

Water quality and growth of juvenile specimens in giant freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879) cultures applied in recirculation and biofloc systems

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Abstract

*Biofloc research commenced in the late 1970s, with the white shrimp *Litopenaeus vannamei* and the Nile tilapia *Oreochromis niloticus* the most commonly studied species. The present study evaluated *M. rosenbergii* cultures in recirculation and biofloc systems by comparing the water quality and productive performance of juveniles in both systems. The study was based on a simple randomized experimental design with a recirculation and biofloc treatment, each conducted in triplicate. The initial length and weight of the organisms was 1.04 cm and 0.31 g (recirculation) and 1 cm and 0.30 g (biofloc), respectively, while the bioassay, which lasted nine weeks, was undertaken in a salinity of 5 ppm with a natural photoperiod (12:12). The present study monitored basic variables corresponding to the water, survival, growth (in weight), and the composition of the plankton.*

The basic variables of the water (temperature, salinity, and pH) were kept within the recommended range for the culture. Of the plankton observed in the biofloc, nematodes, rotifers, cyanobacteria, ciliates, heliozoa, and dinoflagellates predominated. The organisms grown in the recirculation system reached 5.35 cm in length and 1.28 g in weight, while those grown in the biofloc reached 5.18 cm and 2 g, with significant differences in individual weight observed. Survival in the recirculation and biofloc systems was 73% and 60%, respectively, with significant differences observed. It is concluded that, although the survival rate was higher in the recirculation system, the weight of individual organisms was higher in the biofloc treatment.

Keywords: Biofloc, *Macrobrachium rosenbergii*, physicochemical parameters.

Introduction

Aquaculture has great economic potential in Mexico, bringing with it social benefits and providing an important source of food (Álvarez *et al.*, 2012; Campos *et al.*, 2016). In light of population growth, climate change, and globalization, the search for methods that promote the development of sustainable aquaculture is of the utmost importance (Campos *et al.*, 2016).

Biofloc is an aggregate comprising bacteria, algae, and other microorganisms associated with particulate organic matter. In its initial stages, biofloc is formed by the colonization of heterotrophic bacteria, which secrete exopolysaccharides, which, in turn, facilitate the adhesion of microorganisms and organic matter (Crab *et al.*, 2007; Ahmad *et al.*, 2017; Venegas, 2019).

The microorganisms that develop in bioflocules play a key role in the nutrition of cultivated animals, as they are a rich natural source of proteins and lipids that is available *in situ* 24 hours a day. An interaction is produced in the water column among organic matter, the physical substrate, and a wide range of microorganisms, such as phytoplankton, free and attached bacteria, the aggregates of particulate organic matter, and herbivores, such as rotifers, ciliates, flagellates, protozoa, and copepods.

This natural productivity plays an important role in the recycling of nutrients and the maintenance of water quality (Emerenciano *et al.*, 2013).

Among the main benefits of biofloc are water savings and the reduction of pelleted feed use (García-Ríos *et al.*, 2019), with this system also increasing culture density and minimizing the use of space, thus guaranteeing a higher quality product than traditional systems (Mancipe *et al.*, 2019).

In biofloc technology (BFT) systems, one of the most efficient methods for eliminating potentially toxic nitrogenated compounds occurs via the nitrification process, which consists in two phases: first, the bacteria of the genera *Nitrosomonas sp* and *Nitrosococcus sp* act on the ammonia generated by feces, urine, and food, oxidizing these residues into nitrite; and, second, the nitrite is converted into nitrate by bacteria of the genera *Nitrobacter sp* and *Nitrospira sp*, although, in some cases, the nitrate is reduced to nitrogen gas (via anoxic denitrification) by bacteria of the genera *Achromobacter sp* and *Pseudomonas sp*, thus completing the abovementioned cycle (Pérez-Rostro *et al.*, 2014).

Among the most important parameters in biofloc cultures are dissolved oxygen, pH, temperature, ammonium levels, and salinity. While the optimal level of dissolved oxygen for shrimp is usually higher than 3 mg / l (D'abramo *et al.*, 2003), it has recently been shown that shrimp can tolerate levels of 2 mg / l for short periods and survive. However, for farms that operate with BFT systems, it has been reported that the minimum dissolved oxygen levels must be higher than 4 mg / l (Miranda-Baeza *et al.*, 2018).

The pH values in biofloc systems can vary from 6.8 to eight units, although values lower than seven are normal, and, if not properly controlled, could negatively impact the nitrification process (Emerenciano *et al.*, 2017). Temperature is an important factor for the growth of organisms, which present better growth levels when the water temperature is within the optimal range, which, in the case of tropical species, varies from 28 to 30 ° C (Emerenciano *et al.*, 2017).

Ammonium, in traditional cultures, should exceed 1.2 mg / l (Frías-Espericueta *et al.*, 2000), although in BFT systems, this value should be lower than 20 mg / l (Emerenciano *et al.*, 2017; Miranda-Baeza *et al.*, 2018), while nitrites should not exceed 1 mg / l once the levels are considered stable, as high levels could be difficult to control (Emerenciano *et al.*, 2017). Nitrates at concentrations lower than 100 mg / L do not present a high level of risk for organisms grown in biofloc (Miranda-Baeza *et al.*, 2018).

As an alternative source of live food, flocs and their various benefits are of interest to aquaculture nutrition. Few studies have been conducted in Mexico on juvenile *M. rosenbergii*, despite it being a species with great commercial potential (Pérez-Fuentes *et al.*, 2013). The present study aimed to evaluate *M. rosenbergii* cultures in recirculation and biofloc systems by comparing the water quality and the productive

performance of juveniles in both systems.

Materials and methods

The present study was carried out in the aquaculture bioassay laboratory of the Faculty of Biological and Agriculture and Livestock Sciences at the University of Veracruz, campus Tuxpan, in the state of Veracruz. The organisms were donated by the Postgraduate College, based at the Veracruz campus of the university, and acclimatized for 15 days on arrival at the laboratory.

Experimental design

The bioassay comprised two treatments (recirculation and traditional) conducted, in triplicate, using 300/L plastic tanks as the experimental units, into which 90 *M. rosenbergii* juveniles (the equivalent of 270 ind/m³) were placed for each replica. The initial length and weight for the recirculation treatment was 1.04 cm and 0.31 g, respectively, and 1.01 cm and 0.30 g, respectively, for the biofloc treatment.

The water in the recirculation system was driven by an *airlift*-type system, while the experimental units were connected to a biological filter, with aeration provided by means of a ½ Hp electric blower. Prior to seeding, the system remained in operation for three weeks in order for the seedstock to mature, while, after seeding, a 50% water change was undertaken every week to guarantee optimal water quality conditions.

The experimental biofloc units were fitted with air diffusers on the tank floor to ensure that the matter remained suspended/keep the matter in suspension. Prior to seeding in the biofloc system, a three-week maturation period was applied, in which 5 g of unrefined sugar and 2 g of ground pelleted feed were added per m³ of water on a weekly basis. During the culture period, the carbon-nitrogen (C:N) ratio was 9: 1, while unrefined sugar was added as a complementary source of carbon, with the corresponding estimations made according to Avnimelech (2009). Both treatments used natural photoperiods of 12 h light and 12 h darkness.

Water quality monitoring

The water variables (salinity, temperature, and pH) were measured twice a day at 09:00 and 21:00, with the salinity measured using a Brix refractometer, the pH measured using an API saline water kit, and the dissolved oxygen levels measured using a HANNA® model HI9747 oximeter.

The ammonium and nitrite levels in the biofloc system were monitored weekly for both treatments, while the nitrogen compounds were measured using a HANNA model HI83300 field photometer.

The amount of suspended solids was measured every week, using an Imhoff cone, with a 1L biofloc sample taken, for each measurement, and left to stand for 30 minutes prior to the reading.

Monitoring of microorganisms in the biofloc system

Every week, water samples (500 mL) were taken in the biofloc tanks and left to settle in the laboratory for a period of 60 min, fixed in 100 mL containers with 4% formalin, and then viewed under an optical microscope.

Feeding

In both treatments, the organisms were fed twice a day, at 09:00 and 19:00. The proportion of feed was calculated based on the estimated living biomass and adjusted weekly, from 10% at the beginning of the experiment to 5% by the end. Commercial feed, 0.5 mm in diameter and comprising 45% crude protein,

4% fiber, 9% fat, 13% ash, and 12% moisture (of the brand Pedregal®), was used.

Growth in length and weight was calculated biometrically. The length of the individuals was measured from rostrum to telson with a transparent ruler, while their weight was taken with a digital scale precise to a tenth of a gram. At the end of the bioassay, the organisms were counted to estimate the survival rate.

Data processing

The data obtained was subject to an analysis of the statistical assumptions of normality via the Shapiro Wilks test, while the homogeneity of variances was analyzed using Levene's test, with a significance level of $P < 0.05$. To ascertain the differences between both treatments, Student's t tests were performed, with data not complying with the assumptions analyzed via the nonparametric Mann-Whitney test. All statistical analyses were performed using the Minitab 10 software.

Results

The salinity levels in the biofloc treatment presented a minimum value of 4 psu and a maximum value of 6 psu, while minimum and maximum values of 5 psu and 7 psu, respectively, were observed for the recirculation treatment. In both treatments, the average salinity level observed throughout the experiment was 5 psu, with no statistically significant differences observed.

In the mornings, the dissolved oxygen level for the biofloc treatment was recorded, giving a minimum value of 6 mg / l and a maximum value of 9.6 mg / l, while, for the recirculation treatment, a minimum value of 5.3 mg / l and a maximum value of 7.5 mg / l were observed. During the night, the biofloc treatment presented a minimum value of 5.9 mg / l and a maximum value of 9.2 mg / l, while the recirculation treatment presented a minimum value of 5.1 mg / l and a maximum value of 7.7 mg / l. No statistically significant differences were observed for dissolved oxygen between the treatments.

The biofloc system presented a minimum and maximum morning temperature of 18 ° C and 28.2 ° C, respectively, while this was 22.2 ° C and 27 ° C, respectively, for the recirculation system. At night, the minimum temperature for the biofloc treatment was recorded at 19.5 ° C with a maximum temperature of 30.9 ° C, while, for the recirculation system, the minimum value was 23 ° C and the maximum was 28.7 ° C. The student's t test did not show statistically significant differences.

For both treatments, the minimum pH value in the morning was 8.2 units, while the maximum was 8.4 units. From Day 5 onwards, it was noted that a pH value of 8.2 became constant for both treatments, between which no significant differences were observed.

The ammonium levels in the biofloc treatment presented minimum and maximum levels of 0.25 mg / l and 0.36 mg / l, respectively, with stable concentrations close to 0.2 mg / L then obtained from Week 3 onwards. Minimum and maximum ammonia values of 0.25 mg / l and 0.55 mg / l, respectively, were obtained for the recirculation system. The student's t test did not show significant differences between the two treatments for this parameter.

A minimum nitrite level 1 mg / l was observed for the biofloc treatment, with a maximum peak of over 20 mg / l, while this variable remained at zero for the recirculation system due to the weekly water replacements carried out. The minimum and maximum nitrate levels observed for the biofloc treatment were 5 mg / l and 17.5 mg / l, respectively, with this variable beginning to increase once the nitrite levels

began to decrease. The nitrate level was 0 mg / l for the recirculation system.

The levels of suspended solids in the biofloc system ranged from 5 mg / L to a maximum of 8 mg / L and were found to be close to 1 mg / L for the recirculation system. The student's t test showed significant differences between treatments for this variable.

The microorganisms that presented during the first two weeks of the biofloc treatment were *Spirostomum sp*, *Epistylis sp*, and microalgae, with *Euplotes sp*, *Oscillatoria sp*, *Epistylis sp*, and *Paramecium sp* appearing in weeks three and four. At the end of the culture, *Paramecium sp*, nematodes, *Aspidisca sp*, *Oscillatoria sp*, and rotifers were also observed (Figure 1).

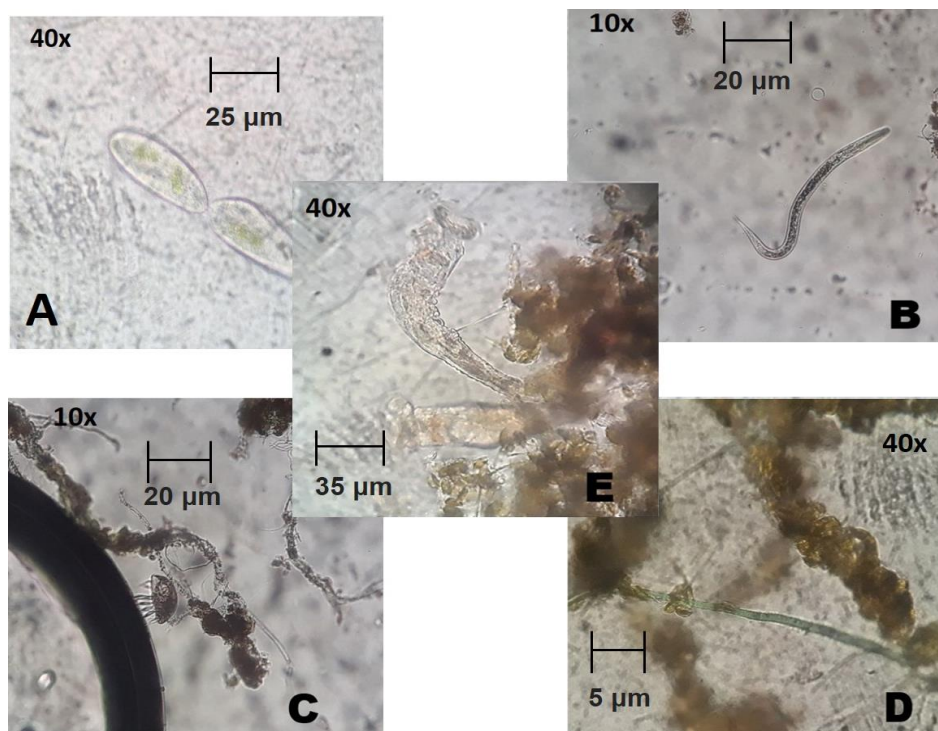
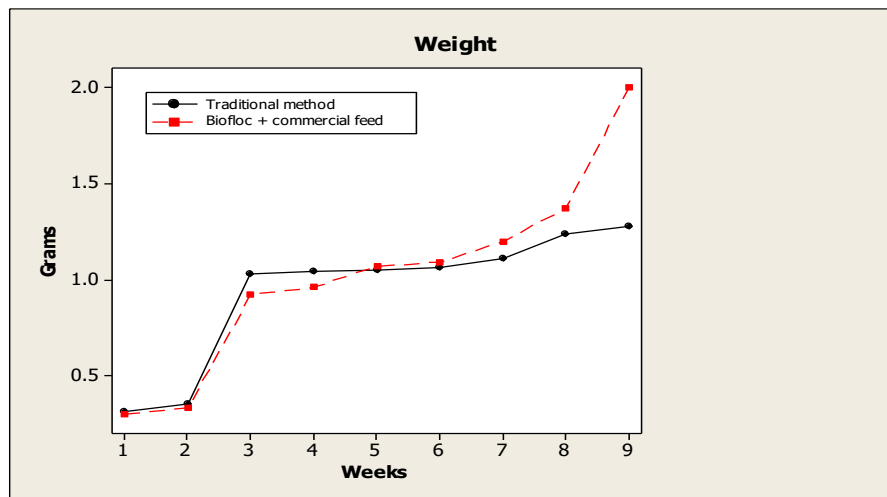


Figure 1. Some microorganisms present in the biofloc treatment at the end of the culture period: *Paramecium sp* (A); nematodes (B); *Aspidisca sp* (C); *Oscillatoria sp* (D); and, rotifers (E)

The daily weight gain observed for the recirculation treatment was 0.01 g, while this was 0.02 g for the biofloc treatment, with the student's t test showing significant differences. By the end of the experiment, a length of 5.35 cm and a weight of 1.28 g were observed for the recirculation treatment, while these variables were 5.18 cm and 2 gm, respectively, for the biofloc treatment. The student's t test showed significant differences (Figure 2).

Figure 2. Average individual weight of *M. rosenmebergii* grown in the recirculation and biofloc systems.

The survival rate was found to be 73% and 60% for the recirculation and biofloc treatments, respectively, with significant differences observed between the treatments ($U < 0.5$).



Discussion

With dissolved oxygen being one of the most important parameters for shrimp culture, the average levels obtained by the present study for this parameter were 6.9 mg / l (recirculation) and 7.9 mg / l (biofloc). Miranda-Baeza *et al.* (2018) recommend minimum values of over 4 mg / l for biofloc culture systems. With D'abramo *et al.* (2003) suggesting that this parameter should remain above 3 mg / l, both treatments were found to be within the optimal range. With PH also an important variable, a study conducted on juvenile *M. rosenbergii* by Tidwell *et al.* (2003) found pH values of 9.0 units, a level considered critical. The average pH value observed in the present study was below 9.0 units for both treatments, which concurs with that recommended by D'abramo *et al.* (2003), who indicate optimal pH values of between 6.5 and 7.5 units. For biofloc systems, Emerenciano *et al.* (2017) recommend a pH of over 7.0 units in order to enable nitrifying bacteria to develop adequately in the flocs and carry out the nitrification process.

With regard to temperature, Singholka (1984) recommends an optimal temperature of between 18 and 34 ° C. As the bioassay conducted in the present study obtained an average temperature of 24.4 ° C for the recirculation treatment and 24.7 ° C for the biofloc treatment, these values were found to be within the optimal range for the development of the organisms.

While *M. rosenbergii* larvae require brackish water of 12 ups, when entering the near-juvenile stage, they need freshwater and can withstand a change in salinity of 12 to 0 ups. López (2004) recommends that this change in salinity be gradual, although this point does not appear in the literature cited. The present study maintained salinity at between 5 and 8 ups, thus staying within the recommended range.

Ammonium results from the metabolic waste produced by organisms (Emerenciano *et al.*, 2017). Miranda-Baeza *et al.* (2018) indicate that, for commercial cultures produced via biofloc systems, ammonium levels should not exceed 20 mg / l; however, for traditional cultures, Frías-Espericueta *et al.* (2000) recommend levels of below 1.2 mg / l. As, in the present study, ammonium levels remained at an average of 0.26 mg / l (biofloc) and 0.28 (recirculation), they were within the optimal range.

Boyd (2001) suggests a nitrite concentration below 0.23 mg / l for traditional systems, while, for biofloc systems, Emerenciano *et al.* (2017) recommend that said concentration does not exceed 1 mg / l once the medium is stable, as it is normal for this parameter to be high while still in the maturation process. A peak of over 20 mg / l was recorded in the fourth week for the biofloc treatment, after which the levels dropped

to and remained at levels close to 0 mg / l until the end of the experiment, while, in the recirculation treatment, this variable remained at levels close to zero due to the water changes. The nitrate levels remained well below 100 mg / l, at which level and above the organisms may be damaged (Miranda-Baeza *et al.*, 2018).

In BFT systems, floc levels tend to increase with the age of the culture and in accordance with the amount of feed added and the effect of adding external carbon sources (Emerenciano *et al.*, 2011). Together, these factors increase the amount of particulate organic matter and microorganisms, which should be monitored in order that suspended solids remain at adequate levels for the species cultured. In this regard, Avnimelech and Suryakumar (2017) recommend concentrations of between 200 to 300 mg / l, concentrations which may vary depending on the species and the age of the organisms cultured. Emerenciano *et al.* (2017) recommend levels of sedimentable solids of 5 to 15 ml / l for shrimp grown in biofloc, a suggested range with which the present study complied, maintaining levels of 6 mg / l to 8 mg / l.

The diversity of microorganisms that develop naturally in the biofloc system tends to increase with the age of the culture. Various authors have monitored the succession of the main groups of microorganisms, among whom, Emerenciano *et al.* (2011) report four main groups, including protozoa (ciliates), rotifers, cyanobacteria (filamentous and unicellular), and pennate diatoms. Moreover, Monroy-Dosta *et al.* (2013) described five groups, comprising nematodes, microalgae, ciliates, rotifers, and bacteria. Jiménez *et al.* (2017) recorded seven phytoplankton genera, twelve ciliate genera, and one rotifer genus when molasses was used as a carbon source, while, when molasses and polished rice were used, three phytoplankton genera, five ciliate genera, and one group of rotifers were recorded. The present study registered six groups of microorganisms at the end of the culture period, including nematodes, rotifers, cyanobacteria, ciliates, heliozoa, and dinoflagellates, results which, in general, concur with previous studies and reinforce the importance of biofloc as a permanent source of live food.

Among the most important benefits of biofloc culture is its contribution to the nutrition of organisms. Studies conducted on stable isotopes indicate that biofloc contributes, by up to 18%, to tissue formation in shrimp (Ray *et al.*, 2017). The foregoing concurs with the results obtained by the present study and may explain the difference found in final weight between the organisms grown in the recirculation and biofloc systems (1.28 g / ind and 2.0 g / ind, respectively).

Although biofloc provides live food of high nutritional quality via high-density systems, it is necessary to supplement the organisms' diet with pelleted food (Moreno-Arias *et al.*, 2018). Emerenciano *et al.* (2011) found that postlarvae of the pink shrimp *Farfantepenaeus paulensis* fed with biofloc plus commercial feed presented a significantly higher weight (235 mg) than those cultured with biofloc without a feed supply (218 mg).

Previous studies conducted on shrimp and prawn have shown that organisms cultured in biofloc reach higher individual weights than those cultured in traditional systems. Among said studies, Pérez-Rostro *et al.* (2014) reported that *M. rosenbergii* adults reached a weight of 15.17 ± 8.2 g / ind in biofloc, while weights of 12.57 ± 7.89 g / ind were obtained by cultures using traditional methods. Furthermore, Kim *et al.* (2014) reported a final average weight of 13.298 g/ind for *Litopenaeus vannamei* cultured in biofloc and 7.767 g/ind for the control treatment.

Finally, Emerenciano et al. (2011) reported a higher survival rate (84.8%) for the postlarvae of the pink shrimp *Farfantepenaeus paulensis* in the control treatment than in biofloc treatments applied both with and without commercial feed (81.5% and 67.0%, respectively). Pérez-Rostro *et al.* (2014) recorded an 85% survival rate for *M. rosenbergii* via both traditional and biofloc culture methods. The present study obtained a lower survival rate for the biofloc treatment (60%) than for the recirculation system (73%), which could be due to the peak of nitrite levels registered during the fourth week of the culture. As research on *M. rosenbergii* cultures conducted in biofloc systems is incipient in Mexico, it is necessary to continue studying this species to obtain more information that would enable its exploitation in the medium term, due its high commercial potential for inland systems.

Conclusion

M. rosenbergii juveniles cultured in biofloc presented a higher individual weight than those cultured in the recirculation system, which can be attributed to the presence of permanently available live food.

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