Biometric and reproductive characteristics of the King Tiger Plecos *Hypancistrus* sp. "L-333" (Siluriformes: Loricariidae) endemic to the lower Xingu River (Pará, Brasil)

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Abstract

This research analyzed the biometrics and reproductive characteristics of a group of Hypancistrus sp. L-333 (King Tiger Plecos) in order to gather biological information and aid the rearing of the species in *captivity, thus supporting its natural conservation. This species has a natural distribution that is restricted to the lower Xingu River, and is currently threatened by the hydroelectric projects in the region. The acquired specimens were measured, weighed and classified according to sex based on morphometric characteristics (evidence of secondary sexual characteristics) that were later confirmed by dissection. A total of 32 individuals, 16 males and 16 females were identified. Although the length-weight ratio did not present significant differences, the results revealed that the species has secondary sexual dimorphism. The odontodes were more developed in the mid-lateral region of the body, post-dorsal region, caudal peduncle and in the first ray (ossified) of the pectoral fin in males. Histological analyses of the gonads confirmed that all of the fish were adult individuals. The diameter of the oocytes ranged from 0.14 to 2.0 mm, presented three distinct modes, and were synchronous in more than two groups, which evidences batch spawning. Fecundity was low, between 35 and 54 oocytes in the largest batch. This information is novel and important for the identification of reproductive groups, and is aimed at breeding in captivity so that these resources can be exploited in a sustainable way, without harming the already threatened natural stocks.*

Keywords: King Tiger Plecos, *Hypancistrus-zebra*, Reproduction, Conservation, Xingu River.

1. Introduction

Ornamental fish are widely taken from the rivers of clear, muddy and black waters in the different Brazilian states that make up the Amazon basin (Anjos et al., 2009), and the state of Pará is the main exporter of ornamental fish. It is in the state of Para that the species of the family Loricariidae stand out. These are locally known as acaris or bodós and internationally known as “Plecos” or by the L-codes (Seidel, 1996; Prang, 2007). Among the several species exported are the individuals of the genus *Hypancistrus*, which belong to the tribe Ancistrini and the subfamily Hypostominae. *Hypancistrus* is distinguished from the other genera of this subfamily by having the presence of teeth in greater number along the premaxilla and in the maxilla (Armbruster, 2004; Armbruster et al., 2007).

Currently, eight species of the family Loricariidae are formally described; *Hypancistrus zebra* Isbrücker and Nijssen (1991), which is endemic to the stretch of the Xingu River known as Volta Grande, Pará state, Brazil, *H. inspector* Armbruster (2002), which occurs in the regions of the upper Orinoco River and the Negro River, between Venezuela and Brazil; the species of *Hypancistrus contradens, H. debilittera, H. furunculus*, and *H. lunaorum* Armbruster et al. (2007) are distributed in the Orinoco River, Venezuela, and *H. margaritatus* and *H. phantasma* Tan and Armbruster (2016) from the Negro River, Amazonas state, Brazil.

Six other morphospecies of *Hypancistrus* (with distribution restricted to the Xingu River) are in the process of taxonomic confirmation and description, and are part of the list of species and morphospecies that allowed to be exported for ornamental purposes (Interministerial Normative Instruction n° 01 of the Ministry of Fisheries and Aquaculture-MPA and the Ministry of the environment - MMA of January 3rd, 2012, which establishes norms, criteria and standards for the exploitation of native or exotic fish from inland waters for ornamental or aquaristic purposes). Among these morphospecies that are organized with the L-code, we highlight *Hypancistrus* sp. L-004, L-066, L-136, L-260, L-262 and L-333.

Hypancistrus sp. L-333, known locally as "Acari pão" and internationally with the code-L or as "King Tiger Plecos", is endemic to rocky outcrops in the lower Xingu River, downstream of Cachoeira Grande, in the state of Pará, Brazil (Camargo et al., 2004). It is abundant in this region, although specimens have already been released in places upstream of Cachoeira Grande, as a result of the disposal of ornamental fish exporting companies in the city of Altamira-PA (Anatole et al., 2008). This morphospecies, in conjunction with the other Loricarideos, are in danger of local extinction, which may happen before they are known and identified, mainly as a result of the loss of their habitats resulting from the construction of the hydroelectric dam of Belo Monte (Buckup and Santos, 2010; Khatri, 2013; Haglund, 2014), which interrupts and alters the ecology of vast stretches of the Xingu river and makes the environment unsuitable for this and other species that depend on the lotic environment of the rapids and the periphyton for their survival (Haglund, 2014).

Due to its great ornamental value, the King Tiger Plecos is highly sought after by the international market, mainly in Europe, Asia and the United States. Although its capture has been allowed since 2012 (MPA and MMA, 2012), the demand seems to be higher than the available stock, which is found in the lower Xingu River.

Studies are being conducted to find ways to reproduce *Hypancistrus* sp. L-333 in captivity. However, these initiatives are usually carried out by exportation companies operating in the city of Belém, Pará state (personal observation by F. Souza), or in other countries, such as Singapore, Germany and New Zealand (Seidel, 2005; Mick, 2005; Stevens, 2014a; 2014b). Nevertheless, despite initiatives to reproduce *Hypancistrus* sp. L-333, the techniques currently used have not yet been fully mastered, which, once mastered, would minimize the fishing pressure on natural stocks.

As such, primary information for the development of captive fish reproduction techniques is still limited especially that regarding phenotypic character, such as sex differentiation, specimen size, coloration and secondary sexual dimorphism (Godinho et al., 2007), and the main external morphological characteristics, such as the presence of urogential papilla, modifications of pelvic and anal fins, body dichromatism, behavioral change, presence or hypertrophy of odontodes, among others (Moreira et al., 2001; Py-Daniel and Cox-Fernandes, 2005; Saurabah et al., 2013). These differentiations contribute to the attraction of animals that form groups or couples for reproductive purposes, and also aid in genetic improvement, which facilitates the choice of desired characteristics to raise the quality and commercial value of the offspring (Cnaani and Levavi-Sivan, 2009; Saurabah et al., 2013; Martínez et al., 2014).

Unfortunately, there is lack of studies on the species of Loricariidae, mainly regarding the genus *Hypancystrus*, which have not yet been formally described, and which are already strongly threatened with extinction, especially by the anthropogenic actions in the Xingu River region (its endemic region). Thus, the objective of this research was verify the existence of phenotypic differences that can be used for the differentiation between male and female individuals of *Hypancistrus* sp. "L-333" and, in this way, contribute to the identification and formation of couples in breeding periods, thereby assisting in the maintenance and development in captivity. In addition, during the study, useful information can be gathered for the conservation of the species in its natural environment.

2. Methods

2.1 Study area

The region of the Xingu River between the locations of Vitória do Xingu (2° 53’ 5.11” S, 52° 0’ 45.24” W) and Porto de Moz (1° 45’ 6.29” S, 52° 14’ 14.53” W), both in the state of Pará, is a distribution site of the target species of the study (Figure 1).

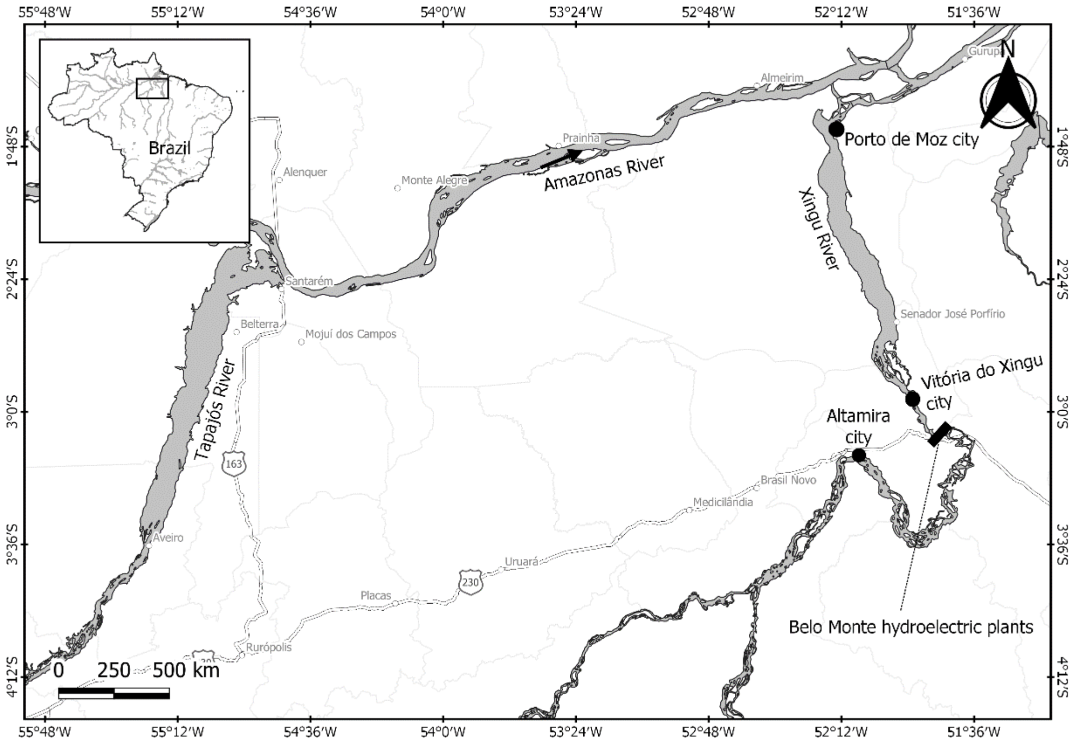

Figure 1. Map of Brazil showing details of the geographical location of the region of the lower Xingu River (area of distribution of the species *Hypancistrus* sp. “L-333”), downstream of the Belo Monte hydroelectric dam.

2.2 Sample collection and preparation

In total, 32 specimens of the King Tiger Plecos (*Hypancistrus* sp. “L-333”) were acquired from catches made by fishermen in the month of July 2014, under the zoological material collection license number 7119-1 (Authorization and Information System in Biodiversity – SISBIO/ICMBio). The individuals were transported to Belém, Pará state in accordance with the norms and criteria established in the Normative Instruction MPA and MMA Nº 1 of 03/01/2012 (MPA and MMA, 2012) and MPA Nº 21 of 11/09/2014 (MPA, 2014).

When the specimens arrived, they were quarantined for observation and then acclimatized in 110 liter aquariums (70 cm long x 45 cm deep x 35 cm wide) with a water recirculation system and constant temperature control, which was maintained around 28 °C (±0.7) and under natural lighting (12 h of light/12 h of darkness). The fish were fed with commercial feed in tablets, which was exclusive for Plecos or ornamental acari in general, and offered twice a day (9 h and 15 h), and corresponded to 2% of the biomass of each group.

After the quarantine period (30 days), the morphological analysis of the specimens to verify the existence of secondary sexual differences was performed. For this purpose, the fish were anesthetized with Tricaine-

S® (Ms 222 - tricaine methanesulfonate, Western Chemical Inc., USA), at a concentration of 100 mg per liter of water, which aided in handling. For the morphological differentiation criteria between the sexes, evidence of secondary sexual characteristics was considered, as described by Seidel (1996), Roman (2011), Py-Daniel and Cox-Fernandes (2005) for species belonging to the family *Loricariidae* of the Amazon.

For males, the characteristics included the predominance of long spines or odontodes in the mid-lateral region of the body, in the pectoral and intraopercular fin regions, as well as having larger fins, a more elongated body and a triangular head. For females, the presence of shorter spines or odontodes, a more robust body, shorter fins and a more rounded head were observed. The total length (TL) in cm and the total weight (TW) in grams were also recorded for each specimen.

After the procedures and obtention of biometric data, the specimens were euthanized with a lethal dose of anesthetic, as recommended by the American Veterinary Medical Association (AVMA, 2013). The preparations of the histological slides were performed using the techniques described by Prophet et al. (1995). Fragments of the gonads (ovaries and testicles) measuring 0.5 cm were immersed in Bouin fixative solution (saturated aqueous solution of picric acid, 36-40% formalin and glacial acetic acid) for 24 hours and submitted to the paraffin inclusion technique. The 5 µm thick gonad sections were stained in hematoxylin/eosin (HE) for observation and documentation using a Zeiss® PRIMO STAR® trinocular microscope with a Zeiss® camera. The histological procedures for the gonads were performed at the Federal Rural University of Amazonia - UFRA.

2.3 Analysis of biometric and histological data

The biometric information of the specimens were tabulated and submitted first to descriptive analysis to obtain the mean values and standard deviations of the weights and lengths of females and males. The growth models considered were of the type: $TW = aTL^b$, where TW is the total weight (g), TL is the total length (cm), a is a constant and b is the allometric coefficient (slope). In this study, the parameters a and b were determined by linear regression: $log TW = log a + b . log TL$. The 95% confidence interval (CI) was determined for parameters a and b. The Pearson correlation coefficient r-squared (r^2) was estimated. The allometric condition factor (K_{rel}) was calculated according to the equation: $K_{rel} = TW/(aTL^{b})$ (Le Cren, 1951; Froese, 2006). The differences between females and males was obtained with a covariance analysis (ANCOVA), by analyzing significant differences ($\alpha = 0.05$) between the slopes. All statistical analyses were performed in the R 4.0.0 program (R core team, 2020).

In the histological analysis, morphometric observations were performed and subsequently, microscopic analyses of the gonads (ovaries and testes) to confirm the visually observed phenotypic characteristics, according to the classification established by Vazzoler (1996), which is defined as:

Stage I: immature individuals (females with large amount of oogonia and previtellogenic oocytes; males with large amount of spermatogonia);

Stage II: maturing individuals (females with oocytes at the beginning of endogenous vitellogenesis; males with all types of cells: spermatogonia, spermatids, spermatocyte and few spermatozoa);

Stage III: mature (females with larger oocytes in protein vitellogenesis, with cytoplasm filled with globules of vitello and a membrane with two layers, the chorion and the theca; males with large amount of sperm in the lumen);

Stage IV: exhausted or spawned (females with few oocytes and large amount of empty follicles and cells in atresia; males with totally empty lumen).

3. Results

Of the 32 specimens analyzed, 16 specimens were classified as males and 16 as females (Table 1). For males, the minimum and maximum values of total length were 10.1 and 13.7 cm (mean of 11.86 cm ± 0.85) and for females the lengths were 8.0 and 10.4 cm (mean of 9.11 cm ± 0.78). The minimum and maximum weight of males was 19.5 and 38 g (mean 27.01 g ± 5.31) and for females weights were 8.9 and 16.0 g (mean 13.10 g ± 3.57). Length – weight relationships for the separate sexes and both together had significant coefficients of determination that were above 0.91. There was no significant difference in the slope between males and females (ANCOVA; $p = 0.076$). The allometric condition factor (K_{rel}) was slightly higher for females.

Table 1. Length – weight relationship parameters estimated for females, males and the total number of *Hypancistrus* sp. "L-333" in the lower Xingu River region, Pará state, Brazil. N – number of individuals; TL – total length; TW – total weight; LW – length – weight; CI – confidence interval; r^2-Pearson r – squared for log – log regression, SD – standard deviation; K_{rel} – allometric condition factor; a – intercept; b – slope.

Sex		**TL**		**TW**		**Length-weight relationship (LWR) parameters**			
	N	**Min**	**Max**	**Min**	**Max**	**a (95% CI)**	**b (95% CI)**	**r^2 (95 % CI)**	**K_{rel} (SD)**
Female	16	8.0	10.4	8.9	22.5	0.034 (0.008 – 0.142)	2.685 (2.038 – 3.332)	0.922 (0.785 – 0.973)	1.004 (0.099)
Male	16	10.1	13.7	19.5	38.0	0.061 (0.014 – 0.274)	2.458 (1.851 – 3.065)	0.918 (0.776 – 0.972)	1.003 (0.078)
Total	32	8.0	13.7	8.9	38.0	0.031 (0.019 – 0.051)	2.730 (2.521 – 2.939)	0.971 (0.942 – 0.986)	1.004 (0.089)

The growth curve and the result of the model with the two aggregate sexes are presented in Figure 2, and in most cases the males were observed at the top of the curve, while the females were at the bottom.

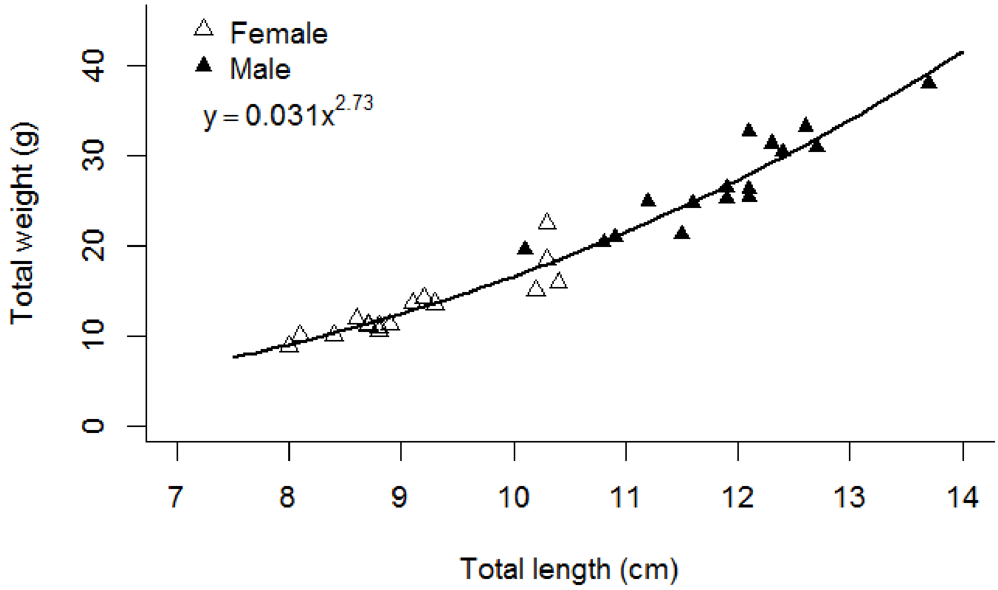

Figure 2. Length – weight relationship of all *Hypancistrus* sp. “L-333” individuals caught.

The standard coloration of males and females were similar, and they presented a pattern of black bands on a white background or light yellow background, which were observed along the entire length of the body of the fish. These bands are arranged randomly, and do not follow a pattern. They are usually arranged in six dark bands present in the dorsal fin, five to six in the pectoral fin and four to five in the pelvic fin. Six to eight dark vertical bars are also present on the caudal fin (Figure 3). In both sexes, the ventral part of the body presented a clear to light gray surface from the mouth to the urogenital region.

Characteristics of secondary sexual dimorphisms were apparent in all captured specimens, and correspond to the class of total length amplitude between 8.0 cm and 13.7 cm. Adult males have a slightly wider head than the females, with a more pronounced elevation of the supraoccipital process. There is a presence of longer odontodes in the interopercular region and in the entire distal extension of the first ray (ossified) of the pectoral fin (Figure 3A). The adipose fin of these individuals presents developed odontodes, which are more easily seen if observed horizontally (Figure 3). Males also have longer and more pronounced odontodes in the mid-lateral region of the body, especially in the post-dorsal area and caudal peduncle. These present an epidermis (bone plates with odontodes) that is dry and rough. On the other hand, in females, the odontodes are poorly developed throughout the body, presenting a less dry or rough epidermis. In males, the first rays of the caudal fin (both upper and lower) are more elongated and in the form of a small filament, while in females these caudal rays are shorter (Figure 3B).

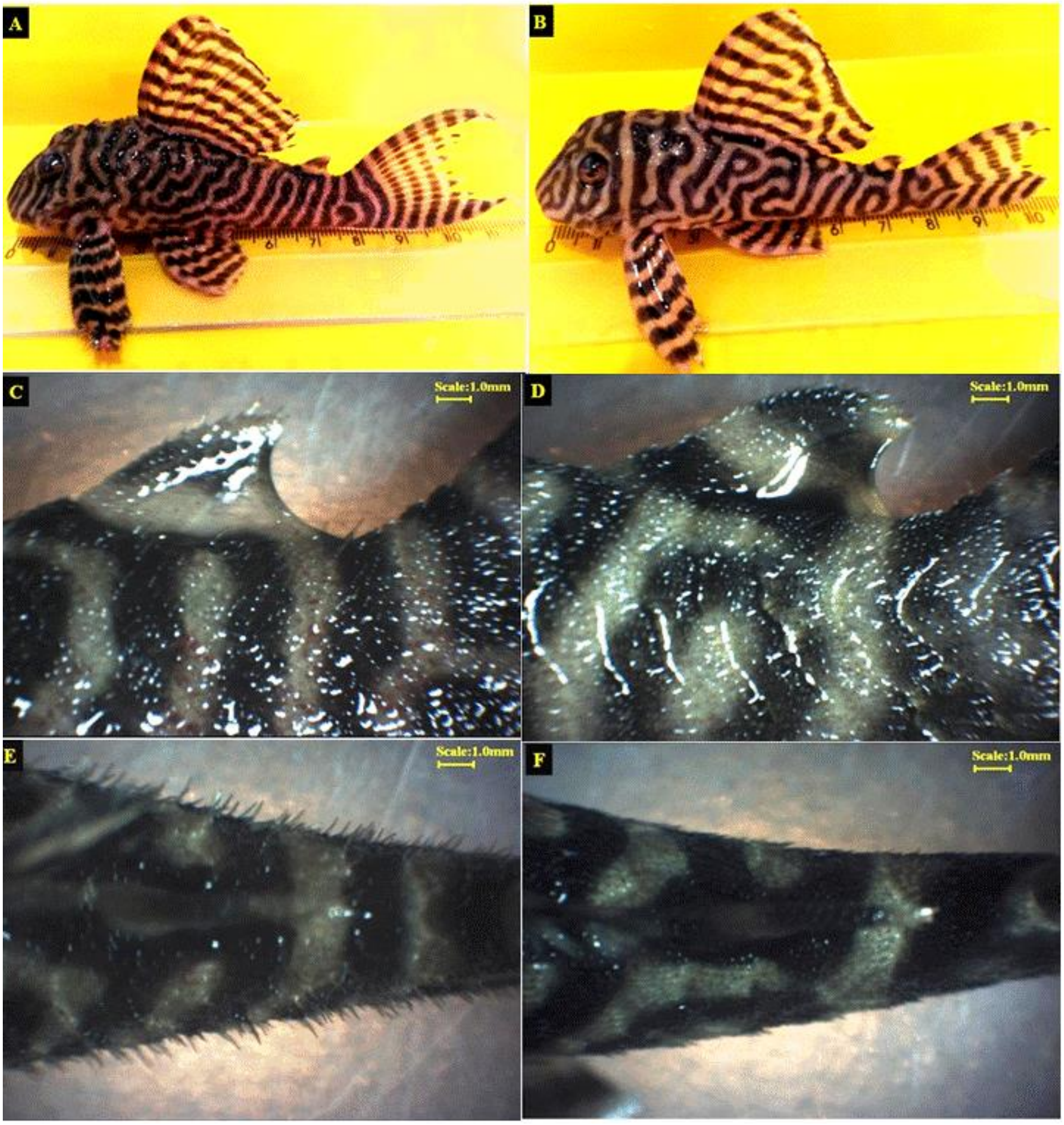

Figure 3. Male (A) and female (B) specimens of *Hypancistrus* sp. “L-333”; caudal peduncle of male (C) and female (D) observed horizontally and post dorsal region of male (E) and female (F) observed from above.

Regarding the morphology of the gonads in male individuals, the testes of *Hypancistrus* sp. L-333 are even, dorso-ventral flattened organs and are located in the posterior ventral cavity of the body, just above the intestine. They are a lobular structure consisting of seminiferous tubules, which join in the terminal portion near the urogenital opening. For female specimens, the ovaries are saculiform with opaque yellow coloration, and are covered externally by a thin peritoneum, consisting of dense, connective tissue. This membrane is directed towards the inside of the ovary and forms structures called ovule lamellae, in which the oocytes at different stages of development are located.

All male subjects analyzed were in the process of maturation (Stage II) or were mature (Stage III). Through microscopic observation of the testicles, it was possible to identify, in the seminiferous tubules, a few spermatogonias on the periphery of the tubules, exhibiting rounded cells with well-defined nucleus, which were the largest of the spermatogenic lineage (Figure 4A). It was also possible to observe spermatid cells, which are smaller than spermatogonias and are located on the margins of the seminiferous tubules (Figure 4C). In addition, most of the males analyzed had testicles with the lumen completely occupied by spermatozoa, which are smaller cells with a highly condensed nucleus and are found in the central part of the seminiferous tubule (Figure 4).

All the females analyzed were in maturation (Stage II) or were mature (Stage III) and presented oocytes in different stages of development, with diameters ranging from 0.14 to 2.0 mm. The oocytes presented three distinct diameters, and were synchronous in more than two groups, evidencing spawning of the batch type. Fecundity was low, being between 35 and 54 oocytes in the largest batch (n = 5), however the number of oocytes in the lower diameters was not counted.

In microscopic observations of the ovaries, it was possible to identify three main types of oocyte cells. The previtellogenic oocytes (PVG) were characterized by having small size (0.14 -0.20 mm) and homogeneous basophilic cytoplasm, usually a central or sub-central nucleolus. Oocytes were also found in the gonads at the beginning of vitellogenesis (IVG), with diameters of 0.38-0.74 mm, which were easily distinguished from PVG oocytes by the presence of lipid globules in the peripheral cytoplasm (endogenous vitellogenesis), and cortical alveoli that presented visible nuclei that were located in the central position (Figure 4). A large amount of oocytes were also found in protein vitellogenesis (VGP), with a diameter of 1.7 to 2.0 mm, and these presented larger oocytes, in which it was possible to observe two membrane structures: the chorion (zona radiata) and the theca (granular layer), which also presented cytoplasm that was completely filled with globules of vitello (Figure 4). No empty or postovulatory follicles were found, which would indicate specimens with gonads in Stage IV (spawned). These structures, which are visible only after spawning, are ovulatory lamellae that surround the oocytes. In addition, no cells were found in atresia (which are generally oocytes, unspawned and remain in the ovaries, have an irregular appearance, and have lost their rounded configuration) (Figure 4).

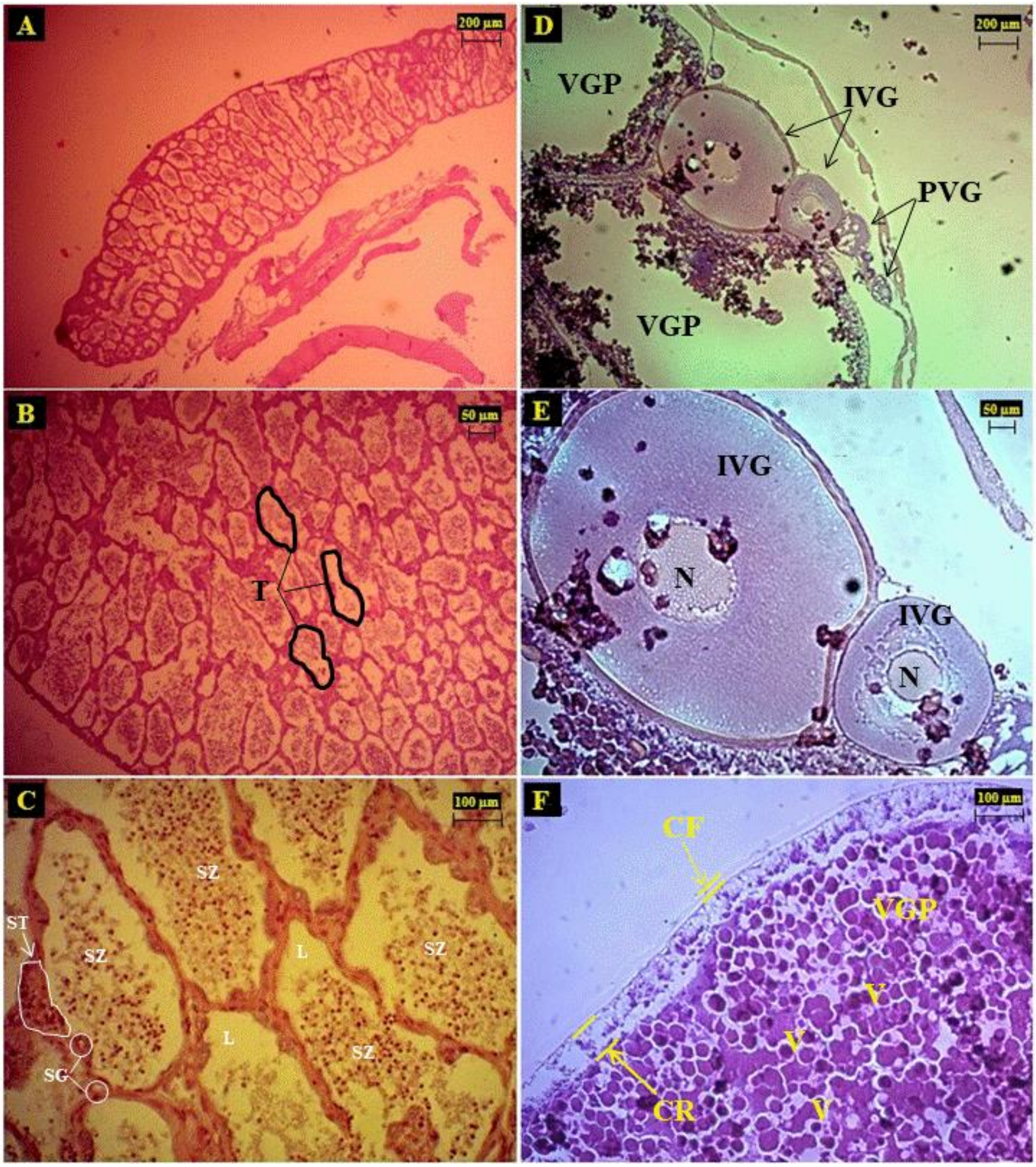

Figure 4. Testicular and oocyte photomicrography of *Hypancistrus* sp. L-333. (A) transverse aspect of the flattened form of the testicle (40x magnification); (B) presence of a large amount of seminiferous tubules (T) as exemplified in the image (100x); (C) detail of seminiferous tubules with the presence of different cell types: spermatogonia (SG), spermatids (ST) and large amount of spermatozoa (SZ) scattered in the lumen (L) (400x); (D) ovary fragment with the presence of previtellogenic oocytes (PVG), beginning of endogenous vitellogenesis (IVG) and oocytes in protein vitellogenesis (VGP) (40x); (E) detail of oocytes at the beginning of vitellogenesis (IVG) with diameters between 0.38 to 0.74 mm and cytoplasm filled with vitello – the nucleus is still visible and located in a central position (N) (100x); and (F) details of mature oocyte in protein vitellogenesis (VGP) in which it is possible to observe large amount of globules of vitello (V) and two membrane structures, the chorion or corona radiata (CR) and follicular cells (CF) (400x) with hematoxylin-eosin (HE) staining.

4. Discussion

All values for parameter B of the length – weight relationship estimates were within the expected limits (2.5 to 3.5), as suggested by Froese (2006). For the condition factor, which has been widely used to indicate changes in the well-being of fish, since it reflects the physiological condition, food availability and adaptation of fish the seasonal fluctuations that occur in the environment (Barbieri and Verani, 1987; Chellappa et al., 1995), *Hypancistrus* sp. L-333 showed no significant differences in the condition factor between the sexes. Food availability seems to have been sufficient to provide good trophic conditions and ensure the necessary energy supply to the species for the reproductive period (at least for the observed sample period).

Regarding body size, the males of *Hypancistrus* sp. L-333 were generally larger than females with classes of lengths greater than 11 cm (TL). However, the slope between the sexes was not distinct. The larger size of the male may have occurred as a result of older individuals having been captured compared to the females or be a biological attribute of the species, since it confers a competitive advantage for reproduction since the males of the family Loricariidae present parental care (Tos et al., 1997; Suzuki et al., 2000).

In males, odontodes developed along the body were also found, and was a striking feature between the sexes and, mainly in the breeding season, one that can serve in the selection of individuals. Among several genera of Loricariinae, a subfamily of Loricariidae, although there are different forms of secondary sexual dimorphism, the main ones are related to the pronounced development of odontodes in the pectoral, pelvic, and anal regions, as well as in the snout, head bones and in the caudal peduncle region (Covain and Fisch-Muller, 2007). In the present research, these characteristics were also highlighted in the studied species.

In reproductive season, the secondary sexual dimorphism, which was quite evident between the sexes of *Hypancistrus* sp. L-333, can be a useful tool in the selection and formation of couples for reproduction in captivity. The sister species, *Hypancistrus zebra*, does not present obvious sexual dimorphisms or dichromatism, which hinders the identification of the sexes (Roman, 2011). This is also the case for *H. furunculus,* which has distribution restricted to the Orinoco River, Venezuela (Armbruster et al., 2007). However, in a study conducted in the Orinoco River, sexual dimorphism was observed for the species *H. contradens, H. debilittera* and *H. lunaorum,* and nuptial males have slightly hypertrophied odontodes on the lateral plates of the head and in the caudal region (the size of the odontodes subsequently increases in the second half of the body).

Secondary sexual dimorphisms in fish are external characteristics generated by the sexual instinct that are usually easily visible and, as such, are a useful tool for gender classification among individuals. These sexual differences, depending on the species, can be observed in the morphology of the body; more specifically, in the urogenital region, modifications in the pelvic and anal fins, more pronounced coloration in one of the sexes, protrusions in the cephalic region, behavioral differences, among others (Rotta, 2004; Py-Daniel and Cox-Fernandes, 2005; Anjos and Anjos, 2006; Godinho, 2007; Godinho et al., 2010).

Several species of the family Loricariidae have structures that may indicate characteristics of secondary sexual dimorphism (Py-Daniel and Cox-Fernandes, 2005). However, many authors mention that the presence of odontodes in different parts of the body is the most striking characteristic of secondary sexual dimorphism that exist in species of Loricariidae (Rodríguez and Miquelarena, 2005; Covain and Fisch-

Muller, 2007; Pereira et al., 2007), and can be found in large species, such as the *Panaque schaeferi* that can reach a standard length of 60 cm (Lujan et al., 2010) as well as in miniature species such as *Nannoplecostomus eleonorea*, which reaches a total length of close to 3 cm (Ribeiro et al., 2012).

Odontodes can be found in both males and females of several species of the family Loricariidae (Py-Daniel and Cox-Fernandes, 2005), however, some authors mention that sexing should be done using the presence of odontodes to distinguish males and females within this family (Rodríguez and Miquelarena, 2005; Covain and Fisch-Muller, 2007; Pereira et al., 2007). Male and female *Pareiorhaphis nasuta*, for example, have odontodes in the pectoral fin region, however, these odontodes are larger in adult males and smaller in juvenile males and females, thus indicating a form of secondary sexual dimorphism (Pereira et al., 2007). A similar pattern occurs in the species *Dolichancistrus atratoensis*, *D. carnegiei*, *D. cobrensis* and *D. fuesslii*, which have odontodes in both sexes, and these structures are noticeably more developed in males compared to females (Ballen and Vari, 2012). These characteristics were also observed in *Hypancistrus* sp. L-333, in individuals above 8.0 cm (TL).

In the species of the genus *Rineloricaria*, the odontodes are also more pronounced in males during the period of reproductive activity, particularly on the the sides of the head, in the opercular region, on the dorsal surface of the pectoral fins, and in the pre-dorsal and the caudal peduncle regions (Rodríguez and Miquelarena, 2005; Covain and Fisch-Muller, 2007). This is clearly visible in the species of *Rineloricaria osvaldoi*, especially in relation to immature males and females (Fichberg and Chamon, 2008). However, there are also some species of acari of the Xingu and Tapajós Rivers in the state of Pará that present hypertrophy of the odontodes in males in reproductive age, as is the case of *Baryancistrus chrysolomus* (Py-Daniel et al., 2011) and *Peckoltia feldbergae* (Oliveira et al., 2012).

In addition to odontodes, the histology of the reproductive organs is an important tool for confirming the sex of the specimens and determining the stage of gonadal maturation, and provides direct visualization of the reproductive structures of females (oogonia, oocytes, etc.) and males (spermatogonia, spermatids, etc.) and has greater accuracy, regardless of their degree of maturation (Vazzoler, 1996; Solis-Murgas et al., 2011; Andrade et al., 2015). The present research shows that the individuals of the analyzed species captured in the flood period (July) were in the process of gonadal development. However, it is important to point out that the reproductive period of the species was not the object of the study, since the samples came from a single month of collection.

Although there were no differences in the pattern of coloration between males and females, secondary sexual dissimilarities (odontodes developed along the body and in the distal part of the first ossified ray of the pectoral fin) that are essential in the differentiation of males and females were verified. The ease in the differentiation of the sexes of *Hypancistrus* sp. L-333 in the reproductive season may present advantages for the cultivation of this species in captivity, and thus minimize the fishing pressure of these individuals in their natural environment, since this species has not yet been formally described, despite being considered abundant in the recent past (Anatole et al., 2008). On the other hand, the natural environment of this species is threatened due to the Belo Monte hydroelectric dam on the Xingu River, which affects precisely the area of the geographical distribution of the species. In this region, the flow of the Xingu River has lost its hydrological and seasonal characteristics, influencing the decrease in flow and depth (Haglund, 2014). These factors put at imminent risk the fish populations that inhabit these environments, especially the populations of small Loricariidae such as *Hypancistrus* sp. L-333.

The impacts of the Xingu River dam are already reflecting in the congener species *H. zebra*, which now has a diminished distribution in the Volta Grande stretch of the Xingu River. Although since 2004 it is forbidden to catch *H. zebra* (Normative Instruction Nº. 5, May 21st, 2004), this sister species is the most sought after among the Loricariidae in aquarism around the world, and is currently critically endangered, due to the loss of its natural habitat and illegal fishing. This can lead, in a short period of time, to the decline and or extinction of the natural population of this species (Roman, 2011).

Even though *H. zebra* is recognized by the federal government as being critically endangered, and is prohibited for exploitation (Ordinance Nº. 445, December 17th, 2014) and is listed in the Red Book of Endangered Species of Brazil (ICMBio, 2016), specimens of this species are smuggled to other countries, such as Peru and Colombia. According to Anatole et al. (2008), there are estimates that annually about 250,000 specimens of this species are smuggled from the Xingu River region, and are sold in pet shops in Europe and the United States, and have a market value of around U$ 400 dollars (Pedersen, 2016).

The results presented here can serve as basic information to aid the cultivation of *Hypancistrus* sp. L-333 in captivity, and thus minimize the impacts on the natural stocks of this species, which is threatened by the existence of the hydroelectric dam on the Xingu River. Even if this species presents low fecundity, due to the small amount of oocytes in complete vitellogenesis (between 35-54 oocytes), there are indications that it performs more than one spawning throughout its reproductive period.

The present study details an imminent reproduction phase of the species *Hypancistrus* sp. L-333 in nature, due to the larger batch of oocyte found in the females, which were in the phase of complete vitellogenesis, and a large amount of spermatozoa in the males, which characterizes preparation for the reproductive process of the species in its natural habitat. However, only the continuity of monitoring of the reproduction of the species after the full operation of the Belo Monte hydroelectric power plant, can elucidate the gaps in information on the permanence, or not, of this and other species of Loricariidae that are endemic to the Xingu River region, in the area of influence of the project, so that sustainability indicators can be analyzed for the best management of the natural stocks of King Tiger Plecos.

5. Conclusion

In the present study it was possible to determine the sex of individuals of *Hypancistrus* sp. "L-333" by using the phenotypic characteristics (confirmed by histological analysis) that exist between males and females of the King Tiger Plecos and indicate which groups of reproductive individuals can be identified through external characteristics. As such, males and females of the King Tiger Plecos can be selected for their most striking reproductive characteristics (more developed odontodes in the post-dorsal region of the body and the first ossified ray of the pectoral fin with well-developed odontodes in males), and these characteristics can be applied in obtaining groups suitable for reproduction in captivity.

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