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Changes in ichthyofauna composition along a gradient from clearwaters to blackwaters in coastal streams of Atlantic forest (southeastern Brazil) in relation to environmental variables

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The lack of knowledge of the freshwater ichthyofauna of coastal streams in the State of São Paulo (Brazil) is a cause of concern, as these streams are inserted in the Atlantic forest, a hotspot highly threatened. The aim of the present study is to investigate the freshwater ichthyofauna composition of clear and blackwater streams in a preservation area of Brazilian Atlantic forest. Fish samples were taken using electrofishing. A total of 20 species were registered, with *Astyanax ribeirae*, *Hollandichthys multifasciatus*, and *Mimagoniates microlepis* (Characiformes, Characidae) as the more representative. In general, the observed pattern of occurrence and distribution of fish species varied according to habitat characteristics, due to the longitudinal gradient in clearwaters, and among clearwaters and blackwaters. In clearwater streams, the headwater stretches had lower species diversity, while the opposite occurred in the middle and lower sites. These longitudinal variations of ichthyofauna were related with habitat characteristics (depth, stream flow, and bottom type) in which they were found, since the diversity of habitats was higher in headwaters and lower in downstream reaches (middle and lower sites). The physical and chemical variables of water do not seem to have influenced the distribution of species in clearwater streams, but the clear and blackwater fish composition was influenced mainly by pH concentration. Unlike the spatial differences, significant temporal differences were not registered in fish assemblages, probably due to the absence of a pronounced dry season in the studied region.

A falta de conhecimento dos peixes de água doce em riachos costeiros no estado de São Paulo é preocupante, considerando que estes riachos estão inseridos em um bioma brasileiro extremamente ameaçado, a floresta Atlântica. O objetivo deste trabalho é investigar a composição da ictiofauna de riachos de águas claras e pretas em uma área preservada da Mata Atlântica. A pesca elétrica foi utilizada para amostrar a ictiofauna. Foram coletadas 20 espécies, sendo *Astyanax ribeirae*, *Mimagoniates microlepis* e *Hollandichthys multifasciatus* (Characiformes, Characidae), as mais representativas. De modo geral, o padrão de ocorrência e distribuição da ictiofauna observado variou de acordo com as características dos habitats, tanto no gradiente longitudinal nos riachos de águas claras, quanto entre os riachos de águas claras e o de águas pretas. Nos riachos de águas claras, os trechos de cabeceiras apresentaram riqueza e diversidade de espécies menor, enquanto o inverso ocorreu nos pontos médios e inferiores. Estas variações longitudinais da ictiofauna provavelmente estão associadas às características dos ambientes (profundidade, correnteza e substrato) em que foram encontradas, visto que a diversidade de habitats foi maior nos trechos de cabeceiras e menor nos trechos médios e inferiores. As variáveis físicas e químicas da água parecem não ter influenciado tanto a distribuição das espécies nos riachos de águas claras, porém as diferenças na composição da ictiofauna entre riachos de águas claras e de águas pretas foram influenciadas principalmente pelo pH. Ao contrário das diferenças espaciais, não foram registradas diferenças temporais nas ictiocenoses, provavelmente devido à ausência de um período seco pronunciado na região de estudo.

Key words: Fish structure, Habitat diversity, Jureia-Itatins, pH concentration.

Introduction

One of the challenges for stream fish ecologists until today is to understand the mechanisms and processes responsible for fish structuring (Gido & Jackson, 2010). The structure of fish assemblages in streams can be influenced by factors that act on the temporal and spatial dimensions. In the first case, fish abundance and distribution are influenced by the combination of historical, evolutionary and biogeographical processes, including contemporaneous aspects (daily, seasonally, and yearly variations), while the spatial dimension considers global, regional and local scales (Matthews, 1998; Winemiller *et al*., 2008).

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Several studies conducted both in temperate (*e.g*. Sheldon, 1968; Horwitz, 1978; Angermeier & Schlosser, 1989) and tropical (*e.g*. Angermeier & Karr, 1983; Angermeier & Schlosser, 1989; Gilliam *et al*., 1993) environments, have shown that ichthyofauna diversity increases from upstream to downstream areas and that there is a positive relationship between fish diversity and habitat diversity (Gorman & Karr, 1978; Ibañez *et al*., 2009). In Brazil, such patterns were also found for several inland streams (*e.g.*, Braga & Andrade, 2005; Casatti, 2005). In coastal drainages, the influence of the longitudinal gradient in the structure of fish assemblages of clearwater streams was investigated from the production rate (Mazzoni & Lobón-Cerviá, 2000), habitat (Mazzoni & Iglesias-Rios, 2002; Gerhard *et al*., 2004; Barreto & Aranha, 2005; Guimarães *et al*., 2010), species composition (Uieda & Uieda, 2001; Ferreira & Petrere Jr., 2009), and trophic (Esteves & Lobón-Cerviá, 2001) perspectives, among others. Blackwater streams occur in temperate and tropical forests (Meyer, 1990), and in Brazil, the hydrochemical differences of blackwaters from clearwaters were highlighted in Amazon rivers (Por & Lopes, 1994), but not in Atlantic forest streams. Little attention was given to the relationship between habitat *versus* fish diversity in freshwater coastal streams, considering the water variables differences between streams of clear and blackwaters.

The lack of knowledge of the ichthyofauna in coastal streams in the State of São Paulo is a cause of concern (Menezes *et al*., 2007), as they are inserted in the Atlantic forest, a highly threatened hotspot due to deforestation (Myers *et al*., 2000; Ribeiro *et al*., 2009). The popular or technical literature often neglects the conservation of aquatic environments (Menezes *et al*., 2007), despite the high endemism and diversity of fish in streams in this threatened Brazilian biome (Bizerril, 1994, 1995; Ribeiro, 2006; Menezes *et al*., 2007; Abilhoa *et al*., 2011; Oyakawa & Menezes, 2011). Several species have been described, however, few ecological studies have been conducted so far (*e.g*., Buck & Sazima, 1995; Esteves & Lobón-Cerviá, 2001; Deus & Petrere Jr., 2003; Gerhard *et al*., 2004; Ribeiro *et al*., 2006; Serra *et al*., 2007; Ferreira & Petrere Jr., 2009; Mattox & Iglesias, 2010).

The Jureia-Itatins Ecological Station (JIES) is one of the few protected areas in the State of São Paulo which harbor a significant portion of this biome, but unfortunately, knowledge about ichthyofauna diversity is incipient (*e.g*., Sabino & Silva, 2004; Oyakawa *et al*., 2006). The aim of the present study is to investigate the freshwater ichthyofauna composition of clear and blackwater streams in JIES. Thus, we addressed the following specific questions: (1) Does the fish fauna of clearwater streams differ in relation to the blackwater stream? (2) What is the relationship between habitat diversity and ichthyofauna diversity in clearwater streams? (3) Does the fish fauna of clearwater streams and the blackwater stream vary over 1-year period? We hypothesized that habitat characteristics will reflect the spatial distribution and the diversity of fish species in clear and blackwater streams. Considering the absence of a pronounced dry season in the studied region, temporal variations in ichthyofauna composition are not expected.

Material and Methods

Study area

The Juréia-Itatins Ecological Station (JIES) is a conservation area (80.000 ha) located on the south coast in the State of São Paulo, between 24°18'- 24°32'S and 47°00'- 47°30'W. The climate is classified as humid subtropical without a dry season. The hotter and rainier season occurs from October to April, and a less pronounced rainy season occurs from May to September. The average annual rainfall and temperature are 2,277 mm and 21.4ºC, respectively. JIES is formed by rocks of the Precambrian Coastal Complex which are predominant and occur in part of the coastal mountains of the Itatins mountain range and in the coastal plains in the State of São Paulo south coast. Several elevations occur along the long stretch of the coastal plain of the eastern lowlands of Ribeira de Iguape River, especially in the Jureia mountain range (altitude around 870 m). Although the Atlantic rainforest covers many vegetation types, the dense ombrophilous forest and "restinga" forest are predominant in mountain and lowland regions, respectively, of the studied area. Rupestrian fields are present at the top of the hills, and mangroves are found along large river estuaries (Marques & Duleba, 2004). "Restinga" is the usual name that classifies the vegetation of the Brazilian coastal plains formed by sediments of marine origin (Sampaio *et al*., 2005).

The JIES has a diverse river system including different types of water, classified according to hydrological and chemical characteristics as: mountain streams (clearwaters), lowland or "restinga" streams (blackwaters), and small estuarine areas (transition zones between the marine and freshwater environments) (Por, 1986; Por & Lopes, 1994). The differences are primarily related to topography, soil type and the vegetation type predominant in the drainage system. Thus, mountain streams that drain the dense ombrophilous forest (Precambrian soils) have clear but nutrient-poor waters (pH ~ 5), while the lowland streams have blackwaters rich in humic substances (pH ~ 4), due to the presence of "restinga" forest (alluvial, podzolic, and hydromorphic soils). The estuarine regions are influenced by the tidal rhythm fluctuations and therefore have brackish waters (Por, 1986; Por & Lopes, 1994). The JIES area is drained mainly by the middle and lower stretches of Una do Prelado River (or "Comprido" River), a lowland and medium-sized river (100 m), which runs parallel to the sea shore surrounding the Jureia mountain range (Por, 1986; 2004). Additionally, the JIES has a number of smaller streams that drain directly into the sea, as the selected ones in the present study.

Sampling

Sampled sites included six clearwater streams (ribeirão das Antas, ribeirão Tetequera, ribeirão Grajaúna, "Ponte 2", rio Verde, "Riacho 1"), and one blackwater stream (rio Preto). Quotation marks indicate fictitious names to unnamed streams. The sampled sites were selected to include the upper (site 1), middle (site 2) and lower (site 3) stretches of each stream (except rio Preto). The choice of sampled sites was based on topographic maps, considering the structural characteristics of habitat and the altitude of each site. Additional blackwater streams were not selected because they were larger than the other selected streams and/or because of marine influence. Streams near estuaries were avoided because of the strong tidal influence, but despite this, some lower stretches possibly have been affected by brackish waters during high tides. Thus, collections were practiced during low tide whenever possible (periods of waxing and waning moons) to minimize marine influence. Therefore, 19 sampling sites were selected (Table 1). Samplings were made during four occasions (autumn: 08-14 April 2009; winter: 28 July to 04 August 2009; spring: 05-12 November 2009, and summer: 02-09 February 2010).

Environmental variables

The pH, conductivity, dissolved oxygen and water temperature were measured at each sampling site during the year (four samples). The Winkler method was used to measure the dissolved oxygen, according to Moraes (2001). The other variables were measured in the field using a digital Marte analyzers, models MB 10P and MB 11P.

Habitat structure was evaluated at each site measuring stream width (m), flow ($m.s^{-1}$), depth (cm), and the predominant bottom type (clay: < 0.05, sand: 0.05-2, gravel: 2-10, pebble: 10-100, rock: 100-300, boulder: > 300 mm, bed rock, plant debris and silt). A weighted mean was calculated to bottom type, according to the formula: $x = \Sigma(f_i * x_i)/\Sigma f_i$, considering the numerical frequency (f) and weight (x) of i bottom categories (clay = 1, sand = 2, gravel =

Table 1. Description of the sampled sites in Jureia-Itatins Ecological Station, indicating the clear (*) and blackwaters (**) streams, geographic location, channel order (scale 1:50,000), altitude (m), shading and state of preservation of riparian vegetation (P: preserved, PD: partially deforested, D: deforested). Range and average (in parentheses) values of pH, conductivity ($\mu S.cm^{-1}$), water temperature (°C), dissolved oxygen ($mg.L^{-1}$), and Shannon diversity index considering habitat structure ($H'_{habitat}$) at each sampled site, between April 2009 and February 2010. Upper (site 1), middle (site 2) and lower (site 3) stream stretches. Measures of habitat structure were taken during June 2010.

Streams	Sites	Coordinates	Order	Altitude	Shading	Riparian vegetation	pH	Conductivity	Water temperature	Dissolved oxygen	$H'_{habitat}$
Antas*	1	24°21’26.90”S 47°02’15.60”W	2	49	> 76%	PD	5.4-6.2 (5.9)	44.1-57.5 (50.4)	17.5-25.5 (22.2)	8.3-10.3 (9.3)	2.09
	2	24°21’37.00”S 47°02’15.20”W	2	19	> 76%	PD	5.1-6.1 (5.7)	49.9-58.4 (52.8)	17.3-25.4 (22.5)	8.2-10.4 (9.1)	1.93
	3	24°21’43.20”S 47°02’15.34”W	2	15	> 76%	PD	5.2-7.1 (6.0)	43.9-57.0 (49.9)	17.5-27.5 (23.0)	8.0-9.0 (8.6)	1.71
Tetequera*	1	24°23’18.00”S 47°05’53.70”W	1	41	26-50%	PD	5.3-5.8 (5.5)	26.1-45.2 (39.0)	17.0-26.3 (22.3)	8.0-14.4 (10.3)	2.38
	2	24°23’25.70”S 47°05’49.30”W	2	25	0-25%	D	4.9-5.6 (5.2)	36.9-46.8 (42.9)	17.5-25.8 (22.3)	7.8-16.8 (11.4)	2.21
	3	24°23’00.20”S 47°04’50.30”W	2	16	51-75%	PD	4.8-6.0 (5.5)	40.2-50.7 (45.4)	17.5-28.2 (23.9)	8.2-9.6 (9.3)	2.12
Grajaúna*	1	24°31’03.10”S 47°12’48.90”W	1	60	> 76%	P	5.4-6.6 (6.3)	44.8-62.3 (55.7)	18.0-25.7 (22.4)	7.2-10.3 (8.5)	2.13
	2	24°31’08.10”!S 47°12’42.90”W	2	30	51-75%	P	4.9-7.1 (6.1)	46.0-61.5 (56.9)	18.0-24.7 (22.4)	7.8-10.1 (9.0)	2.12
	3	24°31’17.30”S 47°12’04.40”W	2	15	> 76%	P	4.6-6.8 (5.7)	61.2-67.2 (63.3)	18.0-26.5 (22.8)	6.8-9.3 (7.9)	1.84
“Ponte 2”*	1	24°32’18.80”S 47°12’46.40”W	1	45	> 76%	P	5.2-7.9 (6.5)	64.6-87.0 (74.2)	18.2-25.7 (21.8)	8.6-9.9 (9.2)	1.89
	2	24°32’23.00”S 47°12’44.90”W	2	30	> 76%	P	5.4-7.6 (6.3)	59.9-85.4 (71.8)	17.9-26.2 (22.1)	6.8-10.1 (8.0)	1.89
	3	24°32’30.00”S 47°12’39.60”W	2	18	> 76%	P	5.4-8.1 (6.3)	62.4-88.0 (71.5)	17.9-27.9 (22.8)	7.4-13.3 (9.5)	1.66
rio Verde*	1	24°32’52.60”S 47°14’37.20”W	2	90	26-50%	P	6.0-7.7 (6.7)	39.7-48.4 (43.9)	18.0-25.5 (21.8)	8.0-10.5 (9.2)	2.09
	2	24°32’52.10”S 47°14’17.50”W	3	60	26-50%	P	6.2-7.5 (6.9)	44.6-50.5 (47.0)	18.9-27.5 (22.9)	7.2-10.3 (8.9)	2.07
	3	24°32’54.10”S 47°14’04.50”W	3	35	26-50%	P	5.5-6.3 (6.0)	46.5-51.8 (49.2)	17.7-25.5 (21.4)	8.0-10.5 (9.4)	1.76
“Riacho 1”*	1	24°33’05.50”S 47°13’55.70”W	1	35	> 76%	P	5.3-7.2 (6.4)	81.3-94.5 (88.3)	20.2-22.2 (21.3)	7.6-20.5 (11.2)	1.72
	2	24°33’02.50”S 47°13’55.90”W	2	21	> 76%	P	5.5-7.3 (6.6)	81.1-93.0 (86.8)	20.1-24.6 (22.3)	8.0-9.0 (8.1)	1.78
	3	24°32’59.40”S 47°13’57.90”W	2	18	> 76%	P	5.5-8.0 (6.7)	86.9-89.2 (88.1)	20.0-25.6 (22.5)	6.6-8.9 (7.3)	1.26
rio Preto**	-	24°31’17.30”S 47°12’04.40”W	1	14	> 76%	P	3.2-4.3 (3.9)	49.6-80.0 (66.7)	17.5-27.3 (23.1)	3.3-5.7 (4.7)	1.80

3, pebble = 4, rock = 5, boulder = 6, and bed rock = 7). The shading provided by riparian vegetation cover (0-25%, 26-50%, 51-75% and above 76%) and its preservation status (preserved, partially deforested and deforested) were also registered. Habitat structure measures followed Gorman & Karr (1978), with modifications, *i.e*, data was obtained in stretches of 100 m, which included the 50 m stretches where the ichthyofauna was sampled. Ten linear transects were set along stream stretches (one every 10 m). Replicate measurements of stream width and flow, channel depth and bottom type were taken every meter along each transect. Stream width and depth were measured with a measuring tape, and stream flow with a mechanical General Oceanics flowmeter. Bottom type was classified visually, considering the established categories. Habitat structure measures were conducted once during 11-20 June 2010 at all sampling sites to provide an environmental characterization of selected streams.

Ichthyofauna

Fish specimens were caught with electrofishing in stretches of 50 m in one passage of the equipment (without block seine). Electrofishing gear was used according to the field conditions. In sites easier to access, the electrofisher consisted of a rectifier current (connected to two dip nets) powered by a gasoline-driven Yamaha generator. Sampled sites which were difficult to reach were sampled with a Smith-Root backpack electrofisher (model LR-24, 500V DC), connected to two dip nets. The dip nets were handled by two people who walked slowly upstream in the opposite direction to the stream flow to the upper limit (50 m). Because of the greater width of the lower point (site 3) in rio Verde and in order to make sampling more efficient, this stretch was split in two and sampling performed first in the right bank, and then on the left shore. In April 2009, electrofishing was not performed at the upper stretch (site 1) of the "Riacho 1" due to the low water volume.

Fish specimens were anesthetized with benzocaine, fixed in 10% formalin for around 48 hours, and then kept in 70% alcohol until the analysis. Fish specimens were measured to obtain the total and the standard length (mm), and weighted (g).

Identification of fish species was made by specialists, and voucher specimens of freshwater species are deposited in the fish collection of the Coleção de Peixes do Departamento de Zoologia e Botânica do Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista, São José do Rio Preto, SP (DZSJRP 13234-13258).

Data analysis

Environmental variables. The numerical frequencies from categories of structural variables of the habitat, *i.e*., stream width, depth (shallow: 0-30, moderate: 30-100, deep: > 100 cm), flow (very slow: < 0.05, slow: 0.05-0.2, moderate: 0.2-0.4, fast: 0.4-1.0, very fast: > 1.0 $m.s^{-1}$), and bottom type (clay: < 0.05, sand: 0.05-2, gravel: 2-10, pebble: 10-100, rock: 100-300, boulder: > 300 mm, bed rock, plant debris), were used to estimate the habitat diversity of each sampling site (1, 2, 3) within each stream (Gorman & Karr, 1978). The Shannon index was calculated to assess habitat diversity ($H'_{habitat}$), using the software PAST (Hammer *et al*., 2001).

A multivariate approach was used to identify the most important factors for fish community structure, considering habitat (width, depth, flow, and bottom type) and water variables (pH, conductivity, dissolved oxygen and temperature) at each sampled site. The dimensionality of data was reduced in the Principal Component Analysis (PCA) and data was log (x+1) transformed in order to linearize relationships (Legendre & Legendre, 1998). Data was treated as continuous both for habitat and water variables, and the mean values were used to water variables. This analysis was performed using the software PAST (Hammer *et al*., 2001).

Ichthyofauna. The α diversity of ichthyofauna was estimated to each site by the Shannon index ($H'_{ichthyofauna}$), considering the six clearwater streams (three sites in each one) and the blackwater stream, during the four temporal collections, using the software PAST (Hammer *et al*., 2001). In order to verify the differences of $H'_{ichthyofauna}$ between sites (1, 2, 3) and seasons (four temporal collections), we used the following model: $H'_{ichthyofauna} = S_t + S_i + S_e + H'_{habitat} + S_i * S_e + S_i * H'_{habitat} + S_e * H'_{habitat} + S_i * S_e * H'_{habitat}$, where $H'_{ichthyofauna}$ is the ichthyofauna diversity estimated by Shannon index, S_t is the effect of the streams, S_i is the effect of the sites, S_e is the effect of the seasons and $H'_{habitat}$ is the habitat diversity estimated by Shannon index. The rio Preto was excluded from this analysis due to the absence of replicate sites in this stream.

A post-hoc test (LSD) was used to verify fish fauna variations among sites (1, 2, 3) considering the observed species richness and ichthyofauna diversity registered in each clearwaters' stream stretch.

Simple linear regressions were used to verify the dependence of ichthyofauna diversity on habitat diversity to each site (1, 2, 3), according to the model: $H'_{ichthyofauna} = a + b H'_{habitat} + e$, were $H'_{ichthyofauna}$ is the ichthyofauna diversity estimated by Shannon index, $H'_{habitat}$ is the habitat diversity estimated by Shannon index, e is the error (with normal distribution and constant variance), and a and b are the model parameters representing the intercept and the slope, respectively.

To univariate analysis, we considered p-values less than 0.05 statistically significant.

To ordinate the sample sites hierarchically, a cluster analysis was applied to the ichthyofauna abundance matrix, considering the six clearwater streams (three sites in each one) and the blackwater stream. The Bray-Curtis index and the single linkage method were used. Data was log (x+1) transformed in order to linearize relationships (Legendre & Legendre, 1998). This analysis was performed using the software PRIMER 6 (Clarke & Gorley, 2006).

To analyze species-environment relationships, a multivariate approach was used relating the fish species abundance with habitat structural variables (stream width, depth, flow, and bottom type) and the physical and chemical variables of water (pH, conductivity, dissolved oxygen, and temperature), considering the six clearwater streams (three sites in each one) and the blackwater stream. The dimensionality of data was reduced in Canonical Correspondence Analysis (CCA) and data was log (x+1) transformed in order to linearize relationships (Legendre & Legendre, 1998). Data was treated as continuous both for habitat and water variables, and the mean values were used to water variables. This analysis was performed using the software R, version 2.15.1 (R Development Core Team, 2012).

Results

Environmental variables

The characteristics of the sampled sites, such as the preservation status of riparian vegetation and the shading that it provides to streams are presented in Table 1.

Overall, data of physical and chemical water variables were similar among clearwater streams (Table 1). PCA evidenced the differences between clearwater streams and the blackwater stream: blackwaters had low dissolved oxygen and pH concentration, lentic waters and small-sized bottom categories, whereas clearwaters had the opposite characteristics (Fig. 1). The lowest pH values were associated to the blackwaters of rio Preto (ranged from 3.2 to 4.3). In most clearwater streams, the concentration of dissolved oxygen decreased from upstream to downstream, while the water temperature increased (Table 1).

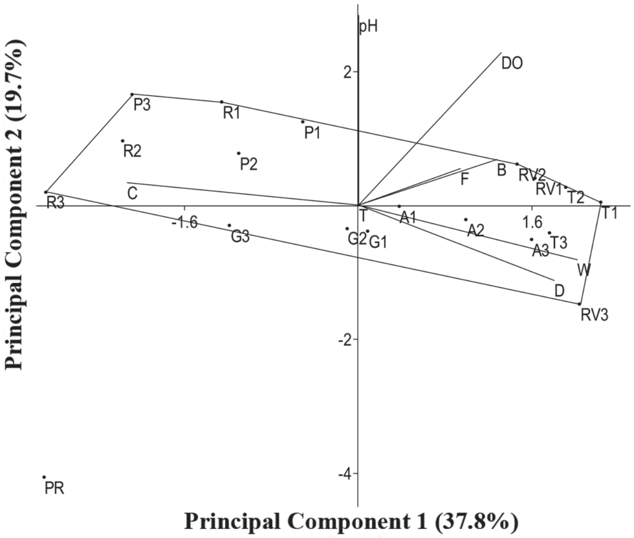

Fig. 1. Ordination resulted from principal components analysis, considering the structural habitat variables (width: W, depth: D, flow: F, and bottom: B), and the physical and chemical water variables (pH, conductivity: C, dissolved oxygen: DO, and temperature: T) in sites of Jureia-Itatins Ecological Station. Streams: Antas (A), Tetequera (T), Grajaúna (G), "Ponte 2" (P), rio Verde (RV), "Riacho 1" (R), and rio Preto (PR). Stretches: upper (1), middle (2), and lower (3). Percentages of explanation of the axes are given in parentheses. Envelope designates clearwater streams.

The habitat diversity was higher in the headwaters (upper stretches), and decreased gradually toward downstream (middle and lower stretches) in clearwater streams, according to the Shannon index (Table 1).

Ichthyofauna

A total of 3,650 specimens of six orders, 11 families and 20 species of freshwater fish were captured (Table 2). The family Characidae predominated, mainly due to the abundance of *Astyanax ribeirae*, *Mimagoniates microlepis*, and *Hollandichthys multifasciatus*. Considering each of the 19 sampled sites, the observed species richness ranged from zero (upper reach of "Ponte 2") to 14 species (lower reach of ribeirão Grajaúna) (Table 2). Upper stream stretches had much lower species richness, whereas middle and lower sites were more species-rich but had similar values (Table 2, Fig. 2a). The same pattern was observed for ichthyofauna diversity among stream stretches: according to Shannon index, ichthyofauna was much less diverse in the upper reaches, whereas middle and lower stretches were more diverse (Table 2, Fig. 2b).

Differences in ichthyofauna diversity using the Shannon index values were found among sites (1, 2, 3) ($F = 3.368$, $df = 2$, $p = 0.046$), but not between seasons ($F = 0.768$, $df = 3$, $p = 0.520$). The significant interaction between sites and habitat diversity ($F = 3.587$, $df = 2$, $p = 0.038$) indicated that the relationship between ichthyofauna diversity and habitat diversity among sites (1, 2, 3) were not the same. In the upper sites (1), species diversity decreased as the habitat diversity increased ($p = 0.035$), while in the lower sites (3) there was an opposite trend ($p = 0.205$). In the middle sites (2) there was a trend similar to that found for the upper sites, but it was not significant ($p = 0.821$).

Overall, the similarity was greater among stretches of the same stream according to ichthyofauna abundance, as showed by cluster analysis (Fig. 3). The similarity was also greater between streams of same drainages: middle and lower stretches of ribeirão Grajaúna were ordinate closer to rio Preto, stretches of "Riacho 1" were closer to rio Verde, and ribeirão Tetequera was nearer to ribeirão das Antas stretches. The upper stretches of studied streams had lower species similarity among them, which evidence the presence of a few species in common between these headwaters environments.

The CCA ordered the ichthyofauna according to their abundance in each site in relation to habitat characteristics (Fig. 4). In general, the CCA evidenced the differences in species composition among clear and blackwater streams: the low pH concentration of blackwaters explained the abundance of *Mimagoniates microlepis*, *Characidium pterostictum*, *Pseudotothyris obtusa*, *Hyphessobrycon griemi*, *H. reticulatus*, and *Scleromystax macropterus* at rio Preto. The abundance of some of these species approached the rio Preto with middle and lower Grajaúna clearwater stretches. To "Riacho 1" and "Ponte 2" streams, the high conductivity and pH concentration were determinant to abundance of *Hollandichthys multifasciatus* in these streams. The occurrence of *Characidium* cf. *schubarti* and *Kronichthys*

Table 2. Total number of individuals (N), biomass (B), and average standard length (SL) of fish species from all sites, species richness, Shannon diversity index considering the ichthyofauna ($H'_{ichthyofauna}$) of each sampled site in Jureia-Itatins Ecological Station, between April 2009 and February 2010. Streams: Antas (A), Tetequera (T), Grajaúna (G), "Ponte 2" (P), rio Verde (RV), "Riacho 1", and rio Preto (PR). Stretches: upper (1), middle (2), and lower (3). Site-specific columns represent the number of individuals for each species.

Species (Acronym)	N	B (g)	SL (cm)	A1	A2	A3	T1	T2	T3	G1	G2	G3	P1	P2	P3	RV1	RV2	RV3	R1	R2	R3	PR
Astyanax ribeirae (Arib)	1032	1900.8	4.0		106	103	205	569	32		8	9										
Mimagoniates microlepis (Mmic)	853	384.1	3.0		38	54		2			198	231										330
Phalloceros cf. *reisi* (Prei)	660	206.4	2.5		62	90		131	22	1	53	16		157	11		45	13	13	18	27	1
Hollandichthys multifasciatus (Hmul)	353	2025.9	6.1		10			11	2	3	18	4		86	46			1	66	61	29	16
Pseudotothyris obtusa (Pobt)	112	32.9	2.4								1	40										71
Characidium cf. *pterostictum* (Cpte)	100	63.9	3.3								19	39										42
Acentronichthys leptos (Alep)	94	152.9	6.0		10	9		1	2	9	16	20		11	10							6
Characidium lauroi (Clau)	71	23.9	2.8	2	21	5	3	21			3	14					1				1	
Rhamdia quelen (Rque)	66	3377.6	14.7		19	1				4	7	2		6	26							1
Schizolecis guntheri (Sgun)	58	24.7	2.9					13	29		9						7					
Gymnotus pantherinus (Gpan)	48	625.4	17.7						6		9	6		8	5	2	5			2		5
Hyphessobrycon griemi (Hgri)	41	9.1	2.1									3								2		36
Antlantirivulus santensis (Rsan)	39	29.8	3.7									1		9					7	15	7	
Characidium cf. *schubarti* (Csch)	35	65.6	4.5	8												24	3					
Hyphessobrycon reticulatus (Hret)	27	18.7	3.0								1	2										24
Kronichthys heylandi (Khey)	24	16.8	3.3													1	22	1				
Geophagus brasiliensis (Gbra)	21	307.9	6.5		6	8		5				1										1
Scleromystax macropterus (Smac)	11	19.8	3.6																			11
Hoplias malabaricus (Hmal)	3	253.5	15.7		1			1	1													
Synbranchus marmoratus (Smar)	2	41.3	30.0			1		1														
Total	3650	9581.0		10	273	271	208	755	94	17	342	389	0	277	98	27	83	15	86	98	64	543
Species richness				2	9	8	2	10	7	4	12	14	0	6	5	3	6	3	3	5	4	12
$H'_{ichthyofauna}$				0.50	1.71	1.39	0.08	0.82	1.46	1.15	1.50	1.49	-	1.11	1.34	0.42	1.24	0.49	0.69	1.05	1.03	1.38

heylandi in rio Verde showed the difference between this site in relation to other clearwater streams. Overall, clearwater streams had larger bottom categories, higher flow velocity, dissolved oxygen and pH concentration in comparison to the blackwater stream.

Discussion

Differences between clearwater streams and the blackwater stream

The physical and chemical water variables do not seem to have influenced the distribution of species in clearwater streams, but the difference between fish assemblages composition of clearwater streams and the blackwater stream may have been influenced mainly by pH, as evidenced by PCA and reinforced by CCA. Although our study covered only one blackwaters' stream due to limitations in the field, it is known that blackwaters invariably exhibit different characteristics from clearwaters, *i.e.*, blackwaters are very acidic (pH between 3 and 4.5) as a result of high concentration of humic substances (Janzen, 1974). Low pH concentration has strong effects on fish communities (Jackson *et al.*, 2001), and lowland streams at JIES have acidic waters (pH ~ 4.0) (Por, 1986), similar to that found in the rio Preto (3.2 - 4.3). This condition can be stressful for most fish species; however, species that inhabit blackwater environments naturally tolerate low pH (Dunson *et al.*, 1977) due to long-term selection for such tolerance (Jackson *et al.*, 2001).

The second highest species richness in JIES streams was recorded in the rio Preto: *Mimagoniates microlepis*, *Pseudotothyris obtusa*, *Characidium* cf. *pterostictum*, *Hyphessobrycon griemi* and *H. reticulatus* were abundant species in blackwaters. These species were also sampled in two clearwater sites, but near rio Preto (middle and lower stretches of ribeirão Grajaúna, with slightly acid clearwaters). According to Menezes & Weitzman (2009), *M. microlepis* is much more common in blackwater streams than previously thought. In fact, this species was more abundant in the rio Preto and at adjacent sites (ribeirão Grajaúna), than in clearwater streams distant from blackwater streams. At Itanhaém River in the coastal plain in the State of São Paulo, close to the JIES, Leung & Camargo (2005) found this species only in blackwater streams.

In coastal streams, *P. obtusa*, *C. pterostictum*, *H. griemi* and *H. reticulatus* occur in clearwaters (Aranha *et al.*, 1998; Leung & Camargo, 2005; Becker *et al.*, 2008), but some of these species are also abundant inhabiting blackwater streams (Leung & Camargo, 2005). In addition, *Scleromystax macropterus* occurred exclusively in the rio Preto, which supports the assertion of Oyakawa *et al.* (2006) that the species shows a preference for lentic and blackwater streams. The high similarity found between fish assemblages of rio Preto and ribeirão Grajaúna is related to the fact that the first stream is a tributary of the latter, which may promote the mixing of ichthyofauna and the sharing of species, such as

P. obtusa, *Characidium* cf. *pterostictum*, *H. griemi* and *H. reticulatus*. Thereby, the highest species richness was recorded at these sites.

The remarkable difference in the pH concentration between blackwaters and clearwaters, as widespread in the literature (*e.g*. Janzen, 1974; Por, 1986) and also obtained during this study, makes our results reliable although samples were taken in only one blackwater stream. We consider that it is extremely important to know the fish fauna of these habitats for their conservation, since blackwater streams are inserted into the coastal plain, which is highly threatened by deforestation due to increased urbanization.

Clearwater's fishes *vs.* habitat structures

In clearwater streams, the headwater stretches had lower richness and species diversity, while the opposite occurred in the middle and lower sites. Overall, the observed pattern of occurrence and distribution of fish species varied according to habitat structure, due to the longitudinal gradient in clearwater streams. In JIES, the mountain streams are high-gradient as they are inserted in mountain regions that may reach 900 m. As the topography influences the geomorphology of coastal streams, the stream's reaches exhibit particular features (Por, 1986). Considering that biological communities change in a predictable way according to the geomorphological changes of the stream channel (Vannote *et al*., 1980), the differences in fish assemblage composition among stretches of clearwater's streams (upper, middle and lower) are probably related to habitat characteristics (stream depth, flow and bottom type) within stream's reaches.

The ichthyofauna is deeply influenced by depth, stream flow and bottom type (Angermeier & Schlosser, 1989), which should therefore influence the structure of the ichthyofauna due to species' addition and substitution processes along the longitudinal gradient of distribution (Sheldon, 1968). In JIES, the headwaters (high altitude) characteristically had high current, deep pools, and bottom dominated by boulders and bedrocks; included riffles and rapids, which selects species able to withstand the strong current, such as *Characidium* spp. and *Kronichthys heylandi*. The middle stretches (moderate elevation) had a wider diversity of bottom types, and pools and riffles mesohabitat alternated. Fish species occurring in these sites tend to segregate according to the mesohabitat availability, and the occurrence of many species with different characteristics in the middle stretches of the sampled streams was probably due to this. In general, the occurrence of characids and poecilids species is related to pools or marginal backwater areas, while the presence of crenuchids and loricarids species is related to riffles (Sabino & Silva, 2004). *Astyanax ribeirae*, *Mimagoniates microlepis*, *Hollandichthys multifasciatus* (characids), *Phalloceros* cf. *reisi* (poeciliid), *Characidium* cf. *pterostictum*, *C.* cf. *schubarti*, *C. lauroi* (crenuchids), *Schizolecis guntheri*, and *Kronichthys heylandi* (loricarids) occurred in those habitats in JIES. Finally, the lower stretches (low altitude) had slow flow, backwater areas with sandy bottom, pools and runs. These characteristics may have favored the occurrence of *Acentronichthys leptos*, *Gymnotus pantherinus*, *Geophagus brasiliensis*, *Hoplias malabaricus*, and *Synbranchus marmoratus* at lower sites.

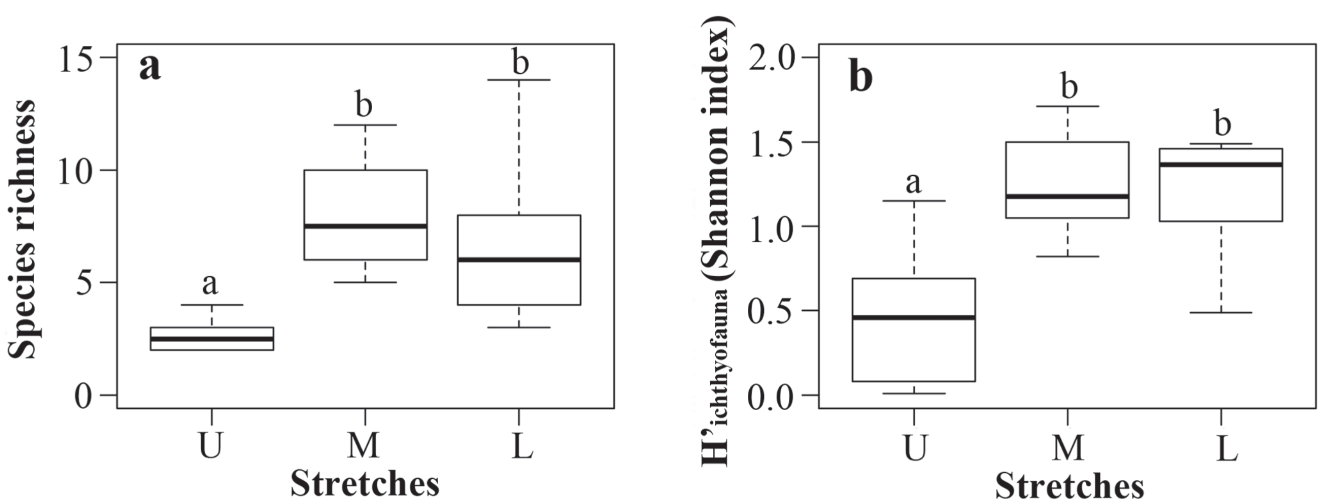

Fig. 2. Box-plots showing median (thick lines), first, and third quartiles (boxes), minimum and maximum values (whiskers) to upper (U), middle (M) and lower (L) stream stretches, according to ichthyofauna richness (A) and ichthyofauna diversity (B) in Jureia-Itatins Ecological Station. Different letters above each box-plot represents $p < 0.05$ to post-hoc tests, and same letters represents $p > 0.05$.

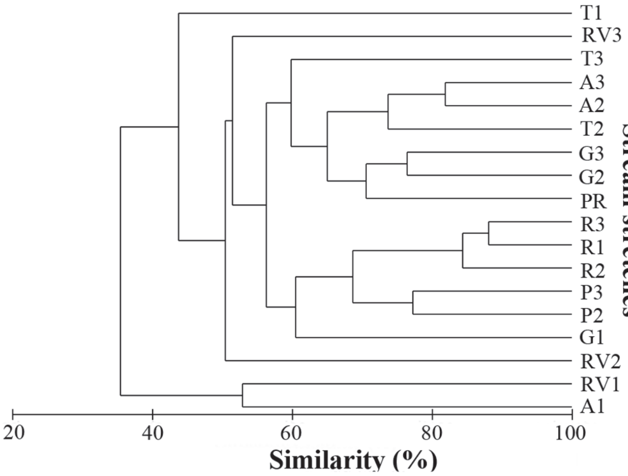

Fig. 3. Dendrogram of similarity produced by cluster analysis, according to ichthyofauna abundance data in each site of Jureia-Itatins Ecological Station. Streams: Antas (A), Tetequera (T), Grajaúna (G), "Ponte 2" (P), rio Verde (RV), "Riacho 1" (R), and rio Preto (PR). Stretches: upper (1), middle (2), and lower (3).

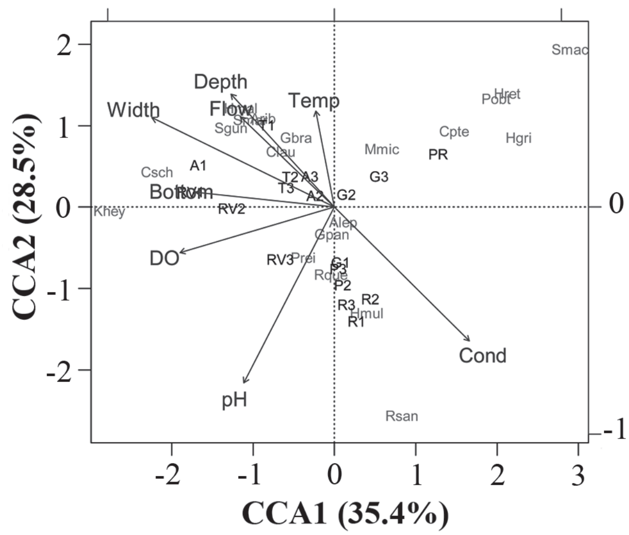

Fig. 4. Ordination resulted from canonical correspondence analysis according to ichthyofauna abundance in each site in relation to physical and structural characteristics of the habitat. Habitat variables: temperature (Temp), pH concentration (pH), dissolved oxygen (DO), conductivity (Cond), stream width (Width), stream flow (Flow), bottom type (Bottom), and stream depth (Depth). Streams: Antas (A), Tetequera (T), Grajaúna (G), "Ponte 2" (P), rio Verde (RV), "Riacho 1" (R), and rio Preto (PR). Stretches: upper (1), middle (2), and lower (3). Percentages of explanation of the axes are given in parentheses. The bottom and left-hand scales are for the sites and the fish species, the top and right-hand scales are for the habitat variables. The species acronyms are presented on Table 2.

Seasonal distribution of species

Temporal differences were not found in JIES's fish assemblages. In the study area, rainfall is high throughout the year without the presence of a pronounced dry season but a less rainy period (Marques & Duleba, 2004), and thus possibly the species diversity is not seasonally different. According to Esteves & Lobon-Cerviá (2001), torrential rains in coastal streams are common throughout the year and can influence fish's population size. These authors found that *Mimagoniates*, *Schizolecis* and *Deuterodon* were more abundant during the rainier season, and this may be related to the recruitment of young individuals in this period. The same possibly may have occurred in clearwater streams to *Mimagoniates microlepis* (also at rio Preto), and *Hollandichthys multifasciatus* which were more abundant in this study during the rainier season. However, some species such as *Acentronichthys leptos*, *Characidium lauroi*, *C. pterostictum*, *Schizolecis guntheri*, and *Atlantirivulus santensis* (clearwaters), and *Pseudotothyris obtusa*, *Hyphessobrycon griemi*, *H. reticulatus*, and *Scleromystax macropterus* (blackwaters), were more abundant during the drier season. In this case, the efficiency of electrofishing should be taken into consideration, since the sampling efficiency may be lower during the rainier season due to increased flow (and decreased conductivity), which consequently affects the electric field and facilitates the escape of fish (Zalewski & Cowx, 1990).

Concluding remarks

In comparison to other Brazilian drainages, studies on coastal stream fish are scarce, which hinders the understanding of several aspects of ichthyofauna composition and distribution. This study showed the importance of pH concentration for organization of fish assemblages when comparing clearwater streams and the blackwater stream. We also showed evidences for different relationships between habitat diversity and species diversity among stream stretches, indicating that habitat structure influences fish diversity in clearwater streams. However, we believe that the altitudinal gradient and even the isolation between streams can be also responsible for structuring the fish fauna in streams of clearwaters; therefore, these spatial issues should be focused on future studies. Finally, we concluded that temporal variation (seasonality) is not an important factor for structuring fish assemblages in coastal streams of the Atlantic forest.

Considering that the studied streams are located in a conservation area and exhibit pristine features, the results of this study should provide subsidies for species management in degraded areas. Furthermore, undisturbed environments have become increasingly scarce. Thus, our data may be used as references for other studies requiring such information. The presence of many endemic fish species (and some endangered) in JIES, reinforces the importance of legally protected areas from human impacts within the Atlantic forest biome.

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