

PRE-NURSERY OF SHRIMP POST-LARVAE REARED IN BIOFLOC SYSTEM UNDER DIFFERENT STOCKING DENSITIES

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ABSTRACT

This study evaluated different stocking densities during pre-nursery of Pacific white shrimp post-larvae (PL) reared in a biofloc system. The tanks (60 L) were stocked with PL stage 5 (PL 5) under five densities (80, 100, 120, 140 and 160 PLs L⁻¹), in triplicate, resulting in 15 experimental units. PLs were fed nine times a day using commercial feed. Molasses was added in all treatments four times a day at an average carbon: nitrogen ratio of 14.7: 1. The experiment was carried out until the PLs reached PL 20 stage, and during this time, water quality variables, survival, weight gain and survival to salinity stress were all evaluated. For treatments above 100 PLs L⁻¹, total suspended solids were higher than recommended (700 mg L⁻¹). Also, the treatment with 160 PL L⁻¹ had higher total ammonia nitrogen peaks (>10 mg L⁻¹), resulting in lower survival in this treatment. No differences were observed between treatments in the other performance parameters evaluated (final weight and survival to salinity stress). It was concluded that pre-nursery of Pacific white shrimp can be performed using densities up to 140 post-larvae L⁻¹ in a biofloc system without compromising shrimp growth performance.

Key words: *Litopenaeus vannamei*; post-larvae; stress test; sustainability.

PRÉ-BERÇÁRIO DO CAMARÃO EM BIOFLOCOS SOBRE DIFERENTES DENSIDADES DE ESTOCAGEM

RESUMO

O estudo avaliou diferentes densidades de estocagem no pré-berçário de camarão-branco-do-pacífico em sistema de bioflocos. Tanques (60 L) foram estocados com pós-larva estágio 5 (PL 5) em cinco densidades (80, 100, 120, 140 e 160 PLs L⁻¹), em triplicata, resultando em 15 unidades experimentais. PLs foram alimentados nove vezes ao dia usando ração comercial. O melão foi adicionado em todos os tratamentos quatro vezes ao dia em uma proporção média de carbono: nitrogênio de 14,7: 1. O experimento foi conduzido até que as PLs atingissem o estágio PL 20, nesse período, a qualidade da água, sobrevivência, ganho de peso e sobrevivência ao estresse salino foram avaliados. Para tratamentos acima de 100 PLs L⁻¹, o total de sólidos suspensos foi superior ao recomendado (700 mg L⁻¹). Além disso, o tratamento com 160 PL L⁻¹ apresentou maiores níveis de nitrogênio amoniacal total (> 10 mg L⁻¹), resultando em menor sobrevivência neste tratamento. Não houve diferença nos demais parâmetros de desempenho avaliados (peso final e sobrevivência ao estresse salino) entre os tratamentos. Concluiu-se que o pré-berçário de camarão-branco-do-pacífico pode ser realizado utilizando densidades de até 140 pós-larvas L⁻¹ em sistema de bioflocos sem comprometer o desempenho zootécnico do camarão.

Palavras-chave: *Litopenaeus vannamei*; pós-larva; teste de estresse; sustentabilidade.

INTRODUCTION

Shrimp farming is strongly affected by low temperatures, limiting growth and survival of cultured organisms during the colder months in subtropical regions (Peixoto Junior et al., 2003; Barbieri et al., 2016; Pontinha et al., 2018). In tropical regions, cycles can be repeated, allowing production throughout the year; however, in subtropical regions, low temperatures may restrict shrimp farming to periods between six and eight months per year (Peixoto Junior et al., 2003; FAO, 2009; Krummenauer et al., 2010; Barbieri et al., 2014).

The use of limited water-exchange production systems in greenhouses, such as that employed in biofloc technology (BFT), is an alternative to increase the period

of farming in subtropical regions, reducing water exchange and minimizing heat loss (McAbee et al., 2003; Arnold et al., 2009; Crab et al., 2009; Li et al., 2009). The intensive production of shrimp post-larvae has been gaining more attention worldwide, with the potential to improve production in aquaculture through the application of a transitional nursery system. This system can be defined as an intermediate step between hatchery and shrimp farms, thereby improving production mainly by increasing survival rates by producing post-larvae more resistant to environmental variations (Samocha et al., 2002).

The technology for intensive production of juvenile shrimp is already successful. This type of crop has high stocking density. However, a well-known negative correlation exists between shrimp performance and stocking density (Wyban and Sweeney, 1991; Sandifer and Hopkins, 1996; Moss and Moss, 2004; Naranjo-Paramo et al., 2004; Li et al., 2007; Arnold et al., 2009). Shrimps stocked at high densities generally grow less and have a lower survival than shrimps stocked at low densities. This reduction is a result of increased competition for food and space and also for cannibalism events (Krummenauer et al., 2006; Arnold et al., 2006). On the other hand, it is known that low densities do not favor biofloc formation, while higher densities can accelerate the bacterial stabilization process (McIntosh et al., 2001).

Therefore, the objective of this study was to evaluate the effect of stocking density on water quality variables and shrimp post-larvae performance in the pre-nursery phase of a biofloc system.

MATERIAL AND METHODS

Biological material

The *Litopenaeus vannamei* larval lineage utilized in the experiment was free of any pathogens that would require notification of the International Organization of Epizootics (Aquatec LTDA, Rio Grande do Norte, Canguarateda, Brazil). First, nauplii were raised in a 20 m³ semi-cylindrical hatchery tank at a stocking density of 100 larvae L⁻¹, using seawater at salinity of 35 g L⁻¹, until reaching post-larvae stage 5 (PL 5). The microalga *Chaetoceros muelleri* (5×10^6 cells mL⁻¹) was added daily in the tanks culture. When post-larvae reached PL 5 stage, they were transferred to the experimental units, which were initially filled with water from the hatchery tank.

Experimental conditions

Post-larvae were reared under five treatments with different stocking densities (80, 100, 120, 140 and 160 PLs L⁻¹). The experimental tanks were filled with water from the previous autotrophic larval hatchery with the following parameters: *Chaetoceros muelleri* (5×10^6 cells mL⁻¹), oxygen = 5.7 mg L⁻¹, pH = 8.2, temperature = 29.2 °C, salinity = 35.0 g L⁻¹, total ammonia nitrogen = 0.2 mg L⁻¹, free ammonia = 0.02 mg L⁻¹, nitrite = 0.00 mg L⁻¹, nitrate = 0.00 mg L⁻¹, phosphate = 0.001 mg L⁻¹, total suspended solids (TSS) = 267.4 mg L⁻¹, volatile suspended solids (VSS) = 59.0 mg L⁻¹ and alkalinity = 125.3 mg L⁻¹.

The experiment was conducted until PLs reached the PL 20 stage, fifteen days after stocking. During the experimental period, water was not exchanged, but rather replaced with fresh water owing to evaporation. No suspended solids were removed from the water during the experiment.

PLs were fed nine times a day (0800, 1000, 1200, 1400, 1600, 1800, 2100, 2300, and 0300), using microencapsulated commercial diets (INVE) based on the manufacturer's recommendation for each stage (Table 1).

Artificial substrate (Needlona® Renner PE 251 Black: 100% polyester fiber, 250 g m⁻² weight, 1.4 mm thickness, density of 0.18 g cm⁻³) was added to the experimental units. Substrates were fixed in rigid PVC frames used for attachment to the rearing tanks. Six substrates (0.16 m² each) were uniformly distributed in each tank in order to reach 100% of the tank area (0.89 m²). They were fixed vertically in the water column, 5 cm below the surface and 10 cm above the tank bottom (Rezende et al., 2018).

Water fertilization

The rearing tanks were filled with 54 liters of water at 35 g L⁻¹ and inoculated with six liters of the microalga *Chaetoceros muelleri*, resulting in the density of 5×10^6 cells mL⁻¹ for each tank. Later, molasses was added at carbon: nitrogen (C: N) 14.7: 1 (Avnimelech, 1999). Fertilization with organic carbon was done in two ways. First, the amount of carbohydrate necessary to neutralize the ammonia excreted by shrimp was estimated assuming that shrimp would absorb about 25% of the dietary nitrogen, and that 75% of this nitrogen would be converted into ammonia released in the water (total ammonia). Carbon sources were added to each tank at a rate of 20 g carbohydrate per gram of total ammonia formed. Second, when total ammonia exceeded 1.0 mg L⁻¹, molasses was added at a ratio of 20 g carbohydrate for each 1.0 mg of total ammonia (Avnimelech, 1999).

Chemical and physical variables of the water

Dissolved oxygen, temperature (YSI 55, YSI Incorporated, Yellow Springs, OH, USA) and pH (YSI 100, YSI Incorporated, Yellow Springs, OH, USA) were measured twice a day. Salinity (YSI 30, YSI Incorporated, Yellow Springs, OH, USA), nitrite and total ammonia nitrogen were analyzed daily (APHA, 2005). Alkalinity (APHA, 2005-2320 B), TSS and VSS were assessed every two days (APHA, 2005-2040 D and 2005-2540 E) using 0.6 µm glass fiber microfilters (GF-6, Macherey-Nagel, Düren, Germany).

Two hundred milliliters of water were collected from each tank. Samples were frozen until nitrate (HACH method 8039, cadmium reduction) and orthophosphate analysis. TAN, nitrite, nitrate and orthophosphate analyses were carried out using a spectrophotometer following Strickland and Parsons (1972), as well as guidelines contained in APHA (2005).

Table 1. Inputs of feed, molasses and the resulting C:N ratio in tanks of Pacific white shrimp (PL 5 to 20) reared during 15 days in the pre-nursery phase of a biofloc system under different stocking densities (80, 100, 120, 140 and 160 post-larvae L⁻¹).

Input (g)	Days															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
80	Diet	2.2	2.2	2.2	2.2	2.2	2.2	2.6	2.6	2.6	2.6	3.2	3.2	3.2	3.2	3.7
	Mol.	4.4	4.4	4.4	4.4	4.4	4.4	5.3	5.3	5.3	5.3	6.5	6.5	6.5	6.5	7.5
	+ Mol.	0.0	0.6	2.1	1.6	7.6	0.7	4.5	9.2	2.6	6.9	9.6	1.7	4.3	3.3	1.6
	C/N	14.7	15.4	17.2	16.6	23.1	15.6	19.4	23.4	17.5	21.5	22.2	16.2	18.3	17.5	17.0
100	Diet	2.7	2.7	2.7	2.7	2.7	2.7	3.2	3.2	3.2	3.2	4.0	4.0	4.0	4.0	4.6
	Mol.	5.5	5.5	5.5	5.5	5.5	5.5	6.5	6.5	6.5	6.5	8.1	8.1	8.1	8.1	9.3
	+ Mol.	0.0	0.8	1.3	3.8	4.8	4.9	5.5	4.6	5.8	3.4	2.9	1.5	4.7	3.4	1.6
	C/N	14.7	15.6	16.1	18.6	19.5	19.6	19.3	18.6	19.5	17.6	22.7	15.8	17.9	17.1	15.7
120	Diet	3.2	3.2	3.2	3.2	3.2	3.2	3.9	3.9	3.9	3.9	4.9	4.9	4.9	4.9	5.5
	Mol.	6.5	6.5	6.5	6.5	6.5	6.5	7.9	7.9	7.9	7.9	9.9	9.9	9.9	9.9	11.1
	+ Mol.	0.0	0.5	1.8	4.0	6.0	5.3	6.0	9.8	4.0	7.2	14.4	1.4	5.8	5.8	1.3
	C/N	14.7	15.2	16.3	18.1	19.7	19.1	18.9	21.2	17.6	19.6	22.1	15.5	17.9	17.9	15.3
140	Diet	3.9	3.9	3.9	3.9	3.9	3.9	4.5	4.5	4.5	4.5	5.7	5.7	5.7	5.7	6.4
	Mol.	7.9	7.9	7.9	7.9	7.9	7.9	9.1	9.1	9.1	9.1	11.5	11.5	11.5	11.5	12.9
	+ Mol.	0.0	0.5	4.7	3.9	6.3	9.8	3.4	11.4	3.8	8.1	22.0	0.5	5.3	5.8	3.3
	C/N	14.7	15.0	18.0	17.5	19.0	21.2	16.8	21.2	17.0	19.5	24.0	14.9	17.3	17.5	16.1
160	Diet	4.3	4.3	4.3	4.3	4.3	4.3	5.1	5.1	5.1	5.1	6.5	6.5	6.5	6.5	7.3
	Mol.	8.7	8.7	8.7	8.7	8.7	8.7	10.3	10.3	10.3	10.3	13.1	13.1	13.1	13.1	14.8
	+ Mol.	0.0	0.0	1.8	6.2	6.1	16.9	1.0	13.1	6.3	7.8	28.1	0.9	5.1	6.9	7.5
	C/N	14.7	14.7	15.8	18.6	18.5	21.4	15.2	21.3	18.1	18.8	24.9	15.1	16.9	17.6	17.6

Total ammonia nitrogen (TAN) was kept around 1.0 mg L⁻¹ in the water by adding sugarcane molasses (mol.) when this limit was exceeded (+ mol. = + molasses). PLs 5 to 13 (day 1 to 9) were feed with EPAC PL, from PL 14 to 16 (day 10 to 12), a diet containing 25% / 75% (Day 10), 50% / 50% (11) and 75% / 25% (day 12) of EPAC PL and XL, respectively. From PL 17 to 20 (day 13 to 15), EPAC XL feed was used. Composition of the diets: EPAC PL (100 to 300µm) and EPAC XL (300 to 600µm) - Minimum crude protein (45%), maximum moisture (10%), maximum crude fiber (3.0%), maximum mineral matter (15%), ether extract min (7.0%), maximum calcium (2.2%), low calcium (1.0%), low phosphorus (1.0%).

Growth performance and stress survival

Every day, 10 PLs from each tank were analyzed at macro- and microscopic levels to assess larval quality. We observed the following parameters: swimming activity, lipid reserves and color of hepatopancreas, intestinal contents, deformities, presence of epibionts, adhered particles, muscle necrosis and opacity, and survival to salinity stress (%) (FAO, 2009). Performance parameters evaluated were final survival (%) and final wet weight (mg).

Statistical analysis

A one-factor ANOVA, followed by Newman-Keuls test (Zar, 1984), was used to compare treatments at a significance level of 0.05. Shapiro-Wilk and Levene's tests assessed normality and homoscedasticity, respectively (Zar, 1984). Data expressed as a percentage underwent angular transformation before analysis.

Total ammonia nitrogen levels over time were analyzed by one-way ANOVA with repeated measures. Treatment was considered the main factor and rearing time the additional factor. Significant differences were analyzed by a Newman-Keuls test (Zar, 1984) with a significance level of 0.05.

RESULTS

Temperature and dissolved oxygen were kept in 29.4 ± 0.2 and 5.7 ± 0.2 in all treatments, respectively. The pH was maintained between 8.2 and 8.3 for all treatments, and it was significantly different among treatments and days of cultivation. Salinity was maintained 35.4 ± 0.2 , and it was significantly different just between the days of cultivation (Table 2).

Alkalinity were significantly different among treatments and days, increased in all groups during the experiment (Figure 1). Treatments with higher stocking density presented higher alkalinity. However, on the last day of rearing, a reduction in alkalinity was observed in the treatment with stocking density of 140 and 160 post-larvae L⁻¹ (Figure 1). Orthophosphate was significantly different among days of cultivation.

Total ammonia nitrogen (TAN) was significantly different among treatments, days and the interaction between these factors, increased in all groups during the experiment. TAN increased on day 10 of the experiment in all treatments (Figure 2), and the tanks stocked with 160 post-larvae L⁻¹ presented the highest levels (9.53 ± 4.27 mg L⁻¹), while toxic ammonia (N-NH₃) reached 1.03 mg L⁻¹. From day 11 of rearing, ammonia spikes began to

Table 2. Water quality variables in pre-nursery tanks of Pacific white shrimp reared during 15 days under different stocking densities (80, 100, 120, 140 and 160 post-larvae L⁻¹) in a biofloc system.

Parameter	Treatments (pl L ⁻¹)					Valor - p		
	80	100	120	140	160	p - T	p - D	p - D x T
Alkalinity (mg L ⁻¹)	266.4 ± 4.9 ^a	279.5 ± 10.9 ^b	304.5 ± 10.0 ^b	343.5 ± 18.5 ^c	353.1 ± 18.0 ^c	<0.001	<0.001	0.439
pH	8.3 ± 0.1 ^a	8.3 ± 0.1 ^a	8.4 ± 0.1 ^b	8.2 ± 0.1 ^a	8.3 ± 0.1 ^a	0.006	<0.001	0.207
Salinity (g L ⁻¹)	35.0 ± 0.3	35.6 ± 0.1	35.4 ± 0.2	35.8 ± 0.1	35.4 ± 0.2	0.804	0.003	0.116
PO ₄ ³⁻ (mg L ⁻¹)	0.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.834	0.020	0.129
TSS (mg L ⁻¹)	372.1 ± 26.7 ^a	391.7 ± 31.9 ^a	467.7 ± 36.1 ^b	509.1 ± 28.2 ^b	581.3 ± 22.5 ^c	<0.001	<0.001	<0.001
N-NH _{3,4} (mg L ⁻¹)	1.9 ± 0.1 ^a	1.9 ± 0.1 ^a	2.3 ± 0.2 ^{ab}	2.6 ± 0.2 ^{bc}	2.9 ± 0.5 ^c	0.003	<0.001	0.013
N-NO ₂ (mg L ⁻¹)	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3154	<0.001	0.784
N-NO ₃ (mg L ⁻¹)	0.5 ± 0.4	0.6 ± 0.5	0.5 ± 0.7	1.1 ± 1.9	1.6 ± 1.8	0.774	0.017	0.716

Mean ± standard deviation data (maximum and minimum). ANOVA with repeated measurements, T (treatment), D (days), T x D (interaction between treatment and days). Means in the same column followed by different letters indicate significant difference between treatments by Newman-Keuls test (p < 0.05).

Table 3. Zootechnical parameters in pre-nursery tanks of Pacific white shrimp reared during 15 days under different stocking densities (80, 100, 120, 140 and 160 post-larvae L⁻¹) in a biofloc system.

Parameters	Treatments (pl L ⁻¹)					Valor - p
	80	100	120	140	160	
Survival (%)	95.67 ± 2.89 ^a	89.67 ± 3.51 ^{ab}	86.33 ± 2.31 ^b	90.00 ± 2.00 ^{ab}	55.00 ± 7.94 ^c	<0.0001
Final wet weight (mg)	8.67 ± 0.29	9.33 ± 0.58	8.67 ± 1.53	9.67 ± 0.58	9.33 ± 1.53	0.7011
Salinity stress survival (%)	95.33 ± 1.15	97.33 ± 1.15	92.67 ± 4.16	94.00 ± 4.00	96.67 ± 1.15	0.2399

Mean values ± SD, n = 3. One-way ANOVA. Means in the same row followed by different letters indicate significant difference between treatments by Newman-Keuls test (P < 0.05).

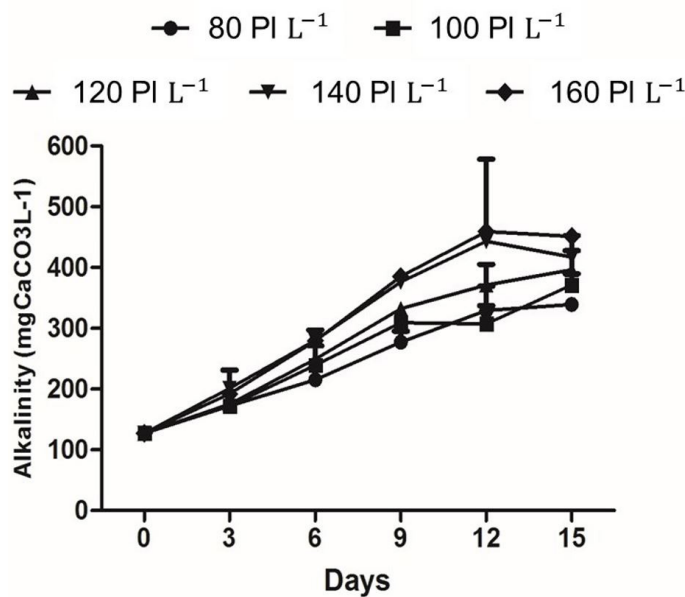


Figure 1. Alkalinity in pre-nursery tanks of Pacific white shrimp reared during 15 days under different stocking densities (80, 100, 120, 140 and 160 post-larvae L⁻¹) in a biofloc system.

decrease. In the present study, it was possible to observe the presence of nitrite in the treatment with 140 post-larvae L⁻¹ as soon as the sixth day of rearing, well before it appeared in other treatments. In other treatments the presence of nitrate was only observed on the ninth day of rearing (Figure 3).

TSS was significantly different among treatments, days and the interaction between these factors. The treatments presented variation according to the stocking density such that increased density correlated with increased TSS in the water (Table 2). TSS presented gradual growth during the culture (Figure 4). TSS was similar between treatments up to the sixth day of rearing, followed by differentiation from the ninth day.

Survival was higher in tanks with lower stocking density, but no significant difference was observed between treatments with 100 and 140 post-larvae L⁻¹ (Table 3). In tanks with higher stocking densities, e.g., 160 post-larvae L⁻¹, shrimp survival rate was lower (Table 3).

During the experiment, larval quality was not different among treatments, given not performed statistically, only through observation. All larvae were active (high swimming activity), had lipid reserves, normal hepatopancreas color and full intestines. We found no deformities, epibionts, adhered particles, or muscular opacity and necrosis. Survival to salinity stress and final weight did not differ statistically among groups (Table 3).

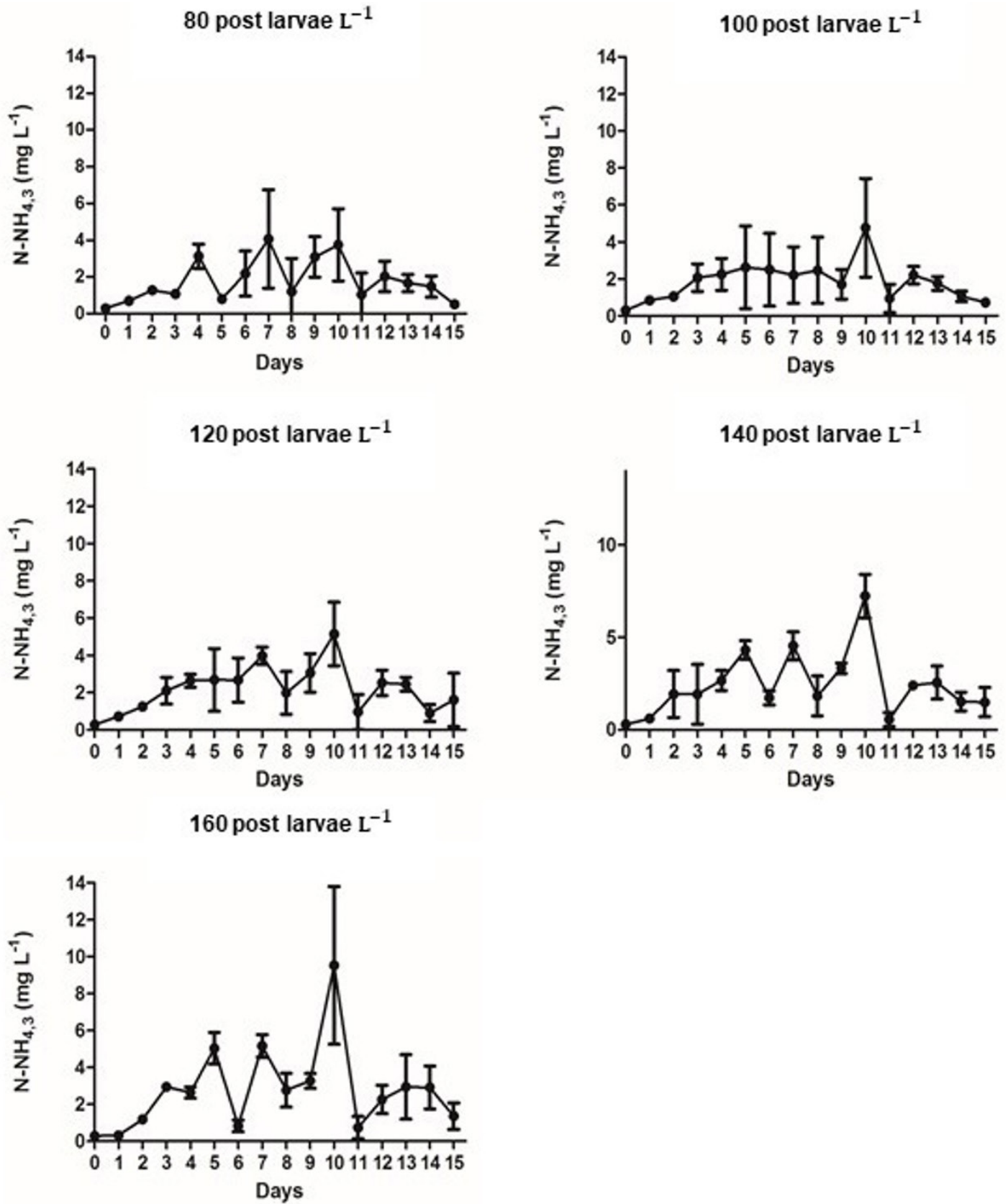


Figure 2. Total ammonia nitrogen (TAN) in pre-nursery tanks of Pacific white shrimp reared during 15 days under different stocking densities (80, 100, 120, 140 and 160 post-larvae L⁻¹) in a biofloc system.

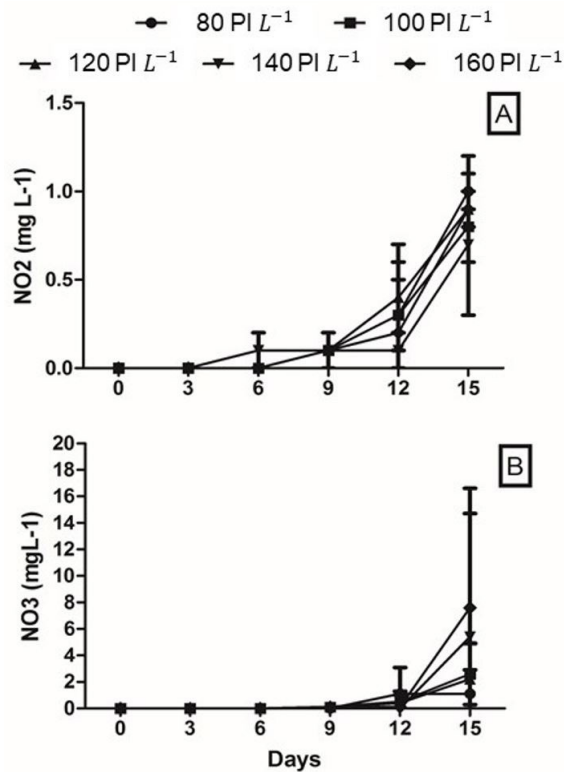


Figure 3. Nitrite (A) and nitrate (B) in pre-nursery tanks of Pacific white shrimp reared during 15 days under different stocking densities (80, 100, 120, 140 and 160 post-larvae L⁻¹) in a biofloc system.

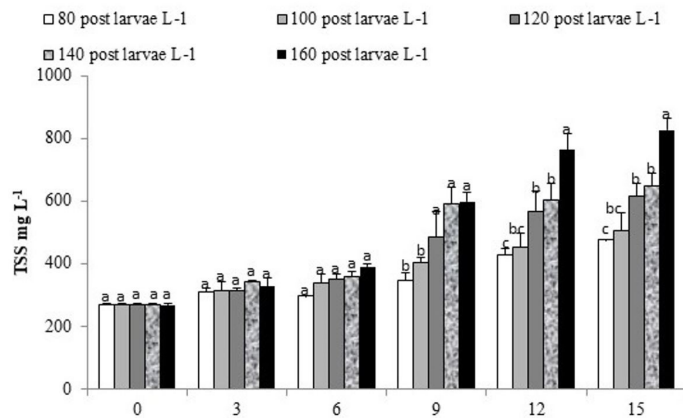


Figure 4. Total suspended solids - TSS in pre-nursery tanks of Pacific white shrimp reared during 15 days under different stocking densities (80, 100, 120, 140 and 160 post-larvae L⁻¹) in a biofloc system.

DISCUSSION

In general, water quality parameters varied according to shrimp stocking density, but still remained within the appropriate range for *L. vannamei* rearing (Van Wyk and Scarpa, 1999). Our results corroborated those of Arnold et al. (2009) who evaluated different

stocking densities with zero water exchange for *P. monodon* nursery and observed variation in water quality parameters according to the increase in density.

The range of pH maintained during the experiment favors the growth of nitrifying bacteria (Avnimelech, 2014). The progressive increase of phosphate concentration likely resulted from the constant feed and molasses input (Barak et al., 2003).

Levels of toxic ammonia were above recommended (0.19 mg L⁻¹). According to Cobo et al. (2012), the median lethal concentration for TAN and toxic ammonia post larvae in salinity 34 g L⁻¹, temperature 26° and pH 8.5 are 13.2 mg L⁻¹ and 1.9 mg L⁻¹, respectively. Tolerance to toxic ammonia decreases with exposure time. Studies have shown that shrimps exposed to lethal levels of toxic ammonia experienced a decrease in tolerance up to 64.7% after 96 hours of TAN exposure. The toxicity of ammonia is also influenced by some parameters, such as temperature, salinity and pH (Lin and Chen, 2001). The decrease in ammonia from the 11th day of farming may be directly related to ammonia transformation into nitrite in the system.

Alkalinity showed growth up to the 12th day, owing to constant input of molasses in the system. Because of its ash content, sugarcane molasses can increase alkalinity in the biofloc system (Avnimelech, 2014). The presence of nitrite and the consumption of alkalinity indicate the presence of nitrification (Cohen et al., 2005). Avnimelech (2009) reports an average of four weeks for the establishment of a chemoautotrophic bacterial community and, consequently, the beginning of nitrification in a BFT system. Also, higher stocking densities of *L. vannamei* during rearing stimulate the earlier stabilization of the chemoautotrophic community (McIntosh, 2000). In the present study, increase in density may have favored the acceleration of nitrification, through the observation of decrease in ammonia and alkalinity and also presence of nitrite in the higher density treatments.

Samocha et al. (2007) recommend maintaining TSS for *L. vannamei* nursery <500 mg L⁻¹. However, Schweitzer et al. (2017) observed that TSS maintained between 500 and 700 mg L⁻¹ for PL (PL₆-PL₁₈) of *L. vannamei* did not affect rearing. In this study, TSS around 650 mg L⁻¹ did not affect survival in treatments. According to Schweitzer et al. (2013), high values (800 to 1000 mg L⁻¹) increased the presence of particles in the gills, reducing survival and yield. Only treatment with 160 post-larvae L⁻¹ did not maintain TSS close to the recommended standards.

Salinity stress survival is an important parameter to evaluate larval quality, ensuring that larvae will be resistant to transportation and allow grow-out in the farm. PLs need to get above 75% survival values in salinity stress to be released from the laboratory to the farm (FAO, 2009). All treatments in this study had acceptable levels in the salinity stress test.

In a study with artificial substrate and different stocking densities in the nursery of *L. vannamei*, no significant difference was observed for final weight with densities varying between 778 to 1556 post-larvae m⁻¹ (Moss and Moss, 2004). Similarly, with a zero-water exchange system, Arnold et al. (2009) reported no significant difference for growth in tanks with artificial substrate during the nursery of *P. monodon* with densities varying

between 2.500 and 5.000 post-larvae m^{-3} . However, these stocking densities are lower than those used in our work, ranging from 80.000 to 160.000 post-larvae m^{-3} .

Total ammonia of up to $7.24 \pm 1.18 \text{ mg L}^{-1}$ did not cause mortality of PLs for treatments up to 140 post-larvae L^{-1} , resulting in high survival in these treatments. However, mortality was observed around 45% in treatments stocked with 160 PLs L^{-1} when TSS values reached values above 700 mg L^{-1} and total ammonia was above 8.0 mg L^{-1} . Therefore, it appears that both parameters acted together to increase mortality.

It can be concluded that BFT combined with artificial substrates can tolerate an increase in stocking density up to 133% (140 PLs L^{-1}) based on the acceptable stocking density of about 60 post-larvae L^{-1} in traditional pre-nursery systems with no artificial substrate. The use of the substrate artificial enables shrimp rearing up to 140 post larvae L^{-1} with low water exchange and no need of clarifiers for removal of solids from the water, resulting in higher yield.

CONCLUSION

Pre-nursery of Pacific white shrimp can be performed with a stocking density of up to 140 post larvae L^{-1} in a BFT system without water exchange and with the use of the artificial substrate without the need for decanters and without compromising the growth performance of PLs.

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