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Isotopic profile and mercury concentration in fish of the lower portion of the rio Paraíba do Sul watershed, southeastern Brazil

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The aim of this study was to evaluate the isotopic profile and mercury (Hg) concentrations in fish (carnivores, omnivores and detritivores) in the lower portion of the rio Paraíba do Sul watershed, southeastern Brazil. Carbon ($\delta^{13}C$) isotopic analyses revealed that pelagic and benthonic sources are part of the feeding of the fish from the different guilds (-14.0 to -24.8 ‰). The benthic sources are usually enriched in $\delta^{13}C$ (-16.9‰, from watershed runoff) compared to pelagic sources because the phytoplankton, important primary producer that supports several pelagic chains, has $\delta^{13}C$ signature lighter (-23.9‰). The nitrogen ($\delta^{15}N$) isotopic signatures indicated that most guilds were at the same trophic position (10.0 to 15.5 ‰), except for pelagic omnivorous fish, which had a lower trophic position. Niche overlap was observed among pelagic and demersal carnivorous fish, demersal omnivorous fish, and demersal detritivorous fish. The lower isotopic niche breadth of pelagic carnivorous fish reveals the specialized resource use by this guild. Hg concentrations (ng g^{-1} dry weight) differed significantly between demersal carnivorous fish (185.3 dry weight; 27.8 wet weight) and demersal omnivorous fish (277.9 dry weight; 41.7 wet weight) and between pelagic omnivorous fish (197.2 dry weight; 29.6 wet weight) and demersal omnivorous fish due to (1) differences in food sources: guilds that fed on bottom resources were more affected by contamination because the sediment is an important Hg accumulator in the study area, and (2) because of its trophic positions. Considering that the fish consumed prey of similar trophic positions, the guilds did not show a well-defined food hierarchy. Therefore, in this study, there was no clear relationship between Hg and $\delta^{15}N$.

O objetivo deste estudo foi avaliar o perfil isotópico e as concentrações de mercúrio (Hg) em peixes (carnívoros, onívoros e detritívoros) na bacia inferior do rio Paraíba do Sul, sudeste do Brasil. As análises isotópicas de carbono ($\delta^{13}C$) revelaram participação de fontes pelágica e bentônica na alimentação dos peixes das diferentes guildas (-14,0 a -24,8 ‰). As fontes bentônicas são usualmente mais enriquecidas em $\delta^{13}C$ (-16,9‰, derivado do escoamento superficial da bacia de drenagem) comparadas às fontes pelágicas pois, o fitoplâncton, importante produtor primário que suporta inúmeras cadeias pelágicas, tem assinatura de $\delta^{13}C$ mais leve (-23,9‰). As assinaturas isotópicas de nitrogênio ($\delta^{15}N$) indicaram que a maioria das guildas estava no mesmo nível trófico (10,0 a 15,5 ‰), exceto os peixes onívoros pelágicos cuja posição trófica foi inferior. Observou-se sobreposição de nicho entre peixes carnívoros pelágicos e demersais, onívoros demersais, e detritívoros demersais. A menor amplitude de nicho isotópico dos peixes carnívoros pelágicos revela o uso de recursos especializados por esta guilda. A concentração de Hg (ng g^{-1} peso seco) diferiu significativamente entre peixes carnívoros demersais (185,3 peso seco; 27,8 peso úmido) e onívoros demersais (277,9 peso seco; 41,7 peso úmido), e entre peixes onívoros pelágicos (197,2 peso seco; 29,6 peso úmido) e onívoros demersais devido a (1) diferenças nas fontes alimentares: guildas que utilizaram recursos de fundo foram mais afetadas pela contaminação pois o sedimento é um importante acumulador de Hg na área de estudo, e (2) por causa das suas posições tróficas. Tendo em vista que os peixes consumiram presas com níveis tróficos semelhantes, as guildas não apresentaram uma hierarquia alimentar definida. Desse modo, no presente estudo não se verificou uma relação clara entre Hg e $\delta^{15}N$.

Keywords: Carbon, Freshwater, Hg, Nitrogen, Trophic guilds.

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Introduction

Trophic dynamics between sympatric species can be understood from their isotopic compositions (Benedito-Cecílio *et al*., 2000; Post, 2002; Layman *et al*., 2007). Trophic niche can be defined from the relationship between carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic signatures, which is called the isotopic niche (Bearhop *et al*., 2004; Layman, 2007; Jackson *et al*., 2011). Isotopic niche uses measures of $\delta^{13}C$ and $\delta^{15}N$ dispersal to indicate how species and organisms explore environmental resources. In general, $\delta^{13}C$ values are used to trace the origin of the different food sources (DeNiro & Epstein, 1978; Vander Zanden *et al*., 1997; Fry, 2008), whereas $\delta^{15}N$ values are useful to evaluate the species' trophic position, considering the fractionation along the food chain (Minagawa & Wada, 1984; Post, 2002).

Mercury (Hg) is a trace element with a recognized capacity for bioaccumulation in organisms and biomagnification along the trophic positions of aquatic food chains (Malm *et al*., 1995; Wren *et al*., 1995; Svodobová *et al*., 1999; Di Beneditto *et al*., 2012; Zhang *et al*., 2012). Trophic transfer is the main Hg source for consumer organisms, which is verified by the positive relationship between Hg concentration and $\delta^{15}N$ values along the links of the food chains (Di Beneditto *et al*., 2012, 2013). Therefore, Hg is efficient as a trophic marker.

The rio Paraíba do Sul watershed, located between coordinates 20º26' and 23º38' S and 41º00' and 46º30' W, extends over 62.074 km^2, of which 26.851 km^2 falls within the state of Rio de Janeiro, southeastern Brazil. This river is formed by the confluence of the Paraitinga and Paraibuna rivers and runs through 1.150 km to its mouth on the north coast of the state of Rio de Janeiro (21º35'S; 041º03'W) (Associação Pró-gestão da Bacia Hidrográfica do Rio Paraíba do Sul (AGEVAP) & Comitê de Integração da Bacia Hidrográfica do Rio Paraíba do Sul (CEIVAP), 2011). In the 1980s, human activities such as the use of organomercurial pesticides and gold prospecting were initiated in the lower portion of the rio Paraíba do Sul watershed and contributed significantly to the Hg contamination of the water resources (Câmara, 1990; Lima, 1990). Lacerda *et al*. (1993) estimated that 150-300 kg/year of Hg were released into the rivers of the region during this period. The same authors detected Hg concentrations in the fluvial sediment up to eleven times higher than the average (50 µg.kg^{-1}) for non-contaminated areas. The fish fauna of the region are affected by Hg, and mean concentrations of 301 µg.kg^{-1} and 151 µg.kg^{-1} were recorded for *Salminus maxillosus* (Valenciennes, 1850) and *Hoplias malabaricus* (Bloch, 1794), respectively (Yallouz *et al*., 2000).

According to Bizerril & Primo (2001), the rio Paraíba do Sul watershed houses more than 160 fish species. Omnivory is the predominant feeding habit of this ichthyofauna, followed by iliophagy. Approximately 40 fish species are of economic importance for the riverine communities along this watershed, with emphasis on *H. malabaricus, Leporinus conirostris* (Steindachner, 1875), *Astyanax bimaculatus* (Linnaeus, 1758)*, Pimelodus maculatus* (Lacepède, 1803), *Rhamdia quelen* (Quoy & Gaimard, 1824), *Hypostomus affinis* (Steindachner, 1880), *Loricariichthys* sp. and *Cichla ocellaris* (Bloch & Schneider, 1801).

The aim of this study was to evaluate and compare the trophic niches of fish of different guilds using trophic markers (isotopic signatures and Hg concentration) to understand the habitat use by this group of organisms in the lower portion of the rio Paraíba do Sul watershed.

Materials and methods

Study site and sampling. The study site was the lower portion of the rio Paraíba do Sul watershed, including the Pomba and Dois Rios tributary rivers, located between the north and northwest regions of the state of Rio de Janeiro. Sampling points were distributed among five locations: São Sebastião do Paraíba (SSP), Itaocara (ITA) and São Fidélis (SFI), in the rio Paraíba do Sul; Baltazar and Santo Antônio de Pádua, in the rio Pomba (RPO); and Guaraní, in the rio Dois Rios (RDR) (Fig. 1). The rio Paraíba do Sul and its tributaries, the Pomba and Dois Rios rivers, are characterized as shallow environments. The depth at sampling points did not exceed 5 m (SSP: 4.6 m, SFI: 3.4 m, ITA: 3.1 m, RPO: 2.6 m, RDR: 1.2 m).

Fish samplings were performed bimonthly between December 2012 and October 2013 (December, January, March, May, July, October) with different fishing artifacts (sieve, trawl, cast net, and gillnet). Fish were identified and grouped into different trophic guilds (carnivores, omnivores and detritivores) and habitats (pelagic and demersal) according to literature data (Meurer & Zaniboni Filho, 1997; Silva & Oliveira, 1997; Lowe-McConnell, 1999; Durães *et al*., 2001; Araújo *et al*., 2005; Pereira & Resende, 2006; Botelho *et al*., 2007; Gomiero & Braga, 2007; Santos *et al*., 2009; Bozza & Hahn, 2010; Mazzoni *et al*., 2010; Moraes *et al*., 2013). To confirm the taxonomy, it was used fish identification guides from Atlantic Forest (Oyakawa *et al*., 2006; Menezes *et al*., 2007) and specific papers for some groups (Bizerril & Araujo, 1992; Albert *et al*., 1999; Reis & Schaefer, 1998; Albert & Cramptom, 2003; Ribeiro & Lucena, 2006).

Most species were small in size (Table 1), and the largest possible quantity of muscle tissue was extracted from each specimen. Muscle tissue was frozen, lyophilized, and homogenized using a mortar and pestle for the isotopic composition and Hg concentration analyses.

In October 2013, 5 L of water and a sediment sample were collected from each sampling point for Hg determination. Suspended particulate matter (SPM) was obtained from the total water volume by the saturation of three filters in each sampling point (GF/F, 0.7 µm). Sediment was sifted from a < 63 µm fraction and frozen, lyophilized, and homogenized using a mortar and pestle.

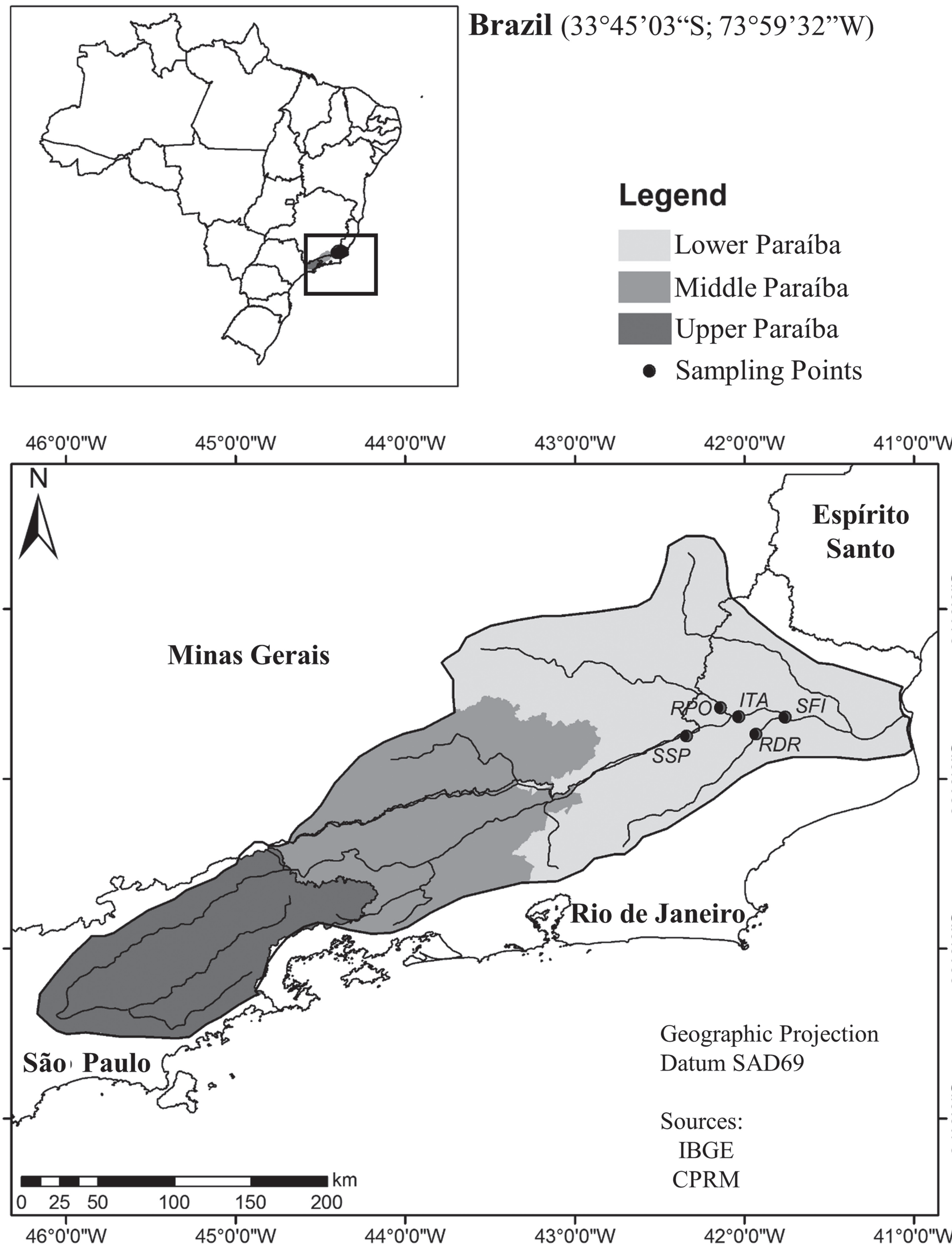

Fig. 1. Lower portion of the rio Paraíba do Sul watershed with the distribution of the five sampling points: rio Paraíba do Sul in Itaocara (ITA), São Sebastião do Paraíba (SSP) and São Fidélis (SFI); rio Pomba in Baltazar (RPO); rio Dois Rios in Guarani (RDR).

$\delta^{13}C$ and $\delta^{15}N$ isotopic compositions in fish. The isotopic analyses were performed in a Thermo Finnigan Delta V Advantage mass spectrometer attached to a Flash 2000 element analyzer. Reference values for $\delta^{15}N$ and $\delta^{13}C$ were, respectively, atmospheric nitrogen and Pee Dee Belemnite (PDB). The results were expressed in parts per thousand (‰). Analytical precision was ±0.3‰ for $\delta^{15}N$ and ±0.2‰ for $\delta^{13}C$, determined in triplicate for each 20 samples. Analytical precision for the elemental and isotopic composition was determined by certified standards (Protein OAS/Isotope Cert 114859; Elemental Microanalysis), organic carbon and total nitrogen (99%), and $\delta^{13}C$ and $\delta^{15}N$ (100%).

The C:N ration ≤ 3.5 in muscle samples indicated low lipid levels (C:N); therefore, the interpretation of $\delta^{13}C$ was not compromised (Post *et al*., 2007).

Trophic position (TP) was determined using phytoplankton as baseline ($\delta^{15}N$= 6.4‰). It was assumed they represent the primary producer occupying TP_{phyto}=1. Trophic position of consumers was estimated according to:

$$TP_{consumer} = [(\delta^{15}N_{consumer} - \delta^{15}N_{phyto})/3.4‰] + TP_{phyto}.$$

Where $TP_{consumer}$ and $\delta^{15}N_{consumer}$ are, respectively, the trophic position and the $\delta^{15}N$ signature of an organism or group of organisms. $\delta^{15}N_{phyto}$ is the $\delta^{15}N$ signature of baseline organism (phytoplankton) and 3.4‰ represents the trophic enrichment factor (Post, 2002; Di Beneditto *et al*., 2012).

Hg determination in fish, sediment and SPM. The digestion of the fish muscle samples was performed in a digestion block from an acid mix (HNO_3:H_2SO_4, 1:1) with the addition of potassium permanganate ($KMnO_4$ 5% w/v) and titration with hydroxylamine hydrochloride ($HONH_3Cl$ + NaCl 12% w/v), following Bastos *et al.* (1998). Analytic quality control and digestion efficiency were verified using certified reference material (DORM-3 and iaea-436), with percent recovery between 90.6 and 91.9%. Analytic method precision was evaluated in triplicate for each 10 samples. The detection limit was 1.0 ng g^{-1}.

The digestion of the SPM and sediment samples was performed in a digestion block and consisted of a mix of Milli-Q H_2O, aqua regia (3HCl:1HNO_3), and potassium permanganate ($KMnO_4$) (Bastos *et al*., 1998). SPM was represented by a composite sample of three filters from the sampling point. Sediment was digested in triplicate. Digestion efficiency was verified using certified reference materials (iaea-405). The detection limit was 0.5 ng g^{-1} and the percent recovery was 93.8%.

For all samples, Hg determination was performed using a Flow Injection Mercury System (Perkin Elmer FIMS-400), and the coefficient of variation among replicas was lower than 10%. The dry weight basis concentration of Hg was converted to wet weight considering 75% of water lost during freeze-dry (Di Beneditto *et al*., 2013).

Statistical analyses. Data normality and homogeneity of variance were tested, respectively, with the Shapiro-Wilk and Levene tests. Differences in the isotopic niches of the fish trophic guilds regarding the values of $\delta^{13}C$, $\delta^{15}N$ and TP were tested with analysis of variance (ANOVA) and an *a posteriori* Tukey test. Isotopic niche breadth was quantified using the corrected standard ellipse area (SEAc), calculated with the Stable Isotope Bayesian Ellipses tool in R (SIBER - Jackson *et al*., 2011), available in the Stable Isotope Analysis package in the software R (SIAR- Parnell & Jackson, 2013) (R Development Core Team, 2014).

Isotopic variances among the fish trophic guilds were verified using the F test. The p values provided additional data for the niche overlap results, indicating whether there was overlap in the breadth of resources explored in functions of similarity in the use of basal resources and/or in the diversity of trophic positions in the diet.

To meet the assumptions of the ANOVA, the Hg data were transformed with a maximum likelihood function (BoxCox). The *a posteriori* Tukey test was performed for multiple means comparisons. The difference in Hg concentrations between the abiotic components (SPM and sediment) was verified with the t test. The linear regression between the log-transformed Hg concentrations and the $\delta^{15}N$ values of the fish trophic guilds was used to evaluate the relationship between these variables.

All statistical analyses were performed in the software R (R Development Core Team 2014), and the value of $p \leq 0.05$ was chosen as an indicator of significance between differences.

Results

The 22 fish species sampled were grouped into the following trophic guilds: 10 carnivorous fish (6 pelagic and 4 demersal), 9 omnivorous fish (4 pelagic and 5 demersal), and 3 demersal detritivorous fish (Table 1).

The demersal omnivorous fish showed the heaviest $\delta^{13}C$ isotopic signatures, followed by pelagic carnivorous fish and demersal detritivorous fish, whose $\delta^{13}C$ values were similar. The demersal carnivorous fish showed intermediate $\delta^{13}C$ values, similar to those of pelagic omnivorous fish (Tables 1-2; Fig. 2).

Regarding the $\delta^{15}N$ values, the demersal omnivorous fish showed isotopic signatures similar to those of pelagic and demersal carnivorous fish and of demersal detritivorous fish. Pelagic omnivorous fish showed lighter $\delta^{15}N$ signatures than the remaining trophic guilds (Tables 1-2; Fig. 2). Pelagic omnivorous fish showed the lowest TP value (2.68), which was significantly different for remaining trophic guilds ($p < 0.001$) (Table 1).

Table 1. Distribution of the fish species of the lower portion of the rio Paraíba do Sul watershed into trophic guilds, with total abundance of specimens (n total), number of specimens used for the statistical analyses regarding Hg concentration (n Hg), number of specimens used for the isotopic analyses (n isotope), total length (cm), $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures (‰), trophic position (TP) and Hg concentrations ($ng.g^{-1}$) in dry weight (DW) and in wet weight (WW). SD = standard deviation; Min = minimum value; Max = maximum value. Lower-case letters statistically differ the parameters among trophic guilds.

Trophic guild	Species	n (total)	n (Hg)	n (isotope)	Total length (mean ± SD) (Min - Max)	$\delta^{13}C$ (mean ± SD) (Min - Max)	$\delta^{15}N$ (mean ± SD) (Min - Max)	TP (mean ± SD)	TP (mean ± SD)	Hg DW (mean ± SD) (Min - Max)	Hg WW (mean ± SD) (Min - Max)
Pelagic carnivore	*Oligosarcus hepsetus* (Cuvier, 1829)	67	62	25	16.3 ± 4.2 (8.1 to 23.4)	-18.7 ± 1.0 (-17.0 to -20.4)	13.7 ± 0.6 (12.4 to 14.9)	3.16 ± 0.18	3.19^{a} ± 0.19	216.3^{ab} ± 82.4 58.5 to 452.6	32.5^{ab} ± 12.4 8.8 to 67.9
	Salminus brasiliensis (Cuvier, 1816)	2	2	1	32.5 ± 17.0 (20.5 to 44.5)	-17.9	15.1	3.55			
	Cichla ocellaris (Bloch & Schneider, 1801)	2	2	2	24.6 ± 10.5 (17.2 to 32.0)	-18.6 ± 1.22 (-17.7 to - 19.4)	14.5 ± 0.89 (13.9 to 15.1)	3.39 ± 0.26			
	Gymnotus sylvius Albert & Fernandes-Matioli, 1999	1	1	1	21.6	-15.4	14.1	3.26			
	Gymnotus carapo (Linnaeus, 1758)	1	1	1	24.2	-17.8	13.3	3.02			
	Aequidens tetramerus (Heckel, 1840)	1	1	1	7.9	-17.0	14.1	3.25			
Demersal carnivore	*Crenicichla lacustris* (Castelnau, 1855)	20	18	21	17.1 ± 3.5 (12.5 to 29.2)	-19.0 ± 1.82 (-14.0 to -22.3)	13.7 ±0.6 (12.6 to 14.8)	3.14 ± 0.17	3.03^{a} ± 0.27	185.3^{a} ± 134.7 26.0 to 539.0	27.8^{a} ± 20.2 3.9 to 80.9
	Trachelyopterus striatulus (Steindachner, 1877)	7	4	7	20.6 ± 2.0 (16.8 to 22.5)	-21.2 ±1.3 (-18.6 to -22.7)	12.2 ±0.8 (11.2 to 13.4)	2.69 ± 0.24			
	Rhamdia quelen (Quoy & Gaimard, 1824)	2	2	1	27.6 ± 0.9 (26.9 to 28.3)	-20.7	12.6	2.82			
	Hoplias malabaricus (Bloch, 1794)	2	2	1	31 ± 8.1 (25.4 to 36.6)	-18.5	13.8	3.17			
Pelagic omnivore	*Astyanax parahybae* Eigenmann, 1908	43	40	10	11.2 ± 0.9 (9.6 to 13.1)	-21.7 ± 2.2 (-19.1 to -24.8)	13.1 ± 0.8 (11.6 to 14.1)	2.96 ± 0.23	2.68^{a} ± 0.34	197.2^{a} ± 85.6 43.1 to 432.0	29.6^{a} ± 12.9 6.5 to 64.8
	Astyanax giton Eigenmann, 1908	16	16	7	11.4 ± 1.03 (9.5 to 13.8)	-19.1 ± 0.9 (-17.9 to - 20.9)	11.5 ± 1.14 (10.0 to 13.1)	2.51 ± 0.34			
	Astyanax janeiroensis Eigenmann, 1908	16	16	7	12.6 ± 1.2 (10.4 to 14.5)	-20.7 ± 0.97 (-18.6 to -21.5)	11.3 ± 0.5 (10.8 to 12.2)	2.44 ± 0.15			
	Astyanax hastatus Myers, 1928	1	1	0	11.7	-	-	-			
Demersal omnivore	*Pimelodella lateristriga* (Lichtenstein, 1823)	59	58	7	14.3 ± 2.2 (11.2 to 23.0)	-17.3 ± 13.6 (-14.3 to -18.4)	13.4 ± 0.74 (12.4 to 14.3)	3.06 ± 0.22	3.20^{a} ± 0.21	277.9^{b} ± 181.7 22.3 to 746.4	41.7^{b} ± 27.3 3.4 to 112.0
	Pimelodus fur (Lütken, 1874)	39	38	13	19.6 ± 3.4 (14.1 to 24.4)	-18.1 ± 1.3 (-15.4 to -19.5)	14.0 ±0.3 (13.3 to 14.3)	3.24 ± 0.07			
	Pachyurus adspersus Steindachner, 1879	32	30	9	18.5 ± 3.7 (14.1 to 26.0)	-16.9 ± 1.71 (-14.2 to -19.1)	14.0 ± 1.1 (11.8 to 15.5)	3.23 ± 0.32			
	Leporinus conirostris Steindachner, 1875	3	3	2	26.3 ± 0.7 (25.8 to 26.8)	-20.0 ± 0.03 (-20.0 to -20.0)	14.1 ±0.29 (13.9 to 14.3)	3.27 ± 0.09			
	Apareiodon piracicabae (Eigenmann, 1907)	2	2	1	12.4	-15.3	14.4	3.35			
Demersal detritivore	*Loricariichthys castaneus* (Castelnau, 1855)	8	7	7	30.4 ± 5.8 (23.6 to 36.0)	-18.4 ± 0.58 (-17.5 to -19.4)	13.8 ± 0.38 (14.4 to 13.3)	3.19 ± 0.11	3.04^{b} ± 0.20	253.9^{ab} ± 155.0 75.9 to 483.5	38.9^{ab} ± 23.3 11.4 to 72.6
	Hypostomus affinis (Steindachner, 1877)	4	4	4	32.2 ± 8.4 (21.5 to 40.6)	-18.5 ± 4.5 (-14.3 to -22.5)	12.9 ± 0.3 (12.4 to 13.2)	2.91 ± 0.1			
	Hypostomus auroguttatus (Kner, 1854)	2	2	2	24.8 ± 1.1 (24.0 to 25.6)	-18.0 ± 0.53 (-17.6 to -18.6)	12.5 ±0.38 (13.3 to 14.4)	2.79 ± 0.08			

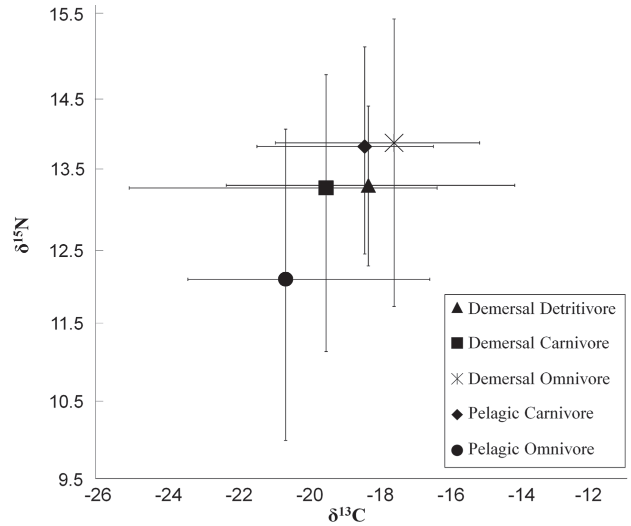

Fig. 2. $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of the trophic guilds of fish of the lower portion of the rio Paraíba do Sul watershed. Bars represent standard deviations.

The isotopic niche area represented by the SEAc showed the following values for the fish trophic guilds: pelagic omnivores (3.15‰²) > demersal carnivores (2.76‰²) > demersal detritivores (2.66‰²) > demersal omnivores (1.86‰²) > pelagic carnivores (1.03‰²) (Fig. 3).

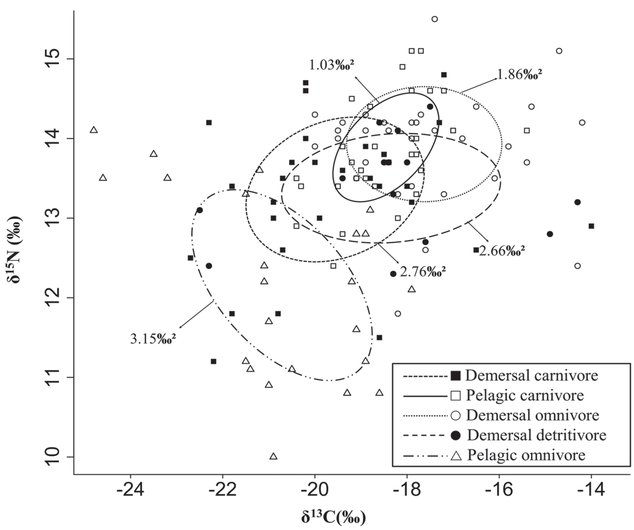

Fig. 3. Isotopic niche areas of the trophic guilds of fish of the lower portion of the rio Paraíba do Sul watershed. Values in ‰² indicate the corrected standard ellipse areas (SEAcs).

Pelagic carnivorous fish showed a smaller isotopic niche area than the remaining trophic guilds (Fig. 3). This fish guild shared a large part of its niche with the demersal guilds (Fig. 3). Despite the ellipse overlap, there were significant differences in the $\delta^{13}C$ isotopic variances among these trophic guilds (Table 2). Pelagic omnivorous fish showed the largest niche breadth, and their isotopic variances were high (1.37‰ for $\delta^{15}N$ and 3.57‰ for $\delta^{13}C$) (Fig. 3; Table 2). This trophic guild shared only a small portion of its niche with detritivores and demersal carnivores (Fig. 3). Although the ellipse overlap area was small, there was no significant difference between the isotopic variances of pelagic omnivorous fish in relation to demersal detritivorous fish and demersal carnivorous fish (Table 2).

Table 2. $\delta^{13}C$ and $\delta^{15}N$ signatures and their variances among the trophic guilds of fish. Lower-case letters statistically differ the parameters among trophic guilds. Statistical differences among trophic guild for $\delta^{13}C$ and $\delta^{15}N$ signatures resulted in a *p* value < 0.001 and for their variances had a *p* value ≤ 0.04.

Trophic guilds	Mean ± variance $\delta^{15}N$ (‰)	Mean ± variance $\delta^{13}C$ (‰)
Pelagic carnivore	$13.8^{ab} \pm 0.43^{b}$	$-18.4^{ac} \pm 1.23^{b}$
Demersal carnivore	$13.3^{ab} \pm 0.81^{ab}$	$-19.5^{ab} \pm 3.69^{a}$
Pelagic omnivore	$12.1^{b} \pm 1.37^{a}$	$-20.7^{b} \pm 3.57^{a}$
Demersal omnivore	$13.9^{a} \pm 0.49^{b}$	$-17.6^{c} \pm 2.61^{a}$
Demersal detritivore	$13.3^{ab} \pm 0.45^{ab}$	$-18.3^{ac} \pm 5.31^{a}$

Hg concentration in the fish trophic guilds. The Hg concentrations in the muscle tissue of the fish did not show a clear relationship with the trophic hierarchy of the guilds and were distributed as follows: demersal omnivore (277.9 ng g^{-1}) > demersal detritivore (253.9 ng g^{-1}) > pelagic carnivore (216.3 ng g^{-1}) > pelagic omnivore (197.2 ng g^{-1}) > demersal carnivore (185.3 ng g^{-1}) (Table 1). Demersal omnivorous fish showed higher Hg concentrations than demersal carnivorous fish and pelagic omnivorous fish ($p \leq 0.05$).

The relationship between the Hg concentrations and the $\delta^{15}N$ values was positive and significant ($p = 0.02$), indicating that Hg concentrations increased with higher isotopic values. However, only 24% of the Hg concentration varied as a function of the $\delta^{15}N$ values, as indicated by the low R value (0.24) (Fig. 4). Considering the abiotic components, the Hg level was higher in the sediment (126.5 ± 25.7 ng g^{-1}) than in the SPM (6.96 ± 2.6 ng g^{-1}) ($p \leq 0.05$).

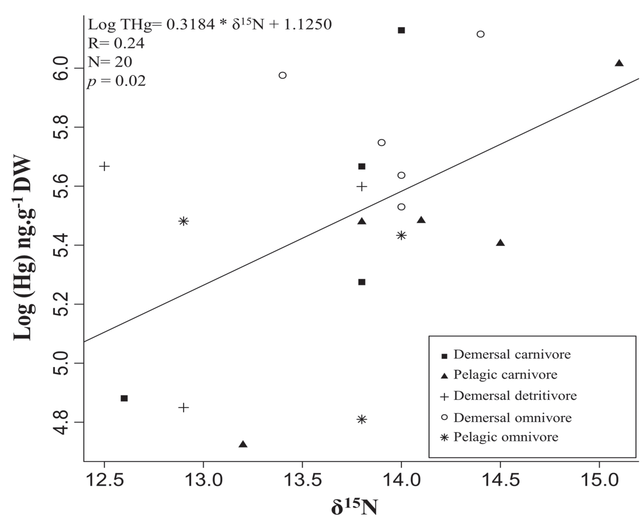

Fig. 4. Relationship between Hg concentrations and $\delta^{15}N$ values of the trophic guilds of fish of the lower portion of the rio Paraíba do Sul watershed.

Discussion

Isotopic signatures as indicators of food source and trophic position. The $\delta^{13}C$ isotopic signatures recorded in demersal omnivorous fish and pelagic omnivorous fish confirm their participation in the benthonic and pelagic food chain, respectively. These findings reflect the preferred habits of the fish that comprise these two trophic guilds. The differences in the $\delta^{13}C$ isotopic signatures between demersal omnivorous fish and demersal carnivorous fish indicate that the feeding of the second group consists mainly of pelagic sources, although its preferred occurrence area is associated with the bottom. Conversely, pelagic carnivorous fish showed $\delta^{13}C$ signatures similar to those of the demersal trophic guilds, indicating the importance of benthonic sources for their feeding.

Connection between pelagic and demersal habitats is a possibility in shallow environments. The low depth allows species to transit between habitats to obtain food and/or allows prey to migrate between habitats.

In this study, the rio Paraíba do Sul and its tributaries are shallow environments, a fact that facilitates the migration of species through the water column. In general, phytoplankton is an important primary producer and the main carbon source for pelagic food chains, with lighter isotopic values (-20 to -32‰) (France, 1995; Hobson, 1999; Vander Zanden & Vadeboncouer, 2002; Martinelli *et al*., 2009). Some authors analyzed the $\delta^{13}C$ compositions in some compartments of the lower portion of the rio Paraíba do Sul watershed. Carvalho (1998) evaluated the phytoplankton, whose mean $\delta^{13}C$ value was -23.6‰ and evaluated the bioproducts of the sugarcane industry (-12.5 to -15‰). Mazurec (2003) evaluated plantation soils (-17.5 to -22.4‰). These last two sources are important carbon sources for the benthonic food chain of this region, with an overall mean of 16.9‰.

The $\delta^{15}N$ isotopic values were similar between the pelagic and demersal carnivores, demersal detritivores, and demersal omnivores, indicating that these guilds are at the same trophic position. The similarity between demersal omnivores and carnivores (pelagic and demersal) in terms of $\delta^{15}N$ signatures reveals their condition of non-voracious predators. The fish species *Rhamdia quelen*, *Crenicichla lacustris*, and *Trachelyopterus striatulus*, whose feeding consists of insects, crustaceans, mollusks, and small-sized fish (Dias *et al*., 2005; Gomiero & Braga, 2007; Santos *et al*., 2009), had a high abundance among demersal carnivorous fish. The species *Oligosarcus hepsetus*, the most abundant among the pelagic carnivorous fish, feeds on small-sized fish belonging to lower trophic positions (Araújo *et al*., 2005; Botelho *et al*., 2007; Santos *et al*., 2014). Furthermore, the low numeric representation of piscivorous fish belonging to the top of the food chain (*Hoplias malabaricus*, *Salminus brasiliensis*, and *Cichla ocellaris* - Suarez *et al*., 2001; Bozza & Hahn, 2010) in the sampling of this study likely contributed to the lower trophic positions of this guild in comparison to the remaining guilds (Table 1).

The $\delta^{15}N$ signatures of the demersal omnivorous and detritivorous fish were similar to those of carnivorous fish (both pelagic and demersal), indicating the preference for animal food sources. However, light $\delta^{15}N$ signatures place the species of the genus *Astyanax* at a lower trophic position than the remaining guilds. In general, the species of the genus *Astyanax* feed on insects and plant matter (Esteves, 1996; Vilella *et al*., 2002; Mazzoni *et al*., 2010).

Isotopic niche size and isotopic variance of the fish trophic guilds. Pelagic carnivorous fish showed the smallest isotopic niche area, indicating that this trophic guild explored more specific food resources. Food selectivity was reflected on both the exploration of a smaller breadth of basal resources and the low diversity of preys, considering the smaller variances of $\delta^{13}C$ and $\delta^{15}N$.

Therefore, pelagic carnivorous fish seem to be more susceptible to food pressure because their isotopic niche is contained within the isotopic niche of the trophic guilds of demersal fish. The food specificity of pelagic carnivorous fish may be a successful condition if food is renewable and widely available. However, if food resources are not maintained, these fish might become vulnerable and might then explore alternative food resources as a competition strategy (Roughgarden, 1974; Begon *et al*., 2007).

The trophic guilds of demersal fish showed wider isotopic niches. The higher trophic plasticity derived mainly from the $\delta^{13}C$ isotopic variances, which were significantly higher ($p < 0.04$) than those of pelagic carnivorous fish. The higher variability in the use of carbon sources indicates differences between demersal fish guilds and pelagic carnivorous fish in the consumption of preys at the base of the food chain. This strategy reduces the trophic redundancy between these groups of fish and minimizes the effects of competition by differentiating a range of resource exploration.

Food variability between demersal fish was not significant, considering the $\delta^{13}C$ and $\delta^{15}N$ isotopic variances ($p > 0.05$). The trophic similarities were more marked between carnivores and omnivores that feed on live preys, in contrast to detritivores. Despite the isotopic redundancy, these fish trophic guilds may coexist if the food resources shared are not limiting. In general, tropical fluvial ecosystems show a great availability of food resources because of their hydrological regime. During the flood periods, rivers expand, flooding the marginal vegetation and increasing the diversity of preys and habitats for fish and remaining organisms (Abelha *et al*., 2001).

The larger isotopic niche area of pelagic omnivorous fish (3.15‰^{2}) indicates that this guild has a larger trophic breadth than the remaining guilds. A large niche breadth can be a strategy adopted by pelagic omnivorous fish to decrease food redundancy, considering the trophic similarity among the species of the genus *Astyanax* (Esteves, 1996; Vilella *et al*., 2002; Mazzoni *et al*., 2010). Pelagic omnivorous fish showed the smallest degree of food similarity among the fish trophic guilds because their niche space was little shared and the areas of overlap with other guilds were small.

Hg concentrations in the fish trophic guilds. Hg bioaccumulation in organisms can be affected by their biological characteristics, such as body size, age, and the physiology of the excretion of the contaminant (Kasper *et al*., 2007). In this study, the lengths of the fish that compose the trophic guilds do not seem to affect Hg concentrations, in contrast to reports from other authors (Costa & Lacerda, 2009; Gewurtz *et al*., 2011; Kehrig & Malm, 2011). The breadth of Hg concentration in the trophic guilds was not proportional to the size variability of the fish. Pelagic carnivorous fish showed the smallest variations in Hg concentration, despite their highest variation in body length (40.23%). Furthermore, the highest breadths of Hg concentrations were recorded in the trophic guilds with the smallest variations in body length (demersal omnivores and demersal carnivores). These findings indicate that the body lengths of the fish do not affect the variation of Hg concentrations in the different trophic guilds.

The relationship between Hg concentration and $\delta^{15}N$ isotopic values in the muscle tissue of the fish of the trophic guilds analyzed was not clear. In general, it is expected that organisms of higher trophic positions would exhibit higher Hg concentrations than those of lower trophic positions (*e.g*., Carvalho *et al*., 2008; Eagles-Smith *et al*., 2008; Kehrig & Malm, 2011; Di Beneditto *et al*., 2012, 2013). This occurred because the guilds are not distributed following a food hierarchy. Their similarities in resources use was a determining factor for overlaps in Hg levels and $\delta^{15}N$. The composition of carnivorous guild (pelagic and demersal) by non-voracious predators and few top predators contributed to equate its $\delta^{15}N$ signature to demersal omnivores and demersal detritivores. In other words, the carnivorous guild had a most generalized representation. The species distribution in more specialized trophic categories could reduce the width of resources used and niches overlap. Thus, this could characterize more strictly the food habits of trophic guilds and the relationship between Hg and $\delta^{15}N$ could verify more clearly.

The Hg concentrations were comparable between pelagic carnivores, demersal omnivores, and demersal detritivores. The preference of fish for resources of the benthonic food chain may have contributed to the similarities in Hg concentration of this element because their preferred food sources are affected by the sediment. According to data from this study, this abiotic compartment retains a considerable concentration of the Hg present in the water column and is an important repository of this element in the lower portion of the rio Paraíba do Sul watershed.

Regarding demersal omnivorous and carnivorous fish, the trophic dissimilarities may have contributed to the differences in the Hg concentrations. Demersal omnivorous fish prefer resources associated with the bottom, whereas demersal carnivores demonstrated a preference for food resources from the pelagic environment, as indicated by the $\delta^{13}C$ values.

The lower portion of the rio Paraíba do Sul watershed hosts fish trophic guilds with diversified diets, integrating preys of different habitats (pelagic and demersal) and trophic positions. Carnivorous fish (pelagic and demersal) are at the same trophic position as demersal detritivorous and demersal omnivores because of the absence of voracious predators in this carnivore guild. The similar trophic positioning among the fish guilds resulted in a weak Hg x $\delta^{15}N$ relationship. Although the Hg concentration of the trophic guilds was not distributed along a well-defined food hierarchy, the importance of the trophic positioning in relation to the concentration of this contaminant in the muscle tissue of some fish species and the influence of the sediment on the contamination of demersal fish could be verified.

Except for pelagic carnivorous fish, the remaining guilds exhibited large trophic niches, demonstrating the capacity of these fish to explore the resources available in the environment. This food plasticity and the availability of resources provided by this fluvial system help minimize the effects of potential competition for resources.

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