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Induced spawning and reproductive variables of the catfish *Lophiosilurus alexandri* Steindachner, 1876 (Siluriformes: Pseudopimelodidae)

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Lophiosilurus alexandri is an endemic fish from the São Francisco River basin, Brazil. The aim of this study was to induce *L. alexandri* to spawn and to obtain data on several reproductive variables for this species. For induced spawning, adults were submitted to *Cyprinus carpio* pituitary homogenate (CPH). Nine of the 12 females (75%) responded positively to the treatment. The stripping of oocytes was performed 8.4 h after the second dose of CPH with the water temperature maintained at 26ºC. The number of stripped oocytes per gram of ova was 74 ± 5 oocytes g^{-1}, and the mean oocyte diameter was 3.1 ± 0.2 and 3.6 ± 0.2 mm, before and after hydration, respectively. The oocytes were opaque, yellowish, demersal, highly adhesive, and covered by a gelatinous coat. The total fecundity was 4,534 ± 671 oocytes, and the fertilization rate was 59%. The initial and final fertilities were 2,631 ± 740 and 1,542 ± 416 embryos, respectively. Larval hatching occurred up to 56 h after fertilization, and the larvae had a total length of 8.4 ± 0.1 mm. This work provides important biological information for *L. alexandri* that can be used for management and conservation of this species.

Lophiosilurus alexandri é um peixe endêmico da bacia do rio São Francisco, Brasil. O objetivo do trabalho foi induzir *L. alexandri* à desova e obter dados sobre várias variáveis reprodutivas para esta espécie. Para desova induzida, adultos foram submetidos ao homogeneizado de hipófise de *Cyprinus carpio* (HHC). Nove das 12 fêmeas (75%) responderam positivamente ao tratamento. A extrusão dos ovócitos aconteceu 8,4 h após a segunda dose de HHC com a temperatura da água mantida a 26ºC. O número de ovócitos liberados por grama de ova foi de 74 ± 5 ovócitos g^{-1} e a média do diâmetro ovocitário foi de 3,1 ± 0,2 e 3,6 ± 0,2 mm, antes e depois da hidratação, respectivamente. Os ovócitos foram opacos, amarelo-castanho, demersais, altamente adesivos e revestidos por capa gelatinosa. A fecundidade total apresentou 4.534 ± 671 ovócitos e a taxa de fertilização foi de 59%. As fertilidades inicial e final foram de 2.631 ± 740 e 1.542 ± 416 embriões, respectivamente. A eclosão das larvas aconteceu até 56 h após a fertilização e as larvas tiveram comprimento total de 8,4 ± 0,1 mm. Este trabalho fornece informações biológicas importantes para *L. alexandri*, que podem ser utilizadas para o manejo e conservação desta espécie.

Key words: Artificial reproduction, Fecundity, Fertility, Hypophysation assay, Neotropical catfish.

Introduction

The Siluriformes constitutes a teleost group of 37 families with 3569 species described (Eschemeyer & Fong, 2013) The majority of the species from this order occur mainly in tropical regions such as South America, Africa, and Southeast Asia (de Pinna, 1998). Pseudopimelodidae is a family of freshwater Neotropical catfish representing 37 species that are restricted to South America (Eschemeyer & Fong, 2013). *Lophiosilurus alexandri* Steindachner, 1876, is popularly known as "pacamã" and is a member of the Pseudopimelodidae that is endemic to the São Francisco River basin. It is a sedentary and piscivorous fish that preferentially inhabits lentic environments, with the males exhibiting parental care. *Lophiosilurus alexandri* reaches gonadal maturation from October to February, coinciding

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with the local rainy season (Sato *et al.*, 2003a, 2003b). This fish is very important in sport and craft fishing because it reaches up to 8 kg in body weight (Sato *et al.*, 2003a). Moreover, it has economic potential in aquaculture due to its fillet quality, market price, and international use in ornamental aquaria (Santos & Luz, 2009).

Natural fish populations have declined over the last several decades because of environmental degradation and overfishing. This has resulted in an increased effort to develop techniques for hatchery production of fish. The hypophysation has been used for induction and synchronization of ovulation and spawning in several species of fish in captivity (Woynarovich & Horvat, 1980; Sato *et al*., 1999; Sato *et al.*, 2003a; Sampaio & Sato, 2006; Nogueira *et al.*, 2012; Santos *et al*., 2013). Moreover, data on reproductive variables, such as fecundity and fertility, can be obtained from induced spawning; such variables are needed for species conservation and efficient production of embryos, larvae, and fingerlings.

The number of oocytes eventually released by an individual during its breeding period is considered total fecundity. The relative fecundity, calculated in relation to body weight or length, is indicative of the reproductive capacity (Arantes *et al.*, 2011). Therefore, fecundity studies are important for the successful management and conservation of fish stocks (Shatunovskiy, 1988; Arantes *et al.*, 2011). Another biologically important variable is fertility, which is of practical importance in hatcheries. Initial fertility indicates the number of stripped eggs, while final fertility is the number of viable embryonic eggs estimated after blastopore closure (Sato *et al.*, 2000; Sato *et al.*, 2003a, 2003b; Sampaio & Sato, 2006). Knowledge of the fecundity and fertility of a particular species is an important tool that can be applied in aquaculture and fish farming.

Because biological information about artificial reproduction and other reproductive aspects of *L. alexandri* is limited, the goal of this work is to induce spawning by hypophysation and to describe the reproductive variables of *L. alexandri*. This will provide essential data that can be subsequently applied to the management, conservation, and aquaculture of this species.

Material and Methods

Breeding management. All experiments were performed at the Hydrobiology and Hatchery Station of Três Marias (18°11'58"S 45°15'07"W), Minas Gerais, Brazil, in accordance with the Guidelines for Animal Experimentation established by the Brazilian College for Animal Experimentation (COBEA). Adult specimens of *L. alexandri* were captured in the São Francisco River and kept for at least one year in a 600-m^2 pond at a stocking rate of 1 kg of fish per 5 m^2. The experiment was performed in January 1997, and the animals were fed, *ad libitum*, live fish such as tilapia *Oreochromis niloticus* and curimatãs *Prochilodus argenteus*. A voucher specimen was deposited in the Museu de Zoologia of the Universidade de São Paulo (MZUSP 105894).

Breeding selection for induced reproduction was performed using secondary sex characters of females and males observed in reproduction. Then, animals selected were transferred to treatment tanks (3 x 1 x 0.8 m). The tanks were supplied with running water having the following physical characteristics that were maintained throughout the experiment: temperature = 26°C, dissolved oxygen = 5.9-6.2 mg L^{-1}, conductivity = 58-65 µS cm^{-1}, and pH = 6.8-7.2. The rate of water flow in the tanks was approximately 15 L min^{-1}.

Induced spawning. For the hypophysation experiment, twenty-four specimens (males and females) of *L. alexandri* were induced to spawn with crude extract of *Cyprinus carpio* pituitary. The carp pituitary homogenate (CPH) was prepared by macerating dried pituitaries (Agrober, Budapest, Hungary) in glycerin followed by homogenization in physiological saline (0.7% NaCl). The females that presented bulging of the coelomic cavity and urogenital papilla reddish received two intraperitoneal injections of CPH (0.8 ± 0.2 and 6.0 ± 0.6 mg kg^{-1} body weight) with a 14 h interval between injections. The males received a single dose containing 2.4 ± 0.2 mg kg^{-1} body weight of the same CPH at the time that the second dose was given to the females (Woynarovich & Horvat, 1980; Sato *et al.*, 2003a). The water temperature of the tank was measured every hour after the second dose of hormone to calculate the degree-hours (average water temperature x number of hours until spawning) and to estimate the time of spawning in *L. alexandri*.

Fecundity, fertilization rate, fertility, and hatching. For the purpose of estimating the total fecundity, samples of spawned oocytes were collected and weighed. Total fecundity was determined considering the total number of oocytes (stripped oocytes plus released oocytes and oocytes retained in the ovaries). The number of oocytes per gram of ova was obtained by counting fresh oocytes present in approximately 2 g of ova samples. Additionally, the stripped ova index was calculated using the following formula (SOI = 100 x stripped ova weight x BW^{-1} where BW = body weight). After stripping, the animals were killed by transversal section of the spinal cord, and for each female, the gonadosomatic index was also calculated (GSI = 100 x GW x BW^{-1}, where GW = weight of free oocytes released + weight of ovaries after extruded).

The oocytes and sperm were obtained by stripping, and dry fertilization was performed (Sato *et al*., 2003a). After fertilization, 20 g of fertilized eggs of each female was rinsed with water to allow hydration, and then the embryos were transferred to funnel-type fiberglass incubators with 20 L capacity (Woynarovich & Horvat, 1980). The water supplying the incubators had the following

physicochemical characteristics: temperature = 23.0-25.0°C, dissolved oxygen = 5.9-6.2 mg L^{-1}, pH = 6.8-7.2 and conductivity = 58-65 µS cm^{-1}. For each female that responded to induced spawning, the fertilization rate of the eggs (*i.e.*, relative number of viable and dead embryos) was calculated from a sample of 200 eggs after blastopore closure, which were collected from the middle section of the incubator. For each female that responded to induced spawning, the initial fertility (the number of stripped eggs) and the final fertility (the number of viable embryonic eggs counted after blastopore closure, Sato *et al.*, 2000) were determined using 200 eggs, which were collected from the middle section of the incubator with a glass tube. Finally, a linear relationship of total fecundity, initial and final fertility in relation to body weight or total length was determined for *L. alexandri*.

The number of degree-hours for hatching was calculated using the average incubator water temperature (recorded every hour) multiplied by the time period spanning the moment the hydrated eggs were dropped into the incubator until hatching.

Morphology and morphometry. The morphology of the ovaries and testes were recorded for *L. alexandri*. After spawning, the animals were killed, and the gonads were removed. For histology, ovary and testis samples were fixed in Bouin's solution for 6-8 h at room temperature. The specimens were embedded in paraffin, sectioned at a thickness of 3-5 µm, and stained with hematoxylin-eosin (Michalany, 1980).

Twenty eggs for each female (non-hydrated and hydrated) were used to measure the vitelline sac diameter, perivitellinic space width, and chorion thickness using an ocular micrometer coupled to an Olympus stereomicroscope. Egg pigment and the presence or absence of a gelatinous coat were also described. The total length (mm) of newly hatched larvae was measured using 180 specimens (*i.e.*, 20 larvae from each incubator). The presence or absence of a larval adhesive organ was also determined using a stereomicroscope, and the initial larval displacement in the water column was also recorded.

Statistical analyses. Descriptive statistics and linear regression for biological variables such as total fecundity, initial and final fertility, total length, and body weight were performed using GraphPad InStat (Software Inc., Version 3.05, San Diego, CA, USA), and the values were expressed as the means ± standard deviation (SD).

Results

The females did not differ in size from males, but when abdominal pressure was applied, the females released oocytes whereas for the males, semen was obtained with difficulty. Ten males (Table 1) responded positively to hypophysation (*i.e.*, approximately 83%); they were easily handled, and secondary sex characters such as emission sounds were not observed while they were kept in the experimental tanks. Nine females (Table 1) responded positively to hypophysation (*i.e.*, 75%).

Table 1. Means, standard deviation and range of the total length (TL), body weight (BW) and animals number (N) of *Lophiosilurus alexandri* that responded to induced spawning.

	Females (N = 9)		Males (N = 10)	
	Mean ± SD	Range	Mean ± SD	Range
TL (cm)	62.7 ± 2.4	57.0 - 70.0	61.9 ± 4.0	56.0 - 67.0
BW (kg)	3.2 ± 0.9	2.2 - 4.8	2.7 ± 0.5	2.1 - 3.8

Reproductive variables. Data on biological variables such as the mean number of oocytes per gram of ova, the stripped ova index, the gonadosomatic index, fertilization rate, total fecundity, and initial and final fertility after induced spawning in *L. alexandri* are listed in Table 2. Ovulation occurred approximately 8.1-8.7 h after the second dose of CPH at 26°C, corresponding to 210-225 degree-hours (218 ± 6). The linear relationship showed that total fecundity, initial fertility, and final fertility increase proportionally with body weight and total length in *L. alexandri* (Fig. 1).

Egg biology. The eggs of *L. alexandri* were opaque, light yellow, demersal, adhesive and covered with a gelatinous coat. After fresh stripping, the non-hydrated oocyte diameter was approximately 3.0 mm and increased to 3.5 mm after hydration. Other findings about the egg biology of *L. alexandri* such as perivitellinic space width, chorion thickness and yolk sac diameter are summarized in Table 3.

Gonad morphology. Macroscopically, *L. alexandri* ovaries and testes were paired organs, and the ovarian ducts opened into the urogenital papilla. The mature ovaries were bulky, highly vascularized, and yellowish and had large vitellogenic oocytes that were observable macroscopically

Table 2. Means of the oocytes number per gram of ova, stripped ova index, gonadosomatic index, fertilization rate, total fecundity, and initial and final fertility after induced spawning in females of *Lophiosilurus alexandri* (N = 9).

Variables	Mean ± SD	Range
Oocytes number per gram of ova	74 ± 5	68 - 81
Stripped ova index (%)	1.2 ± 0.2	0.9 - 1.4
Gonadosomatic index (%)	2.0 ± 0.4	1.6 - 2.9
Egg fertilization rate (%)	59 ± 5	51.8 - 68.4
Total fecundity	4,534 ± 671	3,519 - 5,467
Initial fertility	2,631 ± 740	1,501 - 3,920
Final fertility	1,542 ± 416	898 - 2241

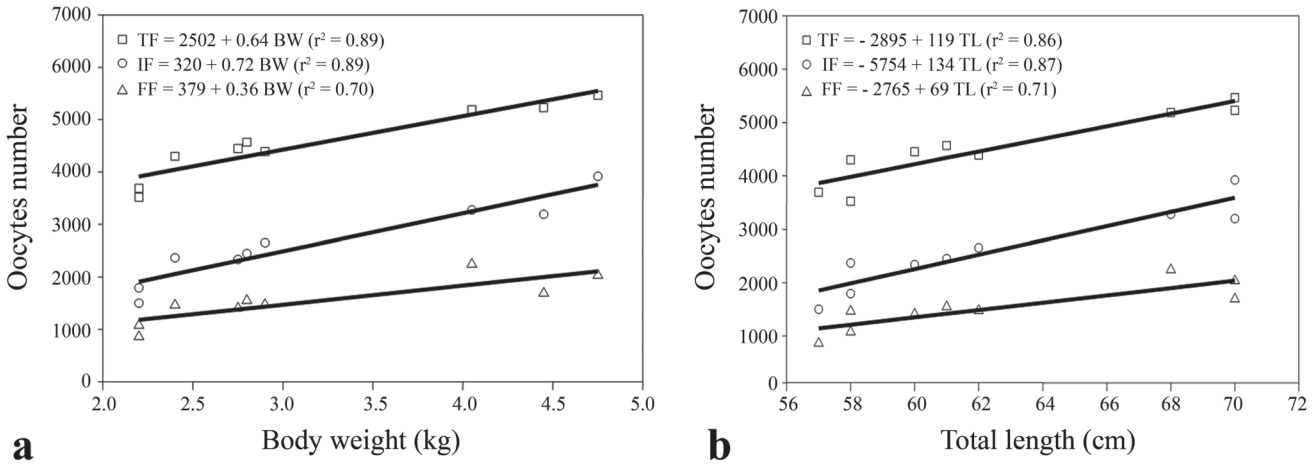

Fig. 1. Linear relationships of total fecundity (TF), initial fertility (IF) and final fertility (FF) to variations in body weight (BW) (**a**) and total length (TL) (**b**), obtained simultaneously from nine *Lophiosilurus alexandri* females submitted to hypophysation at Três Marias Hydrobiology and Hatchery Station in January 1997.

(Fig. 2a). Microscopically, the mature ovaries mainly exhibited vitellogenic oocytes, which contained acidophilic yolk globules that were widespread throughout the ooplasm (Fig. 2b). Mature testes were whitish, voluminous, turgid, and highly vascularized and had well developed fringes (Fig. 2c). The histological analyses of the mature fringed testis showed seminiferous tubules filled with sperm (Fig. 2d).

The larvae of *Lophiosilurus alexandri*. Hatching time ranged from 54.8-58.0 h at 24°C, corresponding to 1,260-1,450 degree-hours (1,358 ± 60). The larvae of *L. alexandri* had no adhesive organs and an absence of vertical movements in the water column. The newly hatched larvae had an average total length of 8.4 ± 0.1 mm.

Table 3. Means of the non-hydrated and hydrated oocytes diameter, yolk sac diameter, perivitellinic space width and chorion thickness after induced spawning in females of *Lophiosilurus alexandri* (N = 180).

Egg measurements	Mean ± SD	Range
Non-hydrated oocytes diameter	3.1 ± 0.2 mm	2.8 - 3.3 mm
Hydrated oocytes diameter	3.6 ± 0.2 mm	3.3 - 3.8 mm
Yolk sac diameter	2.7 ± 0.1 mm	2.4 - 2.9 mm
Perivitellinic space width	0.2 ± 0.02 mm	0.1 - 0.3 mm
Chorion thickness	0.3 ± 0.01 mm	0.25 - 0.29 mm

Discussion

This manuscript provides important biological data regarding artificial reproduction and reproductive variables of pacamã *L. alexandri*, an endemic fish from the São Francisco River basin with great economic potential for aquaculture and for restocking operations. Essential biological information about wild populations, such as reproductive cycles, fecundity, and fertility, are needed to manage native and endangered species and for aquaculture purposes (Sato *et al.*, 1998; Sato *et al.*, 2003a; Caneppele *et al.*, 2009; Arantes *et al.*, 2011). Although *L. alexandri* is potentially useful in aquaculture and important in sport and craft fishing, only a few studies about its reproductive biology have been published, and they describe the reproductive apparatus, gametogenesis, and adhesive eggs (Rizzo *et al.*, 2002; Barros *et al.*, 2007). In the present study, *L. alexandri* responded well to treatment with CPH; the rate of positive responses in females submitted to hypophysation (75%) was high compared to other Siluriformes species: 58.3% in *Pseudoplatystoma corruscans* (Sato *et al.*, 2003a) and 67% in *Trachelyopterus galeatus* (Santos *et al.*, 2013), or was similar to 70.4% in *Pimelodus maculatus* (Sato *et al.*, 1999), 71% in *Rhamdia sapo* (= *Rhamdia quelen*) (Espinach Ros *et al.*, 1984), 75% in *Pseudopimelodus charus*, and 80% in *R. quelen* (Sampaio & Sato, 2006). Induced spawning by hypophysation has been used with success in several migratory and sedentary fish from the São Francisco River basin (Sato *et al.*, 2003a). These studies of artificial reproduction are important for gaining knowledge of reproductive variables, embryogenesis, and larval and fingerling

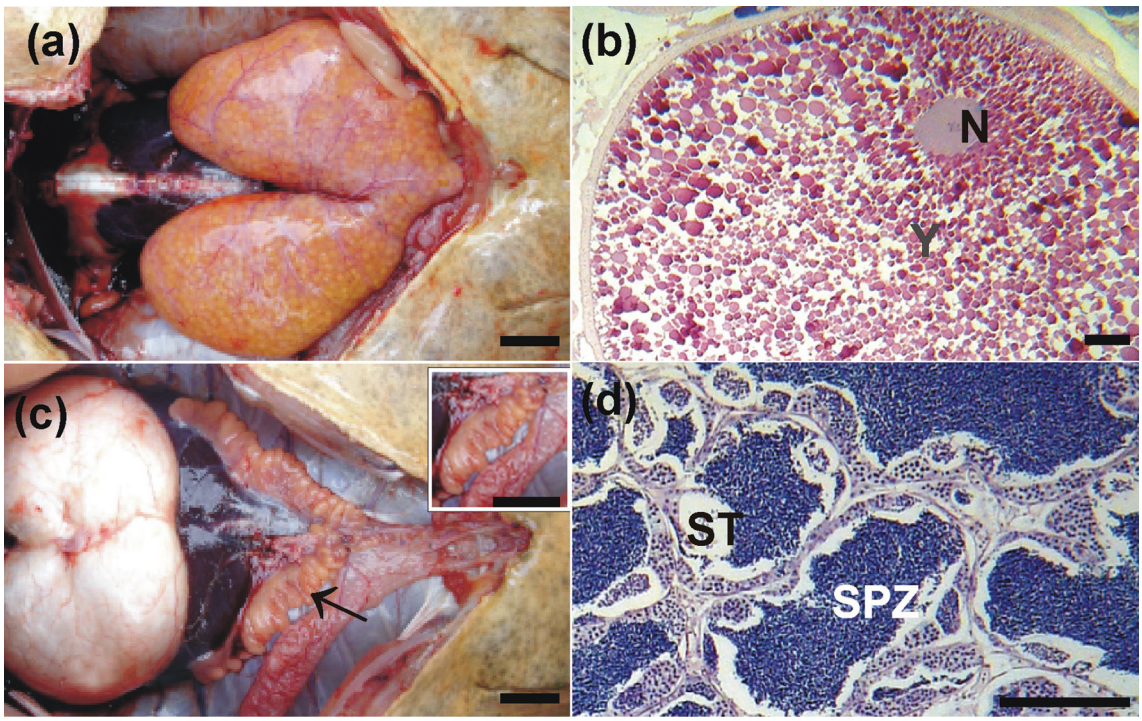

Fig. 2. Macro and microscopic morphology of the ovaries (**a-b**) and testis (**c-d**) mature of the *Lophiosilurus alexandri*. (**a**) The mature ovaries were bulky, highly vascularized, yellowish and presenting large vitellogenic oocytes. (**b**) Histological section of the vitellogenic oocyte characterized by the presence of acidophilic yolk globules (Y) throughout the ooplasm and nucleus (N) migrating toward the animal pole. (**c**) Mature testis (arrow) are whitish, turgids, vascularized and with well developed fringes (Insert showing fringes). (**d**) Mature testis showing seminiferous tubules (ST) filled by spermatozoa (SPZ). (a, c and insert) bars 1cm; (b and d) stained with hemotoxylin-eosin and bars 100 µm.

development of these species in aquaculture at the Três Marias hatcheries, Minas Gerais, Brazil (Sato *et al.*, 2003a).

The relationship between temperature and time can be expressed in degree-hours that is used in several fields, including agriculture and meteorology, for to predict temperature dependent processes. In fish farming, the concept of degree-hours is a valuable tool for estimating the time of ovulation and hatching because temperature is an important abiotic factor that influences the speed of physiological processes of organisms. The stripping of the oocytes from *L. alexandri* was performed at an average of 218 degree-hours (approximately 8.4 h) after the second dose of CPH at 26°C; these results were similar to those obtained in other Siluriformes submitted to the same water temperature: 212 degree-hours in *Rhinelepis aspera* (Sato *et al.*, 1998), 213 in *Pimelodus maculatus* (Sato *et al.*, 1999), and 226 in *Pseudoplatystoma corruscans* (Sato *et al.*, 2003a). Hatching in *L. alexandri* was on average 1358 degree-hours (approximately 56.6 h) after egg fertilization at 24°C, which is a relatively long time but is similar to that of other Siluriformes that have adhesive and large eggs: 1099 degree-hours (approximately 45.8 h) in *Franciscodoras marmoratus* and 1022 degree-hours (approximately 42.6 h) in *Rhinelepis aspera* (Sato *et al.*, 2003a). However, in Siluriformes with free eggs, the hatching period is shorter: 27 h at 23°C in *Rhamdia hilarii* (= *R. quelen*) (Godinho *et al.*, 1978); 30-45 h at 22-24 °C in *R. sapo* (= *R. quelen*) (Cussac *et al.*, 1985); 30.5 h at 24ºC in *R. quelen* (Riehl & Patzner, 1998); 16.2 h at 24.2ºC in *Pimelodus maculatus* (Sato *et al.*, 1999); and 20 h at 24.4ºC in *Pseudoplatystoma corruscans* (Sato *et al.*, 2003a).

Lophiosilurus alexandri had spherical, opaque, demersal, yellowish eggs that had a gelatinous coat and great adhesiveness, which are properties that have also been reported in several Siluriformes species (Godinho *et al.*, 1978; Espinach Ros *et al.*, 1984; Sato *et al.*, 2003b; Santos *et al.*, 2013). The presence of a gelatinous coat appears to be a common feature in Siluriformes eggs (Pereira *et al.*, 2006). The gelatinous coat provides the eggs the ability to bind to submerged vegetation or objects as well as other eggs (Riehl & Patzner, 1998). However, there are eggs that have a gelatinous coat without adhesiveness as described

for *Pseudoplatystoma corruscans*, *Pseudopimelodus charus*, *R. quelen*, and *Pimelodus maculatus* (Sato *et al.*, 2003a). Non-hydrated eggs of *L. alexandri* measured on average 3.1 mm and after hydration this increased to 3.6 mm, an increase of approximately 16%, which can be considered small compared to eggs from other Siluriformes species (Sato *et al.*, 2003b) and high compared to the egg diameter after hydration in the reared catfish *Zungaro jahu* (Nogueira *et al*., 2012).

The gonad morphology of *L. alexandri* was similar to those described for pacamã from the Santo Antônio River, Minas Gerais, Doce River basin (Barros *et al*., 2007). However, in the present study, histological ovary sections from the females that were induced to spawn had vitellogenic oocytes exhibiting nucleus migration toward the mycropilar apparatus in the region of the animal pole. This cellular event is a feature of final maturation, and it was also recorded in vitellogenic oocytes of *Prochilodus argenteus* submitted to CPH (Arantes *et al*., 2011). Morphometric data regarding the perivitellinic space width (approximately 190 µm), yolk sac diameter (approximately 2650 µm), and chorion thickness (approximately 270 µm) were obtained for *L. alexandri*. The chorion thickness in newly fertilized eggs of *L. alexandri* was relatively larger compared to other teleosts such as *Astyanax bimaculatus* and *Tetragonopterus chalceus* (Sato *et al.*, 2003a), and the value was close to that described for other catfish species such as *Pimelodus maculatus* (Sato *et al.*, 1999), *Pseudopimelodus charus*, and *R. quelen* (Sampaio & Sato, 2006). A very thick chorion, as observed in *L. alexandri*, may represent a defense mechanism for the embryo against environmental adversities and may contribute to higher survival rates because this species has a relatively low fecundity. The average diameter of the yolk sac in *L. alexandri* was high compared with other catfish species; for example, the diameter was 694 µm in *Pimelodus maculatus* (Sato *et al.*, 1999), 1058 µm in *Pseudopimelodus charus*, and 955 µm in *R. quelen* (Sampaio & Sato, 2006).

Regarding the number of oocytes in relation to the weight of the ova, *L. alexandri* had an average of 74 oocytes g^{-1}, a value which is much smaller than other Siluriformes including *Pseudoplatystoma corruscans* (Sato *et al.*, 2003a), *Pimelodus maculatus* (Sato *et al.*, 1999), *Z. jahu* (Nogueira *et al.*, 2012), *T. galeatus* (Santos *et al.*, 2013), *R. quelen*, and *Pseudopimelodus charus* (Sampaio & Sato, 2006). The low fecundity (*i.e.*, 324 oocytes per gram of ovulated ovary mass) was also described in another Siluriformes species that was induced to spawn: the endangered *Steindachneridion parahybae* from the Paraíba do Sul River, Brazil (Caneppele *et al.*, 2009). The average GSI for mature females of *L. alexandri* was 2%. In general, mature females of Siluriformes species exhibit GSI values between 7 and 20% (Sato *et al.*, 2003b; Godinho, 2007). The maximum reported SOI (stripped ova index) for *L. alexandri* was 1.4% with a mean value of 1.2%. Commonly, Siluriformes species have SOI ranging from 3 to 16% (Sato *et al.*, 1998; Sato *et al.*, 1999; Sampaio & Sato, 2006).

Fecundity is an important biological variable for aquaculture, species management, and conservation, and it estimates the reproductive potential of a fish species (Godinho, 2007; Arantes *et al.*, 2011). Migratory fish generally have high fecundities while sedentary fish release a much smaller number of eggs per spawning, which is compensated by spawning multiple times throughout the reproductive period (Godinho, 2007). The total fecundity of pacamã ranged from 3,519 to 5,467 oocytes, which was similar to the range recorded for *T. galeatus* (*i.e.*, 1,505 to 4,651 oocytes, Santos *et al*., 2013) but was much smaller than the fecundities measured in other Neotropical catfishes: 24,640-134,176 in *Pseudopimelodus charus*; 16,750-79,886 in *R. quelen* (Sampaio & Sato 2006); 80,120-205,206 in *Pimelodus maculatus* (Sato *et al.*, 1999); 21,813 in *F. marmoratus* (Sato *et al.*, 2003b); and 81,900-347,604 in *Rhinelepis aspera* (Sato *et al.*, 1998).

The fertilization rate (*i.e.*, the relative number of viable and dead embryos) is another important tool in fish farming and is important to the management and conservation of fish species (Sato *et al.*, 2003a; Godinho, 2007). *Lophiosilurus alexandri* had a 59% fertilization rate, which is similar to that obtained in other Siluriformes: 61.6% in *Rhamdia sapo* (= *R. quelen*) (Espinach Ros *et al.*, 1984), 70.4% in *Pseudoplatystoma corruscans* (Sato *et al.*, 2003a), 64.8% in *Pimelodus maculatus* (Sato *et al.*, 1999), 72.4% in *Rhinelepis aspera* (Sato *et al.*, 1998), 75% in *Rhamdia quelen* and *Pseudopimelodus charus* (Sampaio & Sato, 2006). However, *Z. jahu* submitted to CPH presented high fertilization rate (*i.e.*, 92%, Nogueira *et al.*, 2012). In *Pseudoplatystoma fasciatum*, fertilization rates of approximately 53% and 56% were described for the animals' first and second gonadal maturations submitted to CPH, respectively. In this work, the authors also reported that the fertilization rate in induced spawning using human chorionic gonadotropin (hCG) was significantly higher (*i.e.*, approximately 82%) than in animals treated with CPH in the second gonadal maturation (Leonardo *et al.*, 2004). The initial fertility rate of *L. alexandri* averaged 58%, and while the final fertility rate reached 34% in relation to total fecundity, these values were low compared to other Siluriformes species (Sato *et al.*, 1998; Sato *et al.*, 1999; Sampaio & Sato, 2006).

Regarding larval biometry, *L. alexandri* larvae measured on average 8.4 mm in total length, which is much higher than other Siluriformes larvae (4.0 mm in *Rhamdia hilarii* (= *R. quelen*); 2.6 mm in *Pimelodus maculatus*; 2.8-3.5 mm in *R. quelen*; and 3.0 mm in *Pseudopimelodus charus*) (Godinho *et al.*, 1978; Sato *et al.*, 2003b; Sampaio & Sato 2006). The cement gland, or adhesive organ, is found

on the dorsal and lateral head region of some fish larvae and is important to attach the larvae to a substrate during early development (Sato *et al*., 2003b; Gomes *et al.*, 2007). In this work, the larvae of *L. alexandri* did not present a larval adhesive organ; the absence of an adhesive organ is a common feature in the larvae of some Siluriformes species (Sato *et al.*, 2003b; Sampaio & Sato, 2006; Santos *et al.*, 2013).

In conclusion, the results obtained here for *L. alexandri* demonstrated the success of artificial reproduction for this species. Because there are few studies on the reproductive biology of *L. alexandri*, this work provides important biological information regarding induced spawning and reproductive variables that can be applied to aquaculture, management and conservation of this species.

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