

RESEARCH ARTICLE

## Fish habitat associations along a longitudinal gradient in a preserved coastal Atlantic stream, Brazil

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**ABSTRACT.** Habitat conditions at multiple scales are one of the major factors structuring ichthyofauna. Thus, we analyzed the fish habitat associations along the headwater-mouth gradient of a coastal Atlantic stream. We categorized the sampling sites into habitat units, so that in the middle reach these categories were statistically differentiated into riffles, runs and pools. Samplings were carried out quarterly from May 2009 to February 2010 using electrofishing. Principal component analysis (PCA) indicated an environmental gradient from higher water velocity and rocky bottom to deeper and sandy areas in the headwater-mouth direction. A total of 1,495 individuals belonging to 27 species were captured, being 13, 18 and 22 from headwater, middle and mouth reaches, respectively. Shannon diversity was slightly higher in the middle reach, while beta diversity showed higher rates of addition than turnover in species along the longitudinal gradient. Fish structure, evaluated by DCA (detrended correspondence analysis) scores, showed significant differences between upper reaches and mouth reach, but the middle riffles did not differ from headwater habitats. In the middle reach, mesohabitat analysis distinguished riffles, with higher abundance of fast-water crenuchids, from pools, with a higher abundance of lentic-water characids. These results suggest that environmental differences along the stream determine the wider structural patterns. However, the middle reach amassed species from upper areas and lowlands in structured fish mesohabitat associations, possibly implying distinct local ecological interactions. These findings contribute to the assessment of stream conservation status and to recognize eventual direct impacts on fish structures along longitudinal gradients.

**KEY WORDS.** Abiotic gradient, diversity, ichthyofauna, mesohabitat, multiple scales.

### INTRODUCTION

A major challenge in ecology of freshwater environments is understanding the functioning of river systems and identifying the mechanisms that underlie the structure of communities (Melles et al. 2012). A coherent approach to understanding these mechanisms is to compare communities along rivers, since fluvial and geomorphological differences occur longitudinally (Angermeier and Karr 1983, Ibañez et al. 2007, Silva et al. 2013). In this sense, the composition and diversity of the aquatic fauna have showed longitudinal changes, with adjustments at each local environmental conditions to its functioning along river system networks (Vannote et al. 1980, Oberdorff et al. 1993, Ferreira and Petrere Jr 2009, Suvarnaraksha et al. 2012).

Longitudinal changes in the structure and richness of ichthyofauna have been widely recorded both in temperate (Oberdorff et al. 1993, Pekárik et al. 2011) and tropical streams (Casatti 2005, Petry and Schulz 2006, Ferreira and Petrere Jr 2009, Silva et al. 2013). Several findings, summarized in Ibañez et al. (2009) have showed similar longitudinal increases of fish richness and proportional shifts of trophic guilds along rivers. Therefore, fish community structures converge to similar patterns along biogeographically distinct rivers, regardless of their phylogenetic and historical constrains (Ibañez et al. 2009). In this sense, geomorphological parameters, such as altitude, discharge, water velocity and channel depth, have more influence in predicting the increases in local fish richness/diversity and changes in fish community composition along the headwater-mouth direction,

than physicochemical water variables (Lorion et al. 2011, Suvarnaraksha et al. 2012). For example, headwater reaches with fast water and low environmental heterogeneity has shown few species that are able to survive under such conditions, while larger and more diversified downstream habitat reaches tend to support a richer and more diverse ichthyofauna (Ibañez et al. 2007, Suvarnaraksha et al. 2012). Moreover, according to local adaptations and species composition, studies have proposed that upper, middle and lower reaches of rivers are discrete zones of fish colonization (Ferreira and Petreire Jr 2009, McGarvey 2011).

However, longitudinal changes in composition and richness of fish species need a detailed assessment because their structural differences may also occur at reduced spatial scales (Rahel and Hubert 1991). At local scales, especially at moderate slopes gradients, streams are structurally complex and can be divided into mosaics of riffles, pools and runs mesohabitats (Allan 1995, Rezende et al. 2010), which are clearly delimited by different combinations of water velocity, depth and substrate composition (Angermeier and Schlosser 1989, Langeani et al. 2005). As mesohabitats have significant differences in their specific sets of environmental conditions, they play an important role in structuring the ichthyofauna (Rezende et al. 2010, Teresa and Casatti 2012), because fish communities have different strategies for foraging, breeding and obtaining refuge from predators (Matthews 1998).

Recently, efforts have been made to detect differences in functional and taxonomic diversity (Teresa and Casatti 2012), interspecific variation in habitat selection (Kano et al. 2013), guild composition (Pegg et al. 2014), and structure and complexity of food webs (Worischka et al. 2014) at mesohabitat scale. These studies have improved the knowledge on fish ecological differences among riffles, pools, and runs, and highlighted the importance of spatial mesoscale in assessing environments with contrasting conservation status (Teresa and Casatti 2012). On the other hand, since smaller spatial units are nested within broader environmental characteristics of basin network, the main challenge in understanding fish spatial structuration is assess the effects of multiple spatial scales interaction (i.e. micro and mesohabitat, stream reaches and landscape) because connections among different scales might create emergent patterns that transcend local processes (Cheek et al. 2016).

Atlantic forest is one of the world's hotspots for biodiversity conservation and, despite its threatened fauna and flora (Myers et al. 2000), coastal Atlantic streams located in this biome harbor one of the richest and ecologically most complex ichthyofaunas in the Neotropical Region (Vari and Malabarba 1998). These streams are represented by small and medium sized water bodies that run along upper cascaded channels, passing through intermediate reaches, which usually include combinations of riffles, pool and runs to slow lowlands moving channels (Rincón 1999, Oyakawa et al. 2006, Rezende et al. 2010). Coastal Atlantic streams support a high diversity of small-sized fish species with limited geographical distributions and high endemism (Esteves

and Lobón-Cerviá 2001, Barreto and Aranha 2005, Gonçalves and Braga 2012).

Upper courses of coastal Atlantic streams from southern and southeast Brazil are widely located in preserved and topographically irregular areas of Serra do Mar mountains (Oyakawa et al. 2006), making it difficult to carry out integrative spatial studies about distribution patterns of ichthyofauna (Ferreira and Petreire Jr 2009). Furthermore, most studies on fish community structure along coastal streams (Mazzoni and Lobón-Cerviá 2000, Ferreira and Petreire Jr 2009, Gonçalves and Braga 2012) have not considered the local fish-habitat associations and their influences on fish patterns in river segments. In this sense, knowledge about the fish ecology of coastal streams is still insufficient to infer accurate structuring patterns and processes, particularly at multiple scales, since fish composition and diversity are ultimately determined at both fine and coarse spatial scales (Cheek et al. 2016). Thereby, we expect that both environmental characteristics along the longitudinal gradient and local habitats induce changes on the ichthyofauna structure in a preserved Brazilian coastal Atlantic stream. We addressed the following questions: (1) Does fish structure, in density and composition, differ among headwater, middle and mouth reaches? (2) What are the relationships among local habitats and ichthyofauna along longitudinal gradient? (3) What are the patterns of fish distribution among riffle, pool, and run mesohabitats?

## MATERIAL AND METHODS

The study was carried out in the Vermelho River, eastern Atlantic basin, Brazil (Fig. 1). The Vermelho River begins in the mountainous part of the Reserva Particular do Patrimônio Natural – RPPN Morro da Mina, between the coastal municipalities of Antonina and Morretes, state of Paraná, and flows into the Xaxim River, which connects the river basin network to the estuarine complex of the Paranaguá Bay. The confluence of the Vermelho and Xaxim rivers is located approximately 15 km from Antonina and 40 km from Paranaguá in a preserved area of the Serra do Mar mountains. According to the Strahler classification (Allan 1995), the Vermelho River is a first to third-order stream along its course and it is a typical stream found in the coastal Atlantic forest of southern-southeast Brazil (Oyakawa et al. 2006). The Vermelho River is about 3 km long and between 2 and 5 m wide, with a steep slope at its upper course, riffles alternating with runs and shallow pools at the intermediate course and lentic habitats in the lowland reaches. As defined by Rincón (1999), riffles are reaches of great slope with fast running waters on a rocky bed; runs are areas with relatively slow running waters, with smaller slope and no turbulence, and are usually deeper than riffles; and pools are deeper than riffles and runs, with slow flow and sandy substrate. According to the Koeppen classification (Maack 1981), the regional climate is Af (tropical rainforest, fully humid), a transitional tropical climate, with annual rainfall between 2,500 and 3,000 mm

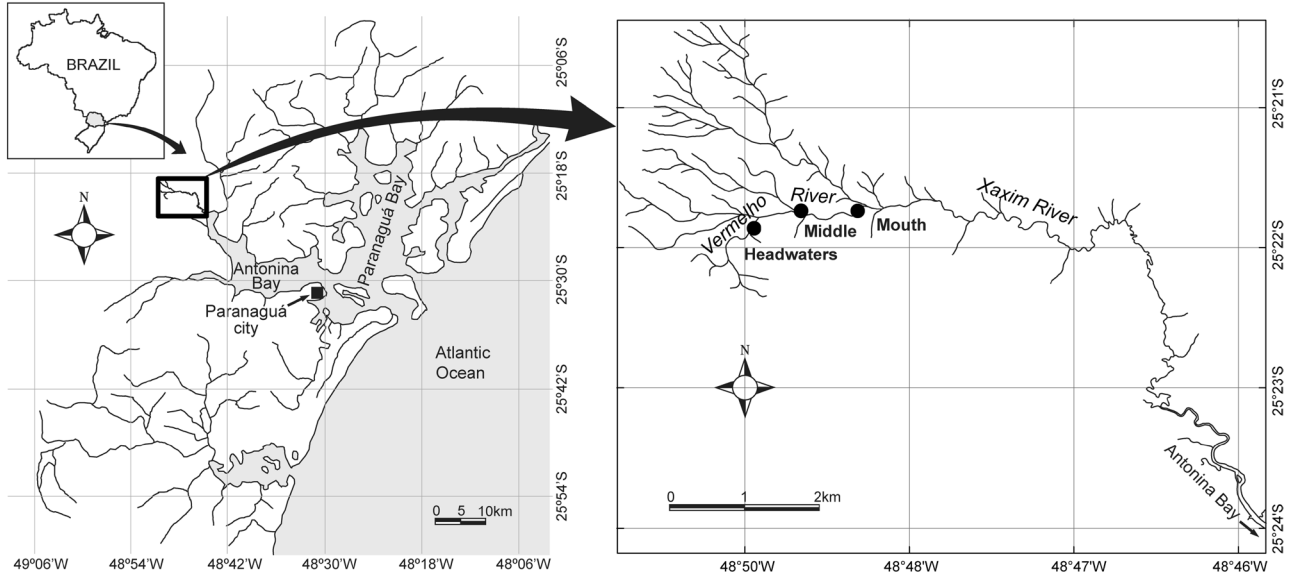


Figure 1. Location of the Vermelho River, eastern Atlantic basin, Antonina, Paraná state, Brazil. Black circles indicate the sampling reaches; arrow indicates the flow direction. Source: PEREIRA Jaime Luiz Lopes, 2015.

and mean temperature in the coldest month above 18°C. The vegetal formations of the RPPN are mangrove and 'restinga' (an herbaceous/shrubby community of coastal dunes) in lowlands and submontane Dense Ombrophilous Forest in highland areas where Vermelho River is localized (Velloso et al. 1991).

Samples of fish and abiotic variables were all taken in third-order stream reaches of headwater, middle and mouth of Vermelho River with approximately 50 m long each (Fig. 1). The headwater reach in the upper course (25°21'54.12"S, 48°49'55.56"W, 160 m a.s.l., 47.7 m long) was characterized as a cascaded channel, with the predominance of riffles, rocky bottom and a dense riparian vegetation canopy. The middle reach at the intermediate gradient (25°21'45.12"S, 48°49'35.58"W, 80 m a.s.l., 50.2 m long) had moderate water velocity riffles and areas with runs and pools, with the predominance of sandy/rocky substrate and dense/moderate vegetation canopy. On the other hand, the mouth reach at the lower course (25°21'42.10"S, 48°49'11.08"W, 55 m a.s.l., 48 m long) was characterized by slow-moving waters, with a predominance of sandy/clayey bottom and moderate vegetation canopy (Suppl. material 1).

Each reach was subdivided into different sampling sites according to their hydrological and structural characteristics. These sampling sites were provisionally classified in mesohabitats along the headwater-mouth direction in the following order: pool, riffles, riffles and run (headwater reach); pool, run, riffles, run, riffles and pool (middle reach) and pool, riffles and run (mouth reach). However, the sampling sites classification in mesohabitats was only validated in middle reach (Fig. 2), because it presented *a posteriori* significant differences in hydrological and environmental characteristics. Environmental differences

among mesohabitats were tested with a discriminant analysis using the matrix of abiotic variables transformed as log (x+1). The Wilks' lambda statistic was used to evaluate the efficiency of environmental variables in discriminating the mesohabitats *a priori*. Values range from 1 (no discriminant power) to 0 (perfect discriminant power) (Manly 2008). The discriminant analysis assumptions were checked by the Shapiro-Wilk test for normality and Levene's test for homogeneity of variances among the groups.

Fish were collected by electrofishing using a TOYAMA® power generator (127 volts AC and 5 A) from May 2009 to February 2010. As fish species are able to move among habitats and migrate short distances seasonally (Esteves and Aranha 1999), we considered the samplings as a snapshot study (Gotelli and Ellison 2004) of existing ichthyofauna. We blocked each sampling site with 3-mm mesh seines to reduce the spatial dependence of data and prevent fish from escaping (Fig. 2), and samples were taken every three months, in order to reduce the temporal dependence of data. We performed three successive passes of electrofishing in the upstream direction, and samples included all fish captured by dip nets as well as those retained in block seines. Fish were euthanized with 10% benzocaine and after fixed in 10% formaldehyde. In the laboratory, specimens were identified and counted. Fish were collected with authorization of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), license number 16475-1, and the procedures for manipulation of biological material are approved by Ethics Committee on Animal Use of the Universidade Estadual de Maringá (ECAU) in accordance with protocols of ethical and methodological aspects, for the use of fish. Due to possible character introgression in

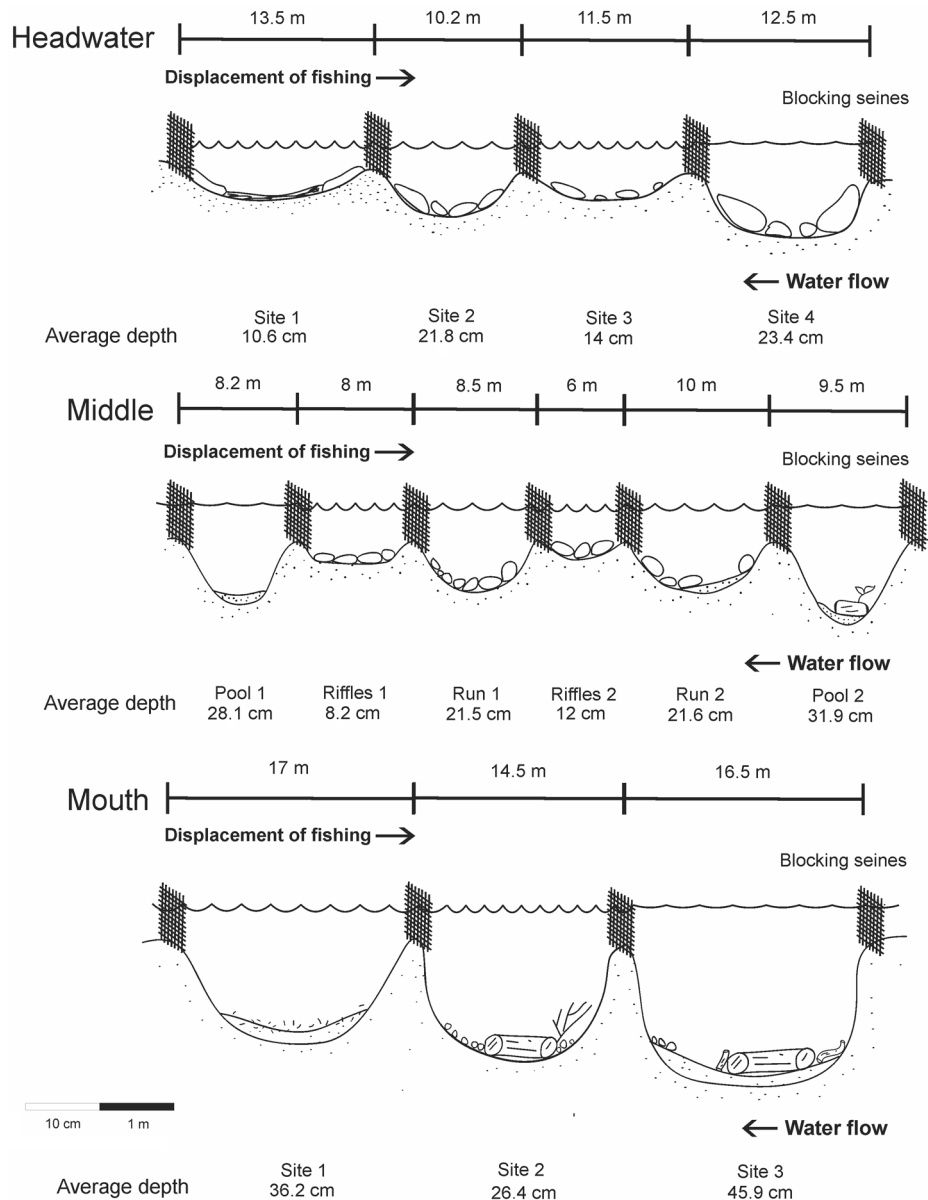


Figure 2. Fish sampling procedure. Length (above) and average depth (below) of each blocked (with seines) sampling site are showed. Middle sites were clustered into mesohabitats (see text). Displacement of fishing indicates the upstream direction of passage of electro-fishing dip nets with electrodes.

the area, identification of the species belonging to *Characidium* is difficult (Paulo A. Buckup, pers. comm.), we were unable to identify two individuals of this genus with confidence, and they have been tentatively identified as *Characidium lanei* Travassos, 1967 based on photographs. Voucher specimens of 25 out of 27 species (Table 3), were deposited in the Museu de Peixes of Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura

(Nupélia) of the Universidade Estadual de Maringá (NUP9465, NUP9524-NUP9529, NUP9541, NUP9567-NUP9572, NUP9576-NUP9595, NUP9742 and NUP19193-NUP19198), available on the SpeciesLink website (<http://www.splink.org.br>). For *Gymnotus pantherinus* (Steindachner, 1908) and *Hisonotus leucofrenatus* (Miranda Ribeiro, 1908) we uploaded photographs on the Fish-Base website (<http://www.fishbase.org>).

Water temperature ( $^{\circ}\text{C}$ ), dissolved oxygen ( $\text{mg}\cdot\text{l}^{-1}$ ), pH and electrical conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ) were measured at each sampling site where fish were captured and determined as the arithmetic mean of four observations during the year. For hydraulic variables, the total length of each sampling site was measured and then three equidistant transversal sections along the site were marked. Channel width (m) was measured along each of these transversal sections, being determined as the arithmetic mean of three observations. Mean depth (cm) was estimated through five equidistant measurements along each transversal section and then determined as the arithmetic mean of 15 observations for each site. Similarly, water velocity ( $\text{m}\cdot\text{s}^{-1}$ ), measured with a General Oceanics<sup>®</sup> mechanical flowmeter was determined from the arithmetic mean of three observations taken at the center of each transversal section at each site. The substrate bottom composition was categorized into different size classes and quantified by visual inspection. A total percentage of 100% was established for the sum of all substrate size categories at each sampling site. Size classes were based on Gordon et al. (1992): boulders (>80 mm diameter), cobbles (50–80 mm), pebbles (25–50 mm), gravel (5–25 mm), sand (<5 mm), and sand/clay (mixture of sand with silt). Organic structures, including leaf litter and tree trunks/branches were also quantified.

We applied a principal component analysis (PCA) to the Pearson correlation matrix of environmental variables to describe the patterns of environmental variability among headwater, middle and mouth reaches. The variables were log ( $x+1$ ) transformed in order to linearize the correlations. The Broken-Stick model was used for retention and interpretation of the axes, in which only axes with eigenvalues greater than those randomly generated are interpreted (Jackson 1993). Species richness, Shannon diversity and Pielou's evenness (Magurran 1988), calculated from species abundance matrix, were used as ichthyofauna descriptors. Since species richness could increase in direct proportion to the number of individuals sampled, we used the rarefaction method, with an algorithm from Krebs (1989), to compare headwater, middle and mouth reaches. Beta diversity ( $\beta_2$ ), calculated to evaluate the proportional addition and replacement of species along the longitudinal gradient, was obtained using Harrison index (Harrison et al. 1992). Beta diversity ( $\beta_2$ ) measures the amount by which the regional diversity exceeds the maximum diversity obtained locally and reaches zero if adjacent sampling sites share all species, while highest  $\beta_2$  values indicate an increase in the degree of species replacement (Petty and Schulz 2006).

To assess the volume of each sampling site, we used the following formula:  $\text{Volume}_{\text{site}} = \text{Length}_{\text{site}} * \text{Mean depth}_{\text{site}} * \text{Mean channel width}_{\text{site}}$ . Furthermore, in order to convert fish abundance data into density (i.e.,  $\text{individuals}\cdot\text{m}^{-3}$ ), we divided the number of individuals of each species by its respective sampling site volume. From these data, longitudinal and local patterns of ichthyofauna were described by a detrended correspondence analysis (DCA), combining the densities of each

sampling site among the stream reaches. Fish densities were log ( $x+1$ ) transformed to linearize the relationships. In this analysis, only axes with eigenvalues > 0.20 were retained and interpreted (Manly 2008). DCA has been used as an alternative ordination method to CA (correspondence analysis), which eliminates the arch effect (Hill and Gauch 1980) and presumably shows more accuracy in the relationships of interest (Gotelli and Ellison 2004). Moreover, DCA preserves the distances among sites and among species observations in the multivariate space after the dimensional reduction and, therefore, allow realistic *a posteriori* tests of axis scores. Scores of the first significant DCA axis were subjected to a non-parametric Kruskal-Wallis analysis of variance to check for possible significant effects of the reach on ichthyofauna structure. Subsequently, a multiple comparison test was applied to determine which averages, and thus which reaches, differed from each other. Similarly, a DCA was employed to verify fish distribution patterns among mesohabitats of middle reach. Multivariate analyses, calculation of diversity indices and species rarefaction were performed in PAST 2.01 software. Drawing of scatter and line plots, discriminant and Kruskal-Wallis analyses and the tests of multiple comparisons were performed in Statistica 7.0.

## RESULTS

### Environmental variables

Most of the variations in environmental parameters were related to water velocity, which decreased along the stream, and to the average depth and channel width, which increased (Table 1). Boulders were the main substrate at headwater sites, while in the middle reach, heterogeneous substrate with cobbles, pebbles and gravel predominated in different mesohabitats, and at mouth reach, fine granulometric substrate with trunks and leaf litter predominated. Temperature, dissolved oxygen, pH and conductivity had low variation in headwater-mouth direction.

The first PCA axis retained for interpretation (eigenvalue = 5.2) explained 35% of total variance and primarily distinguished the reaches (Fig. 3). With more negative scores, all sites from the headwater reach and riffles from the middle reach showed higher current velocity and rocky bottoms, while at the positive end, habitats from the mouth reach were deeper and had wider channels, with sand and sand/clay substrates, and also higher quantity of trunks/branches and leaf litter. Runs and pools from middle reach were located at the intermediate environmental gradient of PCA.

### Mesohabitats determined *a priori*

The discriminant analysis distinguished the groups of riffle, pool and run mesohabitats predefined according to hydraulics and structural characteristics from middle reach (Wilks' lambda = 0.0017,  $F = 10.96$ ,  $p < 0.001$ ). Average depth and cobble substrate percentage were the variables that significantly influenced the separation of groups (Table 2). Riffles had

Table 1. Mean  $\pm$  standard deviation of abiotic variables and main substrate bottom for sampling sites in headwater, middle and mouth reaches of the Vermelho River, Paraná state, Brazil. Temp. = temperature, DO = dissolved oxygen, Cond. = conductivity. Deviations represent environmental variation between May 2009 and February 2010. \* Sites at middle reach were classified as riffles, runs and pools.

	Sites	Water vel. (m.s <sup>-1</sup> )	Depth (cm)	Channel width (m)	Temp. (°C)	DO (mg.l <sup>-1</sup> )	pH	Cond. (µS/cm <sup>-1</sup> )	Boulders (%)
Headwater	Site 1	7.0 $\pm$ 2.1	10.6 $\pm$ 2.9	3.1 $\pm$ 0.9	20.0 $\pm$ 2.4	7.7 $\pm$ 1.2	7.0 $\pm$ 0.4	45.1 $\pm$ 4.9	55 $\pm$ 8.7
	Site 2	4.4 $\pm$ 2.2	21.8 $\pm$ 4.8	4.1 $\pm$ 1.0	20.1 $\pm$ 2.5	7.8 $\pm$ 0.9	7.2 $\pm$ 0.4	41.6 $\pm$ 2.6	36,3 $\pm$ 6.5
	Site 3	6.6 $\pm$ 1.8	14 $\pm$ 5.9	2.9 $\pm$ 1.0	20.1 $\pm$ 2.3	7.4 $\pm$ 1.4	7.3 $\pm$ 0.3	42.1 $\pm$ 2.3	37,5 $\pm$ 8.3
	Site 4	5.5 $\pm$ 1.8	23.4 $\pm$ 8.3	2.6 $\pm$ 1.0	20.1 $\pm$ 2.3	7.9 $\pm$ 1.5	7.4 $\pm$ 0.3	41.8 $\pm$ 1.2	75 $\pm$ 5
	Pool1	1.7 $\pm$ 1.8	28.1 $\pm$ 5.1	2.7 $\pm$ 0.4	20.6 $\pm$ 2.4	8.0 $\pm$ 0.7	6.5 $\pm$ 0.5	40.7 $\pm$ 1.6	1.3 $\pm$ 2.2
Middle*	Riffles1	6.4 $\pm$ 1.8	8.2 $\pm$ 2.2	2.6 $\pm$ 0.3	20.5 $\pm$ 2.3	7.8 $\pm$ 0.5	7.0 $\pm$ 0.6	39.3 $\pm$ 4.2	1.3 $\pm$ 2.2
	Runs1	2.0 $\pm$ 1.5	21.5 $\pm$ 8.2	2.8 $\pm$ 0.4	20.6 $\pm$ 2.2	7.1 $\pm$ 0.4	7.1 $\pm$ 0.4	39.4 $\pm$ 4.0	0 $\pm$ 0
	Riffles2	6.5 $\pm$ 3.1	12.0 $\pm$ 5.2	2.0 $\pm$ 0.8	20.7 $\pm$ 2.4	7.5 $\pm$ 0.7	7.2 $\pm$ 0.3	39.4 $\pm$ 4.8	0 $\pm$ 0
	Runs2	1.2 $\pm$ 1.5	21.6 $\pm$ 4.7	3.7 $\pm$ 0.2	20.6 $\pm$ 2.3	7.3 $\pm$ 0.7	7.2 $\pm$ 0.4	39.3 $\pm$ 4.3	0 $\pm$ 0
Mouth	Pool2	0.7 $\pm$ 0.6	31.9 $\pm$ 6.4	3.5 $\pm$ 0.3	20.7 $\pm$ 2.3	7.6 $\pm$ 1.0	7.2 $\pm$ 0.4	39.3 $\pm$ 4.4	0 $\pm$ 0
	Site 1	2.1 $\pm$ 1.0	35.2 $\pm$ 20.0	3.9 $\pm$ 0.8	20.4 $\pm$ 3.3	7.5 $\pm$ 1.1	6.1 $\pm$ 0.2	38.8 $\pm$ 4.4	0 $\pm$ 0
	Site 2	4.2 $\pm$ 1.4	26.4 $\pm$ 22.7	4.2 $\pm$ 1.0	20.5 $\pm$ 3.3	7.3 $\pm$ 1.0	6.7 $\pm$ 0.6	36.6 $\pm$ 5.2	0 $\pm$ 0
	Site 3	1.4 $\pm$ 1.5	45.9 $\pm$ 29.7	5.4 $\pm$ 1.1	20.5 $\pm$ 3.3	7.6 $\pm$ 0.9	6.7 $\pm$ 0.5	39.9 $\pm$ 1.7	0 $\pm$ 0

	Sites	Boulders (%)	Cobbles (%)	Pebbles (%)	Gravel (%)	Sand (%)	Sand/Clay (%)	Trunk/branch (%)	Leaf litter (%)
Headwater	Site 1	55 $\pm$ 8.7	30 $\pm$ 0	8.8 $\pm$ 4.1	6.3 $\pm$ 5.4	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
	Site 2	36,3 $\pm$ 6.5	35 $\pm$ 6.1	15 $\pm$ 5	13.8 $\pm$ 8.1	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
	Site 3	37,5 $\pm$ 8.3	33.8 $\pm$ 10.8	6.3 $\pm$ 2.1	22.5 $\pm$ 14.4	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
	Site 4	75 $\pm$ 5	17.5 $\pm$ 4.3	1.3 $\pm$ 2.1	6.3 $\pm$ 5.4	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
	Pool1	1.3 $\pm$ 2.2	3.8 $\pm$ 2.2	3.8 $\pm$ 2.2	57.5 $\pm$ 17.9	2.5 $\pm$ 4.3	23.8 $\pm$ 12.9	0 $\pm$ 0	7.5 $\pm$ 13
Middle*	Riffles1	1.3 $\pm$ 2.2	26.3 $\pm$ 13.9	67.5 $\pm$ 10.9	2.5 $\pm$ 2.5	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	2.5 $\pm$ 2.5
	Runs1	0 $\pm$ 0	28.8 $\pm$ 11.3	35 $\pm$ 5	23.8 $\pm$ 9.6	0 $\pm$ 0	2.5 $\pm$ 4.3	5 $\pm$ 3.5	5 $\pm$ 3.5
	Riffles2	0 $\pm$ 0	72.5 $\pm$ 4.3	18.8 $\pm$ 4.1	1.3 $\pm$ 2.2	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	7.5 $\pm$ 5.6
	Runs2	0 $\pm$ 0	27.5 $\pm$ 8.3	12.5 $\pm$ 4.3	46.3 $\pm$ 4.1	1.3 $\pm$ 2.2	0 $\pm$ 0	5 $\pm$ 6.1	7.5 $\pm$ 5.6
Mouth	Pool2	0 $\pm$ 0	16.3 $\pm$ 8.2	8.8 $\pm$ 2.2	57.5 $\pm$ 4.3	0 $\pm$ 0	0 $\pm$ 0	6.3 $\pm$ 4.1	11.3 $\pm$ 7.4
	Site 1	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	2.5 $\pm$ 4.3	28.8 $\pm$ 18.2	31.3 $\pm$ 20.1	17.5 $\pm$ 2.5	20 $\pm$ 6.1
	Site 2	0 $\pm$ 0	0 $\pm$ 0	15 $\pm$ 15	35 $\pm$ 8.7	12.5 $\pm$ 8.3	2.5 $\pm$ 4.3	28.5 $\pm$ 7.4	6.3 $\pm$ 4.1
	Site 3	0 $\pm$ 0	0 $\pm$ 0	13,8 $\pm$ 6.5	5 $\pm$ 0	1.3 $\pm$ 2.1	26.3 $\pm$ 6.5	25 $\pm$ 3.5	28.8 $\pm$ 5.4

higher percentage of cobbles, while pools were deeper and had the bottom covered mostly by leaf litter, and runs presented intermediate abiotic conditions between riffles and pool.

#### Richness and diversity

A total of 1,495 fish individuals were captured, belonging to 27 species, 12 families and six orders. Siluriformes contributed with 14 (51.9%), Characiformes with seven (25.9%), and the other orders had only one or two species (Table 3).

Fish species richness increased along the stream, with 13, 18 and 22 species in headwater, middle and mouth reaches, respectively (Table 3). Rarefaction analysis showed that the expected richness was also higher at the mouth reach, although an asymptote was not attained in its rarefaction curve (Fig. 4). Species composition also differed among reaches: from 27

species, only nine, particularly the characids, *Deuterodon langei* Travassos, 1957, *Mimagoniates microlepis* (Steindachner, 1876), *Characidium lanei* Travassos, 1967 and *Characidium pterostictum* Gomes, 1947 occurred throughout the stream.

In the headwater reach, *C. lanei*, *D. langei* and *Rhamdioglanis frenatus* Ihering, 1907 were the most abundant, while *Ituglanis proops* (Miranda Ribeiro, 1908) was rare and exclusive. In the middle reach, in addition to *C. lanei* and *D. langei*, *M. microlepis* also predominated. *Rivulus luelingi* Seegers, 1984, with only one individual, was exclusive. At the mouth reach, abundances were higher for *M. microlepis*, *Pseudotothyris obtusa* (Miranda Ribeiro, 1911) and *C. lanei*, while *Astyanax* aff. *ribeirae* Eigenmann, 1911, *Hoplias malabaricus* (Bloch, 1794) and *Gymnotus* spp. had only one individual each (Table 3).

Shannon diversity and evenness had their highest absolute values in the middle reach (Table 3). Beta diversity

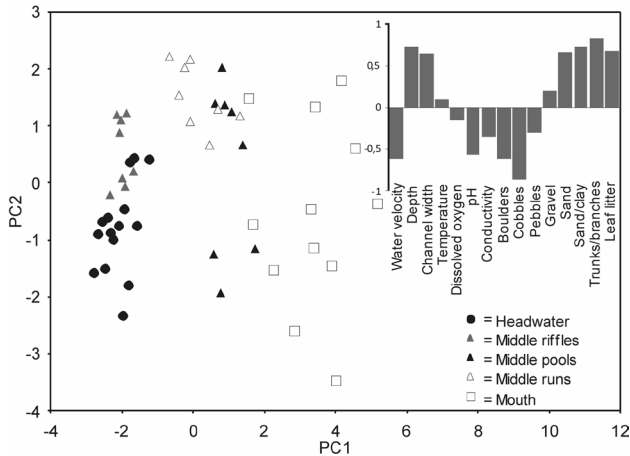


Figure 3. Scatter plot of sampling sites and mesohabitats in the Vermelho River, Paraná state, Brazil, along the environmental gradient produced by the first two PCA axes (PC1 and PC2). Inner figure shows Pearson correlations of variables with PC1.

Table 2. Results of discriminant analysis with environmental variables that contributed to separation of groups of mesohabitats (i.e., riffles, pools and runs) in the middle reach of the Vermelho River. Bold values are significant ( $p < 0.05$ ).

	Axis 1	Axis 2	Wilks' Lambda	Partial Lambda	F	p
Depth	<b>-2.187</b>	-0.059	<b>0.0052</b>	<b>0.321</b>	<b>7.39</b>	<b>0.019</b>
Water velocity	-0.369	-0.240	0.0017	0.983	0.06	0.942
Cobbles	<b>1.647</b>	2.591	<b>0.0053</b>	<b>0.314</b>	<b>7.65</b>	<b>0.017</b>
Pebbles	0.417	1.142	0.0028	0.593	2.40	0.161
Leaf litter	-0.144	-2.953	0.0032	0.526	3.15	0.106
Sand	1.604	0.828	0.0035	0.474	3.88	0.074
Gravel	-0.220	1.042	0.0028	0.597	2.36	0.165
Sand/clay	-0.384	1.619	0.0023	0.730	1.29	0.333
pH	1.577	1.277	0.0034	0.488	3.67	0.081
Dissolved oxygen	0.119	-1.352	0.0025	0.673	1.70	0.251
Trunks/branches	-1.192	-0.646	0.0028	0.598	2.36	0.165
Temperature	-1.506	0.622	0.0027	0.622	2.13	0.190
Conductivity	-2.440	2.088	0.0028	0.595	2.38	0.163
Boulders	0.725	-0.355	0.0022	0.761	1.10	0.385
Channel width	-1.410	0.520	0.0022	0.772	1.03	0.405
Eigenvalues	89.2	5.6				
%	94	100				

indicated greater addition than replacement of fish species along the stream. The index values were low when comparing headwater with middle ( $\beta_2 = 0.23$ ) and middle with mouth reaches ( $\beta_2 = 0.30$ ). Poeciliid, gymnotid and callichthyid species were the main additions, while *Ancistrus multispinnis* (Regan, 1912) and *R. frenatus* were the main replacements downstream (Table 3).

Table 3. Number of individuals per fish species collected and ichthyofauna descriptors in headwater, middle and mouth reaches in the Vermelho River, Paraná state, Brazil. In bold, predominant species.

Species	Voucher specimens	Headwater	Middle	Mouth
<b>Characiformes</b>				
<b>Characidae</b>				
<i>Deuterodon langei</i> Travassos, 1957	NUP9541	<b>105</b>	<b>155</b>	33
<i>Hollandichthys multifasciatus</i> (Eigenmann & Norris, 1900)	NUP9529	4	36	13
<i>Mimagoniates microlepis</i> (Steindachner, 1876)	NUP9526	10	<b>73</b>	<b>202</b>
<i>Astyanax aff. ribeirae</i> Eigenmann, 1911	NUP9742	–	2	1
<b>Crenuchidae</b>				
<i>Characidium lanei</i> Travassos, 1967	NUP9525	<b>117</b>	<b>111</b>	<b>38</b>
<i>Characidium pterostictum</i> Gomes, 1947	NUP9588	29	22	30
<b>Erythrinidae</b>				
<i>Hoplias malabaricus</i> (Bloch, 1794)	NUP19193	–	–	1
<b>Cyprinodontiformes</b>				
<b>Poeciliidae</b>				
<i>Phalloceros harpagos</i> Lucinda, 2008	NUP9527	–	27	6
<b>Rivulidae</b>				
<i>Rivulus luelingi</i> Seegers, 1984	NUP19198	–	1	–
<b>Gymnotiformes</b>				
<b>Gymnotidae</b>				
<i>Gymnotus pantherinus</i> (Steindachner, 1908)	–	–	4	1
<i>Gymnotus carapo</i> Linnaeus, 1758	NUP19194	–	–	1
<b>Perciformes</b>				
<b>Gobiidae</b>				
<i>Awaous tajassica</i> (Lichtenstein, 1822)	NUP19195	–	–	4
<b>Siluriformes</b>				
<b>Callichthyidae</b>				
<i>Scleromystax barbatus</i> (Quoy & Gaimard, 1824)	NUP9570	–	16	21
<b>Loricariidae</b>				
<i>Ancistrus multispinnis</i> (Regan, 1912)	NUP13626	12	9	–
<i>Schizolecis guntheri</i> (Miranda Ribeiro, 1918)	NUP9465	7	70	5
<i>Schizolecis</i> sp. 1	NUP10903	–	–	32
<i>Schizolecis</i> sp. 2	NUP10904	8	41	–
<i>Hisonotus leucofrenatus</i> (Miranda Ribeiro, 1908)	–	–	–	3
<i>Kronichthys cf. lacerta</i> (Nichols, 1919)	NUP10900	–	–	11
<i>Pseudotothyris obtusa</i> (Miranda Ribeiro, 1911)	NUP10902	–	–	<b>59</b>
<i>Rineloricaria</i> sp.	NUP10898	–	12	12
<b>Heptapteridae</b>				
<i>Acentronichthys leptos</i> Eigenmann & Eigenmann, 1889	NUP9592	19	17	2
<i>Rhamdioglanis frenatus</i> Ihering, 1907	NUP13614	<b>32</b>	9	–
<i>Pimelodella pappenheimi</i> Ahl, 1925	NUP13613	–	–	6
<i>Rhamdia quelen</i> (Quoy & Gaimard, 1824)	NUP9524	4	35	4
<b>Trichomycteridae</b>				
<i>Ituglanis proops</i> (Miranda Ribeiro, 1908)	NUP19197	2	–	–
<b>Synbranchiiformes</b>				
<b>Synbranchidae</b>				
<i>Synbranchius marmoratus</i> Bloch, 1795	NUP9567	4	5	12
Richness		13	18	22
Abundance		353	645	497
Shannon diversity		1.87	2.35	2.17
Evenness		0.73	0.81	0.70

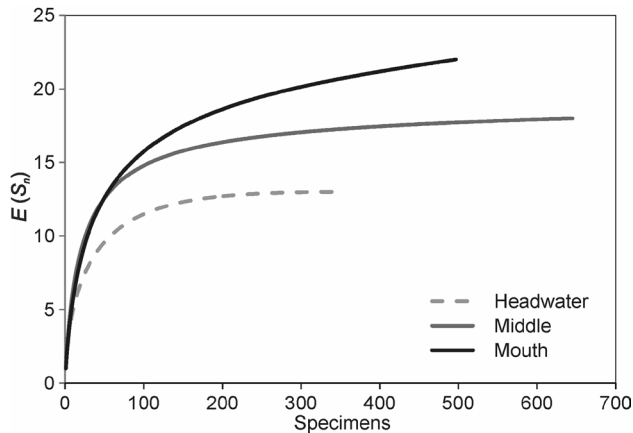


Figure 4. Species rarefaction curves for reaches in the Vermelho River, Paraná state, Brazil.  $E(S_n)$  denotes the expected number of species according to abundance.

#### Longitudinal and local fish community structure

The first DCA axis retained for interpretation ( $\lambda_1 = 0.50$ ) classified the ichthyofauna structure into headwater, middle and mouth reaches and explained 21.3% of total variance (Fig. 5). *Kronichthys cf. lacerta* (Nichols, 1919), *Awaous tajasica* (Lichtenstein, 1822) and *Schizolecis* sp. 1 were positively correlated with DCA1, while *A. multispinnis*, *Acentronichthys leptos* Eigenmann & Eigenmann, 1889 and *R. frenatus* were negatively correlated. For DCA2, *I. proops* and *Gymnotus carapo* Linnaeus, 1758 were positively correlated, while *Kronichthys cf. lacerta* and *R. luelingi* were negatively correlated. For the DCA1 scores, there was a significant difference in fish structure only between the upper reaches (headwater, middle) and the mouth reach (Kruskal-Wallis test = 31.66,  $p < 0.05$ ; multiple comparisons  $p < 0.05$ ). Headwater and middle reaches did not differ because of the similarity in fish structures between middle riffles and headwater sites. These habitats shared many lotic species, such as *A. multispinnis*, *A. leptos* and *Characidium* spp. Middle pools showed a greater similarity in regard to fish structure with lentic sites of the mouth reach, sharing exclusive species such as *Scleromystax barbatus* (Quoy & Gaimard, 1824) and *Phalloceros harpagos* Lucinda, 2008 in addition to a great number of individuals of *M. microlepis*.

In the middle reach, fish richness and abundance were higher in pools and runs (Table 4). *Phalloceros harpagos*, *Gymnotus pantherinus* (Steindachner, 1908), *S. barbatus*, *Rhamdia quelen* (Quoy & Gaimard, 1824) and *Synbranchus marmoratus* Bloch, 1795 were exclusive to pools and runs; *Acentronichthys leptos* to riffles and runs; *Astyanax aff. ribeirae* to pools and *R. luelingi* to riffles. Other species occurred throughout all mesohabitats. However, we observed a clear pattern, where lentic habitat fishes (i.e. *D. langei*, *M. microlepis* and *Hollandichthys multifasciatus*

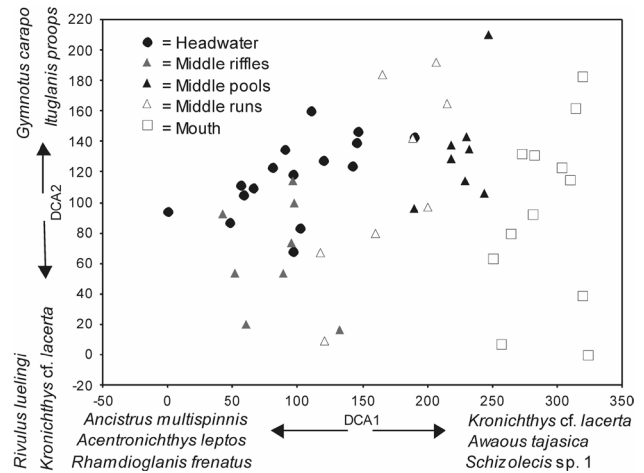


Figure 5. Ordination of the sampling sites through a detrended correspondence analysis (DCA) applied to the density matrix of fish species in the Vermelho River, Paraná state, Brazil.

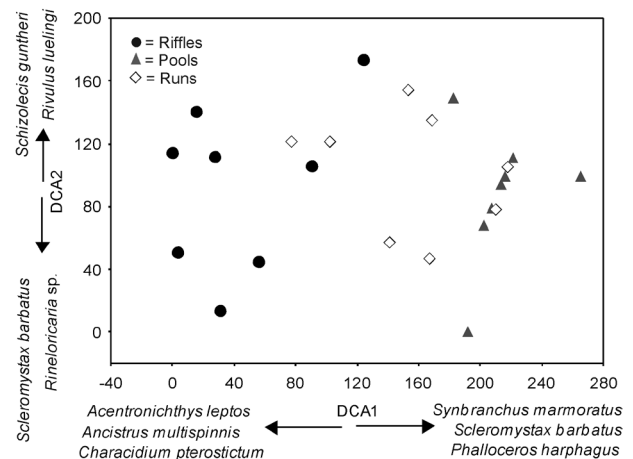


Figure 6. Ordination of sampling mesohabitats in the middle reach through a detrended correspondence analysis (DCA) applied to density matrix of fish species in the Vermelho River, Paraná state, Brazil.

(Eigenmann & Norris, 1900)), were more abundant in pools, while fast-current dwelling (i.e. *Characidium* spp., *A. leptos* and *A. multispinnis*), were more abundant in riffles. Runs tended to present intermediate abundance for most of the species. For mesohabitat fish structure, the first DCA axis retained ( $\lambda_1 = 0.49$ ), explained 33% of total variance, segregating pools (positive scores) from riffles (less positive scores) (Fig. 6). *Synbranchus marmoratus*, *S. barbatus* and *P. harpagos* were positively correlated with DCA1, while *A. leptos*, *A. multispinnis* and *C. pterostictum* were negatively correlated.



Table 4. Number of individuals per fish species collected and richness in riffle, pool and run mesohabitats of middle reach in the Vermelho River, Paraná state, Brazil. In bold, predominant species.

Species	Riffles	Pools	Runs
<b>Characiformes</b>			
<i>Deuterodon langei</i> Travassos, 1957	7	<b>103</b>	45
<i>Hollandichthys multifasciatus</i> (Eigenmann & Norris, 1900)	1	<b>27</b>	8
<i>Mimagoniates microlepis</i> (Steindachner, 1876)	1	<b>62</b>	10
<i>Astyanax aff. ribeirae</i> Eigenmann, 1911	–	2	–
<i>Characidium lanei</i> Travassos, 1967	<b>64</b>	17	30
<i>Characidium pterostictum</i> Gomes, 1947	<b>12</b>	2	8
<b>Cyprinodontiformes</b>			
<i>Phalloceros harpagos</i> Lucinda, 2008	–	22	5
<i>Rivulus luelingi</i> Seegers, 1984	1	–	–
<b>Gymnotiformes</b>			
<i>Gymnotus pantherinus</i> (Steindachner, 1908)	–	1	3
<b>Siluriformes</b>			
<i>Scleromystax barbatus</i> (Quoy & Gaimard, 1824)	–	11	5
<i>Ancistrus multispinnis</i> (Regan, 1912)	5	1	3
<i>Schizolecis guntheri</i> (Miranda Ribeiro, 1918)	25	18	27
<i>Schizolecis</i> sp. 2	10	16	15
<i>Rineloricaria</i> sp.	1	5	6
<i>Acentronichthys leptos</i> Eigenmann & Eigenmann, 1889	<b>13</b>	–	4
<i>Rhamdioglanis frenatus</i> Ihering, 1907	3	3	3
<i>Rhamdia quelen</i> (Quoy & Gaimard, 1824)	–	<b>30</b>	5
<b>Synbranchiiformes</b>			
<i>Synbranchus marmoratus</i> Bloch, 1795	–	4	1
Richness	12	16	16
Abundance	143	324	178

## DISCUSSION

Based on the literature, between 20 and 50 fish species are estimated to occur in coastal Atlantic streams of Paraná state (Fogaça et al. 2003, Barreto and Aranha 2005, Abilhoa and Bastos 2009, Guimarães et al. 2010). Channels located on steep slopes of the Serra do Mar mountains and geographical isolation due the subdivision of eastern Atlantic basin in several sub basins, have conferred to fish smaller sizes and higher degree of endemism (Vari and Malabarba 1998, Menezes et al. 2007). Here, the Vermelho River presented similar fish richness (27 species) and composition similarities to 30 species recorded by Barreto and Aranha (2005) in a stream on the northern coast of Paraná, which is similar in length. As expected to fish communities in coastal Atlantic streams, we sampled fish with small-sized bodies and species such as *D. langei*, *M. microlepis* and *H. multifasciatus* which are not endemic but typically occur in coastal basins (Menezes et al. 2007).

We also registered three species that are probably new. *Rineloricaria* sp. corresponds to *Rineloricaria* sp. 1 from Abilhoa and Bastos (2009), with three dark-brown bands on dorsal region of the body between dorsal and caudal fins. *Schizolecis guntheri*

presents a large dark stripe along the sides of the body and a small light area within a blotch on the base of the caudal fin (Abilhoa and Bastos 2009). However, *Schizolecis* sp. 1 and *Schizolecis* sp. 2 differed because both do not present a continuous dark stripe. The first has a large blotch above the pectoral fin and another one in the base of the caudal fin, while the latter has similar large blotches, but with irregular dots and longitudinal small bars along the body. We also initially identified two specimens as a distinct species of *Characidium*. However, this genus includes probable cases of interspecific hybridization (Pansonato-Alves et al. 2014), generating individuals with intermediate morphologies. In the study region, *C. lanei* and *C. pterostictum* live in sympatry, which increases the probability of hybridization between this two species (Paulo A. Buckup, pers. comm.). After a review of photographs, the two specimens were tentatively identified as *C. lanei* because of the higher color similarity (Abilhoa and Bastos 2009). Reanalysis of the data, after this reidentification, presented slight, but not significant differences in outputs, which did not change the interpretation of ecological patterns.

The richness and species rarefaction increased in headwater-mouth direction, while Shannon diversity was higher in the middle reach. Similar results were reported by Casatti (2005), who argued that the increase in richness and in diversity is likely related to higher structural complexity downstream. The middle reach of the Vermelho River showed typical characteristics of a transitional environment between the headwater reach (rocky bottom and high water velocity) and mouth reach (sandy substrate and low water velocity). Thus, the sampling sites of middle reach, which were scattered at the intermediate abiotic gradient of the PCA, presented higher environmental variability, which provides a more diversified meso- and microhabitats, allowing a more diversified fish fauna to inhabiting this reach.

The Vermelho River had higher rates of species addition than replacement along its course, corroborating results of Petry and Schulz (2006), who reported higher addition over replacement in the Sinos River, southern Brazil. Higher additions occur in rivers with few abrupt geomorphological transitions, enabling a gradual adjustment in fish composition to different environmental conditions downstream (Rahel and Hubert 1991). For the Vermelho River, which shows a pronounced slope between headwater and mouth reaches, the most frequent species additions likely related to increased volume and availability of different habitats downstream and, also, to the ingress of estuarine species, such as *A. tajasica*, in the mouth reach. However, the replacements of *A. multispinnis* and *R. frenatus*, which did not occur in the mouth reach, are likely associated with the longitudinal decrease of water velocity and changes from a rocky substrate to sandy bottom downstream. *Ancistrus multispinnis* inhabits moderate to fast currents dwelling on boulders and cobbles (Barreto and Aranha 2005) and presents larger ventral sucker mouth than other loricariids, which allows it to adhere to the substratum (Breda et al. 2005). A congeneric of *R. frenatus*, *Rhamdioglanis transfaciatus* Miranda Ribeiro, 1908 with

similar elongated body shape, inhabits upper fast-water reaches (Guimarães et al. 2010), indicating the capacities of these species of swimming among boulders and cobbles.

The DCA and statistical tests showed that fish community structure differed only between upper and mouth reaches. Fast-water sites from headwater reach did not differ from middle riffles because these sites share similar composition and density of fish. On the other hand, the middle pool ichthyofauna was more similar to lentic habitats of the mouth reach. According to Frissell et al. (1986), due to the hierarchical nature of river systems, processes at large scales will ultimately influence fish structures at smaller spatial scales. However, the similarity in fish fauna between fast- and slow-moving water habitats in different reaches of the Vermelho River might reflect the low influence of large-scale processes, such as human activities, in its surrounds. In turn, different reaches could be offering similar conditions to the input of allochthonous matter and filtering mechanisms to reduce sedimentation along the entire stream, maintaining similarity in some of the fish-local habitats associations at a larger scale. Additionally, we observed higher environmental similarity, such as rocky substrate and fast waters between headwater and middle riffles habitats, which is an indicative of similar fish structures in function of their habitat (Taylor 2000, Langeani et al. 2005).

Similarly, Cheek et al. (2016) observed that landscape- and stream reach-scale variables had a low degree of explanation for the variation of the fish structure in a minimally disturbed stream when compared to other mesoscale variables. These findings open the discussion about the strength that finer spatial scales might have on ichthyofauna structure in preserved systems, since environmental homogenization in impacted streams (Olden et al. 2004) could obscure the local fish patterns included in broader scales. For Vermelho River, which is a preserved stream, the middle reach has higher local environmental heterogeneity when compared with the headwater and mouth reaches, creating some combinations of fish-mesohabitat associations. At middle reach, the mesohabitat scale seems to transcend longitudinal processes, not completely differentiating its fish structure from the others reaches, but mixing fish composition from upper and lower reaches in the structuring of local mesohabitats. Therefore, results might be interpreted as an alternative to mesohabitat-specific fish distributions among riffles, runs and pools according to fish adaptations. In this sense, it is expected that from middle reach to upstream or to downstream, the fish-habitat associations are more homogeneous in function of the greater longitudinal scale influence.

Analyzing only longitudinal scale, similar patterns in fish distribution among river segments were identified by Ferreira and Petrer Jr (2009), who established altitudinal zones with a predominance of different fish families in streams of the Atlantic coast, São Paulo. At reach scale, fish communities in the Vermelho River seem to reflect adjustments in the specific composition according to the abiotic gradient. Therefore, the stream might be

characterized by a headwater reach with a poorer ichthyofauna, with species such as *A. multispinnis* and *Characidium* spp., which are adapted to high water velocity; middle reach with the highest diversity, fish-mesohabitat structuring and an increase in abundance of water column dwelling species, such as characids and poeciliids; and a mouth reach, within boundaries of the coastal plain, with slow water velocity and deeper environments, that favor colonization by characids such as *M. microlepis*, loricariids of lentic habitats, and eventually estuarine species.

Focusing only on the mesohabitat scale, several authors recognize that within fish community, some species preferably inhabit riffles or runs while others inhabit pools (Taylor 2000, Langeani et al. 2005). Likewise, Rezende et al. (2010) identified and established fish species as indicators of mesohabitats according to their relative densities in riffles and pools in a coastal stream. In the Vermelho River, although headwater and mouth reaches are the most environmentally homogeneous habitats, the middle reach was geomorphologically structured into riffles, pools and runs. In riffles, which are hydrologically more dynamic mesohabitats, the ichthyofauna probably directs large amounts of energy to maintain their position against the current (Matthews and Styron Jr 1981). Energy-saving strategies could explain the high relationship of some species, such as *A. multispinnis*, and *Characidium* spp., with the middle riffles, since these species have depressed and fusiform bodies, minimizing the energy cost of swimming in fast waters (Breda et al. 2005). Barreto and Aranha (2005) observed *A. multispinnis* and *C. pterostictum* in moderate to torrential currents, the latter maintaining themselves by standing on their pectoral and pelvic fins in protected areas behind cobbles, possibly to reduce energy expenditure.

On the other hand, pools have been considered as mesohabitats with the highest habitat availabilities and environmental complexity, which may support a greater number of species (Taylor 2000, Langeani et al. 2005). We also reported higher richness and habitat preferences for *P. harpagos*, *S. barbatus* and *S. marmoratus* in pools. Similar to our findings, *P. harpagos* was defined as an indicator of pools by Rezende et al. (2010), while *S. barbatus* occurs in areas with slow currents on sandy substrate, and *S. marmoratus* occurs in holes of clay margins (Barreto and Aranha 2005). In addition, pools presented higher amounts of trunks, branches, leaf litter and were deeper, that favors the colonization by characids with laterally compressed body, such as *D. langei* and *M. microlepis*. Since pools were more environmentally structured, they might also represent a refuge from predators (Matthews 1998).

Different from riffles and pools, totally segregated from one another, fish community structure of runs was slightly similar in composition with other mesohabitats. However, runs harbor a high number of lentic species such as *D. langei*, *M. microlepis* and *H. multifasciatus* in relation to riffles, and an intermediate abundance of fast-water fish, such as *Characidium* spp. Similar to our study, where the ichthyofauna was not completely distinct in composition, but mainly in proportional abundance, Rezende

et al. (2010) found no indicator species for runs. As there are no boundaries between mesohabitats to prevent fish movement, species may be using runs to forage or as transient areas to access their preferential sites. In this sense, these runs would act as ecotones between riffle and pool mesohabitats (Jowett 1993).

In short, our results showed differences in composition and structure of ichthyofauna in the Vermelho River, related both to reach and mesohabitat scales. These results reveal differences in distribution, abundance and ecological requirements of species and suggest that environmental differences along stream determine the wider structural patterns of ichthyofauna. However, combining reach and habitat spatial scales, some patterns in fish similarities showed that the middle reach is a transitional heterogeneous area that congregates species from the headwater and mouth reaches, but is locally structured in fish-mesohabitat associations. Additionally, these local patterns revealed distinct fish structures between riffles and pools, which likely imply distinct local ecological interactions. Our findings reflect the preserved conservation status of the Vermelho River, contributing to identifying possible direct impacts on fish fauna along longitudinal gradients in other Atlantic coastal streams.

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### Supplementary material 1

#### Figure S1. Hydrological and structural characteristics of the sampling reaches of the Vermelho River, state of Paraná, Brazil

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Data type: specimens data

Explanation note: (A) Cascaded channel in the headwater; (B, C and D) riffles, pool and runs mesohabitats, respectively, in the middle; (E-F) slow-moving waters habitats in the mouth.

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