Hormonal induction of the reproduction in jaú (*Zungaro jahu*): case report

Carp pituitary, induced reproduction, migratory fish.

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ABSTRACT

This case report divulges the results of two studies conducted to evaluate the reproduction induction in males and females of jaú (*Zungaro jahu*) submitted to different concentrations and number of doses of crude extract of carp pituitary (CECP). The study I was conducted during the reproductive period between the years of 2006 and 2007, whereas the study II was carried out between 2007 and 2008. In the study I, the males (number of individuals, N = 3) were submitted to two doses of CECP (0.50 and 5.00 mg/kg of body weight – BW) while the females (N = 3) received three doses (0.25, 0.50 and 5.00 mg/kg of BW). In the study II, the male (N = 1) was submitted to four doses of CECP (0.30, 0.30, 0,30 and 6.00 mg/kg of BW) while the female (N = 1) received three doses (0.30, 0.60 and 6.00 mg/kg of BW). In both studies were evaluated the parameters: weight of the spawning, number of eggs per gram of spawning, individual and relative fecundity, gonadosomatic index for the females; and spermiation volume and sperm concentration for the males. The fertilization rate also was determined. For all evaluated parameters, the doses of CEPC used in the study II resulted in the better hormonal induction of the reproduction in *Zungaro jahu*.

Keywords: Bird, complex, enzyme, phytate, phosphorus, nutrition.

INDUÇÃO HORMONAL DA REPRODUÇÃO EM JAÚ (*ZUNGARO JAHU*): RELATO DE CASO

RESUMO

Este relato de caso divulga os resultados de dois estudos conduzidos para avaliar a indução da reprodução em machos e fêmeas de jaú (*Zungaro jahu*) submetidos à diferentes concentrações e número de doses de extrato bruto de hipófise de carpa (EBHC). O estudo I foi conduzido durante o período reprodutivo entre os anos de 2006 e 2007, enquanto que o estudo II foi realizado entre 2007 e 2008. No estudo I, os machos (número de indivíduos, N = 3) foram submetidos a duas doses de EBHC (0,50 e 5,00 mg/kg de peso corporal - PC) enquanto as fêmeas (N = 3) receberam três doses (0,25; 0,50 e 5,00 mg/kg de PC). No estudo II, o macho (N = 1) foi submetido a quatro doses de EBHC (0,30; 0,30; 0,30 e 6,00 mg/kg de PC), enquanto a fêmea (N = 1) recebeu três doses (0,30; 0,60 e 6,00 mg/kg de PC). Em ambos os estudos foram avaliados os parâmetros: peso corporal na desova, número de ovos por grama durante a desova, fecundidade individual e relativa, índice gonadossomático paras as fêmeas e volume de espermiação e concentração de esperma para os machos. A taxa de fertilização também foi determinada. Para todos os parâmetros avaliados, as doses de EBHC utilizadas no estudo II resultaram na melhor indução hormonal da reprodução em *Zungaro jahu*.

Palavras-chave: hipófise de carpa, reprodução induzida, peixe migratório.

INTRODUCTION

The high number of species in the aquaculture involves a high plasticity of reproductive traits, which means that an induction technique successful in a particular species may have different effects in another. Thus, so that we can properly handle the reproduction of the specie to be propagated it is necessary to know their reproductive physiology, age and place of spawning, and physicochemical characteristics of the environment (ANDRADE et al., 2015).

The jaú (*Zungaro jahu*) is a Neotropical freshwater fish that belongs to the Siluriformes order and Pimelodidae family. It is considered a large fish reaching almost 1.5 m of length and weighing over of 100 kg (AGOSTINHO et al., 2003; MATEUS & PENHA, 2007). It is a typical migratory species that moves principally along of the rivers Paraná, Paraguay and Uruguay (AGOSTINHO et al., 2003; RESENDE, 2003; ZANIBONI FILHO & WEINGARTNER, 2007). However, the population of this species has suffered a decline due to factors that interfere in its migration behavior, such as the deforestation of marginal rivers, pollution, overfishing and construction of hydroelectric dams (AGOSTINHO et al., 2003; MASCARENHAS-ALVES et al., 2007). As a consequence, it is officially listed as a threatened species in the Brazilian States of Minas Gerais (GODINHO, 1998) and Paraná (MIKICH & BÉRNILS, 2004).

Like many migratory species that do not reproduce naturally in captivity (ANDRADE & YASUI, 2003), the Z. jahu needs to be induced to reproduce using hormonal applications (DRUMOND, 2008). Reproduction of this species in captivity is difficult and is necessary more scientific work in this area because the production of fry is important for restocking in addition to collaborate for viability of their use in the animal production. The spawning induction by different hormone therapies has been widely studied. However, the administration of extract of carp pituitary is still the most frequently used being that this extract provides gonadotropin for the reproduction induction. Nevertheless, is necessary establishes the concentrations and number of doses required for reproduction induction (LEGENDRE et al. 1996). Therefore, this case report divulges two studies conducted to evaluate the reproduction induction in males and females of jaú (*Zungaro jahu*) submitted to different concentrations and number of doses of crude extract of carp pituitary.

MATERIAL AND METHODS

Two studies were conducted with *Zungaro jahu* at different times and places, being evaluated the use of different concentrations and number of doses of crude extract of carp pituitary (CECP). The study I was conducted at the Fish Culture Station Jupiá located between the cities of Ilha Solteira (São Paulo, Brazil) and Três Lagoas (Mato Grosso do Sul, Brazil) during the reproductive period between the years of 2006 and 2007. The study II was carried out at the Fish Culture Station of Volta Grande located in the city of Conceição das Alagoas (Minas Gerais, Brazil) during the reproductive period between the years of 2007 and 2008. The use of specimens from two different locations was in order to increase the fish sampling number.

For both studies, the fishes were selected according to their secondary sexual characteristics, such as a swollen abdomen for the females and the redness of the urogenital papilla for both sexes. To ensure preparation for reproduction, gonadal maturation was accompanied fortnightly through ovarian biopsy and visualization of germinal vesicle migration for females and spermiation by celomatic compression for males. After the selection they were sexed and transferred to tanks of masonry and glass with capacity of 1500 L, where they were kept for the hormonal induction of spawning and subsequent reproduction. The CECP doses were administered by intraperitoneal injection at the base of the dorsal fin.

In the study I, three males were submitted each one to two doses of CECP (0.50 and 5.00 mg/kg of body weight – BW) while three females received each one three doses of CECP (0.25, 0.50 and 5.00 mg/kg of BW). These induction protocols were tested in this study because they were successfully used to induce the reproduction in other species of native Siluriformes. For the females, the time intervals between the first and second dose of CECP and between the second and third were, respectively, of 24 and 12 hours. On the other hand, for the males, the time interval between the first and second dose was of 12 hours with the first dose occurring of synchronized manner

in the same time that the second dose was realized for the females.

Already in the study II, one male was submitted to four doses of CECP (0.30, 0.30, 0,30 and 6.00 mg/kg of BW) while one female received three doses (0.30, 0.60 and 6.00 mg/kg of BW). For the male, the CECP doses of number one, two and three were given with an interval of 24 hours each while a fourth dose was administered 12 hours after the third. The first dose of CECP was administered for the female in the same time that the male received the second dose. This assured that the administration of the last dose was synchronized for both sexes.

In both studies, after the extrusion of the gametes, the following parameters were evaluated for females: weight of the spawning, number of eggs per gram of spawning, individual fecundity (number of eggs spawned/female), relative fecundity (number of eggs spawned/body weight), gonadosomatic index (weight of spawning x 100/body weight).

Already for the males the spermiation volume and sperm concentration were determined. When none male showed positive reproduction results by hormonal induction, he was sacrificed by cerebral concussion and his testicles were removed to obtain sperm via their maceration. For the determination of the sperm concentration, the semen was diluted in a solution of formaldehyde citrate (2.9 g of sodium citrate, 4 ml of formaldehyde 35% and distilled water q.s.p. 100 mL). Then, the number of spermatozoa was counted in a Neubauer chamber. Thus, the sperm concentration was calculated using the equation:

$$SC = N \times CF,$$

where, SC is the sperm concentration (spermatozoa/mm^3), N is the number of cells counted in a Neubauer chamber and CF is a correction factor given by:

$$CF = (q \times df) / d,$$

where: q is 5 because represents the ratio between the total number of small squares of the Neubauer chamber (25) and the number of small squares counted (5), df is the dilution factor of the semen (= 104) and d corresponding to depth from the coverslip to the bottom of the Neubauer chamber (= 0.1 mm).

Posteriorly, there was the procedure of "dry" fertilization and the newly hydrated eggs were transferred to funnel-type incubators with constant aeration (Figure 1).

To test the effectiveness of the reproduction induction, three samples from each incubator were collected in order to calculate the fertility rate (FR), given by the following equation: FR = [E /(E + i)] x 100, where "E" is the number of viable embryos and "i" is the number of unviable eggs.

RESULTS AND DISCUSSION

Approximately 67% of the females of Zungaro jahu induced by crude extract of carp pituitary (CECP) in the study I spawned (females 1 and 2). The single female induced in the study II also spawned (named now as female 3). The spawning rate observed in the study II of the present work was higher than the values related in the literature for several other Siluriformes species: 58% for *Pseudoplatystoma corruscans*, 60% for *Conorhynchos conirostris*, 70% for *Franciscodoras marmoratus* and for *Pimelodus maculatus*, 71% for *Ramdia sapo*, 75% for *Lophiosilurus alexandri* and for *Pseudopimelodus charus*, 80% for *Ramdia quelen* and 82% for *Rhinelepis aspera* (ESPINACH ROS et al., 1984; SAMPAIO & SATO, 2006; SANTOS et al., 2013).

The reproductive parameters of the females submitted to different doses of CECP are shown in the Table

FIGURE 1. Funnel-type incubators for fertility evaluation.

1. It is possible verify that the hormonal induction evaluated in the study II provides the better results. For example, there was an improvement of up to 1700% for the parameter relative fecundity (ranged from 2.95 to 52.27).

The number of oocytes/g determined in this study was higher than for Clarias gariempinus and Pseudoplatystoma corruscans (CARDOSO et al., 1995; LEGENDRE et al., 1992) being a portion of this variation due to differences commonly observed between different species of fish because according to Legendre et al. (1996), the size and number of oocytes produced in a spawning is highly variable among Siluriformes species.

Analyzing the results of the study I is possible verify that the weight of the spawning was not dependent of the initial weight of the female. In addition, the weight of the female does not influence the number of oocytes/g of spawning because the results for this parameter were similar between the two females evaluated during the same reproduction period (683 versus 657). Already the results of weight of the spawning and, consequently, of the gonadosomatic index varied greatly between the study I and II corroborating with the results of Sato et al. (2003), that also demonstrated influence of the hormonal dose about the reproduction induction.

None male in the study I responded to the treatment of induction of the reproduction by CECP. Thus, one male was sacrificed to obtain the intratesticular semen. Already in the study II, the single evaluated male (named now as male 2) showed spontaneous spermiation during the procedure of abdominal massage. Therefore, the results indicates that application of low daily doses and a last dose higher, synchronized with the application of the last dose in females, triggers internal processes in the male that allow spermiation upon abdominal massage. According to Woynarovich & Horváth (1983) the use of parceled doses produces better reproduction results than a single dose of hormonal inductor. The results of spermiation volume and sperm concentration are shown in Table 2.

In males, hormonal treatments are not used to stimulate maturation of the gametes, but rather they are used to increase the fluidity and seminal volume (VERMEIRSSEN et al. 2004). In the present case report was observed that the increase in the spermiation volume and sperm concentration was probably caused by increase of the number of CECP doses and/or type of semen obtainment (intratesticular semen or from spermiation spontaneous by abdominal massage).

The fertilization rate varied from 5% to 90% (Table 3). There were large numerical differences between incubators fertilized with intratesticular sperm (study I) and ejaculated semen (study II). This probably occurred because the quality and quantity spermatic is usually impaired when the spermiation is not spontaneous by abdominal massage (LEGENDRE, 1992), and is important consider that the spermatic quality and quantity affects the success of the fertilization process, the hatching rate and also the production rate of healthy larvae (RURANGWA et al., 2004).

TABLE 1. Reproductive parameters of females of jaú (*Zungaro jahu*) submitted to different doses of crude extract of carp pituitary

	Doses of crude extract of carp pituitary (mg/kg of body weight)		
	0.25, 0.50 and 5.00 (Study I)		**0.30, 0.60 and 6.00 (Study II)**
Parameter	**Female 1**	**Female 2**	**Female 3**
Initial weight (g)	13,400	15,600	16,500
Weight of the spawning (g)	260	70	997
Number of oocytes/g of spawning	683	657	865
Individual fecundity[1]	177,580	45,990	862,405
Relative fecundity[2]	13.25	2.95	52.27
Gonadosomatic index[3]	1.94	0.45	6.04

[1] Number of eggs spawned/female. [2] Number of eggs spawned/body weight. [3] Weight of the spawning x 100/body weight.

TABLE 2. Reproductive parameters of males of jaú (*Zungaro jahu*) submitted to different doses of crude extract of carp pituitary

	Doses of crude extract of carp pituitary (mg/kg of body weight)	
	0.50 and 5.00 (Study I)	0.30, 0.30, 0,30 and 6.00 (Study II)
Parameter	**Male 1***	**Male 2**
Initial weight (g)	13,400	16,500
Spermiation volume (mL)	07	15
Sperm concentration (sptz x 109)	08	12

*Intratesticular semen.

TABLE 3. Fertilization rate (%) for females of jaú (*Zungaro jahu*) for each reproductive period evaluated.

	Reproductive period	
	Between 2006 and 2007 (Study I)	Between 2007 and 2008 (Study II)
Female 1 × Male 1	5	-
Female 2 × Male 1	15	-
Female 3 × Male 2	-	90

Considering only the study II, the fertilization rate obtained was higher than related for other species of Siluriformes (range from 59 to 79%) (ESPINACH ROS et al., 1984; SAMPAIO & SATO, 2006, SANTOS et al., 2013).

CONCLUSION

In this case report, the concentrations and dose numbers of crude extract of carp pituitary evaluated in the study II resulted in the better hormonal induction of the reproduction in females and males of jaú (Zungaro jahu).

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