

Original article

## Food resource partitioning among species of *Astyanax* (Characiformes: Characidae) in the Lower Iguaçú River and tributaries, Brazil

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Resource partitioning allows for interspecific coexistence and is frequently reported for similar species. Here, we predicted the existence of resource partitioning among species of *Astyanax* that co-occur in the Low Iguaçú River and tributaries in Brazil. A total of 848 stomachs of five species of *Astyanax* were analyzed. Algae, terrestrial plant and fruit/seed were the most consumed resources. *Astyanax bifasciatus* and *A. dissimilis* had predominantly herbivorous diets, *A. gymnodontus* and *A. lacustris* were omnivorous, and *A. minor* was mainly algivorous. Permutational analysis of variance showed the species had different diets, and similarity percentage analysis indicated that fruit/seed and terrestrial plant contributed the most to this differentiation. A paired comparison indicated that the trophic breadth of *A. gymnodontus* differed from that of other species. The food overlap was low for 55% of *Astyanax* pairs. These results showed alignment with the niche theory, in which differentiation in the use of food resources facilitates the coexistence of species and minimizes competition. These adjustments to coexistence become relevant in the context of endemic species in a highly isolated basin under intense threat (dams, species introduction, deforestation, and pollution) as is the case for the Iguaçú River basin.

**Keywords:** Coexistence, Congeneric species, Diet, Diet overlap, Niche theory.

O particionamento de recursos permite a coexistência interespecífica e é frequentemente relatado para espécies semelhantes. Predizemos a existência de partição de recursos entre espécies de *Astyanax* que co-ocorrem no baixo rio Iguaçú. O total de 848 estômagos de cinco espécies de *Astyanax* foi analisado. Algas, plantas terrestres e frutos/sementes foram os recursos mais consumidos. *Astyanax bifasciatus* e *A. dissimilis* apresentaram dietas predominantemente herbívoras, *A. gymnodontus* e *A. lacustris* foram onívoras e *A. minor* foi principalmente algívora. As espécies apresentaram diferentes dietas (PERMANOVA) e a análise SIMPER indicou que frutos/sementes e plantas terrestres tiveram maior contribuição para esta diferenciação. A comparação pareada mostrou que a amplitude trófica de *A. gymnodontus* diferiu das outras espécies. A sobreposição alimentar foi baixa para 55% dos pares de *Astyanax*. Nossos resultados mostraram-se alinhados com a teoria de nicho, em que a diferenciação no uso de recursos alimentares facilita a coexistência de espécies e minimiza a competição. Estes ajustes para coexistência tornam-se relevantes no contexto de espécies endêmicas em uma bacia altamente isolada e sob intensa ameaça (barramentos, introdução de espécies, desmatamento e poluição), como é o caso da bacia do rio Iguaçú.

**Palavras-chave:** Coexistência, Dieta, Espécies congêneres, Sobreposição de dieta, Teoria de nicho.

### Introduction

Unlike terrestrial species or those with aerial or wind-dispersed life stages, species confined to freshwater environments generally cannot disperse from one catchment to another, unless corridors of aquatic habitats exist to

connect them (Abell *et al.*, 2008). Thus, the connections and disconnections between adjacent river basins have long been recognized as essential for the diversification of freshwater fish in the Americas (Buckup, 2011). In contrast, tectonic movements have in some cases established barriers to dispersal (vicariance) between adjacent river basins,

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allowing for further speciation and giving rise to endemic fish fauna (Cox *et al.*, 1980; Ricklefs, Schluter, 1993; Albert *et al.*, 2011). The Iguaçu River, a left-bank tributary of the Paraná River in Brazil, has been isolated from the main Paraná valley for approximately 22 million years by a geological lift, which gave rise to the Iguaçu Waterfalls (about 70 m in height; Maack, 1981). The geographic isolation promoted by this natural barrier has led to the occurrence of many endemic species, which represent more than 50% of the fish species in the Iguaçu River basin (Baumgartner *et al.*, 2012). Threats to the integrity of this fish fauna diversity (dams, species introduction, deforestation, and pollution) (Baumgartner *et al.*, 2012), coupled with the high endemism, were recognized by Abell *et al.* (2008), who included this basin as one of the 426 global freshwater ecoregions requiring urgent conservation planning efforts.

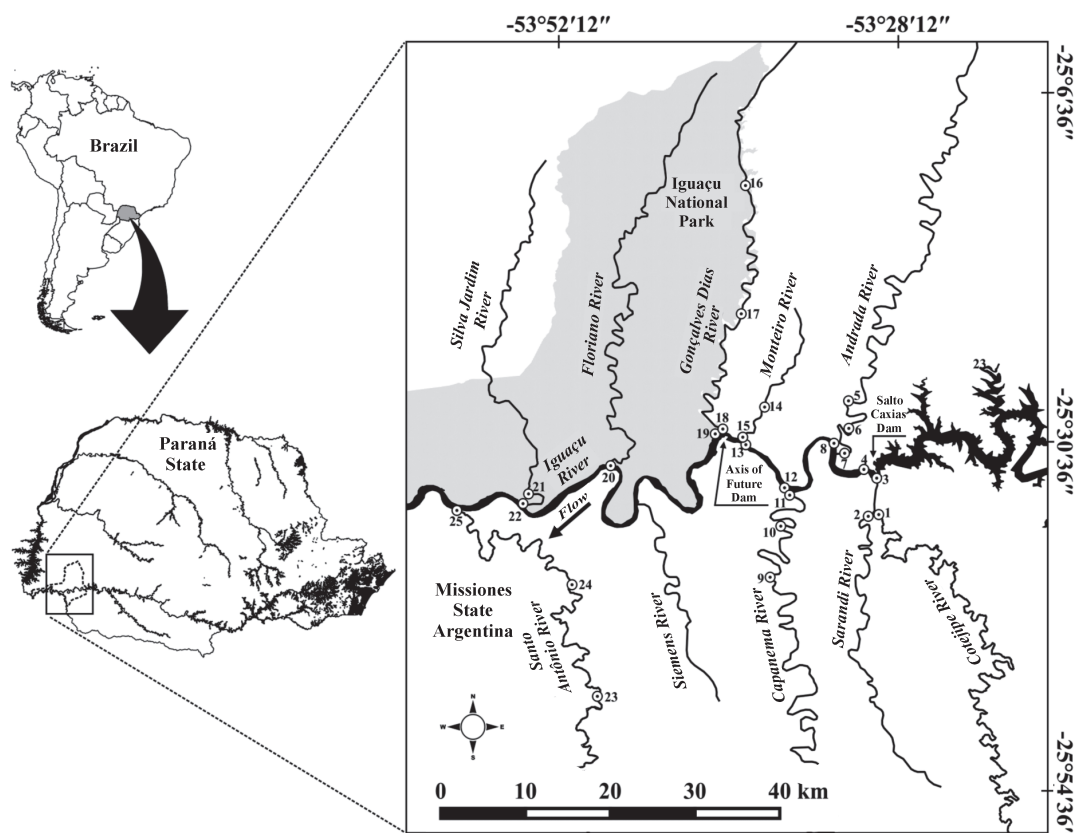
In this basin, *Astyanax* Baird, Girard 1854 stands out for its richness (Pavanelli, Oliveira, 2009), with 11 described species, of which 10 are endemic (Baumgartner *et al.*, 2012). The occurrence of a large number of congeneric species living in sympatry raises a central ecological question, which is to understand how morpho-physiologically similar species minimize competition for resources (Chesson, 2000; Kylafis, Loreau, 2011; Juncos *et al.*, 2015). According to the niche theory, when sympatric species overlap in the use of resources along one dimension, they must differentiate in another resource in order to coexist (Hutchinson, 1957). Species differ mainly in the use of habitat, food, and time

(Schoener, 1974; Wiens *et al.*, 2010; Wang *et al.*, 2015). This differentiation is called resource partitioning (Schoener, 1974) and enables interspecific coexistence (Ross, 1986). Moreover, resource partitioning is widely reported for congeners (Barreto, Aranha, 2006; Neves *et al.*, 2018).

In this context, our study was guided by the following question: Is the coexistence of *Astyanax* species in the Iguaçu River basin favored by food resource partitioning? We predict that *Astyanax* species of the Low Iguaçu River consume different resources, presenting low food overlap. Thus, the aims of this study were to i) evaluate the food items used by *Astyanax* species that co-occur in the Low Iguaçu River; ii) investigate possible differences in the consumption of food resources and in the niche breadth; and iii) infer the degree of overlap among species.

## Material and Methods

The Iguaçu River rises in the Serra do Mar and flows through a geological fault throughout Paraná State (Maack, 1981). The hydrographic basin covers the Paraná and the Santa Catarina states in Brazil and the Misiones region in Argentina (Dalla Corte *et al.*, 2015). The study area comprised the Low Iguaçu River and its main tributaries in the stretch between the Salto Caxias dam and the Santo Antônio River mouth (Iguaçu National Park), where 25 sampling sites were established (the first sampling site: 25°35'17.04"S–53°29'56.58"W; the last sampling site: 25°35'17.16"S–53°59'25.20"W; Fig. 1).



**Fig.1.** Study area showing the sampling sites (numbers 1 to 25) in the Lower Iguaçu River and tributaries in Brazil.

Fish were collected monthly from September 2013 to August 2014, using gill nets (2.5–14.0 cm mesh) exposed for 24 h and inspected every six hours, as well as electrofishing in the morning. Caught fish were euthanized with 250 mg/L benzocaine [State University of Western Paraná (UNIOESTE) Ethics Committee-Protocol 62/09] and later fixed in 10% neutralized formaldehyde.

Fish were identified according to Garavello, Sampaio (2010) and Baumgartner *et al.* (2012), measured (total and standard length), weighed (g), eviscerated, and the stomachs were collected. All the analyzed individuals were considered adults based on macroscopic evaluation of gonadal development. Voucher specimens were deposited in the fish collection of the Zoology Museum/State University of Londrina (MZUEL) (Tab. 1).

Stomach contents were analyzed using optical and stereo microscopes. The items were quantified according to the volumetric method (volume percentage of each item in relation to the total volume of the stomach contents) (Hyslop, 1980). The volume of each food item was determined using graduated test tubes; for volumes lower than 0.1 mL, a counting chamber was used (Hellowell, Abel, 1971). Food items were identified according to Bicudo, Bicudo (1970) for algae and McCafferty (1981), Borrer, DeLong (1988), and Mugnai *et al.* (2010) for invertebrates.

To identify possible differences among the diets of *Astyanax* species, a permutational analysis of variance (PERMANOVA, Bray-Curtis index) with 9999 permutations was applied to the volume matrix of food items. The data were transformed using the square root to reduce asymmetry and satisfy the premise of homogeneity. When significant differences were detected, pairwise tests using the *pseudo t*-statistic were applied to determine which species showed different diets. The food items that most contributed to distinguish the species pairs were verified using similarity percentage (SIMPER) analysis (Clarke, 1993).

Interspecific differences in diet breadth were evaluated by permutational multivariate analysis of dispersion (PERMDISP) (Anderson, 2006). This analysis was used to measure the distance from the centroid of a group defined *a priori*, in this case the species, and is calculated by principal co-ordinates analysis (PCoA). Calculation of the centroid of the group was performed using Bray-Curtis dissimilarity index values to compare the average dissimilarity among the *n* individual observations within the group. In this case, the mean distances of the samples in relation to the group mean (centroid) correspond to the niche breadth. In order to test the null hypothesis that the niche breadth did not differ among species, *F*-statistics were calculated using the Monte Carlo method with 9999 randomizations. The pairwise comparison by Tukey's honestly significant difference method was used to indicate differences in the interspecific variability of individuals of each species.

The diet overlap per sample was calculated based on the volume matrix of food items for each pair of *Astyanax* species at each site and month of collection using the Pianka index (Pianka, 1973) described by the following equation:

$$O_{jk} = \frac{\sum_i^n P_{ij} \times P_{ik}}{\sqrt{\sum_i^n P_{ij} \times \sum_i^n P_{ik}}}$$

where,

- $O_{jk}$  = Pianka niche overlap between species *j* and *k*;
- $P_{ij}$  = proportion of food resource *i* in the diet of species *j*;
- $P_{ik}$  = proportion of food resource *i* in the diet of species *k*;
- n* = total number of food resources.

The Pianka index ranges from 0 to 1 and indicates low (0.00–0.39), intermediate (0.40–0.60), or high overlap (0.60–1.00), where high overlap values may be indicative of competition or resource partitioning (modified from Grossman, 1986; Novakowski *et al.*, 2008). The overlap index values were transformed to a percentage, ranging from 0 to 100.

The PERMANOVA and SIMPER analyses were calculated using PRIMER v.6 (Clarke, 1993; Clarke, Gorley, 2006). The PERMDISP was performed with R (Oksanen *et al.*, 2009; R Development Core Team, 2017). The food overlap analysis was performed using EcoSim 7.0 (Gotelli, Entsminger, 2010). The level of significance in all analyses was 0.05.

## Results

We analyzed 848 stomachs belonging to five species (Tab. 1): *Astyanax bifasciatus* Garavello, Sampaio, 2010; *Astyanax dissimilis* Garavello, Sampaio, 2010; *Astyanax gymnodontus* (Eigenmann, 1911); *Astyanax minor* Garavello, Sampaio, 2010; and *Astyanax lacustris* (Lütken, 1875). The first four species are endemic to the Iguaçu River basin, while *A. lacustris* is distributed throughout the basin of the Upper Paraná River.

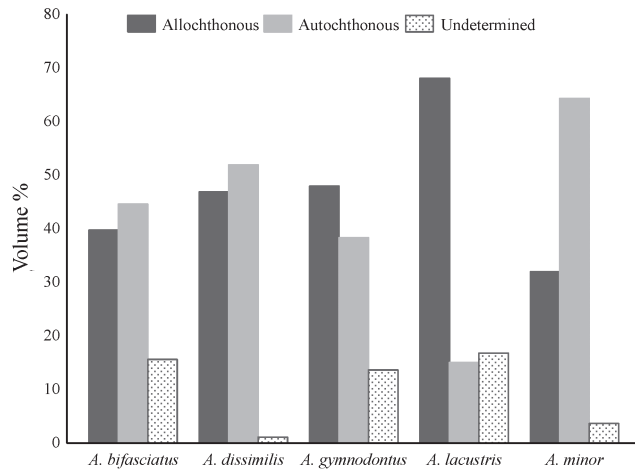
We identified 40 food items consumed by the species that, according to their origin, were classified as allochthonous, autochthonous, and undetermined (Tab. 1). The food items were grouped into 10 categories: terrestrial plant, fruit/seed, terrestrial insect, terrestrial invertebrate, algae, aquatic plant, aquatic insect, fish, aquatic invertebrate, and detritus (Tab. 1).

Algae, terrestrial plant, fruit/seed, and detritus were the most consumed resources. *Astyanax bifasciatus* and *A. gymnodontus* foraged on 82.5% and 72.5% (33 and 29 items) of the 40 identified items, respectively. *Astyanax lacustris* and *A. minor* ingested 24 different food resources, and *A. dissimilis* consumed approximately 60% of the listed food items (23 out of 40) (Tab. 1).

Items of allochthonous and autochthonous origin appeared in different proportions in the diets of the fish species (Fig. 2). Allochthonous resources (fruit/seed) were predominant in the diet of *A. lacustris* (Tab. 1). On the other hand, autochthonous resources (algae) dominated the diet of *A. minor* (Fig. 2). The other species consumed similar proportions of both sources (Fig. 2).

**Tab. 1.** Volumetric percentage (mL) of food items consumed by five species of *Astyanax* and their niche breadth in the Lower Iguaçu River and tributaries in Brazil. L=larvae; P=pupae; A=adult.

| Species  | <i>A. bifasciatus</i> | <i>A. dissimilis</i> | <i>A. gymnodontus</i> | <i>A. lacustris</i> | <i>A. minor</i> |
|--|-----------------------|----------------------|-----------------------|---------------------|-----------------|
| Number of stomachs   | 402                   | 54                   | 153                   | 129                 | 110             |
| Diet breadth   | 0.63                  | 0.60                 | 0.65                  | 0.62                | 0.61            |
| Voucher specimens  | MZUEL16267            | MZUEL16339           | MZUEL16353            | MZUEL16359          | MZUEL16346      |
| Standard length range (cm)                                     | 5.7 – 14.0            | 5.6 – 12.2           | 5.3 – 14.4            | 4.6 – 11.5          | 5.4 – 12.3      |
| <b>ALLOCHTHONOUS</b>   |                       |                      |                       |                     |                 |
| <b>Terrestrial plant</b>                                       |                       |                      |                       |                     |                 |
| Leaves and stems   | 31.57                 | 12.52                | 11.34                 | 6.75                | 19.36           |
| Fruit/seed   | 5.77                  | 16.30                | 28.36                 | 43.16               | 8.42            |
| <b>Terrestrial insect</b>                                      |                       |                      |                       |                     |                 |
| Coleoptera A   | 0.48                  | 1.49                 | 2.52                  | 11.72               | 0.89            |
| Diptera A  | 0.07                  |                      | 0.01                  | 0.03                |                 |
| Hymenoptera A  | 1.10                  | 9.04                 | 4.34                  | 1.11                | 2.93            |
| Isoptera A   | 0.01                  | 0.34                 |                       |                     | 0.02            |
| Fragments of terrestrial insects                               | 0.50                  | 1.21                 | 1.18                  | 2.80                | 0.36            |
| <b>Terrestrial invertebrate</b>                                |                       |                      |                       |                     |                 |
| Aranae   | 0.04                  |                      |                       | 0.53                |                 |
| Oligochaeta  | 0.19                  | 5.98                 | 0.19                  | 1.98                |                 |
| <b>AUTOCHTHONOUS</b>   |                       |                      |                       |                     |                 |
| <b>Algae</b>   |                       |                      |                       |                     |                 |
| Bacillariophyta  | 0.39                  | 0.01                 | 0.03                  |                     | 3.17            |
| Chlorophyceae  |                       |                      |                       |                     | 0.85            |
| Cyanophyceae   | 2.41                  |                      | 0.14                  | 2.89                |                 |
| Oedogoniophyceae ( <i>Oedogonium</i> )                         | 3.24                  | 1.63                 | 4.14                  |                     | 5.68            |
| Rhodophyceae (Audouinellaceae)                                 | 0.85                  | 1.58                 | 0.47                  |                     | 0.33            |
| Rhodophyceae (Compsopogonaceae, <i>Compsopogon caeruleus</i> ) | 22.03                 | 38.13                | 6.29                  | 3.53                | 44.54           |
| Zygnemaphyceae   | <0.01                 |                      |                       |                     | 0.96            |
| Zygnemaphyceae ( <i>Spirogyra</i> )                            | <0.01                 |                      |                       |                     |                 |
| <b>Aquatic plant</b>   |                       |                      |                       |                     |                 |
| Aquatic insect   | 1.42                  | 7.04                 | 0.89                  | 3.42                | 6.12            |
| Chironomidae   | 0.08                  | 0.05                 | 0.02                  | 0.94                |                 |
| Coleoptera L   | 0.03                  | 1.98                 | 0.27                  | 0.32                | 1.69            |
| Diptera L  | 0.05                  |                      | 0.01                  | <0.01               | 0.04            |
| Diptera P  | 0.06                  | 0.69                 | 0.01                  | 0.30                | 0.62            |
| Ephemeroptera A  | 0.43                  | 1.82                 |                       | 0.05                | 0.26            |
| Hemiptera A  | 0.17                  | 0.10                 | 0.27                  | 0.09                |                 |
| Lepidoptera L  | 0.09                  | 0.21                 | 0.04                  |                     | 1.19            |
| Lepidoptera P  | <0.01                 |                      |                       |                     |                 |
| Odonata A  | 0.03                  | 0.17                 | 0.06                  | 1.07                | 0.85            |
| Plecoptera A   | 0.02                  |                      |                       | 0.03                |                 |
| Simuliidae L   | <0.01                 |                      |                       |                     |                 |
| Trichoptera L  | 0.13                  | 1.09                 | 0.03                  | <0.01               | 0.01            |
| Fragments of aquatic insects                                   | 0.31                  | 0.91                 | 0.16                  | 0.60                | 1.45            |
| <b>Fish</b>  |                       |                      |                       |                     |                 |
| Scale  | <0.01                 |                      | 10.86                 | 0.67                |                 |
| Fragments of fish  | <0.01                 |                      | 0.06                  | 0.67                |                 |
| <b>Aquatic invertebrate</b>                                    |                       |                      |                       |                     |                 |
| Aeglidae   | 0.03                  | 1.34                 | 8.38                  | 0.06                | 1.69            |
| Bivalvia   |                       |                      | 3.93                  |                     |                 |
| Gastropoda   |                       |                      | 1.88                  |                     |                 |
| Microcrustacean  |                       |                      | 2.45                  |                     |                 |
| Porifera   |                       | 1.34                 | 0.13                  |                     | 1.69            |
| Testate amoebae  |                       |                      |                       | 0.06                |                 |
| <b>UNDETERMINED</b>  |                       |                      |                       |                     |                 |
| Detritus   | 15.61                 | 1.13                 | 13.66                 | 16.80               | 3.67            |



**Fig. 2.** Volumetric percentage of the origin of food items in the diet of *Astyanax* species in the Low Iguaçu River and tributaries in Brazil.

*Astyanax gymnodontus* and *A. lacustris* exhibited an omnivorous diet in which fruit/seed was the main resources, but the species complemented this with different foods (detritus, fish, and algae for the former and detritus and terrestrial insect for the latter; Tab. 1). The other species showed an herbivorous diet. *Astyanax bifasciatus* predominantly consumed terrestrial plant and algae. *Astyanax dissimilis* principally consumed algae and fruit/seed while *A. minor* mainly consumed algae (Tab. 1).

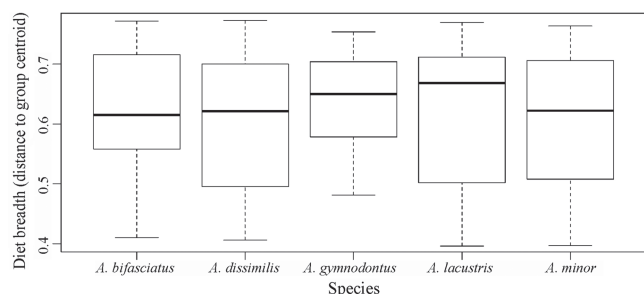
The PERMANOVA ( $pseudo-F = 12.73$ ;  $p < 0.01$ ) indicated differences among the diets of the species pairs, except for the pair *A. dissimilis* × *A. minor* that showed similar diet preferences ( $pseudo-t$  statistic;  $p > 0.05$ ) (Tab. 2). The observed dissimilarity, verified through SIMPER, indicated terrestrial plant and fruit/seed as the main food items that distinguished *A. lacustris* from the other species. In addition, the Rhodophyceae *Compsopogon caeruleus* was responsible for the dissimilarity in the diets of *A. bifasciatus*, *A. dissimilis*, *A. gymnodontus*, and *A. minor* (Tab. 2).

**Tab. 2.** Dissimilarity of *Astyanax* species diet in Lower Iguaçu River and its tributaries in Brazil as verified by SIMPER and PERMANOVA ( $pseudo-t$ ). Cc=*Compsopogon caeruleus*; Tp=terrestrial plant; Fs=fruit/seed; De=detritus.

| Species                                       | SIMPER Results    |       |                  |                                      | PERMANOVA<br>Pair-wise Tests |
|---|-------------------|-------|------------------|--------------------------------------|------------------------------|
|   | Dissimilarity (%) | Items | Contribution (%) | Mean abundance A<br>Mean abundance B |                              |
| <i>A. bifasciatus</i> × <i>A. dissimilis</i>  | 83.10             | Cc    | 24.36            | 0.17                                 | 0.21<br>$t = 1.92; p < 0.01$ |
|   |                   | Tp    | 21.59            | 0.22                                 |                              |
|   |                   | Fs    | 10.22            | 0.05                                 |                              |
| <i>A. bifasciatus</i> × <i>A. gymnodontus</i> | 87.90             | Tp    | 20.06            | 0.22                                 | 0.12<br>$t = 4.04; p < 0.01$ |
|   |                   | Fs    | 16.85            | 0.05                                 |                              |
|   |                   | Cc    | 14.61            | 0.17                                 |                              |
| <i>A. bifasciatus</i> × <i>A. lacustris</i>   | 89.66             | Fs    | 20.45            | 0.22                                 | 0.05<br>$t = 5.41; p < 0.01$ |
|   |                   | Tp    | 19.66            | 0.06                                 |                              |
|   |                   | Cc    | 14.25            | 0.02                                 |                              |
| <i>A. bifasciatus</i> × <i>A. minor</i>       | 82.93             | Cc    | 24.71            | 0.17                                 | 0.21<br>$t = 2.31; p < 0.01$ |
|   |                   | Tp    | 22.85            | 0.22                                 |                              |
|   |                   | De    | 9.91             | 0.09                                 |                              |
| <i>A. dissimilis</i> × <i>A. gymnodontus</i>  | 89.76             | Cc    | 19.60            | 0.21                                 | 0.06<br>$t = 2.87; p < 0.01$ |
|   |                   | Fs    | 17.99            | 0.08                                 |                              |
|   |                   | Tp    | 14.18            | 0.11                                 |                              |
| <i>A. dissimilis</i> × <i>A. lacustris</i>    | 89.94             | Fs    | 21.92            | 0.22                                 | 0.08<br>$t = 3.65; p < 0.01$ |
|   |                   | Cc    | 20.49            | 0.02                                 |                              |
|   |                   | Tp    | 12.82            | 0.06                                 |                              |
| <i>A. dissimilis</i> × <i>A. minor</i>        | 79.54             | Cc    | 28.06            | 0.21                                 | 0.21<br>$t = 0.97; p = 0.45$ |
|   |                   | Tp    | 17.40            | 0.11                                 |                              |
|   |                   | Fs    | 11.80            | 0.08                                 |                              |
| <i>A. gymnodontus</i> × <i>A. lacustris</i>   | 84.96             | Fs    | 25.86            | 0.22                                 | 0.20<br>$t = 1.88; p < 0.01$ |
|   |                   | De    | 16.03            | 0.09                                 |                              |
|   |                   | Tp    | 13.18            | 0.06                                 |                              |
| <i>A. gymnodontus</i> × <i>A. minor</i>       | 89.64             | Cc    | 19.33            | 0.06                                 | 0.21<br>$t = 3.72; p < 0.01$ |
|   |                   | Fs    | 17.74            | 0.20                                 |                              |
|   |                   | Tp    | 15.37            | 0.12                                 |                              |
| <i>A. lacustris</i> × <i>A. minor</i>         | 90.50             | Fs    | 21.67            | 0.22                                 | 0.05<br>$t = 4.45; p < 0.01$ |
|   |                   | Cc    | 19.90            | 0.02                                 |                              |
|   |                   | Tp    | 14.02            | 0.06                                 |                              |

*Astyanax* species showed average centroid distance values above 0.6 (Tab. 1; Fig. 3), indicating a wide trophic niche breadth. *Astyanax gymnodontus* presented the largest niche (0.65) (Tab.1; Fig. 3) significantly different from that of other species (PERMDISP,  $F = 3.78$   $p < 0.01$ ) (Tab. 3).

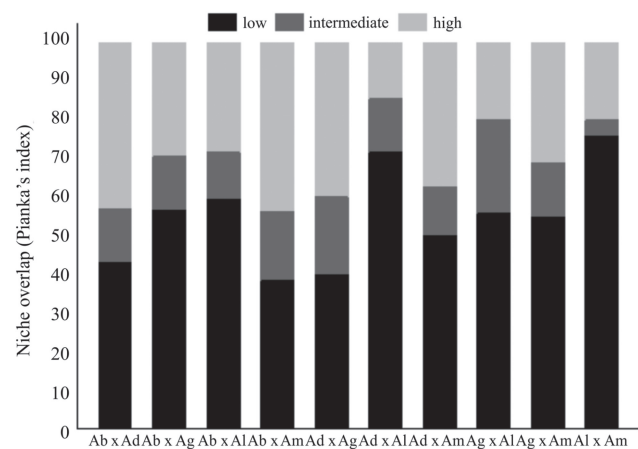
The diet overlap was low for 55% of the *Astyanax* pairs, especially those composed of *A. lacustris*, as well as the pairs *A. bifasciatus* × *A. gymnodontus*, *A. gymnodontus* × *A. minor*, and *A. dissimilis* × *A. minor* (Fig. 4).



**Fig. 3.** Variation in diet breadth based on 40 food items consumed by five *Astyanax* species in the Low Iguaçú River and tributaries in Brazil. Diet breadth was evaluated by dispersion of the diet of species in the multivariate space by using PERMDISP (large spatial distance from the centroid indicates wide dispersion, i.e., wide trophic breadth). Lower and upper bars of the box represent 25 and 75 quartiles, respectively. The horizontal bar within each box represents the mean diet breadth.

**Tab. 3.** P-values of PERMDISP analyses of the diet breadth of five *Astyanax* species in the Low Iguaçú River and its tributaries in Brazil.

|                       | <i>A. dissimilis</i> | <i>A. gymnodontus</i> | <i>A. lacustris</i> | <i>A. minor</i> |
|-----------------------|----------------------|-----------------------|---------------------|-----------------|
| <i>A. bifasciatus</i> | 0.067                | <b>0.009</b>          | 0.400               | 0.157           |
| <i>A. dissimilis</i>  |                      | <b>0.004</b>          | 0.366               | 0.577           |
| <i>A. gymnodontus</i> |                      |                       | <b>0.003</b>        | <b>0.002</b>    |
| <i>A. lacustris</i>   |                      |                       |                     | 0.688           |



**Fig. 4.** Relative frequency (%) of the diet overlap index of *Astyanax* species pairs in the Low Iguaçú River and tributaries in Brazil. Ab=*A. bifasciatus*; Ad=*A. dissimilis*; Ag=*A. gymnodontus*; Al=*A. lacustris*; Am=*A. minor*. Low < 0.39; intermediate =0.40-0.59; high > 0.60.

## Discussion

Our findings corroborated our prediction. *Astyanax* species consumed a variety of resources, exhibiting trophic segregation in co-occurrence. The observed consumption of foods of different trophic levels and origins (autochthonous and allochthonous) revealed a more generalist and opportunistic diet for *A. gymnodontus* and *A. lacustris*, while the other species had herbivorous dietary preferences. *Astyanax* is known for its opportunistic feeding habits (Lobón-Cerviá, Bennemann, 2000) and the use of different feeding strategies (Abelha *et al.*, 2001, 2006; Mazzoni *et al.*, 2010) that enable the species to forage at almost all trophic levels (Abelha *et al.*, 2006). Most of the previous dietary information about *Astyanax* species from Iguaçú River is from pre- and post-impoundment studies in which the trophic plasticity of this fish group is revealed by their opportunistic feeding behavior in response to environmental changes (see Cassemiro *et al.*, 2002, 2005; Loureiro-Crippa, Hahn, 2006; Delariva *et al.*, 2013; Mise *et al.*, 2013).

This peculiarity explains the wide array of food items of different origins (autochthonous and allochthonous) explored by the studied species and corroborates their wide trophic niche. The riparian forest in the sample sites, especially inside the Iguaçú National Park (almost pristine), may play an essential role in the abundance of these distinct food resources. This vegetation is a source of allochthonous food, such as terrestrial plant, fruit/seed and terrestrial insect, besides being a substrate represented by trunks, branches, and other structures that fall into the river bed and are colonized by algae and aquatic invertebrates, increasing the availability of autochthonous food resources to fish fauna.

The expressive consumption of fruit/seed and/or terrestrial plant by *A. bifasciatus*, *A. gymnodontus*, and *A. lacustris* and algae by *A. dissimilis* and *A. minor* emphasized the preference toward foods of plant origin. Consistent with our results, most *Astyanax* species were previously described as herbivorous: *A. bifasciatus*, *A. dissimilis* (Delariva *et al.*, 2013; Mise *et al.*, 2013), and *A. gymnodontus* in the pre-impoundment period of Salto Caxias Reservoir (Cassemiro *et al.*, 2005; Delariva *et al.*, 2013) and *A. lacustris* (Cassemiro *et al.*, 2002, 2005; Loureiro-Crippa, Hahn, 2006), whose preference for fruit/seed was also reported by Andrian *et al.* (2001) and Silva *et al.* (2014). Additionally, *A. minor* was described as detritivorous in the Segredo Reservoir (Mise *et al.*, 2013) and as herbivorous in lotic environments in the region of Salto Caxias Reservoir, showing a diet mainly composed of algae and plants (Delariva *et al.*, 2013).

Although the species fed predominantly on terrestrial plant, fruit/seed and algae, the proportion of these main resources and those that were complementary (e.g., aquatic plant, terrestrial insect, detritus, and fish) varied among the different *Astyanax* species. These results highlight the ability of the species to differentiate their diets despite the usual tendency of *Astyanax* populations to adopt a generalist diet.

The low values of food overlap may indicate trophic segregation among species (Corrêa *et al.*, 2011). This is an expected result for similar species since the niche theory (Hutchinson, 1957) predicts that coexistence among species depends on a single difference or limiting similarity in order to minimize competition. Under these conditions, resource partitioning becomes an important mechanism for fish species segregation (Zaret, Rand, 1971; Edlund, Magnhagem, 1980), which is apparently operating among the populations of *A. bifasciatus*, *A. gymnodontus*, *A. lacustris*, *A. dissimilis*, and *A. minor* in the Low Iguaçu River. The similarity found in the diet of the pair *A. minor* × *A. dissimilis* was the only counterpoint to this result, but it does not discard ecological separation between these species since we investigated only one niche dimension. Usually congeneric species inhabiting the same area avoid competition through three possible ecological differences: they exploit different habitats or microhabitats, they consume different foods, or they are active at different times (Pianka, 2000). Another point to consider is that the similarity in the diet was mainly influenced by the high consumption of the alga *Compsopogon caeruleus*. Although the availability of food resources was not measured in the sampled sites, there must be high algal abundance in the sites owing to the rocky consistency of most of the sampled lotic stretches of the Iguaçu River and its tributaries, which may provide substrates for algal colonization, thus, reducing food limitations for *A. minor* × *A. dissimilis*.

Despite the low values, the overlap of terrestrial plant, fruit/seed and *Compsopogon caeruleus* reinforced the importance of these food items in maintaining the studied *Astyanax* populations. This information gains relevance considering the landscape threats over the Iguaçu basin (Baumgartner *et al.*, 2012; Kliemann *et al.*, 2018), especially by dam construction and removal or degradation of the riparian vegetation. The loss of habitat heterogeneity in the conversion of lotic environments to reservoirs (Agostinho *et al.*, 2007) and the limitation of allochthonous resources can be decisive for the interactions among species, with increasing competition and possible displacement of species with low fitness.

In conclusion, the wide range of food resources consumed by the evaluated *Astyanax* species demonstrates the potential of these species to exploit all environmental compartments. However, differences in the proportions and main resources consumed by each species indicate that trophic segregation promotes the co-occurrence of *Astyanax* species in the Low Iguaçu River.

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