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Isotopic variation in five species of stream fishes under the influence of different land uses

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The aim of this study was to test if changes in land use alter the isotopic signature of fish species, promoting changes in the trophic position and food resource partitioning between these consumers. Three different systems were investigated: pasture streams ($n = 3$), streams in sugar cane plantations ($n = 3$) and reference streams ($n = 3$). Fish species *Aspidoras fuscoguttatus*, *Astyanax altiparanae*, *Characidium zebra*, *Hisonotus piracanjuba* and *Knodus moenkhausii* were selected, and their nitrogen and carbon isotopic compositions were estimated to assess changes in the trophic level and partitioning of food items consumed. The composition of δ^{13}C (‰) only differed among the land use categories for *A. altiparanae*, *H. piracanjuba* and *K. moenkhausii.* Resource partitioning was different for all species, with changes in the sources or proportions they consumed in each land use category, but only *A. altiparanae* introduced new food sources in large quantity in altered land uses. It is important to note, however, that the results from the resource partitioning analysis are limited due to large overlapping of isotopic signatures between the analysed food resources. All fish species exhibited variation in δ^{15}N (‰), with the highest values found in streams under sugar cane or pasture influence. Despite the variation in nitrogen isotopic values, only *C. zebra* and *H. piracanjuba* displayed changes in trophic level. Therefore, it is believed that the increase in the δ^{15}N (‰) value of the individuals collected in streams under the influence of sugar cane or pasture was due to the greater influence of livestock dung and chemical and organic fertilizers. The results also highlight the importance of studying consumer species along with all forms of resources available at each location separately, because the signatures of these resources also vary within different land uses.

Key words: carbon; food resources; nitrogen; stable isotopes; trophic position; trophic webs.

INTRODUCTION

Aquatic environments are altered by a variety of external factors, including those of natural origin, such as floods and droughts, and also by anthropogenic ones, such as

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the construction of dams, urbanization, monoculture and pasture systems (Malmqvist & Rundle, 2002; Pompeu *et al.*, 2005; Cunico *et al.*, 2006; Barletta *et al.*, 2010). In the Cerrado, which is the second major Brazilian biome, the main threats to biodiversity are related to two economic activities: intensive grain monoculture and extensive cattle ranching (Diniz-Filho *et al.*, 2009). These two activities have great potential for altering aquatic communities because they may trigger a series of effects that adversely affect aquatic ecosystems.

The replacement of native vegetation by pastures and monoculture systems has resulted in the reduction or even removal of riparian vegetation, which can directly affect the flow of carbon in aquatic environments (Silva *et al.*, 2007). This substitution can also significantly alter the incidence of solar energy and the exchange of organic and inorganic material between aquatic and terrestrial ecosystems (Pusey & Arthington, 2003). Changes in riparian vegetation cover interfere with nutrient supply, allochthonous material, autochthonous production and the quality and quantity of available food resources, which may alter the trophic webs of affected environments and have an effect on aquatic biodiversity (Pusey & Arthington, 2003; Thomas *et al.*, 2004; Meynendonckx *et al.*, 2006; Ferreira *et al.*, 2012). Thus, one way to observe the effects of anthropogenic changes on aquatic communities is to assess how fish respond to changes in the sources of available resources.

Tropical freshwater fishes exploit food resources in a variety of ways, and many species can change their diet opportunistically in response to the relative abundance of food (Peterson & Winemiller, 1997; Abelha *et al.*, 2001). This ability to take advantage of the food resources that are more abundant at any given time is characterized as trophic adaptability, and these changes in resource use can be temporary or permanent depending on the circumstances (Gerking, 1994). The capacity to exploit available resources will also vary according to the strategies adopted by each species, including generalist species (with no strong preference for a food resource, using a variety of resources), specialists (with a diet restricted to a relatively small number of items and usually presenting remarkable morphological adaptations) and opportunists (that feed on a resource that is not common to their diet or make use of an abundant and unusual food source) (Gerking, 1994).

Another important factor is that the ability to change the sources consumed can result in changes in the trophic positions occupied by fishes. Most tropical species of fish alter their trophic position during ontogeny, and in the same populations, individuals may have food preferences or make use of different dietary tactics (Abelha *et al.*, 2001). Thus, one way to observe the dynamics of aquatic communities is to estimate the positions occupied by stream fishes in trophic webs with different anthropogenic influences.

Studies investigating the diet and feeding ecology of fishes are conducted primarily through stomach contents analysis (Pompeu & Godinho, 2003; Maroneze *et al.*, 2011; Gandini *et al.*, 2012). New approaches, however, such as the use of stable isotopes, allow for the quantification of the carbon sources effectively entering into a system (Forsberg *et al.*, 1993; Benedito-Cecílio *et al.*, 2000; Ferreira *et al.*, 2012), in addition to determining the relative assimilation of some resources that are poorly quantified in the analysis of stomach contents, as is the case for detritus (Keough *et al.*, 1998). The use of this tool has yielded progress in studies of aquatic environments, especially in trophic ecology studies, and carbon (^{13}C) and nitrogen (^{15}N) isotopes are the most commonly used isotopes (Jepsen & Winemiller, 2002; Hoeinghaus *et al.*, 2007; Jardine,

2014). The transfer of the carbon isotope signature along the trophic web is conservative and can be used to trace the flow of energy in systems where there are several types of foods with different ^{13}C values (Jardine *et al.*, 2003; Manetta & Benedito-Cecílio, 2003). The ^{15}N isotope, in turn, is consistently fractionated throughout the trophic web, allowing inferences about the trophic relationships of consumers with their diet (Vander Zanden *et al.*, 1997; Post, 2002; Vanderklift & Ponsard, 2003). Therefore, these two isotopes are useful to trace the transfer of carbon and nitrogen from plants and detritus to primary and secondary consumers (Peterson & Fry, 1987; Ferreira *et al.*, 2012; Jardine, 2014).

Assuming that anthropogenic actions promote significant changes in the dynamics of energy flow in aquatic communities, the hypotheses that fishes exhibit different isotopic signatures of carbon and nitrogen in streams under influence of different land use were tested. Confirming this hypothesis, the aim becomes to assess whether some or all fish species display a similar trend and whether this could be accounted for by: (1) shift in the isotope ratios of resource or (2) utilization of a novel resource. The possible variation in the trophic level of each species between streams under the influence of different land uses was assessed. The isotopic composition of carbon and nitrogen of five fish species and the available food sources present in streams with different degrees of anthropogenic influence (streams with natural cover, streams under the influence of sugar cane cultivation and streams located in pastures) were used to detect possible changes in the consumption of food sources and to determine the trophic position of the consumers studied.

MATERIALS AND METHODS

STUDY AREA AND LAND COVER CLASSIFICATION

This study was carried out in tributary streams of the São Simão reservoir, located in the sub-basin of the Paranaíba River, south-eastern Brazil. The Paranaíba River basin is the second largest hydrographic unit of the Paraná Basin, encompassing 25·4% of its area, which corresponds to a drainage area of 222·8 km^2, covering parts of the states of Goiás (65%), Minas Gerais (30%), Federal District (3%) and Mato Grosso do Sul (2%). Most parts of the Paranaíba River basin are located in Cerrado, the second largest Brazilian biome, but much of this area has been deforested as a result of anthropogenic activities. The hydrological regime of the rivers in this basin is governed by two seasons: rainy from October to March and with episodic rainfall in the remaining months of the year (CBH-Paranaíba, 2012).

Nine second and third-order streams (located in the states of Goiás and Minas Gerais) were selected from 110 previously visited tributary streams of the São Simão reservoir (Fig. 1). The 110 sampling points were chosen according to the methodology proposed by Olsen & Peck (2008), in which points are defined by a spatially balanced and ranked selection algorithm. The nine streams were selected according to the different types of land use in which they were located, with three located in pastures and three in sugar cane cultivation areas. Three streams defined as reference streams were selected as controls for having representative riparian vegetation and good water quality (Brazilian Water Quality Resolution, CONAMA 357/2005, Brazilian National Environmental Council). The anthropogenic stream characteristics included the total absence (streams located in pastures) or low presence (streams with influence from sugar cane cultivations) of riparian vegetation.

The land use surrounding the sampled streams was evaluated according the oriented mapping method described in Lima *et al.* (2010), in eight multi-spectral RapidEye images (https://apollomapping.com) between September and October 2011, with five spectral bands. The percentage of natural vegetation, pastures and plantations of sugar cane was determined for

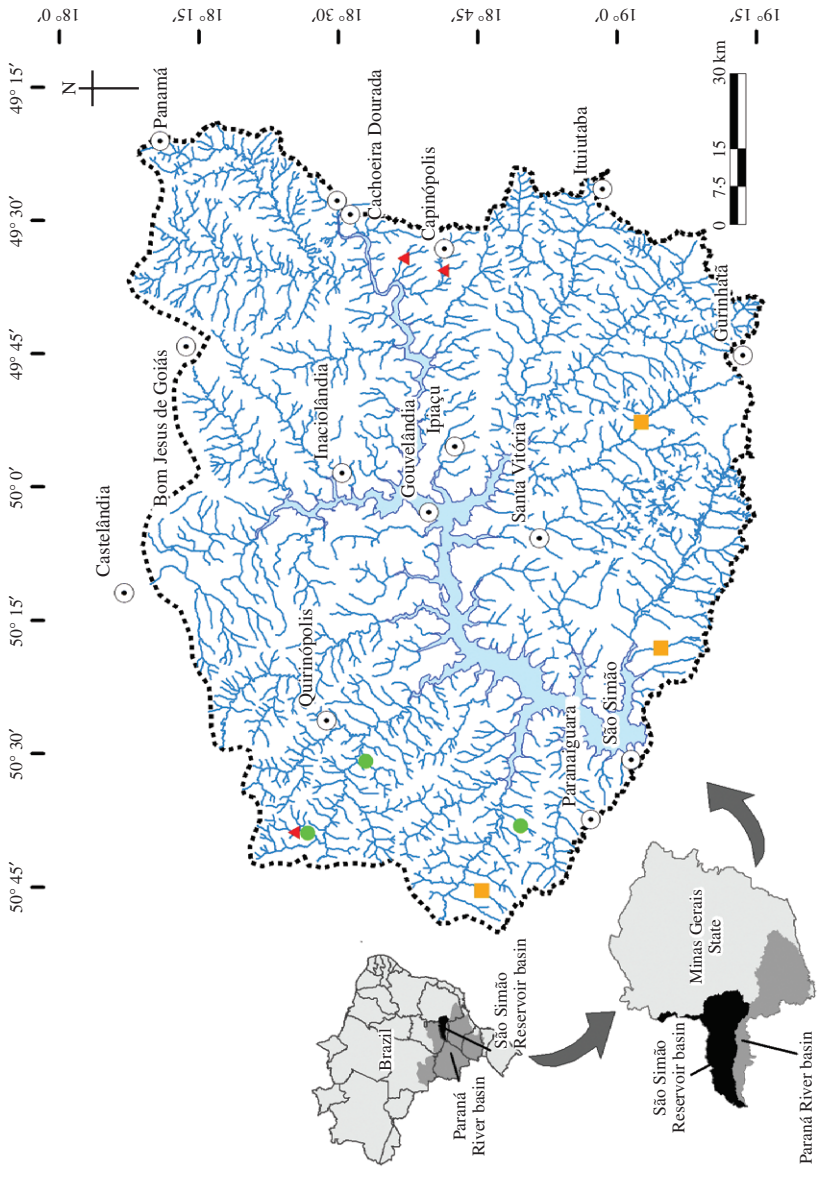

FIG. 1. Locations of the nine tributary streams of the São Simão reservoir in the Paranaíba River basin where sampling was performed in September 2012 (⁂, study area ; 🗺, reservoir; ∼, drainage; ⊙, cities; ■, pasture; ▲, sugar cane; ●, natural cover. Geographic Projection Datum WGS-84. Cartographic Data: IBGE, 1979, 2000 Geominas, 2004; ANA, 2008.).

the nine streams accordingly, in a 150 m radius buffer, around the upstream limit of the sampled stretch (Table I). Ortho-rectified and atmospherically corrected images were obtained through a partnership between the Federal University of Lavras (UFLA) and the Ministry of Environment (MMA). Acquisition errors, clouds and shadows were removed in the pre-processing phase (Coppin *et al.*, 2004), which also included visual evaluation of image registration. To validate the results of the classification, an array of errors was generated, measuring the global and kappa accuracy (Shanmugam *et al.*, 2006). Real field data collected in September 2012 were used to verify the accuracy of mapping. The mapping results in a high kappa and global accuracy with values of 96 and 98%.

The length of the section sampled in each stream was proportional to its width, being defined as 40 times the mean width of the stream and encompassing a minimum of 150 m of sampling. Each stream was sampled (fishes and resources) and evaluated only once in the dry season, in September 2012. To illustrate the variation in the physical habitat of streams with different anthropogenic influences, environmental variables were quantified according to the protocols proposed by Lazorchak *et al.* (1998), and the metrics were calculated according to Kaufmann *et al.* (1999). In this study, the percentages of fine substrata, vegetation cover, rapid flow, algae, aquatic plants (macrophytes) and leaf banks were evaluated. These variables were assessed because they consistently reflected the effects of different land uses on the physical habitat of streams, especially with regard to the availability of resources (Table I).

COLLECTION AND PROCESSING OF FISHES

Fish collection was performed in the downstream–upstream direction with nets made with insect screen (80 cm in diameter, 1 mm mesh) and trawls (3 m long, 5 mm mesh). Each stream was subdivided into 10 sections for the collection of ichthyofauna, with a sampling time of 12 min per section, totalling 2 h of collection per stream. The collected samples were immediately killed and stored on ice for further processing in the laboratory and subsequent analysis of the isotopic composition. In the laboratory, the collected organisms were taxonomically identified with the aid of identification keys of Paraná Basin fishes. Adults individuals of the species *Aspidoras fuscoguttatus* Nijssen & Isbrücker 1976, *Astyanax altiparanae* Garutti & Britski 2000, *Characidium zebra* Eigenmann 1909, *Hisonotus piracanjuba* Martins & Langeani 2012 and *Knodus moenkhausii* (Eigenmann & Kennedy 1903) were selected. The factors that determined the choice of these five species are: (1) presence and abundance in all of the streams under the influence of the different land uses, (2) similar ontogenetic stage, (3) absence of migratory behaviour, (4) representation in different trophic guilds and (4) feeding in different positions in the water column (Table II). For larger specimens (>5 cm total length, L_T), such as *A. altiparanae* and *C. zebra*, a part of the muscle was removed for isotopic analysis. Smaller fishes (<5 cm), such as *A. fuscoguttatus*, *H. piracanjuba* and *K. moenkhausii*, were analysed whole, and only the digestive tract was removed. There was no change in the methodology used in the processing of each species. Fish samples were lyophilized for 24 h and ground to a fine and homogeneous powder using a mortar and pestle and stored in Eppendorf tubes. For the isotopic analysis, *c.* 2–5 mg of dry animal tissue was used.

The species were deposited in the Ichthyological Collection of the UFLA (CI-UFLA), with the following catalogue numbers: *A. fuscoguttatus* (CI-UFLA 0806), *A. altiparanae* (CI-UFLA 0807), *C. zebra* (CI-UFLA 0815), *H. piracanjuba* (CI-UFLA 0823) and *K. moenkhausii* (CI-UFLA 0839).

COLLECTION AND PROCESSING OF FOOD RESOURCES

Samples of periphyton, filamentous algae, coarse particulate organic matter (CPOM), vegetation (grasses and sugar cane) and benthic macroinvertebrates were collected. Sampling of resources was carried out in parallel to fish collecting, standardizing the collection of a minimum five samples of each resource per stream sampled. The selection of these resources was based on best-practice guidelines proposed by Phillips *et al.* (2014) and the food preferences and feeding guilds of the five species were taken into account. This information was obtained through studies (documented in the literature) that used analyses of stomach contents (Table II).

TABLE I. Physical characteristics, land use and environmental variables of the nine tributary streams of the São Simão reservoir, Brazil. The numbers 1, 2 and 3 correspond to each of the three streams sampled in each land use category (see Fig. 1).

	Geographic co-ordinates			Characteristics of streams				Land use				Environmental variables					
Streams	S	W	Alt. (m)	Order[a]	Mean depth (m)	Mean width (m)	Mean area (m^2)	Natural cover (%)	Pasture (%)	Cane (%)	Others (%)	Fine sub. (%)	Veg. cover (%)	Rapid flow (%)	Aquatic plant (%)	Leaf banks (%)	Algae (%)
Natural cover																	
1	18° 47′ 19·49″	50° 39′ 40·86″	482	2	0·20	3·42	0·68	53·14	0	46·86	0	<0·01	0·94	0·69	<0·01	0·17	<0·01
2	18° 30′ 56·82″	50° 31′ 51·62″	434	3	0·19	7·29	1·36	39·13	57·87	0	3	<0·01	0·47	0·46	<0·01	0·14	<0·01
3	18° 24′ 20·08″	50° 39′ 49·89″	509	3	0·19	7·35	1·43	50·86	49·14	0	0	<0·01	0·9	0·51	<0·01	0·18	<0·01
Sugar cane																	
1	18° 22′ 51·60″	50° 39′ 42·74″	516	2	0·23	3·98	0·93	14·05	36·56	45·81	3·59	0·01	0·73	0·38	<0·01	0·11	<0·01
2	18° 36′ 28·65″	49° 35′ 09·14″	441	3	0·19	1·85	0·35	14·63	0	85·37	0	<0·00	0·99	0·17	0·04	0·16	<0·01
3	18° 40′ 41·51″	49° 36′ 46·50″	463	2	0·27	1·27	0·35	0	43·39	56·61	0	0·03	1	0·67	0·02	0·07	<0·01
Pasture																	
1	19° 03′ 03·40″	50° 20′ 06·81″	425	3	0·64	3·49	2·25	8·57	62·35	0	29·08	0·3	0·33	0·43	0·66	0·02	0·22
2	19° 01′ 38·55″	49° 54′ 32·40″	461	3	0·13	2·00	0·25	0	97·69	0	2·31	0·21	0·01	0·45	0·05	<0·01	0·14
3	18° 42′ 59·17″	50° 46′ 52·77″	479	2	0·17	4·80	0·82	16·41	65·35	0	18·24	0·02	0·38	0·15	0·02	0·02	<0·01

Alt., altitude; [a]rank of the streams orders according to Strahler (1957); sub., substratum; Veg. cover, vegetation cover. All environmental variables were calculated according to Kaufmann *et al.* (1999) and indicate the percentage in which they occur in each assessed stream.

TABLE II. Feeding habits of five studied species. Information drawn from studies using analyses of stomach contents. In some cases, the information known for the genus was used (few details about the species)

Species	Feeding guilds	Feeding preference	Feeding tactics	References
Aspidoras fuscoguttatus	Invertivore	Autochthonous items (mainly larvae of aquatic insects)	Grubbers (hunting especulation)	Casatti *et al.* (2009)
Astyanax altiparanae	Invertivore herbivory omnivore	Allochthonous items associated with riparian vegetation	Picking items in the water column	Casatti (2002), Cassemiro *et al.* (2002)
Characidium zebra	Invertivore	Mainly immature forms of aquatic insects	Stalking predator	Casatti (2002), Cetra *et al.* (2011), Silva *et al.* (2012)
Hisonotus piracanjuba	Periphitivore detritivore	Mainly diatoms, Chlorophyta and organic matter	Grazers	Casatti (2002), Silva *et al.* (2012)
Knodus moenkhausii	Invertivore	Autochthonous (algae, nymphs and larvae of macroinvertebrates) and allochthonous items (fragments of terrestrial insects)	Drift feeding, picking at relatively small prey, surface picking	Ceneviva-Bastos & Casatti (2007)

Periphyton was collected by scraping rocks and storing the scrapings with distilled water in a plastic container. After collection, the samples were stored on ice until transfer to a freezer where they were immediately frozen for preservation. In the laboratory, samples were filtered using a filtration apparatus attached to a vacuum pump using calcined 45 μ Millipore (http://www.merckmillipore.com) glass fibre filters. Filamentous algae were manually collected, stored in plastic containers and stored on ice. For the collection of vegetation (grasses and sugar cane) and CPOM, the streams were divided into 10 sections (like the methodology for collecting fishes) and the collection of these resources was made in interspersed sections. The samples were packed in paper bags and kept in plant presses until further processing in the laboratory. Benthic macroinvertebrates were collected through qualitative collection using kick-nets (0·5 mm mesh size) along the streams.

In the laboratory, all samples were kept in a 60° C oven for a period of 48 h, then they were ground to a fine and homogeneous powder using a mortar and pestle, and stored in Eppendorf tubes. For the isotopic analysis, the amount for food resources required was *c*. 5–10 mg.

ISOTOPIC ANALYSIS

After preparation of the material, the samples were sent to the Isotopic Ecology Laboratory, Centre for Nuclear Energy in Agriculture (CENA) at the University of São Paulo (USP), in the city of Piracicaba, for isotopic analysis. Samples of biological material were analysed for their isotope ratios (^{13}C:^{12}C and ^{15}N:^{14}N) in addition to their total carbon and nitrogen contents. To determine the isotope ratio, a mass spectrometer system in the continuous-flow mode (CF-IRMS) was used with a Carlo Erba elemental analyser (CHN 1110) coupled to a Delta Plus mass spectrometer (Thermo Scientific; www.thermoscientific.com). The results were expressed as the difference of international reference standards, in the delta notation (*δ*‰), and calculated using the following formula: $\delta X = [(R_{\text{sample}} R_{\text{standard}}{}^{-1}) - 1]\ 10^3$, where X is ^{13}C or ^{15}N and R represents the isotopic ratio ^{13}C:^{12}C or ^{15}N:^{14}N (Barrie & Prosser, 1996).

DATA ANALYSIS

A non-parametric multivariate analysis of variance (NPMANOVA), a non-parametric test of significant difference between two or more groups based on any distance measure (Anderson, 2001), was performed to check changes in the carbon (^{13}C) and nitrogen (^{15}N) signatures of each of the five fish species among the different land uses. The same procedure was used to test whether there was variation in the isotopic signatures of the resources present in all types of land uses. These analyses were performed in the PAleontological STatistics (PAST) software (http://folk.uio.no/ohammer/past/) and the pair-wise NPMANOVAs between all pairs of groups are provided as a *post hoc* test.

The isotopic values of nitrogen alone cannot be used to represent the trophic position of consumers because the $\delta^{15}N$ of primary producers (which convert inorganic N into organic N) is highly variable within and between systems over time (Vander Zanden *et al.*, 1997). Therefore, to estimate the trophic position (T_P) of fishes, the method proposed by Vander Zanden *et al.* (1997) was used: $T_{\text{Pfish}} = [(\delta^{15}N_{\text{fish}} - \delta^{15}N_{\text{resources}}) \div 3 \cdot 0] + 1$, where $\delta^{15}N_{\text{fish}} = \delta^{15}N$ values of fish, $\delta^{15}N_{\text{resources}}$ = mean values of $\delta^{15}N$ of basal resources (macroinvertebrates were not considered), 3·0 represents the fractioning per trophic level and 1 is the position of producers within the food chain. The trophic position was calculated for each individual of the five species studied, and for this calculation, the $\delta^{15}N$ values of the basal resources of each of the nine streams were considered separately. To determine whether there was variation in the trophic levels occupied by the species in the different land use categories, NPMANOVA was performed, followed by a *post hoc* test.

The Bayesian mixing model stable-isotope analysis in R (SIAR) developed by Parnell *et al.* (2010) was used to determine the relative contribution of each carbon source to the diet of the fish. Besides being able to work with underdetermined systems (number of food sources is greater than the number of isotopic signatures), SIAR also accounts for uncertainties associated with sample variability and trophic enrichment. *Aspidoras fuscoguttatus*, *A. altiparanae*, *C. zebra*, *H. piracanjuba* and *K. moenkhausii* were considered consumers, and periphyton,

filamentous algae, CPOM, vegetation (sugar cane and grasses of pasture) and benthic macroinvertebrates were considered food resources.

In this study, not all streams had the same carbon sources. Grasses were present only in pastures and sugar cane was present only in streams under that land use. All other resources were common to all land use categories. Thus, the resources present in each land use category were considered separately in all analyses performed in this work. The fractionation values used were 1‰ for carbon (Oliveira *et al.*, 2006) and 3‰ for nitrogen (Ferreira *et al.*, 2012). The assumed significance level was $P < 0{\cdot}05$.

RESULTS

A total of 144 samples of consumers were analysed, comprising *A. fuscoguttatus* (29), *A. altiparanae* (21), *C. zebra* (27), *H. piracanjuba* (17) and *K. moenkhausii* (50). Additionally, 388 samples of food resources were analysed. Most resources showed variations in the isotopic signatures of carbon and nitrogen among the stream categories (Table III). It was also possible to observe a large overlap of isotopic signatures, especially between algae and CPOM and between benthic macroinvertebrates and periphyton in reference streams and in streams under influence of sugar cane (Fig. 2).

All fish species exhibited variation in δ^{15}N mean values, with the highest values found in streams under the influence of pasture or sugar cane cultivation. In contrast, the carbon signature only varied in *A. altiparanae*, *H. piracanjuba* and *K. moenkhausii*, suggesting that these species fed on different food resources under the influence of each type of land use (Table III and Fig. 2).

For *A. fuscoguttatus*, δ^{15}N values differed between reference streams and the other streams, and the highest values were found in streams under the influence of sugar cane cultivation. The carbon signature of *A. fuscoguttatus* did not vary among the land use categories (Table III and Fig. 2). The δ^{15}N values of *A. altiparanae* differed between streams under the influence of pasture and reference streams, with higher values observed in streams located in pastures. Differences were also observed in the carbon signature of *A. altiparanae*, which also differed between streams under the influence of pasture and reference streams (Table III and Fig. 2).

The carbon isotopic signature of *C. zebra* did not change, but its nitrogen signature varied, differing between streams under the influence of sugar cane and those in pastures, being higher in streams located in pastures (Table III and Fig. 2). The same pattern was observed for *H. piracanjuba*, whose nitrogen signature also differed between streams with influences of sugar cane and pasture, being higher in streams located in pastures. For this species, there was a slight variation in the carbon isotopic signature (Table III and Fig. 2). *Knodus moenkhausii* exhibited the greatest variation in both δ^{15}N and δ^{13}C. Nitrogen isotopic signatures were lower in reference streams compared with the others, whereas carbon values differed within streams under the influence of pastures (Table III and Fig. 2).

Even though all species exhibited differences in δ^{15}N values according to the trophic level determination model, only *C. zebra* and *H. piracanjuba* showed variation in their trophic position among the stream categories, with individuals of these species occupying higher trophic levels in reference streams and lower levels in pasture and sugar cane streams, respectively. The other species occupied the same trophic levels in all stream categories (Table IV). Regarding the proportion of resources consumed in each stream category, all species exhibited variation (however small) in the items

TABLE III. Mean ± S.D. isotopic signatures of resources and fishes sampled in the three land use categories. The letters a, b and c indicate which signatures are different according to *post hoc* test

	δ^{15}N (‰)				δ^{13}C (‰)			
	Natural cover	Sugar cane	Pasture	*P*	Natural cover	Sugar cane	Pasture	*P*
Algae	6·21 ± 2·43 (a)	4·52 ± 1·56 (a)	6·32 ± 1·98 (a)	>0·05	−29·96 ± 5·44 (a)	−30·10 ± 2·83 (a)	−32·76 ± 5·81 (a)	>0·05
CPOM	0·68 ± 0·56 (a)	4·13 ± 2·46 (b)	2·19 ± 1·03 (b)	<0·001	−30·15 ± 0·55 (a)	−30·08 ± 0·62 (a)	−30·17 ± 0·46 (a)	>0·05
Benthic	5·90 ± 1·70 (a)	7·99 ± 2·16 (b)	7·14 ± 1·53 (b)	<0·001	−27·28 ± 1·63 (a)	−25·71 ± 1·72 (b)	−28·20 ± 5·41 (a)	<0·001
Periphyton	4·60 ± 0·85 (a)	6·71 ± 1·94 (b)	6·15 ± 0·77 (b)	<0·001	−27·16 ± 1·93 (a)	−25·84 ± 0·97 (a)	−25·86 ± 4·71 (a)	>0·05
Sugar cane	–	4·46 ± 0·75	–		–	−13·11 ± 0·25	–	
Pasture (grasses)	–	–	4·90 ± 2·74		–	–	−16·83 ± 3·55	
Aspidoras fuscoguttatus	9·88 ± 1·31 (a)	11·59 ± 1·06 (b)	11·10 ± 0·59 (b)	<0·01	−28·19 ± 2·15 (a)	−25·28 ± 1·94 (a)	−27·18 ± 4·20 (a)	>0·05
Astyanax altiparanae	7·73 ± 0·61 (a)	8·96 ± 1·43 (ab)	9·18 ± 1·24 (b)	<0·05	−22·53 ± 1·49 (a)	−21·19 ± 0·86 (ab)	−19·97 ± 1·45 (b)	<0·01
Characidium zebra	10·28 ± 0·63 (ab)	9·86 ± 0·27 (a)	10·80 ± 0·62 (b)	<0·01	−26·36 ± 0·92 (a)	−26·32 ± 0·95 (a)	−26·32 ± 0·79 (a)	>0·05
Hisonotus piracanjuba	10·47 ± 0·53 (ab)	10·22 ± 0·28 (a)	11·12 ± 0·62 (b)	<0·05	−29·83 ± 0·86 (a)	−26·83 ± 0·62 (b)	−29·56 ± 2·66 (a)	<0·05
Knodus moenkhausii	8·58 ± 1·01 (a)	9·88 ± 0·94 (b)	10·51 ± 0·93 (b)	<0·001	−24·62 ± 1·19 (a)	−23·37 ± 0·91 (b)	−29·95 ± 2·73 (c)	<0·001

CPOM, coarse particulate organic matter.

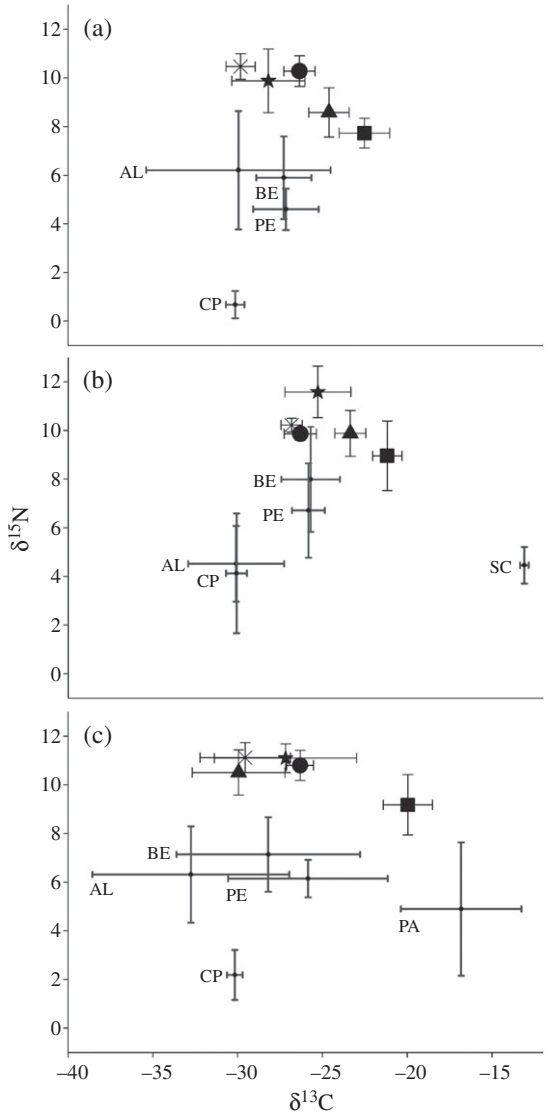

FIG. 2. Representation of the δ^{15}N and δ^{13}C variations in each species and resources collected in different stream categories: (a) natural cover, (b) sugar cane and (c) pasture. Values are means ± S.D. Fish species: *Aspidoras fuscoguttatus* (★), *Astyanax altiparanae* (■), *Characidium zebra* (●), *Hisonotus piracanjuba* (▲) and *Knodus moenkhausii* (×). Resources: algae (AL), benthic macroinvertebrates (BE), periphyton (PE), coarse particulate organic matter (CP), sugar cane (SC) and pasture (PA).

assimilated. *Aspidoras fuscoguttatus* consistently consumed algae, benthic macroinvertebrates and periphyton varying only in their proportions (Table V and Fig. 3).

The species *A. altiparanae* consumed especially periphyton and benthic macroinvertebrates in all stream categories, although the relative importance of each item cannot be established due to the overlap between their signatures. Its most striking characteristic was the incorporation in large proportions of carbon derived from sugar

TABLE IV. Estimated trophic positions for each species in each stream category according to the method proposed by Vander Zanden *et al.* (1997). Values in bold correspond to trophic levels that varied among stream categories according to NPMANOVA analyses ($P < 0{\cdot}05$). The letters a, b and c indicate which signatures are different according to *post hoc* test. The calculation of the mean ± S.D. δ^{15}N resources was made using the basal resources: algae, coarse particulate organic matter and periphyton (macroinvertebrates were not considered)

Stream categories	δ^{15}N fishes (‰)	δ^{15}N resources (‰)	Trophic level	*P*
Aspidoras fuscoguttatus				>0·05
Natural cover	9·88 ± 1·31	3·53 ± 2·67	3·12 ± 0·44	(a)
Sugar cane	11·59 ± 1·06	5·10 ± 2·19	3·16 ± 0·35	(a)
Pasture	11·10 ± 0·59	5·14 ± 2·33	2·99 ± 0·20	(a)
Astyanax altiparanae				>0·05
Natural cover	7·73 ± 0·61	3·53 ± 2·67	2·40 ± 0·20	(a)
Sugar cane	8·96 ± 1·43	5·10 ± 2·19	2·29 ± 0·48	(a)
Pasture	9·18 ± 1·24	5·14 ± 2·33	2·35 ± 0·41	(a)
Characidium zebra				<0·001
Natural cover	10·28 ± 0·63	3·53 ± 2·67	**3·25 ± 0·21**	**(a)**
Sugar cane	9·86 ± 0·27	5·10 ± 2·19	**2·58 ± 0·09**	**(b)**
Pasture	10·80 ± 0·62	5·14 ± 2·33	**2·89 ± 0·21**	**(c)**
Hisonotus piracanjuba				<0·001
Natural cover	10·47 ± 0·53	3·53 ± 2·67	**3·31 ± 0·18**	**(a)**
Sugar cane	10·22 ± 0·28	5·10 ± 2·19	**2·70 ± 0·09**	**(b)**
Pasture	11·12 ± 0·62	5·14 ± 2·33	**3·00 ± 0·21**	**(c)**
Knodus moenkhausii				>0·05
Natural cover	8·59 ± 1·00	3·53 ± 2·67	2·69 ± 0·33	(a)
Sugar cane	9·88 ± 0·94	5·10 ± 2·19	2·59 ± 0·31	(a)
Pasture	10·51 ± 0·93	5·14 ± 2·33	2·79 ± 0·31	(a)

cane and grasses in streams under the influence of sugar cane cultivation and pastures, respectively (Table V and Fig. 3). Periphyton and benthic macroinvertebrates were also the most important resources for *C. zebra* in all streams (Table V and Fig. 3).

Hisonotus piracanjuba incorporated carbon from algae, benthic macroinvertebrates and periphyton in reference streams and streams under the influence of pasture (Table V and Fig. 3). *Knodus moenkhausii* exhibited large variations in the proportion of food resources assimilated in the three stream categories. In reference streams and streams under the influence of sugar cane, the signature was based on benthic macroinvertebrates and periphyton, and in streams under the influence of sugar cane also there was incorporation of vegetation derived from sugar cane. In streams under a pasture influence, algae were incorporated in large amounts for this species, with a significant reduction in the assimilation of periphyton (Table V and Fig. 3).

DISCUSSION

The interest in studying the feeding ecology of fishes is primarily related to the role of species in the trophic ecology of aquatic ecosystems (Braga *et al.*, 2012). By supporting the hypotheses that land use alters the carbon and nitrogen signatures of the

TABLE V. Stable Isotope Analysis in R (SIAR) results of the food source proportions in the diet of the five species of fish showing the calculated monthly distribution range from low 95% to high 95% and the mean

Category	Resources	*Aspidoras fuscoguttatus* Mean (±95% range)	*Astyanax altiparanae* Mean (±95% range)	*Characidium zebra* Mean (±95% range)	*Hisonotus piracanjuba* Mean (±95% range)	*Hisonotus piracanjuba* Mean (±95% range)
Natural cover	Algae	0·38 (0·15–0·63)	0·16 (0·00–0·33)	0·13 (0·00–0·31)	0·38 (0·09–0·66)	0·10 (0·00–0·28)
	Benthic	0·33 (0·02–0·60)	0·30 (0·04–0·51)	0·57 (0·27–0·89)	0·27 (0·00–0·51)	0·52 (0·26–0·80)
	Periphyton	0·19 (0·00–0·42)	0·41 (0·10–0·77)	0·25 (0·00–0·50)	0·19 (0·00–0·42)	0·36 (0·09–0·61)
	CPOM	0·09 (0·00–0·27)	0·14 (0·01–0·27)	0·04 (0·00–0·13)	0·16 (0·00–0·42)	0·03 (0·00–0·07)
Sugar cane	Algae	0·11 (0·00–0·29)	0·14 (0·00–0·31)	0·21 (0·02–0·38)	0·21 (0·01–0·39)	0·08 (0·00–0·18)
	Benthic	0·52 (0·19–0·85)	0·21 (0·00–0·40)	0·32 (0·13–0·52)	0·28 (0·03–0·51)	0·40 (0·21–0·58)
	Periphyton	0·23 (0·00–0·47)	0·20 (0·00–0·39)	0·25 (0·02–0·46)	0·23 (0·01–0·44)	0·29 (0·03–0·52)
	CPOM	0·10 (0·00–0·27)	0·13 (0·00–0·31)	0·18 (0·00–0·35)	0·25 (0·00–0·46)	0·07 (0·00–0·17)
	Sugar cane	0·05 (0·00–0·13)	0·32 (0·17–0·46)	0·03 (0·00–0·08)	0·03 (0·00–0·08)	0·16 (0·11–0·21)
Pasture	Algae	0·24 (0·00–0·46)	0·07 (0·00–0·18)	0·22 (0·04–0·39)	0·34 (0·08–0·58)	0·41 (0·17–0·64)
	Benthic	0·43 (0·10–0·82)	0·15 (0·00–0·34)	0·28 (0·04–0·50)	0·33 (0·02–0·60)	0·43 (0·16–0·73)
	Periphyton	0·20 (0·00–0·44)	0·20 (0·00–0·40)	0·23 (0·02–0·43)	0·18 (0·00–0·38)	0·10 (0·00–0·26)
	CPOM	0·05 (0·00–0·14)	0·05 (0·00–0·14)	0·12 (0·00–0·31)	0·10 (0·00–0·28)	0·03 (0·00–0·09)
	Pasture	0·08 (0·00–0·21)	0·52 (0·35–0·69)	0·15 (0·01–0·27)	0·06 (0·00–0·16)	0·03 (0·00–0·08)

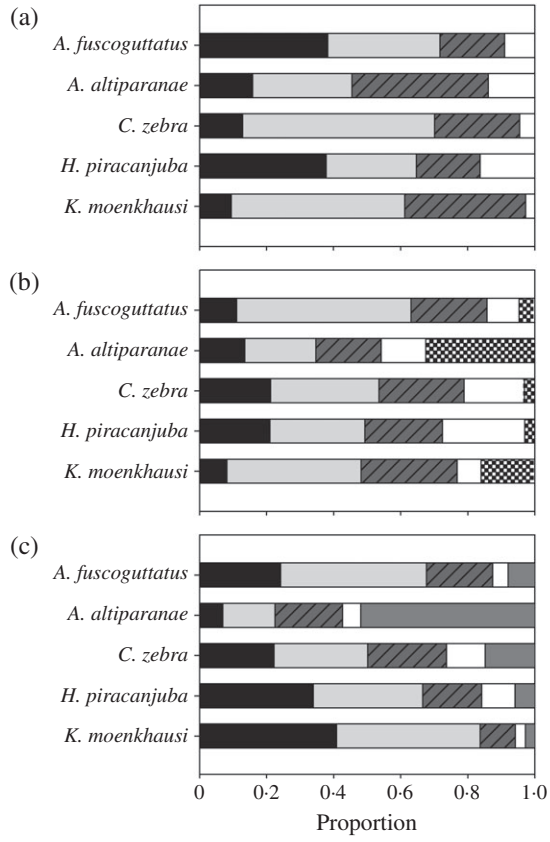

FIG. 3. Stable isotope analysis in R (SIAR) output results. Mean of the proportion of food resources used by each species: *Aspidoras fuscoguttatus*, *Astyanax altiparanae*, *Characidium zebra*, *Hisonotus piracanjuba* and *Knodus moenkhausii* in each land use category: (a) natural cover, (b) sugar cane and (c) pasture. Resources: algae (■), benthic macroinvertebrates (▭), periphyton (▨), coarse particulate organic matter (▭), sugar cane (▩) and pasture (■). See more results in Table V.

studied fish species, this study illustrated how changes in land use in the area around streams affect trophic dynamics in fish communities. The variation in isotopic signatures of consumers occurred primarily in response to variations in signatures of resources, particularly the isotopic composition of nitrogen, which was different in streams with anthropogenic influences for all the analysed species. The results highlight the importance of studying consumer species along with all forms of resources available at each location separately, because the signatures of these resources also vary with changes in land use (Cabana & Rasmussen, 1996). Although the majority of the species showed only variation in the proportions of assimilated resources among land uses, *A. altiparanae* introduced new food resources into the diet when in altered streams. It is important to note, however, that the results related to the resource partitioning analysis are limited due to large overlapping of isotopic signatures among the analysed food resources.

The fact that all fish species showed variation in their nitrogen signature (with higher values observed in streams under the influence of sugar cane cultivations or pastures)

did not translate into a large variation in the trophic levels occupied in each land use category (only the trophic level occupied by *C. zebra* and *H. piracanjuba* changed). These changes may have occurred due to the greater influence of chemical and organic fertilizers in these streams. The use of vinasse as a fertilizer in sugar cane plantations is a practice that has been used in Brazil since the mid-1980s as an alternative to the disposal of this waste, which was previously routed to surface springs (sacrifice areas) (Corazza, 2006). Vinasse is a by-product of the distillation of alcohol and can be used as an organic fertilizer in the production of sugar cane because it has high organic matter content, potassium and water, which are important for this crop (Lyra *et al.*, 2003). It is important to emphasize, however, that this practice can be damaging, as it may affect surface springs, soil and groundwater (Lyra *et al.*, 2003; Corazza, 2006). Pastures also release an excessive amount of waste into rivers and natural watercourses, and influence the input of nutrients in aquatic environments through livestock dung. This may lead to serious ecological imbalances, polluting rivers and springs due to the reduction of the dissolved oxygen content of the water and the excessive inputs of nutrients.

It is known that management practices and the chemical and organic fertilizers applied in these land use categories can modify the δ^{15}N of the soil available to plants, nitrate (NO_3^-), nitrite (NO_2^-) or ammonium ions (NH_4^+), and the waste products may occasionally be carried to waterways. Several studies have shown the effects of agriculture on the concentrations of nutrients in aquatic environments (Meynendonckx *et al.*, 2006; Riseng *et al.*, 2011; Broderius, 2013). Silva *et al.* (2007), for example, found that sugar cane cultivation is an important factor modifying the chemistry of small catchments by changing the flows of carbon, nitrogen and major ions. Therefore, a possible nutrient enrichment in streams under the influence of sugar cane cultivations and pastures may be the explanation for the higher nitrogen values observed in the resources and the consumers. This effect has been documented in several studies reporting how anthropogenic land use increases the heavy isotope concentration of nitrogen, δ^{15}N in basal resources (Broderius, 2013) and consumers (Harrington *et al.*, 1998).

All the studied fish species modified the proportions of different resources consumed, although the overlap of the signature among some resources hinders more in depth conclusions. Only *A. altiparanae* showed large variations in diet composition, incorporating new resources in large quantities in streams with anthropogenic influence. These changes, particularly the incorporation of new allochthonous items (sugar cane and grasses) into the food webs, are a result of changes in their surrounding land use. Additionally, there could be a restriction of some items that are more available in reference streams, such as natural riparian vegetation, resulting in a reduction in the use of coarse and particulate organic matter as resources. The generalist behaviour exhibited by these fish species was expected because these were the only fish species present in all stream categories. Importantly, the ability to modify diet can be an advantage because, in some cases, trophic adaptability is more important for survival than dependence on a specialized feeding habit (Gerking, 1994).

Despite the limitation of the results regarding the overlap of isotopic signatures, the partition of resources used by each species reinforces other studies that investigated their feeding preferences using stomach content analyses (Table II). According to Casatti *et al.* (2009), *A. fuscoguttatus*, although classified as invertivorous, has the ability to consume a variety of food items, predominantly autochthonous items, with chironomid larvae and other insects being the most consumed items. The same pattern

was observed in this study, with most of the carbon incorporated by *A. fuscoguttatus* originating from benthic macroinvertebrates in all stream categories.

The species *A. altiparanae* is considered by some authors as invertivorous (Casatti, 2002) and by others as herbivorous or omnivorous (Cassemiro *et al.*, 2002; Braga & Gomiero, 2003). According to Casatti (2002), this species has a preference for allochthonous organic matter and collects organic matter in the water column. It is interesting to note that the isotopic signatures of sugar cane and pasture (grasses) were also incorporated into the signature of *A. altiparanae* in streams under the influence of these types of land use. This fact suggests a more generalist than specialist behaviour, aside from showing obvious opportunism. Other studies have also indicated the trophic plasticity of this species, often observing a change in diet after some effect on the physical habitat of aquatic environments (Cassemiro *et al.*, 2002).

Importantly, the isotopic signature of vegetation varies in accordance with the decarboxylation processes employed, categorizing plants as C3, C4 or CAM (crassulacean acid metabolism; Manetta & Benedito-Cecílio, 2003). In preserved streams, *i.e.* those with representative riparian vegetation, most resources are derived from C3 sources. In the areas with sugar cane cultivation and pasture, plants are C4. Despite their disadvantages (because they are less nutritious and difficult to digest), recent studies have suggested that the contribution of C4 plants can be substantial for aquatic communities (Hoeinghaus *et al.*, 2007; Ferreira *et al.*, 2012). Of the species assessed here, *A. altiparanae* was the one that most utilized C4 sources as dietary items.

The great overlap between the signature of periphyton and benthic macroinvertebrates hinders the analysis of eventual changes of the diet of *C. zebra*. The species is considered a generalist insectivore, however (Silva *et al.*, 2012), with characteristic morphological attributes (Casatti & Castro, 2006). Some stomach content studies have confirmed this dietary preference for aquatic insects, especially immature stages of Trichoptera, Ephemeroptera, Plecoptera and Diptera (Silva *et al.*, 2012). EPTs (Ephemeroptera, Plecoptera and Trichoptera) are known for being sensitive to environmental disturbances (Callisto *et al.*, 2001; Bispo *et al.*, 2006). Thus, a possible reduction in abundance of benthic macroinvertebrates sensitive to environmental disturbances could eventually be a reason for the lower consumption of macroinvertebrates by *C. zebra* in streams under the influence of sugar cane and pasture, and the subsequent reduction in its trophic level.

Identifying the dietary items consumed by detritivorous species such as *H. piracanjuba* has always been a challenge to researchers because this information is more difficult to obtain by analysing stomach contents (Gerking, 1994). Through the analysis of stable isotopes, however, such information is more accessible. Because it is a detritivorous or periphytivorous species (Casatti, 2002; Silva *et al.*, 2012), it was expected that different food resources (animal and plant) would be incorporated into the isotopic signature of *H. piracanjuba*. This was exactly what was observed, with the main resources used being divided into algae, benthic macroinvertebrates, periphyton and, in some cases, CPOM.

For *K. moenkhausii*, also classified as invertivorous, benthic macroinvertebrates were also well represented in the isotopic signature in all categories of land use, but the sugar cane resource was also assimilated by this species in streams under the influence of sugar cane. In streams under the influence of pastures, with a higher incidence of sunlight, which consequently leads to an increase in primary production (Vannote *et al.*, 1980), the importance of algae as resource increased. According to Ceneviva-Bastos

& Casatti (2007), *K. moenkhausii* can be considered an opportunistic feeder due to its adoption of various feeding tactics and the high variety of consumed items (related to habitat and seasonal variations). Such opportunism can be reflected in the abundance of the species, which demonstrates a strong capacity to allocate a significant portion of its energy to reproduction, even in environments that are physically affected by anthropogenic activities (Ceneviva-Bastos & Casatti, 2007).

The findings of this study corroborate the idea that the analysis of δ^{15}N and δ^{13}C isotopes can serve as a biomonitoring tool to investigate the effects of changes caused by anthropogenic activities on aquatic communities. Although isotopic signatures vary with anthropogenic influence, it is important to note that this information is not sufficient to make inferences about variations in carbon flow or the trophic positions of species. Therefore, new methods must be adopted, *e.g.* estimation of the trophic position of consumers used in this study. Resource partitioning analysis can also be an interesting method to investigate trophic changes, but a detailed investigation of species-specific feeding habits is necessary considering the overlap of the isotopic signatures of the resources. The importance of studying both the resources and consumers together, given that resources also exhibit variation in carbon and nitrogen signatures in accordance with changes in land use, was evident. The absence of riparian vegetation was a determining factor in anthropogenically modified landscapes and in those under the pressure of activities such as cultivation and pasture, as it may have a strong influence on the transportation of fertilizers and pesticides to watercourses. Moreover, the fact that most species changed isotopic signature demonstrated the ability of different land uses to alter the trophic dynamics of aquatic communities.

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