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Growth of Amazon ornamental fish *Nannostomus beckfordi* larvae (Steindachner, 1876) submitted to different stocking densities and feeding management in captivity conditions

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Abstract

The study evaluated productive performance of larvae *Nannostomus beckfordi* submitted to feeding management (Experiment one) and different stock density (Experiment two) in captivity conditions. The first experiment evaluated feeding rate (100 and 200 nauplii of artemia larvae^{-1} day^{-1}) and feeding frequency (2 and 4× per day). Second experiment evaluated different stock density (1, 5, 10, 20, 40 larvae per litre). At the end of 15 days, for both experiment, the survival and productive performance such as total length (TL), final weight (FW), specific development rate (SDR), specific growth rate (SGR), uniformity for weight (UW) uniformity for length (UL) and relative condition factor (Kr) was determined. For the main results, there is no significant difference to the rate and feeding frequency. The stock density has no significant difference for UW, Kr and survival. Nonetheless, a reduction in the TL, FW, SDR, SGR and FW with the increase in density (20 for 40 larvae per litre) was observed. Thus, for this species *Nannostomus beckfordi* during to initial stage using exogenous feeding, it is recommended 20 larvae per litre fed with 100 nauplii per larvae having two meals per day.

KEYWORDS
characidium, larviculture, pencil fish, production, zootechnical development

1 | INTRODUCTION

South America has the largest fresh water ichthyofauna on the planet, with more than 5,000 species identified in Brazil. These fish have significant economic value in the international ornamental fish market due to their diverse patterns and colours (Prang, 2008; Reis et al., 2016). The most traded fish are captured in Amazon basin, but this activity has an impact on wild populations (Anjos, Amorim, Siqueira, & Anjos, 2009; Graça & Pavanelli, 2008; Tlusty et al., 2013; Zuanon, Salaro, & Furuya, 2011).

Therefore, the rational and sustainable production of these ornamental species has become an effective option to protect wild stocks and generate income in Brazil. The Lebiasinidae family is among the Amazon species that stand out as having ornamental potential; these fish are distributed from Central America to South America. This family has several species of interest, such as the pencil fish, with its cylindrical body, coloured stripes and superior mouth (Weitzman & Weitzman,).

Within this family, *Nannostomus beckfordi* shows potential in the international market, reaching 3.84 € per fish (Allpondsolutions, 2019; Prang, 2008). It is a peaceful fish, with red coloration and a long black stripe on its body; it lives on shoal, feeding on microcrustaceans or periphytons and decomposed organic matter (Weitzman & Weitzman,). However, there are no scientific reports about its production or management in captivity.

For this reason, to expand the market, it is necessary to develop technological information that determine the ideal management for fish production and reproduction in captivity. Larviculture is one of the critical phases of production in captivity because it encompasses several stress factors, such as changes in water parameters, nutritional deficiencies and infections by pathogens, generating mortality and reducing production (Zuanon et al., 2011).

The feeding frequency, feeding rate and stocking density are important management characteristics for larviculture, influencing survival and performance. Excessive stocking density can promote an excess of nitrogen residue, reducing the water quality and performance, but a low number of individuals implies a bad use of space (Campagnolo & Ruñer, 2006; Luz & Santos, 2008). Nonetheless, the ideal feeding frequency leads to better feed conversion, and handling might be reduced (Jomori, Carneiro, & Martins, 2005; Luz & Portella, 2005). At the ideal feeding rate, feed waste can be avoided and operational costs can be improved.

Thus, the aim of this study was to evaluate different feeding management strategies (feeding frequencies and feeding rates) and the ideal stocking density for the productive and survival of *Nannostomus beckfordi* larvae in captivity.

2 | MATERIALS AND METHODS

Two experiments were carried out using *Nannostomus beckfordi* larvae. The wild adult fish used as the broodstock were captured and maintained in the laboratory (SISBIO Authorization number 25822). After hatching, the larvae were reared until 8 days. On the first 3 days after hatching, they consumed the yolk sac, and on the next 5 days, they were fed with microalgae and infusoria (Abe, Dias, Cordeiro, Ramos, & Fujimoto, 2015). Afterwards, the size of the mouth was checked in a microscope to ensure that the fish would be able to prey on *Artemia nauplii* and start the experiment. The larvae for both experiments had a total length of 0.3 ± 0.01 cm, but they had no measureable weight, due to their small size. Experimental units were conducted in small containers (1 L), with a semi-static system without aeration and water exchange (20% per day after the last feeding).

The first experiment was a 2 × 2 factorial design, with two feeding frequencies (2× per day, at 08:00 and 17:00 hours; 4× per day, at 08:00, 11:00, 14:00 and 17:00 hours), two feeding rates (100 and 200 *Artemia nauplii* per larvae per day) and four replicates, for 15 days. The stocking density used in this experiment was five larvae per litre.

The second experiment was a completely randomized design, with five stocking densities (1, 5, 10, 20 and 40 larvae per litre) and four replicates per stocking density. The larvae were fed for 15 days, using the best results from the feeding frequency and rate experiment.

Artemia cysts were incubated daily in water with a salinity of 30 g per litre, at a temperature of 28°C. After incubation, the cysts were discarded and the nauplii were sampled for quantification. The following water quality parameters were determined every day: oxygen (mg/L), temperature (°C), electric conductivity (µs/cm) and pH. The total ammonia (mg/L) was determined every 3 days. At the end of the experiment, a biometric process was conducted to determine weight and length in order to evaluate the following productivity performance parameters: total length (TL), final weight (FW), specific growth rate (SGR; ln FW or TL—ln initial weight or initial length/days × 100) (Lugert, Thaller, Tetens, Schulz, & Krieter, 2016), relative condition factor (Kr; expected weight—observed weight) (Le Cren, 1951) survival (S) and uniformity (U) (Furuya, Souza, Furuya, Hayashi, & Ribeiro, 1998).

$$U = \frac{X}{X1} \times 100$$

U, Uniformity for Length (UL) or Uniformity for Weight (UW); *X*, Total number of fish into experimental unit; *X*1, Number of fish with Final Weight (FW) or Total length (TL) ±20% inside of the Final Weight Mean or Total Length Mean into experimental unit.

The homoscedasticity and normality of the residuals were tested using the Bartlett and Shapiro–Wilk tests respectively. Data of non-normal distribution were transformed with the arcsine of the square root (x). Afterwards, an analysis of variance was conducted ($p \le 0.01$), with post hoc Tukey tests ($p \le 0.05$), using the statistical program *Assistat* 7.7 (Silva & Azevedo, 2016).

3 | RESULTS

In the first experiment, the feeding rate and frequency did not affect the water quality parameters, namely oxygen (6.2 ± 0.36 mg/L), temperature (27.6 ± 0.4°C), electric conductivity (315.35 ± 0.4 µs/cm), pH (6.2 ± 0.3) and total ammonia (0.5 ± 0.2 mg/L). There was no statistical interaction ($p < 0.05$) between the feeding rate and feeding frequency. The larvae development of the pencil fish, as assessed by the zootechnical parameters, did not differ with the feeding rate or feeding frequency (Table 1).

In the second experiment, there was a statistical difference ($p < 0.05$) in some water quality parameters, namely the dissolved oxygen and total ammonia at the largest stocking densities (Table 2).

With regard to the zootechnical parameters, there was no statistical difference ($p > 0.05$) in terms of uniformity, the relative condition factor or survival. However, an increase in the stocking density (20 and 40 larvae/L) promoted a reduction ($p < 0.05$) in productivity in terms of total length, total weight, specific development rate and specific growth rate (Table 3).

4 | DISCUSSION

In the first experiment, water quality parameters showed adequate values for *Nannostomus beckfordi*, not influencing the zootechnical parameters of the study (Abreu et al., 2014). The feeding frequency (two or four times per day) did not differ between treatments for

TABLE 1 Zootechnical parameters (mean values ± *SD*) of pencil fish *Nannostomus beckfordi* in different rate and feeding frequency

	Feeding frequency (times/day)		Feeding rate (nauplii/fish/day)		*p*-value		
Variables	2	4	100	200	FF	FR	FR × FR
TL (cm)	1.23 ± 0.01	1.26 ± 0.03	1.25 ± 0.01	1.33 ± 0.02	0.5945	0.5980	0.8586
TW (g)	0.0146 ± 0.021	0.0149 ± 0.013	0.0147 ± 0.018	0.0182 ± 0.01	0.2642	0.4614	0.4514
$SGR_{(L)}$ (%/day)	0.22 ± 0.02	0.23 ± 0.01	0.22 ± 0.02	0.23 ± 0.02	0.0651	0.7809	0.0780
$SGR_{(W)}$ (%/day)	0.74 ± 0.03	0.75 ± 0.03	0.75 ± 0.02	0.78 ± 0.04	0.1424	0.0826	0.2400
UL (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	–	–	–
UW (%)	64.2 ± 0.2	68.5 ± 0.1	66.6 ± 0.2	62.5 ± 0.5	0.5987	0.6854	0.5687
Kr	1.00 ± 0.02	1.00 ± 0.01	0.98 ± 0.02	1.00 ± 0.01	0.0842	0.1863	0.6515
S (%)	92.5 ± 9.25	95 ± 9.25	92.5 ± 9.25	95 ± 10.35	0.7825	0.8694	0.7541

Abbreviations: Kr, Relative condition factor; S, Survival; $SGR_{(L)}$, Specific growth rate for length; $SGR_{(W)}$, Specific growth rate for weight; TL, Total length; TW, Total weight; UL, Uniformity for length; UW, Uniformity for weight.

TABLE 2 Mean values and standard deviation of water quality parameters in different stocking densities

Density	DO	Temp	Cond	pH	TA
1 larvae/L	5.96 ± 0.02 a	27.13 ± 0.05	315.37 ± 1.47	5.84 ± 0.005	0.61 ± 0.007c
5 larvae/L	5.93 ± 0.05 a	27.06 ± 0.05	314.07 ± 0.95	5.85 ± 0.01	0.63 ± 0.007bc
10 larvae/L	5.86 ± 0.05 a	27.06 ± 0.05	314.37 ± 4.19	5.85 ± 0.005	0.72 ± 0.070b
20 larvae/L	5.7 ± 0.1 ab	27.03 ± 0.05	318.57 ± 3.32	5.87 ± 0.01	0.79 ± 0.035 ab
40 larvae/L	5.5 ± 0.2 b	27.03 ± 0.05	318.27 ± 4.91	5.87 ± 0.01	0.85 ± 0.084 a

Abbreviations: Cond, Conductivity (µs/cm); DO, Dissolved oxygen (mg/L); pH and TA, Total ammonia (mg/L); Temp, Temperature (°C). Values within the same column followed by different letter were significantly different ($p < 0.05$).

TABLE 3 Mean values ± *SD* at zootechnical parameters of pencil fish larvae *Nannostomus beckfordi* in different stocking densities

	Density (larvas/L)					
Variables	1	5	10	20	40	*p*-value
TL (cm)	1.25 ± 0.06 A	1.22 ± 0.06 A	1.22 ± 0.02 A	1.21 ± 0.01 AB	1.11 ± 0.02 B	0.0029
TW (g)	0.0125 ± 0.01 A	0.0111 ± 0.02 A	0.0120 ± 0.01 A	0.0105 ± 0.013 AB	0.0083 ± 0.005 B	0.022
SDR (%/day)	0.18 ± 0.01 A	0.18 ± 0.01 A	0.18 ± 0.01 A	0.17 ± 0.01 A	0.16 ± 0.01 B	0.0008
SGR (%/day)	0.68 ± 0.01 A	0.65 ± 0.03 A	0.67 ± 0.02 A	0.64 ± 0.02 AB	0.59 ± 0.01 B	0.9916
UL (%)	100 ± 0 A	100 ± 0 A	97.5 ± 5 A	98.75 ± 2.5 A	98.75 ± 2.5 A	0.6839
UW (%)	100 ± 0 A	57.5 ± 9.5 B	55 ± 5.7 B	40 ± 4 B	40 ± 2 B	0.0001
Kr	1.00 ± 0.03 A	0.98 ± 0.03 A	1.00 ± 0.01 A	0.99 ± 0.03 A	0.99 ± 0.02 A	0.7826
S (%)	100 ± 0 A	100 ± 0 A	97.5 ± 5 A	97.5 ± 2.9 A	98.12 ± 2.4 A	0.6254

Abbreviations: Kr, Relative condition factor; S, Survival; SDR, Specific development rate; SGR, Specific growth rate; TL, Total length; TW, Total weight; UL, Uniformity for length; UW, Uniformity for weight. Values within the same line followed by different letter were significantly different ($p < 0.05$).

the dependent variables measured. Similar results were observed in *Hoplias lacerdae* and *Lophiosilurus alexandri* with the same feeding frequencies during larviculture (Luz & Portella, 2005; Santos, Pedreira, & Luz, 2016). However, an increase in the feeding frequency for other two ornamental species, *Pyrrhulina brevis* and *Heros severus*, promoted an improvement in performance (Abe et al., 2015, 2016).

The observed result for feeding frequency used could be explained for the mechanical limitation of fish stomach capacity and time for gastric evacuation of pencil fish larvae. Feeding frequency and rate higher than the ideal limits can reduce the efficiency of digestion due to the inefficiency of digestive enzymes caused by the reduction in feed gastrointestinal transit time (Booth, Tucker, Allan, & Fielder, 2008; Honorato et al., 2016; Lee, Hwang, & Cho, 2000; Luz & Portella, 2005; Navarro-Guillén, Engrola, & Yúfera, 2018; Riche, Haley, Oetker, Garbrecht, & Garling, 2004).

According to Abe et al. (2015), the ornamental species *Pyrrhulina brevis* does not show any alteration in growth with different feeding rates, as was also observed in the present study. During larviculture of another species, 250 nauplii/larvae was an adequate feeding rate for captivity culture of *Heros severus* (Abe et al., 2016); however, an increase in the number of *Artemia* nauplii promoted an improvement in performance, with 900–1600 nauplii being required per larvae of *Hoplias lacerdae* and *Lophiosilurus alexandri* (Luz & Portella, 2005; Santos, Correia & Luz, 2015).

The same productivity performance at the lowest feeding frequency could be explained by the quiet and sedentary behaviour of the pencil fish *Nannostomus beckfordi*, as is also observed in *L. alexandri* and *H. lacerdae* (Salaro et al., 2011; Travassos, 1959). With this behaviour, the fish avoid wasting energy as there is no need to feed several times per day. The species *L. alexandri* and *H. lacerdae* have carnivorous feeding habits and need a larger amount of *Artemia* nauplii than pencil fish (Luz & Portella, 2002, 2005), probably due to the difference in the intestinal tract ingestion capacity between the species.

Thus, the adequate feeding rate and feeding frequency between species are related to different aspects, such as species' physiology, behaviour, developmental stage, feeding habit and position within the water column (Jomori et al., 2005; Luz & Portella, 2005; Santos et al.., 2016; Zuanon et al., 2011). The determination of the ideal feeding rate and feeding frequency is important for captivity management, promoting animal welfare, reducing the labour costs, avoiding waste and reducing production costs (Fujimoto et al., 2016; Santos et al., 2015; Zhao et al., 2016).

In the second experiment, there was a reduction in the water quality due to the high stocking density. This was reflected in the largest dissolved oxygen consumption and the increase in nitrogen compounds produced by the fish (Abe et al., 2015; Abe et al., 2016; Luz et al.., 2012; Santos et al., 2015). In addition to the reduction in water quality, there was also a reduction in the productivity performance as stocking density increased. This has been observed in several species, such as *Horabagrus brachysoma*, *Oreochromis niloticus*, *Trichogaster trichopterus*, *Pterophyllum scalare*, *Carassius auratus* and *Heros severus*, due to the behaviour alterations that lead to aggression for feed or territories at high stocking densities (Abe et al., 2016; Barcellos, Nicolaiewsky, Souza, & Lulhier, 1999; Gonçalves Júnior, Pereira, Matielo, & Mendonça, 2013; Gonçalves Júnior, Mendonça, Pereira, Matielo, & Amorim, 2014; Sahoo, Giri, Chandra, & Sahu, 2010; Syarifuddin & Kramer, 1996).

For this reason, the poorest water, associated with possible competition, promotes a reduction in larvae performance at high stocking densities. However, in the present study, there was no reduction in the survival or relative condition factor at increased stocking densities; this is important for ornamental fish production due to the trade style of this market (sales by the unit, not by weight). Thus, an increase in density (40 larvae/L) can be used by any producer without interfering with fish health and survival.

5 | CONCLUSION

This is the first report about feeding management for species *Nannostomus beckfordi* in captivity conditions. Its larviculture (during 15 days) could use 100 artemia nauplii/fish at two feeding frequency having stocking density 20 larvae/L.

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