FISH SPECIES INDICATORS OF ENVIRONMENTAL QUALITY OF NEOTROPICAL STREAMS IN SOUTHERN BRAZIL, UPPER PARANÁ RIVER BASIN

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Background. Some studies in the Neotropical ecozone evaluated the correlation between the ichthyofauna and the physical and chemical characteristics of streams. This study aimed to estimate the environmental quality of Neotropical streams through quality protocols and diversity indexes. The hypothesis is that it is possible to identify fish species indicators of variables and streams quality associated to environmental conservation and degradation.

Materials and methods. Sampling occurred quarterly from October 2012 to July 2013, totalling four phases of field surveys in three sample units on each stream (Água dos Anjos Stream, Monjolinho Stream, and Ubá Stream, upper Paraná River basin) in southern Brazil. The Indicator Value Method (IndVal) was used to relate the ichthyofauna (abundance and relative frequency of fish species) to physical and chemical variables and streams and their respective environmental characteristics.

Results. A total of 33 species were analysed as possible indicators by the IndVal method, and 21 species showed a significant relation ($P < 0.05$) to one or more environmental variables. The exotic *Poecilia reticulata* Peters, 1859 was best linked to low-oxygen water. *Bryconamericus iheringii* (Boulenger, 1887) was an indicator of the stream with the worst environmental quality among the analysed streams, and also indicated significantly lower extent of riparian vegetation (<6 m) and unstable substrate. *Geophagus brasiliensis* (Quoy et Gaimard, 1824) was also indicator of the stream with inferior environmental quality and low of riparian vegetation (<6 m). On the other hand, *Trichomycterus diabolus* Bockmann, Casatti et de Pinna, 2004 was indicator of diverse habitat environments, as it was linked to higher extent of riparian vegetation (>18 m) and high water oxygenation.

Conclusion. The fish species: *P. reticulata*, *B. iheringii*, and *G. brasiliensis* were indicators of inferior environmental quality, and *T. diabolus* was an indicator of superior quality, and its abundance can characterize as important bio-indicators of environmental characteristics of Neotropical streams.

Keywords: fish conservation, Indicator Value Method, Neotropical fishes, environmental quality of streams

INTRODUCTION

Several studies dealing with the relation of fish communities to levels of environmental variables use indices of biotic integrity (Angermeier and Karr 1984, Casatti et al. 2006, Pinto and Araújo 2007, Machado et al. 2011). In the Neotropics some studies evaluated the correlation between the fish assemblages and the physical characteristics of streams, such as width, depth, type of substrate, flow (Araújo and Tejerina-Garro 2007, Felipe and Súarez 2010, Araújo et al. 2011), presence of the riparian vegetation (Casatti 2010), and the chemical properties of water (Silva 1995, Oliveira and Bennemann 2005, Daga et al. 2012). However, few studies have sought to identify species that directly express the current characteristics of the environment in which they operate, with indicator species index (Alexandre et al. 2010).

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Dufrene and Legendre (1997) proposed an index of indicator species evaluation called Indicator Value Method (IndVal), which takes into account values of abundance and frequency of occurrence of a species, and relating them to an environmental variable. According to McGeoch and Chown (1998), the IndVal has the advantages of robustness to compare data from different sample sizes and derived from different methodologies, and the fact that the groups, or categories, can be classified priori or posteriori, hierarchically or not, when it is compared to some others used to find indicator species.

The IndVal has been employed in studies with different organisms, from bacteria (Shawkey et al. 2009), plants (Bataineh et al. 2007), aquatic invertebrates (Carbonell et al. 2011, Souza et al. 2011), amphibians (Campos et al. 2013), birds (Mikusiński et al. 2001), and fish (Penczak 2009, Penczak et al. 2012, Dukowska et al. 2013).

Karr (1981) considers some advantages of using fish as indicator organisms in environmental monitoring programs: the large amount of information on the life history of many species of fish, the range of trophic levels, the relative easiness of identification, the relative knowledge of the general public to understand the results from scientific information about this group, the possibility of analysing the effects of toxicity and stress along fish growth and reproduction, and the presence of fishes in virtually all types of aquatic environments.

The hypothesis of this study is that it is possible to identify Neotropical fish species indicators of variables and streams associated to environmental conservation and degradation, detected by environmental protocols and ecological diversity indexes.

MATERIALS AND METHODS

Study area. The samplings were performed in the Das Cinzas River basin, located in southern Brazil, in the upper Paraná River system. The total area of the Das Cinzas River basin is 9612.8 km^2 and its length is 240 km. The region is composed by areas of artificial pasture, mixed plantations, grasslands, and forestry. The industries of the region are mainly agro-industrial, such as sugar mills, distilleries, dairies, and slaughterhouses (Pereira and Scroccaro 2015). The physical and chemical characteristics and the fishes were sampled in three streams of Atlantic Forest biome tributaries of the Jacarezinho River, located in the medium portion of the Das Cinzas River. The three streams are: Água dos Anjos Stream and Monjolinho Stream, tributaries of the right margin of the Jacarezinho River, and Ubá Stream, tributary of the left margin. Sampling occurred in three units on each stream—Água dos Anjos Stream: A1: headwater (23°13′15.80″S, 49°57′12.46″W), A2: middle (23°13′24.80″S, 49°57′26.19″W), A3: mouth (23°13′39.60″S, 49°58′32.11″W); Monjolinho Stream: M1: headwater (23°15′56.35″S, 49°54′57.53″W), M2: middle (23°16′28.10″S, 49°55′46.46″W), M3: mouth (23°16′25.46″S, 49°56′42.01″W); and Ubá Stream: U1: headwater (23°14′15.00″S, 50°2′13.00″W), U2: middle (23°13′43.82″S, 50°2′15.25″W), U3: mouth (23°12′59.76″S, 50°2′8.85″W) (Fig. 1). Each sample unit was represented by a stream stretch of 50 m length, previously blocked upstream and downstream with fence made of netting material of 3 mm mesh.

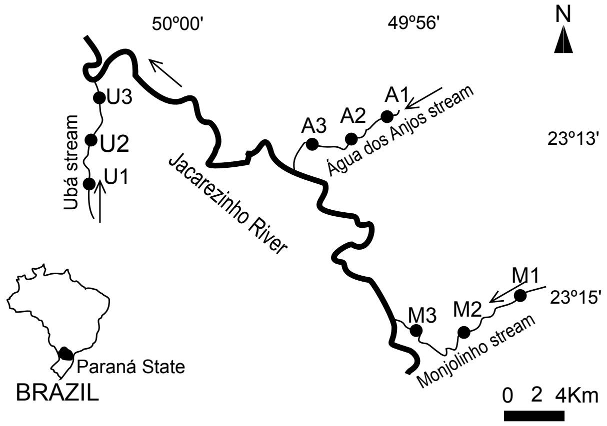

Fig. 1. Sampling units in streams Água dos Anjos, Monjolinho, and Ubá, Das Cinzas River basin, upper Paraná River, southern Brazil; The arrows indicate the direction of water flow

Abiotic variables and environmental quality. The physical and hydrological characteristics measured in each sampling section were: maximum width and depth of the channel, measured with a 10 m measuring tape, water velocity by the float method, flow (Palhares et al. 2007), and temperature (Tecnopon® MPA-210P). The chemical characteristics analysed were: conductivity (Instrutherm® CD-860), dissolved oxygen (Politerm® POL-60), and pH (Tecnopon® MPA-210P). Additionally, a protocol for visual assessment of habitats, modified from Callisto et al. (2001, Appendix A) was used. The protocol considered the properties of the aquatic ecosystems and margins, as substrates, changes in watercourses, presence and extent of riparian vegetation and margin stability. In this protocol, each environmental characteristic received scores from 0 to 3, according to their quality (0 for minimum quality, and 3 for maximum quality). Each stream was classified as environment of superior or inferior quality (high and low values of the protocol summation, respectively). Moreover, parameters of richness, abundance, Pielou Equitability (J') and Shannon diversity (H') were estimated on fish populations. The results of the application of protocol on environmental assessment and of the diversity parameters were used to assess the environmental quality of streams, and later, to relate to their fish species indicators.

Fish collection. Sampling occurred quarterly from October 2012 to July 2013, totalling four phases of field surveys. Fish were collected by electrofishing with alternating current of 127 V and 6 A. Three successive runs of dip nets in each stretch from downstream to upstream direction were performed. Captured individuals were anesthetized and sacrificed by immersion in eugenol (active ingredient: phenolic eugenol, 4-Allyl-2-methoxyphenol-C10H12O2, derived from stems, flowers, and leaves of *Eugenia caryophyllata* and *Eugenia aromatica* trees) (Griffiths 2000), fixed in 10% formalin solution and later preserved in 70% ethanol. The species identifications were performed according to Oyakawa et al. (2006) and Graça and Pavanelli (2007). Voucher species were deposited in the Ichthyological Collection of the Center of Research in Limnology, Ichthyology and Aquaculture (Nupélia) of the University of Maringá State.

Data analysis. Indicator Value Method (IndVal) (Dufrene and Legendre 1997) was used to relate the ichthyofauna (abundance and relative frequency of fish species) to: physical and chemical variables and environmental quality of the streams by modified from Callisto et al. (2001) (Appendix A). The physical and chemical variables and the streams (which were also considered variables) were classified into three categories (Table 1), related to the ecological parameters of environmental quality streams: category 1 for the lowest physical and chemical variables values and for the Água dos Anjos stream, category 2 for the median values and for the Monjolinho Stream, and category 3 for the high values and for the Ubá stream. Moreover, three environmental variables were selected from the modified protocol of Callisto et al. (2001). Each environmental variable was classified into four categories, ranging from 0 (inferior) to 3 (superior environmental quality; Table 2). The IndVal employs the Monte Carlo test with 5000 permutations, and significant values ($P < 0.05$) were used to define potential indicator species of particular categories of the streams, physical and chemical variables and of the environmental characteristics. The formula of the IndVal is

$$\text{IndVal}_{ij} = A_{ij} \times B_{ij} \times 100$$

where

$$A_{ij} = n_{ij} \cdot n_i^{-1}$$

$$B_{ij} = N_{ij} \cdot N_i^{-1}$$

where: A_{ij} is the mean abundance of *i* species in *j* category, B_{ij} refers to the number of categories in the variable *j* where the *i* species is present, n_{ij} is the number of individuals *ji*, n_i is the number of individuals *i*, N_{ij} is the number of sites *ji*, N_i is the total number of categories of variable *i*. This analysis was performed using PC-ORD 3.0 program (McCune and Mefford 1997). Additionally, Spearman analysis was performed to evaluate the possible correlation between the values of physical and chemical variables to the ecological attributes of fish populations: richness, abundance, Pielou Equitability (J') and Shannon Diversity (H'). We assume that when a species is an indicator of a type of physical or chemical variables related to low diversity, it could also be considered indicative of inferior environmental quality, which is also true in reverse. Correlation coefficients and diversity were calculated in the PAST program (Hammer et al. 2001), and the correlation graphs were performed in Statistica 7.1 (Statsoft).

RESULTS

A total of 7102 specimens of 33 species from six orders and 12 families were captured. In the protocol of environmental quality applied, the Ubá Stream scored the highest values (U1 = 39, U2 = 28, and U3 = 27), followed by Monjolinho Stream (M1 = 20, M2 = 29, and M3 = 25), and by Água dos Anjos Stream (A1 = 23, A2 = 13, and A3 = 21). For the diversity, Ubá Stream showed the highest values in indices of Shannon ($H' = 2.053$) and Pielou Equitability ($J' = 0.758$). In turn, the Água dos Anjos Stream was pointed out as the least diverse ($H' = 1.942$), due to high dominance ($D = 0.2366$), and the Monjolinho Stream was intermediary in diversity ($H' = 2.006$, $J' = 0.6231$, $D = 0.2188$).

Concerning the IndVal index, of the 33 species analysed as possible indicators, 22 showed significant relation ($P = < 0.05$) to one or more categories, and eleven were not significantly related to any category of physical or chemistry variable, environmental characteristics or streams. The majority of species were related to four to six categories, each. The Água dos Anjos Stream showed the largest number of significant associations, revealing eleven indicator species to this environment, followed by the Monjolinho Stream with four, and the Ubá Stream, with only two (Table 3).

Table 1

Categories of streams and the physical and chemical variables used in the IndVal analysis with stream fishes of the Das Cinzas River basin, upper Paraná River

Variable	Category 1	Category 2	Category 3
Stream	Água dos Anjos	Monjolinho	Ubá
Conductivity [$mS \cdot cm^{-1}$]	<0.20	0.20–0.22	>0.22
Width [m]	<4	4–6	>6
Oxygen [$mg \cdot L^{-1}$]	<13	13–14	>14
pH	<7.5	7.5–8	>8
Depth [cm]	<50	50–60	>60
Temperature [°C]	<22.7	22.7–24	>24
Flow [$m^3 \cdot s^{-1}$]	<0.20	0.20–0.40	>0.40
Velocity [$m \cdot s^{-1}$]	<0.3	0.3–0.4	>0.4

Category 1 = low values, category 2 = medium values, Category 3 = high values.

Table 2

Environmental categories (modified from Callisto et al. 2001) used in the IndVal analysis of stream fish of the Das Cinzas River basin, upper Paraná River

Variable	Category 0	Category 1	Category 2	Category 3
Type of substrate	Rocky; absence of loose stones	Mainly formed of gravel; presence of some loose stones	Abundance of loose stones and gravel	Abundance of loose stones
Type of stream bottom	Less than 10% stable habitat; substrate unstable or absent	10%–30% of stable habitat; substrates frequently modified	30%–50% of stable habitats, without evidence of alteration by erosion or siltation	More than 50% of stable and diversified habitats (pieces of submerged trunks, gravel)
Riparian vegetation	Width of riparian vegetation less than 6 m; restricted or no vegetation due to anthropogenic activity (pastures, roads, etc.)	Width of riparian vegetation between 6 and 12 m; intense anthropogenic activity	Width of riparian vegetation between 12 and 18 m; minimal anthropogenic activity	Width of riparian vegetation > 18 m; without influence of anthropogenic activities

Category 0 = characteristics of inferior environmental quality, Category 3 = characteristics of superior environmental quality.

Geophagus brasiliensis (Quoy et Gaimard, 1824); *Crenicichla britskii* Kullander, 1982; and *Oligosarcus paranensis* Menezes et Géry, 1983 were the best quality indicators (species with higher IndVal values associated with this stream) of the Água dos Anjos Stream, whereas *Apareiodon piracicabae* (Eigenmann, 1907); *Apareiodon ibitiensis* Amaral Campos, 1944; and *Hypostomus hermanni* (Ihering, 1905) had the highest significant values for the Monjolinho Stream, and finally, *Trichomycterus diabolus* Bockmann, Casatti et de Pinna, 2004 and *Imparfinis mirini* Haseman, 1911 were unique indicators to the Ubá Stream (Table 3). *Trichomycterus diabolus* and *Serrapinnus notomelas* (Eigenmann, 1915) showed significant associations with the largest number of environmental variables (eleven and nine, respectively), and *T. diabolus* was the best indicator to four categories: smaller width, depth and temperature and high dissolved oxygen. In addition, *T. diabolus* was also the best indicator of environments with abundance of loose stones, diverse habitats and high amount of riparian vegetation (width > 18 m). *Apareiodon piracicabae* and *Poecilia reticulata* Peters, 1859 were the best indicators to four and three extreme categories respectively. *Apareiodon piracicabae* indicated highest values of conductivity, width, temperature, and pH, and *P. reticulata* indicated high water speed and high water depth, and low dissolved oxygen.

The species *Characidium zebra* Eigenmann, 1909, *Imparfinis mirini*, and *Phalloceros harpagos* Lucinda, 2008 had the highest values for two categories each: higher flow and engineered substrates for *C. zebra*, lower pH and conductivity for *I. mirini*, and less flow and low amount of riparian vegetation (width < 6 m) for *P. harpagos*. Finally, the species with the largest indicator value for only one extreme category in the environmental variables were: *Bryconamericus iheringii* (Boulenger, 1887) for environment with rocky substrate and absence of rubble; *G. brasiliensis* for abundance of loose stones; *Hypostomus strigaticeps* (Regan, 1908) for 30%–50% of stable habitats; and *Rhamdia quelen* (Quoy et Gaimard, 1824) associated with low water velocity (Table 3). The hydrological variable with highest amount of species significantly associated was the water flow, with 14 associations, seven species were related to the high water flow (category 3), two to the middle (category 2) and five to the low flow (category 1). It was followed by variable river width with 13 associations, and pH with 11.

Moreover, the depth and the substrate type showed a significant relation with only three species each. Two species were related to the high water depth and one to the low depth, whereas two species were related to abundance of loose stones and gravel, and one to abundance of loose stones. Riparian vegetation showed seven species with significant association values of their categories, and width, pH and temperature showed nine.

The Spearman analysis revealed a positive correlation between richness and conductivity ($r = 0.78$, $P = 0.02$), conductivity and abundance ($r = 0.77$, $P = 0.02$), water flow and diversity ($r = 0.68$, $P = 0.05$) and abundance and richness ($r = 0.78$, $P = 0.02$), and negative correlation between abundance and equitability ($r = -0.89$, $P = 0.001$, Fig. 2).

DISCUSSION

The Água dos Anjos Stream was characterized by lower values in the diversity and in the protocol of quality environmental. This stream showed the worst environmental quality among the three studied streams, with lower equitability and higher dominance, which is typical features of impacted environments. Moreover, the Ubá Stream had the best quality, with higher values of diversity and equitability.

The main factor responsible for *Geophagus brasiliensis* as the best indicator of the Água dos Anjos Stream was its high abundance in this environment, and low abundance in the other sampled streams. In addition, the Água dos Anjos Stream was the only with a species of the order Perciformes as the main indicator. The parodontids *Apareiodon piracicabae* and *A. ibitiensis* were the best indicators of the Monjolinho Stream, with the former species also presenting the highest values for high conductivity, width,

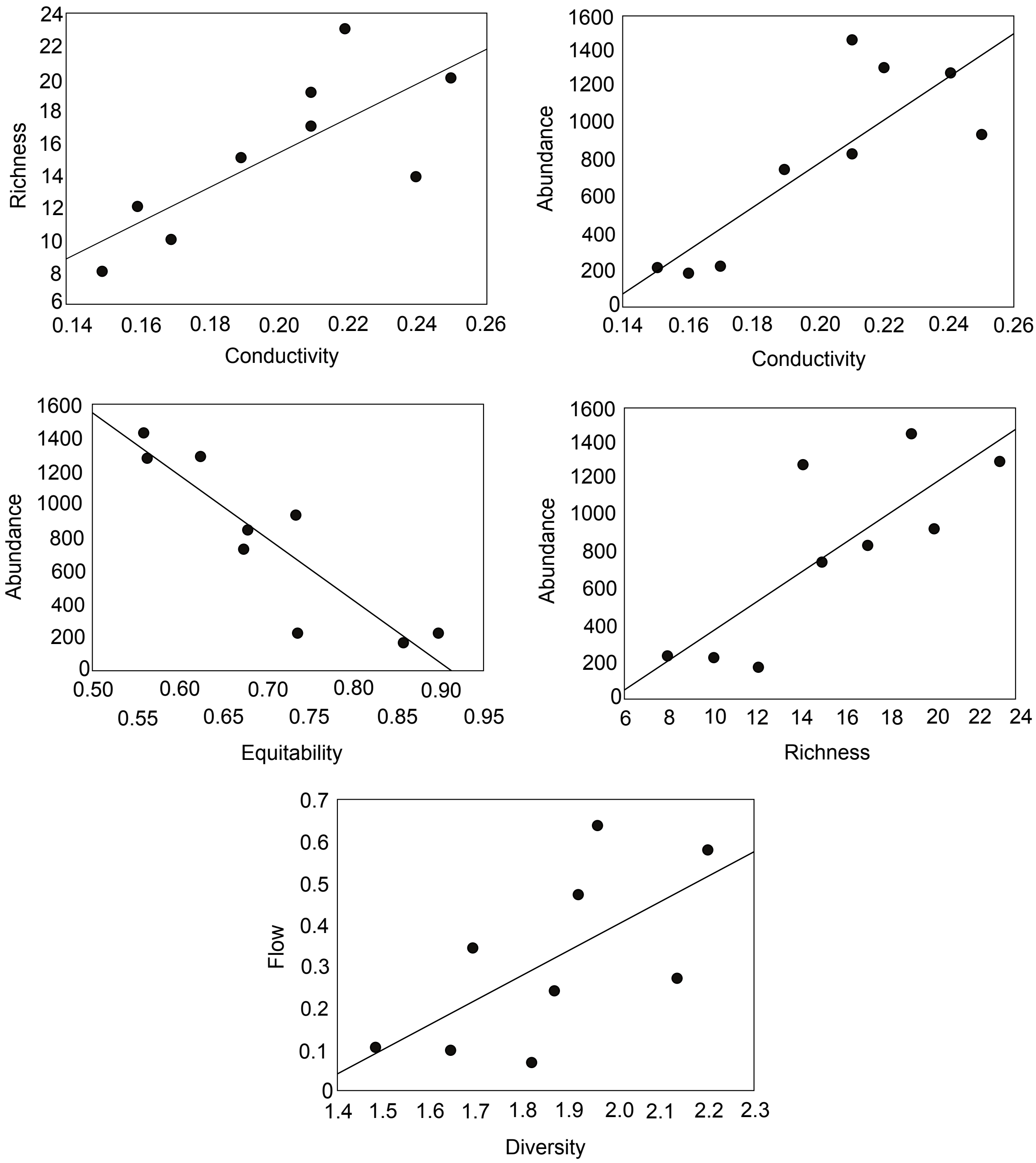

Fig. 2. Significant correlations (Spearman) between the ecological attributes of the fish fauna (richness, abundance, equitability *J′* and diversity *H′*) and environmental variables

temperature and pH. In turn, the Ubá Stream had two catfish (Siluriformes) as indicators, *Trichomycterus diabolus* and *Imparfinis mirini*. *Trichomycterus diabolus* was also associated with low temperatures, high dissolved oxygen, extensive cover of riparian vegetation (riparian vegetation width greater than 18 m) and high habitat diversity (pieces of submerged trunks, gravel). Thus, these two species, particularly *T. diabolus*, were considered dependent to factors as habitat integrity, the integrity of the substrate, oxygenation and water quality (Casatti 2002, Shibatta and Cheida 2003, Oliveira and Bennemann 2005).

Among the species considered tolerant to environmental degradation, *Phalloceros harpagos* can be regarded as a model species, because it was associated with low flow and low riparian vegetation (< 6 m). The absence of native riparian vegetation typically is associated to inferior environmental quality in streams of the Atlantic Forest biome (Casatti 2010). *Geophagus brasiliensis* and *Bryconamericus iheringii* were also significantly associated with lower extent of riparian vegetation. Casatti (2010) refers to the native riparian vegetation as an essential resource for maintaining the diversity of fish, relating the decrease in forest with species loss, faunal homogenization, and biomass reduction of these organisms.

Table 3

Significant values of the Index Indicator Species (IndVal) of each fish species for each category (c) of physical and chemical variables, environmental characteristics and tributaries of the Das Cinzas River, upper Paraná River basin

Species	Variable																							
	Stream		Conductivity		Width		Oxygen		pH		Depth		Temperature		Flow		Velocity		Substrate		Bottom		Riparian V	
	c	IndVal	c	IndVal	c	IndVal	c	IndVal	c	IndVal	c	IndVal	c	IndVal	c	IndVal	c	IndVal	c	IndVal	c	IndVal	c	IndVal
Apareiodon ibitiensis	2	60.2**							3	60.2**			3	60.2**	3	65.8**								
Apareiodon piracicabae	2	__69.1__**	__3__	__64.7__**	__3__	__40.3__*			__3__	__69.1__**			__3__	__69.1__**	3	73.9**							1	49.4**
Astyanax altiparanae	1	60.4**	2	48.3*	2	52.8**			2	52.8**					3	42.9*					1	44.4*		
Astyanax bockmanni	1	51.3*			2	53.9*	2	55.3*							1	53*					0	49*		
Bryconamericus exodon															3	72.4**					1	51.5*		
Bryconamericus iheringii	1	50.4*			2	51.3*	2	53.7*							1	52.8*					__0__	__47.8__**	0	42.8*
Characidium zebra					2	59.8*									__3__	__76.3__**					__1__	__51.3__**		
Corydoras aeneus	1	41.7**	2	41.7**			2	31.2*	2	41.7*			1	31.2*	1	31.2*					0	47.9**		
Crenicichla britskii	1	71.4**					1	48.9*					2	48.9*							1	63**		
Geophagus brasiliensis	1	__98.5__**	2	56.9*	2	54.8*			2	54.8*					1	58.1**			__3__	__57.6__*			0	54.5*
Gymnotus inaequilabilatus	1	41.7**	2	41.7*	2	41.7*			2	41.7*							3	33.1*					1	31.9*
Hoplias sp. 1	1	38.7*	2	37.4*	2	38.7*																		
Hypostomus hermanni	2	54.6**							3	54.6**			3	54.6**	3	54.6**								
Hypostomus strigaticeps	2	50**			2	30*	2	38.7*	3	50**			3	50**	3	40.5*	1	39.4*			__2__	__46.1__**		
Imparfinis mirini	3	59**	__1__	__79__**	1	51.7*			__1__	__54.5__*					2	59**			2	49.7*				
Oligosarcus paranensis	1	67.7**			2	36.5*			2	36.5*											1	37.3*		
Phalloceros harpagos															__1__	__58.3__**	1	39.1*					__0__	__58.3__**
Poecilia reticulata							__1__	__55.9__**			__3__	__56.5__**	2	55.9**			__3__	__44.9__*						
Rhamdia quelen							2	56.9*									__1__	__55__*						
Serrapinnus notomelas	1	48.4**	2	48.8**	2	48.4**	1	34.9*	2	48.4**	3	36.4*	2	34.9*			3	44.6**					1	42.6*
Synbranchus marmoratus	1	42.5*																						
Trichomycterus diabolus	3	__64.2__**	1	47*	__1__	__64.2__**	__3__	__73.6__**			__1__	__47.8__*	__1__	__64.2__**	2	64.2**	3	37.4*	__2__	__81.5__**	__3__	__97__**	__3__	__96.4__**

* = $P < 0.05$, ** = $P < 0.01$; bold type = IndVal larger values for the associated category; For variable “Stream”: 1 = Água dos Anjos stream, 2 = Monjolinho stream and 3 = Ubá stream; For the variables “Conductivity, Width, Oxygen, pH, Depth, Temperature, Flow, and Velocity”: 1 = low values, 2 = medium values and 3 = high values; For the variables (Type of) Substrate, (Type of stream) bottom, and (Riparian V)egetation”: 0 = inferior environmental quality, 1 = medium, 2 = medium to superior, 3 = superior.

The association of *Poecilia reticulata* with the low oxygen level can be explained by the fact that this species have the ability to perform air–water interface respiration, for such environments with hypoxia (Kramer and Mehegan 1981). *Poecilia reticulata* is an exotic species introduced in the upper Paraná River basin in order to combat insect larvae (Langeani et al. 2007), and this fish is constantly linked to degraded environments, as in the work of Oliveira and Bennemann (2005), Pinto and Araújo (2007) and Vieira and Shibatta (2007). Ferreira and Casatti (2006) used *P. reticulata* in the construction of an Index of Biotic Integrity (IBI) correlating the increase in the percentage of its abundance with increasing degradation of habitats.

Characidium zebra was linked to higher flow, which was a characteristic of environments with higher diversity, but also indicated river bottoms with unstable substrates. According to Casatti and Castro (1998), *C. zebra* inhabits environments with relatively high water flow, as inferred from its fusiform body (Casatti and Castro 2006). The occurrence of *Trichomycterus diabolus* indicates low conductivity (characteristic of the Ubá Stream) and superior environmental quality, whose *T. diabolus* was also the best indicator. Usually in rivers and streams as those from the Atlantic Semideciduous Forest in southern Brazil, high conductivity may indicate inferior environmental quality, because as the conductivity measures the concentration of ions, high values may be the result of dumping of organic waste, leading to a decrease in oxygen and consequent homogenization of aquatic communities (Felipe and Súarez 2010).

The fact that 60% of the species analysed in this work as a possible indicator presented significant associations with at least one environmental categories, was primarily due to the use of a lot of variables for the Indicator Species Index, because each variable had a mean value of 8.9 significant interactions. This approach provides knowledge on the relation of fish species with a wider range of environmental characteristics. However, it is necessary to exercise caution at the time of classifying species as indicators of environmental degradation or conservation, because the same species can be relate to categories of both types of habitats. Even considering these practical difficulties, it was possible to diagnose, *Poecilia reticulata*, *Bryconamericus iheringii*, and *Geophagus brasiliensis* as tolerant to environmental degradation. The first two species were related to characteristics of low environmental quality. In addition, *B. iheringii* was abundant along all study sites, which proves its tolerance to different environmental characteristics. *Geophagus brasiliensis*, highly abundant in the Água dos Anjos Stream, indicated significantly well this stream with lower environmental quality. Teresa and Casatti (2012) also reported the highest frequency of *G. brasiliensis* to deforested streams, and Shibatta et al. (2002) and Castro (unpublished[*]) classified *B. iheringii* as constant in several different types of stream environments, inferring his tendency to generalize in the use of resources. Moreover, *Trichomycterus diabolus* may be considered indicative of good environmental quality to all studied streams, mainly to be linked to large amount of riparian vegetation (extension > 18m) and high habitat diversity (pieces of submerged trunks, gravel). Works such as those of Trajano (1997), Oliveira and Bennemann (2005), Galves et al. (2007), and Rondineli et al. (2009) considered the species of the genus *Trichomycterus* as sensitive to environmental degradation and argued that they can be used as indicators of superior environmental quality in Neotropical streams. Therefore, this paper gives knowledge into the monitoring and conservation of aquatic ecosystems through the use of stream fish species as important bio-indicators of environmental characteristics.

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Appendix A

Environmental assessment protocol applied in this study, to verify the environmental quality of the streams studied (modified from Callisto et al. 2001)

Habitat parameters	Great (3 points)	Good (2 points)	Median (1 point)	Poor (0 points)
Fund type	More than 50% of stable and diversified habitats (pieces of submerged trunks, gravel)	30%–50% of stable habitats, without evidence of alteration by erosion or siltation	10%–30% of stable habitat; substrates frequently modified	Less than 10% stable habitat; substrate unstable or absent
Pool width	Rapids (Riffles) and pools well developed; pool as wide as the stream, and with a length equal to twice the width of the stream	Pools with the width of the stream, but less than twice the width of the stream length	Rapids absent; Pools not as wide as the stream and its length less than twice the width of the stream	Pools rapid or non-existent
Pool frequency	Pools frequent; Pool distance divided by the width of the stream between 5 and 7	Pool infrequent; remaining distance divided by the width of the river between 7 and 15	Pools or curves occasional; habitats formed by the contours of the fund; distance between pools divided by the width of the stream between 15 and 25	Pools shallow or absent; distance between pools divided by the width of the stream > 25
Substrate type	Abundance of rubbles	Abundance of rubbles and gravel	Mainly formed of gravel; presence of some rubbles	Rocky; absence of rubbles
Deposition of mud	Between 0% and 25% of the fund covered with mud (silt and clay)	Between 25% and 50% of the fund covered with mud	Between 50% and 75% of the fund covered with mud	Over 75% of the fund covered by mud
Deposition of sediments	Less than 5% of the fund with deposition of sediments; absence of deposition in the pools	Some evidence of change in the background; increase mainly gravel, sand or mud; 5%–30% of the modified fund, soft deposition in the pools	Moderate deposition of gravel, sand or mud on the margins, between 30% and 50% of the modified fund; moderate deposition in the pools	Large deposits of mud, silted margins; more than 50% of the modified fund
Changes in the stream channel	Channelling or dredging absent or minimal; stream with normal pattern	Presence of channelling, usually near building bridges; evidence of changes for over 20 years	Modification present on two margins; 40% to 80% of the modified stream	Cemented margins; over 80% of the modified stream
Characteristics of the flow	Relatively equal flow across the width of the stream; Minimum amount of exposed substrate	Water depth above 75% of the stream channel; or less than 25% of the exposed substrate	Water depth between 25% and 75% of the stream channel, and/or most of the substrate in the "fast" exposed	Water depth sparse and present only in the pools
Stability margins (score for each margin).	Stable margins; minimal or no evidence of erosion; little potential for future problems. Less than 5% of the margin affected	Moderately stable; small areas with erosion. Between 5% and 30% with margin erosion	Moderately unstable; between 30% and 60% with margin erosion. High risk of erosion during floods	Unstable; many areas with erosion; frequent areas uncovered in the stream curves; erosion between 60% and 100% margin
Riparian vegetation	Width of riparian vegetation > 18 m; without influence of anthropogenic activities	Width of riparian vegetation between 12 and 18 m; minimal anthropogenic activity	Width of riparian vegetation between 6 and 12 m; intense anthropogenic activity	Width of riparian vegetation less than 6 m; restricted or no vegetation due to anthropogenic activity (pastures, roads, etc.)