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ABSTRACT

This study evaluated short cycles of feed deprivation on the growth performance and survival of larvae and juveniles of the ornamental fish *Pyrrhulina brevis*. The first experiment used larvae fed with artemia nauplii in a completely randomized design arranged in factorial (2 × 5) distributed in two feeding frequencies (two or four meals a day), five feeding deprivation protocols (7/0: seven days of continuous feeding [DCF] and no feed deprivation; 6/1: six DCF and one day of feed deprivation; 5/2: five DCF and two days of feed deprivation; 4/3: four DCF and three days of feed deprivation; Alt: alternated feeding days), and five replicates. The second experiment used juveniles arranged in a completely randomized design with five treatments (same treatments from previous experiment) and five replicates feeding commercial ration. For larviculture, the feeding deprivation resulted in the worst larvae development rate and survival. The use of feeding deprivation is not recommended for larvae because it is dependent on exogenous feed daily. For juveniles, one day of feeding deprivation a week provided lower ration consumption without negative effects on fish development.

KEYWORDS

Feeding strategy; feeding deprivation; compensatory growth; starvation; larviculture

Introduction

Pyrrhulina brevis is an Amazon ornamental fish from the Lesbiasinidae family with pacific behavior, fusiform body, a maximum length of 7 cm, and a gray color with red shades (Weitzman and Weitzman 2003). Because of these characteristics, the genus is in high demand on the international ornamental fish market, but scientific data that support its captivity rearing to supply the market are scarce (Abe et al. 2015). Studies on reproduction, larviculture, and feeding management at different life stages are important to promote fish farming and to offer animals with better sanitary quality, thereby reducing the pressure on wild stocks (Abe et al. 2019).

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Feed deprivation naturally occurs in the wild and is caused by climatic, spatial, or reproductive changes (Pottinger, Rand-Weaver, and Sumpter 2003). After such events, some fish can compensate for poor growth by returning to normal levels after refeeding, which is known as "compensatory growth" (Ali et al., 2003; Chauvigné et al. 2003; Hagen et al. 2009; Jobling 2010). Further, adequate morphological and physiological changes (e.g., hypertrophy gastrointestinal tract, increase in intestinal villi, liver glycogen reserves) are necessary to obtain greater feeding efficiency and increased growth performance after a feed deprivation period (El-Araby, Amer, and Khalil 2020; Furné et al. 2012; Rønnestad et al. 2013; Urbinati, Sarmiento, and Takahashi 2014). In aquaculture, this approach reduces feed and labor costs in fish farming without damaging growth and health (Urbinati, Sarmiento, and Takahashi 2014).

Studies investigating short cycles of feeding deprivation and refeeding strategies reported better nutrient absorption, promoting greater growth performance in cichlid (Arauco and Costa 2012; Palma et al. 2010), characid (Souza et al. 2003; Urbinati, Sarmiento, and Takahashi 2014), anabantid (Santos et al. 2016), and cyprinid fish (Yengkokpam et al. 2014).

Although there are several scientific reports for freshwater species, data for Amazon species are still missing. In this context, we evaluated the impact of short cycles of feed deprivation on the growth performance and survival of larvae and juveniles of the Amazon ornamental fish *Pyrrhulina brevis*.

Material and methods

All procedures were carried out in accordance with the ethics committee for animal care of the Pio Décimo College (Protocol 16/2018).

First experiment design—larvae

This study used *P. brevis* larvae (3.3 ± 0.14 mm and 0.1 ± 0.05 mg) with an age of 7 days after hatching. The yolk sac was already consumed, and the larvae had 3 days of initial feeding of microalgae, *Paramecium*, and protozoans according to the study by Abe et al. (2015).

The larvae were allocated into polyethylene tanks (1 L) at static conditions with forced aeration and partial water exchange (30%) to remove waste 2 hours after the last daily feeding. The larvae were stocked at a density of 10 individuals/L throughout the experiment, and dead larvae were removed.

The trial tests were arranged in two feeding frequencies (two and four meals a day) and five feeding deprivation strategies: (7/0: seven days of continuous feeding [DCF] and no feed deprivation; 6/1: six DCF and one day of feed deprivation; 5/2: five DCF and two days of feed deprivation; 4/3: four DCF and three days of feed deprivation; Alt: feeding on alternate days). The experiment lasted for 60 days and had five replicates per treatment.

To evaluate the effect of feed deprivation on larvae, we fed the larvae strictly with *Artemia nauplii* for 60 days. They received 150 *A. nauplii*/larvae/day according to the Abe et al. (2015). The cysts of *Artemia* (30 g) remained in salt water (30 g/L) with forced aeration at a temperature of 28°C over a period of 24 h. After hatching, the *Artemia nauplii* were removed and washed in fresh water. Subsequently, 1 mL of *A. nauplii* was placed under a stereomicroscope in triplicate, using a petri dish, and larvae were counted at a magnification of 40x.

The water variables of dissolved oxygen (6.09 ± 0.23 mg/L)(YSI550A), temperature (27.10 ± 0.23°C)(YSI 550A), electric conductivity (404.78 ± 20.15 µs/cm) (YSI 30), pH (6.22 ± 0.14)(YSI 60), and total ammonia (0.90 ± 0.15 mg/L) (Hanna® HI 83,224–02) were maintained ideal for *P. brevis* (Abe et al. 2015).

Second experiment design—juveniles

This experiment used 675 *P. brevis* juveniles (2.74 ± 0.03 cm and 0.177 ± 0.003 g) from another natural reproduction in captivity. The fish were distributed in fifteen 310-L tanks plugged in to a recirculation system (RAS) at a stocking density of 45 fish/tank. The RAS consisted of a mechanical filter (acrylic perlon), a biological filter (gravel nº2), and a submersed pump (Jacuzzi 3b-m 1.5cv 16 m^3/h).

The experimental design was a completely randomized design with five feeding protocols (7/0: seven days of continuous feeding [DCF] and no feed deprivation; 6/1: six DCF and one day of feed deprivation; 5/2: five DFC and two days of feed deprivation; 4/3: four DFC feeding and three days of feed deprivation; Alt: alternate feeding days) and five replicates throughout the 60 days. The fish were fed ad libitum with commercial ration for ornamental fish (crude protein 32.5%, lipid 4.0%, crude fiber 3.0%; minerals 8.0%, and moisture 15.0%) twice a day.

In the second experiment the water quality remained ideal for *P. brevis* (Abe et al. 2015): dissolved oxygen of 7.34 ± 0.34 mg/L, temperature of 27.42 ± 0.24° C, electric conductivity of 354.78 ± 29.15 µs/cm, pH of 6.52 ± 0.14, and total ammonia of 0.21 ± 0.12 mg/L.

Growth parameters

At the end of the first experiment, all larvae underwent biometric procedures to determine final weight (FW), final length (FL), in order to evaluate the following productivity performance parameters:

Specific growth rate (%) for weight and length (SGR_W and SGR_L) according to Lugert et al. (2016):

$$SGR = \left(\frac{ln\left(W_f orL_f\right) - ln\left(W_i orL_i\right)}{t}\right)x100$$

W_f and L_f = final weight and length; W_i and L_i = initial weight and length; t = days of experiment.

Uniformity (U) according to Furuya et al. (1998):

$$U = \frac{X}{X1}x100$$

U = uniformity for weight and for length (UW and UL); X = total number of fish into experimental replicate; X1 = number of fish with final weight (FW) or total length (TL) within standard deviation (SD ± 20%) from final weight or total length of replicate.

Relative condition factor according (Kr) to Le Cren (1951):

$$Kr = \frac{W_{expec}}{W_{obser}}$$

W_{expec} is the logarithmic regression between weight and length; W_{obser} is the natural log of weight.

Final survival (%):

$$S = \left(\frac{N_f}{N_i}\right) * 100$$

N_f = number of larvae at the end of experiment; N_i = number of larvae at the begin of experiment.

At the end of the second experiment, all juveniles underwent biometric procedures to determine final weight (FW), final length (FL), biomass total (BT), apparent ration consumption (ARC), then determinate: SGR_W, SGR_L, UW, UL, Kr, final survival (S).

Weight Gain (WG):

$$G = (Final\ weight - Initial\ weight)$$

Length Gain (LG):

$$LG = (Final\ lenght - Initial\ lenght)$$

Biomass Gain (BG):

$$BG = (Final\ biomass - Initial\ Biomass)$$

Relative Ration Consumption (RRC):

$$RRC = \left(\frac{Apparent\ Ration\ Consumption}{Number\ of\ feeeding\ day}\right)$$

The apparent ration consumption was determined weekly by ration weight before and after the weekly feeding period.

Feed conversion rate (FCR):

$$FCR = \left(\frac{Apparent\ Ration\ Consumption}{Biomass\, gain}\right)$$

Statistical analysis

The data were submitted to normality and homoscedasticity tests of Shapiro-Wilk and Bartlett respectively. Afterward, a two-way ANOVA was performed on the data of the first experiment and one-way ANOVA on the data of the second experiment, followed by a post hoc Tukey test ($p < .05$) for mean comparisons, using the statistical software Past 3.0.

Results

Feed deprivation and feeding frequency showed statistically significant interactions for final weight, final length, specific growth rate for weight, and specific growth rate for length, with the lowest values for larvae receiving two meals a day (Table 1). Feeding on alternate days reduced larval weight and length. However, 1 day of feed deprivation resulted in similar larval weight and length compared to the control (continuous feeding 7/0). For larvae, an increase in feed deprivation and a reduction in feeding frequency caused lower survival rates. Feed deprivation for 3 days (4/3) showed the lowest survival rate. However, 1 day of feed deprivation (6/1) showed a similar survival rate compared to the control (daily feeding 7/0) (Table 1). Relative condition factor, larval weight, and length uniformity did not show any differences among the treatments (Table 2).

For juveniles, at the end of 60 days, 1 day of feed deprivation caused similar larval growth performance when compared to the control group (7/0). Feed deprivation for more than 1 day a week reduced the final weight, the weight gain, the specific growth rate for weight, the biomass total and biomass gain, the apparent ration consumption, and the apparent feed conversion. Further, there were no differences in specific growth rate for length, final length, and length gain in larvae fed 1 or 2 days a week. Uniformity values for weight and length, relative condition factor, and survival rate did not differ statistically among the different management strategies (Table 3).

Table 1. Mean ± standard deviation on growth performance of *Pyrrhulina brevis* larvae subjected to the different feeding frequencies and cycles of feeding deprivations.

	Frequency	7/0	6/1	5/2	4/3	Alt
FL (mm)						
	2	21.68 ± 0.61 Ab	20.36 ± 1.02 Bb	20.60 ± 1.62 BCb	18.84 ± 0.43 Cb	19.54 ± 0.81 Ca
	4	24.32 ± 0.74 Aa	21.94 ± 0.41 Aa	22.07 ± 0.71 Aa	21.02 ± 1.50 Aa	20.46 ± 1.62 Aa
FW (mg)						
	2	145.22 ±14 Ab	124.34 ± 16 Bb	115.53 ± 25 BCb	88.55 ± 55 CDb	85.26 ± 12 Db
	4	182.13 ± 80 Aa	148.92 ± 20 Ba	120.75 ± 13 BCa	125.01 ± 25 CDa	103.22 ± 28 Da
$SRG_{(W)}$ (%)						
	2	1.68 ± 0.41 Ab	2.13 ± 0.75 Ab	1.81 ± 1.13 Aa	2.39 ± 0.53 Ab	1.45 ± 0.71 Ab
	4	2.14 ± 0.63 Aa	2.59 ± 0.44 Aa	1.83 ± 0.22 Aa	2.74 ± 0.71 Aa	2.12 ± 0.24 Aa
$SRG_{(L)}$ (%)						
	2	3.13 ± 0.05 Aa	3.03 ± 0.03 Aa	3.16 ± 0.05 Aa	3.08 ± 0.12 Aa	3.03 ± 0.14 Aa
	4	3.33 ± 0.04 Aa	3.15 ± 0.07 Ba	3.03 ± 0.09 BCa	2.91 ± 0.04 Cb	2.96 ± 0.07 Ca
Survival (%)						
	2	89.51 ± 0.50 Aa	78.35 ± 2.62 Ab	74.54 ± 10.54 Aa	38.12 ± 3.42 Bb	66.65 ± 4.82 ABa
	4	90.68 ± 0.50 Aa	82.65 ± 1.31 Aa	74.68 ± 8.35 ABa	46.24 ± 4.41 Ba	72.35 ± 5.91 ABa
p value						
		Frequency		Deprivation		Interaction
FL (mm)		0.0001		0.0001		0.0001
FW (mg)		0.0001		0.0001		0.0001
SRG(g) (%)		0.0014		0.0548		0.0011
SRG(l) (%)		0.5486		0.0005		0.0001
S (%)		0.0001		0.0174		0.0001

Note. FL = final length, FW = final weight, $SGR_{(L)}$ = specific growth rate for length, $SGR_{(W)}$ = specific growth rate for weight, S = survival. Different letters in the columns (lowercase) and row (uppercase) means statistical difference by Tukey test ($P < 0.05$).

Table 2. Mean ± standard deviation on growth performance of *Pyrrhulina brevis* larvae subjected to the different feeding frequencies and cycles of feeding deprivations.

	Feed frequency (times/day)		Feed deprivation				
Variables	2	4	7/0	6/1	5/2	4/3	Alt
$U_{(L)}$ (%)	96.6 ± 2.41	97.6 ± 2.64	100.00 ± 0.00	98.00 ± 2.82	94.00 ± 0.75	98.00 ± 1.72	95.00 ± 1.43
$U_{(W)}$ (%)	73.37 ± 2.29	74.54 ± 1.48	77.35 ± 1.62	76.77 ± 2.13	76.06 ± 2.75	66.51 ± 2.14	73.01 ± 2.82
Kr	1.00 ± 0.01	1.00 ± 0.01	1.01 ± 0.01	1.01 ± 0.01	0.99 ± 0.01	0.98 ± 0.01	1.00 ± 0.01
P value							
	Feed frequency		Feed deprivation			Interaction	
$U_{(L)}$ (%)	0.8122		0.9211			0.8446	
$U_{(W)}$ (%)	0.8541		0.9654			0.6845	
Kr	0.4585		0.6525			0.6358	

Note. $U_{(L)}$ = uniformity for length, $U_{(W)}$ = uniformity for weight, Kr = relative condition factor.
Different letters in the row means statistical difference by Tukey test ($P < 0.05$).

Table 3. Mean ± standard deviation on growth performance of *Pyrrhulina brevis* fingerlings subjected to the different cycles of feeding deprivations.

	Feeding deprivation					
Variables	7\0	6\1	5\2	4\3	Alt	P value
FL (cm)	4.71 ± 0.14 a	4.55 ± 0.11 a	4.38 ± 0.15 ab	4.18 ± 0.12 b	4.37 ± 0.11 ab	0.0053
FW (g)	0.97 ± 0.06 a	0.83 ± 0.07 ab	0.71 ± 0.04 b	0.66 ± 0.05 c	0.71 ± 0.01 b	0.0002
LG (cm)	1.93 ± 0.14 a	1.78 ± 0.11 a	1.61 ± 0.15 ab	1.41 ± 0.12 b	1.61 ± 0.11 ab	0.0053
WG (g)	0.79 ± 0.06 a	0.65 ± 0.07 ab	0.52 ± 0.04 b	0.48 ± 0.05 c	0.52 ± 0.01 b	0.0002
$SGR_{(L)}$ (%/day)	0.88 ± 0.05 a	0.82 ± 0.03 a	0.76 ± 0.07 ab	0.68 ± 0.04 b	0.76 ± 0.02 ab	0.0053
$SGR_{(W)}$ (%/day)	2.82 ± 0.10 a	2.57 ± 0.13 a	2.28 ± 0.09 b	2.18 ± 0.12 b	2.29 ± 0.01 b	0.0003
BT (g)	37.71 ± 4.14 a	31.08 ± 2.63 ab	24.91 ± 1.53 bc	20.75 ± 2.25 c	24.03 ± 1.94 c	0.0003
BG (g)	29.74 ± 4.14 a	23.11 ± 2.63 ab	16.95 ± 1.53 bc	12.78 ± 2.25 c	16.06 ± 1.94 c	0.0003
ARC (g)	42.54 ± 2.56 a	33.05 ± 3.23 b	29.47 ± 2.57 b	23.13 ± 0.67 c	21.88 ± 0.29 c	0.0001
RRC(g)	0.018 ± 0.001a	0.017 ± 0.001 a	0.018 ± 0.002 a	0.012 ± 0.002 b	0.021 ± 0.001 a	0.0485
FCR	1.44 ± 0.19 a	1.43 ± 0.14 a	1.74 ± 0.17 b	1.88 ± 0.12 b	1.37 ± 0.17 a	0.0466
$U_{(L)}$ (%)	94.92 ± 2.28	99.14 ± 1.52	97.25 ± 2.72	96.11 ± 6.81	95.91 ± 3.55	0.7239
$U_{(W)}$ (%)	62.01 ± 9.16	59.32 ± 11.01	57.76 ± 11.67	62.02 ± 3.61	57.37 ± 8.32	0.9412
Kr	1.00 ± 0.03	0.98 ± 0.08	0.99 ± 0.03	1.00 ± 0.05	1.01 ± 0.06	0.8434
S (%)	86.71 ± 5.88	82.34 ± 2.57	79.26 ± 5.13	70.32 ± 10,96	76.33 ± 6.42	0.1047

Note. FL = final length, FW = final weight, LG = length gain, WG = weight gain, $SGR_{(L)}$ = specific growth rate for length, $SGR_{(w)}$ = specific growth rate for weight, BT = biomass total, BG = biomass gain, ARC = apparent ration consumption, RRC = relative ration consumption, FCR = feeding conversion rate, $U_{(L)}$ = uniformity for length, $U_{(W)}$ = uniformity for weight, Kr = condition factor, S = Survival, Alt = alternated feeding. Different letters in the row means statistical difference by Tukey test ($P < 0.05$).

Discussion

Feed deprivation resulted in high mortality rates in *P. brevis* larvae that were subjected to three days of food deprivation or that were fed on alternate days. The larvae probably have no energy reserves that could be used during feed deprivation, or they had no time to adapt to the changing feeding strategy (Bar 2014; Zaldúa and Naya 2014). Thus, increasing time among the feeding frequencies as well as the intensification of feeding deprivation can affect the normal development and survival of *P. brevis*, reducing the final weight and consequently daily growth rate ($SGR_{(L)}$). This effect also causes reduced development, muscle atrophy, and reduced swimming speed to basal energy for larvae of *Brycon orbignyanus* when the feeding deprivation occurs for more than two days (Melo et al. 2020).

Feed deprivation can reduce growth performance because the fish use the nutrients for basal metabolic processes instead of body development and reproduction (Ali et al., 2003). In addition, the youngest fish have little capacity to adapt to a new condition, and therefore feed deprivation causes several morphophysiological alterations, which are reflected in growth and survival reductions (Ali et al., 2003; Harpaz et al. 2005; Jobling 2010; Krogdahl and Bakke-mckellep 2005).

Some fish species subjected to feed deprivation can reduce their heart rate, thereby saving energy and showing morphological changes, such as a reduction in gut length (Gisbert and Doroshov 2003; Rios et al. 2002; Wang, Hung, and Randall 2006; Zaldúa and Naya 2014; Zeng et al. 2012).

However, during the refeeding, some fish have morphophysiological changes such increased stomach capacity and increased absorption structures in the intestine to reach a "compensatory growth". This feeding strategy named "compensatory growth" allow the fish to grow just like other fish with normal feeding (Ali et al., 2003).

Fish larvae depend on exogenous feed with a high nutritional profile through the larviculture period, and therefore, feed deprivation for larvae is not recommended as it results in low development rates; in addition, food deprivation strategies for periods greater than three days can increase fish mortality (Bolasina, Pérez, and Yamashita 2006; Kojima et al. 2015; Wunderink et al. 2012).

In contrast, the juveniles could manage at least 1 day of feed deprivation without growth and survival losses. This difference between larvae and juveniles can be explained by the greater energy reserves in juvenile fish, making them more resistant to feed deprivation (Bar 2014; Kojima et al. 2015).

According to Won and Borski (2013), after feeding deprivation, fish undergo endocrine alterations such as elevated levels of ghrelin and growth hormone, increasing the appetite. Thus, *P. brevis* juveniles showed lower apparent ration consumption with increased cycles of feed deprivation; however, the relative ration consumption remains similar to continuous feeding, demonstrating hyperphagia, which is a species adaptation strategy.

Feeding deprivation also can increase the ingestion rate of food, as observed for *Brycon amazonicus* and *Acanthopagrus schlegelii*. However, when it remains for a long time, during the refeeding some animals maybe have no compensatory growth due to the poor nutrient absorption (Urbinati, Sarmiento, and Takahashi 2014; Xiao et al. 2013).

However, this hyperphagia promoted the increase of feeding conversion rates in treatments with 2 or 3 days of feed deprivation. Furthermore, in the alternated days feed deprivation treatment no alteration of the feeding conversion rate was observed, demonstrating that for *P. brevis* the feeding deprivation on alternate days would be harmful for its development.

Hyperphagia can cause hypertrophy of the stomach and an increased amount of digestive enzymes, allowing better nutrient absorption (Känkänen and Pirhonen 2009; Xiao et al. 2013). However, in *P. brevis*, hyperphagia was probably not able to increase enzyme activities and nutrient use to promote compensatory growth at more than 1 day of feed deprivation.

Despite the reduced growth performance, feed deprivation had no influence on fish uniformity (weight and length), the relative condition factor, or survival, demonstrating an adequate nutritional profile and health. In addition, feed deprivation reduced feed and labor costs (Fujimoto et al. 2016; Oh et al. 2013; Urbinati, Sarmiento, and Takahashi 2014). Although we did not calculate the production costs, a 22.3% reduction in ration consumption was determinate.

A similar reduction (22.50%) in ration consumption was reported for tilapia (*Oreochromis nilotius*) submitted to feed deprivation (2 days a week) without affecting growth (Palma et al. 2010). The fish fed on alternate feed deprivation days had a better feed conversion rate, despite the reduced performance compared to the control (daily feeding 7/0). Thus, considering the ornamental market, where every fish is sold per unit and not per weight, feeding on alternate days could be more profitable, but this will need to be investigated in detail in further studies.

Therefore, scientific knowledge about the feeding strategies using different management strategies throughout the rearing period is an important factor. Feed deprivation as a feeding strategy can result in reduced labor and feeding costs, making it a potential alternative to optimize captivity rearing.

Conclusion

This study does not recommend feed deprivation in *Pyrrhulina brevis* larviculture. However, juveniles can be subjected to 1 day of feed deprivation a week without impacts on growth performance.

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Disclosure statement

The authors have no any conflict of interest to declare.

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