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Nitrogenous compounds and alkalinity patterns in *Penaeus vannamei* nurseries and pre-grow-out with low salinity water and synbiotic system: a case study

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Abstract
This study aimed to evaluate the effect of different management strategies on the toxic nitrogen compounds and alkalinity patterns in nurseries and pre-grow-out of *Penaeus vannamei* culture with low salinity water (2.5 g L^{-1}) and synbiotic system. A study case is presented using three individual experiments (E): two nurseries (EI and EII: 2000 shrimp m^{-3} or 600 shrimp m^{-2}) and one pre-grow-out (EIII: 300 shrimp m^{-3} or 90 shrimp m^{-2}). Key water quality management strategies included the adoption of artificial substrate and water reuse with a microbial-based synbiotic system. In EI experiment, mean TAN was less than 0.50 mg L^{-1} throughout the culture, NO_2^--N reached a maximum concentration of 0.80 mg L^{-1}, and NO_3^--N showed a decrease over the time. In EII, mean TAN was 0.64 mg L^{-1} with a peak on day 10 of the experimental course, with the same pattern occurring for NO_2^--N. On the other hand, NO_3^--N constantly increased from 0.10 mg L^{-1} at the beginning to 2.32 mg L^{-1} at the end of the trial. In EIII, mean TAN was less than 0.50 mg L^{-1} reaching a maximum concentration of 0.93 mg L^{-1}; NO_2^--N reached a maximum mean concentration of 0.70 mg L^{-1} at the end of the experiment. NO_3^--N had a mean of 1.50 mg L^{-1} at the beginning of the experiment and a reduction to 0.53 mg L^{-1} at the end of the trial. Alkalinity showed mean concentrations of 114.90 mg L^{-1}, 88.04 mg L^{-1}, and 88.27 mg L^{-1} for EI, EII, and EIII, respectively. The results demonstrated that the proposed management protocols adopted in EI, EII, and EIII were efficient for toxic nitrogen control in culture conditions using low salinity water and synbiotic system.

Keywords Oligohaline water · Fermentation · Nitrification · Substrate · Inoculum · Water reuse

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Introduction

In 2020, crustaceans' production in inland waters was 4.48 million tons, representing 8.2% of the total inland world aquaculture production (FAO 2022). Shrimp *Penaeus vannamei* is the most cultured crustacean species in inland waters, being produced in regions far from the coast in several countries around the world, such as Brazil, Thailand, Mexico, Ecuador, and China (including Gobi Desert Northern China, considered one of the most remote in the world) (Nunes and López 2001; Boyd and Thunjai 2003; Roy et al. 2010; FAO 2020). The interests in *P. vannamei* production in inland waters is mainly due to key biological and physiological characteristics of the species, including the broad osmoregulatory capacity that allows *P. vannamei* to be produced in environments with salinity ranging from 0.5 to 45 g L^{-1} (Roy et al. 2010) and adapt to high stocking densities (Samocha 2019).

Currently, synbiotic and biofloc are microbial-based systems that utilize external organic carbon source to boost heterotrophic microorganisms and played a key role in intensive *P. vannamei* farm operations (e.g., water quality management and pathogens exclusion) (Khanjani et al. 2023). These approaches combined with lined ponds, temperature control using greenhouses, solid waste control, and artificial water salinization can help the system's consistency and efficiency (Venero et al. 2009; Kawahigashi 2018; Samocha 2019; Galkanda-Arachchige et al. 2020). The combination of these approaches may increase shrimp yields, even though with pathogen coexistence (El-Sayed 2021), and allow *P. vannamei* farming in inland areas close to large cities (Emerenciano et al. 2022). However, shrimp production in intensive systems with minimal water exchange increases mortality risk due to the greater toxicity of nitrogenous compounds [i.e., un-ionized ammonia (NH_3) and nitrite (NO_2^-)], especially in cultures using low salinity water (Esparza-Leal et al. 2016; Valencia-Castañeda et al. 2019).

This high toxicity of nitrogen compounds at low salinity is related to osmoregulatory processes in crustaceans. The most toxic ammonia nitrogen form is NH_3, as it is transported easily through the animal's gill membranes (Romano and Zeng 2013). Nitrite (NO_2^-) is an intermediate compound resulting from the biological oxidation of ammonia by the nitrifying chemoautotrophic bacteria (e.g., *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*), which use bicarbonate ions (HCO_3^-) and oxygen (O_2) to oxidize these compounds to obtaining energy (Samocha 2019). This compound is also toxic to shrimp, and according to Chen and Chen (1992), the increased concentration of nitrite in the water can lead to a reduction of the growth and impact the molting frequency of the animals. Furthermore, changes on osmoregulatory processes can affect ammonia excretion rate, as well as affect the production of enzymes that transport ions through the shrimp body (Jensen 2003; Abakari et al. 2021).

In marine environments, the issues due to high nitrite concentrations are less recurrent, due to higher concentrations of chloride ion (Cl^-), which competitively inhibits NO_2^- uptake by shrimp (Tomasso 2012). Thus, in environments where there is a low concentration of Cl^- ion (e.g., water with low salinity), there is a greater absorption of NO_2^- through the gills, causing an increase in the concentration of NO_2^- in the animals' hemolymph (Sowers et al. 2004; Tomasso 2012). Controlling the concentration of nitrogenous compounds is one of the key management strategies to ensure water quality maintenance in an intensive culture condition with minimal water exchange for shrimp (Samocha 2019).

Therefore, the use of microbial-based systems helps to control these nitrogen compounds through the heterotrophic and chemoautotrophic bacteria activities, which act by

assimilating total ammonia nitrogen (TAN) and converting into microbial biomass, and by the conversion of NH_3 and NO_2^- into a less toxic form (NO_3^-), respectively (Ebeling et al. 2006; Khanjani et al. 2023). Recently, strategies to control nitrogenous compounds such as the use of artificial substrates (Schveitzer et al. 2013a) and water reuse (Krummenauer et al. 2014) have been tested for shrimp in a high salinity environment in BFT with promising results. In this sense, considering the lack of studies evaluating the use of different strategies to control nitrogenous compounds in nurseries and pre-grow-out in low salinity water, more studies are needed to better comprehend the water quality dynamics in these particular conditions. Therefore, the aim of this study was to describe the patterns of nitrogen compounds and alkalinity using a case study, which included three individual experiments in two different phases of *P. vannamei* culture (nursery and pre-grow-out) using low salinity water and synbiotic system.

Materials and methods

Experimental design

The case study was carried out at Laboratório de Carcinicultura (LACAR), Universidade Federal Rural de Pernambuco (UFRPE), Brazil. Three experiments evaluating the patterns of nitrogenous compounds and alkalinity were carried out in the following phases: (EI) nursery phase for 35 days; (EII) nursery phase for 40 days; and (EIII) pre-grow-out phase for 56 days, both with low salinity water (salinity 2.5 g L^{-1}) and synbiotic system. Experimental units with 60 L of useful volume and 0.2 m^2 of bottom area were used, with constant aeration (dissolved oxygen > 5 mg L^{-1}). A summary of the main characteristics of each experiment is described in Table 1.

For both experiments, *P. vannamei* post-larvae (PL) were acclimated to a salinity close to 2.5 g L^{-1}. In EI and II, PL24 (9.24 ± 1.38 mg and 10.31 ± 2.97 mg, respectively) were used. In these experiments, shrimp were stocked at a density of 2000 PL m^{-3} (600 PL m^{-2}). In EIII, juveniles (0.41 ± 0.01 g) were stocked at a density of 300 shrimp m^{-3} (90 shrimp m^{-2}).

Experimental conditions

The water used in the experiments was obtained from a mixture of seawater with a salinity of 35 g L^{-1} and tap freshwater up to a salinity of 2.5 g L^{-1}. This water was subsequently filtered (30 µm) and chlorinated (13 g m^{-3}). After, the water was fertilized using methodology adapted from Romano et al. (2018). The fertilizer was obtained from a mixture of

Table 1 Characteristics of *Penaeus vannamei* nurseries and pre-grow-out cultures using low salinity water where nitrogenous compounds and alkalinity patterns were analyzed

Experiment	Phase	Time (days)	Culture key features	Number of repetitions
EI	Nursery	35	Synbiotic system, artificial substrate	9
EII	Nursery	40	Synbiotic system, artificial substrate, water reuse	12
EIII	Pre-grow-out	56	Synbiotic system, artificial substrate, water reuse	9

20 g m^{-3} of rice bran, 2 g m^{-3} of molasses/sugar, 0.50 g m^{-3} of commercial bacteria blend [(6.5×10^7 colony forming units g^{-1}) composed of *Bacillus subtilis*, *B. licheniformis*, *Bacillus* sp., enzymes, sodium chloride (NaCl), and magnesium hydroxide ($Mg(OH)_2$)] (Kayros Agrícola e Ambiental, Brazil), 4 g m^{-3} of sodium bicarbonate, 0.25 g m^{-3} of baker yeast (*Saccharomyces cerevisiae*), and water at salinity close to 2.5 g L^{-1} in a proportion of 10 times the amount of rice bran. The fertilizer solution was submitted to fermentation for 24 h (anaerobic condition) and respiration for 24 h (aerobic condition), and then applied into the experimental units. The fertilizer solution was applied daily 24 days prior to stocking in EI, and 7 days (also daily) prior to stocking in EII and EIII. After stocking, the solution was applied three times per week in both EI, EII, and EIII, and it was suspended when the level of settleable solids reached 5.0 mL L^{-1}.

As part of the culture protocol, in EII, a mature microbial-rich inoculum from a previous culture cycle and representing 15% of the tank volume was used to aid the water (microbial) maturation process. The inoculum utilized had the following conditions: TAN = 0.49 ± 0.17 mg L^{-1}, NO_2^--N = 0.70 ± 0.40 mg L^{-1}, NO_3^--N = 0.81 ± 0.32 mg L^{-1}, total alkalinity = 88.33 ± 13.23 mg $CaCO_3$ L^{-1}, and pH = 7.74 ± 0.05. Likewise, in EIII, a 20% inoculum from a previous cycle was used with the following conditions: TAN = 0.40 ± 0.86 mg L^{-1}, NO_2^--N = 0.41 ± 0.19 mg L^{-1}, NO_3^--N = 12.18 ± 8.47 mg L^{-1}, total alkalinity = 140.60 ± 12.36 mg $CaCO_3$ L^{-1}, and pH = 7.76 ± 0.04. Additionally, in all experiments as a part of the culture protocol, artificial substrates composed by *Anomalocardia brasiliana* shells were added into the experimental units to assist the development of the nitrifying bacteria community. Shells were arranged on "pillows-type" device (25 cm × 24 cm × 5 cm—width × height × depth) made of high-density 4-mm hole polyethylene mesh, occupying a bottom area of 28.12% and representing 3.36% of the useful volume of each experimental unit (Fig. 1).

Feed management

During EI (40% crude protein, 11% lipids, 4% crude fiber, and 14% ash; Guabitech Inicial, Guabi, Brazil) and EII (45% crude protein, 9.5% lipids, 4% crude fiber, and 12% ash; ADM Animal Nutrition Company), animals were fed with the commercial feed 4 times a day (8:00 am, 11:00 am, 2:00 pm, and 5:00 pm). In the experiments EI and EII, the amount of

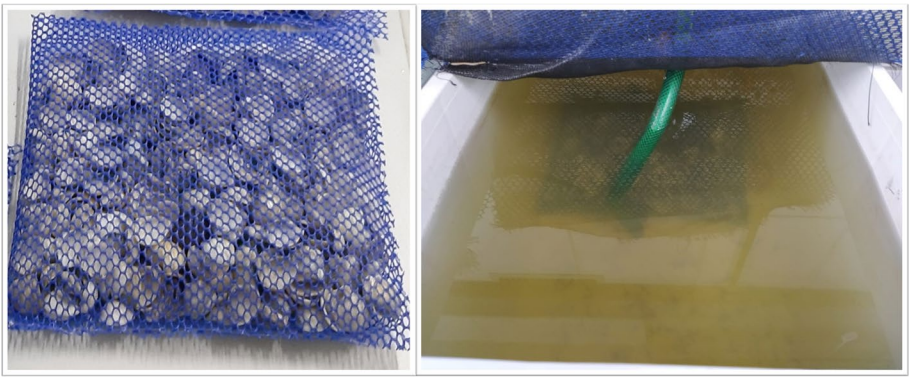

Fig. 1 Substrate model inserted in the experimental units during *Penaeus vannamei* culture using low salinity water and synbiotic systems

feed offered to the shrimp was calculated following Van Wyk et al. (1999). In EIII, shrimp were fed with commercial feed (35% crude protein, 10% lipids, 4% crude fiber, and 14% ash; Guabitech Active, Guabi, Brazil) 3 times a day (8:00 am, 12:00 pm, and 4:00 pm), and the amount of feed offered was calculated following Garza de Yta et al. (2004).

Carbon:nitrogen (C:N) ratio

The C:N ratio was estimated from the total amount of organic carbon and nitrogen source (rice bran, molasses/sugar, and feed) used to experimental units and feed the animals during the trial period. The organic carbon content of the feed and rice bran was estimated from the following equation (Hart et al. 2007):

$$Organic\ carbon = (0.53 \times protein) + (0.8 \times lipids) + (0.42 \times fibre) + (0.42 \times nitrogen\ free\ extract) \quad (1)$$

The total N input was estimated considering the N content in the feed crude protein (16%; Samocha 2019) and the percentage that is available in the system (75%; Avnimelech 2012). The amount of total N was estimated from the following equation (Samocha 2019):

$$N(g) = amount\ of\ feed(g) \times \%\ CP \times \%\ N \quad (2)$$

where:

% CP Feed crude protein percentage (decimal value).
% N Nitrogen (N) percentage in crude protein (decimal value).

Analysis of crude protein, lipids, moisture, ash, and fiber contents of rice bran and molasses was performed in triplicate using standard methods (AOAC 2016). Rice bran used had 41.8% organic carbon and 3.46% nitrogen, sugar had 39.2% organic carbon (Sugar CHO 99%), and molasses had 30% organic carbon and 0.06% nitrogen.

Water quality

During the experimental period in the three experiments, water temperature (°C) and water dissolved oxygen (DO; mg L^{-1}) were monitored daily (YSI 556 MPS, Yellow Springs, OH, USA). TAN, NO_2^--N, NO_3^--N, and total alkalinity (mg L^{-1}) were measured once a week (Fries 1971; APHA 2005, 2012). Settleable solids (SS, mL L^{-1}; Eaton et al 1995) were analyzed three times a week.

Data analysis

Descriptive statistics was performed for production performance and water quality variables with means, standard deviation, and maximum and minimum values. Fluctuations over time of TAN, NO_2^--N, NO_3^--N, and total alkalinity were presented with means and standard deviation. Graphics were made using R software version 4.2.3 (R Core Team 2023) with ggplot2 (Wickham 2016), ggbreak (Xu et al 2021), and Rmisc (Hope 2022) packages.

Results

Production performance

Production performance descriptive statistics are presented in Table 2. In EI, mean final weight was 0.43 g, mean survival was above 80%, FCR equal to 1.83, and final yield was 0.74 Kg m^{-3} (Table 2). In experiment EII, mean final weight was 1.16 g, survival was higher than 90%, FCR was 0.97, and yield was 2.13 Kg m^{-3} (Table 2). Regarding experiment EIII, mean final weight was 4.35 g, survival was higher than 60%, FCR was 1.26, and final yield was 0.93 Kg m^{-3} (Table 2).

Water quality

Water quality descriptive statistics for EI are presented in Table 3. During the experimental period, temperature was maintained at 29.38 °C, with maximum and minimum values of 29.72 °C and 29.13 °C, respectively. Mean DO was 6.16 mg L^{-1}, with maximum and minimum values of 6.60 mg L^{-1} and 5.90 mg L^{-1}, respectively.

Mean TAN concentration reached the maximum concentration of 0.39 mg L^{-1} on day 35 of the experimental time (Fig. 2). Mean NO_2^--N concentration reached 0.41 ± 0.19 mg L^{-1} on day 35 of the experiment (Fig. 3). Mean NO_3^--N was between 30.70 ± 22.35 at the beginning of the experimental time and 11.80 ± 8.40 mg L^{-1} at the end of the experiment (Fig. 4).

Initial total alkalinity was 100.00 ± 7.07 mg $CaCO_3$ L^{-1}, and at the 35th day of the experimental time was 140.60 ± 12.36 mg $CaCO_3$ L^{-1} (Fig. 5). Mean settleable solids was 8.30 mL L^{-1} throughout the experiment (Table 3).

In EII, water temperature was maintained at 30.83 ± 0.17 °C and mean DO was at 5.72 ± 0.05 mg L^{-1}. Maximum and minimum values for temperature and DO were 31.24 °C and 30.65 °C, and 5.79 mg L^{-1} and 5.64 mg L^{-1}, respectively. A TAN increase was observed in the first 10 days of culture, reaching a mean concentration of 1.48 ± 0.22 mg L^{-1} and stabilizing in the following weeks at mean levels of 0.39 ± 0.26 mg L^{-1} (Fig. 2). Regarding NO_2^--N, an increase in the first 10 days of culture was observed, reaching a concentration of 0.99 ± 0.37 mg L^{-1} and stabilizing in the following weeks at mean concentration of 0.38 ± 0.10 mg L^{-1} (Fig. 3). NO_3^--N concentration in EII showed a gradual increase during the experimental time, with a mean of 0.10 ± 0.27 on day 0 of the experimental time and reaching a concentration of 2.32 ± 1.17 mg L^{-1} after 40 days of culture (Fig. 4).

Table 2 *P. vannamei* growth performance in nurseries (EI and EII) and pre-grow-out (EIII) using different strategies to control nitrogenous compounds in low salinity water during 35, 40, and 56 days, respectively

	EI	EII	EIII
Initial weight (mg)	9.24 ± 1.38	10.31 ± 2.97	417.00 ± 10.00
Final weight (g)	0.43 ± 0.09	1.16 ± 0.05	4.35 ± 0.47
Survival (%)	85.37 ± 5.32	91.74 ± 4.18	68.52 ± 17.12
FCR	1.83 ± 0.38	0.97 ± 0.06	1.26 ± 0.10
Yield (Kg m^{-3})	0.74 ± 0.16	2.13 ± 0.13	0.93 ± 0.31

Data are mean ± standard deviation. *EI*, experiment I; *EII*, experiment II; *EIII*, experiment III. *FCR*, feed conversion ratio

Table 3 Water quality variables in *P. vannamei* nurseries (EI and EII) and pre-grow-out (EIII) using different strategies to control nitrogenous compounds in low salinity water during 35, 40, and 56 days, respectively

Variables	EI	EII	EIII
Temperature (°C)	29.38 ± 0.15 (29.72–29.13)	30.83 ± 0.17 (31.24–30.65)	30.56 ± 0.80 (34.50–28.50)
DO (mg L^{-1})	6.16 ± 0.22 (6.60–5.90)	5.72 ± 0.05 (5.79–5.64)	6.28 ± 0.35 (7.30–5.32)
Salinity (g L^{-1})	2.60 ± 0.10 (2.81–2.43)	2.49 ± 0.19 (2.78–2.22)	2.78 ± 0.20 (3.51–2.36)
pH	7.90 ± 0.13 (8.33–7.75)	7.97 ± 0.09 (8.09–7.80)	7.34 ± 0.34 (8.03–6.42)
TAN (mg L^{-1})	0.20 ± 0.44 (2.47–0.00)	0.64 ± 0.53 (1.81–0.00)	0.23 ± 0.18 (0.93–0.04)
NO_2^--N (mg L^{-1})	0.23 ± 0.16 (0.80–0.00)	0.52 ± 0.33 (1.60–0.20)	0.23 ± 0.24 (1.39–0.01)
NO_3^--N (mg L^{-1})	19.55 ± 15.90 (62.44–0.00)	1.21 ± 1.16 (4.26–0.00)	0.62 ± 0.56 (2.26–0.00)
Total alkalinity (mg L^{-1})	114.90 ± 20.22 (160.00–75.00)	88.04 ± 18.63 (120.00–55.00)	88.27 ± 14.10 (115.00–55.00)
SS (mL L^{-1})	8.30 ± 3.55 (20.00–1.20)	2.69 ± 2.11 (6.61–0.41)	6.27 ± 4.28 (20.00–0.50)

Data are mean ± standard deviation (maximum–minimum). *EI*, experiment I; *EII*, experiment II; *EIII*, experiment III. *DO*, dissolved oxygen; *TAN*, total ammonia nitrogen; *NO_2^--N*, nitrite nitrogen; *NO_3^--N*, nitrate nitrogen; *SS*, settleable solids

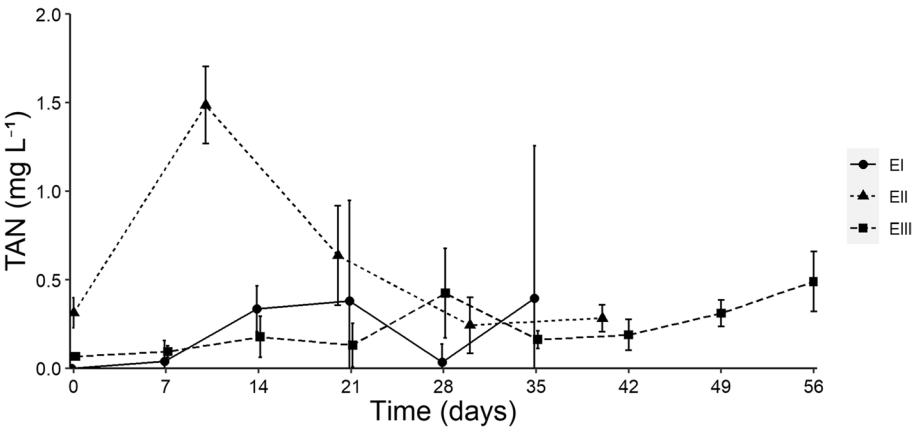

Fig. 2 Total ammonia nitrogen (TAN) concentration during *P. vannamei* nurseries (EI and EII) and pre-grow-out (EIII) using different strategies to control nitrogenous compounds in low salinity water during 35, 40, and 56 days, respectively. EI, experiment I; EII, experiment II; EIII, experiment III

Total alkalinity at the beginning of the culture period was 85.00 ± 0.00 mg $CaCO_3$ L^{-1}, and at the end with a mean total alkalinity of 61.67 ± 6.51 mg $CaCO_3$ L^{-1} (Fig. 5) and mean settleable solids in 2.69 ± 2.11 mL L^{-1}.

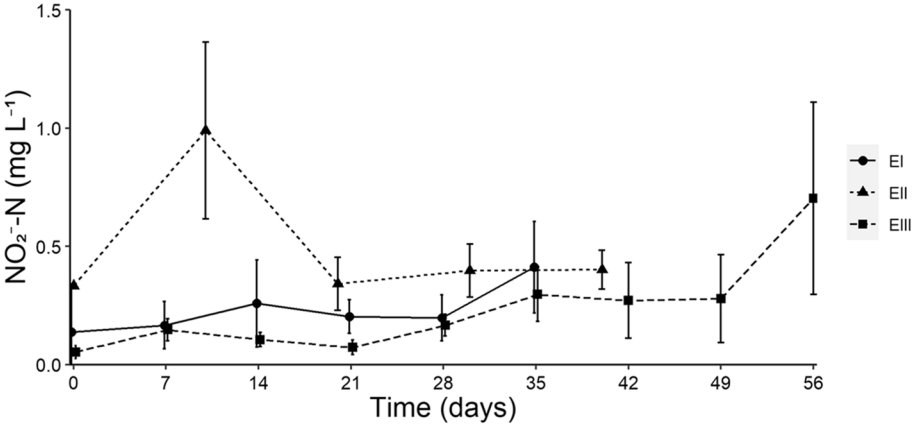

Fig. 3 Nitrite nitrogen (NO_2^--N) concentration during *P. vannamei* nurseries (EI and EII) and pre-grow-out (EIII) using different strategies to control nitrogenous compounds in low salinity water during 35, 40, and 56 days, respectively. EI, experiment I; EII, experiment II; EIII, experiment III

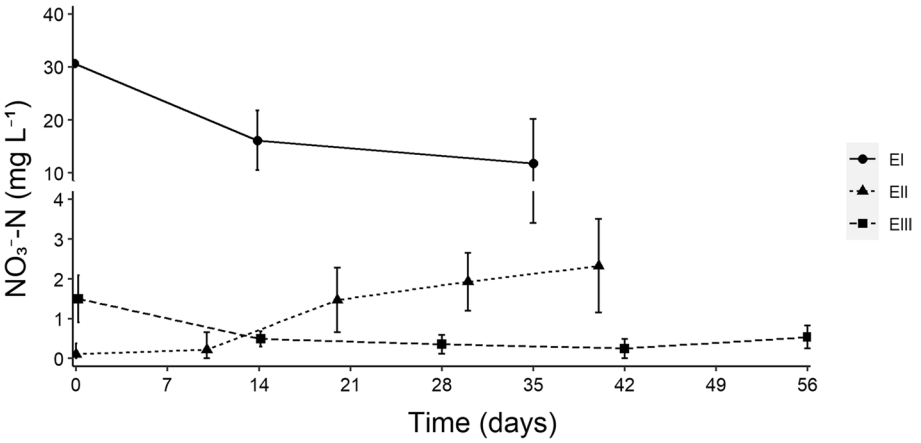

Fig. 4 Nitrate nitrogen (NO_3^--N) concentration during *P. vannamei* nurseries (EI and EII) and pre-grow-out (EIII) using different strategies to control nitrogenous compounds in low salinity water during 35, 40, and 56 days, respectively. EI, experiment I; EII, experiment II; EIII, experiment III

Water quality descriptive statistics for EIII are in Table 3. During the experimental period, water temperature was maintained at 30.56 ± 0.80 °C and mean dissolved oxygen was at 6.28 ± 0.35 mg L^{-1}. Maximum and minimum values for temperature and DO were 34.50 °C and 28.50 °C and 7.30 mg L^{-1} and 5.32 mg L^{-1}, respectively.

Mean TAN concentration was 0.06 ± 0.01 mg L^{-1} on day 0 and gradually increased during the experiment, reaching 0.49 ± 0.17 mg L^{-1} at the end of the experimental time (Fig. 2). Initial NO_2^--N mean concentration was 0.05 ± 0.03 mg L^{-1}, reaching a mean concentration of 0.70 ± 0.40 mg L^{-1} on day 56 of the trial (Fig. 3). Mean NO_3^--N was between 1.50 ± 0.59 mg L^{-1} at the beginning and 0.53 ± 0.29 mg L^{-1} at the end of the experimental time (Fig. 4). Mean total alkalinity was 60.00 ± 4.33 mg $CaCO_3$ L^{-1} at the

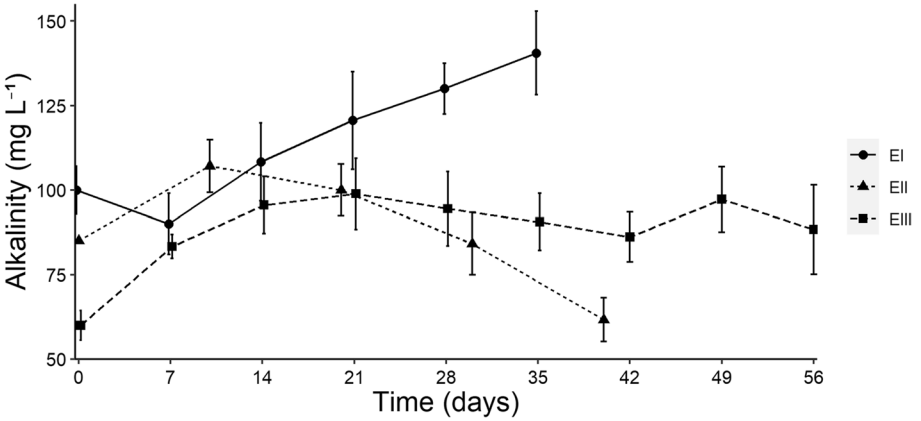

Fig. 5 Alkalinity during *P. vannamei* nurseries (EI and EII) and pre-grow-out (EIII) using different strategies to control nitrogenous compounds in low salinity water during 35, 40, and 56 days, respectively. EI, experiment I; EII, experiment II; EIII, experiment III

beginning of culture, ending the experiment with total alkalinity of 88.33 ± 13.23 mg $CaCO_3$ L^{-1} (Fig. 5) and a mean settleable solids in 6.27 ± 4.28 mL L^{-1}.

C:N ratio

The C:N ratio of experiments EI, EII, and EIII were 7.76:1, 6.81:1, and 7.56:1, respectively (Table 4). Amounts of carbon and nitrogen supplied to the systems during culture time are summarized in Table 4.

Discussion

The literature is scarce regarding studies that demonstrate nitrogenous compounds and alkalinity fluctuation in different phases of shrimp culture in low salinity waters and using synbiotic system. This case study is quite unique as it explored both nursery and pre-grow-out phases, showing that the protocol used (including artificial substrates and inoculum addition) was effective in controlling toxic nitrogenous compounds in this singular culture condition (low salinity and synbiotics). For the three experiments carried out, an efficient nitrification process was observed as per Ray et al. (2011). This can be verified due to the maturation of the system in limited water exchange conditions with an overall NO_3^--N concentration higher than NO_2^--N and TAN concentrations, especially towards the end

Table 4 Composition of the feed, molasses/sugar, and rice bran (C and N) and calculated C:N ratio of experiments EI, EII and EIII

Experiment		C (feed + molasses/sugar + rice bran)	N (feed + rice bran)	C:N
EI	g	327.10	42.13	7.76
EII	g	608.75	89.44	6.81
EIII	g	280.17	37.06	7.56

of the culture period (Lara et al. 2017; Ferreira et al. 2016). The proper establishment of the nitrifying and heterotrophic bacterial community can be attributed due to the protocol applied, which combined the following strategies: (i) use of synbiotic system, (ii) use of artificial substrate, and (iii) use of initial inoculum in EII and EIII (15–20%).

Furthermore, Xu et al. (2016) observed a proper control of nitrogenous compounds with C:N ratios between 9 and 15:1 in minimal water exchange shrimp culture with high salinity (24 to 26 g L^{-1}). In addition, Da Silva et al. (2021) using a synbiotic system (fertilization with rice bran submitted to fermentation processes and respiration by probiotic microorganisms) in high salinity (32 g L^{-1}) and maintaining a C:N ratio close to 10:1 managed to keep TAN < 0.5 mg L^{-1} and NO_2^--N < 1.0 mg L^{-1} throughout the entire experimental period. In our experimental set-up, the use of *A. brasiliana* shell substrate provided a greater surface area for the growth of nitrifying bacteria, improving nitrification process during all the experiments. The incorporation of artificial substrates in intensive culture conditions (e.g., using biofloc technology) improved water quality and supported shrimp growth (Lara et al. 2021). Such strategy can provide a complementary surface area, for example, varying between 200 and 400% of the lateral area of the tank (Ferreira et al. 2016; Lara et al. 2021), boosting the nitrifying community development and assist with the control of toxic TAN and NO_2^--N.

The water reuse from other culture cycles in EII and EIII proved to be an efficient complementary strategy to control toxic nitrogenous compounds during the experimental time. These results were corroborated by Krummenauer et al. (2014), who tested the use of different percentages of inoculum in intensive *P. vannamei* culture using biofloc technology and found that a percentage between 25 and 100% of inoculum provides better nitrifying bacteria development and, consequently, better TAN and NO_2^--N control in the system. Santos et al. (2019) testing smaller volumes of inoculum (10%) managed to maintain mean TAN and NO_2^- at 0.04 mg L^{-1} and 0.14 mg L^{-1}, respectively, and the NO_3^- at 25.06 mg L^{-1}. These results indicate the use of this strategy can contribute to the system's nitrification process and stabilization of toxic nitrogenous compounds.

High concentrations of nitrogenous compounds are toxic to aquatic animals, negatively influencing the animal's performance such as growth, survival, and food intake, enabling changes in the metabolic responses and immune system (Jiann-Chu and Chi-Yuan 1991; Hargreaves 1998; Ebeling et al. 2006; Ray et al. 2011; Cui et al. 2017). TAN and NO_2^--N concentrations were kept within the estimated safe level for intensive shrimp culture using low salinity water where TAN should be kept < 0.81 mg L^{-1} and NO_2^--N < 0.45 mg L^{-1} (Lin and Chen 2001; Gross et al. 2004; Valencia-Castañeda et al. 2018). Peaks in TAN (1.48 mg L^{-1}) and NO_2^--N (0.99 mg L^{-1}) concentrations observed on the 10th day of culture in EII can be attributed to the feed supply with a high percentage of CP (45%) at the beginning of the trial. This occurred even when using the strategy of reducing daily feed intake (i.e., 25% reduction in the amount of feed supplied) to reduce TAN production in the system. In aquaculture environments, the major source of N input comes from the feed supplied (Hargreaves 1998) since CP contains approximately 16% of N in its composition (Samocha 2019). The increase in TAN and NO_2^--N concentrations in some periods occurred likely due to the nitrifying bacteria community that was not yet fully established in the system. As an example, Esparza-Leal et al. (2016) cultured *P. vannamei* at different salinities in a biofloc system with a 25% inoculum and no water exchange found issues in the nitrification process. The authors observed an accumulation of NO_2^--N on the 14th day of culture in treatments with salinity 2 and 4 g L^{-1}, resulting in high mortality rates.

In the EI experiment, NO_3^--N showed a trend to decrease over the experimental period. This pattern could have occurred through the process of denitrification or uptake by algae.

These variations in NO_3^--N patterns over time commonly occur in microbial-based intensive systems due to variations on the water microbial profile and amount of nutrients supplied (Robles-Porchas et al. 2020; Abakari et al. 2021). Alkalinity in EI remained at concentrations above 100 mg $CaCO_3$ L^{-1}, gradually increasing over time. This trend can be explained by the presence of *A. brasiliana* shells in the system, which are constituted of $CaCO_3$ that may have been hydrolyzed and released in the water column (Gosling 2003). Another possible explanation is the occurrence of the denitrification process within the substrate. This similar alkalinity recovery pattern was also observed by Schveitzer et al. (2013b) in a *P. vannamei* culture with different levels of solids in BFT system. On the other hand, in EII and EIII, there was a reduction in alkalinity during the culture period. This reduction can be explained by (i) the use of inoculum strategy and (ii) quicker establishment of the nitrifying community in EII and EIII, with a greater consumption of $CaCO_3$ by the microbial community (Santos et al. 2019), and by the shrimp, especially in higher stocking densities and biomass (Boyd and Tucker 1998; Ebeling et al. 2006).

In intensive shrimp farming systems, maintaining alkalinity below 100 mg L^{-1} and pH below 7 negatively affects water quality conditions and, consequently, animal performance (Furtado et al. 2011). Furtado et al. (2015) found suitable nitrification rates and growth performance in high salinity culture conditions with alkalinity above 150 mg $CaCO_3$ L^{-1}. Under these conditions, there was a minor pH variation throughout the experimental period. The efficiency in the oxidation process of nitrogen compounds in intensive systems is closely related to proper alkalinity levels, as chemoautotrophic bacteria use the inorganic carbon present in the $CaCO_3$ molecule for their growth (Ebeling et al. 2006). It is estimated that to consume 1 g of NH_4^+-N, 7.05 g of alkalinity is consumed (as $CaCO_3$; Ebeling et al. 2006).

Finally, the strategies used to manage toxic nitrogen compounds in low salinity water produced significant effects on shrimp growth. The growth results achieved in this study were superior to those found by other studies for shrimp culture with low salinity water (Maicá et al. 2014; Esparza-Leal et al. 2016; Zacarias et al. 2019). In high salinity conditions, De Lima et al. (2021) using a synbiotic nursery system reached a final weight of 1.13 g and survival of 92.67% (control treatment); and Brito et al. (2018) using a biofloc system for 42 days reached a final weight of 3.94 g, survival of 90%, and FCR of 1.49 (control treatment). Furthermore, we can assume that our findings are comparable to intensive *P. vannamei* culture using higher salinities.

Conclusion

The management of toxic nitrogen compounds in *P. vannamei* nursery and pre-grow-out conditions, especially with low salinity waters, can be a challenge in several commercial shrimp operations. Our study demonstrated that strategies such as synbiotic system, water inoculum from previous cycles (15–20%), and use of artificial substrate proved to be viable tools for the control of toxic nitrogenous compounds without compromising shrimp growth in different phases of culture using intensive system with low salinity water.

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Data availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate The research undertaken complies with the current animal welfare laws in Brazil. *Penaeus vannamei* used in this experimental work does not need approval from the Ethics Committee for Animal Use in Brazil. All the authors agree to participate in this experiment.

Human and animal ethics The authors followed international and institutional animal management guidelines for the experiments.

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