

Assessing the importance of the riparian zone for stream fish communities in a sugarcane dominated landscape (Piracicaba River Basin, Southeast Brazil)

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Received: 4 August 2014 / Accepted: 18 February 2015 / Published online: 11 March 2015
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Abstract In the Neotropics, the effect of agriculture on freshwater habitats is still poorly understood, particularly with respect to sugarcane expansion to meet increased ethanol demands. The variation in taxonomic composition, community attributes and trophic structure of fish assemblages from streams under different riparian zone preservation conditions were studied. Nine stream reaches under the following riparian vegetation conditions were selected: Native Forest (NF), with mostly primary forest; Secondary Forest (SF), which included sites with vegetation in an advanced stage of regeneration surrounded by sugarcane plantations; and Sugarcane sites (SC), located in areas without riparian vegetation, adjacent to sugarcane crops. Forty-one species were collected. The variance partitioning and partial Redundancy Analysis (pRDA) indicated that community composition and trophic structure were significantly explained by riparian condition and spatial variations, while diversity, richness, abundance and biomass were

only explained by season and space, with higher diversity and richness at the SF sites. Abundance-Biomass curves (ABC curves) suggested that sugarcane plantations destabilized fish assemblages, especially at the more intensively managed sites, which seem to influence stream integrity and associated fish fauna. We concluded that both physical conditions of streams and some attributes of fish communities reflected the different levels of preservation of the riparian buffers, but given the influence of regional processes which have a pervasive role in shaping local assemblages, land use at the watershed scale seemed to be important especially to explain the higher richness and diversity found at the SF sites.

Keywords Deforestation · Ichthyofauna · Agro-ecosystem · Neotropical region · Land use

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Introduction

The influence of land cover on the biological and physical conditions of streams has been intensively studied in temperate regions, indicating that when landscapes are converted from forests and wetlands to agricultural and urban areas, biological communities are affected in predictable ways (Stanfield and Kilgour 2013). Such changes often imply in the reduction of the riparian forests, which affect the functioning of stream ecosystems (Allan et al. 1997). Riparian vegetation acts on regulating aquatic food webs, providing material subsidies through inputs of terrestrially derived food, and

providing temperature, light and structural protection of habitats (Naiman and Décamps 1997; Pusey and Arthington 2003; Iwata et al. 2012). In addition, riparian zones help maintaining water quality by retaining sediments and contaminants from surrounding areas (Gregory et al. 1991; Wang et al. 1997; Harding et al. 1998; Dudgeon 2008) and maintain in-stream habitat heterogeneity, by regulating flow and discharge, being an important buffer against the impacts of deforestation (Naiman and Décamps 1997). Therefore, riparian vegetation has a multi-scale environmental influence on aquatic communities (e.g., fish and invertebrates) and their trophic dynamics (Gregory et al. 1991; Rabeni and Smale 1995; Allan and Castillo 2007; Lorion and Kennedy 2009) and has become an integral component of management strategies for stream biodiversity conservation (Naiman and Décamps 1997).

Several studies have found negative effects of deforestation on fish in tropical stream ecosystems (Bojsen and Barriga 2002; Wright and Flecker 2004; Casatti et al. 2009; Lorion and Kennedy 2009; Iwata et al. 2012), observing severe reduction in habitat quality, fish diversity, and changes in food resource availability. In agricultural regions, impacts are even more intense, affecting water quality, biodiversity, sedimentation and nutrient levels (Corbi et al. 2006; Riseng et al. 2011). Fewer insect species and intolerant fish species have also been recorded in agricultural areas (Wang et al. 1997; Genito et al. 2002), with observed changes in the diet of characids as reported by Ferreira et al. 2012a in agricultural regions of the Piracicaba River Basin.

Although agricultural expansion alone cannot explain the deforestation rates observed in the past, both processes have long been connected in Brazil (Gibbs et al. 2010). The biggest threat posed by expanding the amount of land under cultivation for energy, or any other use, is the irreversible conversion of virgin ecosystems, which may affect the hydrologic cycle and climate, reducing regional precipitation and increasing temperatures (Goldemberg et al. 2008). Sugarcane is among the economically most important crops in Brazil, with the largest area under cultivation in the world, accounting for approximately one third of global production (Zuubier and Vooren 2008). In the Southeastern region of the country, specifically in the state of São Paulo, the crop has expanded from ~18,000 km² in 1990 to 52,000 km² in 2011 (MAPPC 2012), occupying approximately 25 % of the state's agricultural area. In several

cases, riparian zones, considered priority areas for biodiversity conservation (Barletta et al. 2010; SMA 2011), are surrounded by sugarcane fields and recent surveys indicate a deficit of riparian vegetation cover in 1,400,000 hectares in the state, of which 300,000 ha are in sugarcane industry land (SMA 2011). The Piracicaba, Capivari and Jundiá River Basins (PCJ Basins) have a long history of human occupation and environmental change, with 72.7 % of the basin conformed by pastures and sugarcane (CBH-PCJ 2011). Between 2004 and 2011 there was an increase of 258,682 ha of sugarcane crop area in these basins (IRRIGART 2007; CBH-PCJ 2011).

Land use change toward sugarcane production has raised a number of questions regarding its negative consequences and sustainability. According to Goldemberg et al. 2008, although there are several positive impacts, its negative aspects are related to the destruction or damage of high biodiversity areas, deforestation, soil damage and degradation, water resource contamination or depletion and diminished food security. Several studies have addressed the environmental sustainability issue of sugarcane cultivation with respect to aquatic systems, focusing on sedimentation (Fiorio et al. 2000), transport of contaminants like pesticides, heavy metals, and herbicides (atrazine) (Corbi et al. 2006), the origin of organic matter (Martinelli et al. 1999) and effects of land use on water chemistry and macroinvertebrates (Ometto et al. 2000). Bunn et al. 1997 and Ferreira et al. 2012b used multiple-stable isotope analysis to identify sources of organic carbon supporting food webs in aquatic systems in catchments with different riparian zones. The latter authors showed that in regions where the buffer zone was not covered by riparian forest, there was a significant increase in C₄ carbon (sugarcane and pasture). Given the number and importance of the links between riparian and lotic ecosystems, spatial and temporal variation in fish assemblage composition and ecological attributes (i.e., species richness, dominance, abundance and biomass) have been linked to the variation in riparian cover (Godinho 2008).

Considering that the way in which species respond to anthropogenic disturbance is usually complex and strongly influenced by several ecological processes (Hayes et al. 1996), it is often difficult to predict how a particular aquatic-riparian ecosystem will change following a management activity (Bisson et al. 1996). Thus, the aims of this study were to investigate which

environmental factors are important in determining the distribution of fish species in streams with riparian zones under different conditions of preservation in the Piracicaba River basin, and the amount of variation that can be assigned solely to the riparian condition or to other factors as season and spatial variability. We hypothesized that streams in sugarcane plantation areas would exhibit altered habitat conditions and fish communities compared with sites with preserved riparian buffers, and that these would support a more balanced community.

Materials and methods

Study area

The Piracicaba River Basin spans 12,568 km², 90.7 % of which are in the State of São Paulo (CBH-PCJ 2010), forming the Piracicaba-Jundiá-Capivari Basins (PCJ basins). This area contains scattered fragments of Atlantic Forest, in an interface with Seasonal Semideciduous Forests of the Plateau (PCJ 2012). The climate is subtropical, with average annual temperatures between 18 and 22 °C and rainfall of 1,405 mm (Caram 2010; CBH-PCJ 2011). The wet season is between October and March, and the dry season from April to September (Ometto et al. 2000). This basin is highly industrialized (CETESB 2010) and the most populous in São Paulo state, with about five million inhabitants. The basin is mainly occupied by pasture (45 %), agriculture (31 %), natural forest and reforestation (16 %) and urban areas (6 %) (Filoso et al. 2003).

The present study focused on two sub-basins of the Piracicaba River Basin, the Atibaia (2,868 km²) and Jaguari River Basins (3,290 km²) (CBH-PCJ 2010). The Atibaia River Basin has widespread eutrophication, is predominantly agricultural land (50.52 %) (Piacente 2005), with 16.4 % native vegetation (IRRIGART 2006). The Jaguari River Basin is intensively deforested due to urbanization and expansion of industrial and agricultural activities (72.4 %) retaining only 17.7 % of its natural vegetation (Whately and Cunha 2007).

Nine stream reaches were selected based on field observations of the condition of the riparian buffer zones. The width of this zone and the degree of complexity of the forest canopy (i.e., presence of multi-strata forest vegetation) were used to classify them into three categories (Native Forest, Secondary Forest and

Sugarcane). The absence of point sources of pollution, similar dimensions and location within the same basin were also considered. The stream reaches were gentle-gradient riffle/pool types (Rosgen 1994) and varied from 1st to 3rd order. Each riparian condition had similar numbers of sites in each order. Percent land cover classes for each watershed and a relief model with a 15 m spatial resolution were obtained using the Geographic Information System (ArcInfo 9.3) based on the digital cartographic databases (Brazilian Institute of Geography and Statistics - IBGE) scale 1:50,000. The direct contribution area was delimited for each watershed based on topography, contour, and hydrographic layers. The land use types included native forests, secondary forests, sugarcane, and the category “others” (pasture, bare soils and perennial crops). Native forest comprised dense ombrophilous forests and lowland vegetation, while secondary forests comprised semi-deciduous and secondary dense ombrophilous vegetation.

Three stream reaches were selected under each of the following riparian conditions (Fig. 1): primary forest buffers within forested areas (Native Forest - NF); riparian vegetation consisting of secondary forest, with sugarcane fields in adjacent areas (Secondary Forest - SF); sparse or absent riparian vegetation, with surrounding sugarcane fields (Sugarcane - SC). Native Forest sites presented dense woody vegetation on both banks, with buffer widths of 12–18 m. The catchment was composed by both secondary and primary forest, and absence of sugarcane fields (Table 1). Secondary forest reaches were located within sugarcane properties in Cosmópolis (SP) showing clear signs of deforestation with a relatively open understory and 6–12 m of riparian buffer zones. This forest type results from the natural regeneration of vegetation in areas where primary forest was cut down. In such cases, land was almost always used temporarily for agriculture or pasture and forest emerged spontaneously after these activities were abandoned. In these zones, vegetation was dominated by sparse scrub, several pioneer species, grasses, bamboos, the castor-oil plant (*Ricinus communis*) and *Cecropia* sp. Sugarcane was abundant in the catchment (35–63 %), followed by a mix of primary native and secondary forests (Table 1).

In Sugarcane sites, riparian vegetation consisted primarily of *Brachiaria* type grasses. Sugarcane fields were sparse and riparian vegetation was composed of shrubs in the Açude Stream (AÇ). Severe erosion, with the presence of gullies, was also observed along this

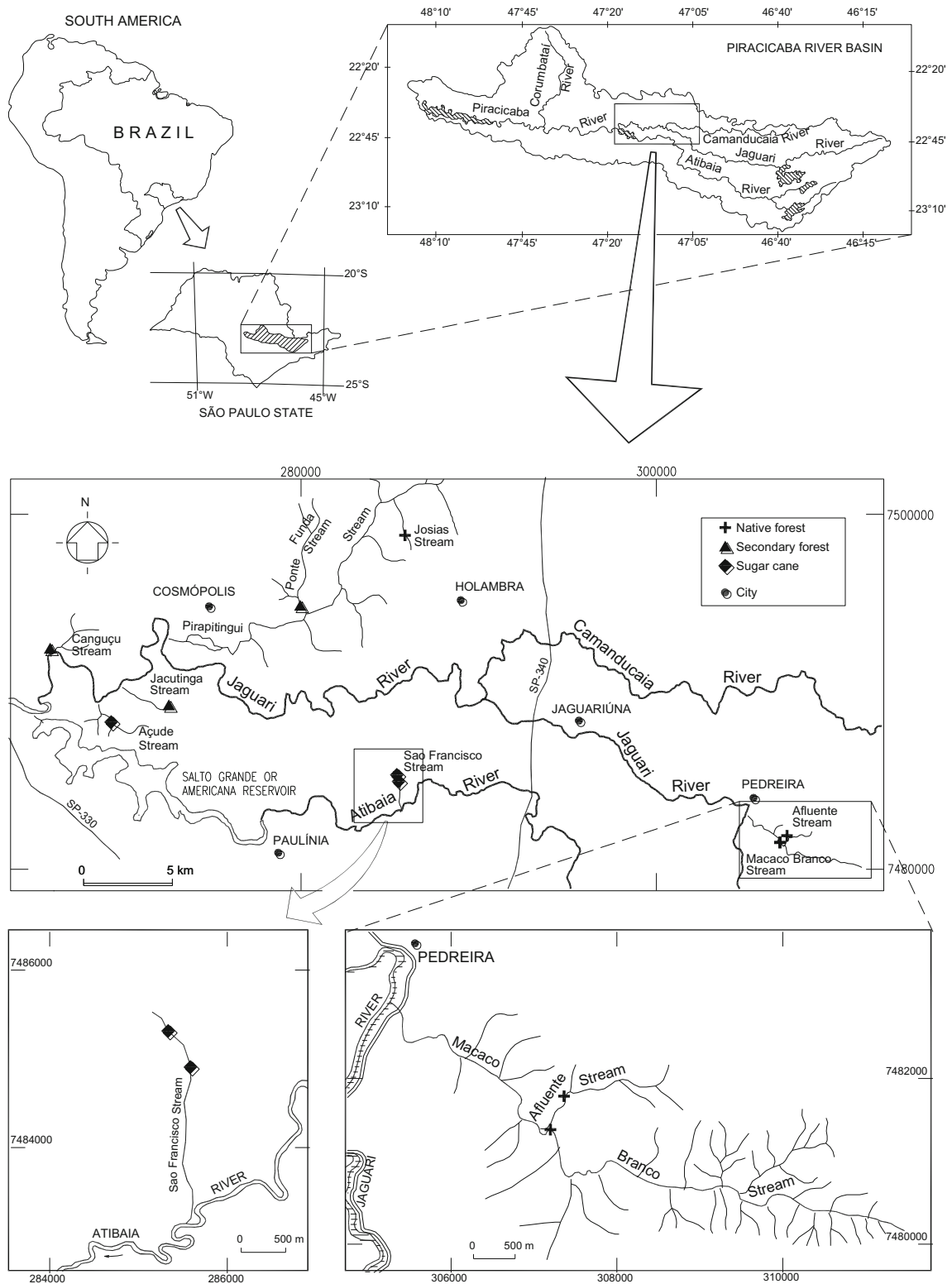


Fig. 1 Location of the study area in the Piracicaba River Basin, São Paulo State, showing the sampling sites in selected streams

Table 1 Sampling locations, catchment size (hectares), and percentage area of the most important land use categories for each watershed. ¹Sugarcane Sites; ²Secondary Forest Sites; ³Native Forest Sites

| Sampling site | Catchment area (ha) | Sugarcane (%) | Secondary forest (%) | Total native forest (%) | Other land uses (%) | Coordinates |
|-------------------------------------|---------------------|---------------|----------------------|-------------------------|---------------------|---------------------------|
| ¹ Açude (AÇ) | 387.14 | 89.9 | 3.2 | 3.2 | 3.70 | 22°41'53.6"S 47°14'39.3"W |
| ¹ S. Francisco 1 (SF1) | 164.3 | 89.4 | 0.0 | 3.2 | 7.4 | 22°43'46.2"S 47°05'10.5"W |
| ¹ S. Francisco 2 (SF2) | 164.3 | 89.4 | 0.0 | 3.2 | 7.4 | 22°43'46.7"S 47°05'13.2"W |
| ² Jacutinga (JA) | 623.6 | 57.4 | 3.3 | 11.9 | 27.4 | 22°41'23.7"S 47°12'48.6"W |
| ² Canguçu (CA) | 496.0 | 62.9 | 4.1 | 22.2 | 10.8 | 22°39'45.1"S 47°16'30.3"W |
| ² Ponte Funda (PF) | 1664.1 | 35.2 | 4.0 | 10.4 | 50.4 | 22°38'28.3"S 47°08'22.7"W |
| ³ Josias (JO) | 186.8 | 0.0 | 14.2 | 15.4 | 70.4 | 22°36'17.2"S 47°04'55.5"W |
| ³ Macaco Branco (MB) | 736.2 | 0.0 | 12.5 | 14.4 | 73.1 | 22°45'51.8"S 46°52'45.7"W |
| ³ T-Macaco Branco (T-MB) | 139.4 | 0.0 | 0.0 | 30.1 | 69.9 | 22°45'51.5"S 46°52'45.7"W |

reach. The other two reaches (SF 1 and SF 2), were subject to different sugarcane management activities. During the study period, the plantation ranged between ratoon cane and reform classes, i.e., the stage at which sugarcane has gone through more than one cut and the stage when natural productivity decreases indicating the need for renovation, respectively (CANASAT 2011; SMA 2011). In the dry season, sugarcane fields next to sample reaches were harvested, while in the rainy season they were in more advanced stages of development. Sugarcane was dominant at the catchment scale, attaining ≈90 % of land cover (Table 1).

All sample reaches were located in the Jaguari River Basin, except for SF 1 and SF 2 sites, which were located in the Atibaia River Basin.

Environmental data and fish sampling

The following physical and chemical parameters were recorded at each site with a HORIBA U-22 multiparameter analyzer during dry (August–September/2011) and rainy (March and December/2011) seasons: temperature (°C), turbidity (NTU), dissolved oxygen (mg.L⁻¹), pH, conductivity (μS.cm⁻¹), and total dissolved solids (mg.L⁻¹). Water samples were also collected and kept on ice for laboratory analysis of nitrate (μg.L⁻¹), nitrite (μg.L⁻¹), ammonium (μg.L⁻¹), total nitrogen (μg.L⁻¹), orthophosphate (μg.L⁻¹), total phosphorus (μg.L⁻¹), and total suspended solids (mg.L⁻¹) (APHA 2005).

In-stream variables were recorded during site surveys within the 50-m long reaches where fish were sampled. These variables included measures of width (m), depth (m) and water velocity (m.s⁻¹) (Marques and Argento

1988). Flow rate (m³.s⁻¹) was measured with a General Oceanics flow meter (2031H/2135) and calculations made according to Santos et al. 2001. The proportion of canopy cover, riffles, pools, trunks and roots (%) and substrate categories were estimated visually at each stream reach. Substrate was coarsely categorized as sand (<3 mm); fine gravel (≈3–49 mm), coarse gravel (≈50–149 mm); small boulders (≈150–300 mm) and large boulders (>300 mm).

A rapid assessment protocol (Habitat Quality Index - HQI) was used to quantify the habitat disturbance level of the sampling sites, based on the methodology proposed by Callisto et al. 2002. Site characteristics were evaluated through different sets of attributes including the type of occupation of the water body, margins and/or erosion on river banks and siltation, anthropogenic changes, vegetation cover, odor, oil, water and substrate quality. The sum of scores gives the final index ranging from 0 to 100, where 0–40 means “impacted”, 41–60 “moderately impacted” and >61 is “natural” habitat.

Fish samples were collected during the dry and rainy seasons using electrofishing equipment (Honda Generator - EU10i 1,000 W, 120 V, 60 Hz). Three successive removals in 50 m stretches were performed at each site, following the 3-catch removal method (Zippin 1958), resulting in a constant fishing effort (~40 min for each removal) at each locality. The reach was set at this length in order to maintain the uniformity of the riparian conditions along the sampling site, avoiding other anthropogenic influences as channelization or dredging. This length is similar to the one used by Teixeira-de Mello et al. 2014 to evaluate the efficiency of electrofishing for assessing richness, density and size distribution in small

streams located in different climatic and biogeographical regions, including neotropical streams. Three passes have also proved to be adequate for obtaining quantitative data in Atlantic Forest streams (Mazzoni et al. 2000), while Humpal and Lusk 2006 have shown that data from two-pass sampling is more precise than one pass only for detecting fish community structure in streams.

Specimens were fixed in 10 % formalin, transferred to 70 % alcohol, identified according to Britski 1972, measured and weighed in the laboratory. Identification was confirmed at the Museum of Zoology, University of São Paulo, Brazil, where voucher specimens were deposited (MZUSP 110954 -110972).

Data analysis

As an initial approach, we examined the species-environment relationships using a Canonical Correspondence Analysis (CCA) on the software CANOCO 4.5 (ter Braak and Šmilauer P 2002), to identify patterns between assemblages. Step-wise forward selection was used to reduce the environmental variables to those most correlated with the axes (ter Braak 1987). A cutoff point of $p=0.05$ was used to incorporate variables into the final models. The significance of each variable for each axis was determined using the null matrices obtained by the Monte Carlo permutation test (Hope 1968) considering 9999 permutations and a significance level of $p=0.05$. Data in percentages were square root transformed, while other values were transformed to $\log(x+1)$. Shannon-Wiener Diversity (H'), Simpson Dominance (λ') (Krebs 1989) and Margalef Richness (d) (Ludwig and Reynolds 1988) were calculated for each site and season using PRIMER 6 software (Clarke and Gorley 2006).

By means of a pRDA we run a variance partitioning analysis (Borcard et al. 1992; Peres-Neto et al. 2006) to understand the influences of riparian condition (Sugarcane, Secondary Forest, Native Forest), season (Dry and Rainy) and space (distance among sampled sites) on abiotic variables and community descriptors. Partial RDA considers the idea that some species and environmental variables may share a common spatial structuring, and is based on pre-existing methods of canonical ordination [RDA (van den Wollenberg 1977) and CCA (ter Braak 1986)]. Recently, variance partitioning has become popular among community ecologists to disentangle the influences of multiple sets of predictors on

community structure (Dray et al. 2012) measuring the fractions assigned to pure and shared effects.

The abiotic variables were represented by a matrix of physical, chemical and in-stream structures, while fish assemblages were described by three matrices of: fish composition (species-site matrix), diversity/abundance (Margalef richness, Shannon-Wiener diversity, Simpson dominance, biomass – g/m^2 , and density – individuals/ m^2), and trophic composition (densities - g/m^2 of omnivores, detritivores, carnivores and insectivores). The classification of trophic groups was based on several studies (Casatti and Castro 1998; Gibran et al. 2001; Esteves and Alexandre 2011; Castro et al. 2004; Ferreira 2007; Braga and Gomiero 2009; Costa and Schulz 2010). The Hellinger transformation of species abundance and trophic composition matrices was applied to the data previously to the pRDA analysis. From IBGE maps (scale 1:50,000) rivers channels were vectorized using AutoCAD 2007, in order to measure geographical distances among sampling sites. Then, a principal coordinate analysis (PCOA) was applied to pairwise-distance matrix among sites and eigenvectors used to describe the spatial configuration of sampled sites. Variance partitioning analyses, pRDA and PCOA were done in R software (R Development Core Team 2014). Permutation procedures were used (1,000 permutations) to test the significance of riparian condition, space and season considering the significance level at 0.05.

Stress levels of fish communities based on species abundance and biomass at each sampling site were compared using Abundance and Biomass Curves (ABC Curves) (Warwick 1986) in PRIMER 6 (Clarke and Gorley 2006). The difference between the two curves is given by the W statistic that ranges from -1 to 1, where a negative sign indicates that the biomass curve lies below the abundance curve and suggests a disturbed community (Clarke and Warwick 2001).

Results

Abiotic stream characteristics

Native Forest sites presented the highest values of conductivity, ammonium, nitrite, nitrate, orthophosphate, total nitrogen, canopy cover, percentage of pools and HQI, while SC sites showed the highest values of total phosphorus and lowest canopy cover (Table 2). The best habitat conditions (HQI) were recorded in the rainy

Table 2 Water quality and habitat structure (range and mean values - dry and rainy season) in the nine sampling sites of streams of the Piracicaba River basin under different riparian conditions ($n=6$ for each riparian condition)

| Variable | Native forest | | Secondary forest | | Sugarcane | |
|---|---------------|--------------|----------------------------|--------------|-----------|--------------|
| | Mean | Range | Mean | Range | Mean | Range |
| Temperature (°C) | 20.0 | 16.0–25.0 | 20.0 | 18.0–22.1 | 20.9 | 19.0–23.6 |
| Turbidity (NTU) | 62.4 | 18.6–86.7 | 85.2 | 20.0–220.0 | 107.5 | 49.5–253 |
| Dissolved Oxygen (mg.L ⁻¹) | 8.3 | 5.6–10.0 | 7.9 | 5.7–10.4 | 8.7 | 7.3–5.8 |
| pH | 6.2 | 5.6–6.8 | 5.8 | 5.1–6.6 | 5.5 | 4.7–23 |
| Conductivity (µS.cm ⁻¹) | 99.7 | 63.0–150.0 | 28.8 | 11.0–51.0 | 16.0 | 10.0–23.0 |
| TDS | 65.0 | 40.0–100.0 | 18.5 | 10.0–33.0 | 12.0 | 10.0–16.0 |
| TSS (mg.L ⁻¹) | 2.0 | 40.0–100.0 | 12.5 | 10.0–33.0 | 10.1 | 10.0–16 |
| Nitrate (µg.L ⁻¹) | 301.0 | 82.8–666.5 | 282.3 | 47.3–640.0 | 194.1 | 103.7–309.3 |
| Nitrite (µg.L ⁻¹) | 4.0 | 2.0–9.1 | 1.1 | 0.01–2.3 | 0.7 | 0.3–1.1 |
| Ammonium (µg.L ⁻¹) | 344.4 | 257.3–500.9 | 168.8 | 52.0–282.0 | 139.6 | 112.2–169.7 |
| Total Nitrogen (µg.L ⁻¹) | 1488.5 | 713.1–2176.0 | 1317.5 | 679.0–1730.0 | 1071.3 | 541.7–1794.0 |
| Ortophosphate (µg.L ⁻¹) | 29.9 | 12.6–55.8 | 5.1 | 0.7–11.9 | 4.7 | 2.2–6.7 |
| Total Phosphorus (µg.L ⁻¹) | 59.9 | 39.8–81.3 | 27.6 | 17.4–34.8 | 53.1 | 13.3–174.2 |
| Depth (m) | 0.2 | 0.1–0.3 | 0.3 | 0.08–0.6 | 0.1 | 0.1–0.2 |
| Width (m) | 2.3 | 1.5–3.6 | 2.4 | 1.75–3.7 | 1.6 | 0.8–2.9 |
| Water velocity (m.s ⁻¹) | 0.2 | 0.0–0.3 | 0.4 | 0.2–1.0 | 0.4 | 0.2–0.8 |
| Flow (m ³ .s ⁻¹) | 0.01 | 0.0–0.02 | 0.07 | 0.0–0.2 | 0.03 | 0.0–0.1 |
| Canopy Cover (%) | 80.0 | 60.0–90.0 | 44.17 | 5.0–70.0 | 0.8 | 0.0–5.0 |
| Riffles (%) | 27.5 | 5.0–60.0 | 20.8 | 0.0–50.0 | 15.3 | 0.0–40.0 |
| Pools (%) | 51.7 | 20.0–90.0 | 20.8 | 10.0–30.0 | 16.7 | 0.0–60.0 |
| HQI | 54.3 | 70.0–83.0 | 44.3 | 41.0–50.0 | 49.0 | 39.0–62.0 |
| Dominant substrate | Sand/Clay | | Sand/Silt Coarse gravel | | sand/silt | |

HQI Habitat quality index, TSS Total dissolved solids, TDS Total dissolved solids

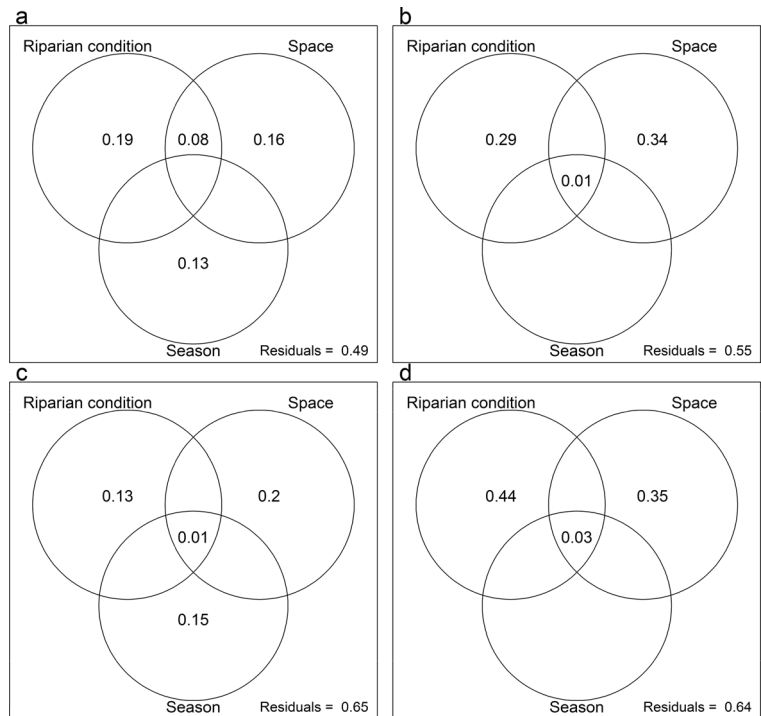
season when NF and SF sites were considered ‘natural’ (>61). Sugarcane sites ranged from ‘impacted’ (0–40) to ‘altered’ (41–60), except in the Açude stream (AÇ) where the habitat was classified as ‘natural’ in the dry season and altered in the rainy season. The mean monthly temperature and rainfall in the study area ranged from 16.5 to 25.8 °C and from 2.9 mm to 500.3 mm in the dry and rainy seasons, respectively (CIIAGRO 2012). The variance partitioning analysis related to the abiotic variables showed that shared effects among riparian condition, space and season were not significant, with only pure effects being observed. These variables were significantly influenced by riparian condition ($r^2_{adj}=0.19$, $F=3.64$, $p=0.002$), seasonality ($r^2_{adj}=0.13$, $F=4.37$, $p=0.001$) and space ($r^2_{adj}=0.16$, $F=3.32$, $p=0.001$) (Fig. 2a). Shared effects were in general not important except for a small fraction of 8 % between riparian

condition and space. An overlap between sugarcane and native forested sites along the first pRDA axis was observed. This axis was predominantly influenced by flow, total suspended solids and water velocity on the negative extremity. The SF sites differed from the other riparian conditions along the second pRDA axis mainly due to its wider and deeper channels and higher flow (Fig. 3a).

Fish assemblages

A total of 1,325 individuals of 41 fish species were collected. *Bryconamericus iheringii* and *Astyanax* sp. were the most abundant species in NF sites. *Gymnotus carapo*, *Astyanax paranae*, *Astyanax altiparanae* and *Imparfinis mirini* were numerically dominant in the SF sites, while *Phallocceros* spp. was the most abundant

Fig. 2 Venn diagram showing the percent of explanation or riparian conditions, space and season on abiotic variables (a), species abundance (b), diversity/abundance measures (c) and trophic composition (d). Zero values are not shown



species in sugarcane reaches. Twenty-two species occurred exclusively in one of the three different riparian conditions, with seven occurring only in NF; 11 in SF; and four in SC reaches (Table 3). The percentage of rare species for each riparian condition (i.e., with ≤ 5 individuals) was higher at the SF (68.9 %) and SC (55.6 %), followed by NF sites (47.4 %).

The first two axes in the CCA explained 48.9 % of variation in the data, most of which (26.8 %) was explained by axis 1 (Fig. 4). Orthophosphate ($p=0.0002$), riffles ($p=0.001$) and depth ($p=0.003$) explained most of the variance in this analysis. Axis 2 explained 22.1 % of data variation, with higher contribution of nitrate ($p=0.0005$) and stream width ($p=0.005$). Species such as *Characidium zebra* and *Apareiodon affinis*, were associated with deep sites with a high proportion of riffles, while *Gymnotus carapo*, and *Hypheosbrycon eques*, among others, showed preference for slow flowing habitats, such as the Tributary of Macaco Branco (NF), Jacutinga (SF) and São Francisco streams (SC). The benthic catfish *Imparfinis mirini*, occurred both in SF sites and the Açude stream (SC), which differed from other SC sites because of the low orthophosphate levels and higher proportion of pebbles, to which *Corydoras aeneus*, *Hypostomus ancistroides*, and *Gymnotus pantherinus* were associated.

Community composition was significantly influenced by riparian condition ($r^2_{\text{adj}}=0.29$, $F=4.78$, $p=0.001$) and space ($r^2_{\text{adj}}=0.34$, $F=5.30$, $F=0.001$), but not by seasonality ($r^2_{\text{adj}}\approx 0$, $F=0.43$, $p=0.924$) (Fig. 2b). The different riparian conditions showed little overlap, suggesting that assemblage composition was a good descriptor of riparian conditions under different preservation conditions. The first axis of pRDA separated the NF sites with predominance of *Geophagus brasiliensis* from SC sites with predominance of *Hypostomus ancistroides* and *Gymnotus pantherinus*. SF sites grouped on the positive extreme of the second pRDA axis and were described mainly by the predominance of *Gymnotus carapo* and *Astyanax altiparanae* (Fig. 3b).

The diversity/abundance matrix was significantly influenced by seasonality ($r^2_{\text{adj}}=0.15$, $F=3.89$, $p=0.022$) and space ($r^2_{\text{adj}}=0.20$, $F=3.17$, $p=0.034$), but not by riparian condition ($r^2_{\text{adj}}=0.13$, $F=2.36$, $p=0.077$) (Fig. 2c). This weak influence of riparian condition reflected on a great overlap among sites on the pRDA. Nevertheless, SF sites were associated to higher richness and Shannon diversity as shown on the negative extreme of the first pRDA axis (Fig. 3c).

As for assemblage composition, trophic structure of fish communities was influenced only by riparian condition ($r^2_{\text{adj}}=0.44$, $F=5.75$, $p=0.011$) and space ($r^2_{\text{adj}}=$

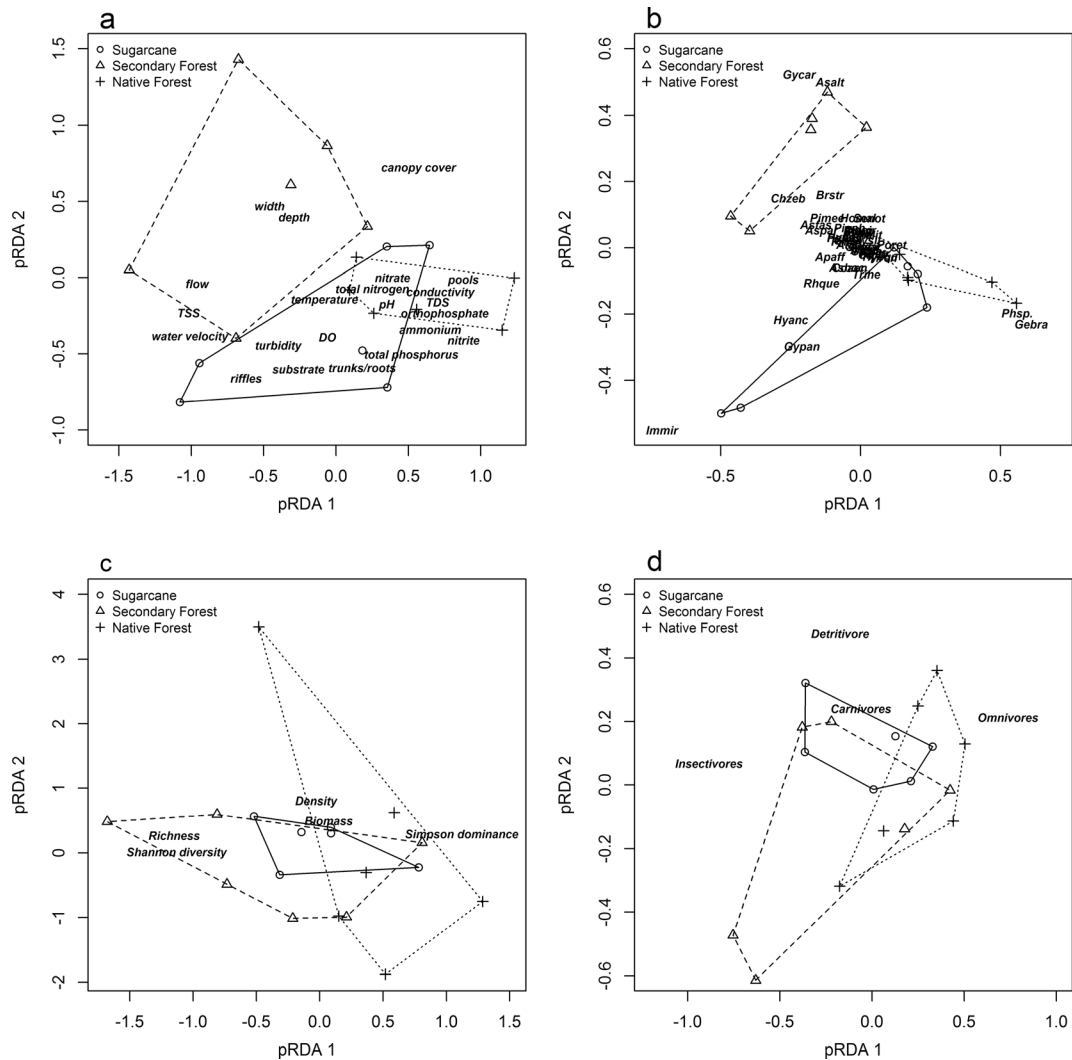


Fig. 3 Biplot of Partial Redundancy Analysis (pRDA) showing the influence of riparian conditions on abiotic variables (a), species abundance (b), diversity/abundance measures (c) and trophic composition (d). The biplots show only pure effects associated with riparian condition, excluding the effects of space and season. See species acronyms in Table 3

0.35, $F=4.77$, $p=0.013$), but not by seasonal variations ($r^2_{adj} \approx 0$, $F=0.76$, $p=0.407$) (Fig. 2d). The first axis of pRDA described a gradient of sites with predominance of omnivores to sites with predominance of insectivores, which were related to SF sites. Despite the statistical significance, there was great overlap among sites with different type riparian conditions (Fig. 3c) suggesting that trophic structure may be a weak predictor of this kind of modification.

The results of the ABC curves and their corresponding W values (Fig. 5), indicated that the NF and SF sites had preserved and moderately impacted communities (W ranging from -0.141 to 0.23), while most impacted

conditions were observed in the SC sites (W ranging from -0.44 to 0.18).

Discussion

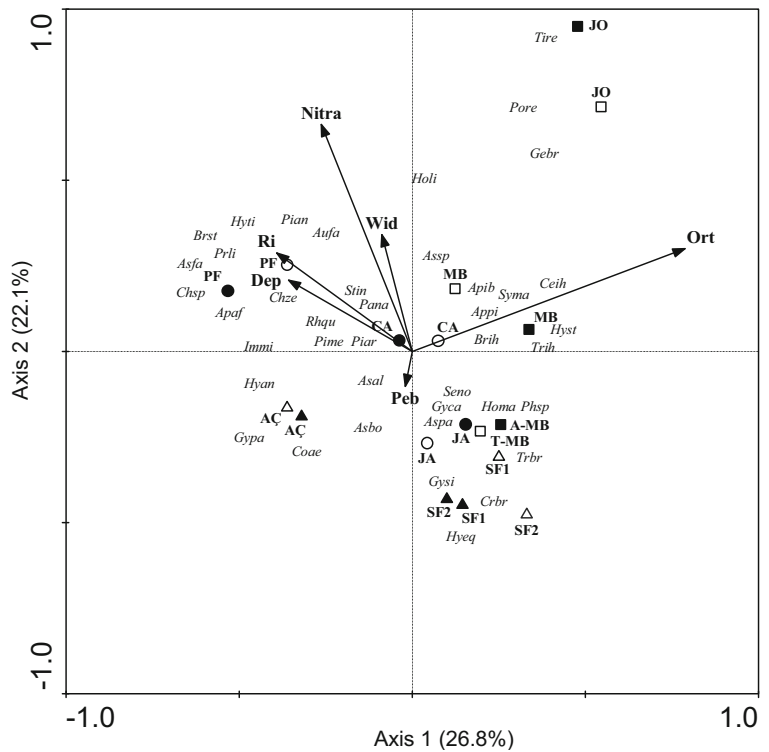
Abiotic variables

Although water quality met the guidelines proposed by the Brazilian regulatory arm of the Ministry of Environment (CONAMA 2005) for aquatic life preservation (class 02), physicochemical variables were different for the three riparian vegetation types. Native Forest

Table 3 Total and relative fish abundance for each riparian condition (grouped seasons) in nine stream reaches of the Piracicaba River Basin

| Species | Native forest | | Secondary forest | | Sugarcane | | | |
|---|---------------|-------|------------------|-------|------------|-------|------------|-------|
| | Total | % | Total | % | Total | % | | |
| <i>Bryconamericus iheringii</i> (Brih) | 232 | 17.51 | 210 | 32.16 | 14 | 3.93 | 8 | 2.53 |
| <i>Phalloceros</i> spp. (Phsp) | 229 | 17.28 | 78 | 11.94 | 5 | 1.4 | 146 | 46.2 |
| <i>Astyanax paranae</i> (Aspa) | 161 | 12.15 | 85 | 13.02 | 62 | 17.42 | 14 | 4.43 |
| <i>Astyanax</i> sp. (Assp) | 128 | 9.66 | 127 | 19.45 | 1 | 0.28 | | |
| <i>Imparfinis mirini</i> (Immi) | 121 | 9.13 | | | 38 | 10.67 | 83 | 26.27 |
| <i>Gymnotus carapo</i> (Gyca) | 77 | 5.81 | 1 | 0.15 | 75 | 21.07 | 1 | 0.32 |
| <i>Astyanax altiparanae</i> (Asal) | 60 | 4.53 | | | 52 | 14.61 | 8 | 2.53 |
| <i>Geophagus brasiliensis</i> (Gebr) | 48 | 3.62 | 48 | 7.35 | | | | |
| <i>Characidium zebra</i> (Chze) | 35 | 2.64 | | | 35 | 9.83 | | |
| <i>Trichomycterus iheringi</i> (Trih) | 27 | 2.04 | 26 | 3.98 | | | 1 | 0.32 |
| <i>Hypostomus strigaticeps</i> (Hyst) | 26 | 1.96 | 26 | 3.98 | | | | |
| <i>Gymnotus pantherinus</i> (Gypa) | 18 | 1.36 | | | | | 18 | 5.7 |
| <i>Astyanax fasciatus</i> (Asfa) | 15 | 1.13 | | | 15 | 4.21 | | |
| <i>Parodon nasus</i> (Pana) | 15 | 1.13 | 10 | 1.53 | 5 | 1.4 | | |
| <i>Hypostomus ancistroides</i> (Hyan) | 14 | 1.06 | | | 3 | 0.84 | 11 | 3.48 |
| <i>Pimelodella meeki</i> (Pime) | 12 | 0.91 | | | 10 | 2.81 | 2 | 0.63 |
| <i>Rhamdia quelen</i> (Rhqu) | 12 | 0.91 | 7 | 1.07 | 2 | 0.56 | 3 | 0.95 |
| <i>Bryconamericus stramineus</i> (Brst) | 11 | 0.83 | | | 11 | 3.09 | | |
| <i>Apareiodon ibitiensis</i> (Apib) | 8 | 0.6 | 8 | 1.23 | | | | |
| <i>Trichomycterus brasiliensis</i> (Trbr) | 8 | 0.6 | 8 | 1.23 | | | | |
| <i>Serrapinnus notomelas</i> (Seno) | 7 | 0.53 | | | 2 | 0.56 | 5 | 1.58 |
| <i>Hyphessobrycon eques</i> (Hyeq) | 6 | 0.45 | | | | | 6 | 1.9 |
| <i>Apareiodon affinis</i> (Apaf) | 6 | 0.45 | | | 5 | 1.4 | 1 | 0.32 |
| <i>Hoplosternum littorale</i> (Holi) | 5 | 0.38 | 2 | 0.31 | 3 | 0.84 | | |
| <i>Cetopsorhamdia iheringi</i> (Ceih) | 5 | 0.38 | 5 | 0.77 | | | | |
| <i>Poecilia reticulata</i> (Pore) | 5 | 0.38 | 4 | 0.61 | 1 | 0.28 | | |
| <i>Astyanax bockmanni</i> (Asbo) | 4 | 0.3 | | | 1 | 0.28 | 3 | 0.95 |
| <i>Apareiodon piracicabae</i> (Appi) | 4 | 0.3 | 3 | 0.46 | 1 | 0.28 | | |
| <i>Hoplias malabaricus</i> (Homa) | 3 | 0.23 | 1 | 0.15 | 2 | 0.56 | | |
| <i>Hypostomus tietensis</i> (Hyti) | 3 | 0.23 | | | 3 | 0.84 | | |
| <i>Crenicichla britskii</i> (Crbr) | 3 | 0.23 | | | | | 3 | 0.95 |
| <i>Gymnotus silvius</i> (Gysi) | 3 | 0.23 | | | 1 | 0.28 | 2 | 0.63 |
| <i>Piabina anhembi</i> (Pian) | 2 | 0.15 | | | 2 | 0.56 | | |
| <i>Characidium</i> sp. (Chsp) | 2 | 0.15 | | | 2 | 0.56 | | |
| <i>Prochilodus lineatus</i> (Prli) | 2 | 0.15 | | | 2 | 0.56 | | |
| <i>Tilapia rendalli</i> (Tire) | 2 | 0.15 | 2 | 0.31 | | | | |
| <i>Synbranchus marmoratus</i> (Syma) | 2 | 0.15 | 2 | 0.31 | | | | |
| <i>Piabina argentea</i> (Piar) | 1 | 0.08 | | | 1 | 0.28 | | |
| <i>Steindachnerina insculpta</i> (Stin) | 1 | 0.08 | | | 1 | 0.28 | | |
| <i>Corydoras aeneus</i> (Coae) | 1 | 0.08 | | | | | 1 | 0.32 |
| <i>Australoheros facetus</i> (Aufa) | 1 | 0.08 | | | 1 | 0.28 | | |
| Total abundance | 1,325 | | 653 | | 356 | | 316 | |

Fig. 4 Representation of the first two axes of the Canonical Correspondence Analysis (CCA) for fish communities from nine stream sites from the Jaguari and Atibaia River Basins. Dry season (open symbols) and rainy season (filled symbols): ■ Native Forest; ● Secondary Forest; ▲ Sugarcane. Vector lines indicate the relationship of the significant environmental variables to the ordination axis; line length is proportional to its relative significance. *Ort* Orthophosphate, *Nitra* Nitrate, *Wid* Width, *Ri* Riffles, *Peb* Pebbles and *Dep* Depth. See species acronyms in Table 3



sites had higher conductivity, total dissolved solids and nitrogen compounds, which can be related to litter accumulation and decomposition on the stream bed (Esteves 1998). According to Nislow 2005, vegetation type may influence nutrient retention processes, with older forests retaining naturally fewer nutrients than younger forests, and releasing them into the river. Similar results were found by Burcham 1988; Lorion and Kennedy 2009, and Riseng et al. 2011, who reported higher conductivity in dense forested sites. Nevertheless, these values are low when compared to other studies in the Piracicaba River Basin, which reported conductivity between 130 to 272.8 $\mu\text{S}\cdot\text{cm}^{-1}$ (Ometto et al. 2000; Esteves and Alexandre 2011).

Native Forest stream reaches were structurally more complex, as reflected by a higher HQI, and proportion of pools and trunks. Forests formed by older vegetation are more vulnerable to being blown down, increasing the supply of large woody debris (LWD) into the channel, as well as the frequency of pools (Nislow 2005). This can significantly increase nutrient and material retention in the stream by creating transient storage zones, thus increasing habitat and flow path complexity (Bilby and Likens 1980). The LWD can also greatly impact fish habitat

by increasing pool habitat area and pool frequency, which is important for survival of aquatic biota, supporting a more diverse and structured fish community (Casatti et al. 2006b).

The variance partitioning analysis of abiotic characteristics showed that the effect of riparian condition that is spatially structured accounted for only 8 % of total variation. This effect is probably due to nearby sites that also have similar riparian conditions. Nevertheless, this shared effect was smaller than those assigned solely to riparian conditions (19 %), space (16 %) or season (18 %). Residual variation accounted for 49 % and this may be attributed to other non-measured underlying factors. In fact, we did not quantify other substances as agrochemicals heavily used during the production of sugarcane that can potentially be harmful to the environment (Smeets et al. 2006) nor the influence of larger scale factors at the watershed scale. The search for other environmental factors (metals and organochlorinated compounds) as those reported by Corbi et al. 2006 in streams located in sugarcane areas could thus be considered in other studies, increasing the explanation of the remaining variation.

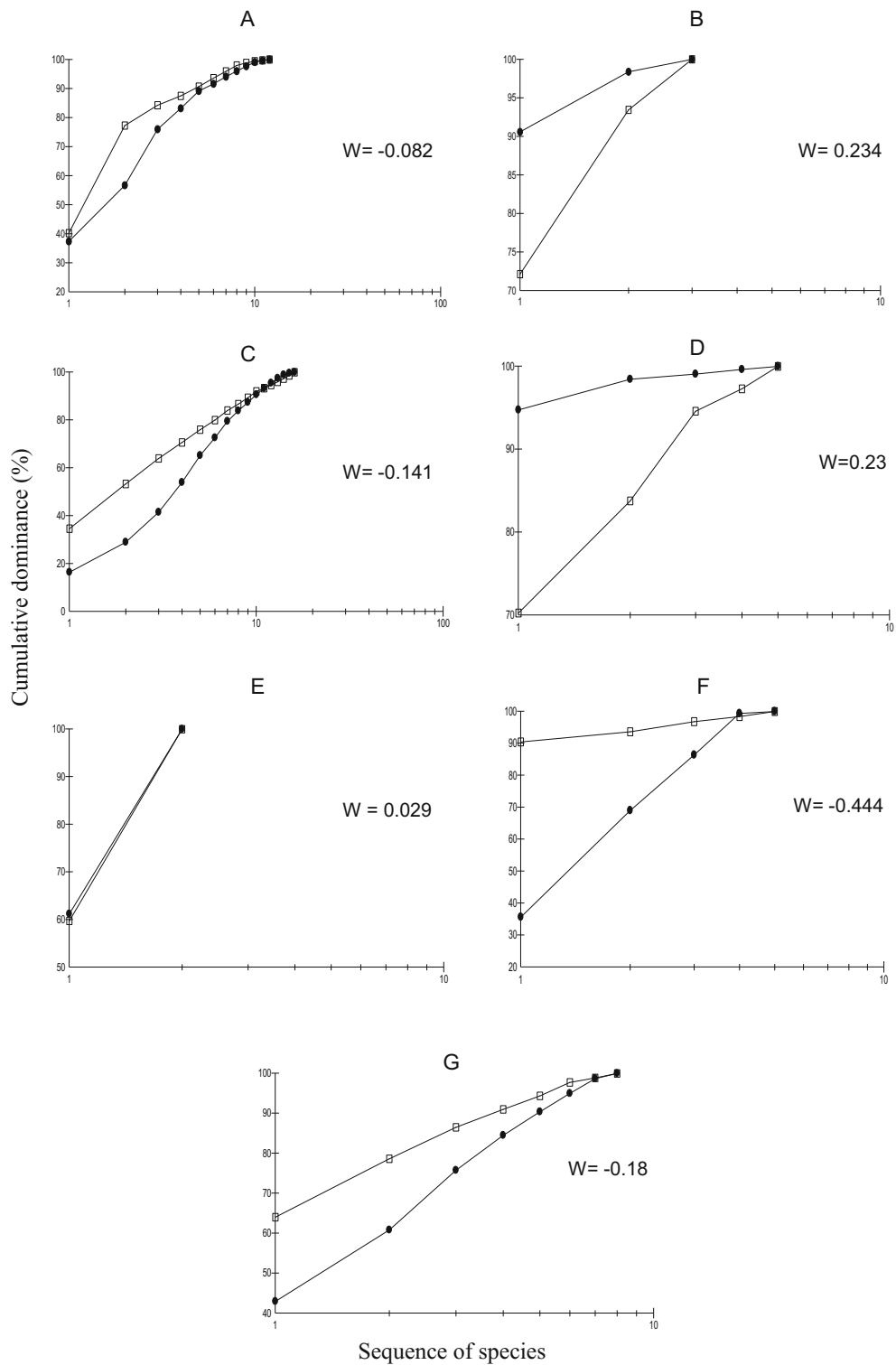


Fig. 5 ABC Curves based on abundance (\square) and biomass (\bullet) of fish sampled at the nine stream reaches from the Jaguari and Atibaia River Basins in the dry season. Streams: *A* Macaco Branco, *B* Tributary of Macaco Branco (Native Forest), *C* Ponte

Funda, *D* Canguçu, *E* Jacutinga (Secondary Forest), *F* São Francisco 1, *G* Açude (Sugarcane). The Josias stream (Native Forest) and São Francisco 2 (Sugarcane) were not included because of single species dominance

Species-environment relationships

The number of species observed in this study (41) was high when compared to those found by Gerhard 2005, who conducted a survey in 60 watersheds in the Corumbataí River, also in the Piracicaba River Basin. This author used electrofishing in low order streams and found 51 species, whereas Esteves and Alexandre 2011, with a comparable sampling effort to our study recorded 35 species in the Guamium River (Piracicaba River Basin). Results indicated the predominance of *Bryconamericus iheringii* in NF sites; this appears to be a species characteristic of more pristine environments (Hued and Bistoni 2005). Individuals of *B. iheringii* are active swimmers, which dwell in flowing environments, and mostly prey on items carried by the current (Ferreira et al. 2012a), which may explain their presence in more heterogeneous environments. *Phalloceros* spp. is an opportunistic species that occupies disturbed habitats (Casatti et al. 2008; Araújo et al. 2009) such as the SC sites where it was most abundant. Several species were associated to specific microhabitats as shown by the CCA. Species of the Parodontidae (e.g., *Apareiodon affinis*) and Crenuchidae (e.g., *Characidium zebra*) families occurred in microhabitats with higher proportion of riffles, as observed in the SF and NF sites. Most species in these families inhabit fast flowing waters and stay close to the bottom where they scrape the substrate in search of food (Marceniuk and Hilsdorf 2010). *Gymnotus carapo* occurred in shallow waters characteristic of SF reaches, particularly in the Jacutinga stream, where grasses were abundant on the river banks. This species appears to prefer river banks and backwaters where submerged marginal vegetation is abundant (Ferreira and Casatti 2006; Oyakawa et al. 2006), which may be related to their ability to tolerate and explore structurally simplified streams (Casatti et al. 2006a). The low HQI, high TSS and high turbidity recorded at the SF and SC sites, indicated that these sites presented altered environmental conditions, with visible effects of erosion. Different species associations were observed at these sites, as well as in the São Francisco stream, where *Hyphessobrycon eques* occurred in shallow flowing waters. This species is known to prefer standing waters with abundant macrophytes and is frequently found in disturbed environments (Oyakawa et al. 2006; Marceniuk and Hilsdorf 2010). In fact, *H. eques* occurred exclusively in stretches where floating pennywort *Hydrocotyle ranunculoides* abundance was high.

This is an invasive macrophyte from the Araliaceae that is currently spreading in waterways; it is highly adaptable to a wide range of environmental conditions, reducing the level of dissolved oxygen (Bonants and Edema 2012) and disrupting natural erosion-deposition processes (DEFRA 2010). *Hypostomus ancistroides*, *Corydoras aeneus*, *Gymnotus pantherinus* and *I. mirini* occurred in the Açude stream, a SC site with a higher HQI, despite heavy siltation. Both *H. ancistroides* and *I. mirini* have been associated with higher siltation related to the removal of riparian vegetation (Casatti et al. 2005; Furlan et al. 2012) and urbanized streams (Tófoli et al. 2013), indicating their adaptability to altered conditions.

Factors influencing fish assemblages

Comparisons among our three riparian conditions suggested that this component had significant effects on some aspects of the fish assemblages, which included species composition and trophic structure, while richness, diversity, biomass and total density were not influenced by riparian condition, but only by seasonality and space. As for the abiotic stream conditions, part of the variability could not be explained by our data set, a situation commonly found in other studies (Borcard et al. 1992; Alexandre et al. 2010; Ferreira et al. 2014). High unexplained variances are not unusual in metacommunity studies (Melo et al. 2011), and although the explained data sets may seem proportionally low, the underlying causes found to be significant can nevertheless be considered as important in the structuring of these communities as stressed by Borcard et al. 1992. This analysis is further complicated by the fact that intrinsic and extrinsic local processes (e.g., competition, predation and disturbance) have been considered important to explain local fish assemblage structure, as well as the role of regional processes which have a pervasive role in shaping local assemblages (Schlosser 1995). More recently, several temperate region studies have shown that the variability in results from studies of landscape influences on streams can be attributed to confounding effects within the predictor data sets, regional variation in responses, catchment size-dependent responses and use of different types of metrics and measurement approaches (Stanfield and Kilgour 2013). However, studies on the effects of riparian vegetation on aquatic communities have stressed the importance of local influence, because riparian buffers help

the preservation of mesohabitats and other in-stream features (Birnbaum et al. 2007; Lorion and Kennedy 2009; Casatti et al. 2012).

Several studies have shown positive correlations between nutrient levels and fish biomass and abundance (Murphy and Meehan 1991; Nislow 2005). Fish abundance can change in agricultural regions (Lammert and Allan 1999), deforested sites (Bojsen and Barriga 2002), and in streams with different intensities of physical habitat degradation and water chemistry (Casatti et al. 2006b). Although some studies have observed a decrease in fish biomass in streams within agricultural areas (Karr et al. 1985) and habitats with impaired environmental conditions (Casatti et al. 2006a), Bojsen and Barriga 2002 and Lorion and Kennedy 2009 found that biomass did not differ between sites with different riparian conditions. Our results agree with the latter findings and may be attributed to the influence of other factors such as space, which might be a limiting factor for total fish biomass (Bojsen and Barriga 2002). A balance between the disappearance of intolerant species at the SC sites, and an increase of tolerant species such as the r strategist *Phalloceros* spp. that may benefit from the larger amounts of food (periphyton and macroinvertebrates) in these altered streams may also explain the obtained results.

Richness and diversity measures, found to be higher at the SF sites could also not be explained by the influence of the riparian conditions, but only by spatial and seasonal factors. Although SF sites were apparently at an advanced stage of riparian vegetation regeneration, at the catchment scale, land use patterns presented relatively high levels of sugarcane cultivation at these sites, which may be considered a disturbance. Considering that when environmental disturbances are frequent, communities are dominated by opportunistic species, with low species diversity and when disturbances are rare, diversity is lower due to resource competition, one may suppose that in environments with intermediate levels of disturbance, diversity is high due to the presence of both opportunistic and intolerant species (Begon and Mortimer 1986), as predicted by the Intermediate Disturbance Hypothesis (Connell 1978). Wang et al. 1997 have shown that agriculture has a small influence on habitat quality or biotic integrity in watersheds with less than 50 % agriculture, suggesting that there may be a threshold level at which agricultural effects begin to manifest themselves.

Results also indicated an important influence of both space and riparian conditions on the trophic structure of

the fish communities, with SF sites presenting higher values of insectivore species. Other guilds as omnivores and carnivores were similar between riparian conditions, while detritivores, especially *Phalloceros* spp. were more abundant at SC sites. Zeni and Casatti 2014 found greater availability of aquatic insects in homogenous streams of the northwest region of the state of São Paulo, and this was associated with the presence of submerged plants such as grasses and the opportunistic behavior of fish. Wichert and Rapport 1998 indicate that a higher proportion of insectivores and piscivores is expected in less disturbed systems than in heavily degraded sites, because as degradation intensifies, those species at the top of the trophic structure are expected to disappear first. In fact, SF sites showed lower canopy cover, which may have favored the development of grasses, to which the insectivorous *G. carapo* was highly associated.

In our analysis of the community attributes, it also has to be considered that conditions within the different riparian areas, especially sugarcane reaches, were not homogeneous with respect to impairment level and intensity of management activity. Unlike the Açude stream, where sugarcane plantations were not intensively managed, two sites (SF-1 and SF-2) were subject to intense management activities where heavy machinery exposed bare soils during harvest. Thus communities and physical conditions may have responded with different intensities to the local land use, suggesting that future sampling schemes could be conducted in order to understand the influence of different sugarcane management activities or site abandonment on stream communities.

The ABC curves reflected the effects of the different riparian conditions on fish assemblages, indicating that SC sites were subject to stress according to Warwick 1986 classification. From an ecological perspective, the interpretation of these plots is based on the r-K selection gradient, where species with long development times and large size are represented by few individuals, but are dominant in terms of biomass. In this case, the biomass curve lies above the abundance curve (Clarke and Warwick 2001; Clarke and Gorley 2006). According to these authors, in moderately affected sites, K strategist species are partially eliminated, reducing inequality between biomass and abundance, so that the curves are close and may even cross. Impacted sites favor the presence of small, r-strategist species, which become numerically dominant, in which case the abundance curve lies above the biomass curve. Forested (NF, SF)

sites showed a dominance of small Characidae like *B. iheringii* (up to 11.4 cm total length), several *Astyanax* species (5.0–11.5 cm total length) and the Gymnotiform *G. carapo* (up to 76 cm total length). Their occurrence in these habitats may be related to the increased heterogeneity of these reaches, where pools, riffles, trunks and grasses may have provided spatial refuge not just from predation but also for the metabolic demands of maintaining position in flowing environments. The shape of ABC curves is influenced by the presence of large bodied species, which in these sites consisted of a large sedentary piscivore (*H. malabaricus*), the armored catfish *H. ancistroides* and the electric knifefish *G. carapo*. Despite the abundance of the siluriform *I. mirini* (up to 9.3 cm total length) in one SC site (AÇ), the dominance of the small viviparous *Phalloceros* spp. (up to 3 cm total length) seemed to account for the shifts in the ABC curves, indicating that agricultural disturbances were major stressors for these streams. Similar results were found for disturbed streams in northeastern São Paulo, where the exotic viviparous *Poecilia reticulata* was characteristic of reaches without canopy cover, with severe siltation, and mesohabitat simplification (Casatti et al. 2006b). Some authors consider the results of ABC curves may be biased by an influx of recruits of dominant species (Yemane et al. 2005), as well as the migration of large bodied species. However, this seems unlikely in this case, as most of the dominant species in the NF and SF sites were small sized Characidae, known to perform only short-distance reproductive migrations (Vazzoler 1996), while no typical large seasonal species occurred at the studied reaches.

Although there is some controversy regarding the relative influence of land use and land cover at the different scales, with some studies favoring the basin level (Wang et al. 1997; Harding et al. 1998) and others favoring the local scale (Stauffer et al. 2000; Birnbaum et al. 2007), this study showed that both physical conditions of streams and some attributes of fish communities reflected the different levels of preservation of the riparian buffers. These findings have important implications for the conservation of fish biodiversity and watershed management practices, especially when considering that only 7.9 % of native forest remains in the PCJ basin (CBH-PCJ 2011). Considering that only 5 % of the sugarcane areas in the State of São Paulo are of riparian vegetation (SMA 2011), a target to be met is natural vegetation restoration along streams on

sugarcane fields, with strips of 30 m for rivers <10 m wide being proposed by the Brazilian Forest Code (n° 12.651, May 2012). Therefore, proper planning, improvement of land use practices, and protection of streams and riparian ecosystems are important issues to consider regarding environmental sustainability of sugarcane production as it was shown that fish assemblages were disrupted in sugarcane sites, probably as a consequence of low environmental integrity.

Acknowledgments The authors thank the Instituto de Pesca (APTA/SAA) for providing infrastructure; its staff and students for their help in field work (Sérgio Luiz da Silva, Paulo Henrique Gusmão, Ludmila Cristina Baldi and Katerine Pereira Carvalho); Fundação de Amparo à Pesquisa do Estado de São Paulo for financial support (Proc. 2010/52069-6); Conselho Nacional de Desenvolvimento Científico e Tecnológico for the MsC scholarship (Proc. 154078/2010-7); Osvaldo T. Oyakawa for fish identification; Cíntia Kameyama for macrophyte identification; Mirna Ferracini for map drawing; Antônio Olinto, André Vaz, Leonardo Tachibana and Eduardo Paes for help with statistical analyses and José Carlos Perdigão and Walter Lima for help in locating sampling sites. This study was approved by the Ethics Committee of the Fisheries Institute of São Paulo (CEEAIIP) (Protocol 07/2010).

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