

The relationship between shrimp (*Litopenaeus vannamei*) size and biofloc consumption determined by the stable isotope technique

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ABSTRACT

Biofloc technology (BFT) is considered one of the most promising methods for the sustainable development of shrimp culture. Bioflocs keep the water quality at good standards and represent a complementary food source, allowing the production of high stocking densities of the white shrimp *Litopenaeus vannamei*. Microorganisms present in flocs are an important source of lipids and proteins; however, laboratory observations indicate that floc consumption is not the same for all shrimp stages. We have, therefore, established the hypothesis that bioflocs are more important food sources for larger shrimps that can better manipulate the flocs and pick up and consume specific microorganisms. The stable isotope technique has been recently applied in aquaculture to quantify the importance of different food sources to the growth of target species. In this study, this technique was employed in two experiments in order to test the established hypothesis, measuring biofloc consumption by 0.01 g *L. vannamei* postlarvae in nursery tanks and by 0.80 g shrimp in the grow-out phase. Both experiments were conducted in greenhouse tanks (12–800 L) with a stocking density of 2000 shrimp/m³ in the nursery phase and 400 shrimp m³ in the grow-out phase. The experiment was designed with two treatments with four replicates each: (I) F, with feed only; and (II) BF, with bioflocs and feed. Shrimp were fed a commercial diet twice a day in the nursery and grow-out stage with 40 and 38% crude protein, respectively. Differences were found between water quality parameters for pH, ammonia, nitrite, nitrate, phosphate, SST and turbidity ($p < 0.05$). Zootechnical performance also showed differences between shrimp of different treatments for final weight, final biomass, survival, yield m³ and m² ($p < 0.05$). The results of the stable isotope technique indicate that bioflocs contributed 22–43% C and 0–43% N to the shrimp tissue composition during the nursery phase, whereas in the grow-out phase, the contribution of bioflocs varied between 63 and 100% C and 35–86% N. Differences in the abundances of *Oocystis* sp., pennate diatoms and flagellates ($p < 0.05$) indicate that these microorganisms were preferentially consumed by larger shrimp. Thus, the results of this study corroborate the hypothesis that the contribution of bioflocs as a supplementary source of natural food depends on the size of the shrimp.

1. Introduction

The traditional intensive production of marine shrimp can impact the environment, especially due to the release of effluents in areas adjacent to cultivation. Salinization of receiving water bodies, damage by the introduction of exotic species and the spread of diseases are other problems caused by the effluents of traditional aquaculture systems (Boyd, 2003; Lightner, 2005). Biofloc technology (BFT) has been highlighted as one of the most suitable and promising systems for the

sustainable development of intensive shrimp culture (Krummenauer et al., 2011; Hargreaves, 2013) since this system is based on shrimp growth with minimal or no water renewal. The system reduces the demand for water and consequently has small emission of effluents, minimizing the impacts to the environment and raising the cultivation biosecurity by reducing the introduction and spread of diseases (McIntosh, 2000; Wasielesky et al., 2006; Samocha et al., 2017).

The BFT system is characterized by the transformation of ammonia, which is toxic at high concentrations, into microbial biomass. This

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system involves the manipulation of the carbon/nitrogen ratio (Avnimelech, 1999), where the heterotrophic bacteria present in bioflocs assimilate inorganic nitrogen after the addition of an organic carbon source, improving the parameters of water quality and reducing the need for constant water exchange (Ebeling et al., 2006). In addition, the formation of a natural biota composed predominantly of heterotrophic organisms represents a complementary food source for the produced organisms, depending on the ability of the reared species to use the natural productivity of the system (Wasielesky et al., 2006; Hargreaves, 2013; Lara et al., 2017). According to Cardona et al. (2015), bioflocs formed in the culture system can provide approximately 37 to 40% of natural productivity and can stimulate digestive enzyme activity; this food availability and increase in enzymatic activity may contribute to the growth of shrimp reared in biofloc systems.

With the establishment of the BFT system, there is a need to know the real contribution of microbial aggregates to the reared organisms and how much of the microbial biomass can be incorporated into the muscle of the shrimp. For example, Burford et al. (2004a) estimated that 29% of the biomass incorporated into shrimp is from bioflocs, and Wasielesky et al. (2006) estimated that 32% of shrimp growth can be attributed to bioflocs. Moreover, the contribution of microbes to BFT changes over time, with a larger contribution to larger shrimp (Abreu et al., 2007; Suita et al., 2016). Thompson et al. (2002) demonstrated that the shrimp *Penaeus paulensis* could selectively feed on microorganisms. In doing so, it is likely that microbes would be a more important food source for larger shrimp, which can better manipulate the bioflocs in the system.

Although penaeid shrimp predation on natural microbiota has been demonstrated using stomach content analysis techniques, there is still no consensus among researchers regarding its contribution. Moreover, the results obtained with this technique are not very accurate, as it only allows the identification of what was consumed but not necessarily what has been digested and incorporated into the tissues and used for growth (Thompson et al., 2002; Soares et al., 2005). To obtain better and more detailed information on this issue, it is possible to use the stable isotope technique and mass-balance mixing model. This technique allows identification of the influence of diet on the growth of a consumer after a given period of time, since the consumer's isotope ratios tend to be similar to those of the assimilated diet (Peterson and Fry, 1987; Gamboa-Delgado et al., 2008).

To use the stable isotope mixing model approach, one assumes that the species has an isotopic profile similar to that of its diet consumed after incorporation into its tissue. However, differences in isotopic composition between animals and their diet depend on chemical reactions that occur inside the organism at the moment of digestion, leading to isotopic fractionation, i.e., isotopic shifts between a food source and its expression in an animal tissue (Pearson et al., 2003). Previous studies with young shrimp have estimated that 15 days are necessary for this balance between the organism and the diet to occur (Gamboa-Delgado and Le Vay, 2009; Gamboa-Delgado et al., 2013) or at the moment when the organism quadruples its weight (Fry, 2006). According to Peterson and Fry (1987), the fractionation between animals and their diets varies between 3 and 5‰ and 0–1‰ for nitrogen and carbon stable isotope ratios, respectively. Stable isotope mixing models are often used to quantify the % contribution of foods to animal (in this case, shrimp) tissue growth (Phillips and Gregg, 2001; Phillips, 2012), which considers the isotopic differences between the sources and the analyzed tissue.

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios can be measured in bioflocs, feed and shrimp tissue in order to determine the contribution of each food source to shrimp (Ray and Lotz, 2017). Previous studies using this technique with *Litopenaeus stylirostris* showed that bioflocs could supply 37 to 40% of C and N necessary for growth (Cardona et al., 2015). Similarly, Thomas and Ray (2018) found that bioflocs may provide 13% of C and 34% of N to the tissue of shrimp in the nursery phase and may originate from bioflocs or some food

recycling. Despite these results, no study has evaluated the possible influence of shrimp size on biofloc consumption in BFT systems.

In this study, we employed the stable isotope technique in order to test the hypothesis that the consumption of bioflocs by shrimp is related to their size. Two experiments were conducted with the shrimp *Litopenaeus vannamei* of different sizes/weights (0.01 and 0.83 g) fed commercial feed with high protein levels. In both experiments, the shrimp had also available a large amount of microorganisms (bacteria and protozoa) present in bioflocs as a supplementary food source.

2. Materials and methods

2.1. Culture conditions

The first experiment in the nursery was conducted over 40 days, whereas the second experiment with larger shrimp (grow-out) took 60 days. Both experiments were conducted in the facilities of the Marine Aquaculture Station (EMA), Oceanographic Institute of the Federal University of Rio Grande, located in Casino, Rio Grande - RS Brazil (32° 19' S, 52° 15' W). The species used in the study was the Pacific white shrimp *L. vannamei* purchased from Aquatec® LTDA (Rio Grande do Norte); shrimp were kept from nauplii to the postlarval phase (PL25) in the Carcinoculture laboratory larviculture sector, Marine Aquaculture Station - IO / FURG. For the nursery experiment, shrimp were stored in 12 rectangular experimental units with 800L useful volume, located in a greenhouse, with a density of 2000 shrimp / m³ and an initial weight of 0.01 ± 0.007 g. For the grow-out phase experiment, a density of 400 shrimp m³ with an initial weight of 0.83 ± 0.53 g was used in the same experimental units previously described for the nursery experiment.

2.2. Experimental design

Both experiments consisted of two treatments with four replicates: (I) F, with feed only; and (II) BF, with bioflocs and feed. In treatment I, water renewals were performed only when the ammonia concentration reached 1.0 mg L⁻¹. In treatment II (BF), a 25% inoculum of mature bioflocs from previous cultures was used, following the methodology proposed by Krummenauer et al. (2014). When the total ammonia nitrogen concentration reached 1.0 mg L⁻¹, corrections were made by adding sugarcane molasses to adjust the C:N ratio to 6:1. In doing so, the nitrogen present as ammonium was converted to bacterial biomass. For each 1.0 g of nitrogen total ammonia, 6.0 g of carbon is necessary (Avnimelech, 1999; Ebeling et al., 2006). A commercial probiotic (Sanolife® PRO-W. INVE, Belgium) was also applied to the water of both experiments once a week at a concentration of 0.5 ppm. This probiotic is composed of *Bacillus subtilis* and *Bacillus licheniformis*, with a bacterial concentration of 5.0×10^{10} colony forming units (cfu) / g.

2.3. Shrimp feeding and monitoring

In the F and BF treatments, the shrimp were fed twice daily in the nursery and growth phase using the commercial Potimar PL GUABI® diet (40 and 38%), respectively. To control consumption, 10% of the amount of feed was placed in the trays, and the rest was distributed for transport in the tank. Adjustments in feeding rates were made following the method of Jory et al. (2001).

2.4. Zootechnical performance

The zootechnical performance of the shrimp was monitored weekly by measuring the mean weight of 30 animals per experimental unit using a digital scale with a precision of 0.01 g (Marte® UX420H). The following metrics were evaluated:

WG = (FW – IW)/n weeks of culture;

where WG = weight gain, IW = initial weight, FW = final weight;

FCR = feed offered/biomass increase;

where FCR = apparent feed conversion rate;

Survival: (final biomass/individual mean weight)/number of individuals stocked) x100;

Productivity: (final biomass – initial biomass)/tank volume.

2.5. Stable isotope analysis and calculation

Samples of feed bioflocs and shrimp were collected every 15 days in all treatments to determine the isotopic values of carbon and nitrogen. Three biofloc samples were taken from each treatment (BF and F). For each muscle sample, four shrimp were randomly taken from each of the 12 experimental tanks, and the shrimp from each tank were pooled and frozen for analysis. To collect biofloc samples, 2L of water from each tank was manually filtered through a 50 µm mesh, after which the material present in the mesh was scraped and stored in flasks. The samples were kept in a deep freezer (Quimis 80v) and freeze-dried in a freeze-dryer (Liotop-L101 / Liobras®) for approximately 10 h at the Seabird and Turtle Laboratory of the Institute of Biological Sciences FURG.

All samples were macerated, weighed (1.0 ± 2.0 mg), placed in tin capsules and sent to the laboratory of the UC Davis Stable Isotope Facility, CA, U.S.A., where the analyses of the isotopic relation of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) were determined by mass spectrometry. The results are presented in delta notation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = ((\text{R sample}/\text{R standard}) - 1) \times 1000$$

where R sample is the ratio of heavy and light isotopes from the sample and R standard corresponds to the ratio between heavy and light isotopes of Pee Dee Belemnite and atmospheric air, the international standards for carbon and nitrogen, respectively. The contribution of nitrogen and carbon from bioflocs and diet to shrimp growth in both experiments was evaluated by solving the system of mass balance and mixing equations for one isotope and two sources (being applied to C and N separately) (Phillips and Gregg, 2001). The model considers the isotopic differences between the sources (feed and bioflocs) and mixture (whole body of shrimp). The contributions of nitrogen and carbon in the bioflocs and diet (feed) to shrimp growth in both experiments were evaluated by solving the mass balance system and mixing equations for one isotope and two sources, as described below:

$$\delta^{15}\text{N}_{\text{shrimp}} = f_{\text{Bioflocs}} \delta^{15}\text{N}_{\text{Bioflocs}} + f_{\text{feed}} \delta^{15}\text{N}_{\text{feed}}$$

$$\delta^{13}\text{C}_{\text{shrimp}} = f_{\text{Bioflocs}} \delta^{13}\text{C}_{\text{Bioflocs}} + f_{\text{feed}} \delta^{13}\text{C}_{\text{feed}}$$

where $\delta^{15}\text{N}_{\text{shrimp}}$ and $\delta^{13}\text{C}_{\text{shrimp}}$ are isotopic signatures of shrimp, whereas $\delta^{15}\text{N}_{\text{bioflocs}}$, $\delta^{13}\text{C}_{\text{bioflocs}}$, $\delta^{15}\text{N}_{\text{feed}}$ and $\delta^{13}\text{C}_{\text{feed}}$ are the isotopic signatures of bioflocs and artificial feeding. In addition, f_{Bioflocs} and f_{feed} are the proportions of sources (bioflocs and feed) in the isotopic signature of the shrimp.

In this study, the adopted value for fractionation was between 0.45 and 1.88 for C and 0.73 and 2.59 for N. Moreover, Cardona et al. (2015) obtained these factors of discrimination distinguishing the sources used with shrimp consuming only bioflocs and with shrimp consuming only the experimental diet.

2.6. Water quality parameters

Physical and chemical water parameters such as temperature and dissolved oxygen were monitored daily using a multiparameter YSI® 55 instrument and a YSI®100 pH meter. The total ammonia concentration ($\text{N}(\text{NH}_4^+ + \text{NH}_3)$) was determined according to UNESCO (1983). The nitrite ($\text{N}(\text{NO}_2^-)$), nitrate ($\text{N}(\text{NO}_3^-)$) and phosphate ($\text{P}(\text{PO}_4)$) concentrations were determined weekly using the methodology described in Strickland and Parsons (1972). Salinity and turbidity were monitored

weekly using an ATC® handheld optical refractometer and a turbidimeter (Hach® 2100P), respectively. Water transparency was determined using a Secchi disk and total suspended solids (TSS) analysis according to the methodology of Strickland and Parsons (1972). Total suspended solids were maintained at the levels recommended by Gaona et al. (2011), namely, 500 mgL^{-1} , and when the values exceeded the recommended value, clarifiers were used (Ray et al., 2010; Gaona et al., 2011).

2.7. Analysis of chlorophyll a

For chlorophyll a concentration analysis, 10 mL water samples were collected once a week from the culture tanks. The samples were filtered through GF 50-A fiberglass microfilters. Photosynthetic pigment extraction was performed by immersing the filters in 10 mL of 90% acetone (Merck® PA), which were stored in vials and kept in a freezer (-12°C). After a 24-h period, the chlorophyll a concentration was read using a fluorimeter (Turner® Designs Trilogy) (Welschmeyer, 1994).

2.8. Statistical analysis

Data were submitted to normality (Shapiro-Wilk) and homoscedasticity (Levene test) tests. After confirmation of these assumptions, simple analysis of variance (one-way ANOVA) was used. If significant differences ($p < .05$) were detected, post hoc Tukey's test was applied, with the confidence level set at 95% ($p < .05$) (Zar, 2010).

3. Results

3.1. Experiment 1 - Nursery

The results obtained for the chemical and physical parameters of the first experiment are shown in Table 1. Variables such as ammonia, nitrite, nitrate, phosphate, TSS, turbidity and Secchi disk showed significant differences between the treatments F and BF ($p < .05$), whereas variables such as dissolved oxygen, pH, temperature, salinity, alkalinity and chlorophyll did not show any significant differences between treatments ($p > .05$).

Fig. 1A shows the ammonia concentrations over 40 days in culture in the F and BF treatments. In the F treatment, maximum ammonia concentrations of 1.3 mgL^{-1} were observed after seven days of the study, whereas in the treatment with bioflocs (BF), the maximum ammonia concentration was 0.3 mgL^{-1} . Fig. 1B shows that the nitrite concentrations in the F treatment peaked at day 42 with 12 mgL^{-1} , and those in the BF treatment remained at an average of 1.8 mgL^{-1} . The highest nitrate levels were measured on day 45 for the BF treatment

Table 1

Physical and chemical parameters of water (average $\pm$ standard deviation) in the feed and bioflocs with feed treatments during the culture of *L. vannamei* in the nursery phase. Different superscripted letters in a row denote significant differences ($p < .05$) between treatments.

Parameters	Feed	Bioflocs + Feed
Dissolved oxygen (mg L^{-1})	6.53 ± 0.81	6.40 ± 0.86
pH	8.11 ± 0.24	8.00 ± 0.31
Temperature ($^\circ\text{C}$)	27.71 ± 2.78	27.25 ± 2.68
Salinity	25.89 ± 3.75	29.54 ± 2.80
Ammonia (mg L^{-1})	0.65 ± 0.60^a	0.06 ± 0.14^b
Nitrite (mg L^{-1})	3.46 ± 4.92^a	0.39 ± 0.73^b
Nitrate (mg L^{-1})	1.57 ± 2.65^a	13.52 ± 18.36^b
Phosphate (mg L^{-1})	0.67 ± 0.28^a	0.38 ± 0.44^b
Alkalinity (mg L^{-1})	159.62 ± 37.28	156.94 ± 46.41
TSS (mg L^{-1})	135.40 ± 68.65^a	578.33 ± 215.42^b
Turbidity (NTU)	46.31 ± 41.71^a	234.65 ± 113.05^b
Secchi (cm)	24.23 ± 7.38^a	9.46 ± 1.61^b
Chlorophyll a ($\mu\text{g L}^{-1}$)	154.16 ± 140.56	227.82 ± 129.60

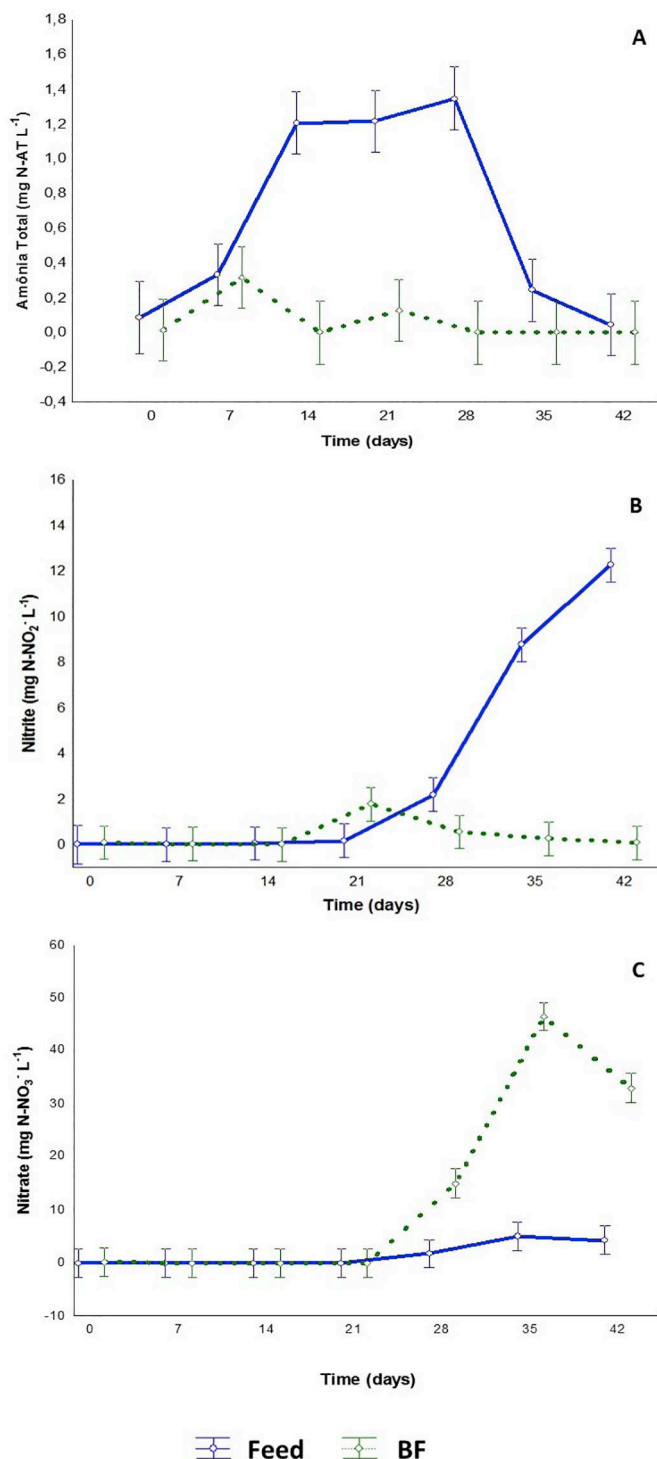


Fig. 1. Variations (means $\pm$ standard deviation) of concentrations (A) ammonia mgL^{-1} , (B) nitrite mgL^{-1} and (C) nitrate mgL^{-1} during the culture of *L. vannamei* in the nursery phase.

(45 mgL^{-1}) and on day 34 for the F treatment (5 mgL^{-1}), as shown in Fig. (1C).

According to the results shown in Fig. 2 for microorganisms at the beginning and end of cultivation, there were significant differences ($p < .05$) for *Oocystis*, *Planktonema*, flagellates and *Pseudanabaenaceae* between treatments. Fig. (2A) shows the abundances of microorganisms at the beginning of culture, with a maximum of 12.99×10^6 flagellates in the biofloc treatment and a minimum of 0.1×10^6 nematodes in the control treatment. As shown in Fig. 2B, there was a

significant difference for *Oocystis* and *Pseudanabaenaceae* between treatments ($p < .05$) at the end of culture. A maximum abundance of 4.61×10^6 was observed for *Pseudanabaenaceae* in the biofloc treatment, and a minimum of 0.1×10^6 was observed for pennate diatoms in the control treatment.

The mean and standard deviation of the carbon and nitrogen isotope ratios of the bioflocs and feed in the F and BF treatments are shown in Table 2.

Fig. 3 presents the isotopic values of shrimp muscle throughout the experiment. This figure shows the carbon (C) (Fig. 3A) and nitrogen isotope values (Fig. 3B). The carbon isotope ratios in the F and BF treatments present intermediary values between feed and biofloc values, although at the end of the study period, the bioflocs showed a decrease in the $\delta^{13}\text{C}$ values, and these were very similar to the values measured in the shrimp tissues. For nitrogen (Fig. 3B), the bioflocs showed a constant increase throughout the experiment. The results of the carbon, nitrogen and carbon/nitrogen contributions as food sources are shown in Table 3.

The zootechnical performance results in the present experiment for *L. vannamei* shrimp during 40 days of study are shown in Table 4. Compared to treatment BF, treatment F showed better zootechnical performance, especially in terms of the final weight, feed conversion, productivity per m^3 and m^2 , with significant differences ($p < .05$).

3.2. Experiment 2 – Grow-out

The results obtained in the present experiment for the chemical and physical water parameters during 60 days of study are shown in Table 5. Variables such as pH, ammonia, nitrite, nitrate, phosphate, TSS and turbidity showed significant differences between the F and BF treatments ($p < .05$), whereas variables such as dissolved oxygen, temperature, salinity, alkalinity, and chlorophyll levels did not show significant differences between treatments ($p > .05$).

Significant differences ($p < .05$) were observed in the total ammonia and nitrite concentrations between the treatments. Fig. 4A shows the ammonia concentrations over 60 days of culture. In the F treatment, maximum ammonia concentrations of 6 mgL^{-1} were observed on the seventh day of the study, while for the treatment with bioflocs (BF), the average observed concentrations were 0.08 mgL^{-1} . Fig. 4B shows that nitrite concentrations in the F treatment peaked at day 35, with 5.3 mgL^{-1} , and in the treatment with biofloc, they remained at an average of 0.30 mgL^{-1} . There was a significant difference in the nitrate concentration between treatments F and BF ($p < .05$) (Fig. 4C). The highest measured nitrate levels were on day 42 (36 mgL^{-1}) in the biofloc with feed treatment, on day 49 (30 mgL^{-1}) in the biofloc treatment without feed, and on day 56 in the control treatment (13 mgL^{-1}).

According to the results shown in Fig. 5 for the microorganisms, it is possible to observe that at the beginning of cultivation, there was a greater abundance of *Pseudanabaenaceae*, with a maximum of 76.06×10^7 , and a minimum of 1×10^7 nematodes both in biofloc treatments. By the end of culture, there was a maximum of 415.2×10^7 flagellates and a minimum of 5×10^7 pennate diatoms.

The mean and standard deviation of the separate carbon and nitrogen isotope ratios of the bioflocs and feed used as a food source in the control and feed biofloc treatments are shown in Table 6. Fig. 6 shows isotopic values of shrimp muscle throughout the experiment. Fig. 6A and B show that both the carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values, respectively, in the and biofloc with feed treatments are closer to values of the feed treatment. The results of carbon, nitrogen and carbon/nitrogen as the food source are shown in Table 7.

The results for zootechnical performance in the present experiment for *L. vannamei* shrimp during 60 days of study are shown in Table 8. Treatment F showed significantly better values of zootechnical performance in terms of survival, feed conversion, and productivity per m^2 ($p < .05$).

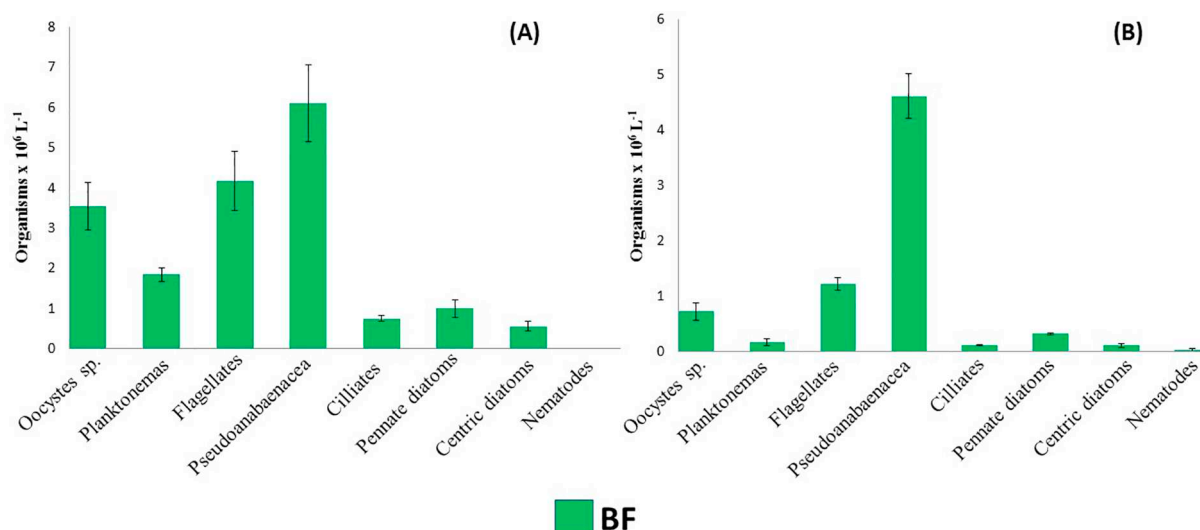


Fig. 2. Mean values $\pm$ standard deviation (A) community of microorganisms at the beginning of culture and (B) community of microorganisms at the end, during the culture of *L. vannamei* in the nursery phase.

Table 2

Carbon and nitrogen isotopic values (mean $\pm$ standard deviation) of the feed and biofloc with feed treatments.

Sources	Treatments	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Bioflocs	Bioflocs w/ feed	-16.77 ± 1.16	4.25 ± 0.55
Feed	Feed	-22.29 ± 0.45	5.70 ± 0.36

Table 3

Contribution values of food sources (%) in isotopic balance during *L. vannamei* culture in the nursery phase in the feed and biofloc with feed treatments.

Sources	Feed	Bioflocs + Feed
Carbon	56–78%	22–43%
Nitrogen	56–100%	0–43%
C and N*	56–78%	22–43%

4. Discussion

Biofloc technology has established a new paradigm in the intensive production of aquatic organisms, such as fish and shrimp. The microbial colonization of suspended solids helps to solve two main problems of the production of organisms at high density. The first problem is related to water quality. This technology can be used to decrease the levels of ammonium, a toxic nitrogen compound, by manipulating the heterotrophic bacterial community. When the ammonium concentration reaches high and dangerous levels, the addition of a carbon source, such as sugarcane molasses, allows the growth of heterotrophic bacteria that

incorporate ammonium into their biomass as protein (Avnimelech, 1999, 2015; Ebeling et al., 2006). However, the speed of biofloc formation is not only related to the sugar cane molasse, but also to the type of aeration employed (Lara et al., 2017).

Molasses addition decreases the ammonium levels in tanks without the necessity of water exchange. However, the supplement of a carbon source is not sufficient to decrease the concentration of nitrite, another toxic nitrogen compound. Total removal of nitrite is only possible by the action of nitrifying bacteria, especially nitrite-oxidizing bacteria (NOB) (Ebeling et al., 2006; Crab et al., 2007). However, bioflocs also

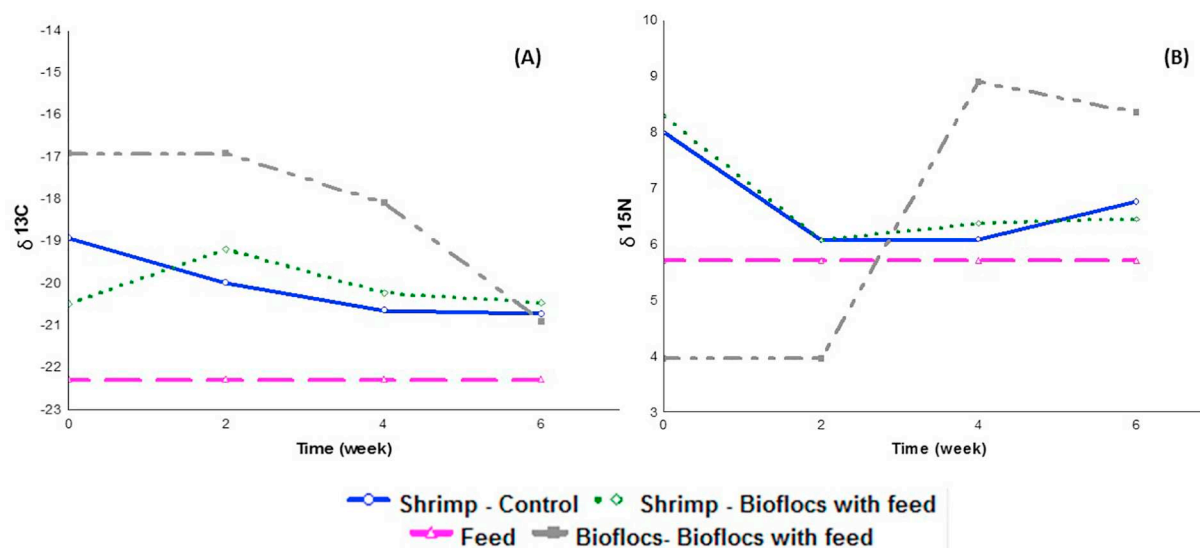


Fig. 3. Changes in the isotopic values (A) of carbon ($\delta^{13}\text{C}$) and (B) ($\delta^{15}\text{N}$) in the tissues of the shrimp according to the control and bioflocs with feed treatments in the nursery phase.

Table 4

Zootechnical performance of the shrimp (average $\pm$ standard deviation) in the control and biofloc treatments during the culture of *L. vannamei* in the nursery phase in the feed and biofloc with feed treatments. Different superscripted letters in a row denote significant differences ($p < .05$) between treatments. Apparent feed conversion rate (AFC) and specific growth rate (SGR).

Parameters	Feed	Biofloc + Feed
Initial weight (g)	0.01 $\pm$ 0.007	0.01 $\pm$ 0.007
Final weight (g)	1.14 $\pm$ 0.49 ^a	0.55 $\pm$ 0.29 ^c
Survival (%)	96.32 $\pm$ 12.58	89.45 $\pm$ 9.15
AFC	0.94 $\pm$ 0.10 ^a	2.11 $\pm$ 0.09 ^b
Productivity (kg m ⁻²)	1.73 $\pm$ 0.18 ^a	0.77 $\pm$ 0.03 ^b
Productivity (kg m ⁻³)	2.16 $\pm$ 0.22 ^a	0.96 $\pm$ 0.04 ^b
SGR (% day ⁻¹)	9.82 $\pm$ 0.27	8.28 $\pm$ 0.28

Table 5

Physical and chemical parameters of water (average $\pm$ standard deviation) in the feed and bioflocs with feed treatments during the culture of *L. vannamei* in the growth phase. Different superscripted letters in a row denote significant differences ($p < .05$) between treatments.

Parameters	Feed	Bioflocs + Feed
Dissolved oxygen (mg L ⁻¹)	6.49 $\pm$ 0.79	6.42 $\pm$ 0.80
pH	7.85 $\pm$ 0.21	7.68 $\pm$ 0.30
Temperature (°C)	26.75 $\pm$ 1.64	26.75 $\pm$ 1.58
Salinity	25.21 $\pm$ 3.05	21.92 $\pm$ 2.81
Ammonia (mg L ⁻¹)	0.74 $\pm$ 1.81 ^a	0.06 $\pm$ 0.06 ^b
Nitrite (mg L ⁻¹)	1.48 $\pm$ 2.10 ^a	0.15 $\pm$ 0.15 ^b
Nitrate (mg L ⁻¹)	6.72 $\pm$ 5.37 ^a	21.25 $\pm$ 12.43 ^b
Phosphate (mg L ⁻¹)	1.55 $\pm$ 0.85 ^a	3.09 $\pm$ 1.27 ^b
Alkalinity (mg L ⁻¹)	164.38 $\pm$ 17.18	151.38 $\pm$ 22.07
TSS (mg L ⁻¹)	123.44 $\pm$ 84.12 ^a	440.88 $\pm$ 218.75 ^b
Turbidity (NTU)	44.23 $\pm$ 30.27 ^a	172.31 $\pm$ 109.36 ^b
Chlorophyll a (µg L ⁻¹)	140.76 $\pm$ 106.48	392.10 $\pm$ 145.99

play an important role in the nitrifying process, serving as substrates for the growth of these microorganisms in BFT systems. In our study, it was possible to see the action of a well-established nitrifying bacterial community present in bioflocs, as indicated by the nitrogen compound measurements (Figs. 1 and 4). The use of mature bioflocs with an active nitrifying bacterial community contributed to maintaining ammonium and nitrite at low levels in BF treatments during both experiments.

Another relevant aspect of biofloc technology is that microorganisms (bacteria and protozoans) found in the flocs represent a complementary food source for the produced organisms. Several studies have demonstrated that microorganisms present in flocs and biofilms are important sources of proteins and lipids (Thompson et al., 2002; Martins et al., 2001; Decamp et al., 2003; Emerenciano et al., 2011; Ballester et al., 2010; Reis et al., 2019), and their consumption contributes to a decrease in the apparent food conversion rate. The smaller consumption of commercial feed leads to a reduction in production costs. Personal observation during the production of *L. vannamei* at different larval stages indicated that larger animals spend more time manipulating the aggregate, probably selecting the preferred microorganisms. On the other hand, tinier shrimp are sometimes smaller than the flocs and therefore do not interact much with the suspended solids. This information led us to formulate the hypothesis that the consumption of flocs and their microbiota during intensive shrimp production would be related to shrimp size.

To evaluate possible microbial consumption by shrimp, an analysis of food items found in the stomach and intestinal tract is normally employed. However, this methodology is hard to use in small animals. Another possibility is the analysis of prey abundance variability; i.e., a decrease in the abundance of a specific food item could be the result of direct consumption by the shrimp. However, one of the best alternatives to identify the consumption of specific food sources is the use of the stable isotopic technique. The stable carbon and nitrogen isotope

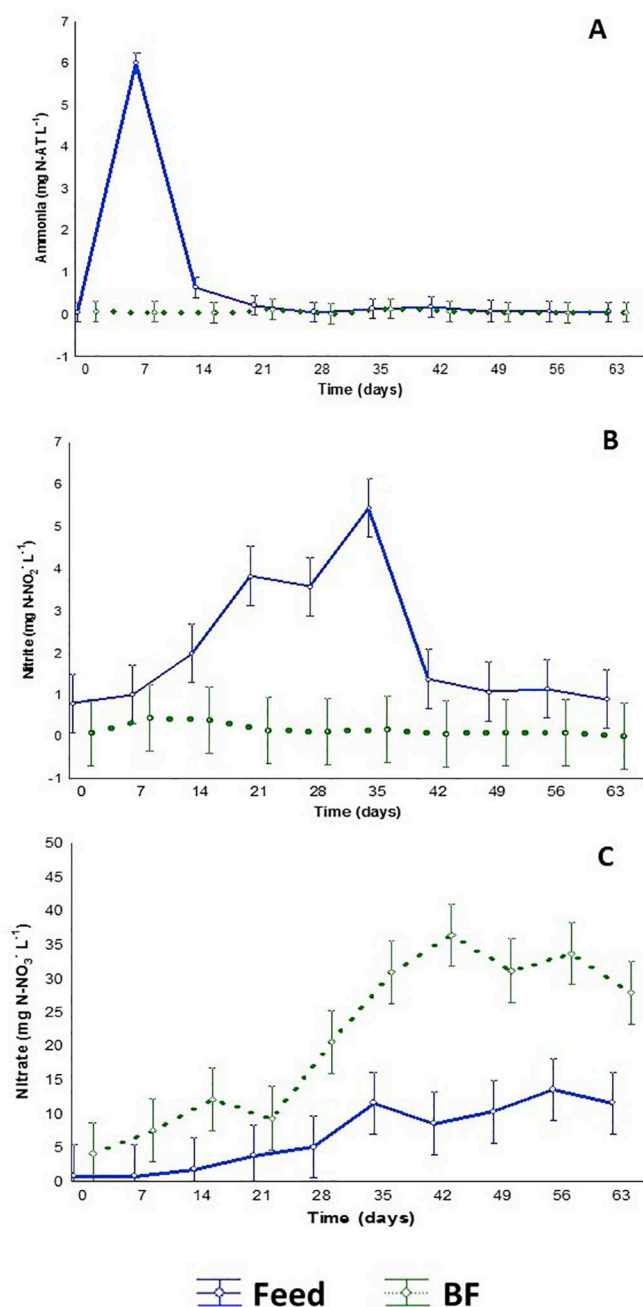


Fig. 4. Variations (means $\pm$ standard deviation) of concentrations (A) ammonia mgL⁻¹, (B) nitrite mgL⁻¹ and (C) concentrations nitrate mgL⁻¹ during the culture of *L. vannamei* in the grow-out phase.

technique ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) has been widely used to help understand which food source is more important to the growth of penaeid shrimp (Nunes et al., 1997; Burford et al., 2004; Thomas and Ray, 2018). In general, the $\delta^{13}\text{C}$ signal provides information about the primary energy source, whereas the $\delta^{15}\text{N}$ ratio allows discrimination of trophic levels and can provide information on nitrogen processing and therefore nitrogen sources (Peterson and Fry, 1987).

The results of this technique presented in Figs. 3 and 6 as well as in Tables 3 and 7 helped to corroborate the work hypothesis of our study. The $\delta^{13}\text{C}$ of bioflocs in the first experiment showed a constant decrease throughout the study, probably because feed was the main carbon source for floc formation (Fig. 3A). On the other hand, the $\delta^{15}\text{N}$ signature showed an increase during the same period (Fig. 3B), probably as the result of the microbial colonization of aggregates, since proteins

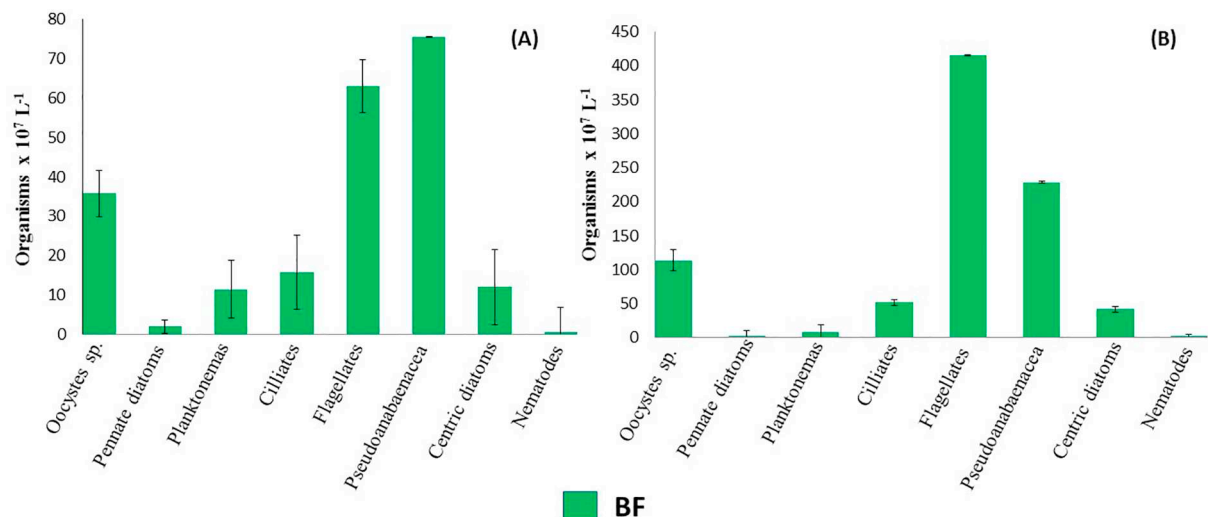


Fig. 5. Mean values $\pm$ standard deviation (A) community of microorganisms at the beginning of culture and (B) community of microorganisms at the end during the culture of *L. vannamei* in the grow-out phase.

Table 6

Carbon and nitrogen isotopic values (mean $\pm$ standard deviation) of the control and bioflocs with feed treatments.

Source	Treatments	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Bioflocs	Bioflocs with feed	-22.71 ± 0.18	6.67 ± 0.37
Feed	Feed	-22.83 ± 0.17	4.40 ± 0.49

of microorganisms that colonized the floc would contribute to the observed $\delta^{15}\text{N}$ enrichment. On the other hand, $\delta^{15}\text{N}$ values in the second experiment (Fig. 6B) shows the opposite trend, with a constant decrease in $\delta^{15}\text{N}$ during the study. This was probably due to the consumption of microorganisms present in flocs by the shrimp.

Similarly, the mass balance and mixing equations indicated that the biofloc contribution was much larger in the second experiment, surpassing the feed as the main sources of carbon (63–100%) and nitrogen (35–86%) (Table 7), whereas in the first experiment, flocs contributed a maximum of 43% of C and N (Table 3).

In a similar experiment studying the species *Litopenaeus stylirostris*,

Table 7

Contribution values of food sources (%) in isotopic balance during *L. vannamei* culture in the grow-out phase.

Source	Feed	Biofloc + Feed
Carbon	0–36%	63–100%
Nitrogen	13–64%	35–86%
C e N ^a	13–36%	63–86%

^a Values referring to the average of the contribution intervals.

Cardona et al. (2015), determined a contribution of 37 to 40% of natural productivity to the shrimp. Gamboa-Delgado et al. (2013) related a level of inclusion of 25% biofloc flour in diets for *L. vannamei*, which resulted in nitrogen originating from biofloc being found in the shrimp muscle, with growth comparable to that with 100% fish flour. However, Burford et al. (2004a) estimated that the nitrogen derived from the bioflocs was restricted to a level between 18 and 29% for young *L. vannamei*; similar values were found during the nursery phase in the present study, which indicated that up to 43% of nitrogen in the shrimp

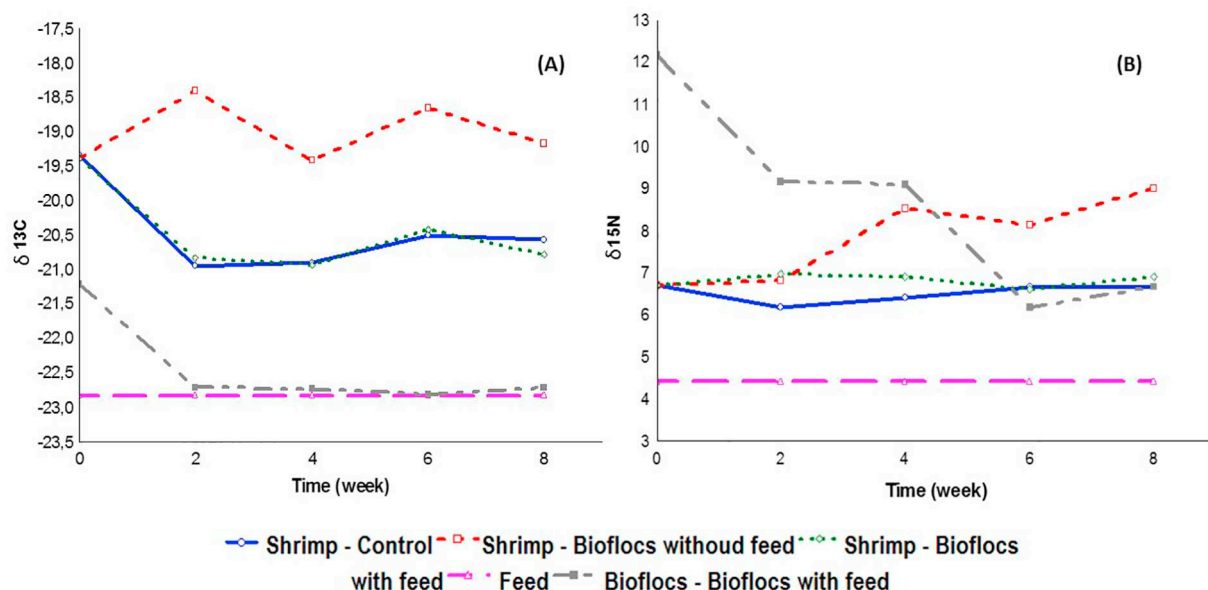


Fig. 6. (A) Changes in isotopic carbon values in shrimp muscle, (B) Changes in isotopic nitrogen values in shrimp muscle in grow-out phase.

Table 8

Zootechnical performance of the shrimp (average $\pm$ standard deviation) in the feed and bioflocs with feed treatments during the culture of *L. vannamei* in the grow-out phase. Different superscripted letters in a row denote significant differences ($p < .05$) between treatments. Apparent feed conversion rate (AFC) and specific growth rate (SGR).

Parameter	Feed	Bioflocs with feed
Initial weight (g)	0.83 $\pm$ 0.53	0.83 $\pm$ 0.33
Final weight (g)	7.97 $\pm$ 2.03	7.69 $\pm$ 2.27
Survival (%)	88.35 $\pm$ 12.58 ^a	78.74 $\pm$ 9.15 ^b
AFC	1.66 $\pm$ 0.23 ^a	1.95 $\pm$ 0.15 ^b
Yield (kg m ⁻²)	2.25 $\pm$ 0.29 ^a	1.90 $\pm$ 0.13 ^c
Yield (kg m ⁻³)	2.82 $\pm$ 0.37	2.37 $\pm$ 0.16
SGR (% week ⁻¹)	0.60 $\pm$ 0.08	0.60 $\pm$ 0.05

was from the bioflocs.

Analyzing only the $\delta^{15}\text{N}$ isotope ratio, Abreu et al. (2007) observed that microorganisms present in the biofilm formed in the culture tanks can supply more than 70% of the nitrogen used by *Farfantepenaeus paulensis*, which is a percentage similar to that found in the grow-out phase, where it can be observed that the bioflocs can supply 35 to 86% of nitrogen used by *L. vannamei*. On the other hand, Silva et al. (2013) determined the nitrogen mass balance in the biofloc system and observed that 38.9% of nitrogen from the supplied feed was not used by the shrimp but was available in the system. The results of the present study confirm the results of these authors, since it was evident that over time, the isotopic signature of the feed was incorporated into the bioflocs. However, it is likely that a parcel feed that would be lost after decomposition could be available as recycled food, in the form of bacterial biomass.

Another line of evidence that also supports the hypothesis that larger shrimp consume more biofloc microbes comes from the direct observations of protozoan abundances in both experiments, although these results deserve closer observation due to some apparent incongruities. A decrease in protozoan abundance throughout the study can be considered the result of shrimp predation on these microorganisms. In this way, we could say that most observed protozoans in the first experiment were consumed (Fig. 2), contradicting the stable isotope results. However, two points must be considered: 1) Microorganism abundances in the first experiment were two orders of magnitude smaller than those in the second experiment. Thus, decreases in abundances observed in the second experiment would be more pronounced than in the first experiment. 2) Another important aspect is the fact that the two microbial items (flagellates and nematodes) showed increased numbers in the first experiment, whereas in the second experiment, only pennate diatoms, *Planktonema* and nematodes showed a significant decrease. In a previous study analyzing the relationship between microorganism abundance and lipid and protein contents in biofilms, Silva et al. (2008) observed that the variation in lipids in the biofilm was closely related to the abundance of free heterotrophic bacteria, filamentous cyanobacteria, flagellates and nematodes, whereas the protein content varied similarly with the abundance of unicellular centric diatoms and nematodes. Similarly, Ballester et al. (2010) observed that the shrimp *F. paulensis* selectively feed on nematodes, tintinnids and rotifers on the biofilm present in pens.

All evidence presented indicates a positive relationship between shrimp size and microorganism consumption. However, it is noteworthy that despite high microbial consumption in the second experiment, the zootechnical parameters were not better in the treatment with biofloc. Besides to be a complementary food source, the consumption of microorganisms can influence other aspects related to shrimp health such as resistance to diseases (Hostins et al., 2017). On the other hand, other microorganisms like cyanobacteria and flagellates can be toxic to shrimp, or cause gills clogging.

Changes in the culture environment, such as nutrients and natural

competition of communities of different species may influence primary productivity as well as the predominance of specific groups of microorganisms more adapted to the established environmental conditions (Irigoien and Castel, 1997; Lourenço and Marques Júnior, 2009; Emerenciano et al., 2011). During this work, it was possible to verify the presence of microorganisms in the culture system at the beginning and end of the study. According to Ray et al. (2010), the abundance of certain microorganisms, mainly cyanobacteria, in the system can affect the growth and survival of shrimp. In the present study, during the nursery phase, a high concentration of filamentous cyanobacteria was verified (Pseudanabaenaceae), and the presence of these organisms may have contributed to the slower growth of the shrimp in this treatment. The same finding was reported by Nunes et al. (2004), where a high concentration of filamentous cyanobacteria trichomes of the same family found in the present work was observed.

According to the same authors, the presence of these organisms was the main vector of contamination in the reared shrimp. Different types of cyanobacteria have caused low productivity in the culture of shrimp; for example, Pacheco (2016) detected a bloom of the cyanobacterium *Nodularia spumigena* in a system of production located in the same laboratory as the present study. Furthermore, the toxicity of a type of filamentous cyanobacterium (*Schizothrix calcicola*) was evaluated in the culture of *L. vannamei* by Pérez-Linares et al. (2003), in which serious damage to the digestive tissue of the animal was detected, affecting the absorption and assimilation of food. Moreover, these filamentous cyanobacteria may interfere with the respiratory process of the animal, causing stress and increasing susceptibility to disease (Lightner et al., 1978). This issue may have occurred in the biofloc treatment with feed in the nursery, since shrimps gill clogging was observed throughout the experiment.

5. Conclusion

The findings of the present study indicate a positive relationship between *L. vannamei* size and the consumption of biofloc microbes. In the nursery phase, the bioflocs contributed 22–43% C and 0–43% N to shrimp tissue composition, whereas in the grow-out phase, C and N of biofloc origin contributed 63–100% and 35–86%, respectively. These results represent an important contribution to the better management of natural and artificial foods and the intensive production of *L. vannamei* using BFT technology.

Declaration of Competing Interest

None.

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ORIGINAL ARTICLE



Growth performance and health of juvenile tambaqui, *Colossoma macropomum*, in a biofloc system at different stocking densities

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Abstract

The present study evaluated the biofloc system (BFT) for rearing of tambaqui (*Colossoma macropomum*) at different densities. Juveniles of 23.74 ± 0.22 g were randomly distributed following a 2×3 factorial design to evaluate three stocking densities (50, 100 and 200 fish/m³) in BFT (BFT50, BFT100 and BFT200) and clear water (CW50, CW100 and CW200) and basal (BS30, with 30 fish/m³), in triplicates for 60 days. Water quality variables, zootechnical performance and blood parameters were evaluated. The dissolved oxygen concentration was significantly lower in BFT and inversely related to stocking density, while electric conductivity was higher for BFT. The fish of BFT50 and BFT100 presented the lowest feed conversion ratio, the greatest final weight, weight gain and specific growth rate among treatments. Blood parameters showed no statistical differences among treatments. BFT is efficient for culturing tambaqui, and 100 fish/m³ is the best stocking density for the tested growth stage, despite presenting greater biomass and productivity in the density of 200 fish/m³.

KEYWORDS

BFT, density, growth performance, production system

1 | INTRODUCTION

Biofloc systems (BFT) are considered environmentally friendly as they operate with minimal water use, which is enhanced by the performance of specific microbial communities effective in controlling nitrogen by-products that are potentially toxic to animals (Avnimelech, 1999). Currently, production technologies in BFT have been made available mainly for shrimp (*Litopenaeus vannamei*) (Ferreira et al. 2020) and Nile tilapia (*Oreochromis niloticus*) (Cao et al., 2020).

Despite prospects for the viability to produce tilapia in BFT, legal restrictions in regions like the Amazon restrain the expansion of aquaculture activity with ruling aspects related to deforestation and,

consequently, occupation of areas and use of water resources, in addition to culturing non-native species (Benatti & Fischer, 2018; Junk, 1984). In line with this, BFT can be considered a strategy to extend aquaculture. However, in order to be economically feasible, production intensification is frequently required as well as the target organism to tolerate higher densities (Krummenauer et al., 2011). Thus, the knowledge on the response of the most important species in the Amazon, such as the tambaqui (*Colossoma macropomum*) in BFT systems, can contribute to develop aquaculture in the tropical region worldwide. Tambaqui is highly representative in South and Central America, the Caribbean and Asia, mainly in China, Indonesia, Malaysia, Myanmar and Vietnam (Wojnárovich & Anrooy, 2019), and is the most important native species produced in the Amazon (Valladão et al., 2018). Its

characteristics, such as tolerance to low dissolved oxygen concentration, pH variations, high concentrations of ammonia and nitrite, and omnivorous habit and/or digestive system that allows to better assimilate microbial particles, are in favour for the adaptation to BFT (Valladão et al., 2018; Woynárovich & Anrooy, 2019).

Tambaqui is being reared in different systems, such as in earthen ponds with minimal water exchange and under aeration regimes (emergency and supplementary) at 12.5 fish/m² (Izel-Silva et al., 2020), incages at densities of 300–480 fish/m³ (Frisso et al. 2020) and also in BFT testing the efficacy of *Bacillus subtilis* at 100 fish/m³ (dos Santos et al. 2020), indicating the species capacity to adapt to different systems. Silva and Fujimoto (2015) also reported this species capacity to tolerate high density, but optimal stocking density in BFT systems is still not well established. To this end, the present study aimed to determine the optimal stocking density of tambaqui in BFT compared with clear water (CW) using as indicators water quality variables, zootechnical and economic performance and fish health condition.

2 | MATERIAL AND METHODS

2.1 | Ethics statement

The present work was undertaken accordingly with the Aquaculture Research ethical policy and with the approval of the Committee for Ethics in Research in the Use of Animals (CEUA) (Prot. 015/2015).

2.2 | Experimental design and procedures

The experiment was carried out at the Aquaculture Experimental Station of the Coordination of Technology and Innovation (COTEL) of the National Amazonia Research Institute (INPA) in Manaus, AM, Brazil, for 60 days.

The experiment followed a completely randomized 2 × 3 factorial design formed by two rearing systems (BFT and CW) and three stocking densities (50, 100 and 200 fish/m³) in triplicates. Additionally, a basal treatment (30 fish/m³) was employed to maintain fish in homeostasis to compare its health conditions with the experimental fish.

Tambaqui juveniles (23.74 ± 0.22 g) were kept in 300-L circular PVC tanks (EU) with constant aeration and a 12-h photoperiod. In BFT, bacteria matured for 30 days from a tambaqui culture were inoculated in each EU by using 100 L of bioflocs. Replenishment of water losses by evaporation and water exchange was performed in the treatment with BFT to maintain the settleable solids (SS) concentration close to 20 mg/L. For the CW treatment, in order to maintain water quality, 25% water exchange was carried out daily. In the basal treatment (BS), constant water renewal (30 L/h) was maintained throughout the experiment to guarantee the best environmental conditions for the fish.

In order to sustain water quality in the BFT treatments, sugar was added as an organic carbon source based on the carbon (C) and nitrogen (N) levels, considering also these nutrients input through the diet.

During the first 7 days of the experiment, sugar was added to maintain a C:N ratio of 20:1 (Avnimelech, 1999). After the first week, a C:N ratio of 6:1 was adopted based on the total ammonia concentrations detected in the water (above 1.0 mg/L) (Ebeling et al., 2006).

2.3 | Water quality

Temperature (°C), dissolved oxygen (mg/L), pH and electrical conductivity (mS/cm) were measured daily with a multiparametric probe (YSI Professional Plus). Total ammonia (mg/L) (Verdouw et al., 1978) and nitrite (mg/L) (Boyd & Tucker, 1992), total hardness (mg/L of CaCO₃) (Boyd & Tucker, 1992) and turbidity (UNT) were established using a turbidimeter (Hanna model HI98703). Total alkalinity (CaCO₃ mg/L) and carbon dioxide (CO₂ mg/L) were determined weekly by a method adapted from Boyd and Tucker (1992).

Total alkalinity was adjusted to 100 mg/L CaCO₃ for treatments BFT and CW (Ebeling et al. 2006) using dolomitic limestone for maintenance purposes (Furtado et al., 2011). Settleable solids (SS) (mg/L) were measured weekly with Imhoff cones (Avnimelech, 2007). Every 15 days, total suspended solids (TSS) (mg/L) were measured following the method adapted by Strickland and Parsons (1972).

2.4 | Diets

Fish were fed four times a day with commercial feed containing 45% crude protein (CP) up to 40 g of mean weight and 36% CP for the remaining of the trial at 5.0% of the total biomass (Silva et al., 2007) of each EU, calculated through biweekly sampling.

2.5 | Growth performance

For growth performance evaluation, 9 fish/EU previously anaesthetized with eugenol (0.2 ml/L) were sampled every 2 weeks and, at the end of the experiment, all fish were used to calculate: final weight (FW); weight gain (WG) = final average weight – average starting weight; specific growth rate (SGR) = [(ln final weight – ln initial weight)/number of days] × 100; survival rate (SR) = (final number of fish/initial number of fish) × 100; feed conversion ratio (FCR) = amount of feed offered/weight gain; biomass = final weight × number of fish in the EU; and productivity = final tank biomass/tank volume.

2.6 | Blood collection and determination of blood parameters

Blood was collected from 3 fish/EU previously anaesthetized (1 ml/L of eugenol) by caudal vessel puncture using 10% EDTA solution as anticoagulant. The following were analysed: number of erythrocytes (RBC – erythrocytes/μl) in the citrate formaldehyde

TABLE 1 Physico-chemical variables of water in systems with biofloc (BFT) and clear water (CW) technology in *Colossoma macropomum* production at different densities (50, 100, 200 fish/m³) for 60 days

				Breeding system	
Water variables		Density (Fish/m ³)		BFT	CW
DO (mg/L)		200		5.07 ± 0.02 bB	5.76 ± 0.07 aC
		100		5.72 ± 0.38 bB	6.24 ± 0.24 aB
		50		6.21 ± 0.17 bA	6.71 ± 0.20 aA
CO ₂ (mg/L)		200		23.66 ± 2.08 aA	16.26 ± 1.39 bA
		100		18.40 ± 1.43 aB	14.37 ± 0.22 bB
		50		17.12 ± 0.71 aB	13.12 ± 0.94 bB
pH		200		7.37 ± 0.12 aA	7.49 ± 0.01 aA
		100		7.50 ±0.07 aA	7.51 ± 0.10 aA
		50		7.56 ± 0.17 aA	7.51 ± 0.14 aA
Temperature (°C)		200		26.02 ± 0.13 aA	25.93 ± 0.06 aA
		100		26.05 ± 0.12 aA	25.95 ± 0.04 aA
		50		26.04 ± 0.12 aA	26.07 ± 0.03 aA
Electric conductivity (μS/cm)		200		374.77 ± 72.59 aA	175.62 ± 0.06 bA
		100		328.37 ± 12.02 aA	133.19 ± 0.24 bA
		50		354.33 ± 56.24 aA	112.96 ± 0.19 bA
ANOVA two-way					
Factors	DO	CO ₂	pH	Temperature	Electric conductivity
Breeding system	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> < 0.05
Density	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05
Interaction	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05

Note: Different lowercase letters between columns (rearing systems) and uppercase letters between rows (stocking densities) indicate statistically significant differences (Tukey's test at the 5% probability level).

Abbreviations: CO₂, carbon dioxide; DO, dissolved oxygen; pH, hydrogen potential.

solution by a haemocytometer; haematocrit (Ht – %) by the micro-haematocrit method; haemoglobin concentration ([Hb] – g/dl) by the cyanmethaemoglobin method; mean corpuscular volume (VCM – fl), mean corpuscular haemoglobin (HCM – pg) and mean corpuscular haemoglobin concentration (CHCM – %) following Wintrobe's methodology (1934). From blood plasma, the following analyses were performed: glucose (mg/dl) by the enzymatic-colorimetric method (glucose oxidase); cholesterol (mg/dl) and triglycerides (mg/dl) by means of the colorimetric system; total proteins (g/dl) following the modified biuret method. To analyse the leukocyte respiratory activity (LRA), fish blood was collected in microtubes containing 10 µl of sodium heparin and centrifuged (Eppendorf, model 5430 R) at 3000 g for 5 min (Siwicki et al., 1994).

2.7 | Statistics

Data as percentages were transformed by the arcsine into values above 70% and below 30%. Data normality was ascertained by Shapiro–Wilk and the homogeneity of variances by Bartlett, analysed by an analysis of variance (two-way ANOVA), followed by Tukey's post hoc test to compare the means among treatments (Zar,

1996). For the results of blood parameters, Dunnett's test and the non-parametric Kruskal–Wallis test were applied according to the data normality and homoscedasticity premises. The level of significance was set at 5% for all the statistical analyses.

3 | RESULTS

3.1 | Water quality

The increase in stocking density resulted in lower water DO and higher CO₂ concentrations in both systems CW and BFT and the latest resulted in higher O₂ use and CO₂ production than in CW (Table 1). BFT200 showed the highest water CO₂ (23.66 ± 2.08 mg/L) (Table 1). No differences were observed for temperature and pH between rearing systems and densities (*p* > 0.05), while electrical conductivity values (*p* < 0.05) in BFT were higher compared with CW (Table 1).

The total alkalinity and hardness values reveal no differences between the evaluated densities (*p* > 0.05), which were, however, significantly higher (*p* < 0.05) for BFT than CW (Table 2). Biofloc formation in BFT system was evidenced by the significantly higher

SS and turbidity values ($p < 0.05$). Results showed that increasing stocking density and organic fertilizations induced higher TSS, as well as differences between the tested systems (BFT and CW) and microbial flocs present in BFT (Table 2). Yet the increase in stocking density significantly influenced the total ammonia and nitrite concentrations ($p < 0.05$), CW presented higher values than BFT for both parameters (Table 2). Differences were not found for water quality parameters regarding the interaction of rearing systems and densities for tambaqui (Tables 1 and 2).

The highest total ammonia concentrations were observed between day 7 and 29 with treatment BFT200 8.74 mg/L (0.56 mg/L of NH_3) and in the last week with treatment CW200 3.86 mg/L (0.24 mg/L of NH_3) (Figure 1a,b). Also, between days 11 and 31, nitrite concentration reached the highest levels recorded in BFT and in CW after day 41 (Figure 2a,b).

3.2 | Growth performance and fish health

The performance index of fish in BFT was significantly higher than in CW. Despite the non-significant differences regarding the interaction between rearing systems and stocking densities for zootechnical parameters of tambaqui, the FW, WG and SGR in both rearing systems were negatively influenced by the increase in stocking density ($p < 0.05$). Regardless of the rearing system, fish kept at 200 fish/m³ presented poorer growth and FCR in both systems ($p < 0.05$) compared with the lowest density (50 fish/m³) (Table 3).

Stocking density influenced the FCR values, but with no difference between the 200 fish/m³ in both rearing systems. For the lower densities of BFT (50 and 100 fish/m³), FCR was significantly lower ($p < 0.05$) than BFT200 and those of CW (Table 3).

TABLE 2 Water physico-chemical variables of the experimental units of *Colossoma macropomum* monitored weekly in biofloc (BFT) and clear water (CW) at different stocking densities (50, 100 and 200 fish/m³) for 60 days

				Breeding system			
				BFT		CW	
Water variables		Densities (fish/m³)					
Total alkalinity (mg/L CaCO ₃)		200		88.46 ± 15.58 aA		71.20 ± 24.61 bA	
		100		97.86 ± 22.87 aA		65.13 ± 21.65 bA	
		50		93.45 ± 10.70 aA		76.93 ± 2.26 bA	
Total hardness (mg/L CaCO ₃)		200		109.76 ± 10.63 aA		52.63 ± 20.54 bA	
		100		96.28 ± 14.71 aA		65.13 ± 31.18 bA	
		50		107.69 ± 6.63 aA		60.58 ± 32.85 bA	
SS (ml/L)		200		12.83 ± 6.79 aA		1.12 ± 0.47 bA	
		100		10.95 ± 6.17 aA		0.70 ± 0.31 bA	
		50		7.44 ± 4.66 aA		0.46 ± 0.48 bA	
Turbidity (UNF)		200		149.56 ± 52.50 aA		37.62 ± 2.40 bA	
		100		140.33 ± 35.24 aA		23.37 ± 1.72 bA	
		50		91.84 ± 15.30 aA		15.96 ± 3.82 bA	
TSS (mg/L)		200		491.19 ± 23.70 aA		122.40 ± 59.74 bA	
		100		355.64 ± 68.59 aB		27.65 ± 6.01 bB	
		50		206.52 ± 67.71 aC		24.65 ± 6.15 bB	
Total ammonia (mg/L NH ₃ +NH ₄)		200		1.88 ± 0.55 bA		2.92 ± 1.19 aA	
		100		1.33 ± 0.81 bB		2.46 ± 1.02 aB	
		50		1.07 ± 0.40 bB		1.70 ± 0.15 aC	
Nitrite (mg/L NO ₂)		200		1.85 ± 0.20 bA		2.05 ± 0.09 aA	
		100		1.29 ± 0.10 bB		1.59 ± 0.06 aB	
		50		1.12 ± 0.13 bC		1.33 ± 0.13 aC	
ANOVA two way							
Factors	Total alkalinity	Total hardness	SS	Turbidity	TSS	Total ammonia	Nitrite
Breeding system	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05
Density	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05
Interaction	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05

Note: Different lowercase letters between columns (rearing systems) and uppercase letters between rows (stocking densities) indicate significant statistical differences (Tukey's test at 5% probability).

Abbreviations: SS, settleable solids; TSS, total suspended solids.

FIGURE 1 Concentrations of total ammonia (mg/L) of systems with biofloc technology—BFT (a) and clear water—CW (b) of *Colossoma macropomum* at different densities (50, 100 and 200 fish/m³) evaluated for 60 days

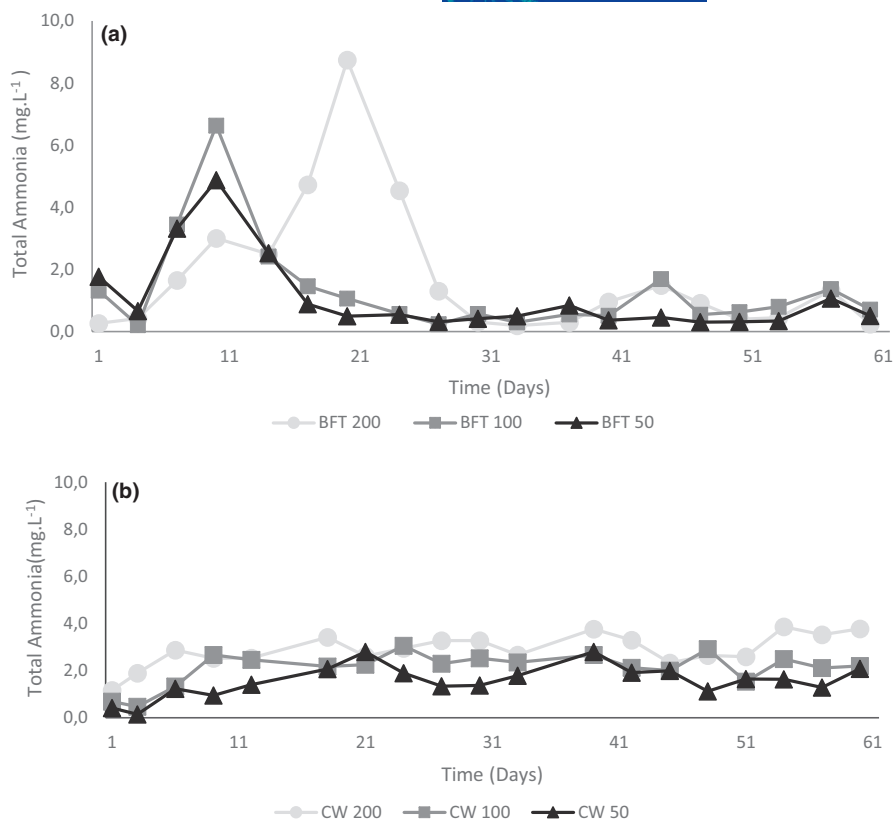
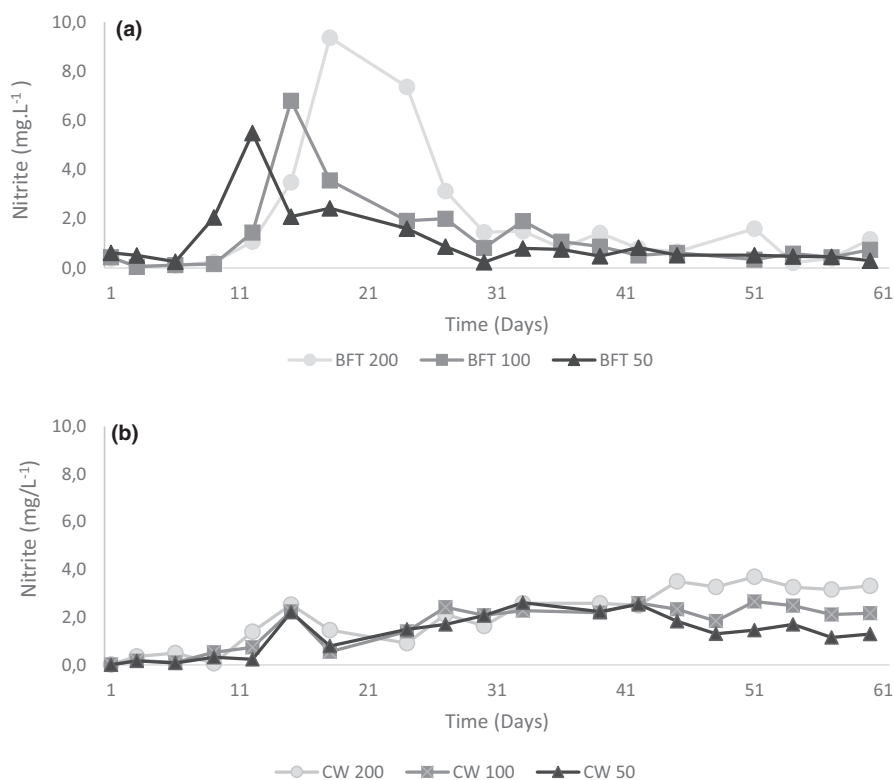


FIGURE 2 Total nitrite concentrations (mg/L) in the water of systems with biofloc technology—BFT (a) and clear water—CW (b) of *Colossoma macropomum* at different densities (50, 100 and 200 fish/m³) for 60 days



The biomass and productivity values were directly influenced by the stocking density, with a significant difference in both systems ($p < 0.05$), and in the densities of 50 and 100 fish/m³, BFT showed better results than CW (Table 3). As an important indicator of animal

welfare in a confinement system, fish survival in the present study did not show significant changes ($p < 0.05$) between treatments (Table 3).

Results of blood parameters of the tambaqui presented no statistically differences ($p > 0.05$) among treatments and compared

with the basal, indicating satisfactory management conditions for the species (Table 4).

4 | DISCUSSION

4.1 | Water quality

The presence of microorganisms in BFT results in higher O₂ demand and CO₂ production through respiratory and chemical processes during the transformation of nitrogen compounds in the

environment (Avnimelech, 2009). In the present study, the aeration system was able to maintain DO concentrations in the comfort zone (>5.0 mg/L) for the species (Saint-Paul, 1984) in all treatments. These results corroborate those described by Ekasari and Maryam (2012), for red *Oreochromis* sp., in which increasing rearing density lowered water DO with acute exposures and CO₂ concentration of 5.0 to 10% caused physiological changes and may have induced blood acidosis (Gilmour et al. 2005). In BFT200, CO₂ concentration close to 12% may have stressed the animals, explaining the poorer performance.

The importance of water temperature to BFT is associated with its influence on the microorganism formation and stabilization

TABLE 3 Growth parameters of *Colossoma macropomum* in biofloc (BFT) and clear water (CW) at different stocking densities (50, 100, and 200 fish/m³) during the 60-day experiment

					Rearing system			
					BFT		CW	
Parameters		Density (Fish/m³)						
Initial weight (g)		200			23.91 ± 0.34 aA		23.74 ± 0.61 aA	
		100			23.88 ± 0.45 aA		23.74 ± 0.52 aA	
		50			23.27 ± 0.97 aA		23.89 ± 0.91 aA	
Final weight (g)		200			58.42 ± 8.60 aC		57.32 ± 7.43 aB	
		100			94.95 ± 8.96 aB		74.30 ± 8.13 bA	
		50			116.43 ± 3.62 aA		85.71 ± 8.30 bA	
WG (g)		200			34.51 ± 0.24 aC		33.58 ± 6.81 aB	
		100			71.07 ± 8.50 aB		50.56 ± 7.60 bA	
		50			93.16 ± 2.64 aA		61.82 ± 7.44 bA	
SGR (%)		200			1.49 ± 0.29 aC		1.46 ± 0.17 aB	
		100			2.36 ± 0.12 aB		1.89 ± 0.15 bA	
		50			2.68 ± 0.02 aA		2.12 ± 0.21 bA	
FCR		200			1.29 ± 0.08 aA		1.31 ± 0.06 aA	
		100			0.89 ± 0.08 bB		1.21 ± 0.05 aAB	
		50			1.03 ± 0.06 bB		1.15 ± 0.04 aB	
Biomass (kg/UE)		200			3.41 ± 0.16 aA		3.34 ± 0.47 aA	
		100			2.89 ± 0.43 aB		2.18 ± 0.31 bB	
		50			1.78 ± 0.18 aC		1.27 ± 0.48 bC	
Productivity (kg/m³)		200			11.38 ± 0.55 aA		11.14 ± 1.19 aA	
		100			9.65 ± 0.81 aB		7.28 ± 1.02 bB	
		50			5.95 ± 0.40 aC		4.25 ± 0.15 bC	
Survival rate (%)		200			97.22 ± 3.47 aA		97.77 ± 2.54 aA	
		100			100.00 ± 0.00 aA		98.90 ± 1.92 aA	
		50			100.00 ± 0.00 aA		95.55 ± 3.85 aA	
ANNOVA two way								
Factors	Initial weight	Final weight	WG	SGR	FCR	Biomass	Productivity	Survival rate
Breeding system	<i>p</i> > 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05
Density	<i>p</i> > 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05
Interaction	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05	<i>p</i> > 0.05

Note: Values are presented as mean ± standard deviation. Different lower cases indicate significant statistical differences (Tukey's test at 5% probability) between cultivation systems and uppercase letters between stocking densities.

Abbreviation: FCR, feed conversion ratio; SGR, specific growth rate; WG, weight gain.

TABLE 4 Range $\pm$ standard deviation of *Colossoma macropomum* blood parameters bred in a biofloc (BFT), clear water (CW) and basal (BS) systems at different densities (50, 100, 200 fish/m³) for 60 days

Blood parameters	Density (Fish/m ³)	Breeding system		
		BFT	CW	BS
Haematocrit (%)	200	18.61 $\pm$ 2.50	19.22 $\pm$ 0.94	20.44 $\pm$ 1.58
	100	19.33 $\pm$ 0.92	18.44 $\pm$ 0.25	
	50	19.94 $\pm$ 1.60	19.55 $\pm$ 1.08	
Erythrocyte (10 ⁶ /μl)	200	1.10 $\pm$ 0.01	1.03 $\pm$ 0.08	1.05 $\pm$ 0.18
	100	1.09 $\pm$ 0.16	1.02 $\pm$ 0.14	
	50	1.04 $\pm$ 0.12	1.09 $\pm$ 0.04	
Haemoglobin (g/dl)	200	5.10 $\pm$ 0.78	4.64 $\pm$ 0.64	5.24 $\pm$ 0.69
	100	5.01 $\pm$ 1.07	4.65 $\pm$ 0.89	
	50	5.27 $\pm$ 1.13	5.24 $\pm$ 0.68	
ACV (fl)	200	171.62 $\pm$ 25.52	190.07 $\pm$ 20.92	196.91 $\pm$ 15.58
	100	180.96 $\pm$ 23.52	186.01 $\pm$ 26.61	
	50	192.52 $\pm$ 17.59	179.68 $\pm$ 17.03	
CCH (g/dl)	200	27.86 $\pm$ 6.07	24.10 $\pm$ 2.24	25.81 $\pm$ 4.15
	100	25.83 $\pm$ 4.44	25.14 $\pm$ 4.49	
	50	26.32 $\pm$ 3.61	29.26 $\pm$ 4.73	
ACH (pg)	200	46.87 $\pm$ 6.07	45.60 $\pm$ 2.24	50.39 $\pm$ 4.15
	100	46.23 $\pm$ 4.44	46.71 $\pm$ 4.49	
	50	50.50 $\pm$ 3.61	52.10 $\pm$ 4.73	
Total proteins (g/dl)	200	3.37 $\pm$ 0.61	3.13 $\pm$ 0.22	3.27 $\pm$ 0.71
	100	4.24 $\pm$ 0.44	3.79 $\pm$ 0.45	
	50	4.56 $\pm$ 0.86	4.66 $\pm$ 0.87	
Glucose (mg/dl)	200	60.45 $\pm$ 12.19	45.85 $\pm$ 11.25	54.14 $\pm$ 8.98
	100	64.89 $\pm$ 13.59	62.08 $\pm$ 12.66	
	50	69.26 $\pm$ 15.58	63.81 $\pm$ 13.98	
Cholesterol (mg/dl)	200	51.22 $\pm$ 8.75	47.34 $\pm$ 5.82	48.31 $\pm$ 6.20
	100	43.71 $\pm$ 8.01	46.12 $\pm$ 8.13	
	50	52.17 $\pm$ 9.45	51.06 $\pm$ 6.27	
Triglyceride (mg/dl)	200	176.89 $\pm$ 28.18	175.81 $\pm$ 23.60	188.88 $\pm$ 24.11
	100	206.84 $\pm$ 30.72	212.37 $\pm$ 17.08	
	50	218.62 $\pm$ 19.27	206.45 $\pm$ 25.05	
RAL (%)	200	11.18 $\pm$ 2.72	8.99 $\pm$ 1.35	11.62 $\pm$ 1.82
	100	9.93 $\pm$ 3.44	7.78 $\pm$ 2.67	
	50	9.37 $\pm$ 4.98	7.51 $\pm$ 5.23	

Note: No letters indicate that no statistical differences appear between breeding systems and densities; Dunnett test at the 5% probability level. Abbreviations: ACH, average corpuscular haemoglobin; ACV, average corpuscular volume; CCH, concentration of corpuscular haemoglobin; RAL, respiratory activity of leukocytes.

time, as well as the environment maintenance (Hostins et al., 2015). In the present study, water temperature (25.87 ± 0.13 to $27.37 \pm 0.05^\circ\text{C}$) was lower than those considered ideal ($29\text{--}32^\circ\text{C}$) for tambaqui (Amanajás et al. 2018), suggesting potential increase in fish performance at higher temperatures such as in outdoor infrastructures.

Total alkalinity of close to 100 mg/L CaCO₃ ensured buffering effect and maintained pH values between 7 and 8, ideal for

tambaqui production (pH between 6 and 8) according to Aride et al. (2007) and Wood et al. (2018). Nonetheless, in this study, pH values between 6 and 8 were also within acceptable limits for the balance of the BFT system, which requires pH between 7 and 9 to ensure its stability and performance of the organisms (Chen et al., 2006). The dolomitic limestone (CaMgCO₃) added to both systems in the present study in order to deliver adequate water alkalinity also increased total hardness as well as its electric conductivity,

which is commonly used to assess nutrient availability in aquatic ecosystems (Boyd, 2015). Holanda Cavalcante et al. (2014) suggest that fish handling is safer and animal performance is better in water with total alkalinity to hardness ratio of 1:1 (between 55 and 150 mg/L of CaCO_3). Although these parameters varied significantly between production systems during the experiment due to the daily water renewal in CW, the supply of CaMgCO_3 maintained the proportion of 1:1 in both systems. Additionally, the transformation of nitrogen compounds into microbial flakes in the BFT explains the differences in TSS and turbidity between the systems tested.

Although the limits of tolerance to SS and TSS for tambaqui have not been determined, these parameters were within the range suggested for tilapia (5–50 mg/L and up to 500 mg/L, respectively) according to Avnimelech (2015). Avoiding excess of TSS is important because it can interfere with water quality and the ability of fish to assimilate nutrients in the system (Zemor et al., 2019) and also can cause instability of variables such as ammonia and nitrite.

Despite the short-term peaks of ammonia observed along the experiment, especially along the initial 20 days in BFT and in CW200 (Figure 1a,b), the mean ammonia concentration during the trial did not reach the tolerance limit for tambaqui ($\text{LC}_{50} = 141.38$ mg/L of total ammonia and 1.09 mg/L of NH_3) (Souza-Bastos et al., 2017). Additionally, the readily removal of the ammonia by the microorganisms in BFT with the application of organic carbon source and the water renewal in CW may have contributed to the high survival rate in all treatment in the present study.

All treatments except for BFT200 and CW200 presented nitrite mean concentrations below the limit recommended for this species according to Costa et al. (2004) ($\text{LC}_{50-96\text{ h}} = 1.82 \pm 0.98$ mg/L) along the experiment (Table 2). However, similarly to the ammonia pattern in BFT, nitrite presented peaks beyond the fish tolerance limit between day 11 and 31 in BFT and cumulatively in CW after day 31 (Figure 2a,b), which could have led to irreversible damage and cell death (Costa et al., 2017). Despite these peaks, their short duration indicates the effect of nitrifying bacteria specially in the BFT environment (Ebeling et al. 2006), preventing fish mortality. This suggests that tambaqui presents good tolerance to the nitrogen compounds present in water, which could benefit its production in BFT, in addition to efficiency in reducing these metabolites.

4.2 | Growth performance and fish health

The growth performance of aquatic organisms is affected by stocking density, where the lowest density commonly allows better food use and reduced risks of stress to the animals (Krummenauer et al. 2011), alleviate competition for space and food resulting in higher productivity (Haridas et al. 2017).

In this study, fish growth performance (FW, WG and SGR) was negatively affected by increasing stocking densities and fish in BFT featured better performance index than in CW. These results corroborate those described by Ekasari and Maryam (2012) and

Haridas et al. (2017), who verified the decrease in the daily average and total weight gain for red and GIFT tilapia (*Oreochromis* sp.) in BFT with increasing densities: 25, 50 and 100 fish/ m^3 and 200, 250, 300 and 350 fish/ m^3 respectively. At higher densities, the greater nutrient supply causes poorer water quality and increases competition for food and space, resulting in reduced growth and survival. This condition also leads to more stress to the animals that impairs their physiology and immune response at higher stocking densities. This could have contributed to the animal increased final weight at lower densities, despite lower biomass and productivity (Haridas et al. 2017).

dos Santos et al. (2020) tested probiotics in BFT and CW system, for tambaqui, and observed WG, FW and SGR of 33.57 g, 56.97 g and 1.52%/day, respectively, which were lower than those of the present study in the same time period of 60 days. Those results may be a consequence of poorer nitrifying bacteria performance considering the lower water temperature (26°C), total alkalinity (<20 mg/L of CaCO_3) and pH (<7) compared with the present study (Ebeling et al. 2006).

The poorest FCR was observed at the highest density (200 fish/ m^3). However, BFT100 and BFT50 presented the best FCR values when compared to CW and BFT200. This suggests a response with energy expenditure for food competition and to maintain homeostasis from stress caused by the larger number of animals. The adequate FCR values obtained for tambaqui in both systems (0.89 ± 0.08 to 1.31 ± 0.06) may be related to the good conditions of the rearing environment and the extra food available in BFT.

This observation corroborates with dos Santos et al. (2020) findings in which tambaqui in BFT presented lower FCR (0.71 ± 0.08 to 0.80 ± 0.10) than in CW, emphasizing the effect of microbial flakes on the zootechnical performance of animals raised in BFT. Results of FCR for tambaqui in net cages under different stocking densities presented by Frisso et al. (2020) were between 2.20 ± 0.16 and 1.65 ± 0.04 , also showing the FCR improvement in BFT system compared with other with constant water renewal, for animals in the same life stage of the present study.

According to Day et al. (2016), FCR of *O. niloticus* in BFT was better (1.01 ± 0.05) than with *O. mossambicus* (2.24 ± 0.16) in the initial phase of BFT, indicating that fish performance in BFT system may vary with species.

The productivity and biomass in the present study at 50 and 100 fish/ m^3 for BFT were higher than CW (Table 3), probably due to the extra food available in BFT. These results corroborate those obtained by Luo et al. (2014) with *O. niloticus* for which average productivity in BFT (44.95 kg/ m^3) was significantly higher than in the RAS (36.87 kg/ m^3).

The high survival rate in all treatments indicates the high rusticity and adaptability of tambaqui to adverse water quality. Frisso et al. (2020) obtained 99% survival for tambaqui in cages and dos Santos et al. (2020) survival rate above 90% in BFT system, which is corroborated by the finds of the present study. However, high stocking densities may affect fish well-being and performance (Aride et al. 2007; Costa et al. 2019).

In addition to growth performance, assessment of blood parameters is important to evaluate farm animal physiological conditions (Affonso et al. 2012; Fazio et al. 2019). The associative analysis of haematological parameters facilitates the development of indicators of fish health status in relation to nutrition, water quality and diseases (Fazio et al. 2019). Increase in fish density may onset haematological responses, as well as changes in water quality and animal behaviour, reducing the physical space available to perform baseline activities (Costa et al. 2019).

In the present study, efficiency in transporting oxygen to the tissues or anaemia was not observed, given the concentrations of Hb, HCM and CHCM, as well as for cholesterol, triglycerides, glucose, total plasma protein and respiratory activity of leukocytes (RAL) in fish under different densities and different rearing systems (Table 4). The steady haematological indices among treatments (Basal, BFT e AC) are compatible with the values obtained in previous studies with tambaqui (Affonso et al. 2002; Costa et al. 2019) and suggest that the breeding systems and stocking densities tested did not compromise fish homeostasis and corroborates with the results obtained by Long et al. (2015) and Haridas et al. (2017) for tilapia in BFT. However, Frisso et al. (2020) observed increase in tambaqui blood glucose and cortisol with increase in stocking density in net cages. Thus, the steady blood parameters of fish kept in BFT systems are probably a result of this rearing environment capacity in maintaining animal homeostasis.

5 | CONCLUSION

BFT provides better conditions for growth of tambaqui juveniles (23–115 g) than CW and does not affect animal welfare. The optimal density for rearing tambaqui in both systems under the evaluated conditions is 100 fish/m³. However, in the densities of 200 fish/m³, they showed higher biomass and productivity. Tambaqui adapts well to environmental changes being suitable for intensive culture with minimal water exchange.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial/personal interests.

AUTHOR CONTRIBUTIONS

Raphael Brito dos Santos: Conceptualization, methodology, validation, data curation, formal analysis, investigation, writing - original draft, writing - review & editing, visualization. Judá Izel-Silva: Methodology, validation, investigation, writing - review & editing. Michelle Midori Sena Fugimura: Conceptualization, methodology, validation, formal analysis, resources, supervision, writing - review & editing. Sabrina Medeiros Suita: Supervision, writing - review & editing. Eduardo Akifumi Ono: Methodology, validation, resources, data curation, writing - review & editing. Elizabeth Gusmão Affonso: Conceptualization, methodology, validation, formal analysis, resources, data curation, writing - review & editing, supervision, project administration, funding acquisition.

DATA AVAILABILITY STATEMENT

Data that support this study are available from the corresponding author upon request.

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Dietary protein levels for juvenile matrinxã (*Brycon amazonicus*) reared in biofloc and clear water systems

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ABSTRACT - This study evaluated the potential for rearing matrinxã (*Brycon amazonicus*) with different crude protein (CP) levels and using production performance, water quality, and animal welfare as indicators. To achieve this, 720 juvenile fish (3.65 ± 0.17 g) were distributed in 24 experimental units (200 L). The study, which lasted for 60 days, followed a factorial design (2×4) with two production systems: biofloc technology (BFT) and clear water (CW) and four CP levels (24, 30, 36, and 42%) in the diet, with three repetitions for each. We found that the BFT system showed a lower total ammonia level (0.45 ± 0.15 mg/L) compared with CW (1.16 ± 0.50 mg/L). Fish produced in the BFT system exhibited better performance in terms of apparent feed conversion (1.06 ± 0.15), productivity (7.68 ± 2.38 g/m³), and survival ($76.95 \pm 9.15\%$). The type of production system affected total leukocytes and neutrophils in the blood. Juvenile matrinxã can be fed a diet containing 30% CP in both production systems. Furthermore, the results demonstrated that bioflocs do not confer a direct benefit to juvenile matrinxã in terms of their feeding; however, the BFT system is a promising alternative for increasing the survival and improving animal welfare during the rearing phase.

Keywords: feeding, fish production, ideal protein, production system

1. Introduction

Among current production technologies, the biofloc system (BFT) can be considered sustainable in terms of the use of water, physical space, and its generation of effluents (McCusker et al., 2023; Khanjani et al., 2024a). This technology guarantees an additional source of food for aquatic organisms through the conversion of nitrogenous waste into microbial flocs (Silva et al., 2018; Khanjani et al., 2023a; Dos Santos et al., 2021, 2023, 2024; McCusker et al., 2023), in addition to contributing to the improvement of water quality, which has a direct influence on the performance and reproduction of aquatic organisms (Dos Santos et al., 2023; Khanjani et al., 2024b). The water in the system can be reused in subsequent production cycles due to the presence of a microbiota that acts in the cycling of nitrogenous waste, which, when kept in suspension in the water column by continuous aeration, can be used as a nutritional source for the farmed animals (Vadhel et al., 2020; Dos Santos et al., 2023; McCusker et al., 2023).

The microbial floc present in the BFT system can serve as an additional food source, thereby reducing the feed supply and/or the amount of crude protein (CP) in the feed offered. This makes the system more economical and contributes to fish performance (Lara et al., 2017; Dos Santos et al., 2023, 2024; Khanjani et al., 2023b). The potential application of this technology has already been evaluated using juvenile tambaqui (*Colossoma macropomum*), a fish native to the Amazon and Orinoco basins, as demonstrated by Dos Santos et al. (2021, 2023, 2024). The use of the BFT system can be a valuable strategy for increasing the production of fish species of great economic interest. This method has already been successfully implemented in other countries for shrimp species, as demonstrated by Emerenciano et al. (2013b,c), for the Pacific white shrimp (*Litopenaeus vannamei*), and red tilapia (*Oreochromis niloticus*), as demonstrated by Widanarni et al. (2012).

The matrinxã, *Brycon amazonicus*, has high commercial interest due to its good acceptance in the consumer market and easy adaptation to confined systems (Nascimento et al., 2020). According to Mattos et al. (2013), it has great potential in the Amazon region and other regions of Brazil due to its omnivorous feeding habits and to its excellent production performance rates in various farming systems. However, despite being a species with great productive/economic potential (IBGE, 2021), its production technology still needs to be better structured, especially in the larviculture and rearing phase of the species, which presents intraspecific cannibalism and aggressive behavior (Carvalho et al., 2018; Ferreira et al., 2020; Izel-Silva et al., 2024).

As previously demonstrated by Izel-Silva et al. (2024), the reduction in luminosity due to the turbidity of the water in the biofloc system contributes to a reduction in aggressive behavior and an increase in the productivity of the species. A reduction in light intensity in the water leads to an increase in melatonin production, which in turn reduces the aggressive behavior of larval-stage or juvenile matrinxã (Lopes et al., 2018; Amaral et al., 2020; Cornélio et al., 2021). It may also contribute to a reduction of protein levels in the feed, as already found with tilapia (*Oreochromis* sp.) (Day et al., 2016; Luo et al., 2017; Silva et al., 2018) and carp (*Cyprinus* sp.) (Castro et al., 2016; Najdegerami et al., 2016) in the juvenile phase.

Furthermore, despite the species' market potential, matrinxã production is still in its early stages. It is therefore important to study production management strategies, such as the BFT system, which can increase productivity and reduce production costs by providing additional nutrients to the fish such as microbial protein. Dos Santos et al. (2023, 2024) discovered that it is feasible to decrease the CP levels in tambaqui feed without compromising performance or animal welfare, as well as improving the digestibility and absorption of food. Although matrinxã is not a filter-feeding species like tambaqui, it does consume zooplankton in its juvenile stage, and in the natural environment, fish between 25-50 mm standard length, for example, can have up to 80% of their diet made up of zooplankton microorganisms (mainly microcrustaceans) and insect larvae (Leite, 2004). As a result, it is not really known how much of the microorganisms present in the BFT the matrinxã could consume or how significant this intake could be in reducing the amount of feed offered or the protein in the diet. Thus, the objective of this study was to determine the optimal percentage of CP in the diet of juvenile matrinxã that would promote the best animal performance and welfare for the species, without compromising water quality in the clear water (CW) and BFT systems.

2. Material and methods

2.1. Ethical approval

The experiment was carried out at the Laboratório de Fisiologia Aplicado à Piscicultura. (LAFAP), of the Estação Experimental de Aquicultura of the Coordenação de Tecnologia e Inovação (COTEI) at the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM, and was approved by the Ethics Committee on the Use of Animals (CEUA) at INPA (No. 040/2020, SEI 01280.001374/2020-68).

2.2. Experimental design

Juvenile matrinxã weighing approximately 0.5 g were obtained from a commercial fish farm and acclimated for 40 days in 2,000-L fiberglass tanks with bioflocs and constant aeration. After the

acclimatization period, 720 Juvenile matrinxã (weighing 3.6 ± 0.1 g) were distributed in a factorial design (2×4) that involved two production systems (BFT and CW) and four CP levels in the diet (24, 30, 36, and 42%), with three repetitions each. Matrinxã juveniles ($n = 30$, equivalent to 150 fish/m^3) were distributed in 24 circular polyethylene tanks of 500 L (filled with 200 L of water) with intense and continuous aeration, using microperforated hoses to suspend particulate matter in the water column. Each experimental unit was maintained in isolation with no interconnectivity between them in both the BFT and CW systems. The study was conducted for 60 days.

The experimental unit in the BFT system received an inoculum of bioflocs that had been matured in the laboratory and corresponded to 50% of the volume used in each experimental unit, following the methodology of Krummenauer et al. (2012). In the CW treatments, a 50% water exchange occurred daily throughout the experimental period.

2.3. Experimental diets

The feeds (Table 1) used in the experiment were isocaloric; the ingredients of which were ground to 1 mm, homogenized in a mixer, and processed in a commercial extruder (INBRA MX-80, Inbramaq, Ribeirão Preto, Brazil), and the 2–4 mm pellets were dried using forced ventilation (55°C). The animals were fed three times a day (8:00, 12:00, and 17:00 h) until apparent satiety, with the amount adjusted daily by observing the leftover feed 15 min after offering the feed.

Table 1 - Formulation and nutritional composition of the experimental diets with different crude protein levels for juvenile *Brycon amazonicus* in biofloc and clear water systems for 60 days

Ingredient (%)	Crude protein level			
	24%	30%	36%	42%
Soybean meal	10.45	25.35	29.72	30.58
Corn grain	44.42	30.48	16.17	3.49
Corn gluten	12.00	8.00	13.00	19.50
Corn starch	10.00	10.00	10.00	10.00
Offal meal	6.96	11.50	15.00	19.50
Fish meal	5.00	5.00	5.00	5.00
Meat and bone meal	2.00	2.00	2.00	2.00
Wheat flour	2.00	2.00	2.00	2.00
Soy oil	2.80	1.77	1.34	0.50
Dicalcium phosphate	1.80	1.05	0.60	0.00
Limestone	1.05	0.83	0.65	0.46
Inert	0.00	0.50	3.00	5.45
L-lysine	0.60	0.60	0.60	0.60
DL-methionine	0.40	0.40	0.40	0.40
Mineral supplement	0.50	0.50	0.50	0.50
BHT	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00
Centesimal composition (%)				
Dry matter	91.24	90.23	94.13	90.64
Crude protein	24.40	30.20	36.90	42.80
Ether extract	6.80	4.80	4.20	4.00
Crude fiber	1.30	1.50	1.90	2.00
Ash	4.80	4.58	6.84	6.82
Gross energy (kcal)	4,086.85	4,086.85	4,086.85	4,086.84

Premix: folic acid (minimum), 100.00 mg/kg; nicotinic acid (minimum), 10.00 g/kg; pantothenic acid (minimum), 5,000.00 mg/kg; BHT (minimum), 5,000.00 mg/kg; biotin (minimum), 50.00 mg/kg; cobalt (minimum), 30.00 mg/kg; copper (minimum), 1,000.00 mg/kg; choline (minimum), 80.00 g/kg; iron (minimum), 7,500.00 g/kg; iodine (minimum), 250.00 mg/kg; inositol (minimum), 4,000.00 g/kg; manganese (minimum), 2,000.00 mg/kg; magnesium (minimum), 600.00 mg/kg; potassium (minimum), 2,000.00 mg/kg; selenium (minimum), 70.00 mg/kg; vitamin A (minimum), 2,000,000.00 IU/kg; vitamin B1 (minimum), 2,000.00 mg/kg; vitamin B2 (minimum), 4,000.00 mg/kg; vitamin B12 (minimum), 10,000.00 mcg/kg; vitamin C (minimum), 80.00 g/kg; vitamin B6 (minimum), 5,000.00 mg/kg; vitamin D3 (minimum), 600,000.00 IU/kg; vitamin E (minimum), 15,000.00 IU/kg; vitamin K3 (minimum), 700.00 mg/kg; zinc (minimum), 7,500.00 mg/kg.

2.4. Water quality analyses

Temperature, pH, dissolved oxygen, and salinity were checked daily using a multiparameter probe (Akso, AK88). The total ammonia concentration (Verdouw et al., 1978) and nitrite (Boyd and Tucker, 1992) in the water were assessed three times a week. Nitrate (adapted from Papaspyrou et al., 2014) and turbidity (NTU; portable turbidimeter - Hanna, HI93703C) were measured once a week.

Total suspended solids levels were checked every week by filtering 20 mL of water through GF 50-A glass fiber filters (Strickland and Parsons, 1972). The volume of settleable solids was monitored twice a week using Imhoff cones (Avnimelech, 2007). The alkalinity (mg/L of CaCO_3) was monitored three times a week using acid-base titration and the methyl-orange indicator (APHA, 1998), with a target of maintaining a level above 50 mg/L, and, when necessary, corrections were made with sodium bicarbonate (Avnimelech, 2009). In the CW system, the alkalinity was always adjusted after changing the water in the system.

2.5. Analyses of animal performance and somatic indices

To evaluate productive performance, the weight of each fish was measured using biometric assessments at the beginning and end of the experiment. For this, the animals were anesthetized in a benzocaine solution (100 mg/L) and, from the weight obtained, the following was calculated: average final weight (FW) = final weight of all fish/number of fish; daily weight gain (DWG) = (average final weight (g) – average initial weight (g))/days; specific growth rate (SGR) = $[(\ln \text{final average weight (g)} - \ln \text{initial average weight (g)})/\text{days}] \times 100$; apparent feed conversion (AFC) = feed intake (g)/weight gain (g); survival (S%) = (number of animals at the end of the experiment/number of animals at the beginning of the experiment) $\times 100$, and productivity (g/m^3) = (total biomass/tank volume)/1,000; protein efficiency ratio (PER%) = average weight gain/(CP in the diet $\times$ average feed intake).

For the analysis of the somatic indices, three fish from each experimental unit were euthanized in a benzocaine solution (300 mg/L) for dissection of the total viscera and liver, and weighted. The following calculations were used: viscerosomatic index (%) = (viscera weight / live weight) $\times 100$ and hepatosomatic index (%) = (liver weight / live weight) $\times 100$.

2.6. Blood collection and determination of blood parameters

To evaluate the blood parameters, three fish from each experimental unit were previously anesthetized in a benzocaine solution (100 mg/L) and had blood collected through puncture of the tail vein using a syringe with heparin anticoagulant (10 μL). The following parameters were analyzed: hematocrit (Ht), using the microhematocrit method; hemoglobin ([Hb]) concentration, using the cyanmethemoglobin method and commercial reagents; and erythrocyte count (RBC), using a Newbauer chamber and a formaldehyde-citrate solution. The total and differential leukocyte count was performed using blood samples previously stained with May-Grünwald-Giemsa-Wright (MGGW) stain according to the methodology in Tavares-Dias and Moraes (2004). The number of leukocytes and thrombocytes were quantified from the 2,000 erythrocyte count and, for the differential blood cell count, 200 leukocytes were identified and classified as lymphocytes, monocytes, or neutrophils.

From the Ht, [Hb], and RBC results, the Wintrobe indices were calculated: mean corpuscular volume (MCV) = hematocrit $\times 10/\text{number of erythrocytes (fl)}$; mean corpuscular hemoglobin (MCH) = $[\text{Hb}] \times 10/\text{RBC (pg)}$; and mean corpuscular hemoglobin concentration (MCHC) = $[\text{Hb}] \times 100/\text{Ht (g/dL)}$. The values of cholesterol, triglycerides, and glucose in the plasma were determined using the enzymatic colorimetric method using commercial kits, and total proteins were determined using the modified biuret method.

2.7. Assessments of the nutritional composition of fish and bioflocs

To determine the proximal composition of fish, three animals were collected per experimental unit, then euthanized in benzocaine (300 mg/L) and subsequently stored in a freezer at -18°C . The samples

were dehydrated in a ventilated oven at 55 °C until constant weight and ground in a multiprocessor to obtain uniform samples. Dry matter (%) was determined in an oven at 105 °C, CP was obtained using the micro-Kjehldal method, ether extract was analyzed through continuous extraction with petroleum ether in a Soxhlet extractor, and ash was obtained via burning in a muffle furnace at 550 °C (AOAC, 2012).

For the proximal analysis of the flocs, at the end of the experiment, 80 L of suspended bioflocs were collected from each experimental unit and transferred to conical tanks with a capacity of 100 L. After decanting for 3 h, approximately 1 L of sedimented material was collected and stored at -18 °C. This material was dried in a ventilated oven at 55 °C and macerated to obtain uniform samples for the determination of CP, ether extract, and ash (AOAC, 2012).

2.8. Statistical analysis

For all water quality and performance evaluations, each cage containing 30 juvenile matrinxã was considered an experimental unit. For somatic indices and hematological variables, each fish was considered an experimental unit, with nine repetitions of each treatment. Data were first checked for normality of studentized residuals using the Shapiro-Wilk test with the InfoStat statistical program (version 2020), and homogeneity of variances using the Bartlett test with R software. Only for the proximal composition of the bioflocs, one-way analysis of variance (one-way ANOVA) was performed. All other data was subjected to two-way analysis of variance (two-way ANOVA), and the means were compared by Tukey's test. The significance level accepted for all the tests carried out was $\alpha = 0.05$.

The statistical model for one-way ANOVA used to test the effect of treatments was:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

in which Y_{ij} = is the observation of experimental units ($j = 1, 2, 3$) in the factor protein level ($i = 24, 30, 36$ and 42%), μ = the overall mean, τ_i = fixed effect of factor i protein levels ($i = 24, 30, 36$ and 42%), and ε_{ij} = the random error associated with each observation Y_{ij} .

The statistical model for two-way ANOVA used to test the effect of treatments was:

$$Y_{ijk} = \mu + \tau_i + \gamma_j + (\tau\gamma)_{ij} + \varepsilon_{ijk}$$

in which Y_{ijk} = value observed, μ = the overall mean, τ_i = effect of production system (Biofloc and Clear Water), γ_j = effect of protein level (24, 30, 36 and 42%), $\tau\gamma$ = effect of interaction between production system and protein level, and ε_{ijk} = random error associated with each observation Y_{ijk} .

3. Results

3.1. Water quality

No significant differences in temperature were observed between the treatments. The concentration of total ammonia in the water was only affected by the production system, with lower levels observed in the biofloc system (0.45 ± 0.15 mg/L; $P < 0.0001$). The production system had no effect on pH or nitrite concentration. Salinity (1.45 ± 0.13 ppt; $P < 0.0001$) was higher and dissolved oxygen ($DO = 6.03 \pm 0.10$ mg/L; $P < 0.0015$) was lower in the BFT system. There were no significant differences in pH and nitrite levels at the three lowest CP levels. Dissolved oxygen was higher in the water with fish fed 24% CP and the two lowest CP levels resulted in lower salinity (Table 2).

The study found a significant interaction between the CP level and the production system in relation to nitrate, alkalinity, total suspended solids (TSS), settleable solids (SS), and turbidity ($P < 0.05$). However, only alkalinity was affected by the different CP levels in the CW system. On the other hand, reducing the CP level in the diet resulted in lower concentrations of nitrate, TSS, SS, and turbidity and an increase in the concentration of alkalinity in the BFT system (Table 3).

Table 2 - Mean $\pm$ standard deviation of water quality variables of the biofloc (BFT) and clear water (CW) systems verified over 60 days during the production of juvenile matrinxã (*Brycon amazonicus*) fed diet with different crude protein levels

Variable	Temperature (°C)	DO (mg/L)	pH	Salinity (ppt)	Ammonia (mg/L)	Nitrite (mg/L)
BFT	27.04 $\pm$ 0.13	6.03 $\pm$ 0.10B	7.19 $\pm$ 0.28	1.45 $\pm$ 0.13A	0.45 $\pm$ 0.15B	0.49 $\pm$ 0.22
CW	27.04 $\pm$ 0.12	6.16 $\pm$ 0.11A	7.27 $\pm$ 0.18	0.06 $\pm$ 0.03B	1.16 $\pm$ 0.50A	0.38 $\pm$ 0.30
Protein level (%)						
24	26.98 $\pm$ 0.14	6.26 $\pm$ 0.10b	7.43 $\pm$ 0.06b	0.69 $\pm$ 0.68a	0.53 $\pm$ 0.25	0.22 $\pm$ 0.09a
30	27.08 $\pm$ 0.08	6.08 $\pm$ 0.11a	7.26 $\pm$ 0.08ab	0.75 $\pm$ 0.76ab	0.80 $\pm$ 0.35	0.39 $\pm$ 0.17ab
36	27.06 $\pm$ 0.12	6.07 $\pm$ 0.12a	7.20 $\pm$ 0.14ab	0.83 $\pm$ 0.82b	0.83 $\pm$ 0.50	0.46 $\pm$ 0.27ab
42	27.03 $\pm$ 0.17	5.98 $\pm$ 0.08a	7.03 $\pm$ 0.24a	0.79 $\pm$ 0.79ab	1.07 $\pm$ 0.77	0.68 $\pm$ 0.28b
BFT						
Protein level (%)						
24	26.96 $\pm$ 0.13	6.23 $\pm$ 0.04	7.44 $\pm$ 0.09	1.31 $\pm$ 0.09	0.31 $\pm$ 0.06	0.24 $\pm$ 0.04
30	24.08 $\pm$ 0.07	5.99 $\pm$ 0.08	7.24 $\pm$ 0.09	1.44 $\pm$ 0.04	0.51 $\pm$ 0.21	0.40 $\pm$ 0.08
36	27.08 $\pm$ 0.17	5.98 $\pm$ 0.04	7.17 $\pm$ 0.12	1.57 $\pm$ 0.15	0.47 $\pm$ 0.14	0.61 $\pm$ 0.19
42	27.03 $\pm$ 0.25	5.93 $\pm$ 0.07	6.98 $\pm$ 0.16	1.50 $\pm$ 0.11	0.53 $\pm$ 0.09	0.72 $\pm$ 0.72
CW						
Protein level (%)						
24	27.01 $\pm$ 0.17	6.28 $\pm$ 0.15	7.42 $\pm$ 0.01	0.07 $\pm$ 0.03	0.75 $\pm$ 0.10	0.20 $\pm$ 0.13
30	27.09 $\pm$ 0.10	6.17 $\pm$ 0.04	7.28 $\pm$ 0.07	0.05 $\pm$ 0.01	1.09 $\pm$ 0.10	0.38 $\pm$ 0.25
36	27.04 $\pm$ 0.08	6.16 $\pm$ 0.11	7.21 $\pm$ 0.19	0.09 $\pm$ 0.05	1.19 $\pm$ 0.47	0.31 $\pm$ 0.27
42	27.04 $\pm$ 0.11	6.02 $\pm$ 0.05	7.17 $\pm$ 0.26	0.07 $\pm$ 0.01	1.61 $\pm$ 0.76	0.64 $\pm$ 0.42
Two-way ANOVA						
System	NS	<0.0015	NS	<0.0001	<0.0001	NS
Protein	NS	<0.0002	<0.0016	<0.0391	NS	<0.0196
Interaction	NS	NS	NS	NS	NS	NS

DO - dissolved oxygen; NS - not significant.

Means followed by different letters in the same column differ according to ANOVA and Tukey's test ($P < 0.05$).

3.2. Animal performance and somatic indices

No significant differences were found in FW, SGR, DWG, PER, and feed intake between the production systems (Table 4). However, fish produced in the biofloc system showed higher productivity (7.68 $\pm$ 2.38 g/m³; $P < 0.0001$) and survival rates 76.95 $\pm$ 9.15%, and lower apparent feed conversion (AFC = 1.06 $\pm$ 0.15; $P < 0.0424$) (Table 4).

The 24% CP level had generally the worst performance, while there was no significant difference among the 30, 36, and 42% CP levels. Additionally, the CP levels did not affect animal survival. In BFT, the hepatosomatic (HSI = 2.45 $\pm$ 0.48%; $P < 0.0006$) and viscerosomatic (VSI = 11.27 $\pm$ 2.94%; $P < 0.0042$) indices were higher. The CP levels did not have a significant effect on the HSI. However, juveniles that were fed with 42% CP had a lower VSI (7.73 $\pm$ 1.22%; $P < 0.0181$) compared with those fed 24% CP (12.30 $\pm$ 3.82%; Table 4).

3.3. Blood parameters

Blood parameters (Tables 5 and 6) of the fish (in BFT and CW) showed no significant differences between the systems. The blood parameters of the juveniles fed different CP levels in the diet also showed no significant differences, except for Hb concentration ($P < 0.0262$). The 42% CP level in the diet showed a higher (9.28 $\pm$ 0.78 g/dL) hemoglobin concentration than those fed the 24% CP diet (8.26 $\pm$ 3.88 g/dL), while there was no difference among the three highest CP levels.

Table 3 - Mean ± standard deviation of water quality variables of the biofloc (BFT) and clear water (CW) systems verified over 60 days during the production of juvenile matrinxã (*Brycon amazonicus*) fed diet with different crude protein levels

Variable	Nitrate (mg/L)	Alkalinity CaCO ₃ (mg/L)	TSS (mg/L)	SSV (mg/L)	Turbidity (UNF)
BFT	129.98±36.83A	50.59±11.16A	192.54±44.07A	12.15±8.25A	76.47±19.11A
CW	9.61±4.09B	45.34±3.55B	25.33±6.80B	0.19±0.08B	4.73±1.37B
Protein level (%)					
24	52.13±49.09c	55.24±8.71c	80.33±69.00c	1.70±1.87a	26.93±26.25c
30	58.60±51.92bc	52.20±4.35c	101.25±85.02b	5.55±6.47b	38.94±37.28b
36	80.70±79.50ab	45.04±3.61b	117.33±99.04ab	6.57±7.08ab	45.44±45.57ab
42	87.78±89.05a	38.68±3.97a	136.83±99.0a	10.87±13.22a	51.08±49.41a
BFT					
Protein level (%)					
24	96.49±10.76Ab	63.79±1.78Aa	141.00±27.37Ad	3.30±1.04Ac	50.32±8.88Ad
30	104.35±19.68Ab	56.07±1.28Ab	177.50±24.07Ac	10.90±4.35Ab	72.74±6.78Ac
36	152.29±20.00Aa	46.05±4.95Ac	207.00±19.83Ab	12.90±2.19Ab	86.73±8.79Ab
42	166.82±32.80Aa	36.48±4.64Ad	244.67±15.73Aa	21.50±9.87Aa	96.08±5.09Aa
CW					
Protein level (%)					
24	7.76±0.58Ba	48.11±1.64Ba	19.67±10.67Ba	0.10±0.10Aa	3.53±1.41Ba
30	12.84±8.01Ba	48.32±0.80Ba	25.00±7.05Ba	0.20±0.001Ba	4.16±1.08Ba
36	9.11±0.53Ba	44.04±2.24Aab	27.67±2.93Ba	0.23±0.06Ba	5.14±1.09Ba
42	8.74±2.17Ba	40.87±1.86Ab	29.00±2.63Ba	0.23±0.06Ba	6.07±0.64Ba
Two-way ANOVA					
System	<0.0001	<0.0003	<0.0001	<0.0001	<0.0001
Protein	<0.0037	<0.0001	<0.0002	<0.0082	<0.0001
Interaction	<0.0027	<0.0001	<0.0010	<0.0092	<0.0001

TSS - total suspended solids; SSV - settleable solids volume; NS - not significant.
Means followed by different letters, uppercase for comparison of production systems and lowercase for protein levels, differ according to ANOVA and Tukey's test (P<0.05).

We found an interaction (P<0.0223) between production systems and CP level for the variable Ht. Juveniles produced in the BFT system had a lower average Ht value (30.06±2.05%) than those produced in the CW system and feeding the 24% CP diet (33.22±4.32%). Hematocrit was not affected by CP levels in juveniles in the CW system, and there was no significant difference in Ht in the higher CP levels in the BFT (Table 5). The profile of defense cells (Table 6) showed differences only between the BFT and CW systems in terms of total leucocytes and neutrophils (P<0.0001), with higher averages in the juvenile matrinxã reared in BFT (total leucocytes = 36.31±12.54 ×10³/μL and neutrophils = 0.81±1.11 ×10³/μL) compared with the CW system (total leucocytes = 29.74±9.74 ×10³/μL and neutrophils = 0.09±0.22 ×10³/μL).

3.4. Nutritional composition of the fish and the bioflocs

For the mean composition values of the juvenile matrinxã at the end of the study (Table 7), there was no significant difference in CP content in the carcass. However, the ether extract content was higher (P<0.0058) in juveniles reared in the BFT system (22.04±2.36%) compared with those in the CW system (20.35±1.69%). Additionally, a significant interaction was observed with dry matter (P<0.0365) and ash (P<0.0327). No difference was found in the dry matter content in the nutritional composition of matrinxã reared in the BFT system and fed 30 to 42% CP levels. The nutritional composition of the biofloc did not show any significant differences in CP or ether extract at different levels of CP. However, the mineral matter content was lower (24.53±2.16%; P<0.0003) when the percentage of CP in the diet was higher (Table 8).

Table 4 - Mean $\pm$ standard deviation of productive parameters and somatic indices of juvenile matrinxã (*Brycon amazonicus*) produced in biofloc (BFT) and clear water (CW) systems and fed diet with different crude protein levels for 60 days

Variable	FW (g)	SGR (%/day)	DWG (g/day)	AFC	Productivity (g/m ³)	Survival (%)	Feed intake (g)	VSI (%)	HSI (%)	PER (%)
BFT	66.16 $\pm$ 18.33	4.78 $\pm$ 0.53	1.04 $\pm$ 0.30	1.06 $\pm$ 0.15A	7.68 $\pm$ 2.38B	76.95 $\pm$ 9.15A	1458.72 $\pm$ 374.34A	11.27 $\pm$ 2.94A	2.45 $\pm$ 0.48A	3.00 $\pm$ 0.48
CW	64.34 $\pm$ 24.83	4.62 $\pm$ 0.75	1.02 $\pm$ 0.41	1.16 $\pm$ 0.13B	4.57 $\pm$ 1.39A	50.00 $\pm$ 12.31B	929.48A $\pm$ 309.71B	8.32 $\pm$ 2.25B	1.59 $\pm$ 0.44B	2.90 $\pm$ 0.69
Protein level (%)										
24	41.23 $\pm$ 19.19b	3.94 $\pm$ 0.67b	0.62 $\pm$ 0.32b	1.25 $\pm$ 0.07a	3.60 $\pm$ 1.41b	60.00 $\pm$ 14.46	758.20 $\pm$ 328.86b	12.30 $\pm$ 3.82a	2.13 $\pm$ 0.94	3.61 $\pm$ 0.27a
30	71.81 $\pm$ 15.436a	4.95 $\pm$ 0.36a	1.14 $\pm$ 0.26a	1.09 $\pm$ 0.13ab	6.69 $\pm$ 2.05a	62.78 $\pm$ 15.83	1303.68 $\pm$ 285.43a	9.56 $\pm$ 2.28ab	2.04 $\pm$ 0.48	3.19 $\pm$ 0.40a
36	66.88 $\pm$ 17.10ab	4.78 $\pm$ 0.49a	1.06 $\pm$ 0.28ab	1.06 $\pm$ 0.10b	6.74 $\pm$ 2.27a	68.33 $\pm$ 18.23	1295.29 $\pm$ 452.90a	9.59 $\pm$ 2.55ab	2.03 $\pm$ 0.64	2.69 $\pm$ 0.32b
42	81.07 $\pm$ 12.38a	5.13 $\pm$ 0.72a	1.29 $\pm$ 0.21a	1.04 $\pm$ 0.17b	7.46 $\pm$ 2.46a	62.78 $\pm$ 23.52	1419.23 $\pm$ 381.00a	7.73 $\pm$ 1.22b	1.89 $\pm$ 0.51	2.31 $\pm$ 0.30b
BFT										
Protein level (%)										
24	38.32 $\pm$ 6.63	3.93 $\pm$ 0.16	0.57 $\pm$ 0.12	1.27 $\pm$ 0.08	4.17 $\pm$ 0.95	72.22 $\pm$ 3.85	945.69 $\pm$ 254.38	14.62 $\pm$ 3.99	2.83 $\pm$ 0.73	3.38 $\pm$ 0.18Aa
30	73.41 $\pm$ 10.46	5.01 $\pm$ 0.17	1.17 $\pm$ 0.17	0.98 $\pm$ 0.09	8.31 $\pm$ 1.07	75.56 $\pm$ 1.93	1516.74 $\pm$ 102.91	11.52 $\pm$ 0.68	2.38 $\pm$ 0.30	3.46 $\pm$ 0.32Aa
36	72.28 $\pm$ 2.87	5.00 $\pm$ 0.11	1.15 $\pm$ 0.05	1.05 $\pm$ 0.05	8.56 $\pm$ 1.39	78.89 $\pm$ 11.70	1665.41 $\pm$ 214.82	10.37 $\pm$ 0.95	2.37 $\pm$ 0.29	2.64 $\pm$ 0.10Ab
42	80.62 $\pm$ 8.65	5.19 $\pm$ 0.14	1.29 $\pm$ 0.14	0.95 $\pm$ 0.10	9.68 $\pm$ 0.94	81.11 $\pm$ 15.40	1729.84 $\pm$ 237.76	8.60 $\pm$ 1.05	2.23 $\pm$ 0.49	2.51 $\pm$ 0.26Ab
CW										
Protein level (%)										
24	44.14 $\pm$ 29.18	3.96 $\pm$ 1.04	0.68 $\pm$ 0.48	1.23 $\pm$ 0.08	3.03 $\pm$ 1.74	47.78 $\pm$ 7.70	593.50 $\pm$ 376.33	9.98 $\pm$ 2.09	1.42 $\pm$ 0.46	3.38 $\pm$ 0.02Aa
30	70.21 $\pm$ 21.86	4.88 $\pm$ 0.55	1.11 $\pm$ 0.37	1.19 $\pm$ 0.07	5.08 $\pm$ 1.23	50.00 $\pm$ 11.55	1090.60 $\pm$ 238.57	7.60 $\pm$ 1.05	1.70 $\pm$ 0.37	2.91 $\pm$ 0.28Bb
36	61.48 $\pm$ 25.20	4.56 $\pm$ 0.66	0.98 $\pm$ 0.42	1.02 $\pm$ 0.16	4.91 $\pm$ 0.95	57.75 $\pm$ 18.95	925.17 $\pm$ 235.96	8.80 $\pm$ 3.68	1.70 $\pm$ 0.78	2.74 $\pm$ 0.10Abc
42	81.53 $\pm$ 17.55	5.07 $\pm$ 0.45	1.30 $\pm$ 0.29	1.18 $\pm$ 0.15	5.25 $\pm$ 0.65	44.44 $\pm$ 11.71	1108.60 $\pm$ 130.17	6.88 $\pm$ 0.58	1.54 $\pm$ 0.23	2.11 $\pm$ 0.20Ac
Two-way ANOVA										
System	NS	NS	NS	<0.0424	<0.0001	<0.0001	<0.0001	<0.0042	<0.0006	NS
Protein	<0.0082	<0.0052	<0.0083	<0.0092	<0.0001	NS	<0.0007	<0.0181	NS	<0.0001
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.0197

FW - final weight; SGR - specific growth rate; DWG - daily weight gain; AFC - apparent feed conversion; VSI - viscerosomatic index; HSI - hepatosomatic index; PER - protein efficiency ratio. Means followed by different letters, uppercase for comparison of production systems and lowercase for protein levels, differ according to ANOVA and Tukey's test (P<0.05).

Table 5 - Mean $\pm$ standard deviation of blood parameters of juvenile matrinxã (*Brycon amazonicus*) produced in biofloc (BFT) and clear water (CW) systems and fed diet with different crude protein levels for 60 days

Variable	Ht (%)	Hb (g/dL)	RBC ($\times 10^6/\mu\text{L}$)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)	Total protein (g/dL)
BFT	34.79 $\pm$ 4.68	8.80 $\pm$ 2.42	1.71 $\pm$ 0.22	202.23 $\pm$ 35.57	54.75 $\pm$ 16.37	26.74 $\pm$ 8.18	201.71 $\pm$ 33.62	613.75 $\pm$ 166.58	106.11 $\pm$ 9.06	3.11 $\pm$ 0.42
CW	36.03 $\pm$ 4.13	8.91 $\pm$ 1.01	1.72 $\pm$ 0.27	215.23 $\pm$ 44.87	53.10 $\pm$ 10.41	24.90 $\pm$ 3.03	204.53 $\pm$ 47.07	585.40 $\pm$ 188.19	107.62 $\pm$ 11.7	3.31 $\pm$ 0.09
Protein level (%)										
24	33.22 $\pm$ 4.3b	8.26 $\pm$ 3.88a	1.71 $\pm$ 0.20	197.00 $\pm$ 34.56	53.50 $\pm$ 20.16	27.96 $\pm$ 11.24	222.74 $\pm$ 43.59	689.39 $\pm$ 145.04	102.71 $\pm$ 4.14	3.08 $\pm$ 0.44
30	36.44 $\pm$ 4.70a	8.25 $\pm$ 0.93ab	1.78 $\pm$ 0.21	205.75 $\pm$ 26.82	50.08 $\pm$ 6.39	24.51 $\pm$ 2.82	197.78 $\pm$ 46.39	599.84 $\pm$ 195.99	106.38 $\pm$ 2.35	3.17 $\pm$ 0.30
36	34.50 $\pm$ 4.11b	9.02 $\pm$ 1.11ab	1.65 $\pm$ 0.29	216.93 $\pm$ 56.70	53.77 $\pm$ 14.54	26.30 $\pm$ 3.16	193.82 $\pm$ 35.05	566.03 $\pm$ 160.22	110.15 $\pm$ 16.29	3.09 $\pm$ 0.16
42	37.47 $\pm$ 3.55a	9.28 $\pm$ 0.78b	1.71 $\pm$ 0.26	223.23 $\pm$ 37.65	55.33 $\pm$ 9.63	24.98 $\pm$ 3.19	198.13 $\pm$ 32.58	543.03 $\pm$ 180.70	110.20 $\pm$ 3.19	3.51 $\pm$ 0.33
BFT										
Protein level (%)										
24	30.06 $\pm$ 2.05Bb	7.68 $\pm$ 1.02	1.65 $\pm$ 0.17	158.40 $\pm$ 22.46	47.86 $\pm$ 9.04	25.62 $\pm$ 3.05	216.58 $\pm$ 38.59	710.14 $\pm$ 175.28	104.49 $\pm$ 34.04	3.59 $\pm$ 0.96
30	36.56 $\pm$ 5.54Aa	8.99 $\pm$ 0.94	1.78 $\pm$ 0.15	205.88 $\pm$ 26.50	50.75 $\pm$ 4.98	24.90 $\pm$ 3.10	203.07 $\pm$ 20.76	606.94 $\pm$ 129.52	102.32 $\pm$ 28.70	3.39 $\pm$ 1.05
36	35.17 $\pm$ 4.64Aa	9.17 $\pm$ 1.20	1.67 $\pm$ 0.27	216.05 $\pm$ 48.59	56.84 $\pm$ 14.53	26.30 $\pm$ 3.68	202.77 $\pm$ 36.00	611.24 $\pm$ 210.65	118.78 $\pm$ 27.98	3.21 $\pm$ 1.09
42	37.39 $\pm$ 1.75Aa	9.35 $\pm$ 0.54	1.76 $\pm$ 0.27	217.85 $\pm$ 37.17	54.79 $\pm$ 12.26	25.07 $\pm$ 2.01	184.40 $\pm$ 33.76	526.67 $\pm$ 210.65	101.90 $\pm$ 29.45	3.04 $\pm$ 0.01
CW										
Protein level (%)										
24	36.39 $\pm$ 3.58Aa	8.83 $\pm$ 1.13	1.78 $\pm$ 0.22	208.59 $\pm$ 41.55	50.40 $\pm$ 9.08	24.29 $\pm$ 2.14	228.90 $\pm$ 49.63	668.65 $\pm$ 114.07	100.93 $\pm$ 34.12	2.88 $\pm$ 0.96
30	36.33 $\pm$ 4.04Aa	8.71 $\pm$ 0.96	1.79 $\pm$ 0.27	205.63 $\pm$ 28.74	49.42 $\pm$ 7.81	24.11 $\pm$ 2.64	192.50 $\pm$ 63.87	592.73 $\pm$ 254.44	110.42 $\pm$ 28.14	2.97 $\pm$ 1.10
36	33.83 $\pm$ 3.66Aa	8.86 $\pm$ 1.06	1.63 $\pm$ 0.32	217.81 $\pm$ 66.85	56.70 $\pm$ 15.44	26.30 $\pm$ 2.78	184.87 $\pm$ 33.68	520.83 $\pm$ 198.67	101.52 $\pm$ 35.54	2.98 $\pm$ 0.82
42	37.56 $\pm$ 4.88Aa	9.21 $\pm$ 0.99	1.67 $\pm$ 0.26	288.87 $\pm$ 39.49	55.87 $\pm$ 6.79	24.89 $\pm$ 4.18	211.85 $\pm$ 26.29	559.39 $\pm$ 156.25	118.51 $\pm$ 28.07	3.74 $\pm$ 0.90
Two-way ANOVA										
System	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Protein	<0.0092	<0.0262	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	<0.0223	NS	NS	NS	NS	NS	NS	NS	NS	NS

Ht - hematocrit; Hb - hemoglobin; RBC - erythrocyte count; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration; NS - not significant. Means followed by different letters, uppercase for production systems and lowercase for protein levels, differ according to ANOVA and Tukey's test ($P < 0.05$).

Table 6 - Mean $\pm$ standard deviation of the total and differential leukocytes counts of juvenile matrinxã (*Brycon amazonicus*) produced in biofloc (BFT) and clear water (CW) systems and fed diet with different crude protein levels for 60 days

Variable	Thrombocytes ($\times 10^3/\mu\text{L}$)	Total leukocytes ($\times 10^3/\mu\text{L}$)	Lymphocytes ($\times 10^3/\mu\text{L}$)	Monocytes ($\times 10^3/\mu\text{L}$)	Neutrophils ($\times 10^3/\mu\text{L}$)
BFT	1.21 $\pm$ 1.45	36.31 $\pm$ 12.54A	29.35 $\pm$ 9.38	6.16 $\pm$ 3.47	0.81 $\pm$ 1.11A
CW	1.11 $\pm$ 0.89	29.74 $\pm$ 9.74B	24.83 $\pm$ 8.03	4.82 $\pm$ 2.48	0.09 $\pm$ 0.22B
Protein level (%)					
24	1.12 $\pm$ 1.46	31.28 $\pm$ 11.38	26.15 $\pm$ 7.51	4.62 $\pm$ 3.57	0.51 $\pm$ 1.27
30	1.15 $\pm$ 0.97	29.17 $\pm$ 13.04	23.70 $\pm$ 9.62	4.99 $\pm$ 3.49	0.48 $\pm$ 0.82
36	0.74 $\pm$ 0.78	34.63 $\pm$ 10.70	28.18 $\pm$ 8.25	6.26 $\pm$ 2.99	0.19 $\pm$ 0.35
42	1.60 $\pm$ 1.42	37.42 $\pm$ 10.86	30.60 $\pm$ 9.78	6.20 $\pm$ 2.01	0.63 $\pm$ 0.83
BFT					
Protein level (%)					
24	1.37 $\pm$ 1.86	32.73 $\pm$ 15.78	26.76 $\pm$ 9.49	5.16 $\pm$ 4.82	0.81 $\pm$ 1.79
30	0.74 $\pm$ 0.70	36.02 $\pm$ 11.50	28.47 $\pm$ 7.41	6.72 $\pm$ 4.07	0.83 $\pm$ 1.08
36	0.72 $\pm$ 0.79	32.23 $\pm$ 12.98	26.05 $\pm$ 10.2	5.85 $\pm$ 3.14	0.34 $\pm$ 0.43
42	1.99 $\pm$ 1.95	44.27 $\pm$ 7.74	36.11 $\pm$ 8.79	6.91 $\pm$ 1.82	1.25 $\pm$ 0.76
CW					
Protein level (%)					
24	0.87 $\pm$ 1.02	29.83 $\pm$ 5.36	25.54 $\pm$ 5.77	4.07 $\pm$ 2.03	0.22 $\pm$ 0.39
30	1.56 $\pm$ 1.07	22.33 $\pm$ 11.38	18.93 $\pm$ 9.71	3.26 $\pm$ 1.75	0.14 $\pm$ 0.17
36	0.76 $\pm$ 0.87	37.50 $\pm$ 7.53	30.73 $\pm$ 4.81	6.77 $\pm$ 3.06	0.00 $\pm$ 0.00
42	1.20 $\pm$ 0.50	30.59 $\pm$ 9.33	25.09 $\pm$ 7.77	5.50 $\pm$ 2.09	0.00 $\pm$ 0.00
Two-way ANOVA					
System	NS	<0.0001	NS	NS	<0.0001
Protein	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS

NS - not significant.

Means followed by different letters in the same column differ according to ANOVA and Tukey's test ($P < 0.05$).

4. Discussion

4.1. Water quality

The benefits of BFT technology include improved performance, welfare, water quality maintenance, and a source of protein (Ogello et al., 2021; Dos Santos et al., 2023, 2024). This system allows for a reduction in CP levels in feed and an increase in productivity (Dos Santos et al., 2023).

The treatments with higher CP levels (30, 36, and 42%) resulted in a lower DO concentration in the water because of the CP in the diet and the accumulation of nitrogenous excreta. The bioflocs contain heterotrophic bacterial biomass, which, along with the oxidation processes of organic matter, consumes DO and reduces its concentration (Manduca et al., 2021). However, the DO levels remained within the acceptable range for fish farming (Boyd, 1982).

The disparity in the pH values between the protein levels can be attributed to the consumption of alkalinity by the chemoautotrophic bacteria and the alkalinity adjustments made with sodium bicarbonate to maintain optimal bacterial activity within the system. Furthermore, the metabolic processes of the animals and microorganisms within the BFT system result in elevated CO_2 levels, which subsequently lead to a reduction in pH (Vinatea et al., 2010; Widanarni et al., 2012). However, these pH values remained within the ideal range for production systems, maintaining homeostasis, animal performance, and the nitrification process (Ebeling et al., 2006; Emerenciano et al., 2017; Figueroa-Espinoza et al., 2022).

Table 7 - Mean $\pm$ standard deviation of the proximal composition of juvenile matrinxã (*Brycon amazonicus*) produced in biofloc (BFT) and clear water (CW) systems and fed diet with different crude protein levels for 60 days (values in % dry basis)

Variable	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)
BFT	90.44 $\pm$ 1.85	54.31 $\pm$ 1.52	22.04 $\pm$ 2.36A	8.37 $\pm$ 0.67B
CW	89.99 $\pm$ 2.18	55.03 $\pm$ 1.70	20.35 $\pm$ 1.69B	8.76 $\pm$ 0.71A
Protein level (%)				
24	89.34 $\pm$ 1.72a	53.94 $\pm$ 1.07	21.18 $\pm$ 2.88	8.64 $\pm$ 0.68
30	91.25 $\pm$ 1.94a	54.35 $\pm$ 1.90	21.27 $\pm$ 1.53	8.39 $\pm$ 0.81
36	89.85 $\pm$ 1.95b	55.50 $\pm$ 1.34	20.90 $\pm$ 2.49	8.78 $\pm$ 0.71
42	90.68 $\pm$ 2.03ab	55.09 $\pm$ 1.77	21.50 $\pm$ 1.95	8.46 $\pm$ 0.66
BFT				
Protein level (%)				
24	88.44 $\pm$ 0.96Ab	54.10 $\pm$ 0.82	22.08 $\pm$ 3.90	8.37 $\pm$ 0.47Aa
30	91.27 $\pm$ 2.09Aa	54.28 $\pm$ 2.53	21.33 $\pm$ 2.13	7.84 $\pm$ 0.39Bb
36	90.07 $\pm$ 1.24Aab	55.18 $\pm$ 1.19	22.85 $\pm$ 1.28	8.76 $\pm$ 0.80Aa
42	91.97 $\pm$ 0.50Aa	54.08 $\pm$ 1.81	22.03 $\pm$ 1.61	8.07 $\pm$ 0.43Aab
CW				
Protein level (%)				
24	90.24 $\pm$ 1.92Aa	53.78 $\pm$ 1.33	20.28 $\pm$ 1.04	8.45 $\pm$ 0.85Aa
30	91.23 $\pm$ 1.97Aa	54.42 $\pm$ 1.25	21.20 $\pm$ 0.79	8.94 $\pm$ 0.74Aa
36	89.09 $\pm$ 2.50Aa	55.82 $\pm$ 1.51	18.95 $\pm$ 1.71	8.80 $\pm$ 0.70Aa
42	89.40 $\pm$ 2.20Ba	56.10 $\pm$ 1.81	20.97 $\pm$ 2.21	8.84 $\pm$ 0.65Ba
Two-way ANOVA				
System	NS	NS	<0.0058	<0.0473
Protein	<0.0389	NS	NS	NS
Interaction	<0.0365	NS	NS	<0.0327

NS - not significant.

Means followed by different letters, uppercase for comparison of production systems and lowercase for protein levels, differ according to ANOVA and Tukey's test ($P < 0.05$).**Table 8** - Mean $\pm$ standard deviation of the proximate composition of the bioflocs collected from the biofloc system used for the production of juvenile matrinxã (*Brycon amazonicus*) fed diet with different crude protein levels for 60 days (values in % dry basis)

Protein level (%)	Crude protein (%)	Ether extract (%)	Ash (%)
24	31.07 $\pm$ 0.76	4.30 $\pm$ 0.52	28.87 $\pm$ 1.33a
30	32.07 $\pm$ 1.78	5.30 $\pm$ 0.46	29.37 $\pm$ 1.05a
36	32.30 $\pm$ 2.17	4.50 $\pm$ 0.56	29.67 $\pm$ 3.70a
42	33.57 $\pm$ 0.38	4.70 $\pm$ 0.62	24.53 $\pm$ 2.16b
One-way ANOVA	NS	NS	<0.0003

NS - not significant.

Means followed by different letters in the same column differ according to ANOVA and Tukey's test ($P < 0.05$).

The use of sodium bicarbonate to stabilize water alkalinity and pH in BFT systems (Emerenciano et al., 2017) resulted in an increase in salinity. The alkalinity concentrations in both the BFT and CW systems were influenced by the CP level in the feed. The decrease in alkalinity values in the CW system may be attributed to the daily water renewal, which causes ion losses, unlike the BFT system (Dos Santos et al., 2023). The observed variation in alkalinity values among the studied systems is likely attributed to their distinct characteristics, as observed by Dos Santos et al. (2023). In their study, which tested different levels of CP in the feed in two production systems (BFT and CW) using tambaqui, the authors found higher alkalinity values in BFT.

Frequent monitoring of the concentration of total ammonia is necessary as it tends to increase with the amount of feed provided, with the accumulation of biomass in the tanks and according to the level of protein in the diet (Leira et al., 2017). Total ammonia results from the decomposition of organic matter, feed, and excreta. The BFT system showed statistically lower levels of ammonia, thus indicating the efficiency of ammonia assimilation by nitrifying and heterotrophic bacteria in the system (Ebeling et al., 2006). Similar results were reported by Dos Santos et al. (2021, 2023).

There were no significant differences in nitrite concentrations between the BFT and CW production systems. This may be attributed to the changes of water and stabilization of nitrifying bacteria. However, higher average values were observed in the BFT system and in diets with higher protein levels, which was likely due to the increased nitrogen intake from the feed. The interaction between the factors reflected these effects, with the BFT system providing a greater amount of nitrate than the CW system. This was due to the presence of chemoautotrophic bacteria, which promoted a greater conversion of nitrogen compounds. The effect was intensified by the greater amount of nitrogen in the feed. The nitrate concentrations in the factors studied (Table 3) support the findings of Mansour and Esteban (2017) and Dos Santos et al. (2023), who reported higher nitrate concentrations in the BFT system compared with the CW system. This was observed in tilapia raised using different carbon sources and protein levels, as well as in tambaqui in two production systems (BFT and CW) and with three CP levels, respectively.

In a BFT system, it is crucial to maintain TSS levels below 500 mg/L and SS volume below 50 mL/L to ensure proper functioning of the system (Hargreaves, 2013). The mean values of the experiment remained within the range used for other species, such as Nile tilapia raised in a biofloc system (Avnimelech, 2009) and juvenile tambaqui (Dos Santos et al., 2023). These species did not experience any mortality or clinical-behavioral alterations. The BFT system used in this study showed that higher protein diets led to an increase in TSS and SS, resulting in greater nitrogen excretion and microbial floc formation (Silva et al., 2018). The reduction of light entering the BFT system had the effect of increasing the turbidity of the water. This may suggest better animal welfare, as lower light intensity has been shown to reduce aggressive behavior in the species (Lopes et al., 2018; Amaral et al., 2020; Cornélio et al., 2021).

4.2. Animal performance, somatic indices, and blood parameters

Several studies have shown that the BFT system can enhance the productive performance of fish. This is because the microorganisms present in the biofloc system become bioavailable as a complementary food source (Dos Santos et al., 2021; Pires et al., 2022; Dos Santos et al., 2023, 2024). The study found that the performance of juvenile matrinxã in the two production systems evaluated was mainly related to the formation of microbial flocs and water quality in the BFT system. This suggests that the fish may be utilizing the flocs and experiencing better welfare, resulting in better performance compared with those reared in the CW system (Dos Santos et al., 2023).

The apparent feed conversion of the juveniles in the BFT system was found to be lower. This may have been due to the animals exhibiting a more optimal use of the food provided, given that the high turbidity of the system reduces aggressive behavior and stress among the animals, which also contributes to a higher survival rate of the species (Amaral et al., 2020; Cornélio et al., 2021; Izel-Silva et al., 2024). This supports the findings of Luo et al. (2014), who observed a decrease in the feed conversion ratio of Nile tilapia from 1.4 in a recirculating aquaculture system to 1.2 in a biofloc system, resulting in a 22% increase in the final weight of the fish. Nevertheless, the outcomes of this study indicate that matrinxã, despite not being filter feeders like the tilapia, may ingest particles from the BFT system. This is evidenced by the higher productivity and survival of the fish in the BFT system than in the CW system. The quantities of bioflocs consumed by the matrinxã are insignificant for their growth or for the reduction of protein in the diet; however, there are potential benefits for the health and welfare of the animals, as indicated by the survival rate and some of the physiological parameters discussed below.

Based on the results for performance, it is evident that a diet containing 24% CP does not meet the nutritional requirements of juvenile matrinxã in either of the systems. This leads to lower feed intake and productivity, as well as higher feed conversion. Mattos et al. (2018) evaluated the effect of different CP levels (30, 35, 40, and 45%) on juvenile matrinxã (approximately 3 g) in a continuous flow system and found no significant difference in the performance of the animals fed the three highest CP levels in the diet. The difference observed in this study may be related to the production system and the composition of the nutrients in the diet. Sgnaulin et al. (2021) evaluated the impact of reducing dietary protein on the production of *Piaractus mesopotamicus* and observed that the fish reared in the BFT system had a lower requirement for CP in their feed due to the utilization of nutrients from the bioflocs. Despite this, the present study found no interaction between the production system and the level of protein in the feed. This suggests that the juvenile matrinxã did not consume enough microbial flocs to increase or compensate for their growth in relation to the diets. Nevertheless, there was a noticeable improvement in feed conversion.

In addition to performance parameters, body indices have been used to assess factors affecting nutrient assimilation and nutritional status in fish (Silva et al., 2018). This study found that the BFT and CW systems had a statistically significant effect on the viscerosomatic and hepatosomatic indices of the juvenile matrinxã. However, the effect of CP levels did not affect the differences in the hepatosomatic index.

The blood parameters of the juvenile matrinxã in the BFT and CW systems did not indicate any effect on the homeostasis of fish. This suggests that the water quality and microbiological action did not induce stress in the animals. These results support the findings for Nile tilapia (*O. niloticus*) and tambaqui (*C. macropomum*) in BFT systems compared with CW systems (Dos Santos et al., 2021, 2024; Figueroa-Espinoza et al., 2022). The reduction in protein levels in the diet did not harm the physiological homeostasis of the fish, which supports the findings of Dos Santos et al. (2024). They also observed no significant effects on the metabolism and/or welfare indicators of juvenile tambaqui when reducing protein levels from 32 to 24% in BFT and CW systems.

Nonetheless, the fish showed a significant reduction in hemoglobin levels when they received the feed with the lowest protein content. This suggests a nutritional insufficiency that may have decreased oxygen demand and, as a result, reduced weight gain. Furthermore, the hematocrit level exhibited an interaction between the production systems and protein content, and the BFT system intensified the effects of protein restriction in the diet, resulting in a lower hematocrit level in fish feed with the lowest protein level.

Total leucocytes and neutrophils showed statistical differences between the juveniles in the BFT and CW systems, with higher averages in the matrinxã reared in the BFT system. As observed by Dos Santos et al. (2024), this difference can be attributed to prolonged contact with the microorganisms present in the BFT system. These authors found not only a higher number of monocytes in tambaqui (*C. macropomum*) raised in BFT, but also an increase in their respiratory activity.

4.3. Nutritional composition of the fish and the bioflocs

The nutritional composition of the feed of aquatic organisms can be a determinant of nutrient assimilation (Silva et al., 2018). The biofloc system can achieve a balance between protein, lipids, and ash in feed (Marinho-Pereira et al., 2020). The production of microorganisms in the system is affected by the type of feed and the CP level of the diets (Pires et al., 2022). This study shows that the nutritional composition of the animals was not affected by the production system. The levels of composition were similar between the BFT and CW systems in terms of crude protein. The results suggest that the CP in the feed can be reduced without compromising the efficiency of bioflocs as a feed supplement in BFT systems (Dos Santos et al., 2023). The results for CP are comparable to those reported by Eid et al. (2021), but lower than the values reported by Figueroa-Espinoza et al. (2022), who evaluated juvenile Nile tilapia in a biofloc system with experimental diets.

The bioavailability of protein, lipids, mineral matter, and microorganisms in microbial flocs within the BFT system can vary depending on the feed, stage of development of the cultivated organisms, and production management in the environment (Pires et al., 2022; Figueroa-Espinoza et al., 2022). The protein levels in the flocs, ranging from 31.0 to 33.5%, fall within the recommended range of 12 to 49% in the literature (Emerenciano et al., 2013a; Dos Santos et al., 2023). Therefore, the protein content in bioflocs can vary depending on the farming system (age and C:N ratio) and the formulation of the added feed (Figueroa-Espinoza et al., 2022).

5. Conclusions

The study found that juvenile matrinxã can be fed a diet containing 30% CP in both the BFT and CW systems. The results demonstrate that microbial flocs do not confer a direct benefit to juvenile matrinxã in terms of their feeding. However, the presence of the bioflocs and the increased turbidity in the water lead to an increase in the survival rate of the juveniles and improves animal welfare. Consequently, it is crucial to monitor the system to ensure that it is functioning optimally and prevents future losses, thereby promoting greater availability of juveniles in the BFT system for production.

Data availability

The entire dataset supporting the results of this study is available upon reasonable request to the corresponding author.

Author contributions

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Conflict of interest

The authors declare no conflict of interest.

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Brycon amazonicus larviculture cannibalism is reduced in biofloc systems

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Keywords:	BFT, larval cannibalism, Amazonian fish, turbidity.
Abstract:	<p>This study evaluated matrixã Brycon amazonicus larviculture performance at different total suspended solid (TSS) concentrations in a biofloc technology (BFT) system, for 5 and 10 days. Larvae 24 hours after hatching were used at a stocking density of 10 larvae L⁻¹ for 10 days in four treatments: clear water (CW – control, 100% water renewal per day and no bioflocs), and three TSS concentrations in BFT systems: Low BFT (BFTL) - up to 200 mg L⁻¹, Medium BFT (BFTM) - 200 to 350 mg L⁻¹ and High BFT (BFTH) - 350 to 500 mg L⁻¹. The matrixã larvae were fed artemia nauplii until the 5th day and artemia nauplii plus mash feed containing 55% crude protein from the 6th to the 10 th day. Water quality, performance, morphology, and type II cannibalism (complete larvae ingestion) (n= 30) were evaluated after 5 and 10 days. Water quality, although altered, remained within the appropriate matrixã limits in all treatments, with about a 90% water consumption decrease observed in the BFT systems. The BFT treatments resulted in larval survival increases of over 170% compared to the CW group, at 5 (p<0.05) and 10 (p<0.05) days of rearing, with CW = 31.4 and 17.9%; BFTL = 81.5 and 39.4%; BFTM = 88.9 and 59.9% and BFTH = 87 and 53.5% respectively. Larvae growth (p<0.05) and morphological development (p<0.05) at 5 days were lower in the BFTH treatment, due to the presence of more developed cannibalistic larvae compared to the other treatments, evidencing greater predation capacity. Cannibalism was observed after 5 and 10 days in all treatments, except for the BFTH group at 5 days and BFTM group at 10 days, proving the influence of high TSS levels and, consequently, turbidity, in reducing this behavior. The findings reported herein indicate matrixã larviculture BFT system efficiency and suggest the use of TSS concentrations between 200 and 350 mg L⁻¹ as an alternative to reduce cannibal behavior and increase matrixã productivity, in addition to maintaining good quality and reducing water use.</p>

Highlights

- Biofloc technology (BFT) increases matrinxã larvae survival
- Higher total suspended solids (TSS) reduce type II cannibalism
- BFT maintains water quality and reduces water use in fish hatcheries
- Matrinxã larvae perform better between 200 and 350 mg L⁻¹ TSS
- Larval survival decreased 40% between 5 and 10 days of rearing

***Brycon amazonicus* larviculture cannibalism is reduced in biofloc systems**

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Abstract

This study evaluated matrinxã *Brycon amazonicus* larviculture performance at different total suspended solid (TSS) concentrations in a biofloc technology (BFT) system, for 5 and 10 days. Larvae 24 hours after hatching were used at a stocking density of 10 larvae

L⁻¹ for 10 days in four treatments: clear water (CW – control, 100% water renewal per day and no bioflocs), and three TSS concentrations in BFT systems: Low BFT (BFTL) - up to 200 mg L⁻¹, Medium BFT (BFTM) - 200 to 350 mg L⁻¹ and High BFT (BFTH) - 350 to 500 mg L⁻¹. The matrinxã larvae were fed artemia nauplii until the 5th day and artemia nauplii plus mash feed containing 55% crude protein from the 6th to the 10th day. Water quality, performance, morphology, and type II cannibalism (complete larvae ingestion) (n= 30) were evaluated after 5 and 10 days. Water quality, although altered, remained within the appropriate matrinxã limits in all treatments, with about a 90% water consumption decrease observed in the BFT systems. The BFT treatments resulted in larval survival increases of over 170% compared to the CW group, at 5 (p<0.05) and 10 (p<0.05) days of rearing, with CW = 31.4 and 17.9%; BFTL = 81.5 and 39.4%; BFTM = 88.9 and 59.9% and BFTH = 87 and 53.5% respectively. Larvae growth (p<0.05) and morphological development (p<0.05) at 5 days were lower in the BFTH treatment, due to the presence of more developed cannibalistic larvae compared to the other treatments, evidencing greater predation capacity. Cannibalism was observed after 5 and 10 days in all treatments, except for the BFTH group at 5 days and BFTM group at 10 days, proving the influence of high TSS levels and, consequently, turbidity, in reducing this behavior. The findings reported herein indicate matrinxã larviculture BFT system efficiency and suggest the use of TSS concentrations between 200 and 350 mg L⁻¹ as an alternative to reduce cannibal behavior and increase matrinxã productivity, in addition to maintaining good quality and reducing water use.

Keywords: BFT, larval cannibalism, Amazonian fish, turbidity.

1. Introduction

Cannibalism in fish occurs in more than 30 families, and is associated with drastic survival declines, reflecting in substantial economic losses for the productive sector (Baras and Jobling, 2002; Naumowicz et al., 2017). The Bryconidae family is an example of this, in which several species belonging to the *Brycon* genus, such as *B. moorei* (Baras et al., 2005), *B. sinuensis* (Jiménez-Velásquez et al. 2021), *B. vonoy* (Coraspe-Amaral et al., 2017) and *B. amazonicus* (Cornélio et al., 2021), all of commercial interest, present this behavior.

Cannibalism in *Brycon amazonicus*, popularly known as matrinxã, is influenced by environmental characteristics, such as light intensity, which modulates behavioral interactions and aggressiveness in this species (Lopes et al., 2018; Muller et al., 2019) and high water turbidity, which limits light penetration and contributes to the increased survival of this species (Cornélio et al 2021). This has also been observed for *Sander vitreous* (Perciformes) larvae, in which increased turbidity is an essential environmental requirement for the intensive production of this species (Bristow et al., 1996; Clayton et al., 2009). This trait has been observed in biofloc (BFT) technology systems, especially when associated with high total suspended solids (TSS) levels, affecting both water turbidity and the number of microorganisms present in the medium (Ray et al., 2010; Poli et al., 2015).

The BFT system gathers bacteria, organism remains and debris, among others, forming a microbial community highly efficient in nutrient cycling and reuse, which maintains water quality, also serving as a source of supplementary food for the reared organisms (Wasielesky et al., 2006; Wang et al., 2015; Besen et al., 2021). In addition, nutritional biofloc characteristics improve fish resistance and immunity (Ekasari et al.,

2015; Dos Santos et al., 2021; 2022). The use of this technology in larviculture has been applied to several fish species, such as tilapia *Oreochromis niloticus* (Ekasari et al., 2015; Li et al., 2018), African catfish *Clarias gariepinus* (Ekasari, et al., 2016), catfish *Rhamdia quelen* (Poli et al., 2015), goldfish *Carassius auratus* (Besen et al., 2021) and pirapitinga *Piaractus orinoquensis* (Lasso, et al., 2022).

Up to 90% larval mortality rates are noted in current matrinxã larviculture systems with continuous water flows (Barros et al., 2019), despite this being an important Brazilian aquaculture species (IBGE, 2022). This species production is compromised by its aggressive behavior and cannibalism, limiting the supply of fingerlings. In this context, the aim of the present study was to define TSS concentrations capable of improving matrinxã larviculture in a BFT system, by assessing water quality, zootechnical performance, cannibalism frequency and larvae morphology.

2. Material and methods

2.1 Study site and Ethics Committee

All experiments were carried out at the National Institute of Amazonian Research (*Instituto Nacional de Pesquisas da Amazônia - INPA*) Aquaculture Experimental Station, in Manaus, AM, Brazil, approved by the Committee on Ethics in the Use of Animals (CEUA) (Prot. 067/2018), SEI 01280.000076/2019-17.

2.2 Experimental protocol

Matrinxã larvae 24 hours after hatching (HAH) (1.16 ± 0.29 mg and 5.8 ± 0.3 mm), devoid of pigments, with endogenous energy reserves (yolk phase) and limited swimming

capacity were employed in the experiments. Larvae were quantified by volumetric estimation and randomly distributed at density of 10 larvae L⁻¹ in 12 70 L experimental units (EU) under a controlled 12/12 h photoperiod and continuous aeration for 10 days.

Four treatments were carried out: control - Clear Water (CW), with 100% daily water renewal (without bioflocs), and three total suspended solid (TSS) concentrations in the biofloc systems, as follows: Low BFT (BFTL) = up to 200 mg L⁻¹; Medium BFT (BFTM) = 200 to 350 mg L⁻¹ and High BFT (BFTH) = 350 to 500 mg L⁻¹, comprising three replicates each, using mature inoculum from three intensive tambaqui (*Colossoma macropomum*) rearing tanks without water changes and with controlled nitrogenous compound levels due to the presence of bioflocs. Sodium bicarbonate (NaHCO₃) as an alkalizer and common salt without iodine were added to correct alkalinity, pH and salinity values.

The larvae of all treatments until the 5th day were fed 50 artemia nauplii larvae⁻¹ meal¹, every 4 hours. From the 6th to the 10th day, they were fed both artemia nauplii and mash feed containing 55% crude protein (CP) four times a day, 300 mg EU⁻¹, three times a day.

2.3 Water quality monitoring

Temperature, dissolved oxygen (DO), electrical conductivity, salinity and pH were evaluated daily employing a digital multiparameter YSI Professional Plus probe (Yellow Spring Instruments®). Every two days, the following parameters were analyzed: total ammonia (Verdouw et al., 1978), nitrite and total alkalinity (Boyd and Tucker, 1992), suspended solids (SS) using Imhoff cones (Avnimelech, 2007), turbidity using a digital Hanna turbidimeter and TSS by the gravimetric method (Strickland and Parsons, 1972). Every five days, nitrate concentrations were determined by spectrophotometry (García-Robledo et al., 2014) and total hardness, by titration (Boyd and Tucker, 1992).

Organic fertilization was performed by adding sugar (42% C) to the BFT treatments when total ammonia concentrations were $\geq 1 \text{ mg L}^{-1}$. Regarding fertilization, a 6:1 C:N ratio was applied based on Ebeling et al. (2006). The limit of 0.86 mg L^{-1} nitrite was established as the maximum acceptable concentration per BFT EU (Avilez et al., 2004). When nitrite concentrations exceeded this value, partial water changes (5%) were performed. Total alkalinity maintenance $> 100 \text{ mg L}^{-1}$ was performed using NaHCO_3 (Furtado et al., 2011) and TSS levels were maintained by clarification to preserve predetermined treatment levels, by removing 25% of EU volumes (17.5 L), 1 h in a static system (sedimentation) and return of the same water without the precipitated material (Martins et al., 2017).

2.4 Zootechnical larvae performance

After 5 and 10 days, 10 larvae per EU (n=30) were evaluated concerning zootechnical performance, measuring their wet weight (mg) on an analytical balance and total length (mm) using an optical Zeiss Discovery V12 stereomicroscope. Specific growth rates (%/day) were calculated for each EU as $\text{SGR} = 100 \times [(\ln \text{ final value} - \ln \text{ initial value})]/\text{cycle in days}$. Survival was estimated by volumetric method, by sampling three 600 mL water aliquots under intense aeration from each EU (2.5% of the total volume), performing total larvae counts in that volume. Productivity was calculated based on the survival means multiplied by the initial stocking larvae density.

2.5 Larvae morphology

Following larvae euthanasia (n=30) with an alcoholic 0.5% eugenol solution (Souza et al., 2014), macroscopic evaluations were performed under an optical Zeiss Discovery V12 stereomicroscope using a computerized imaging system available in the

Zen software. These evaluations were carried out to identify type II cannibalism (totally ingested prey) (Baras and Jobling 2002; Ribeiro and Portella, 2021), while a morphological analysis was carried out to characterize larvae development levels and population aspects employing the notochord and swim bladder inflation as indicators (Kendall et al., 1983; Bristow and Summerfelt, 1994; Lima et al., 2021).

2.6 Statistical analyses

To compare the effects of the different treatments on the evaluated variables, data normality and homoscedasticity at a 95% significance level ($p > 0.05$) were determined using the Shapiro-Wilk and Bartlett tests, respectively. An analysis of variance (ANOVA One-way) was performed ($P < 0.05$), followed by the Tukey test when differences were detected ($P < 0.05$). Student's t test was performed to compare larvae survivals after 5 and 10 days of rearing per EU ($P < 0.05$).

3. Results

3.1 Water quality

Among the evaluated variables, turbidity, TSS and SS were significantly different ($p < 0.05$) among all treatments, lower in the control treatment (CW), followed by the BF_{TL}, BF_{TM} and BF_{TH} groups, respectively. Furthermore, total hardness, nitrate, electrical conductivity, and salinity differed between all treatments ($p < 0.05$). No significant differences were observed for temperature and DO, although pH and alkalinity were significantly lower in the BF_{TH} group ($p < 0.05$) compared to the other treatments. With regard to nitrogenous compounds in the CW and BF_{TL} treatments presented the

highest total ammonia concentrations, while nitrite levels were lower in the CW group (Table 1).

Changes comprising 25% of the total water volume plus a 25% clarification were performed in the BFT treatments on the 8th day of the experiment, due to high nitrite ($4.04 \pm 0.66 \text{ mg L}^{-1}$) and TSS concentrations in the BFTL (322.5 mg L^{-1}), BFTM (428.7 mg L^{-1}) and BFTH (612.5 mg L^{-1}) groups. Concerning the CW group, 100% daily renewals were performed to maintain water quality, although this represents an almost 9-fold higher water use than in the BFT treatments considering the initial supply (Table 1).

3.2 Zootechnical larvae performance

Survival of larvae reared in the BFT systems ($85.8 \pm 3.8\%$ and $50.9 \pm 10.5\%$) was significantly higher than in the control group ($31.4 \pm 6.4\%$ and $17.9 \pm 8.2\%$) at 5 and 10 days respectively, with a 173% increase on the 5th day and 185% increase on the 10th day when comparing the means of BFT treatment with the CW group. However, the BFTH group presented the lowest values in relation to final weight and SGR. Comparing survival at 5 and 10 days, a significant decrease was observed in all experimental units, with no difference between treatments and a mean reduction of $41.3 \pm 8.3\%$ (Figure 1).

3.3 Larvae morphology and cannibalism

At the end of the 5th day, the BFTL and BFTM groups presented more flexed larvae than the other treatments ($p < 0.05$). On the 10th day, despite no significant differences, only the BFTL and CW groups contained post-flexion larvae. The swim bladder presented significant differences only on the 10th day, with more larvae with an empty bladder in the BFTH treatment compared to the CW group and the occurrence of larvae with a full bladder noted on the 5th day only in the CW group. The presence of type

II cannibalism on the 5th and 10th days was observed in all treatments except for BFTH larvae at 5 days and BFTM larvae at 10 days (Table 2). Figure 3 depicts the size and morphology of matrinxã larvae in each treatment, indicating larvae presenting and not presenting type II cannibalism (A, B, C, E, F and H).

4. Discussion

4.1 Water quality

Total suspended solids are mainly associated with the presence of the microbial community developed in the BFT system, used both as a food source and in the processes of nitrogenous compound transformation in water (Avnimelech, 1999; Besen et al., 2021). In the present study, the different TSS concentrations in each treatment resulted in specific system features. A positive relationship between TSS and turbidity has also been observed by Ray et al. (2010) and Poli et al. (2015) when evaluating different TSS concentrations in BFT systems, demonstrating a direct relationship between biofloc concentrations and light water penetration.

Excess TSS increases oxygen consumption and may result in negative effects on animals reared in BFT systems (Hargreaves, 2006; Ray et al., 2010; Avnimelech, 2015). However, TSS concentrations of up to 500 mg L⁻¹ in the BFT treatments investigated herein did not negatively influence matrinxã larvae survival, with no effect on water oxygen concentrations. These results corroborate other assessments conducted on fish larvae displaying high tolerance to TSS concentrations in BFT systems, such as *Rhamdia quelen*, of up to 1000 mg L⁻¹ TSS (Poli et al., 2015) and *Piaractus orinoquensis* larvae above 1000 mg L⁻¹ (Lasso et al., 2022).

Temperature and DO remained within the limits considered adequate for matrinxã (24 to 28°C and > 5 mg L⁻¹) (Barros et al., 2019). The highest alkalinity consumption was determined in the BFTH system, resulting in lower pH values, despite the management adopted in the correction of this variable having been the same for all treatments. This suggests that BFT systems, with higher TSS concentrations, present higher maintenance costs due to nutrient recycling in different trophic levels and alkalinity consumption by bacteria present in the medium (Avnimelech, 2007; Ray et al., 2010; Hargreaves, 2013). The maintenance of high alkalinity values is important for the buffer system balance, which minimizes pH variations, in addition to enabling bacterial nitrogenous compound metabolism (Ebeling et al., 2006).

Nitrogenous compounds were maintained within the comfort limit for matrinxã (< 0.86 mg L⁻¹ NO₂ and <0.88 mg L⁻¹ N-TA) (Avilez et al., 2004; Souza-Bastos et al., 2017), except on the 8th day in the BFT group (NO₂ > 0.86 mg L⁻¹), due to the water renewal and clarification management carried out during the experiment, in addition to TSS level maintenance, thus increasing water use efficiency (Ebeling et al., 2006 , Ray et al., 2010; Avnimelech 1999, 2015; Zemor et al., 2019).

The high water consumption in commercial larviculture laboratories when employing native Amazon fish, due to continuous water exchanges (Honczaryk and Inoue, 2009; Woynárovich and Van Anrooy, 2019), restricts this practice to areas with adequate and abundant water sources or with efficient water treatment system conditions. However, the BFT system allowed larvae rearing for 10 days with only 25% water renewal. Minimal water exchange during the production cycle due to the presence of biofloc bacteria, is therefore, advantageous, leading to lower water use compared to conventional aquaculture production systems (Hargreaves, 2006; Dos Santos et al., 2021; 2022)

Despite treatment differences concerning salinity, and consequently conductivity, values were close to those recommended for matrinxã at this stage (2 g L⁻¹) (Jomori et al., 2013). The difference between total hardness the values among all treatments took place since the beginning of the experiments, due to the employed inoculum.

4.2 Larval performance

The highest matrinxã larvae survival observed in the BFT systems at 5 and 10 days of rearing may be associated with decreased light intensity due to high turbidity, which reduces aggressiveness and cannibalism (Lopes et al., 2018; Muller, et al., 2019; Cornelius, et al., 2021). This effect is attributed to increased melatonin production at low light levels, which affects the dopaminergic and serotonergic systems of these animals, promoting an anti-stress effect and reduced locomotor activity (López-Patiño et al., 2013; Amaral et al., 2020).

The survival of matrinxã larvae under laboratory conditions ranges from 8.5 to 79%, depending on the type of live food (Atencio-Garcia et al., 2003), water salt concentration (Jomori et al., 2013), temperature and storage density (Barros et al., 2019), light intensity (Muller, et al., 2019) and water turbidity (Cornélio et al., 2021). Similar values were observed for matrinxã larvae in the CW group in the present study, although lower than those noted for the BFT group in the same period (5 days). Thus, biofloc technology, in addition to maintaining water quality, seems to be efficient for matrinxã larvae survival. These results corroborate other BFT larviculture studies carried out for *O. niloticus* (Ekasari et al., 2015), *Clarias gariepinus* (Ekasari, et al., 2016), *Rhamdia quelen* (Poli et al., 2015) and *Carassius auratus* (Besen et al., 2021).

The larvae rearing period under super-intensive conditions in reproduction laboratories is important, as it influences productivity and operational costs (Jomori et al.,

2003; Baras et al., 2005; Mai and Zaniboni-Filho, 2005; Muller et al., 2019). The ideal is a minimum period for the larvae to become apt for nursery conditions, for example in the production of *Salminus brasiliensis*, from 4 to 6 days (Mai and Zaniboni-Filho, 2005) and *Piaractus mesopotamicus*, from 6 to 9 days (Jomori et al., 2003). The *B. amazonicus* larvae performance in the present study, with a significant survival decrease between 5 and 10 days of rearing in all treatments, suggests that the rearing time for this species under laboratory conditions is up to 5 days.

High TSS concentrations in the BFTH system reduced matrinxã larvae growth (Figure 1). It is possible that the obtained high survival rate and, consequently, higher stocking density, influenced food:larvae ratio decreases compared to larvae reared in the CW system. This corroborates previous assessments reporting reduced fish larvae growth with increased stocking density (Gomes et al., 2000; Kupren et al., 2011; Besen et al., 2021), as well as the effect of lesser light conditions caused by increased water turbidity, decreasing matrinxã aggressiveness and predation (Lopes et al., 2018; Muller et al., 2019). These results corroborate Poli et al. (2015) who observed a similar result for *R. quelen* larvae when evaluating different TSS concentrations in a BFT system, with lower average weights in treatments with higher TSS concentrations (400-1000 mg L⁻¹), and higher survival compared to the CW group.

The average final weight of *B. amazonicus* larvae under super-intensive conditions described in the literature is similar to that obtained in the present study and those reported by Barros et al. (2019) (1.7 - 2.7 mg), and lower than those indicated by Jomori et al. (2013) (7 – 15 mg). However, larvae fed for 24 h and transferred to nurseries (final weight ± 2 mg), represented over a 400% increase in juvenile survival compared to those transferred without this management (Atencio-Garcia et al., 2003). Furthermore,

when the aim is to produce fingerlings, survival is more important, mainly because this product is sold per unit (Gomes et al., 2000; IBGE, 2022).

4.3 Larvae morphology and cannibalism

Larval development is directly associated to their nutritional condition, as any morphological change will depend on nutrient and energy availability (Pelster, 2011; Neumann et al., 2020). An important larval formation event in fish comprises notochord flexion, as this is directly linked to the development of the swimming system, determining predation and escape behaviors, and essential in defining the larval stages (Kendall et al., 1983; Lima et al., 2021). The notochord flexion period takes place from 60 to 83 HAH (Neumann, 2008; Lima et al., 2021). However, in the present study, larvae at this stage were noted up to 244 HAH (10 days), indicating delayed development, mainly in the BFTH system. The high turbidity and, consequently, lower light intensity in this treatment reduced larval activity, as well as predation, maintaining less developed larvae longer within the system. 

The time to form structures and the size of the fish in each stage are associated to environmental factors, such as food supply and temperature, but it is mainly determined by genetics (Kendall et al., 1983; Jomori et al., 2013). In the present study, as the larvae had the same genetics, food management and temperature, it seems that the amount of ingested food during different development stages in the BFT group at different TSS concentrations was the main influencing factor, mainly due to decreased activity/aggressiveness in low-light environments (Lopes et al., 2018; Muller et al., 2019), as well as a higher storage density in relation to the CW group.

A similar behavior was also noted in relation to the swim bladders formation, which maintains animal buoyancy, thus reducing swimming energy expenditure (Pelster,

2011). In matrinxã, which is a physostomic fish species, this organ is permanently in contact with the feeding canal (esophagus), which allows it to inflate when emerging (Pelster, 2011; Neumann et al., 2020). The filling of the swim bladder in this species takes place at 48 to 63 HAH, according to Neumann, (2008) and Lima et al. (2021). However, in the present study, matrinxã larvae without inflated swim bladders were observed in all treatments up to 10 days after rearing (244 HAH), more frequent in the BFTH system compared to the CW group. The presence of larvae with a full swim bladder observed only in the 5-day CW group is due to the presence of more developed cannibals (Figure 2). Despite this morphological development being slower than those described for matrinxã by Neumann (2008) and Lima et al. (2021), this did not compromise the BFT treatment results, mainly by maintaining the batch more homogeneous, resulting in greater survival and productivity.

5. Conclusion

The BFT system improves the survival and zootechnical performance of the matrinxã larvae and reduces the presence of type II cannibalism, suggesting the use of TSS between 200 and 350 mg L⁻¹. Due to the significant decrease in survival between rearing times (5 and 10 days), a period of 5 days under the proposed conditions should not be exceeded without removing more developed cannibal larvae, requiring further studies concerning size selection, feeding rates and stocking density in this system. In addition, the BFT system allows for more efficient water use, maintaining adequate quality for the species at this development stage.

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7. Tables

Table 1. Physico-chemical *Brycon amazonicus* larviculture water quality variables in clear water (CW) and biofloc systems (BFT) under different total suspended solids concentrations for 10 days.

Variables	Treatments			
	CW	BFTL	BFTM	BFTH
Temperature (C°)	26.94 ± 0.02	26.87 ± 0.03	26.90 ± 0.07	26.88 ± 0.04
DO (mg L ⁻¹)	5.58 ± 0.04	5.60 ± 0.11	5.53 ± 0.16	5.64 ± 0.06
EC (µs cm ⁻¹)	2659 ± 5.84 ^a	2867 ± 43.8 ^b	3294 ± 18.6 ^c	3087 ± 16.1 ^d
Salinity	1.34 ± 0.0 ^a	1.47 ± 0.01 ^b	1.67 ± 0.01 ^c	1.56 ± 0.01 ^d
pH (-log ₁₀ [H ⁺])	8.05 ± 0.04 ^a	8.02 ± 0.02 ^a	8.03 ± 0.01 ^a	7.57 ± 0.04 ^b
TA (CaCO ₃ mg L ⁻¹)	115.2 ± 8.08 ^a	114.8 ± 6.19 ^a	123 ± 4.02 ^a	70.8 ± 4.41 ^b
Hardness (CaCO ₃ mg L ⁻¹)	21.78 ± 1.02 ^a	42.22 ± 1.17 ^b	67.2 ± 1.64 ^c	119.1 ± 7.04 ^d
Ammonium (N-AT mg L ⁻¹)	1.10 ± 0.22 ^a	0.80 ± 0.24 ^{ab}	0.27 ± 0.07 ^c	0.45 ± 0.01 ^{bc}
Nitrite (N-NO ₂ mg L ⁻¹)	0.04 ± 0.01 ^a	0.81 ± 0.07 ^b	0.69 ± 0.11 ^b	0.98 ± 0.17 ^b
Nitrate (N-NO ₃ mg L ⁻¹)	3.13 ± 0.21 ^a	25.00 ± 3.44 ^b	56.12 ± 2.92 ^c	109.6 ± 3.81 ^d
SS (mL L ⁻¹)	0.09 ± 0.05 ^a	6.85 ± 1.45 ^b	13.22 ± 0.40 ^c	16.06 ± 1.42 ^d
Turbidity (NTU)	9.05 ± 2.80 ^a	40.99 ± 3.53 ^b	88.63 ± 8.65 ^c	119.9 ± 3.19 ^d
TSS (mg L ⁻¹)	7.67 ± 4.51 ^a	150.3 ± 21.1 ^b	301.5 ± 37.2 ^c	401.7 ± 3.51 ^d
Water renewal (%)	1000 ± 0 ^a	25 ± 0 ^b	25 ± 0 ^b	25 ± 0 ^b
Water use (L UE ⁻¹)	770 ± 0 ^a	87.5 ± 0 ^b	87.5 ± 0 ^b	87.5 ± 0 ^b

Low BFT (BFTL) up to 200 mg L⁻¹; Medium BFT (BFTM) from 200 to 350 mg L⁻¹ and high BFT (BFTH) from 350 to 500 mg L⁻¹. DO= dissolved oxygen, EC= electrical conductivity, TA= total alkalinity, SS= suspended solids, TSS = total suspended solids. Different letters indicate statistical difference by Tukey's test (p<0.05).

Table 2. Frequency (%) of *Brycon amazonicus* larvae (n=30) per ontogenetic development event, and presence of type II cannibalism on the 5th and 10th day of rearing, in clear water (CW) and biofloc systems with different total suspended solid (TSS) concentrations.

		5 days (144 HPH)				10 days (264 HPH)			
Parameters		CW	BFTL	BFTM	BFTH	CW	BFTL	BFTM	BFTH
Notochord flexion	Pre-flexion	83±15 ^a	43±25 ^b	63±6 ^{ab}	90±0 ^a	0±0	7±12	17±29	27±24
	Flexion	17±15 ^a	57±25 ^b	37±6 ^{ab}	10±0 ^a	80±20	83±15	90±17	73±24
	Post-flexion	0±0	0±0	0±0	0±0	20±20	10±10	0±0	0±0
Swim bladder	Empty	20±10	30±52	63±15	83±21	3±6 ^a	7±12 ^{ab}	17±21 ^{ab}	56±26 ^b
	Inflating	63±6	70±52	37±15	17±21	33±49	43±25	77±6	42±24
	Full	17±15	0±0	0±0	0±0	63±55	50±36	13±15	3±5
Type II cannibalism		**	**	*	-	***	**	-	*

HPH= hours post-hatching; BFTL-BFT up to 200 mg L⁻¹; BFTM-BFT from 200 to 350 mg L⁻¹ and BFTH - BFT from 350 to 500 mg L⁻¹ TSS. Different letters indicate statistical differences between treatments according to ANOVA and Tukey's test (p<0.05). (*) presence in 1/3 of the experimental units (EU), (**) presence in 2/3 of the EU, (***) presence in 3/3 of the EU.

8. Figures

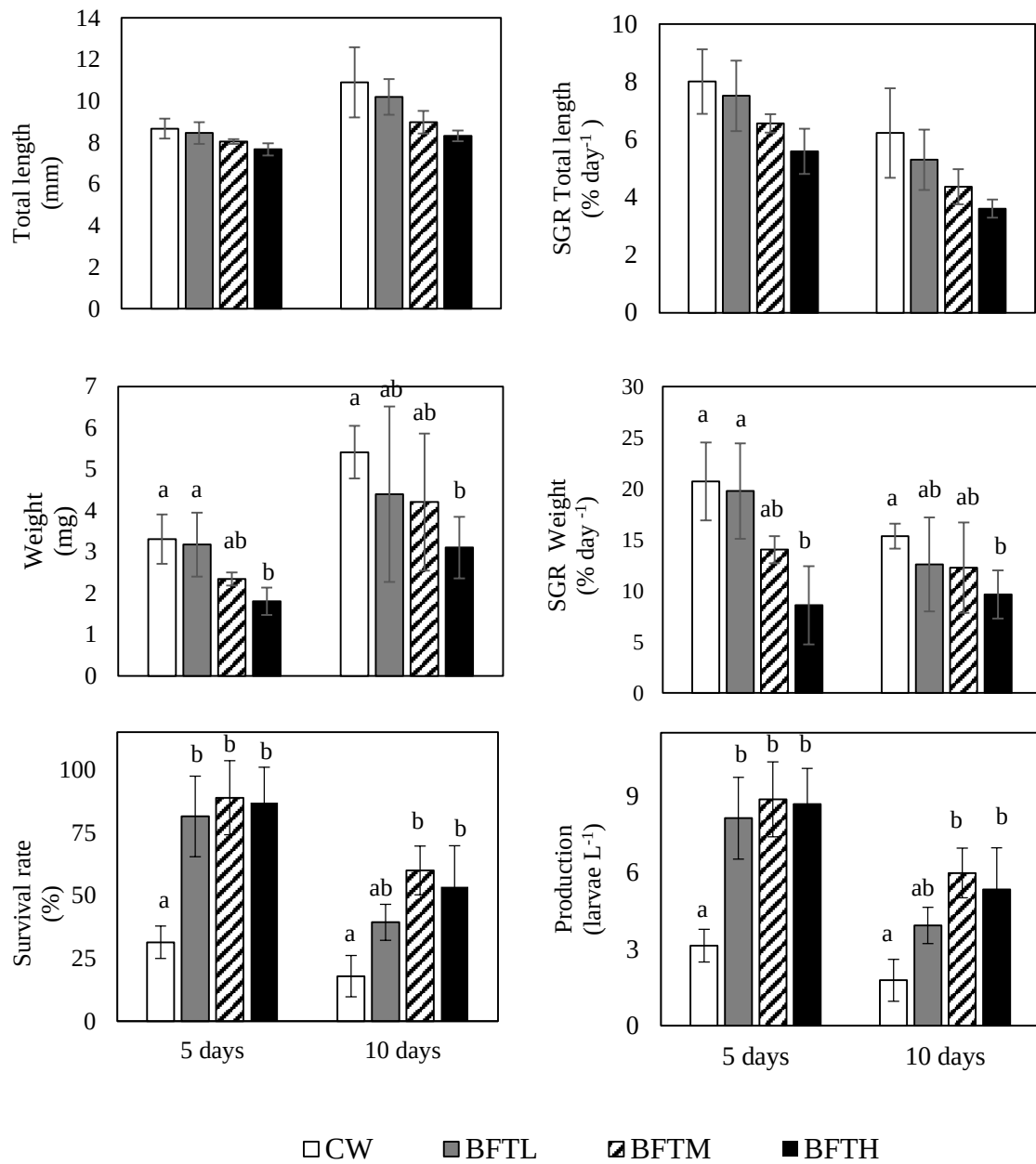
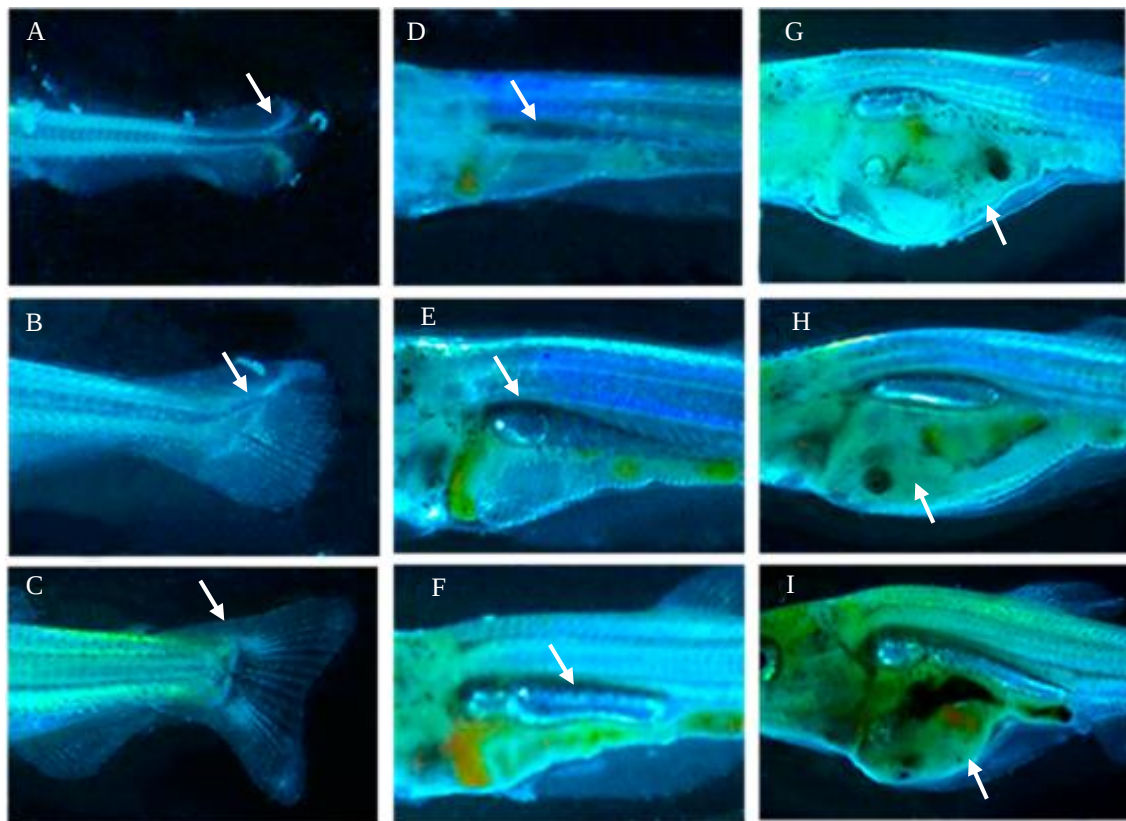


Figure 1. Zootechnical performance of *Brycon amazonicus* larvae reared in clear water (CW) and biofloc systems: low BFT (BFTL) up to 200 mg L⁻¹; Medium BFT (BFTM) from 200 to 350 mg L⁻¹ and high BFT (BFTH) from 350 to 500 mg L⁻¹. SGR= specific growth rate. Different letters indicate statistical differences between treatments by Tukey's test (p<0.05).



591

592 Figure 2. Ontogeny of *Brycon amazonicus* larvae as development indicators. Notochord
 593 flexion: pre-flexion (A), flexion (B) and post-flexion (C). Swim bladder inflation: empty
 594 (D), inflating (E) and full (F). Presence of type II cannibalism (G, H and I).

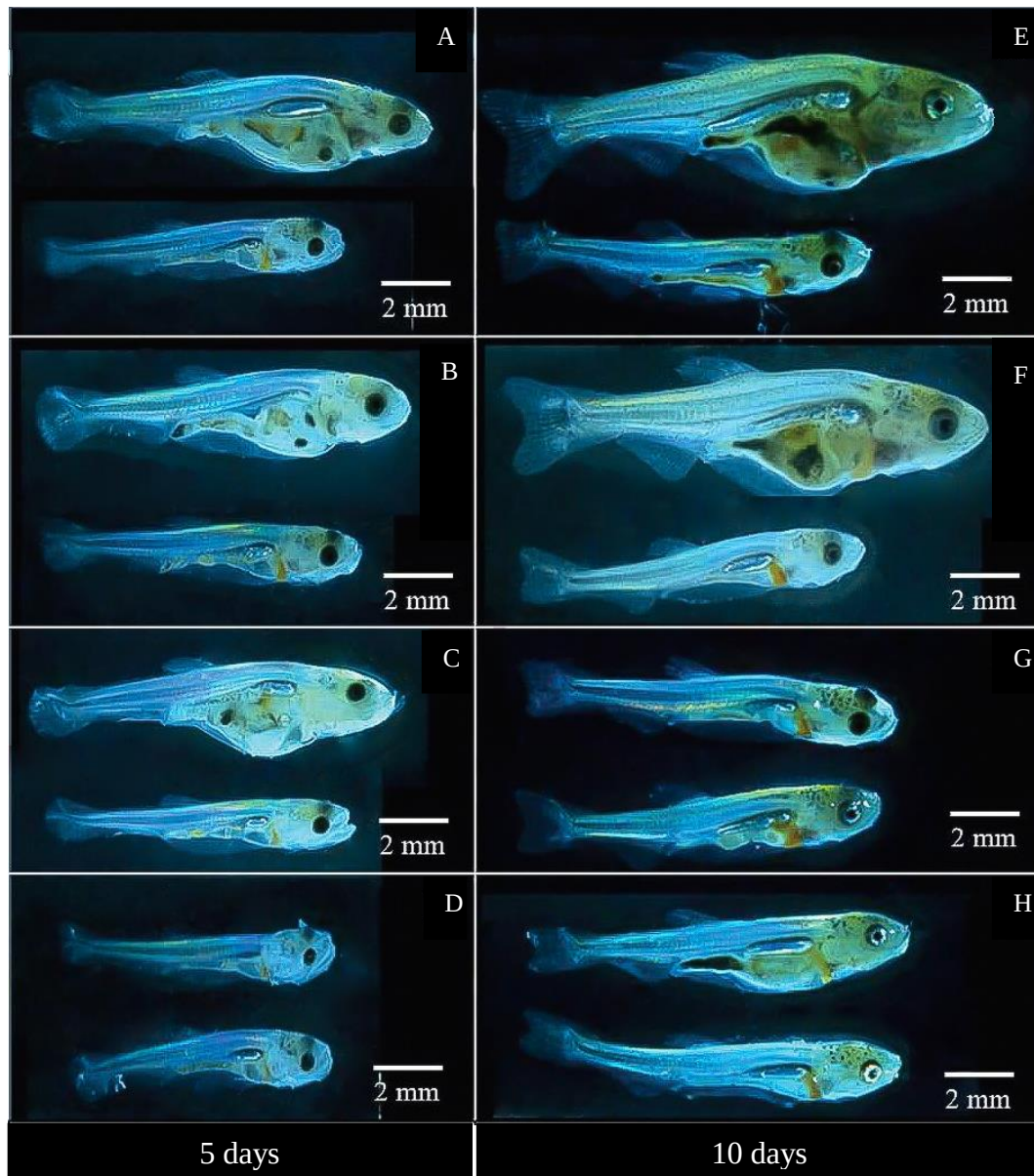


Figure 3. *Brycon amazonicus* larvae reared in a clear water system (A and E) and under different total suspended solid (TSS) concentrations in bioflocs systems: up to 200 mg L⁻¹ (BFTL) (B and F), 200 to 350 mg L⁻¹ (BFTM) (C and G) and 350 to 500 mg L⁻¹ (BFTH) (D and H) at 5 and 10 days of rearing, and 144 and 264 hours post-hatching.

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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