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Short communication

Short periods of food restriction do not affect growth, survival or muscle development on pacu larvae

J.T. Kojima [a], N.J. Leitão [a], O.C.C. Menossi [b], T.M. Freitas [b], M. Dal-Pai Silva [c], M.C. Portella [b,*]

[a] UNESP–Univ Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane, Jaboticabal, São Paulo 14884-900, Brazil
[b] UNESP–Univ Estadual Paulista, Centro de Aquicultura (Caunesp), Via de Acesso Prof. Paulo Donato Castellane, Jaboticabal, São Paulo 14884-900, Brazil
[c] UNESP–Univ Estadual Paulista, Instituto de Biociências, Departamento de Morfologia e Centro de Aquicultura (Caunesp), Botucatu, São Paulo 18618-970, Brazil

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ABSTRACT

The present study evaluated the effects of different fasting periods on survival rate, growth, muscle development and compensatory growth in pacu (*Piaractus mesopotamicus*) larvae. In Phase I, larvae (5 days post hatching, dph) reared under an intensive system in laboratory conditions were subjected to 0, 2, 4, 6 or 8 days fasting followed by 10 days of regular feeding with live feed. In Phase II, the same larvae were transferred to outdoor ponds, in a semi-intensive system, where post-fasting growth was investigated for 30 days. After fasting, larvae exhibited weight reduction, loss of muscle mass and smaller-diameter fibers, but after they were regularly fed for 10 days, growth performance and morphometric analysis of muscle fibers showed that muscle mass and weight were recovered. At the end of the Phase II, juvenile survival was higher in fish subjected to 0 (control) and 2 days fasting than those subjected to 4 days fasting. With respect to the distribution of juveniles into size classes, those subjected to 4-day fasting yielded a higher proportion (63%) of super small-size individuals, whereas most juveniles from 0- to 2-day fast treatments were medium-sized. In short, pacu larvae under different fasting periods are capable of compensatory growth, and if fasting does not exceed 2 days, subsequent growth performance and muscle development are not affected. This short fasting regime can therefore serve as an emergency feeding strategy to produce juveniles of this important South American fish species.

1. Introduction

The production of juvenile fish in Brazil is conducted primarily in semi-intensive systems (Jomori et al., 2003), in which larvae are stocked in fertilized ponds immediately after mouth opening. The success of fish production in this system depends on a number of factors that are difficult for farmers to control, such as quality and amount of live organisms produced in the ponds and environmental conditions. Delay in the first larval feeding compromises feeding behavior (Peña and Dumas, 2005), nutritional conditions (Gisbert et al., 2004), growth (Shan et al., 2009), muscle development (Leitão et al., 2011), survival (Shan et al., 2008; Zhang et al., 2009) and organogenesis of digestive tract (Menossi et al., 2012; Xiong et al., 2006).

Food deprivation can trigger behavioral and physical changes in juvenile and adult fish, but it does not impair their capacity to grow if adequate feeding conditions are reestablished within a short period of time (Souza et al., 1997). Fish can show exceptionally rapid growth, called compensatory growth, when favorable feeding conditions are recovered. This response is affected by many factors such as severity of food restriction, age, sex, sexual maturity, temperature and nutritional composition of the diet provided during refeeding (Ali et al., 2003).

Muscle mass corresponds to 60–70% of the body weight of a fish and represents the highest nutritional and commercial value of these animals (Ayala et al., 2010; Sänger and Stoiber, 2001). Fish muscle grows by hypertrophy and hyperplasia throughout all the development phases (Dal Pai-Silva and Carvalho, 2007; Rowlerson and Veggetti, 2001), whereas in most other vertebrates, postnatal muscle growth occurs mainly by hypertrophy (Carpenè and Veggetti, 1981; Egginton and Johnston, 1982). The balance between hypertrophy and hyperplasia determines growth rate and size and depends on a number of biotic and abiotic factors such as temperature (Assis et al., 2004), light–dark cycle, feeding regime and diet (Fauconneau and Paboeuf, 2001; Johnston, 2006). Pacu larvae subjected to fasting or undernourishment suffer weight loss and muscle fiber atrophy (Leitão et al., 2011). However, the effects of post-fasting feeding on muscle growth recovery have yet to be studied in pacu larvae.

The present study evaluated how different food deprivation periods affect the productive performance, muscle growth and compensatory growth of pacu larvae and juveniles in the short term (in laboratory) and after a growth recovery period in a farming system (outdoor ponds). Our findings show that a delay of up to 2 days in the first feeding

* Corresponding author at: UNESP - Univ Estadual Paulista, Centro de Aquicultura. Via Prof. Paulo Donato Castellane, s/n. 14884-900, Jaboticabal, SP, Brazil. Tel.: +55 16 32097198.
E-mail address: portella@caunesp.unesp.br (M.C. Portella).

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does not compromise muscle growth or the development of pacu juveniles in their later growth phases.

2. Materials and methods

2.1. Experimental design

The 120 thousand pacu (*Piaractus mesopotamicus*) larvae used in the experiment were obtained from a commercial hatchery (Colpani Piscicultura; Mococa, São Paulo, Brazil). They were transferred to our laboratory 3 days post hatching (dph), and the experiment was initiated when they started exogenous feeding, at 5 dph (0.59 ± 0.13 mg wet weight and 6.20 ± 0.08 mm total length).

The experiment was carried out in two phases. In Phase I, larvae were kept in an intensive system (in laboratory) and subjected to different fasting periods before being fed *Artemia* nauplii for 10 days. In Phase II, larvae were transferred to a semi-intensive system (outdoor ponds) for growth recovery. The treatments were named according to the number of fasting days before larvae received the first feeding, as follows: F2, F4, F6 and F8 (2, 4, 6 and 8 days, respectively). One treatment without food deprivation (F0) and another imposing fasting for the entire experiment (Fn) were used as positive and negative controls. The two phases were conducted using a completely randomized design, the first with 5 replicates and the second with 3 replicates of each treatment.

In Phase I, larvae were stocked at 20 larvae L^{-1} in thirty 200-L tanks, supplied with a continuous flow of water (400% daily renewal rate, 29.9 ± 0.5 °C and 4.4 ± 0.1 mg L^{-1} dissolved oxygen). After fasting, larvae were supplied with increasing quantities of *Artemia* nauplii (300 to 1500 nauplii larvae^{-1}) for 10 days, five times a day (8, 11, 14, 17 and 20 h). Leftovers on the bottom of the tanks were removed daily by siphoning.

In Phase II, larvae from each treatment were grouped and randomly redistributed in three 40 m^2 ponds with brick walls and earthen bottom, at a density of 100 larvae m^{-2}. Larvae from treatments F8 and Fn died before the end of Phase I, and these treatments were therefore excluded from Phase II. The ponds were protected against bird attack with a net and supplied with a continuous flow of spring water (1.5 to 2.0 L min^{-1}, 28.8 ± 2.4 °C, 4.4 ± 0.5 mg L^{-1} dissolved oxygen and 82.7 ± 10.7 cm transparency). Larvae were fed commercial feed for fish larvae containing 55% crude protein and 7% crude lipid; feed was distributed over the pond surface four times a day. Before larvae introduction, the ponds underwent prophylactic treatment with CaO (60 g m^{-2}) and received one dose of 178 g m^{-2} of organic fertilizer followed by weekly administration of 45 g m^{-2} to promote natural plankton production.

We adopted the terminology proposed by Kendall et al. (1984), considering *larvae* as being individuals under differentiation and morphological transformation, and *juveniles* those exhibiting the main morphological characteristics of adults, particularly fin and scale formation.

2.2. Performance

Throughout Phase I, samples were collected at the end of the fasting periods and 4 and 10 days after the onset of live feed offer. Larvae were randomly sampled ($n = 40$ per replicate) before they received the first meal of the day, anesthetized and euthanized with benzocaine (0.15 g L^{-1}), fixed in 10% formaldehyde and preserved in 70% ethanol solution for further determination of weight (W, mg) and total length (L, mm). The parameters evaluated were as follows: weight gain (WG) = final weight − initial weight; specific growth rate (SGR) = 100 × (Ln final weight − Ln initial weight) / number of days between the biometric measurements; condition factor (K) = W/ L^b. The *b* value was estimated in each phase by $W = aL^b$ (*a* is the linear coefficient of weight–length relationship), after log transformation of data and fitting by the least-squares method, according to Santos (1978). Survival rate (S) was considered as S = 100 × NF/(NI−NC), where N is the number of fish in each tank, F and I represent the final and initial values, and C represents the fish in each tank collected throughout the experiment. The tanks were checked daily for dead fish (*M*), which were recorded and removed. Apparent cannibalism rate (C) was calculated by the equation $C = 100 - [S + M]$, where *S* is survival rate at the end of the indoor phase (in percent value) and *M* is fish death throughout the indoor phase (in percent value).

At the end of Phase II, a sample of 1000 fish from treatment F0 was biometrically evaluated, and average total length was used to establish size classes, whose ranges were defined based on the standard deviation of these animals. Five size classes (in cm) were established: class SS: <3.8 (super small); class S: 3.8 ≤ S < 4.1 (small); class M: 4.1 ≤ M < 5.2 (medium); class L: 5.2 ≤ L < 5.5 (large); and class XL:≥5.5 (extra-large). All fish from each treatment were classified according to size class and 10% from each class per treatment were evaluated by biometrics. Biometric data of each size class weighted by the frequency of occurrence were used to calculate weight gain (WG), specific growth rate (SGR) and condition factor (K). Survival rates were determined by a fish count at the end of each experimental phase.

2.3. Morphological and morphometric evaluation of muscle fibers

Concomitant to growth evaluation, fish samples were also collected for histological analyses. In Phase I, whole fish were fixed ($n = 8$ per treatment); in Phase II, sample fish were dissected and a section of the epaxial muscle was used ($n = 8$ per size class). The fixing protocol consisted of sample immersion in Karnovsky solution for 24 h, washing with Sorensen's phosphate buffer (pH 7.2), preservation in 70% ethanol solution and embedding in histological resin (Historesin®, Leica, Germany), according to manufacturer's instructions. Samples were cut transversally and histological cross sections (4 μm) were stained with hematoxylin–phloxine B.

An image analysis system (Leica IM50, Germany) was used to calculate the smallest diameter of 200 muscle fibers per animal in a deep portion of the epaxial region in order to evaluate muscle growth. In Phase I, every deep muscle fiber in the section was measured. Determining the smallest diameter avoids assessment errors if the cross sectioning of the specimen is not properly performed (Dubowitz, 1985).

Muscle fibers were distributed in classes according to their diameter (in μm) in order to evaluate hypertrophic and hyperplastic growth. Diameter classes were determined based on results obtained by Assis et al. (2004) for pacu larvae: class 10: $d \leq 10$; class 20: $10 < d \leq 20$; class 30: $20 < d \leq 30$; class 40: $30 < d \leq 40$; and class 50: $d > 40$.

2.4. Statistical analysis

Data were checked for normality by the Cramer–von Mises test, and for homoscedasticity by Levene's test. Percentage data were log-transformed ($y = \arcsin\sqrt{x/100}$, where *x* is the percent value before ANOVA application). Data on growth, survival and fiber diameter were evaluated by one-way ANOVA followed by the Tukey test ($\alpha = 0.05$). Data transformation and analysis were performed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

3. Results and discussion

3.1. Survival and growth performance

In the present study, we observed that 100% of pacu larvae were dead after 8 days fasting (13 dph). Earlier studies showed total mortality of pacu larvae subjected to food deprivation at 16 (Tesser et al., 2005), 18 (Leitão et al., 2011) and 19 dph (Menossi et al., 2012). Larval resistance to food deprivation can change according to fish species, development stage (Shan et al., 2008), egg quality (Rana, 1985; Zhao et al., 2001) and temperature (Shan et al., 2008). Mean post-fasting weight decreased with an increase in the food deprivation period, and larvae subjected to 6-day fasting suffered 50.8% weight loss. However, fasted