Received: 28 March 2020 | Revised: 13 May 2020 | Accepted: 22 May 2020

DOI: 10.1111/are.14734

ORIGINAL ARTICLE

Aquaculture Research WILEY

Applying biofloc technology in the culture of juvenile of *Piaractus brachypomus* (Cuvier, 1818): Effects on zootechnical performance and water quality

Leydy Y. Sandoval-Vargas[1,2] | Martha N. Jiménez-Amaya[1] | José Rodríguez-Pulido[1] | Diana N. Guaje-Ramírez[1] | Juan A. Ramírez-Merlano[1,3] | Víctor M. Medina-Robles[1]

[1]Grupo de Investigación sobre Reproducción y Toxicología de Organismos Acuáticos – GRITOX, Facultad de Ciencias Agropecuarias y Recursos Naturales, Instituto de Acuicultura de los Llanos, Universidad de los Llanos, Villavicencio, Colombia

[2]Doctorate in Agricultural Sciences, Faculty of Natural Resources, Temuco Catholic University, Temuco, Chile, Temuco, Chile

[3]Programa de pós graduação em Aquicultura, Laboratório de Genética e Biologia Molecular Comparada, Universidade Nilton Lins, Manaus, Brazil

Correspondence
Víctor M. Medina-Robles, Instituto de Acuicultura de los Llanos, Universidad de los Llanos, Kilómetro 12 Vía Puerto López, Villavicencio, Meta, Colombia.
Email: vmmedinarobles@unillanos.edu.co

Funding information
This study is part of the project with code FCARN-13-2014 and was funded by the General Directorate of Research at Universidad de los Llanos. Finally, we thank to *Capital Semilla 2017*, Faculty of Agricultural Science and Natural Resources at Universidad de los Llanos for the financial support for the translation of the manuscript.

Abstract

The aim of this study was to assess the zootechnical performance and water quality of a cachama blanca (*Piaractus brachypomus*) culture using biofloc technology (BFT) versus a system with daily water exchange (DWE). To do this, 180 juveniles (mean initial weight: 5.40 ± 0.19 g) were distributed in 12 circular plastic tanks with stocking densities of 20 or 40 individuals m^3; then, they were cultured for 91 days. BFT treatments kept a C:N ratio approximately of 15:1. Temperature, pH and oxygen were monitored daily, while the other variables were measured weekly. Most productive variables were significantly influenced by both culture system and stocking density with significantly higher values of daily weight gain, total weight gain and total length for fish kept in DWE 20. However, only minor differences were observed within the BFT system. With the exception of the toxic nitrogen compounds (NH_4^+ and NO_2^-), all the other water quality parameters were within the acceptable ranges for the cultivation of tropical fish. Microorganisms started to settle from the first week. A total of 23 genera were present, the most outstanding of which being seven genera of ciliates and three rotifers, rhizopods and chlorophytes. In conclusion, both systems BFT and DWE are useful for increasing the production of *P. brachypomus* in captivity. Additionally, the BFT system can potentially be applied for growing juveniles of this specie in regions with scarce water resources.

KEYWORDS

aquaculture, biofloc, cachama blanca, intensive systems, microorganism characterization

1 | INTRODUCTION

Cachama blanca, *Piaractus brachypomus*, (Order: Characiformes, Family: Characidae, Sub-family: Serrasalminae) is a freshwater fish native to the Orinoco and Amazon basins. It is currently cultured and marketed for local consumption in more than five countries in South America and in several Asian countries (Valladão, Gallani, & Pilarski, 2018; Abraham, Sarker, Dash, Patra, & Adikesavalu, 2017; Kumar et al., 2018). In the context of Colombian fish farming, *P. brachypomus* is the second most produced fish and the first among the native species (Merino, 2014). Traditionally, the production cycle of *P. brachypomus* is carried out in semi-intensive systems using earth ponds and stocking densities ranging from 1 to 3 fish m^3, thus reaching fish biomasses from 1.5 to 2 kg m^3 after a culture process spanning 6 months. Such process uses a single preparation with commercial feed containing 45 to 24% of crude protein (Merino, Bonilla, & Bages, 2013). Even though this approach has an acceptable production performance, its stability and, consequently, the projections regarding production increases might be affected mostly by factors such as low availability and water quality. Regarding the first factor, Puentes, Escobar, Polo, Gutiérrez, Castaño, Amado, Alonso, Suárez, Ramírez. (2015) stated that, during the past 2 years, there had been water shortages in most of Colombia, a phenomenon that could be strengthened by global climate change. As for water quality, it is the main factor limiting the expansion of aquaculture in general. Furthermore, the Ministry of Environment, Housing and Territorial Development (MAVDT - Ministerio de Ambiente, Vivienda y Desarrollo Territorial, 2010) reported that 45% of the water resources in Colombia were in poor conditions, while only 4% were in a good state. The interior ecosystems on which Colombian continental fisheries and aquaculture depend are being threatened by the increasing pollution and contamination generated by agriculture and mining (Ajiaco-Martínez, Ramírez-Gil, Sánchez-Duerte, Lasso, & Trujillo, 2012).

In this context, the continuity and growth of the Colombian fish farming industry depend on the adoption of new production technologies that are more efficient in the usage of water resources. In fact, biofloc technology (BFT) has already been applied in species with high distribution and production values such as tilapia (Luo, Zhang, Cai, Tan, & Liu, 2017; Manduca et al., 2020; Martins, Tarouco, Rosa, & Robaldo, 2017; Pérez-Fuentes, Hernández-Vergara, Pérez-Rostro, & Fogel, 2016). This tool is considered water resource friendly, as it requires less water when compared with traditional culture systems (Avnimelech, 2015; Emerenciano, Ballester, Cavalli, & Wasielesky, 2012). Biofloc is a relatively new technology based on the management of microbial communities via the recycling and reuse of nutrients encouraged by a high carbon/nitrogen ratio (C/N ratio) (Avnimelech, 2015; Luo et al., 2017). Normally, C/N ratios between 10 and 25 with constant aeration promote the growth of microorganisms which are highly efficient in the degradation of toxic nitrogen compounds generated by feed leftovers and fish excretions. Moreover, these microorganisms are transformed into microbial protein (Avnimelech, 2015), which may be consumed by omnivores and detritivores species (Azim, Verdegem, Singh, Van Dam, & Beveridge, 2003; Faizullah, Rajagopalsamy, Ahilan, & Francis, 2015; Wang et al., 2015).

Besides preventing the formation of toxic metabolites and being a source of additional feed, BFT minimize the proliferation of pathogens and makes it possible to intensify production by increasing stocking density (Ekasari et al., 2015; Magondu, Charo-Karisa, & Verdegem, 2013). Undoubtedly, the latter advantage together with the small amount of water used is the main benefit of BFT.

Despite this fact and the popularity of this tool in some countries where it is used in large commercial production settings mainly with shrimp and tilapia (Emerenciano, Cuzon, Arévalo, & Gaxiola, 2014; Zhang, Luo, Tan, Liu, & Hou, 2016), there are still few studies focusing on other commercial and native species. Likewise, studies at the laboratory level are scarce. In the field of fish farming, tilapia was the first species to be included in the system (Azim & Little, 2008; Crab, Kochva, Verstraete, & Avnimelech, 2009; Martins et al., 2017). In the last decade, some studies have been conducted as well in *P. brachypomus* with variables zootechnical results (Abad, Rincón, & Poleo, 2014; Alzate-Díaz & Pardo-Carrasco, 2016; Brú-Cordero, Pertúz-Buelvas, Ayazo-Genes, Atencio-García, & Pardo-Carrasco, 2017; Chaverra, García, & Pardo, 2017; Poleo, Aranbarrio, Mendoza, & Romero, 2011). Therefore, more studies are necessary in this species. The aim of this study was to assess the zootechnical performance and water quality in a culture of *P. brachypomus* using biofloc technology (BFT) versus a daily water exchange using densities of 20 years 40 fish m^3. In addition, we have examined the main groups of microorganisms associated with the biofloc system.

2 | MATERIALS AND METHODS

2.1 | Location

The study was conducted at the Aquaculture Institute of Universidad de los Llanos in Villavicencio, Colombia. All animal handling procedures complied with the rules and regulations concerning laboratory animals described by the Committee on Care and Use of Laboratory Animal Resources—National Research Council (US), (1996).

2.2 | Preparation of the biofloc system

The experiment was carried out in 12 low circular plastic tanks (Colempaques®) with an effective water volume of 500 L. Aeration was supplied by a 1.5 horsepower blower attached to a PVC® pipe which was connected inside each tank to an aeration hose installed at the bottom forming a circular shape. The system was installed under a roof with a natural photoperiod of 12 hr.

Five days before starting the experiments, all tanks were filled 95% with water from an underground well. Usually, the water obtained from this source is lacking in carbonates and salinity; this was corrected by adding 100 g of dolomite lime, 10 g of sodium bicarbonate ($NaHCO_3$) and 10 g of sodium chloride (NaCl) for each m^3 of water (values were determined in previous tests). During the second day, the volume was completed to 500 L with water from a previously established BFT system. Additionally, each tank was fertilized with 20 g of commercial feed consisting of 34% crude protein and 5 g of refined brown sugar, exito® brand. This was done from the second to fourth days.

2.3 | Experiment conditions

A total of 12 tanks were set up as follows: six had minimal water exchange and used an integrated biofloc production system with stocking densities of 20 and 40 fish m^3 (three tanks for each stocking density). The amount of evaporated water was calculated by measuring the height of the water column (H) every 8 days and replacing that value in the cylinder formula (LR2H). The remaining six tanks contained the same stocking densities (20 and 40 fish m^3) with daily water exchange (DWE) ranging from 5 to 15 for the first stocking density and 10 to 30% for the second density (Table 1). Thus, two biofloc treatments (BFT 20 and BFT 40) and two daily water exchange treatments (DWE 20 and DWE 40) were established, each with three repetitions. Based on the densities described above, 180 fingerlings of *P. brachypomus* obtained through artificial reproduction, with a weight of 5.40 ± 0.19 g and a length of 7.10 ± 0.89 cm were randomly distributed among the experimental units and cultured for 91 days (13 weeks).

2.4 | Feeding plan and carbohydrate addition

During the whole experiment, the fish received a commercial feed with 34% CP, supplied in two daily rations (at 8:00 and 15:30 hr). The amount of feed provided was calculated according to the fish biomass and the percentage feed rate described by the SINCHI y PEDICP (2014). Thus, 50% of the fish population was weighted for this purpose every 14 days.

To optimize the feed, the water quality and to adjust the daily carbon nitrogen ratio of the BFT system, each ration was weighed daily on a digital scale (Ohaus Scout Pro). Afterwards, the feed was supplied to satiety without exceeding the percentage calculated with the biomass. Finally, the amount of exceed feed was recorded.

The BFT system units maintained a C/N ratio approximately 15:1 throughout the experiment. To achieve this, the carbon and nitrogen contribution was calculated daily after administering the feed. The calculation was done through the equations proposed by Avnimelech (2012) and De Schryver, Crab, Defoirdt, Boon, and Verstraete, (2008) which made it possible to perform the corresponding correction using refined brown sugar. Besides the carbon source, commercial bacteria strains (EcoPro™) were also added every third day at a rate of 50 µl/L of culture water. The strains were activated according to the protocol suggested by the manufacturer.

2.5 | Zootechnical parameters

All the fish were sampled at the end of the trial and total length and weight measurements were determined according to Bicudo, Sado, and Cyrino, (2009).

- Weight gain = Wf−Wo
- Specific growth rate (SGR) = 100 [Ln Wf (g)−Wo (g)]/T, where, Wf = final weight and Wo = initial weight and T is the experimental period (91 days);
- Length gain = Ltf−Lto, where: Ltf = final total length and Lto = initial total length.
- Feed conversion ratio (FRC) = feed intake/Weight gain.
- Survival rate (%) = 100 × (final fish count/ initial fish count)

In order to estimate the viscerosomatic indices, a sample of 6 animals per treatment (2 fish per replica) was randomly selected and sacrificed after being tranquilized in cold water.

2.6 | Water quality

The dissolved oxygen, temperature (YSI EcoSense DO200A, USA) and pH of the water were measured twice a day (at 8.30 and 15:00 hr) prior to each feeding. The remaining parameters were determined once a week. The values for pH, conductivity and total dissolved solids (TDS) were measured directly in each tank using a multiparameter measuring device (ExStik® EC500, Extech instruments). In addition, alkalinity, hardness, nitrites, nitrates and ammonium were determined through photometric analysis (YSI 9,500). Finally, for measuring floc volume, a sample

TABLE 1 Amount of water used during the culture period of *Piaractus brachypomus* in BFT and DWE

Treatment	Period	Replacement rate (%)	Number of replenishments per period	Volume recovered (L)	Volume recovered/ period	Volume recovered during the 13 weeks (L)
DWE 20	1	5	27	25	675	4,700
	2	10	31	50	1,550	
	3	15	33	75	2,475	
DWE 40	1	10	27	50	1,350	9,400
	2	20	31	100	3,100	
	3	30	33	150	4,950	
BFT 20	1–3	3	13	15	195	195
BFT 40	1–3	3	13	15	195	195

Note: Calculations were made to replenish the volume of water in a tank with an effective volume of 500 L.
Periods: 1) August 23 to September 19, 2) September 20 to October 18, 3) October 19 to November 23.
For DWE 20 and DWE 40 treatments, the replacement rate and volume recovered (L) are expressed as daily values.
For BFT 20 and BFT 40 treatments, the replacement rate and volume recovered (L) are expressed as weekly values.

TABLE 2 Growth performance and survival rate of *Piaractus brachypomus* reared BFT and DWE system. (Mean ± *SEM*)

Variable	Culture System	Stocking density (fish m^3) 20	40	Two-way ANOVA (*P*-value) Culture system	Density	Interaction C. system × S. density
Total weight gain(g)	BFT	125.60 ± 1.28^{aB}	119.60 ± 8.19^{aA}	.0036**	.0065**	.0449*
	DWE	155.90 ± 3.52^{aA}	127.50 ± 2.85^{bA}			
Daily weight gain(g)	BFT	1.32 ± 0.01^{aB}	1.24 ± 0.09^{aA}	.0050**	.0142*	.3112
	DWE	1.66 ± 0.03^{aA}	1.34 ± 0.02^{bA}			
Total length gain (cm)	BFT	98.83 ± 0.86^{aB}	91.93 ± 3.48^{aA}	.0018**	.0025**	.3112
	DWE	110.80 ± 2.26^{aA}	99.3 ± 0.26^{bA}			
Feed conversion ratio	BFT	1.08 ± 0.02^{aA}	1.12 ± 0.01^{aA}	.5519	.9794	.7346
	DWE	1.06 ± 0.01^{aA}	1.03 ± 0.19^{aA}			
Specific growth rate	BFT	3.46 ± 0.03^{aB}	3.20 ± 0.05^{bB}	.0001**	.0002**	.9793
	DWE	3.80 ± 0.00^{aA}	3.50 ± 0.05^{bA}			
Survival rate (%)	BFT	96.67 ± 3.33^{aA}	100.00 ± 0.00^{aA}	.7152	.7152	.0954
	DWE	100.00 ± 0.00^{aA}	95.00 ± 2.88^{aA}			

$^{a, b}$Within a row indicate significant differences ($p \le .05$) between stocking densities.

$^{A, B}$Within a column and end point indicate significant differences ($p \le .05$) between the same density for different culture systems.

*$P < 0.05$; **$P < 0.01$.

of 1,000 ml of culture water was taken and allowed to settle for 20 min in an Imhoff cone according to the methodology described by Avnimelech (2012).

2.7 | Microorganism characterization

In order to characterize the microorganisms (microfauna and microalgae) associated with the biofloc, samples of 10 ml were taken every 8 days and fixed in 1% formalin. The samples were observed under an optical microscope attached to a photographic camera (Nikon Digital Sight, DS-5M) used to make the corresponding photographic records. The taxonomic classification of the microorganisms was conducted up to genus level with the assistance of experts. The keys used for taxonomic identification of the microorganisms were as follows: Streble & Krauter, 1987; Borror & Hill, 1995; Foissner, Chao, & Katz, 2007; Thorp & Covich, 2009 and the web page: https://www.itis.gov/standard.html.

2.8 | Statistical analysis

Production and water quality variables were analysed through descriptive statistics (95% reliability), and their values were expressed as mean ± standard error of the mean (*SEM*). The assumptions of normality (Kolmogorov–Smirnov test) and of homogeneity of variances (Levene's test) were previously verified. To determine the effects of the system and stocking density on the production parameters, a two-way analysis of variance (ANOVA) was conducted, followed by a Tukey test. In all cases, $p \le .05$ was used as the statistical criterion to reveal significant differences. All statistical tests were conducted by using the R software, version 3.6.3 for Windows (R Core team, 2020), while Graphics were constructed in GraphPad Prism 5.0 for Windows (GraphPad PRISM version 5.0, GraphPad Software Inc.).

3 | RESULTS

3.1 | Zootechnical parameters

The performance of fish cultured for 91 days is shown in Table 2. According to the results of the two-way ANOVA, there were no significant effects of culture system or stocking density for survival and feed conversion. However, all the other variables were significantly influenced by both factors, with significantly higher values of daily weight gain, total weight gain and total length for fish kept in DWE 20. Nevertheless, a significant interaction ($p = .04$) was only found for total weight gain. The analysis within the BFT system shows that all productive variables (with the exception of SGR) were similar whether lower or higher density was used.

Although DWE 20 obtained better results at the end of the experiment, the analysis of weight gain data recorded every 14 days made it possible to identify that there were no significant differences between BFT and DWE system or within the same system until day 42. The mean weights of the 4 groups on day 42 ranged from 52 to 57 g (data not shown); therefore, the best zootechnical performance observed in the DWE 20 individuals occurred approximately after the first half of the experimental phase.

Regarding the viscerosomatic (VSI) and hepatosomatic (HSI) indices along with the intraperitoneal fat ratio (IFR), there were no

TABLE 3 Somatic indices of *Piaractus brachypomus* reared in BFT and DWE system. (Mean ± *SEM*)

	Stocking density			
	20 fish m^3		40 fish m^3	
Somatic indices	BFT	DWE	BFT	DWE
Viscerosomatic Index (VSI %)	5.70 ± 0.13	5.10 ± 0.28	5.40 ± 0.18	4.20 ± 0.13
Hepatosomatic index (HSI %)	2.40 ± 0.08	2.50 ± 0.14	2.50 ± 0.06	1.90 ± 0.19
Intraperitoneal fat ratio (IFR %)	2.20 ± 0.20	2.70 ± 0.18	2.40 ± 0.16	2.30 ± 0.12

significant differences between the fish cultured using either system (Table 3).

Regardless of the previous results, fish cultured with BFT showed a more tranquil behaviour when fed and weighed. Likewise, they had more mucus on the skin and better coloration, that is a more intense and reddish tone in the anal and caudal fins and in the anterior part of the abdomen. Moreover, the rest of their bodies had a bright and clear colouring. Conversely, fish cultured in the system with daily water exchange had a yellowish, rather than reddish, colour and their bodies were dark and not very bright, similar to the *Colossoma macropomum* species. The latter characteristic is of special interest for freshwater species such as catfish, tilapia and species from the *Piaractus* genus, since colour is one of the most valued attributes at the time of purchase by consumers.

Finally, the amount of water necessary to replenish the loss by evaporation in a unit BFT (tank) varied between 13 and 15 L per week. Therefore, the weekly reposition rate was expressed as 3%. Taking into account the initial volume of water plus that of replacement by evaporation or replenishment, the mean volume of water used to produce 1 kg of fish biomass at densities of 20 and 40 fish m^3 in the system BFT was 265.3 and 139 L respectively. In contrast, the daily water exchange system used 1611.9 and 1862.3 L to produce the same amount of fish biomass at the same densities. Values were calculated considering the information shown in Table 1 and the final weight.

3.2 | Water quality

Figure 1 shows the mean values of the water quality variables recorded in both culture systems over the 13 weeks. The most stable water quality parameter, both between treatments and throughout the culture period, was temperature, with an average value of 25°C, while oxygen levels showed a decreasing trend in all the assessed treatments, no values below 6.5 mg/L were observed.

pH and alkalinity showed a decreasing trend in all the experimental units starting from the second week. However, the pH of the DWE groups was above 7.3 throughout the experiment without the need for corrections other than those added to the replenished water. In contrast, in the treatments using BFT, specifically in the BFT 40, pH and alkalinity dropped to values close to 6.0 and 23 mg/L of $CaCO_3$ respectively.

Hardness in the DWE treatments ranged from 40 to 60 mg/L of $CaCO_3$ throughout the experiment. In contrast, it had a tendency to increase in the BFT treatments, reaching values close to 200 mg/L of $CaCO_3$ in the BFT 40 treatment. The values for conductivity and TDS also had minimal variations over time in the DWE units but increased progressively in the BFT treatments.

All nitrogen compounds showed drastic fluctuations and increases during the first five weeks of the culture. During this period, ionized ammonium (NH_4^+) reached maximum values of 3.8, 2.6 and 2.3 mg/L for DWE 20, BFT 20 and DWE 40 respectively. In turn, nitrite (NO_2^-) levels ranged from 0.00 to 13 mg/L. Both variables were above the maximum levels reported for fish farming; however, there was no fish mortality or abnormal behaviour. To cope with this situation, we decided to add the double of sugar calculated to BFT units and increase the rate of water exchange for the DWE units. Consequently, after the sixth week, the decrease and stability of the above mentioned values was within the acceptable ranges for fish farming.

Even though the ammonium and nitrite values decreased after the sixth week, nitrates (NO_3^-) continued to fluctuate in all units except for the BFT 40 treatment, which was more stable and did not exceed 10 mg/L.

Floc volume in the BFT units decreased between the first and third weeks of culture and increased from the third week to the end of the experiment. The highest volume was observed in the BFT 40 treatment, reaching a maximum value of 40 ml during the last week of the experiment. The BFT 20 treatment, in turn, obtained a maximum of 14 ml at the end of the 13-week culture period. Furthermore, the units with daily water exchange did not show floc accumulations greater than 1 ml, since residues were eliminated daily via hose suction.

3.3 | Microorganism characterization

Table 4 describes the taxonomic composition of the microorganisms found in the treatment with BFT during the 13 weeks of the experiment. In general, microorganisms began to settle from the first week in both stocking densities. During this period, rotifers and microalgae were present. Between the second and third weeks, some of these genera disappeared and reappeared along with new genera from other phyla between the fourth and fifth weeks. Following this

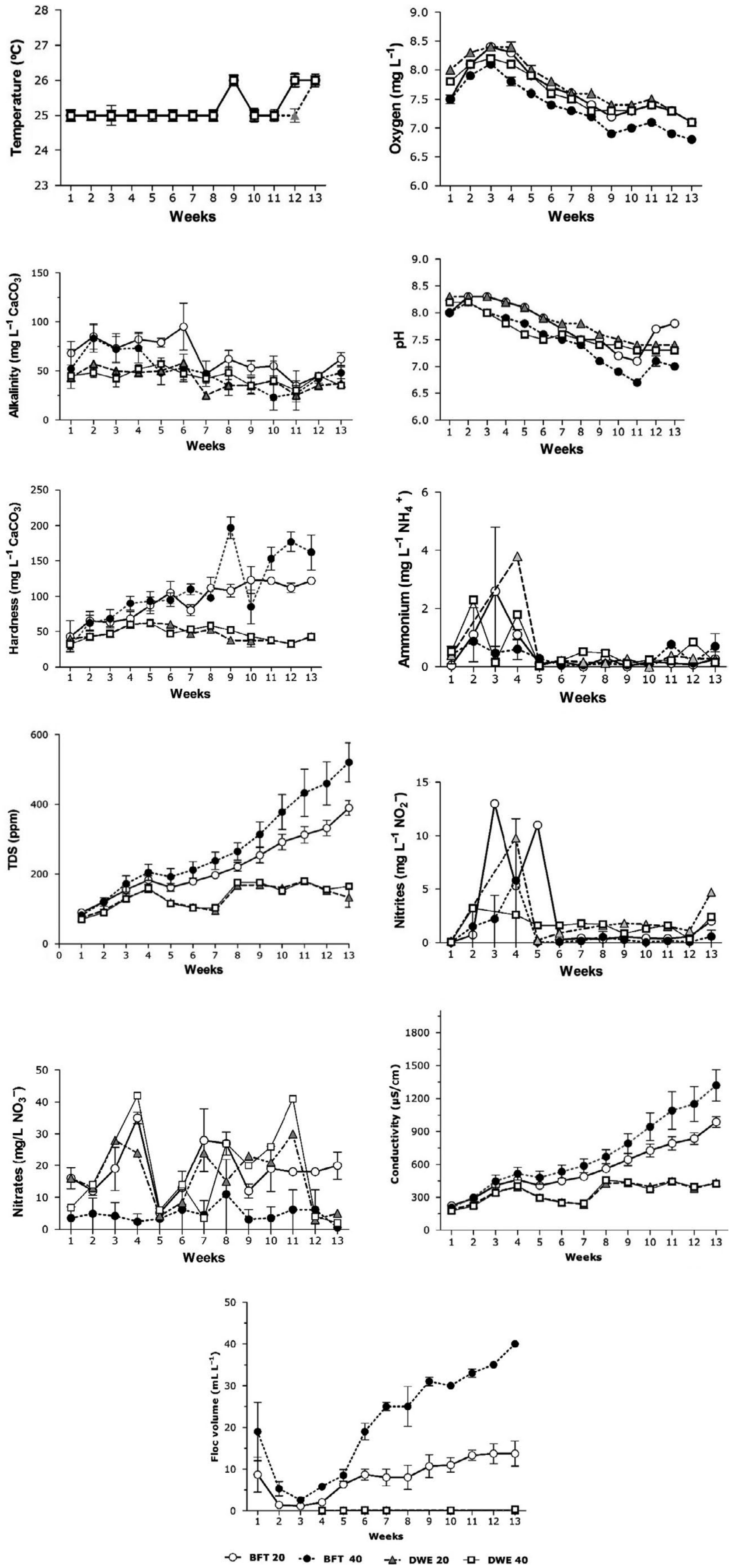

FIGURE 1 Weekly mean values of water quality parameters on the culture of *Piaractus brachypomus* in biofloc system (BFT) and daily water exchange (DWE). Two stocking densities were used 20 and 40 fish m^3 (BFT 20, BFT 40, DWE 20 and DWE 40). TDS (Total Dissolved Solids Total)

TABLE 4 Microfauna and microflora groups found during the on-growing of *Piaractus brachypomus* in the BFT system

Microfauna	Weeks												
Phylum													
Genus	1	2	3	4	5	6	7	8	9	10	11	12	13
Annelida													
Aelosoma		+	+	+	+	+	+	+	+	+	+	+	+
Pristina								+	+	+	+	+	+
Ciliates													
Euplotes			+	+	+	+	+	+	+	+	+	+	+
Vorticella	+	+	+	+	+	+	+	+	+	+	+	+	+
Blepharisma								+	+	+	+	+	+
Epistylis					+	+	+	+	+	+	+	+	+
Zoothamnium				+	+	+	+	+	+	+	+	+	+
Podophrya										+	+		
Tokophrya											+	+	
Rotifers													
Lecane	+	+	+	+	+	+	+	+	+	+	+	+	+
Philodina	+			+	+	+	+	+	+	+	+	+	+
Keratella	+												
Arthropods													
Canthocamptus								+	+	+	+	+	+
Gastrotrichs													
Chaetonotus	+			+	+	+	+	+	+	+	+		
Nematodes													
Panagrolaimus		+		+		+	+						
Rhizopods													
Assulina	+	+	+	+	+	+	+	+	+	+	+	+	+
Arcella								+	+	+	+	+	+
Amoeba					+	+	+	+	+	+	+	+	+
Tardigrades													
Hypsibius								+	+	+	+	+	+
Platyhelminthes													
Stenostomum					+	+				+	+	+	+
Microflora													
Division													
Genus													
Chlorophyta													
Scenedesmus	+	+			+	+	+	+	+	+	+	+	+
Acutodesmus	+				+	+	+	+	+	+	+	+	+
Golenkinia	+	+	+	+	+	+			+	+	+		

Note: + Presence of microorganisms

dynamic, the maximum proliferation of genera occurred between 8th and 12th weeks.

Microfauna was the most diverse group, represented by 20 genera included in 9 phyla, whereas microalgae were represented by three genera of chlorophytes (green algae). The most representative phylum within the microfauna was that of the ciliates, composed by 7 genera, of which *Vorticela* was present during the entire experiment; its maximum increase having been observed during the sixth week. Similarly, the genera *Assulina* and *Lecane* belonging to the rhizopods and rotifers phyla, respectively, were present throughout the whole period. The fourth predominant genus was *Aelosoma* from the annelids phylum. This genus appeared from the second week onwards and was present until the end of the experiment. For their part, microalgae were also present for most of the study. Figure 2 shows some of the described genera.

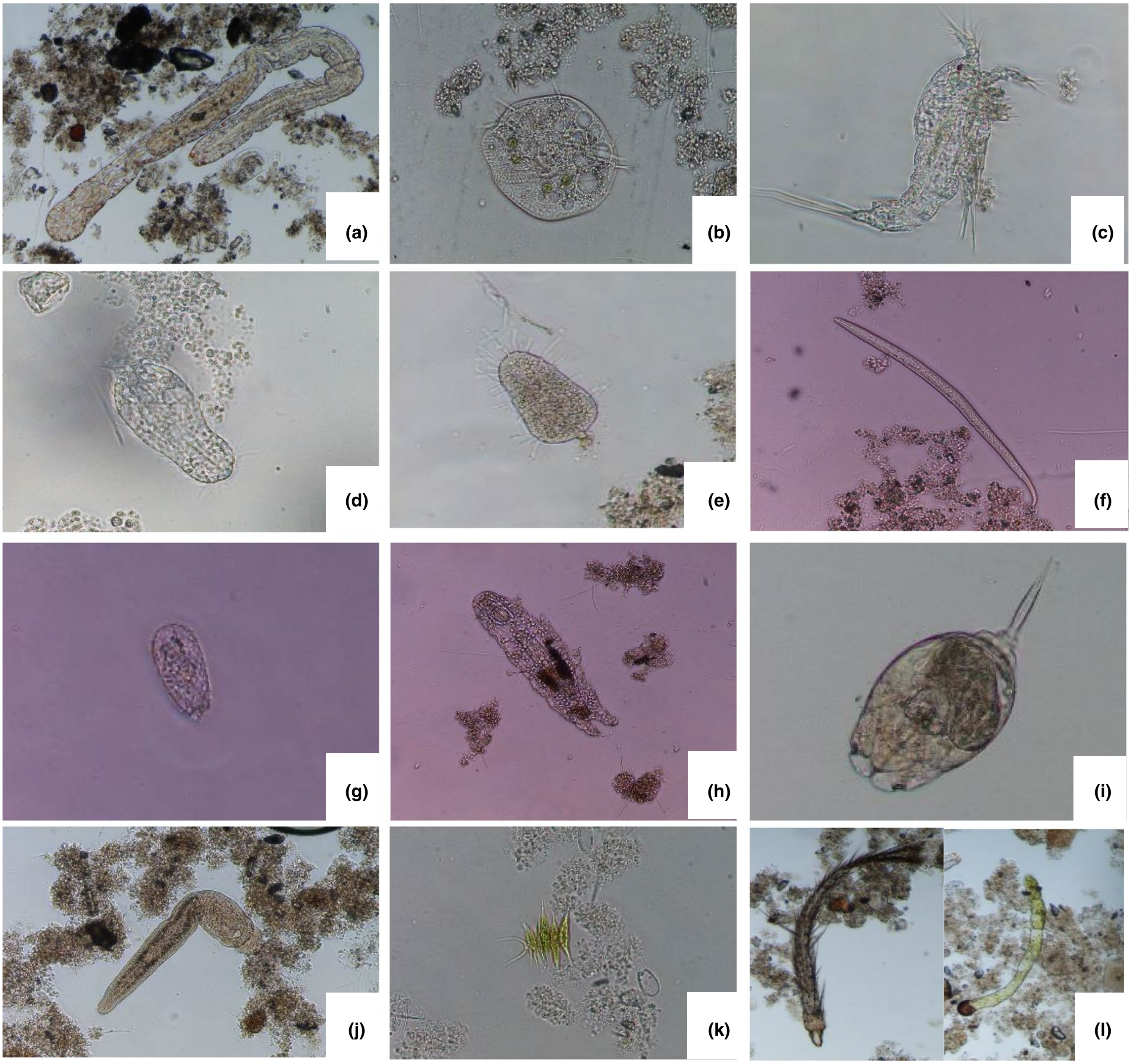

FIGURE 2 Microorganisms found in the culture of *Piaractus brachypomus* in the BFT system. Classification by genus: A. Aelosoma (10x) B. Euplotes (20x) C. Canthocamptus (20x) D. Chaetonotus (40x) E. Podophrya (20x) F. Panagrolaimus (20x) G. Assulina (20x) H. Hypsibius (10x) I. Lecane (20x) J. Stenostomum (10x) K. Acutodesmus (10x) L. Other microorganisms (10x y 20x) [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

4.1 | Zootechnical parameters

The tendency of the current animal production systems is to use technologies which maximize productivity per area, volume and time unit while reducing environmental impact. In this sense, BFT systems are an intensive production model for both fish and shrimp (Azim & Little, 2008; Emerenciano et al., 2014; Poli, Schveitzer, & de Oliveira, 2015; Ekasari et al., 2016). However, the inclusion of new species in the system requires determining whether the organisms can truly adapt and develop in accordance with production criteria.

In this study, the best results in terms of productive performance at the end of the experiment were observed in DWE 20. However, it is important to highlight that there were no differences within BFT system, indicating that *P. brachypomus* can be cultured under this technology using a stocking density of 40 fish m^3. Furthermore, it is also important to note that we observed high survival rates both for the individuals cultured with BFT and with DWE. Our results are in agreement with previous findings, regarding *P. brachypomus* survival in BFT (Alzate-Díaz & Pardo-Carrasco, 2016; Chaverra et al., 2017;

Poleo et al., 2011). Together, these findings demonstrate that this species have the ability to adapt to culture systems with higher stocking density compared with traditional systems.

Nevertheless, special attention should be paid to the relation between stocking density and weight gain. In this study, the weight gain in all experimental groups showed a slight tendency to decrease when stocking density was higher and even was significant in the DWE system. Interestingly, data in the literature show that at densities among 17 and 42 fish m^3 the daily weight gain varied from 1.6 to 2.33 g/day (Abad et al., 2014; Poleo et al., 2011, Alzate-Díaz, & Pardo -Carrasco, 2016); however, another study shows that at density of 80 fish m^3 this variable was among 0.4 and 0.5 g depending on the culture system (Chaverra et al., 2017). Thus, the best productivity (15.9 kg m^3) of this specie was reached after 84 days using a stocking density of 42 fish m^3 (Alzate-Díaz & Pardo-Carrasco, 2016), followed by 12.96 kg m^3 after 192 days with a stocking density of 31 fish m^3 (Poleo et al., 2011), in both mentioned studies, the initial mean weight was similar (44–54 g). In the present study, juveniles of *P. brachypomus* with an initial weight of 5.4 ± 0.19 g at a stocking density of 40 fish m^3 produced 5.0 kg m^3 of biomass at the end of the 90-day period. Although this last value is lower than those reported in the above-mentioned studies, it is similar to the results described by Abad et al. (2014) who reported final fish biomass of 7.89 kg m^3 after 210 days with a stocking density of 17 fish m^3. In other words, the values for production increased per unit of volume and over time. However, additional studies are required to determine which is the best density or biomass by unit of volume.

Although the daily weight gain for BFT treatments in this study was around one gram below the value described by Poleo et al. (2011) and Abad et al. (2014), and only 3 mg below the values reported by Deza-Taboada, Quiroz, Rebaza-Alfao, & Rebaza-Alfao, (2002) for extensive systems, the feed conversion ratio obtained in the present study was better than the value reported by the first author and similar to the one described by Piñeros-Roldan, Gutiérrez-Espinosa, & Castro-Guerrero, (2014) for similar juveniles (in terms of development) cultured for 51 days. Therefore, the incompatibilities may be attributed, on the one hand, to the differences in the stages of development of the fish evaluated in the three studies and, on the other hand, to the negative effect of the high levels of toxic nitrogen compounds predominant during the first weeks of the experiment. A previous work concluded that exposition to high levels of ammonium has physiological effects such as the decrease in feed consumption, which in turn results in reduced weight gain (Atwood, Tomasso, Ronan, Barton, & Renner, 2000; Ortega, Renner, & Bernier, 2005). Likewise, high nitrite levels have been linked to low growth rates (Kroupova et al., 2008; Siikavuopio & Sæther, 2006). From another perspective, the performance of the zootechnical parameters at the end of the present experiment could have been influenced by the percentage of protein supplied in the diet. According to Vásquez-Torres, Hernández-Arévalo, Gutiérrez-Espinosa, and Yossa, (2012) crude protein values above 31.6% significantly affect the specific growth rate (SGR), feed conversion ratio (FCR), protein retention rate and protein efficiency ratio (PER) of juveniles of *P. brachypomus* with a weight beyond 60 grams.

As for somatic indices, the literature reports scarce and varied values for *P. brachypomus*. Regardless, the results of this study were within the wide range reported for the species (Abad et al., 2014; Rodríguez & Landines, 2011). In addition to their usefulness as indicators of the development and physiological status of the organisms, these indices have also been proposed as approximate adaptation indicators (Páez-Martíinez, Cruz, & López-Rubalcava, 2003; Wang et al., 2015); thus, it is important to use them for assessing the organisms cultured using BFT systems. Similar to the results of this study, Ekasari et al. (2016) found no differences between the HSI of *Clarias gariepinus* fingerlings cultured using BFT and that of the controls. On the contrary, supplementing the feed with 10% BFT reduced the HSI significantly and improved the hepatic antioxidant capacity of *Carassius auratus* specimens (Wang et al., 2015).

Regarding water resources, implementing BFT made it possible to reduce the water required to produce 1 kg of fish biomass in 85 to 93%. In this sense, the high efficiency of the system was demonstrated. Thus, and considering that traditional farming conditions require daily water exchange rates between 5% and 15%, BFT is a valuable alternative for farming *P. brachypomus* in regions where water resources are limited. Nevertheless, further studies are required which evaluate the profitability of the system, given the high energy cost generated by aeration.

4.2 | Water quality

One of the critical points in the implementation of BFT is maintaining water quality. Each water quality variable alone can significantly affect the behaviour of the other variables and thus the health of the species being farmed (Boyd, 2017). In this study, most water quality parameters (with the exception of toxic nitrogen compounds) were within the permissible ranges for farming tropical fish (Weingartner & Zaniboni, 2004). The pH and alkalinity values were lower in BFT tanks than in the DWE, while the production of nitrogen compounds was more elevated for the BFT treatment. This trend was more visible in the treatment with the highest stocking density (BFT 40), which is in agreement with the results obtained in channel catfish (Green, 2015) and Nile tilapia using a similar system (Azim & Little, 2008; Pérez-Fuentes et al., 2016).

According to Avnimelech (2015), this behaviour is characteristic of systems where nitrification is occurring and implies the presence of nitrifying microorganisms and the release of H^+ ions, which leads to a decrease in the alkalinity and pH of the culture water (De Holanda Cavalcante et al., 2014). Consequently, the gradual decrease in alkalinity and pH observed during the early weeks of the culture using BFT and the dramatic drop seen during its last weeks were probably influenced by the growth of communities of nitrifying organisms.

Systems requiring minimal water exchange such as BFT require the addition of carbonates, typically sodium bicarbonate, to maintain alkalinity within a range of 100 to 150 mg/L of $CaCO_3$ and thus supply the demand for carbonates of the nitrifying bacteria involved in the dynamics of the culture (Ebeling, Timmons, & Bisogni, 2006). In the present study, the alkalinity ranged from 23 to 95 mg/L of $CaCO_3$, with the lowest values in BFT 40. Low alkalinity levels after the fifth week were corrected by addition of sodium bicarbonate and dolomite lime at a ratio of 1:5 until the $CaCO_3$ concentrations reached values higher than 60 mg/L and a pH over 7.0. In this sense, the setting of this parameter for future studies should be taking into account these findings and those of Poleo et al. (2011) who conducted experiments on the same species using an intensive system with no water exchange and Poli, Schveitzer, and de Oliveira Nuñer (2015) who studied *Rhamdia quelen* cultured in BFT. Maintaining BFT cultures with these alkalinity standards is fundamental since, on the one hand, it renders the system more stable by avoiding daily pH oscillations and, on the other hand, it helps maintain the physiological status of the fish (Martins et al., 2017).

However, maintaining high alkalinity values in closed systems implies the constant addition of calcareous materials, this in turn leads to increased hardness resulting from the release of divalent calcium cations (Ca^{2+}) (De Holanda Cavalcante et al., 2014). This effect was evident in this study mainly in the treatments demanding the highest amount of sodium bicarbonate and dolomite lime.

Under traditional culture conditions, hardness values above 20 mg/L of $CaCO_3$ are considered optimal for the growth and zootechnical performance of freshwater aquatic organisms (De Andrade et al., 2007). However, these values may increase over time for BFT systems. In this way, the maximum level of hardness observed in the present study was 197 mg/L of $CaCO_3$ for the BFT 40 treatment, while Poleo et al. (2011) reported values between 348 and 570 mg/L of $CaCO_3$ after a period of 192 days. Consequently, and considering that *P. brachypomus* is native to bodies of water with hardness values below 20 mg/L of $CaCO_3$ (Sánchez & Vásquez, 1986), we can then conclude that this species is able to adapt to a wide range of hardness, which in turn could facilitate its inclusion in culture systems using BFT.

The conductivity showed values similar to those reported by Poli et al. (2015) for *Rhamdia quelen* larvae. The treatments with BFT had a tendency to increase due to the sodium bicarbonate and dolomite lime used when correcting alkalinity and pH. Likewise, the sodium chloride that was added to the replenished water gradually increased the values of these variables. Conversely, the DWE treatments had a stable behaviour resulting from the elimination of salts upon exchanging water.

The values of ionized ammonium (NH_4^+) and nitrites (NO_2^-) detected in our experiment during the first five weeks are in agreement with those found by Avnimelech (2012), who stated that ammonium and nitrite levels are usually high for BFT systems during the first three to five weeks, and increase in a parallel manner. Similarly, nitrates (NO_3^-) had high values during the same period. The presence of the three nitrogen compounds simultaneously and the decrease in pH and alkalinity suggest predominance of autotrophic organisms (Ebeling et al., 2006). In this regard, autotrophic nitrification took place during the entire experiment and in most treatments. It was, however, less evident in the BFT 40 treatment indicating a higher presence of heterotrophic communities when compared to other experimental groups.

The high levels of toxic nitrogen compounds observed during the first weeks in the treatments using BFT were probably related to the initial fertilization, which had more nitrogen than carbohydrates. Likewise, these levels could have been influenced by the system's short maturation period. However, no fish mortality or erratic movements were observed in fish of that treatment. Likewise, the strong aeration was likely to facilitate the volatilization of unionized ammonia (NH_3) (Hall & Tank, 2003; Passell, Dahm, Bedrick, (2007), while the stability of the temperature and pH prevented the toxicity (Timmons, Ebeling, Wheaton, Summerfelt, & Vinci, 2002).

As for the tanks with DWE, the high levels of ammonium and nitrites observed during the first weeks matched the low rate of exchange. Thus, stability within the normal ranges was achieved as exchange volume increased.

The acceptable level of unionized ammonia (NH_3) in aquaculture systems is only 0.025 mg/L. A good rule of thumb is values of 1 mg/L for total ammonia nitrogen (TAN) for cool water and 2 or 3 1 mg/L for warmwater fish (Ebeling & Timmons, 2012), while nitrites (NO_2^-) should not surpass 1 mg/L (Timmons et al., 2002). Nevertheless, the values for toxic nitrogen compounds determined in the present study are far above these and those reported by most experimental studies using BFT (Bakar et al., 2015; Poleo et al., 2011; Wang et al., 2015).

The floc volume obtained during the first three weeks is attributed to the initial addition of commercial feed, since it became hydrated and formed agglomerations that were read as floc. However, true floc was formed from the third week onward as a result of fish excretions, concentrate residues and the metabolic activity of the microorganisms emerging in the water (Lee et al., 2013). The highest weekly increase in the floc volume after the 5th week was found in the BFT 40, probably due to the higher amount of organic matter produced by the system, as it implies higher microbial metabolic activity (Bakar et al., 2015); on the other hand, Sobeck and Higgins (2002) and Luo, Avnimelech, Pan, and Tan, (2013) demonstrated that floc formation is influenced by the concentration of divalent cations of calcium which bond with negatively charged functional groups (biopolymers produced by the microorganisms). According to this, the high hardness values for the BFT treatment might have improved floc formation.

4.3 | Microorganism characterization

Microorganisms play a very important role in aquaculture systems. Their benefits have been closely related to primary production, production of high-quality feed for other organisms, nutrient recycling and water quality (Avnimelech, 2012; Ballester et al., 2010). The proliferation of microbial communities in BFT systems is mediated mainly by the strong aeration and addition of carbon sources; thus, the microorganisms, together with the organic matter in suspension, form the bioflocs (Burford, Thompson, McIntosh, Bauman, &

Pearson, 2003; Haslun, Correia, Strychar, Morris, & Samocha, 2012). In addition to this, the proliferation of microorganisms in this study was influenced by the initial inoculation; this is demonstrated by the presence of microfauna and microalgal communities during the first week of culture and the number of phyla and genera that emerged in subsequent weeks, which was higher when compared with the findings of other studies (Azim & Little, 2008; Ekasari & Maryam, 2012; Emerenciano et al., 2012; Ray et al., 2010). Normally, microalgae are the first to grow, thus becoming the basic source of feed for the subsequent development of zooplankton communities and, at the same time, provide nutrients which aid bacterial growth (Wei, Liao, & Wang, 2016).

The most representative microorganism groups in this study were ciliates and rhizopods, which belong to kingdom Protista. Likewise, rotifers and chlorophytes, which belong to kingdoms Animalia and Plantae, respectively, were also representative. Similar studies highlight the presence of protozoa, ciliates and rotifers (Azim & Little, 2008; Effendy, Al Deen, & Chithambaran, 2016; Emerenciano et al., 2014), which means that BFT systems are an optimal culture medium for that organisms. Among these, protozoa and ciliates are an important source of enrichment for the floc aggregates, as they have a higher protein–energy ratio and are capable of synthesizing long-chain polyunsaturated fatty acids by feeding on bacteria (Zhukova & Kharlamenko, 1999).

Regarding microfauna genera, the results of this study are similar to those of a research conducted on a macrocosm-microcosm system associated with a tilapia culture where seven genera of ciliates were identified and two described: *Vorticella* and *Epystilis*. In addition, a group of rotifers from the *Philodina, Lecane and Keratella* genera was also present (Monroy-Dosta, de Lara, Castro-Mejía, Castro-Mejía, & Coelho-Emerenciano, 2013).

In relation to other studies, the organisms found in this study are comparable in terms of phyla but not in terms of genera. Out of four genera described by Azim and Little (2008) only *Lecane* is common. Likewise, of the four protists described by Ekasari and Maryam, (2012) only *Arcella* is common. Variations in the settling and growth of microorganism groups are attributed to factors such as salinity, temperature, light intensity, photoperiod, stocking density and nutrient availability (Pinho, Molinari, de Mello, Fitzsimmons, & Emerenciano, 2017). In conclusion, the results of this study indicate that both systems BFT and DWE are useful for increasing the production of *P. brachypomus* in captivity. Additionally, juveniles of this specie can be cultured successfully in regions with scarce water resources using BFT technology with a stocking density of 40 fish m^3. Future experiments should be designed to determine the optimal biomass per unit of volume, implement measures for reducing the formation of toxic nitrogen compounds and adjust alkalinity values from the start of the culture.

ACKNOWLEDGMENTS

The authors would like to thank the Aquaculture Institute of Universidad de los Llanos and the members of the GRITOX Research Group, particularly to Ginna Urrego, Laura Marin, Cesar Morales and Yeferson Moreno for the logistic support provided during the experiment. We also thank to John Jairo Díaz for assisting us in the statistical analysis of the data.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Conceived the project, contributed reagents/materials/analysis tools:Mauricio Medina. Conceived and designed the experiments: Mauricio Medina, Juan Ramírez, Martha Jimenez and Leydy Sandoval. Performed the experiments: Leydy Sandoval, Martha Jiménez, José Rodríguez, Diana Guaje and Mauricio Medina. Analyzed the data and drafted paper: Leydy Sandoval and Mauricio Medina. All the authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support this study are available from the corresponding author upon reasonable request.

ORCID

Leydy Y. Sandoval-Vargas https://orcid.org/0000-0003-1846-9563
Martha N. Jiménez-Amaya https://orcid.org/0000-0002-6918-0973
José Rodríguez-Pulido https://orcid.org/0000-0002-6735-3885
Diana N. Guaje-Ramírez https://orcid.org/0000-0001-6519-6372
Juan A. Ramírez-Merlano https://orcid.org/0000-0001-9712-6678
Víctor M. Medina-Robles https://orcid.org/0000-0002-4871-2715

REFERENCES

Abad, D., Rincón, D., & Poleo, G. (2014). Índices de rendimiento corporal en morocoto *Piaractus brachypomus* cultivado en sistemas Biofloc. *Zootecnia Tropical*, *32*, 119–130.

Abraham, T. J., Sarker, S., Dash, G., Patra, A., & Adikesavalu, H. (2017). Chryseobacterium sp. PLI2 and Aeromonas hydrophila co-infection in pacu, Piaractus brachypomus (Cuvier, 1817) fries cultured in West Bengal, India. *Aquaculture*, *473*, 223–227. https://doi.org/10.1016/j.aquaculture.2017.02.016

Ajiaco-Martínez, R. E., Ramírez-Gil, H., Sánchez-Duerte, P., Lasso, C. A., & Trujillo, F. (2012). IV. Diagnóstico de la Pesca Ornamental en Colombia. C. A Lasso (Ed.), *Editorial Recursos Hidrobiológicos y Pesqueros Continentales de Colombia. Instituto de Investigación de los Recursos Biológicos*. Bogotá, DC: Alexander von Humboldt.

Alzate-Díaz, H. A., & Pardo-Carrasco, S. C. (2016). Evaluación de fuentes proteicas para el desempeño productivo de cachama blanca *Piaractus brachypomus* en sistema biofloc. *Orinoquia*, *20*(2), 50–59. https://doi.org/10.22579/20112629.441

Atwood, H. L., Tomasso, J. R., Ronan, P. J., Barton, B. A., & Renner, K. J. (2000). Brain monoamine concentrations as predictors of growth inhibition in channel catfish exposed to ammonia. *Journal of Aquatic Animal Health*, *12*(1), 69–73. https://doi.org/10.1577/1548-8667(2000)012<0069:BMCAPO>2.0.CO;2

Avnimelech, Y. (2012). Y. Avnimelech (Ed.), *Biofloc Technology - A Practical Guide Book* (2nd ed.). Baton Rouge, LA: The World Aquaculture Society.

Avnimelech, Y. (2015). *Biofloc technology – A practical Guide Book* (3rd ed.). Baton Rouge, LA: The World Aquaculture. Society.

Azim, M. E., & Little, D. C. (2008). The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *283*(1–4), 29–35. https://doi.org/10.1016/j.aquaculture.2008.06.036

Azim, M. E., Verdegem, M. C. J., Singh, M., Van Dam, A. A., & Beveridge, M. C. M. (2003). The effects of periphyton substrate and fish stocking density on water quality, phytoplankton, periphyton and fish growth. *Aquaculture Research*, *34*(9), 685–695. https://doi.org/10.1046/j.1365-2109.2003.00867.x

Bakar, N. S. A., Nasir, N. M., Lananan, F., Hamid, S. H. A., Lam, S. S., & Jusoh, A. (2015). Optimization of C/N ratios for nutrient removal in aquaculture system culturing African catfish, (*Clarias gariepinus*) utilizing Bioflocs Technology. *International Biodeterioration & Biodegradation*, *102*, 100–106. https://doi.org/10.1016/j.ibiod.2015.04.001

Ballester, E. L. C., Abreu, P. C., Cavalli, R. O., Emerenciano, M., De Abreu, L., & Wasielesky, W. Jr (2010). Effect of practical diets with different protein levels on the performance of *Farfantepenaeus paulensis* juveniles nursed in a zero exchange suspended microbial flocs intensive system. *Aquaculture Nutrition*, *16*(2), 163–172.

Bicudo, Á. J. A., Sado, R. Y., & Cyrino, J. E. P. (2009). Dietary lysine requirement of juvenile pacu *Piaractus mesopotamicus* (Holmberg, 1887). *Aquaculture*, *297*(1–4), 151–156. https://doi.org/10.1016/j.aquaculture.2009.09.031

Borror, A. C., & Hill, B. F. (1995). The order Euplotida (Ciliophora): Taxonomy, with division of Euplotes into several genera. *Journal of Eukaryotic Microbiology*, *42*(5), 457–466. https://doi.org/10.1111/j.1550-7408.1995.tb05891.x

Boyd, C. E. (2017). General relationship between water quality and aquaculture performance in Ponds. J. Galina (Ed.), In *Fish diseases* (pp. 147–166). London, UK.: Academic Press.

Brú-Cordero, S. B., Pertúz-Buelvas, V. M., Ayazo-Genes, J. E., Atencio-García, V. J., & Pardo-Carrasco, S. C. (2017). Bicultivo en biofloc de cachama blanca -*Piaractus brachypomus*-y tilapia nilótica -*Oreochromis niloticus*- alimentadas con dietas de origen vegetal. *Revista de la Facultad de Medicina Veterinaria y de Zootecnia*, *64*(1), 44–60..

Burford, M. A., Thompson, P. J., McIntosh, R. P., Bauman, R. H., & Pearson, D. C. (2003). Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture*, *219*(1–4), 393–411. https://doi.org/10.1016/S0044-8486(02)00575-6

Chaverra, G. S. C., García, G. J. J., & Pardo, C. S. C. (2017). Biofloc effect on juvenils Cachama blanca *Piaractus brachypomus* growth parameters. *CES Medicina Veterinaria y Zootecnia*, *12*(3), 170–180.

R Core Team (2020). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Crab, R., Kochva, M., Verstraete, W., & Avnimelech, Y. (2009). Bio-flocs technology application in over-wintering of tilapia. *Aquacultural Engineering*, *40*(3), 105–112. https://doi.org/10.1016/j.aquaeng.2008.12.004

De Andrade, L. S., de Andrade, R. L. B., Becker, A. G., Rossato, L. V., da Rocha, J. F., & Baldisserotto, B. (2007). Interaction of water alkalinity and stocking density on survival and growth of silver catfish, *Rhamdia quelen*, juveniles. *Journal of the World Aquaculture Society*, *38*, 454–458. https://doi.org/10.1111/j.1749-7345.2007.00118.x

De Holanda Cavalcante, D., Caldini, N. N., da Silva, J. L. S., dos Santos Lima, F. R., & do Carmo, M. V. (2014). Imbalances in the hardness/alkalinity ratio of water and Nile tilapia's growth performance. *Acta Scientiarum Technology*, *36*(1), 49–54.

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. (2008). The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture*, *277*(3–4), 125–137. https://doi.org/10.1016/j.aquaculture.2008.02.019

Deza-Taboada, S. A., Quiroz, S., Rebaza-Alfaro, M., & Rebaza-Alfaro, C. (2002). Efecto de la densidad de siembra en el crecimiento de *Piaractus brachypomus* (Cuvier, 1818) "paco" en estanques seminaturales de Pucallpa. *Folia Amazónica*, *13*(1–2), 49–64.

Ebeling, J. M., & Timmons, M. B. (2012). Recirculating Aquaculture Systems. En J. H. Tidwell (Ed.), *Aquaculture Production Systems*, (pp. 245–277). New Delhi, India: Wiley & Sons, Inc.

Ebeling, J. M., Timmons, M. B., & Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture*, *257*(1–4), 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019

Effendy, I., Al Deen, S., & Chithambaran, S. (2016). Semi intensive and semi Biofloc methods for the culture of Indian White Prawn, *Fenneropenaeus indicus* in high-density polyethylene liner ponds. *Hayati Journal of Biosciences*, *23*(3), 106–110. https://doi.org/10.1016/j.hjb.2016.06.004

Ekasari, J., & Maryam, S. (2012). Evaluation of biofloc technology application on water quality and production performance of red tilapia *Oreochromis* sp. cultured at different stocking densities. *Hayati Journal of Biosciences*, *19*(2), 73–80.

Ekasari, J., Rivandi, D. R., Firdausi, A. P., Surawidjaja, E. H., Zairin, M., Bossier, P., & De Schryver, P. (2015). Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. *Aquaculture*, *441*, 72–77. https://doi.org/10.1016/j.aquaculture.2015.02.019

Ekasari, J., Suprayudi, M. A., Wiyoto, W., Hazanah, R. F., Lenggara, G. S., Sulistiani, R., … Zairin, M. (2016). Biofloc technology application in African catfish fingerling production: The effects on the reproductive performance of broodstock and the quality of eggs and larvae. *Aquaculture*, *464*, 349–356. https://doi.org/10.1016/j.aquaculture.2016.07.013

Emerenciano, M., Ballester, E. L. C., Cavalli, R. O., & Wasielesky, W. (2012). Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). *Aquaculture Research*, *43*(3), 447–457. https://doi.org/10.1111/j.1365-2109.2011.02848.x

Emerenciano, M., Cuzon, G., Arévalo, M., & Gaxiola, G. (2014). Biofloc technology in intensive broodstock farming of the pink shrimp *Farfantepenaeus duorarum*: Spawning performance, biochemical composition and fatty acid profile of eggs. *Aquaculture Research*, *45*(10), 1713–1726. https://doi.org/10.1111/are.12117

Faizullah, M., Rajagopalsamy, C. B. T., Ahilan, B., & Francis, T. (2015). Impact of biofloc technology on the growth of goldfish young ones. *Indian Journal of Science and Technology*, *8*(13), 1–8. https://doi.org/10.17485/ijst/2015/v8i12/54060

Foissner, W., Chao, A., & Katz, L. A. (2007). Diversity and geographic distribution of ciliates (Protista: Ciliophora). W. Foissner & D. Hawksworth (Eds.), In *Protist diversity and geographical distribution* (pp. 111–129). Dordrecht: Springer.

Green, B. W. (2015). Performance of a temperate-zone channel catfish biofloc technology production system during winter. *Aquacultural Engineering*, *64*, 60–67. https://doi.org/10.1016/j.aquaeng.2014.11.001

Hall, R. J. O., & Tank, J. L. (2003). Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. *Limnology and Oceanography*, *48*(3), 1120–1128. https://doi.org/10.4319/lo.2003.48.3.1120

Haslun, J. A., Correia, E., Strychar, K., Morris, T., & Samocha, T. (2012). Characterization of bioflocs in a no water exchange super-intensive system for the production of food size pacific white shrimp *Litopenaeus vannamei*. *International Journal of Aquaculture*, *2*(1), 29–38.

Kroupova, H., Machova, J., Piackova, V., Blahova, J., Dobsikova, R., Novotny, L., & Svobodova, Z. (2008). Effects of subchronic nitrite exposure on rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicology*

and Environmental Safety, 71(3), 813–820. https://doi.org/10.1016/j.ecoenv.2008.01.015
Kumar, A., Pradhan, P. K., Das, P. C., Srivastava, S. M., Lal, K. K., & Jena, J. K. (2018). Growth performance and compatibility of pacu, *Piaractus brachypomus* with Indian major carps in polyculture system. *Aquaculture*, *490*, 236–239. https://doi.org/10.1016/j.aquaculture.2018.02.052
Lee, J., Cho, D. H., Ramanan, R., Kim, B. H., Oh, H. M., & Kim, H. S. (2013). Microalgae-associated bacteria play a key role in the flocculation of *Chlorella vulgaris*. *Bioresource Technology*, *131*, 195–201. https://doi.org/10.1016/j.biortech.2012.11.130
Luo, G. Z., Avnimelech, Y., Pan, Y. F., & Tan, H. X. (2013). Inorganic nitrogen dynamics in sequencing batch reactors using biofloc technology to treat aquaculture sludge. *Aquacultural Engineering*, *52*, 73–79. https://doi.org/10.1016/j.aquaeng.2012.09.003
Luo, G., Zhang, N., Cai, S., Tan, H., & Liu, Z. (2017). Nitrogen dynamics, bacterial community composition and biofloc quality in biofloc-based systems cultured *Oreochromis niloticus* with poly-β-hydroxybutyric and polycaprolactone as external carbohydrates. *Aquaculture*, *479*, 732–741. https://doi.org/10.1016/j.aquaculture.2017.07.017
Magondu, E. W., Charo-Karisa, H., & Verdegem, M. C. J. (2013). Effect of C/N ratio levels and stocking density of *Labeo victorianus* on pond environmental quality using maize flour as a carbon source. *Aquaculture*, *410*, 157–163. https://doi.org/10.1016/j.aquaculture.2013.06.021
Manduca, L. G., da Silva, M. A., Alvarenga, É. R. D., Alves, G. F. D. O., Fernandes, A. F. D. A., Assumpção, A. F., … Turra, E. M. (2020). Effects of a zero exchange biofloc system on the growth performance and health of Nile tilapia at different stocking densities. *Aquaculture*, *521*, 735064. https://doi.org/10.1016/j.aquaculture.2020.735064
Martins, G. B., Tarouco, F., Rosa, C. E., & Robaldo, R. B. (2017). The utilization of sodium bicarbonate, calcium carbonate or hydroxide in biofloc system: Water quality, growth performance and oxidative stress of Nile tilapia (*Oreochromis niloticus)*. *Aquaculture*, *468*, 10–17. https://doi.org/10.1016/j.aquaculture.2016.09.046
Merino, M. (2014). *Plan Nacional para el desarrollo de la Acuicultura sostenible en Colombia-PlaNDAS*. Bogotá: Colombia: Ministerio de Agricultura y Desarrollo Rural-MADR.
Merino, M. C., Bonilla, S. P., & Bages, F. (2013). Diagnóstico del estado de la acuicultura en Colombia. M.C Merino (Ed.), *Plan Nacional de Desarrollo de la Acuicultura Sostenible en Colombia AUNAP-FAO*. Bogotá, Colombia: Ministerio de Agricultura y Desarrollo Rural.
MAVDT - Ministerio de Ambiente, Vivienda y Desarrollo Territorial (2010). *Política Nacional para la Gestión Integral del Recurso Hídrico* (p. 124). Bogotá, Colombia. Ministerio de Ambiente, Vivienda y Desarrollo Territorial.
Monroy-Dosta, M. D. C., Lara-Andrade, D., Castro-Mejía, J., Castro-Mejía, G., & Coelho-Emerenciano, M. G. (2013). Composición y abundancia de comunidades microbianas asociadas al biofloc en un cultivo de tilapia. *Revista de Biología Marina y Oceanografía*, *48*(3), 511–520. https://doi.org/10.4067/S0718-19572013000300009
National Research Council (US) (1996). *Institute for Laboratory Animal Research. Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academies Press (US).
Ortega, V. A., Renner, K. J., & Bernier, N. J. (2005). Appetite-suppressing effects of ammonia exposure in rainbow trout associated with regional and temporal activation of brain monoaminergic and CRF systems. *Journal of Experimental Biology*, *208*(10), 1855–1866. https://doi.org/10.1242/jeb.01577
Páez-Martíinez, N., Cruz, S. L., & López-Rubalcava, C. (2003). Comparative study of the effects of toluene, benzene, 1, 1, 1-trichloroethane, diethyl ether, and flurothyl on anxiety and nociception in mice. *Toxicology and Applied Pharmacology*, *193*(1), 9–16. https://doi.org/10.1016/S0041-008X(03)00335-1
Passell, H. D., Dahm, C. N., & Bedrick, E. J. (2007). Ammonia modeling for assessing potential toxicity to fish species in the Rio Grande, 1989–2002. *Ecological Applications*, *17*(7), 2087–2099. https://doi.org/10.1890/06-1293.1
Pérez-Fuentes, J. A., Hernández-Vergara, M. P., Pérez-Rostro, C. I., & Fogel, I. (2016). C: N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. *Aquaculture*, *452*, 247–251. https://doi.org/10.1016/j.aquaculture.2015.11.010
Piñeros-Roldan, A. J., Gutiérrez-Espinosa, M. C., & Castro Guerrero, S. R. (2014). Sustitución total de la harina de pescado por subproductos avícolas suplementados con aminoácidos en dietas para juveniles de Piaractus brachypomus, Cuvier 1818. *Orinoquia*, *18*(2), 13–24. https://doi.org/10.22579/20112629.298
Pinho, S. M., Molinari, D., de Mello, G. L., Fitzsimmons, K. M., & Coelho Emerenciano, M. G. (2017). Effluent from a biofloc technology (BFT) tilapia culture on the aquaponics production of different lettuce varieties. *Ecological Engineering*, *103*, 146–153. https://doi.org/10.1016/j.ecoleng.2017.03.009
Poleo, G., Aranbarrio, J. V., Mendoza, L., & Romero, O. (2011). Cultivo de cachama blanca en altas densidades y en dos sistemas cerrados. *Pesquisa Agropecuária Brasileira*, *46*(4), 429–437. https://doi.org/10.1590/S0100-204X2011000400013
Poli, M. A., Schveitzer, R., & de Oliveira Nuñer, A. P. (2015). The use of biofloc technology in a South American catfish (*Rhamdia quelen*) hatchery: Effect of suspended solids in the performance of larvae. *Aquacultural Engineering*, *66*, 17–21. https://doi.org/10.1016/j.aquaeng.2015.01.004
Puentes, V., Escobar, F. D., Polo, C. J., Gutiérrez, J., Castaño, F., Amado, C., Alonso, J. C., Mojica, D. F., Suárez, A. M., Ramírez, J.G. (2015). Evaluación Integral y Perspectivas del Sector Acuícola y Pesquero de Colombia 2015 – 2040. En Ortega-Lara O, Amado AC, Córdoba-Rojas DF, Barbosa LS (Eds.), *Avances de Acuicultura y Pesca Vol. I*. Autoridad Nacional de Acuicultura y Pesca (pp. 51 – 70). Oficina de Generación del Conocimiento y la Información – AUNAP ©, FUNINDES ©, Bogotá, DC.
Ray, A. J., Seaborn, G., Leffler, J. W., Wilde, S. B., Lawson, A., & Browdy, C. L. (2010). Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. *Aquaculture*, *310*(1–2), 130–138. https://doi.org/10.1016/j.aquaculture.2010.10.019
Rodríguez, L., & Landines, M. A. (2011). Evaluación de la restricción alimenticia sobre el desempeño productivo y fisiológico en juveniles de cachama blanca, *Piaractus brachypomus*, en condiciones de laboratorio. *Revista de la Facultad de Medicina Veterinaria y de Zootecnia*, *58*(III), 141–155.
Sánchez, L., & Vásquez, E. (1986). Estudio estacional y longitudinal de la hidroquímica y fitoplancton en una sección del bajo Orinoco (Venezuela). *Memoria Sociedad Ciencias Naturales La Salle*, *46*, 69–93.
Siikavuopio, S. I., & Sæther, B. S. (2006). Effects of chronic nitrite exposure on growth in juvenile Atlantic cod, Gadus morhua. *Aquaculture*, *255*(1–4), 351–356. https://doi.org/10.1016/j.aquaculture.2005.11.058
SINCHI y PEDICP (2014). *Guía para el manejo de peces en cautiverio. Modelos prácticos de producción piscícola. CESCAN II –Cooperación UE-CAN. Proyecto Especial Binacional Desarrollo Integral de la Cuenca del Río Putumayo – PEDICP* (p. 38). Bogotá, DC: Instituto Amazónico de Investigaciones Científicas. SINCHI.
Sobeck, D. C., & Higgins, M. J. (2002). Examination of three theories for mechanisms of cation-induced bioflocculation. *Water Research*, *36*(3), 527–538. https://doi.org/10.1016/S0043-1354(01)00254-8
Streble, H., & Krauter, D. (1987). *Atlas de los microorganismos de agua dulce: La vida en una gota de agua. ed*. Barcelona: Omega.
Thorp, J. H., & Covich, A. P. (Eds.) (2009). *Ecology and classification of North American freshwater invertebrates*, London, UK: Academic press.
Timmons, M. B., Ebeling, J. M., Wheaton, F. W., Summerfelt, S. T., & Vinci, B. J. (2002). *Recirculating Aquaculture Systems*, (2nd ed., p. 769). New York, NY: Cayuga Aqua Ventures.

Valladão, G. M. R., Gallani, S. U., & Pilarski, F. (2018). South American fish for continental aquaculture. *Reviews in Aquaculture*, *10*(2), 351–369. https://doi.org/10.1111/raq.12164

Vásquez-Torres, W., Hernández-Arévalo, G., Gutiérrez-Espinosa, M. C., & Yossa, M. I. (2012). Effects of dietary protein level on growth and serum parameters in cachama (*Piaractus brachypomus). Revista Colombiana de Ciencias Pecuarias*, *25*(3), 450–461.

Wang, G., Yu, E., Xie, J., Yu, D., Li, Z., Luo, W., ... Zheng, Z. (2015). Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus*. *Aquaculture*, *443*, 98–104. https://doi.org/10.1016/j.aquaculture.2015.03.015

Wei, Y., Liao, S. A., & Wang, A. L. (2016). The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs. *Aquaculture*, *465*, 88–93. https://doi.org/10.1016/j.aquaculture.2016.08.040

Weingartner, M., & Zaniboni Filho, E. (2004). Efeito de fatores abióticos na larvicultura de pintado amarelo *Pimelodus maculatus* (Lacépède, 1803): Salinidade e cor de tanque. *Acta Scientiarum Animal Sciences*, *26*(2), 151–157. https://doi.org/10.4025/actascianimsci.v26i2.1859

Zhang, N., Luo, G., Tan, H., Liu, W., & Hou, Z. (2016). Growth, digestive enzyme activity and welfare of tilapia (*Oreochromis niloticus*) reared in a biofloc-based system with poly-β-hydroxybutyric as a carbon source. *Aquaculture*, *464*, 710–717. https://doi.org/10.1016/j.aquaculture.2016.08.013

Zhukova, N. V., & Kharlamenko, V. I. (1999). Sources of essential fatty acids in the marine microbial loop. *Aquatic Microbial Ecology*, *17*(2), 153–157. https://doi.org/10.3354/ame017153

How to cite this article: Sandoval-Vargas L, Jiménez-Amaya M, Rodríguez-Pulido J, Guaje-Ramírez D, Ramírez-Merlano J, Medina-Robles V. Applying biofloc technology in the culture of juvenile of *Piaractus brachypomus* (Cuvier, 1818): Effects on zootechnical performance and water quality. *Aquac Res*. 2020;51:3865–3878. https://doi.org/10.1111/are.14734