







# Comparative analysis of the reproductive activity of *Leporinus piau* (Characiformes: Anostomidae) in lentic and lotic environments

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Submitted May 29, 2020  
Accepted October 30, 2020  
by Paulo Pompeu  
Epub December 4, 2020

In this study, we determined the main reproductive parameters of piau gordura, *Leporinus piau*, in two sections of the São Francisco River basin. Between May 2015 and April 2016, a total of 573 specimens were captured from a lentic environment (section 1), the Três Marias Reservoir (TMR), and a lotic environment (section 2), downstream of the TMR at the confluence of the São Francisco River (SFR) with the Abaeté River. Analysis of reproductive activity showed that *L. piau* from both sections reproduced, but females and males from section 1 exhibited higher total length, body weight, Fulton condition factor, and gonadosomatic index values, as compared to section 2. Sexual dimorphism was evident in the species, with females being larger than males. Moreover, males reached first gonadal maturation at a smaller size than females. The peak maturation/mature stage was observed in November/April for females and males in section 1 and in November/December in section 2, coinciding with high temperatures and precipitation in the region. In both sections of the river, *L. piau* exhibited the typical characteristics of partial spawning, with a prolonged spawning period, and preferential reproduction in lentic environments.

**Keywords:** Fecundity, Follicles, Gonadal maturation, Gonadosomatic index.

Online version ISSN 1982-0224  
Print version ISSN 1679-6225

Neotrop. Ichthyol.  
vol. 18, no. 4, Maringá 2020

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Neste estudo, determinamos os principais parâmetros reprodutivos do piau gordura, *Leporinus piau*, em duas seções da bacia do rio São Francisco. Entre maio de 2015 e abril de 2016, um total de 573 espécimes foram capturados de um ambiente lêntico (seção 1), o reservatório de Três Marias (RTM), e um ambiente lótico (seção 2), à jusante da RTM na confluência do rio São Francisco (RSF) com o rio Abaeté. A análise da atividade reprodutiva mostrou que *L. piau* se reproduz nas duas seções, mas fêmeas e machos da seção 1 apresentaram maiores valores de comprimento total, peso corporal, fator de condição de Fulton e índice gonadosomático em comparação com a seção 2. O dimorfismo sexual foi evidente na espécie com as fêmeas sendo maiores do que os machos. Além disso, os machos atingiram a primeira maturação gonadal em tamanho menor do que as fêmeas. O pico do estágio de maturação/maduro foi observado em novembro/abril para fêmeas e machos na seção 1 e em novembro/dezembro na seção 2, coincidindo com altas temperaturas e precipitação na região. Em ambas as seções do rio, *L. piau* apresentou características típicas de desova parcelada com período de desova prolongada e reproduz preferencialmente em ambientes lênticos.

**Palavras-chave:** Fecundidade, Folículos, Índice gonadosomático, Maturação gonadal.

## INTRODUCTION

The São Francisco River (SFR) is an important source of fish for Brazil. However, in recent decades, the fish populations have been dwindling due to the construction of hydroelectric dams. The Três Marias Reservoir (TMR) becomes thermally stratified in the summer; the deepest water (hypolimnion) is the coldest and is considered a lentic environment. This cold water is released by the hydroelectric plant (Sato *et al.*, 2005) and subsequently, there are several impacts on fish in the receiving river system. The main impacts seem to be on fish reproduction, as the release of this cold water acts as a flood event, which is critical for triggering reproductive migration and spawning (Arantes *et al.*, 2010; Olden, Naiman, 2010). Negative impacts have been detected immediately downstream of several hydroelectric dams in rivers around the world (Donaldson *et al.*, 2008). Further downstream, after confluence with a medium-sized tributary (Abaeté River), the SFR has a higher temperature ( $24.31 \pm 0.71$ ), a greater amount of dissolved oxygen ( $7.97 \pm 0.55$ ), and a faster flow rate ( $630.8 \pm 60.4$ ). The SFR is considered to be a lotic environment, which is favorable for fish reproduction (Bazzoli *et al.*, 2019).

Reproduction is one of the most important parameters of fish biology (Ratton *et al.*, 2003; Froese, 2006) and its success depends on the reproductive potential of the species (*i.e.*, the gonadosomatic index, condition factor, and fecundity). Reproductive potential provides useful information for the conservation of a species and the maintenance of viable natural populations (Normando *et al.*, 2009; Melo *et al.*, 2011). In the SFR Basin, the fish family Anostomidae has three genera: *Leporellus* Lütken, 1875, *Leporinus* Agassiz, 1829, and *Schizodon* Agassiz, 1829. The species piau gordura,

*Leporinus piau* (Fowler, 1941), is a medium-sized fish of importance to commercial and sport fishing in the region of the TMR (Garavello, Britski, 2003).

Previous studies of this species have only investigated lentic environments (Tavares, Godinho, 1994; Silva Filho *et al.*, 2012). Given that there have been no published studies to date comparing the reproductive activity of *L. piau* in lentic and lotic environments, the objective of this work was to analyze and compare the reproductive activity of this species in two distinct sections of the SFR basin: the TMR, a lentic environment, and the SFR, a lotic environment.

## MATERIAL AND METHODS

**Study area.** A total of 573 specimens of *L. piau* were captured from two sections of the SFR Basin: section 1 = the TMR (18°23'27"S 45°13'12"W); section 2 = 34 to 54 km downstream of the TMR, after the confluence of the SFR with the Abaeté River (18°00'49"S 45°10'51"W). Fish were sampled bimonthly from May 2015 to April 2016 using gill nets with meshes ranging from 3.0 to 7.0 cm between opposite knots. The gill nets were remained submerged for 12 h for three consecutive nights every two months. Sampling at both sites occurred in the same week. If captured alive, the fish were euthanized according to the standards of the Ethical Principles of the Animal Experimentation Guide, CONCEA (MCTI – CONCEA, 2013). The research was approved by the Committee on Ethics in the Use of Animals (CEUA PUC Minas protocol No. 021/2015).

**Sex ratio, biological indices, and biometry.** The sex ratio, being the ratio of the absolute frequency of females to males, was determined for *L. piau* samples retrieved from the two sections of the basin. All fish from each river section were dissected and measured for total length (TL), body weight (BW), and gonad weight (GW). These biometric data were used to calculate the gonadosomatic index ( $GSI = GW \times 100 / BW$ ) and the Fulton condition factor ( $K = BW \times 100 / TL^3$ ) due to the isometric growth of the species (Tavares, Godinho, 1994; Padilha *et al.*, 2013; Araújo *et al.*, 2016).

**Histology, gonadal maturation stage, and spawning type.** For histological analysis, fragments from the middle region of the ovaries and testes of samples were fixed in Bouin's fluid for 24 h. Samples were then dehydrated, embedded in paraffin, sectioned at 5  $\mu$ m thickness, and stained with hematoxylin-eosin (HE). Stages of gonadal maturation, spawning type, and frequency distributions were established based on the macro- and microscopic characteristics of the gonads and on variations in the GSI in order to determine the better breeding period and breeding site (section 1 or section 2) (Weber *et al.*, 2013; Normando *et al.*, 2014; Brandão *et al.*, 2017). The size of fish samples at the first gonadal maturation was taken as the smallest total length of females and males with gonads in the maturing/mature stage from each river section (Boncompagni-Júnior *et al.*, 2013; Brandão *et al.*, 2017).

**Histometry and fecundity.** The diameters of 50 vitellogenic follicles with little shrinkage and intact spherical shape, at the maturing/mature stage of development, were measured in samples from each section. Follicles were examined on histological slides using an Olympus BX 50 light microscope with Olympus CellSens Standard 1.9 software (Arantes *et al.*, 2010; Marcon *et al.*, 2015; Bazzoli *et al.*, 2019). To determine

fecundity, sub-samples of mature ovaries ( $n = 10$  from each section) were collected. Samples from the middle region of the ovaries were fixed in a modified Gilson solution (100 mL of 60% ethanol, 880 mL of distilled water, 15 mL of 80% nitric acid, 18 mL of glacial acetic acid, and 20 g of mercuric chloride). Dissociated vitellogenic follicles were separated and counted under a stereoscopic microscope. The number obtained in the sub-sample was extrapolated to determine the total weight of the ovaries according to the simple rule of three. Absolute fecundity (AF) was calculated using the equation:  $AF = NFO \times GW$ , where NFO is the number of vitellogenic follicles per gram of ovary. Relative fecundity (RF) was calculated using the equations TL ( $AF/TL$ ) and GW ( $AF/GW$ ) (Brandão *et al.*, 2017; Bazzoli *et al.*, 2019).

**Statistical analysis.** The average GSI for each bimester in section 1 of the TMR was compared using analysis of variance (ANOVA). After testing for normality, the data from each bimester were compared using a one-way ANOVA test followed by Duncan's test. T-tests were used to compare the biological indices (GSI and K), total length, body weight, follicular diameter, as well as absolute fecundity and relative fecundity, between sections 1 (lentic environment) and 2 (lotic environment). A significance level of  $P < 0.05$  was employed. The chi-square test ( $X^2$ ;  $P < 0.05$ ) was applied to detect possible differences in the proportions of males and females.

## RESULTS

A total of 364 specimens were collected from section 1 (222 females, 142 males) and 209 from section 2 (105 females, 104 males). There were more females than males in section 1 ( $X^2 = 17.59$ ,  $P < 0.05$ ). In section 2, there were slightly more females than males, but this difference was not statistically significant ( $X^2 = 0.005$ ,  $P > 0.05$ ).

Females and males had higher TL, BW, K, and GSI values in the TMR section (section 1) than downstream of the TMR (section 2) (Tabs. 1–2). Females had significantly higher TL and BW values (section 1, TL=  $23.10 \pm 3.10$ ; BW=  $225.50 \pm 95.60$ ; section 2, TL=  $21.60 \pm 3.30$ ; BW=  $179.00 \pm 89.30$ ) than males (section 1, TL=  $20.5 \pm 1.5$ ; BW=  $178.6 \pm 126.1$ ; section 2, TL=  $17.5 \pm 2.0$ ; BW=  $119.4 \pm 64.3$ ) in both sections. In section 1, the GSI values were higher at the bimester sample collection period (Tab. 3).

The following stages of gonadal maturation (SGM) were established for males and females: 1 = rest, 2 = maturation/mature, and 3 = spent for males and spawned for females (Figs. 1–2). Analysis of the bimonthly distribution of gonadal maturation stages showed high frequencies of resting females in July/August and males during January/February in section 2. The peak maturation/mature stage was observed in November/April for females and males in section 1 and in November/December in section 2. The spawned stage was observed during all months for section 1, while the spent stage was observed in January/February. In section 2, no samples in the spawned stage were observed in July/August and none in the spent stage were observed in November to February and July/August (Fig. 3).

The smallest male in section 1, observed in SGM 2, was 19.8 cm in TL, while in section 2, the smallest male was 17.3 cm. The smallest female in section 1 was 22.6 cm in TL, while the smallest female was 18.6 cm in section 2. All values were within the estimated size at first gonadal maturation. The mean vitellogenic follicle diameter was

significantly higher in section 1 than in section 2 (Tab. 1). AF and RF, calculated using TL and GW, were greater in section 1 ( $P > 0.05$ ) than in section 2 (Tab. 1).

**TABLE 1** | Biological parameters measured for female *Leporinus piau* captured in two sections of the São Francisco River Basin (SFR) from May 2015 to April 2016: section 1 - Três Marias Reservoir (TMR); section 2 - SFR downstream of TMR at the confluence of the SFR with the Abaeté River. N = number of fish caught; TL = total length; BW = body weight; GSI = gonadosomatic index at maturation/mature stage; K = Fulton condition factor; DF = diameter of vitellogenic follicle; AF = absolute fecundity; RF/TL = relative fecundity by TL; RF/GW = relative fecundity by gonadal weight (GW).

	Section 1 (N=222)		Section 2 (N=105)	
	Mean ± SD	Range	Mean ± SD	Range
TL	23.10 ± 3.10a	14.50 – 30.40	21.60 ± 3.30b	14.8 – 29.5
BW	225.50 ± 95.60a	35.60 – 495.00	179.00 ± 89.30b	22.50 – 429.0
GSI	12.50 ± 4.40a	3.00 – 14.20	10.70 ± 2.80b	2.00 – 14.10
K	1.70 ± 0.30	0.90 – 2.40	1.60 ± 0.30	0.20 – 2.30
DF	748.20 ± 62.70b	604.20 – 865.00	676.80 ± 732.0a	486.50 – 829.20
AF	43.23 ± 37.11	14.71 – 85.20	41.89 ± 26.21	21.36 – 78.05
RF = AF/TL	1.87 ± 855	895 – 2.69	1.61 ± 1.22	621 – 2.98
RF = AF/GW	1.98 ± 755	571 – 2.32	1.96 ± 354	170 – 2.36

Data expressed as mean ± standard deviation (SD); different letters in the same row indicate statistical differences between sampling sections (t-test;  $P < 0.05$ ).

**TABLE 2** | Biological parameters of male *Leporinus piau* captured in two sections of the São Francisco River basin (SFR) from May 2015 to April 2016: section 1 - Três Marias Reservoir (TMR); section 2 - SFR downstream of TMR at the confluence of the SFR with the Abaeté River. N = number of fish caught; TL = total length; BW = body weight; GSI = gonadosomatic index at maturation/mature stage; K = Fulton condition factor.

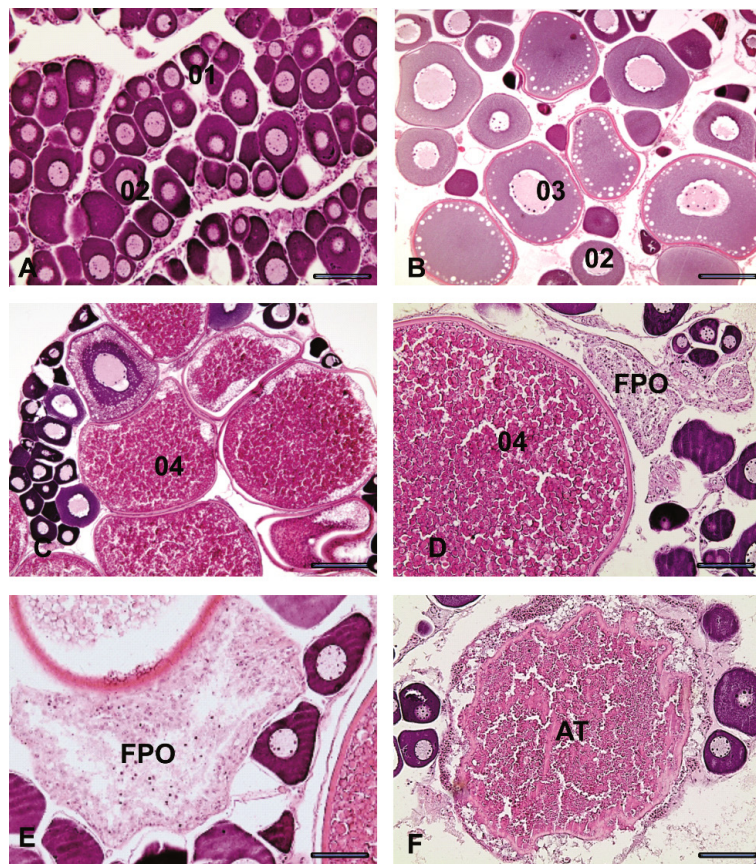
	Section 1 (N=142)			Section 2 (N=104)	
	Mean ± SD	Range		Mean ± SD	Range
TL	20.5 ± 1.5a	12.5 – 25.5		17.5 ± 2.0b	11.0 – 24.0
BW	178.6 ± 126.1a	39.0 – 2080.0		119.4 ± 64.3b	20.5 – 265.0
GSI	3.1 ± 1.5	1.18 – 7.93		3.0 ± 1.8	1.1 – 6.1
K	1.67 ± 0.9	0.5 – 17.0		1.56 ± 0.2	1.1 – 2.1

Data expressed as mean ± standard deviation (SD); different letters in the same row indicate statistical differences between sampling sections (t-test;  $P < 0.05$ ).

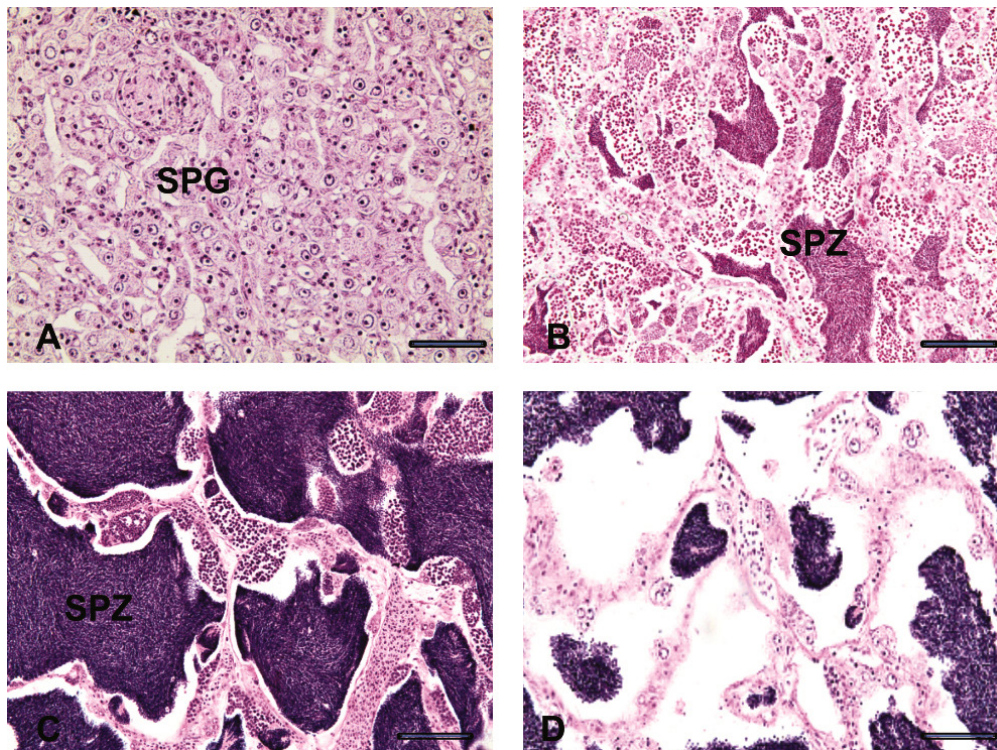
**TABLE 3** | Average gonadosomatic index (GSI) values each bimester of *Leporinus piau* females and males captured in the Três Marias Reservoir (TMR) of the São Francisco River basin (SFR) from May 2015 to April 2016.

Bimester	Females (N=222)	Males (N=142)
	IGS	IGS
May/Jun	1.75 ± 1.74 <sup>a</sup>	0.26 ± 0.15 <sup>a</sup>
Jul/Aug	0.33 ± 0.21 <sup>a</sup>	0.13 ± 0.09 <sup>a</sup>
Sep/Oct	1.08 ± 0.94 <sup>a</sup>	0.69 ± 0.46 <sup>a</sup>
Nov/Dec	9.26 ± 3.90 <sup>b</sup>	4.85 ± 1.53 <sup>b</sup>
Jan/Feb	10.50 ± 5.00 <sup>b</sup>	2.64 ± 1.42 <sup>b</sup>
Mar/Apr	5.25 ± 5.18 <sup>b</sup>	1.46 ± 1.53 <sup>a</sup>

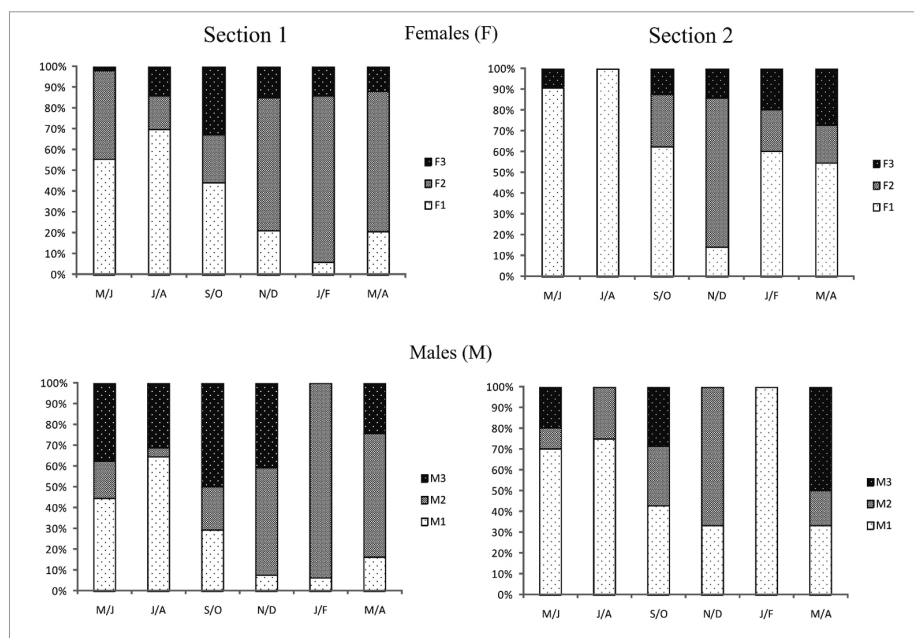
Data expressed as mean ± standard deviation (SD). Different letters in the same column indicate differences between bimesters (Duncan test;  $P < 0.05$ ).



**FIGURE 1** | Histological sections of ovaries of *Leporinus piau* stained by HE. (A) Ovary in rest (F1) with initial perinucleolar oocytes (O1) containing basophilic cytoplasm and nucleus with various nucleoli and advanced perinucleolar oocytes (O2) containing finely granular cytoplasm and nucleus with nucleoli close to the nuclear envelope. (B) Beginning of maturation with the appearance of pre-vitellogenic follicles with characteristic cortical alveoli (O3) in the peripheral ooplasm. (C) Maturation/mature (F2) with vitellogenic oocyte (O4) and cytoplasm filled with yolk globules, thin zona radiata (ZR), and squamous follicular cells. (D) Spawned (F3) with post-ovulatory follicles (POF) alongside follicles at all stages of development. (E) Detail of post-ovulatory follicle (POF). (F) Detail of atresic follicle (AF). Bars: **A** and **B** = 200µm; **C** = 300µm; **D** and **F** = 150µm; **E** = 50µm.



**FIGURE 2** | Histological sections of testes of *Leporinus piau* in different stages of gonadal maturation stained by HE. (A) At rest (M1), containing only spermatogonia (SPG) and lumen of closed seminiferous tubules. (B) Initiation of maturation with a small number of spermatozoa (SPZ) in the lumen of the seminiferous tubules. (C) Maturation/mature (M2), with seminiferous tubules filled with spermatozoa (SPZ). (D) Spent (M3), with the lumen of the seminiferous tubules open and an appreciable amount of spermatozoa. Bars: A and D = 40µm; B and C = 200µm.



**FIGURE 3** | Bimonthly distribution of stages of gonadal maturation of female and male *Leporinus piau* in sections 1 and 2 of the São Francisco River (SFR) basin from May 2015 to April 2016.

## DISCUSSION

In general, females were more predominant than males in section 1, with slightly more females than males also observed in section 2. The sex ratio in fish can vary over the life cycle, but it is usually 1:1 in a population (Vazzoler, 1996) as observed in *L. piau* in section 2 and in other fish species in the SFR (Cruz *et al.*, 1996; Ferreira *et al.*, 1996). The predominance of females in section 1 may be related to different growth percentages between the sexes, the selectivity of fishing devices, and/or population stratification (Hojo *et al.*, 2004). Similar to the findings of the present study, in areas under the influence of dams on the upper Uruguay River, females of *Acestrorhynchus pantaneiro* Menezes, 1992, are more predominant than males, indicating that the species has adapted to colonize lentic environments (Meurer, Zaniboni-Filho, 2012). Similarly, Nikolsky (1978) reported that areas with an abundance of food have higher proportions of females.

Females and males from section 1 had greater TL, BW, K, and GSI values than those in section 2, indicating negative environmental impacts downstream of the dam (Paukert, Rogers, 2004; Donaldson *et al.*, 2008), as has also been observed for *Schizodon knerii* (Steindachner, 1875) (Brandão *et al.*, 2017) and *Serrasalmus brandtii* Lütken, 1875 (Bazzoli *et al.*, 2019). According to Froese (2006), growth depends on the health of the fish as well as seasonal and environmental factors. In this study, females of both sections had greater TL and BW values than males, indicating sexual dimorphism in the species, which is typical for fish of the order Characiformes (Lowe-McConnell, 1999). Such sexual dimorphism is advantageous because fertility increases exponentially with length. In the present study, the highest GSI values for females and males were recorded in section 1, where water temperature and oxygen levels are more favorable (Freitas *et al.*, 2013; Weber *et al.*, 2013; Normando *et al.*, 2009). This finding confirms that *L. piau* preferentially favor lentic environments.

The largest females and males at gonadal maturation were recorded in section 1, which may be related to food availability and differences in environmental conditions between the studied sections (Bazzoli *et al.*, 2019). This finding could also be related to the production of sex hormones such as 17- $\beta$  oestradiol, which is responsible for both somatic growth and gonadal development (Arantes *et al.*, 2010). The present study found that males reached first gonadal maturation at a smaller size than females, as observed for other species in the SFR Basin (Brandão *et al.*, 2017; Bazzoli *et al.*, 2019).

The histological characteristics of the ovaries and testes of *L. piau* are similar to those of other species of the family Anostomidae (Rizzo *et al.*, 1996; Ricardo *et al.*, 1997; Brito *et al.*, 1999; Weber *et al.*, 2013; Brandão *et al.*, 2017). As reported for other fish, the mature ovaries of *L. piau* exhibit asynchronous development of follicles in different growth stages (*i.e.*, perinucleolar, pre-vitellogenic, and vitellogenic follicles) (Honorato-Sampaio *et al.*, 2009; Marcon *et al.*, 2015; Bazzoli *et al.*, 2019). The present study established three stages of gonadal maturation for females and males, according to the classification criteria used for other species of Anostomidae (Weber *et al.*, 2013; Brandão *et al.*, 2017). The peak maturation/mature stage was observed in November/April for females and males in section 1 and in November/December in section 2, which coincides with high temperatures and rainfall (Normando *et al.*, 2014; Brandão *et al.*, 2017; Bazzoli *et al.*, 2019).



Favorable biotic and abiotic factors are essential for triggering gametogenesis, determining gonadal maturation, and determining the spawning periods of fish (Lowe-McConnell, 1999). However, in this study, the diameter of vitellogenic oocytes and GSI differed between the analyzed sections, indicating that the environment in section 2 affects the vitellogenic follicle diameter and GSI of *L. piau*, as observed in the species *S. brandtii* (Bazzoli *et al.*, 2019). The morphological changes in vitellogenic oocytes may be caused by differences in the vitellogenin concentrations due to unfavorable environmental factors (Chakrabarty *et al.*, 2012; Marcon *et al.*, 2015). In addition, the migration of fish during the reproduction period from areas near the dam, which have lower availability of food (Albrecht, Pellegrini-Caramaschi, 2003), lower temperature, and less dissolved oxygen in the water (Abdo *et al.*, 2018), to places where environmental conditions are more favorable (Hatanaka, Galetti Jr., 2003) can cause loss of energy and can affect reproduction (Arantes *et al.*, 2010), as seen in the GSI and the diameter of the follicles; although, reproduction occurred throughout the year in *L. piau*.

The present study found relative fecundity be higher in section 1 compared to section 2. These data indicate that, as with higher GSI and vitellogenic follicle diameter, fecundity may be influenced by the more favorable reproductive conditions for *L. piau* in section 1. Physical and chemical conditions of water are known to be the main factors influencing reproductive potential, as evidenced by vitellogenic follicle number and diameter (Arantes *et al.*, 2010; Weber *et al.*, 2013). The wide range of fecundity observed in this study may be related to the release of oocytes in batches - an opportunistic reproductive characteristic of fish that spawn several times throughout the year, *i.e.*, partial spawners (Brandão *et al.*, 2017).

The results of the present study demonstrate that 1) *L. piau* reproduces in the two studied sections of the SFR Basin; 2) females and males have lower TL, BW, K, and GSI values in the lotic environment of section 2; 3) sexual dimorphism occurs in this species, with females being larger than males; 4) males reach first gonadal maturation at a smaller size than females; 5) the peak maturation/mature stage is in November/April for females and males in section 1 and in November/December in section 2, which coincides with increased temperatures and rainfall in the region; and 6) the species exhibits typical characteristics of partial spawning with a prolonged spawning period. Therefore, the two sections studied here are important for the reproduction of the species since the stretches upstream and downstream of the dam are important in the maintenance of the species. In addition, the dam environment proved to be the most favorable for the development and reproduction of the species.

## ACKNOWLEDGMENTS

The authors thank to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support, and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the concession of a scholarship.

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**Nilo Bazzoli:** Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

#### ETHICAL STATEMENT

This study was carried out according to the standards of the Ethical Principles of the Animal Experimentation Guide, CONCEA (Brazil, 2013). The sampling protocol was approved by the Committee on Ethics in the Use of Animals (CEUA PUC Minas protocol No. 021/2015).

#### COMPETING INTERESTS

We declare that we have no conflicts of interest.

#### HOW TO CITE THIS ARTICLE

Nascimento AV, Marcon L, Santos JE, Santiago KB, Rizzo E, Bazzoli N. Comparative analysis of the reproductive activity of *Leporinus piau* (Characiformes: Anostomidae) in lentic and lotic environments. *Neotrop Ichthyol.* 2020; 18(4):e200091. <https://doi.org/10.1590/1982-0224-2020-0091>

## Neotropical Ichthyology



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Official Journal of the Sociedade Brasileira de Ictiologia