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ORIGINAL ARTICLE

Effect of feeding frequency and water salinization on early development of *Pyrrhulina brevis*, an Amazonian ornamental fish

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ABSTRACT

Pyrrhulina brevis is an endemic fish from the Amazon basin that is valued in the ornamental fish market. In larviculture, fish are very sensitive to stressors and have a high mortality rate. Salinized water may provide more energy to overcome stress and feeding frequency is an important strategy to improve productive performance. Therefore, the aim of this study was to evaluate the best feeding frequency and the best level of water salinization for larval development of *P. brevis*. A total of 360 post-larvae of *P. brevis* (5.26 ± 1.65 mg, 5.57 ± 0.68 mm) were randomly distributed in 36 aquariums (1 L) in a 3x4 factorial scheme, to evaluate three concentrations of salt in water (0, 1 and 2 g L^{-1}) and four feeding frequencies (once, twice, three and four times a day). We offered 150 *Artemia* nauplii per day and per post-larvae during 15 days. The fish kept at 1 g L^{-1} salinized water, and those fed 3 and 4 times a day presented significantly greater final length and weight, weight gain, and specific growth rate for length and weight. An interaction between salinized water and feeding frequency was observed for survival rate. We conclude that, during the initial rearing phase of *P. brevis*, feeding frequency of three times a day and salinized water at 1 g L^{-1} is recommended.

KEYWORDS: productive performance, nutritional management, live foods, ornamental fish farming

Efeito da frequência alimentar e da salinização da água sobre o desenvolvimento inicial de *Pyrrhulina brevis*, um peixe ornamental da Amazônia

RESUMO

Pyrrhulina brevis é um peixe endêmico da bacia amazônica que apresenta bons valores no mercado de peixes ornamentais. Na larvicultura, os peixes são muito sensíveis aos estressores e apresentam alta taxa de mortalidade. A água salinizada pode fornecer mais energia para superar o estresse e a frequência alimentar é uma estratégia importante para melhorar o desempenho produtivo. Portanto, o objetivo deste trabalho foi avaliar a melhor frequência alimentar e o melhor nível de salinização da água para o desenvolvimento larval de *P. brevis*. Um total de 360 pós-larvas de *P. brevis* (5,26 ± 1,65 mg, 5,57 ± 0,68 mm) foram distribuídos aleatoriamente em 36 aquários (1 L) em esquema fatorial 3x4, para avaliar três concentrações de sal na água (0, 1 e 2 g L^{-1}) e quatro frequências de alimentação (uma, duas, três e quatro vezes ao dia). Foram oferecidas 150 náuplios de *Artemia* por dia e por pós-larva, durante 15 dias. Os peixes mantidos com 1 g L^{-1} de água salinizada e os alimentados 3 e 4 vezes ao dia apresentaram comprimento e peso finais, ganho de peso e taxa de crescimento específico para comprimento e peso significativamente maiores. Uma interação entre água salinizada e frequência alimentar foi observada para a taxa de sobrevivência. Concluímos que, durante a fase inicial de criação de *P. brevis*, recomenda-se uma frequência alimentar de três vezes ao dia e água salinizada a 1 g L^{-1}.

PALAVRAS-CHAVE: desempenho produtivo, manejo nutricional, alimento vivo, piscicultura ornamental

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INTRODUCTION

During fish production, larviculture is one of the most critical phases (Abe *et al*. 2015). Post-larvae are more sensitive to stressors such as changes in water quality and nutritional management (Zuanon *et al.* 2011). The initial feeding directly influences the growth performance of the post-larvae and the supply of live food during larviculture has been the most used strategy to ensure the well-being and development of animals in the first days of life (Fosse *et al.* 2013).

Among live foods, *Artemia sp*. stands out for its ease of production and storage, in addition to its nutritional quality (Silva and Mendes 2006; Kestemont *et al*. 2007). Fish fed *Artemia* nauplii in the first days of life have good growth rates and low mortality (Campelo *et al*. 2020). However, it is important to control the daily food supply so that it can be used more efficiently by the post-larvae. Lack of food is harmful to the development of fish, and excess results in an unfavorable cost-benefit relation to the producer, as manpower for *Artemia* nauplii processing and fish feeding is a considerable part of larvicultuure expenses, and is harmful for the post-larvae, since excess food can decrease water quality (Oliveira *et al*. 2020).

Feeding frequency represents the number of meals offered to fish per day and is directly related to the improvement in growth performance variables (Xie *et al*. 2011). HHHowever, the ideal feeding frequency depends on the species, life stage and water quality (Thomassen and Fjaera 1996). Since post-larvae have a short gastrointestinal tract, the time spent by the food in the tract is also short (Lee *et al*. 2000). Providing food at an inappropriate frequency can promote intestinal overload, which results in lower nutrient absorption (Riche *et al*. 2004). Thus, determining the optimal feeding frequency is important to optimize food utilization and production costs (Abe *et al*. 2016).

Another relevant point to be considered during the larviculture of freshwater fish is the salinization of breeding water, through the addition of common salt (NaCl). The salinized water can help the initial development of the post-larvae, reducing ion losses and excessive water absorption due to the osmotic differences between the fish plasma and the external environment (Varsamos *et al*. 2005; Fabregat *et al*. 2015). This makes it possible to redirect energy to other physiological processes such as growth (Baldisserotto *et al.* 2007).

Salinized water also benefits *Artemia* nauplii, making this microcrustacean live longer and increasing its availability time as fresh food for the post-larvae (Beux and Zaniboni-Filho 2018). Adding salt to the water is also a good alternative to reduce stress in the larviculture environment (Jomori *et al.* 2012), since the increase in salt concentration in the water allows fish to reduce energy expenditure with the maintenance of osmotic balance (Sampaio and Bianchini 2002; Salaro *et al*. 2012). However, the use of inadequate concentrations of salt in the water can compromise animal welfare and generate physiological and behavioral changes (Luz 2007; Luz and Santos 2008; Dias *et al.* 2016), that can result in reduced growth and survival of freshwater fish (Luz *et al*. 2013).

Pyrrhulina brevis Steindachner, 1876 (Characiformes: Lebiasinidae) is an endemic species of the Amazon basin that has a cylindrical, elongated body and an upturned mouth, with gregarious habit, normally occurring in shoals, which makes it attractive to the ornamental market, and showing territorial behavior during the reproductive period (Weitzman and Weitzman 2003; Arias and Rossi 2005; Silva *et al*. 2016). These characteristics, as well as its rapid adaptation to rearing conditions, place *P. brevis* in the spotlight of the international ornamental fish trade (Abe *et al*. 2021).

Since *P. brevis* is an ornamental Amazonian fish that reproduces easily under captive conditions and has a promising consumer market, it is important to optimize protocols for the production of young forms, such as the best way to offer live food and to salinize the water to reduce stress and increase the viability of *Artemia* nauplii used as live food. Other studies have already evaluated the use of salinized water (Abe *et al.* 2015), prey concentrations (Oliveira *et al.* 2020) and feed deprivation cycles (Abe *et al.* 2021) for *P. brevis*. However, no study so far has tested the interaction of the water salinization level with the feeding frequency for this species. Thus, the present study aimed to evaluate the best feeding frequency and water salinization level during larviculture of *P. brevis* both in terms of fish growth and labor production costs.

MATERIAL AND METHODS

The experiment was approved by the ethics committee on the use of animals of Universidade Federal do Pará – UFPA (Process # 7656100517 CEUA/UFPA) and carried out at the Pisciculture Laboratory (LAPIS) of UFPA, Bragança campus, Pará state, Brazil.

Fish and experimental conditions

Post-larvae of *P. brevis* were obtained from four breeding pairs held at the Pisciculture Laboratory under controlled conditions. After hatching, the larvae were observed daily to monitor the consumption of the yolk sac. On the seventh day after hatching, the yolk sac was completely consumed and the mouth opening had reached the ideal size for the acceptance of exogenous food, characterizing the beginning of the post-larvae phase. For the initial biometrics, due to the small size and fragility of the fish, a sample of 40 individuals was used to estimate the initial mean weight and total length (5.26 ± 1.65 mg and 5.57 ± 0.68 mm, respectively). A total of 360 post-larvae were randomly selected and distributed in 36 aquariums (1L volume), in a 3x4 factorial scheme with three replications per treatment. The factor water salinization consisted of three concentrations of sodium chloride in the water (0, 1 and 2 g L^{-1}) and the factor feeding frequency consisted of four levels: feeding live *Artemia* nauplii once a day (08:00), twice a day (08:00 and 14:00), three times a day (08:00, 10:00 and 14:00) and four times a day (08:00, 10:00, 14:00 and 16:00). The experiment duration was of 15 days, corresponding to the duration of larval development of *P. brevis*.

Artemia hatching

Overall, each post-larva was fed 150 nauplii of *Artemia* per day (1500 nauplii per replicate per day), as indicated by Abe *et al.* (2015). This amount was distributed among the number of daily feeding bouts corresponding to each treatment level. A total of 5 g L^{-1} of *Artemia* cysts were hatched daily in a transparent container containing 2 L of salinized water at a concentration of 35 g L^{-1}. The containers for the hatching of the *Artemia* were kept at room temperature under artificial lighting of fluorescent lamps (15 W) and constant aeration for 24 hours, the period necessary for the hatching of the nauplii. After hatching, the nauplii were removed by siphoning to separate the cyst residues, then washed in running water to remove the saline water, so as not to influence the experimental treatments, and transferred to a 200-ml beaker. The density of nauplii was estimated by collecting 0.5 ml from the beaker and counting the number of nauplii in this sample under a stereomicroscope with 40x magnification (QUIMIS 14 Q714Z-2, Diadema, Brazil). From this sample, the volume to be supplied to each aquarium was calculated.

Water quality parameters

The water quality parameters were measured every three days. Temperature (26.28 ± 0.04 ºC), pH (6.53 ± 0.05), dissolved oxygen (6.28 ± 0.06 mg L^{-1}) and electrical conductivity (1.83 ± 0.03 µS cm^{-1}) were measured with the aid of a multiparameter device (Horiba Advanced Techno Co. Ltd., Kyoto, Japan). Total ammonia (0.70 ± 0.03 mg L^{-1}) was measured using a Labcon Test Kit (Alcon Ltda., Brazil). All parameters remained in appropriate conditions for Amazonian fish farming (Silveira *et al.* 2009). Two hours after the last feeding of the day, 50% of the water of each aquarium was siphoned out and replaced to remove feces and possible food residues, to ensure water quality and the well-being of the post-larvae.

Growth performance

At the end of the experimental period, all fish were counted, measured and weighed with the aid of an analytical balance (Gehaka AG200, Real Parque, São Paulo, Brazil, precision 0.0001 g) and a digital caliper (Vonder, 0.01 mm), We determined the following growth performance parameters:

- Final length (FL) (mm);
- Final weight (FW) (mg);
- Weight gain (WG) (mg) = final weight – initial weight;
- Length gain (LG) (mm) = final length – initial length;
- Specific growth rate in weight (SGR_W) (% day^{-1}) = [(ln FW – ln initial weight)/15] *100, where 15 is the duration of the experiment in days;
- Specific growth rate in length (SGR_L) (% day^{-1}) = [(ln FL – ln initial length)/15] *100;
- Batch uniformity for weight (UW) (%) (Furuya *et al.* 1998) = (number of fish with weight ± 20% of the mean per experimental unit)/total number of fish per experimental unit) *100;
- Batch uniformity for length (UL) (%) = (number of fish with length ± 20% of the mean per experimental unit)/total number of fish per experimental unit) *100;
- Survival rate (SR) (%) = final number of fish/initial number of fish*100.

Labor cost

The labor cost was estimated based on the Brazilian minimum monthly wage in April 2021 (USD 200.20, with an exchange rate of 1 USD = BRL 5.49). Considering a workload of eight hours per day and 24 days per month, the labor cost per hour was USD 1.15. The total feeding time of the post-larvae in hours was calculated by multiplying the feeding frequency by a feeding time of 30 minutes for a standard 1,000 post-larvae, adjusted according to the survival rate among treatments. The labor cost (USD $month^{-1}$) was calculated as the ratio between the total feeding time for a 15-day trial period and the hourly labor value (Mankiw 2020).

Statistical analysis

The normality and homoscedasticity of the response variables were confirmed by the Lilliefors and Bartlett tests, respectively. Each growth performance variable was analyzed with two-way analysis of variance. When no interaction between salinity and feeding frequency was detected, the variable was analyzed with single-factor ANOVA. The labor cost was analyzed only as a single-factor ANOVA, since the salinization of water was not considered for the calculation. For significant ANOVAs, the post-hoc Tukey test was used for pairwise comparison of means. The significance level was 5% P < 0.05) for all analysis. The data were analyzed using the SAEG 9.1 software.

RESULTS

The growth performance variables of FL, LG, FW, WG, SGR_W and SGR_L varied significantly with the feeding frequency and with the water salinization level, but there was no interaction between the factors (Table 1). No significant differences were observed for UW and UL (Table 1). For the fish survival rate, an interaction between feeding frequency and salinized water was observed (Table 2).

Table 1. Growth performance parameters of *Pyrrhulina brevis* post-larvae submitted to different feeding frequencies and water salinization levels. FL = final length; LG = length gain; FW = final weight; WG = weight gain; SGR_W = specific growth rate for weight; SGR_L = specific growth rate of length; WU = weight uniformity; LU = length uniformity; SR = survival rate.

Treatment	Growth performance parameters								
	FL (mm)	LG (mm)	FW (mg)	WG (mg)	SGR_W (% dia^{-1})	SGR_L (% dia^{-1})	WU (%)	LU (%)	SR (%)
Feeding frequency (N feedings per day)									
1	9.9 ± 0.5C	5.7 ± 0.5C	7.5 ± 1.0B	6.0 ± 1.0B	10.73 ± 0.9B	5.7 ± 0.3B	46.4 ± 18.0	96.5 ± 4.6	97.8 ± 3.5
2	10.4 ± 0.3B	6.1 ± 0.3B	8.9 ± 0.5A	7.4 ± 0.5A	11.93 ± 0.4A	6.0 ± 0.2B	51.1 ± 14.6	95.6 ± 4.9	100.0 ± 0.0
3	10.8 ± 0.2A	6.5 ± 0.2A	9.1 ± 0.5A	7.6 ± 0.5A	12.04 ± 0.4A	6.2 ± 0.1A	53.3 ± 12.1	95.6 ± 5.9	100.0 ± 0.0
4	10.9 ± 0.2A	6.7 ± 0.2A	9.2 ± 0.5A	7.7 ± 0.5A	12.14 ± 0.4A	6.3 ± 0.2A	54.4 ± 12.1	98.8 ± 2.2	97.8 ± 3.5
Water salinization (g L^{-1})									
0	10.1 ± 0.5C	5.9 ± 0.5C	7.8 ± 0.9B	6.3 ± 0.9B	11.0 ± 0.8B	5.8 ± 0.4C	96.6 ± 4.6	96.6 ± 0.0	98.3 ± 2.8
1	19.9 ± 0.3A	6.7 ± 0.3A	9.4 ± 0.5A	7.9 ± 0.5A	12.2 ± 0.4A	6.3 ± 0.2A	91.1 ± 5.9	100.0 ± 0.0	100.0 ± 0.0
2	10.5 ± 0.5B	6.3 ± 0.3B	7.4 ± 0.5C	7.4 ± 0.5C	11.9 ± 0.4C	6.0 ± 0.2B	95.7 ± 5.0	95.7 ± 5.0	98.3 ± 2.8
Feeding frequency	***	***	***	***	***	***	NS	NS	NS
Water salinization	***	***	***	***	***	***	NS	NS	NS
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	**

Mean values within each variable and factor with different letters differ significantly according to a Tukey test at 5% probability. ANOVA: NS: not significant (P > 0.05); *P < 0.05; **P < 0.01; ***P < 0.001.

Table 2. Survival rate of *Pyrrhulina brevis* post-larvae in different levels of water salinization and feeding frequency. Values are the mean ± standard deviation of three replicates.

Feeding frequency (N feedings per day)	Water salinization (g L^{-1})		
	0	1	2
1	93.3 ± 4.4 bB	100 ± 0.0 aA	100 ± 0.0 aA
2	100 ± 0.0 aA	100 ± 0.0 aA	100 ± 0.0 aA
3	100 ± 0.0 aA	100 ± 0.0 aA	100 ± 0.0 aA
4	100 ± 0.0 aA	100 ± 0.0 aA	93.3 ± 4.4 bB

Means with different lowercase letters in the same line indicate significant pairwise difference (P < 0.05) according to a Tukey test (n=3). Means with different capital letters in the same column indicate significant pairwise difference (P < 0.05) according to a Tukey test (n=3).

Post-larvae subjected to salinized water of 1 g L^{-1} showed significantly higher values for FL, LG, FW, WG, SGR_W and SGR_L compared to the other salinization levels. FL, LG and SGR_L were significantly higher for feeding frequencies of three and four times a day, while FW, WG and SGR_W were significantly higher for feeding frequencies of two, three and four times a day (Table 1). The survival rate was 100% in all treatment combinations, except for fish fed once a day at 0 g L^{-1} salinity and fish fed four times a day at 2 g L^{-1} salinity, with two dead larvae each. The post-larvae were found dead with no signs of injury or any trace of disease.

As expected, the labor cost increased directly and significantly with the increase of the feeding frequency. The labor cost was of USD 8.0 ± 0.3, 16.3 ± 0.0, 24.5 ± 0.0 and 31.9 ± 1.1 for the feeding frequencies of one, two, three and four times a day, respectively.

DISCUSSION

In the present study, *P. brevis* post-larvae submitted to feeding frequencies of three and four times a day presented significantly higher values of final length, length gain, final weight and weight gain. Our results confirm that providing food at the appropriate feeding frequency can improve the development of *P. brevis* post-larvae. Similar results were observed for post-larvae of *Heros severus* Heckel, 1840, which had higher final weight, weight gain, specific growth rate, final length and length gain when fed three, four and five times per day (Paixão *et al.* 2019). Likewise, post-larvae of *Pterophyllum scalare* Schultze, 1823, had higher values for weight gain, specific growth rate and survival rate when fed four times a day (Eiras *et al.* 2019). On the other hand, a feeding frequency of twice times a day is recommended in *Betta splendens* Regan, 1910, larviculture in order to obtain better growth, uniformity and survival (Sales *et al.* 2016).

Generally, fish in the early stages show better development when subjected to a higher feeding frequency, due to the higher metabolic rate of the animals at this stage of development (Pouey *et al.* 2012). A low feeding frequency implies a larger amount of food being supplied per meal, resulting in a suboptimal food consumption and nutrient absorption, due to the fast passage of the food through the gastrointestinal tract of the post-larvae (Luz and Portella 2005; Salaro *et al*. 2012; Abe *et al*. 2016). Post-larvae usually have more opportunities to obtain sufficient food at higher feeding frequencies (Xie *et al.* 2011). However, very high feeding frequencies can also cause hierarchy problems, generating food dispute and favoring the consumption of food by dominant fish, due to the competition for the smaller amount of food per feeding bout. In addition, higher feeding frequencies can significantly increase the production cost and labor cost (Luz and Portella 2015; Okomoda *et al.* 2019).

In the present study, post-larvae subjected to salinized water of 1 g L^{-1} had the best values for final length and weight, length and weight gain, and specific growth rate for length and weight, when compared to fish kept in non-salinized water or water salinized at 2 g L^{-1}. Similarly, other studies that tested the effect of water salinization on post-larvae of *P. brevis* obtained best production parameters at 1 g L^{-1} (Oliveira *et al.* 2020) and at 2 g L^{-1} (Abe *et al.* 2015), although the latter did not test concentrations lower than 2 g L^{-1} and neither of the two studies related the salinized water with the feeding frequency of the fish. The use of salinized water at concentrations higher than 2 g L^{-1} also resulted in negative effects on growth and survival of post-larvae of *Colossoma macropomum* (Cuvier, 1816)*, Brycon amazonicus* (Spix and Agassiz, 1829)*, Astronotus ocellatus* (Agassiz, 1831) (Jamori *et al.* 2013), *Hypsolebias radiseriatus* Costa, 2012 (Araújo *et al.* 2021), *Oreochromis niloticus* (Linnaeus, 1758) (Luz *et al.* 2013) and *Brycon vonoi* Lima, 2017 (Coraspe-Amaral *et al.* 2017). Therefore, our results confirm that freshwater fish species perform better when raised in slightly higher salinity levels compared to their natural conditions (Boeuf and Payan 2001), but generally lower than 2 g L^{-1}. The use of inadequate concentrations of salt in the water can lead to reduced development and survival of freshwater fish raised in captivity (Luz *et al.* 2012). High concentrations of salt in the water compromise animal welfare and generate physiological and behavioral changes (Luz 2007; Luz and Santos 2008; Dias *et al*. 2016). These effects may be related to osmoregulatory imbalance when the concentration of salts in water exceeds the limits of homeostasis (Barton 2002).

In this study, an interaction between feeding frequency and salinized water was observed for the survival rate of post-larvae, with the lowest survival being for fish submitted to a 2 g l^{-1} treatment and feeding four times a day. The fish in this treatment could be suffering stress caused by the high salinized water and very fractional feeding (Hayashi *et al*. 2004; Luz *et al*. 2012), which may have benefited the dominant fish, so the combination of the two stressors harmed the fish's survival. In the same way, fish submitted to the treatment of one feeding per day in water without the addition of salt also had impaired survival, probably because the *Artemia* nauplii supplied in this treatment showed faster mortality and ended up being less attractive to the fish (Portella *et al*. 2000; Cardoso and Igarashi 2009). The reduced survival rate of fish fed once a day in association with fresh water confirms the hypothesis that salt can benefit post-larvae, probably due to reduced stress and increased survival of *Artemia* nauplii (Beux and Zaniboni-Filho 2018). The lower survival of fish fed four times a day and kept in salinized water with 2 g l^{-1} confirms that the excess of salt together with the excessive feeding frequency is also harmful to the post-larvae. These results confirm the importance of determining the ideal amount of salt in the water according to the frequency of feeding used.

The post-larvae production cost was significantly higher for higher feeding frequencies. This result was expected, as the labor increases with the frequency of feeding. These results corroborate those found for breeding male *B. splendens*, with fish fed at higher frequencies having the highest production costs (Santos *et al.* 2019). Labor cost results were used to determine the best feeding frequency for *P. brevis*, since the fish length variables allowed the choice of three or four feedings per day. Furthermore, when taking into account the fish weight variables, two, three or four feedings per day can be used. However, labor costs were 23.3% lower with three feedings per day compared to four feedings per day, and 33.4% lower with two feedings per day compared to three feedings per day. Therefore, our results indicate that the best feeding frequency for *P. brevis* post-larvae is three feedings per day, which maximizes growth performance parameters of the post-larvae while minimizing the production costs.

CONCLUSIONS

A feeding frequency of three times a day in salinized water at 1 g L^{-1} optimized the early post-larval development of *Pyrrhulina brevis.* Our results suggest that, although higher feeding frequencies imply higher labor costs, the cultivation in lower levels of water salinization (in the tested range of 0 to 2 g L^{-1}) and intermediate feeding frequency (in the tested range of one to four bouts per day) may also usefully favor increased survival rates of the post-larvae.

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