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ZOOLOGY

Reproduction and fecundity of invader of internal insemination *Trachelyopterus galeatus* in a Neotropical floodplain

Amanda Borges dos Santos Schmidt[1], Vanessa de Brito Pereira[1], Arthur Henrique de Sousa Antunes[1], Ana Luiza Faria Bernardes[1], Herick Soares de Santana[2] and Claudenice Dei Tos [1*]

[1]Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura, Departamento de Biologia, Centro de Ciências Biológicas, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. [2]Departamento de Áreas Acadêmicas, Instituto Federal de Goiás, Águas Lindas de Goiás, Goiás, Brazil. *Author for correspondence. E-mail: claudenicedeitos@uol.com.br

ABSTRACT. After the formation of Itaipu Reservoir, the invader *Trachelyopterus galeatus* colonized the upper Paraná River. Light microscopy was used to describe gametogenesis and the reproductive phases of females and males. The following data were verified: diameter of the oocytes, spawning type, batch fecundity by ovary weight, standard length, and total weight of the fish, along with the regions where this species reproduced in the upper Paraná River floodplain. A total of 470 specimens were collected quarterly in 2016, 2018 and 2019, and bimonthly in 2017. The gonads were fixed in a Karnovsky solution, dehydrated, infiltrated, and embedded in historesin. The histological slides were stained using PAS + iron hematoxylin + metanil yellow, analyzed and photographed under an image-capturing microscope. As regards diameter of the oocytes and fecundity estimates, ovaries whose oocytes were measured under a stereomicroscope were sampled. In the oogenesis, undifferentiated and differentiated oogonia, early primary growth oocytes, secondary growth oocytes, full-grown oocytes and maturing oocytes were recorded. In the spermatogenesis, primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa were recorded. The reproductive phases found for females and males were: immature, early development, late development, spawning/sperm-releasing capable, regression, and regeneration. *Trachelyopterus galeatus* prefers to occupy and reproduce in the Ventura, Patos, Guaraná, Fechada, Garças, and Pau Véio lagoons. The diameter of the oocytes varied from 0.4 to 2.9 mm. Females spawn, on average, 113 oocytes per batch. Batch fecundity variation shows that the larger the ovary, standard length, and total weight, the larger the number of oocytes to be spawned. This invader possesses reproductive success in the upper Paraná River floodplain, especially in lagoons.

Keyword: Oogenesis; spermatogenesis; fish fecundity; spawning type; reproductive phases.

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Introduction

Among the main threats to biodiversity are biological invasions, which can cause great damage to native species in both terrestrial and aquatic environments (Ehrenfeld, 2010; Simberloff et al., 2013). Species habitat change can have diverse causes, such as the release of animals held in captivity, livestock farming and exotic plant cultivation, or even removal of environmental barriers (Vitule, 2009).

Once a species arrives in a new habitat and manages to establish and maintain viable populations, it will probably cause serious damage to this environment, such as reduction in native populations, extinctions, exacerbated levels of competition, and rapid occupation of the environment (Suarez & Tsutsui, 2008; Pysek, Blackburn, García-Berthou, Perglová, & Rabitsch, 2017), as has occurred with the invasive species of piranha *Serrasalmus marginatus* in the upper Paraná River (Rodrigues, Santana, Baumgartner, & Gomes, 2018).

Another example of a fish invader is *Trachelyopterus galeatus* (Linnaeus, 1766) (Siluriformes, Auchenipteridae) (Reis, Kullander, & Ferraris, 2003; Ferraris, 2007), synonym of *Parauchenipterus galeatus* (Graça & Pavanelli, 2007). This species occurs in several freshwater ecoregions of South America (Graça & Pavanelli, 2007; Ota, Depra, Graça, & Pavanelli, 2018). It occupied the Lower Parana ecoregion in the Paraná River basin; and after the formation of Itaipu Reservoir and the flooding of Saltos de Sete Quedas, the species invaded and colonized the Upper Parana ecoregion (Júlio Jr, Dei Tos, Agostinho, & Pavanelli, 2009; Garcia, Vidotto-Magnoni, & Orsi, 2019), becoming the third most abundant species in the upper Paraná River floodplain (Tonella et al., 2018). This sedentary species has internal fertilization (Meisner, Burns, Weitzman, & Malabarba, 2000; Suzuki, Vazzoler, Marques, Lizama, & Inada, 2004; Lahnsteiner & Patzner, 2009; Sousa, Mendes, Pereira, Fernandes, & Bentes, 2016; Lemes, Vizioli, Marcon, & Bazzoli, 2016), omnivorous feeding habits, and feeds, in the upper Paraná River floodplain, on terrestrial and aquatic plants, algae, fish, bivalves, gastropods, detritus, and arthropods (especially insects) (Tonella et al., 2018).

Trachelyopterus galeatus reaches sexual maturity at 10.8 cm (females) and 11.3 cm (males) standard length, and the population, investigated through macroscopic studies of the gonads, reproduces from October to April, during the flood period in the upper Paraná River floodplain (Suzuki et al., 2004). The reproductive period, and occurrence and reproduction sites, can vary from year to year due to the prevailing environmental conditions. Macroscopic evaluation of the male and female gonads was done using gonadal indices to determine the spawning period of this species, which reproduces in January and February during the rainy season in Pereira de Miranda Weir in the municipality of Petecoste (Ceará State) (Silva & Viana, 2003). On the other hand, macroscopic studies of the male and female gonads carried out in Extremoz Lagoon in Rio Grande do Norte state revealed that this species reproduces in May, June and July during the rainy period (Medeiros, Chellappa, & Chellappa, 2003). However, in the Marine Extractive Reserve of Tracuateua, in the municipality of Tracuateua (Pará state), macroscopic analyses of the gonads showed that both sexes reached sexual maturity at 10.5 cm total length and reproduced during the rainy season from July to September (Sousa et al., 2016). Evaluation of the gonadal maturation phases carried out under light microscopy, in Irapé Reservoir (Jequitinhonha River basin/Minas Gerais state), showed that this species reproduces from November to February during the rainy season (Lemes et al., 2016). The development patterns of the oocytes can be synchronous, group-synchronous or asynchronous (Murua et al., 2003; Lowerre-Barbieri, 2009). The development of synchronous oocytes is typical of semelparous species, i.e. those that present a single reproductive event over their lifetime, with all of the oocytes developing at the same time. On the other hand, iteroparous species, i.e. those that present multiple reproductive events over their lifetime, can have synchronous (determinate fecundity) or asynchronous (indeterminate fecundity) oocyte development. Fish with determinate fecundity can be total spawners, i.e. release eggs in a single event in the reproductive season, or batch spawners over the reproductive season. Fish with indeterminate fecundity release eggs in batches over an extensive reproductive season (Vazzoler, 1996; Lowerre-Barbieri, 2009). Reproductive aspects of these species were studied by Melo et al. (2011), Chiarini-Garcia, Vieira, and Godinho (2014) and Lemes et al. (2016), who show reproductive characteristics that allow the maintenance of the viable male gametes stored by the female until the moment of fertilization. Therefore, this study intends to: i) characterize gametogenesis through light microscopy and verify the reproductive phases of this species based on this information; ii) estimate oocyte diameter and verify if oocyte development is synchronous (determinate fecundity) or asynchronous (indeterminate fecundity); iii) verify the reproduction sites in the Paraná, Baía, and Ivinheima rivers; iv) estimate batch fecundity by gonad weight, total length, and total weight of the fish.

Material and methods

The study was carried out in the upper Paraná River floodplain. The floodplain possesses three conservation units (*Parque Nacional de Ilha Grande*, *Área de Proteção Ambiental das Ilhas e Várzeas do rio Paraná*, and the *Parque Estadual do Ivinheima*), characterized by being one of the few remaining lotic areas of the upper Paraná River (Agostinho & Zalewski, 1996). They are essential for the maintenance of the biological communities, which depend on the flow and hydrodynamics of the Paraná River to carry out their specific and ecological functions.

Samplings were carried out at nine sites: Baía River (Rbai) (1) 22°43'23.16"S; 53°17'25.5"W; Ivinheima River (Rivi) (2) 22°47'59.64"S; 53°32'21.3"W; Paraná River (RPar) (3) 22°45'39.96"S; 53°15'7.44"W; Guaraná Lagoon (Lgua) (4) 22°43'16.68"S; 53°18'9.24"W. Patos Lagoon (Lpat) (5) 22°49'33.66"S; 53°33'9.9"W. Garças Lagoon (Lgar) (6) 22°43'27.18"S; 53°13'4.56"W; Pau Véio Lagoon (Lpve) (7) 22°44'50.76"S; 53°15'11.16"W; Fechada Lagoon (Lfec) (8) 22°42'37.92"S; 53°16'33.06"W and Ventura Lagoon (Lven) (9) 22°51'23.7"S; 53°36'1.02"W (Figure 1).

The sampling period covered February, June/July, September and November/2016, March and September/2017 and March, June, September and November/2018 and March, June, September and November/2019. The specimens were caught using gillnets (length: 20 m; meshes: 2.4 to 16 cm between opposite knots), which were exposed for 24 hours and inspected at 8 a.m., 4 p.m. and 10 p.m. The samples were sent to the *Base Avançada do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura* (Nupélia) of the

Universidade Estadual de Maringá (UEM), situated in the municipality of Porto Rico (Paraná State). The fish were euthanized using a benzocaine solution, according to the protocols of the Animal Ethics Committee (*Comissão de Ética no Uso de Animais* (CEUA) (Protocol n° 051/2010-PPG/UEM).

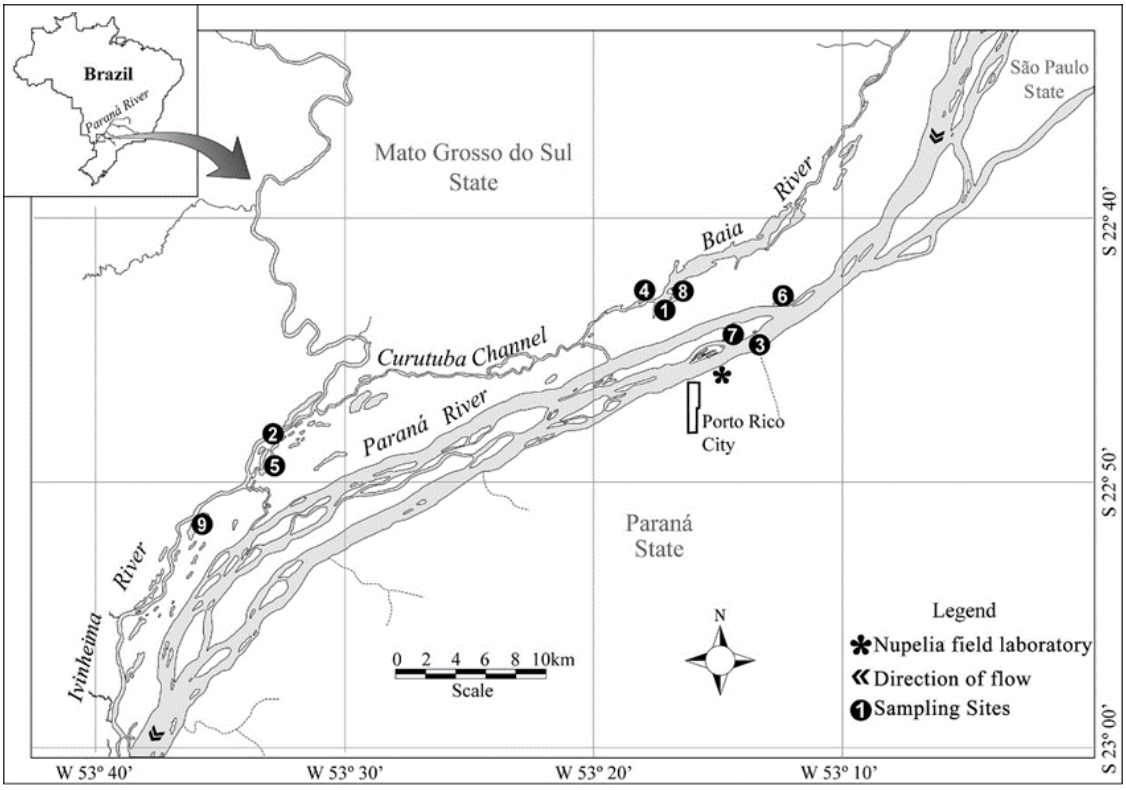

Figure 1. Sampling areas of the invader *Trachelyopterus galeatus*: Ivinheima River [Patos Lagoon (Lpat) (5), Ventura Lagoon (Lven) (9), Ivinheima River (Rivi) (2)], Baía River [Fechada Lagoon (Lfec) (8), Guaraná Lagoon (Lgua) (4), Baía River (Rbai) (1)] and Paraná River [Garças Lagoon (Lgar) (6), Pau Véio (Lpve) (7), Paraná River (RPar) (3)].

Standard length (cm), total weight (g), gonad weight (g) and gonad development phase were later recorded from each specimen. The reproductive phases were attributed through macroscopic characteristics of the gonads (color, vascularization, gonad shape, occupation of the abdominal cavity, flaccidity, and oocyte visualization). Histological analyses were carried out by immersing gonad samples in a modified Karnovsky solution (Karnovsky, 1965) using 2% glutaraldehyde, 4% paraformaldehyde, and Sorensen's phosphate buffer (0.2M at pH 7.2) for 24 hours. The fixed gonad samples were routinely processed by dehydration in a graduated ethanol series (70% to 95%) and embedded in historesin (glycol metacrylate) + 95% alcohol for 12 hours and later infiltrated in historesin (glycol metacrylate). The samples were sectioned using a microtome with a glass knife (5 µm thick). The histological sections were stained using periodic acid-Schiff + iron hematoxylin + metanil yellow (Quintero-Hunter, Grier, & Muscato, 1991). Histological assessment of the gonad sections was carried out under light microscopy. Digital documentation of the gonad images was acquired using a microscope equipped with a Nikon Eclipse camera. These images were used to identify and describe the germ cells of the males and females and the reproductive phases, as well as verify standardized scales using the measurement program Image-Pro Plus (v. 6.0, Media Cybernetics, Bethesda, MD, USA). Terminologies to describe the morphology of the male germ cells follow Grier, Aranzábal, and Patiño (2009a) and Quagio-Grassiotto, Wildner, and Ishiba (2013). The terminologies to describe the morphology of the oogonia and oocytes follow Quagio-Grassiotto, Grier, Mazzoni, Nobrega, and Amorim (2011). The terminologies of the reproductive phases follow Brown-Peterson, Wyanski, Saboridorey, Macewicz, and Lowerre-Barbieri (2011), Quagio-Grassiotto et al. (2013) and Dei Tos, Santana, Antunes, and Bernardes (2021). The reproductive areas were evaluated through the number of individuals in different phases of reproduction in different sampling locations in the upper Paraná River floodplain. Fecundity evaluation was carried out on 29 ovaries fixed in 10% buffered formaldehyde. Batch fecundity was estimated and evaluated by ovary weight (g), standard length (cm), and total fish weight (g) according to Vazzoler (1996). The quantitative data of the batch fecundity were evaluated through variations in the size range, mean, and standard deviation using the program Statistica 7.0.

Results

A total of 470 (242 female; 228 male) gonads were histologically evaluated using light microscopy. The germ cells of the females are illustrated and described in Figure 2.

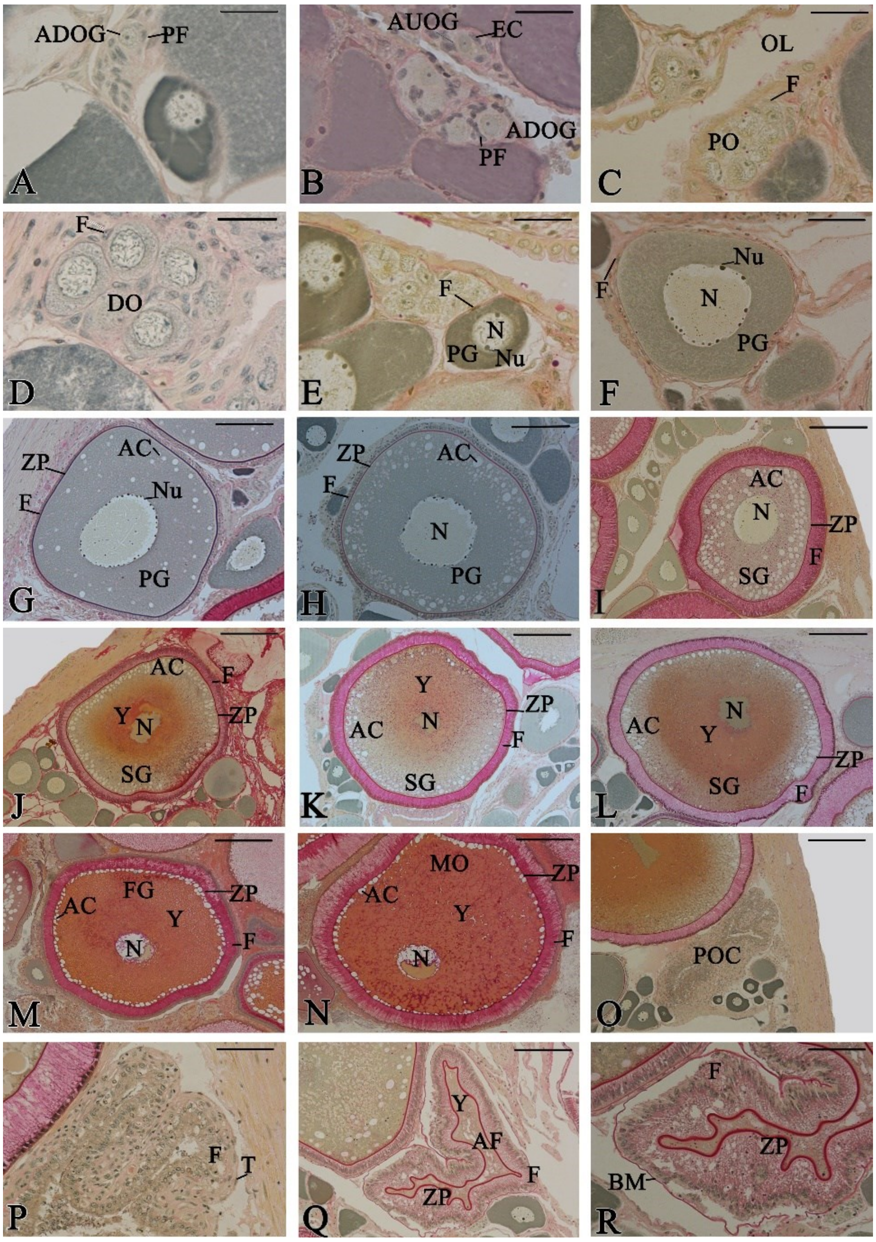

Figure 2. Oocyte development stages of the invader *Trachelyopterus galeatus* sampled in the upper Paraná floodplain. Staining: PAS + iron hematoxylin + metanil yellow. (A) Differentiated oogonia with prefollicle cells, bar = 25 µm. (B) Undifferentiated and differentiated oogonia with prefollicle cells, bar = 25 µm. (C) Cyst with pachytene oocytes surrounded by follicle cells, bar = 25 µm. (D) Cyst with diplotene oocytes surrounded by follicle cells, bar = 25 µm. (E) Primary growth oocytes with basophilic ooplasm and nucleus with two peripheral nucleoli, bar = 25 µm. (F) Primary growth oocyte with basophilic ooplasm and nucleus with perinuclear nucleoli, bar = 62.5 µm. (G) Early primary growth oocyte with basophilic ooplasm, early formation of cortical alveoli, and zona pellucida, bar = 125 µm. (H) Early primary growth oocyte with basophilic ooplasm and increase in the number of cortical alveoli, bar = 125 µm. (I) Secondary growth oocyte with ooplasm replete with cortical alveoli, vestigial yolk, zona pellucida, and well-developed follicle cells, bar = 250 µm. (J) Secondary growth oocyte showing early vitellogenesis, bar = 250 µm. (K) Secondary growth oocyte with intermediate vitellogenesis, bar = 250 µm. (L) Secondary growth oocyte with late vitellogenesis, bar = 250 µm. (M) Full-grown oocyte with ooplasm replete with yolk, peripheral cortical alveoli, nucleus slightly eccentric, bar = 250 µm. (N) Maturing oocyte with nucleus migrating to the animal pole and almost reaching the periphery of the ooplasm, bar = 250 µm. (O) Postovulatory follicle forms after the release of the mature oocyte into the ovarian lumen, bar = 125 µm. (P) Detail of a postovulatory follicle showing follicular cells and theca, bar = 62.5 µm. (Q) Atretic follicle, bar = 125 µm. (R) Detail of atretic follicle showing the zona pellucida and vestiges of yolk, bar = 62.5 µm. OL, ovarian lumen; AUOG, undifferentiated oogonia; ADOG, differentiated oogonia; EC, epithelial cell; PF, pre-follicular cell; PO, pachytene oocyte; DO, diplotene oocyte; N, nucleus; Nu, nucleolus; PG, primary growth oocyte; CA, cortical alveoli; F, follicular cells; Y, yolk; SG, secondary growth oocyte; FG, full-grown oocyte; MO, maturing oocyte; POC, postovulatory follicle complex; BM, basal membrane; AF, atretic follicle.

The following were recorded in the evaluation of the reproductive phases of *T. galeatus* females: immature (Figures 3A-C), early development (Figures 3D-F), late development (Figures 3G-I), spawning capable (Figures 3J-L), regressing (Figures 3M-O), and regenerating (Figures 3P-R) ovaries.

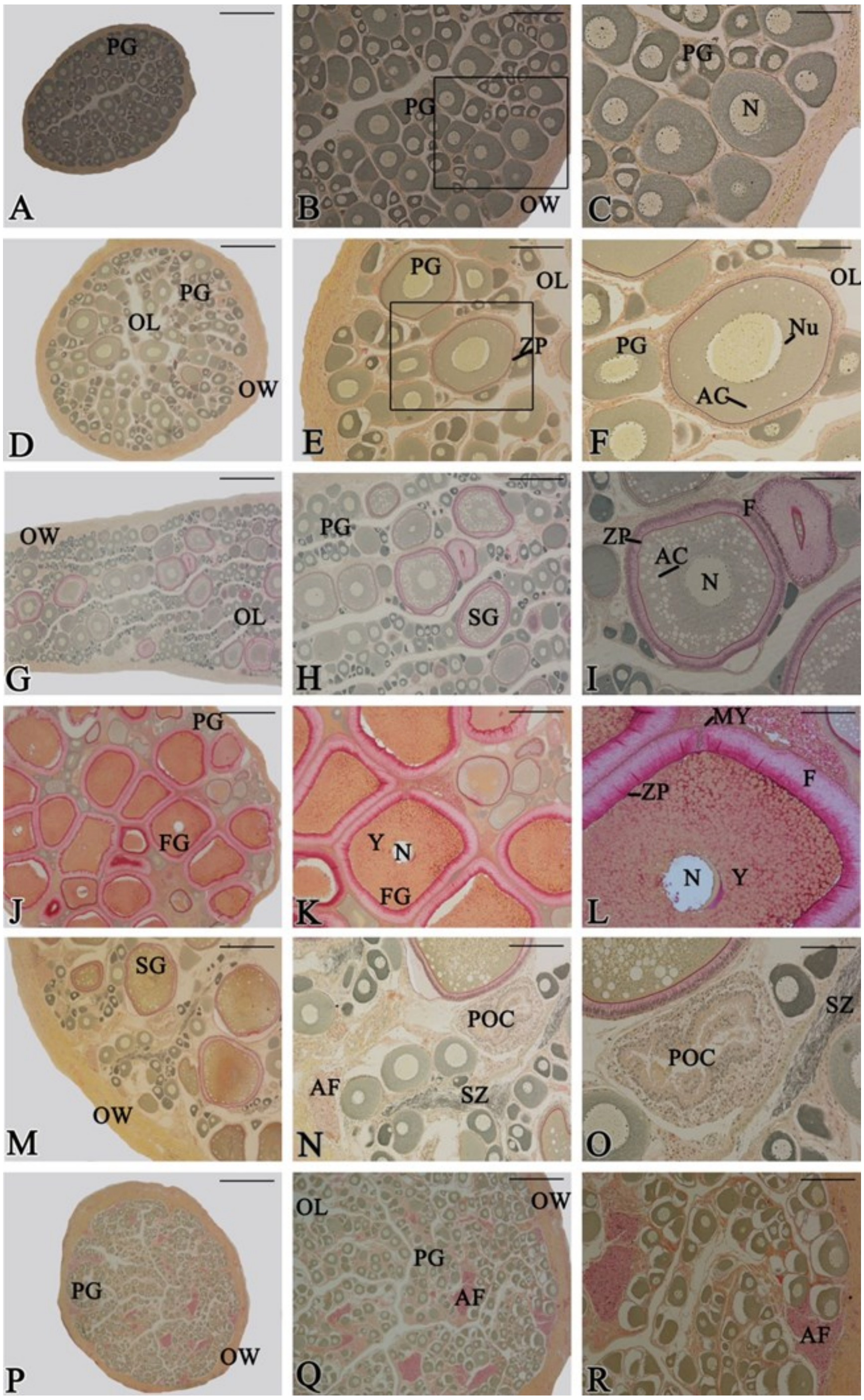

Figure 3. Reproductive phases of invader *Trachelyopterus galeatus* females sampled in the upper Paraná River floodplain. Staining: PAS + iron hematoxylin + metanil yellow. (A-F, J-R, cross section; G-I, longitudinal section). (A, B, C) Immature, Primary growth oocytes with basophilic ooplasm, (A) bar = 615 μm, (B) bar = 250 μm, (C) bar = 125 μm. (D, E, F) Early development, Primary growth oocytes with initial formation of cortical alveoli and zona pellucida, (D) bar = 615 μm, (E) bar = 250 μm, (F) bar = 125 μm, (G, H, I) Late development, Primary growth oocytes and secondary growth oocytes replete with cortical alveoli and developed follicular cells, (G) bar = 1250 μm, (H) bar = 615 μm, (I) bar = 250 μm. (J, K, L) Spawning capable, predominance of late vitellogenic and full-grown oocytes, with detail of the micropyle, (J) bar = 1250 μm, (K) bar = 615 μm, (L) bar = 250 μm. (M, N, O) Regression, Primary growth oocytes predominate, with a few secondary growth oocytes, postovulatory follicles, and spermatozoa, (M) bar = 1250 μm, (N) bar = 615 μm, (O) bar = 250 μm. (P, Q, R) Regeneration, Occurrence of only primary growth oocytes from atretic follicles, (P) bar = 1250 μm, (Q) bar = 615 μm, (R) bar = 250 μm. OL, ovarian lumen; PF, pre-follicular cell; OG, oogonia; OW, ovarian wall; N, nucleus; Nu, nucleolus, PG, primary growth oocyte; CA, cortical alveolus; F, follicular cells; Y, yolk; SG, secondary growth oocyte; FG, full-grown oocyte; POC, postovulatory follicular complex; AF, atretic follicle.

Characterizing the germ cells of the testicles of *T. galeatus*, the present study found the following: primary spermatogonia (Figures 4A, G), secondary spermatogonia (Figures 4B), primary spermatocytes (Figures 4C, G), secondary spermatocytes (Figures 4D, G), spermatids (Figures 4E, G), and spermatozoa (Figures 4F, G).

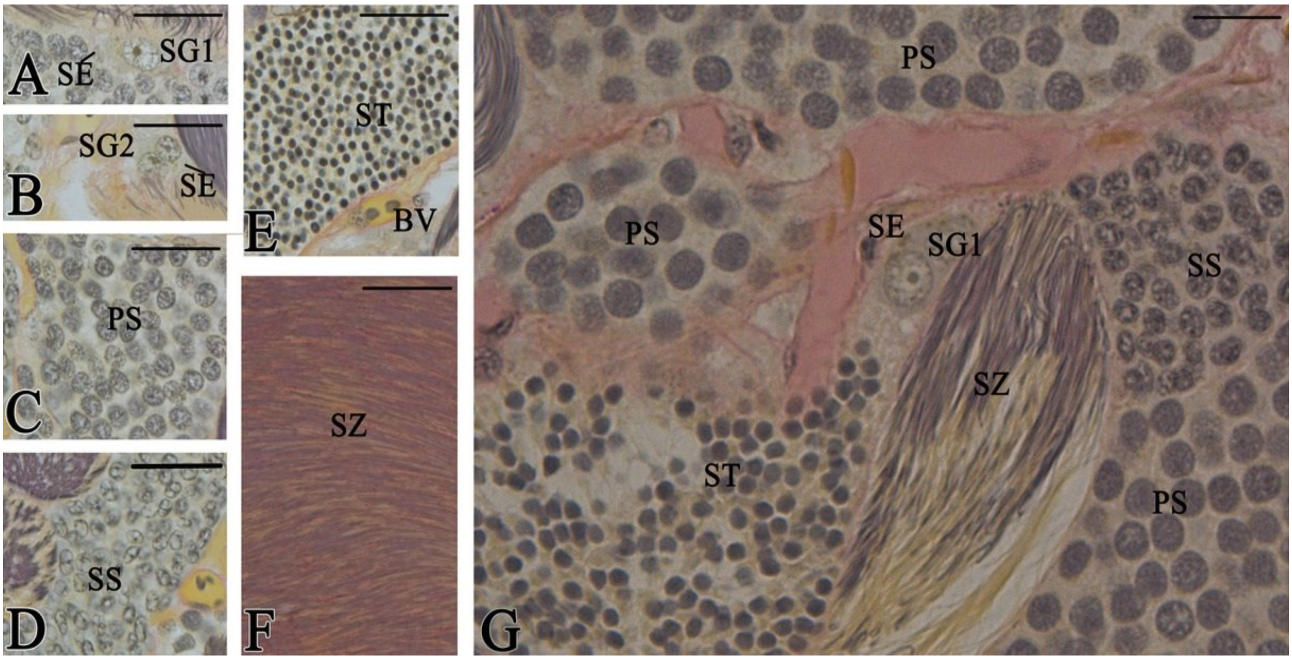

Figure 4. Types and morphology of germ cells during the spermatogenesis of the invader *Trachelyopterus galeatus* in the upper Paraná River floodplain. Staining: PAS + iron hematoxylin + metanil yellow. Scales (A to F) bar = 25 μm and (G) bar = 62.5 μm. (A) Primary spermatogonia are the largest cells of the germinative epithelium, surrounded by Sertoli cells, with granular cytoplasm, a spherical central nucleus, and a nucleolus. Spermatogonia proliferate mitotically and originate secondary spermatogonia. (B) Secondary spermatogonia, slightly smaller than the primary spermatogonia, spherical and surrounded by Sertoli cells, with a spherical nucleus and a nucleolus. They divide mitotically several times before beginning meiosis. (C) Primary spermatocytes are slightly smaller than the secondary spermatogonia, without Sertoli cells; and after completing the first division of meiosis, originate secondary spermatocytes. (D) Secondary spermatocytes are slightly smaller than the primary spermatocytes, spherical, divide rapidly through the second meiotic division, giving origin to spermatids. (E) Spermatids are smaller than secondary spermatogonia, spherical, do not divide, but transform into spermatozoa through spermatogenesis. (F) Spermatozoa are smaller than spermatids and become mobile through the development of the flagellum. SG1, primary spermatogonia; SG2, secondary spermatogonia; SE, Sertoli cells; PS, primary spermatocytes; SS, secondary spermatocytes; ST, spermatids; SZ, spermatozoa.

Evaluating the reproductive phases of *T. galeatus* males, the present study found: immature (Figures 5A-C), early development (Figures 5 D-F), late development (Figures 5G-I), sperm-releasing capable (Figures 5J-L), regressing (Figures 5M-O), and regenerating (Figures 5P-R) testicles.

In the lumen of the ovary in the late development phase (Figure 6) the spermatozoa were found associated with ovarian lamellae forming spermatozeugmata.

Trachelyopterus galeatus occurred in every environment sampled in the floodplain and reproducing females and males were recorded in the Ventura, Patos, Guaraná, Fechada, Garças, and Pau Véio lagoons (Figure 7). Lower reproduction was recorded in the Ivinheima and Baia rivers. Juveniles were more abundant in the lentic environments of the Ventura, Guaraná, Fechada, and Patos lagoons, while few occurred in the rivers (Figure 7).

The relative frequency distribution per diameter class of oocytes from *T. galeatus* ovaries (Figure 8) shows that the diameter of the oocytes varied from 0.4 to 2.9 mm (n = 6852 oocytes; average diameter = 1.3 mm; standard deviation = 0.3) and confirms the hypothesis that this species has synchronous oocyte development, with determinate fecundity and batch spawning over its reproductive period.

The variation of batch fecundity by gonad weight shows that the larger the gonad (Figure 9A), fish length (Figure 9B), and total fish weight (Figure 9C), the larger the number of oocytes found in the batch to be spawned. On average, there were 113 oocytes per batch (minimum = 52; maximum = 249).

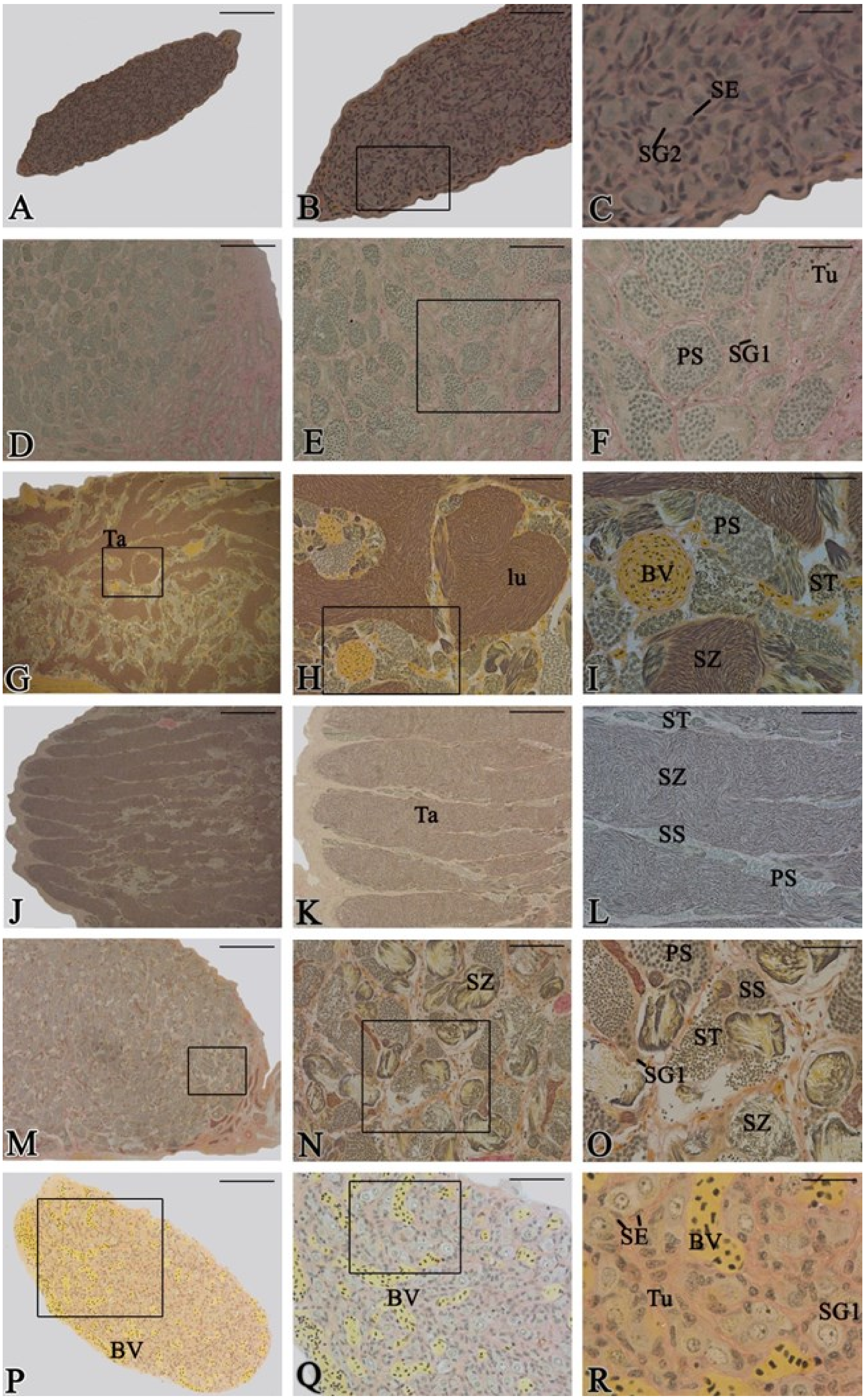

Figure 5. Reproductive phases of invader *Trachelyopterus galeatus* males in the upper Paraná River floodplain. Staining: PAS + iron hematoxylin + metanil yellow. (A-R, longitudinal section). (A, B, C) Immature, secondary spermatogonia predominate, (A) bar = 125 µm, (B) bar = 62.5 µm, (C) bar = 25 µm. (D, E, F) Early development, with primary and secondary spermatocytes, (D) bar = 250 µm, (E) bar = 125 µm, (F) bar = 62.5 µm. (G, H, I) Late development, with primary and secondary spermatocytes, spermatids, and initial formation of spermatozoa, (G) bar = 615 µm, (H) bar = 125 µm, (I) bar = 62.5 µm, (J, K, L) Sperm-releasing capable, anastomosing seminiferous tubules are replete with spermatozoa, having cysts with primary and secondary spermatocytes and spermatids, (J) bar = 615 µm, (K) bar = 250 µm, (L) bar = 125 µm. (M, N, O) Regression, the seminiferous tubules are emptier, with spermatozoa, (M) bar = 615 µm, (N) bar = 125 µm, (O) bar = 62.5 µm. (P, Q, R) Regeneration, discrete seminiferous tubules with empty lumen, primary spermatogonia, and smaller blood vessels between the seminiferous tubules, (P) bar = 125 µm, (Q) bar = 62.5 µm, (R) bar = 25 µm. SG1, primary spermatogonia; SG2, secondary spermatogonia; SE, Sertoli cells; PS, primary spermatocytes; SS, secondary spermatocytes; ST, spermatids; SZ, spermatozoa, AT, anastomosing tubules; BV, blood vessel; LU, seminiferous tubule lumen.

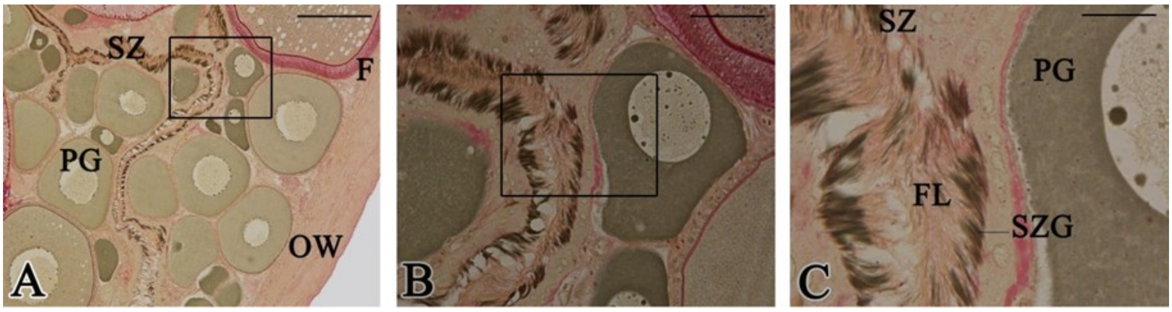

Figure 6. Ovary section in the late development phase of *Trachelyopterus galeatus* in the upper Paraná River floodplain. Staining: PAS + iron hematoxylin + metanil yellow. (A-C, cross section). (A, B, C) Ovary showing (SZG) spermatozeugmata associated with ovarian lamellae. (A) bar = 250 µm, (B) bar = 62.5 µm, (C) bar = 25 µm. PG, primary growth oocyte; SZ, spermatozoa; FL, flagellum; OW, ovarian wall; F, follicular cells.

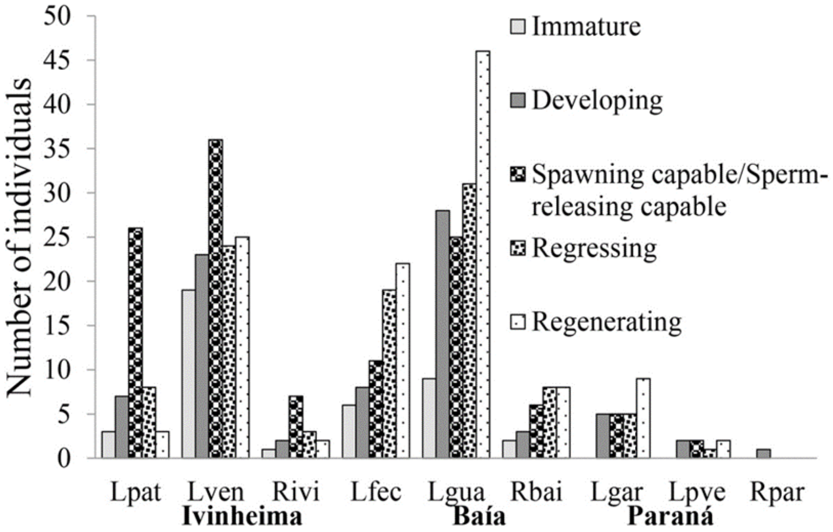

Figure 7. Number of individuals per reproductive phase of invader *Trachelyopterus galeatus* females and males in the upper Paraná River floodplain. Rivers: Ivinheima [(Patos Lagoon (Lpat), Ventura Lagoon (Lven), Ivinheima River (Rivi)], Baía [(Fechada Lagoon (Lfec), Guaraná Lagoon (Lgua), Baía River (Rbai)], and Paraná [(Garças Lagoon (Lgar), Pau Véio Lagoon (Lpve), Paraná River (Rpar)].

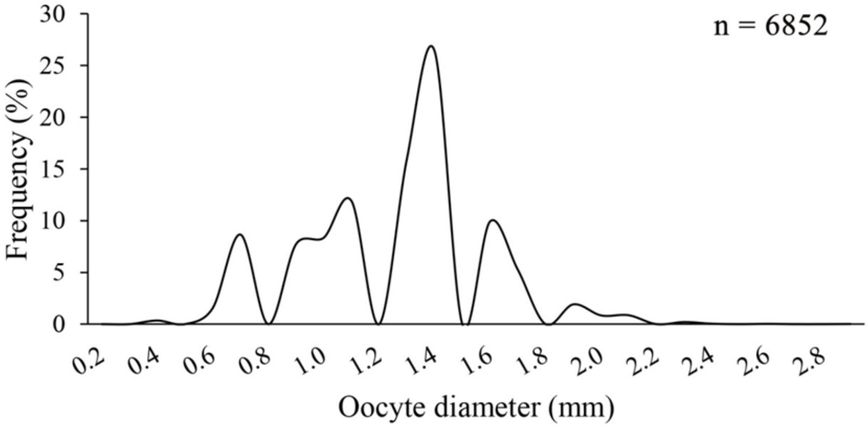

Figure 8. Frequency distribution of invader *Trachelyopterus galeatus* oocytes sampled in the upper Paraná River floodplain.

Discussion

The formation of Itaipu Reservoir led to the invasion and colonization of the upper Paraná River by 33 species that had previously been limited to the lower Paraná River (Julio Jr et al., 2009), while more recent records show the occurrence of 64 invasive species (Ota et al., 2018). The new introductions occurred after the 2002 construction of the Piracema Canal, an ecological corridor [(plus a combination of other factors such as fish escapes, aquaculture cages, and aquarists that release fish into natural environments, like those recorded by Langeani et al. (2007)] for fish to reach the reproductive areas of the upper Paraná River. After the formation of

Itaipu Reservoir, *T. galeatus* was recorded among its 10 most abundant species (Agostinho, Gomes, & Pelicice, 2007) and is still among the most abundant species of the upper Paraná River (Tonella et al., 2018).

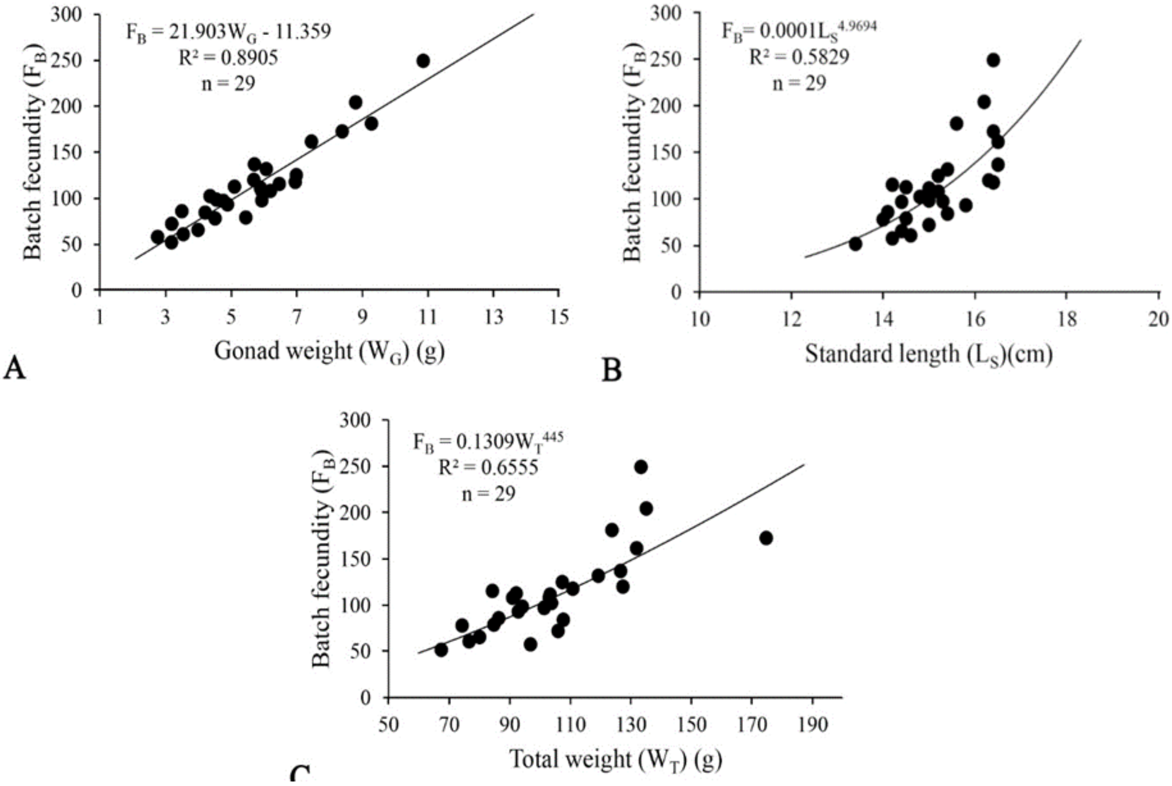

Figure 9. (A) Linear relationship between batch fecundity and weight. (B) Exponential relationship between relative batch fecundity and standard length. (C) Total weight of the invader *Trachelyopterus galeatus* sampled in the upper Paraná River floodplain.

The studies, based on macroscopic analysis of gonads of *T. galeatus*, evaluated for five annual cycles from October 1986 to September 1988 and from May 1992 to February 1995, recorded individuals reproducing in the Baía, Paraná, Ivinheima and Iguatemi rivers, in the Corutuba and Cortado channels, and in the Patos and Guaraná lagoons, from October to April in the rainy period (Suzuki et al., 2004). The results of the present study show intense reproductive activity for this species in the Garças and Pau Véio lagoons and in the Baía and Ivinheima rivers. The lentic environments in the upper Paraná River floodplain are generally more favorable to the reproduction of this invader.

This species has sexual dimorphism by internal fertilization. In the sperm-releasing capable males, the anal fin is modified into an intromittent organ with an internal canal (the gonopodium), which corresponds to the first anal fin ray; whereas in the spawning-capable females, the anal fin is unmodified (Loir, Cauty, Planquette, & Le Bai, 1989; Meisner et al., 2000). The genital system of *T. galeatus* males is composed of the testicles (testicular lobules or spermatogenic lobes), efferent duct (testicular duct), and seminal vesicle (Loir et al., 1989; Meisner et al., 2000; Lahnsteiner & Patzner, 2009; Melo et al., 2011).

The ovary pattern of the *T. galeatus* females follows that of most ovarian-cyst teleosts that present two saculiform ovaries suspended dorsally by the mesovary inside the celomatic cavity, with a cavity in its interior, in which ovarian lamellae are projected (Hoar, 1969; Grier, Uribe-Aranzábal, & Patiño, 2009b; Melo et al., 2011). Internally, the ovaries possess lumen that is continuous with the gonoducts and open in the urogenital papilla (Grier, Uribe, & Parenti, 2007; Grier et al., 2009b).

The ovarian sections in the late development, spawning-capable and regression phases show spermatozoa with elongated heads dispersed in the ovarian lumen and forming spermatozeugmata associated with ovarian lamellae. A well-developed zona pellucida was recorded in ovaries with secondary growth, full-grown, and mature oocytes, typical of species with adhesive eggs. Spermatozoa dispersed in the ovarian lumen and adhesive eggs were also recorded from *T. galeatus* in other hydrographic systems (Meisner et al., 2000; Melo et al., 2011; Lemes et al., 2016). The spermatozoa of *T. galeatus* have elongated heads and may be free in the ovarian lumen or attached to the ovarian lamellae organized in groups forming spermatozeugmata. This represents an important strategy to maintain the viability of the spermatozoa for long periods (Melo et al., 2011; Chiarini-Garcia et al., 2014).

The male and female germ cells allowed the characterization of gonadal development phases according to the proposal of Brown-Peterson et al. (2011), which was adopted in the upper Paraná River floodplain in the study of the reproduction of other invaders *Serrasalmus marginatus* (Melo, Santana, & Dei Tos, 2017), *Loricariichthys platymetopon* (Cardim, Pereira, Santana, & Dei Tos, 2019) and *Auchenipterus osteomystax* (Dei Tos et al., 2021). This scale has been adopted for investigations of freshwater fish reproduction in Brazil since 2013 for *Hoplias malabaricus* and *Sorubim lima* (Quagio-Grassiotto et al., 2013), *Pimelodus maculatus* and *Serrasalmus maculatus* (Wildner, Grier, & Quagio-Grassiotto, 2013), and more recently for *Hyphessobrycon igneus* (Longoni, Giora, & Fialho, 2018), *Devario aequipinnatus* (Jesus-Silva, Oliveira, Ribeiro, Ninhaus-Silveira, & Veríssimo-Silveira, 2018) *Astyanax* aff. *bimaculatus* (Araújo, Nascimento, Gomes, Sales, & Oliveira, 2019) and *Rhamdia quelen* (Mazzoni, Bombardelli, & Quagio-Grassiotto, 2020).

Artificial reproduction studies with *T. galeatus* show that the non-hydrated oocyte diameter varied from 2.2 to 2.4 mm (mean = 2.3 mm) (Santos, Arantes, Sampaio, & Sato, 2013). The results of this study with populations from different environments of the upper Paraná River floodplain corroborate the non-hydrated oocyte diameter. The number of oocytes per gram of ovary was, on average, 351 (varied from 342 to 358) (Santos et al. 2013). These values are above those found in natural populations of *T. galeatus* in the floodplain and vary depending on the size classes and weight of the analyzed individuals. The reproductive strategies of this non-migratory species with internal fecundity, group-synchronous oocyte development in multiple batches, retention of spermatozoa in the ovarian lumen, and adhesive oocytes guarantee its successful occupation of the lentic environments of the upper Paraná River floodplain.

The results indicate that reproductive activity is more intense in lentic environments than in lotic. In addition, low reproductive activity in the Ivinheima River is especially noticeable, demonstrating the importance of areas of preservation for the support and maintenance of biological communities. These areas are characterized by being among the most integral of the upper Paraná River floodplain, corroborating hypotheses that higher diversity and complexity may be a factor in resistance to biological invasions (Beaury, Finn, Corbin, Barr, & Bradley, 2019). Levine (2000), Stachowicz and Byrnes (2006), and Henriksson, Yu, Wardle, and Englund (2015) demonstrate the importance of other factors in invasion success.

Conclusion

Lastly, long-term monitoring of invasions, made possible by long-term projects, furnishes results that facilitate interpretation of the reasons for the success/failure of certain species. *Trachelyopterus galeatus*, specifically, possesses a high reproductive capacity, enabling its dispersion and occupation of diverse habitats (excluding rivers), which explains its high abundance and rates of colonization after the removal of a natural barrier in the construction of Itaipu Reservoir.

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