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Research Article

Effect of fasting on freshwater angelfish *Pterophyllum scalare* (Lichtenstein; Pisces: Cichlidae) development

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ABSTRACT. We tested the effect of fasting on the development of juvenile freshwater angelfish (*Pterophyllum scalare*). The juveniles were subjected to different fasting periods (0, 3, 6, 9, 12, and 15 days of fasting). Fish from all treatments were fed for 30 days after the fasting period. No difference ($P > 0.05$) was observed between treatments for final weight and length. There was a significant difference ($P < 0.05$) in treatments 12 and 15 days fasting for liver and intestine weight. These results may prove that 12 and 15 days of fasting force juveniles to use energy reserves, and these adaptations may also represent a saving energy strategy. The fish subjected to treatments 6, 9, 12, and 15 days of fasting showed different vacuolization in hepatocytes from the ones subjected to 0 and 3 days of fasting ($P < 0.05$). These results show that during the lack of feeding, juveniles (*P. scalare*) mobilizes energy stored in the liver. No difference ($P > 0.05$) was observed between treatments for survival rate. However, the increase in the number of deaths from the eighth day of fasting may indicate that periods with more than 15 days of fasting could be fatal to this species during this life stage. According to our findings, it can be assumed that juvenile freshwater angelfishes have strategies (e.g. a decrease in liver and intestine weight) to resist 15 days of fasting.

Keywords: *Pterophyllum scalare*; Cichlidae; food deprivation; hepatocytes; phenotypic plasticity; aquaculture

INTRODUCTION

Fasting is common in nature for many species, like salmonids (Brett 1995), eels (Schmidt 1923), birds (Liknes et al. 2014), and snakes (Lignot et al. 2005). These animals obtain the energy necessary for their basal metabolism, physical activities, growth, and reproduction from food intake (Wang et al. 2006). When food is not available, they must use their energy reserves to survive (Urbinati et al. 2014, Hernández et al. 2018).

The animals have different internal sources of energy to access during fasting (e.g. muscle glycogen; adipocytes); among these internal sources of energy, the energy reserves of the liver and intestine play significant roles. Changes in these organs during fasting have been reported by many authors (Rios et al. 2004, Gaucher et al. 2012, Alix et al. 2017). The liver acts in the catabolism of energy substrate and also stores lipids, glycogens, and proteins that can be used during fasting periods (Black & Love 1986). Whereas the intestine has an essential function regarding energy maintenance since this organ is energetically expensive (Funes et al. 2014, Wang et al. 2006), demanding oxygen consumption (Cant et al. 1996) and high cellular turnover rate of the intestinal epithelium (Secor 2005).

Experiments of fasting on fish at the juvenile stage have shown that these animals are adapted to support prolonged starvation. However, these experiments have usually been carried out on large species, with a preference for using juveniles at an advanced stage of development (Pérez-Jiménez et al. 2007, Urbinati et al. 2014). In contrast, the effect of fasting on fish that had recently changed for the juvenile stage has only rarely been tested.

The freshwater angelfish (*Pterophyllum scalare*) is a cichlid from the Amazon basin and is one of the most

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important species for the ornamental fish trade. For ornamental purposes, fish are transported for trading inside bags, and in some cases, they experience long trips and many days of fasting. Thus, the present experiment was carried out to elucidate the effects of fasting on the phenotypic plasticity of organs with increased activity during such periods (e.g. the liver and intestine) and the likelihood of survival after such events, concerning the development of juveniles of the Amazon cichlid *P. scalare*.

MATERIALS AND METHODS

Water quality and maintenance system parameters

The experiment was carried out in a recirculating aquaculture system, composed of 18 tanks (20 L each), with mechanical filtration based on an acrylic blanket and expanded clay; a 200 L biological filter with ceramic rings for bacterial fixation and a 30 ppi foam filter; an ultraviolet reactor (36 W lamp) coupled to a pump of 2000 L h^{-1} capacity; a sump of 80 L with a pump of 4800 L h^{-1} capacity for water return; and a heater with thermostat (300 W) to assure the optimal temperature for *Pterophyllum scalare* development. A digital timer was installed to turn on/off twenty 20 W fluorescent lamps to ensure a 12:12 h photoperiod.

The water flow rate was regulated so that the total volume of each tank would be renewed at least 20 times a day. Feces and feed that had not been consumed were removed daily from the tanks and the mechanical filter through a siphoning process. During this cleaning process, up to 10% of the total water volume of the system was changed daily.

Dissolved oxygen and temperature were measured daily (YSI 550 A ± 0.01), pH three times a week (pHtek PHS-3E ± 0.02), and total ammonia nitrogen (NH_3 + NH_4^+) once a week (Hanna HI83203). These parameters were sampled randomly in four tanks of the experimental system and always at the same time (10 h). The following water quality values were obtained from the recirculating aquaculture system over the experimental period (mean ± standard error): 6.74 ± 0.05 mg L^{-1} for dissolved oxygen; 29.3°C for temperature; 6.6 ± 0.3 for pH; and 0.029 ± 0.013 mg L^{-1} for total ammonia nitrogen.

Design, animals, feeding, and body indexes

Six treatments (T0, T3, T6, T9, T12, and T15) were developed to evaluate the effect of fasting on juvenile freshwater angelfish. The treatments of fasting periods were designed as follows: T0: zero days fasting, T3: three days fasting, T6: six days fasting, T9: nine days fasting, T12: 12 days fasting, and T15: days fasting. After the respective fasting periods, the fish from all treatments were fed for 30 days. Treatment 0 (T0) was assumed to be the positive control, and so, the fish subjected to this treatment did not go through any fasting period.

The experiment was divided into two sequential periods: a fasting period, in which duration varied according to the proposal for each treatment, and the feeding period, with the same duration for all treatments. Thus, juveniles (*P. scalare*) were analyzed for the fasting period, the effect of the feeding period, and the combined effect of these two periods (treatment effect).

The juveniles used in this experiment were obtained through natural spawning from three different couples that were kept in an indoor laboratory. The time difference between these three spawnings did not exceed two days. Larvae from these three couples were grown together in a single tank.

Two hundred twenty-four juveniles, with no significant difference ($P > 0.05$) in weight (1.09 ± 0.12 mg) and total length (26.51 ± 0.71 mm), were used in the experiment. From these, 20 specimens were killed (desensitized using clove oil and ice) the day before the onset of the experiment to obtain the initial body indexes (length; weight; liver weight; intestine weight). Also, from this group of 20 fish, liver, and intestine samples (n = 6) were collected for histological analysis on these organs before the fasting period. These data were used as initial body indexes for T0.

For T0, 24 juveniles were distributed randomly into three different tanks, eight fish per tank. For the other treatments (T3, T6, T9, T12, and T15), 180 juveniles were distributed randomly into 15 different tanks, totaling 12 fish per tank. Thus, 18 tanks (six treatments with three repetitions each) were used in this experiment; each represents an experimental unit.

At the end of each treatment's fasting period, all fish from the respective treatment were measured and weighed to obtain body indexes; four animals from each tank were killed to obtain liver and intestine weight values. The liver and the initial portion of the intestine were fixed in formalin for further histological analyses. Thus, at the beginning of the feeding period, all treatments had eight fish per tank.

In the final of the experimental period of each treatment, the fish from the respective treatment were measured and weighed to obtain length and weight data. Three fishes from each tank were killed to obtain liver and intestine weight; the liver and the initial portion of the intestine were fixed in formalin for further histological analyses.

Deaths were recorded daily to obtain values for the fasting effect on the estimated survival rate. It is important to mention that fish that were euthanized at the end of each fasting period (T3: third day; T6: sixth day, T9: ninth day, T12: 12th day, T15: 15th day), were not counted as mortality since they did not die through the influence of the treatments.

The hepatosomatic index (HSI) was calculated from the ratio of liver weight (g) and fish weight (g), following the equation proposed by Wingfield & Grimm (1977):

$$HSI = \frac{Liver\ weight(g)}{Fish\ weight(g)} \times 100$$

The data of feeding (DF) day 0 (0 DF) of these treatments were compared with the initial data of T0 (0 DF) to obtain data (i.e. weight, length) regarding the effect of fasting period on juveniles for treatments T3, T6, T9, T12, and T15 since all 224 fish came from the same tank and had no significant difference ($P < 0.05$) among them.

The variable's value to be analyzed at 0 DF was compared with the same variable at 30 DF in the same treatment to observe the development of juveniles during the feeding period; the variables were compared between treatments at the time of 30 DF, to compare the effect of the treatment (combined effect of the two periods) at the end of the experimental period.

During the feeding period, the fish were fed, *ad libitum*, four times a day (08:00, 11:00, 14:00, and 17:00 h), using a commercial feed (Aquaxcel Purina® 4512) of 0.8 mm (feed chemical composition: protein: 45 g kg^{-1}, fat: 12 g kg^{-1}, crude fiber: 5 g kg^{-1}, moisture: 11 g kg^{-1}). Each experimental unit had a vessel to keep the commercial feed to control the feed used during the feeding period. Each vessel was weighed at 0-DF and 30-DF. The difference between these two measures was considered as the total feed intake per experimental unit. Each empty vessel's weight (before filling with feed) was obtained, and this value was subtracted to obtain the feed intake.

An analytical scale (Shimadzu AUX 220 ± 0.001 g) and a digital caliper (Western 6" (150 mm) ± 0.01 mm) were used to obtain all weight and length values, respectively.

Histological analyses

For subsequent analysis under an optical microscope, the liver and intestine samples were fixed in 10% buffered formalin for one week. They then were dehydrated in an increasing ethyl alcohol series, clarified with xylol, and embedded in paraffin, following the routine techniques of the histopathology laboratory. Serial sections of 5 μm of thickness were obtained with the aid of a microtome and were then subjected to routine dewaxing, hydration, and staining techniques (Humason 1972). It is important to emphasize that transverse sections were cut through the intestine to ensure the integrity of the villi and their size in the lumen.

The histological slides were photographed under an optical microscope (Zeiss® Axio Vert. A1) with a coupled camera. The degree of vacuolization was quantified by counting vacuolated hepatocytes per slide using the Image J software, developed by Wayne Rasband (http://imagej.nih.gov/ij/docs/index.html); and the intestinal villus length measurements were performed using the Axio Vision 4.9 software.

Statistical analysis

For the variables of final weight and length, liver and intestine weight, hepatosomatic index, feed intake, and intestinal villi length, mixed linear models were analyzed using the MIXED procedure of the statistical analysis system (SAS System, Inc., Cary, NC, USA). In cases of significant difference, the Tukey test at 0.05 probability was applied. Before the analyses using the MIXED procedure, the variables were evaluated for normality and homoscedasticity by the tests Shapiro Wilk and Breusch-Pagan, using the UNIVARIATE and MODEL procedures of the analytical system (SAS). Final weight and feed intake correlation were investigated using Pearson's correlation analysis. Concerning the degree of vacuolization in the liver, the theory of generalized linear-model Poisson distribution was used. The procedure adopted in this case was the GLIMMIX of the statistical analysis system (SAS System, Cary, NC, USA), and, in cases of significant difference, the Tukey test at 0.05 probability was applied. The survival estimate was calculated using the model proposed by Kaplan & Meier (1958).

RESULTS

Body indexes and feed intake

A slight decrease in weight between the juveniles (*Pterophyllum scalare*) subjected to fasting was observed (Table 1), but the fasting periods tested in the present experiment were not sufficient to cause any significant weight loss ($P > 0.05$).

After 15 days of feeding (15 DF), there was no significant difference in weight ($P > 0.05$) between treatments. The same result ($P > 0.05$) was observed regarding this parameter after 30 days of feeding (30-DF) (uppercase letters; Table 1). It is possible to realize that fish of treatments T0 and T3 do not present a significant difference ($P > 0.05$) in weight between

Table 1. Total length and weight of juveniles (*Pterophyllum scalare*) during different periods of fasting and feeding. Uppercase letters in the same column mean a significant difference between treatments ($P < 0.05$) and, lowercase letters on the same line mean a significant difference ($P < 0.05$) during the feeding period. 0 DF: zero days of feeding; 15 DF: 15 days of feeding; 30 DF: 30 days of feeding. T0, T3, T6, T9, T12 and T15, represents, respectively: 0, 3, 6, 9, 12 and 15 days of fasting.

Treatment (fasting days)	Total length (mm)		
	0 DF	15 DF	30 DF
T0	26.5 ± 0.7^{Ab}	28.3 ± 0.7^{Ab}	34.0 ± 0.7^{Aa}
T3	26.9 ± 0.6^{Ab}	29.3 ± 0.7^{Ab}	34.1 ± 0.7^{Aa}
T6	26.8 ± 0.6^{Ac}	31.5 ± 0.7^{Ab}	35.1 ± 0.7^{Aa}
T9	26.3 ± 0.6^{Ac}	31.8 ± 0.7^{Ab}	35.6 ± 0.7^{Aa}
T12	26.3 ± 0.6^{Ab}	30.5 ± 0.8^{Aa}	34.1 ± 0.8^{Aa}
T15	25.3 ± 0.6^{Ab}	30.9 ± 0.9^{Aa}	33.8 ± 0.9^{Aa}
Treatment (fasting days)	Weight (g)		
	0 DF	15 DF	30 DF
T0	0.1096 ± 0.012^{Ab}	0.1303 ± 0.012^{Ab}	0.2269 ± 0.012^{Aa}
T3	0.1047 ± 0.011^{Ab}	0.1551 ± 0.012^{Ab}	0.2259 ± 0.012^{Aa}
T6	0.1008 ± 0.011^{Ac}	0.1737 ± 0.012^{Ab}	0.2501 ± 0.012^{Aa}
T9	0.0893 ± 0.011^{Ac}	0.1899 ± 0.012^{Ab}	0.2629 ± 0.012^{Aa}
T12	0.0888 ± 0.011^{Ac}	0.1680 ± 0.013^{Ab}	0.2370 ± 0.012^{Aa}
T15	0.0862 ± 0.011^{Ab}	0.1644 ± 0.014^{Aa}	0.2167 ± 0.012^{Aa}

periods 0 DF and 15 DF. Nevertheless, they present a significant difference ($P < 0.05$) in the 30 DF period (uppercase letters; Table 1); while for T6, T9 and T12, there is a significant difference ($P < 0.05$) from 0 DF to 15 DF, and from 15 DF to 30 DF (lowercase letters; Table 1). For T15, a significant difference was observed ($P < 0.05$) of 0 DF to15 DF, but not from 15 DF to 30 DF (lowercase letters; Table 1).

Similar results to those observed for weight were noticed for the length ($P > 0.05$) (Table 1). Despite the similarity with weight data, the total lengths of the juveniles shortly after the fasting period (0 DF) did not demonstrate even a slight decrease (Table 1).

The values obtained for feed intake (g) per treatment were (mean ± standard error): T0: 5.4460 ± 0.7356 g; T3: 5.2807 ± 0.7356 g; T6: 5.8077 ± 0.7356 g; T9: 6.8120 ± 0.7356 g; T12: 5.9950 ± 0.7356 g; T15: 4.582 ± 0.7356 g. There was no significant difference ($P > 0.05$) between feed intakes. However, a high correlation ($r = 0.93$, $P < 0.05$) between feed intake and fish weight was observed in the experimental units.

Phenotypic plasticity

The information presented in Table 1 demonstrates the adaptability of juveniles to the effects of fasting. For intestine weight (IW), periods of three, six, and nine days of fasting (T3, T6, and T9, respectively) were not enough to affect IW (mg) ($P > 0.05$), despite the observation of a slight decrease in the means, compared with the control treatment. However, for treatments T12 and T15 (12 and 15 days of fasting, respectively), a significant influence ($P < 0.05$) compared to T0 was observed. Thus, attesting that under such conditions, the juveniles presented a loss of mass in this organ.

It was observed that 30 DF were not enough for the juveniles subjected to the treatments T12 and T15 to return to normal IW (mg) ($P < 0.05$) (IW 30 DF; Table 2).

Subsequently, the fasting periods, the liver weight (LW) in treatments T3, T6, and T12 showed decreases compared with T0, but without any significant difference ($P > 0.05$) (LW 0 DF, Table 2). The fish subjected to the treatments T12 and T15 had significantly lower LW (mg) than the fish subjected to T0 ($P < 0.05$) shortly after the fasting period.

After 30 DF, the LW (mg) of the fish that had been subjected to the treatments T12 and T15 did not become normal and significant differences were observed ($P < 0.05$) compared with T0 ($P < 0.05$).

It was observed that fasting influenced the hepatosomatic index ($P < 0.05$). Figure 1 shows that three days of fasting (T3) were sufficient to influence this variable. Also, it can be observed that the treatments that were longer than T3, i.e. T6, T9, T12, and T15, did not modify this condition ($P > 0.05$).

Figure 1 also shows that, for the hepatosomatic index, fish that went through the fasting periods of the T3, T6, T9, and T12 treatments, followed by 30 DF period, were able to recover their normality, as observed in fish from the control treatment (T0) ($P >$

Table 2. Phenotypic plasticity of juveniles (*Pterophyllum scalare*) during fasting and feeding (mean ± standard error). IW: intestine weight; LW: liver weight; 0 DF: zero days of feeding; 30 DF: 30 days of feeding. Different letters in the same column mean a significant difference among treatments. T0, T3, T6, T9, T12 and T15, represents, respectively: 0, 3, 6, 9, 12 and 15 days of fasting.

Treatment (fasting days)	IW (mg)		LW (mg)	
	0 DF	30 DF	0 DF	30 DF
T0	57.35 ± 6.4^{A}	97.25 ± 6.4^{A}	36.96 ± 4.3^{A}	65.39 ± 4.3^{A}
T3	42.13 ± 9.0^{AB}	92.72 ± 6.4^{AB}	16.45 ± 6.0^{AB}	57.13 ± 4.3^{AB}
T6	36.20 ± 9.0^{AB}	91.03 ± 6.4^{AB}	11.47 ± 6.0^{AB}	50.96 ± 4.3^{AB}
T9	36.32 ± 9.0^{AB}	91.81 ± 6.4^{AB}	13.40 ± 6.0^{AB}	49.16 ± 4.3^{AB}
T12	32.73 ± 9.0^{BC}	68.14 ± 6.4^{BC}	10.00 ± 6.0^{B}	45.14 ± 4.3^{B}
T15	33.75 ± 9.0^{C}	46.43 ± 6.4^{C}	9.33 ± 6.0^{B}	24.81 ± 4.3^{C}

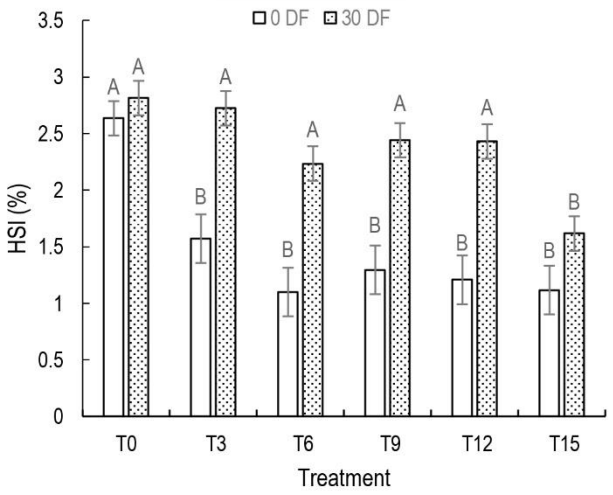

Figure 1. Hepatosomatic index (HSI) of juveniles (*Pterophyllum scalare*) during periods of fasting and feeding. 0 DF: zero days of feeding; 30 DF: 30 days of feeding. Different letters (A and B) mean a significant difference ($P < 0.05$). T0, T3, T6, T9, T12 and T15, represents, respectively: 0, 3, 6, 9, 12 and 15 days of fasting.

0.05). On the other hand, even after 30 DF, the fish subjected to T15 were unable to compensate for the damage caused during the fasting period and presented a significant difference for this variable, compared with the other treatments ($P < 0.05$).

Vacuolated hepatocytes were observed in the livers of the juveniles subjected to feeding. They are composed of polygonal structures, with significant free areas, indicating energy reserves ("lipid and glycogen drops," which were washed during histological procedures), with a nucleus at the periphery of the cell. Polygonal hepatocytes were observed without free areas, the nucleus was centralized, and a smaller cytoplasmic area in the livers of the juveniles that went through fasting periods.

The data concerning the degree of vacuolization are shown in (Fig. 2). The juveniles subjected to the treatments T0 and T3 presented hepatocytes with clear and vacuolated cytoplasm (Fig. 3). The same appearance was not observed in the hepatocytes of the fish that received T6, T9, T12, and T15, after fasting periods. Nevertheless, the juveniles from T6, T9, T12, and T15 presented vacuolated hepatocytes after the period of 30 DF.

The histological analysis made it possible to observe the serosal, muscle, submucosal, and mucosal layers (Fig. 4) of the intestine of freshwater angelfish. Goblet cells can be seen in the intestinal villi. Fasting influenced the villi length in the initial fraction of the intestine ($P < 0.05$). This comparison, shown in Figure 5, within the treatment between the start and end of the feeding period (0-30 DF), indicates that intestinal villi still enter the intestinal lumen at this point of the juvenile phase.

The effect of different periods of fasting on the villi can be seen in Figure 5 (0 DF). These data show the regression of villi, which tended to decrease the length from 6 fasting days onwards. After 30 DF, none of the intestinal villi of the juveniles in any of the treatments presented any significant difference in length ($P > 0.05$).

Survival estimate

There was no significant difference in the survival estimates for the juveniles (*P. scalare*) subjected to different fasting periods ($P > 0.05$). From 144 fish (sampling was not considered in this calculation), only 14 fish died during the experimental period (Table 3).

Most deaths occurred between days eight to fifteen of fasting, which can be called a critical period. The survival estimates for T15 dropped from 100 to 86.5% over this period. For this treatment, deaths occurred on days 8, 9, 14, and 15. In this critical period, deaths in treatments T9 and T12 were observed, with one death

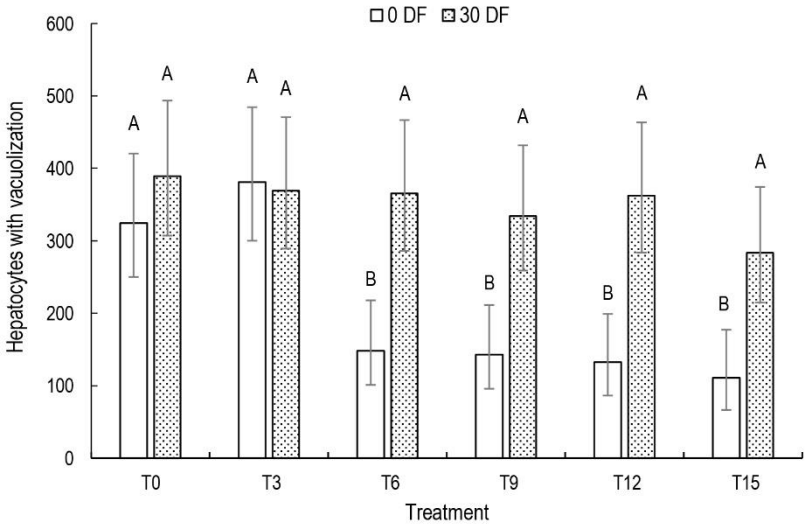

Figure 2. The degree of vacuolization of the hepatocytes of juveniles (*Pterophyllum scalare*) subjected to different periods of fasting. 0 DF: zero days of feeding; 30 DF: 30 days of feeding. Different letters (A and B) mean a significant difference ($P < 0.05$). T0, T3, T6, T9, T12 and T15, represents, respectively: 0, 3, 6, 9, 12 and 15 days of fasting.

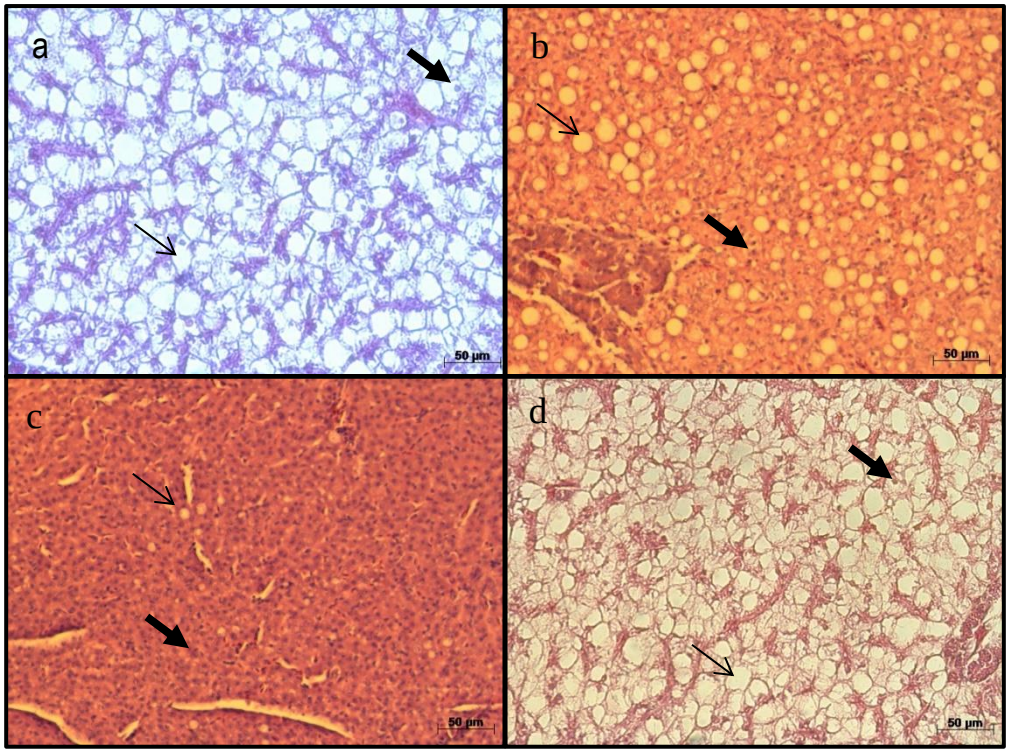

Figure 3. Liver images of juveniles (*Pterophyllum scalare*) subjected to different fasting and feeding periods. a) Fish without feed deprivation; b) fish that went through nine days of fasting; c) fish that went through 15 days of fasting; d) fish that went through nine days of fasting and then were fed for 30 days; thick arrow: hepatocyte nucleus; slim arrow: vacuolated hepatocyte.

occurring on the eighth day in T9 and one death on the eighth day in T12, along with two deaths on the 10th day in T12.

Although the critical starvation period started on the eighth day of fasting, no fish deaths were observed in T9 and T12 during the feeding period. The same cannot be said for T15, which presented two deaths even after

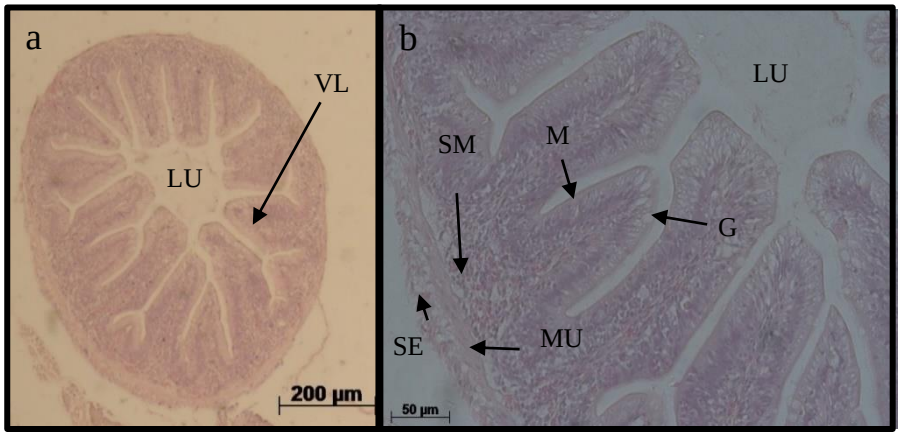

Figure 4. Microscopy images of freshwater angelfish (*Pterophyllum scalare*) intestine. a) 5x, and b) 20x cross-section of the initial portion of the gut of juvenile freshwater angelfish. LU: lumen; VL: intestinal villus; SE: serosa; MU: muscle layer; SM: submucosa; M: mucosa; GC: goblet cell.

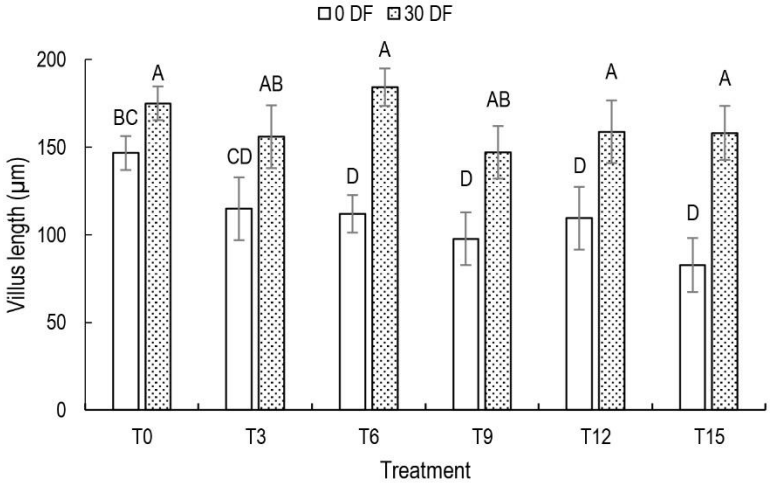

Figure 5. Intestinal villi length of juveniles (*Pterophyllum scalare*) subjected to periods of fasting and feeding. 0 DF: zero days of feeding; 30 DF: 30 days of feeding. Different letters (A and B) mean a significant difference ($P < 0.05$). T0, T3, T6, T9, T12 and T15, represents, respectively: 0, 3, 6, 9, 12 and 15 days of fasting.

the end of the fasting period: one on day 19, the fourth day of the feeding period, and another on day 23, the eighth day of the feeding period. The deaths observed in the treatments T0, T3 and T6 occurred randomly throughout the feeding period.

DISCUSSION

Body indexes and feed intake

In the present experiment, it was observed that the fasting periods tested did not influence the lengths of the juvenile fish. This information is mainly relevant to the ornamental fish trade since changes in the fish's length result in losses of quality and price.

The results observed in the present experiment regarding fish length corroborate the ones reported by Gaucher et al. (2012) and Power et al. (2000), demonstrating that reduction of body length is not a common adaptative strategy of fish. For some invertebrates, changes in body length are a strategy for dealing with variations in food availability; this has been reported about the sea urchin, *Diadema antillarum*, by Levitan (1989); and for Pacific krill *Euphausia pacifica*, which also reduces the body size when they are in feed stress situations (Marinovic & Mangel 1999).

Fasting periods up to 15 days were not enough to influence the weight of these fish. However, the decrease in the mean weight values between these juve-

Table 3. Survival probability (mean % ± standard error) and the number of dead fish (per treatment) of juveniles (*Pterophyllum scalare*) submitted to different treatments. All fish were considered per treatment, except sampling. ND not applicable. T0, T3, T6, T9, T12 and T15, represents, respectively: 0, 3, 6, 9, 12 and 15 days of fasting.

Treatment (fasting days)	Survival probability (%)	Experimental period (days)	Number of dead fish		Survival probability (%)	
			Fasting period	Feeding period	Fasting period	Feeding period
T0	96.00 ± 0.04	30	ND	1	ND	96.00 ± 0.04
T3	96.77 ± 0.03	33	0	1	100	96.77 ± 0.03
T6	96.77 ± 0.03	36	0	1	100	96.77 ± 0.03
T9	96.77 ± 0.03	39	1	0	96.77 ± 0.03	100
T12	90.90 ± 0.05	42	3	0	96.00 ± 0.04	100
T15	81.10 ± 0.06	45	5	2	86.50 ± 0.06	94.60 ± 0.06

niles with the increasing length of the fasting period may indicate that periods longer than 15 days of fasting may affect the weight of juvenile freshwater angelfish.

Periods of fasting are common in nature, and animals exhibit behavioral and physiological adaptations that allow them to go through such periods without significant weight loss. Fu et al. (2011), working with *Silurus meridionalis* juveniles, observed that these fish did not present any significant weight loss after one week of fasting, but two and four weeks of feed restriction affected their weight. Those results, like these of the present study, show that fish can endure specific periods of fasting without significant weight changes.

Several authors have observed an increase in feed intake in fish that have been subjected, before, to periods of fasting (Nikki et al. 2004, Fang et al. 2014, Urbinati et al. 2014), and this phenomenon is called hyperphagia. In the present experiment, we opted for *ad libitum* feeding, and orts were frequently observed. The option to carry out this type of feeding, *ad libitum*, during the feeding period ensured unrestricted access to feed to all fish in all treatments. Therefore, all fish were able to demonstrate their maximum potential during the feeding period. Nevertheless, because of this feeding regime (*ad libitum*) and the orts, it was challenging to observe hyperphagia. However, the significant weight increase observed between periods 0 DF to 15 DF for treatments T6, T9, T12, and T15 may indicate hyperphagia. These fish that went through these fasting periods, when they had access to food, probably ate more than was customarily observed, characterizing hyperphagia.

Phenotypic plasticity

The decrease in intestine weight observed between juveniles subjected to the treatments T12 and T15 may represent an adaptation of the species to long periods of fasting.

The reduction in the intestine mass, an organ that expends a large amount of energy (Wang et al. 2006) and is less commonly used during fasting periods, may reduce maintenance energy and rearrangement of energy to organs of more significant importance during fasting. The reduction in intestinal villus length may also be considered a strategy for dealing with seasonal food shortage in this species' habitat.

Other authors have already reported modifications in the gut of fish during fasting periods. Vidal et al. (2018) observed significant changes in the intestine weight of *Jenynsia multidentata* maintained for seven days in a fasted state. Rios et al. (2004) reported reduced gut length and thickness in adult *Hoplias malabaricus* maintained for 30 days in a fasted state.

Cramp et al. (2005) mentioned that changes in the gut of *Cyclorana alboguttata* after periods of fasting were a strategy of this species for reducing maintenance energy. The fact that, in the present experiment, the fish subjected to the treatments T12 and T15 were unable to reach the expected intestine weight after 30 DF may be an indication that under such circumstances, these fish prefer to normalize the state of other organs, as observed for liver weight in the fish subjected to T12.

The influence of feeding on intestine development has been reported by some authors (Day et al. 2014, Funes et al. 2014). The act of feeding and the need to digest food for energy supply induces the intestine development, which may occur with more intensity in the early stages of life (larval and juvenile stages). It may even involve the penetration of villi towards the lumen of the intestine, thereby increasing the organ's absorption surface. However, fasting seems to have the opposite effect, with regression for the intestinal villi observed. Similar results were observed in birds (Chediack et al. 2012), reptiles (do Nascimento et al. 2016), fish (Hall & Bellwood 1995, Day et al. 2014, Chen et al. 2017), and mammals (Dunel-Erb et al. 2001). For fish, Day et al. (2014) reported that a

reduction in microvilli size occurred soon after the onset of the fasting period and highlighted the relevance of this phenotypic adaptation to this environmental difficulty.

The decrease in liver weight and hepatocytes vacuolization over the fasting period indicates that juvenile (*P. scalare*) uses the energy reserves contained in this organ during these periods. Qian et al. (2016) indicate that metabolic pathways such as fat digestion and absorption, and glycolysis/gluconeogenesis are accessed during fasting periods. As in the present experiment, Gaucher et al. (2012) observed no decrease in liver weight in *Hyphessobrycon luetkenii* subjected to nine days of fasting, but when these fish were subjected to 16 days of fasting, a significant difference in liver weight was observed.

The reduction of the HSI in fasting indicates that this variable is directly related to the use of the fish's energy reserves. This hypothesis has also been put forward for other species. Querol et al. (2002) suggested, regarding the *Locariichthys platymetopon*, that there was a direct relationship between HSI and the accumulation of energy reserves. These authors suggest that the increase in HSI is a consequence of a strategy used by this species to overcome periods of stress during winter. Power et al. (2000) demonstrated a rapid decrease in HSI values among juvenile sea bream (*Sparus aurata*) during fasting and correlated this variable with the use of liver storage energy.

The rapid reduction of HSI values, followed by stability, may indicate that for juveniles freshwater angelfish, this energy reserve is used in short periods of fasting, but if the fasting persists, another energy source should be used. Regarding this other energy source, the intestinal mass loss may indicate that visceral fat is one of the energy sources when fasting periods become too long.

Other authors have already reported a decrease in visceral fat in an increased fasting period. Gaucher et al. (2012) reported a decline in mean visceral fat values in *Hyphessobrycon luetkenii* subjected to nine and sixteen days of fasting, but these authors mentioned that despite the decline in mean values, no significant difference was observed between the fish subjected to fasting and those that were not. Vidal et al. (2018) reported a significant difference in the levels of visceral fat in *Jenynsia multidentata* on fasting for seven days.

For the freshwater angelfish (*P. scalare*) juveniles subjected to the treatment T3, T6, T9, and T12, followed by 30 DF, the normalization of HSI showed the maintenance of liver energy storage importance for fish metabolism. Even after 30 DF, the fish subjected to T15 treatment had low HSI values, demonstrating the severity of the fasting duration's damage.

Survival estimate

The values obtained for the survival estimate demonstrate that the species' adaptive strategies against food deprivation challenges are efficient. In both juvenile and adult phases, fish have adaptations and strategies to withstand long periods of food deprivation (Wang et al. 2006). Even after 15 days of fasting, these survival data are of great interest to the ornamental fish market since ornamental fish are transported in plastic bags and usually go through fasting periods. This ability to withstand fasting probably influenced the survival values observed in the present experiment. As in the present experiment, several other authors have reported little or no mortality in fasting experiments conducted on fish (Power et al. 2000, Hernández et al. 2018).

However, it is important to highlight the increase in the number of deaths observed from the eighth day of fasting onwards, which may indicate that for freshwater angelfish at this phase of development, periods of fasting longer than eight days may exceed the limit of energy reserve of individuals that are less adapted to this challenge. Several authors (Xiwu et al. 2009, Sun & Li 2014, Lima et al. 2017) have reported that there is a critical starvation point, called point-of-no-return (PNR), at which some animals lose the ability to feed and consequently die (Blaxter & Hempel 1963) or even when they manage to feed, fail to resume life-essential metabolic activities and die (Lasker et al. 1970). Despite the different interpretation methodologies of PNR, most of them consider that this point is only reached when within a population, 50% of mortality occurs, which did not occur in any of the present experiment treatments. Although fish are ectothermic animals and do not require nutritional support to maintain thermal homeostasis, phenotypic changes and increased death rates from relatively short fasting periods may mean that they are more abruptly in the early juvenile period, affected by environmental changes. However, it can be concluded that juveniles (*P. scalare*) could tolerate fasting periods of up to 15 days. Fasting periods longer than 15 days may result in financial implications in cases of commercial production. Further experiments need to be conducted on catabolism and energy pathways to elucidate which precursors of energy (e.g. hepatic glycogen, muscle glycogen, hepatic lipid, liver protein, or visceral fat) are essential during different periods of fasting.

REFERENCES

Alix, M., Bloundeau-Bidet, E., Grousset, E., Shirangui, A., Vergnet, A., Guinand, B., et al. 2017. Effects of fasting and re-alimentation on gill and intestine

morphology and indicators of osmoregulatory capacity in genetically selected sea bass (*Dicentrarchus labrax*) populations with contrasting tolerance to fasting. Aquaculture, 468: 314-325. doi: 10.1016/j.aquaculture.2016.10.016

Black, D. & Love, M. 1986. The sequential mobilization and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. Journal of Comparative Physiology, 156: 469-479.

Blaxter, J.H.S. & Hempel, G. 1983. The influence of egg size on herring larvae (*Clupea harengus* L.). Journal du Conseil International pour l' Exploration de la Méditerranée, 28: 211-214.

Brett, J.R. 1995. Energetics. In: Margolis, L. & Groot, C. (Eds.). Physiological ecology of Pacific salmon. UBC Press, Vancouver, 1-68 pp.

Cant, J., McBride, B. & Croom, W.J. 1996. The regulation of intestinal metabolism and its impact on whole animal energetics. Journal of Animal Science, 74: 2541-2553. doi: 10.2527/ 1996.74102541x

Chediack, J.G., Funes, S.C., Cid, F.D., Filippa, V. & Caviedes-Vidal, E. 2012. Effect of fasting on the structure and function of the gastrointestinal tract of house sparrows (*Passer domesticus*). Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 163: 103-110. doi: 10.1016/ j.cbpa.2012.05.189

Chen, C.H., Tan, Q., Liu, M., Wu, F., Chen, J. & Xie, S.H. 2017. Effect of starvation on growth, histology and ultrastructure of digestive system of juvenile red swamp crayfish (*Procambarus clarkia* Girard). Iranian Journal of Fisheries Sciences, 16(4): 1214-1233.

Cramp, R.L., Franklin, C.E. & Meyer, E.A. 2005. The impact of prolonged fasting during aestivation on the structure of the small intestine in the green-striped burrowing frog, *Cyclorana alboguttata*. Acta Zoologica, 86: 13-24. doi: 10.1111/j.0001-7272.2005.00180.x

Day, R.D., Tibbetts, I.R. & Secor, S.M. 2014. Physiological responses to short-term fasting among herbivorous, omnivorous, and carnivorous fishes. Journal of Comparative Physiology - Part B: Biochemistry & Molecular Biology, 184: 497-512.

do Nascimento, L.F.R., da Silveira, L.C., Nisembaum, L.G., Colquhoun, A., Abe, A.S., Mandarim-de-Lacerda, C.A. & de Souza, S.C.R. 2016. Morphological and metabolic adjustments in the small intestine to energy demands of growth, storage, and fasting in the first annual cycle of a hibernating lizard (*Tupinambis merianae*). Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 195: 55-64. doi: 10.1016/j.cbpa.2016.02.002

Dunel-Erb, S., Chevailer, C., Laurent, P., Bach, A., Decrock, F. & LeMaho, Y. 2001. Restoration of the jejunal mucosa in rats refed after prolonged fasting. Comparative Biochemistry and Physiology - Part A: Molecular & integrative Physiology, 129: 933-947. doi: 10.1016/j.cbpb.2010.09.010

Fang, J., Tian, X., Dong, S., Fang, J. & Zhang, J. 2014. An experimental study on the compensatory growth of tongue sole, *Cynoglossus semilaevis* (Günther, 1873), following lower temperature manipulation. Aquaculture Research, 45: 1523-1532. doi: 10.1111/are.121101

Fu, S.J., Pang, X., Cao, Z.D., Peng, J.L. & Yan, G. 2011. The effects of fasting on the metabolic interaction between digestion and locomotion in juvenile southern catfish (*Silurus meridionalis*). Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 158: 498-505. doi: 10.1016/j.cbpa.2010.12.013

Funes, S.C., Filippa, V.P., Cid, F.D., Mohamed, F., Caviedes-Vidal, E. & Chediack, J.G. 2014. Effect of fasting in the digestive system: Histological study of the small intestine in house sparrows. Tissue and Cell, 46: 356-362. doi: 10.1016/j.tice.2014.06.007

Gaucher, L., Vidal, N., Alejandro, D. & Naya, D.E. 2012. Digestive flexibility during fasting in the Characid fish *Hyphessobrycon luetkenii*. Journal of Morphology, 273: 49-56. doi: 10.1002/jmor.11005

Hall, K.C. & Bellwood, D.R. 1995. Histological effects of cyanide, stress and starvation on the intestinal mucosa *Pomacentrus coelestis*, a marine aquarium fish species. Journal Fish Biology, 47: 438-454.

Hernández, D.R., Barrios, C.E., Santinón, J.J., Sánchez, S. & Baldisserotto, B. 2018. Effect of fasting and feeding on growth, intestinal morphology, and entero-endocrine cell density in *Rhamdia quelen* juveniles. Aquaculture Research, 49: 1512-1520. doi: 10.1111/are.13606

Humason, G.L. 1972. Animal tissue techniques. W.H. Freeman, San Francisco.

Kaplan, E.L. & Meier, P. 1958. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association, 53: 457-481. doi: 10.2307/2281868

Lasker, R., Feder, H.M., Theilacker, G.H. & May, R.C. 1970. Feeding, growth, and survival of *Engraulis mordax* larvae reared in the laboratory. Marine Biology, 5: 345-353.

Levitan, D.R. 1989. Density-dependent size regulation in *Diadema antillarum*: effects on fecundity and survivorship. Ecology, 70: 1414-1424. doi: 10.2307/1938200

Lignot, J-H., Helmstetter, C. & Secor, S.M. 2005. Postprandial morphological response of the intestinal epithelium of the Burmese python (*Python molurus*). Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 141: 280-291.

Liknes, E.T., Guglielmo, C.G. & Swanson, D.L. 2014. Phenotypic flexibility in passerine birds: seasonal variation in fuel storage, mobilization and transport. Comparative Biochemistry and Part A: Molecular & Integrative Physiology, 174, 1-10. doi:10.1016/j.cbpa. 2014.03.017

Lima, A.F., Andrade, F.F., Pini, S.F.R., Makrakis, S. & Makrakis, M.C. 2017. Effects of delayed first feeding on growth of the silver catfish larvae *Rhamdia voulezi* (Siluriformes: Heptapteridae). Neotropical Ichthyology, 15: e160027. doi: 10.1590/1982-0224-20160027

Marinovic, B. & Mangel, M. 1999. Krill can shrink as an ecological adaptation to temporarily unfavorable environments. Ecology Letters, 2: 338-343.

Nikki, J., Pirhonen, J., Jobling, M. & Karjalainen, J. 2004. Compensatory growth in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum), held individually. Aquaculture, 235: 285-296. doi: 10.1016/j.aquaculture.2003.10.017

Pérez-Jiménez, A., Guedes, M.J., Morales, A.E. & Olivia-Teles, A. 2007. Metabolic responses to short starvation and refeeding in *Dicentrarchus labrax*. Effect of dietary composition. Aquaculture, 265: 325-335. doi: 10.1016/j.aquaculture.2007.01.021

Power, D.M., Melo, J. & Santos, C.R.A. 2000. The effect of food deprivation and refeeding on the liver, thyroid hormones, and transthyretin in sea bream. Journal of Fish Biology, 56: 374-387. doi: 10.1111/j.1095-8649.2000.tb02112.x

Qian, B., Xue, L. & Huang, H. 2016. Liver transcriptome analysis of the large yellow croacker (*Larimichthys crocea*) during fasting by using RNA-Seq. Plos One, 11: e0150240. doi: 10.1371/journal.pone.0150240

Querol, M.V.M., Querol, E. & Gomes, N.N.A. 2002. Fator de condição gonadal, índice hepatossomático e recrutamento como indicadores do período de reprodução de *Loricariichthys platymetopon* (Osteichthyes, Loricariidae), Bacia do Rio Uruguai Médio, Sul do Brasil. Iheringia, Série Zoologia, 92: 79-84. doi: 10.1590/S0073-47212002000300008

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Rios, F.S., Kalinin, A.L., Fernandes, M.N. & Rantin, F.T. 2004. Changes in gut gross morphology of Traíra *Hoplias malabaricus* (Teleostei, Erythrinidae) during long-term starvation and after refeeding. Brazilian Journal of Biology, 64: 683-689. doi: 10.1590/S1519-69842004000400017

Secor, S.M. 2005. Evolutionary and cellular mechanisms regulating intestinal performance of amphibians and reptiles. Integrative and Comparative Biology, 45: 282-294. doi: 10.1093/icb/45.2.282

Sun, X. & Li, Q. 2014. Effects of delayed first feeding on larval growth, survival and development of the sea cucumber *Apostichopus japonicus* (Holothuridae). Aquaculture Research, 45: 278-288. doi: 10.1111/j. 1365-2109.2012.03224.x

Urbinati, E.C., Sarmiento, S.J. & Takashi, L.S. 2014. Short-term cycles of feed deprivation and refeeding promote full compensatory growth in the Amazon fish matrinxã (*Brycon amazonicus*). Aquaculture, 433: 430-433. doi: 10.1016/j.aquaculture.2014.06.030

Vidal, N., Gonzáles-Bergonzoni, I. & Naya, D.E. 2018. The effect of fasting on nutritional status, organs size, and isotopic composition in a Neotropical fish species (*Jenynsia multidentata*). Hydrobiologia, 828: 73-82. doi: 10.1007/s10750-018-3803-9

Wang, T., Hung, C.C.Y. & Randall, D.J. 2006. The comparative physiology of food deprivation: from feast to famine. Annual Review of Physiology, 68: 223-251.

Wingfield, J.C. & Grimm, A.S. 1977. Seasonal changes in the plasma cortisol, testosterone and estradiol-17β in the plaice, *Pleuronectes platessa* L. General and Comparative Endocrinology, 31: 1-11. doi: 10.1016/ 0016-6480(77)90184-8

Xiwu, Y., Yuehuan, Z., Zhongming, H., Feng, Y. & Guofan, Z. 2009. Effects of starvation on larval growth, survival, and metamorphosis of Manila clam *Ruditapes philippinarum*. Acta Ecologica Sinica, 29: 327-334. doi: 10.1016/j.chnaes.2009.09.012