



## Effects of stocking density and artificial substrates on yield and water quality in a biofloc shrimp nursery culture

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**ABSTRACT** - The objective of this study was to evaluate the effect of different stocking densities and the presence/absence of two artificial substrates on water quality and production rates of marine shrimp in a biofloc shrimp nursery culture. Two experiments were performed: different stocking densities with mosquito netting substrate and the presence/absence of polyester-type substrate. The first experiment lasted 38 days, and shrimp at an initial weight of  $0.013 \pm 0.010$  g were stocked in 24 tanks. The second experiment lasted 35 days, and shrimp at an initial weight of  $0.037 \pm 0.002$  g were stocked in six tanks. Weekly biometric measurements were performed to adjust the amount of feed. Suspended solids were higher at a density of  $6000 \text{ PL m}^{-3}$  and mosquito netting substrate. Final weight and specific growth rate were higher in treatments with mosquito netting substrate. However, survival was significantly lower with this substrate. Yield was significantly higher at a density of  $6000 \text{ PL m}^{-3}$ . Polyester-type substrate had no significant effect on production rates or variables of water quality. However, this substrate could reduce the production of sludge. The results indicate that it is possible to culture shrimp in nursery stage up to  $6000 \text{ PL m}^{-3}$  in a biofloc system.

Key Words: artificial substrates, biofloc technology, *Litopenaeus vannamei*, stocking density, yield

### Introduction

Aquaculture is a sector with rapid growth and represents almost 50% of global seafood products, growing approximately 6.3% from 2010 to 2015. Shrimp farming contributed 6.9% of total production (FAO, 2016; FAO-FIGIS, 2018). However, new diseases, such as the White Spot Syndrome Virus, and now Acute Hepatopancreatic Necrosis Disease, have encouraged the development of new production systems to ensure biosecurity (Lightner et al., 2012). One such super-intensive system with low or no water renewal is known as biofloc technology (BFT) (Crab et al., 2012). Biofloc systems consist of clusters of algae, protozoa, bacteria, and organic and inorganic detritus (Avnimelech, 2015), which, in addition to controlling the nitrogen compounds in the water, can serve as a food supplement for animals such as shrimp (Avnimelech and Kochba, 2009).

A nursery stage is an intermediate step between the shrimp hatchery stage and the beginning of on-growing. The aim of the nursery stage is to keep larvae at high densities and in a controlled system to reach approximately 1 g (Cohen et al., 2005). However, implementation costs of such nursery are high, owing to the construction of small tanks coated with geomembrane, the use of continuous aeration systems, greenhouse, specialized labor, high-quality post-larva, and control of water quality. Therefore, increasing productivity through nurseries is a strategy that has been used in several farming production systems (Mishra et al., 2008). Many studies have defined the initial stocking densities in clear-water culture systems (Moss and Moss, 2004; Cohen et al., 2005; Mishra et al., 2008). However, for new production systems that incorporate BFT, it is necessary to establish densities and system load capacities. Nevertheless, the effects of stocking densities on water quality in a biofloc-based nursery are unknown. Consequently, increasing densities have been studied relative to the use of artificial substrates in biofloc for rearing shrimp (Schweitzer et al., 2013a) and clear-water system nursery (Moss and Moss, 2004).

Therefore, this study aimed to evaluate the effect of four different stocking densities (3000, 4000, 5000, and  $6000 \text{ PL m}^{-3}$ ) and the presence/absence of two artificial

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substrates (mosquito netting and artificial polyester) on water quality and production rates of marine shrimp in a biofloc shrimp nursery farming.

## Material and Methods

The experiments took place in Florianópolis, Santa Catarina, in southern Brazil (27° 34' 55" S and 48° 26' 28" W).

Nauplii of *L. vannamei* were obtained, SPEEDLINE-HB12 (high performance for growth, uniformity in size), from a commercial laboratory (Aquatec Ltd., Canguaretama, RN, Brazil). The nauplii were raised in 15 m<sup>3</sup> larval rearing tanks, and when they reached post-larval stage 10 (PL<sub>10</sub>), they were transferred to 50 m<sup>3</sup> biofloc tanks in a greenhouse without water renewal. Subsequently, the shrimps were transferred to experimental units at PL<sub>20</sub> and PL<sub>30</sub> stages for experiments 1 and 2, respectively.

The first experiment consisted of cultivating shrimp at different stocking densities with artificial mosquito netting substrate. Four stocking densities (3000, 4000, 5000, and 6000 PL m<sup>-3</sup>), with and without mosquito netting, were established, resulting in eight treatments. The experiment was completely randomized in a bifactorial design (4 × 2), with three replications, totaling 24 experimental units.

The second experiment consisted of cultivating shrimp at the highest density of the experiment 1 (6000 PL m<sup>-3</sup>), with and without artificial polyester-type substrate: Control (without polyester-type substrate) and Substrate (with polyester-type substrate). The units were randomized in a one-factor experimental design with three replications, totaling six experimental units.

In each experiment, the experimental units consisted of circular 800-L tanks with an aeration center ring (Aerotube™) to keep the solids in suspension and maintain the level of dissolved oxygen in the water at the recommended concentration for *L. vannamei* larval cultivation (as described by Schweitzer et al., 2013a). The water temperature was kept constant, between 29 and 30 °C, using 800-W heaters connected to a thermostat. The tanks were covered with black shading net and positioned inside a greenhouse with natural light. A 90-L settling chamber (adapted from Ray et al., 2010) was attached to each tank and was eventually operated at a flow rate of 650 L h<sup>-1</sup> when the total suspended solids (TSS) reached a concentration of 560 mg L<sup>-1</sup> to maintain the required TSS levels for shrimp (Ray et al., 2010; Schweitzer et al., 2013a). Two types of artificial substrate were used: in the experiment 1, mosquito netting (polyethylene screen

with a 1 mm mesh size); in experiment 2, a polyester-type substrate (100% polyester, grammage 250 g m<sup>-2</sup>, 1.44 mm thickness, 0.18 g cm<sup>-3</sup> density, and a continuous resistance and temperature of 150 °C, Needlona®). Both substrates incremented 100% of the useful area of the tank (six substrates per tank of 0.47 × 0.55 m) and were oriented vertically. The substrates were made following the methodology described by Schweitzer et al. (2013a).

Three days before the stocking, each 800 L tank was inoculated with 400 L of biofloc (see physical and chemical characteristics in Tables 1 and 3) and 400 L of seawater. The experimental units were stocked with shrimp at an initial weight of 0.013±0.010 g in experiment 1 and 0.037±0.002 g in experiment 2. The tanks were stocked with post-larvae according to formula (1):

$$N_{\text{initial}} = \frac{\text{biomass (g)}}{\text{average PL weight (g)}} \quad (1)$$

The biomass was obtained by weighing all the shrimp. To obtain the average weight, a sample of shrimp was collected, weighed, and counted. Subsequently, the weight (with a precision scale of 0.01 g Bel®) was divided by the number of animals.

During the 38 and 35 days of respective experiments, shrimp were fed four times a day (8:30, 11:30, 14:00, and 17:00 h) with commercial feed (Guabi Potimar, 40% crude protein). The amount of feed was calculated in accordance with a feed table (Van Wyk, 1999) and adjusted every week according to biomass biometrics.

Controlling fertilization with white sugar to regulate total ammonia nitrogen (TAN) was accomplished in the following two ways: first, during the first three experimental days, the amount of carbohydrate necessary to neutralize TAN excreted by the shrimp was estimated by assuming that shrimp absorb about 25% of the nitrogen added to the feed and that 75% of this nitrogen is converted into TAN dissolved in water (Avnimelech, 1999; 2015). White sugar (99% reducing sugars, 39.6% carbon) was added to each tank at a ratio of 20 g carbohydrate for each gram of TAN targeting C:N ratio of 13:1. Second, when TAN surpassed 1 mg L<sup>-1</sup>, additional carbohydrate (white sugar) was added to the system at a ratio of 20:1 (carbohydrate:TAN) (Avnimelech, 1999).

Alkalinity was corrected with calcium hydroxide, which was added according to feeding (~20% calcium hydroxide in relation to feed input) to maintain alkalinity greater than 120 mg L<sup>-1</sup>. Total suspended solids were maintained in the range of 400 to 600 mg L<sup>-1</sup>, as recommended for shrimp (Schweitzer et al., 2013b), and were controlled by 90-L individual conical bottom settling tanks, adapted from Ray et al. (2010). The water

was not renewed during the experiment. Fresh water was replenished only from loss by evaporation.

Dissolved oxygen and water temperature (dissolved oxygen meter, model YSI Pro20, assembled in USA, CF parts made in China) were measured twice a day (8:00 and 16:30 h), and pH (pH meter Thermo Scientific™, model Orion Star™ A211, Indonesia), salinity (conductivity meter EcoSense®, model EC300A, made in China for YSI Inc.), TSS (APHA, 2005 – 2540D), volatile (VSS) and fixed suspended solids (FSS) (APHA, 2005 – 2540E), settleable solids (Imhoff cone), alkalinity (APHA, 2005 – 2320B), ammonia, and nitrite were analyzed twice a week (Strickland and Parsons, 1972). Nitrate was analyzed once a week using the Hach kit NitraVer 5 reagent pillow.

Moreover, the amount of solids removed from the system was evaluated in the second experiment.

In experiment 2, the initial and final TSS concentration of each tank was calculated to estimate the amount of sludge produced per tank. In addition, the amount of sludge withdrawn from the system was measured each time sludge was removed from the clarifier. To find the total quantity of sludge produced by each experimental tank, the following formula was used:

$$\text{Sludge produced (g tank}^{-1}\text{)} = \frac{(\text{TSS final} \times v_1) - (\text{TSS initial} \times v_1)}{1000} + \Sigma(\text{TSS clarifier} \times v_2), \quad (2)$$

in which TSS final is the concentration of total suspended solids in  $\text{mg L}^{-1}$  at the end of experiment 2;  $v_1$  is the tank volume in liters; TSS initial is the concentration of total suspended solids in  $\text{mg L}^{-1}$  at the start of experiment 2; TSS clarifier is the concentration of total suspended solids in the sludge removed with the settling chamber in  $\text{mg L}^{-1}$  – samples of 5-15 mL of the sludge from the graduated bucket were filtered to determine the concentration of TSS (APHA, 2005);  $v_2$  is the volume containing the sludge in liters (measured in graduated buckets); and  $\Sigma$  is the sum of the solid in times the solids were removed by settling chamber.

At the start of the experiment, random samples in quadruplicate were collected for the PL average weight of shrimp from the matrix tank. The initial stocking biomass was calculated with the following formula (3).

$$\text{Stocking biomass (g)} = \text{PL average weight of shrimps (g)} \times \text{number of animals} \quad (3)$$

For each experimental unit, 30 shrimps were sampled and weighed in group weekly. At the end of the experiment, all post-larvae were weighed in group, and an average of the weight of the shrimp of each tank was calculated to estimate production rates with their respective formulas: final number of animals (4), estimated survival (5), feed conversion ratio (FCR; 6), yield (7), and the specific growth rate (SGR; 8).

$$N_{\text{final}} = \frac{\text{Final biomass (g)}}{\text{individual shrimp average (g)}} \quad (4)$$

$$\text{Estimated survival (\%)} = \frac{N_{\text{final}}}{N_{\text{initial}}} \times 100 \quad (5)$$

$$\text{FCR apparent} = \frac{\text{Total offered feed (g)}}{\text{Increase biomass (g)}} \quad (6)$$

$$\text{Yield (kg m}^{-3}\text{)} = \frac{\text{Final biomass (kg)}}{\text{tank volume (m}^3\text{)}} \quad (7)$$

$$\text{SGR} = 100 \times \left[ \frac{\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}}{\text{days of culture}} \right] \quad (8)$$

Two-way ANOVA with repeated measures was applied in the analysis of the water quality parameters, and Two-way ANOVA was applied to shrimp performance. The presence or absence of artificial substrates and shrimp stocking density were the main factors (experiment 1). Significant differences between the treatments and between weeks of cultures were analyzed with Tukey's test (Zar, 2010). Homoscedasticity was tested using the Bartlett test. The t test was applied in experiment 2 for shrimp performance. Significance level used for all tests was 0.05.

## Results

Dissolved oxygen showed significant differences among the different densities evaluated, involving measured periods (AM, PM), while temperature did not show significant differences between treatments (Table 1).

The pH at a density of 6000 PL  $\text{m}^{-3}$  was significantly lower, and over time, the alkalinity and pH tended to decrease for all treatments. Salinity was maintained at  $34.16 \pm 0.39$  ppt (Table 1).

Total ammonia nitrogen had three peaks at 10, 14, and 35 days of culture. Nitrite was higher in the treatment with mosquito netting and increased throughout the experiment (Table 1). Nitrate also accumulated throughout the experiment ( $P < 0.05$ ).

Total suspended solids were higher at the higher density (6000 PL  $\text{m}^{-3}$ ). Treatments with mosquito netting had a higher concentration of solids (Table 1). The VSS levels were significantly higher at a density of 6000 PL  $\text{m}^{-3}$  (Table 1), increasing during the experiment ( $P < 0.05$ ).

In experiment 1, final weight and SGR were higher in treatments with artificial substrate but with no differences among densities (Table 2). The yield was significantly higher at 6000 PL  $\text{m}^{-3}$  density and higher in treatments with artificial substrate (Table 2). Survival was significantly higher in treatments without substrate (Table 2). Apparent FCR, on average, was  $1.11 \pm 0.08$ , and no significant difference in density or the substrate was observed (Table 2).

Table 1 - Water quality parameters of *L. vannamei* nursery cultured for 38 days in a biofloc technology system in four densities (3000, 4000, 5000, and 6000 PL m<sup>-3</sup>) with and without artificial mosquito netting as substrate (S)

Parameter	Treatment								Inoculum
	3000	3000 + S	4000	4000 + S	5000	5000 + S	6000	6000 + S	
DO (mg L <sup>-1</sup> ) AM	5.65±0.05a	5.54±0.07a	5.59±0.04ab	5.52±0.11ab	5.53±0.06ab	5.56±0.04ab	5.44±0.12b	5.44±0.03b	-
DO (mg L <sup>-1</sup> ) PM	5.48±0.11a	5.51±0.04a	5.48±0.07ab	5.42±0.11ab	5.39±0.08ab	5.39±0.09ab	5.42±0.17b	5.41±0.02b	-
Temperature (°C) AM	28.87±0.14	28.88±0.51	28.93±0.16	29.18±0.61	29.27±0.26	29.23±0.12	28.9±0.31	28.89±0.59	-
Temperature (°C) PM	30.47±0.21	30.59±0.19	30.54±0.16	30.86±0.42	30.83±0.45	30.86±0.43	30.60±0.04	30.64±0.09	-
Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	141.58±4.94	138.06±0.76	139.09±2.31	132.15±2.91	138.42±5.78	133.70±2.73	137.33±6.56	138.06±5.30	154.67±2.31
pH	8.06±0.06b	8.08±0.01b	8.03±0.01ab	8.03±0.03ab	7.97±0.03a	8.04±0.03a	7.95±0.03c	7.95±0.02c	8.19±0.01
Salinity (ppt)	34.79±0.79	34.24±0.39	34.17±1.29	33.84±0.52	34.35±0.25	33.95±0.55	34.43±0.72	33.51±0.52	34.57±0.81
TAN (mg L <sup>-1</sup> )	0.36±0.05	0.37±0.02	0.56±0.28	0.36±0.02	0.48±0.16	0.44±0.09	0.45±0.03	0.45±0.04	0.10±0.01
N-NO <sub>2</sub> (mg L <sup>-1</sup> )	0.59±0.01A	0.63±0.03B	0.54±0.06A	0.69±0.04B	0.57±0.06A	0.71±0.04B	0.65±0.07A	0.68±0.07B	0.01±0.01
N-NO <sub>3</sub> (mg L <sup>-1</sup> )	43.47±22.50	53.30±8.80	41.12±15.23	52.10±2.50	50.12±5.55	48.60±6.00	48.63±6.10	47.80±5.00	8.90±3.12
TSS (mg L <sup>-1</sup> )	487.73±58.10aA	502.45±6.34aB	479.50±30.02aA	522.18±14.49aB	509.48±21.72aA	583.09±38.58aB	584.30±32.38bA	625.82±46.54bB	388.33±19.60
VSS (mg L <sup>-1</sup> )	202.70±25.11a	207.12±12.75a	207.59±15.62a	222.94±6.79a	218.73±9.06a	247.09±27.39a	257.12±20.24b	282.79±31.72b	164.00±13.75

PL - post-larvae; DO - dissolved oxygen; TAN - total ammonia nitrogen; TSS - total suspended solids; VSS - volatile suspended solids.

Mean values ± standard deviation; n = 3.

Averages in the same column followed by different letters indicate significant differences by Tukey test (P<0.05).

Lowercase letters represent density (D) differences, and uppercase letters represent the presence or absence of substrate.

Table 2 - Shrimp production in tanks with *L. vannamei* nursery cultured for 38 days in a biofloc technology system at densities of 3000, 4000, 5000, and 6000 PL m<sup>-3</sup>, with and without mosquito netting as substrate (S)

Parameter	Treatment								ANOVA <sup>1</sup>		
	3000	3000 + S	4000	4000 + S	5000	5000 + S	6000	6000 + S	D	S	D × S
Final weight (g)	0.78±0.22A	1.23±0.22B	0.67±0.20A	1.06±0.25B	0.79±0.15A	0.88±0.01B	0.68±0.08A	0.94±0.06B	ns	*	ns
Yield (kg m <sup>-3</sup> )	2.89±0.32aA	3.42±0.22aB	3.30±0.42abA	3.96±0.39abB	4.47±0.43bA	4.40±0.39bB	4.97±0.51cA	5.32±0.12cB	*	*	ns
FCR-A	1.05±0.17	1.06±0.03	1.03±0.06	1.11±0.04	1.04±0.14	1.23±0.22	1.10±0.04	1.23±0.10	ns	ns	ns
Estimated survival (%)	108.90±17.62A	79.82±10.73B	107.55±18.19A	81.01±10.70B	104.12±21.99A	85.27±8.98B	103.18±2.71A	80.54±5.63B	ns	*	ns
SGR (% day <sup>-1</sup> )	10.69±0.70A	11.95±0.47B	10.31±0.78A	11.53±0.61B	10.79±0.47A	11.08±0.20B	10.41±0.31A	11.25±0.17B	ns	*	ns

PL - post-larvae; SGR - specific growth rate; FCR-A - apparent feed conversion ratio.

Mean ± standard deviation; n = 3.

Different letters in the same column indicate significant differences by Tukey test (P<0.05).

Lowercase letters represent differences in density, while uppercase letters represent the presence or absence of substrate.

<sup>1</sup> Two-way ANOVA was used to determine the effect of stocking density (D), substrates (S), and D × S interaction (D × S).

In experiment 2, temperature and dissolved oxygen were constant during the experiment and were not significantly different between treatments (Table 3). The pH was significantly higher in the treatment with artificial substrate with a tendency to decrease with time. No difference in alkalinity was noted between treatments, but it decreased throughout the days of experiment. Salinity, TSS, VSS, ammonia, nitrite, nitrate, and alkalinity remained at normal levels between treatments.

On the other hand, the average amount of sludge produced for each 800-L experimental unit was 945 g for control and 733 g for polyester substrate. The solids produced did not show significant differences between treatment with substrate and control ( $P>0.05$ ); however, the amount of sludge produced in treatment with polyester substrate was 19% lower than control.

The estimated survival, final weight, apparent FCR, yield, and SGR in treatment with artificial polyester substrate were  $95.84\pm 8.52\%$ ,  $0.81\pm 0.14$  g,  $1.21\pm 0.04$ ,  $4.73\pm 0.50$  kg  $m^{-3}$ , and  $8.79\pm 0.49\%$   $day^{-1}$ , respectively. For control, these parameters were  $85.98\pm 12.34\%$ ,  $0.88\pm 0.21$  g,  $1.20\pm 0.06$ ,  $4.60\pm 0.50$  kg  $m^{-3}$ , and  $9.00\pm 0.72\%$   $day^{-1}$ , respectively. Therefore, the polyester-type substrate did not affect the production rates ( $P>0.05$ ), as shown by the results presented.

## Discussion

Temperature, dissolved oxygen concentration, salinity, alkalinity, and pH remained within the limits considered appropriate for *L. vannamei* (Van Wyk and Scarpa, 1999).

However, the dissolved oxygen in the first experiment showed differences among densities, as expected, owing to the presence of more individuals competing for oxygen. Nevertheless, this parameter always stayed above 5 mg  $L^{-1}$ . The pH was influenced by the stocking density and was lower at 6000 PL  $m^{-3}$  density, most likely influenced by respiration and  $CO_2$  production, as well as the degradation of organic matter.

Total suspended solids is one of the most important variables of biofloc cultures (Ebeling et al., 2006). In the first experiment, TSS was higher at higher density, probably due to the quantity of offered feed, sugar, calcium hydroxide, as well as the number of animals. Values of TSS did not exceed the limit considered for this species (Schveitzer et al., 2013b). In treatments with mosquito netting, a higher amount of TSS was observed owing to greater growth, as shown by weekly biometric measurements, considering 100% survival.

Even controlled by settling chamber from 460 to 200 mg  $L^{-1}$  (Ray et al., 2010), an increase in the amount of TSS was observed during the days of experiment, which is expected in a biofloc system (Schveitzer et al., 2013a).

Settleable solids in both experiments were maintained below 15 mL  $L^{-1}$ . Schveitzer, et al. (2013a) observed obstruction in shrimp gill with more than 15 mL  $L^{-1}$ . Similarly, VSS were higher in treatment with higher density. Ebeling et al. (2006) suggested that a growing environment with a greater amount of VSS could be considered a predominantly heterotrophic system.

In the first experiment, TAN showed three peaks (days 10, 14, and 35) and was controlled by addition

Table 3 - Water quality parameters in nursery of marine shrimp stocked at a density of 6000 PL  $m^{-3}$  in a biofloc technology system with and without artificial substrate (polyester) during 35 days in culture

Parameter	Treatment		Inoculum	ANOVA <sup>1</sup>		
	Substrate	Control		S	T	S × T
DO (mg $L^{-1}$ ) AM	5.54±0.08	5.53±0.06	-	ns	-	-
DO (mg $L^{-1}$ ) PM	5.41±0.13	5.41±0.13	-	ns	-	-
Temperature (°C) AM	28.97±0.43	28.47±0.44	-	ns	-	-
Temperature (°C) PM	29.38±0.53	28.92±0.50	-	ns	-	-
Alkalinity (mg $CaCO_3$ $L^{-1}$ )	147±11	128±11	129±14	ns	*	ns
pH	8.35±0.03	7.99±0.01a	7.93±0.03b	*	*	ns
Salinity (ppt)	35.00±0.00	35.72±0.23	35.77±0.67	ns	*	*
TAN (mg $L^{-1}$ )	0.18±0.05	0.18±0.01	0.17±0.04	ns	*	ns
N- $NO_2$ (mg $L^{-1}$ )	0.50±0.05	0.70±0.10	0.60±0.10	ns	*	ns
N- $NO_3$ (mg $L^{-1}$ )	37.65±8.44	105.19±2.40	96.82±19.81	ns	*	ns
TSS (mg $L^{-1}$ )	388±66	500±30	508±43	ns	*	ns
VSS (mg $L^{-1}$ )	115±19	212±19	216±14	ns	*	ns
SS (mg $L^{-1}$ )	-	6.35±1.31	7.58±1.35	ns	-	-

PL - post-larvae; DO - dissolved oxygen; TAN - total ammonia nitrogen; TSS - total suspended solids; VSS - volatile suspended solids; SS - settleable solids; ns - not significant. Mean values ± standard deviation; n = 3.

Means with different letters in the same row indicate significant differences by Tukey test ( $P<0.05$ ).

<sup>1</sup> One-way ANOVA with repeated measures was used to determine the effect of substrates (S), time in days (T), and S × T interaction (S × T).

\*  $P<0.05$ .

of white sugar. The major peak of total ammonia was  $4.5 \text{ mg L}^{-1}$ . Using the calculation suggested by Emerson et al. (1975), toxic ammonia ( $\text{NH}_3$ ) reached a maximum value of  $0.25 \text{ mg L}^{-1}$ , whereas the lethal concentration ( $\text{LC}_{50}$ ) of  $\text{NH}_3$  to *Litopenaeus vannamei* is  $2.78 \text{ mg L}^{-1}$  (Lin and Chen, 2001), and the safety level for a water pollutant is 10%  $\text{LC}_{50}$  (Sprague, 1969). Therefore, ammonia reached no sublethal levels for shrimp, and it did not apparently affect animal performance. In the second experiment, ammonia was less than  $1 \text{ mg L}^{-1}$  throughout the experiment, and the addition of a carbon source was not required. Ammonia peaks present in the first experiment predominantly resulted from heterotrophic inoculum, which is different from the second experiment, in which the initial water inoculum was predominantly chemoautotrophic.

In both experiments, nitrite was, on average, less than  $1 \text{ mg L}^{-1}$  and was, therefore, within the acceptable range for this species (Lin and Chen, 2003). Nitrate accumulated over cultivation time in both experiments, as previously noted by Ray et al. (2011), but these values did not affect the performance of shrimp and were lower than reported toxic nitrate values for shrimp according to Kuhn et al., (2010), who reported nitrate toxicity only in lower salinities than the evaluated in the present study.

In the second experiment, treatment with polyester substrate was observed to produce less sludge, probably due to the sludge adhered in the substrate, giving it a sludge retention potential, as also observed by Samochoa et al. (1993), when vertical netting was used as substrate in the nursery.

In the first experiment, the treatments with mosquito netting had lower survival than treatments without substrate, probably because the post-larvae were trapped in the net. However, final weight and SGR of shrimp were higher with mosquito netting, regardless of density. The increased growth with AquaMats™ (high surface area polymer filter, Meridian Aquatic Technology, LLC, Calverton, Maryland, USA) substrate in the nursery was also observed by Moss and Moss (2004), as well as Schweitzer et al. (2013a), who reported increased growth with mosquito net substrate in grow-out phase system for *Litopenaeus vannamei*. In some treatments, survival was over 100%, because the number of animals stocked is estimated by weight. Similar results were reported by Cohen et al. (2005). In the second experiment, the survival was 10% higher in treatment with polyester, but it was statistically similar for both treatments and was within the range reported for shrimp nursery (Moss and Moss, 2004;

Cohen et al., 2005; Mishra et al., 2008; Wasielesky et al., 2013; Correia et al., 2014).

In the first experiment, SGR was similar to that obtained by Correia et al. (2014) in a biofloc system over the course of 62 days in a *L. vannamei* nursery with 5000  $\text{PL m}^{-3}$  density. However, in the second experiment, the presence of polyester-type substrate had no influence on final weight or SGR, and this rate was lower than the observed by Correia et al. (2014) in a study on *L. vannamei*. Apparent FCR was statistically similar between treatments with average values of 1.11 in the first experiment and 1.20 in the second experiment. These values were similar to other studies of white shrimp nursery resulting in final weights of 0.33 and 1.0 g (Wasielesky et al., 2013; Correia et al., 2014).

Finally, yield was significantly higher in treatment with 6000  $\text{PL m}^{-3}$  and treatments with mosquito netting, as also observed by other authors reporting on the increase in stocking densities with this species (Moss and Moss, 2004; Zhang, 2011; Wasielesky et al., 2013). Mosquito netting was also found to increase yield, thereby increasing system capacity.

## Conclusions

It is possible to produce juvenile *Litopenaeus vannamei* at densities of up to 6000  $\text{m PL}^{-3}$  without compromising survival, growth, or productivity. The mosquito netting substrate decreases shrimp survival; however, it results in a higher final weight of animals and, hence, system capacity and final biomass.

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## References

- APHA - American Public Health Association. 2005. Standard methods for the examination of water and wastewater. 21st ed. American Water Works Association and Water Pollution Control Association, Washington, DC.
- Avnimelech, Y. 2015. Biofloc technology: a practical guide book. 3rd ed. The World Aquaculture Society, Baton Rouge, LA, USA.

- Avnimelech, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture* 176:227-235. [https://doi.org/10.1016/S0044-8486\(99\)00085-X](https://doi.org/10.1016/S0044-8486(99)00085-X)
- Avnimelech, Y. and Kochba, M. 2009. Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks, using N-15 tracing. *Aquaculture* 287:163-168.
- Cohen, J. M.; Samocha, T. M.; Fox, J. M.; Gandy, R. L. and Lawrence, A. L. 2005. Characterization of water quality factors during intensive raceway production of juvenile *Litopenaeus vannamei* using limited discharge and biosecure management tools. *Aquacultural Engineering* 32:425-442. <https://doi.org/10.1016/j.aquaeng.2004.09.005>
- Correia, E. S.; Wilkenfeld, J. S.; Morris, T. C.; Wei, L. Z.; Prangnell, D. I. and Samocha, T. M. 2014. Intensive nursery production of the Pacific white shrimp *Litopenaeus vannamei* using two commercial feeds with high and low protein content in a biofloc-dominated system. *Aquacultural Engineering* 59:48-54. <https://doi.org/10.1016/j.aquaeng.2014.02.002>
- Crab, R.; Defoirdt, T.; Bossier, P. and Verstraete, W. 2012. Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture* 356:351-356. <https://doi.org/10.1016/j.aquaculture.2012.04.046>
- Ebeling, J. M.; Timmons, M. B. and Bisogni, J. J. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture* 257:346-358. <https://doi.org/10.1016/j.aquaculture.2006.03.019>
- Emerson, K.; Russo, R. C.; Lund, R. E. and Thurston, R. V. 1975. Aqueous ammonia equilibrium calculations - Effect of pH and temperature. *Journal of the Fisheries Research Board of Canada* 32:2379-2383. <https://doi.org/10.1139/f75-274>
- FAO-FIGIS - Fisheries and Aquaculture Information and Statistics Branch. 2018. Fishery Statistical Collections, Global Aquaculture Production. Available at: <<http://www.fao.org/fishery/statistics/global-aquaculture-production/query/em>>. Accessed on: Jan. 15, 2018.
- FAO - Food and Agriculture Organization of the United Nations. 2016. The State of World Fisheries and Aquaculture Contributing to food security and nutrition for all. Food and Agriculture Organization of the United Nations, Roma.
- Kuhn, D. D.; Smith, S. A.; Boardman, G. D.; Angier, M. W.; Marsh, L. and Flick, G. J. 2010. Chronic toxicity of nitrate to Pacific white shrimp, *Litopenaeus vannamei*: Impacts on survival, growth, antennae length, and pathology. *Aquaculture* 309:109-114. <https://doi.org/10.1016/j.aquaculture.2010.09.014>
- Lightner, D. V.; Redman, R. M.; Pantoja, C. R.; Noble, B. L. and Tran, L. 2012. Early mortality syndrome affects shrimp in Asia. *Global Aquaculture Advocate*, p.40.
- Lin, Y. C. and Chen, J. C. 2001. Acute toxicity of ammonia on *Litopenaeus vannamei* boone juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology* 259:109-119.
- Lin, Y. C. and Chen, J. C. 2003. Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. *Aquaculture* 224:193-201.
- Mishra, J. K.; Samocha, T. M.; Patnaik, S.; Speed, M.; Gandy, R. L. and Ali, A. M. 2008. Performance of an intensive nursery system for the Pacific white shrimp, *Litopenaeus vannamei*, under limited discharge condition. *Aquacultural Engineering* 38:2-15. <https://doi.org/10.1016/j.aquaeng.2007.10.003>
- Moss, K. R. K. and Moss, S. M. 2004. Effects of artificial substrate and stocking density on the nursery production of pacific white shrimp *Litopenaeus vannamei*. *Journal of the World Aquaculture Society* 35:536-542. <https://doi.org/10.1111/j.1749-7345.2004.tb00121.x>
- Ray, A. J.; Farno, C. C.; Bailey, B.; Breland, V. M.; Dillon, K. S. and Lotz, J. M. 2011. Differences in chemical dynamics between chemoautotrophic and three different heterotrophic biofloc-based shrimp (*Litopenaeus vannamei*) culture systems. *Journal of Shellfish Research* 30:546-546.
- Ray, A. J.; Lewis, B. L.; Browdy, C. L. and Leffler, J. 2010. Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. *Aquaculture* 299:89-98. <https://doi.org/10.1016/j.aquaculture.2009.11.021>
- Samocha, T.; Lawrence, A. and Biedenbach J. M. 1993. The effect of vertical netting and water circulation pattern on growth and survival of *Penaeus vannamei* post larvae in an intensive raceway system. *Journal of Applied Aquaculture* 2:55-64.
- Schveitzer, R.; Arantes, R.; Baloi, M. F.; Costodio, P. F. S.; Arana, L. V.; Seiffert, W. Q. and Andreatta, E. R. 2013a. Use of artificial substrates in the culture of *Litopenaeus vannamei* (Biofloc System) at different stocking densities: Effects on microbial activity, water quality and production rates. *Aquacultural Engineering* 54:93-103. <https://doi.org/10.1016/j.aquaeng.2012.12.003>
- Schveitzer, R.; Arantes, R.; Costodio, P. F. S.; Santo, C. M. D.; Arana, L. V.; Seiffert, W. Q. and Andreatta, E. R. 2013b. Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. *Aquacultural Engineering* 56:59-70. <https://doi.org/10.1016/j.aquaeng.2013.04.006>
- Sprague, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. *Water Research* 3:793.
- Strickland, J. D. and Parsons, T. R. 1972. A practical handbook of seawater analysis. 2nd ed. Bulletin 167. Fish Research Board of Canada, Ottawa, Ontario, Canada.
- Van Wyk, P. 1999. Nutrition and feeding of *Litopenaeus vannamei* in intensive culture systems. p.125-139. In: Farming marine shrimp in recirculating freshwater systems. Van Wyk, P.; Davis-Hodgkins, M.; Laramore, R.; Main, K. L.; Mountain, J. and Scarpa, J., eds. Florida Department of Agriculture and Consumer Services, Florida.
- Van Wyk, P. and Scarpa, J. 1999. Water quality requirements and management. p.141-162. In: Farming marine shrimp in recirculating freshwater systems. Van Wyk, P.; Davis-Hodgkins, M.; Laramore, R.; Main, K. L.; Mountain, J. and Scarpa, J., eds. Florida Department of Agriculture and Consumer Services, Tallahassee, FL, USA.
- Wasielesky, W.; Froes, C.; Foes, G.; Krummenauer, D.; Lara, G. and Poersch, L. 2013. Nursery of *Litopenaeus vannamei* reared in a biofloc system: The effect of stocking densities and compensatory growth. *Journal of Shellfish Research* 32:799-806. <https://doi.org/10.2983/035.032.0323>
- Zar, J. H. 2010. Biostatistical analysis. 5th ed. Prentice Hall, New Jersey.
- Zhang, B. 2011. Influence of the artificial substrates on the attachment behavior of *Litopenaeus vannamei* in the intensive culture condition. *International Journal of Animal and Veterinary Advances* 3:37-43.