Stream fish communities and their associations to habitat variables in a rain forest reserve in southeastern Brazil

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Synopsis

This paper describes the spatial variability of fish communities and identifies patterns of association between fish communities and habitat variables, including anthropogenic factors. We sampled streams inside and in the surroundings of a rain forest reserve in the southeast of Brazil in the rainy season. We could distinguish three main groups of streams: upland streams (draining the upland, flat portions of the mountain ridge of Serra de Paranapiacaba), adventitious streams of clearwater mountain torrents (small streams draining confined valleys in the slopes of this sierra), and large streams of clearwater mountain torrents (relatively unconstrained large streams close to the foots of the mountains). Despite the high variability of fish communities associated with these streams we identified some patterns using exploratory statistical analyses. These patterns were corroborated by additional field observations and information from the scientific literature. The main differences in fish community composition and diversity among the three groups of streams are probably related to large-scale factors such as elevation and position of the stream in the watershed. However, differences within these three groups seem to be mostly due to site-specific factors. Differences of instream characteristics are likely to be caused by natural variability of the ecosystems but also, in some cases, by human disturbances like pollution from human settlements, agriculture and mining.

Introduction

Considerable variability in aquatic ecosystem characteristics (e.g. river flow, temperature, nutrient concentration) occurs naturally (Vadas & Orth 2000). Human intervention in their patterns of variability (e.g., dam building, clear-cutting of riparian vegetation, discharge of contaminants) may cause important ecological effects such as the decline of natural populations (Gonzalez-Oreja & Saiz-Salinas 1998, Fitzgerald et al. 1998, Smith et al. 1999). Due to the range of natural variability it is difficult to detect when human-induced changes result in significant ecological effects outside the boundaries of the baseline range for a particular ecosystem (Doeward-King et al. 2001). This consideration is also crucial before planning any environmental management actions towards preservation, since different causes may require different solutions.

One possible way to evaluate the baseline condition and its natural range of variation is to use simultaneous measurements or descriptions of several adjacent ecosystems and their associated fish communities (Ford 1989). The comparison should be based not only on site-specific features

of an ecosystem (river bed complexity, shading, marginal vegetation, turbidity etc.), but also on features related to larger scale characteristics (latitude, position in a catchment area, elevation from sea level, river order etc.).

Distinguishing between patterns of anthropogenically disturbed and non-disturbed communities applying indices based on taxonomic composition data, such as Margalef's or Berger–Parker index (Magurran 1988) is common. Regardless of the index chosen, the method consists of a comparison of index values between supposedly 'disturbed' and 'less disturbed' sites. Another approach for preliminary distinction between disturbed and nondisturbed communities is the use of exploratory data analyses (Gentile et al. 1999). For instance, cluster analysis may be useful in identifying similar groups of sampled communities. Nonmetric mul-

Figure 1. Map of Betari, Iporanga and Pilões watersheds showing distribution of human settlements, mines (active and inactive limestone mines and inactive lead and gold mines), and agricultural activities, and limits of the rain forest reserve PETAR.

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tidimensional scaling may represent similarities among community compositions (Schiffman et al. 1981). Exploratory data analyses generate hypotheses that can then be tested using confirmatory statistics such as multivariate hypothesis testing, etc. For instance, it is possible to test whether observed differences between groups of communities sampled are mainly associated with the natural variability of ecosystems or with changes caused by anthropogenic factors.

The aim of this paper is, firstly to describe spatial variability of fish communities of a rain forest reserve located in the southeast of Brazil and, secondly, to identify patterns of association between fish communities and habitat variables, including anthropogenic factors.

Methods

Study area

We conducted the study in three independent watersheds (Betari, Iporanga and Pilões) belonging to the Alto Ribeira watershed, located approximately 350 km southwest of the city of São Paulo. The study area comprises the natural reserve known as the Parque Estadual Turístico Alto do Ribeira (PETAR) and parts of other three environmentally protected areas of Atlantic Rain Forest: the Parque Estadual Intervales, the Area de Proteção Ambiental da Serra do Mar and the Área de Proteção Ambiental Furnas (Figure 1). The study area is located in the Serra de Paranapiacaba mountain ridge, and elevations of the hilly terrain of the park varies from 100 to 1000 m above the sea level. The climate is subtropical humid without a typical dry season and with a mean annual precipitation over 1700 mm. Most of the precipitation is concentrated between October and March.

Several human activities are present in the study area and may induce changes in the aquatic ecosystems (Shimada 1999, Moraes et al. 2003a). They are the release of non-treated domestic liquid waste near human settlements, the contamination of streams by heavy metals from piles of waste rock of former gold and lead–silver mines, and the

Figure 2. Map of Betari, Iporanga and Pilões watersheds showing sampling sites location, hydrography and limits of the rain forest reserve PETAR.

release of particles during explosions of quarries for limestone mining. Furthermore, the application of pesticides by the health authorities to control vector-borne diseases or by workers to control pests in middle scale agriculture can also cause ecological effects. The distribution of the main pollution sources in the region is shown in Figure 1.

Site selection

We sampled 14 sites on 11 streams (Figure 2) during three different sampling campaigns in the rainy season, in November 1998, March 1999 and January 2000. We took a total of 31 samples (Table 1). This sampling program was based on an environmental risk assessment of the PETAR reserve (Moraes et al. 2003a). Sampling site selection was primarily based on the presence or absence of pollution sources, but optimal site selection was hindered by the limited accessibility of streams due to topography and the bad conditions of the roads. Stream features such as substrate type, riparian vegetation, flow intensity, shading, and channel width were also taken into consideration during stream reach selection.

Fish sampling

The stream segments for sampling fish communities were 30–35 m long. Sampling protocol, logistic constraints and site-specific characteristics were considered in establishing the length of each section to be sampled. The width of the sampled segment varied between 1 and 11 m, producing different sampling areas (Table 1). Sampling width was the same as stream width, except when this exceeded about 10 m, as indicated in Table 2. Two of the streams wider than 10 m presented a braided pattern (B10 and P9). In these cases only one channel was selected for sampling, but the sum of all stream channels widths are given in Table 2. Other large streams (B4, I4 and P9) contained either non-wadeable pools, which precluded electrofishing, or areas with swift currents that could not ensure collector's safety. In these cases, we sampled a lateral portion of the channel of the width reported in Table 2. We blocked sampled segments in small streams with 6 mm mesh nets at

both ends, while we blocked segments in streams wider than 10 m only at the downstream end.

We performed sampling of stream fish communities by electrofishing with an AC shocker powered by a 1000 W (Honda) generator placed on the riverbank. The same operators of the electrofishing apparatus were used throughout and they had extensive experience and knowledge about the fishing gear. In November 1998, a two – person crew conducted fishing, one person carrying the hand-held electrified pole, and the other a handheld electrified dip-net. In March 1999 and January 2000, the crew consisted of three persons. The third person carried a robust non-electrified pole, wading together with the fishing crew, turning up cobbles, moving large boulders and blocks and removing small snags, in order to dislodge hiding fish. On all occasions, three downstream passes of equal effort were done in a slow zig-zag pattern. At the end of each pass the downstream blocking net was checked for stunned fish. Fish captured in each pass were kept separate, sacrificed in a lethal solution of anesthetic, fixed in 10% formalin, and then preserved in 70% ethanol. At the laboratory, we identified all fish, counted them and later deposited them at the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Stream physical measurements and site descriptions using GIS

We did stream characterization in conjunction with fish sampling. We measured stream width with a tape measure in the beginning, middle and end of the reach segment (the average of the three measures was reported to the nearest 0.5 m). We measured stream reach depth by taking up to 10 measures with a calibrated pole throughout the reach sampled (the average was reported to the nearest 0.1 m). We estimated reach complexity on the basis of a visual estimate of the diversity of flow patterns, substrate categories and instream structure, such as leaf packs, immersed vegetation, and large organic debris (LOD). This is a relative estimate assigning a score from one to four to each sampled stream segment. We assigned a reach a complexity score of one when its water flow was unidirectional, only one or two substrate categories dominated the streambed, little or no structure occurred and LOD and overhanging vegetation

Table 2. Habitat features of sites sampled along streams in Pilões, Iporanga, and Betari watersheds.

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were nearly absent. A reach scored four when flow occurred in a diverse array of speeds and directions, had more than four substrate categories (and low dominance), and its instream structure was complex due to the presence of LOD, leaf packs or immersed vegetation. Shading, i.e., stream canopy cover on the reach sampled, was visually estimated and recorded to the nearest 10%.

We measured temperature, pH, dissolved oxygen and electrical conductance three times at each site (Horiba U-10 Water Quality Checker). Unfortunately, it was not possible to record these water parameters in January 2000. We compared the means of measurements taken from all samples in November 1998 and March 1999 by paired ttests, to evaluate differences related to seasonality. Results showed no significant differences among sampling occasions thus we selected only 1998 values to characterize sites in the following analysis.

We used topographic maps (1:50,000) produced by Instituto Brasileiro de Geografia e Estatística (1987) to determine elevation (20 m resolution), river order (Horton Strahler system) and the downstream link (D-link), that is, the magnitude of the link of the next downstream confluence of each of the sampled reaches (Fitzpatrick et al. 1998). Bedrock description was based on geological maps (Shimada 1999).

Sources of pollution included agriculture, human settlements, abandoned and active limestone mines, and abandoned lead–silver and gold mines. To count the number of sources upstream from each sampling site, we compiled maps of hydrology and human settlements (Instituto Brasileiro de Geografia e Estatística 1987), mining activities (Shimada 1999) and vegetation coverage (Secretaria do Meio Ambiente do Estado de São Paulo 1997) using Arc View®, Arc Info® and Cad®. We calculated the number of limestone, gold and lead mines or houses located 2.5 km upstream along the streams of the entire drainage network of each sampling site (including 500 m wide buffer zone along each margin) (method modified from Dyer et al. 2000). Additionally, we estimated the percentage of agricultural land (i.e. percentage of bare soil plus crop fields and pastures) in an area defined by a circle of 2.5 km diameter upstream of the sites (the circle was positioned upstream the

sampling site covering most of the drainage area occurring there). The choice of 2.5 km distance or diameter was based on previous field studies and represents approximately the furthest distances from the sources where pesticides, nutrients, particles and metals were detected in samples from PETAR streams (Moraes et al. 2001, 2003b, c).

Data analysis

Total number of species (S) , number of species normalized to sampled area (S_a) , total number of individuals (N), number of individuals normalized to sampled area (N_a) , the Margalef's diversity in- dex (D_{Mg}) and the Berger–Parker dominance index (d) were calculated for each fish community sample according to Magurran (1988). To assess how the 13 surveyed habitat variables and community indices were interrelated, we constructed a correlation matrix using the Spearman rank correlation coefficient (r_s) . Habitat and fish data used in this analysis were always those collected at the first sampling occasion of each sampled reach. We assessed the significance level of each r_s at 0.05 according to the Pearson-curve approximation (Zar 1999).

For subsequent analyses, we log transformed fish catch data to give more weight to rare species, as these species abundances are likely to be underestimated due to the small sampled reach length. We standardized the transformed fish abundance data by dividing these by the sampled area, since the sampled reaches differ in size up to 10 times. Then we multiplied the resulting figures by 100 to facilitate data visualization;

$$
N_{ij} = \frac{\log_{10}(n_{ij} + 1) \times 100}{A_j} \tag{1}
$$

where A_i is the sampled area in each *j* site $(A, \text{ in } \mathbb{R})$ $(m²)$ and n_{ij} is the number of fish of species i sampled at site j.

For identification of similar communities, we carried out a cluster analysis using the fish community data (transformed according to equation (1)), with help of the Statistica Software[®] Program. The linkage distance we chose for defining clusters was 0.5. The analysis was based on the reciprocal of the Pearson correlation as a distance

measure and on the unweighted pair-groups method (UPGMA) as the algorithm. We tried other distance measures, algorithms, and different linkage distances for defining clusters to assess their impact on the dataset and robustness of the groups. We made the final choice on the best interpretability of results (Fowler et al. 1998).

In order to identify patterns of association between habitat variables and fish community composition, we used a three-step procedure. First, we converted transformed fish community data into a matrix of similarity of fish data per sites. The original matrix of species \times sites contained 38 species and 31 samples. We calculated the distances matrix applying the Bray and Curtis index, as recommended by Faith et al. (1987) and Clarke (1993), as the majority of cells in the fish data sets possessed zero values. Clusters produced with the reciprocal of the Pearson correlation coefficient have virtually the same identity of groups pro-

Table 3. Fish species collected in Pilões, Iporanga and Betari watersheds, Southeast Brazil.

Order	Family	Species name			
Characiformes	Characidae	Astyanax janeiroensis Astyanax sp. Bryconamericus microcephalus Deuterodon cf. iguape Hollandichthys multifasciatus Mimagoniates microlepis			
	Crenuchidae	Characidium cf. pterostictum Characidium sp. n.			
Siluriformes	Callichthyidae	Corydoras barbatus			
	Loricariidae	Ancistrus multispinis Harttia kronei Hypostomus interruptus Kronichthys sp. Neoplecostomus ribeirensis Otocinclus gibbosus Paratocinclus maculicauda Isbrueckerichthys spp. Rineloricaria sp. 1 Rineloricaria sp. 2			
	Heptapteridae	Acentronichthys leptos Chasmocranus lopezi Imparfinis sp. Microglanis sp. n. Pimelodella transitoria Rhamdia sp. Rhamdioglanis frenatus			
	Trichomycteridae	Trichomycterus davisi Trichomycterus sp. 1 Trichomycterus sp. 2 Ituglanis proops			
Gymnotiformes	Gymnotidae	Gymnotus sp.			
Cyprinodontiformes	Poecilidae	Cnesterodon sp. Phalloceros caudimaculatus			
Perciformes	Cichlidae	Crenicichla sp. n. Geophagus brasiliensis			

duced with the Bray-Curtis matrix of similarity with a 0.8 linkage distance.

Second, we interpreted the similarity matrix using a nonmetric multidimensional scaling (MDS) with the help of the Statistica Software[®] Program. According to James & McCulloch (1990), MDS is a robust ordination method for reducing the dimensions of data based on rank order of inter-object (in this case, sampling sites) distances. The results are often similar to those of principal component analysis (PCA) but MDS does not require that the data meet numerous assumptions like PCA and other parametric multivariate methods often do (e.g., multivariate normal distribution of data and linearity of relationships). MDS analysis gives a graphical representation of dissimilarities among community compositions with proximity indicating similarity. Its interpretation should be qualitative and subjective. The criterion for the number of dimensions was based on the goodness of fit with change of dimensionality (Shiffman et al. 1981). Despite the final number of dimensions obtained for describing the data were higher than two, the presentation of results were in two-dimensional MDS graphs, because of their increased clarity of the ordination plot. The grouping of similar sites was based on the clusters produced by the UPGMA algorithm on the Pearson's reciprocal distance matrix.

The third and final step in this procedure was the calculation of Spearman rank correlation coefficients between the MDS scores and the fol329

lowing 13 habitat variables for each site. The variables were: elevation, downstream-link, reach complexity, mean stream reach width, temperature, pH, dissolved oxygen, water conductance, number of houses, number of mines (lead, gold and limestone), and percentage of bare soil, crop fields and pastures (see details above). Significant correlations ($p \leq 0.05$) were represented in the MDS two-dimensional graphs.

Results

Fish community characterization

The 31 fish samples obtained from 14 sites during the three campaigns contained 4 614 individuals, belonging to 35 species, nine families, and five orders (Tables 3 and 4). The Siluriformes was the most important order in terms of individuals (68%) and species (63%) , followed by the Characiformes (30 and 23%, respectively), the Cyprinodontiformes (1 and 6%), Perciformes (1 and 6%) and the Gymnotiformes (0.1 and 3%). Although sampling sites and numbers of sites per campaign differed, Siluriformes were always the most abundant and species – rich order. These results are due to the large number of species in the families Loricariidae (mailed catfishes) and Heptaperidae (new family formerly included in the 'antenna catfishes' Pimelodidae, Bockmann & Guazzelli 2003). Most Characiformes species found in this

Table 4. Number of fish species (S) and individuals (N) obtained in 31 samples in streams from the Betari, Iporanga and Pilões watersheds, Southeast Brazil, depicting theirs distributions in orders and families in 1998, 1999 and 2000 sampling campaigns.

Orders	Families	S			\boldsymbol{N}				
		1998	1999	2000	Total	1998	1999	2000	Total
Characiformes	Characidae Crenuchidae	4 2	5 2	$\overline{4}$ $\overline{2}$	6 2	202 103	466 424	49 146	717 673
Siluriformes	Callichthyidae Loricariidae Heptapteridae Trichomycteridae	9 5	9 $\overline{7}$	10 $\overline{7}$ 4	10 4	21 428 101 39	64 877 185 111	26 1037 187 64	111 2342 473 214
Gymnotiformes	Gymnotidae			$\mathbf{0}$		3	3	θ	6
Cyprinodontiformes	Poecilidae		2		$\overline{2}$	9	18	24	51
Perciformes	Cichlidae Total	25	2 30	$\overline{2}$ 31	$\overline{2}$ 35	16 922	$\overline{4}$ 2152	7 1540	27 4614

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study are tetras (family Characidae), but the Crenuchidae (South American darters) dominated the 1999 and 2000 samples in terms of individuals (Table 4).

The dominance of siluriforms, particularly Loricariidae, may be due to the general characteristics of the sampled sites. Most sampled reaches were shallow $(< 0.5$ m deep) with a preponderance of riffle or run habitats and rocky substrate. Pool development is poor in most areas, and most channels are flat, without well defined river banks. Open water habitats and shelters along the channel margins (e.g. undercut banks, backwater pools) are nearly absent in our sampled reaches. Such channels may be especially suitable to benthic species. An alternative hypothesis to explain the large proportion of Siluriformes, and the also the benthic Crenuchidae found in this study is that the sampling gear have biased catches towards the Siluriformes and other benthic species. That is because these species are more cryptic and slower than the Characiformes, and usually adopt hiding behaviors as a defense, rather than escaping from the nearby area being electrofished (P. Gerhard, personal observations).

We found large differences in fish abundance, richness and diversity among sampling sites (Table 5). The richest sites (S) were B10 in the Betari River, with 19 species, followed by I4 in the Iporanga River, I5 in the Soarez Stream and P9 in the Pilões River, all three sites with 17 species but during different sampling campaigns. The number of species per site varied from 2 to 19 with a bimodal distribution; one around 2 and other around 14 species. Although site selection was not random, these species richness modes correspond roughly to the mean species number of two sets of samples; one containing the large streams with about 10 m wide channels, and the other containing small streams with a channel width of about 3 m. The small streams set had surveyed areas much smaller than the large streams group but it included more habitat units (see comment below). So it is possible that comparing surveyed area of narrower streams to large ones would not result in an proportional increase in species numbers. The number of species per unit area (S_a, S_a) Table 5) does not clearly relate to mean stream width (Table 6), indicating that this variable should be related to other factors. In terms of fish

abundance (N) , the number of fish captured varied by two orders of magnitude, from six individuals in P5, a small headwater tributary of the Pilões River, to 649 individuals in B1, in the Betari River. Even if these numbers were normalized by sampled area (N_a) , the difference is about the same: 0.05 vs. 1.71 individuals per m². As for the number of species reported per sample, diversity is also related to surveyed area and mean stream width (Table 6). Thus, the most diverse stream reaches, according to the Margalef's diversity index (D_{Mg}) were the ones containing the largest number of species (S) , as this index is heavily weighted toward species richness (Magurran 1988). The highest dominance Berger–Parker index (d) were calculated for sites in small streams with only a few species (e.g. I2 and P5) or sites in streams dominated by a few species (e.g. B11 in 2000).

Habitat variables and community indexes correlations

Table 6 shows the correlation coefficients of 16 habitat variables and 6 community indices measured or derived from samples taken from the 14 stream reach samples. There are three groups of pairs of variables that showed significant correlations. The first group includes five habitat variables: elevation, Horton–Strahler stream ordering, the downstream link metric, stream width, percent shading and dissolved oxygen. The first four variables are correlated by simple geomorphological phenomena (e.g., low-order streams are generally located at higher elevations), as mean stream width and percent shading are obviously related. Dissolved oxygen presented significant correlations with elevation, stream order and D-link, stream depth, width and substrate complexity. This is mostly due to the distribution of sampling sites, that is, to the presence of the upland streams, sluggish and relatively deep, and the larger and more turbulent clearwater mountain torrents.

The second group of significant correlations among variables relates some community metrics to habitat measurements. Number of species (S), individuals (N) and both the Margalef and Berger– Parker diversity indices were clearly related to site elevation, stream ordering, the downstream link, percent shading and stream width. The trend is that species diversity is higher in stream reaches

wid = stream reach mean width, dep = mean stream reach depth, comp = reach habitat complexity, T = temperature, DO = dissolved oxygen, cond = water conductance, hset = human settlements, gold = gold mines, lead = lead mines, lime = limestone mines, agri = agriculture fields, S = number of species, S_a = species per sampled area (in species m- 2), $N =$ individuals, $N_a =$ individuals per area (in individuals m⁻ 2), D_{Mg} = Margalef's diversity index, and d = Berger Parker's dominance index. located in relatively low areas, where channels are wide, unshaded, and both the stream order and Dlink scores are high.

The third group of significantly interrelated variables refers to high correlations among community indices. As noted above, the Margalef diversity index is positively correlated to species number, the opposite occurring with the dominance based Berger–Parker diversity index.

Similarity between fish communities

Figure 3 presents a graphic representation of the cluster analysis based on fish species abundance. The first six clusters (G1–G6) presented relatively low species richness (S) and diversity (D_{Mg}) . The definition of some clusters were highly dependent on the occurrence of one or a few species: G1 was defined by the occurrence of Astyanax sp., Rhamdia sp. and G. brasiliensis; G2 by Characidium sp. n. (absent from all samples but I3 and P5); G3 and G5 by P. caudimaculatus and R. frenatus; G4 by H. interruptus and T. davisi; G6 by H. kronei, C. lopezi, R. frenatus and P. transitoria.

Cluster G7 was formed by samples taken from large streams containing from 13 to 19 species. Four species appear as dominants in this cluster: C. pterostictum, K. subteres, Rineloricaria sp. 1 and R. frenatus. One species, A. multispinis, appeared solely in this group. Cluster G8 showed very low species richness, and an association of Astyanax sp. and Isbrueckerichthys spp. Cluster G9 was defined by the occurrence of Isbrueckerichthys spp., H. kronei, C. lopezi, R. frenatus and B. microcephalus.

Some of the species occurrences can be broadly related to elevation, resembling cases of substitution and zonation. For instance, there seems to be a substitution of Characidium sp. n. at high elevations (upland headwaters above 800 m, e.g. I3, P5) by C. pterostictum at lower elevations (e.g. I4, P9). At high altitudes (900– 400 m) Astyanax sp. (e.g. I3, P8), and at intermediate elevations (around 700–240 m, e.g. B1, B9), B. microcephalus and Isbruesckerichthys spp. dominate the fish communities. Below 200 m (e.g. B10, I4, P9), more species are added to the communities, such as C. barbatus, A. multispinis and Rineloricaria spp.

Fish communities in relation to habitat variables

Figures 4a–d show similarities among fish communities sampled and their patterns of association with habitat variables. MDS scores along dimension 1 (Figure 4a) indicated that fish samples at sites belonging to clusters G1, G2 and G8 clearly differed from fish samples from other sites. These three clusters were composed of fish from upland streams located in the headwaters of the Iporanga and Pilões Rivers. Spearman rank correlations between MDS scores along dimension 1 and hab-

Figure 3. Clusters produced by the unweighted pair-group average (UPGMA) method on the reciprocal of Pearson coefficients calculated from data on fish species abundance. Discontinuous line represents the linkage distance chosen for defining clusters (G1– G9). Samples collected in November 1998 (n), March 1999 (m) and January 2000 (j).

Figure 4. Multidimensional Scaling (MDS) of fish data. Habitat factors correlated with MDS dimensions (and their Spearman rank correlations with the MDS dimensions) are shown. Arrows indicate directions of increasing magnitude. Aggregation of sites was based on cluster analysis (see Figure 3). In Figure 4A, discontinuous lines limit cluster of upland rivers.

itat variables indicated that fish samples from clusters G1, G2 and G8 were associated with high elevations, areas of intense agricultural activities, low downstream-link scores. The five sampling sites contained in these clusters are in streams draining the upland portions of the Serra de Paranapiacaba, above 700 m above the sea level. They are narrow, occur in low gradient areas and their dissolved oxygen content and substrate complexity are relatively low. The gentle gradient of the terrain facilitates the establishment of agriculture in these upland areas. Sampling site I2, is one of the uplands streams directly affected by the exploitation of limestone inside PETAR. Only two fish species were found at this site during all three campaigns and most individuals were small. A quarry for limestone mining is located nearby I2. During explosions of limestone, considerable

amounts of particles are spread to the surface water, increasing water turbidity. In fact, during the field campaign performed in 1999, turbidity at the site was extremely high compared to other campaigns and sites.

Fish samples from the remaining clusters (excluding G1, G2 and G8) were differentiated along dimension 2 of Figure 4a. The differences were primarily associated with D-link scores and stream water parameters (T, pH, conductivity). Fish samples from sites belonging to clusters G6 and G9 were associated with lower D-link scores, and streams with lower temperatures, higher pH and conductivity than sites of clusters G3, G4, G5 and G7. Spearman rank correlations between MDS scores along dimension 3–7 and habitat variables were not statistically significant and were not further considered.

A new MDS analysis was performed that excluded fish samples from the upland streams of the Pilões and Iporanga watersheds (clusters G1, G2 and G8). This was done to further clarify patterns of association between fish communities sampled from the remaining sites and habitat variables (Figure 4b–d). Spearman rank correlations between MDS scores along dimension 1 and habitat variables (Figure 4b and c) indicated that fish samples from sites that clustered in G6 and G9 were associated with higher elevations, lower Dlink score and stream water with higher pH and conductivity than clusters G4 and G7.

Correlations between the MDS scores along dimension 2 and habitat variables (Figure 4b and d) indicated that fish community of clusters G3, G5 and G6 were associated with streams of lower substrate complexity, and lower water conductivity than G4, G7 and G9. These correlations pointed to associations between fish samples from clusters G4, G7 and G9 and the presence of human settlements.

Spearman rank correlations between MDS scores along dimension 3 and habitat variables (Figure 4c and d) indicated that fish communities of cluster G4 were associated with narrow streams and low DO. Spearman rank correlations between MDS scores along dimension 4–7 and habitat variables were not statistically significant and are not presented.

Excluding upland streams of Pilões and Iporanga watersheds, the remaining surveyed streams can be considered clearwater mountain torrents, according to the classification scheme proposed by Por (1986). These clearwater mountain torrents may be further divided into two groups: adventitious streams flowing in confined valleys (streams where sites B4, B7, B11 and I5 are located) and large streams running through relatively narrow (ca. 100–200 m) floodplains (sites B1, B9, B10, I4 and P9). These two groups of clearwater mountain torrents can be distinguished by variables associated to the relative stream reach position: elevation, downstream link, and channel width. As the elevation diminishes, the downstream link increases (following the addition of first-order tributaries to the drainage network) and the drainage area increases, and so does channel width. Streams located at lower elevations have higher species diversity.

Though the main differences between fish communities of the two groups of clearwater mountain torrents can to a large extent be explained by the relative position of the sampling sites in the watershed, differences found within the two groups may be related to instream characteristics. For instance, the Passagem do Meio Stream (site B11) is one of the adventitious streams flowing in confined valleys. The fish community of B11 showed relatively low species richness and diversity, despite the absence of visible physical barriers for fish dispersion from the Betari River (sampled reach was about 300 m upstream to the confluence of the two streams). This stream had the lowest water conductivity and pH of all the streams sampled. Additional data on this site (Moraes et al. 2001) showed that B11 water was very soft and had zero alkalinity. These water characteristics, which probably reflect bedrock composition (phyllite), may act as a chemical barrier to fish dispersion from the Betari River. An alternate explanation for the low diversity and abundance is the possibility of oligotrophic conditions. However, measurements of nutrients (Moraes et al. 2001) and observations of submerged organic material do not support the hypothesis that the waters are oligotrophic.

Another adventitious stream flowing in a confined valley is the Monjolos Stream (site B7), which receives discharges of non-treated domestic sewage from a village. This system has low dissolved oxygen and high water conductivity (Table 2) combined with high concentrations of ammonia and total nitrogen (Moraes et al. 2001). The observed abundance of algae and an algivoredetritivore fish species (*H. interruptus*) further underpin the hypothesis of human impacts in the stream.

The Furnas Stream (site B4) is also an adventitious stream flowing in a confined valley. Fish composition in this stream was different from the Betari River regardless of the fact that B4 was only about 500 m upstream of the confluence of the two streams and no physical barriers for fish migration were apparent. The differences may be related to instream features such as percentage of shading which influence the primary production, and also possibly due to the presence of a chemical barrier to fish dispersion from the Betari River (e.g., pollution). In the Furnas Stream, changes in com-

munity trophic structure, fish condition, and decrease of fish diversity, and fish abundance are most likely the result of exposure of fish to metals from an abandoned mine (Moraes et al. 2003c).

The Soarez Stream (site I5) is another narrow clearwater mountain torrent. Its fish diversity was the highest among all adventitious streams and even higher than the indices calculated for large clearwater mountain torrents. One plausible explanation of this high diversity is a combination of three factors: absence of human disturbances, absence of physical or chemical barriers to fish dispersion between the Iporanga River (reach sampled was about 1900 m upstream to the confluence of the two streams) and the high score on downstream link.

Sampling methods and fish catch results

The choice of 30–35 m long sample reaches was based on logistic constraints. In smaller streams (e.g. B4, B7, I3) this length usually included more than two repetitions of pools and riffles habitats types present in the reach. This probably assured a representative sample of these local fish communities. On the other hand, larger streams presented some difficulties associated with electrofishing, as pointed above, so that only specific habitat units were surveyed. Consequentially only the fish communities associated with slow riffles and runs were sampled at these locations. This standpoint is raised from results of samples taken by different gears (seines, fyke nets, baited traps and gillnets) and more than 50 h of snorkeling in riffles, runs and deep pools mostly in Betari River during 1996–1999 (P. Gerhard, unpublished data). According to these observations and additional sampling procedures, there is a consistent group of species occurring mainly in deeper habitats, such as Hypostomus interruptus, Harttia kronei, Rhamdia quelen, Hoplias cf. malabaricus, Oligosarchus hepsetus and Geophagus brasiliensis (see Table 3 for species list), which was not properly represented or not even detected on samples collected by electrofishing.

In the last two campaigns, there were an increased sampling effort applied by the three-person electrofishing crew. Total catches were more than 100% higher in some sites in the 1999 and 2000 campaigns compared to the 1998 campaign.

However, little or no gross effect on fish species composition and diversity indices estimation were observed (Table 3). This is an indication that the addition of a third person in the electrofishing procedure did not change the proportional yield of each species.

Discussion

The estimates for species richness found in this study are in accordance with Matthews (1998) regarding the number of fish species typically found in sampled reaches. Matthews concluded that most localities (lakes and streams worldwide) contain fewer than 20 species, while some tropical streams may contain up to 100 species. Studies conducted in both tropical and subtropical ecosystems in southeastern Brazil found from eight to 19 fish species for stream reaches of tens of meters (e.g. Esteves & Lobón-Cerviá 2001, Mazzoni & Lobón-Cerviá 2000, Castro & Casatti 1997, Sabino & Castro 1990, Costa 1987, Uieda 1984), or from 38 to 40 species for small watersheds (Garutti 1988, Cláudia Pereira de Deus, personal communication). Apart from local characteristics regarding site complexity, stream productivity, canopy cover or climate, there seems to be a limit to the numbers of fish species within any given community (Matthews 1998).

Most of the fish collected in this study are Siluriformes and Characiformes, two of the most species – rich orders in South America (Lowe-McConnell 1987). In terms of species numbers, our results are consistent with those of Esteves & Lobón-Cerviá (2001), who surveyed stream reaches in an Atlantic watershed (ca. 500 km northeast of PETAR) and by Oyakawa (2002), who investigated headwater streams in the Ribeira watershed (including sampling sites in PETAR). However we found Characiformes less abundant than other studies conducted in comparable streams in the Neotropical region, where tetras dominates catfishes in species and abundance (Angermeier & Karr 1984, Costa 1987, Sabino & Castro 1990, Sabino & Zuanon 1998, Esteves & Lobón-Cerviá 2001 and Oyakawa 2002). Siluriformes are probably the most adapted group for using low flow refuges (sensu Seddell et al. 1990) among boulders and cobbles, in reaches where sand or silt substrate is not prevalent (step-pool or plane-bed reaches, Bisson & Montgomery 1996). On the other hand, the relatively small volume to area habitat ratio implied less opportunity for pelagic fish, here mostly Characiformes species, but also Perciformes and Cyprinodontiformes. Mazzoni and Lobón-Cerviá (2000) also observed less water column species in their shallower sites.

The high number of species in the family Loricariidae may also be a response to habitat conditions in relation to its dietary habits; most are grazers, feeding on substrate attached periphyton (Sabino & Castro 1990, Buck & Sazima 1999). Many of the reaches sampled here are dominated by riffles with minimal shading in wide, canopy open streams. Riffles are long recognized as high productivity areas within the channel (Hynes 1970). Besides this, most of these sampled streams drain limestone areas, presenting high pH and alkalinity values (see Moraes et al. 2001), besides rather transparent waters. These are factors conveying to explain some of the large Loricariidae density in certain riffles, such as that of Kronichthys sp., with 0.63 individuals m^2 , or that of Rineloricaria sp. 1, with 0.22 in B10 (March 1999), compared to $0.09-0.04$ of K. heylandi, and 0.02 in similar streams and seasons in the study of Esteves and Lóbon-Cerviá (2001). In fact, Pareiorhaphis spp. attained densities as high as 1.48 individuals \hat{m}^2 , suggesting that, overall, many of the studied streams in PETAR are highly productive systems.

Some general patterns of fish diversity and community composition were detected. As shown earlier for temperate and tropical streams (Garutti 1988, Ibarra & Stewart 1989, Matthews 1998), these patterns are partially related to a longitudinal/altitudinal distribution of species along rivers. In general this pattern relates to a gradual increase in fish species numbers as stream order increases, mostly due to the addition of species than to their replacement (Roux & Copp 1996). The addition of species downstream may be explained by an increase of ecosystem productivity (Vanotte et al. 1980) combined with higher habitat complexity (Angermeier & Schlosser 1989, Schlosser 1990), mostly in the lateral dimension of the lotic system (Ward 1989). In fact, the sites studied here lie between the extremes of first or second order cascade reaches to fifth–sixth order pool-riffle reaches

presenting some sections of braided channels (Frissel et al. 1986).

However, some natural irregularities occur in the downstream addition of fish species in any river system. One of these may be due to the pattern of stream linkages throughout an entire watershed. According to Osborne & Wiley (1992), the downstream link appeared as a better descriptor of fish species diversity than the most used Horton-Strahler ordering system. Gorman (1986) pointed out that adventitious streams are influenced by migration or colonization of fish species moving from the main river to its tributaries, so that a small creek may bear many more species than if simply predicted by its relative size. In this study, such a discontinuity in species richness was observed in a correlation among D-link and number of species in a sample, indicating that the relative position of a stream in a watershed matters (Osborne & Wiley 1992).

Another sort of natural discontinuities is the presence of geographical barriers imposed by the downstream cascading reaches in the same rivers. It is possible that such phenomena occurred in determining possible colonists for the upland streams studied here. However, it is certain that factors influencing water quality and habitat structure also intervene here. Increased erosion due to clear-cutting of forest for agricultural use and application of fertilizers may increase the amount of inorganic nutrients and decrease the concentration of dissolved oxygen in the water (Cullen 2001). Pesticides used on crops can reach the rivers through surface run-off or through the soil from the site of application (Moraes et al. 2003b). Sensitive fish populations may decline if the concentration of pesticides in water increases to levels high enough to cause effects. In the present study, however, it is not possible to disentangle all of the variables correlated to species occurrence in the upland streams, and more studies are needed on the natural variability of this stream category.

In summary, three main groups of streams could be distinguished: upland streams, adventitious streams of clearwater mountain torrents, and large streams of clearwater mountain torrents, following the scheme proposed by Por (1984) for Atlantic Rainforest streams. The main differences in fish composition and diversity among the three groups

of streams are possibly related to large-scale factors such as elevation, gradient, and position of the stream in the watershed. Differences within the three groups seem to be due to site-specific factors, as reported by May & Brown (2002). Factors describing variability within the same type of stream include instream structure (habitat complexity), physiological parameters (temperature and pH) and human impacts (agriculture, mining and sewage discharge), among others. These differences in stream characteristics may be caused by natural temporal and spatial variability of the ecosystems (e.g. gradual natural changes in physicochemical and physiographic stream parameters downstream) (Vanotte et al. 1980), but also by human influence on the variability of these parameters.

Data on fish habitat preferences, diet, reproduction mode as well as tolerance to siltation, mine waste pollution, or pesticides are not known for most species sampled during this study. It is therefore difficult, for example, to relate how surface and drift-feeders such as Characidae species (Sabino & Castro 1990) would be affected by the increased concentration of suspended particles in water. Along the same line, mailed catfish populations may be severely affected by substrate filling, in a manner similar to those discussed by Berkman & Rabeni (1987). As most of the headwaters of Betari, Iporanga and Pilões watersheds are located outside PETAR reserve boundaries (see Figures 1 and 2), water quality and conservation issues became urgent, specially after it has been shown that aquatic organisms are menaced by sub-lethal doses of pesticides in most of the studied streams (Moraes et al. 2003b). Although the present study included most of the types of stream reaches occurring in PETAR, there is still a need to focus on many issues. For instance, this study did not take into account species typically found in large pools of clearwater mountain torrents, nor does it includes sites representing upland streams with no significant human impacts. An assessment of optimal stream reach length for the estimation of species numbers and abundance may be needed, as none of the studies using electro fishing as a sampling tool in the Atlantic rainforest presented any discussion on accuracy of the methods. The link between fish and habitat might be explored with more detailed approaches, such

as those in the studies of Moyle & Senanayake (1984). It is the information on the interactions between fish and habitat, together with further investigation of fish natural history and their tolerance to pollution that may enhance our ability to understand stream ecosystem change, and then, contribute to species conservation.

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