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Effect Of Stocking Density On The Production Parameters And Water Quality Of Macrobrachium Acanthurus Culture In A Biofloc System

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Abstract
One of the factors influencing the farming of aquatic species is stocking density, which is directly related to organism growth. For this reason, the present study evaluated, via two experimental tests, different stocking densities in Macrobrachium acanthurus cultures undertaken in biofloc systems. The first test evaluated four densities (60, 120, 180, and 240 Ind/m^3) for 14 weeks, while the second evaluated two densities (120 and 240 Ind/m^3) for an eight-week culture period. The best results for the first test were obtained at densities of 60 Ind/m^3, finding weight gain of 0.159±0.002 g, a growth rate of 638.6±11.54 %, a specific growth rate of 2.04±0.01% day^{-1}, and a survival rate of 90.47±4.12 %. The best results obtained by the second test presented at densities of 120 Ind/m^3, with weight gain of 0.886±0.008 g, a specific weight gain of 5.83±0.01% day^{-1}, and a survival rate of 91.02±5.87%. The highest biomass observed for both tests was reported at densities of 240 Ind/m^3, with 5.27±0.51 and 35.77±1.71 g for the first and second tests, respectively. The water temperature observed in the first test was 24.43±3.55 °C, while the second found higher temperature values, corresponding to 27.37±0.71 °C. Moreover, both experimental tests revealed that the concentration of sedimentable solids increased in line with increased density. Finally, the composition of the biofloc was mainly dominated by four groups of microorganisms—protozoa, rotifers, bacteria, and nematodes.

Key words: *Density, biofloc, production parameters, growth rate*

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I. Introduction

According to the United Nations Food and Agriculture Organization (FAO), aquaculture is a production activity with a high growth rate, wherein production is predicted to reach 106 million tons by 2030, while an increase in the trade and consumption of aquatic organisms has also been observed in recent years (FAO, 2022).

Currently, prawn production is conducted on a small scale for local and family consumption, due to various problems such as seed collection, aggressive or territorial behavior, and low growth rates compared to other crustacean species (Espinosa *et al.* 2011; Valverde and Varela, 2020).

Studies conducted on the tilapia *Oreochromis niloticus*, the marine shrimp *Penaeus vannamei*, *Penaeus indicus*, and *Penaeus stylirostris*, and the prawns *Macrobrachium rosenbergii*, *M. americanum*, and *M. tenellum* report that high culture densities can directly affect growth and survival, due to competition for space and food (Ronald *et al.* 2014; Lisen *et al.* 2021; Emmerson and Andrews, 1981; Murcia-Mena and Paz-Quevedo, 2020; Aragón-Noriega *et al*. 2000; Cupertino *et al.* 2017; Paul *et al.* 2016; Ponce-Palafox *et al.* 2018; Peña-Herrejón *et al.* 2019).

The discharge occurring during the replacement of water in aquaculture farms generates contamination due to the high amount of organic material and nitrogenated compounds present (Martínez-Córdova *et al.* 2010; Anaya and Bückle, 2012). For this reason, biofloc technology is being increasingly used to maintain product quality and save water, given that the chemoautotroph and heterotrophic bacteria present in the system are able to metabolize the different forms of nitrogen, further to reducing feed costs and feed conversion rate and increasing production (Avnimelech, 1999; Ebeling *et al*. 2006; De Schryver *et al*. 2008; Emerenciano *et al.* 2013; Collazos and Arias, 2015; Hernández *et al*. 2019).

II. Material And Methods

Organism handling and experimental design

The organisms were captured in the Pantepec River, in Tuxpan, Veracruz, using a net with a mesh width of 2 mm. Once captured, the individuals were transported to the aquaculture bioassay unit at the Faculty of Biological and Agricultural Sciences of the Veracruzana University.

Two experimental tests were undertaken, with the first using a system of four plastic 1000-L tanks

divided into three with a plastic mesh. The experimental design of this test comprised four treatments conducted in triplicate, with stocking densities of 60, 120, 180, and 240 Ind/ m^3 evaluated. The second test used a system of six plastic 60-L tanks, evaluating two treatments conducted in triplicate with stocking densities of 120 and 240 Ind/ m^3.

A total of two thousand organisms with an average weight of 25mg were selected and acclimatized for five days prior to the beginning of the experimental tests, wherein they were provided commercial feed, containing 35% protein, two times per day, with the proportions corresponding to 16% biomass per tank. Each tank was supplied with 24-hour airflow via a system comprising a 1 hp-blower, tubes, hoses, and diffusing rings.
Prior to stocking, the tanks were filled with biofloc water previously matured at a carbon-nitrogen ratio of 12:1.

Both experimental tests monitored the physical and chemical parameters of temperature, dissolved oxygen, pH, total ammonium, and sedimentable solids.

Production parameters

On completion of the tests, weight gain, biomass, growth rate, specific growth rate, and survival rate were calculated, which involved obtaining 25% of the total quantity of organisms per tank and recording the total weight with a digital scale with 0.1g precision.
Said parameters were calculated using the following equations:

Weight gain (g**)—**in grams

$$Wg = fw - iw$$

where $_f$w represents the final weight and $_i$w the initial weight.

Growth rate (%**)—**average weight increase expressed as a percentage

$$GR = \frac{fw - iw}{iw}\ x100$$

where $_f$w represents the final weight and $_i$w the initial weight.

Specific growth rate (SGR)—average daily growth

$$\text{SGR}\ (\%/day^{-1}) = \frac{100\text{x}(\text{in fw} - \text{in iw})}{\text{t}}$$

where $_f$w represents the final weight, $_i$w the initial weight, and t the culture period.

Survival (S)—percentage of organisms alive by the end of the culture period

$$S = \frac{Fn}{In}\ x100$$

where $_f$N is the final number of organisms by the end of the culture period and $_i$N is the initial number of organisms.

The data obtained was subject to Shapiro Wilk normality tests, with a one-way ANOVA and Kruskal Wallis statistical analyses also applied. All the test results were analyzed using the InfoStat-Statitstical Sofware statistical program, version 5.13.

III. Results

The temperature, dissolved oxygen, pH, and total ammonium levels were observed to be similar among treatments for the first test (Table 1). The temperature values were found to be below those established for organism growth for different crustacean species.

While the total ammonium concentration found for the treatment conducted at a density of 240 Ind/m^3 was the highest, significant differences were not observed (Kruskal Wallis, $p>0.05$).

An increase in the quantity of sedimentable solids was observed in line with increased organism density, with the lowest quantity reported at densities of 60 Ind/m^3, with 7 ml/L, while the highest quantity presented at densities of 240 Ind/m^3, with 11 ml/L.

Table 1. Variation in the water quality parameters for *M. acanthurus* over the 14-week culture period

Parameters	Treatments				*p* value
	T1 (60 Ind/m^3)	T2 (120 Ind/m^3)	T3 (180 Ind/m^3)	T4 (240 Ind/m^3)	
Temperature (°C)	24.23±3.56[a]	24.32±3.45[a]	24.27±3.52[a]	24.43±3.55[a]	0.951
Dissolved oxygen (mg/L)	7.55±0.58[a]	7.44±0.52[a]	7.52±0.49[a]	7.40±0.54[a]	0.232

pH (UI)	7.44±0.08[a]	7.47±0.09[a]	7.43±0.07[a]	7.46±0.08[a]	0.161
Total ammonium (mg/L)	0.12±0.11[a]	0.20±0.19[a]	0.21±0.22[a]	0.22±0.20[a]	0.115

Values are median ± DS, with n= 99 per treatment for temperature and dissolved oxygen and n=34 for pH and total ammonium. Results of the Kruskal Wallis statistical analysis. Non-significant differences (p>0.05).

The values obtained for weight gain, biomass, growth rate, specific growth rate, and survival were significantly different among treatments (p<0.05). The highest values were reported for the treatment conducted at a density of 60 Ind/m^3; however, the highest biomass value was reported for the treatment conducted at a density of 240 Ind/m^3 (Table 2).

Table 2. Variation in the production parameters for *M. acanthurus* over the 14-week culture period

Parameters	Treatments				*p* value
	T1 (60 Ind/m^3)	T2 (120 Ind/m^3)	T3 (180 Ind/m^3)	T4 (240 Ind/m^3)	
Weight gain (g)	0.159±0.002[a]	0.153±0.002[ab]	0.141±0.004[c]	0.149±0.003[bc]	<0.01
Biomass (g)	1.98±0.12[a]	3.34±0.27[ab]	3.93±0.54[c]	5.27±0.51[bc]	<0.01
GR (%)	638.6±11.54[a]	614.6±8.32[ac]	566.6±16.16[b]	596±12[bc]	<0.01
SGR (% day^{-1})	2.04±0.01[a]	2.0±0.01[ac]	1.93±0.02[b]	1.97±0.01[bc]	<0.01
Survival (%)	90.47±4.12[a]	80.95±5.45[ab]	71.42±9.52[b]	68.45±4.49[b]	0.010

Values are median ± DS, with n=3 per treatment. Results of the ANOVA statistical analysis. The different letters indicate significant differences to the data obtained via the Tukey test (p<0.05)

Second experimental test

The second test found significant differences for the average temperature, dissolved oxygen, and total ammonium values for both treatments evaluated (Kruskal Wallis, p<0.05) (Table 3), while the pH levels were observed at similar concentrations with no significant differences (p>0.05).

Table 3. Variation in the production parameters of *M. acanthurus* over the eight-week culture period

Parameters	Temperature (°C)	Dissolved oxygen (mg/L)	pH (UI)	Total ammonium (mg/L)
T1 (120 Ind/m^3)	27.37±0.71	6.57±0.53	7.71±0.12	0.15±0.16
T2 (240 Ind/m^3)	27.19±0.73	6.33±0.54	7.72±0.14	0.21±0.17
p value	0.010	<0.001	0.441	0.037

Values are median ± DS, with n= 171 per treatment for temperature and dissolved oxygen, while n=48 for pH and total ammonium. Results of the Kruskal Wallis statistical analysis. Significant difference (p<0.05)

The values obtained for weight gain and specific growth rate presented significant differences between the two treatments (ANOVA, p<0.05). The highest values were found at a density of 120 Ind/m^3 (Table 4). The values obtained for survival were similar for both treatments, while the highest biomass value was obtained at a density of 240 Ind/m^3.

Table 4. Variation in the production parameters for *M. acanthurus* over the eight-week culture period

Parameters	Weight gain (g)	Biomass (g)	SGR (% day^{-1})	Survival (%)
T1 (120 Ind/m^3)	0.886±0.008	20.89±1.53	5.83±0.01	91.02±5.87
T2 (240 Ind/m^3)	0.794±0.008	35.77±1.71	5.65±0.01	87.17±4.83
p value	<0.001	<0.001	<0.001	0.430

Values are median ± DS, with n=3 per treatment. Results of the ANOVA statistical analysis. Significant differences observed in the results of the Tukey test ($p<0.05$).

Finally, the composition of the biofloc in both experimental tests was mainly dominated by protozoa, rotifers, nematodes, and bacteria.

IV. Discussion

According to various studies, temperature is an important parameter for prawn development, growth, and survival and must remain at 28-30°C (Ponce *et al.* 2006; Vega *et al.* 2011; Arana *et al.*, 2013; Cupertino *et al.*, 2017). Although the results obtained for these parameters by the first test showed similar behavior among treatments, they remained at lower levels that were not apt for the culture, while these levels were raised in the second test via the use of aquarium heaters.

Various studies have reported that low levels of dissolved oxygen promote the manifestation of stress in organisms, triggering decreased ingestion of food, slower growth, and increased susceptibility to disease (Arana *et al.* 2013; Cupertino *et al.* 2017).

The present study found acceptable or similar dissolved oxygen levels for both experimental tests.

In the system of interest, the pH must remain at optimal levels to enable the nitrifying bacteria to develop and then adequately perform their function in the biofloc (Miranda *et al.* 2020). In both tests, the levels of these bacteria were recovered via the constant addition of sodium bicarbonate.

Despite the elevated densities used in both tests, the biofloc enabled the total ammonium levels total to be maintained at adequate concentrations, ones similar to those reported by other studies (Cupertino *et al.* 2017; Ponce-Palafox *et al.* 2018; Cruz-Cruz *et al.* 2021).

In terms of the production variables, it was observed that the weight gain, growth rate, specific growth rate, and survival values obtained were higher at low densities (60 and 120 Ind/m^3), while, at densities of 240 Ind/m^3 in both experimental tests, higher biomass values were reported, although with smaller organisms.

These values were lower than those obtained by Emmerson and Andrews (1981), Murcia-Mena and Paz-Quevedo (2020), Arana *et al*. (2013), Cupertino *et al.* (2017), Paul *et al.* (2016), Ponce-Palafox *et al.* (2018), Peña-Herrejón *et al.* (*2019*), and Cruz-Cruz *et al.* (2021).

Monrroy *et al*. (2013) describe communities of organisms associated with biofloc, finding microalgae, ciliates, rotifers, nematodes, bacteria, and the yeast *Rhodotorula sp* to be the main groups observed in tilapia culture. Cruz-Cruz *et al.* (2021) reported nematodes, rotifers, cyanobacteria, ciliates, heliozoa, and dinoflagellates for *M. rosenbergi*, while Gómez-Mundo *et al.* (2020) observed microalgae, ciliates, paramecium, protozoa, amoeba, gastrotrichs, heliozoa, and oligochaetes in *P. setiferus* culture. The two experimental tests applied by the present study revealed that the composition of the biofloc was determined by four groups, among which the main microorganisms found were protozoa, rotifers, bacteria, and nematodes.

The levels of sedimentable solids were observed to increase, for both tests, in line with increased stocking density, due to the increased quantity of commercial feed provided, despite which the values obtained were observed to be within acceptable ranges. This behavior is similar to that reported by Gómez-Mundo *et* a*l*. (2020) and Cruz-Cruz *et al.* (2021) in *P. setiferus* and *M. rosenbergi* cultures.

V. Conclusions

Water temperature was found to influence prawn growth. The temperatures recorded during the first test were below those reported and recommended for the culture of interest. However, by maintaining higher levels, the second test obtained an improvement in growth values.

The dissolved oxygen and pH concentrations remained at adequate concentrations in both tests. The biofloc system kept ammonium concentrations at acceptable levels, which were below toxic levels for the organisms.

Both tests obtained higher weight gain, growth rate, specific growth rate, and survival values at densities of 60 and 120 Ind/m^3; however, the highest biomass value was obtained at densities of 240 Ind/m^3.

Despite the fact that the elevated densities applied promoted an increased presence of sedimentable solids, these values were reported at levels that are non-lethal for the organisms.

Finally, the composition of the biofloc used in both experimental tests was determined by four groups of microorganisms—protozoars, rotifers, bacteria, and nematodes.

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