

Research Article

Stocking density for freshwater prawn *Macrobrachium rosenbergii* (Decapoda, Palaemonidae) in biofloc system

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ABSTRACT. The objective of this study was to evaluate the effect of stocking densities on productive performance of the freshwater prawn *Macrobrachium rosenbergii* in biofloc system. Experimental tanks (microcosms) with 0.20 m² area were used as experimental units. The tanks were connected to two 300 L matrix tanks (macrocosm) with biofloc technology, used as recirculating units. *M. rosenbergii* juveniles, with an initial weight of 0.315 ± 0.06 g and initial length of 33.34 ± 2.26 mm, was randomly distributed in the experimental tanks at different stocking densities (50, 100, 150, 200 and 250 ind m⁻²) and reared during 60 days. The total biomass at the end of the experiment was significant higher ($P < 0.05$) with the use of higher stocking density (250 ind m⁻²). However, prawns stocked at the density of 50 m⁻² showed significant higher ($P < 0.05$) survival (73%) and significantly lower values ($P < 0.05$) for feed conversion rate (1.28). The different stocking densities evaluated did not affect the weight and length of prawns. The recommended density for growing *M. rosenbergii* in the biofloc system is 50 ind m⁻².

Keywords: *Macrobrachium rosenbergii*, prawn farming, BFT, water exchange, productive performance, aquaculture.

INTRODUCTION

Freshwater prawn farming occupies a prominent place among aquaculture activities. The latest data by FAO (2016) showed production of approximately 500,000 ton of freshwater prawn, with highlights for the species *Macrobrachium rosenbergii* (De Man, 1879) and *Macrobrachium nipponense* (De Haan, 1849). The freshwater prawn *M. rosenbergii* is considered an aggressive and territorial animal, so supposedly it would be inadvisable culture this species in high densities. However, recent studies demonstrated the possibility of intensifying the freshwater prawn farming (Moraes-Valenti & Valenti, 2007; Kimpara *et al.*, 2013; Dutra *et al.*, 2016), making it interesting to evaluate the farming of *M. rosenbergii* in superintensive system with microbial flocs (Biofloc Technology-BFT) (De Schryver *et al.*, 2008). The grow-out of freshwater prawns is generally carried out in earthen ponds with fertilization and supplemental

feeds, considered a semi-intensive system, and the stocking is accomplished with post-larvae or juveniles, in densities which vary from 4 to 20 ind m⁻² (Valenti *et al.*, 2010). Stocking densities for rearing *M. rosenbergii* in super intensive Biofloc system still need to be established.

Biofloc systems do not generate wastewater and can be conducted in structures completely isolated from the natural environment, their sustainability and biosafety characteristics make their use very attractive (Wasielesky Jr. *et al.*, 2006; Ray *et al.*, 2009; Samocha *et al.*, 2011), allowing the production of freshwater prawns in regions of temperate climate throughout the year (Stokstad, 2010). This technique was initially developed for the production of tilapia (Avnimelech *et al.*, 1995) and has shown promising results for the marine shrimp farming (Krummenauer *et al.*, 2011; Wasielesky Jr. *et al.*, 2013). The bioflocs are formed by organic particles that remain suspended in the water column, they are composed of a large amount of hetero-

trophic bacteria, flagellates, ciliates, cyanobacteria, microalgae, small metazoans and fungi (Avnimelech, 2012), microorganisms that are known for the high content of nutrients essential to shrimp, such as polyunsaturated fatty acids and essential amino acids (Ballester *et al.*, 2007).

The microorganisms that compose the biofloc assimilate and recycle nitrogen compounds dissolved by excretion and remains of food in decomposition (Burford *et al.*, 2003; Wasielesky *et al.*, 2006; Crab *et al.*, 2007), allowing the water to be reused by several cycles (Avnimelech, 2009). In this type of system, the use of probiotics was also evaluated and showed promising results (Souza *et al.*, 2012; Krummenauer *et al.*, 2014).

Based on the above considerations, the purpose of the present study was to evaluate the effect of stocking density on productive performance of juvenile freshwater prawn *M. rosenbergii* reared in a super-intensive Biofloc system without water renewal.

MATERIALS AND METHODS

Experimental conditions

The experiment was conducted at the Prawn Culture Laboratory of the Federal University of Paraná - UFPR - Palotina Sector, Paraná, Brazil. To evaluate the effect of stocking density on productive performance of the freshwater prawn *M. rosenbergii*, an experimental system with bioflocs was used consisting of 15 rectangular experimental microcosms with 0.20 m² of area and volume of 47.25 L, built in a closed room with 14 m². In each experimental unit, two plastic screens were transversally attached to the bottom and the sides of the tanks. Such structures were used as substrates for the development of natural biota, representing an additional source of feed for the prawns, assisting in nitrification and distribution of prawns and reducing the relative density (Ballester *et al.*, 2007). All tanks were covered with 50% shading cloth, to prevent animals from escaping.

The experimental tanks were connected to two matrix tanks (300 L) each with a bottom area of 0.43 m². From one of the matrix tanks, the water was pumped to the experimental units; the other acted as the water receptor from the experimental units. A total of 1,308.75 L of water was used in the experiment, with input and output flow rate of 1.2 L min⁻¹ in each experimental unit. Total water recirculation rate in the system was 1,050 L h⁻¹. When necessary, the volume of evaporated water from the system was added to the matrix tank. The details of the experimental system are shown in Figure 1.

Thirty days before the start of the experiment, the matrix tanks were filled with clear water and 50 L of water rich in microorganisms (green water) from external tanks. Then 100 prawns were stocked in each tank (230 ind m⁻²), with mean weight of 0.873 ± 0.287 g and mean length of 43.613 ± 12.058 mm to stimulate and maintain the development of bioflocs (Avnimelech, 1999). Furthermore, a daily addition of 7.5 g of probiotic Sanolife PROW (Inve®) in the matrix tanks was performed. The probiotic was matured for 8 h in a container with 10 L of water, previously dechlorinated with powdered ascorbic acid (1g 1,000 L⁻¹), and then added to the system water.

The reception and distribution tanks of the experimental system water received illumination throughout the trial period. The illumination system had four fluorescent lamps, two of 25 W and two of 36 W, and two 30 W halogen lamps. The lamps were placed at 50 cm from the water surface. The use of light on the distribution tanks aimed to maintain the photosynthetic activity of the microorganisms present in the microbial flocs. The adopted photoperiod was 12:12 (light:dark; light from 6:00 to 18:00 h) as recommended by Araújo & Valenti (2007).

To maintain suitable oxygen concentration in the water a rectangular air stone of 15 cm was used in each experimental unit. Aeration was moderate to keep the bioflocs suspended without causing stress to the animals, preventing them from being thrown against the tank walls due to excessive turbulence. At each of the matrix tanks, three air stones of 15 cm were used.

Electric heaters maintained the temperature in the rearing system with thermostats. To keep the pH above 7.0 sodium bicarbonate was added (NaHCO₃) at a ratio of 0.06 g L⁻¹, to keep alkalinity above 100 mg L⁻¹ was added 0.20 g L⁻¹ of dolomitic limestone, as recommended by Furtado *et al.* (2011).

Experimental design and feed management

M. rosenbergii post-larvae were acquired from the commercial laboratory Fazenda Santa Helena, Rio de Janeiro, Brazil, and kept under adaptation for a period of 30 days until the start of the experiment. After the adaptation period, 450 juveniles of *M. rosenbergii*, with initial mean weight of 0.315 ± 0.06 g and initial mean length of 33.34 ± 2.26 mm, were distributed in the experimental units, with different stocking densities (50, 100, 150, 200 and 250 ind m⁻²) in a completely randomized design with three replications per treatment (density). The experiment lasted 60 days.

Prawns were fed three times a day, with an initial feeding rate equivalent to 7% of their biomass. The commercial diets Guabi® (Potimar 40-J), INVE® (XL

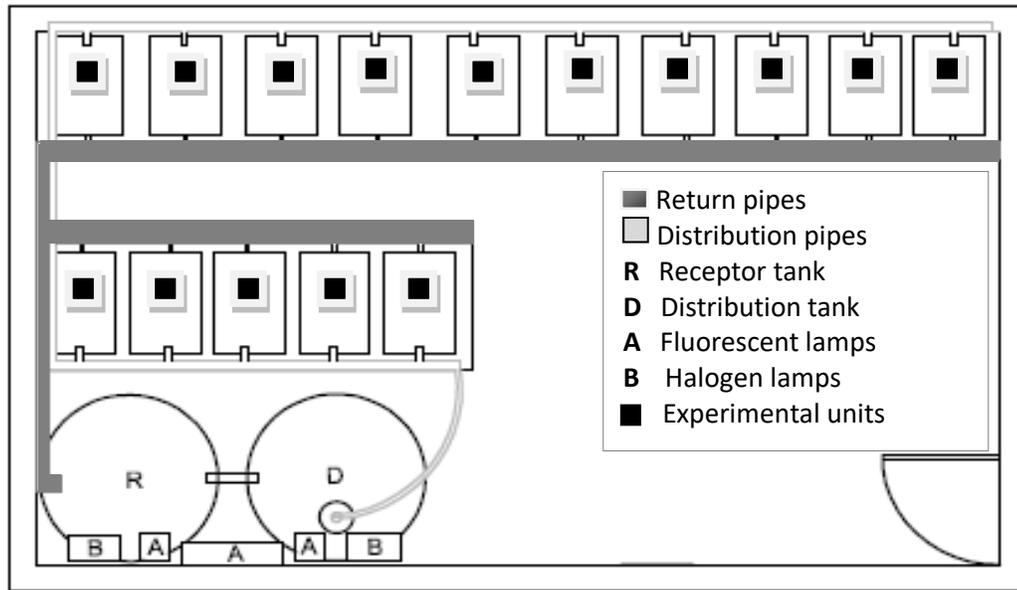


Figure 1. Illustration of the experimental system.

and Stress Pac) with 40, 48 and 42% crude protein, respectively, were used. In the morning (8:00 am) prawns were fed the J-40 diet in the proportion of 30% of the established daily amount; in the afternoon (13:30) they received another 30% of the daily amount with XL diet, and at 17:30 pm received the remaining 40% with Stress Pac diet. Guabi[®] J-40 diet was supplemented with the probiotic Sanolife Pro-W (Inve[®]) in the proportion of 5.0 g kg⁻¹ of diet. The use of three different artificial diets was a strategy to guarantee the quality of the feed offered. The amount of feed was adjusted daily according to the consumption in each experimental tank. Observations in the consumption were made with the aid of a waterproof flashlight.

Evaluation of the productive performance

Biometrics were performed at the beginning and at the end of the experiment, intermediate biometrics were not performed to avoid stressing the prawns. At the end of the experiment the remaining prawns in all the experimental units were counted to determine the survival rate, additionally, prawns were individually weighed to the nearest 0.01 g (analytical scale, AY 220 Marte[®]) and measured (digital caliper with 150 mm and precision of 0.01 mm, 402.150BL, King Tools[®]) to evaluate the productive performance. The following production indexes were evaluated: final weight, weight gain, total length, feed conversion rate, specific growth rate and biomass gain. The feed conversion rate was apparent and calculated based on the amount of

feed supplied, not being considered the leftovers. The following equations were used:

$$\text{Survival, } S (\%) = (N_f / N) \times 100$$

$$\text{Weight gain, WG (mg)} = W_f - W_i$$

$$\text{Specific growth rate, SGR (\% d}^{-1}\text{)} = 100 \times [\ln (W_f) - \ln(W_i)] / \Delta t$$

$$\text{Biomass gain, BG (g)} = B_f - B_i$$

$$\text{Feed conversion rate, FCR} = \text{feed offered (g)/BG (g)}$$

where N = number of prawns stocked at the beginning of the experiment; N_f = number of living prawns at the end of the experiment; B_f = final biomass (g); B_i = initial biomass (g); W_f = final weight (mg); W_i = initial weight (mg); Δt duration of experimental period (days).

Water quality monitoring

Water quality variables were monitored daily using specific equipment: temperature with a digital thermometer (CE[®]), dissolved oxygen with an oximeter AT-170 (Afakit[®]), pH with a pH meter AT-315 (Alfakit[®]), conductivity with a conductivimeter AT-230 (Alfakit[®]) and turbidity with turbidimeter AP2000 (PoliControl[®]). Additionally, water samples were daily collected to quantify the concentrations of total ammonia nitrogen (TAN) and nitrite (N-NO), according to the methodology proposed by Mackereth *et al.* (1978) and every five days to measure water hardness and alkalinity (Walker, 1978) and concentrations of nitrate (N-NO₃) (Mackereth *et al.*, 1978). All analyses were carried out in a spectrophotometer 2000UV (BE Photonics[®]). In addition, a 1 L water sample was

collected two times a week to determine the settleable solids in an Imhoff cone according to the methodology proposed by Eaton *et al.* (1995) and adapted by Avnimelech (2007).

Statistical analysis

The data of the productive performance were evaluated by analysis of variance (ANOVA one way, $\alpha = 0.05$) after being confirmed the homoscedasticity of variances (Levene's test) and normality of the data distribution (Shapiro-Wilk test). When there were significant differences ($P < 0.05$) the HSD-Tukey test was applied. Data of final density and final biomass were evaluated by linear regression.

RESULTS

The values of water quality variables monitored during the experiment are presented in Table 1. The results of productive performance of *M. rosenbergii* reared in a biofloc system with different stocking densities are shown in Table 2.

There were no significant differences among treatments ($P > 0.05$) for the final weight, final length, weight gain, and specific growth rate. The density of 50 ind m⁻² presented significant higher survival ($P < 0.05$) and a significantly lower feed conversion ratio ($P < 0.05$). However, the final biomass gain was significantly higher ($P < 0.05$) in the treatment with the stocking density of 250 ind m⁻².

The relationship between biomass gain and final density (considering the final survival), was evaluated by linear regression and is shown in Figure 2. The final density was 36, 52, 77, 87, and 97 ind m⁻² for the experimental treatments with an initial stocking density of 50, 100, 150, 200 and 250 ind m⁻², respectively.

DISCUSSION

Water quality variables monitored in the experimental tanks remained within the suitable range for *M. rosenbergii* culture. Temperature, dissolved oxygen concentration and pH recorded in this study were adequate, considering that this species has the best performance in temperatures between 28 and 30°C (New, 2002), that the optimal values of dissolved oxygen should be above 5.0 mg L⁻¹ (Cheng *et al.*, 2003) and that the pH should be between 7.0 and 8.5 (Tavares, 1995).

The alkalinity in the biofloc system should be maintained between 100 and 150 mg L⁻¹, to prevent low pH values that may compromise the growth of the farmed organisms (Ebeling *et al.*, 2006). In the present

study the values of alkalinity were slightly below the recommended level, however, pH was not compromised. According to Wasielesky *et al.* (2007), alkalinity and pH tend to naturally decrease in biofloc farming systems, while the concentrations of nitrogen compounds tend to increase.

In biofloc systems, the maintenance of the ammonia concentration and nitrite within acceptable limits for the farming of aquatic organisms is usually performed by adding a carbon source (Wasielesky *et al.*, 2006; Ballester *et al.*, 2010; Crab *et al.*, 2012). The ammonia and nitrite levels observed during the experimental period did not exceed the 0.5 mg L⁻¹ limit recommended by New (2002) even without the addition of a carbon source in the system water. The main factors that probably were related to the maintenance of these low concentrations of nitrogen compounds were the use of green water at the beginning of the formation of flocs and the use of probiotics. The illumination system used in the experiment allowed the maintenance of microalgae within the bioflocs, contributing to the maintenance of low ammonia concentration (Ray *et al.*, 2009). Furthermore, the use of probiotic supplement in the rearing water stimulates growth of suspended materials, phytoplankton, microbial aggregates and particulate organic matter (Hargreaves, 2006), supporting the heterotrophic bacteria conversion of nitrogen compounds such as ammonia and nitrite in microbial protein, which is ingested by the prawns, keeping the quality of the rearing water (Avnimelech, 1999; Burford *et al.*, 2004).

At the end of the experiment, the higher survival rate was observed at the stocking density of 50 ind m⁻², all other stocking densities evaluated showed significantly lower survival values ($P < 0.05$). Several researchers also found decreased survival rate with increasing stocking density in the production of freshwater prawn (El-Sherif & Mervat, 2009; Paul *et al.*, 2016) and marine shrimp (Krummenauer *et al.*, 2011; Fróes *et al.*, 2013; Wasielesky *et al.*, 2013) in different farming systems. The increase in stocking density interferes with hierarchical competition and disputes for space and food, resulting in higher cannibalism (Sampaio & Valenti, 1996; Arnold *et al.*, 2006; Moraes-Valenti *et al.*, 2010). According to David *et al.* (2015), *M. rosenbergii* shows higher performance at lower stocking densities. El-Sherif & Mervat (2009) evaluated the effects of different stocking densities for *M. rosenbergii* (50; 100; 150 and 200 ind m⁻²) in experimental tanks with clear water, observing higher survival rate (72.2%) using the density of 50 ind m⁻² at the end of 90 days of rearing, corroborating the results observed in the present study. Additionally, despite having influenced survival, increased stocking density evaluate in this study, did not affect the results of speci-

Table 1. Water quality variables monitored in recirculation system with microbial flocs in the farming of freshwater prawn *Macrobrachium rosenbergii* during 60 days. Data presented as mean ± SD. The reference values are cited as ideal for prawn farming.

Variables	$\bar{X} \pm S$	Reference
Temperature (°C)	30.34 ± 0.78	28-30°C New (2002)
Oxygen (mg L ⁻¹)	8.05 ± 0.42	> 5 mg L ⁻¹ Arana (2004)
pH	7.95 ± 0.13	8.0 New (2002)
Alkalinity (mg L ⁻¹)	93.17 ± 19.86	>120 mg L ⁻¹ New (2002)
Hardness (mg L ⁻¹)	65.17 ± 16.44	60-120 mg L ⁻¹ New (2002)
Orthophosphate (mg L ⁻¹)	0.22 ± 0.38	-
Turbidity (ntu)	4.55 ± 1.71	-
Conductivity (µS cm ⁻¹)	0.55 ± 0.19	-
Imhoff Cone (mL L ⁻¹)	0.16 ± 0.08	< 10 mL L ⁻¹ Samocha <i>et al.</i> (2007)
Ammonia (mg L ⁻¹)	0.08 ± 0.05	< 0.5 mg L ⁻¹ New (2002)
Nitrite (mg L ⁻¹)	0.38 ± 0.03	< 0.5 mg L ⁻¹ New (2002)
Nitrate (mg L ⁻¹)	3.22 ± 0.88	-

Table 2. Means and standard deviations of survival (S), final weight (FW), final length (FL), weight gain (WG), biomass gain (BG), feed conversion rate (FCR) and specific growth rate (SGR) of *Macrobrachium rosenbergii* juveniles reared in different stocking densities in a biofloc system during 60 days. *In the same line, different superscript letters represent significant differences (HSD-Tukey, $P < 0.05$); P , test value ANOVA single factor.

Variables	Density (ind m ⁻²)					P
	50	100	150	200	250	
S (%)	73 ± 6 ^a	52 ± 3 ^b	51 ± 5 ^b	43 ± 10 ^b	39 ± 3 ^b	0.00032
FW (g)	2.22 ± 0.24	2.46 ± 0.28	2.15 ± 0.12	2.38 ± 0.27	2.54 ± 0.20	0.29853
FL (mm)	61.52 ± 3.60	61.39 ± 3.11	59.07 ± 1.61	59.95 ± 1.67	61.17 ± 1.59	0.69954
WG (g)	1.91 ± 0.24	2.14 ± 0.28	1.83 ± 0.12	2.06 ± 0.27	2.23 ± 0.20	0.28595
BG (g)	13.13 ± 1.15 ^d	19.07 ± 2.60 ^{cd}	23.47 ± 2.32 ^{bc}	27.99 ± 5.44 ^{ab}	33.28 ± 1.17 ^a	0.00008
FCR (g)	1.28 ± 0.11 ^c	1.77 ± 0.24 ^{bc}	2.06 ± 0.19 ^{ab}	2.48 ± 0.45 ^{ab}	2.51 ± 0.09 ^a	0.00113
SGR (%)	3.25 ± 0.18	3.41 ± 0.18	3.20 ± 0.00	3.36 ± 0.10	3.47 ± 0.10	0.27492

fic growth rate, final weight and the final length of the prawns. In crowded conditions, prawn demonstrates a strategy of reduction in the number of individuals rather than affecting weight and growth performance validating the results of Begon *et al.* (2007) which determined that the intraspecific competition can influence the regulation of population size.

The feed conversion ratio at the end of the experiment was significantly lower ($P < 0.05$) at the stocking densities of 50 and 100 ind m⁻². Paul *et al.* (2016) and El-Sherif & Mervat (2009) also observed less efficient feed conversion rate with increasing stocking density in the farming of *M. rosenbergii*. In this study, the best results of feed conversion rate observed (1.28) demonstrate the efficiency of the biofloc system for freshwater prawn farming as long as the proper stocking density is used. Prawns in biofloc system have better feed conversion rate compared to traditional farming systems (Pérez-Rostro *et al.*, 2014) because bioflocs allow prawn to reduce energy costs in

the search for feed, providing greater energy storage in muscles and tissues and contributing to better nutrition and feed efficiency (Ballester *et al.*, 2010). Prawn find in bioflocs a source of supplementation to their diet, given that it is an autochthonous food and is permanently available, unlike artificial diets, which loses its nutritional value when in contact with water for long periods of exposure.

Linear regression analysis showed a strong association between biomass gain and final density of the prawns. Experimental treatments with higher stocking densities (200 and 250 ind m⁻²) showed significant higher biomass gain ($P < 0.05$) at the end of the experiment, even with the lowest survival rates; in these treatments the weight and length of the prawns were similar to the other evaluated stocking densities. However, the high mortality and feed conversion rate of prawns in these treatments suggest that the use of high stocking densities are not suitable for the farming of *M. rosenbergii* in the biofloc system, due to higher

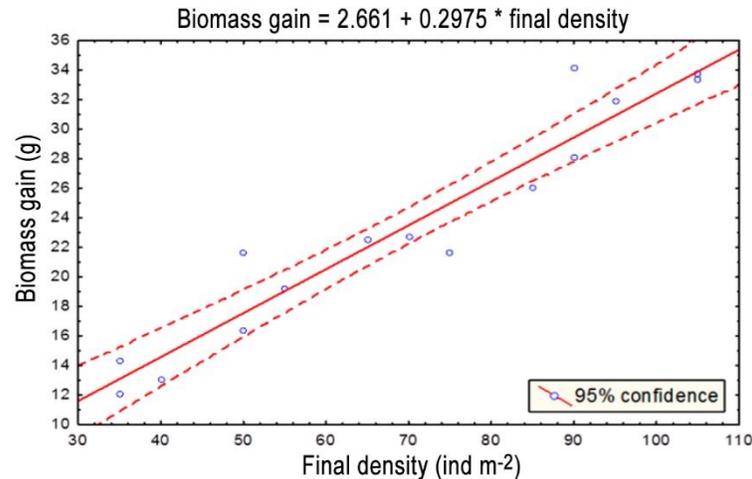


Figure 2. Scattergram (IC 95%) of the linear regression model of biomass gain (y) and final density (x) of juvenile *Macrobrachium rosenbergii* reared in a biofloc system.

costs (investments to acquire post-larvae) and food expenses during the rearing. The stocking densities of 200 and 250 ind m⁻² showed significant higher feed conversion rate ($P < 0.05$), with averages values of 2.48 and 2.51, respectively. According to Wasielesky *et al.* (2013), the shrimp ability to take advantage of the microbial community present in biofloc may decline or be less efficient at high stocking densities. Therefore, the density of 50 ind m⁻² is more suitable for the rearing of *M. rosenbergii* in Biofloc system, because it showed higher survival rate (73%) and better feed conversion rate (1.28).

Regardless of the assessed stocking densities, prawns reared in the biofloc system had an average weight gain of 2.03 g, higher than in clear water systems for prawn farming. Mancebo (1978), in a study with *M. rosenbergii* juveniles in tanks with clear water, observed average weight gain of 0.57 g after 60 days of rearing, and Sandifer & Smith (1977) observed for the same species 0.84 g weight gain. In these studies, the initial weight of the prawn was similar to that used in the present study. According to Wasielesky *et al.* (2006), animals reared within the microbial flocs show an increase in weight gain due to the nutritional benefits present in these rearing systems. In addition to the benefits as a nutritional source, the microorganisms present in the bioflocs also contributed to the maintenance of water quality during the experimental period (Ballester *et al.*, 2010).

The use of probiotic in the feed and water may also have contributed to the positive results of productive performance observed in this study. Probiotic compounds of *Bacillus subtilis* (Ehrenberg, 1835) and *Bacillus licheniformes* (Weigmann, 1898), such as

those used in this study are effective in inhibiting the action of pathogenic bacteria, and improve the assimilation of food nutrients, collaborate with the immune system, accelerate the degradation of waste and nutrient recycling (Decamp *et al.*, 2008). According to Silva *et al.* (2008), the presence of these microorganisms in the water improves the feed conversion rate of the shrimp, increasing productivity due to the availability of nutrients. Krishna *et al.* (2009) noted that in tanks with the addition of probiotic shrimp showed better use of the food, probably due to the influence of microorganisms in digestion and absorption of nutrients.

In conclusion, according to the results, *M. rosenbergii* juveniles reared in the biofloc system have a better productive performance with the use of a stocking density of 50 ind m⁻².

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