Neotropical Ichthyology, 7(4):759-762, 2009
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Scientific Note

Induced spawning of the endangered Neotropical species *Steindachneridion parahybae* (Siluriformes: Pimelodidae)

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The "surubim do Paraíba" (*Steindachneridion parahybae*) is a freshwater catfish endemic to the Paraíba do Sul River basin, Brazil. This species has been seriously threatened by environmental disturbances in the last several decades. Wild *Steindachneridion parahybae* males and females were collected in 2003 and taken to the hatchery of a power plant of the Companhia Energética de São Paulo (CESP). *Steindachneridion parahybae* broodstocks were artificially induced to reproduce in December 2003 using a combination of carp pituitary extract (CPE) and human chorionic gonadotropin (hCG). Oocytes and milt were stripped; the fertilized eggs were transferred to 60-liter conical incubators and hatched larvae distributed in nine horizontal trays. Exogenous feed was started just after yolk sac absorption. A high rate of cannibalism and photophobia were observed during the larval period, resulting in a 26% survival rate from larvae to fingerlings.

O "surubim do Paraíba" (*Steindachneridion parahybae*) é um bagre de água doce, endêmico da bacia do rio Paraíba do Sul, Brasil. Esta espécie foi seriamente ameaçada por distúrbios ambientais nas últimas décadas. Machos e fêmeas selvagens de *Steindachneridion parahybae* foram coletados em 2003 e transferidos para a piscicultura da CESP (Companhia Energética de São Paulo). Reprodutores de *S. parahybae* foram induzidos à reprodução artificial em dezembro de 2003 usando uma combinação de extrato hipofisário de carpa (CPE) e gonadotropina coriônica humana (hCG). Após a extrusão dos óvulos e do sêmen, os ovos fertilizados foram transferidos para incubadoras cônicas de 60 litros e, em seguida, as larvas eclodidas distribuídas em nove incubadoras horizontais. Após a absorção do saco vitelino, a alimentação exógena foi iniciada. Uma alta taxa de canibalismo e fotofobia foram observados durante o período larval, resultando em uma taxa de sobrevivência de 26% de larvas para os alevinos.

Key words: Surubim do Paraíba, Paraíba do Sul River, Artificial reproduction.

The "surubim do Paraíba" (*Steindachneridion parahybae*) (Fig. 1) is a medium-sized siluriform freshwater species, endemic to the Paraíba do Sul River basin (Oliveira & Moraes Jr., 1997; Garavello, 2005). This basin is an isolated hydrographic basin located in southeast Brazil (20°26' and 23°39'S, and 41° and 46°30'W), and *S. parahybae* was commonly captured in the commercial fisheries along the Paraíba do Sul River during the 1950's (Machado & Abreu, 1952).

The surubim do Paraíba species is critically threatened (Brasil, 2004; Caneppele *et al*., 2008; São Paulo, 2008; Honji *et al*., 2009), and presently, there are no records of this species in the southern rivers of the Paraíba do Sul basin (São Paulo, 2008). A few populations still remain in northern tributaries, but their survival faces constant threats from pollution, river damming, sand extraction from riverbeds and floodplains, predatory fishing, and the introduction of exotic fish species (Hilsdorf & Petrere, 2002). The biological information that is available about the surubim do Paraíba is very scarce. Its spawning season occurs from late November until March, and its feeding habit is mainly carnivorous (ichthyophagous). Their

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endangered status (Rosa & Lima, 2005) makes any biological study of surubim do Paraíba extremely laborious, especially during sample collection stages. Therefore, the present difficulty of capturing the species may contribute to the paucity of information about this species, which is at risk of becoming extinct without being fully described.

In the past five years, this species has been included in the conservation program undertaken by the Companhia Energética de São Paulo (CESP, 2006). The first goal of the program was to develop an artificial reproduction protocol that would be applied to *S. parahybae* fingerling production. The objective of this study was to present the results of the first reported artificial spawning of *S. parahybae*.

Information from local artisanal fishermen made it possible for us to locate a population that still survives in a small tributary of the Paraíba do Sul River. During 2003, fifteen individuals were caught in the Paraíba do Sul River, near the town of Rio das Flores in the state of Rio de Janeiro (22°13'54"S 43°25'15"W), and brought to the CESP hatchery in order to start the first trials of induced hormonal reproduction. The broodstock (100% survival) were maintained in 200 m^2 ponds and fed commercial food (40% crude protein, Purina TC 40).

In December 2003, the animals were selected according to the typical morphological characteristics of sexual maturity (Leonardo *et al*., 2004), and based on these characteristics, two females and two males were chosen for artificial reproduction. The females were selected by external characteristics according to the hyperemic genital pore (and swollen abdomen), and the males were chosen according to the white color and high fluidity of milt when the abdominal region was squeezed. The selected broodstock were transferred to the laboratory and kept in 1,000 liter tanks.

The animals were induced to reproduce by combining whole acetone-dried carp pituitary extract (CPE) (Fish Braz) with a protocol proposed by von Ihering & Azevedo (1934, 1936) but using CPE (as proposed for the pimelodid *Pseudoplatystoma fasciatum*, by Leonardo *et al*., 2004) and human chorionic gonadotropin (hCG) (Pregnyl - Organon), was performed as follows. Two CPE doses (0.6 mg and 5.4 mg CPE per kg of fish body weight, dissolved in 0.9% sodium chloride solution, considering 0.6 ml in each dose) were used for females, with a 12 h interval between the CPE doses. For males, a single dose was applied at the same time as the females' second dose; this dose contained 3 mg CPE per kg and was also diluted in 0.9% sodium chloride solution. A single dose of hCG was given at the same time as the second CPE application. The hCG concentration used was 2 IU per kg for females and 1 IU per kg for males (Harvey & Carolsfeld, 1993). A summary of the doses used in this protocol is shown in Table 1.

Fig. 1. *Steindachneridion parahybae*, Paraíba do Sul River basin, Paraibuna, São Paulo State, Brazil. Lateral view (image by Caneppele).

Table 1. Protocol of *Steindachneridion parahybae* induced reproduction

Animal	Weight (g)	hCG (IU/kg)	First CPE dose (mg/kg)	Second CPE dose (mg/kg)	Eggs spawned weight (g)
Female 1	2400	2	0.6	5.4	66
Female 2	1300	2	0.6	5.4	40
Male 1	1800	1		3.0	
Male 2	1100	1		3.0	

The use of hCG combined with pituitary extracts has been used in fish species (Thalathiah *et al*., 1988; Kucharczyk *et al*., 1997; Zohar & Mylonas, 2001; Leonardo *et al*., 2004) and has proven to be a very successful and consistent strategy in catfish species (Thalathiah *et al*., 1988).

After hormone administration, each couple was placed in a 1,000 liter glass tank to facilitate the observation of their sexual behavior during spawning. The time necessary for gamete elimination, counted from the second hormone administration until spawning, was calculated as accumulated thermal units (ATU), or degree-hours. Therefore, the ATU is the sum of the water temperature per hour, from the second hormonal application until spawning (Weingartner & Filho, 2005). After 200 ATUs (average temperature of 24°C), it was possible to observe an increase in the animals' activity, including aggressive behavior that could harm the partner. This behavior was important to determine when to separate males and females and to strip their gametes in plastic containers. Fertilization was performed by gentle mixing using the "dry" method (von Ihering & Azevedo, 1936). Spawning occurred between 240 and 255 ATU. The ATU value for *S. parahybae* was similar to other siluriform species, such as *Rhamdia quelen*, where the ATU value was 220 - 240 at 22-27°C (Baldisserotto & Neto, 2005), *Pseudoplatystoma corruscans* (200 - 280 ATU at 28°C) (Campos, 2005) and *Steindachneridion melanodermatum* (240 - 280 ATU at 27°C) (Ludwig *et al*., 2005).

The estimated number of ovules in *S. parahybae* was 324 ovules per gram of ovulated ovary mass (calculated by weighing the ovulated eggs and counting subsamples in triplicate). Considering the body weight of both females (Table 1), we can conclude that the number of released ovules ranged from 9,000 to 10,000 eggs kg^{-1} of female body weight. This value is lower than the number found in *R. quelen*, in which each kilogram of female body weight corresponds to approximately 216,000 eggs (Baldisserotto & Neto, 2005). Higher numbers of eggs are also found in *P. corruscans*, which produces between 135,000 and 220,000 eggs kg^{-1} of female body weight (Campos, 2005), and consequently, *S. parahybae*

releases a smaller mass of eggs (27 - 30 g kg^{-1}, calculated from Table 1) than does *P. corruscans*. During the 2007-2008 spawning season, after the beginning of physiological studies on the pituitary-gonad axis of *S. parahybae*, two females were also stripped using the same protocol described here for hormonal induction, and the amount of eggs released increased to about 45 g kg^{-1} (data not shown).

After spawning and hydration, the eggs were gently transferred from the plastic containers to the 60-liter conical incubators (built at CESP) (Table 1). In general, *S. parahybae* eggs were floating, yellowish, measuring ca. 190 mm. After an incubation of 42 h at an average temperature of 24ºC, approximately 29,200 hatched larvae from both females were divided among nine horizontal trays (built at CESP; 180 x 40 x 8 cm) (3,200 larvae per tray), where they were maintained for 8 days. The yolk reserve was completely absorbed within the first 24 h, *i.e.*, one day after hatching (DAH). The congeneric species *S. melanodermatum* showed a shorter egg incubation period, 18 h, but a longer yolk absorption period, 48 h, at 27ºC (Ludwig *et al*., 2005).

The first feeding started on the 2nd DAH; cannibalism was observed during larval development, as was heterogeneous larval growth and photophobia. Cannibalism has already been observed in many siluriform species (Luz *et al*., 2001; Baldisserotto & Neto, 2005; Campos, 2005; Ludwig *et al*., 2005; Schutz *et al*., 2008) and is one of the main bottlenecks responsible for reducing larval survival. Reducing the amount of cannibalism during this phase of fingerling production will require specific attention. Different photoperiods were proposed to reduce cannibalism in *Steindachneridion scriptum*. Schutz *et al*. (2008) suggested a photoperiod of 14 h of light per day to be the best to achieve a higher survival with good growth. Photoperiod studies with the same species suggest that *S. scriptum* can easily acclimate to the light, displaying higher growth during the photoperiod with continuous light when compared with darkness (Zaniboni-Filho *et al*., 2008).

During the incubation period - from the absorption of the yolk sac to the 3rd DAH - larvae were fed a combination (in the same proportion) of *Astyanax* spp. larvae and *Daphnia* spp. For *S. scriptum* larvae, the best growth rate was achieved using fish larvae (*Prochilodus lineatus*) as initial feeding (Schutz *et al*., 2008). Beginning on the 4th DAH, all larvae were fed a commercial powdered diet containing 55% crude protein (CP), mixed with fish meal in a proportion of 4:1 (commercial diet: fish meal). The larvae accepted the artificial diet, and this transition was considered successful as previously suggested for *S. scriptum* larvae, in which feed transition was implemented between the second and eighth days of cultivation (Adamante *et al*., 2007).

On the 8th DAH, larvae measuring 1.2 cm were transferred to two 200 m^3 earthen ponds provided with partial shade. The same feeding protocol was maintained (commercial powdered diet) to feed the larvae from the 4th DAH in the laboratory trays to approximately 30 days after hatching in the ponds. Animals were maintained for a further five months in the fish ponds with commercial extruded diet with a pellet size of 26 mm and 45% CP. After five months, there were 7,800 surviving fingerlings, each measuring around 12 cm and weighing 23 g, yielding an estimated 26% rate of survival from the larval to fingerling phase.

This first trial of induced reproduction in *S. parahybae* is an important step towards understanding the reproductive biology of this endangered species, as well as contributing to its conservation. Captive breeding followed by introduction of captive-bred fish into the environment are techniques used widely to recover endangered fish species (Philippart, 1995; Brannon *et al*., 2004). The production of *S. parahybae* fingerlings in captivity on a regular basis will allow the establishment of a more effective re-introduction program, as well as an investigation of its potential for fish farming. The protocol reported is viable for induced spawning in *S. parahybae*, but other hormones and doses should be investigated. Nevertheless, some basic aspects of the reproductive biology of *S. parahybae* remain unknown, such as the annual gonad histomorphology, which are important factors for establishing an accurate ovarian development cycle and determining the best month for artificial reproduction. Additionally, the annual variation of the gonadosomatic index and relative fecundity, which is important for predicting egg masses and amounts during spawning, has to be investigated.

Acknowledgements

The authors would like to thank the local fishermen who helped us find and collect broodstock, as well as FAPESP (07/55494-7) for the financial support. The authors also wish to recognize the contribution made by the CESP staff, Benedito P. P. Barros and Milton M. Rosa.

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Accepted October 11, 2009
Published December 18, 2009