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Induced reproductive responses of the neotropical anostomid fish *Leporinus elongatus* Val. under captive breeding

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Abstract

Experiments on induced spawning of the anostomid fish *Leporinus elongatus* Val. 1849, an important neotropical migratory fish, were carried out at Três Marias Fish Hatchery Station, State of Minas Gerais, Brazil, to develop a reliable protocol for inducing breeding in this species. *L. elongatus* matures reliably in earthen ponds to a point at which final maturation can be induced with carp pituitary extract. The following data were recorded: number of extruded eggs (g ova^{-1}) = 2444 ± 740; stripped ova weight:body weight = 13.1 ± 2.9%; retained (ovulated but not stripped) ova weight:body weight = 5.8 ± 2.6%; initial fertility = 318.7 × 10^3 ± 149.2 × 10^3; final fertility = 229.3 × 10^3 ± 129.3 × 10^3; egg fertilization rate = 63.8 ± 16.4% and ≈ 229 000 larvae per female. The relationships between stripping time and water temperature and between hatching and water temperature were given by the same exponential equation $t = ae^{-bT}$. The relationships between body weight (Wt) and initial (IF) and final fertility (FF) were expressed by the equations: IF = –9382 + 322 047 × Wt (r^2 = 0.84); FF = –40 780 + 265 062 × Wt (r^2 = 0.75).

Introduction

Leporinus elongatus Val. 1849, the largest known Anostomidae fish, is native to two of the largest South American rivers – the transnational Paraná and the São Francisco. It can reach up to 7.5 kg in body weight (Godoy 1975) and is important in commercial and sport fisheries. 'Piapara' or 'piau-verdadeiro', as it is known in the Paraná and São Francisco, respectively, is mainly herbivorous–insectivorous, but also feeds on crustaceans and plankton (Godoy 1975; Fontenele & Vasconcelos 1977). It matures, but does not spawn, in captivity. In this respect, it resembles other neotropical freshwater species that undergo reproductive migration in their native habitat (for a review, see Lamas 1993).

Hydroelectric developments, agriculture, pollution and other environmental degradation are resulting in a steady decline in most stocks of migratory fish, including *L. elongatus*, in south-eastern Brazil (Godinho 1998). Restocking of these river basins has been carried out for some time, but relatively few *L. elongatus* have been included in this programme. However, its low position in the food web, its large size and the decreasing availability of wild stocks have recently aroused a renewed interest in this species for both stocking and aquaculture.

Reproductive data for *L. elongatus* are scarce (Godoy 1975; Fontenele & Vasconcelos 1977) and are needed to develop reliable protocols for breeding this fish in captivity. In this paper, we summarize the data obtained from routine hypophysation of this fish over various reproductive seasons in a state-run Brazilian hatchery and develop recommendations for

such a protocol. These data are relative to egg production and to the effect of water temperature on ovulation and embryonic development.

Materials and methods

Broodstock management

Fish were captured in the São Francisco River and maintained in 0.06–0.1 ha earthen ponds at Três Marias Fish Hatchery Station, Três Marias, MG, Brazil (18° 12′S, 45° 15′W) at a stocking rate of 1 kg of fish 7 m^{-2}. They were fed on commercial feed (22% crude protein), 1.5% of body weight day^{-1}, 5 days $week^{-1}$. Water was supplied to the ponds from the Três Marias Reservoir in the São Francisco River. A total of 118 females (0.98 ± 0.35 kg body weight) and 122 males (0.62 ± 0.18 kg body weight) were used for the work reported here during the reproductive season of 1989–93.

Induced breeding

Fish were seined a few times in the holding ponds during the reproductive season and assessed for readiness for induced breeding. Females with a large, flaccid abdomen and protruding, reddish urogenital papilla and males that released milt easily with slight abdominal pressure and vocalized were considered to be ready for treatment. Those selected for use were weighed to the nearest gram and transferred to 3.0 × 1.0 × 0.8 m brick-lined tanks with running water, where they remained throughout the induction process.

Induced breeding (hypophysation) was carried out with crude carp pituitary extract (CCPE), as described by Ihering (1937), with some modifications. This is the standard procedure applied to other species at Três Marias Fish Hatchery Station. Briefly, acetone-dried pituitaries were macerated with mortar and pestle and extracted into 0.8–1.0 mL of 0.7% NaCl solution kg^{-1} fish body weight. This slurry was injected intraperitoneally as a single injection for the males (2.5 ± 0.3 mg CCPE kg^{-1} body weight) and as two injections for the females (0.9 ± 0.2 and 5.9 ± 0.3 mg of CCPE kg^{-1} body weight) separated by an interval of 14.1 ± 0.8 h. Pituitary extracts were prepared fresh before each use. Progress of maturation was monitored by observing the behaviour of the fish and by regular palpation of the female abdomen to check for ready release of ovulated eggs. Eggs were gently stripped manually just after ovulation into a dry plastic bowl. Fertilization was performed 'dry' with fresh milt from one to three males for a few minutes. Time to ovulation/egg stripping and hatching were monitored for each treatment, as was the water temperature.

Egg production

The weight (to the nearest 0.01 g) of the freshly stripped ova was determined for 70 females. A subsample of the eggs of ≈ 2 g (to the nearest 0.01 g) from 28 of these fish was fixed in modified Gilson's solution (Simpson 1951) for subsequent enumeration and estimation of number of eggs g^{-1} stripped ova and the number of eggs per stripped ova (initial fertility). The weight (to the nearest 0.01 g) of the retained ova (ovulated but not stripped eggs) in 58 of the stripped females was also determined after killing the fish and carefully dissecting out their ovaries. Thus, the ratios of stripped ova weight:body weight (%) as well as the retained ova weight:body weight (%) were calculated. The diameter of 20 eggs, before and after hydration, in each of 19 females was measured (to the nearest 0.1 mm) under a stereomicroscope.

Incubation and fertilization

The fertilized eggs were rinsed several times with water to remove excess milt and allow the beginning of hydration before transferring to 60-L and 200-L funnel-type upwelling fibreglass incubators; 70–80 g and 120–130 g of eggs were placed in each incubator respectively.

Water in both brick-lined tanks and incubators originated from Três Marias reservoir and had temperatures between 23 °C and 25 °C, dissolved oxygen of 5.5–6.5 mg L^{-1}, pH of 6.0–6.5 and conductivity of 50–80 μS cm^{-2}. The fertilization rate of the eggs (i.e. relative number of normal and dead embryos) of 73 females was determined after blastopore closure from a sample of ≈ 200 eggs collected from the middle section of the incubator with a glass tube. The number of viable embryonic eggs (final fertility) was estimated after blastopore closure.

Broodstock survival

The survival of 56 male and 53 female fish was monitored during the induced breeding period and for up to 1 week after treatment.

Statistics

Times to ovulation/stripping and hatching were clustered within the nearest full degree of temperature they experienced (23 °C, 24 °C and 25 °C). The distinctness of these clustered data were assessed with ANOVA followed by Tukey's test for comparing multiple means. In addition, a curve was fitted to both stripping and fertilization time as a function of water temperature during treatment to generate a generalized predictive model for these parameters.

Results

Breeding season, behaviour and survival of spawners

Brooders were found to be mature enough for hypophysation between December and February. Of the 118 female fish selected for treatment during this time, 74 (62.7%) responded positively and produced viable eggs. The females generally remained immobile in the treatment tank throughout the inducing process but, shortly before ovulation, started swimming back and forth repeatedly in the tank. The survival rate of brooders was 87% for males and 62% for females.

Egg production

L. elongatus eggs are opaque, light grey in colour, demersal and free. Freshly stripped egg diameter was about 1 mm, increasing to 2.2 mm after hydration (Table 1). Other characteristics of egg production of this species submitted to hypophysation are summarized in Table 1. The relation between initial and final fertility in relation to body weight is shown in Fig. 1.

Effect of water temperature on ovulation and embryonic development

Both time to ovulation after treatment and time to hatching were significantly ($P < 0.001$) accelerated by increased water temperature. Ovulation/stripping occurred at 9.8–8.4 h after the second CCPE injection, with a water temperature of 23–25 °C (Fig. 2), corresponding to cumulative thermal units of 300–200 hour-degrees. Hatching times ranged from 22.6 to 17.2 h, in water of 23–25 °C, corresponding to 520–430 hour-degrees (Fig. 3).

For both stripping and hatching times, the relationship with water temperature was shown to be of the exponential type $t = ae^{-bT}$.

Discussion

The reproductive period of *Leporinus elongatus*, maintained at Três Marias Fish Hatchery Station, coincided with its natural spawning season, i.e. within the rainy reason in south-eastern Brazil (for a review, see Lamas 1993). It also coincided with that of most species induced to reproduce at the same hatchery station (Y Sato, unpubl. obs.). As

Table 1 Data on egg production of *L. elongatus* obtained through hypophysation with crude carp pituitary extract, at Três Marias Fish Hatchery Station, Três Marias, MG, Brazil

Parameter	*n*	Mean ± SD	Range
Egg size (diameter, μm)			
Prehydration	380*	1.0 ± 0.1	0.9–1.2
Post-hydration	380*	2.2 ± 0.1	2.0–2.5
Eggs g of stripped ova^{-1} (× 10^3)	28	2.44 ± 0.74	2.32–2.58
Stripped ova weight: body weight (%)	70	13.1 ± 2.9	7.16–19.8
Retained ova weight: body weight (%)	58	5.8 ± 2.6	1.8–13.3
Egg fertilisation rate (%)	73	63.8 ± 16.4	23.0–98.6
Initial fertility† (× 10^3)	28	318.7 ± 149.2	136.7–680.9
Final fertility‡ (× 10^3)	28	229.3 ± 129.9	84.5–537.2

n, number of observations; SD, standard deviation.

*Measurements made in 20 eggs in each of 19 females.

†Number of eggs stripped per ova.

‡Number of viable embryonic eggs estimated after blastopore closure.

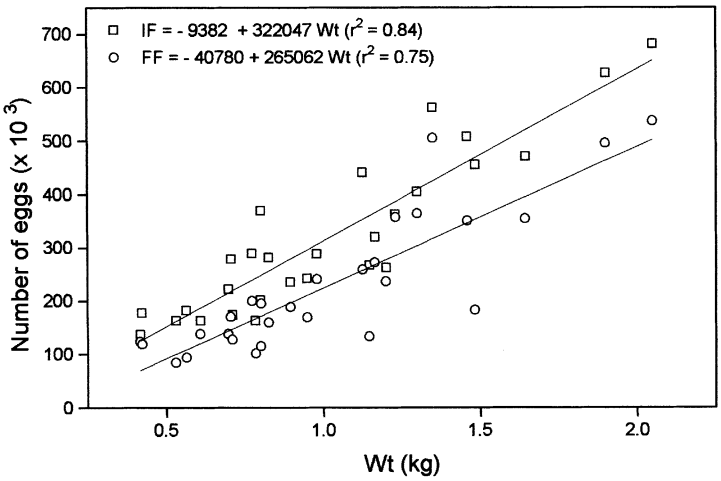

Figure 1 Initial fertility (IF) and final fertility (FF) on body weight (Wt) obtained from 28 females of *L. elongatus* induced to spawn under hypophysation.

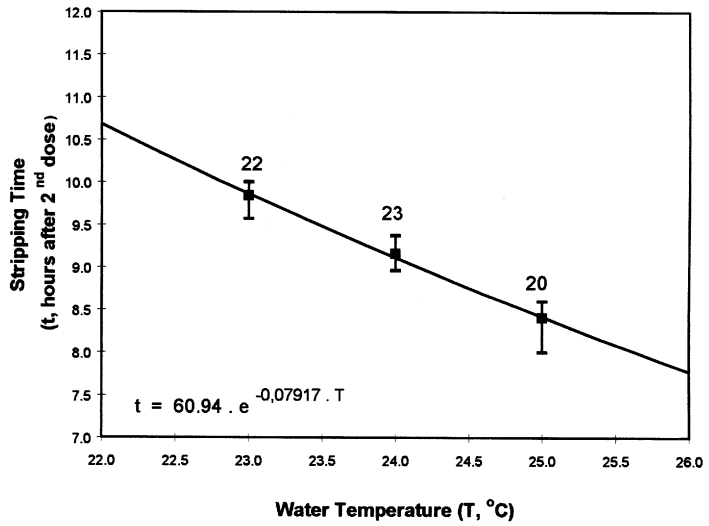

Figure 2 Stripping time of *L. elongatus* subjected to hypophysation at different water temperatures. Means (black squares), range (vertical line) and number of females (numerals above the vertical lines).

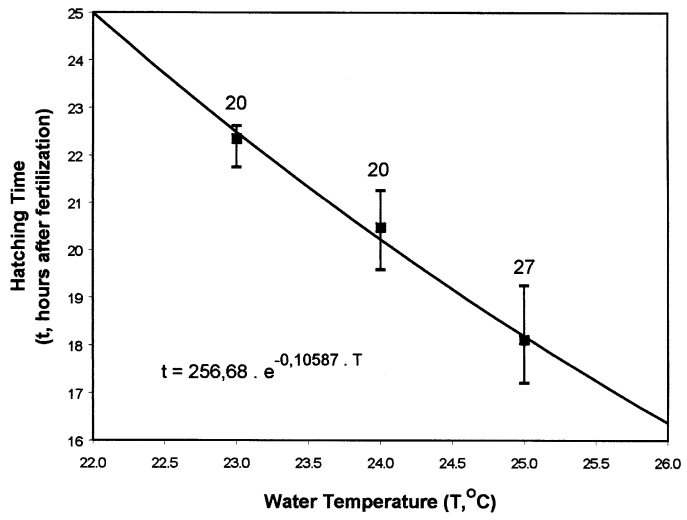

Figure 3 Hatching time of *L. elongatus* eggs at different water temperatures. Means (black squares), range (vertical line) and number of females (numerals above the vertical lines).

reported for some Prochilodontidae (Azevedo & Canale 1938; Fontenele 1953; Sato, Cardoso, Godinho & Godinho 1996), female *L. elongatus* showed a distinctive increase in swimming activity at the time of ovulation that assisted in timing the stripping of eggs appropriately.

The size of the egg before and after hydration was comparable with that of other neotropical, migratory species with group-synchronous spawning (Lamas 1993). Increasing water temperature during treatment revealed a similar pattern of oocyte final maturation acceleration observed in various species of carp (Horváth 1978), in *Rhamdia sapo* (C. & V.) (Espinhach Ros, Amuty, Arceredillo, Orti & Nani 1984) and in *Prochilodus marggravii* (Walbaum 1792) (Sato *et al.* 1996).

Godoy (1975) estimated slightly shorter hatching times of 15.5–17 h for *L. elongatus* in the Mogi Guaçu River, south-eastern Brazil, with water temperatures of 24–26 °C and cumulative thermal units to hatching of 400 hour-degrees. The exponential relationship for both stripping and hatching time with water temperature registered in the present work proved to be similar to that observed in salmonids (Kamler 1992).

Approximately 229 000 larvae per female were produced, as judged by the number of eggs stripped and fertilization rates. This represents less than half of the eggs per female available for stripping. Losses were attributed equally to incomplete stripping (≈ 30% of the eggs were retained in the ovaries after stripping) and fertilization failure and/or embryonic fatality (an average of about 70% of the eggs survived to closure of blastopore).

Thus, the standard technology used for induced breeding at Três Marias Fish Hatchery Station was effective for *L. elongatus*, but the efficiency could be improved, as judged by the large variation in the proportions of eggs retained in the female after stripping and the relatively low fertilization rate of the eggs.

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