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How seasonality and anthropogenic impacts can modulate the structure of aquatic benthic invertebrate assemblages

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Abstract: We studied a benthic invertebrate assemblage of a stream that passes through pristine, rural, suburban and urban areas of a municipality located in southeastern Brazil to investigate a possible relationship between this assemblage structure and urbanization. The environmental variables and fauna structure were analyzed in a spatial and temporal scale, sampling the four sites in a dry and wet season. We found a clear spatial pattern, with higher similarity between sites from rural and suburban area that presented intermediate environmental characteristics. The pristine site showed in both seasons the lowest values of alkalinity and fecal coliform. On the other hand, the site located in the urban area showed the lowest concentration of dissolved oxygen and higher of suspended solids, ammonia and fecal coliform. The extreme values of these three variables occurred in the wet season, probably related to the high rainfall values. The benthic invertebrate fauna structure followed the same longitudinal and seasonal pattern found for the environmental variables. The site in urban area showed the lowest richness, diversity and evenness, with a dominance of two groups resistant to adverse environmental conditions (Oligochaeta and Orthocladiinae) and absence of more sensitive groups (Coleoptera, Ephemeroptera and Trichoptera). The increase drag of the substrate and associated invertebrates can be responsible for the lower abundance and richness observed in the wet season. The environmental variables that best defined the differentiation between sites (dissolved oxygen, organic suspended solids and fecal coliform) related directly to urbanization effects, like dump effluents and removal of riparian vegetation.

Nomenclature: Domínguez and Fernandez (2009).

Abbreviations: acar–Acarina, amer–*Americabaetis*, ANOSIM–ANalysis Of SIMilarity, Anne–Annelida, argi–*Argia*, baet–*Baetodes*, came–*Camelobaetidius*, Chel–Chelicerata, chir–Chironominae, clad–Cladocera, Cnid–Cnidaria, Cole–Coleoptera, Coll–Collembola, cope–Copepoda, Crus–Crustacea, Dipt–Diptera, Ephe–Ephemeroptera, hage–*Hagenulopsis*, hete–*Heterelmis*, Hete–Heteroptera, Hexa–Hexapoda, hiru–Hirudinea, macr–*Macrelmis*, Mega–Megaloptera, metri–*Metrichia*, Moll–Mollusca, Nema–Nematoda, Neme–Nemertea, neoe–*Neoelmis*, neot–*Neotrichia*, NMDS–Non-metric Multi-Dimensional Scaling, Odon–Odonata, olig–Oligochaeta, orto–Orthocladiinae, OTU–Operational Taxonomic Unit, Plat–Plathyhelminthes, Plec–Plecoptera, psep–*Psephenus*, SIMPER–SIMilarity PERcentages, simu–Simuliidae, smic–*Smicridea*, tany–Tanypodinae, thra–*Thraulodes*, trav–*Traverhyphes*, Tric–Trichoptera, worm–*Wormaldia*.

Introduction

In recent decades, rapid population growth and urban expansion also promoted the increase and diversification in the uses of water, resulting in a multiplicity of negative impacts on water resources. As a result, there is a significant drop in water quality and loss of aquatic biodiversity, due to changes in physical and chemical characteristics of the environment and natural dynamics of biological communities (Goulart and Callisto 2003, Carpenter et al. 2011).

This scenario is also found in Brazil, as presented by Agostinho et al. (2005) in a review of the conservation status of the biodiversity of Brazil´s inland waters. The authors emphasized that the insufficient number of studies, researchers and infrastructure required for sampling, combined with the dispersal of existing information, also contributed to the fact that this diversity remains poorly known.

The pollution of aquatic systems results from diffuse sources in the watershed through land use activities, as well as from direct discharge of pollutants, such as industrial and domestic sewage dump (Carpenter et al. 2011). In southeastern Brazil, the principal threats to freshwater biodiversity, especially in regions with high human population density, such as São Paulo State, is due to large urban areas, agriculture and industry (Agostinho et al. 2005).

The environmental degradation of rivers and streams is also commonly associated with the removal or modification of riparian vegetation. According to Barrella et al. (2001), this vegetation contributes to the structural preservation of aquatic habitats by preventing erosion of the adjacent soil and maintain the stability of banks. This vegetation also acts as a filter in the land-water transition zone by regulating the discharge and water flow, and thereby controlling the entry of sediment (Barrella et al. 2001). Over time, the input of sediment in riverbeds promotes homogenization and reduces the availability of habitats, altering the structure of aquatic assemblages (Watzen 2006, Vasconcelos and Mello 2008). The presence of riparian vegetation, even in urban streams, can help to mitigate some of the impacts of urbanization, such as loss of biodiversity (Moore and Palmer 2005) and maintenance of ecosystem function (Yule et al. 2015).

Regarding seasonality, the increased discharge and flow in the wet season can influence the benthic invertebrate fauna, directly by causing their displacement or indirectly through changes in the substrate, which can lead to changes in food availability (Allan 1995).

The study of the ecological integrity of ecosystems may use the response of the organisms to changes in the environment where they live, which can be of anthropogenic or natural origin (Buss et al. 2003). This use of biological variables in monitoring programs enables a wide and integrated characterization of the effects of various disorders, providing data on the environmental conditions over time (Iliopoulou-Georgudaki et al. 2003, Cortezzi et al. 2009). Among the aquatic communities, invertebrates are good indicators of environmental conditions (Callisto et al. 2001), and so are widely used in environmental impact assessments and biological monitoring (Goulart and Callisto 2003, CETESB 2013). Unfortunately, in Brazil the monitoring and implementation of management actions are often inadequate and conducted for a short period of time (Agostinho et al. 2005).

The aim of this study was to investigate the effect of anthropogenic impacts and seasonality on the structure of benthic invertebrate assemblages. Physical changes in the environment, like high flow and discharge during the wet season, are supposed to act strongly on the structure of the fauna by dragging the animals and their potential refuges. On the other hand, chemical changes should have more impact in the dry season due to lower dilution and higher concentration of substances derived from the urban area.

Studies that evaluate the effect of anthropogenic impacts due to urbanization near the freshwater bodies can provide data to support scientific and governmental actions aimed at the recovery and conservation of this essential good for human life as the quality of drinking water. The area chosen for the development of the present study represents well the situation found in most municipalities of small to medium size, in which the main stressors of urbanization are disposals of domestic and animal origin. Not only the area, but also the choice of methodology allows that sanitary agents of the municipality use the results obtained and implement future measures to control and mitigate the effects of degradation.

Materials and methods

Study area

The Ribeirão Grande stream is one of the tributaries of the left bank of the Guareí River that belongs to Paranapanema River basin, and is located in the municipality of Angatuba, in São Paulo State, southeastern Brazil (Fig. 1). We selected four sampling sites along the stream (Fig. 2), one of 1st order (S1) and the others of 3rd order (S2, S3 and S4), all with substrate composed predominantly by cobble and pebble in the runs and sand in the pools, but differing in relation to the characteristics of marginal vegetation and anthropogenic impacts.

Site 1 (23º29'38,9"S; 48º20'8,4"W) has preserved riparian vegetation and no obvious human disturbances. Site 2 is located in the rural area (23º29'24,5"S; 48º21'52,6"W); it has a pasture with livestock in the left margin and bamboo grove in the right bank. Site 3 is inserted in the suburban area (23º29'20,6"S; 48º23'48,6"W) and has riparian vegetation partially preserved and organic material dump originated from domestic sewage and from livestock located near the river bank. Site 4 is located in the urban area, after the municipal sewage treatment plant (23º28'44,6"S; 48º25'37,4"W); it presents marginal vegetation composed mainly by scrub and *Eucalyptus* and water with change in color and strong odor.

Sampling and data analysis

We collected data of environmental variables and of benthic invertebrates in run mesohabitats in each of the four sites studied along the Ribeirão Grande stream in September

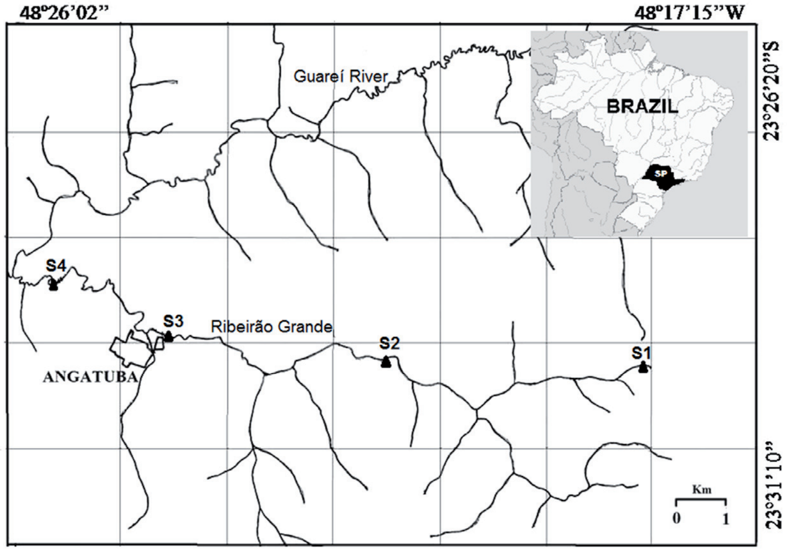

Figure 1. Location of the four sites sampled in Ribeirão Grande stream, located in the municipality of Angatuba, in São Paulo State (SP), southeastern Brazil: S1–pristine area, S2–rural area, S3–suburban area, S4–urban area.

Figure 2. Overview of the four sites studied in Ribeirão Grande Stream: site 1 (A), site 2 (B), site 3 (C), site 4 (D).

20, 2013 (dry season) and January 22, 2014 (wet season). Although September showed a total rainfall above 100 mm, the date of collection on this month was preceded by two months with low rainfall (July and August) and the most rain recorded in September was concentrated between September 22 and 29. For January, the precipitation recorded was 170 mm.

The water variables measured in the field with a Technical Kit for potability analysis (Alfakit, Cod 1065) were chloride, hardness, alkalinity, ammonia, chlorine, iron and fecal coliform. We chose this kit rather than more sophisticated laboratory tools because it is cheaper, easier to handle and is recommended for quality control of water for human consumption by sanitary agents of municipalities.

Additionally, we collected water samples for analysis of pH, suspended solids (organic and inorganic) and concentration of dissolved oxygen (Winkler method modified by the addition of azide, Golterman et al. 1978). To characterize the habitat physical structure, the surface current velocity was measured by the method of floating objects driven by the current (Schwoerbel 1975), with the average speed obtained by correcting the surface current velocity in function of the substrate granulometry (Leopold and Souza 1979). The discharge was determined using the method proposed by Leopoldo and Souza (1979), and the width and depth means were determined on each site in upstream and downstream transects.

Invertebrates were collected with Surber sampler (mesh 250 µm, 900 cm^2), in a total of ten replicates per site and season, and packed in containers with 70% alcohol until the screening in the laboratory. The animals were identified to the lowest possible taxonomic level, using general keys (Pennak 1978, Lopretto and Tell 1995, Merritt and Cummins 1996, Domínguez and Fernandez 2009), supplemented by specific literature for the identification of insects.

The abundance data of total OTU's (operational taxonomic units) was analyzed for assemblage differences between sites and seasons (Primer v.6, Clarke and Gorley 2006). Exploratory multivariate analyses were applied to abundance data (log (x+1) transformed and using Bray-Curtis similarity coefficient) including: a) a hierarchical classification technique (using the group average linkage option) for the grouping of samples, b) an ordination technique (NMDS - non-metric multi-dimensional scaling) representing the samples as points in a two-dimensional space.

An analysis of similarity (ANOSIM) was applied to a resemblance matrix constructed with the ten replicates of each site and season (data log(x+1) transformed, Bray-Curtis similarity coefficient), using a two-way crossed layout (Primer v.6). The null hypothesis of no differences is rejected based on Global R value (no differences with values equal or near 1), at a significance level of 1%. When the global test suggests that there are differences, the program presents a pairwise test to verify which site comparison shows the major differences. Also, in this case a similarity percentage routine (SIMPER) is performed to interpret such differences and the role of individual OTU's in contributing to the closeness of samples within a group (percentage of similarity on each site and season) and to the separation between two groups of samples (percentage of dissimilarity between sites and seasons).

The ANOSIM and SIMPER analyses were also applied considering only the OTU's with relative abundance >1% (OTU's represented by less than 1% of relative abundance in all sites/seasons were considered rare) to verify if the high percentage of rare species found could influence the results of spatial and seasonal comparisons.

The abundance values of all identified OTU's were used to plot dominance curves (or ranked species abundance plots) for the four sites in each season (Primer v.6). These curves display evenness and richness assemblage components, with species ranked in decreasing order of abundance (x-axis on a log scale).

To verify the species–environment relationship in the separation of sample sites, we made a Canonical Correspondence Analysis (software PAST), using the environmental variables data (Table A1 in Appendix) and the absolute abundance only of non-rare OTU´s (relative abundance >1%, Table A2 in Appendix). Environmental and benthic fauna data were transformed (log(x+1)), and a stepwise forward selection (software CANOCO) was used to reduce the environmental variables to those most correlated with the axes. A cutoff point of p = 0.05 was used to incorporate variables into the final models. To test for the significance of an axis, a Monte Carlo permutation test was made considering 9999 permutations and significance level of 5% (Hope 1968).

Results

The total OTU's sampled ranges from 20 (D4) to 40 (W2), with a high percentage of rare groups (with relative abundance <1%) (Table A2 in Appendix).

Among the sampled invertebrates, two groups stood out in abundance in all sites and seasons, Arthropoda-Hexapoda as the most dominant, followed by Annelida-Oligochaeta. A dominance of Oligochaeta occurred only in both seasons of site 4 (Fig. 3). Comparing the percentage of these two groups per season, it is evident that similarity is larger between sites during the wet season (Fig. 3).

When the abundance of hexapod orders were analyzed (Fig. 4), there were spatial and seasonal differences. In dry season, Diptera had the highest abundance in most sites, except for D1 where Ephemeroptera showed higher percentage (Fig. 4). On the other hand, in the wet season four orders showed extreme abundance, except for W4 where Diptera predominated with 90% of abundance (Fig. 4).

The cluster analysis used to compare the abundance of all OTU's per site (Fig. 5) highlighted higher similarity between sites 2 and 3 (70%) and lower similarity of site 4 with the remaining (45%). Sites 2 and 3 showed a seasonal distinction, with the dominance of Diptera-Chironomidae (Chironominae) and Diptera-Simuliidae in the dry season and of Annelida-Oligochaeta and Trichoptera-Hydropsychidae (*Smicridea*) in the wet season (Table A2). For site 4, the two seasons were joined with 60% of similarity, having in com-

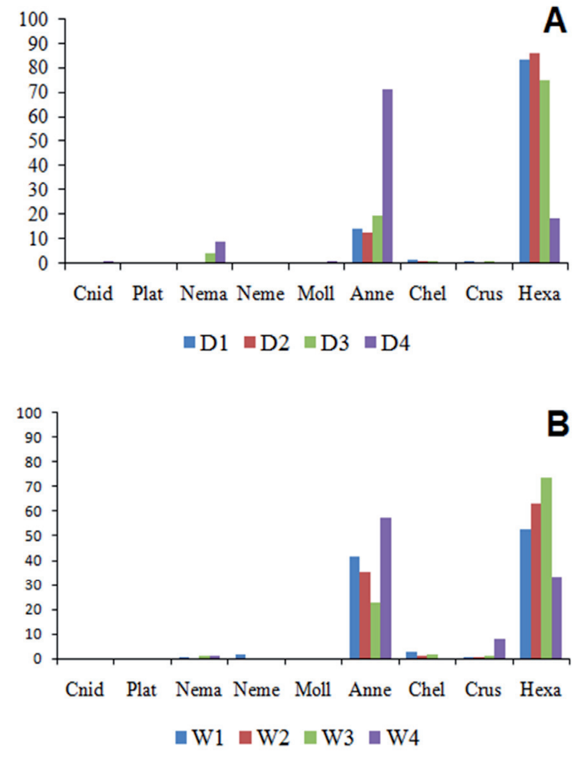

Figure 3. Relative abundance of invertebrates sampled in four sites of Ribeirão Grande stream, in the dry (A) and wet (B) season.

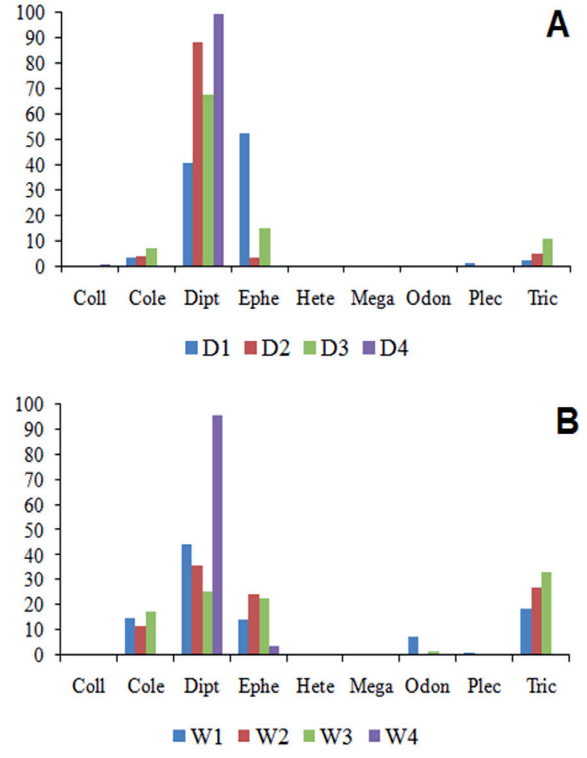

Figure 4. Relative abundance of Hexapoda orders sampled in four sites of Ribeirão Grande stream, in the dry (A) and wet (B) season.

mon a dominance of Annelida-Oligochaeta (>50% of relative abundance).

The analysis of total abundance of OTU's by a NMDS ordination confirmed a higher similarity between sites 2 and 3 sampled in both seasons, the isolation of site 4 (with a distinct separation of seasons), and a dispersion of site 1 replicates, although well delimited from the other sites (Fig. 6).

The species dominance curve (Fig. 7) highlights the results obtained in the Cluster and NMDS analysis, demonstrating the dominance and low evenness in site 4 due to the high abundance of Annelida-Oligochaeta.

The results of the analysis of similarity (ANOSIM) indicate significant differences on the absolute abundance of invertebrates sampled in the four sites and two seasons, considering the total operational taxonomic units (OTU's) sampled and only the ones with relative abundance >1% (Table 1).

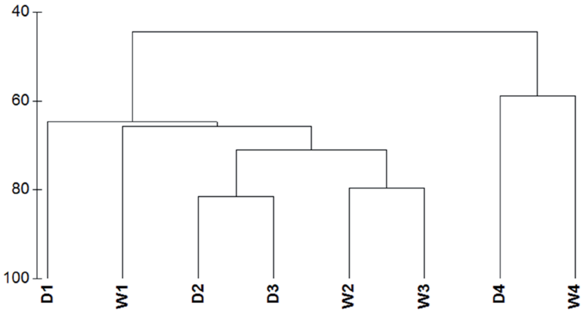

Figure 5. Results of the hierarchical cluster analysis (sum of 10 replicates per site/season) applied to the abundance data of 65 invertebrate OTU's sampled in four sites of Ribeirão Grande stream, in the dry (D) and wet (W) season.

The analysis of seasons shows that the percentage of dissimilarity was lower than 50% (Table 1), although significantly different, what can explain the high values of similarity between seasons found in the exploratory multivariate analysis (cluster and NMDS). The groups with the highest percentage of contribution to seasonal dissimilarity (SIMPER results) were Simuliidae and Chironominae, most abundant in sites 2 and 3 during the dry season.

For the factor site, the pairwise test indicates a greater differentiation between sites 1 and 4 and lower between sites 2 and 3, although yet significantly different (Table 1), in agreement with the results of exploratory multivariate analysis (clustering and NMDS). The groups with the highest percentage of contribution to the dissimilarity between sites 1 and 4 (SIMPER results) were Nematoda, Oligochaeta and Orthocladiinae, most abundant in site 4.

The stepwise approach of CCA selected only dissolved oxygen, organic suspended solids and fecal coliform as the environmental variables that most correlated with the axes (p = 0.001). For CCA analysis (Fig. 8), only the first axis was significant (p = 0.038) explaining 83.4% of the variance. On the positive quadrant of this axis, site 4 separated due to the highest concentration of organic suspended solids and fecal coliform and to the lowest concentration of dissolved oxygen. Five invertebrate groups related better to site 4 (Oligochaeta, Nematoda, Hirudinea, Cladocera and Copepoda), where they were most abundant. The other sites were located in the negative quadrant of this axis, site 1 mainly related to the greater abundance of *Baetodes* and sites 2 and 3 with the greater abundance of Chironominae and Simuliidae (Fig. 8, Table A2).

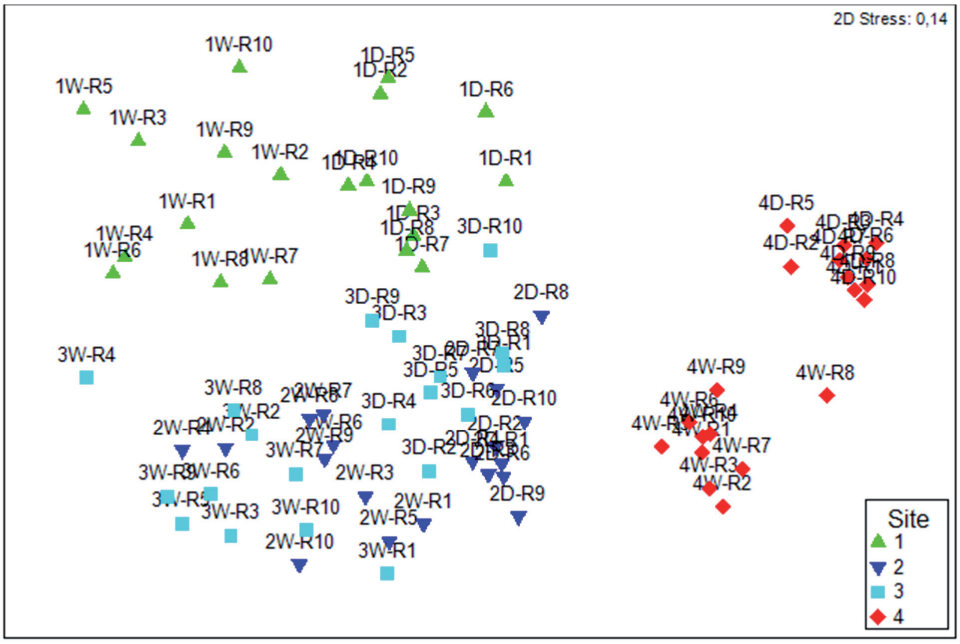

Figure 6. Results of non-metric multi-dimensional scaling (NMDS) applied to the abundance data of 65 invertebrate OTU's sampled in four sites of Ribeirão Grande stream, in the dry (D) and wet (W) season, with the plot of all replicates per site and season (R1 to R10).

Table 1. Results of ANOSIM and SIMPER tests applied to verify the existence of significant differences on the absolute abundance of invertebrates sampled in four sites and two seasons of Ribeirão Grande stream, considering the total taxonomic operational units (OTU's) sampled and only the ones with relative abundance >1%. Values of R near or equal to 1 indicate significant differences between the factors analyzed (seasons and sites).

TESTS	Total (65 OTU's)	RA>1% 26 OTU's)
ANOSIM – between seasons		
Global R	0.849	0.826
SIMPER – between seasons (across sites)		
Average similarity - dry	74.60%	78.69%
Average similarity - wet	69.56%	72.95%
Average dissimilarity (dry x wet)	44.50%	39.36%
ANOSIM – between sites		
Global R	0.885	0.891
Pairwise test R sites 1,2	0.909	0.914
Pairwise test R sites 1,3	0.843	0.846
Pairwise test R sites 1,4	1	1
Pairwise test R sites 2,3	0.390	0.416
Pairwise test R sites 2,4	0.999	0.999
Pairwise test R sites 3,4	0.994	0.992
SIMPER – between sites (across seasons)		
Average similarity – S1	66.53%	71.10%
Average similarity – S2	71.93%	75.8%
Average similarity – S3	65.72%	69.61%
Average similarity – S4	84.12%	86.74%
Average dissimilarity sites 1,2	48.93%	45.81%
Average dissimilarity sites 1,3	47.58%	43.67%
Average dissimilarity sites 1,4	65.12%	61.65%
Average dissimilarity sites 2,3	36.70%	33.28%
Average dissimilarity sites 2,4	56.24%	52.36%
Average dissimilarity sites 3,4	59.94%	55.61%

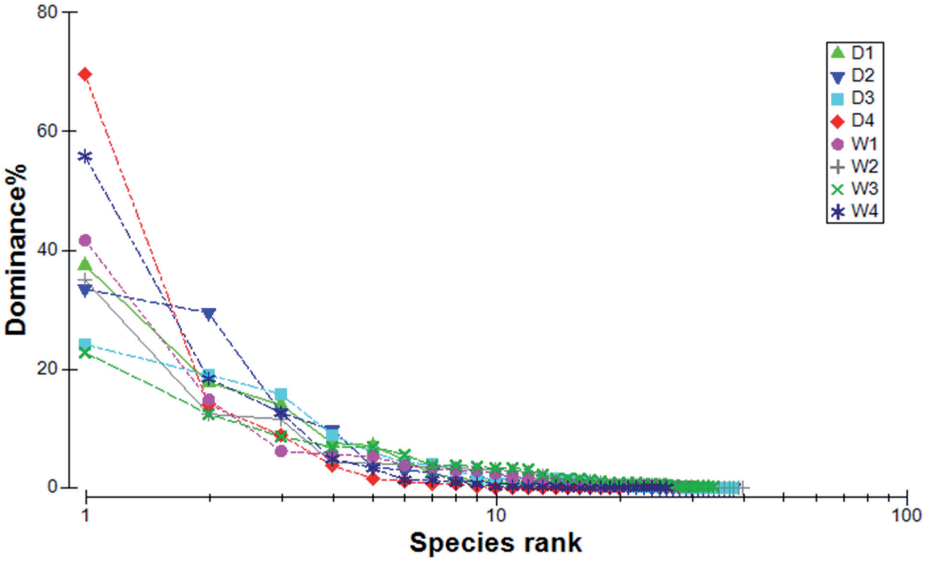

Figure 7. Curves of species dominance plotted with the invertebrate OTU's sampled in four sites of Ribeirão Grande stream, in the dry (D) and wet (W) season, ranked in decreasing order of abundance.

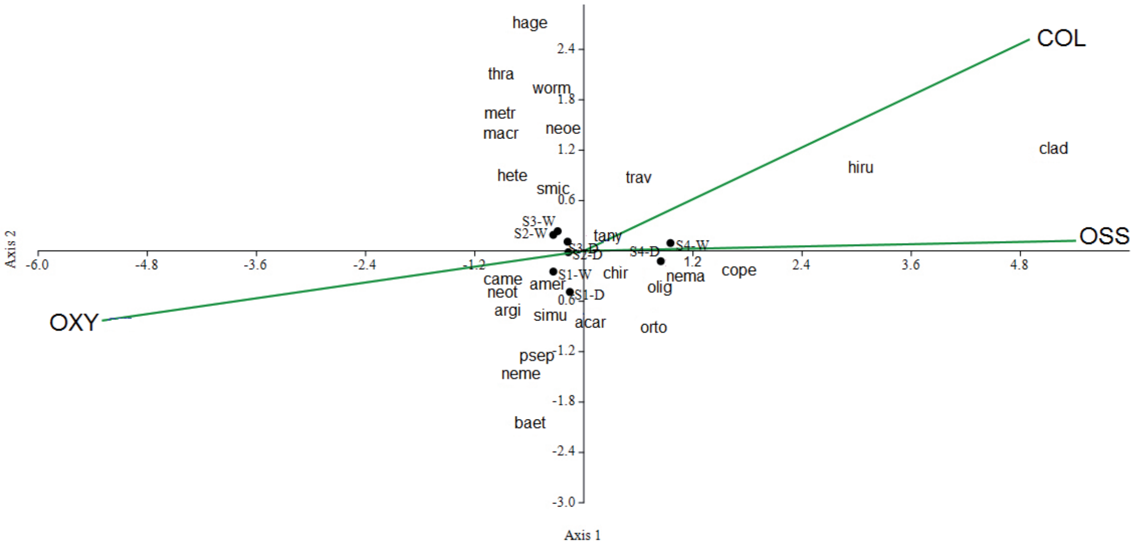

Figure 8. Canonical Correspondence Analysis (CCA) showing the distribution of the four sites (S1, S2, S3 and S4), sampled during the dry (D) and wet (W) seasons, and of the fauna along the first two axes. Environmental variables most correlated with the axis are OXY–dissolved oxygen, OSS–organic suspended solids, COL–fecal coliform.

Discussion

The environmental characterization showed a clear spatial pattern, with greater similarity between sites 2 and 3, probably because they exhibit intermediate environmental characteristics when compared to sites 1 and 4 with extreme conditions. In both seasons, site 1 differentiated by its smaller size (lower values of depth and flow) and pristine environmental conditions, highlighted by the low alkalinity and fecal coliform values. On the other hand, site 4 was related mainly to the lower concentration of dissolved oxygen and the higher of suspended solids, ammonia and fecal coliform. The release of domestic sewage in this segment can be related with more extreme values for these variables found during the wet season, probably due to the increase in water volume during the month of heavy rains. In general, urban streams receive a large amount of rubbish, generated by inadequate garbage-collection service, illegal dumping of domestic waste directly into the rivers, common in our cities and also in much of southeastern Asia, as highlighted by Yule et al. (2015).

The benthic invertebrate fauna structure followed the same pattern of differentiation between sites. Site 1, located farthest from the urban area, presented the lowest total abundance probably due to its smaller size. The ecological indices also highlighted the differentiation of site 4 severely impacted by the disposal of urban waste, with the lowest richness, diversity and evenness and the highest abundance in the two seasons, the latter related to the dominance of Annelida–Oligochaeta and Chironomidae-Orthocladiinae. The predominance of those two invertebrate groups indicates a great resistance to adverse environmental conditions, especially when the low level of dissolved oxygen was considered (Azrina et al. 2006). The absence of aquatic insect representatives of Coleoptera, Ephemeroptera and Trichoptera orders also contributed to the differentiation of site 4. These groups are usually described as sensitive to environmental disturbances (Hepp et al. 2013) and are commonly associated with high diverse habitats, as well as high concentration of dissolved oxygen in the water (Goulart and Callisto 2003, Souto et al. 2011).

The low values of abundance and richness in wet season can be the result of heavy rains and the consequent increase in the drag of the substrate and associated invertebrates in this season. Moreover, this drag is related by some authors to a rearrangement on the fauna structure (Yokoyama et al. 2012), which may be the cause of Diptera dominance reduction and of a uniform distribution in abundance of four orders of insects in sites 1 to 3. Site 4 presented the lowest seasonal similarity and the best discrimination between seasons in the NMDS ordination, probably related to differences in the value of absolute abundance of Annelida-Oligochaeta and Diptera-Chironomidae, twice in the dry season.

The greatest abundance and richness of benthic invertebrates during the dry season has been pointed out by several authors (Uieda and Gajardo 1996, Kikuchi and Uieda 2005, Bispo et al. 2006, Mesa et al. 2009, Ríos-Touma et al. 2011, Yokoyama et al. 2012). However, no evidence of seasonal influence in the benthos structure can also occur, as found by Melo and Froehlich (2001), who considered that once the benthic fauna is adapted to seasonal variations, only drastic events will alter its structure.

The three environmental variables that best defined the differentiation between sites are directly related to urbanization, like dump effluents and removal of riparian vegetation. This vegetation can protect the water bodies from sediment input for promoting greater stability of the surrounding soil and retaining some of the material dragged into the channel (Gomi et al. 2006). The large amount of compounds of organic origin commonly associated with the disposal of sewage promotes the reduction of dissolved oxygen level in the water due to their high consumption during biological oxidation processes (CETESB 2013).

The use of fecal coliforms in the classification of bathing water bodies is extremely important as it indicates the possible existence of pathogens that can cause some diseases (Tonon et al. 2013). In addition, the concentration of fecal coliforms is an important variable for the classification of aquatic systems into quality classes, with the worst class found in site 4, identified as class III in the dry season and IV in the wet and considered unsuitable for bathing in both periods (see Brasil, CONAMA n^0 274). The low environmental quality observed in site 4 may be indicative of the inefficiency of the sewage treatment system, since even though located after the municipal station this site showed alarming levels of coliforms, as well as very low oxygen values.

Cortezzi et al. (2009), analyzing the benthos of Brazilian Midwestern streams crossing protected, agricultural and urban areas, found low species richness in localities highly impacted by human activities, while other points subject to different intensities of impacts presented similar richness. We found similar results: the structure of benthic fauna was similar in sites with similar environmental characteristics and intermediate anthropogenic impacts (sites 2 and 3), with seasonal variation being the main factor responsible for the dissimilarities found. On the other hand, in sites 1 and 4 with extreme environmental conditions we observed low seasonal influence on the fauna, with the spatial differences contributing more significantly to the differentiation found.

The problems caused by urbanization on stream ecosystems are accentuated in developing regions located in the tropics by problems related to culture, infrastructure and governmental actions (Agostinho et al. 2005, Yule et al. 2015). Unfortunately, little attention has been given to the ecological effects of urbanization on tropical rivers and streams (Yule et al. 2015); also, few studies had been carried out in Brazilian urban streams, whether analyzing only the water quality (Cunha et al. 2011, Oliveira et al. 2014) or the structure of the benthic fauna (Cortezzi et al. 2009, Souto et al. 2011). More studies of urban stream ecology are vital for a better understanding of the processes involved and for implementation of better management actions.

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Electronic appendix

Table A1. Mean and standard deviation of 15 abiotic variables measured in four sites of Ribeirão Grande stream in the dry and wet season.

Table A2. Relative abundance of the invertebrates sampled in four sites of Ribeirão Grande stream in the dry and wet season.

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