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# Downstream impacts of a dam and influence of a tributary on the reproductive success of *Leporinus reinhardti* in São Francisco River

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**ABSTRACT:** Downstream impacts of dams on fish fauna are poorly studied, despite causing thermal and hydrological changes that affect the reproductive activity of the fish community. The present study aims to evaluate the reproduction of *Leporinus reinhardti* in 2 sections of the São Francisco River, Brazil, downstream from the Três Marias Dam. Section 1 (S1) is the first 34 km downstream from Três Marias Dam and section 2 (S2) is 34 to 54 km downstream from the dam, where the São Francisco River receives a mid-sized tributary called the Abaeté River. In S1, the values of temperature, dissolved oxygen and flow were lower than those obtained in S2. In S1, females and males did not complete reproduction, a fact proven by the absence of spawned females and males. In S2, reproductive success was proven due to the presence of spawned females and males. Furthermore, the values of total length, body weight, gonadosomatic index, fecundity and vitellogenic oocytes were statistically higher than those obtained in S1. The canonical correlation test indicated that the reproduction of females is more dependent on environmental factors than that of males. In S2, *L. reinhardti* found appropriate conditions for reproduction, probably influenced by the Abaeté River, confirming the need of preserving this tributary for the reproductive success of this species.

**KEY WORDS:** Downstream impacts · Tributary · *Leporinus reinhardti* · São Francisco River · Spawning

## INTRODUCTION

Approximately two-thirds of the largest rivers in the world have some kind of damming along their courses that has several negative impacts, the less visible of which being hydrologic alteration through the selective release of water from reservoirs (Nilsson et al. 2005, Poff et al. 2007, Olden & Naiman 2010). The changes in downstream water parameters depend on the level of stratification of the reservoir and

the depth from which water is released through the turbines (Olden & Naiman 2010).

Fish reproduction is regulated by environmental conditions that stimulate the production of sex hormones during gonadal development through the neuroendocrine-gonadal axis (Nagahama & Yamashita 2008). Changes in environmental conditions downstream from dams may negatively affect fish populations and their reproduction (Lessard & Hayes 2003, Sato et al. 2005), mainly due to the decrease in

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temperature, which may cause a delay in spawning and embryo development and, hence, a reduction in the distribution of a species (Arantes et al. 2010, Olden & Naiman 2010). The effects on fish populations downstream are not always negative. Unlike observations made in neotropical fishes, some temperate-water species experience reproductive success due to physico-chemical changes in the hypolimnion (e.g. temperature, dissolved oxygen, pH, turbidity and conductivity; Miranda et al. 2012).

During the rainy season in the neotropical region, the Três Marias Dam, located on the São Francisco River, releases water downstream 2 to 3°C colder (hypolimnic) than temperatures that generally occurred prior to its construction in 1960 (Esteves et al. 1985). Recent studies in the area have shown that *Prochilodus argenteus*, a large-sized migratory species, suffers reproductive failure as a result of this change imposed by the dam (Sato et al. 2005, Arantes et al. 2010, Domingos et al. 2012, Thomé et al. 2012). However, no studies have yet analysed whether this reproductive failure also affects mid-sized species such as some fish species from the family Anostomidae.

The Anostomidae family is widely distributed in Central and South America (Garavello & Britski

2003). The piau-três-pintas *Leporinus reinhardtii* (Lütken 1875) is a mid-sized fish endemic to the São Francisco River basin. It is abundant in the basin and an important commercial and sport-fishing species.

The aim of the present study was to compare the reproductive activity of *Leporinus reinhardtii* in 2 sections of the São Francisco River downstream from the Três Marias dam and to analyse the impact of this dam and the importance of the Abaeté River on its reproduction.

## MATERIALS AND METHODS

The study analysed fish from 2 sections of the São Francisco River downstream from the Três Marias Dam: section 1 (S1), the first 34 km downstream from the dam (18° 11' S, 45° 14' W) and section 2 (S2), 34 to 54 km downstream from the dam, after the confluence with the Abaeté River (18° 02' S, 45° 11' W; Fig. 1). Fishes and water parameters were collected from May 2011 to April 2012 every month from different points on each section. Fishes were collected with the aid of gillnets with a mesh size of 40 to 70 mm. A total of 562 *Leporinus reinhardtii* were collected, 92 females and 84 males in S1 and 190 females and 196 males in S2. The fish, if alive, were killed by transversal section of the cervical medulla, following the ethical principles of animal handling established by the Brazilian College for Animal Experimentation (COBEA, www.cobea.org.br). For each specimen, total length (TL; to the nearest 0.01 cm), body weight (BW; 0.01 g) and gonadal weight (GW; 0.01 g) were measured.

The following parameters were recorded for each section every month: pH, temperature (°C), dissolved oxygen ( $\text{mg l}^{-1}$ ) and specific conductance ( $\mu\text{S cm}^{-1}$ ), measured with a Horiba model W-10 probe. Water physico-chemical parameters were measured in the 2 sections of the river every month for one year. The water flow ( $\text{m}^3 \text{s}^{-1}$ ) measurements were supplied by the Energy Company of Minas Gerais (CEMIG).

For each specimen, we determined the gonadosomatic index ( $\text{GSI} = \text{GW}/\text{BW} \times 100$ ) and Fulton's condition factor ( $K = \text{BW}/\text{TL}^3 \times 100$ ). The length-weight ratio ( $\text{BW} = a\text{TL}^b$ ) was determined for the females and males of each section. In order to analyse the gonadal development stages, fragments of ovaries and testes were collected and fixed in Bouin's fluid for 24 h, embedded in paraffin, sectioned at a thickness of 4  $\mu\text{m}$  and stained with haematoxylin-eosin for histological analyses.

Based on the microscopic characteristics of the gonads, the following development stages were identi-

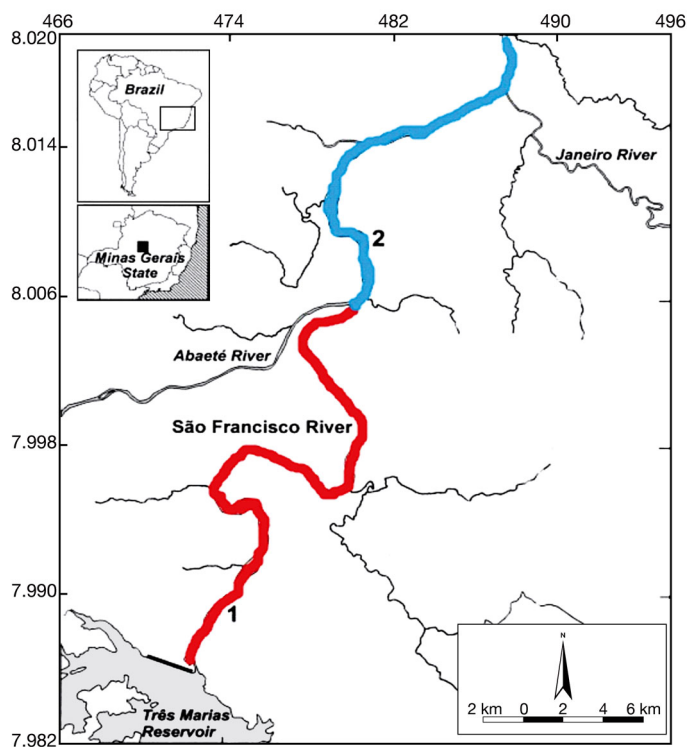


Fig. 1. Location of the study sections of the São Francisco River, downstream from the Três Marias dam. Section 1, immediately following the dam; Section 2, immediately after the confluence with the Abaeté River. UTM coordinates

fied for females (F) and males (M): resting (F1/M1), fully grown gonads (F2/M2) and spawned (F3/M3) (De Carvalho et al. 2009) (Fig. 2). The diameters of 50 vitellogenic oocytes (VO) from ovaries of 10 fully grown females from each section were measured using a camera attached to a light microscope and Motic Image Plus version 2.0 software. Fifteen females with fully grown ovaries, from each section of the river, were used for fecundity estimates. Absolute fecundity (AF) was estimated using the equation:  $AF = OVA \times GW$ , where OVA is the number of oocytes per gram ovary. Relative fecundity (RF) was estimated using the equation:  $RF = AF/BW$ .

In order to compare abiotic parameters of the water of the 2 sections, we used Student's *t*-test, and to compare biological parameters we used the Mann-Whitney *U*-test. We conducted canonical correlation analysis between biological parameters (TL, BW and GSI) and environmental factors (temperature, dis-

solved oxygen, pH, flow and electrical conductivity) for females and males of the 2 sections, and for this analysis the data were log transformed. The software used was Bioestat 5.3 and all tests had a significance level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

The physical and chemical parameters of water, temperature, dissolved oxygen and flow (mean  $\pm$  SD) during the reproductive season (November to February) were significantly higher in S2 compared with S1: temperature (S1 =  $23.1 \pm 0.57^\circ\text{C}$  and S2 =  $24.4 \pm 0.79^\circ\text{C}$ ;  $t = -2.83$ ,  $p = 0.03$ ,  $df = 6$ ), dissolved oxygen (S1 =  $5.5 \pm 1.67 \text{ mg l}^{-1}$ ; S2 =  $8.0 \pm 0.55 \text{ mg l}^{-1}$ ;  $t = -3.06$ ,  $p = 0.01$ ,  $df = 6$ ) and flow (S1 =  $666.7 \pm 94.3 \text{ m}^3 \text{ s}^{-1}$  and S2 =  $812.9 \pm 60.59 \text{ m}^3 \text{ s}^{-1}$ ;  $t = -2.60$ ,  $p = 0.04$ ,  $df = 6$ ).

Females and males of *Leporinus reinhardtii* captured in S2 showed statistically higher values of TL (females,  $U = 2.34$ ,  $p = 0.02$ ,  $df = 257$ ; males,  $U = 7.13$ ,  $p < 0.0001$ ,  $df = 244$ ), BW (females,  $U = 2.17$ ,  $p = 0.02$ ,  $df = 257$ ; males,  $U = 6.12$ ,  $p < 0.0001$ ,  $df = 244$ ) and GSI (females,  $U = 2.62$ ,  $p = 0.01$ ,  $df = 85$ ; males,  $U = 3.10$ ,  $p = 0.01$ ,  $df = 20$ ) compared with females and males captured in S1 (Table 1). In S1, no spawned females and males were found (Fig. 3). The results of this study showed that *L. reinhardtii* presents reproductive success only in S2 after the confluence with the Abaeté River and in S1 the reproduction of these species is not successful. This is probably due to the unfavourable conditions for somatic growth and gonadal development in S1, since, under altered thermal conditions, production and release of sex hormones such as  $17\text{-}\beta$  estradiol, which is responsible for somatic growth and gonadal development, decrease (Blázquez et al. 1998, Arantes et al. 2010). Moreover, several studies have also shown that immediately downstream from dams, the release of cold water is responsible for impaired fish reproductive activity (Clarkson & Childs 2000, Preece & Jones 2002, Todd et al. 2005).

VO and AF showed statistically significant differences between sections,

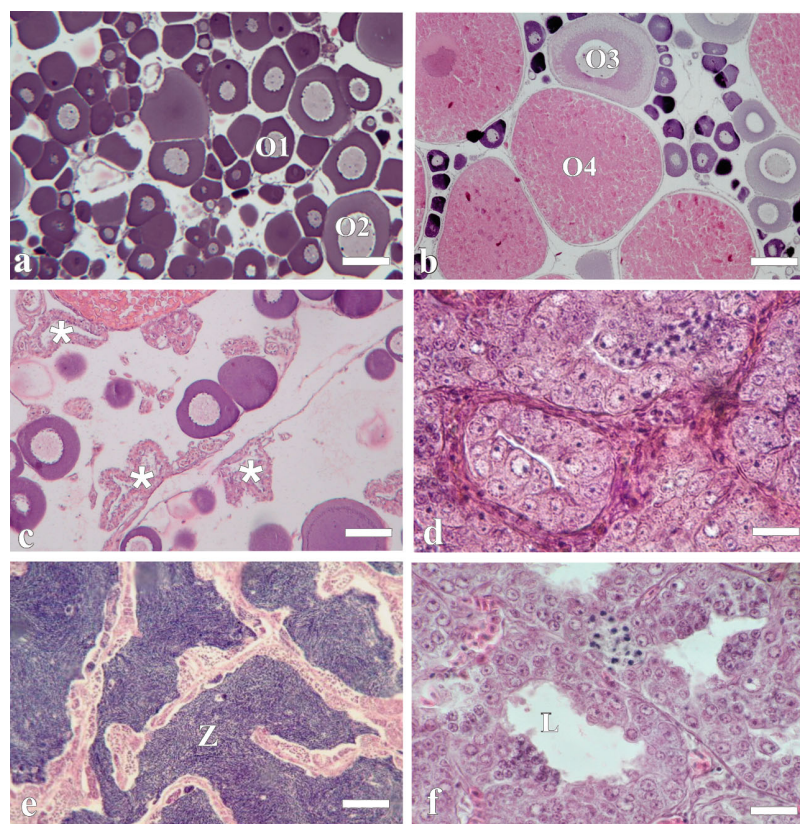
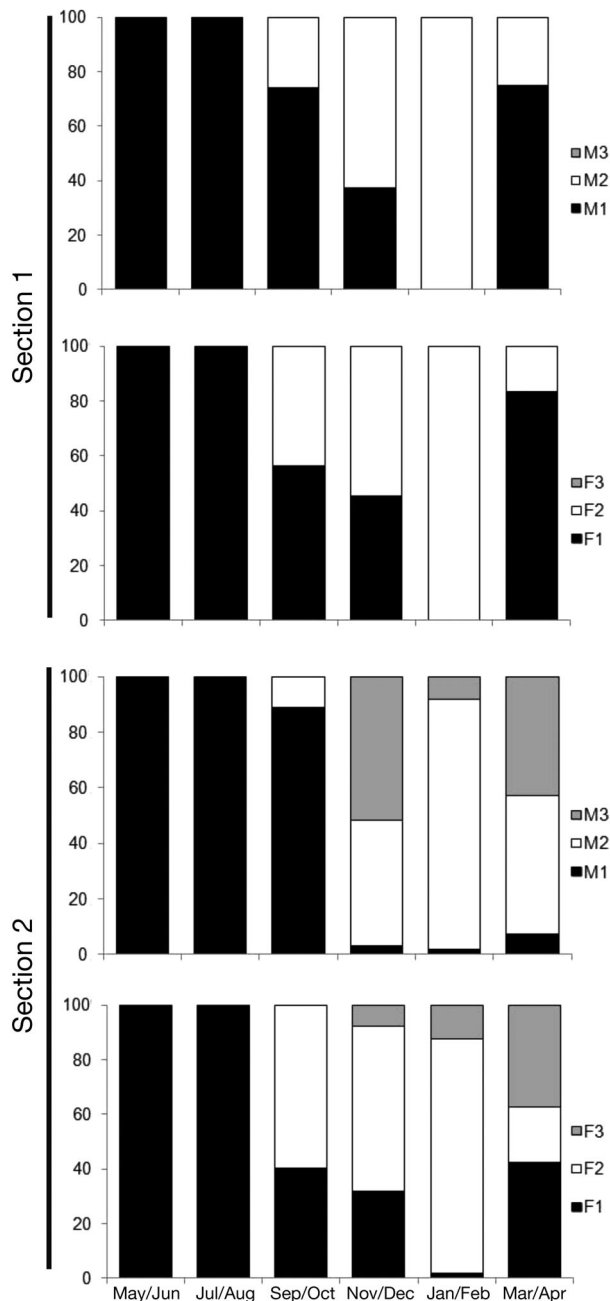


Fig. 2. *Leporinus reinhardtii*. Gonadal development stages stained with haematoxylin-eosin. (a) Resting ovaries presented early (O1) and late (O2) perinucleolar follicles; (b) fully grown ovary emphasizing previtellogenic follicles with cortical alveoli (O3) and vitellogenic follicles (O4); (c) spawned ovaries with predominance of postovulatory follicles (asterisk); (d) resting testis with seminiferous tubules closed; (e) fully grown testis with seminiferous tubules full of spermatozoa (Z), (f) spent testis open and without spermatozoa in the lumen (L). Scale bars: (a,b,d) 70  $\mu\text{m}$ , (c) 180  $\mu\text{m}$ , (e,f) 30  $\mu\text{m}$

Table 1. *Leporinus reinhardtii*. Biological variables (mean  $\pm$  SD) of fish captured in 2 sections of the São Francisco River, downstream of Três Marias Dam. TL: total length; BW: body weight; GSI: gonadosomatic index (of fully grown females and males); *K*: Fulton's condition factor; VO: vitellogenic oocytes; AF: absolute fecundity; RF: relative fecundity. Different superscripts indicate differences between river sections ( $p < 0.05$ )

	Section	TL (cm)	BW (g)	GSI (%)	<i>K</i>	VO ( $\mu\text{m}$ )	AF ( $\times 10^3$ )	RF ( $\times 10^2$ )
Females	S1	19.73 $\pm$ 4.27 <sup>a</sup>	114.53 $\pm$ 86.18 <sup>a</sup>	11.98 $\pm$ 5.25 <sup>a</sup>	1.34 $\pm$ 0.24 <sup>a</sup>	545.99 $\pm$ 61.74 <sup>a</sup>	50.68 $\pm$ 21.96 <sup>a</sup>	2.92 $\pm$ 1.34 <sup>a</sup>
	S2	20.93 $\pm$ 3.77 <sup>b</sup>	135.86 $\pm$ 37.26 <sup>b</sup>	16.40 $\pm$ 5.02 <sup>b</sup>	1.32 $\pm$ 0.22 <sup>a</sup>	571.05 $\pm$ 51.55 <sup>b</sup>	73.20 $\pm$ 22.28 <sup>b</sup>	4.09 $\pm$ 1.79 <sup>a</sup>
Males	S1	14.15 $\pm$ 2.67 <sup>a</sup>	48.49 $\pm$ 24.35 <sup>a</sup>	0.53 $\pm$ 0.19 <sup>a</sup>	1.19 $\pm$ 0.11 <sup>a</sup>	–	–	–
	S2	17.41 $\pm$ 2.60 <sup>b</sup>	61.18 $\pm$ 26.91 <sup>b</sup>	0.68 $\pm$ 0.32 <sup>b</sup>	1.09 $\pm$ 0.17 <sup>b</sup>	–	–	–



and the highest values were found in S2 (VO,  $U = 2.02$ ,  $p = 0.04$ ,  $df = 124$ ; AF,  $U = 1.96$ ,  $p = 0.04$ ,  $df = 18$ ; Table 1). In fact, higher temperatures and higher dissolved oxygen rates are contributory factors for the production of oocytes that are larger in diameter and quantity (Landry et al. 2007).

The GSI peak for females was in January/February and for males in November/December. For both sexes, the average GSI was higher in S2 than in S1 during the spawning season. The largest number of mature females and males was recorded in S2 and the largest number of resting females and males was in S1 (Fig. 3). Thus, the reproductive activity of *Leporinus reinhardtii* coincided with the rainy season in this neotropical region, when water temperatures and river flow are higher, according to reports of Lowe-McConnell (1987). Only in S2 were postovulatory follicles observed. These structures remain in fish ovaries after ovulation and are used for confirmation of spawning (Sato et al. 2005). For *Prochilodus argenteus*, under the influence of a tributary, the frequency of postovulatory follicles observed was higher than observed in an area impacted by a dam (Thomé et al. 2012).

Fulton's condition factor (*K*) showed a statistical difference between males ( $U = 3.66$ ,  $p = 0.01$ ,  $df = 113$ ) from the 2 sections of the river. For the females, however, this difference was not observed ( $U = 0.30$ ,  $p = 0.76$ ,  $df = 326$ ; Table 1). Regarding the length-weight ratio, females and males from the 2 sections presented a growth factor  $>3$ , indicating positive allometric growth for this species according to the classification of Froese (2006).

Fig. 3. *Leporinus reinhardtii*. Relative frequency (%) of gonadal development stages of fish captured in 2 sections of the São Francisco River, downstream from the Três Marias Dam. F1: resting ovary; M1: resting testis; F2: fully grown ovary; M2: fully grown testis; F3: spawning ovary; M3: spawning testis

The canonical correlation ( $R_c$ ) for females showed high correlation and significance between biological and environmental factors for the 2 sections (S1,  $R_c = 0.86$ ,  $p < 0.0001$ ,  $df = 25$ ; S2,  $R_c = 0.91$ ,  $p < 0.0001$ ,  $df = 25$ ). However, males of the 2 sections showed low correlation and did not show significance between biological and environmental factors (S1,  $R_c = 0.25$ ,  $p = 0.93$ ,  $df = 20$ ; S2,  $R_c = 0.23$ ,  $p = 0.15$ ,  $df = 20$ ). The canonical correlation shows that reproduction in males is less susceptible to environmental factors than that of females, as has also been reported for *Oryzas latipes* (Koger et al. 1999) and *Prochilodus argenteus* (Sato et al. 2005).

The results of this study showed a negative impact of the dam on the reproduction of *Leporinus reinhardti* in S1, and favourable conditions for the reproduction of this species in S2 of the São Francisco River. These results indicate the need for taking measures to minimise the impacts imposed by the dam, such as destratification of the water column of the Três Marias reservoir and preservation of downstream tributaries. Such measures have been taken in some countries to control the temperature of the water released downstream of dams (Vinson 2001). The conservation of dam-free tributaries is important as an alternative route for fish reproduction because these watercourses have not had their hydrological regime and physical and chemical conditions of the water changed (Pelicice & Agostinho 2008). The degree to which a tributary helps to minimise thermal effects imposed by a dam depends on its size and its characteristics, such as flow, sediment load and water temperature (Petts 1986).

In summary, this study showed that the physical and chemical conditions of the water in S1 of the São Francisco River cause reproductive failure and that the Abaeté River is very important for the reproduction of *Leporinus reinhardti*.

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