Explaining dissimilarities in macroinvertebrate assemblages among stream sites using environmental variables

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ABSTRACT. The relationship between community structure and environmental factors usually varies according to ecosystem type, group of organisms, and spatial scale. In this study I assessed whether dissimilarities among assemblages of stream macroinvertebrates are related to differences in environmental variables. Data consisted of macroinvertebrate samples of 10 stream sites during the dry season. Seven environmental variables were assessed. The relationship among dissimilarities in assemblage structure and dissimilarities in environmental variables was assessed using the BioEnv approach. Conductivity and measures related to stream size were the most important variables. However, part of the correlation with conductivity was due to the high value observed in a single stream site, which presented a relatively distinct macroinvertebrate fauna. There was an abrupt change in assemblage structure between 4th and 5th order streams. Although the study included a single 5th order site and thus only weak generalizations are possible, this finding corroborates scattered evidence observed in previous studies. The finding that nearby sites may harbor distinct macroinvertebrate assemblages implies whole-catchment conservation strategies. As most of the remaining Atlantic Rain Forest is restricted to small fragments, restoration projects near fragments should be implemented so as to properly conserve lotic ecosystems.

KEY WORDS. Aquatic insects; BioEnv; Brazil; community structure; Neotropical Region.

Streams usually contain a diverse fauna of macroinvertebrates and in a single stream site more than 70 species can coexist (MELO & FROEHLICH 2001a, b). Insects comprise most of the macroinvertebrate fauna either in terms of abundance and number of species (ALLAN 1995, BAPTISTA *et al.* 1998a, KIKUCHI & UIEDA 1998, BUENO *et al.* 2003, BUCKUP *et al.* 2007). Many insect orders are present in streams and together they usually represent around 95% of the macroinvertebrate fauna in a site (MELO & FROEHLICH 2001a). Other macroinvertebrate groups present in streams are crustaceans, mollusks and planarians. Many stream macroinvertebrates present low tolerance to human-induced disturbance and thus are widely used in biomonitoring programs (BARBOUR *et al.* 1999, BAPTISTA *et al.* 2007, COUCEIRO *et al.* 2007, TUPINAMBÁS *et al.* 2007).

The relationship between community structure and environmental factors usually varies according to ecosystem type, group of organism and spatial scale (WU & LOUCKS 1995). In stream ecosystems, VILELLA *et al.* (2004) evaluated a range of environmental factors and concluded that stream megafauna (amphibians, crustaceans and fishes) were most affected by structural environmental factors, particularly the presence of waterfalls. In small scales (e.g. microhabitat), stream macroinvertebrates communities can differ due to differences in substrate composition (KIKUCHI & UIEDA 1998, BUSS *et al.* 2004), availability of food resources (DOBSON 1999, BÜCKER *et al.* 2008) and flow velocity (BOUCKAERT & DAVIS 1998, CRISCI-BISPO *et al.* 2007a). In higher spatial scales, differences in macroinvertebrate communities among stream sites can result from differences in water chemistry and human or natural disturbances (DINIZ-FILHO *et al.* 1998, BUSS *et al.* 2002, DEATH 2002).

The assessment of the relationships among community structure and candidate environmental factors is not an easy task. Many factors are correlated in space and act simultaneously on species. Given the difficulties to carry out field experiments, most studies addressing the effects of environmental factors on community structure rely on observational approaches (GRAHAM 2003). One suitable method to be used in these observational studies is the BioEnv analytical approach proposed by CLARKE & AINSWORTH (1993) that, despite its popularity in marine sciences (the article was cited 484 times until December 2008 in the Science Citation Index), have rarely been used in freshwater ecosystems (but see SOLDNER *et al.* 2004).

In a previous work, MELO & FROEHLICH (2001a) assessed whether macroinvertebrate communities in Neotropical streams conform to predictions of the River Continuum Concept (VANNOTE *et al.* 1980, MINSHALL *et al.* 1985). Specifically, they tested the hypotheses that mid-sized streams (orders 3-4) are the richest in the catchment and that communities in the dry season presents higher equilibrium (sensu MINSHALL *et al.* 1985) than in the rainy season due to the presence of disturbances by spates in the later. In the present study I extend their findings and, using the same data, assessed which environmental factors best explain differences in dissimilarities among stream sites.

MATERIAL AND METHODS

Data were obtained in 10 stream sites of the Rio do Carmo catchment, Parque Estadual Intervales, SP, Brazil (24º18'S, 48º25'W). The vegetation is tropical ombrophilous sub-montane forest (Atlantic Tropical Rain forest) and remains mostly preserved. The area receives 1500-2000 mm of rainfall unevenly distributed in two periods, with 130-270 mm/mo during the rainy season (summer; September-March) and 60-95 mm/mo during and dry (winter; April-August).

Studied streams were of orders 1-5 (Fig. 1) and had the streambed composed mostly by gravel and stones. Stream 3 crosses a calcareous cave and a small swamp. The largest studied stream (10) receives tributaries that pass through calcareous caves. Table I includes information on physical characteristics of the studied stream sites.

Samples were obtained during the dry season of 1997. I obtained 25 sampling units in each stream site. Sampling units consisted of the macroinvertebrate fauna associated to individual stones around 18 cm maximum diameter. Stones were removed from the streambed using a U-net sampler (Melo & Froehlich 2001a). The material collected in the net was transferred to a white tray and all visible invertebrates removed and fixed in 80% ethanol. Stones were examined for attached individuals, particularly cased caddisflies. Additional information on stream sites and sampling, including a list of families, can be found elsewhere (Melo & Froehlich 2001a, 2004, Crisci-Bispo *et al.* 2007a, b).

Previous studies on stream macroinvertebrate communities in South America usually have identified individuals to genus or family. This reflects the poor taxonomical knowledge of the aquatic fauna and consequent lack of regional keys. The use of genus and family level identifications has proved to be useful in many cases. This is particularly true when differences among samples are large, such as those observed in the comparisons of stream faunas among biogeographic provinces or in gradients of human-induced disturbance (Marchant *et al.* 1995). However, small differences in community structure among sites can only be detected using species or morphospecies (Lenat & Resh 2001, also see Melo 2005). As stream sites are located in the same catchment and do not differ largely in environmental conditions, macroinvertebrates were sorted in morphospecies. Because of difficulties in separation in morphospecies, chironomids and acari were not included.

Sampling units (stones) collected in a stream site were pooled to form a sample. The study thus consisted of 10 samples. Dissimilarities among stream sites were estimated using the Bray-Curtis index on log(x+1) transformed samples. This dissimilarity matrix was used to obtain a Non-Metric Multidimensional Scaling (NMDS) ordination of stream sites. The relationship of dissimilarities among stream sites and dissimilarities in environmental factors was assessed using the BioEnv approach (Clarke & Ainsworth 1993). The approach is related to the Mantel test, where a biological dissimilarity matrix is correlated with a second dissimilarity matrix of environmental variables (Diniz-Filho & Bini 1996, Diniz-Filho *et al.* 1998). However, in the BioEnv approach the environmental matrix is constructed using different numbers and combinations of environmental variables. The biological matrix of dissimilarities is first correlated with each environmental dissimilarity matrix obtained using a single variable and the highest correlation selected. Next, the procedure is repeated but now with environmental dissimilarity matrices constructed using two variables. The combination producing the high correlation is selected. The procedure is repeated but using, three, four or more environmental variables up to the total number available, recording in each step the highest correlation. The Spearman rank coefficient was used to measure the correlation of the dissimilarity matrices. The environmental dissimilarities were computed using the Euclidean distance on variables standardized to unit standard deviation. The following variables were used in the BioEnv analysis: I) stream order, II) link magnitude, III) width, IV) baseflow discharge, V) canopy cover, VI) altitude and VII) conductivity (Tab. I).

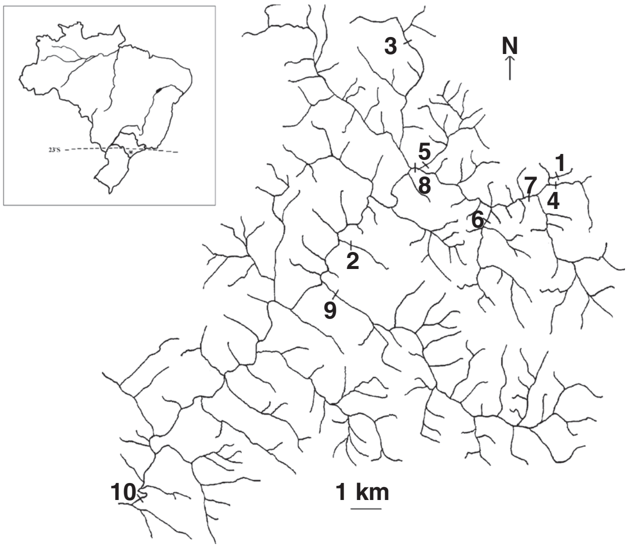

Figure 1. Location of the Rio do Carmo catchment in Brazil and the location of the 10 stream sites studied.

Different from a linear model (e.g. Multiple Regression), where the correlation of the response with the set of predictors always increase or remains the same as more terms are included in the model, in the BioEnv approach the correlation can decrease after the inclusion of variables. This happens because no additional term is included in the statistical model. Instead, the additional variable can deteriorate the correlation pattern between the biological and environmental dissimilarity matrices. This feature thus provides a natural stopping rule in the selection of the best set of environmental variables. All analyses were done using functions in the packages 'vegan' (Oksanen *et al.* 2008) and 'MASS' (Venables & Ripley 2002) of the statistical environment R (R Development Core Team 2007).

RESULTS

The 10 samples contained 10767 individuals and 142 morphospecies. The NMDS ordination of the samples was effective in the reduction of the data dimensionality (stress 0.07) (Fig. 2). The largest stream sites (8, 9 and 10) received high scores in the second axis of the ordination.

The BioEnv analyses indicated that the best correlation was obtained with the dissimilarity matrix built using conductivity, number of stream links and stream order (r = 0.62, Tab. II). Number of stream links and stream order are correlated (r = 0.71) and thus are mostly redundant. In fact, the model including conductivity and number of stream links produced similar correlation to that including the three variables (r = 0.59). The model including conductivity only resulted in low correlation (r = 0.46), indicating that stream size was important. In fact, the NMDS ordination of the samples assigned high scores in the second axis for the largest stream sites (8, 9 and 10) (Fig. 2). The high importance of conductivity is due mostly to the high values observed in site 3 and, to a lesser extent, in site 5 (Tab. I). In the NMDS ordination these two sites received the highest scores in the first axis.

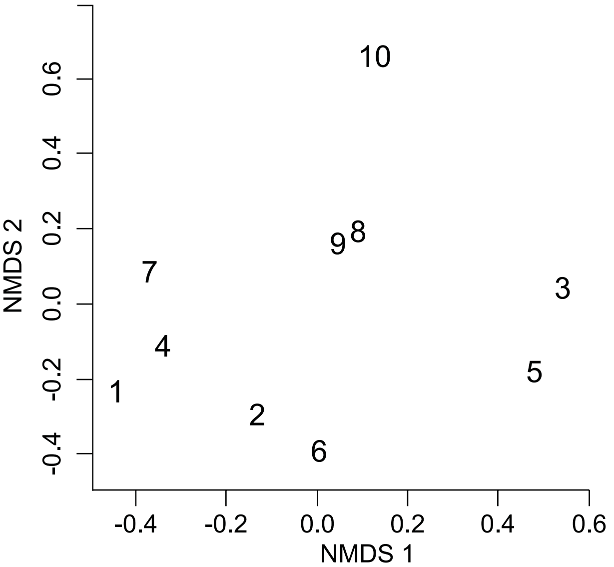

Figure 2. Non-Metric Multidimensional Scaling ordination of the 10 stream sites. Number refers to stream sites shown in figure 1. Stress = 0.07.

Table I. Physical characteristics of the 10 sampling sites in Parque Estadual Intervales. [a] Number of first order streams included in the subcatchment; [b] Obtained using a Presto-Tek (California, USA) conductivimeter.

Stream sites	Stream order	Link magnitude [a]	Width (m)	Discharge (m^3/s)	Canopy cover (%)	Altitude m)	Condutictivity (µs/cm) [b]
1	1	1	1.0	0.005	100	680	51
2	1	1	2.5	0.010	100	540	33
3	2	3	2.5	0.030	94	800	180
4	2	2	3.5	0.059	100	680	48
5	3	11	2.5	0.137	98	530	63
6	3	6	4.0	0.092	98	700	45
7	4	15	6.0	0.238	90	650	40
8	4	43	10.0	0.657	94	520	30
9	4	36	10.0	0.750	84	400	36
10	5	175	21.0	2.729	72	200	75

Table II. Results of the BioEnv analysis for samples collected in 10 stream sites in the Parque Estadual Intervales. (alt) Altitude, (cond) conductivity, (cover) canopy cover, (flow) discharge, (links) link magnitude, (order) stream order, (width) stream width. Units of variables can be found in table I. Correlation for the best model shown in bold.

Model size	Model	Correlation
1	cond	0.4570
2	cond + links	0.5887
3	cond + links + order	0.6190
4	cond + links + order + width	0.6030
5	cond + links + order + width + flow	0.5906
6	cond + links + order + width + flow + alt	0.5657
7	cond + links + order + width + flow + alt + cover	0.5149

DISCUSSION

The results agree with previous works that indicated the importance of stream size and conductivity for the macroinvertebrate fauna (Froehlich & Oliveira 1997, Strieder 2002). For instance, Vannote *et al.* (1980) predicted a continuous change in relative importance of functional feeding groups along a stream size gradient (Baptista *et al.* 1998b). Soldner *et al.* (2004) evaluated the effects of a range of environmental variables on macroinvertebrate fauna in 26 streams in the Dominican Republic. They found that altitude (a proxy for temperature and stream size), percentage of sand/silt and average chemical rank (a metric composed of conductivity, hardness, NH_4, NO_3, PO_4 and dissolved oxygen) were among the most important factors, but stated that such findings may be confounded by human impacts in lowland sites. In Brazil, Baptista *et al.* (2001) studied the macroinvertebrate fauna in 10 stream sites (orders 1-6) in Macaé River catchment and found that conductivity and stream size were among the most important environmental variables structuring assemblages.

The largest stream site (site 10, order 5) presented a relatively distinct assemblage and was scored far from the remaining stream sites in the NMDS ordination. This was the single 5th order stream in the study and thus only weak generalizations are possible. However, previous studies have also shown an abrupt change in macroinvertebrates assemblages among 4th and 5th order streams. For instance, Hynes (1971) presented a similar result in table I of his zonation study in a Neotropical stream in Trinidad, West Indies. Similarly, Minshall & Robinson (1998) found abrupt changes in community structure among large (> 4th order) and small streams. Baptista *et al.* (2001) showed that 5th order sites presented distinct communities and apparently this was not a result from human activities. Perry & Schaeffer (1987) presented evidence of serial discontinuities in stream assemblages. Statzner & Higler (1986) suggested that abrupt change in stream assemblages should likely reflect changes in stream hydraulics. Although specific measures of stream hydraulics are not available, the suggestion of Statzner & Higler (1986) can only in part explain the observed pattern because the sampled site is similar to the 4th order sites in terms of slope and substrate size and composition. Differences in relation to 4th order sites are restricted to discharge, width and longer pools. These scattered evidences suggest that this abrupt change in community structure between 4th and 5th order streams is not due to local idiosyncrasies, but a spread pattern. This topic deserves further investigation, not only to test the strength of the pattern but also its generating causes (Statzner & Higler 1986).

Despite the high correlation observed among differences in conductivity and dissimilarities in stream assemblages, care should be taken in the interpretation of this result. The high relative importance of conductivity was mostly due to the high value observed in stream site 3, which receives water from a calcareous cave. This site presented a relatively distinct community and all site pairs including this stream produced high dissimilarities. The observed result is thus based mostly in one stream site, which provides weak evidence for a strong effect of conductivity. However, this weak evidence is corroborated by the study of Baptista *et al.* (2001), who found that conductivity was among the best environmental factors structuring stream macroinvertebrate assemblages in the Macaé River. A similar case is cited in Allan (1995: 38).

The finding that distinct macroinvertebrate assemblages are present in streams differing in size and conductivity in the same catchment highlights the importance of conservation strategies aimed at the whole drainage basin. However, the current conservation status of the Atlantic Rain Forest poses a challenge for implementation of acts based on such approach.

The Atlantic Rain Forest has long been subjected to intense clearance for human activities (Dean 1997). Currently, only 5% of its original area was not destroyed. Most of the remaining Atlantic Rain Forest is constituted by small fragments and large areas are restricted to steep terrain (Oliveira-Filho & Fontes 2000). Accordingly, few of these fragments are large enough to harbor whole catchments including small- or medium-sized rivers (> 4th order). This implies the urgency of restoration programs in areas adjacent to Atlantic Rain Forest fragments.

ACKNOWLEDGEMENTS

José Flóido, Elias Mota, Benedito Amaral, Eliseu Cordeiro, Faustino Ribeiro, Luiz Ribeiro, Fábio Vanini, Anders Silva, Kátia Pisciotta, and the Intervales staff provided helpful assistance and facilities during field work. Field work was facilitated by a student fellowship from CAPES. I was supported by grants from CNPq (proccess 476304/2007-5) and International Foundation for Science (A/4107-1) during the preparation of the manuscript. This work is part of the BIOTA/FAPESP – The Biodiversity Virtual Institute Program (www.biota.org.br).

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Submitted: 15.VII.2008; Accepted: 09.III.2009.
Editorial responsibility: Paulo Lana