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# Reproductive biology of the Neotropical catfish *Iheringichthys labrosus* (*Siluriformes: Pimelodidae*), with anatomical and morphometric analysis of gonadal tissues

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## ABSTRACT

The reproduction of *Iheringichthys labrosus* (Lütken, 1874) from the Turvo River, Brazil, was studied using anatomical, biometric, histological, and ultrastructural techniques. Between April 2014 and March 2015, a total of 278 males and 512 females were captured bimonthly. The testes of *Iheringichthys labrosus* are fringed and possess a cranial spermatogenic region and an exclusively secretory caudal region. Histologically, the cranial region is composed of seminiferous tubules with spermatogenesis being completed in cysts. The spermatozoa are of the primitive type with a spherical head and have a rudimentary intermediate piece and a long tail with an axonemic arrangement of 9 + 2. The caudal region does not form an individualized gland, and cells in this testis area have characteristics of protein secretion. A variable density electron-dense secretion accumulates in the cisternae of the rough endoplasmic reticulum in the lumen of the seminiferous tubules and in the testicular ducts during maturation. The cortical alveoli are discontinuous, and the zona pellucida consists of three layers crossed by pore canals, and the follicular cells are squamous in the early stages of oogenesis and cuboidal in advanced stages. The gonadosomatic index was associated with the maturation of the gonads while the condition factor indicated that the fish feed less and utilize adipose reserves during the reproductive period. Males and females reproductively functional throughout the year with spawning being partial or multiple, similar to that reported in studies of the species in lentic environments.

## 1. Introduction

Studies focusing on the reproductive dynamics of fish populations in natural environments are fundamental for understanding reproduction and are valuable for the development of conservation measures for fish species (Freitas et al., 2013). The stimuli from abiotic factors, such as temperature, rainfall, and photoperiod, can induce physiological processes resulting in the start of

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reproduction (Shiota et al., 2003; Dzyuba et al., 2019). The study of anatomical and cellular differences in the reproductive system of teleosts is fundamental for understanding the ecological and ethological cycles, as well as helping to establish phylogenetic relationships among related species (Uribe et al., 2016). Allied to this, biological indices such as the gonadosomatic index (GSI) and the Fulton condition factor (K) can provide important information related to reproduction. The gonadosomatic index is a good indicator of fish reproductive function and can also be applied when determining the stages of the reproductive cycle (De Vlaming et al., 1982). Furthermore, the K factor is important to understand the life cycle of fish, and it reflects the interaction between biotic and abiotic factors as well as being indicative of the physiological state of fish populations (Lemma et al., 2015).

The order Siluriformes, one of the most widely distributed and diversified groups of ostariophysan fish, is comprised of about 40 families and 3730 species. The siluriform family *Pimelodidae* has approximately 32 genera and 112 species widely distributed in rivers of South and Central America, the Caribbean islands, and southern Mexico (Nelson et al., 2016).

Some Siluriformes species possess fringed testes with spermatogenic lineage cells distributed throughout the testes (Lopes et al., 2004), while others have a caudal region with seminal vesicles or accessory structures (Santos et al., 2014b) that do not have spermatogenic functions but can store spermatozoa (Legendre et al., 1996). The caudal region of testes of other Siluriformes species have secretory functions without forming an individualized gland (Santos et al., 2001, 2010). Histologically, fish testes have tubular and interstitial compartments, and the tubular compartment is composed of germ and Sertoli cells supported on the basement membrane that defines the boundaries of the interstitium, which is composed of connective tissue and Leydig cells (Santos et al., 2014b).

The ovaries of Siluriformes are paired organs of the cystovary form and possess a germinal compartment in lamellae that protrudes from the ovarian capsule towards the lumen (Sales et al., 2013). Folliculogenesis begins within the germinal epithelium when oogonia enter meiosis; after maturation to the oocyte stage, these cells are surrounded by follicular cells that are supported on the basal membrane and are surrounded by theca of a conjunctive nature (Meijide et al., 2016). Primary growth begins in this phase with the proliferation of organelles in the ooplasm of the oocyte, differentiation of the yolk nucleus, appearance of the zona pellucida, and the appearance of cortical alveoli dispersed in the ooplasm (Grier et al., 2017). Secondary growth involves vitellogenesis, which contains the energy reserve for embryonic and larval development, and there is migration of the nucleus towards the micropyle, which is important for fertilization and successful reproduction (Jalabert, 2005).

The pimelodid *Iheringichthys labrosus* (Lütken, 1874) is a small, non-migratory Neotropical freshwater fish with a benthic feeding habit, and it is the second most important species for commercial fishing in the Itaipu reservoir in Brazil (Abes et al., 2001). *Iheringichthys labrosus* spawn in the Itaipu reservoir from October to December, has a first maturation size of 16.0 cm, and does not perform parental care (Agostinho and Júlio, 1999). The species has a wide geographical distribution and is dominant in Brazilian reservoirs but mainly in the Paraná River basin (Agostinho et al., 2007). Studies on the reproductive biology of this species have been conducted on specimens from some reservoirs, such as the Marimbondo and Itumbiara (Bazzoli et al., 1997), Furnas (Santos et al., 2004), Itaipu (Agostinho et al., 2007), and Capivara (Suzuki et al., 2013). Studies focusing on the reproduction of the species in rivers, however, are still in the initial stages (Suzuki et al., 2013). Due to the lack of adequate information regarding the reproduction of *I. labrosus* in lotic environments, therefore, the present study was conducted to investigate and determine the main reproductive variables of this species by applying anatomical and morphometric analyses using both light and transmission electron microscopy.

## 2. Materials and methods

### 2.1. Sampling, biological indices, and anatomy

Adult *I. labrosus* were collected bimonthly between April 2014 and March 2015 from the Turvo River (20°38′52″S; 46°13′30″ W), Minas Gerais, Brazil, using gillnets with mesh sizes varying from 3.0 to 12.0 cm between opposite knots that remained submersed for 12 h for three nights every 2 months. If captured alive, the fish were euthanized following the standards of the Ethical Principles of the Animal Experimentation Guide, CONCEA (Brazil, 2013), and the collection was permitted through a license granted by the State Forestry Institute (N°. 183085-1561-2012). Standard length (SL) in cm, body weight (BW) in g, and weight of the gonads (GW) in g were recorded for each specimen collected. The biometric data obtained was used to calculate the gonadosomatic index (GSI = GWx100 / BW) and the Fulton condition factor (K = BWx100 / SL$^3$) for fish from each bimester. Macroscopic and microscopic analyses were used to determine the bimonthly frequency distribution of gonadal maturation stages (Bazzoli, 2003). Water temperature (º C) was recorded at the time of collection using an analogue thermometer, while rainfall (mm) and photoperiod (clear hours) for the respective days when collections occurred were obtained from Furnas Centrais Elétricas Meteorological Station (Table 1).

For the anatomical study of gonads, 30 specimens of each sex at different stages of gonadal maturation were fixed in 4% formalin for 24 h and preserved in 70% alcohol. The total number of fringes and their length in the cranial and caudal regions were measured using a digital caliper (DIGIMESS - accuracy of 0.01 mm).

### 2.2. Light microscopy

For light microscopy, another ten specimens of each sex at each stage of maturation were dissected, and fragments of the cranial and caudal regions of the gonads were collected. The fragments were fixed in Bouin's solution for 8 h and submitted to routine histological techniques: embedding in paraffin, sectioning at 5-μm by microtome, and staining with hematoxylin-eosin. The

**Table 1**
Abiotic factors recorded from April 2014 to March 2015 for *I. labrosus* in the Turvo River, Minas Gerais.

| 2-Month Periods | Abiotic factors | | |
|---|---|---|---|
| | Temperature (°C) water | Precipitation (mm) | Photoperiod (hours of light)* |
| Apr-May | 25.11 ± 0.5 | 222.01 ± 19.56 | 11:30 |
| Jun-Jul | 20.41 ± 0.4 | 59.21 ± 11.15 | 10:00 |
| Aug-Sep | 21.01 ± 0.6 | 131.81 ± 14.28 | 11:30 |
| Oct-Nov | 25.41 ± 0.7 | 179.61 ± 19.34 | 13:30 |
| Dec-Jan | 30.91 ± 1.2 | 430.61 ± 26.52 | 14:00 |
| Feb-Mar | 27.31 ± 0.9 | 178.91 ± 21.05 | 13:30 |

* Photoperiod refers to the number of daylight hours for the respective sampling days.

traditional histochemical techniques of periodic acid-Schiff (PAS), amylase + PAS, Alcian blue pH 2.5, and Alcian blue pH 0.5 (Pearse, 1985) were used to detect the presence of carbohydrates and protein secretion in the testes.

### 2.3. Morphometric analysis

The diameters of the nucleus in 50 cells of the spermatogenic lineage and in 50 cells of the ovogenic lineage were recorded, as well as the zona pellucida thickness and the height of the follicular cells of vitellogenic oocytes. Measurements were taken from five to ten histological slides using the free Image J software and reported as mean and standard deviation values.

### 2.4. Transmission electron microscopy

For transmission electron microscopy (TEM), gonad fragments from ten specimens of each sex were fixed in 2.5% glutaraldehyde with 0.1 M phosphate buffer pH 7.2 and post-fixed in 1% osmium tetroxide with 1.5% potassium ferrocyanide. The specimens were embedded in epon/araldite, cut into ultrathin sections, contrasted with uranyl acetate and lead citrate, and examined using a Zeiss EM-10 transmission electron microscope. The morphological characteristics of the cells of the gametogenic lineage and of the stages of gonadal maturation were defined based on the description previously described by Bazzoli (2003).

### 2.5. Statistical analysis

For statistical purposes, bimonthly values of GSI and K were assessed for normality (Shapiro-Wilk), followed by analysis of variance to test for significant differences in mean values. Tukey's parametric test was performed to compare mean values, and when normality was not met, even after appropriate transformations, the Kruskal-Wallis non-parametric test was used with a significance level of $P < 0.05$.

## 3. Results

Males ($n = 278$) had a mean standard length of 11.5 ± 1.46 cm (range = 9.10–15.60 cm) and a mean body weight of 22.50 ± 10.04 g (range = 11.90–39.50 g), while females ($n = 512$) measured 13.78 ± 2.58 cm (range = 12.70–20.50 cm) and weighed 42.78 ± 26.51 g (range 12.10–51.50 g).

The morphological characteristics of gonadal maturation stages for males and females are described (Table 2) and the relative bimonthly frequencies of gonadal maturation stages are depicted in Figs. 1 and 2. The reproductive quiescent stage for males (M1) and females (F1) occurred from April to July, a period when the water temperature and rainfall were less and there was a shorter photoperiod. The mature stage for males (M3) was recorded from August to March and for females (F3) from August to January, however, when there were greater values for the abiotic factors. Partially (F4A) and fully (F4B) spawning females were observed from October to May. Partially depleted males (M4A) were observed from October to March while completely depleted males (M4B) were more frequent in the February to March and April to May.

The testes of *I. labrosus* are paired and fringed with digitiform fringes distributed throughout these structures and were an average of 182 ± 24 mm long (Fig. 3A) (range = 132–224 mm). When reproductively active, the fringes of the cranial region measured 4.32 ± 1.02 mm (range = 3.14–9.5 mm) in length and contained cells of the spermatogenic lineage at different developmental stages. Furthermore, there were spermatozoa in the lumen of the seminiferous tubules (Fig. 3B). The caudal fringes had an average length of 1.60 ± 0.45 mm (range = 1.12–2.78 mm) and had secretory cells on the walls of seminiferous tubules and secretion into the lumen (Fig. 3C), which tested positive for neutral glycoproteins using the PAS technique as well as the control with amylase salivar + PAS. Testes in the quiescent state had rudimentary fringes, cranial fringes containing only spermatogonia on the walls of the seminiferous tubules, and a closed lumen. The caudal fringes contained secretory cells in the walls of the tubules that were closed and without any indications of secretion. Even though there is a fringed aspect of the testes, the testes are externally covered by the tunica albuginea, while internally there are tubular and interstitial compartments with the boundaries being defined by the basement membrane. The tubular compartment is formed by Sertoli cells and isogenic cysts of germ cells distributed throughout the extension

**Table 2**

Macroscopic and microscopic characteristics of the gonadal maturation stages of males and females of *I. labrosus* captured in the Turvo River, Minas Gerais, from April 2014 to March 2015.

| Reproductive cycle stage | Males (M) | Females (F) |
|---|---|---|
| Resting (M1/F1) | Testes with reduced and discrete fringes. Histologically, the walls of the seminiferous tubules are comprised of Sertoli cells and spermatogonia while the lumen is closed | Ovaries whitish, translucent and with little evident vascularization. Histologically, oogonia, initial perinucleolar, advanced perinucleolar oocytes and thick tunica albuginea are present |
| Maturation (M2/F2) | Testes milky-white in color with evident fringes. Histologically, cysts of lineage cells at different stages of development are present. The lumen of seminiferous tubules of the cranial region possess variable amounts of spermatozoa, while in the caudal region they possess small quantities of acidophilic secretion | Ovaries yellowish, initial perinucleolar, advanced perinucleolar oocytes and some pre-vitellogenic oocytes are present |
| Mature (M3/F3) | Testes milky-white in color with prominent fringes. The lumen of seminiferous tubules and spermatic ducts are filled with spermatozoa (LM). The lumen of the tubules of caudal fringes are filled with acidophilic secretion | Ovaries with greater volume, occupy a large part of the coelomic cavity, and have a yellowish-brown color, while vitellogenic oocytes can be seen with the naked eye. Histologically, initial perinucleolar, advanced perinucleolar pre-vitellogenic oocytes and vitellogenic oocytes are present |
| Partly spermiated/spawned (M4A/F4A) | Testes still milky-white, but now with flaccid fringes. Microscopically, lumen of the seminiferous tubules broad with small amounts of spermatozoa. Spermatogonia cysts more predominant than those of other cells of the lineage. Included in this stage are testes with seminiferous tubules, partially empty spermatic ducts. Tubules in the caudal region possess acidophilic secretion | Ovaries with reduced volume, becoming flaccid, and with oocytes still evident to the naked eye. Initial perinucleolar, advanced perinucleolar pre-vitellogenic, vitellogenic oocytes and post-ovulatory follicles are present, along with remnants of spawning including follicular cells, basement membrane, and theca (LM) |
| Fully spermiated/spawned (M4B/F4B) | Testes with very flaccid fringes and hemorrhagic regions. Histologically, the seminiferous tubules and spermatic ducts possess open lumen with residual spermatozoa. The cells of the lineage begin to reorganize into cysts on the wall of the seminiferous tubules. Residual acidophilic secretion is still present in the tubules of the caudal region | Ovaries are flaccid and hemorrhagic. Oocytes, initial perinucleolar, advanced perinucleolar oocytes, post ovulatory follicles, and rare vitellogenic oocytes in the process of atresia are present, along with clefts in the zona pellucida, yolk liquefaction, and follicular cell hypertrophy (LM) |

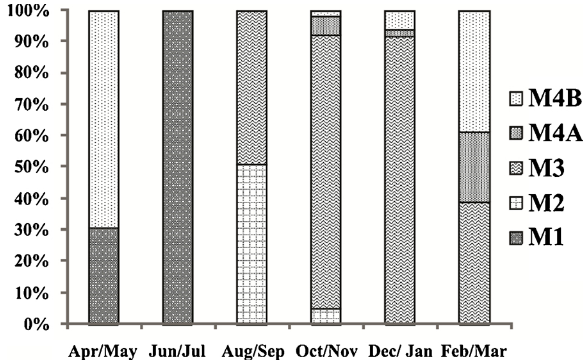

**Fig. 1.** Bimonthly relative frequency distributions of gonadal maturation stages of male *I. labrosus* captured in the Turvo River, Minas Gerais, from April 2014 to March 2015; Quiescent (M1), maturation (M2), mature (M3), partial spermiation (M4A) and complete spermiation (M4B).

of the walls of seminiferous tubules. There is information in Table 3 for the characteristics of the spermatogenic lineage cells detected using transmission electron microscopy (TEM) (Figs. 3D-I). Analysis with the use of TEM revealed cytoplasmic extensions of the Sertoli cells with the boundaries being defined by spermatogenic cysts, which contain cisternae of the rough endoplasmic reticulum and a triangular or ovoid vesicular nucleus with loose chromatin for which there can be aggregations detected in some locations. The interstitial tissue is composed of connective tissue containing Leydig cells. The connective tissue contains collagenous fibrils, blood capillaries, nerves, and myoid cells, which have an elongated, fusiform nucleus and are organized in concentric, discontinuous layers around the seminiferous tubules. Leydig cells contain organelles such as the smooth endoplasmic reticulum, mitochondria with tubular ridges, and a vesicular nucleus.

The ovaries of *I. labrosus* are paired organs, of the cystivary type, oriented longitudinally in the coelomic cavity dorsal to the intestine. The ovaries are covered with the tunica albuginea and composed of connective tissue, while internally, ovuligerous lamellae project towards the ovarian lumen, which contains the germinative epithelium with nests of oogonia and oocytes at different stages of development. During folliculogenesis, oocytes become surrounded by follicular cells, a basement membrane, and theca that originate from undifferentiated ovarian stromal cells. These structures form the follicular complex, which, during development, will is characterized by the primary and secondary growth phases, maturation, and ovulation. Primary growth involves the differentiation

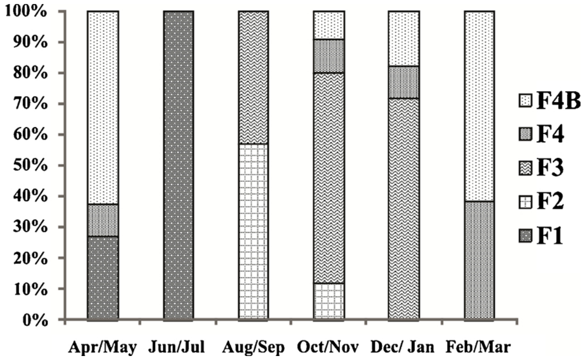

**Fig. 2.** Bimonthly relative frequency distributions of gonadal maturation stages of female *I. labrosus* captured in the Turvo River, Minas Gerais, from April 2014 to March 2015; Quiescent (F1), maturation (F2), mature (F3), partly spawned (F4) and fully spawned (F4B).

of early and advanced perinucleolar oocytes (previtellogenic). Secondary growth involves the incorporation of yolk of hepatic origin that occurs in vitellogenic follicles. The final maturation phase is characterized by the displacement of the nucleus towards the animal pole near the micropyle. During ovulation, the oocytes are released from the follicular complexes and enter the ovarian lumen, and subsequently the ovarian duct, and ultimately are released into the external environment. Information is included in Table 4 for the characteristics of the oogenic lineage cells (Figs. 4A-J). After ovulation of the follicular complex, post-ovulatory follicles occur that consist of follicular and micropylar cells, a basement membrane, and theca with an irregular lumen (Fig. 4K). Oocytes that were not released as a result of ovulation undergo a degenerative process (i.e., follicular atresia; Fig. 4L).

Mean values of GSI and K for males and females during each 2-month period are shown in Table 5). The mean GSI for both males and females were greater ($P < 0.05$) during the August to January and October to January periods, respectively, compared to other 2-month periods. There were greater mean values ($P < 0.05$) of K for males during the June to September period than during the February to July period. While for females, the values were greater ($P < 0.05$) for the June to September and December to January than the February to May period.

## 4. Discussion

The testes of most Siluriformes with external fertilization, such as *I. labrosus* are of the unrestricted spermatogonial type, where spermatogenic cysts are distributed along the seminiferous tubules (Santos et al., 2001), similar to testes of other Siluriformes species (Guimarães-Cruz et al., 2005; Barros et al., 2007; Santos et al., 2010; Melo et al., 2011; Veloso-Junior et al., 2013). The chemical composition of the secretions in the caudal region of testes can vary among species and seasons (Santos et al., 2010). In *I. labrosus,* this secretion is composed of neutral glycoproteins and was detected only in reproductively functional testes (Van Den Hurk et al., 1987; Santos et al., 2001).

The length of the fringes of maturing testes varies between the cranial and caudal regions and among siluriform species (Santos et al., 2010; Guimarães-Cruz and Santos, 2004). These differences between species can occur as a consequence of the continuous physical growth even after sexual maturity (Pudney, 1993), and these differences can also be used as characteristics for phylogenetic analysis among species.

In L. *labrosus*, primary spermatogonia were observed in the germinal epithelium throughout the sampling period, similar to the observations of Uribe et al. (2014). The mitochondrial cement observed in the cytoplasm of spermatogonia is composed of ribosomal RNA and proteins (Peruquetti et al., 2010), and this structure was surrounded by mitochondria that were present from cell differentiation to the formation of spermatozoa. In the spermatozoan phase, the synaptonemic complexes recorded in the primary spermatocytes of *I. labrosus* were composed of paired homologous chromosomes, with pairing occurring during the first meiotic division (Uribe et al., 2014). In the spermiogenic phase there was chromatin condensation, nucleus displacement to the cell pole, cytoplasmic reduction, and differentiation of the intermediate piece and flagellum (Schulz et al., 2010), similar to what was observed in the present study. The nuclear diameters of the spermatogenic cells of *I. labrosus* were greater in primary spermatogonia and decreased gradually in subsequent stages, to the smallest diameter in the spermatozoa. This size reduction may serve to adjust the shape of the spherical head of a sperm cell at the time of its passage through the micropyle channel during fertilization, which would also serve as a natural selection process for hybridization avoidance (Melo et al., 2011).

Analysis of the cystovarian morphology of *I. labrosus* indicated oogonia differentiate into oocytes in the germinal epithelium and then transition through the phases of primary and secondary growth until the gametes are mature (Grier et al., 2016; Meijide et al., 2016; Uribe et al., 2016). The diameter of the vitellogenic oocytes of *I. labrosus* increased about 500 times in relation to the diameter of the oogonia. The diameter of vitellogenic oocytes varies among teleost species, resulting in more desirable conditions for the development of the embryo and the larva (Melo et al., 2011). In addition, sedentary species, such as *I. labrosus* of the present study, generally have larger and adhesive oocytes, while migratory species have smaller eggs without the adhesive properties (Rizzo et al., 2002; Melo et al., 2011).

The morphology of the zona pellucida of teleosts depends on the species, reflects adaptations to different ecological conditions,

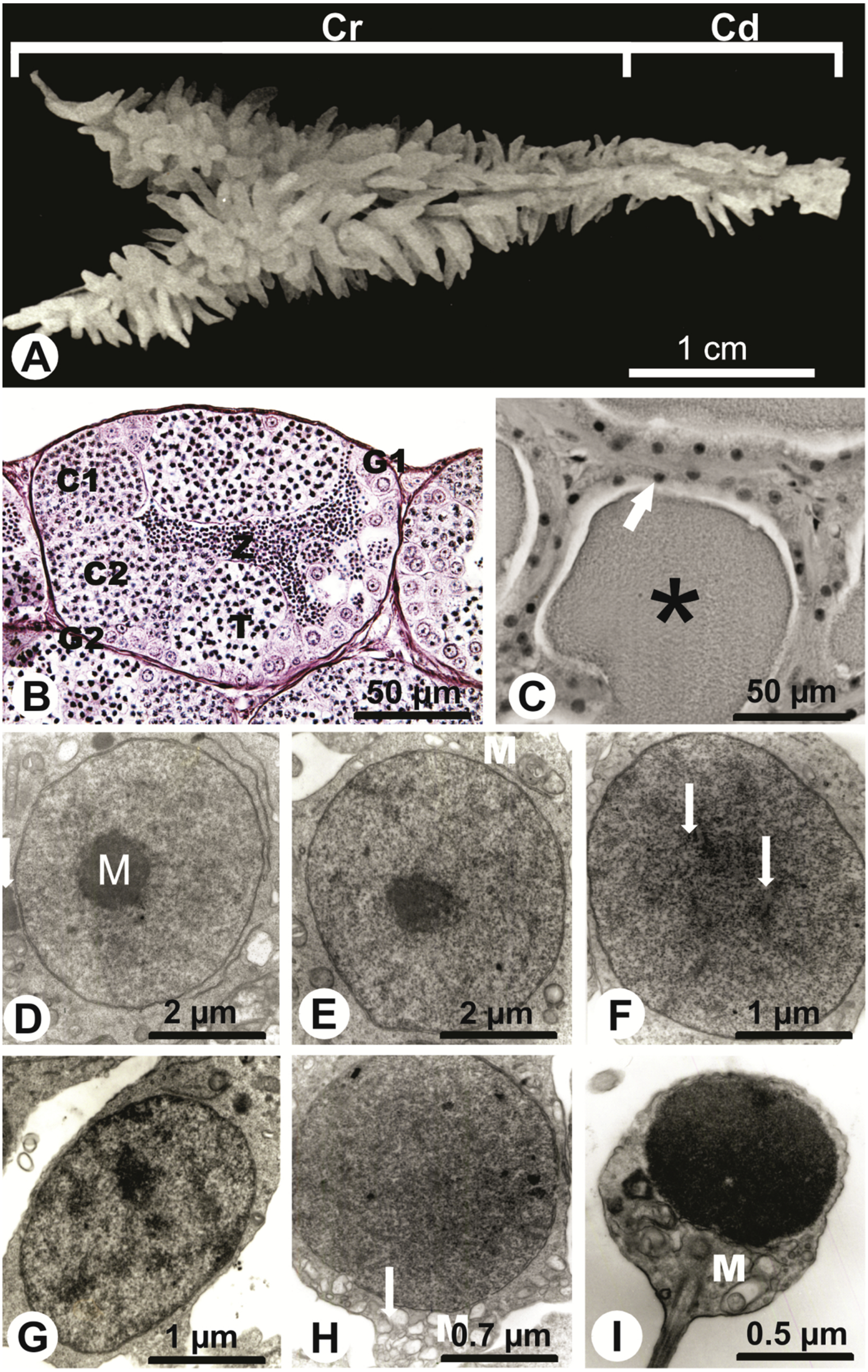

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**Fig. 3.** Histological sections of *I. labrosus* testes at different stages of the reproductive cycle; A) Tissue fringes in maturation with different regions: cranial (Cr) and caudal (Cd); B) Spermatogenic activity in the seminiferous tubules of the cranial region, with cysts of spermatogenic lineage cells at different stages of development with primary spermatogonia (G1), secondary spermatogonia (G2), primary spermatocyte (C1), secondary spermatocyte (C2), spermatid (T), and spermatozoa (Z); C) Secretory activity in the tubules of the caudal region of the testis, showing cuboidal cells in the wall (arrow) and accumulation of secretion in the lumen (asterisk); D) G1: vesicular nucleus and cytoplasm with mitochondria joined by mitochondrial cement (arrow); E) G2: nucleus with loose chromatin and cytoplasm sparse numbers of organelles, but with mitochondria (M); F) C1: nucleus with characteristic synaptonemic complexes (arrows); G) C2: nucleus with chromatin forming electron-dense clumps; H) T: eccentric nucleus and cytoplasm with electron-lucid vesicles (arrow) and mitochondria (M); I) Z: spherical head with dense nucleus, intermediate part with mitochondria (M), and long tail.

**Table 3**
Morphological characteristics of the spermatogenic lineage cells and their respective mean cellular nucleus diameter.

| Spermatogenic cell Ø nuclear µm | Morphological characteristics |
|---|---|
| Primary spermatogonia Ø = 8.87 ± 1.28 | The largest cell of the spermatogenic lineage, with only one per cyst occurring. These cells have an abundant cytoplasm; a spherical, central, vesicular nucleus; and an evident nucleolus. Ultrastructurally, mitochondria are isolated or grouped, joined by mitochondrial cement (Fig. 3D). |
| Secondary spermatogonia Ø = 6.48 ± 0.65 µm | The cyst is formed of two to four cells. They possess cytoplasm with few organelles, a nucleus with chromatin forming lumps, and an electron-dense nucleolus (Fig. 3E). |
| Secondary spermatocyte Ø = 3.08 ± 0.56 | The secondary spermatocyte originates from the first meiotic division of the primary spermatocytes. It has scarce cytoplasm, early vacuolization, and a central nucleus with chromatin forming scattered electron-dense masses (Fig. 3G). |
| Spermatid Ø = 2.18 ± 0.58 | Spermatids possess scarce cytoplasm and a spherical and dense nucleus. Spermiogenesis occurs within the spermatid cysts and when the cystic wall is complete, it breaks, releasing spermatozoa into the lumen of the seminiferous tubules. Ultrastructurally, the cytoplasm possesses intense vacuolization and the nucleus is slightly electron-dense and eccentric; the implantation fossa is being formed and the tail undergoing differentiation (Fig. 3H). |
| Spermatozoon Ø = 1.61 ± 0.13 | The smallest spermatogenic cell line, which occurs on the lumen of the seminiferous tubules and the testicular ducts. It possesses scarce cytoplasm and a spherical and dense nucleus. Spermatozoa possess a spheroidal head and have no acrosome. The intermediate piece is short and poor in mitochondria. The flagellum is single, long and contains microtubules in the axonemic arrangement 9 + 2 (Fig. 3I). |

**Table 4**
Morphological characteristics of the oogenic lineage cells and the respective cell diameters (mean ± standard deviation).

| Oogenic cell Ø cell in µm | Morphological characteristics |
|---|---|
| Oogonia Ø = 13.98 ± 3.78 | The smallest cells of the lineage and are grouped in nests (Fig. 4A). Cells are rounded, with scarce cytoplasm and a vesicular nucleus with prominent nucleoli. Transmission electron microscopy (TEM) shows an ovoid nucleus with finely granular chromatin, a spherical nucleolus, and electron-dense nuclei (Fig. 4B). |
| Initial perinucleolar Ø = 104.14 ± 11.38 | Ooplasm strongly basophilic with a vitreous aspect, a central vesicular nucleus with peripheral nucleoli (Fig. 4C). TEM shows that the follicular wall consists of a single layer of follicular cells, a basement membrane, and theca (Fig. 4D). |
| Advanced perinucleolar Ø = 171.14 ± 16.38 | Basophilic ooplasm with a finely granular appearance, vesicular nucleus with nucleoli adhered to the nuclear envelope. In some oocytes, the yolk nucleus is observed densely stained basophilic structure (Fig. 4E), while the follicular cells remain squamous. TEM shows that the basement membrane is thin, the follicular cells remain squamous, and the theca consists of fibroblast-like cells (Fig. 4F). |
| Pre-vitellogenicØ = 339.02 ± 28.17 | Cells characterized by having a cortical alveolus formed by small discontinuous vesicles in the ooplasm. Cells are slightly basophilic, with finely-granular cytoplasm, a central nucleus, a vesicle, and peripheral nucleoli (Fig. 4G). The zona pellucida has an acellular, acidophilic, and refringent structure. The micropylar apparatus is evident, and the follicular cells are cubic. TEM reveals that the zona pellucida is crossed by pores and with oocyte microvilli interacting with follicular cells (Fig. 4H). The follicular cells are cuboidal and have electron-lucid granules in the apical region; the basal membrane and theca remain thin. |
| Vitellogenic Ø = 562.16 ± 52.16 | Largest cell in the lineage characterized by the presence of acidophilic yolk globules in the ooplasm. At this stage, the cortical vesicles are organized at the periphery of the ooplasm, forming the cortical alveoli. The zona pellucida remains thick (6.21 ± 0.92 µm), acellular, and acidophilic. The follicular cells are prismatic and are 36.46 ± 4.93 µm in height (Fig. 4I). The micropylar device of vitellogenic oocytes consists of a large vestibule, a short micropylar canal, and micropylar cell (Fig. 4I: upper insert). TEM with the ooplasm possessing electron-dense yolk globules and electron-lucid cortical vesicles. The zona pellucida is thickened, crossed by pores, which are partially occluded in this phase. The cytoplasm of the follicular cells is filled with electron-lucid granules (Fig. 4J). The basal membrane and theca show characteristics similar to the previous stage. |

and can include accessory structures which have been reported on the surface of oocytes of several Neotropical teleosts (Rizzo et al., 2002). Some Neotropical siluriform species such as *Rineleps aspera* and L. *alexandri* have adhesive eggs (Rizzo et al., 2002), similar to *I. labrosus*. Micropyle morphology varies among species (Rizzo et al., 2002; Weber et al., 2012) and in *I. labrosus*, micropyle corresponded to type 1, according to the classification proposed by Ricardo et al. (1996).

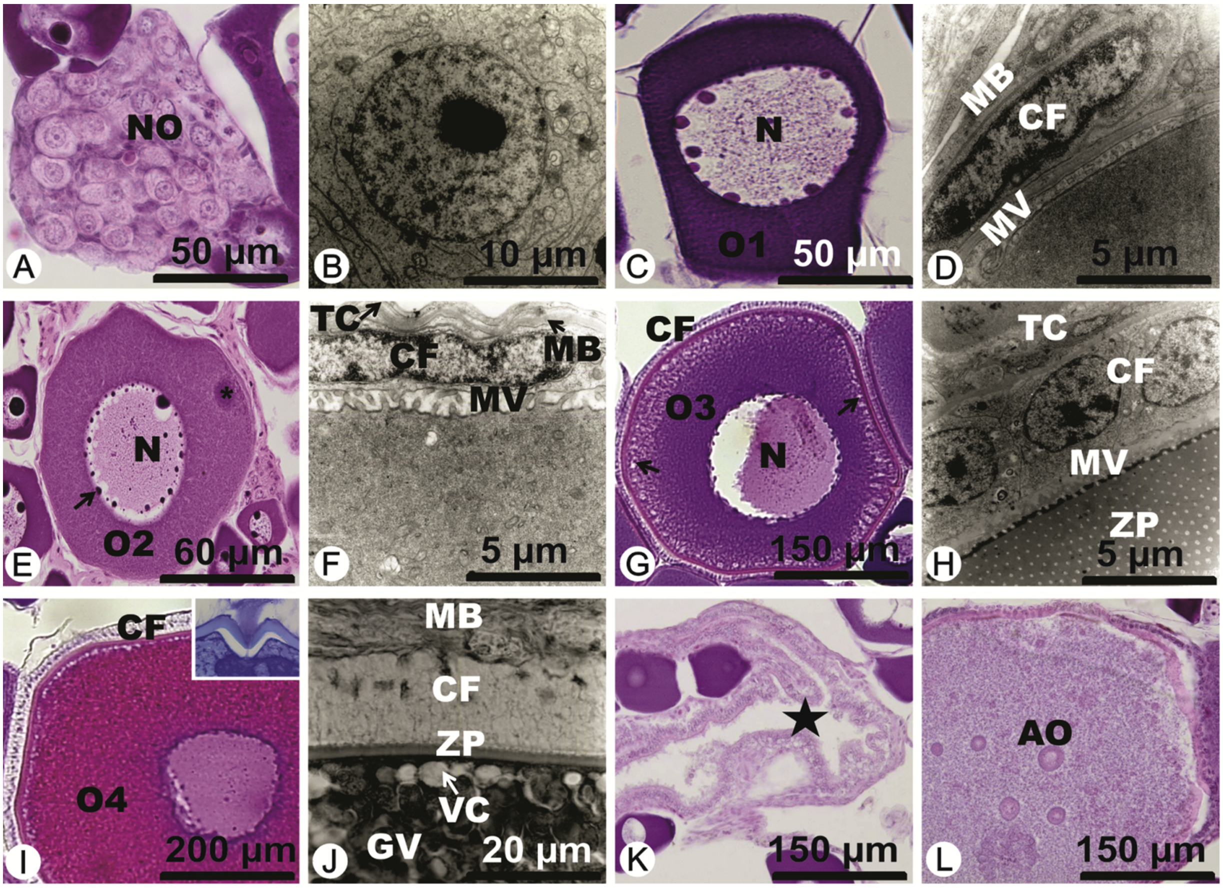

**Fig. 4.** Histological sections of *I. labrosus* ovaries at different stages of the reproductive cycle; A) Nests of oogonia (N0) under light microscopy: embedded in paraffin and stained with hematoxylin-eosin; B) Oogonia under transmission electron microscopy with ovoid nucleus, spherical and electron-dense nucleolus; C) Young oocyte (O1) using light microscopy, indicating nucleus (N); D) Follicular wall and ooplasm of young oocyte using transmission electron microscopy with microvilli (MV) on the oocyte wall, follicular cell nucleus (FC), and basement membrane (BM); E) Pre-vitellogenic oocytes (O2) using light microscopy, with nuclei (N) and small vesicles (arrow); F) Pre-vitellogenic oocyte with follicular cells (CF), basement membrane (MB), theca (TC), and microvilli (MV); G) Oocyte with cortical vesicles (O3) using light microscopy, with nuclei (N), follicular cells (CF), and cortical vesicles (arrow); H) Ultra-structure of oocyte follicular wall with cortical vesicles (O3), also showing zona pellucida (ZP) crossed by microvilli oocyte, follicular cell (CF), and theca (TC); I) Vitellogenic oocyte (O4) with prismatic follicular cells (CF) with granule-filled cytoplasm; Upper part - micropylar apparatus with micropillary cell; J) Vitellogenic oocyte with basement membrane (MB), prismatic follicular cells (CF) - with cytoplasm filled with electron-lucid granules - zona pellucida (ZP) and ooplasm with cortical vesicles (arrow, VC), yolk globules (GV); K) Ovary in stage 4B (fully spawned). Post-ovulatory follicle identified by star; L) Detail of atretic follicle in ovary from which there was total ovulationwith disorganization of the follicular wall and liquefaction of the yolk.

**Table 5**

Gonadosomatic index (GSI) and condition factor (K) during 2-month intervals for males and females of *I. labrosus* captured in the Turvo River, Minas Gerais, from April 2014 to March 2015.

| Bimester 2-Month Intervals | GSI | K | | GSI | K | |
|---|---|---|---|---|---|---|
| | Males | | *n* | Femals | | *n* |
| April-May | 0.34 ± 0.19a | 1.30 ± 0.13a | 26 | 0.68 ± 0.40a | 1.35 ± 0.20a | 59 |
| Jun-Jul | 0.20 ± 0.13a | 1.52 ± 0.16bc | 19 | 0.62 ± 0.17a | 1.48 ± 0.10bcd | 41 |
| Aug-Sep | 1.76 ± 0.59b | 1.52 ± 0.13b | 57 | 1.93 ± 1.40c | 1.56 ± 0.18c | 116 |
| Oct-Nov | 2.20 ± 0.89b | 1.37 ± 0.17ac | 92 | 4.13 ± 2.58b | 1.44 ± 0.15ade | 136 |
| Dec-Jan | 1.88 ± 0.74b | 1.37 ± 0.09ac | 48 | 3.51 ± 2.09b | 1.46 ± 0.12be | 85 |
| Feb-Mar | 0.73 ± 0.53a | 1.33 ± 0.09a | 36 | 1.10 ± 0.97a | 1.37 ± 0.11a | 75 |
| Total | | | 278 | | | 512 |

Values in the same column with different letters are differ ($P < 0.05$).

The follicular cells may have different morphologies, depending on the species and stage of oocyte development (Grier et al., 2017). The follicular cells of *I. labrosus* are squamous in early and advanced perinucleolar oocytes, cuboidal in previllogenic oocytes, and prismatic in vitellogenic oocytes, which is similar to the descriptions of Melo et al. (2011) for other Siluriformes. In addition, results of studies conducted with other Neotropical fish by Rizzo et al. (2002) indicate oocytes containing prismatic follicular cells, such as those reported in the present study: produce a layer of mucus that covers the oocyte making it adhesive.

The basal membrane further serves as a support for follicular cells, functions as a selective filter due to the permeability of molecules and ions, and provides a boundary externally for the theca that is derived from the ovarian stroma (Grier, 2000). In the present study, the basement membrane was ultra-structurally observed at all stages of oocyte development, which is similar to that reported for other Siluriformes species (Melo et al., 2011). The oocyte theca of *I. labrosus* is similar to most teleosts in that it originates from undifferentiated ovarian stromal cells and can be comprised of a single layer (present study) or two to three layers (Grier, 2000). Results from ultrastructural studies indicate some theca cells have mitochondria with tubular ridges and a smooth endoplasmic reticulum, indicating that these cells, referred to a special theca cells, synthesize steroids (De Vlaming, 1983; Santos et al., 2014a).

After spawning, non-released structures of the follicular complex remain in the ovary, constituting the post-ovulatory follicle (Grier et al., 2017). In *I. labrosus*, the post-ovulatory follicle had a large and an irregular lumen and morphological changes as a result of its involution due to cell apoptosis, similar to the ultrastructural assessments of Drummond et al. (2000) for *Astyanax bimaculatus lacustris*. Follicular atresia is common in vertebrate ovaries and can occur at any stage of oogenesis but is more frequent in vitellogenic oocytes (Santos et al., 2008), as observed in the present study and previously reported for the siluriforms (Barros et al., 2007; Sales et al., 2013). In this degenerative process, as reported by Uribe et al. (2016), there is a large amount of phagocytic activity by follicular cells, fragmentation of the zona pellucida, and liquefaction of yolk globules and cortical alveoli develop. Miranda et al. (1999) reported that follicular atresia can be affected by factors such as altered hormonal concentrations, inadequate temperature, biocidal agents, fasting, and inadequate confinement conditions.

Variation in male gonadosomatic index (GSI) throughout the reproductive cycle was more discrete than that observed for females, which may be related to the greater proportion of spermatogenic lineage cells (Bazzoli, 2003). The Fulton (K) condition factor provides important information about the physiological state of fish, assuming that individuals with greater K values have a greater health status (Weber et al., 2017). In the present study, the variation in the GSI and K among the gonadal maturation stages occurs subsequent to the variation in water temperature, rainfall, and photoperiod, and it can, therefore, be inferred that the abiotic factors have an important function in the final maturation and spawning of *I. labrosus*. Similar results have been reported for other migratory Siluriformes species (Melo et al., 2011; Normando et al., 2014).

The results of the present study provide evidence that the *I. labrosus* has a long reproductive period, which occurs from August to March for males and August to January for females. The presence of post-ovulatory follicles next to vitellogenic oocytes indicate that spawning of *I. labrosus* is of the parceled type, which is similar to what has been reported for studies conducted with this species in lentic environments.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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## References

Abes, S.D.S., Agostinho, A.A., Okada, E.K., Gomes, L.C., 2001. Diet of *Iheringichthys labrosus* (Pimelodidae, Siluriformes) in the Itaipu reservoir, Parana river, Brazil-Paraguay. Braz. Arch. Biol. Technol. 44, 101–105. https://doi.org/10.1590/S1516-89132001000100014.

Agostinho, A.A., Júlio Jr, H.F., 1999. Peixes da bacia do Alto rio paraná. In: Lowe-McConnell, R.H. (Ed.), Estudos ecológicos de comunidades de peixes tropicais, São Paulo, pp. 374–400.

Agostinho, A.A., Gomes, L.C., Pelicice, F.M., 2007. Ecologia e Manejo de Recursos Pesqueiros em Reservatórios do Brasil. EDUEM, Maringá, pp. 501.

Barros, M.D.M., Veloso-Junior, V.C., Guimarães-Cruz, R.J., Santos, J.E., 2007. Reproductive apparatus and gametogenesis of Lophiosilurus alexandri Steindachner (Pisces, Teleostei, Siluriformes). Rev. Bras. Zool. 24, 213–221. https://doi.org/10.1590/S0101-81752007000100028.

Bazzoli, N., 2003. Parâmetros reprodutivos de peixes de interesse comercial na região de Pirapora. In: Godinho, H.P., Godinho, A.L. (Eds.), Águas, peixes e pescadores do São Francisco das Minas Gerais, Belo Horizonte, pp. 291–306.

Bazzoli, N., Cangussú, L.C.V., Rizzo, E., Santos, G.B., 1997. Reprodução e desova de mandis *Pimelodus maculatus* e *Iheringichthys labrosus* (Pisces, Pimelodidade) nos reservatórios e Furnas. Marimbondo e Itumbiara. Bios 5, 43–51.

Brazil, 2013. Ministério da ciência, tecnologia e inovação. Diretrizes da prática de eutanásia do CONCEA, Brasília 54p.

De Vlaming, V.L., 1983. Oocyte development patterns and hormonal involvements among teleost. In: Rankin, J.C., Pitcher, T.J., Duggon, R.T. (Eds.), Control Process in Fish Phisiology, London, pp. 176–199.

De Vlaming, V., Grossman, G., Chapman, F., 1982. On the use of the gonadosomatic index. Comp. Biochem. Physiol. 73, 31–39.

Drummond, C.D., Bazzoli, N., Rizzo, E., Sato, Y., 2000. Postovulatory follicle: a model for experimental studies of programmed cell death or apoptosis in teleosts. J. Exp. Zool. 287, 76–182. https://doi.org/10.1002/1097-010X(20000701)287:2<176::AID-JEZ8>3.0.CO;2-2.

Dzyuba, B., Legendre, M., Baroiller, J.F., Cosson, J., 2019. Sperm motility of the Nile tilapia (Oreochromis niloticus): Effects of temperature on the swimming characteristics. Anim. Reprod. Sci. 202, 65–72. https://doi.org/10.1016/j.anireprosci.2019.01.010.

Freitas, L.J.A., Prado, P.S., Arantes, F.P., Santiago, K.B., Sato, Y., Bazzoli, N., Rizzo, E., 2013. Reproductive biology of the characid dourado *Salminus franciscanus* from the São Francisco river. Brasil. Anim. Reprod. Sci. 139, 145–154. https://doi.org/10.1016/j.anireprosci.2013.03.013.

Grier, H.J., 2000. Ovarian germinal epithelium and folliculogenesis in the common snook, *Centropomus undecimalis* (Teleostei: Centropomidae). J. Morphol. 243, 265–281. https://doi.org/10.1002/(SICI)1097-4687(200003)243:3<265::AID-JMOR4>3.0.CO;2-I.

Grier, H.J., Neidig, C.L., Quagio-Grassiotto, I., 2017. Development and fate of the postovulatory follicle complex, postovulatory follicle, and observations on folliculogenesis and oocyte atresia in ovulated common snook, *Centropomus undecimalis* (Bloch, 1792). J. Morphol. 278, 547–562. https://doi.org/10.1002/jmor.20652.

Grier, H.J., Uribe, M.C., Lo Nostro, F., Mims, S., Parenti, L.R., 2016. Conserved form and function of the germinal epithelium through 500 million years of vertebrate evolution. J. Morphol. 277, 1014–1044. https://doi.org/10.1002/jmor.20554.

Guimarães-Cruz, R.J., Santos, J.E., 2004. Testicular structure of three species of neotropical fresh water pimelodids (Pisces: Pimelodidae). Rev. Bras. Zool. 21, 267–271. https://doi.org/10.1590/S0101-81752004000200016.

Guimarães-Cruz, R.J., Santos, J.E., Santos, G.B., 2005. Gonadal structure and gametogenesis of Loricaria lentiginosa Ibsbrucker, (1979) (Pisces: Siluriformes). Rev. Bras. Zool. 22, 556–564. https://doi.org/10.1590/S0101-81752005000300005.

Jalabert, B., 2005. Particularities of reproduction and oogenesis in teleost fish compared to mammals. Reprod. Nutr. Dev. 45, 261–279. https://doi.org/10.1051/rnd:2005019.

Legendre, M., Linhart, O., Billard, R., 1996. Spawning and management of gametes, fertilized eggs and embryos in Siluroidei. Aquat. Living Resour. 9, 59–80. https://doi.org/10.1051/alr:1996042.

Lemma, A., Getahun, A., Lemma, B., 2015. Assessment of length-weight relationship, sex ratio and condition factor of common carp (*Cyprinus carpio* Linnaeus, 1758) in Lake Ziway, Ethiopia. Glob. Res. J. Fish. Sci. Aquacult. 3, 192–197.

Lopes, D.C.J.R., Bazzoli, N., Brito, M.F.G., Maria, T.A., 2004. Male reproductive system in the South American catfish *Conorhynchus conirostris*. J. Fish Biol. 64, 1419–1424. https://doi.org/10.1111/j.0022-1112.2004.00377.x.

Meijide, F.J., Rey Vazquez, G., Grier, H.J., Lo Nostro, F.L., Guerrero, G.A., 2016. Development of the germinal epithelium and early folliculogenesis during ovarian morphogenesis in the cichlid fish *Cichlasoma dimerus* (Teleostei, Perciformes). Acta Zool. 97, 18–33. https://doi.org/10.1111/azo.12101.

Melo, R.M.C., Arantes, F.P., Sato, Y., Santos, J.E., Rizzo, E., Bazzoli, N., 2011. Comparative morphology of the gonadal structure related to reproductive strategies in six species of Neotropical catfishes (Teleostei: Siluriformes). J. Morphol. 272, 525–535. https://doi.org/10.1002/jmor.10931.

Miranda, A.C.L., Bazzoli, N., Rizzo, E., Sato, Y., 1999. Ovarian follicular atresia in two teleost species: a histological and ultrastructural study. Tissue Cell 31, 480–488. https://doi.org/10.1054/tice.1999.0045.

Nelson, J.S., Grande, T.C., Wilson, M.V.H., 2016. Fishes of the World, five ed. John Wiley, Sons New Jersey, pp. 752.

Normando, F.T., Santiago, K.B., Gomes, M.V.T., Rizzo, E., Bazzoli, N., 2014. Impact of the Três Marias dam on the reproduction of the forage fish *Astyanax bimaculatus* and *A. fasciatus* from the São Francisco River, downstream from the dam, southeastern Brazil. Environ. Biol. Fish. 97, 309–319. https://doi.org/10.1007/s10641-013-0153-3.

Peruquetti, R.L., Taboga, S.R., Azeredo-Oliveira, M.T.V., 2010. Nucleolar cycle and its correlation with chromatoid bodies in the *Tilapia rendalli* (Teleostei, Cichlidae) Spermatogenesis. Anat. Rec. 293, 900–910. https://doi.org/10.1002/ar.21099.

Pudney, J., 1993. Comparative cytology of the non-mammalian vertebrate sertoli cell. In: Russell, L.D., Griswold, M.D. (Eds.), The Sertoli Cell, Clearwater, pp. 611–657.

Ricardo, M.C.P., Aguiar, C.A., Rizzo, E., Bazzoli, N., 1996. Morfologia da micrópila e da célula micropilar em teleósteos neotropicais de água doce. Arq. Bras. Med. Vet. Zoo. 48, 17–24.

Rizzo, E., Sato, Y., Godinho, H.P., 2002. Adhesiveness and surface patterns of eggs in neotropical freshwater teleosts. J. Fish Biol. 61, 615–632. https://doi.org/10.1111/j.1095-8649.2002.tb00900.x.

Sales, N.G., Santos, S.A., Arantes, F.P., Hojo, R.E.S., Santos, J.E., 2013. Ovarian Structure and Oogenesis of Catfish *Pimelodella vittata* (Lütken, 1874) (Siluriformes, Heptapteridae). Anat. Histol. Embryol. 42, 213–219. https://doi.org/10.1111/ahe.12004.

Santos, H.B., Thomé, R.G., Arantes, F.P., Sato, Y., Bazzoli, N., Rizzo, E., 2008. Ovarian follicular atresia is mediated by heterophagy, autophagy and apoptosis in *Prochilodus argenteus* and *Leporinus taeniatus* (Teleostei: Characiformes). Theriogenology 70, 1449–1460. https://doi.org/10.1016/j.theriogenology.2008.06.091.

Santos, J.E., Bazzoli, N., Rizzo, E., Santos, G.B., 2001. Morphofunctional organization of the male reproductive system of the catfish *Iheringichthys labrosus* (Lutken 1874) (Siluriformes: Pimelodidae). Tissue Cell 33, 533–540. https://doi.org/10.1054/tice.2001.0207.

Santos, J.E., Rizzo, E., Bazzoli, N., Santos, G.B., 2004. Reproduction of catfish Iheringichthys labrosus (Lütken, 1874) (Pisces: Siluriformes) from Furnas reservoir, Minas Gerais, Brazil. Rev. Bras. Zool. 21, 193–2004. https://doi.org/10.1590/S0101-81752004000200006.

Santos, J.E., Padilha, G.E., Boncompagni Junior, O., Rizzo, E., Bazzoli, N., 2014a. Ovarian follicle growth in the catfish Iheringichthys labrosus (Siluriformes: Pimelodidae). Tissue Cell 38, 303–310. https://doi.org/10.1016/j.tice.2006.07.002.

Santos, M.L., Sales, N.G., Pessali, T.C., Bazzoli, N., Santos, J.E., 2014b. Anatomical and Histological Organization of the Testes of the Inseminating Catfish *Trachelyopterus striatulus* (Steindachner, 1877) (Siluriformes: Auchenipteridae). Anat. Histol. Embryol. 43, 310–316. https://doi.org/10.1111/ahe.12082.

Santos, J.E., Veloso-Júnior, V.C., Oliveira, D.A.A., Hojo, R.E.S., 2010. Morphological characteristics of the testis of the catfish *Pimelodella vittata* (Lütken, 1874). J. Appl. Ichthyol. 26, 942–945. https://doi.org/10.1111/j.1439-0426.2010.01490.x.

Schulz, R.W., França, L.R., Lareyre, J.J., Le Gac, F., Chiarini-Garcia, H., Nóbrega, R.H., Miura, T., 2010. Spermatogenesis in fish. Gen. Compar. Endocrinol. 165, 390–411. https://doi.org/10.4161/21565562.2014.983400.

Suzuki, F.M., Garcia, D.A.Z., Orsi, M.L., 2013. Reproductive dynamics of *Iheringichthys labrosus* (Lütken, 1874) (Teleostei, Pimelodidae), in the Capivara Reservoir, Paranapanema River, Parana, Brazil. Dataset Papers Biol. 2013, 1–7. https://doi.org/10.7167/2013/607541/dataset.

Uribe, M.C., Grier, H.J., García-Alarcón, A., Parenti, L.R., 2016. Oogenesis: From oogonia to ovulation in the flagfish, *Jordanella floridae* Goode and Bean, 1879 (Teleostei: Cyprinodontidae). J. Morphol. 277, 1339–1354. https://doi.org/10.1002/jmor.20580.

Uribe, M.C., Rosa-Cruz, G.D., Grier, H.J., 2014. Proliferation of oogonia and folliculogenesis in the viviparous teleost *Ilyodon whitei* (Goodeidae). J. Morphol. 275, 1004–1015. https://doi.org/10.1002/jmor.20277.

Van Den Hurk, R., Resink, J.W., Peute, J., 1987. The seminal vesicle of the African catfish, *Clarias gariepinus*: a histological, histochemical, enzyme-histochemical, ultrastructural and physiological study. Cell Tissue Res. 247, 573–582.

Veloso-Junior, V.C., Arantes, F.P., Santos, J.E., 2013. Population structure and reproduction of the Neotropical catfish *Pimelodus pohli*, Ribeiro and Lucena, (Teleostei: Pimelodidae). J. Appl. Ichthyol. 30, 399–402. https://doi.org/10.1111/jai.12270.

Weber, A.A., Sato, Y., Santos, J.E., Rizzo, E., Bazzoli, N., 2012. Eggs ultrastructure and early development of *Franciscodoras marmoratus* (Pisces: Doradidae). Anat. Histol. Embryol. 41, 177–183. https://doi.org/10.1111/j.1439-0264.2011.01121.x.

Weber, A.A., Moreira, D.P., Melo, R.M.C., Vieira, A.B.C., Prado, P.S., Da Silva, M.A.N., Bazzoli, N., Rizzo, E., 2017. Reproductive effects of oestrogenic endocrine disrupting chemicals in *Astyanax rivularis* inhabiting headwaters of the Velhas river, Brazil. Sci. Total Environ. 592, 693–703. https://doi.org/10.1016/j.scitotenv.2017.02.181.