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Reproduction of *Pimelodus maculatus* (Siluriformes: Pimelodidae) in three section of Grande River basin, downstream Porto Colombia dam, south-eastern Brazil

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The alterations to the hydrologic regime downstream from hydroelectric dams may cause an impact on the reproductive success of fishes. This study aimed to analyse the influence of the physical and chemical parameters of the water of the Grande and Pardo Rivers on gonadal maturation, oocyte diameter, follicular atresia and biological indices of *Pimelodus maculatus* collected from three river sections: Grande River, downstream from the Porto Colômbia dam (S1), Grande River, downstream from the confluence with the Pardo River (S2) and in the Pardo River channel (S3). Males and females captured in S1 presented significantly higher average values for total length and body weight than those captured in S2 and S3. The gonadosomatic index values were significantly higher in fish collected in S3 and the Fulton condition factor did not show significant differences in fish collected from the three sections. The oocyte diameter, the follicular cells height and the zona pellucida thickness did not show any statistical differences between the sections. Conductivity presented a significant difference between S1 and S3 and during the reproductive period, water transparency presented similar values in the two sampling sections of the Grande River, but a much lower value in the Pardo River. A low frequency of fish with reproductive activity was registered in S1, whereas in S2 and S3 higher frequencies were recorded, emphasising the need of preserving the tributaries for the reproductive success of *P. maculatus* of the Grande River in south-eastern Brazil.

A jusante das barragens hidrelétricas ocorrem alterações no regime hidrológico que podem causar impactos sobre o processo reprodutivo dos peixes. O objetivo do presente estudo foi analisar a influência de parâmetros físico-químicos da água do rio Grande e rio Pardo sobre a maturação gonadal, diâmetro ovocitário, atresia folicular e índices biológicos de *Pimelodus maculatus* coletados em três trechos: a jusante do reservatório de Porto Colômbia (P1), a jusante da confluência do rio Grande com rio Pardo (P2) e na calha do rio Pardo, a aproximadamente 100 km de P2 (P3). Machos e fêmeas capturados no ponto 1 apresentaram valores médios de comprimento total e peso corporal significativamente maiores do que aqueles capturados nos pontos 2 e 3. Os valores de IGS foram significativamente maiores nos peixes coletados no ponto 3 e o fator de condição de Fulton não apresentou diferenças significativas entre os peixes coletados nos três pontos. O diâmetro ovocitário, a altura das células foliculares e a espessura da zona pelúcida dos ovócitos vitelogênicos não mostraram diferenças estatísticas entre os pontos. A condutividade apresentou diferença significativa entre os pontos 1 e 3 e a transparência da água durante o período reprodutivo apresentou valores próximos nos dois pontos amostrais do rio Grande, e valor bem menor no ponto amostral do rio Pardo. Em P1 registrou-se baixa frequência de peixes em atividade reprodutiva, e em P2 e P3 maiores frequências de peixes reproduzindo. Os resultados encontrados enfatizam a necessidade de conservação de tributários para o sucesso reprodutivo de *P. maculatus*, do rio Grande no sudeste do Brasil.

Key words: Abiotic factors, Dam impacts, Environmental impacts, Gonadosomatic index.

Introduction

Several physical and chemical factors affect the behaviour and the reproduction of freshwater fishes, such as: water temperature, rainfall, turbidity, feeding, water level, xenobiotic agents, environmental stress, photoperiod, and population density (Janz *et al*., 2001; Weber *et al*., 2002). Water temperature and rainfall are critical factors which trigger spawning migration and, therefore, the reproductive success of a species may be related to temperature and an increase in water volume (Parkinson *et al*., 1999; Rizzo *et al*., 2003).

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Alterations to the hydrologic regime downstream from hydroelectric dams, such as a decrease in the flood peaks, may result in unstable thermal and hydrologic conditions (Baxter, 1977; Agostinho *et al*., 1992). Such impacts seem to especially affect the reproductive process of neotropical fish since, in tropical regions, the flood regime is considered to be critical for trigging reproduction and spawning migration (Sato *et al*., 2003). Therefore, several impacts are reported relating to fish communities downstream from dams, such as a change in the ichthyofauna's composition before and after the dam, interruption of the migration process, reproduction inhibition, facility to introduce exotic species, changes in behaviour, immobilisation and death, an increase in vulnerability to predation, interference in feeding areas and juvenile recruitment (Welcomme, 1979; Ruane *et al*., 1986; Agostinho *et al*., 1993; Sato *et al*., 2003). In the Paraná River, Brazil, the reproductive behaviour of migratory fish was altered in the first kilometres after the Itaipu dam, where fish could not spawn, and thus presented an intensive process of follicular atresia (Agostinho *et al*., 1993). Likewise, the colder water released by the dams of the Colorado River, in the USA, resulted in a decline of the local ichthyofauna (Paukert & Rogers, 2004).

The so called mandi-amarelo, *Pimelodus maculatus* Lacepède, 1803, is considered by some authors to be a migratory species, requiring shorter sections of rivers in which to reproduce, differing from other neotropical migratory fishes which migrate for long distances (Agostinho *et al*., 2003). This fish represents an important species, both for commercial and recreational fishing (Lundberg & Littmann, 2003) and some studies on this species have been performed in the Paraná River basin (Dei Tos *et al.*, *2002*; Maia *et al*., 2007). Considering that there are no studies analysing the importance of tributaries for the reproduction of the mandi-amarelo in the area influenced by the Porto Colômbia dam, the objective of the study was to comparatively analyse the reproduction of *P. maculatus* in the Grande River, downstream from the Porto Colômbia dam, and its tributary, the Pardo River, in south-eastern Brazil.

Materials and Methods

Study site and Fish. The Porto Colômbia dam is located in middle third of the Grande River, between the municipalities of Planura (Minas Gerais State) and Colômbia (São Paulo State), in south-eastern Brazil. The samples were obtained bimonthly from three sections of the Grande River basin, between January/2010 and February/2011. The first section was located in the Grande River, immediately downstream from the Porto Colômbia dam (S1) (20º07'44"S 48º34'34"W), the second section, downstream from the confluence of the Grande River and the Pardo River (S2) (20º10'04"S 48º38'44"W), and the third, in the channel of the Pardo River (S3) (20º81'48"S 48º23'41"W) (Fig. 1). The fish were captured by fishermen from FURNAS Centrais Elétricas S/A, using gillnets (6 cm mesh size) and casting nets (6 cm mesh size) with the same effort between the sites. The animals were sacrificed by cross-section of the cervical medulla in accordance with the Animal Experimentation Guidelines, established by the Brazilian College of Animal Experimentation (COBEA). For all specimens, we registered the following biometric data: total length (TL), standard length (SL), body weight (BW) and gonad weight (GW). From the biometric data obtained, we calculated the gonadosomatic index (GSI = GW/BW*100) and the Fulton condition factor ($K = BW \times 100/SL^3$). Voucher specimens were sent to Natural Sciences Museum of Pontifícia Universidade Católica de Minas Gerais, Brazil (MCNI-0507).

Histology, gonadal maturation stages and follicular diameter. Gonad fragments were fixed in Bouin's fluid for 8 to 12 hours, kept in alcohol 70% and were submitted to the routine histological techniques: paraffin embedding, 3-5 µm thick microtomy sections, and haematoxylin and eosin stain (HE). The gonadal maturation stages were established based on the oocyte distribution, spermatogenic lineage cells, and variations of the GSI (Santos *et al.*, 2004; Carvalho *et al.*, 2009; Arantes *et al.*, 2010). The diameter of the vitellogenic follicles (follicular cells, zona pellucida and oocyte) was determined from histological slides of the mature ovaries using a micrometric ocular attached to a light microscope.

Fecundity. In order to estimate fecundity, samples from the middle region of the mature ovaries were fixed in a modified Gilson's solution (100 ml of alcohol 60%, 880 ml of distilled water, 15 ml of nitric acid 80%, 18 ml of glacial acetic acid and

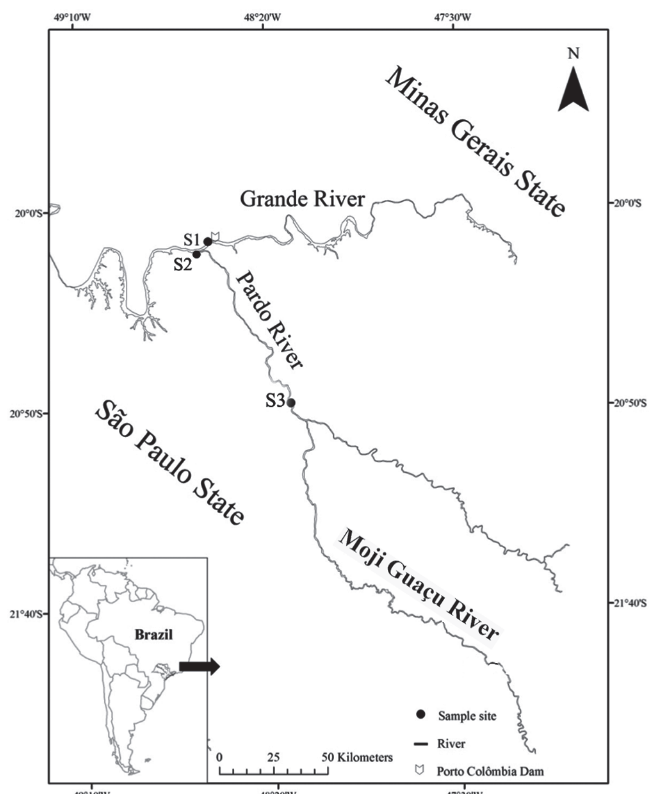

Fig. 1. Study area with indication of the sample sections.

20 g of mercury chloride) until complete dissociation of the follicles had occurred. The vitellogenic follicles were separated from the others by the use of sieves whose meshes presented a diameter smaller than the minimum diameter of the vitellogenic oocytes. Absolute fecundity (Af) was determined from the following expression: Af = NFG.GW; where NFG = number of follicles per ovary gram and GW = gonad weight. The relative fecundity (RF) was calculated in order to eliminate the interference of body weight (BW) on the fecundity estimates, using the expression: RF = Af/BW.

Physical and chemical parameters of the water. A multiparameter surface probe (YSI brand) was used for measuring the following physical and chemical parameters of the water: temperature, dissolved oxygen concentration, pH and electrical conductivity. The water transparency was measured with a Secchi disc.

Statistical Analysis. All results were expressed as means ± standard deviation and were considered significant with $p<0.05$. The comparison of follicular diameters, morphometric measurements, abiotic factors and biological indices of the three river sections were done through the Kruskal-Wallis non-parametric test followed by the Dunn post-test. For all tests, a degree of freedom of H = 2 was obtained.

Results

Morphologically, the ovaries of the *P. maculatus* are sacciform in shape, yellow in colour and the testes are elongated organs with filiform projections containing anastomosed seminiferous tubules. In the caudal region of the testes in advanced maturation/mature and spent stages, the lumen of the seminiferous tubules presented globular acidophilic secretions (Fig. 2).

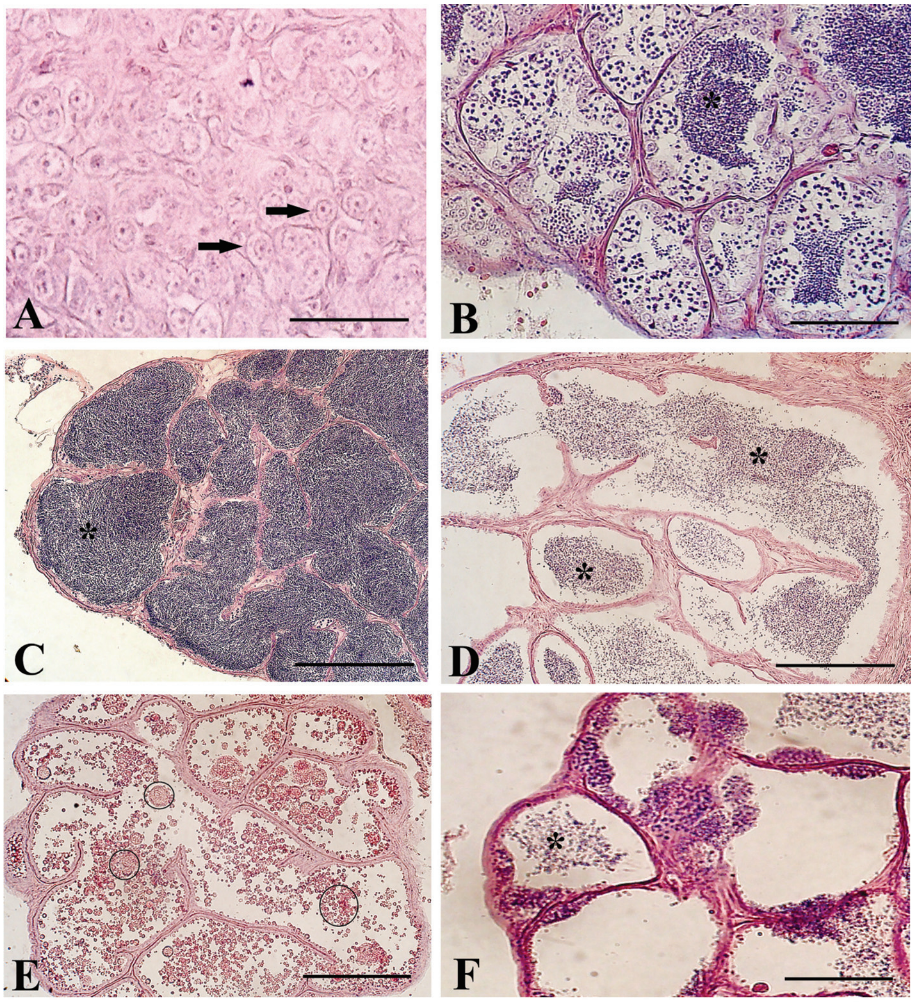

Fig. 2. Histological sections of *P. maculatus* testes, in different stages of gonadal maturation, stained with HE. **A**: Resting with seminiferous tubules closed containing only spermatogonia; **B**: Initial maturation, with seminiferous tubules containing all spermatogenic lineage cells and a small amount of sperm; **C**: Advanced maturation/mature, with seminiferous tubules filled with sperm; **D**: Partially spent, with open seminiferous tubules containing a considerable amount of sperm; **E**: Caudal region of a partially spent testis with globular acidophilic secretion (circle); **F**: fully spent, with open seminiferous tubules containing residual sperm in the lumen. * = Sperm; Arrow = Spermatogonia. Scale bars = 20 µm (**A**), 50 µm (**B** and **F**), 100 µm (**C-E**).

A total of 302 females and 254 males (N = 556) specimens of *P. maculatus* were captured from the three sections. Males and females captured from S1 presented average values of total length and body weight higher than those captured in S2 and S3. The GSI values were significantly higher in fish collected in S3 and the Fulton condition factor did not show significant differences in fish collected in the three sections (Tables 1-2).

Through macro and microscopic analyses of the gonads and GSI variations, four gonadal maturation stages were determined: 1 = resting, 2 = initial maturation, 3 = advanced maturation/mature and 4 = spawned for females and spent for males (Figs. 2-3). During the whole sampling period, there was a predominance of resting females in the three sections and of resting males in S1. Males and females in stages M3 and F3 were recorded in the three sampling sections although females in that stage were rare. Spawned females (F4) were only registered in S3, whereas spent males (M4) were found in all three sections (Fig. 4). The highest values for absolute fecundity and GSI were recorded in S2 and S3 (Tables 1-2). The oocyte diameter, the follicular cells height and the zona pellucida thickness did not show any statistical differences between the sampling sections (Table 3). Despite the low number of spawned females, we observed atretic follicles in the ovaries of females captured in S3. This process was characterised by the fragmentation of the zona pellucida, liquefaction of the yolk, hypertrophy of the follicular cells and yellow body formation. Three phases of follicular atresia were determined in *P. maculatus*: 1 = initial, 2 = intermediate, 3 = final (Fig. 3).

Among the chemical and physical parameters of the water, there were no statistical differences between the three sections regarding the pH, dissolved oxygen concentration and temperature (Fig. 5). In all three sections, the temperature was higher from October to March. The conductivity presented the highest values in S3 and the lowest ones in S1, with statistically significant differences between them. The conductivity peak in S2 and S3 occurred in October, followed by a gradual decrease until February, during the reproductive period. Water transparency in the reproductive period, from November to January, presented similar values from the two sampling sections of the Grande River (S1= 2.45 ± 0.05m, S2= 2.35 ± 0.05m), and a much smaller value in the Pardo River site (S3= 0.22 ± 0.02m).

Discussion

This study showed that the construction of the Porto Colômbia dam on the Grande River resulted in alterations to the reproduction process of *P. maculatus*, in the section downstream from the dam, and that the Pardo River is an important tributary for reproductive success of this species.

Table 1. Biological variables of *P. maculatus* males, collected between January/2010 and February 2011, from three different sections of the Grande River basin, downstream from the Porto Colômbia power plant. Section 1: Grande River, downstream from the Porto Colômbia power plant; Section 2: confluence of the Grande and Pardo rivers; Section 3: the Pardo River channel; N: number of fishes captured; GSI: Gonadosomatic index; K: Fulton condition factor. Data expressed as mean ± standard deviation (SD); different letters indicate statistical differences between the sampling sections ($p < 0.05$).

	Section 1 (N=70)		Section 2 (N=81)		Section 3 (N=101)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
TL (cm)	27.51 ± 2.25a	23 - 33.6	25.42 ± 2.53b	20 - 33	26.31 ± 2.60c	16.5 - 33.5
BW (g)	254.59 ± 78.8a	140 - 527	217.51 ± 60.22b	115 - 415	238.48 ± 72.63a	52 - 533
GSI (%)	0.17 ± 0.13a	0.004 - 0.54	0.29 ± 0.10b	0.10 - 0.58	0.33 ± 0.26b	0.003 - 1.01
K	2.12 ± 0.42a	1.18 - 4.33	2.40 ± 0.40a	1.69 - 4.39	2.28 ± 0.40a	1.15 - 3.25

Table 2. Biological variables of *P. maculatus* females, collected between January/2010 and February 2011, from three different sections of the Grande River basin, downstream from the Porto Colômbia power plant. Section 1: Grande River, downstream from the Porto Colômbia power plant; Section 2: confluence of the Grande and Pardo rivers; Section 3: the Pardo River channel; N: number of fishes captured; n: number of mature ovaries; GSI: Gonadosomatic index; K: Fulton condition factor; Af: Absolute fecundity; RF: Relative fecundity. Data expressed as mean ± standard deviation (SD); different letters indicate statistical differences between the sampling sections ($p < 0.05$).

	Section 1 (N=140)		Section 2 (N=66)		Section 3 (N=96)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
TL (cm)	30.34 ± 3.10a	23.0 - 39.0	27.98 ± 3.72bc	14.5 - 39.0	29.65 ± 2.49ac	24.0 - 36.0
BW (g)	364.57 ± 125.04a	168 - 935	296.69 ± 125.6bc	38 - 873	350.73 ± 88.8ac	178 - 587
GSI (%)	0.74 ± 0.36a	0.22 - 2.25	1.18 ± 1.13ac	0.31 - 4.62	1.30 ± 1.42bc	0.36 - 7.55
K	2.16 ± 0.34a	1.49 - 3.85	2.31 ± 0.33a	1.49 - 2.89	2.29 ± 0.31a	1.55 - 3.2
Af (n=12)	25136 ± 1259.7a	9038 - 26812	45763 ± 1377.6b	7274 - 47289	27095 ± 6731.9a	17165 - 62857
RF	47.40 ± 7.56a	40.9 - 55.7	93.66 ± 58.78a	27.98 - 167.1	75.38 ± 35.04a	31.55 - 125.72

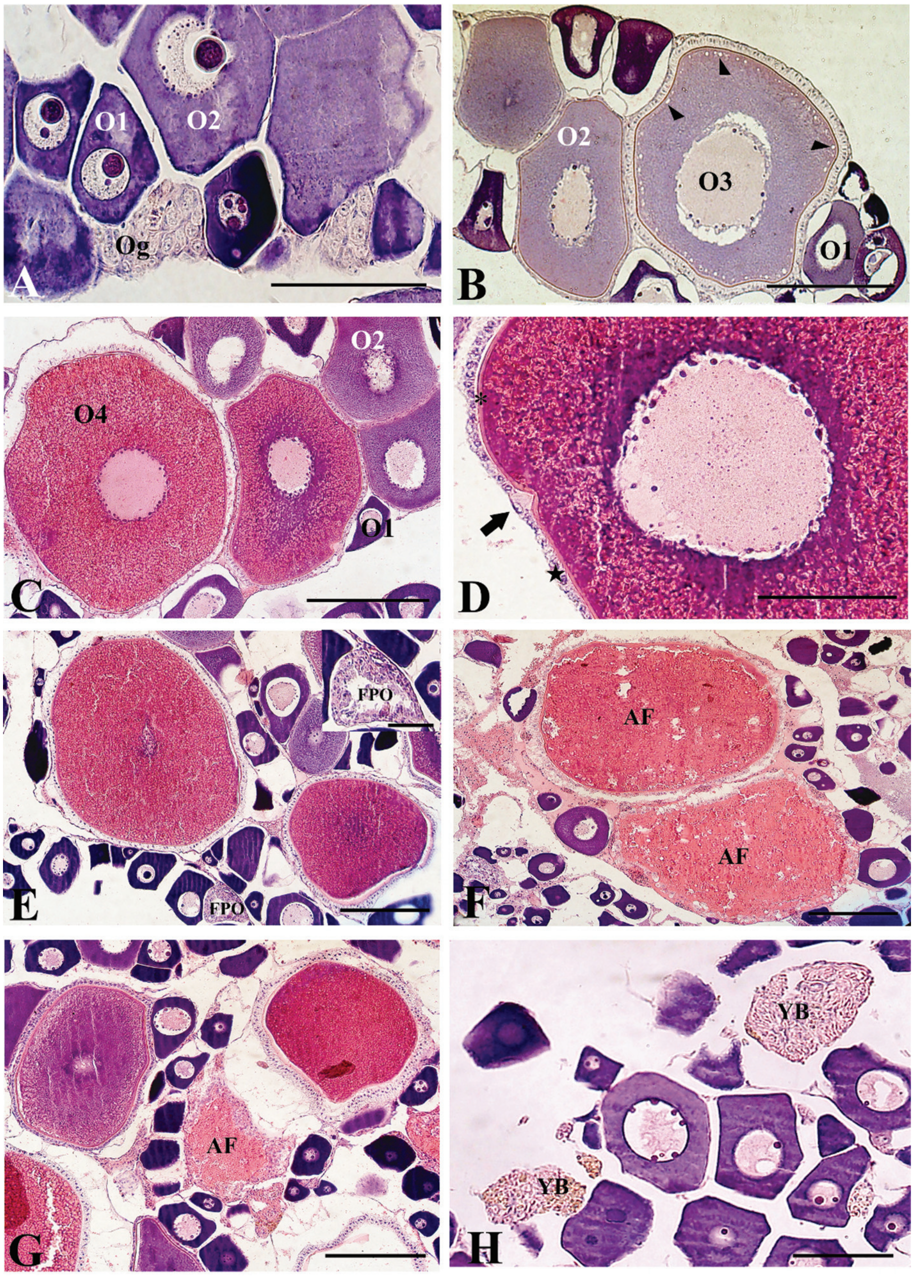

Fig. 3. Histological sections of *P. maculatus* ovaries, in different stages of gonadal maturation, stained with HE. **A**: Resting, with oogonia nests (Og), initial perinucleolar oocyte (O1) with basophilic cytoplasm and advanced perinucleolar follicles (O2) with granular cytoplasm; **B**: Initial maturation, with O1, O2 and pre-vitellogenic oocyte (O3) presenting cortical alveoli (arrow head) in the peripheral ooplasm; **C**: Advanced maturation/mature with O1, O2 and O3 and vitellogenic oocytes with ooplasm filled with acidophilic yolk globules; **D**: Detail of vitellogenic oocyte with cubic follicular cells (star), thin zona pellucida (*) and funnel-shaped micropile (arrow); **E**: Partially spawned, with O1, O2, O3, O4 and post-ovulatory follicles (FPO); insert of post-ovulatory follicle with wide lumen and wall of follicular cells and theca; **F**: Atretic follicles (AF) in the initial phase with yolk liquefaction and fragmentation of the zona pellucida; **G**: Partially spawned ovary with atretic follicle (AF) in the intermediate phase with hypertrophy of the follicular cells and an almost fully reabsorbed yolk; H: Fully spawned ovary with O1, O2 and atretic follicle in the final stage, forming yellow bodies (YB). Scale bars = 50 µm (A, D, insert of POF and H), 100 µm (B, C), 200 µm (E-G).

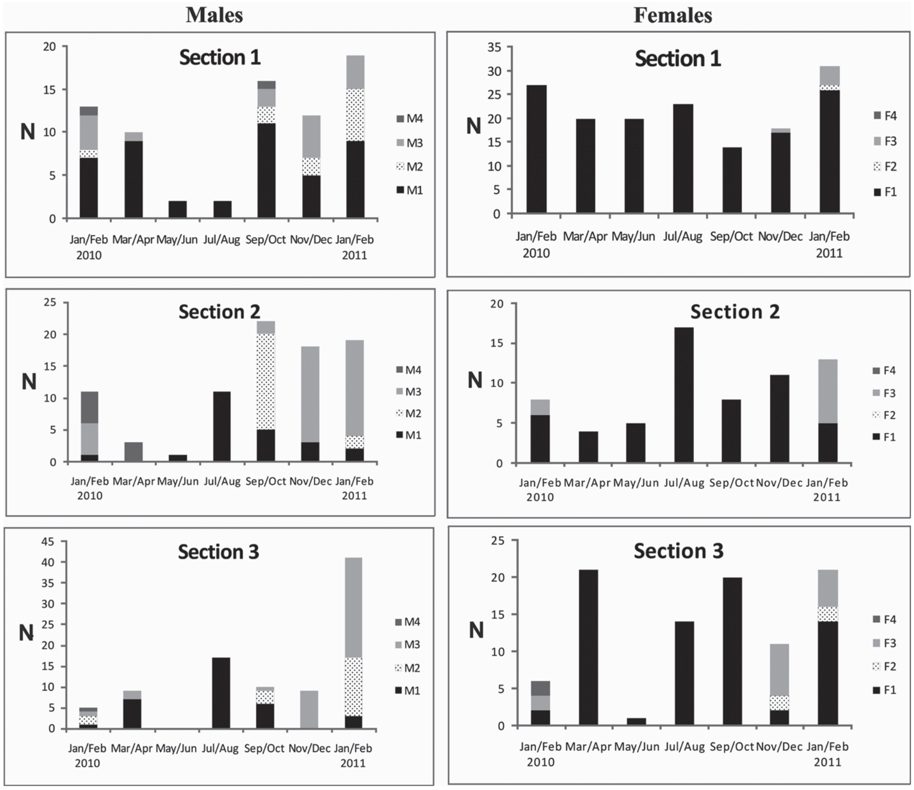

Fig. 4. Bimonthly frequency of the gonadal maturation stages of *P. maculatus* males and females from three sampling sections of the Grande River basin, downstream from the Porto Colômbia dam. Section 1: Grande River, downstream from the Porto Colômbia dam; Section 2: confluence of the Grande and Pardo Rivers; Section 3: the Pardo River channel.

In this study, fish in S1 presented statistically higher body weight and total length averages than the ones in S2 and S3. This may be explained by the fact that the area immediately downstream from the Porto Colômbia dam, a length of approximately 500 meters, is a protection area, where fishing is completely prohibited and, therefore, favouring a larger size for those fish that remain there. In the other areas of the Grande River and in the Pardo River, there is intensive commercial and recreational fishing, and *P. maculatus* is one of the most captured species, thus preventing growth as in S1, similar to Bianchi *et al.* (2000) who found that fish communities subjected to intense fishing pressure tends to be smaller sized.

The morphological features of the fish ovaries and testes captured in the three sampling sections were similar. The yellowish colour of the ovaries and the histological features of the follicular cells and zona pellucida of the vitellogenic oocytes are similar to what has been reported by Melo *et al.* (2011). The funnel-shaped micropyle observed in *P. maculatus* in this study seems to be a common pattern for fish of the Pimelodidae family (Rizzo *et al.*, 2002). In this study, *anastomosis* of the seminiferous tubules, a characteristic of testes of the anastomotic tubular type as described by Parenti & Grier (2004), was observed. In the caudal portion of the spent testes of *P. maculatus*,

Table 3. Measurements of *P. maculatus* vitellogenic follicles from the three sampling sections. Section 1: Grande River, downstream from the Porto Colômbia power plant; Section 2: confluence of the Grande and Pardo rivers; Section 3: the Pardo River channel. N: number of follicles analysed. Data expressed as mean ± standard deviation (SD); different letters indicate statistical differences between the sampling sections ($p < 0.05$).

	Section 1 (N=20)		Section 2 (N=20)		Section 3 (N=40)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Oocyte diameter (µm)	429.7 ± 53.7a	337.8 - 518.1	446.3 ± 70.7a	263 - 563.3	457.5 ± 62.5a	351.2 - 547.8
Follicular cells (µm)	15.01 ± 3.40a	9.60 - 21.50	26.18 ± 12.47a	11.90 - 40.30	18.13 ± 7.41a	7.20 - 35.00
Zona pellucida (µm)	2.63 ± 0.46a	1.80 - 3.50	2.54 ± 0.73a	1.60 - 4.00	2.81 ± 0.62a	1.70 - 4.10

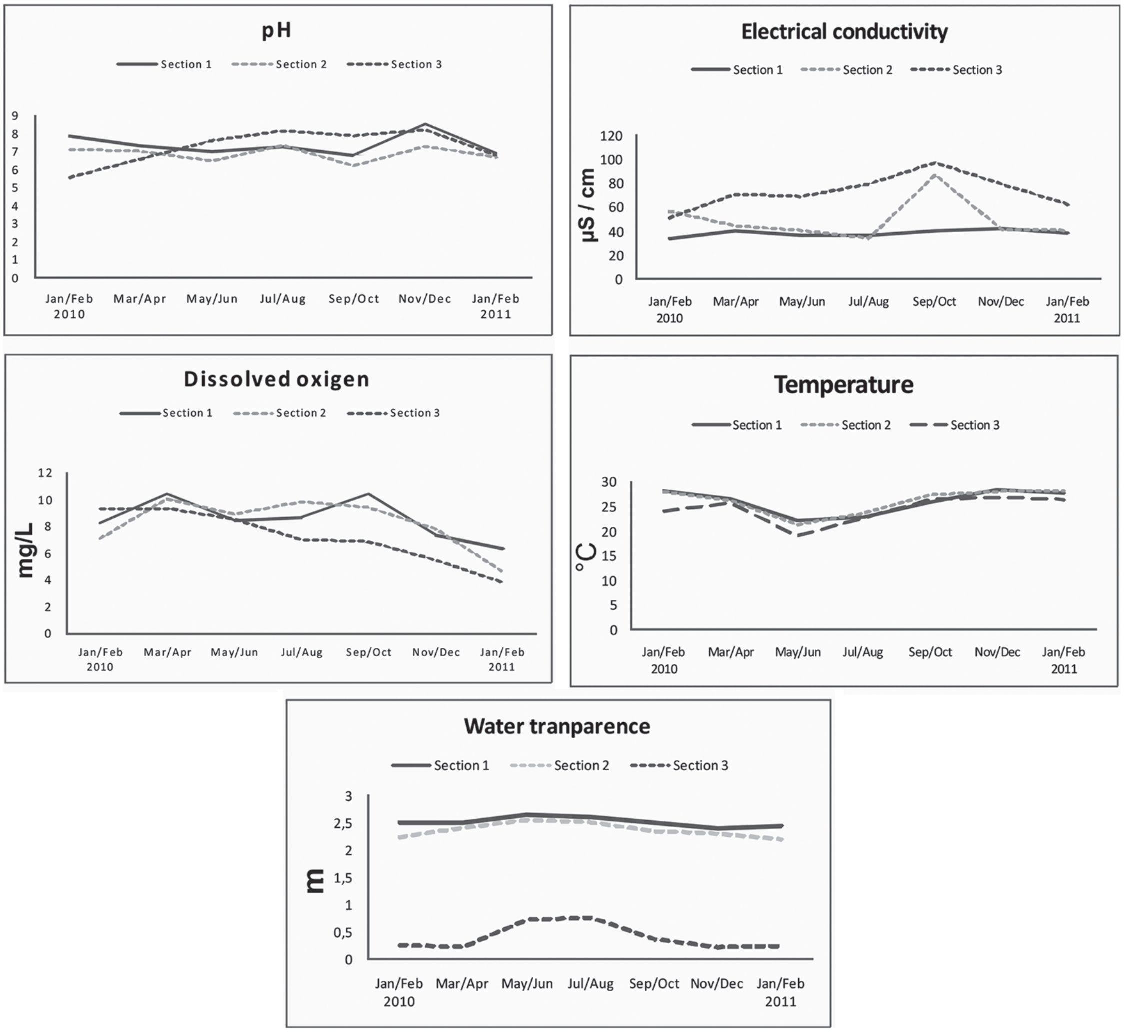

Fig. 5. PH, dissolved oxygen, electrical conductivity, temperature and water transparency in three sections of the Grande River basin, downstream from the Porto Colômbia dam. Section 1: Grande River, downstream from the Porto Colômbia dam; Section 2: confluence of the Grande and Pardo Rivers; Section 3: the Pardo River channel.

globular acidophilic secretion was observed, as has also been observed by Melo *et al.* (2011). Some studies showed that this secretion may be related to sperm maturation and nutrition and may play a crucial role in fertilisation (Mazzoldi *et al*., 2005; Chowdhury & Joy, 2007).

In this study, we observed that the *P. maculatus* reproduces between October and February, which coincides with the rainy season, confirming the observations of Braga (2000). The low variation of the Fulton condition factor in the three sampling sections suggests that the fish's health is not affected by the reproductive period. A similar result was found for this same species in the Piracicaba River, São Paulo State, by Lima-Junior & Goitein (2006). Analysing only the reproductive period, from October to February, it was observed that *P. maculatus* presented a low frequency of fish in reproductive activity (19.70%) from S1, whereas in S2 and S3, higher frequencies, 56.25% and 55.55% respectively, were registered. A similar situation was observed with fish captured during the reproductive period from the São Francisco River, downstream from the Três Marias dam (Sato *et al*., 2005).

Impacts on the ichthyofauna downstream from dams have been reported by several authors (Agostinho *et al*., 1993; Clarkson *et al*., 2000; Paukert & Rogers, 2004; Todd *et al*., 2005; Olden & Naiman, 2010). A recent study showed that, in the São Francisco River immediately downstream from the Três Marias dam, the species *Prochilodus argenteus* presented high rates of vitellogenic oocyte atresia and alterations to the endocrine system due to the physical and chemical changes of the water, especially the colder water temperature of this river section (Arantes *et al*., 2010). Contrarily, in the present study, the mean water temperature in the three sections was not statistically different, thus this is probably not the cause of the low frequency of fish in reproductive activity immediately downstream from the Porto Colômbia dam.

In this study, it was verified that in S3, during the reproductive period, there is a sharp decrease in conductivity and water transparency values, indicating that this decrease may act as a reproductive trigger for *P. maculatus*. This assumption is borne out by Godoy's (1972) reports who also observed in the Grande/Pardo/Moji Guaçu Rivers system a decrease in conductivity and transparency during the reproductive period and considered them to be critical factors for triggering fish reproduction. Even though spent/spawned fish were captured from the Pardo River, the small number of fish in these stages, in relation to the total number of collected fish, leads us to believe that S3 is not a reproduction site, and may only be a migratory route for fish which spawn in the Moji Guaçu River, as described by Godoy (1967, 1972).

In S1, a low frequency of mature and spawned/spent fish was observed. Furthermore, a significantly lower GSI and significantly higher body weight was observed when compared to S2 and S3. These findings may be related to the environmental parameters after dams which are not favourable to the reproductive success of fish (Olden & Naimam, 2010). On the other hand, the higher number of mature and spawned/spent fish captured in S2 and S3 reinforces the idea that *P. maculatus*, in its reproductive migration, makes this potamodromous movement between the Grande, Pardo and Moji Guaçu Rivers up to Cachoeira de Emas (21º92'54"S 48º23'41"W), where it probably finishes its reproductive migration (Godoy 1967, 1972).

It is known that the construction of dams harms the aquatic biota (Agostinho *et al*., 1993; Sato *et al*., 2003; Sato *et al*., 2005; Arantes *et al*., 2010; Barletta *et al*., 2010), thus emphasising the importance of tributaries on the reproductive process since these water bodies may present a natural hydrological system and abiotic factors still unaltered by anthropic action, or within a tolerable threshold for the maintenance of the local biota (Antonio *et al*., 2007). Therefore, our results emphasise the need to preserve the Pardo and Moji Guaçu Rivers for the reproductive success of *P. maculatus*, since that rivers has a crucial hole in the reproductive migration of this species downstream Porto Colômbia dam.

Acknowledgments

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