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Population structure and natural diet of *Astyanax* cf. *paranae* Eigenmann 1914 a typical species of neotropical headwaters streams

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ABSTRACT. This work describes the habits of individuals of the species *Astyanax* cf. *paranae* that inhabit Ribeirão do Ferro, a bedside stream. Overall, 92 specimens were analyzed (53 females and 39 males), and the size ranged from class 4.7-5.7 cm to class 10.7-11.7 cm. The females consisted in the larger portion of the samples, except for classes of 4.7-5.7 and 5.7-6.7 cm. The condition factor between males and females ($t = 2.1545$; $p < 0.05$) shows distinguished values, with females having higher averages. Fragments of allochthonous insects and plant matter make up the basis of the species' diet. The study showed that the population of *A.* cf. *paranae* is predominantly females and have the highest standard for length and weight, which may favor biological diversity and increased larvae and juveniles, maximizing the chance of individuals reaching adulthood. The species prefers an environment with predominance of rocks and logs, which favors shelters. Its diet comes mostly from the riparian forest, with a high dependence on the allochthonous items, thus, these vegetations are very important for maintaining populations of the species.

Keywords: biological parameters; feeding; Ipanema National forest; natural history; riparian forest.

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Introduction

South American freshwater fish fauna is probably the one with the highest species variety amongst all zoogeographic regions (Reis et al., 2016). In Brazil there are approximately 2,587 species of fish, of which 15% occur in the State of São Paulo (Oyakawa & Menezes, 2011). To date, 71 species of fish have been identified in the Sorocaba River Basin (Smith, Petrere Jr., & Barrella, 2007) and 50 for the Ipanema National Forest (Smith, Biagioni, & Halcsik, 2013). Despite this, knowledge of the basic biology of its species is still one of the major challenges of ichthyology (Lowe-McConnell, 1999), and small species that are found mainly in small watercourses are the least known, considering the taxonomy and population ecology (Sanna-Kaisa & Jukka, 2004).

More than 50% of stream species are medium and small, usually up to 150 mm in length, (Castro, 1999). These species have a high ratio of endemism and dependence on alien materials from riparian vegetation (Buckup, 1999; Lowe-McConnell, 1999). The presence of aquatic macrophytes and submerged marginal vegetation is relevant as it also serves as shelter, being a source of food that provides greater richness for the communities of brook fish (Fernandes, Lourenço, Ota, Moreira, & Zawadzk, 2013).

The genus *Astyanax*, is one of the genera with greater abundance of species of Characidae family. Garutti and Britski (2000) stated that *A. scabripinnis paranae*, described by Eignmann 1914, is a common species in the upper Paraná basin, with several populations isolated from each other in headwaters of rivers. Based on a new taxonomic analysis, the subspecies *A. scabripinnis paranae* was treated as *A. paranae* Eigenmann 1914 (Lima et al., 2003).

In recent years there has been an increase in studies and published works on the species complex *A. scabripinnis* (Abilhoa, 2007). Studies carried out with *A.* cf. *paranae* indicate a distribution restricted to small tributaries, especially sections of headwaters (Benedito-Cecilio et al., 2004; Abelha, Goulart, Kashiwaqui, & Silva, 2006). The food habits are predominantly omnivorous (Abelha et al., 2006). Considering the lack of

biological data for species of streams (Azevedo-Santos et al., 2018), especially those occurring in the Sorocaba river basin, and its importance for the conservation unit where it is found, the present work aims to describe the population structure of *A.* cf. *paranae* and the environment in which it lives, the Ribeirão do Ferro, a stream of the Ipanema National Forest, Sorocaba river basin, State São Paulo, Brazil.

Material and methods

Study area

The study area, the stream Ribeirão do Ferro, is located in the Ipanema National Forest (REC; 23° 25' 50,611" S, 47° 39' 54,000" W) 120 km from the city of São Paulo and occupies an area of 5,069.73 hectares covering part of the municipalities of Iperó, Araçoiaba da Serra and Capela do Alto (Figure 1) inserted in a transition area between Cerrado vegetation and Atlantic forest. It is one of the few forest strongholds in the countryside of State São Paulo state where, despite its history of disturbances, holds the greatest biodiversity in the region.

It is cut in its southern part by the Tropic of Capricorn, thus located in a transition zone, from tropical to temperate zone, presenting two types of predominant climates, type Cfa (to the south) limiting with Cwa (to the north), Cfa: warm subtropical climate, constantly humid, with a less dry winter (in this the precipitation oscillates between 30 and 60mm), maximum temperatures superior to 22°C and minimum temperatures below 18°C; and Cwa: warm subtropical climate, with drier winter (precipitation less than 30 mm). The average annual precipitation of the region is of the order of 1400 mm, with a minimum of 800 mm and a maximum of 2,200 mm, the less humid months go from August to November and the humid months go from March to June (Brasil, 2008). It occupies an area of the Ipanema and Ribeirão Iperó and Ribeirão do Ferro sub-basins, integrating the Sorocaba river basin (Smith & Regalado, 2008).

The Iron Stream is born within the limits of the Ipanema Flona, in its southwestern part (Morro Araçoiaba), is 8km long, and forms, at the beginning of its course, the Lagoa da Cobra. Near the northern border of Flona, the Ribeirão do Ferro receives another stream, called by residents as stream of the Onça, that also runs through the seasonal forest in the interior of Flona. Downstream of the stream of the Onça, the Ribeirão do Ferro, already outside of Flona, passes through areas with agriculture and livestock, going to the Sorocaba river, near Yuri Farm (*Instituto Chico Mendes de Conservação da Biodiversidade* [ICMBio], 2017).

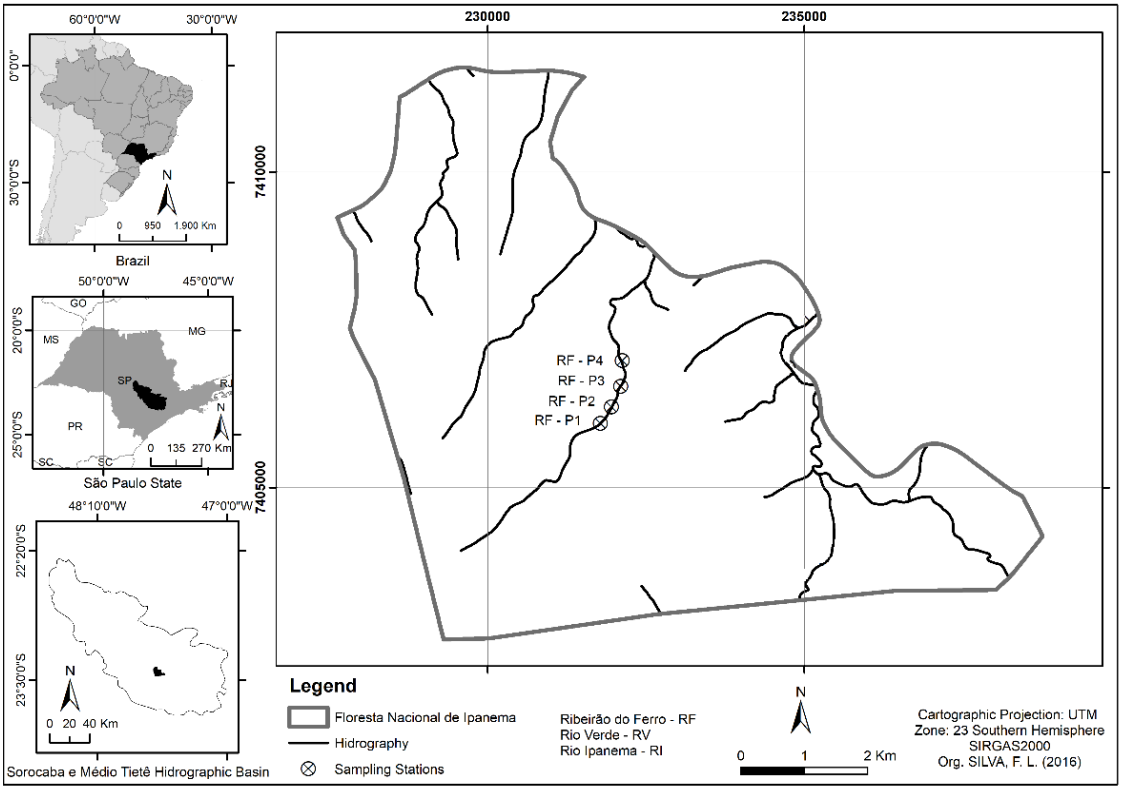

Figure 1. Location of collection points in the Ribeirão do Ferro stream, Ipanema National Forest, State São Paulo, Brazil.

The area of study in Ribeirão do Ferro is located within the reserve in a forest section, with varying depths and widths, most of it narrow and shallow, and in some stretches with waterfalls followed by deep pockets and wide, presenting riparian forest in all of the extension section studied, in both margins.

Structure of streams

The stream was characterized in situ in 4 points located in upper and middle stretch, throughout physical and chemical parameters of water (temperature, dissolved oxygen, pH, electrical conductivity and total dissolved solids) using an OAKTON PCD650 multiparameter probe. The analyzes were performed in July 2012 and January 2013, in a total of two campaigns. The physical parameters of the stream such as rocks and logs were measured through a quadrant within the stream and counting the items, thus estimating the total amount of each item at each point, while sludge, silt, sand, gravel, pebbles and vegetable matter were only checked for presence or absence status at each point. The minimum and maximum depth and width were measured using a metric scale.

Fish sampling

Fish were collected using aid of a trawl of three meters in length, mesh of 1 cm and two sieves of 50x50 cm, mesh of 5 mm, with 30 min. for each sampling method per point, between July 2012 and January 2013. In the laboratory the specimens were identified, counted and measured, obtaining the total length, weighed (using a precision scale) and dissected for removal of the digestive tract. Sexing was done by observing the gonads of the individuals (Vazzoler, 1996). The digestive tract of the specimens in each collection was removed and fixed in 4% formalin solution, and then stored in 70% alcohol solution. The stomach contents were analyzed under a stereoscopic microscope, the identification of the items was performed with the aid of specialized bibliography.

Data analysis

The environmental data of rock, log, silt, sand, gravel, pebble, vegetal matter, minimum depth, maximum depth, average depth, minimum width, maximum width and average width were evaluated using Principal Component Analysis (PCA), using PAST version 1.32 (Hammer, Harper, & Ryan, 2004). For the analysis of data from the population structure regarding size, the frequency distribution of the full-length classes for both sexes was obtained by the rule of Sturge. The ratio between weight and total length was obtained for males and females of the analyzed species, being expressed by the equation: $Weight = k\,.\,total\ length^{b}$. The parameters k and b were obtained by adjusting the logarithm of the dependent (weights) and independent variables (total lengths) by the least squares method (Vazzoler, 1981), we also obtained the Pearson correlation coefficient and the relationship equation between the variables weight and the total length from the diagram of dispersion made with the help of a spreadsheet software. The value of b is the relative growth constant, and tends to assume values closer to 3, when growth is isometric (Weatherley & Gill, 1987), the condition factor was calculated for males and females from the equation: $k = weight\,.\,total\ length^{b}$, and the differences between the main values for each parameter considered were evaluated by the t-test, considering that the values presented normal distribution according to the Shapiro-Wilk test, using a significance level of 0.05. For the analysis of relevance of the identified food items, the frequency of occurrence method was used, evaluating the percentage of stomachs in which a given food item occurs (Hyslop, 1980).

Results

The studied stream presented mild temperature in the two studied seasons, with lower values in the dry season. The pH values indicated acidity in the two seasons of the year, while the dissolved oxygen was below 6 mg L^{-1} (Table 1). The stream has a small width and depth, whereas the bed of the stream is stony/rocky almost in all its extension with the presence of large amounts of foliage, branches and trunks.

The Main Components analysis explained for the first two axes 98.41% of the total data variance. Axis 1, Main Component 1, explained 61.56% and Axis 2, Main Component 2, explained 36.85% of the variation in environmental data (Table 2). The formation of axis 1 was positively influenced by conductivity and total solids dissolved and in minor importance by the rocky substrate. The formation of axis 2 was influenced positively by the presence of rocks and negatively by the conductivity and total dissolved solids. In addition,

in the rainy season there is an elevation of conductivity and total dissolved solids, which is attributed to the transportation of particles to the stream, this being a natural condition that does not affect the population (Figure 2).

A total of 92 adult specimens were analyzed, 53 females and 39 males, and the size range from class 4.7-5.7 cm to class 10.7-11.7 cm. Individuals smaller than 4.7 cm were not considered. The modal values were different between the sexes, with males being more abundant in the 4.7-5.7 length class and females among the 8-7-10.7 length classes (Figure 3). The length distribution by classes showed that male catches were more frequent in class 5.7-6.7 and female catches between classes 9.7-10.7. The species presented weight between 1.13 and 22.71 g, with males between 1.13 and 17.40 g and females 2.74 to 22.71 g.

The weight-to-weight ratio for males and females is shown in Figure 4a and b, as well as their mathematical expressions. It is verified that the value of parameter b was 3.2 for males and 3.1 for females. As the condition factor values presented a normal distribution for male specimens (Shapiro-Wilk W test = 0.8975; $p > 0.0001867$) and females (Shapiro-Wilk W test = 0.242; $p > 4.741E-15$), the means were evaluated through t-test, and the confidence limits were compared for the identification of differences. Significant differences were found for the values of the condition factor between males and females ($t = 2.1545$; $p < 0.05$), and females showed higher averages.

The diet of the species was composed of 10 alimentary, allochthonous and autochthonous items. In the allochthonous items, were found: Blattodea, Coleoptera, Formicidae, Hymenoptera, Lepidoptera and fragments of insects, while autochthonous were fish fragments, gastropod, sediment and vegetal matter. The most frequent items were fragments of alien insects, Formicidae and plant material (Figure 5).

Table 1. Average values and standard deviation for the physical and chemical variables of the water, depth and width of the Ribeirão do Ferro stream, Ipanema National Forest, State São Paulo, Brazil. Temp: temperature, DO: dissolved oxygen, Min dep: minimum depth, Dep med: depth average, Dep max: depth max, Min Wid: minimum width, Wid med: average width, Wid max: maximum width, RF: Ribeirão do Ferro stream, S: dry and C: rain.

Point	Temp (°C)	DO (mg L^{-1})	pH	Min dep (m)	Dep med (m)	Dep max (m)	Min Wid (m)	Wid med (m)	Wid max (m)
RF1 S	17.5 ± 0.45	5.6±0.31	5.93±0.2	0.26±0.05	0.43±0,057	0.6±0.1	1±0.26	1.41±0.20	1.83±0.15
RF2 S	18.46 ±0.89	5.64±0.1	5.93±0.2	0,26±0.11	0.41±0.11	0.56±0.11	1.06±0.20	1.46±0.17	1.83±0.15
RF3 S	16.86±0.05	6.64±0.2	5.33±0.8	0.30±0.1	0.45±0.1	0.6±0.1	1±0.2	1.41±0.33	1.85±0.49
RF4 S	18.66±1.05	5.91±0.7	5.76±0.1	0.23±0.05	0.45±0.086	0.66±0.20	1.56±0.05	2.15±0.37	2.73±0.75
RF1 C	19.23±0.05	4.84±0.2	5.83±0.5	0.26±0.05	0.36±0.057	0.46±0.05	1±0.3	1.4±0.25	1.8±0.3
RF2 C	19.7±0.1	5.61±0.1	5.76±0.1	0.26±0.05	0.33±0.057	0.43±0.05	1.06±0.32	1.45±0.31	1.83±0.35
RF3 C	19.9±0.1	5.10±0.1	5.8±0.1	0.26±0.15	0.36±0.10	0.46±0.05	0.96±0.32	1.38±0.27	1.8±0.34
RF4 C	20.16±0.25	4.9±0.15	5.7±0.1	0.13±0.05	0.26±0.028	0.54±0.4	1.43±0.20	2.03±0.35	2.63±0.51

Table 2. Results of main components analysis: values obtained for each environmental variable, in the first two components, which presented the two highest percentages of variance. Values in bold show the variables with highest correlations within the main component.

Attribute	Code	CP1	CP2
pH	pH	0.0033256	0.0020165
Dissolved oxygen	OD	-0.010589	0.0002549
Conductivity	Cond	**0.85118**	**-0.16768**
Total solids dissolved	Tds	**0.47526**	**-0.14924**
Temperature	Temp	0.01503	-0.00071525
Rock	Roc	**0.22108**	**0.97216**
Trunk	Tron	-0.01887	0.066391
Mud	Lo	-0.0016628	8.75E-01
Silt	Sil	0.00052035	0.004236
Sand	Are	0.0026718	-0.0018188
Gravel	Cas	0.00050316	-0.002174
Pebble	Sei	0.0038792	-0.00037624
Vegetable matter	M veg	0.00047074	-0.0077264
Depth Minimum	Prof min	-0.00029932	-0.0003674
Depth maximum	Prof max	-0.001702	-0.00060897
Depth media	Prof med	-0.0010007	-0.00048818
Minimum width	Larg min	-0.0012973	0.0028208
Maximum width	Larg max	-0.0025731	0.0010638
Width Average	Larg med	-0.0019352	0.0019423
Variance in percentage per factor		61.56%	36.85%
Cumulative variance			98.41%

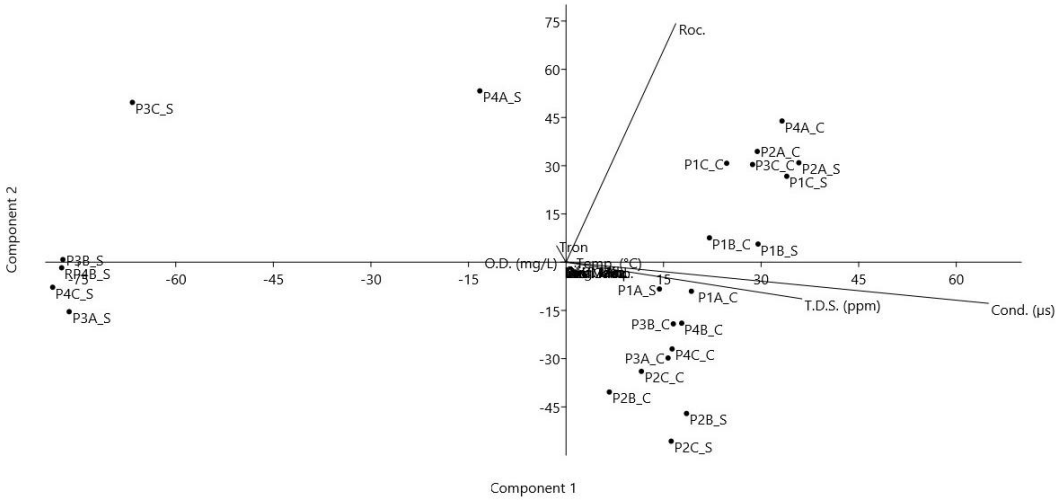

Figure 2. Graph generated from Main Components Analysis on environmental data., P: dot, A B C: replicas, S: dry and C: rain. Legends of the environmental variables see Table 2.

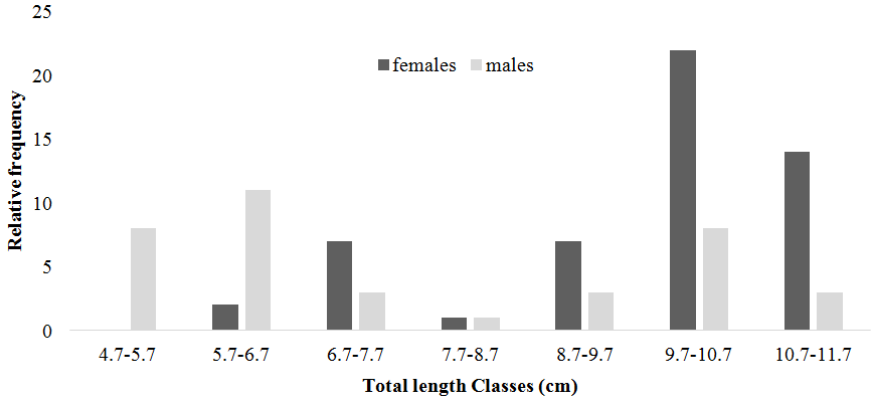

Figure 3. Frequency distribution by length class of males and females.

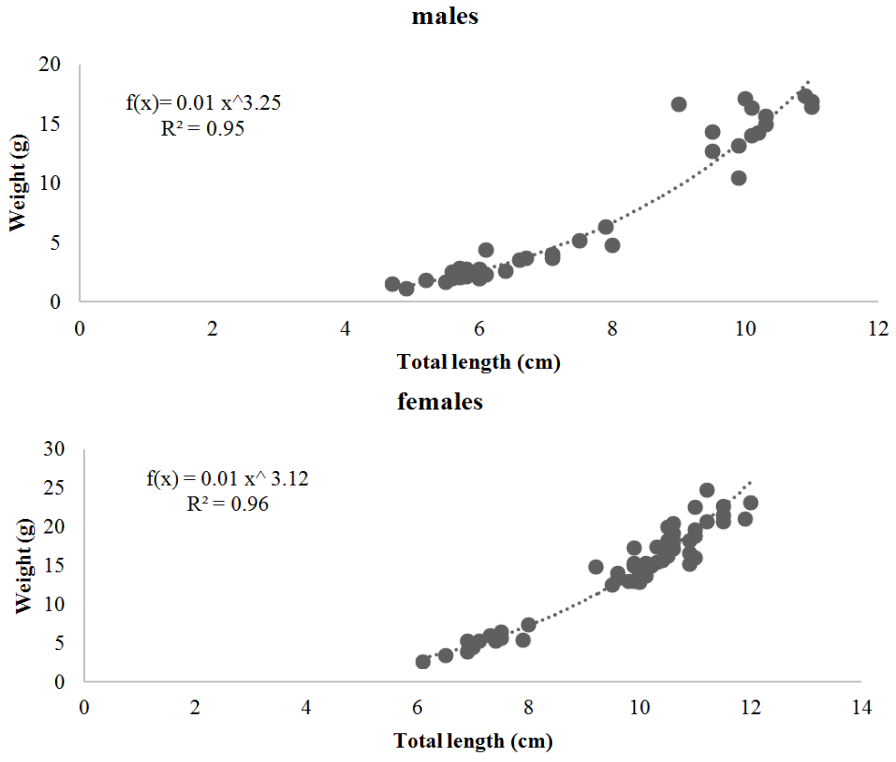

Figure 4. Scatter diagram, trend line, Pearson correlation coefficient and relationship equation between weight/total length (CT) variables; for males and females.

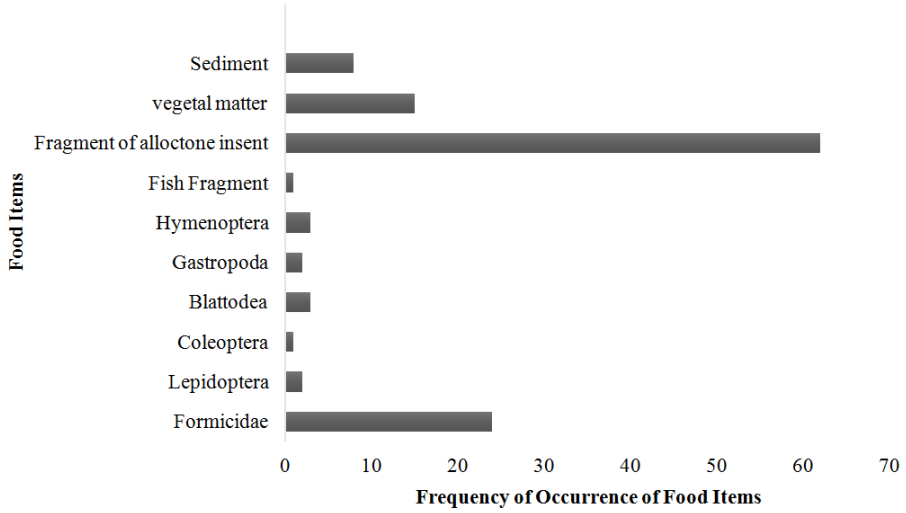

Figure 5. Frequency of occurrence of food items used by *A.* cf. *paranae* in Ribeirão do Ferro, National Forest of Ipanema.

Discussion

According to Kleerekoper (1990), physical and chemical factors are of great importance in the ecological structuring of aquatic environments, determining the occurrence and distribution of organisms. Regarding the physical and chemical variables of water, pH directly influences the physiology of organisms present in aquatic ecosystems (Silva, Angelis, Machado, & Waichaman, 2008).

The low pH values can be linked to a large amount of decomposed organic matter from the riparian forest, the concentration of dissolved oxygen was below 6 mg L^{-1} and this is also associated with a high rate of organic matter decomposition in the water. The mild temperature presented is characteristic of first order streams as they are shaded by the riparian forest, thus preventing greater solar incidence, these streams also have small dimensions of width and depth.

The rocky substratum, with an abundance of trunks and branches pointed out by Main Components Analysis as relevant, leads to the creation of backwaters that end up being used as shelter to the species, furthermore, trunks and branches can still attract insects, which are a relevant food resource for the species. *A.* cf. *paranae* shows a preference for first-order streams with rocky beds, with trunks and hidden portions. This was also observed by Abilhoa (2007) and Veregue and Orsi (2003).

The population of *A.* cf. *paranae* is formed predominantly by females, which was also verified by Abilhoa (2007) within the genus *Astyanax*. This can be linked to several factors, such as distinct growth rates, higher mortality of males, greater longevity of females or even susceptibility to the fishing apparatus used (Abilhoa, 2007).

Regarding the total length, the females presented the highest values except in the classes of 4.7 to 6.7 cm. This may be related to a distinct growth rate, since they require a larger space for the storage of gonads that later became eggs. The highest values for weight were also presented by females. These differences can be interpreted as indication for several biological characteristics and may be related to the weight of the gonads (Benedito-Cecílio & Agostinho, 1997; Abilhoa, 2007). As for parameter b, in general the values approximate 3, which was verified in the present study, characterizing the allometric growth of this species. Le Cren (1951) states that the values of parameter b vary from 2.0 to 4.0, assuming the value 3.0 for an 'ideal fish', i.e. isometric growth, which maintains the same shape during ontogenetic growth. Values lower or higher than 3.0 indicate allometric growth, suggesting individuals that, throughout growth, become more 'elongated' or 'round', respectively (Araújo & Vicentini, 2001).

Astyanax cf. *paranae* presented an omnivorous food pillar, tending to allochthonous towards insects, but also feeds on autochthonous items to a lesser extent, such as insect larvae and gastropods. The food habits presented here are consistent with other studies that demonstrate a predominant insectivorous diet (Esteves, Lobo, & Faria, 2008; Ferreira, Gerhard, & Cyrino, 2012) and are probably related to the occurrence of hanging vegetation on the stream, which provides allochthonous resources for the ichthyofauna. The role of food resources from riparian forests has been constantly noticed (Casatti, 2002; Mazzoni & Rezende, 2003; Rezende & Mazzoni, 2006; Abilhoa, 2007). Thus, in these systems, riparian forests have a relevant

role either as a source of food, in making habitats and refuges, which are important factors to maintain the diversity of ichthyofauna (Vono & Barbosa, 2001).

Conclusion

Given the results presented, the habitat of the species consists of rocky substrates with trunks and branches. The population has a predominance of females which are larger in size and weight. It was also verified the great importance of the ciliary forest for *A.* cf. *paranae*, being of extreme importance its conservation, considering that this provides its food base.

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