



# Effect of artificial seawater and feeding frequency on the larval culture of freshwater Amazonian ornamental fish banded cichlid *Heros severus* (Heckel, 1840) and angelfish *Pterophyllum scalare* (Schultze, 1823)

Bruno J. C. F. Eiras<sup>1</sup>, Galileu C. Veras<sup>2</sup>, Adriana X. Alves<sup>1</sup> and Rauquীরio M. da Costa<sup>2</sup>

<sup>1</sup>Universidade Federal do Pará, Campus de Castanhal, Instituto de Medicina Veterinária. BR-316, km 61, 68740-970 Castanhal, Pará, Brazil.

<sup>2</sup>Universidade Federal do Pará, Campus de Bragança, Instituto de Estudos Costeiros. Av Leandro Ribeiro s/n. 68600-000 Bragança, Pará, Brazil.

## Abstract

The present study aimed to evaluate the effect of salinity and feeding frequency on zootechnical performance of *Pterophyllum scalare* and *Heros severus* five-day-old post-larvae. Two experiments were performed in a completely randomized experimental design in a  $5 \times 2$  factorial scheme, with 5 different NaCl concentrations (0, 2, 4, 6 and 8 g/L) and 2 feeding frequencies (2 and 4 times a day). *P. scalare* showed the highest survival rates ( $p < 0.05$ ) when subjected to salinities of 0, 2 and 4 g/L (97.50–96.25%), and higher values ( $p < 0.05$ ) for standard length (13.22 mm), weight (64.64 mg) and specific growth rate (15.41% per day) when fed 4 times a day. For this species, feeding frequency did not influence survival rates. *H. severus*, in turn, showed higher survival rates in water without the addition of salt (96.25%) and the highest standard length in salinity of 2 g/L (11.80 mm). *H. severus* fed 4 times a day and presented the highest values ( $p < 0.05$ ) for most of the growth variables (weight: 57.28 mg, specific growth rate: 18.30% per day). The results of the present study suggest that banded cichlid post-larvae showed higher survival rates in water without salt addition, however, the best growth of this species occurred at the salinity of 2 g/L. In contrast, angelfish post-larvae can be cultivated in salinities of up to 4 g/L NaCl. A feeding frequency of 4 times per day is recommended for both species.

**Additional keywords:** freshwater ornamental fish; larviculture; salt addition; food supply; zootechnical performance.

**Abbreviations used:** CV (coefficient of variation); DO (dissolved oxygen); EC (electrical conductivity); K (allometric condition factor); LG (length gain); pH; SGR (specific growth rate); SL (standard length); SR (survival rate); T (temperature); USL (uniformity in standard length); UW (uniformity in weight); W (weight); WG (weight gain).

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**Correspondence** should be addressed to Bruno J. C. F. Eiras: [bruno\\_eiras@hotmail.com](mailto:bruno_eiras@hotmail.com)

## Introduction

The angelfish (*Pterophyllum scalare*, Schultze, 1823) and the banded cichlid (*Heros severus* Heckel, 1840) are endemic cichlid species from South America, occurring in the Negro, Amazonas, Solimões and Orinoco rivers and inhabiting calm waters containing extensive vegetation. The angelfish can grow up to 13 cm in length with dorsal, anal and pelvic fins that are extremely long and of a varied range of colors, such as silvery and darker shades. The banded cichlid, in

turn, can grow up to 20 cm in length and are most often a greyish color with eight dark vertical stripes. Both species reproduce in vegetated areas and display good parental care (Lowe-McConnell, 1969; Kullander, 2003).

Many ornamental cichlids such as the angelfish and the banded cichlid are highly valued in the national and international aquarium market due to their extravagant beauty. Knowledge of the cultivation techniques for these and most ornamental fish from South America is still rudimentary. One of the critical points in the development of ornamental fish is the culture of larvae,

which is a stage of great animal fragility and a low survival rate. At this stage, live food is often used for the nutrition of the cultivated organisms, especially *Artemia* sp. nauplii (Concei o *et al.*, 2010). *Artemia* sp. are naturally distributed in saline environments and their survival in freshwater is extremely low, which leads to a reduction in its availability (due to increased mortality) in freshwater fish post-larvae cultures, with negative outcomes in food consumption, larval development and water quality (Beux & Zaniboni-Filho, 2006).

Previous studies have shown that low concentrations of NaCl in freshwater can produce a significant increase in *Artemia* sp. nauplii survival (Beux & Zaniboni-Filho, 2006), thus stimulating consumption of this organism by post-larvae of some neotropical freshwater fish species of commercial importance to human consumption (Luz & Portella, 2002; Beux & Zaniboni-Filho, 2007; Luz & Santos, 2010; Jomori *et al.*, 2013).

In addition, the use of low NaCl concentrations in fish farming has demonstrated therapeutic properties, such as the reduction of stress in cultivation (Sampaio & Bianchini, 2002) and transport (Diniz & Honorato, 2012).

Among the factors that affect the development and survival of fish post-larvae, one of the most important is the feeding frequency, although it has been poorly studied in native fish larviculture (Luz & Portella, 2005). In general, fish larvae present higher growth rates and lower digestive capacities compared to other life stages, therefore requiring a higher frequency of feeding (Xie *et al.*, 2011). However, this frequency varies according to species (Cho *et al.*, 2003). Determining the best feeding frequency is important for avoiding appetite reduction, loss of weight, growth reduction, batch dissimilarity, impairment of water quality, and for promoting animal welfare (Veras *et al.*, 2016a).

The hypothesis of the present study is that the use of low concentrations of NaCl coupled with an adequate feeding frequency in larviculture of the angelfish (*P. scalare*) and banded cichlid (*H. severus*) improves larvae performance and enhances growth, uniformity, and survival rates, thus contributing to the development of cultivation techniques that favor the larviculture of these valuable Amazonian ornamental fish.

## Material and methods

### Experimental design

Research on animals was conducted according to the Institutional Animal Care and Use Committee (License no.: 7656100517). The experiments were performed

with angelfish and banded cichlid five-day-old post-larvae for a period of 15 days, the transitional period from post-larvae to fingerlings. For each species, a total of 400 five-day-old post-larvae from the same spawning were used. Larvae were directly transferred to different salinities one day before the beginning of experiment. Post-larvae's first biometrics were taken on the first day of the experiment, using a representative sample of ten specimens of each species in the experiment. The angelfish post-larvae had weight of  $6.40 \pm 1.46$  mg and standard length of  $5.75 \pm 0.36$  mm; related values for the banded cichlid were  $3.60 \pm 0.63$  mg and  $5.48 \pm 0.27$  mm, respectively. For each experiment, 40 transparent plastic containers (henceforth referred to as experimental units) with a 1 L volume were used, containing 10 post-larvae each.

The experiments were developed using a completely randomized design with a  $5 \times 2$  factorial scheme with five different NaCl concentrations (0; 2; 4; 6 and 8 g/L) and two feeding frequencies (2 or 4 times per day), with four replicates each. Post-larvae were fed twice a day at 08:00 and 17:00 h or four times a day at 08:00; 11:00; 14:00 and 17:00 h, respectively.

Post-larvae of both species were fed 1,000 *Artemia* sp. nauplii per day (Veras *et al.*, 2016b), divided between the different feeding periods. One hour after the final daily feeding, the bottom of all the experimental units were siphoned for the elimination of feces and food remnants.

### Experimental conditions

A photoperiod of 12 h light and 12 h dark was used during the experiments, a condition similar to that observed in equatorial regions. Concentrations of dissolved oxygen (DO), pH, temperature (T) and electrical conductivity (EC) of water were monitored daily using a multiparameter probe (Horiba U-10, USA). The concentrations of ammonia and nitrite were measured every 3 days using commercial water analysis kits (LabconTest, Brazil). The initial values for water quality in the larviculture of banded cichlid and angelfish are shown in Table 1.

### Biometrics

A count of the post-larvae and biometrics were performed at the beginning and end of the experimental period. For this, the post-larvae were anesthetized with eugenol (100 mg/L) according to Vidal *et al.* (2008), counted and measured with a trinocular stereoscopic microscope equipped with a micrometer eyepiece (QUIMIS Q740SZ-T, Brazil), and then weighed with an

**Table 1.** Initial physical-chemical variables of water (mean values  $\pm$  standard deviation) in *Heros severus* and *Pterophyllum scalare* larviculture.

Salinity (g/L)	DO (mg/L)	T (°C)	EC (mS/cm)	pH	Ammonia (mg/L)	Nitrite (mg/L)
<i>Heros severus</i>						
0	3.60 $\pm$ 0.35	27.34 $\pm$ 0.26	0.17 $\pm$ 0.02	6.12 $\pm$ 0.21	0	0
2	3.91 $\pm$ 0.42	27.06 $\pm$ 0.10	4.07 $\pm$ 0.28	6.17 $\pm$ 0.13	0	0
4	4.07 $\pm$ 0.40	27.06 $\pm$ 0.06	7.49 $\pm$ 0.09	6.14 $\pm$ 0.05	0	0
6	4.07 $\pm$ 0.40	27.06 $\pm$ 0.06	7.49 $\pm$ 0.09	6.14 $\pm$ 0.05	0	0
8	4.95 $\pm$ 0.31	27.11 $\pm$ 0.05	15.83 $\pm$ 0.10	6.01 $\pm$ 0.16	0	0
Feeding						
2 times/day	3.89 $\pm$ 0.55	27.20 $\pm$ 0.21	7.62 $\pm$ 5.40	6.19 $\pm$ 0.06	0	0
4 times/day	4.48 $\pm$ 0.44	27.10 $\pm$ 0.11	7.77 $\pm$ 5.42	6.03 $\pm$ 0.17	0	0
<i>Pterophyllum scalare</i>						
0	7.60 $\pm$ 0.08	27.65 $\pm$ 0.08	2.24 $\pm$ 0.05	6.70 $\pm$ 0.02	0	0
2	7.58 $\pm$ 0.04	27.93 $\pm$ 0.08	4.61 $\pm$ 0.03	6.19 $\pm$ 0.12	0	0
4	7.55 $\pm$ 0.05	27.85 $\pm$ 0.09	8.19 $\pm$ 0.13	6.50 $\pm$ 0.04	0	0
6	7.63 $\pm$ 0.04	27.88 $\pm$ 0.08	11.23 $\pm$ 0.09	6.55 $\pm$ 0.02	0	0
8	7.68 $\pm$ 0.04	27.85 $\pm$ 0.17	14.23 $\pm$ 0.13	6.77 $\pm$ 0.03	0	0
Feeding						
2 times/day	7.61 $\pm$ 0.05	27.85 $\pm$ 0.14	8.04 $\pm$ 4.32	6.54 $\pm$ 0.24	0	0
4 times/day	7.63 $\pm$ 0.09	27.80 $\pm$ 0.15	8.16 $\pm$ 4.35	6.54 $\pm$ 0.17	0	0

DO: dissolved oxygen; T: temperature; EC: electrical conductivity.

analytical balance with 0.1 mg readability (GEHAKA AG200, Brazil).

Post-larvae measurements of standard length (SL) and weight (W) were used to calculate several parameters as follows.

— Survival rate (%):

$$SR = \left( \frac{N_e}{N_i} \right) \times 100 \quad (1)$$

where  $N_e$  is the number of live post-larvae for each treatment at the end of the experiment and  $N_i$  is the number of post-larvae at beginning of the experiment.

— Weight gain (mg):

$$WG = W_e - W_i \quad (2)$$

where  $W_e$  is the final average weight of the post-larvae for each treatment at the end of the experiment, and  $W_i$  is the initial average weight of the post-larvae.

— Length gain (mm):

$$LG = L_e - L_i \quad (3)$$

where  $L_e$  is the average length of the post-larvae in each treatment group at the end of the experiment and  $L_i$  is the average length of the post-larvae at the beginning of the experiment.

— Specific growth rate (% per day):

$$SGR = \left[ \frac{(\ln W_e - \ln W_i)}{t} \right] \times 100 \quad (4)$$

where t is the duration of the experimental period.

— Uniformity in weight (%):

$$UW = \left( \frac{N_{\pm 20\%}}{N_t} \right) \times 100 \quad (5)$$

where  $N_{\pm 20\%}$  is the number of post-larvae with weight varying by  $\pm 20\%$  of the average value for each experimental unit, and  $N_t$  is the total number of post-larvae in each experimental unit (Furuya *et al.*, 1998).

— Uniformity in standard length (%):

$$USL = \left( \frac{N_{\pm 20\%}}{N_t} \right) \times 100 \quad (6)$$

where  $N_{\pm 20\%}$  is the number of post-larvae with standard length varying by  $\pm 20\%$  of the average value for each experimental unit adapted by Furuya *et al.* (1998).

— Allometric condition factor:

$$K = \frac{W}{SL^b} \quad (7)$$

where  $W$  is the weight,  $SL$  is the standard length and  $b$  is the angular coefficient obtained by the equation of linear regression between weight and

standard length transformed by logarithm to base 10 (Vazzoler, 1996).

### Statistical analysis

Data obtained for the studied variables were analyzed for normality and homogeneity of variance using Levene's Test. Normally distributed and homoscedastic data were submitted to an analysis of variance (ANOVA), followed by Tukey's post-hoc test to verify the existence of differences between the treatments used. When necessary, the data were transformed (exponential or arc sine) to achieve normality. For non-normal data, even after the transformations referred above, Kruskal-Wallis and Mann-Whitney (U) nonparametric tests were used. For statistical analyses, a significance level of 5% was used. All analyses were performed using the ASSISTAT 7.7 program (Silva & Azevedo, 2016).

## Results

In the case of treatment with salinity of 8 g/L, it was not possible to analyze the post-larvae development for either species tested (angelfish and banded cichlid), since the reduced number of individuals during the experiments did not allow statistical analysis, except for the inference of survival rate-SR (Tables 2 and 3).

### *Heros severus*

The highest SRs ( $p < 0.05$ ) were recorded for post-larvae maintained in freshwater, while there was a reduction in for individuals maintained at salinities between 2 and 6 g/L. Individuals maintained in a concentration of 8 g/L of NaCl demonstrated a lower SR ( $p < 0.05$ ) than other treatments (Table 2).

The uniformity in weight (*UW*) and uniformity in standard length (*USL*) of banded cichlid post-larvae cultivated at salinities of 0, 2 and 4 g/L did not differ from each other ( $p > 0.05$ ), although they presented higher values than specimens maintained at a salinity of 6 g/L ( $p < 0.05$ ; Table 2). Individuals cultivated in a salinity of 0, 2 and 4 g/L showed similar allometric condition factor (*K*) values ( $p > 0.05$ ). Exposure to a salinity of 6 g/L produced a lower *K* value in relation to the 2 g/L treatment ( $p < 0.05$ ).

The standard length (*SL*) and length gain (*LG*) of banded cichlid post-larvae were similar ( $p > 0.05$ ) at salinities of 0, 2 and 4 g/L, with lower values observed for both variables at 6 g/L in comparison to the 2 g/L treatment (Table 2). No effect of salinity ( $p > 0.05$ ) was observed on the weight (*W*), weight gain (*WG*) and the specific growth rate (*SGR*) of banded cichlid post-larvae in different treatment.

The same pattern was observed for feeding frequency ( $p > 0.05$ ) on *SL*, *LG*, *UW*, *USL* and *SR* of banded cichlid post-larvae. However, the feeding frequency showed a significant effect ( $p < 0.05$ ) on *W*, *WG*, *K* and *SGR* of banded cichlid post-larvae, and individuals that were

**Table 2.** Productive performance (mean values  $\pm$  standard deviation) of *Heros severus* post-larvae at the end of the 15-day experiment.

Salinity (g/L)	<i>SL</i> (mm)	<i>LG</i> (mm)	<i>W</i> (mg)	<i>WG</i> (mg)	<i>SGR</i> (%/day)	<i>SR</i> (%)	<i>UW</i> (%)	<i>USL</i> (%)	<i>K</i>
0	11.63 $\pm$ 0.40 <sup>ab[2]</sup>	6.15 $\pm$ 0.40 <sup>ab</sup>	54.41 $\pm$ 5.62 <sup>a</sup>	50.74 $\pm$ 5.62 <sup>a</sup>	17.95 $\pm$ 0.67 <sup>a</sup>	96.25 $\pm$ 1.77 <sup>a</sup>	77.85 $\pm$ 6.05 <sup>a[3]</sup>	100.00 <sup>a</sup>	1.88 $\pm$ 0.16 <sup>ab</sup>
2	11.80 $\pm$ 0.31 <sup>a</sup>	6.31 $\pm$ 0.31 <sup>a</sup>	57.60 $\pm$ 3.58 <sup>a</sup>	53.94 $\pm$ 3.58 <sup>a</sup>	18.35 $\pm$ 0.41 <sup>a</sup>	87.50 $\pm$ 3.54 <sup>b</sup>	78.41 $\pm$ 6.10 <sup>a</sup>	100.00 <sup>a</sup>	1.92 $\pm$ 0.14 <sup>a</sup>
4	11.59 $\pm$ 0.49 <sup>ab</sup>	5.92 $\pm$ 0.49 <sup>ab</sup>	55.16 $\pm$ 3.18 <sup>a</sup>	51.50 $\pm$ 3.18 <sup>a</sup>	18.06 $\pm$ 0.38 <sup>a</sup>	87.50 $\pm$ 8.29 <sup>b</sup>	76.51 $\pm$ 10.70 <sup>a</sup>	100.00 <sup>a</sup>	1.88 $\pm$ 0.15 <sup>ab</sup>
6	10.65 $\pm$ 0.89 <sup>b</sup>	5.17 $\pm$ 0.89 <sup>b</sup>	51.72 $\pm$ 0.45 <sup>a</sup>	48.05 $\pm$ 8.62 <sup>a</sup>	17.55 $\pm$ 1.05 <sup>a</sup>	62.50 $\pm$ 10.61 <sup>c</sup>	36.28 $\pm$ 25.62 <sup>b</sup>	71.3 $\pm$ 19.91 <sup>b</sup>	1.73 $\pm$ 0.44 <sup>b</sup>
8	-	-	-	-	-	8.57 $\pm$ 10.61 <sup>d</sup>	-	-	-
Feeding									
Two times/day	11.25 $\pm$ 0.67 <sup>a</sup>	5.76 $\pm$ 0.67 <sup>a</sup>	52.51 $\pm$ 6.15 <sup>b</sup>	48.85 $\pm$ 6.15 <sup>b</sup>	17.70 $\pm$ 0.75 <sup>b</sup>	67.89 $\pm$ 39.15 <sup>a</sup>	66.18 $\pm$ 26.35 <sup>a[4]</sup>	92.75 $\pm$ 15.57 <sup>a</sup>	1.82 $\pm$ 0.26 <sup>b</sup>
Four times/day	11.55 $\pm$ 0.75 <sup>a</sup>	6.06 $\pm$ 0.75 <sup>a</sup>	57.28 $\pm$ 5.29 <sup>a</sup>	53.61 $\pm$ 5.29 <sup>a</sup>	18.30 $\pm$ 0.64 <sup>a</sup>	72.00 $\pm$ 33.42 <sup>a</sup>	70.48 $\pm$ 25.06 <sup>a</sup>	92.90 $\pm$ 16.27 <sup>a</sup>	1.91 $\pm$ 0.22 <sup>a</sup>
Salinity <sup>[5]</sup>	0.01*	0.01*	0.29 <sup>NS</sup>	0.29 <sup>NS</sup>	0.19 <sup>NS</sup>	<0.01*	<0.01*	<0.01*	0.01*
Feeding <sup>[5]</sup>	0.07 <sup>NS</sup>	0.07 <sup>NS</sup>	0.03**	0.03**	0.03**	0.14 <sup>NS</sup>	0.5 <sup>NS</sup>	>0.05 <sup>NS</sup>	0.04**
Interaction <sup>[5]</sup>	0.06 <sup>NS</sup>	0.06 <sup>NS</sup>	0.52 <sup>NS</sup>	0.52 <sup>NS</sup>	0.57 <sup>NS</sup>	0.68 <sup>NS</sup>	0.79 <sup>NS</sup>	-	0.19 <sup>NS</sup>
CV (%) <sup>[6]</sup>	42.98	42.84	10.53	11.29	3.85	8.43	25.41	17.16	2.6

*SL*: standard length; *LG*: length gain; *W*: weight; *WG*: weight gain; *SGR*: specific growth rate; *SR*: survival rate; *UW*: uniformity in weight; *USL*: uniformity in standard length; *K*: allometric condition factor. <sup>[2]</sup>In each column, values followed by the same letter do not differ from each other according to Tukey's test at 5% probability. <sup>[3]</sup>In each column, values followed by the same letter do not differ from each other using Kruskal-Wallis test at 5% probability. <sup>[4]</sup>Data analysed using the Mann-Whitney test (U). <sup>[5]</sup>*p* value. \*  $p < 0.01$ , \*\*  $p < 0.05$ . <sup>NS</sup>: not significant ( $p > 0.05$ ). <sup>[6]</sup>Coefficient of variation.

**Table 3.** Productive performance (mean values  $\pm$  standard deviation) of *Pterophyllum scalare* post-larvae at the end of the 15-day experiment.

Salinity (g/L)	SL (mm)	LG (mm)	W (mg)	WG (mg)	SGR (%/day)	SR (%)	UW (%)	USL (%)	K
0	13.02 $\pm$ 0.16 <sup>[2]</sup>	7.27 $\pm$ 0.16 <sup>a</sup>	64.55 $\pm$ 4.10 <sup>a</sup>	58.15 $\pm$ 4.10 <sup>a</sup>	15.39 $\pm$ 0.42 <sup>a</sup>	97.50 $\pm$ 5.00 <sup>a</sup>	73.13 $\pm$ 6.58 <sup>a</sup>	100	2.13 $\pm$ 0.16 <sup>a</sup>
2	13.04 $\pm$ 0.27 <sup>a</sup>	7.29 $\pm$ 0.27 <sup>a</sup>	61.14 $\pm$ 4.47 <sup>a</sup>	54.74 $\pm$ 4.47 <sup>a</sup>	15.02 $\pm$ 0.50 <sup>a</sup>	97.50 $\pm$ 5.00 <sup>a</sup>	83.75 $\pm$ 17.28 <sup>a</sup>	100	2.11 $\pm$ 0.15 <sup>a</sup>
4	12.93 $\pm$ 0.14 <sup>a</sup>	7.18 $\pm$ 0.14 <sup>a</sup>	60.07 $\pm$ 2.67 <sup>a</sup>	53.67 $\pm$ 2.67 <sup>a</sup>	14.90 $\pm$ 0.31 <sup>a</sup>	96.25 $\pm$ 5.39 <sup>a</sup>	65.28 $\pm$ 25.70 <sup>a</sup>	100	2.09 $\pm$ 0.18 <sup>a</sup>
6	13.12 $\pm$ 0.31 <sup>a</sup>	7.37 $\pm$ 0.31 <sup>a</sup>	62.06 $\pm$ 4.67 <sup>a</sup>	55.66 $\pm$ 4.67 <sup>a</sup>	15.13 $\pm$ 0.50 <sup>a</sup>	62.50 $\pm$ 15.58 <sup>b</sup>	69.85 $\pm$ 26.18 <sup>a</sup>	100	2.12 $\pm$ 0.17 <sup>a</sup>
8	-	-	-	-	-	3.75 $\pm$ 7.50 <sup>c</sup>	-	-	-
Feeding									
Two times/day	12.83 $\pm$ 0.24 <sup>b</sup>	7.08 $\pm$ 0.24 <sup>b</sup>	59.27 $\pm$ 4.89 <sup>b</sup>	52.87 $\pm$ 4.89 <sup>b</sup>	14.81 $\pm$ 0.55 <sup>b</sup>	71.50 $\pm$ 6.74 <sup>a</sup>	73.62 $\pm$ 26.81 <sup>a</sup>	100	2.07 $\pm$ 0.16 <sup>b</sup>
Four times/day	13.22 $\pm$ 0.35 <sup>a</sup>	7.47 $\pm$ 0.20 <sup>a</sup>	64.64 $\pm$ 3.07 <sup>a</sup>	58.24 $\pm$ 3.07 <sup>a</sup>	15.41 $\pm$ 0.32 <sup>a</sup>	71.50 $\pm$ 8.65 <sup>a</sup>	72.38 $\pm$ 14.73 <sup>a</sup>	100	2.15 $\pm$ 0.16 <sup>a</sup>
Salinity <sup>[3]</sup>	0.47 <sup>NS</sup>	0.47 <sup>NS</sup>	0.20 <sup>NS</sup>	0.20 <sup>NS</sup>	0.20 <sup>NS</sup>	<0.01*	0.42 <sup>NS</sup>	-	0.41 <sup>NS</sup>
Frequency <sup>[3]</sup>	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	1 <sup>NS</sup>	0.88 <sup>NS</sup>	-	<0.01*
Interaction <sup>[3]</sup>	0.16 <sup>NS</sup>	0.16 <sup>NS</sup>	0.30 <sup>NS</sup>	0.30 <sup>NS</sup>	0.29 <sup>NS</sup>	1 <sup>NS</sup>	0.46 <sup>NS</sup>	-	0.26 <sup>NS</sup>
CV (%) <sup>[4]</sup>	1.83	3.27	6.75	7.53	3.01	13.14	30.81	0	2.72

SL: standard length; LG: length gain; W: weight; WG: weight gain; SGR: specific growth rate; SR: survival rate; UW: uniformity in weight; USL: uniformity in standard length; K: allometric condition factor. <sup>[2]</sup>In each column, values followed by the same letter do not differ according to Tukey's test at 5% probability. <sup>[3]</sup>p value. \*  $p < 0.01$ , \*\*  $p < 0.05$ . <sup>NS</sup> Not significant ( $p > 0.05$ ). <sup>[4]</sup>Coefficient of variation.

fed four times a day presented with the highest values for these variables when compared to those fed twice a day (Table 2).

### *Pterophyllum scalare*

With regard to angelfish post-larvae, the highest ( $p < 0.05$ ) SRs were obtained at salt concentrations of 0, 2 and 4 g/L. The lowest SRs were obtained with salinities of 6 and 8 g/L (Table 3).

No significant differences were observed ( $p > 0.05$ ) for the variables of UW, USL, K, SL, LG, W, WG and SGR for angelfish post-larvae.

Feeding frequency did not significantly influence ( $p > 0.05$ ) SR, UW and USL of angelfish post-larvae. However, feeding frequency significantly influenced K, SL, LG, W, WG and the SGR of angelfish post-larvae, presenting the highest values ( $p < 0.05$ ) in animals fed four times daily (Table 3).

## Discussion

In the present study, a negative effect of salinity at 6 g/L was observed on the UW and USL of banded cichlid post-larvae. This result corresponds to the reduced SR of this species at this salinity, which may be an indication of osmotic diffusion stress. This effect may also be related to some individuals in the experimental units being more sensitive to the effects of salinity, thus becoming more debilitated and, consequently, feeding less than others, previously demonstrated in some studies of salt tolerance in larviculture of some freshwater fish species (Fabregat *et al.*, 2008; 2015; 2017).

The angelfish post-larvae showed similar UW in the different salinities used, also presenting 100% USL with all treatments. In the case of ornamental fish, uniformity in the fish facilitate their handling and commercialization because length is one of the qualities that adds value to the commercialization of these organisms (Veras *et al.*, 2016a).

In relation to SR, angelfish post-larvae have proven to be more tolerant to variations in salinity than banded cichlid, with up to a salinity of 4 g/L causing no developmental problems or changes in their SR. Similar results were observed in experiments with post-larvae of the Siamese fighting fish (*Betta splendens*) (Fabregat *et al.*, 2017), giant trahira (*Hoplias lacerdae*) (Luz & Portella, 2002), yellow-mandi catfish (*Pimelodus maculatus*) (Weingartner & Zaniboni-Filho, 2004), pacamã catfish (*Lophiosilurus alexandri*) (Luz & Santos, 2008), tambaqui (*Colossoma macropomum*), matrinxã (*Brycon amazonicus*), oscar (*Astronotus ocellatus*) and piauçu (*Leporinus macrocephalus*) (Jomori *et al.*, 2013). These studies demonstrated that the post-larvae of these species could tolerate salinities from 2–4 g/L without problems in their development and their SRs. However, in the present study, a reduction in the SR of banded cichlid post-larvae was observed at a salinity of 2 g/L. This suggests that salinity tolerance is a species-specific response (Jomori *et al.*, 2013) and that, in general, post-larvae of this species show a lower tolerance to variations in salinity than many other species.

The increase in NaCl concentrations from 6 to 8 g/L caused a negative effect on post-larvae survival in both species studied, resulting in an extremely low SR and

indicating its non-viability for larviculture of these species. This finding is consistent with previous studies conducted with post-larvae of angelfish (*Pterophyllum scalare*) (Fabregat *et al.*, 2008), silver catfish (*Rhamdia quelen*) (Fabregat *et al.*, 2015) and Siamese fighting fish (*B. splendens*) (Fabregat *et al.*, 2017). As a rule, when salt concentrations in water exceed homeostatic limits it generates an osmoregulatory imbalance, promoting stress through neuroendocrine responses and the occurrence of metabolic and osmotic disorders (Barton, 2002). The organism attempts to adapt to the stressful condition by producing catecholamines and corticosteroids. If the conditions persist, stress becomes chronic and the organism loses its adaptive capacity, thus causing immunosuppression that leads to animal death (Bœuf & Payan, 2001; Barton, 2002), as observed in the present study.

Jomori *et al.* (2013) studied the effect of increased water salinity on neotropical fish larviculture and did not observe significant differences in the total length of piauçu (*L. macrocephalus*) post-larvae at salinities of 0–4 g/L, in accordance with the results obtained in the present study with angelfish post-larvae. These same authors also observed that the post-larvae of tambaqui (*C. macropomum*), matrinxã (*B. amazonicus*) and oscar (*A. ocellatus*) had the highest total length in 2 g/L salinity, consistent with our results for banded cichlid post-larvae. In the present study, however, the best development of banded cichlid post-larvae could also be attributed to the lower population density observed within this treatment at the end of the experiments, since the post-larvae that were less resistant to the 2 g/L salinity died during the experiments.

Beux & Zaniboni-Filho (2007) investigated the development of pintado catfish (*Pseudoplatystoma corruscans*) post-larvae and found that the *K* was influenced by the different salt concentrations employed in their experiments, concluding that the *K* value was inversely proportional to the increase of NaCl in the water. In the present study, similar results were obtained for the angelfish post-larvae, although this was not observed for the banded cichlid post-larvae. These results can be explained by the intrinsic capacity of each species to adapt to abrupt environmental variations during acclimation under experimental conditions (Castillo-Vargasmachuca *et al.*, 2013).

In general, uniformity and *SR* may be affected by feeding frequency. Food supplied at periodic intervals may increase disputes between the fish. Under these conditions, dominant fish can feed better than the others, leading to an increase in heterogeneity and greater susceptibility to mortality (Hayashi *et al.*, 2004). However, in the present study, as well as in studies performed with *Pyrrhulina brevis* (Veras *et*

*al.*, 2016a), this influence of feeding frequency on survival, *UW* and *USL* was not observed in angelfish or banded cichlid post-larvae. These results corroborate those obtained by Veras *et al.* (2016a), which suggest that dominance is not a common phenomenon during larviculture. The results also suggest that the amount of feed and the feeding frequency used in the experiments were sufficient to meet the nutritional requirements of post-larvae in both species.

The zootechnical performance of both species improved when their post-larvae were fed four times a day; this corroborates the results obtained for post-larvae of the cichlid (*Herichthys cyanoguttata*) (Montajami *et al.*, 2012), Persian sturgeon fingerlings (*Acipenser persicus*) (Zolfaghari *et al.*, 2011) and zebra fish (*Danio rerio*) larvae (Nekoubin *et al.*, 2013). Conversely, the feeding frequency did not show a significant effect on the performance of the cascudo-preto catfish post-larvae (*Rhinelepis aspera*) (Luz & Santos, 2010), *P. brevis* post-larvae (Veras *et al.*, 2016a) and Siamese fighting fish post-larvae (*B. splendens*) (Sales *et al.*, 2016), or on the condition factor of angelfish juveniles (*P. scalare*) (Kasiri *et al.*, 2011). These findings demonstrate that feeding frequency affects fish growth differently, depending on the species used and the life cycle phase, and it is therefore very difficult to determine a fixed pattern for this variable.

When fish are fed with a low or high feeding frequency, growth and feed conversion may be impaired and increase production costs and possibly decrease water quality (Lee *et al.*, 2000). According to Xie *et al.* (2011), the growth rates during the larval phase are higher than those observed in other phases of the life cycles, despite a smaller digestive capacity. For this reason, the use of a higher feeding frequency may promote post-larval growth because it provides a constant energy source for the development of fish post-larvae. Nevertheless, a very high feeding frequency reduces the amount of food offered during each feeding period, which can generate more disputes for this resource and harm the development of cultivated animals (Hayashi *et al.*, 2004). In the latter case, the ability of digestive enzymes to act on food may be limited. This could impair feed conversion, since the high number of feeds can attenuate the nutrients' absorption and increase the energy required for digestion, with a corresponding increase in oxygen consumption and metabolite release (Rabe & Brown, 2000; Zeytin *et al.*, 2016). A high feeding frequency may also promote greater excretion, which would negatively affect the efficiency of digestive enzymes on food and promote the deterioration of water (Biswas *et al.*, 2006). In addition, the increase in the number of daily feeds could lead to an increase in production

costs due to a greater need for manpower and greater food waste, which are two of the most expensive components of aquaculture (Liu & Liao, 1999; Rabe & Brown, 2000; Biswas *et al.*, 2006; Zeytin *et al.*, 2016).

The feeding frequency used during larviculture is also important when freshwater fish post-larvae are fed with organisms from saline environments — such as *Artemia* sp. nauplii — because this microcrustacean survives in freshwater for a brief time (Beux & Zaniboni-Filho, 2006). Depending on the feeding frequency, there will be a greater opportunity for the fish post-larvae to consume the live nauplii, thus improving nutrient absorption, larval growth (Portella *et al.*, 2000).

The results of the present study suggest that banded cichlid post-larvae showed higher survival rates in water without salt addition (freshwater), however, the best growth of this species occurred at the salinity of 2 g/L. In contrast, angelfish post-larvae can be cultivated in salinities of up to 4 g/L NaCl without compromising their development or survival. A feeding frequency of 4 times per day is recommended for both species — *P. scalare* and *H. severus* — producing better post-larvae development and higher survival rates.

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