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Influence of environmental integrity on the reproductive biology of *Astyanax altiparanae* Garutti & Britski, 2000 in the Ivinhema river basin

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ABSTRACT. In this study, it was examined the reproductive biology of *Astyanax. altiparanae*, in streams with different levels of environmental disturbance in the Ivinhema river basin (Mato Grosso do Sul State, Brazil). Samplings were conducted between July 2001 and November 2011, at 101 sites, using sieves, seining nets, gill nets and electrofishing. Streams were classified into three levels of environmental integrity. We analyzed 735 specimens, 410 females and 325 males. Females predominated in impacted streams and both sexes presented nearly the same pattern of length distribution in the three levels of environmental integrity. Females reached gonadal maturity with greater lengths (L_{50} = 55.67mm) in altered and impacted streams and had higher reproductive activity between October and December. The species showed higher Condition Factor and higher reproductive intensity in the impacted streams. Our results evidenced that in impacted sites the species exhibited a good ability to allocate a significant portion of its energy to reproduction, probably due to the greater supply of food.

Keywords: environmental conditions, fish, reproductive ecology.

Influência da integridade ambiental na biologia reprodutiva de *Astyanax altiparanae* Garutti & Britski, 2000 na bacia do rio Ivinhema

RESUMO. Neste estudo analisamos a biologia reprodutiva de *Astyanax. altiparanae* em riachos com diferentes intensidades de integridade ambiental, na bacia do rio Ivinhema, (Mato Grosso do Sul, Brasil). As amostragens foram realizadas entre julho/2001 e novembro/2011, em 101 locais, utilizando peneiras, rede de arrasto, rede de espera e pesca elétrica. Os riachos foram categorizados em três níveis de integridade ambiental. Foram analisados 735 exemplares, sendo 410 fêmeas e 325 machos. Houve maior predominância de fêmeas nos riachos impactados e ambos os sexos apresentaram aproximadamente o mesmo padrão de distribuição por comprimento nos três níveis de integridade ambiental. Constatamos que as fêmeas atingiram maturidade gonadal com maiores comprimentos (L_{50} = 55,67 mm) nos riachos alterados e impactados apresentaram maior atividade reprodutiva entre outubro e dezembro. A espécie apresentou maior Fator de Condição e maior intensidade reprodutiva nos riachos impactados. Os resultados evidenciaram que em locais impactados a espécie demonstra boa capacidade em alocar parte significativa de sua energia à reprodução, provavelmente em decorrência da maior oferta de alimento.

Palavras-chave: condições ambientais, peixes, ecologia reprodutiva.

Introduction

Currently one of the leading concerns about aquatic ecosystems is to maintain its environmental integrity, which has the goal to sustain the ability of a community to preserve its richness, species composition and organization functionally comparable to that of undisturbed ecosystems, by any unnatural change and/or by human activities (JARAMILLO-VILLA; CARAMASCHI, 2008). However, the integrity and quality of aquatic ecosystems have been subjected to strong disturbances derived from anthropic activities, especially urban sprawl and intensive agriculture (ALEXANDRE et al., 2010; CUNICO et al., 2011). In this way, the effects of degradation in aquatic environments involving several watersheds are significantly affecting the aquatic fauna, mainly fish that are a major component of stream ecosystems, with significant impact on the energy flow and functioning of streams (FLORES-LOPES et al., 2010; TEJERINA-GARRO et al., 2005).

Changes in the aquatic environment through modifications in abiotic and biotic factors can influence the dynamics of fish populations and all their life history traits (LOWE-MCCONNELL, 1999; WOOTTON, 1998), such as reproductive potential, reducing or inhibiting the propagation of species (FIALHO et al., 2008). Thus, knowledge on fish reproductive biology in disturbed ecosystems can provide important information about their adaptability through their phenotypic plasticity, which responds to the characteristics of the environment, allowing them to increase the chances of success against environmental variations (MÉRONA et al., 2009). In addition, it can provide relevant contribution to guide conservation and management of organisms in various types of environments, since reproduction is the most important stage of the life cycle of fish, as it ensures the maintenance of viable populations (VAZZOLER, 1996).

Astyanax altiparanae Garutti & britski, 2000, popularly known as yellow-tailed lambari, belongs to the family Characidae and is widely distributed throughout the Upper Paraná River basin. It has predominantly omnivorous feeding habit and can live in a variety of environments (AZEVEDO et al., 2007). Because of its great ecological plasticity, and for being an opportunistic species, this species has been recorded in both streams with high level of environmental integrity as in streams receiving domestic and industrial effluents (ABES et al., 2001; ONORATO et al., 2000; ORSI et al., 2002, 2004).

In this context, the present study aimed to evaluate the reproductive biology of *A. altiparanae* in streams with different levels of environmental integrity, in the Ivinhema river basin, responding the following questions: 1) Is there any difference in the sex ratio and standard length? 2) Is there any difference in the first maturity length of females? 3) Is there variation in the gonadosomatic index between the levels of environmental integrity? 4) Is there any difference in the reproductive period? 5) Is there variation in the condition factor of females and males between the levels of environmental integrity and over time? 6) At what level of environmental integrity females and males have the best condition factor?

Material and methods

Study area

The study was developed in the Ivinhema river basin, located on the central-southern region of the Mato Grosso do Sul State, to the south of the Upper Paraná River basin, between latitudes 21° and 23°S and longitudes 52°30' and 56°W (SEPLAN, 1990). The intense human intervention has caused some potential and effective environmental impacts on ecosystems of Ivinhema river basin. According to Oliveira et al. (2000) the disordered occupation has implicated major changes in the natural landscape, causing different impacts on natural resources, such as destruction of vegetation, especially riparian forest, land degradation and soil erosion, siltation and contamination of water sources by agrochemicals, producing relevant environmental and social damages.

Samplings were conducted between July 2001 and November 2011, at 101 sites in the Ivinhema River basin (Figure 1), which were chosen for presenting different phytophysiognomies and different levels of environmental integrity.

Data collection

Fish data came from various research projects with different periodicities. Fish were collected during the daytime, at least three days after a rainy day, using a rectangular sieve measuring 0.8 x 1.2 m, with 2 mm aperture. It was also used seining nets (1.5 x 5 m), gillnets with mesh size varying from 1.5 to 5.0 cm and electrofishing.

In the samplings were evaluated the following environmental variables: water electrical conductivity (µS cm^{-1}), turbidity (NTU), dissolved oxygen (mg L^{-1}) and riparian vegetation index (qualitative scale: 1 – without vegetation; 2 – degraded vegetation; 3 – preserved vegetation, based on RUTHERFORD et al., 2001). In the field, fish were fixed in 10% formaldehyde and later identified and preserved in 70% alcohol.

In the laboratory, some biometric data were obtained for each individual: total length (mm), standard length (mm), total weight (g) and sex. For females we also recorded the gonad maturity stages and gonad weight (g). The maturity stages were determined macroscopically according to the classification adapted from Vazzoler (1996): A (immature gonads), B (maturing gonads), C (mature gonads), D (semi-spent gonads) and E (spent gonads).

Data analysis

In order to determine the environmental variables with higher influence on the differentiation of categories of degradation of streams, to classify the levels of environmental integrity, was performed a Principal Component Analysis (PCA), applied according to Manly (1994), and subsequent comparison of variables between categories obtained by the Kruskal-Wallis test.

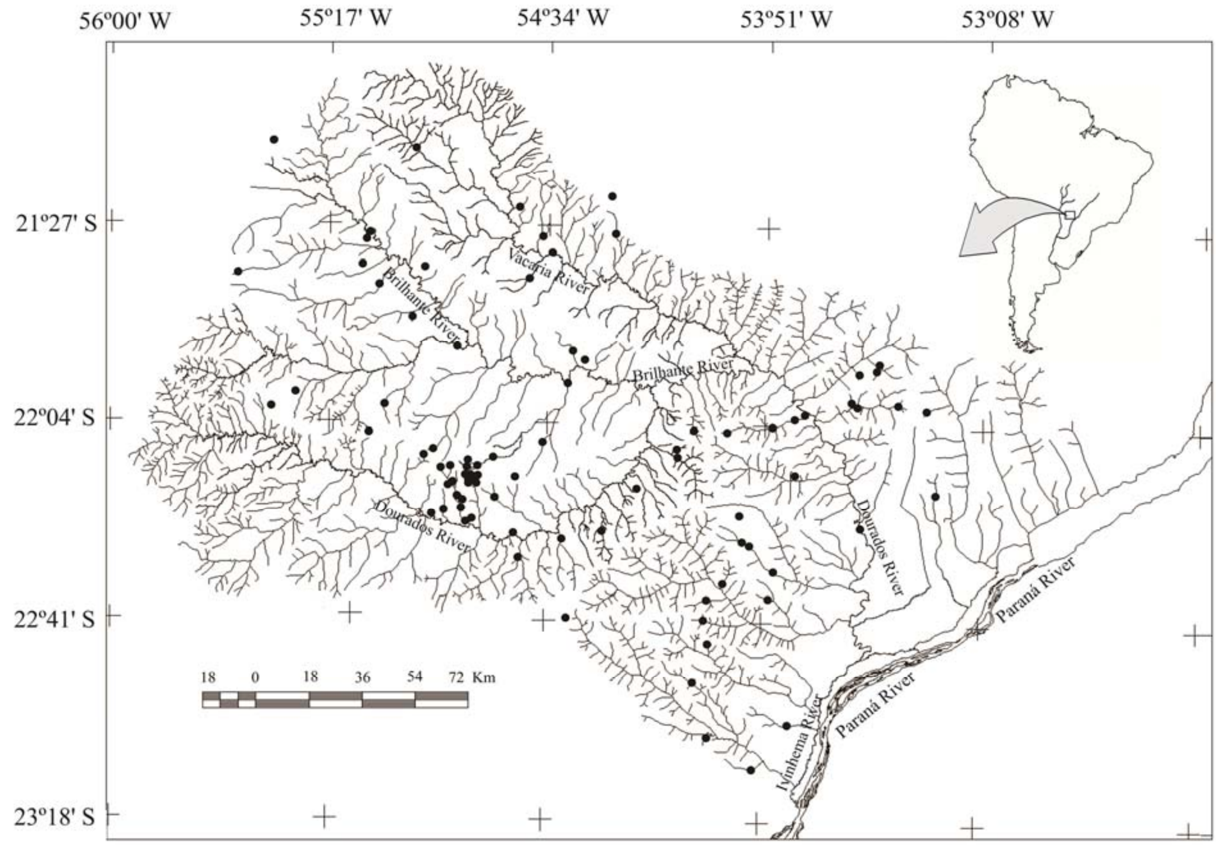

Figure 1. Location of sampled sites in the Ivinhema River basin, Upper Paraná river.

Any differences on the sex ratio of the population between streams with different levels of environmental integrity were verified by a Yates corrected chi-square test (χ^2). Therefore, streams that presented the same level of integrity were pooled. The distributions of standard length of males and females were compared by visual inspection

The probability of *A. altiparanae* females of being adult at a given length was obtained through logistic regression, using the gonads to define whether females are reproducing or not. Females that presented stage A gonad were considered non-reproductive (0) and those that presented stages B, C, D and E, were classified as reproductive animals (1). The frequency of mature females was used as the response variable and the standard length was used as the explanatory variable, according to Roa et al. (1999). The estimated mean standard length at first maturity (L_{50}) represents the point at which 50% of the fish are mature (BARBIERI, 1994), and (L_{100}) represents the length at which all fish are mature. We used the overlap of the Confidence Interval (CI) for these values to check the difference in L50 between streams with different levels of environmental integrity.

To identify the reproductive period of *A. altiparanae* in streams with different levels of environmental integrity we used information of the temporal distribution of frequencies of gonad maturity stages and the temporal variation of the gonadosomatic index (GSI = gonad weight/total weight.100). The GSI calculated for all quarters sampled were compared between seasons and between levels of environmental integrity through the Kruskal-Wallis test complemented by a posteriori Dunn's test. Samplings were divided into quarters according to the seasons of the year.

At each level of environmental integrity and at each quarter, the condition factor was calculated for each sex, using the equation CF = Total weight/Standard length^b (VAZZOLER, 1996). Given the lack of normality, the variation between levels of environmental integrity and over time was analyzed by a Kruskal-Wallis test and Dunn's test.

Statistical analyses were run in the software Systat 12, BioEstat 5.0 (AYRES et al., 2007) and in the platform R (R DEVELOPMENT CORE TEAM, 2009). For all statistical tests aforementioned, the significance level adopted was $p < 0.05$.

Results

The PCA results identified the water electrical conductivity and vegetation index as variables that better differentiated the streams into classes of environmental integrity. The first PCA axis explained 39.86% of variance, with eigenvalue of 1.59. The second axis explained 26.26% of variance, with eigenvalue of 1.05. The conductivity was negatively correlated with the first principal component, whereas the vegetation index was positively correlated with this same axis (Table 1).

Table 1. Loadings of environmental variables with the first two principal components generated by the PCA.

Environmental variables	PC1	PC2
Turbidity	-0.005803	**1.7002**
Dissolved Oxygen	0.644186	**-1.2472**
Conductivity	**-1.794600**	-0.2419
Vegetation index	**1.807878**	0.2097
Explained variance (%)	39.86	26.26

The significant correlation between conductivity and vegetation index ($r = -0.4782$ and $p < 0.0001$) indicated that both variables can represent separately the first principal component. In this way, once the conductivity is a quantitative variable and reveals more information about the water quality, it was selected as reference to distinguish the sites. So, streams with lower conductivity values were considered as less impacted, while higher values of conductivity were indicative of impacted streams.

Streams were grouped into three classes of environmental integrity: least impacted (conductivity < 50 $\mu S\ cm^{-1}$), altered (conductivity between 50 and 100 $\mu S\ cm^{-1}$) and impacted (conductivity > 100 $\mu S\ cm^{-1}$). The establishment of these classes was based on the results obtained by Lima-Junior et al. (2006) and Cetesb (2009). Afterwards, the comparison of these three levels as for the other environmental variables allows a more complete characterization, as listed in Table 2.

Table 2. Medians of the variables turbidity, dissolved oxygen and vegetation index of the three levels of environmental integrity. Different letters indicate significant difference between the levels of environmental integrity (Kruskal-Wallis test).

Environmental integrity	Turbidity	Dissolved Oxygen	Vegetation index
Less impacted sites (conductivity <50 $\mu S\ cm^{-1}$)	6.48^a	7.09^a	2^a
Altered sites (conductivity 50 to 100 $\mu S\ cm^{-1}$)	12.60^b	6.97^a	2^a
Impacted sites (conductivity >100 $\mu S\ cm^{-1}$)	19.29^b	6.24^a	1^b

We analyzed 735 exemplars of *A. altiparanae*, including 410 females and 325 males. Females prevailed throughout the sampling period, and the sex ratio differed significantly in two of the three levels of environmental integrity of the studied streams. The least impacted streams presented a greater proportion of females (1.26:1 female/male, $p = 0.0350$), as well as the impacted streams (1.49:1 female/male, $p = 0.0153$). Streams classified as altered showed a balanced sex ratio ($p = 0.4014$).

The frequency distribution of standard length between sexes showed that both sexes presented approximately the same pattern of length distribution between the three levels of integrity of the streams sampled (Figure 2).

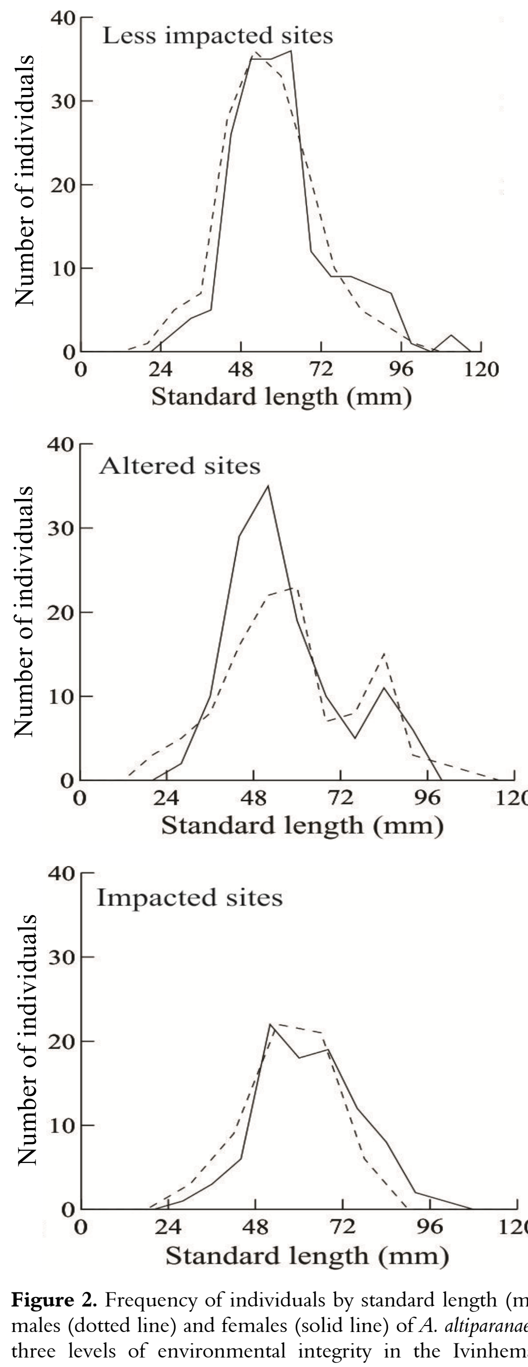

Figure 2. Frequency of individuals by standard length (mm) for males (dotted line) and females (solid line) of *A. altiparanae* in the three levels of environmental integrity in the Ivinhema river basin, upper Paraná river, between July 2001 and November 2011. Number of males: 151, 113 and 61, respectively. Number of females: 191, 127 and 92, respectively.

In the least impacted streams females reached sexual maturity (L_{50}) with 50.37 mm, with confidence intervals $CI_{(\alpha=0.05)}$ = 47.95 to 52.34 mm, whereas 100% of females should reach sexual maturity (L_{100}) with 82.95 mm ($CI_{(\alpha=0.05)}$ = 75.40 to 97.30 mm). In the altered sites, the L_{50} was estimated at 55.67 mm ($CI_{(\alpha=0.05)}$ = 53.65 to 58.22 mm), while all females were able to spawn (L_{100}) with 78.95 mm ($CI_{(\alpha=0.05)}$ = 71.84 to 94.78 mm). In turn, in the impacted sites 50% of females were sexually mature (L_{50}) with 54.47 mm ($CI_{(\alpha=0.05)}$ = 52.77 to 56.14 mm), whereas the size at which all females were adult (L_{100}) was 64.02 mm ($IC_{(\alpha=0.05)}$ = 60.40 to 76.62 mm) (Figure 3, A and B). Thus, by overlapping the confidence intervals we observed that the L_{50} of the least impacted streams is lower than observed in altered and impacted streams (Figure 3B).

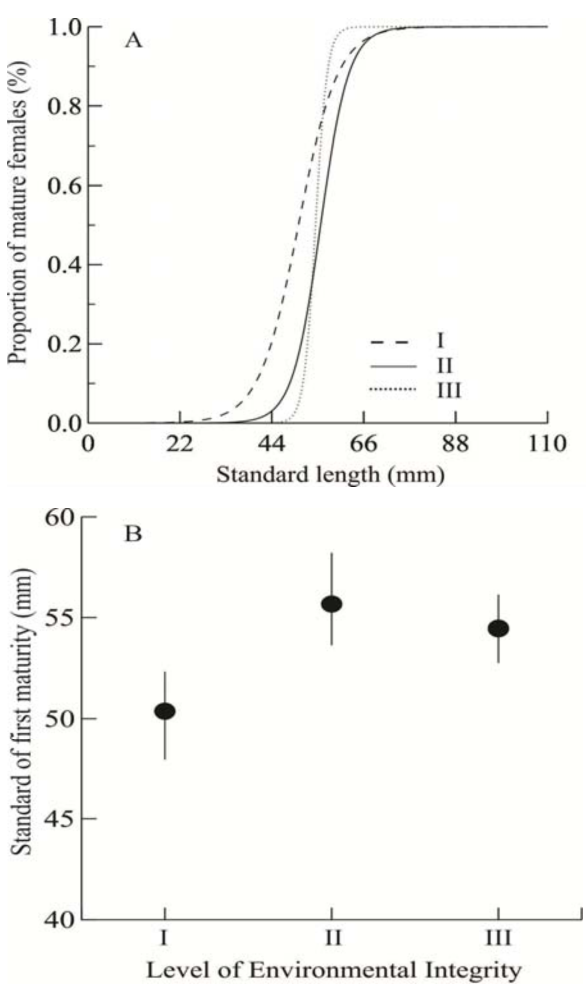

Figure 3. A) Proportion of *A. altiparanae* mature females in relation to standard length between the three levels of environmental integrity (I) Least impacted sites; (II) Altered sites; (III) Impacted sites in the Ivinhema river basin, upper Paraná river, between July 2001 and November 2011. B) Average standard length at first maturity (L_{50}) of *A. altiparanae* in each level of environmental integrity. Bars represent the confidence interval.

No significant variation was detected for the gonadosomatic index (GSI) between the levels of environmental integrity in the first quarter (H = 0.19; df = 2; p = 0.900) and in the fourth quarter (H = 0.093; df = 1; p = 0.075) in the comparison of streams with different levels of environmental integrity. However a significant variation between levels of environmental integrity was observed in the second quarter (H = 7.82; df = 2; p < 0.020) and in the third quarter (H = 21.42; df = 2; p < 0.0001), emphasizing that females showed higher GSI values in altered and impacted streams (Figure 4).

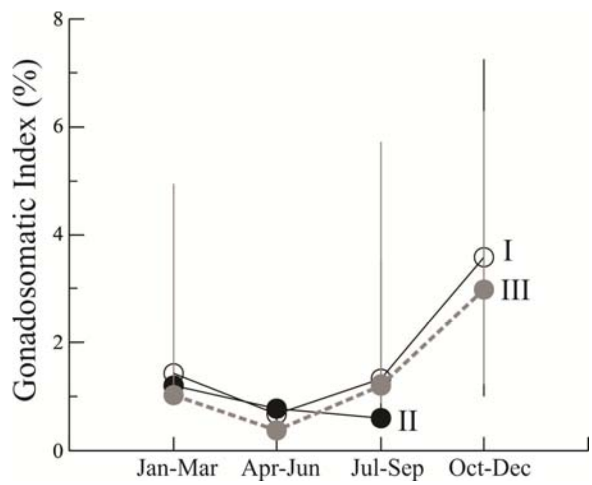

Figure 4. Quarterly distribution of the values of Gonadosomatic Index (GSI) of *A. altiparanae* females in each level of environmental integrity, (I) Least impacted sites; (II) Altered sites; (III) Impacted sites in the Ivinhema river basin, upper Paraná river between July 2001 and November 2011. Circles represent medians and bars represent the respective interquartile deviations (25-75%).

A significant seasonal variation in the GSI was verified in the least (H = 38.24; df = 3; p < 0.0001) and in the impacted streams (H = 23.19; df = 3; p < 0.0001). In the altered sites, there was no significant temporal variation (H = 5.15; df = 2; p = 0.0759). Lower values of GSI were registered between April and June, in the three levels of environmental integrity. Nevertheless, for the least and the impacted streams, GSI values rose progressively from July to September until reaching the highest values between October and December, suggesting that the reproductive period of *A. altiparane* occurs in this period (Figure 4). It is noteworthy that no females were collected with mature gonads (stage C) in October and December in the altered streams.

There was a significant variation in the condition factor of males and females of *A. altiparanae* between the levels of environmental integrity (p < 0.0001). For both sexes, individuals showed a better condition in the altered and impacted streams (Figure 5).

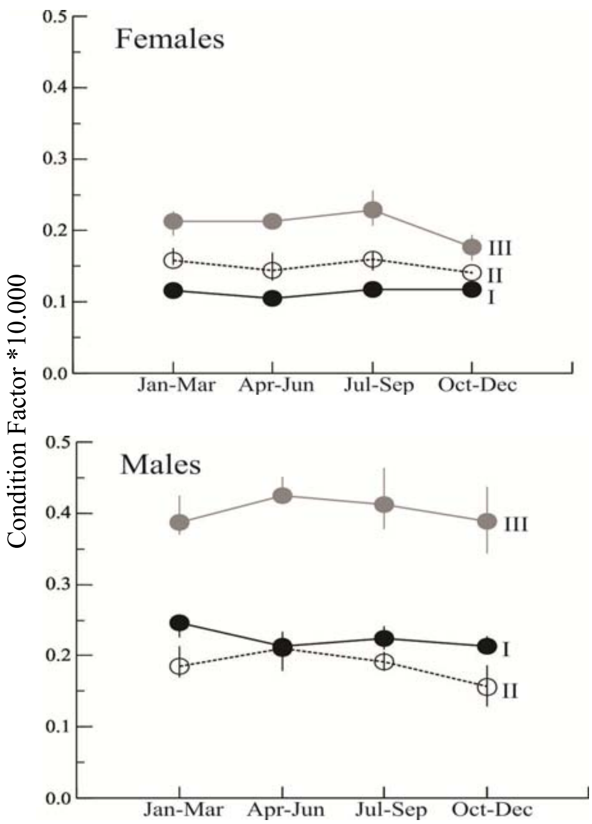

Figure 5. Mean values and confidence intervals for the Condition Factor of females and males of *A. altiparanae* in the three levels of environmental integrity, (I) Least impacted sites; (II) Altered sites; (III) Impacted sites in the Ivinhema river basin, upper Paraná river between July 2001 and November 2011.

Females presented a significant seasonal variation in the condition factor in the least impacted ($H = 24.68$; df = 3; $p < 0.0001$) and impacted streams ($H = 21.43$; df = 3; $p < 0.0001$), and in the altered sites, no significant seasonal variation was observed ($H = 7.55$; df = 3; $p = 0.050$). For males, the condition factor showed seasonal variation in the least impacted streams ($H = 16.39$; df = 3; $p < 0.0009$). There was no significant seasonal variation in the altered ($H = 7.67$; df = 3; $p = 0.05$) and impacted streams ($H = 4.20$; df = 3; $p = 0.240$).

Discussion

The results found in the present study indicate that the electrical conductivity was the variable indicated to distinguish the streams into quality classes. High levels of conductivity are probably resulting from high concentrations of total dissolved solids, which impair the water quality and are harmful to fish communities (KIMMEL et al., 2009). According to Lima-Junior et al. (2006) in the Corumbataí river (São Paulo, State), the least impacted site located on the river presented conductivity <50 μS cm^{-1}, while the most degraded, >100 μS cm^{-1}. Besides that, the Cetesb (2009) sets that electrical conductivity above 100 μS cm^{-1} is usually associated with impacted environments, providing a good indicative of changes in water composition, mainly in its mineral concentration. In agreement with Kimmel and Arzent (2009), the electrical conductivity is an environmental factor important to detect the toxicity to aquatic life, which allow checking the influence of various sources of pollution by human activities in aquatic environments, like the discharges of domestic sewage, industrial and animal wastes, in which the result of contamination can be detected by an increase in electrical conductivity in the watercourse. Therefore, the use of electrical conductivity to classify the levels of environmental integrity was effective to depict the environmental conditions.

In the present study, females were more representative in number, especially in the impacted streams. This predominance of females may be probably due to the conditions favorable to reproduction, i.e., when the food was abundantly available. The higher proportion of females in an animal population is usually considered a strategy for a rapid population growth, and occurs more often when the food supply is abundant (CETRA et al., 2011; FERNANDEZ et al., 2003). The frequency distribution between males and females of *A. altiparanae* per classes of standard length evidences a similarity between sexes in the three levels of environmental integrity, suggesting that males and females have responded similarly to the same environmental pressures, even if growth rates between the sexes may differ (BLANCK; LAMOUROUX, 2007; TONDATO et al., 2012), although females reach longer lengths as a result of the need to accumulate more energy for reproduction (MARCUCCI et al., 2005).

The size at first maturity seems to be adapted to the type of unstable environment that this species lives. In the altered and impacted streams, females reached sexual maturity with greater lengths. Adding this information, we believe that *A. altiparanae* is investing more in growth and body proportions at these sites, due to the greater availability of organic matter and therefore food items in these locations. In this way, greater length at first maturity also indicates a large investment in fecundity, once larger females have higher quantity of oocytes (WOOTTON, 1992), suggesting a higher reproductive investment, presenting an adaptation to ensure the population balance (LOWE-McCONNELL, 1999).

Astyanax altiparanae also presented better body condition and greater reproductive intensity in the altered and impacted streams, indicating that it accumulates energy given the availability of food, but also invests in reproduction by being subjected to a degraded environment (ALEXANDRE et al., 2010; CUNICO et al., 2006; ORSI et al., 2002). As mentioned above, this relationship can be explained by the likely increased supply of food in the impacted sites. As a strategy to overcome environmental stress, the population may be investing in growth and reproduction (OOST et al., 2003). Similar results were found by Alberto et al. (2005) who verified an increase in the Gonadosomatic Index and Condition Factor of *A. fasciatus* in the most polluted stretch of the Camanducaia River (São Paulo, State). According to Santos et al. (2006), the highest condition factor indicates the best environmental conditions for the development of fish, reflecting the abundant availability of food resources.

The reproductive period of *A. altiparanae* took place between October and December coinciding with the rise in temperature, considering that the event should occur when the habitat condition is more favorable to this species (ABILHOA, 2007; BARBIERI; BARBIERI, 1988; LOURENÇO et al., 2008). The quarters including the months from January to March and October to December represent the warmer and wetter periods of the year, whereas the two quarters between April and September are characterized by lower mean temperatures and lower rainfall (SOUZA, 2012). The condition factor in this same period presented a drop related to the use of body reserves for gonadal development (GURGEL, 2004), pointing a greater spawning of this species during these months. Lizama and Ambrósio (2002) still emphasized that the increase in reproductive activity usually coincides with a reduction in feeding activity, which contributes to decreasing values of condition factor during this period.

Conclusion

Based on this study, females of *A. altiparanae* were more numerous than males, especially in the impacted streams, in addition to presenting higher L_{50} in these locations. Also, variation in the Gonadosomatic Index and Condition Factor between the levels of environmental integrity always revealed higher values in the most degraded sites of the basin. These results demonstrate that in the study area, the species exhibits a great adaptation to local conditions of the habitat, possibly for being an opportunistic species able to successfully occupy environments moderately impacted by human activities.

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