

HYPOPHYSATION PARAMETERS OF THE FISH *Prochilodus marggravii* OBTAINED IN ROUTINE HATCHERY STATION CONDITIONS

YOSHIMI SATO¹, ELIZABETH LOMELINO CARDOSO¹,
ALEXANDRE LIMA GODINHO² and HUGO PEREIRA GODINHO²

¹Três Marias Hydrobiological and Fishculture Station, CODEVASF,
P. O. Box 11 - 39205-000 Três Marias, MG

²Institute of Biological Sciences, Federal University of Minas Gerais,
P. O. Box 486 - 30161-970 Belo Horizonte, MG, Brazil

(With 4 figures)

RESUMO

Dados de Hipofisacão do Peixe *Prochilodus marggravii* Obtidos Durante a Rotina de Estação de Piscicultura

Prochilodus marggravii, um importante peixe comercial brasileiro, foi hipofisado no período de 1983 a 1993, na Estação de Hidrobiologia e Piscicultura de Três Marias, em Três Marias, MG. As fêmeas receberam duas doses de extrato bruto de hipófise de carpa contendo, respectivamente $0,8 \pm 0,2$ e $6,0 \pm 0,6$ mg/kg de peso corporal, com $15,8 \pm 2,2$ h de intervalo entre doses. Os machos receberam dose única contendo $2,8 \pm 0,5$ mg/kg de peso corporal do mesmo material. O método "a seco" foi utilizado para fertilizar os ovos. A extrusão dos ovos foi feita $227,3 \pm 9,3$ horas-grau após a aplicação da segunda dose, à temperatura da água de $24,1 \pm 0,7$ °C. As horas-grau à extrusão e à eclosão tendem a diminuir com o aumento da temperatura da água. Fêmeas hipofisadas à temperatura da água abaixo de 23°C não responderam ao tratamento. Durante o período de 10 anos, 194,3kg de fêmeas hipofisadas responderam positivamente ao tratamento. Elas produziram 32×10^6 ovos resultando em $21,7 \times 10^6$ ovos viáveis, os quais corresponderam, respectivamente, a 165×10^3 e 112×10^3 ovos extrusados e viáveis por kg de fêmea.

Palavras-chave: hipofisacão, *Prochilodus marggravii*.

ABSTRACT

Prochilodus marggravii, an important Brazilian commercial fish, was induced to spawn through hypophysation in the period of 1983 to 1993 at Três Marias Hydrobiological and Fishculture Station, Três Marias, MG. The females received two doses containing, respectively, 0.8 ± 0.2 and 6.0 ± 0.6 mg/kg of body weight of dried carp pituitary, with 15.8 ± 2.2 h of

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Correspondence to: H. P. Godinho

interval between doses. The males received a single dose which contained 2.8 ± 0.5 mg/kg of body weight of the same material. The 'dry' method was used to fertilize the eggs. Stripping was performed at 227.3 ± 9.3 degree-hours after the application of the second dose, at the water temperature of $24.1 \pm 0.7^\circ\text{C}$. The degree-hours at stripping and at hatching tended to decrease with increase in the water temperature. Females hypophysed at water temperatures below 23°C did not respond to the treatment. During the 10 year period, 194.3kg of hypophysed females responded positively to the treatment. They produced 32×10^6 eggs resulting in 21.7×10^6 viable eggs, which corresponded to 165×10^3 and 112×10^3 of stripped eggs and viable eggs, respectively, per kg of female.

Key words: hypophysation, *Prochilodus marggravii*.

INTRODUCTION

Among the prochilodontid fish there are species of great importance to Brazilian river fisheries. Almost 20% of the Brazilian freshwater fisheries yields are made of fish belonging to this family (IBGE, 1988).

Prochilodus marggravii (Walbaum, 1792), an endemic species to the São Francisco river basin, eastern Brazil, is the largest fish of the family, reaching 15kg of body weight. It is a mud eater, total spawner and performs migratory journeys during the reproductive period, which extends from November to January within the rainy season (Fontenele, 1953). This period coincides with large floodings, higher water temperature, and longer photoperiod.

Although still being the main species in the São Francisco river fisheries, representing over 50% of the captures at Três Marias region, southeastern Brazil, there has been a steady decline in its yields, with the risk of extinction in various regions of that river basin.

In this work, we present data on induced spawning of *P. marggravii*, obtained through hypophysation during a period of 10 years.

MATERIAL AND METHODS

We utilized data gathered from the routine work at Três Marias Hydrobiological and Fishculture Station, CODEVASF, at Três Marias, MG, Brazil (Lat. $18^\circ 11'S$, Long. $45^\circ 13'W$), using 190 females and 197 males of *P. marggravii* during the period from 1983 to 1993.

The brooders were maintained in 0.1ha, 1m deep ponds, at 1kg of fish/8m² of pond. They received 1.5-2% commercial feed daily which con-

tained a minimum of 22% crude protein, as indicated by the manufacturer. The ponds were fertilized fortnightly with 75kg/ha of ammonium sulfate and 19kg/ha of superphosphate. Since *P. marggravii* does not naturally spawn in captivity, the fishes were hypophysed (Ihering, 1937) with crude carp pituitary extract (CCPE). During the hypophysation treatment, males and females were kept apart in concrete tanks measuring $3.0 \times 1.0 \times 0.8$ m.

Doses (one in the males and two in the females), estimation of time for egg stripping and hatching (in degree-hour) and rate of egg fertilization (in %, estimated after blastopore closure) followed Woynarovich and Horváth (1980). CCPE was injected in the coelomic cavity. The females received two doses containing, respectively, 0.8 ± 0.2 and 6.0 ± 0.6 mg/kg of body weight of CCPE with 15.8 ± 2.2 h of interval between doses. The males received a single dose which contained 2.8 ± 0.5 mg/kg of body weight of a material.

The 'dry' method was used to fertilize the eggs, using semen of one male for the eggs of one female. The fertilized eggs were placed in funnel-type fiberglass incubators with 60 or 200 liter capacity which received about 70g or 150g of eggs, respectively. In the 60 liter incubators, the water flow was at 2 liters/min up until the blastopore closure and then raised to and maintained at 5 liters/min until hatching. In the 200 liter incubators those values were, respectively, 4 liters/min and 8 liters/min. The water supplying the incubators had the following characteristics: temperature = 23.0 - 25.0°C , dissolved oxygen = 5.1 - 6.8 mg/liter, pH = 6.1 - 7.2 , and conductivity = 47 - 105 $\mu\text{S}/\text{cm}^2$.

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The total length (cm), body weight (g), the weight (g) of ova (= extruded eggs), the number of eggs/g of ova and the egg diameter (mm) before and after hydration were obtained.

Fecundity, initial fertility and final fertility were obtained in 22 randomly selected females. Fecundity was calculated as the number of the remaining vitelline oocytes within the ovaries after stripping plus the number of extruded eggs obtained at stripping. Initial fertility was considered as the number of eggs obtained at stripping and final fertility, the number of viable eggs counted after blastopore closure. Relative fecundity and relative fertilities were estimated based on the body weight.

The rate of mortality during hypophysation and within the following week was established using 87 females and 95 males.

The statistical analyses were performed following Sokal and Rohlf (1979) and SAS Institute Inc. (1985).

RESULTS

In the conditions of the present work, part of the cultivated *P. marggravii* broodstock was sexually mature at 1 year and 2 years of age, respectively, for males and females. They accepted pelleted feed besides feeding on organisms present in the ponds. They were ready for hypophysation from November to February. At the time of reproduction, the females showed flaccid, swollen abdomen and reddish and oedematous genital papilla. The males snored and oozed semen through the genital papilla when handled. During treatment, the females remained quiet except at the moment of ovulation when they moved from one side to the other in the concrete tanks.

The data are summarized in Table I. We obtained positive response to the CCPE treatment stripping viable eggs in 148 females, corresponding to 77.9% of the females used. There was a large variation in size of the fish used. The females were heavier ($t = 6.05$; $P < 0.001$; $df = 385$) than the males. Smaller females had higher rates of positive response to the treatment than the larger ones (Fig. 1).

Females ($n=40$) hypophysed at temperatures below 23°C, whose data were not included in this work, did not respond to the treatment. At 23°C, 70% of the females reacted positively ($n=40$), at

24°C they were 74% ($n=100$) and at 25°C, 92% ($n=50$). The degree-hours at stripping tended to decrease when the water temperature increased from 23°C to 25°C ($F = 5.48$; $P = 0.005$; $df = 147$) (Fig. 2).

P. marggravii eggs are free, demersal and gray. During hydration, egg diameter increased from $1.6 \pm 0.1\text{mm}$ to $3.1 \pm 0.2\text{mm}$, characteristically of migratory and total spawning species.

The relationship between water temperature and degree-hours at hatching followed that between water temperature and degree-hours at stripping, i.e., hatching time was also shorter at higher temperatures in *P. marggravii* ($F = 10.93$; $P < 0.001$; $df = 67$) (Fig. 3).

The relationships between fecundity, initial fertility and final fertility and body weight are indicated in Figure 4. These parameters increased exponentially with body weight increase. The lower value of $r^2 (= 0.74)$ for final fertility indicates the wide variation in the egg fertility rates obtained in the present work.

There was no significant correlation of relative fecundity ($r = 0.40$; $P = 0.06$; $n = 22$), relative initial fertility ($r = 0.01$; $P = 0.96$; $n = 22$) and relative final fertility ($r = 0.03$; $P = 0.88$; $n = 22$) with body weight.

There was no significant correlation between egg diameter and body weight ($r = 0.27$; $P = 0.71$; $n = 34$). Although a high variation in the individual egg fertilization rates has been registered, the overall rate of fertilization for the period of study reached almost 70%. Egg fertilization rates show significant correlation with body weight ($r = -0.25$; $P < 0.01$; $n = 40$).

The females were more sensitive to the hypophysation than the males since 27.6% of those which were followed died within 1 week after treatment whereas only 9.5% of the males died within that period.

DISCUSSION

At northeastern Brazil, *P. marggravii* could be hypophysed in 9 different months of the year (Fontenele, 1953). Different climatic conditions between northeastern Brazil and the southeastern (where the present study was conducted) may be responsible for the variations in its reproductive period.

TABLE I
Hypophysation data of *P. marggravii* using crude carp pituitary extract, at the Três Marias Hydrobiological and Fishculture Station, Três Marias, MG, Brazil, during the period of 1984-1993.

| Parameter | N | Mean \pm SD | Range |
|---|-----|-------------------|------------|
| Males | | | |
| Body weight (kg) | 197 | 1.05 \pm 0.51 | 0.25-4.0 |
| Dose (pituitary dry weight, in mg/body weight) | 197 | 2.8 \pm 0.5 | 2.5-4.5 |
| Females | | | |
| Body weight (kg) | 190 | 1.44 \pm 0.72 | 0.37-4.9 |
| Doses (pituitary dry weight, in mg/body weight) | | | |
| — first dose | 190 | 0.8 \pm 0.2 | 0.5-1.5 |
| — second dose | 190 | 6.0 \pm 0.6 | 5.0-8.1 |
| Interval between doses (h) | 190 | 15.8 \pm 2.2 | 12.5-19.0 |
| Water temperature at stripping ($^{\circ}$ C) | 190 | 24.1 \pm 0.7 | 23.0-25.0 |
| Degree-hours at stripping | 148 | 227.0 \pm 8.4 | 210-250 |
| Ovary weight: body weight (%) | 83 | 22.9 \pm 4.1 | 9.5-31.5 |
| Ova weight (g) | 148 | 228.5 \pm 111.1 | 45-644 |
| Ova weight: body weight (%) | 148 | 17.3 \pm 3.3 | 8.0-24.2 |
| Eggs | | | |
| Eggs/g of ova (n) | 56 | 980 \pm 93 | 811-1200 |
| Egg diameter (mm) | | | |
| — Pre-hydration | 34 | 1.6 \pm 0.1 | 1.3-1.8 |
| — Pos-hydration | 34 | 3.1 \pm 0.2 | 2.8-3.8 |
| Egg fertility rate (%) | 148 | 68.8 \pm 18.7 | 16.0-99.6 |
| Fecundity ($n \times 10^3$) | 22 | 277.3 \pm 188.2 | 54.9-790.8 |
| Initial fertility ($n \times 10^3$) | 22 | 219.6 \pm 128.7 | 51.1-536.4 |
| Final fertility ($n \times 10^3$) | 22 | 177.9 \pm 111.3 | 32.4-426.8 |
| Water temperature at hatching ($^{\circ}$ C) | 68 | 24.0 \pm 0.7 | 23.0-25.0 |
| Degree-hours at hatching | 68 | 456.0 \pm 22.4 | 420-500 |

N = number of observations; SD = standard deviation.

The same characteristics employed at Três Marias Fishculture Station to select the brooders for hypophysation were also used by Fontenele (1953) to select *P. argenteus* (= *P. marggravii*) and by Castagnolli and Cyrino (1980) in the case of *P. scrofa*. The behavior of the females in the concrete tanks just prior to ovulation was also registered by Azevedo and Canale (1938) in *P. argenteus* (a different species from *P. marggravii*). The males also moved about in the concrete tanks and snored at this time similarly to the observations of Fontenele (1953).

Apparently, variations in the amount of CCPE given to the females and in the dose interval did not influence the treatment response nor the fertilization rates.

Increase in water temperature tend to shorten the values of degree-hour at stripping of *P. marggravii*. Similar pattern was observed by Horváth (1978) in various carp species and by Espinach Ros *et al.* (1984a) in *Rhamdia sapo*. Increased egg volume following hydration was also recorded in this species (Fontenele, 1953) and in *P. argenteus* (Ihering and Azevedo, 1934).

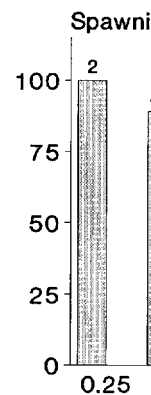


Fig. 1 — Spawning response of *P. marggravii* to hypophysation with crude carp pituitary extract, at the Hydrobiological Station, Três Marias, MG, Brazil, during the period of 1984-1993. Spotted bars = fish with positive response; white bars = fish with negative response.

The duration of the hypophysation treatment and the water temperature-dependent response also occurred. The results are shown in Table I. Since the relative fecundity and the egg weight and ova weight were lower in the control treatment, it

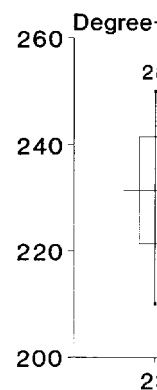


Fig. 2 — Degree-hours at stripping of *P. marggravii* at the Hydrobiological Station, Três Marias, MG, Brazil, in the period of 1984-1993. The box represents the standard deviation; the horizontal line represents the mean; the number 2 indicates the dose.

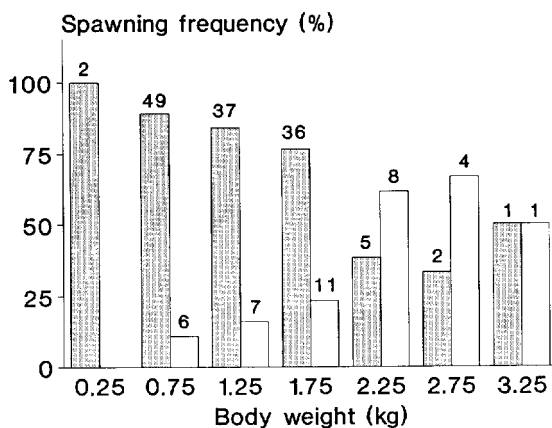


Fig. 1 — Spawning frequency (%) as related to body weight of *P. marginatus* at Três Marias Hydrobiological and Fishculture Station, Três Marias, MG, Brazil, in the period of 1984 to 1993. Spotted bars = frequency of females which responded positively to hypophyisation; open bars = frequency of females with negative response. Numerals above bars = number of females used.

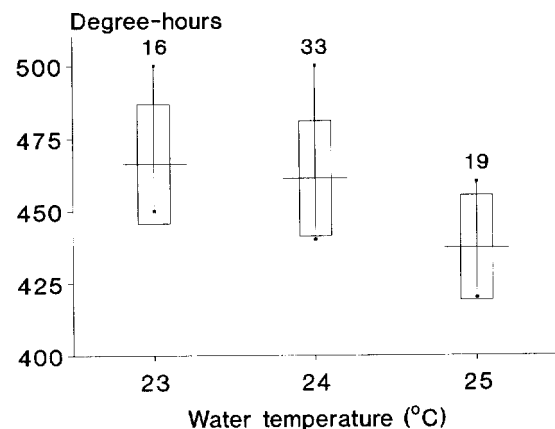


Fig. 3 — Degree-hours at hatching at different water temperature of *P. marginatus* hypophyised at Três Marias Hydrobiological and Fishculture Station, Três Marias, MG, Brazil, in the period of 1984 to 1993. Mean (horizontal line), standard deviation (vertical box) and range (vertical line) are presented; numerals above bars = number of females.

The duration of the embryogenesis is temperature-dependent in *P. marginatus*. This relationship also occurs among the species of *Prochilodus* shown in Table II.

Since there was no significant variation in relative fecundity and fertilities for different body weight and considering that large females tend to give lower number of positive responses to the treatment, it appears that females weighing less

than 2kg are indicated to constitute *P. marginatus* broodstocks.

In summary, 194.3kg of females *P. marginatus* were hypophyised in the period of 1983 to 1993 with the production of 32×10^6 eggs resulting in 21.7×10^6 viable eggs. These data correspond to 165×10^3 and 112×10^3 of stripped eggs and viable eggs, respectively, per kg of female.

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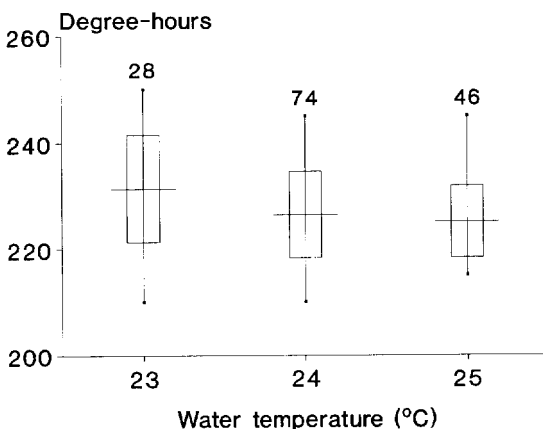


Fig. 2 — Degree-hours at stripping at different water temperature of *P. marginatus* hypophyised at Três Marias Hydrobiological and Fishculture Station, Três Marias, MG, Brazil, in the period of 1984 to 1993. Mean (horizontal line), standard deviation (vertical box) and range (vertical line) are presented; numerals above bars = number of females.

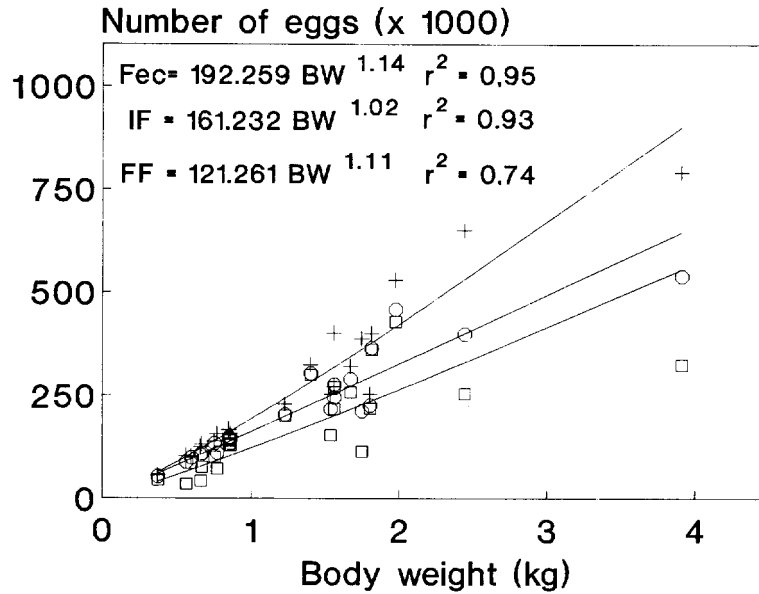


Fig. 4 — Fecundity (+), initial fertility (O) and final fertility (*) on body weight, obtained simultaneously from 22 females *P. marggravii* hypophyised at Três Marias Hydrobiological and Fishculture Station, MG, Brazil, in the period of 1984 to 1993. Fec = fecundity; IF = initial fertility; FF = final fertility.

TABLE II
Relationship between water temperature and degree-hours at hatching in *Prochilodus*.

| Species | Water temperature (°C) | Degree-hours | Author |
|------------------------|------------------------|--------------|------------------------------------|
| <i>P. platensis</i> | 28 | 336 | Espinach Ros <i>et al.</i> (1984b) |
| <i>P. marggravii</i> * | 27.5-29.5 | 371 | Fontenele (1953) |
| <i>P. nigricans</i> | 26.5 | 477 | Eckman (1980) |
| <i>P. marggravii</i> | 24 | 456 | Present work |

*This species was mentioned by the author as *P. argenteus*.

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