Using environmental and spatial filters to explain stonefly occurrences in southeastern Brazilian streams: implications for biomonitoring

Utilizando filtros ambientais e espaciais para explicar a ocorrência de plecópteros em córregos do sudeste Brasileiro: implicações para o biomonitoramento

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Abstract: Many biomonitoring programs of aquatic systems are based on knowledge of species' geographic distributions and on how different groups respond facing different sources of impact. In this study, we asked if plecopteran occurrences at genus level can be explained using environmental conditions of low-order Southeastern Brazilian streams. Additionally, we asked if plecopteran nymphs discriminate reference streams from streams under diffuse anthropic impacts. We sampled plecopteran nymphs in 44 first- and second-order reference streams and in 14 test streams representing three of the most common land uses in the State of São Paulo (*Eucalyptus*, sugar-cane plantation and pasture). Our data set included incidence of Plecoptera genera, and genus richness as response variables, and environmental and spatial variables as predictors. We adopted a variation partitioning technique and estimated the unique and common effects of the two sets of explanatory variables on the response variables using multiple regression and RDA analyses. We found no relation between our response variables and environmental factors. Our results also revealed that only spatial variables significantly explained the variation on distributional patterns of Plecoptera in reference streams. This result seems to characterize differences between mountain areas and the sedimentary basin. Defining regions and reference streams for biomonitoring is an essential step to establish effective biomonitoring in the State of São Paulo, because it may contribute to separate natural variability from anthropogenic influences.

Keywords: autocorrelation, bioindicators, environmental predictors, plecoptera, macroinvertebrates.

Resumo: Muitos programas de biomonitoramento de sistemas aquáticos são baseados no conhecimento das distribuições geográficas das espécies e em como grupos diferentes respondem a diferentes fontes de impacto. Neste estudo, nós perguntamos se ocorrências de plecópteros, em nível de gênero, podem ser explicadas usando condições ambientais de córregos de baixa ordem do Sudeste Brasileiro. Adicionalmente, nós perguntamos se ninfas de plecópteros discriminam córregos referência de córregos que sofrem impactos antrópicos difusos. Nós coletamos ninfas de plecópteros em 44 córregos referência de primeira e segunda ordem e 14 córregos teste, os quais representam três dos mais comuns usos do solo no Estado de São Paulo (*Eucalyptus*, cana-de-açucar e pastagem). Nosso conjunto de dados incluía ocorrência de gêneros de Plecoptera e riqueza de gêneros como variáveis resposta e variáveis ambientais e espaciais como preditoras. Nós adotamos uma técnica de partição de variância e estimamos os efeitos comuns e únicos dos dois conjuntos de variáveis explanatórias nas variáveis resposta através de análise de regressão múltipla e RDA. Nós não encontramos relação entre as variáveis resposta e os fatores ambientais. Nossos resultados também revelaram que apenas variáveis espaciais explicaram significativamente a variação nos padrões de distribuição de Plecoptera em córregos referência. Este resultado parece caracterizar diferenças entre áreas de montanhas e a bacia sedimentar. Definir regiões e córregos de referência para biomonitoramento é um passo essencial para o estabelecimento de um biomonitoramento efetivo no Estado de São Paulo, por que pode contribuir para separar variabilidade natural de influências antropogênicas.

Palavras-chave: autocorrelação espacial, bioindicadores, preditores ambientais, plecoptera, macroinvertebrados.

1. Introduction

Knowledge of species' geographic distributions and the degree to which communities are predictable have major implications for biomonitoring and conservation (Ferrier, 2002). The cornerstone of many biomonitoring programs of aquatic systems, such as RIVPACS, BEAST, and AusRivAS, is that aquatic communities can be predicted from environmental factors. However, as these are empirical approaches, their applicability are regionally restricted because occurrence data for most aquatic communities are sparse for many regions of the world (e.g., Neotropical), resulting in insufficient information for many applications.

The effects of antropogenic impacts on aquatic insects have been documented in different parts of the world (Allan, 2004; Bonada et al., 2006), yet there is still much debate on how predictable are the answers of different groups facing different impacts. In general, results from literature suggest very predictable responses of aquatic insects to point sources of impact like organic sewage, although diffuse impacts show more complex and variable responses.

Stoneflies are one of the most widely used groups for aquatic assessment (Rosenberg and Resh, 1993). The representatives of the order Plecoptera are mainly associated with clean, cool, running waters, although a number of species are adapted to oligotrophic, alpine, and boreal lakes, and to lowland streams. Stonefly nymphs tend to have specific requirements of water temperature, substrate type, and stream size, reflecting their distribution and succession along the course of streams and rivers (Stewart and Harper, 1996). Although stoneflies are sensitive to pollution, or more precisely to low oxygen concentrations accompanying organic breakdown processes, they seem rather tolerant to acidic conditions (Giller and Malmqvist, 1998).

A growing number of studies on the distribution and ecology of plecopteran nymphs at genus and species level in Brazil have been published in recent years (e.g., Froehlich and Oliveira, 1997; Bispo and Oliveira, 1998; Bispo et al., 2002a, 2002b; Bobot and Hamada, 2002; Bispo et al., 2006), but stonefly diversity remains unknown over vast areas. Regarding the response of stoneflies to antropogenic impacts, few empirical data are available at genus and species level, although it is widely assumed that the nymphs are very sensitive. In many cases, they suffer from confounding effects of natural variation and antropogenic influences on plecopteran distributions (e.g., Roque et al., 2003). In general, studies have shown that the stoneflies were absent or have suffered large reduction in nymph density in stream stretches with a pronounced anthropogenic influence (Bispo et al., 2002a).

In this study, we asked if plecopteran occurrences at genus level can be predicted from environmental conditions in low-order Southeastern Brazilian streams, and if plecopteran nymphs discriminate reference streams from streams under diffuse anthropic impacts, such as *Eucalyptus* plantation, pasture and sugar-cane culture. We test here the hypotheses that stream-site conditions can predict genus occurrences (presence/absence). Given the current knowledge of taxonomy and natural history of the species, mainly of adults, recorded for the region studied (State of São Paulo), we expected compositional changes between "montane" and "non-montane" sites. The identification at genus level is justifiable in this work because there is not enough information on plecopteran fauna to allow identification at species level based only on nymphs, and because it is a practical taxonomic level for the purposes of biomonitoring. The approach behind our study is similar to that of reference conditions (Bailey et al., 2004). We intend to focus on the first steps (prediction of taxa in reference areas) used to develop predictive models for stream assessment using aquatic insects in the State of São Paulo.

2. Material and Methods

2.1. Studied area and selection of streams

The State of São Paulo has an approximate area of 248,800 km^2 (95,700 mi^2), and a human population of about 40 million (22% of the Brazilian population). The climate varies from tropical to subtropical.

Four major vegetation types are recorded in São Paulo, the coastal forest or Atlantic Rain Forest, the tropical seasonal forest or Atlantic semi-deciduous forest, the Atlantic Mixed Rain Forest, and the Cerrado (savanna). The Atlantic Rain Forest grows at low to medium elevations (<1000 m a.s.l.) on the eastern slopes of mountain chains running close to the coastline from southern to northeastern Brazil. The Atlantic semi-deciduous forest and cerrado extend across the plateau (usually > 600 m a.s.l.) in the interior central and southeastern parts of the country. The Atlantic Rain Forest and the Mixed Atlantic Rain Forest experience a warm and wet climate without a marked dry season, while the Atlantic semi-deciduous forest and cerrado have a more seasonal climate with a comparatively severe dry season, generally from April to September. The Atlantic Forest and Cerrado are among the most threatened tropical forests in the world (Myers et al., 2000), having been reduced to less than seven percent of its original cover in São Paulo State (SOS Mata Atlântica/INPE, 1993), and most remnants are found in sheltered, steep mountain slopes or small fragments.

The present study was conducted in 44 first- and second-order reference streams in the State of São Paulo (Figure 1). We selected areas using reference approach criteria (Bailey et al., 2004) trying to select representative streams of the less impacted areas. These streams are typical of forested headwater streams, with water depths less than 50 cm, tree canopy coverage exceeding 70% of the channel, absence of macrophytes, high dissolved oxygen levels, low conductiv-

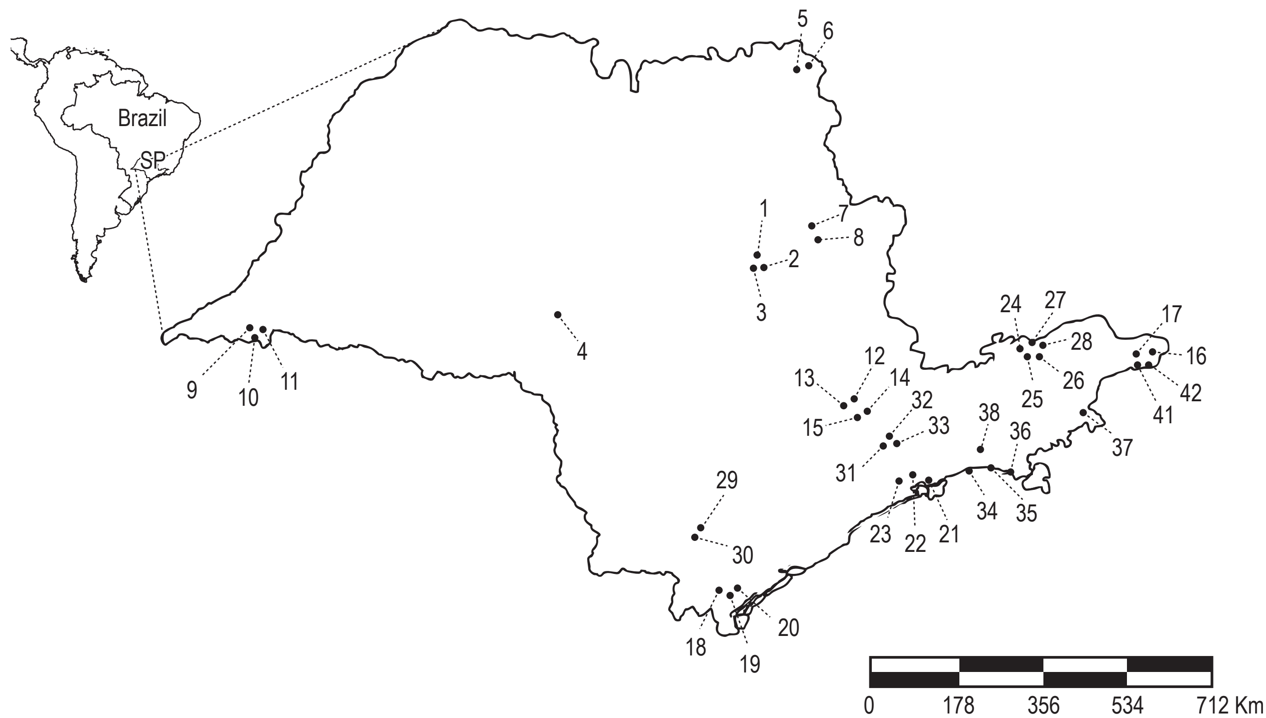

Figure 1. Geographical location of sampled streams in the State of São Paulo. Solid circles denote the locations of the reference streams.

ity, and slightly alkaline or acid water. Water temperature ranged between 15-23 °C. The riparian vegetation along all streams is well preserved.

Fourteen test streams were also sampled, representing three of the most common land uses in the state of São Paulo: *Eucalyptus* plantation (n = 4), sugar-cane plantation (n = 5), and pasture (n = 5). The watersheds of all test streams were completely covered by the land use class, without riparian forest vegetation.

2.2. Plecoptera sampling and identification

During the dry seasons of 2001, 2005, and 2006, plecopteran nymphs were collected on a single occasion with a Surber sampler. Six samples were randomly taken along 100 m of each stream, with three samples from pools and three from riffle sites. For each stream, the samples were pooled prior to statistical analyses.

Nymphs were identified to genus level following original descriptions and Olifiers et al. (2004). The specimens are deposited in the Collection of the Laboratório de Entomologia Aquática da Universidade Federal de São Carlos (SP).

2.3. Environmental measurements

Environmental measures were made at each site to characterize habitat conditions and degree of human disturbance. Conductivity, pH and dissolved oxygen were measured in situ using a Horiba U-10 or a Yellow Springs-556 water checker equipped with a multiple probe. Predominant substrates were estimated subjectively as the proportion of the stream bottom covered by boulder and cobble (>256 mm), gravel (2-255 mm), sand (0.125-2 mm), and mud (<0.125 mm). To assess the physical and biological condition of riparian zone and stream channel morphology, we applied the metrics 1 (land use), 2 (width), 3 (completeness), 4 (vegetation), 5 (retention), 7 (channel), and 8 (bank structure) of the Riparian, Channel, Environmental - RCE Protocol (Petersen, 1992), while the other metrics were not included because our streams have a wide range of substrate characteristics. The following potential environmental variables unaffected by human activity were also used to predict plecopteran occurrences: altitude, latitude, and longitude.

2.4. Data analyses

We carried out the following steps for analyzing possible relationships between environmental and spatial variables, and plecopteran assemblage structure.

We used a principal components analysis (PCA) to reduce the dimensionality and collinearity among environmental variables. The Broken-Stick method was used as a stopping-rule in the PCA. This method only retains the components associated with eigenvalues larger than the values given by the broken stick distribution, providing an accurate estimation of the dimensionality of the data (for details see Jackson, 1993). We created spatial predictors by using the eigenfunction analyses (Griffith and Peres-Neto, 2006). This approach is based on principal coordinate analysis, where eigenvectors are extracted from a connectivity (or distance, see Diniz-Filho and Bini, 2005) matrix among sampling units, and these eigenvectors (called filters), which describe the spatial structure of the region under study at different scales, are used as additional predictors of the response variable. This way, any remaining spatial structures in regression residuals would be taken into account, overcoming the problem of spatial autocorrelation.

A set of ordinary least-squares multiple regressions (OLS) and a variation partitioning technique (Legendre and Legendre, 1998) were carried out to partition the variance explained by environmental variables and space. Thus, we could obtain the unique fractions of variation explained solely by environment (component [a]) and space (component [c]), the common fraction shared by environment and space (component [b]), and the residual non explained variation (d). Genus richness was the response variable. PCA axes, and spatial filters with Moran's I coefficients larger than 0.1 were the predictor variables. This way, only filters that in fact contain important parts of the geometry of the study area will be used in the analysis, avoiding excess of predictors in the multiple regression. The Moran's I is a measure of association of the variable of interest with itself, given a measure of distance (for details see Legendre and Legendre, 1998). Eigenfunction, PCA and multiple regression analyses were made in the freely available package Spatial Analysis in Macroecology v. 2.0, SAM (Rangel et al., 2006). Results of variation partitioning were based on adjusted fractions of variation (Peres-Neto et al., 2006) and were performed using the R-language function "varpart" in the vegan library (Oksanen et al., 2005).

To evaluate possible relationships between incidence (presence and absence) of Plecoptera genera and PCA axes and spatial filters, we adopted a variation partitioning technique based on a redundancy analysis (RDA; Legendre and Legendre, 1998). The RDA is best understood as a method for extending multiple regression, which has a response variable (e.g., compositional data) and multiple predictors (e.g., environmental and spatial data). RDA analysis was made using the R-language and variation partitioning analyses were based on adjusted fractions of variation performed using VarCan software v.1 for Matlab (Peres-Neto et al., 2006).

3. Results

The six principal components axes selected by the Broken Stick criteria accounted for 96% of the variability in stream environmental variables (Table 1). PC1 tended to segregate "high gradient streams", which were mostly covered by boulder, cobble, and gravel from "lowland streams", where sand and mud dominated. Most sites with lower PC1 values were in the Atlantic forest mountain range (eastern coast) while those with higher PC1 values were in the sedimentary plate of the state (western part), but there were some exceptions because we sampled high gradient streams and lowland streams in both areas (Figure 2). Streams with lower PC1 value, related to higher pH values, were located in a carstic area in the State of São Paulo (Parque Estadual de Intervales).

Seven plecopteran genera were found in the 44 reference streams sampled, from two families recorded in Brazil, Gripopterygidae and Perlidae (Froehlich, 1981; 1999): the gripopterygids *Gripopteryx* Pictet 1841, *Guaranyperla* Froehlich 2001, *Paragripopteryx* Enderlein 1909, *Tupiperla* Froehlich 1969 (Froehlich, 2001), and the perlids *Anacroneuria* Klapálek 1909; *Kempnyia* Klapálek 1914, and *Macrogynoplax* Enderlein 1909 (Froehlich, 1981).

Multiple regression analysis revealed that genus richness was not significantly related to any of the environmental variables, but to spatial predictors (r^2adj: 0.294, F: 2.185, P: 0.031) (Table 2). After partitioning the variation

Table 2. Coefficients of the multiple regression for Plecoptera genus richness regressed against environmental (PCA axes) and spatial (spatial filters) predictors. * p < 0.05.

	Richness
PCA1	–0.017
PCA2	–0.304
PCA3	–0.167
PCA4	–0.244
PCA5	0.389
PCA6	–0.237
Spatial Filter nº 1	–3.881*
Spatial Filter nº 2	–0.034
Spatial Filter nº 3	2.935*
Spatial Filter nº 4	–0.177
Spatial Filter nº 5	1.103
Spatial Filter nº 6	1.358

Table 1. Summary of environmental variables and results of Principal Component Analysis from the different studied streams, and correlation coefficients between PCA axes and environmental variables. (Cond: Conductivity, DO: dissolved oxygen, RCE: Riparian Channel Environmental Protocol).

Variables	Mean	SD	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6
pH	6.932	0.841	0.691	–0.404	0.449	0.040	–0.131	0.021
Cond	28.845	22.862	0.546	–0.162	0.630	0.087	0.154	0.415
DO	8.345	1.385	–0.433	–0.446	–0.388	–0.262	0.262	0.554
RCE	182.045	6.847	0.303	0.586	0.143	–0.291	0.650	–0.151
% cobble	21.363	20.583	0.639	0.474	–0.394	–0.300	–0.283	0.192
% gravel	27.613	17.202	0.368	–0.618	–0.428	0.365	0.331	–0.222
% sand	37.25	20.367	–0.525	–0.389	0.547	–0.471	–0.034	–0.209
% mud	14.909	16.702	–0.545	0.519	0.311	0.528	0.064	0.195
Variance explained %	-	-	27.100	22.000	18.800	11.100	9.100	0.084

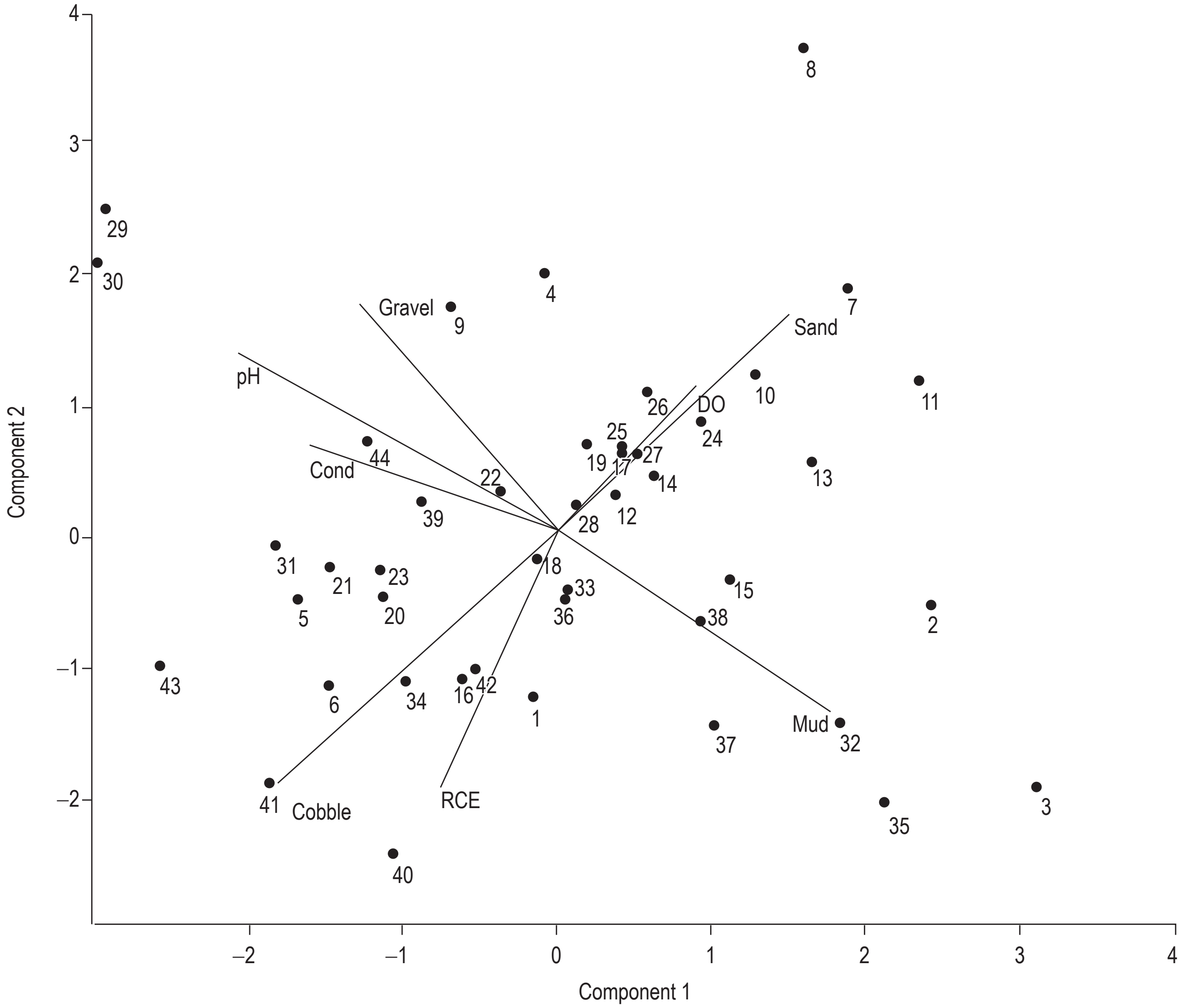

Figure 2. Principal components analysis (PCA) ordination of 44 streams in the State of São Paulo, Brazil, based on measured physical and chemical variables. 1st and 2nd axes explained 27.1 and 22% of variability, respectively.

explained by this full model, we verified that only the spatial component explained variation on genera richness ([c] = 0.122).

Variation in stonefly genus occurrences was also best explained by spatial variables (Figure 3, Table 3). The clearest pattern evidenced by RDA analysis was a trend to discriminate streams characterized by *Kempnyia, Macrogynoplax*, and *Paragripopteryx* occurrences from those with *Anacroneuria*.

Although *Guaranyperla* and *Gripopteryx* showed weak or no relationship with the predictors used, it is important to note that they were absent or rare in streams located in the sedimentary plate of the state. The most frequently collected genera in impacted streams were *Anacroneuria* and *Tupiperla*, whereas *Gripopteryx*, *Guaranyperla,* and *Paragripopteryx* were absent in all test sites. No stoneflies were found in streams located in sugar cane plantations (Table 4).

4. Discussssion

4.1. Stoneflies in streams of references sites

In our study we evidenced a spatially structured pattern in the distribution of plecopteran genera. These findings agree with previous studies that found associations between the distribution patterns of aquatic insects and the geographical position through which streams flow, especially where marked differences in topography are found (see Hawkins and Norris, 2000). Spatial variables are generally more influential across- than within-ecoregions (Heino, 2007), portraying climatic and historical effects on taxa distributions. This pattern seems corroborated by our data set, where the spatial filter 1 accounted for the highest level of spatial autocorrelation, containing a pattern with two major groups of similar values based on Moran's I coefficient values: one group with relatively high values

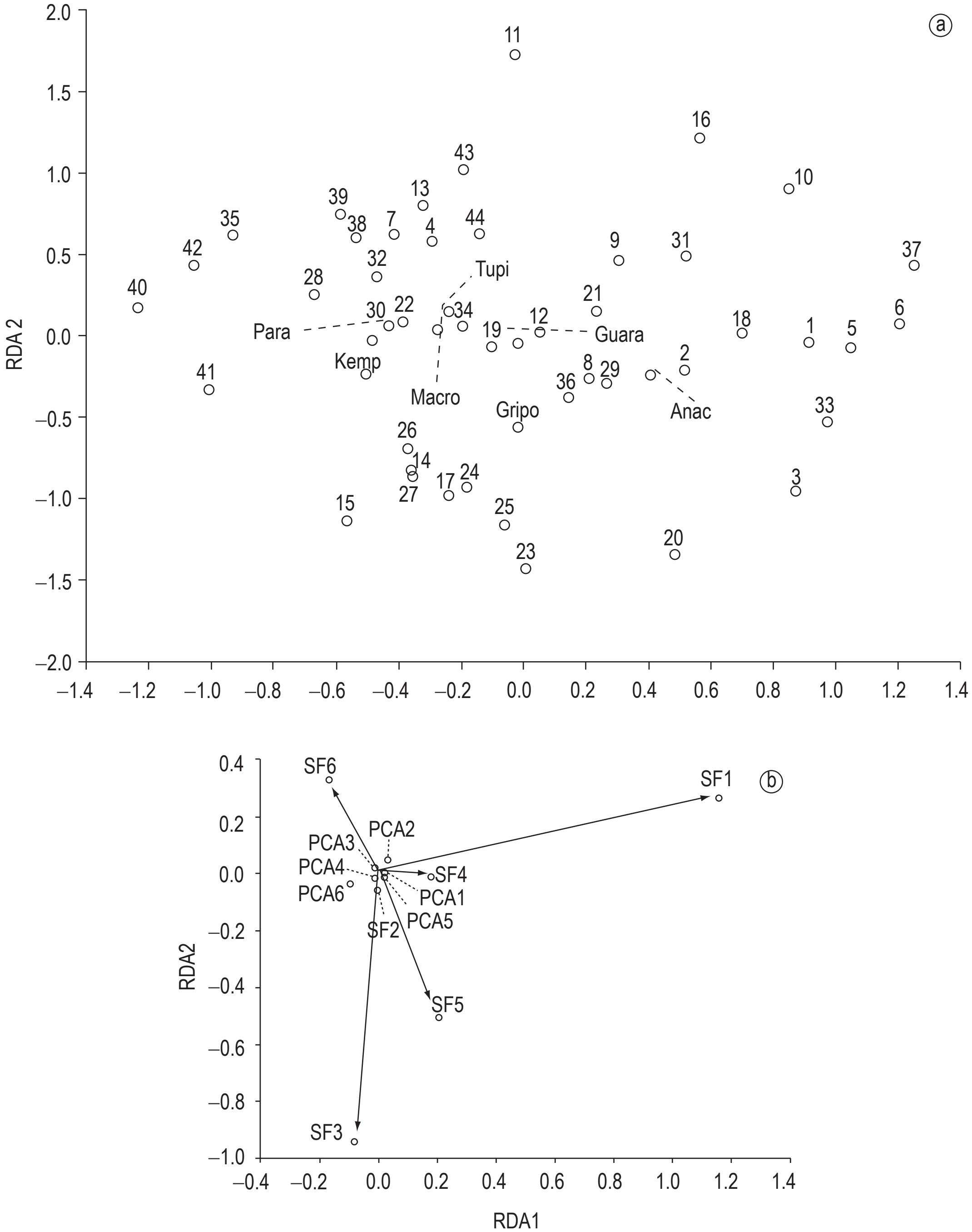

Figure 3. a) RDA ordination diagram of the streams and plecopteran genera and b) RDA ordination diagram of the environmental and spatial predictors. Codes in Figure 1.

Table 3. Relative proportions of variation explained in stonefly occurrences attributable to environmental variables, spatial variables, and shared components between two sets of variables. The component [a] is the pure effect of environmental factors, [c] is the sole effect of spatial predictors, and [b] is the overlap between environmental and spatial predictors.

Total Contribution	Percentg. Explanation	Percentg. Exp - Adjusted	Probability
Environmental and spatial matrixes	0.367	0.122	0.0210
Environmental matrix	0.134	–0.005	0.5280
Spatial matrix	0.310	0.198	0.0001
Partitioning:			
[a]	0.056	–0.076	0.9940
[b]	0.078	0.071	-
[c]	0.232	0.127	0.0060
Residuals	0.632	0.877	-

Table 4. Stonefly occurvrences across impacted streams (different land uses) in the State of São Paulo, Brazil.

	Gripopteryx	*Paragripopteryx*	*Tupiperla*	*Guaranyperla*	*Anacroneuria*	*Macrogynoplax*	*Kempnyia*
Sugar Cane 1	-	-	-	-	-	-	-
Sugar Cane 2	-	-	-	-	-	-	-
Sugar Cane 3	-	-	-	-	-	-	-
Sugar Cane 4	-	-	-	-	-	-	-
Sugar Cane 5	-	-	-	-	-	-	-
Pasture 1	-	-	-	-	-	-	-
Pasture 2	-	-	-	-	-	-	-
Pasture 3	-	-	-	-	-	-	-
Pasture 4	-	-	-	-	1	-	-
Pasture 5	-	-	1	-	1	-	1
Reforestation 1	-	-	-	-	-	1	1
Reforestation 2	-	-	-	-	-	-	-
Reforestation 3	-	-	1	-	-	-	-
Reforestation 4	-	-	1	-	-	-	-

and the other with relatively low values (about 250 km apart from each other). This distance grossly corresponds to the distance among streams within the mountain areas (Serra da Mantiqueira, Serra do Mar and Serra do Japi) and streams in the sedimentary basin. However, it is important to note that a spatial filter is not a variable with ecological meaning, it is a mathematical construction. The amount of variation attributed to spatial variables is probably related to an "unexplored" variable (e.g., dispersal) that could generate the observed pattern. According to Sanderson et al. (2005) space is one important variable for predicting stoneflies due to the influence of neighbouring sites on the species present at a local site, and it is possible that this reflects weak dispersal abilities of species in that order.

Differences in stonefly genus composition between the mountain areas (Serra do Mar and Serra da Mantiqueira) and the sedimentary basin evidenced in our study were consistent with the well-established pattern of aquatic insect changes between "mountain" and "non-mountain" sites. This pattern has already been detected in different parts of the world, and described for stoneflies in Southeastern Brazil based on the natural history of the taxa (Froehlich, 1981, 1999).

The pattern of *Kempnyia, Macrogynoplax*, and *Paragripopteryx* vs. *Anacroneuria* occurrences were congruent with our expectation that stonefly genus occurrences can be related to large-scale conditions. To date, *Kempnyia, Macrogynoplax, Paragripopteryx,* and *Gripopteryx* were mainly reported in mountain streams from Serra do Mar and Mantiqueira, whereas *Tupiperla* and *Anacroneuria* have a wider distribution and are common in lowland streams, typical from the sedimentary basin (Froehlich, 1981, 1999).

Several studies suggested that the occurrence of some stonefly genera can be predicted from environmental variables. For example, Olifiers (2005) studied 16 streams in the Atlantic forest (Rio de Janeiro) and found positive correlations between *Kempnyia, Gripopteryx* and *Paragripopteryx* densities and environmental variables related to low-impaired streams, weak correlation between *Macrogynoplax, Tupiperla* and *Guaranyperla* densities and environmental variables, and lack of relationship between *Anacroneuria* densities and environmental variables. Bobot and Hamada (2002) found that the number of *Macrogynoplax* specimens was negatively correlated with impacted areas, whereas *Anacroneuria* densities did not respond to suspended sediment or deforestation in streams in Central Amazonia. *Anacroneuria, Kempnyia, Macrogynoplax,* and *Gripopteryx* have been collected in low impacted streams in mountainous areas in Central Brazil, but only *Anacroneuria* was found in streams under strong anthropogenic influences (Bispo et al., 2002a, 2002b; Bispo et al., 2006). In our study, environmental variables were not important in controlling stonefly occurrences, so one question remains: why spatial predictors were significant in explaining plecopteran patterns and environmental variables did not?

There are several reasons to explain why stoneflies did not respond to the environmental variables in our reference data set. First, we included only variables from well-preserved streams, so variability could not be strong enough to cause sensible changes in stonefly composition. Second, we possibly did not measure all relevant environmental variables that could affect Plecoptera distribution, or our variables were not good surrogates for them. Third, in general, presence-absence data are less explained by spatial and environmental variables than abundance data when using multiple regressions (e.g., RDA) (Beisner et al., 2006). Fourth, fine taxonomic resolution can be important for detecting ecological patterns for highly diverse taxa such as *Anacroneuria* which has species with very different ecological requirements in the Neotropical region (Tomanova and Tedesco, 2007). Finally, the role of environmental variables in shaping stonefly distributions may be overestimated in previous studies by neglecting spatial autocorrelation, a common problem in ecological studies (Legendre, 1993; Dormann, 2007).

According to McCreadie and Adler (1998; 2006), taxa predictability depends on scale, season, and taxonomic resolution. Although species identification may be an important factor to better understand the associations between stonefly distribution and environmental conditions, we consider that genus level was sufficient to detect large-scale distributional patterns – mountain and non-mountain areas (at least for biomonitoring perspectives), whereas the identification to species level should be more important to detect patterns at smaller scales.

4.2. Stoneflies in streams of test sites and biomonitoring perspective

It is particularly important to understand both from an ecological and biomonitoring perspective whether there is any boundary among regions in the State of São Paulo, or a transition zone between adjacent ecological systems, because they can act or have acted as "filters" regulating the dispersal of taxa, potentially influencing assembly rules. However it is difficult to determine the ultimate factors influencing patterns of plecopteran distribution, because the remaining reference areas in the State of São Paulo are fragmented and embedded in an anthropogenic landscape matrix, which makes it difficult to separate diffuse anthropogenic, historical, and contemporaneous ecological influences on current stonefly distribution. Despite these constraints, the variability among reference streams detected in our study is probably more related to evolutionary and ecological processes than to current or past anthropogenic influences, because the genera that discriminate mountain areas from lowlands (*Kempnyia*, *Macrogynoplax*, *Paragripopteryx*) have been mostly recorded in mountain streams in different parts of the Neotropical region (Froehlich, 1981).

Anthropogenic impacts are not randomly distributed in the State of São Paulo. Most degraded areas are in the sedimentary basin, where intensive land conversion for agriculture (sugar cane, coffee) and pasture has occurred. This landscape pattern, associated with few reference areas remaining in this region, and the plecopteran fauna more characteristic of lowland streams (such as *Anacroneuria*), imply that the evaluation of stream integrity in lowland areas of the State is more problematic than in mountainous areas when using only stonefly occurrences data.

Different levels of distinctiveness between reference and test streams were evidenced in our data; we have not employed statistical analysis due to the zero inflated matrix which requires very complex statistical approaches. The absence of stoneflies in streams flowing through sugar cane plantations clearly indicates the effects of this land use on aquatic insects, corroborating findings by Corbi and Trivinho-Strixino (2007). Streams located in pasture and deforested areas show different compositions of stoneflies, similar to those found in some reference streams in the sedimentary region of the State (only *Anacroneuria* and *Tupiperla* occurrences). These results imply that plecopteran fauna at the genus level would be good indicators of high degree of impairment (e.g., sugar cane), but that detecting impacts of *Eucalyptus* plantation and pasture demand other approaches.

Defining regions and reference streams for biomonitoring is an essential step to establish effective biomonitoring in the State of São Paulo, because it may contribute to separate natural variability from anthropogenic influences. Our study showed that plecopteran assemblages and some genera occurrences are non-random distributed in the São Paulo State presenting a spatially structured pattern. These findings imply that the biotic variation among streams can be partitioned regionally, which would improve the accuracy of bioassessments.

Acknowledgements

We thank Dr. Marcel O. Tanaka and Dr. Pitágoras da C. Bispo for several suggestions that greatly improved earlier versions of this manuscript. We are grateful to all our friends who assisted with sample collection and sorting. The State of São Paulo Research Foundation sponsored this work within the BIOTA/FAPESP–The Biodiversity Virtual Institute Program (www.biota.org.br).

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Received: 20 December 2007
Accepted: 15 April 2008