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Long-term effects of a rural landscape on the structure and trophic organization of a fish assemblage

Efeitos de longo prazo de uma paisagem rural sobre a estrutura e organização trófica de uma assembleia de peixes

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Abstract: Aim: We investigated the long-term effects of a rural landscape on the structure and trophic organization of a fish assemblage. **Methods:** We compared environmental data and data from fish assemblage structure and trophic organization sampled in a stream located in a rural area, within a time gap of 20 years. **Results:** We observed only punctual changes in the environmental variables and fish structure, which may not be related to the rural landscape. In fish diet, insectivory remained predominant in all sample periods. However, when we analyzed the groups of hexapods consumed by the fish species, we found a substitution of Ephemeroptera and Trichoptera by Diptera over time. **Conclusions:** Although the fish assemblage structure was not affected, the insectivore diet analysis showed that the stream stretch could be in an ongoing process of environmental quality loss, once the simplification in the macroinvertebrates assemblage structure over time is indicative of environmental degradation.

Keywords: diet; feeding habits; rural landscape; seasonal variation; tropical stream.

Resumo: Objetivo: Investigamos os efeitos a longo prazo de uma paisagem rural sobre a estrutura e organização trófica de uma assembleia de peixes. **Métodos:** Comparamos dados ambientais e dados da estrutura e organização trófica da assembleia de peixes, amostrados em um riacho localizado em uma área rural, em um intervalo de tempo de 20 anos. **Resultados:** Observamos apenas mudanças pontuais nas variáveis ambientais e na estrutura da ictiofauna, as quais podem não estar relacionadas com o ambiente rural. Na dieta dos peixes, a insetivoria se manteve predominante em todos os períodos amostrais. Entretanto, quando analisamos os grupos de hexápodes consumidos pelas espécies de peixes, encontramos uma substituição de Ephemeroptera e Trichopetra por Diptera ao longo do tempo. **Conclusões:** Embora a estrutura da assembleia de peixes não tenha sido afetada, a análise da dieta insetívora mostrou que o trecho do riacho pode estar em um processo contínuo de perda de qualidade ambiental, uma vez que a simplificação na estrutura da assembleia de macroinvertebrados ao longo do tempo é indicativa de degradação ambiental.

Palavras-chave: dieta; hábito alimentar; ambiente rural; variação sazonal; riacho tropical.

1. Introduction

The South American freshwater fish fauna is considered the most diverse in the world, with a high level of endemism and at least 50% of its species living in streams (Castro, 1999). For the São Paulo State, the brazilian most populous, 66 fish species are under extinction threat in different levels, the vast majority of which are small, typical of headwater streams, and many are susceptible to various impacts that lead to environmental degradation, such as pollution from industrial and domestic sewage and siltation (Oyakawa & Menezes, 2011).

Aquatic environments have a high dependence on the terrestrial landscape, which may influence aquatic ecosystem processes as food chain and habitat features (Fausch et al., 2002). Changes in the natural landscape caused by agriculture and livestock can harmfully affect streams, causing alterations in the physical environment, which results in habitat degradation and impacts on the fish communities, as decrease in diversity and variations in the composition (Roth et al., 1996).

Studies on the Brazilian stream fish fauna have shown a great flexibility and adaptability of this fauna due to spatial and temporal changes in the availability of food resources (Esteves & Aranha 1999; Uieda & Motta 2007). According to Casatti et al. (2006), changes in geomorphological features and in landscape, resulting from the agriculture and livestock activities, can also contribute to the decrease in the feeding resources supply, which may cause an increase of species with generalist diet.

There are many studies showing that changes in landscape affect the fish fauna, however most of them are in a spatial and/or short time scale (e.g. Cunico et al., 2012; Casatti et al., 2015; Ferreira et al., 2015). Long-term studies are extremely rare, but they are crucial for biological monitoring and conservation plans for allowing the distinction between natural variability and that caused by environmental changes (Leung & Dudgeon, 2011).

Here we investigated the long-term effects of a rural landscape on a fish assemblage structure and trophic organization. Our hypothesis was that the maintenance of the pasture area and the increase in *Eucalyptus* plantation in the surrounds of the river over 20 years could have caused major changes in the aquatic environment, which would result in a simplification on the fish assemblage structure and on its trophic organization.

2. Material and Methods

2.1. Study area and landscape characterization

The study was carried out on a fourth order stretch of Capivara River (22°53'53"S, 48°23'9'W), an important tributary on the left bank of the Middle Tietê River Basin, located in the central-west region of São Paulo State, Brazil. The studied stretch is located in a rural area and undergoes by anthropogenic activities over the last decades.

To evaluate changes in landscape along the last decades, we made land use maps by means of remote sense image classification, with aid of visual interpretation of Google Earth high spatial resolution imagery and fieldwork knowledge (Figure 1). To create the maps, we collected remotely sensed images of the years 1984, 1993 and 2013 from Thematic Mapper (TM) and Operational Land Images (OLI) sensors, onboard Landsat-5 and Landsat-8 satellites, respectively. Although in our study there is a fish assemblages comparison between the years 1992/1993 and 2013, we also consider it pertinent to analyze the characteristics of the land uses in a moment before that sample period. Therefore, we also included the analysis of the map of the year 1984 in a way to verify previous changes in the landscape that could influence the fauna. We used only green, red and near infra-red bands, which correspond to TM bands 3, 4 and 5, and OLI bands 4, 5 and 6. We downloaded TM images from National Institute for Space Research's (INPE) database (http://www.dgi.inpe.br/CDSR/) and acquired OLI images from USGS's database (http://www.earthexplorer.usgs.gov/). In order to scale the radiometry of the multitemporal images (Song et al., 2001), we perform a Dark Object Subtraction (DOS) normalization with the Semi-Automatic Classification Plugin (SCP) for QGIS. This algorithm performs DOS1, as described by Chavez (1996), using parameters from Chander & Markham (2003).

We obtained Normalized Difference Vegetation Index (NDVI) to enhance classification by equation NDVI = IR – R / IR + R, where: IR is the near infra-red band and R the corresponding red band. We classified NDVI and the spectral bands in a pixel by pixel approach with Maximum Likelihood algorithm, provided by SCP. We defined three classes based on field information: Seasonal Semideciduous Forest, Pasture and *Eucalyptus* plantations. Finally, we visually assessed the resulting maps and calculated land uses in hectares and percentage in relation to the total map area.

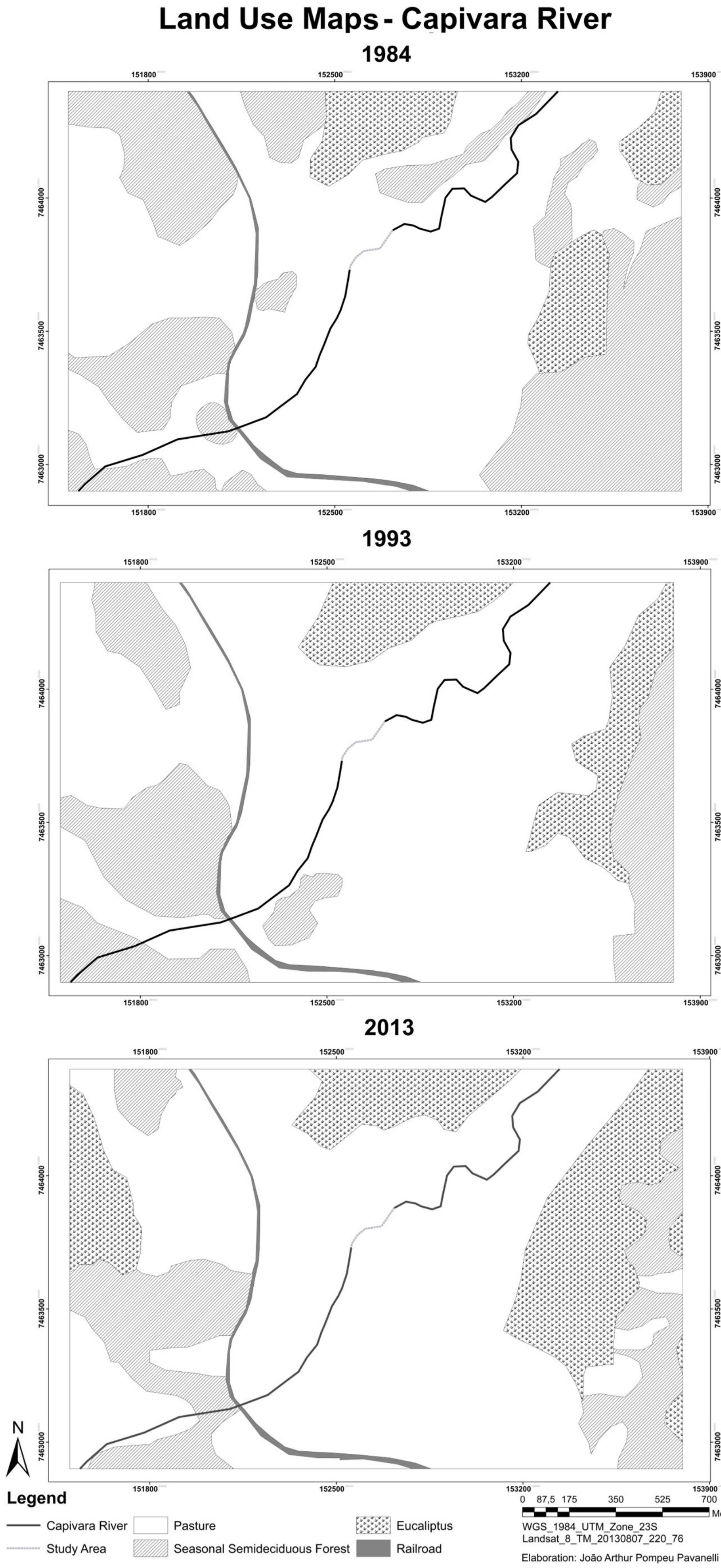

Figure 1. Land use maps of the Capivara River surroundings near the study area, in the years of 1984, 1993 and 2013.

The land use analysis (Table 1) showed the maintenance of pasture, representing over then 60% of the landscape, and a reduction on native forest coverage, replaced by *Eucalyptus* plantation over the past decades in the surroundings of the studied stretch.

2.2. Data collection and analysis

We characterized the limnological variables and fish assemblage along a 150 m stretch of the river in February of 1993 and 2013, representing a month of the wet season, and August 1992 and 2013, representing a month of the dry season. For 1992/1993 analysis, we used the limnological and fish composition data obtained from publications (Barretto & Uieda, 1998; Uieda & Barretto, 1999), while for diet data of this period we analyzed the gut content of the specimens deposited in a scientific collection (MZUSP – Museu de Zoologia, Universidade de São Paulo), following the ideas discussed by Manoel & Azevedo-Santos (2018).

Limnological characterization involved measurements in situ and water samples for laboratory analysis. We measured width and depth (measured in each margin and in the center) every two meters of the total sampled stretch. At the initial, middle and end of the stretch, we measured water temperature, current by the method of floating object driven by the current (Schwoerbel, 1975) and discharge (Leopoldo & Sousa, 1979). We did laboratory analysis of pH, concentration of dissolved oxygen and total suspended solids (Golterman et al., 1978). We used the limnological variables only for comparative analysis, as no statistical analysis could be applied to this comparison due to the lack of replicate data collected in 1992/1993.

We sampled and analyzed the fish assemblage in the two studied periods (1992/1993 and 2013) using the same methodology in a way to make possible the long-term comparison. For fish sample we used hand sieves (mesh 0.3cm) and fish traps in pools with marginal herbaceous vegetation, dip net in riffles and seines in runs. We standardized the sampling effort for number of collectors, sampling area and time spent, which was facilitated by the presence of two members of the field team in the two studied periods.

We analyzed the composition data of the fish assemblage for annual and seasonal similarity in abundance, comparing the data obtained in the wet and dry seasons of 1992/93 and 2013. We also calculated the Abundance, Richness, Shannon-Wiener Diversity and Simpson Equitability for all samples.

We examined the fish gut contents under binocular stereomicroscope and identified the food items to the lowest taxonomic category possible. They were classified as organic matter (remains of decomposed undetermined live material), plant debris (pieces of leaves and roots), algae (filamentous and unicellular), terrestrial hexapods (larvae and adult hexapods of allochthonous origin), aquatic hexapods (larvae and adult hexapods of autochthonous origin), crustaceans, arachnids (aquatic mites), annelids (oligochaetes) and protozoa (testate amoebae). We also identified aquatic hexapods at the order level.

For diet analysis, we used three methods: frequency of occurrence (Hyslop, 1980), biovolume (Esteves & Galetti-Jr, 1995) and numerical method (Hyslop, 1980). To determine the food habit of the species, we combined the frequency of occurrence and biovolume to calculate the alimentary index (Kawakami & Vazzoler, 1980). For the analysis of the consumed aquatic hexapods, we combined the three methods to calculate the Relative Importance Index (Pinkas et al., 1971). We transformed the index values calculated into percentage to allow diet comparison between different fish species and seasons/years sampled. We defined the feeding habits of each species considering the items that represent over than 30% in its diet: a) algivorous - algae, b) carnivorous - animal origin items, c) detritivorous - organic matter, d) insectivorous - aquatic and/or terrestrial insects, e) herbivorous – vegetal debris, f) omnivorous - plant and animal items, g) periphytivorous - algae and organic matter.

Table 1. Number of hectares (ha) and percentage (%) of the different land uses near the study area, in the years of 1984, 1993 and 2013.

	1984		1993		2013	
	ha	%	ha	%	ha	%
Seasonal Semideciduous Forest	106.2	31.0	68.6	20.0	54.3	15.9
Pasture	207	60.5	241.1	70.5	221.6	64.8
Eucalyptus plantation	29	8.5	32.5	9.5	66.3	19.4

We analyzed the fish diet data for annual and seasonal similarity in the overall diet and the consumed aquatic hexapod orders only for species that were present in both seasons (wet and dry) and annual periods (1992/93 and 2013). We could not do this analysis for *Hisonotus depressicauda* because we did not find the fishes of the wet-1993 deposited in the museum.

Statistical analyzes were performed using the software Primer 6.0 (Clarke & Gorley, 2006), using the log (x+1) transformation for fish absolute abundance data and square root for dietary percentage data. We constructed a resemblance matrix using the Bray-Curtis measure of similarity to the composition and diet data. We also performed a Principal Coordinates Analysis (PCoA) with data of hexapod orders consumed by the insectivorous species. This analysis allow us to explore and to visualize differences between species diet over time, where closest points represent species with higher similarity.

3. Results

Although no statistical analysis could be applied to the comparison of limnological data (Table 2) due to the lack of replicate data collected in 1992/1993, some temporal differences could be observed. The current and dissolved oxygen was higher in the dry season of 2013, the highest values of discharge and suspended solids occurred in the wet season of 1993, and in both years larger values of width occurred in the dry season and of depth and temperature in the wet season.

We sampled 12 species, which belong to three orders and eight families, with seven species found in all years and seasons (Table 3). In 1992/93, all 12 species occurred, while in 2013 we did not sampled *Corydoras aeneus* and *Pimelodella meeki*. The structure of the fish assemblage showed punctual differences with no clear patterns. We observed higher values of abundance in the dry-1992 and dry-2013 and lower in the wet-2013. The values of richness and diversity were higher in the wet-1993 and dry-2013, and evenness lower in dry-1992 (Table 3). For the abundance of the fish assemblage (Table 3), the analysis of similarity showed high values among all samples (74%), joining the dry season samples with 82% of similarity and the wet with 76%.

The abundance of most species varied between years and seasons (Figure 2), with only *Characidium zebra* showing values above 10% in all samples (Table 3). Most benthic species remained common in the area over time, as *Characidium zebra*, *Trichomycterus iheringi*, *Hisonotus depressicauda*, *Hypostomus ancistroides*, *Cetopsorhamdia iheringi* and *Imparfinis mirini* (Table 3, Figure 2). The species that showed the highest reduction in abundance when compared the two periods was *Phalloceros harpagos*.

We conducted the temporal comparison of fish diet only for six fish species and a complete temporal change on the main food resource consumed and, consequently, on the feeding habit of fish species was not common (Table 4). Four species, one characiform (*C. zebra*) and three catfishes (*T. iheringi, C. iheringi* and *I. mirini*), presented a constant insectivorous diet, and one armored catfish (*H. ancistroides*) presented a constant detritivorous habit. Only *P. harpagos* showed changes in the overall diet, which alternated between insectivorous and detritivorous diet (Table 4).

When we analyzed the order of hexapod consumed by the four insectivorous species, we found a temporal variation. While in 1992/93 the fishes consumed juveniles of Diptera, Trichoptera and Ephemeroptera, in 2013 the diet was more restricted to Diptera (Table 4, Figure 3).

The PCoA applied to the data of aquatic insect orders consumed by the fish species (Figure 4), differentiated four groups with high similarity based on the higher consumption of: Diptera (Group I),

Table 2. Average value of limnological variables used to characterize the studied stretch of Capivara River, analyzed in 1992, 1993 and 2013, in one month of the dry season (D- August) and one of the wet season (W- February).

Variables	D-1992	W-1993	D-2013	W-2013
Width (cm)	732.7	681.2	782.3	731.5
Depth (cm)	16.2	21.5	15.8	17.0
Water temperature (°C)	19	24	11	23
Current (cm/s)	36	57	64.4	41.7
Discharge (m^3/s)	0.4	1.2	0.6	0.5
Dissolved oxygen (mg/l)	7.8	8.2	9.6	8.5
pH	7.9	6.4	6.7	6.7
Total suspended solids (mg/l)	7.6	19.6	4.7	4.2

Table 3. Absolute (N) and relative (%) abundance of fish species sampled in the Capivara River, during the dry (D) and wet (W) season of 1992, 1993 and 2013.

Sampled species	Acronym	D-1992	W-1993	D-2013	W-2013
		N (%)	N (%)	N (%)	N (%)
Order Characiformes					
Family Parodontidae					
Parodon nasus Kner, 1859	Pnas	2 (0.9)	-	10 (4.0)	3 (2.1)
Family Crenuchidae					
Characidium zebra Eigenmann, 1909	Czeb	48 (21.6)	21 (10.2)	80 (32.0)	18 (12.9)
Family Characidae					
Astyanax paranae Eigenmann, 1914	Apar	7 (3.2)	1 (0.5)	35 (14.0)	-
Order Siluriformes					
Family Trichomycteridae					
Trichomycterus iheringi (Eigenmann, 1917)	Tihe	4 (1.8)	5 (2.4)	9 (3.6)	12 (8.6)
Family Callichthyidae					
Corydoras aeneus (Gil, 1858)	Caen	-	1 (0.5)	-	-
Family Loricariidae					
Hisonotus depressicauda (Miranda Ribeiro, 1918)	Hdep	11 (5.0)	42 (20.5)	31 (12.4)	22 (15.7)
Hypostomus ancistroides (Ihering, 1911)	Hanc	11 (5.0)	30 (14.6)	16 (6.4)	41 (29.3)
Family Heptapteridae					
Cetopsorhamdia iheringi Schubart & Gomes, 1959	Cihe	4 (1.8)	29 (14.1)	19 (7.6)	36 (25.7)
Imparfinis mirini Haseman, 1911	Imir	27 (12.2)	20 (9.8)	23 (9.2)	2 (1.4)
Pimelodella meeki Eigenmann, 1910	Pmee	-	15 (7.3)	-	-
Rhamdia quelen (Quoy & Gaimard, 1824)	Rque	-	3 (1.5)	2 (0.8)	2 (1.4)
Order Cyprinodontiformes					
Family Poeciliidae					
Phalloceros harpagos Lucinda, 2008	Phar	108 (48.6)	38 (18.5)	25 (10.0)	4 (2.9)
Abundance		222	205	250	140
Richness		9	11	10	9
Diversity		2.210	2.959	2.896	2.567
Evenness		0.364	0.631	0.593	0.550

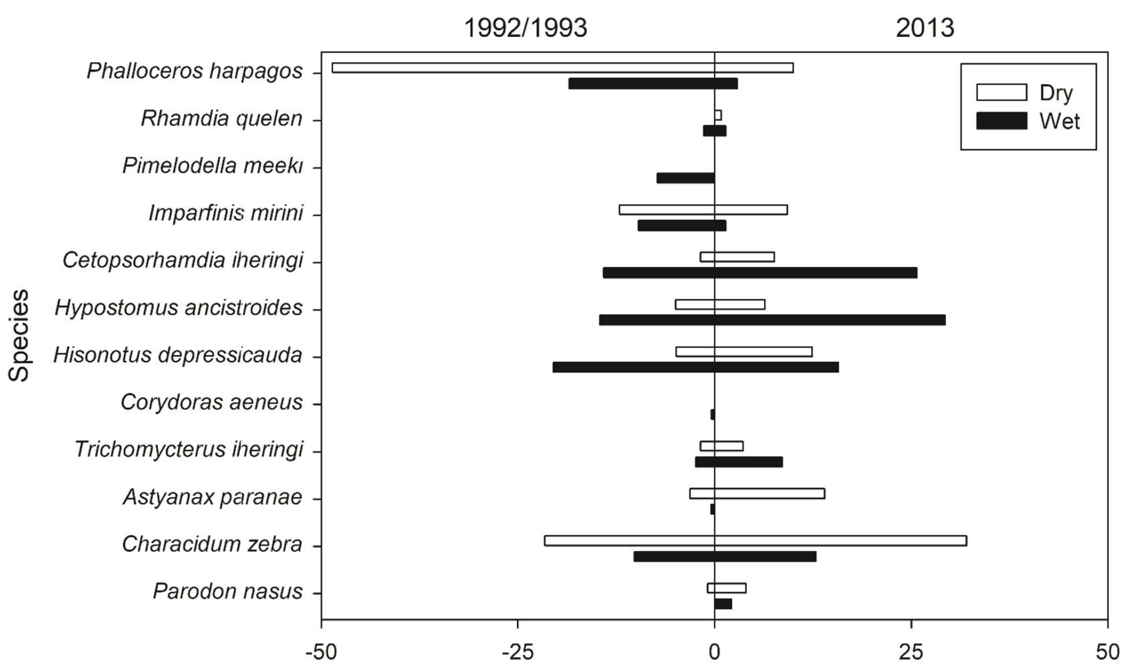

Figure 2. Relative abundance of the fish species sampled in the Capivara River, during the dry and wet season in the years 1992/1993 and 2013.

Table 4. Food habit of the fish species sampled in the Capivara River, during the dry (D) and wet (W) season of 1992, 1993 and 2013. Ins-insectivorous, Det-detritivorous. Predominant Hexapoda orders consumed by the insectivorous species: Dipt-Diptera, Ephe-Ephemeroptera and Tric-Trichoptera.

Fish species	D-1992	W-1993	D-2013	W-2013
Characidium zebra	Ins (Dipt/Ephe)	Ins (Tric)	Ins (Dipt)	Ins (Dipt)
Trichomycterus iheringi	Ins (Tric)	Ins (Dipt/Tric)	Ins (Dipt)	Ins (Dipt)
Hypostomus ancistroides	Det	Det	Det	Det
Cetopsorhamdia iheringi	Ins (Tric/Ephe)	Ins (Dipt/Ephe)	Ins (Dipt/Ephe)	Ins (Dipt)
Imparfinis mirini	Ins (Dipt/Tric)	Ins (Dipt/Ephe)	Ins (Dipt)	Ins (Dipt)
Phalloceros harpagos	Ins (Dipt/Tric)	Det	Det	Ins (Dipt)

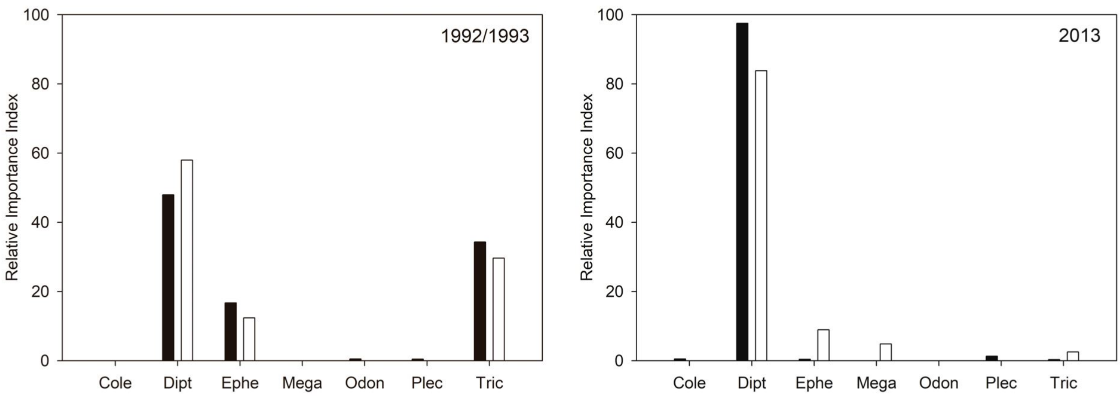

Figure 3. Sum of the relative importance index values of aquatic insects consumed by the four insectivorous fish species sampled in 1992/1993 and 2013 in Capivara River. Black bars represent the wet and white bars the dry season. Insect orders: Cole-Coleoptera, Dipt-Diptera, Ephe-Ephemeroptera, Mega-Megaloptera, Odon-Odonata, Plec-Plecoptera, Tric-Trichoptera.

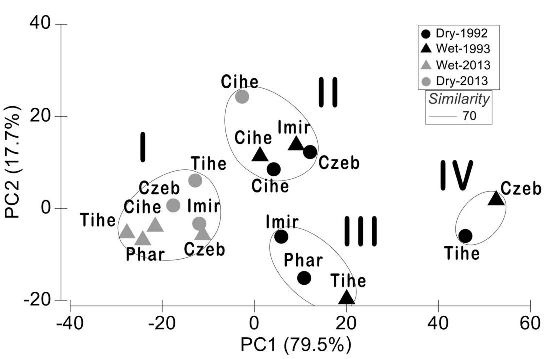

Figure 4. Results of the Principal Coordinates Analysis applied to the diet data based on the relative importance index of the fish species sampled in Capivara River. Species acronym presented in Table 3.

Diptera and Ephemeroptera (Group II), Diptera and Trichoptera (Group III) and Trichoptera (Group IV). The species sampled in 2013 were included mainly in group I, while species collected in 1992/93 were included exclusively in groups II, III and IV. This analysis shows a simplification in the diet of fish sampled in 2013 and a seasonal variation in the diet of most species sampled in 1992/1993, as also shown in Figure 3.

4. Discussion

The land use maps showed that the changes in landscape occurred mainly between 1984 and 1993, with a major reduction on native forest coverage replaced by *Eucalyptus* plantation, which remained between 1993 and 2013. The small variations in the limnological variables indicated that the stream characteristics have remained the same as the characteristics of the landscape and probably the major changes in the stream must have occurred between 1984 and 1993.

The high similarity in the fish fauna composition did not confirm the initial hypothesis of temporal variation on fish fauna structure. Although some species had a remarkable reduction in the abundance, the assemblage in general remained similar, with mainly punctual variations in the composition. The abundance decrease of *P. harpagos* in 2013 may be related to the reduction on the marginal backwaters located in pool mesohabitats, where this species can find refuge. In 1992/93, marginal backwater areas were constant throughout the pool areas in whole stretch sampled; however, in 2013 they were restricted mainly to a total length of no more than four meters. This reduction may be related to the accumulation of sediments in the bedrock, characteristic of streams located in pasture areas, which decrease the depth and affect species as *P. harpagos* (Rabeni & Smale, 1995) that generally inhabits shallower sites in streams (Casatti, 2002).

The absence of major changes in the fish species overall diet , comparing annual or seasonal periods, was similar to that found by Rocha et al. (2009), which showed no significant differences in the overall fishes' diet over a year in a Brazilian deforested stream. Although *P. harpagos* showed variation in the overall diet, these changes occurred only between seasons of each period, thus, this alternation might not be associated with the landscape. A complete change in the overall diet is more common in omnivorous species and would be difficult to occur in detritivores and insectivorous ones due to their evolutionary history. However, some authors (Rocha et al., 2009; Zeni & Casatti, 2014) reported an addition of food items in the diet of detritivorous, such as algae and vegetal debris, or a change in the hexapod orders ingested by insectivorous in impacted aquatic environments. In our study, these changes not occurred in the detritivorous fishes; however, the hexapod orders consumed altered over time in the insectivorous.

The consumption of benthic insects such as Ephemeroptera and Trichoptera were highly reduced in 2013, when most fish species consumed predominantly larvae of Diptera (Chironomidae), which may indicate an expressive simplification in the diversity of food resources. According to Lorion & Kennedy (2009), streams in pasture areas have macroinvertebrate assemblages with low diversity and number of sensitive taxa due to the high substratum embeddedness. Thus, we may infer that a decrease in the macroinvertebrates diversity in the studied stream may be the causal factor of the diet simplification found.

The substitution of Ephemeroptera and Trichoptera by Diptera-Chironomidae in the fish's diet may indicate an ongoing process of environmental quality simplification in the stream, once benthic macroinvertebrates are known as an important tool for biomonitoring programs and this replacement is indicative of possible environmental impact (Buss et al., 2015). The dominance of Chironomidae is generally associated to impacted environments, due to their tolerance to extreme conditions (Bonato et al., 2012), while Trichoptera and Ephemeroptera are indicators of great environmental quality (Barnes et al., 2013). Therefore, the analysis of the insectivorous diet seemed to be a good tool to evaluate environmental changes in long-term studies, since the assemblage composition and overall diet data did not indicate high differences.

Even with the long-term maintenance of the rural landscape, the high similarity in the fish fauna composition and the overall diet showed small effects of this landscape on the fish fauna. However, there was a simplification on the items consumed by insectivorous species, what may be a signal of an ongoing process of environmental quality simplification.

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