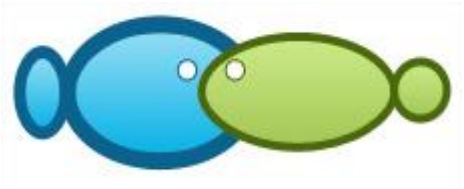

Biofloc application in larviculture of *Pterophyllum scalare* at different stocking densities

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Abstract. The current study aimed to evaluate the productive performance of *Pterophyllum scalare* post-larvae grown in biofloc at different stocking densities. We used 630 angelfish post-larvae of 1.76±0.29 mg of weight and 5.97±0.25 mm of total length, which were acclimated with biofloc water for five hours before stocking. The experiment was performed in a completely randomized design, with two treatments: 25 post-larvae L^{-1} (T25) and 50 post-larvae L^{-1} (T50), and three replicates per treatment. The experimental units consisted of six circular floating containers of 2.8 L useful, arranged directly inside a 7 m^3 tank with biofloc (macrocosm), previously established at a C/N ratio of 10/1. During 40 days, water quality parameters were within the appropriate ranges for both angelfish and biofloc. The T50 was statistically better in terms of weight, length and specific growth rate (131.81 mg, 17.97 mm and 10.97% day^{-1} respectively), however, the highest survival and highest final density were found in T25 (59.05% and 14.76 fingerlings L^{-1}). The biofloc application at a density of 25 post-larvae L^{-1} can be recommended to increase productivity (survival) and profitability in the larval stage of angelfish.
Key Words: angelfish, live food, survival, post-larvae, intensive culture.

Introduction. The fish farming of ornamental species presents a demand with a significant increase, according to Dey (2016) world exports of ornamental fish increased at an annual rate of 6.8% from 2000 to 2014 with a sale for values of US$ 177.7 and US$ 347.5 million respectively, where they mainly represented Asian countries (Singapore, Japan, Thailand, Malaysia and Indonesia), European countries (Czech Republic and Israel) and South American countries (Brazil and Colombia). Due to the biological diversity and variety of fish species with potential for aquarium hobby, Colombia has positioned itself as one of the world's largest providers of ornamental species (Duarte et al 2016). However, about 98% correspond to fish caught from the natural environment, which has led to a massive extraction of ornamental fish, in such a way that many species are classified as highly endangered (Panné & Luchini 2008).

Among the most popular species in the world of aquarium fish is the angelfish (*Pterophyllum scalare*, Lichtenstein 1823), a representative species of the South American ichthyofauna for its wide acceptance in the market (Chapman et al 1997), standing out for its beauty, diversity of varieties (paraiba, blushing, smoke, pearl, ghost, marble, gold, leopard, koi, and others), high price and easy adaptation in captivity, reasons why it has become a species with great productive potential (Ribeiro et al 2007).

The stage considered critical for fish culture is larviculture, an early stage where they face problems such as slow growth and high mortality, mainly due to the limited availability of live food; predator incidence; fluctuation in water quality; and repercussion of diseases during the first stages of life (Jiménez-Rojas et al 2012; Faizullah et al 2015). Generally, angelfish larviculture is developed in conventional systems, which manages low stocking densities, from 0.4 to 5 post-larvae L^{-1} (Deon et al 2017). However, actually, where production systems show a tendency to intensify cultivation, low stocking density could become a problem since it prevents the increase in productivity. Therefore, it is necessary to develop intensive culture that allows improving the productive

characteristics and population density in the larviculture of this species, making effective and efficient use of resources.

An alternative for the development of intensive culture during larviculture, is the implementation of biofloc technology (BFT), which could improve the quality and performance of the larvae (Ekasari et al 2015). This updated technology is based on promoting bacterial growth (autotrophs and heterotrophs) from a high carbon/nitrogen (C/N) ratio in water, in order to degrade organic and inorganic waste and control the water quality with minimal water renewal, with the purpose of producing aquatic organisms at high densities in a sustainable way (Avnimelech 2015; Collazos-Lasso & Arias-Castellanos 2015). Furthermore, the biofloc is a conglomerate of algae, protozoa, rotifers, cladocerans, nematodes and other microorganisms that are aggregated in the flocs and that can be used "in situ" as a source of microbial protein by cultured organisms or they can be processed into food ingredients (De Schryver et al 2008; Kuhn et al 2010; Ekasari 2014; Avnimelech 2015).

Various authors maintain that the application of biofloc during larviculture generates favorable effects on growth, survival and immunity against disease infections, improving the quality of fingerlings of various species such as: the Nile tilapia *Oreochromis niloticus* (Ekasari et al 2015), African catfish *Clarias gariepinus* (Fauji et al 2018), South American catfish *Rhamdia quelen* (Poli et al 2015), bocachico *Prochilodus magdalenae* (Atencio-García et al 2015) and pirarucú *Arapaima gigas* (Dantas 2018). On the other hand, it has been reported that cultivation in biofloc can generate greater productivity in ornamental fish, such as gold fish *Carassius auratus* (Faizullah et al 2015) and guppy *Poecilia reticulata* (Cunha 2016). Furthermore, Diatin et al (2019), demonstrated that biofloc technology allows to increase the initial density of corydoras (*Corydoras aeneus*) up to 6,000 fish m^{-2}, with a survival equivalent to 3,025 fish m^{-2}. Despite being the representative species in the ornamental fish market, the productive performance of angelfish in intensive systems and even more in biofloc systems is little studied. In this context, the present study aimed to evaluate the productive performance of *P. scalare* post-larvae in terms of growth and survival cultivated with biofloc technology at a C/N ratio (10/1) and different stocking densities.

Material and Method. A 40 days trial was conducted in the experimental unit of cultures with bioflocs, of Aquaculture Institute of the Llanos (IALL) attached to the Faculty of Agricultural Science and Natural Resources of the University of the Llanos, Villavicencio - Colombia (4°04'30"N, 73°35'07"W).

Biological material, experimental design and culture condition. The experimental units consisted of six circular plastic containers with an effective volume of 2.8 L, which had windows at the base, covered with 650 µm mesh opening SEFAR® screen and a hose ring at the top to allow buoyancy in the water. These floating containers were placed inside a macrocosm tank (Australian tank lined with high-density polyethylene geomembrane) with 7 m^3 of biofloc.

A total of 630 angelfish post-larvae of 168-hour post-hatching (HPH) with a mean weight of 1.76±0.29 mg and a total body length of 5.97±0.25 mm, obtained from the same reproduction, were used. Five hours prior to the start of the experiment, the post-larvae were acclimated to culture condition with biofloc water. Post-larvae were distributed in a completely randomized experimental design with two treatments and three replicates per treatment. The treatments consisted in two different stocking densities, T25 = 25 post-larvae L^{-1} and T50 = 50 post-larvae L^{-1}. The total number of individuals in each experimental unit of each treatment was 70 and 140 post-larvae respectively.

The biofloc was established and stabilized, at a carbon/nitrogen ratio (C/N) 10/1 following the methodology described by De Schryver et al (2008) and Avnimelech (2015). For this, a commercial fish feed with 32% crude protein was added as a nitrogen source, at an initial concentration of 2 mg L^{-1} of total ammoniacal nitrogen (Fauji et al 2018). At this stage, an organic carbon source was not added taking into account that the fish feed used supplied the necessary C/N ratio. From day 15, once the nitrification

process was verified, that is, a reduction of total ammoniacal nitrogen (TAN = NH_4^+ + NH_3), nitrite ($N\text{-}NO_2$) and an increase of nitrate ($N\text{-}NO_3$), an amount of commercial feed was added daily equivalent to 1 ppm of TAN. To guarantee saturated oxygen and suspended solids, a 0.5 HP blower with Aero-tube TM diffuser hose was used.

The post-larvae were fed with commercial concentrate of 38% crude protein at an initial rate of 40% of the initial biomass, distributed in five rations (08:00, 10:00, 12:00, 14:00 and 16:00 hours). To maintain the C/N ratio, the correction was made according to the amount of feed supplied, applying molasses (33.5% C) as a carbon source. It should be noted that in this study, brine shrimp was not used as the first feeding of the post-larvae, to take advantage of the diversity and abundance of planktonic microorganisms present in the biofloc.

Proximal analysis, characterization and abundance of plankton in the biofloc. At the beginning of the experiment, biofloc samples were collected from the macrocosm tank, filtering the necessary amount on a 100 µm mesh. The samples were dried in an oven at 102°C, stored at a temperature of -20°C, then they were ground and processed for the determination of the proximal composition of the biofloc according to the methodology established by AOAC (2005). Furthermore, the composition of the zooplankton present in the biofloc was analyzed, for which homogeneous subsamples were taken at five different points in the macrocosm tank and conserved in a 4% buffered formalin solution (Thompson et al 2002). The characterization and abundance of plankton was determined in triplicate in 1 mL subsamples, placed and fixed in a Sedgwick-Rafter chamber with Transeau solution, using an inverted microscope with a 20x objective, according to the methodology proposed by Atencio-García et al (2015).

Physical, chemical and biological analysis of water. The parameters of temperature, pH, dissolved oxygen (DO), oxygen saturation (OS) and conductivity were recorded daily in the macrocosm tank at 9:00 hours using the HANNA multiparameter (model HI98194). The parameters of total ammonia nitrogen (TAN), nitrite ($N\text{-}NO_2$), nitrate ($N\text{-}NO_3$), alkalinity and settleable solids (SS) were monitored three times a week. The concentrations of TAN, $N\text{-}NO_2$, $N\text{-}NO_3$ and total alkalinity were measured by photometry (YSI 9500), while the volume of the SS was measured with the Imhoff cone according to the methodology described by Eaton et al (1995).

Zootechnical parameters of angelfish post-larvae. At the end of the culture, the live fish of each experimental unit were quantified, afterwards, a biometry (total length mm and body weight g) was performed on a sample of 30% of the final population with a calibrator and analytical balance (OHAUS DV215CD 210 g/0.0001 g), to determine the final weight (mg); final length (mm); specific growth rate (% day^{-1}) = [(ln final weight – ln initial weight)/time in days] x 100; survival (%) = 100 x (number of final fish/number of initial fish) and final density (fingerlings L^{-1}) = [(number of final fish x 1000)/volume of the experimental unit].

Statistical analysis. Normality and homoscedasticity of the data were analyzed using the Shapiro - Wilk test and the Levene test, respectively. Subsequently, the production parameter data were subjected to a one-way analysis of variance, followed by the comparison of means using the Student's t test. The survival data were transformed (arcsine of the square root) before analysis. In all cases, the results were considered to be significant at 5% probability. For this, the InfoStat software version 2018 was used (Di Rienzo et al 2018).

Results

Proximal composition and abundance of plankton in the biofloc. The mean values of the proximal composition and the abundance of planktonic communities present in the biofloc at the beginning of the *P. scalare* post-larvae culture are shown in Table 1. At the beginning of the experiment, the proximal composition of the biofloc dry sample from the

macrocosm tank presented a value of 38.59±0.35% of crude protein, 1.34±0.11% of lipid, 17.78±1.63% of ash and 3 717.05±103.09 kcal kg^{-1} of gross energy.

According to the analysis of the plankton composition associated with the biofloc, a total of 113.40±21.42 organisms (org) mL^{-1} were identified, consisting mainly of amoebas of the genus Arcella, Centropyxis, Nebela and Amoeba (53.20±11.19 org mL^{-1}); microalgae of the genus Cyclotella, Scenedesmus, Desmodesmus and Microcystis (24.20±7.32 org mL^{-1}); cladocerans of the genus Macrothrix (16.8±10.83 org mL^{-1}) and rotifers of the genus Lecane, Epiphanes and Habrotrocha (16.8±9.66 org mL^{-1}), these four being the most representative groups. In addition, ciliates of the genus Euplotes (2.0±2.45 org mL^{-1}) and annelids such as Aelosoma (0.4±0.89 org mL^{-1}) were identified, these last two were the least abundant groups. The most abundant genera were: Arcella (21.6±13.22 org mL^{-1}), Nebela (20.8±9.01 org mL^{-1}), Macrothrix (16.8±10.83 org mL^{-1}), Lecane (15.2±12.21 org mL^{-1}) and Scenedesmus (12.6±7.3 org mL^{-1}) representing 19.05, 18.34, 14.81, 13.4 and 11.11% of planktonic microorganisms, respectively.

Table 1

Proximal composition and the abundance of planktonic communities present in the biofloc at the beginning of the *Pterophyllum scalare* post-larvae culture

Parameters		*Biofloc*
Proximal composition		% of dry sample
Crude protein		38.59±0.35
Lipid		1.34±0.11
Ash		17.78±1.63
Gross energy (kcal kg^{-1})		3 717.05±103.09
Abundance of microorganisms		Organisms mL^{-1}
Microalgae	Cyclotella	8.4±8.38
	Scenedesmus	12.6±7.3
	Desmodesmus	0.2±0.45
	Microcystis	3.0±3.94
Amoebae	Arcella	21.6±13.22
	Centropyxis	6.4±3.58
	Nebela	20.8±9.01
	Amoeba	4.4±3.29
Ciliates	Euplotes	2.0±2.45
Cladocerans	Macrothrix	16.8±10.83
Rotifers	Lecane	15.2±12.21
	Epiphanes	1.2±1.79
	Habrotrocha	0.4±0.89
Annelids	Aeolosoma	0.4±0.89

Data represent the mean of three replicates±standard deviation.

Water quality. Table 2 shows the physical, chemical and biological parameters of the water presented in the biofloc macrocosm tank during 40 days of scalar post-larvae cultivation. The water quality parameter scopes ranged: 26.24-28.6°C (temperature); 7.6-8.08 (pH); 6.22-7.4 mg L^{-1} (DO); 83.5-97.8% (SO); 2305-2414 µS cm^{-1} (conductivity); 0.12-2.23 mg L^{-1} (NAT); 0.25-1.53 mg L^{-1} ($N\text{-}NO_2$); 149.6-250.0 mg L^{-1} ($N\text{-}NO_3$); 89.5-135.0 mg $CaCO_3$ L^{-1} (alkalinity) and 20.0-34.0 mL L^{-1} (SS).

Table 2

Water quality parameters registered in the biofloc macrocosm tank during 40 days of *Pterophyllum scalare* post-larvae culture

Parameters	*Values*
Temperature (°C)	26.99±0.62
pH	7.60-8.08
OS (%)	86.66±4.17
DO (mg L^{-1})	6.79±0.34
Conductivity (µS cm^{-1})	2337.3±32.0
TAN (mg L^{-1})	0.42±0.49
N-NO_2 (mg L^{-1})	0.37±0.31
N-NO_3 (mg L^{-1})	207.14±47.07
Alkalinity (mg $CaCO_3$ L^{-1})	116.12±11.7
SS (mL L^{-1})	27.41±3.68

OS - Oxygen saturation, DO - dissolved oxygen, TAN - total ammoniacal nitrogen, N-NO_2 - nitrite, N-NO_3 – nitrate, SS - and settleable solids. Data represent the mean of three replicates±standard deviation.

Productive performance of post-larvae. In general, all the productive performance parameters of *P. scalare* post-larvae cultured in biofloc during a period of 40 days, were statistically different ($p<0.05$) between the evaluated treatments (Table 3).

The growth (final weight, final length and specific growth rate) of the post-larvae was significantly higher ($p<0.05$) in treatment T50 (131.81±68.94 mg, 17.97±3.57 mm and 10.97±1.66% day^{-1} respectively), compared to treatment T25 (38.78±19.42 mg, 13.57±1.76 mm and 7.7±1.44% day^{-1} respectively). However, the highest survival and total productivity was observed in the T25 treatment, obtaining 59.05±3.3% and 14.76±0.82 fingerlings L^{-1} respectively. In terms of survival, it was 38.81% higher than the post-larvae produced in the T50 treatment ($p<0.05$).

Table 3

Productive performance of *Pterophyllum scalare* post-larvae grown in systems with BFT during 40 days at different stocking densities [(1)]

Parameters	*Treatments*	
	T25 (25 post-larvae L^{-1})	*T50 (50 post-larvae L^{-1})*
FW (mg)	38.78±19.42[b]	131.81±68.94[a]
FL (mm)	13.57±1.76[b]	17.97±3.57[a]
SGR (% day^{-1})	7.41±1.34[b]	10.40±1.50[a]
Survival (%)	59.05±3.30[a]	20.24±2.97[b]
FD (fingerlings L^{-1})	14.76±0.82[a]	10.12±1.49[b]

[(1)] Means followed by equal letters in the rows do not differ among treatments by the one-way analysis of variance, at 5% probability. FW - Final weight, FL - final length, SGR - specific growth rate, FD - survival and final density. Data represent the mean of three replicates±standard deviation.

Discussion. Biofloc contains high levels of essential nutrients in its proximal composition, especially crude protein, essential fatty acids and amino acids (Azim & Little 2008). At the start of the experiment, the proximal composition of the dry biofloc sample from the macrocosm tank presented a value of 38.59±0.35% crude protein, 1.34±0.11% lipid, 17.78±1.63% ash, and 3717.05±103.09 kcal kg^{-1} gross energy, which was consistent with the levels reported by Crab et al (2010). Protein levels of 34-36% improve the productive performance of the *P. scalare* culture (Arévalo-Ibarra et al 2018), indicating that the protein level of the biofloc analyzed in the present study could supply the protein requirements of the species. However, the nutritional composition and the microbial composition associated with the biofloc can vary according to the organic carbon source (Ekasari 2014), the C/N ratio (De Schryver & Verstraete 2009), the physical, chemical and biological parameters of water (Maica et al 2012) and the population density to be used during cultivation (Widanarni et al 2012).

The success in the production of fish fingerlings depends largely on the availability of adequate microorganisms (size, palatability, digestibility and abundance) for the first feeding during the larval stage (Patra & Ghosh 2015). Regardless of abundance, the microbial composition associated with the biofloc were similar with that reported by Monroy-Dosta et al (2013) and Atencio-García et al (2015) who found bacteria, microalgae, amoebas, ciliates, rotifers, nematodes and annelids. For the present study, a total of 113.40±21.42 organisms (org) mL^{-1} were identified, mainly consisting of amoebae of the genus Arcella, Centropyxis, Nebela and Amoeba (46.91%); microalgae of the genus Cyclotella, Scenedesmus, Desmodesmus and Microcystis (21.34%); rotifers of the genus Lecane, Epiphanes and Habrotrocha (14.81%) and cladocerans of the genus Macrothrix (14.81%). The dominances of the first two groups are attributed to the C/N ratio used to establish the biofloc. Rotifers can generally fragment the flocs, feeding on the attached bacteria and microalgae, consequently, benefiting the productive performance of culture organisms (Thompson et al 2002). The most representative microorganism was the genus Arcella (19.05% of the total planktonic microorganisms) with an abundance of 21.60±13.22 org mL^{-1}, these, in addition to aiding in the elimination of nitrogenous compounds, favor the formation of the floc.

The variables of temperature (Pérez et al 2003), DO concentration (Patra & Ghosh 2015), pH (Ribeiro et al 2007), N-NO_2 concentrations (Serezli et al 2016) remained within acceptable levels for the adequate development of both the angelfish and the biofloc (Emerenciano et al 2017). The water temperature and DO remained stable with minimal variations (26.24-28.46°C and 6.22-7.40 mg L^{-1} respectively), this due to the dome and constant aeration placed in the macrocosm tank. During the experimentation period, the pH and alkalinity values tended to decrease, probably due to the high consumption of inorganic carbon by the dominant autotrophic bacterial communities in the biofloc used (C/N = 10/1), requiring the addition of alkalizers to maintain alkalinity and adequate pH during cultivation (Martins et al 2017). Photosynthetic communities (algae and bacteria) are capable of producing their own food by transforming solar energy into biomass through photosynthesis, during this process algae and bacteria (autotrophs) consume alkalinity and CO_2 to convert into microbial biomass (Avnimelech 2015). The conductivity showed higher values than those recommended for the angelfish culture, due to the application of sodium bicarbonate to maintain alkalinity above 100 mg $CaCO_3$ L^{-1}, however, the culture water in which post-larvae were housed did not significantly affect their development.

The concentration of TAN and N-NO_2 showed a tendency to decrease in the course of the study, on the other hand, the concentration of N-NO_3 and SS increased, indicating the efficiency of the autotrophic communities (photoautotrophs and chemoautotrophs) in the removal of nitrogenous compounds (Ebeling et al 2006). The maximum values of ammonia (N-NH_3) present in TAN in relation to pH and temperature according to the formula proposed by Emerson et al (1975), was 0.11 mg L^{-1}. Probably the NH_3 and NO_2 values did not affect the performance of the post-larvae, because these values were below the toxic levels considered as lethal concentration (LC_{50}) for *P. scalare* (Serezli et al 2016). These authors, when evaluating the toxicity of N-NH_3 and N-NO_2 in juveniles of angelfish exposed in waters with a pH of 7.2±0.2 and a temperature of 24.5±0.5°C, observed that concentrations greater than 0.576 mg N-NH_3 L^{-1} and 6.282 mg N-NO_2 L^{-1} can generate 50% mortality after 96 h of exposure.

The SS found in the macrocosm tank was 27.41±3.68 ml L^{-1}, showing similarities with the values obtained by Lima et al (2015). Furthermore, Avnimelech (2011) recommends for tilapia culture in biofloc systems to maintain the volume of settleable solids between 5 and 50 mL L^{-1}.

In the present study, a trend was observed to reduce survival and the number of fingerlings produced with increased stocking density. High population densities may be associated with stress conditions causing competition for space, greater aggressiveness among fish, and ultimately leading to low survivals due to cannibalism (Barros et al 2019). The survival of angelfish post-larvae cultured with BFT were affected with increasing population density ($p<0.05$). Similar trends were observed in the culture of red tilapia *Oreochromis sp.* (Widanarni et al 2012), Nile tilapia *Oreochromis niloticus*

(Lima et al 2015), African catfish *Clarias gariepinus* (Fauji et al 2018) and *Corydoras aeneus* (Diatin et al 2019). The highest survival rate and final density of angelfish fingerlings was found in the T25 treatment (59.05±3.30% and 14.76±0.82 fingerlings L^{-1}), being higher than the values reported by Gonçalves-Júnior et al (2013) for intensive farming of angelfish with 30% water changes per day. These authors reported survival rates of 100, 50, 58, 43, and 45% for the initial densities of 5, 10, 15, 20, and 25 post-larvae L^{-1} respectively, obtaining in the latter, the highest productivity (11.25 fingerlings L^{-1}).

However, the best growth in terms of final weight, final length and specific growth rate (131.81±68.94 mg, 17.97±3.57 mm and 10.97±1.66% day^{-1} respectively) occurred in the T50. Deon et al (2017), reported the best growth in the highest density (9.33 fish L^{-1}) obtaining a final weight of 1.271 g, final length of 38.92 mm and a specific growth rate of 9.89% day^{-1} in a culture of *P. scalare* with intensive cage systems. It is probable that the high mortalities presented in the treatment with higher densities have generated greater availability of living space and food for the better growth of the angelfish post-larvae. These results create great expectations in the intensive production of ornamental fish in plastic containers arranged in biofloc tanks with other cultivated species as additional income and thus are able to cover the energy cost.

Conclusions. *P. scalare* larviculture developed in bioflocs at a stocking density of 25 post-larvae L^{-1} could be recommended to increase productivity and profitability, obtaining a better survival (59.05±3.30%) and final density (14.76±0.82 fingerlings L^{-1}).

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