinity were observed in TI and especially in TIII, with values occasionally dropping to 80 mg L^{-1}. This phenomenon is likely attributed to the intense metabolic activity of chemoautotrophic bacteria, which utilize the inorganic carbon from alkalinity as a substrate for the oxidation of nitrogenous compounds, consequently depleting alkalinity levels (Ebeling et al., 2006). To counteract this effect and maintain optimal water quality within the closed system, periodic adjustments to alkalinity levels were necessary through the addition of inorganic fertilizers.

The observed patterns of TAN and nitrite concentrations in TI and TII are characteristic of newly established BFT systems. TII exhibited significant fluctuations in ammonia levels, while TI experienced pronounced nitrite fluctuations. These fluctuations can be attributed to the initiation of both cultures with clear water, necessitating the gradual stimulation of microbial growth throughout the experimental period. This gradual establishment of microbial communities, particularly ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), likely contributed to the observed fluctuations. In TI, an initial TAN spike within the first 2 weeks was effectively controlled, however, this caused an accumulation of nitrite for a prolonged period of the trial, which were subsequently controlled from day 35. In TII, the ammonia levels remained elevated until day 35, followed by a subsequent increase in nitrite concentrations. These observations align with findings reported by Reis et al. (2024), who investigated the impact of different green light photoperiods on the water quality parameters during 61 days of the intensive *P. vannamei* rearing (500 shrimp m^{-3}) in a BFT system. Reis et al. (2024) reported complete control of TAN from day 19 and nitrite from day 37 of the culture. Prangnell, Samocha, and Staresinic (2019) observed that in newly established BFT systems, nitrite levels often remain elevated for an extended period compared to ammonia, with complete system maturation typically occurring around 50 days of culture.

In TII, despite the inoculation of probiotic microorganisms belonging to the *Bacillus* genus, a consistent pattern of nitrite control was not observed. The commercial probiotic employed in this trial consisted of diverse species within the *Bacillus* genus, microorganisms well established for their bioremediation capabilities (Hlordzi et al., 2020), including the degradation of organic matter into carbon dioxide and the reduction of nitrogenous compounds and phosphate (Ghosh, 2025). Despite the observed nitrite accumulation in TII, the presence of NOB within the system was evident as indicated by the subsequent accumulation of nitrate. This suggests that the NOB community was not fully established. A plausible explanation for this incomplete NOB establishment lies in the elevated levels of organic matter observed in TII, as evidenced by the increased TSS concentrations. High organic matter loads can exert an inhibitory effect on nitrifying bacteria, thereby slowing down the nitrification process within BFT systems (Abakari et al., 2021).

TIII demonstrated the most effective control of nitrogenous compounds, attributed to the implementation of several key management strategies. These strategies included the reuse of water from previous culture cycles, the inoculation of probiotic microorganisms, and the incorporation of artificial substrates to facilitate the establishment of a robust nitrifying bacterial community within the system. These findings underscore the potential of these integrated management approaches for achieving successful outcomes in intensive *P. vannamei* rearing utilizing BFT systems, particularly in low-salinity environments, such as TIII (salinity 2 g L^{-1}). In low-salinity conditions, the toxicity of nitrogenous compounds, notably ammonia and nitrite, is significantly elevated (Prangnell, Samocha, & Staresinic, 2019), requiring robust control measures to prevent detrimental spikes in their concentration. Krummenauer et al. (2014) demonstrated enhanced ammonia and nitrite removal in *P. vannamei* rearing employing BFT systems at salinity of 31 g L^{-1} and a stocking density of 312 shrimp m^{-3} by reusing water containing mature bioflocs at percentages varying between 25 and 100% of tank volume. Similarly, Moura et al. (2023) reported effective nitrogenous compound control in intensive *P. vannamei* rearing at salinity of 5 g L^{-1} by inoculating the system with mature bioflocs at a concentration of 100 mg L^{-1} of TSS. Water reuse in the BFT system associated with the use of brackish groundwater or estuarine waters allows shrimp farmers to maintain high salinity levels without relying on artificial salt supplementation. Artificial salt can be an expensive addition in closed systems, especially when access to marine water is limited. Understanding the effects of maintaining low-salinity levels in the production system can assist farmers in reducing costs associated with artificial salts, thereby enhancing their market competitiveness.

The use of artificial substrates significantly enhances the growth of nitrifying bacteria within the BFT system by providing an increased surface area for microbial colonization (El-Sayed, 2021). Lara et al. (2021) demonstrated that the inclusion of artificial substrates at 200% and 400% of the tank's lateral surface area significantly improved nitrite control compared to a control group lacking substrates in *P. vannamei* rearing with BFT system at a stocking density of 500 shrimp m^{-3}. Beyond their positive influence on water quality, artificial substrates offer several advantages. They increase the effective tank area mitigating stress associated with high stocking density and facilitating the control of suspended solids (Ferreira et al., 2016; Schveitzer et al., 2013; Silveira, Rosas, Krummenauer, Poersch, & Wasielesky, 2022). The combined application of artificial substrates, probiotic microorganisms, and water reuse positively impacted shrimp growth performance in TII and TIII, as evidenced by favorable final weights, survival rates, and yields. Probiotics supplementation enhances shrimp health by colonizing the intestinal tract, thereby improving feed digestibility and, consequently, promoting growth (Dawood et al., 2019). Naik and Reddy (2020) observed improved final weight, yield, and water quality in *P. vannamei* rearing utilizing BFT systems supplemented with probiotics (*Bacillus licheniformis* and *Lactobacillus rhamnosus*). Furthermore, the presence of artificial substrates enhances shrimp growth by increasing the availability of natural food sources (Ferreira et al., 2016).

The varying durations of the trials, particularly the shorter duration of TIII, may limit direct comparisons of final weight and other growth parameters. Nonetheless, the findings of this case study provide valuable insights into the establishment of intensive *P. vannamei* culture systems, which can be readily applied to the development of aquaculture activities within the state of Virginia, USA. Shrimp production utilizing BFT systems offers several advantages, including the production of high-quality products while minimizing environmental impact. The system's focus on enhancing productivity while reducing environmental impacts aligns with the principles of sustainable aquaculture development (Bossier & Ekasari, 2017).

5 | CONCLUSION

The use of an indoor BFT system with seawater or low-salinity water, coupled with high stocking densities, presents a promising alternative for shrimp production development. The findings of this case study demonstrated that the integration of several key management strategies within BFT system, including the inoculation of probiotic microorganisms, the reuse of water, and utilization of artificial substrates effectively controls toxic nitrogenous compounds without compromising the growth of *P. vannamei* during the grow-out phase.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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