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Reproductive indicators of the endemic species *Astyanax bifasciatus* (Teleostei: Characidae) in a tributary of the Lower Iguaçu River Basin, Brazil

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ABSTRACT. The reproductive process promotes morphophysiological and behavioral changes in fish species throughout their life cycle. Its success is vital to define their resilience in the environment. This study aimed to evaluate the reproductive biology of the endemic fish species *Astyanax bifasciatus* in a tributary of the Lower Iguaçu River Basin, Paraná, Brazil. Fish were collected monthly at four sites along the Jirau Alto River in the city of Dois Vizinhos from October 2015 to September 2016. A standardized catch effort with gill nets and fish traps was used. In the laboratory, standard length and total weight were recorded. After anesthesia, the animals were sectioned to macroscopically determine the sex, sexual maturity stage, and presence of celomatic fat. The gonads and liver were removed to determine the gonadosomatic and hepatosomatic indexes, respectively. A total of 160 individuals (104 females and 56 males) were used. The gonadosomatic index, frequency of the gonadal maturation stages, and condition factor showed a long reproductive period with two investment cycles for both sex. Length at first sexual maturity was 4.57 cm for females and 3.56 cm for males. The reproductive data corroborate the generalist profile of the species and demonstrate a high degree of adaptive capacity, even in smaller tributaries.

Keywords: Gonadossomatic; Auto-ecology; Condition factor; Generalist; Spawning.

Introduction

In natural environments, the population viability of a fish species is entirely dependent on its capacity to adapt its reproductive tactics to the primary environmental conditions or the new conditions brought about by anthropic activities. Depending on the scenario, these tactics may not be enough to ensure a strategy that will maintain and perpetuate the species in the environment (Mérona, Mol, Vigouroux, & Chaves, 2009).

The reproductive tactics of fish are evaluated through multiple indicators, which are obtained from the morphophysiological and behavioral changes that occur in the populations, due to local and seasonal environmental conditions (Winemiller, 1989). The primary indicators used in reproduction studies reflect, for example, the level of investment in the production of gametes, spawning/spermiation or recruitment period (Brewer, Rabeni, & Papoulias, 2008), physiological condition of the animals, dislocation of fat for the incorporation of yolk in the ovarian follicles of females, and sexual maturation sizes of males and females (Brosset et al., 2016), among others.

Given the current scenario of multiple environmental impacts, many anthropic variables interfere with the homeostasis of the systems and, consequently, species fitness (Louiz, Ben-Attia, & Ben-Hassine, 2009), imposing an adaptive pressure on the reproductive tactics in order to ensure population viability. In this context, pollution is one of the many impacts faced by native species (Yamamoto et al., 2016), in addition to the changes in watercourses that generally occur as a result of dams (Weber et al., 2013; De Fries, Rosa, Silva, Vilella, & Becker, 2018).

The Lower Iguaçu River region is a critical example of these impacts since its course is interrupted by six hydroelectric power plants (HPP), five of which are currently in full operation. On the other hand, the ichthyofauna of the Iguaçu River is considered one of the most singular in the world, with more than 75%

endemic species (Baumgartner et al., 2012). The scenario suggests a significant, long-term issue related to the global extinction of species, demanding efficient biological indicators in order to detect changes in the structure of fish assemblages.

In the search for ideal biological indicators, it should be noted that not all species respond negatively to dams (e.g. population decreases/endangerment). There are generalists, or so-called explorer species, with great adaptive capacity, which use the new conditions to reconfigure their reproductive tactics and reach their biotic potential (r_{max}), mediated by resource availability and spawning sites and by the inexistent/low control of the trophic chain (bottom-up and top-down), especially during the first years of flooding (Mérona, Vigouroux, & Horeau, 2003; Silva, Muelbert, Oliveira, & Favaro, 2010).

The fish species *Astyanax bifasciatus* (Garavello & Sampaio, 2010) is popularly known as the red-tail lambari and is restricted to the Iguaçu River Basin; therefore, it is considered endemic to the region. *Astyanax bifasciatus* is a generalist species that has drastically increased its fitness in artificial lakes due to the dams (Silva et al., 2010), demanding the monitoring of its auto-ecology in tributaries to generate data that allow its management or use as bioindicators.

Thus, this study sought to evaluate the reproductive biology of the endemic species *A. bifasciatus* in a tributary of the Lower Iguaçu River Basin, Paraná, Brazil.

Material and methods

Study area

This study was conducted in four sampling points (P1 to P4) along Jirau Alto River, in the city of Dois Vizinhos, southwestern Paraná, Brazil (Figure 1). The Jirau Alto is a second-order river, a tributary of the Dois Vizinhos River, sub-basin of the Chopim River, which is one of the three most important rivers of the Lower Iguaçu River Basin (Pigosso, Bonfante, Farias, Becegato, & Onofre, 2009).

P1 was situated near the headwaters of the Jirau Alto River, with denser riparian vegetation that undergoes periodic flooding. The river is about one meter deep here and the riverbed is muddy (fine sediment). P2 was established approximately 3 km downstream. It is an area with scarce riparian vegetation and a deeper riverbed (1.5 m) with fine sediment. The other points were about 1.5 km from each other. Dense riparian vegetation similar to P1 was verified in P3. In P3, like P4, the river presented greater velocity and its bed was composed of gravel and coarser sediment. P4 was located near the mouth of the Jirau Alto River and its riparian vegetation was restricted to 5 m from the riverbank.

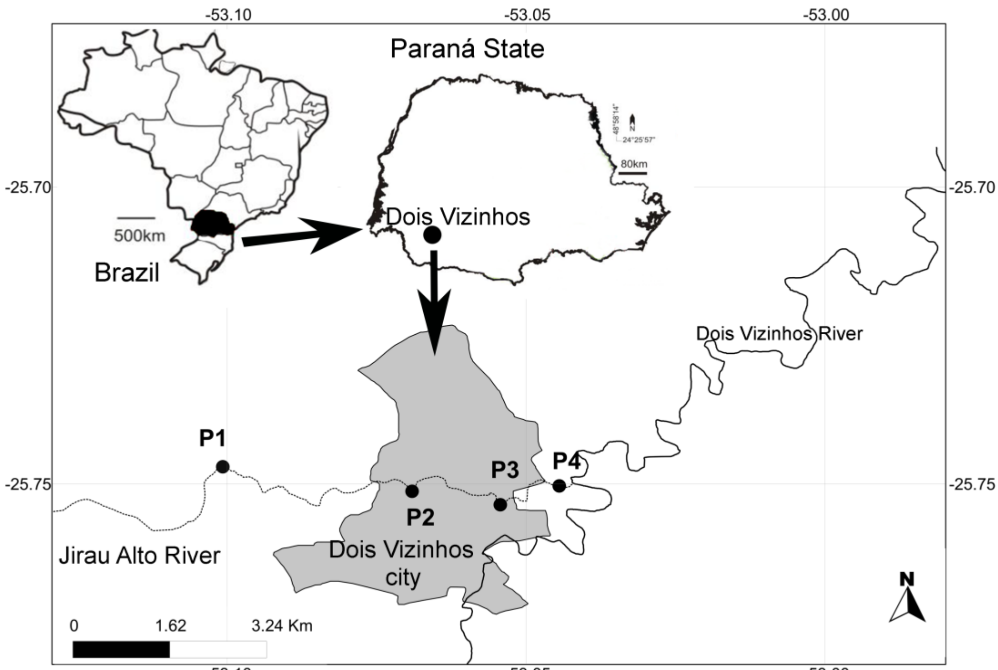

Figure 1. Study area location of the four sampling points (P1, P2, P3, and P4) of *Astyanax bifasciatus* in the Jirau Alto River, Dois Vizinhos, Paraná, Brazil.

The city of Dois Vizinhos has an area of 418 km^2, an average altitude of 509 m, and is located between the geographical coordinates 25°44'35'' S and 53°4'30'' W. Its vegetation is classified as ecotonal, between Semideciduous Seasonal Forest and Mixed Ombrophilous Forest, both presenting phytophysiognomies of the Atlantic Forest (Instituto Brasileiro de Geografia e Estatística [IBGE], 2004).

The climate of the region is characterized as Cfa; humid subtropical mesothermal with hot summers, an undefined dry season, and an average temperature of the coldest and hottest months below 18°C and above 24°C, respectively. Frosts are infrequent and the winds are predominantly south-southeast during mild weather and north-northeast in rainy periods (Maack, 1981). Relative air humidity ranges from 70 to 75% and rainfall is between 1,800 and 2,200 mm $year^{-1}$ (Nitsche, Caramori, Ricce, & Pinto, 2019).

Sampling and laboratory procedures

We performed monthly standardized collections during the full moon from October 2015 to September 2016. The full moon was chosen because there is accumulated experimental evidence that fish from tropical and subtropical waters use moonlight-related periodicities as reliable information for synchronizing the timing of reproductive events (see Ikegami, Takeuchi, Hur, & Takemura, 2014 for more information).

Fish were captured using a set of three gill nets (15, 20 and 25 mm mesh sizes between opposite knots, 10 m long and 2.0 m high). A fish trap was also used (80 cm long and 40 cm in diameter) at each sampling site. The nets were arranged parallel to the riverbank and the fish traps were placed in deeper areas or where there was marginal vegetation. Both types of fishing gear were immersed in water for 24 hours at each site. The set up and removal of the fishing gear were always carried out early in the morning on the same day at all sampling sites.

The captured fish were anesthetized and euthanized using 250 mg L^{-1} benzocaine hydrochloride, according to the CONCEA Directives for Euthanasia Practice, which regulates the procedures and methods of euthanasia in animals (Conselho Nacional de Controle de Experimentação Animal [CONCEA], 2018). The fish were later refrigerated and transported to the Zoology lab of the *Universidade Tecnológica Federal do Paraná* (UTFPR), Dois Vizinhos Campus, for processing.

Authorization to collect the fish was obtained from SISBIO under protocol nº 50414-1. The study was previously approved by the UTFPR Ethical Committee on the Use of Animals, under protocol nº 2015-20.

The taxonomic identification of the fish was confirmed in the laboratory (Baumgartner et al., 2012). Morphometric measurements for total (Lt) and standard (Ls) length (cm) and total weight (Wt) (g) were subsequently determined. The animals were than sectioned to expose the viscera. The liver and gonads were removed and weighed using a semi-analytical balance. Individuals with somatic fat were only counted for frequency estimates.

Before removing the gonads from the abdominal cavity, a previous macroscopic analysis was performed to identify the sex and outline the stage of ovarian and testicular development, as proposed by Bomfim, Peretti, Camillo, Costa, and Nascimento (2015).

Endpoints and Data Analysis

The parameters used to evaluate the reproductive cycle of *A. bifasciatus* were: sex ratio, gonadosomatic index, frequency of gonadal development stages, frequency of individuals with celomatic fat, hepatosomatic index, total and somatic condition factor (allometric model), and sexual maturation length.

The sex ratio was analyzed applying the G-test, evaluating the difference as regards the expected proportion (1:1) of female and males. The results were considered significant when $p < 0.05$.

The individual gonadosomatic index (GSI) was used to calculate the monthly average GSI, which was used to elaborate the maturation curve for females and males. The GSI was obtained by equation 1 (Araújo, Morado, Parente, Paumgartten, & Gomes, 2018):

$$GSI = \frac{\mathrm{Wg}}{\mathrm{Wt}} X\ 100 \qquad (1)$$

where:
Wg = weight of the gonad;
Wt = total weight of the individual.

The percentage frequency of gonadal developmental stages was determined for separate sexes. The developmental stages were initially identified as: immature (IMT); maturing (MTG); mature (MAT);

spawned or recovered (females)/spermiation or emptied (males) (SPN). The monthly percentage of the stages was calculated later, considering the separate sexes.

Only females were used to analyze the frequency of individuals with celomatic fat and the hepatosomatic index because of their ability to incorporate yolk into the ovarian follicles during the reproductive process, which affects their ability to accumulate fat and later make it available. The hepatosomatic index (HIS) was estimated individually and the monthly average HSI was subsequently calculated. The HSI was obtained by equation 2 (Araújo et al., 2018):

$$HSI = \frac{\mathrm{Wl}}{\mathrm{Wt}} X\ 100 \qquad (2)$$

where:
Wl = weight of the liver;
Wt = total weight of the individual.

To correlate the allocation of resources with the reproductive process, the allometric condition factor was calculated for both sexes. The condition factor was determined monthly and for the separate sexes (Lima-Junior, Cardone, & Goitein, 2002) using the total (K) and somatic (K') condition factor. The calculations were performed based on equation 3 (Vazzoler, 1996):

$$K = \frac{\mathrm{Wt\ or\ Ws}}{Ls^{\mathrm{b}}} \qquad (3)$$

where:
Wt = total weight – used to calculate K;
Ws = somatic weight (disregarding the weight of the gonad) – used in K';
Ls = standard length;
b = angular coefficient obtained through the weight-length relationship using the minimum square method, as described by Le Cren (1951).

First maturation size is an estimate based on length classes and the frequency of adults in the population. This analysis generally seeks to estimate the length at which 50% of the population (L_{50}) are adults. The standard length of the individuals was used for this study, which considered adults to be those with gonads in the maturing, mature or spawned/spermiated stage.

The L_{50} were estimated separately for females and males using a curve that correlates the relative frequency of adult individuals with the midpoint of the length classes obtained through the Sturges Postulate. The model used to adjust the curve was expressed by equation 4 (Lemos, Varela-Junior, Velasco, & Vieira, 2011):

$$Fr = 1 - \mathrm{e}^{-\mathrm{a}*\mathrm{Lp}^{\mathrm{b}}} \qquad (4)$$

where:
Fr = relative frequency of adult individuals;
e = base of the Napierian logarithm;
a = intercept value;
b = angular coefficient obtained using the least squares method;
Lp = midpoint of the length classes.

Results

A total of 160 individuals were collected (104 females and 56 males) (Table 1). The prevalence of females occurred throughout the entire collection period, but no significant difference was observed (G test = 15.51; p = 0.16; df = 11).

The maturation curve (Figure 2) demonstrated that, for females, the highest average GSI values occurred in October and November, followed by an abrupt decrease in January and February. A new increase in the GSI between March and May (smaller) was subsequently verified, indicating a long reproductive period with two investment cycles. A progressive increase occurred in the GSI values in later months, suggesting maturation.

Table 1. Number of individuals and length mean of females (F) and males (M) of *Astyanax bifasciatus* in the Jirau Alto River, Dois Vizinhos, Paraná, Brazil. SD = standard deviation.

Month	Number of individuals		Length mean ± SD	
	F	M	F	M
Oct-2015	11	5	8.75 ± 0.77	8.20 ± 0.52
Nov-2015	11	7	9.39 ± 0.54	8.59 ± 0.22
Dec-2015	11	9	8.34 ± 0.45	7.94 ± 0.28
Jan-2016	9	3	9.20 ± 0.93	8.10 ± 0.14
Feb-2016	6	3	7.98 ± 2.34	6.27 ± 1.49
Mar-2016	5	8	7.28 ± 2.44	6.64 ± 0.75
Apr-2016	14	4	5.66 ± 2.41	6.25 ± 2.71
May-2016	11	3	8.10 ± 0.81	8.07 ± 1.54
Jun-2016	5	3	8.74 ± 1.05	6.93 ± 1.34
Jul-2016	5	6	5.78 ± 1.96	6.37 ± 1.52
Aug-2016	10	2	8.04 ± 0.79	7.10 ± 0.50
Sep-2016	6	3	8.55 ± 0.92	7.43 ± 0.87
Total	104	56		

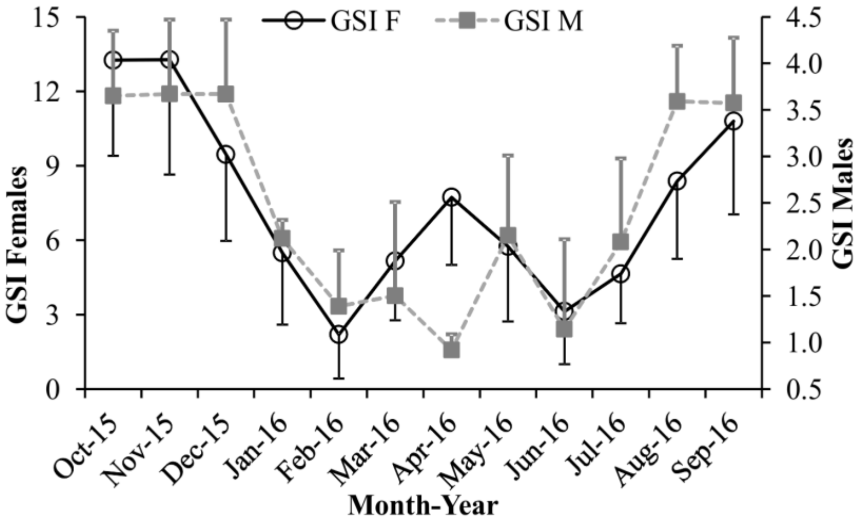

Figure 2. Sexual maturation curve of female (F) and male (M) *Astyanax bifasciatus* in the Jirau Alto River, Dois Vizinhos, Paraná, Brazil. GSI = monthly average of gonadosomatic index.

Male and female gonadal development were similar, with the highest GSI values occurring from August to December, followed by a sudden decline in January and February. Males also appear to have a second investment cycle between March and June. In subsequent months, there was a progressive increase in gonadal development.

The monthly frequency of the gonadal maturation stages of females and males (Figure 3) complements the previously reported GSI results, demonstrating a higher proportion of mature female individuals from September to January and males from October to December. The second period of frequencies of mature individuals was from March to July for males, except in April (no mature males). There was a reduction in the frequency of mature females in February, followed by a new increase from March to May.

Immature individuals were present in February, April, and July, indicating a potencial recruitment and reinforcement of the second reproductive period of the year. There was a higher proportion of females in the spawning stage from December to February and May to June. As regards the males, there was a continuum of spermiated individuals from January to May.

In general, individuals in maturation were observed from June to December for both sexes, with a progressive increase in their number, corroborating the GSI increase during the period.

Based on the frequency of females with celomatic fat, the lowest values were observed from September to October, intermediate values from November to April, and the highest values from May to August. This information agrees with the average hepatosomatic index (HSI) values, which regress during the period in which there are individuals with greater fat accumulation (Figure 4). On the other hand, the highest values of HSI were observed in September, April and May, indicating fat metabolism for the reproductive cycle.

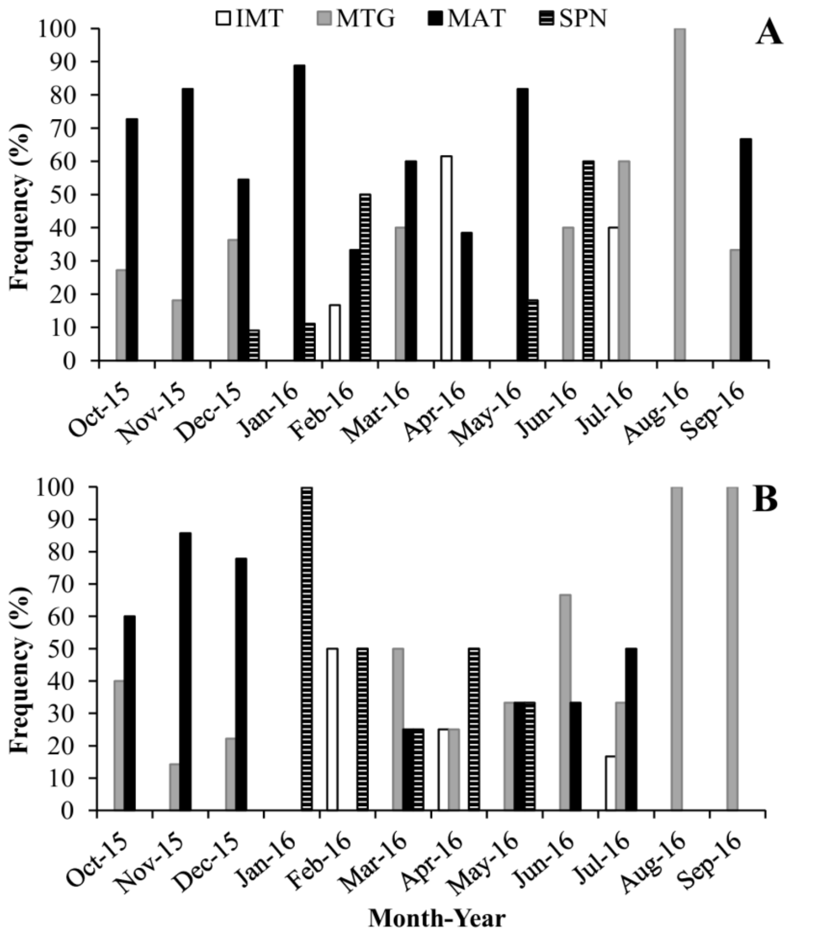

Figure 3. Monthly frequencies for the sexual maturation of female (A) and male (B) *Astyanax bifasciatus* in the Jirau Alto River, Dois Vizinhos, Paraná, Brazil. IMT = immature; MTG = maturing; MAT = mature; SPN = spawned/emptied.

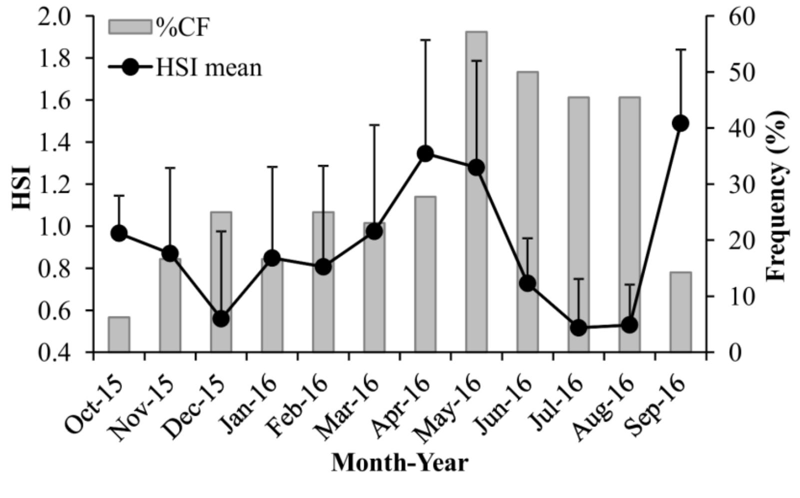

Figure 4. Monthly frequency of females with celomatic fat and average monthly hepatosomatic index (HSI) values for female *Astyanax bifasciatus* in the Jirau Alto River, Dois Vizinhos, Paraná, Brazil.

The condition factor showed that females and males present two periods with the highest average K values throughout the year, from June to September and from February to March. After both periods, a decrease in K values was verified. Moreover, the most relevant differences between K and K' for both sexes were found to have occurred between July and December, suggesting a higher investment in the gonad (Figure 5). These results are consistent with the GSI and frequency of gonadal maturation stages.

Length at first sexual maturity (L_{50}) was estimated to be 4.57 cm for females and 3.56 cm for males. The shortest length at which all individuals were fit for reproduction (L_{100}) was estimated to be 8.36 cm for females and 9.53 cm for males (Figure 6).

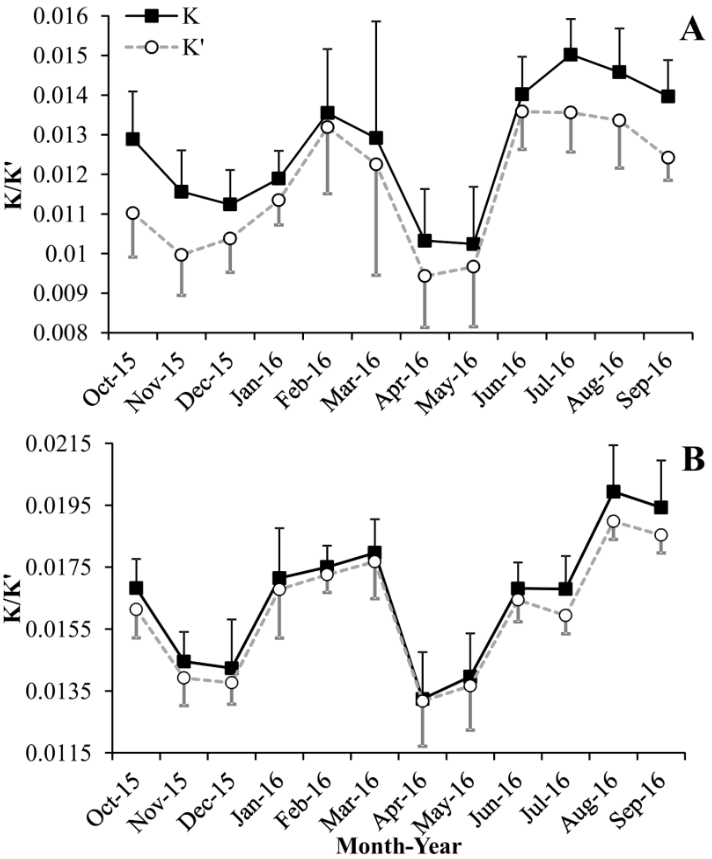

Figure 5. Mean monthly values of the total (K) and somatic (K') condition factors for female (A) and male (B) *Astyanax bifasciatus* in the Jirau Alto River, Dois Vizinhos, Paraná, Brazil.

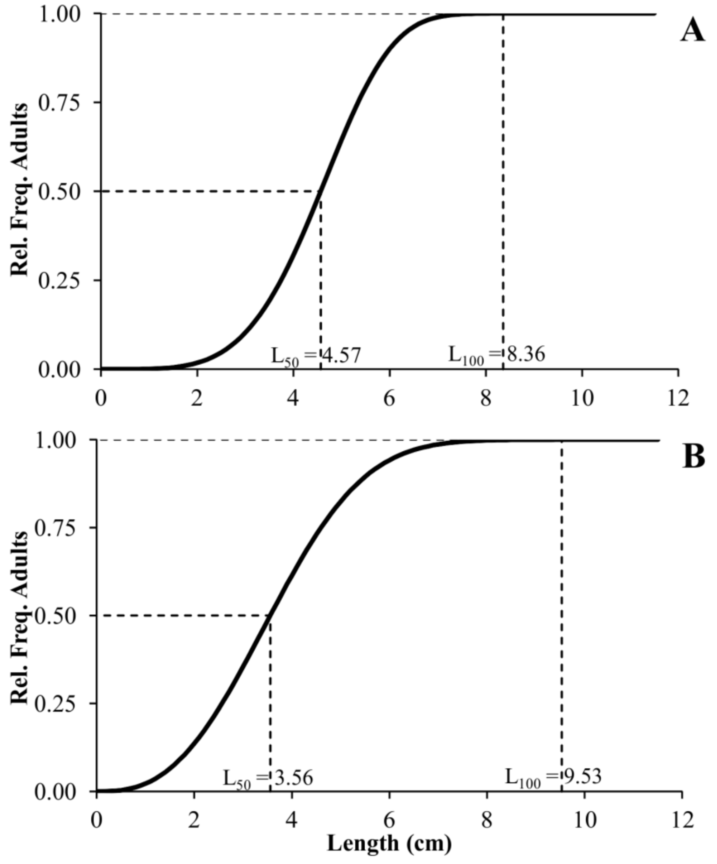

Figure 6. Relative frequency of adults in function of the standard length of female (A) and male (B) *Astyanax bifasciatus* in the Jirau Alto River, Dois Vizinhos, Paraná, Brazil. L_{50} = length at first maturity; L_{100} = shorteest length at which all individuals are adults.

Discussion

The reproductive indicators used in this study were sensitive and responsive, and showed morphophysiological changes that occurred in *A. bifasciatus* individuals collected for one year in the Jirau Alto River, a tributary of the Lower Iguaçu River Basin. The coordination of responses observed from the gonadal development, accumulation and availability of celomatic fat, liver biomass, and physiological status of the individuals support the arguments of the present authors concerning the reproductive tactics adopted by the species for its maintenance in this environment.

The relationship between the GSI indicators and the percentage frequency of the gonadal maturation stages indicated the existence of a long reproductive period, suggesting parceled spawning. This type of spawning has been histologically documented for species in older reservoirs of the Upper Iguaçu River Basin, which shows greater environmental stability (Silva et al., 2010). On the other hand, according to the same authors, species in newly formed reservoirs, the species adopted total spawning as a reproductive tactic, which was related to a more favorable period of the year. In the present study, the species used an adapted reproductive tactic, with two cycles of major investment throughout the year, demonstrating its great adaptative capacity (Abilhoa & Agostinho, 2007), according to the environmental scenario in which it is inserted.

In general, species of the *Astyanax* genus exhibit a long reproductive period and large investments in small and numerous oocytes (Agostinho et al., 1999). However, not all species of this genus have the same adaptive plasticity, due to the variations observed in their ecomorphological attributes and diet composition (Mise, Fugi, Pagotto, & Goulart, 2013). *Astyanax minor*, for example, has a higher colonization capacity in newly formed reservoirs (Oliveira, Santos, Fávaro, & Abilhoa, 2008). This is due to its detritivorous diet and its high capacity to produce numerous and small oocytes, as well as presenting one of the highest fecundities and gonadosomatic relationships observed for the *Astyanax* genus in the Iguaçu River. In older reservoirs, undergoing environmental rebalancing, the species decreases its dominance and yields to the predominance of *Astyanax bifasciatus*, which presents intermediate reproductive characteristics (Bailly, Agostinho, Suzuki, & Luiz, 2005) and a herbivorous or omnivorous diet.

In fact, *A. bifasciatus* has been more successful in 20 to 40-year-old reservoirs in the Iguaçu River (Daga & Gubiani, 2012) and have presented intense and wide reproductive activity, with many spawnings throughout the year, as mentioned above. The oocytes of this species are larger than those of *A. minor* and are produced in smaller quantities (Bailly et al., 2005). On the other hand, the larger size of *A. bifasciatus* (Abilhoa & Agostinho, 2007; Gubiani & Horlando, 2014) compensates, in part, for the production of gametes and favors the swimming performance of the animals (Mise et al., 2013), helping them deal with top-down control and the search for allochthonous food. Thus, *A. bifasciatus* uses a set of reproductive tactics in newly-flooded reservoir environments, with higher productivity and strong environmental fluctuations. Other tactics are adopted in older environments undergoing community rebalancing, where interaction with other species becomes an important variable.

Another coneger species endemic to the Iguaçu River is *Astyanax gymnodontus*, which is worth comparing to *A. bifasciatus*. The characteristics of *A. gymnodontus* are more demanding than the other species of *Astyanax* mentioned, having lower fecundity, larger oocytes (Bailly et al., 2005), larger body size, later first maturity (Baumgartner, Silva, & Baumgartner, 2016) and an insectivorous diet with herbivorous tendencies (Cassemiro, Hanh, & Delariva, 2005). The species is relatively successful and abundant in older reservoirs. Nevertheless, community dominance in these environments is always exercised by *A. bifasciatus* (Daga & Gubiani, 2012), probably due to its intermediate ecomorphological characteristics that favor greater resilience.

In natural environments, such as streams, species would be expected to have adapted to the natural disturbance events and, therefore, would have responded similarly in older reservoirs. This presumption is partially correct since mature individuals are found throughout most of the year. However, the GSI values showed two periods of higher reproductive intensity. This investment in two reproductive seasons is probably related to the intensity of the disturbances, whether originating from natural processes or related to different anthropic activities. In addition to the construction of dams, changes in the habitat/landscape structure, such as the observed suppression of riparian vegetation in the Jirau Alto River (Pigosso et al., 2009), might intensify the effect of floods, making conditions unfavorable for reproduction (Franssen et al., 2006; De Fries et al., 2018). This environment can become more susceptible to the influx effects of urban, industrial or agricultural contaminants, as observed in the Jirau Alto River (Wachtel et al., 2019) and other tributaries of the Lower Iguaçu River Basin (Nimet, Amorim, & Delariva, 2018).

Immature individuals of both sexes were also recorded for a long period in the study area (February to July), suggesting their use in recruitment. This demonstrated the importance of the study area for the entire life cycle of the species, from hatching and larval development to adulthood with gonad maturation and spawning/spermiation. As a species endemic to the Iguaçu River Basin, but with previously-mentioned r-strategist characteristics, it is essential to conserve the habitat and landscape structure to maintain the characteristics of the entire community, controlling species such as *A. bifasciatus*, which presents high biotic potential. A potential indirect effect derived from the population growth of generalist species is their population increase in tributaries (colonization and recolonization), which can cause an increase in interspecific competition and changes in the structure of fish assemblages (Araújo et al., 2013). The species can act as a natural indicator of community vulnerability, responding to changes in the habitat of the Iguaçu River and its tributaries. This positive relationship, due to landscape destructuring in the Iguaçu River Basin, was recently reported by Delariva et al. (2018) and can be used for bioindication (Bueno-Krawczyk et al., 2015; Nimet et al., 2018).

All analyses presented and discussed in this study corroborate the results obtained for the total condition factor (K) of the species. The most significant difference between K and K' in both sexes was consistent with periods of greater gonadal investment and high frequency of mature individuals. The progressive decrease in K values in the subsequent months supports the argument for spawning and spermiation intensification, leading to nutritional stress (Querol, Querol, & Gomes, 2002), due to the physiological changes resulting from the energetic reallocation. Thus, the species accumulated celomatic fat in the fall and winter, and spent it in the early spring with maturation, which precedes the peak of reproductive activity. A sudden HSI increase in the early spring was also observed, suggesting the displacement of the energy reserves to the gonads for storage, as reported by Abelha and Goulart (2008). The pre- and post-spawning/spermiation periods showed animals with the best nutritional conditions, as observed for other species of the genus (Hirt, Araya, & Flores, 2011; Viana, Tondato, Súarez, & Lima-Junior, 2014), indicating the physiological preparation/recovery of the animals for the reproductive process. In this case the variations in K were well-synchronized to the reproductive process, although the relationship is not always linear or exclusive. The physiological condition of the animals is modulated by other factors, such as fluctuations in environmental parameters, food availability, interspecific relationships (parasitism), anthropic pressures and ontogenetic variations (Le Cren, 1951; Datta, Kaur, Dhawan, & Jassal, 2013).

Length at first maturity (L_{50}) showed that the species has a strong generalist characteristic, with rapid growth and maturation of its gonads. Even small individuals (3.5 to 4.5 cm) participate in the reproductive process, even smaller than those already reported for the species in Iguaçu reservoirs (Baumgartner et al., 2012). This divergence is probably associated with the type of environment and intensity of the disturbances. The species submitted to running water environments, with frequent floods associated with habitat degradation and pollution, as observed in the Jirau Alto River, probably presents accelerated sexual maturity as another tactic that aims to ensure reproductive success and its maintenance in the environment. The sexual maturity of females that were larger than males also seems to be a tendency for other abundant endemic *Astyanax* species from the Iguaçu River, such as *A. dissimilis* (Suzuki & Agostinho, 1997), *A. gymnodontus* (Baumgartner et al., 2016) and *A. minor* (Baumgartner et al., 2012). The present study suggests that the divergence in resource allocation between the sexes and the sexual selection process act as the main adaptive forces, making this indicator an important tool to evaluate sexual dimorphism in the genus.

Conclusion

The use of multiple reproductive indicators has allowed a more thorough and integrated view of the reproductive process and techniques adopted by the endemic colonizer, *Astyanax bifasciatus*, in a small tributary of the Lower Iguaçu Basin. The results encourage the adoption of conservationist strategies for the tributaries in the region, with the aim to manage the species as an indicator of habitat changes.

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References

Abelha, M. C. F., & Goulart, E. (2008). Population structure, condition factor and reproductive period of *Astyanax paranae* (Eigenmann, 1914) (Osteichthyes: Characidae) in a small and old Brazilian reservoir. *Brazilian Archives of Biology and Technology, 51*(3), 503-512. doi: 10.1590/S1516-89132008000300009

Abilhoa, V., & Agostinho, A. A. (2007). Aspectos biológicos de duas espécies de *Astyanax* (Teleostei, Characidae) em lagoas marginais do alto rio Iguaçu, Paraná, Brasil. *Estudos de Biologia, 29*(66), 23-32. doi: 10.7213/reb.v29i66.22748

Agostinho, A. A., Miranda, L. E., Bini, L. M., Gomes, L. C., Thomaz, S. M., & Suzuki, H. I. (1999). Patterns of colonization in neotropical reservoirs, and a prognosis on aging. In J. G. Tundisi & M. Straskraba (Eds.), *Theoretical reservoir ecology and its applications* (p. 227-265). São Carlos, SP: International Institute of Ecology.

Araújo, E. S., Marques, E. E., Freitas, I. S., Neuberger, A. L., Fernandes, R., & Pelicice, F. M. (2013). Changes in distance decay relationships after river regulation: similarity among fish assemblages in a large Amazonian river. *Ecology Freshwater Fish*, *22*(4), 543-552. doi: 10.1111/eff.12054.

Araújo, F. G., Morado, C. N., Parente, T. T. E., Paumgartten, F. J. R., & Gomes, I. D. (2018). Biomarkers and bioindicators of the environmental condition using a fish species (*Pimelodus maculatus* Lacepède, 1803) in a tropical reservoir in Southeastern Brazil. *Brazilian Journal of Biology, 78*(2), 351-359. doi: 10.1590/1519-6984.167209.

Bailly, D., Agostinho, A. A., Suzuki, H. I., & Luiz, E. A. (2005). Características reprodutivas de espécies de *Astyanax* e sucesso na colonização de reservatórios do rio Iguaçu, PR. In L. Rodrigues, S. M. Thomaz, A. A. Agostinho & L. C. Gomes (Orgs.), *Biocenose em reservatórios: padrões espaciais e temporais* (p. 243-252). São Carlos, SP: Rima.

Baumgartner, G., Pavanelli, C. S., Baumgartner, D., Bifi, A. G., Debona, T., & Frana, V. A. (2012). *Peixes do Baixo Rio Iguaçu.* Maringá, PR: Eduem.

Baumgartner, M.T., Silva, P.R.L., & Baumgartner, G. (2016). Population structure and reproductive biology of *Astyanax gymnodontus* (Characiformes: Characidae), a poorly known endemic fish of the Iguaçu River basin, Brazil. *Revista de Biologia Tropical, 64*(1), 69-77. doi: 10.15517/rbt.v64i1.18100

Bomfim, A. C., Peretti, D., Camillo, C. S., Costa, S. A. G. L., & Nascimento, R. S. S. (2015). Reproductive biology and variations in the gonadal development of the fish Curimatã (*Prochilodus brevis* Steindachner, 1875) in captivity. *Biota Amazônica, 5*(2), 65-70. doi: 10.18561/2179-5746/biotaamazonia.v5n2p65-70

Brewer, S. K., Rabeni, C. F., & Papoulias, D. M. (2008). Comparing histology and gonadosomatic index for determining spawning condition of small-bodied riverine fishes. *Ecology of Freshwater Fish, 17*(1), 54-58. doi: 10.1111/j.1600-0633.2007.00256.x

Brosset, P., Lloret, J., Munoz, M., Fauvel, C., Van Beveren, E., Marques, V., ... Saraux, C. (2016). Body reserves mediate trade-offs between life-history traits: new insights from small pelagic fish reproduction. *Royal Society Open Science, 3*(10), 1-15. doi: 10.1098/rsos.160202

Bueno-Krawczyk, A. C. D., Guiloski, I. C., Piancini, L. D. S., Azevedo, J. C., Ramsdorf, W. A., Ide, A. H., ... Silva de Assis, H. C. (2015). Multibiomarker in fish to evaluate a river used to water public supply. *Chemosphere, 135*, 257-264. doi: 10.1016/j.chemosphere.2015.04.064

Cassemiro, F. A. S., Hahn, N. S., & Delariva, R. L. (2005). Estrutura trófica da ictiofauna, ao longo do gradiente longitudinal do reservatório de Salto Caxias (rio Iguaçu, Paraná, Brasil), no terceiro ano após o represamento. *Acta Scientiarum. Biological Sciences, 27*(1), 63-71. doi: 10.4025/actascibiolsci.v27i1.1362

Conselho Nacional de Controle de Experimentação Animal [CONCEA]. (2018). *Resolução normativa nº 37: Diretriz da prática de eutanásia do CONCEA.* Brasília, DF: Ministério da Ciência, Tecnologia, Inovações e Comunicações. Retrieved from https://www.mctic.gov.br/mctic/export/sites/institucional/institucional/concea/arquivos/legislacao/resolucoes_normativas/Resolucao-Normativa-n-37-Diretriz-da-Pratica-de-Eutanasia_site-concea.pdf

Daga, V. S., & Gubiani, E. A. (2012). Variations in the endemic fish assemblage of a global freshwater ecoregion: Associations with introduced species in cascading reservoirs. *Acta Oecologica, 41*, 95–105. doi: 10.1016/j.actao.2012.04.005

Datta, S. N., Kaur, V. I., Dhawan, A., & Jassal, G. (2013). Estimation of length-weight relationship and condition factor of spotted snakehead *Channa punctata* (Bloch) under different feeding regimes. *SpringerPlus, 2*, 436-440. doi: 10.1186/2193-1801-2-436

De Fries, L., Rosa, G., Silva, J. P., Vilella, F. S., & Becker, F. G. (2018). Reproduction of two loricariid species in a confined river and implications for environmental impacts of dams. *Neotropical Ichthyology, 16*(4), e170163. doi: 10.1590/1982-0224-20170163

Delariva, R. L., Neves, M. P., Larentis, C., Kliemann, B. C. K., Baldasso, M. C., & Wolff, L. L. (2018) Fish fauna in forested and rural streams from an ecoregion of high endemism, lower Iguaçu River basin, Brazil. *Biota Neotropica, 18*(3), 1-12. doi: 10.1590/1676-0611-BN-2017-0459

Franssen, N. R., Gido, K. B., Guy, C. S., Tripe, J. A., Shrank, S. J., Strakosh, T. R., … Paukert, C. P. (2006). Effects of floods on fish assemblages in an intermittent prairie stream. *Freshwater Biology, 51*(11), 2072-2086. doi: 10.1111/j.1365-2427.2006.01640.x

Garavello, J. C., & Sampaio, F. A. A. (2010). Five new species of genus *Astyanax* Baird & Girard, 1854 from Rio Iguaçu, Paraná, Brazil (Ostariophysi, Characiformes, Characidae). *Brazilian Journal of Biology, 70*(3), 847-865. doi: 10.1590/s1519-69842010000400016

Gubiani, E. A., & Horlando, S. S. (2014). Length-weight and length-length relationships and length at first maturity for freshwater fish species of the Salto Santiago Reservoir, Iguaçu River Basin. *Journal of Applied Ichthyology, 30*(5), 1087-1091. doi: 10.1111/jai.12444

Hirt, L. M., Araya, P. R., & Flores, S. A. (2011). Population structure, reproductive biology and feeding of *Astyanax fasciatus* (Cuvier, 1819) in an Upper Paraná River tributary, Misiones Argentina. *Acta Limnologica Brasiliensia, 23*(1), 1-12. doi: 10.4322/actalb.2011.013

Ikegami, T., Takeuchi, Y., Hur, S. P., & Takemura, A. (2014). Impacts of moonlight on fish reproduction. *Marine Genomics, 14*, 59-66. doi: 10.1016/j.margen.2013.11.007

Instituto Brasileiro de Geografia e Estatística [IBGE]. (2004). *Mapa de biomas do Brasil.* Escala 1:5.000.000. Rio de Janeiro, RJ: IBGE. Retrieved from https://www.ibge.gov.br/geociencias/informacoes-ambientais/15842-biomas.html

Le Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology, 20*(2), 201-219. doi: 10.2307/1540

Lemos, V. M., Varela-Junior, A. S., Velasco, G., & Vieira, J. P. (2011). The reproductive biology of the plata pompano, *Trachinotus marginatus* (Teleostei: Carangidae), in southern Brazil. *Zoologia, 28*(5), 603-609. doi: 10.1590/S1984-46702011000500008

Lima-Junior, S. E., Cardone, I. B., & Goitein, R. (2002). Determination of a method for calculation of Allometric Condition Factor of fish. *Acta Scientiarum. Biological Science, 24*(1), 397-400. doi: 10.4025/actascibiolsci.v24i0.2311

Louiz, I., Ben-Attia, M., & Ben-Hassine, O. K. (2009). Gonadosomatic index and gonad histopathology of *Gobius niger* (Gobiidea, Teleost) from Bizerta Lagoon (Tunisia): evidence of reproduction disturbance. *Fisheries Research, 100*(3), 266-273. doi: 10.1016/j.fishres.2009.08.009

Maack, R. (1981). *Geografia física do Estado do Paraná*. Rio de Janeiro, RJ: José Olympio.

Mérona, B. d., Mol, J., Vigouroux, R., & Chaves, P. d. T. (2009). Phenotypic plasticity in fish life-history traits in two neotropical reservoirs: Petit-Saut reservoir in French Guiana and Brokopondo reservoir in Suriname. *Neotropical Ichthyology, 7*(4), 683-692. doi: 10.1590/S1679-62252009000400018

Mérona, B. d., Vigouroux, R., & Horeau, V. (2003). Changes in the food resources and their utilization by fish assemblages in a large tropical reservoir in South America (Petit-Saut Dam, French Guiana). *Acta Oecologica, 24*(3), 147-156. doi: 10.1016/S1146-609X(03)00065-1

Mise, F. T., Fugi, R., Pagotto, J. P. A., & Goulart, E. (2013). The coexistence of endemic species of *Astyanax* (Teleostei: Characidae) is propitiated by ecomorphological and trophic variations. *Biota Neotropica, 13*(3), 21–28. doi: 10.1590/S1676-06032013000300001

Nimet, J., Amorim, J. P. A., & Delariva, R. L. (2018). Histopathological alterations in *Astyanax bifasciatus* (Teleostei: Characidae) correlated with land uses of surroundings of streams. *Neotropical Ichthyology, 16*(1), e170129. doi: 10.1590/1982-0224-20170129

Nitsche, P. R., Caramori, P. H., Ricce, W. S., & Pinto, L. F. D. (2019). *Atlas Climático do Estado do Paraná*. Londrina, PR: IAPAR. Retrieved from http://www.iapar.br/arquivos/File/zip_pdf/AtlasClimaticoPR.pdf

Oliveira, E. C., Santos, L. E. S., Fávaro, L. F., & Abilhoa, V. (2008). Caracterização da assembleia de peixes em um reservatório recém formado no sul do Brasil. *Estudos de Biologia, 30*(70/72), 125-132. doi: 10.7213/reb.v30i70/72.22817

Pigosso, M., Bonfante, E., Farias, E., Becegato, V., & Onofre, S. B. (2009). Diagnóstico ambiental da bacia hidrográfica do Rio Jirau Alto - Dois Vizinhos - Paraná. *Geoambiente On-line, 13*, 175–193. doi: 10.5216/rev.geoambie.v0i13.25995

Querol, M. V. M., Querol, E., & Gomes, N. N. A. (2002). Fator de condição gonadal, índice hepatossomático e recrutamento como indicadores do período de reprodução de *Loricariichthys platymetopon* (Osteichthyes, Loricariidae), bacia do rio Uruguai Médio, Sul do Brasil. *Iheringia Série Zoologia, 92*(3), 79-84. doi: 10.1590/S0073-47212002000300008.

Silva, J. P. A., Muelbert, A. E., Oliveira, E. C., & Fávaro, L. F. (2010). Reproductive tactics used by the Lambari *Astyanax aff. fasciatus* in three water supply reservoirs in the same geographic region of the upper Iguaçu River. *Neotropical Ichthyology, 8*(4), 885-892. doi: 10.1590/S1679-62252010000400019

Suzuki, H. I., & Agostinho, A. A. (1997). Reprodução de peixes do reservatório de Segredo. In A. A. Agostinho & L. C. Gomes, (Eds.), *Reservatório de Segredo: bases ecológicas para o manejo* (p. 163-182). Maringá, PR: Eduem.

Vazzoler, A. E. A. d. M. (1996). Biologia da reprodução de peixes teleósteos: teoria e prática. Maringá, PR: Eduem.

Viana, L. F., Tondato, K. K., Súarez, Y. R., & Lima-Junior, S. E. (2014). Influence of environmental integrity on the reproductive biology of *Astyanax altiparanae* Garutti & Britski, 2000 in the Ivinhema river basin. *Acta Scientiarum. Biological Sciences, 36*(2), 165-173. doi: 10.4025/actascibiolsci.v36i2.21052

Wachtel, C. C., Oliveira, E. C., Maniglia, T. C., Smith-Johannsen, A., Roque, A. A., & Ghisi, N. C. (2019). Waterborn genotoxicity in Southern Brazil using *Astyanax bifasciatus* (Pisces: Teleostei). *Bulletin of Environmental Contamination and Toxicology, 102*(1), 59-65. doi: 10.1007/s00128-018-2477-3.

Weber, A. A., Nunes, D. M. F., Gomes, R. Z., Rizzo, E., Santiago, K. B., & Bazzoli, N. (2013). Downstream impacts of a dam and influence of a tributary on the reproductive success of *Leporinus reinhardti* in São Francisco River. *Aquatic Biology, 19*, 195-200. doi: 10.3354/ab00531

Winemiller, K. O. 1989. Patterns of variation in life history among South American fishes in seasonal environments. *Oecologia, 8*(2)*1*, 225-241. doi: 10.1007/BF00379810

Yamamoto, F. Y., Pereira, M. V. M., Lottermann, E., Santos, G. S., Stremel, T. R. O., Doria, H. B., ... Oliveira Ribeiro, C. A. 2016. Bioavailability of pollutants sets risk of exposure to biota and human population in reservoirs from Iguaçu River (Southern Brazil). *Environmental Science and Pollution Research, 23*(18), 18111-18128. doi: 10.1007/s11356-016-6924-6