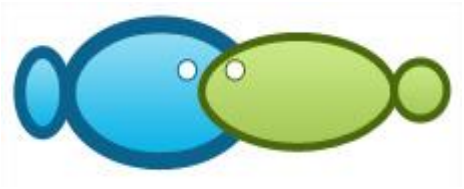

Survival of *Piaractus orinoquensis* larvae (Characiformes: Serrasalmidae) during acclimation to a culture system with biofloc

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Abstract. The fish larvae culture must be preceded by acclimation before stocking, which tries to control the environmental changes and variations in water quality to which the larvae are exposed when they pass from the incubation and yolk sack reabsorption containers to the culture tanks. The aim of this study was to assess the survival of *Piaractus orinoquensis* larvae of 92 hours post-hatching (HPH), at different acclimation times with biofloc water. For this purpose, 100 larvae were placed in a liter of water that came from the incubators where they reabsorbed the yolk sac, and 9 liters of biofloc water were added by dripping in five different times, as follows: treatment one (T1) of 30 minutes, T2 of 1 hour, T3 of 2 hours, T4 of 4 hours and T5 of 8 hours. Each treatment had three replicates and were provided with diffuser stones guaranteeing oxygen saturation. The physical and chemical parameters of the incubator and the biofloc water at the beginning of the experiment were measured and recorded, as was the water in each experimental unit at the end of each acclimation time. There were no differences in larval survival among the different acclimation times, resulting in the following survival rates: 86.3±12.9% (T1), 76.6±10.5% (T2), 90±4.6% (T3), 87.6±6.8 (T4) and 87.3±2.5% (T5). In conclusion, acclimation of 2 hours is enough to guarantee the survival of *P. orinoquensis* larvae in biofloc, as long as the water quality conditions are similar between incubation water and biofloc water where the larvae will be stocked.

Key Words: acclimation, biofloc, fish, larvae, survival, water quality.

Introduction. Larval culture is a critical stage in fish farming, and an understanding of the requirements in the early beginnings is necessary to establish production strategies in confinement (Portella et al 2014). *Piaractus brachypomus* is a South American species known as white cachama, paco, pirapitinga, morocoto, which recently was redescribed and identified a new species from the Orinoco River basin, classified as *Piaractus orinoquensis* by Escobar et al (2019). Its production in early stages presents important limitations, mainly in the outdoor extensive cultivation systems used for larvae and fingerlings culture, which subject small fish to all natural risks, especially adequate feed availability (Atencio 2001; David-Ruales et al 2018).

The *P. brachypomus* larvae have altricial characteristics with indirect ontogeny (Balón 1984), which means that at the beginning of the exotrophy they should in all cases have guaranteed conditions of water quality parameters on comfort scales and live food in permanent and adequate quality and quantity (David et al 2011). Under these conditions, conventional systems in which they continue to be cultivated in ponds on land at low densities (150 postlarvae m^{-3}) with previous fertilization, do not guarantee their greater survival (Atencio 2001; Giacometti-Mai & Zaniboni-Filho 2005). Other research has been reported with water replacement at high densities, 70 *P. brachypomus* larvae L^{-1}, with live food supply, obtaining experimental results that cannot be extrapolated to the productive sector (David et al 2011). Another system that is explored is intensive culture of white cachama with biofloc technology (10 larvae L^{-1}), with interesting results (Ueno et al 2018).

But whatever the system in which the larvae are cultivated, researchers and producers accept that stocking must be preceded by acclimation (Chippari-Gomes et al 1999; Collazos-Lasso & Arias 2007; Baldisserotto 2013; Abu Bakar et al 2015; Martins et al 2017), which tries to control the changes and environmental variations in water quality to which the larvae are exposed when they go from the containers with permanent recirculation (incubators most of the time), to the culture containers. This allows acclimation to be defined as the activity of balancing the water conditions to avoid the greatest possible trauma to the larvae during the larviculture initiation process. For this, gradual adjustments of the water parameters where the fish are contained and the water where they are intended to be cultivated are necessary, these adjustments are routinely only made to equalize temperature (Collazos-Lasso et al 2014).

Fish larvae have a tolerance and adaptation to relatively high variations in environmental conditions, for example thermal tolerance (which gives rise to the generic term "acclimation"), is remarkable if there is no thermal shock (Baldisserotto 2013). In this sense, different studies have determined the different scales of thermal variation for larvae of the genus *Piaractus* as well as pH, ammonium, ammonia, nitrite (Torres-Tabares et al 2007; Collazos-Lasso et al 2014; Barbieri & Vigliar-Bondioli 2015), since, as mentioned, abrupt changes in the parameters can generate extreme physiological responses and metabolic variations that the larvae do not always support.

Biofloc technology farming systems are known to be an alternative for early-stage fish culture (Ekasari et al 2015; Poli et al 2015; Fauji et al 2018), this is because they maintain the regulated water quality parameters from the removal and control of the toxic forms of nitrogen (NH_3 and NO_2^-) mainly (Ebeling et al 2006; Avnimelech 2015). This availability of nutrients generates conditions for the establishment of adequate trophic networks for small fish (Monroy-Dosta et al 2013; Betancur-González et al 2016; Ayazo-Genes et al 2019), the plankton organisms that are produced then serve as food for the ichthyoplankton of cultivation interest. In the case of *P. brachypomus* Ueno et al (2018), presents a preliminary report of water quality conditions in *P. brachypomus* larviculture experiments in a system with biofloc technology.

In this work, different acclimation experiments on 92-hour post-hatching (HPH) *P. orinoquensis* larvae were performed and survival responses at five different times were evaluated.

Material and Method

The experiment was carried out for a week in the rainy season (June 2019) at the Aquaculture Institute of the Llanos University - IALL, Villavicencio - Colombia.

The *P. orinoquensis* larvae were obtained by hormonal induction of the broodstock with carp pituitary extract (CPE, Stoller Fisheries), and international protocol. The fertilized eggs were kept in 200 L cone type upflow incubators supplied with a permanent recirculation system (RAS), with regulated temperature. Once hatched, the larvae were kept in this system until 92 HPH, average time in which they reabsorb the yolk sac by 90% (David-Ruales et al 2018).

Five acclimation times (5 treatments) were evaluated, with three replicates per treatment, and each experimental unit consisted of a cylindrical plastic containers system with a capacity of 10 L. In each replicate, 100 larvae with an average weight of 0.73±0.11 mg were placed in one liter of water (from the incubator), to which 9 liters of water from a biofloc with a C:N ratio of 15:1 was added by macro-drip (venoclysis cannula). This biofloc water was established based on the approaches of De Schryver et al (2008) and Avnimelech (2015), by adding fish feed with a protein level of 32% (humidity of 13%) as a nitrogen source, sugar cane molasses (33.65% of total organic carbon) was used as a carbon source and sodium bicarbonate as an alkalizing source ($NaHCO_3$).

Biofloc water was added at five different times, like this: T1 for 30 minutes, T2 for 1 hour, T3 for 2 hours, T4 for 4 hours and T5 for 8 hours. All the containers where the larvae were arranged, were provided with aeration through diffuser stones. Once the

biofloc water addition times were over, the water inlets were closed and left for two hours, afterwards the larvae were counted.

Water quality parameters of each experimental units were recorded at the beginning and at the end of each experiment, both for incubator and biofloc waters. Temperature, pH and oxygen (DO mg L^{-1} and OS %) were measured with the HANNA HI98194 multiparameter probe; total ammoniacal nitrogen concentrations (TAN = $NH4^+$ + NH_3 mg L^{-1}), nitrite (NO_2^- mg L^{-1}), nitrate (NO_3^- mg L^{-1}) and total alkalinity (TA mg $CaCO_3$ L^{-1}), with YSI photometer (Yellow Springs Instrument™) model 9500; settleable solids (SS ml L^{-1}), with Imhoff cones according to the methodology given APHA (1998), adapted by Avnimelech (2007).

Statistical analysis. An experimental one-way design was used, balancing fixed effect, incorporating the MANOVA technique, determining the dimensionality by means of the maximum likelihood function. The data expressed in percentage (survival rate) were transformed by arcsine technique before analysis. One-dimensional comparisons were made using the Tukey test, considering a significance level of 5%. The SAS University statistical package was used.

Results

Pre-acclimation water quality. Table 1 presents the physical and chemical conditions of the clear water from the recirculation system which was used to maintain the larvae up to 92 HPH, as well as the conditions of the biofloc water to which the acclimation was performed.

Table 1

Physical and chemical parameters of clear water (RAS) and biofloc water at the beginning of acclimation

Parameters	*RAS*	*Biofloc*
T (°C)	31.5±0.6[a]	29.2±0.9[b]
DO (mg L^{-1})	4.6±0.5	5.5±0.4
OS (%)	67.8±1.6[b]	78.2±2.0[a]
pH	7.3-7.6	8.3-8.5
TAN (mg L^{-1})	0.9±0.04[a]	0.04±0.01[b]
NO_2^- (mg L^{-1})	0.02±0.0[b]	0.28±0.02[a]
NO_3^- (mg L^{-1})	117.8±0.7[b]	222.4±2.5[a]
TA (mg $CaCO_3$ L^{-1})	25.0±1.0[b]	195.0±2.6[a]

Data presented as mean±standard deviation. Different letters on the same line indicate statistical difference by the t-test ($p<0.05$). T (temperature), DO (dissolved oxygen), OS (oxygen saturation), TAN (total ammonia nitrogen = NH_4^+ + NH_3), NO_2^- (nitrite), NO_3^- (nitrate) and TA (total alkalinity).

Post-acclimation water quality. Table 2 presents the record for the measured variables, being that there were differences between treatments for T, DO, OS, and TA. The canonical analysis that compared all the physical and chemical variables of water simultaneously, allowed to detect statistical differences among the treatments T1, T2, T3 compared to T4 and T5 treatments.

Table 2

Physical and chemical variables of the water after acclimation of *P. orinoquensis* larvae with biofloc water at five different times

Variables	*Treatments*				
	T1	*T2*	*T3*	*T4*	*T5*
T (°C)	29.5±0.5[a]	28.6±0.3[b]	27.9±0.1[b]	27.0±0.1[c]	25.6±0.1[d]
DO (mg L^{-1})	4.5±0.6[b]	5.6±0.06[a]	5.2±0.2[ab]	5.9±0.5[a]	6.1±0.06[a]
OS (%)	65.0±9.8[b]	79.5±1.5[ab]	71.9±2.8[ab]	80.1±5.6[a]	79.3±3.3[ab]

pH	8.4-8.5	8.5-8.6	8.5-8.6	8.4-8.5	8.4-8.5
TAN (mg L^{-1})	0.6±0.3	0.7±0.4	2.6±1.1	2.2±1.9	2.0±1.8
NO_2^- (mg L^{-1})	0.2±0.02	0.2±0.03	0.2±0.03	0.2±0.01	0.2±0.02
NO_3^- (mg L^{-1})	226.1±68.9	165.6±18.5	204.1±53.1	220.6±35.1	222.9±89.8
TA (mg $CaCO_3$ L^{-1})	153.3±7.6^b	161.6±14.4^{ab}	171.6±15.2^{ab}	151.6±10.4^b	200.0±21.8^a

MANOVA	Multivariate analysis of variance		
Test	Value	F	Pr > F
Wilks' Lambda	0.00015410	3.88	0.0067
Pillai's Trace	3.0415793	2.38	0.0155
Hotelling-Lawley T	129.2850047	18.65	0.0275
Roy's Greatest	117.75849520	88.32	< 0.0001

Comparison	Canonical analysis				
	T1^a	T2^a	T3^a	T4^b	T5^c

Data presented as mean±standard deviation. Different letters in the same line indicate statistical differences by the Tukey test ($p<0.05$). T (temperature), DO (dissolved oxygen), OS (oxygen saturation), TAN (total ammonia nitrogen = $NH_4^+ + NH_3$), NO_2^- (nitrite), NO_3^- (nitrate) and TA (total alkalinity).

Larvae survival. No statistical difference was detected between the treatments when evaluating the percentage of live larvae. See Table 3 and Figure 1.

Table 3

Survival of *P. orinoquensis larvae* acclimated with biofloc water during five different times

Variable	Treatments				
	T1 (1/2 hour)	T2 (1 hour)	T3 (2 hours)	T4 (4 hours)	T5 (8 hours)
Survival (%)	86.3±12.9^a	76.6±10.5^a	90.0±4.6^a	87.6±6.8^a	87.3±2.5^a

Data presented as mean±standard deviation. Different letters indicate significant difference ($p < 0.05$).

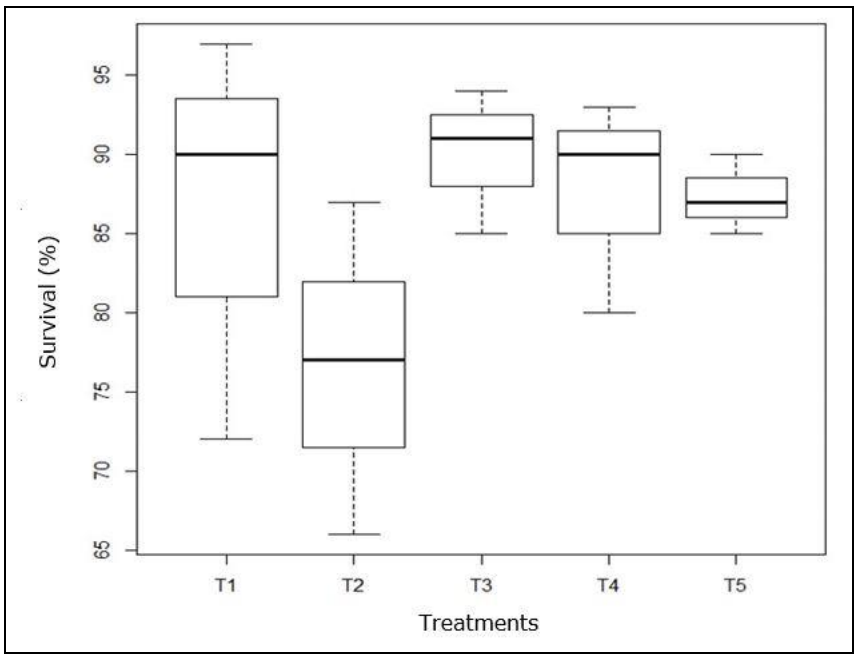

Figure 1. Survival of *P. orinoquensis* larvae after acclimation at five different times.

Discussion. The physical and chemical parameters of the water during the first 92 HPH - RAS compared with those of biofloc presented statistically significant differences ($p < 0.05$), for all the parameters, except DO, being that the mean concentrations of TAN were higher in the RAS and the mean values of pH, TA, NO_2^- and NO_3^- lower, being that both types of water presented adequate conditions and within the recommended ranges for the larvae (Emerenciano et al 2017).

For temperature, differences were found between treatments with an inverse relationship to acclimation time, being that the lowest temperature (25.6±0.1°C) occurred in the longest acclimation time (T5: 8 hours), which allows confirming that at the longer acclimation time, the water tends to cool down considering the small volume of the experimental water. However, the temperatures of the five treatments were on the comfort scales for white cachama larvae (22-29°C) as reported by Collazos-Lasso et al (2014). The pH was statistically similar between treatments, remaining between 8.4-8.6, these values were close to those reported for the cultivation of pacú (*Piaractus mesopotamicus*) by Pellegrin et al (2019). Values for oxygen measurements were on the recommended ranges for fish farming in biofloc (Emerenciano et al 2017).

Nitrogen compound concentrations were statistically similar between treatments. The highest concentrations of TAN were found in T3, T4 and T5, being lower than the lethal concentration 50 (LC50) for fingerlings of *Piaractus mesopotamicus* (1.2±0.3 g), defined as 4.16 mg L^{-1} TAN ($NH4^+$ + NH3) at a pH between 6.78-7.15 and temperature of 25°C (Barbieri & Vigliar-Bondioli 2015). The NO_2^- concentrations for the five treatments had an average value of 0.2 mg L^{-1}, being lower than the report by Torres-Tabares et al (2007), for *P. brachypomus* juveniles (39.5±0.09 g), which at a concentration of 4 mg L^{-1} for 48 hours, presented a mortality of 20% at a pH of 5.7±0.04 and a temperature of 24°C; in this study it is confirmed that the toxicity of nitrite is directly related to pH, being that when it is alkaline the larvae have a higher tolerance than when it is acidic, this explained from the higher percentage of nitrous acid (HNO_2). Another factor that reduces toxicity is the high concentration of chlorine ions (Cl^-), occupying the antiport Cl^-/HCO_3^- and thus preventing the transport and entry of nitrite by the branchial membrane (Baldisserotto 2013).

The NO_3^- and TAN concentrations that were recorded show the dynamics of the nitrification processes that occur in biofloc waters, thus the increase in nitrate decreases the TAN concentrations, with consequent consumption and products (Ebeling et al 2006). On the other hand, the direct relationships found between NO_2^-, pH, and TA, are explained in the nitrification processes in biofloc waters carried out by their autotrophic and heterotrophic communities, responsible for the oxidation processes (nitritation and nitration) and reduction of nitrogen forms (Ray & Lotz 2014; Martins et al 2017; Jiménez-Ojeda et al 2018; Ueno-Fukura et al 2019), with the consequent decrease in the concentrations of total alkalinity (mg $CaCO_3$ L^{-1}), which decreases the buffer capacity that is reflected in changes in pH over time (Kubitza 2017), and establishes the other direct relationship between TA and pH given by oxidation of NO_2^- (Ekasari et al 2015; Luo et al 2017). Considering the above, the survival that occurred in the five acclimation times was not influenced by the NO_2^- concentrations since the levels in the treatments were not lethal for the larvae and the pH values can be attributed to the alkalinities higher than 150 mg $CaCO_3$ L^{-1} in which the experiments were carried out.

The statistical difference shown (canonical analysis) in the physical-chemical variables of water between treatments T1, T2 and T3 compared with T4 and T5, could have been influenced by the lower temperatures in T4 and T5, (Collazos-Lasso et al 2014). Similar situations were recorded in the culture of *Rhamdia sebae* and *Rhamdia quelen* larvae (Chippari-Gomes et al 1999; Collazos-Lasso & Arias 2007).

The values, relationships, and interactions of the water quality parameters before, during and after acclimation had no effect on the survival of the larvae, suggesting great capacity for metabolic adjustments, energy expenditure and behavioral changes of the larvae.

Conclusions. The canonical analysis of the parameters allows to recommend practicing acclimation in any case between 30 minutes and 2 hours, considering that the highest percentage of live larvae occurred in T2 corresponding to 2 hours. If the water quality conditions are similar between the incubation water and the biofloc water in which the *Piaractus orinoquensis* larvae are going to be stocked, they do not require a longer acclimation time.

Acknowledgments. The authors express their gratitude to the General Direction of Research of the Universidad de Los Llanos, for funding the present research.

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Received: 04 July 2020. Accepted: 18 September 2020. Published online: 27 August 2021.
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How to cite this article:
Collazos-Lasso L. F., Ueno-Fukura M., Arias-Castellanos J. A., 2021 Survival of *Piaractus orinoquensis* larvae (Characiformes: Serrasalmidae) during acclimation to a culture system with bioflocs. AACL Bioflux 14(4):2327-2334.