

Research Article

Capturing, induced spawning, and first feeding of wild-caught *Pseudopimelodus mangurus*, an endangered catfish species

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ABSTRACT. This study aimed to describe a procedure for sampling, reproduction, and first feeding of the Neotropical catfish *Pseudopimelodus mangurus*, an endangered fish species. Wild adult *P. mangurus* specimens were collected in the Mogi Guassu River and subsequently induced to spawn in laboratory conditions. After hand-stripping, the females, the average weight of the oocytes was 143 ± 1.6 g, with a fecundity of 718 ± 49.8 oocytes g^{-1} . The diameter of the oocytes non-hydrated was 1226.3 ± 47.7 μm to 1761.2 ± 26.4 μm after hydration. The fertilization rates were $98.00 \pm 0.63\%$, and the hatching rate was $68.94 \pm 11.83\%$. The first feeding was made three days post-hatching with six different treatments, in which the best results arose with sequential feeding with *Artemia* nauplii, *Astyanax altiparanae* and *Prochilodus lineatus* larvae. This condition resulted in a final length of $2,012.7 \pm 44.8$ μm by the 10th day of the experiment, higher survival ($65 \pm 0.9\%$) and lower cannibalism rate ($14 \pm 0.3\%$). The data obtained in this study is important for the establishment of reproductive biotechniques, mass production of *P. mangurus*, and future establishment of *ex-situ* GenBank.

Keywords: *Pseudopimelodus mangurus*; catfish; conservation; GenBank; larvae; reproduction

INTRODUCTION

In the Neotropical region, 1680 fish species are considered endangered and listed in the International Union for Conservation of Nature (Toussaint *et al.*, 2016). Such a panorama suggests research efforts, including artificial propagation, genetics, re-stocking, and biotechniques like chromosome manipulation (Piferrer *et al.*, 2009), chimerism (Yasui *et al.*, 2011; Nascimento *et al.*, 2017), and *ex-situ* GenBank.

Among the endangered species, the bumblebee catfish *Pseudopimelodus mangurus* (Valenciennes, 1835) is a migratory Neotropical catfish that is ecologically relevant, serving as a food source for other species of fish, reptiles, mammals, and birds. *P. mangurus*, also known as "frogfish," belongs to the fa-

mily Pseudopimelodidae and has a carnivorous feeding pattern. It can be found in the Paraná, Uruguay, Paraguay, and La Plata rivers basins (Froese & Pauly, 2015). This species was included in the red list of endangered animals in the São Paulo State, Brazil (Bressan *et al.*, 2009; ALESP, 2014), suggesting that conservation and reconstitution strategies including *ex-situ* GenBank and reproduction are necessary. However, in order to achieve such a preservation protocol, it is first necessary to understand fundamental aspects of this species as reproduction, incubation and first feeding to ensure mass production of juveniles for re-stocking (Luz & Zaniboni-Filho, 2002; Murgas *et al.*, 2012; Arashiro *et al.*, 2018).

Artificial propagation is an important step for mass production, but the procedures to induce spawning that

includes a combination of dosage, hormones, and maturation period are species-specific (Andrade & Yasui, 2003; Godinho, 2007). Also, larval rearing is a critical step, especially within carnivorous fish, as the case of *P. mangurus*. Despite the relevance of such reproductive information, there is no protocol for reproduction and juvenile production in *P. mangurus* among the reviewed literature.

Considering the aspects mentioned above, the objective of this work was to establish protocols for the collection of sexually mature wild specimens, hormonal induction for reproduction and larval feeding of *P. mangurus*.

MATERIALS AND METHODS

All the procedures were conducted during the reproductive season of *Pseudopimelodus mangurus* (October 2015 to February 2018) in agreement with the Guide for the Care and Use of Laboratory Animals of the National Center for Research and Conservation of Continental Fish, Chico Mendes Institute of Biodiversity Conservation (CEUA #010/2015).

Adult specimens were collected from the Mogi Guassu River, Cachoeira de Emas in Pirassununga City, São Paulo State, Brazil (21°55'36.476"S, 47°22'0.836"W) under permission of the same institution (Sisbio #55725-1). The collection was performed using line fishing at the bottom of the river. As *P. mangurus* lives at greater depths and is nocturnal, the sampling was performed from 16:00 until 24:00 h. The equipment for collection sampling included a 0.40 mm line, 4.0 to 7.0 mm hook, dead *Astyanax altiparanae* as bait, and a 2 m fishing rod with a reel. After collection, fish were immediately transported to 2,000 L circular tanks to be induced to spawn.

The females were selected based on morphological appearance, presenting a reddish papilla and a soft, protuberant ventral region (Wojnarovich & Horváth, 1983; Solis-Murgas *et al.*, 2011). Subsequently, the fish were tagged using a 14 mm micro-transponder (ISSO FDC-B 12×2 mm, Animal TAG, USA), weighed, and then transferred to a 2,000 L circular tank with continuous water flow. The spawning was induced by injecting crude carp pituitary extract (CCPE) at 6.6 mg kg⁻¹, divided into two doses, 0.6 and 6 mg kg⁻¹, 6 h after the first dose. The *P. mangurus* males were injected with a single dose of CCPE at 2 mg kg⁻¹ when females received the second dose.

At 165 degrees-hour after the injection, male gametes were sampled before females. Males were euthanized with an overdose of clove oil at 250 mg L⁻¹ (Biodinamica, Ibiporã, Brazil). The testis was removed

and minced in Eagle's Minimum Essential Medium with the pH adjusted to 7.8 (E-MEM, Sigma #M0268, St. Louis, USA). A small aliquot was used to evaluate sperm motility, according to the procedures described by Yasui *et al.* (2015). Females were anesthetized with clove oil at 150 mg L⁻¹, and stripping was performed in a glass container. The weight of total spawn was measured, and a small aliquot was weighed and fixed in 2% glutaraldehyde in Dulbecco's PBS (Sigma #D5773, St. Louis, USA) in order to calculate the number of oocytes per gram, total fecundity (total number of oocytes) and relative weight fecundity (g of oocytes per kg of the female).

For fertilization, the diluted sperm was placed on the oocyte mass and immediately activated by adding water. The fertilized oocytes were incubated at 26°C, as reported by Arashiro *et al.* (2018) for the incubation of *P. mangurus* embryos. A small aliquot (30-80 embryos) was collected randomly and followed until hatching to calculate fertilization and hatching rates. Three spawns of *P. mangurus* were obtained, using a different male and female (three couples), and each spawn was considered as a repetition (eggs were not mixed).

Embryos from each repetition were incubated separately, and three days after hatching, larvae had consumed the yolk sack and started exogenous feeding. Active and healthy larvae from each repetition were divided into six floating plastic containers (20×15 cm) with 30 larvae and a volume of 630 mL of water for each (total of 18 plastic containers). Each container had a 1 mm nylon mesh on the lateral to permit continuous water flowing while the bottom was covered by sand, in order to prevent biofilm formation. The temperature was set at 26°C. The six containers from each repetition received the following feeding T1: *Artemia franciscana* nauplii; T2: plankton; T3: dry food (Laguna larva postlarva, 55% crude protein, Invivo Nutrição e Saúde Animal Ltda., Paulinia, Brazil) T4: *Artemia* nauplii and plankton; T5: *Artemia* nauplii, plankton and dry food; T6: *Artemia* nauplii, *A. altiparanae* and *P. lineatus* larvae.

The plankton used in this experiment were collected in tanks from CEPTA/ICMBio and UV-irradiated at 80 MJ cm⁻² to avoid diseases and frozen for later use. In treatment 6, the larvae of *P. mangurus* were exclusively fed with *Artemia* nauplii during the first three days, and, afterward, the diet was changed to larvae of yellowtail tetra (*Astyanax altiparanae*) and the streaked prochilod (*Prochilodus lineatus*) until satiation.

The containers were cleaned twice a day (08.00 and 18:00 h) with a Pasteur pipette to remove lumps of uneaten food and dead larvae. For evaluation of growth, 10 individuals from each container were daily

collected and returned after measurement, and digital images were obtained using the stereomicroscope (Nikon SMZ 1500, Nikon, Tokyo, Japan) in combination with a CCD camera (Nikon DS-Fi, Nikon, Japan). The software NIS-AR Elements (Nikon, Tokyo, Japan) was used to capture and measure the total length (μm) from the digital images. After 10 days, the survival and cannibalism rates were calculated from each container. The cannibalism rate was calculated as follows: the total number of larvae found dead during the experimentation was added to the number of larvae that survived. This total was subtracted from the number of larvae at the beginning of the experiment.

Statistics

Data are shown as mean \pm standard error. Growth performance was examined using regression analysis and ANOVA followed by the Tukey multiple range tests (for comparison of total length in the last day). In all analyses, the software Statistica (7.0, StatSoft, USA) was used with the probability set at 0.05.

RESULTS

The weight of the captured broodstock ranged from 0.160 to 7.800 kg. From November to December 2017, 19 *Pseudopimelodus mangurus* females were induced to spawn. Eighteen successfully spawned. Oocyte from 10 females was used for me diameter were obtained from 10 females.

Just after spawning and after hydration, oocytes measured $1,226.3 \pm 47.7 \mu\text{m}$ and $1,761.2 \pm 26.4 \mu\text{m}$, respectively. Female weight ranged from 0.803 to 3.492 kg, and these reproductive data were obtained: the number of oocytes per gram (718.0 ± 49.8), fecundity ($257.6 \pm 85.9 \text{ g}$), fertilization rate ($98.0 \pm 0.6\%$), and hatching rate ($68.9 \pm 11.8\%$). The growth of the *P. mangurus* larvae fed with different diets presented a linear regression with a determination coefficient ranging from 0.9918 (fish fed with *Artemia*) to 0.8375 (fish fed with dry food). Based on the angular coefficient of the resultant regression, the best growth was obtained in treatment 6 (fish fed *Artemia* + fish larvae). In this treatment, the estimated daily growth increments were of 1646.1 mm d^{-1} (Fig. 1). The final total length ($20.12 \pm 0.04 \text{ mm}$) was also significantly higher in this treatment (Fig. 2).

As shown in Figure 3, the survival rate of larvae fed with *Artemia* nauplii and fish larvae (treatment 6) was significantly higher ($65.0 \pm 0.1\%$, $P < 0.05$) than the other treatments. The other treatments (T1 to T5) presented low survival rates, ranging from 1.6 to 5%. Also, the cannibalism rate was significantly lower ($P <$

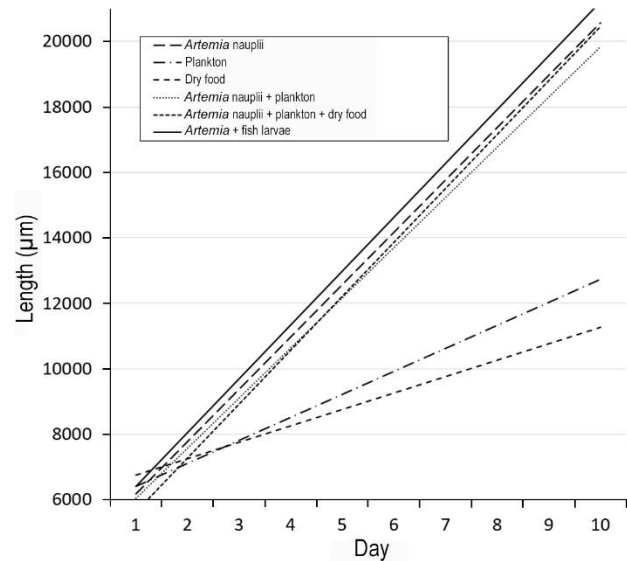


Figure 1. Growth (μm) of *Pseudopimelodus mangurus* larvae fed with different diets. T1: *Artemia*: $y_3 = 1597.9x + 4580.2$; $R^2 = 0.9918$; T2: plankton: $y_4 = 704.21x + 5697.9$; $R^2 = 0.9439$; T3: dry food: $y_5 = 502.13x + 6235.7$; $R^2 = 0.8375$; T4: plankton + *Artemia*: $y_1 = 1532.5x + 4513.2$; $R^2 = 0.9906$; T5: dry food + plankton + *Artemia*: $y_2 = 1648.1x + 3969.3$; $R^2 = 0.9868$; T6: *Artemia* + fish larvae: $y^6 = 1646.1x + 4752.5$; $R^2 = 0.977$.

0.05) when the fish were fed with *Artemia* nauplii and fish larvae ($14.0 \pm 0.03\%$), followed by treatment 4 ($36.6 \pm 4.0\%$). Treatments 1, 2, 3, and 5 showed high rates of cannibalism (ranging from $45.0 \pm 5.5\%$ to $76.6 \pm 1.0\%$).

DISCUSSION

The criteria for selecting females for spawning, including external morphology, abdominal inspection, and redness of the urogenital papilla, was valid for *Pseudopimelodus mangurus* as in the case of other Neotropical migratory species such as *P. charus* and *Rhamdia quelen* (Vieira-Sampaio & Yoshimi, 2006). Similarly, male selection based on semen release after a gently stripping was also an effective selection procedure for mature males.

The protocol of induced spawning involves trial-and-error experiments in which the main factors include the hormone, dose, and ovulation period. In this study, a general protocol using two doses of carp pituitary gland was successfully used as suggested for other fish species such as *Pimelodus maculatus* and *Pseudoplatystoma corruscans* (Arantes *et al.*, 2013), *Pseudoplatystoma fasciatum* (Leonardo *et al.*, 2004), *Steindachneridion melanodermatum* (Ludwig *et al.*, 2005), and *Brycon insignis* (Andrade-Talmelli *et al.*,

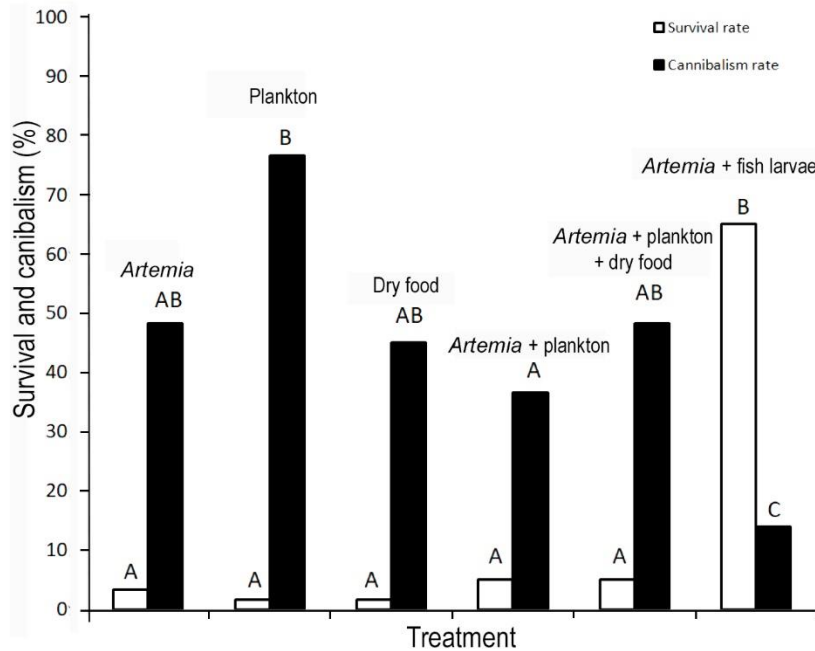


Figure 2. Survival (%) on day 10 and cannibalism over 10 days of *Pseudopimelodus mangurus* larvae fed with different diets. Different letters in survival or cannibalism columns indicate significant differences.

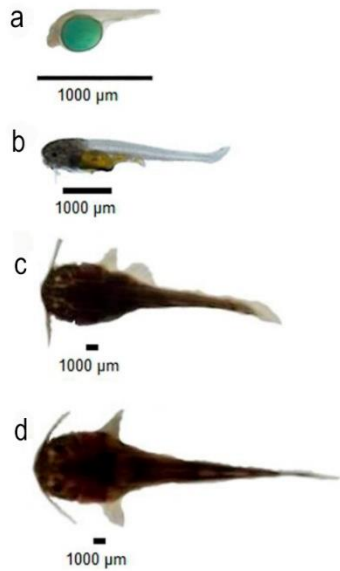


Figure 3. Larvae of *Pseudopimelodus mangurus* from treatment 6 (*Artemia* nauplii in the first three days followed by *Astyanax altiparanae* and *Prochilodus lineatus* larvae in the next days of the experiment). a) Larvae at hatching stage, b) one day, c) fifth days, and d) tenth days of the experiment.

occurs within a more extended period as in the case of *R. quelen* (311 degree-hours), *P. charus* (315 degree-hours) (Vieira-Sampaio & Yoshimi, 2006), *Zungaro jahu* (220-230 degree-hours) (Nogueira *et al.*, 2012), *S. melanodermatum* (260 ± 20 degree-hours) (Ludwig *et al.*, 2005), *P. corruscans* and *P. fasciatum* (180-230 degree-hours) (Inoue *et al.*, 2003).

In most cases, induced reproduction of migratory endangered species is performed in fish maintained in captivity, and maturation occurs during the spawning (rainy) season (Andrade & Yasui, 2003). Such procedures require the previous domestication of the wild-caught specimens, including feeding with commercial diets and the adaptation to artificial ponds. However, the domestication process in captivity until fish reach sexual maturity can take more than two years. In this study, the wild-caught specimens were collected in the spawning areas and promptly induced to spawn, a procedure that has been an effective strategy for the initiation of an *ex-situ* GenBank because juveniles are rapidly obtained after broodstock capture. In addition, higher fertilization rates (>97%) were obtained for *P. mangurus* compared with other siluriform species like *Pimelodus maculatus* (64.80%), *Pseudoplatytoma corruscans* (79.83%) (Arantes *et al.*, 2013), and *Zungaro jahu* (92%) (Nogueira *et al.*, 2012). Hatching rate was also higher (>68%) than in other siluriforms, such as *P. maculatus* (Arashiro *et al.*, 2018) and *Sorubim lima* (Shibatta *et al.*, 2011).

2002). The maturation period was shorter in the case of *P. mangurus* since the final maturation occurred after six hours (165 degree-hours) after the second hormonal dose. In other Neotropical catfish species, ovulation

Such a result is probably related to the hormonal induction of fish at the spawning site. In this environment, fish can find adequate conditions necessary for their gonadal development, including aspects such as nutrition and ideal spawning area.

The oocytes of the *P. mangurus* were smaller than the oocytes of other Neotropical siluriform species such as *Z. jahu* (2,400 μm) (Nogueira *et al.*, 2012), *R. quelen* (2,640 μm), *P. charus* (2,670 μm) (Vieira-Sampaio & Yoshimi, 2006). However, the larval size of *P. mangurus* at hatching was higher than other Neotropical Siluriformes and presented higher growth performance when compared to *P. charus* and *R. quelen* (Vieira-Sampaio & Yoshimi, 2006), *P. maculatus* (Luz & Zaniboni-Filho, 2001), *Pseudoplatystoma corruscans* (Campagnolo & de Oliveira-Nuñer, 2006). First feeding of the majority of carnivorous larvae from Neotropical fishes was achieved successfully using *Artemia* nauplii during the initial stages, as in the case of *Lophisilurus alexandri* (Luz *et al.*, 2011), *Pimelodus maculatus* (Luz & Zaniboni-Filho, 2002), *R. quelen* (Diemer *et al.*, 2012), *P. corruscans* (Segura *et al.*, 2004), *Steindachneridion melanodermatum* (Feiden *et al.*, 2006) and *S. scriptum* (Schutz *et al.*, 2008). *P. mangurus* had a smaller egg size, but the larvae at first feeding were larger than other Neotropical carnivorous larvae. This characteristic probably is the reason why protocols using only *Artemia* nauplii were not successful in this study. Bigger prey such as fish larvae (*A. altiparanae* and *P. lineatus*) had to be added to the diet of *P. mangurus* to reduce cannibalism and to improve growth.

Reproduction using wild-specimens, followed by the rearing of juveniles, may work synergically for GenBank since those juveniles could be easily domesticated and used for reproductive technologies such as surrogate propagation (De Siqueira-Silva *et al.*, 2018).

In conclusion, reproduction and first feeding of *P. mangurus* were successfully achieved using the following procedure: capturing the brood stock in natural environments and immediately inducing spawning with carp pituitary extract (0.6 and 6 mg kg^{-1} for females, and 2 mg kg^{-1} for males); getting testis after sacrificing males and stripping females after 165 h degree-hours after the second dose; doing fertilization with diluted sperm in Eagle's MEM, and feeding the larvae with *Artemia* nauplii in the first three days followed by fish larvae in the next days. These procedures are innovative and are important to establish conservation strategies for this endangered catfish species.

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