*Tropical Zoology, 2021*
Vol. 34 | Issue 1 | 16-23 | doi:10.4081/tz.2021.73

# Evaluation of the reproductive parameters of female neotropical migratory fish from a lotic and lentic environment of a dammed river

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Received for publication: 22 June 2020; Revision received: 22 June 2021;
Accepted for publication: 28 June 2021.

**Abstract:** The fragmentation of watercourses caused by dams is considered to be one of the main threats to aquatic biodiversity worldwide, especially for ichthyofauna. Several studies have shown that the environmental modifications caused by dammed water bodies can change the various reproductive parameters of freshwater fish. Therefore, the present study aims to comparatively analyze the reproductive potential of female *Megaleporinus reinhardti*, a migratory species, sampled in the lentic environment of the Três Marias Reservoir and the lotic environment of the São Francisco River. Biometric data were obtained from 79 females and the biological indices were subsequently calculated. Additionally, the microscopic analysis of the gonads was performed, and the follicular atresia index was compared. The results of the study show that fish from the lotic environment presented higher gonadal volume, fecundity, and oocyte diameter, and a lower follicular atresia index when compared to fish from the lentic environment. In summary, the data suggest that environmental changes, i.e. from a lotic to lentic environment, caused by river damming, may negatively affect the reproductive process of migratory fish, such as *M. reinhardti*, and impair the maintenance of the population of this species in lentic environments.

**Key words:** *Megaleporinus reinhardti*; oogenesis; fecundity; neotropical teleosts; dam impacts.



## Introduction

The fragmentation of rivers caused by dams is considered one of the main threats to aquatic biodiversity worldwide, especially for ichthyofauna (Silva et al. 2017). Approximately two thirds of the world's rivers are obstructed by more than 845,000 dams (Fuggle 2000). In addition to the physical barrier to fish migration, river damming for hydroelectric power generation can cause changes in sediment transport and water quality, as well as thermal and hydrodynamic changes that modify the community structure and composition

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of ichthyofauna species (Arantes et al. 2010; Perini et al. 2013; Freedman et al. 2014).

Fish reproduction is dependent on several environmental stimuli, mainly rainfall, photoperiod, temperature, and river flow. These parameters, when favorable, stimulate the hypothalamic-pituitary-gonadal axis (HPG axis) to release important hormones in the reproductive process (Schulz et al. 2010). Several studies worldwide have shown that the environmental modifications caused by dammed rivers (for example the transformation of a lotic environment - high water flow, in a lentic environment - backwaters, lakes and reservoirs) can give rise to endocrine disorders in freshwater fish, especially in migratory species, resulting in alterations to reproductive parameters such as the gonadosomatic index, fecundity, and gametogenesis (Weber et al. 2013; Normando et al. 2014; Gao et al. 2016).

Fecundity is a parameter used in several studies to assess the reproductive potential of fish, estimating the total number of vitellogenic follicles produced in ovaries (Melo et al. 2011b). To establish the reproductive potential of a fish species, it is necessary to consider the vitellogenic follicle diameter as well as body size and spawning type (Suzuki et al. 2000). In addition, histological analyses are important in the reproductive evaluation of fish species, since the morphological characteristics of vitellogenic follicles may be related to their reproductive strategies. Migratory species usually have small vitellogenic follicles, high fecundity, and non-adhesive oocytes, while non-migratory species have larger oocyte diameters, low fecundity, and may perform parental care (Suzuki et al. 2000; Kolm and Ahnesjö 2005; Melo et al. 2011a).

The family Anostomidae, widely distributed in South and Central America, consists of a group of fish popularly known as "piaus" with three genera occurring in the São Francisco River basin: *Leporellus* Lütken, 1875, *Leporinus* Spix & Agassiz, 1829, and *Schizodon* Spix & Agassiz, 1829 (Garavello and Britski 2003). The species *Megaleporinus reinhardti* (Lütken, 1875), formerly named *Leporinus reinhardti* (Ramirez et al. 2017) and known as "piau três pintas", is a migratory species with full spawning and non-adhesive eggs (Rizzo et al. 2002). The species is endemic to the São Francisco river basin, where it has great importance to the commercial and sport fishing industry (Weber et al. 2013).

In the São Francisco River, southeast Brazil, negative impacts of the Três Marias dam have been detected on the reproductive activity of migratory species such as *Prochilodus argenteus* Agassiz, 1829 (Arantes et al. 2010), *Megaleporinus reinhardti* (Weber et al. 2013) and *Brycon orthotaenia* Günther, 1864 (Nunes et al. 2015). However, these studies focused on impacts in fish inhabiting downstream stretches from Três Marias dam. Due to the ecological and socioeconomic importance of the species, the present study aims to comparatively analyze the reproductive potential of *M. reinhardti* females from the lentic environment of the Três Marias reservoir and the lotic environment of the São Francisco River, downstream the reservoir (natural river environment).

## Material and methods

### *Study area*

The Três Marias Hydropower Plant is located on the São Francisco River, Minas Gerais, Brazil (18°12′51″S, 45°15′51″W). The Três Marias dam was built during 1957–1960 to regulate the São Francisco River, improve navigation, and provide flood control, irrigation, and hydropower production. The dam length is 2.7 km and has a maximum height of 75 m. Reservoir depth usually does not vary by more than 10 m within a year. There are at least 8 free-flowing tributaries upstream from the reservoir (Prado and Pompeu 2014).

***Sampling and biometric data***

Mature *M. reinhardti* females were captured in two different stretches of the São Francisco River basin: 44 specimens from the Três Marias reservoir - stretch 1 (lentic environment) (18º23'27" S, 45º13'12" W), and 52 specimens from the São Francisco River - stretch 2 (natural and lotic environment) (18º07'59" S, 45º14'01" W). All specimens were captured at the same time, during their reproductive period that occurs between November to February (Weber et al. 2013), using gillnets with four to eight cm between opposite knots. The fish were anesthetized with eugenol (200 mg/L) (Paschoalini et al. 2019) and sacrificed by a cross-section of the cervical medulla following the ethical principles established by the National Council for Animal Experimentation Control (CONCEA 2013). From each specimen, the following biometric data were obtained in laboratory: total length (TL), body weight (BW) and gonadal weight (GW). Subsequently, the gonadosomatic index (GSI) was calculated for all females analyzed. The research was approved by the Animal Use Ethics Committee of PUC Minas (CEUA PUC Minas protocol n° 021/2015).

***Histological analysis***

To analyze the morphology of mature ovaries and vitellogenic follicles, ovarian fragments previously fixed in Bouin's solution were submitted to alcohol dehydration, paraffin embedding, five µm thick microtomy, and hematoxylin-eosin (HE) staining. Images of histological structures and diameter measurements of vitellogenic follicles were obtained from 20 specimens of both stretches, using an Olympus BX 50 light microscope coupled with the Olympus CellSens Standard 1.9 software. In addition, the follicular atresia index was also quantified using the following formula: (FAI = VO/AO × 100), where VO = number of vitellogenic follicles and AO = number of atretic follicles (Paschoalini et al. 2019). The variables used to characterize the atresia process were zona radiata fragmentation, yolk liquefaction, and follicular cell hypertrophy.

***Fecundity***

To determine fecundity, ten mature females were randomly selected from each sampling stretch. Ovarian subsamples were collected, weighed, and maintained in modified Gilson's solution until complete dissociation of oocytes was achieved. Dissociated vitellogenic oocytes were separated and counted under a stereomicroscope, and the total number of oocytes in ovaries was then calculated.

***Statistical analysis***

The Lilliefors test was performed to determine the normality of the data. As the biological data did not show a normal distribution, they were analyzed using the nonparametric Mann-Whitney test to compare the sampling sections. The results were expressed as mean ± standard deviation (SD). The significance level for all tests was 95% ($p<0.05$). All graphs and statistical tests were performed using GraphPad Prism 7.0 software for Windows.

## Results

The fish captured from the lotic environment presented statistically lower TL and BW when compared to fish of the lentic environment. However, the gonadosomatic index was higher in fish from the lotic river environment when compared to fish from reservoir – lentic environment (Table 1).

Microscopically, the vitellogenic follicles exhibited ovaries filled with spherical acidophilic yolk globules, squamous follicular cells, and thin zona radiata. Postovulatory follicles were observed only in fish from the lotic environment of the São Francisco River (Figure 1).

A higher frequency of follicular atresia was observed in fish from the lentic environment, with an atresia rate four-fold higher than the lotic environment fish. The follicular diameter of the fish from the reservoir presented lower values (551.8±56.4 µm) when compared to the fish caught in the river (583.7±51.0 µm).

Absolute fecundity (FA), as well as GSI and FD, presented significantly higher values in fish caught in the lotic environment. The AF values observed in both sections were 35619±13697 vitellogenic follicles in fish ovaries from the lentic environment and 59630±19638 vitellogenic follicles in fish ovaries from the lotic environment (Figure 2).

**Table 1.** Range and mean ± standard deviation (SD) of total length (TL), body weight (BW) and gonadosomatic index (GSI) of *M. reinhardti* females captured in the Três Marias reservoir and the São Francisco River from November 2016 to February 2017.

| | Lentic environment (reservoir) | | Lotic environment (river) | |
|---|---|---|---|---|
| | Range | mean ± SD | Range | mean ± SD |
| TL | 22.0 – 29.8 | 25.3 ± 1.8[a] | 18.0 – 27.0 | 22.7 ± 2.1[b] |
| BW | 155.0 – 350.0 | 240.3 ± 46.1[a] | 75.3 – 270.2 | 161.7 ± 43.5[b] |
| GSI | 3.7 – 20.5 | 11.8 ± 4.8[a] | 11.2 – 38.5 | 17.9 ± 5.2[b] |

*Different letters represent statistical differences between sampling stretches ($p<0.05$).*



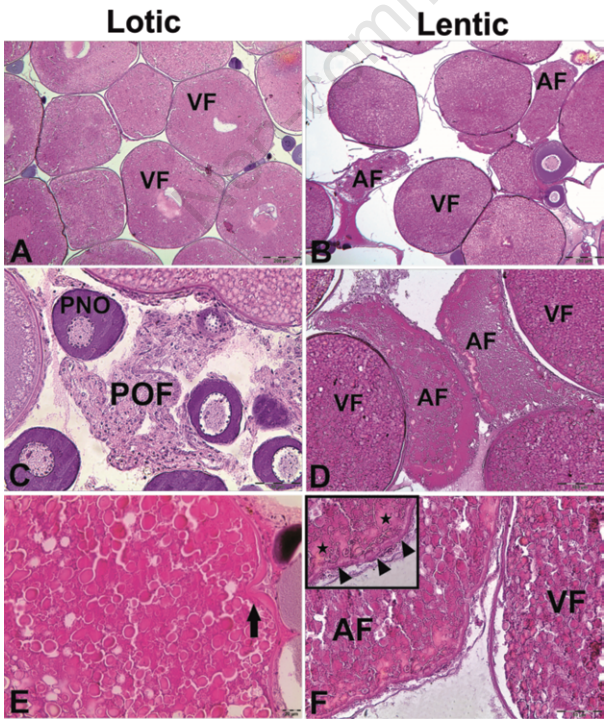


**Figure 1.** Morphological differences in the ovaries of *M. reinhardti* from the lotic environment of the São Francisco River and the lentic environment of the Três Marias Reservoir. (A) Ovary in advanced maturation/mature with abundance of vitellogenic follicles-VF; (B) Advanced maturation/mature ovary containing vitellogenic follicles and some atretic follicles-AF; (C) Detail of postovulatory follicle-POF in ovary sampled from the lotic environment; (D) Atretic follicles at different stages of development; (E) Healthy vitellogenic follicle with funnel-type micropyle ARROW; (F) Detail of the difference between a healthy vitellogenic follicle-VF and atretic follicle-AF. Insert = fragmentation of zona radiata-arrowhead and the beginning of the yolk liquefaction-STAR.

## Discussion and conclusions

The data presented here showed that migratory species inhabiting lentic environments (for example reservoirs), such as *M. reinhardti,* can show reproductive disturbs. Indeed, the reproductive strategy of migratory species is related to the physical and chemical characteristics of lotic environments, and alterations in these parameters, such as the artificial regulation of river flow, may affect and even impair the reproductive process of these species (Sato et al. 2003; Arantes et al. 2010; Cooney and Kwak 2013; Pelicice et al. 2015).

Even in unfavorable environmental conditions or when the migration process is interrupted, migratory fish can usually still achieve advanced maturation of the ovarian follicles (vitellogenic follicles production) at the reproductive season. However, the final maturation of vitellogenic follicles (germinal vesicle migration and breakdown) and subsequent spawning events usually do not occur, making fertilization of follicles impossible (Mylonas and Zohar 2000). This fact corroborates our data once the ovaries in both sampling stretches were detected vitellogenic follicles production with similar characteristics to other *Leporinus* species (Arantes et al. 2017), but structures indicating the conclusion of spawning process, such as postovulatory follicles, were detected only in fish from the lotic environment, confirming that spawning would not occur in the lentic environment.

Although no microscopic alterations were detected in the morphology of yolk globules, follicular cells and zona radiata of vitellogenic follicles, marked differences were observed in some reproductive parameters between the lotic and lentic stretches. The gonadosomatic index is a widely used parameter in reproduction studies, and significant alterations in this index are often related to environmental and/or hormonal alterations (Hojo et al. 2004; Hachfi et al. 2012; Paschoalini et al. 2019). Despite having significantly lower values in total length and body weight, fish from the river showed higher gonadal growth (GSI) when compared to fish from the reservoir. This higher GSI value observed in the lotic environment samples may be related to the greater number and diameter of vitellogenic follicles of the river specimens.

In addition to GSI, the lower values of fecundity and follicular diameter observed in fish from the reservoir reinforce the idea that the reservoir environment, formed by the Três Marias dam, directly impact the reproductive potential of *M. reinhardti* that inhabit these lentic stretches. As detected in our data, the decrease in the number and size of vitellogenic follicles can lead to long-term problems in the size and dynamics of fish populations (Arantes et al. 2013).



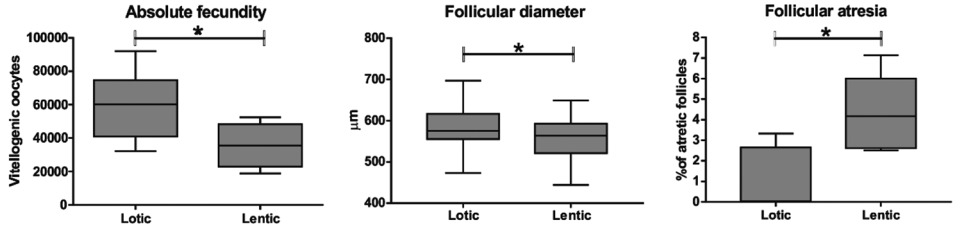


**Figure 2.** Quantification of the absolute fecundity, follicular diameter, and follicular atresia of *M. reinhardti* captured in the lotic environment of the São Francisco River and the lentic environment of the Três Marias Reservoir.

*Statistical difference between lotic and lentic environments ($p<0.05$).

Besides physical barriers interrupting the reproductive migration processes of fish, the thermal and/or hydrological conditions of these artificial reservoirs are inefficient in stimulating the final spawning process of other migratory fish species that inhabit these lentic environments (Parkinson et al. 1999; Kamanga et al. 2004; Arantes et al. 2011). In the present study, this fact was corroborated by the absence of postovulatory follicles in fish ovaries from the lentic environment, which consequently resulted in a four-fold higher rate of follicular atresia than in ovaries collected in the lotic environment. Follicular atresia is a natural process of resorption of unspawned vitellogenic follicles, but this process can be significantly increased by factors such as stress, confinement, hypoxia, extreme temperatures, and inadequate hormone levels (Morais et al. 2012). It is worth mentioning that high rates of atresia can reduce the reproductive potential of fish or even make reproduction unfeasible (Santos et al. 2008).

In summary, our data showed that the Três Marias reservoir has inappropriate conditions to stimulate the final stages of the reproductive process of *M. reinhardti*, a migratory and economically important species in the São Francisco River basin. Thus, the maintenance of the reservoir specimens may be dependent on restocking programs, as well as the upstream lotic portions of the São Francisco River and tributaries populations, such as the Paraopeba River. Finally, the data presented in our study are important to improve the knowledge of *M. reinhardti* reproduction and serve as subsidies for aquaculture, management, and conservation of this important migratory fish.



**Acknowledgments**

The authors would like to thank Rogério Matos for mounting the histological slides and CODEVASF and PUC Minas for their logistical support. This work had the financial support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) under grant (Finance code-001); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) under grant (306946/2016-5 and 407719/2016-4); and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) under grant (APQ03232-15).

**Contributions**

LMAS and GGT: writing original draft, investigation, formal analysis; ER and JES: writing, review and editing; NB: methodology, project administration, funding acquisition; ALP: writing, review and editing, visualization, supervision, project administration.

**Conflict of interest statement**

The authors declare no conflict of interest.

**Data availability statement**

Data will be made available on request.

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