



BENTHIC MACROINVERTEBRATES AS BIOINDICATORS OF ENVIRONMENTAL QUALITY IN THREE STREAMS OF THE AMAMBAI RIVER BASIN, UPPER PARANÁ RIVER, BRAZIL

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Abstract: The objective of this study was to evaluate the environmental integrity of three streams (along 13 sampling sites) of the Upper Paraná River Basin using the benthic macroinvertebrate fauna as bioindicators and to answer the following question: Is there spatial variation, related to environmental quality, in the composition of these taxa in the streams? The invertebrates were sampled with a surber collector and for habitat analysis were determined characteristics as stream depth and width, water velocity, dissolved oxygen, pH, temperature, turbidity, and electrical conductivity. The environmental variables were analyzed by Principal Component Analysis in order to synthesize the data and identify the most important variables for ordering the collection sites. For biological analysis, the BMWP index was used. A Canonical Correlation Analysis of the environmental and biotic data was also performed in order to identify correlations between these two sets of variables. The collected macroinvertebrates belonged to four phyla (Nematoda, Annelida, Mollusca and Arthropoda), with Arthropoda being the most representative (54.93%), with the predominance of the order Diptera (30.29%). The most sensitive orders (Ephemeroptera, Plecoptera and Trichoptera) occurred in four sampling sites, corresponding to less impacted ones. Tolerant organisms, mainly chironomids, were found in all streams, including the most impacted places. These results indicate a spatial variation in the composition of the macroinvertebrates taxa in the streams, in response to variation of environmental quality.

Keywords: biomonitoring; BMWP Index; environmental integrity.

INTRODUCTION

Anthropic activities cause changes throughout the environment in which they occur. In aquatic ecosystems, these changes reduce the quality of water and, consequently, the biodiversity present (Goulart & Callisto 2003). In addition, as pointed out by Flor & Souto (2016), many water bodies pass through urban landscapes and receive domestic

and industrial effluents, which disrupt the quality of aquatic ecosystems.

Biological integrity is directly affected by water quality, and the use of aquatic invertebrates is an important ecological tool for assessing the characteristics of aquatic ecosystems (Mugnai *et al.* 2010). Several biological evaluation techniques have been used to quantify the influence of anthropic activities on the biotic conditions of water resources.

One of them is biomonitoring, which is based on the assumption that biological components respond to environmental degradation by altering their structural and functional characteristics (Gafny *et al.* 2000). Bioindicators are widely used and their application has resulted in the improvement of quality standards for industrial companies through the development of specific legislation for the control of pollution (Silveira *et al.* 2004). According to Silveira *et al.* (2004), the use of benthic macroinvertebrates is advantageous to physical and chemical evaluation of water bodies because they are sensitive to several types of pollutants and physical disturbances.

Among the various benthic macroinvertebrate taxa, the orders of insects considered most sensitive to changes in the aquatic environment, and for this reason used in many studies as bioindicators of good water quality, are Ephemeroptera, Plecoptera and Trichoptera, together referred to as EPT (Mugnai *et al.* 2010). Among the aquatic macrofauna, the role of these orders applies not only to their occurrence in aquatic ecosystems, but also to their abundance and contribution to species diversity in preserved streams (Callisto *et al.* 2001). On the other hand, Diptera (mainly Chironomidae) and worms (Oligochaeta, Hirudinea and Nematoda) are more tolerant to environmental degradation and are considered bioindicators of polluted waters (Bis & Kosmala 2010, Mugnai *et al.* 2010, Trivinho-Strixino 2014, Bem *et al.* 2015, Flor & Souto 2016).

In Paraná River Basin, several studies were conducted in order to understand the influence of environmental predictors over the community of macroinvertebrates (Barbola *et al.* 2011, Souza *et al.* 2014, Bem *et al.* 2015, Schiller *et al.* 2017). However, despite their importance, there are no studies focusing the relation between spatial distribution of benthic macroinvertebrates and environmental quality in rivers or streams of the Amambai River Basin, Upper Rio Paraná Basin. In this context, the present study evaluate the environmental quality of three streams of this basin located in the municipality of Naviraí, Mato Grosso do Sul state, Brazil, using benthic macroinvertebrates as bioindicators of environmental quality, and to answer the following question: Is there spatial variation in the composition of these taxa in the streams related to environmental quality?

MATERIAL AND METHODS

Study area

The present study was performed in the municipality of Naviraí, state of Mato Grosso do Sul, Center-West Brazil (23°03'54" S, 54°11'26" W), with an area of approximately 3,194 km² and a population of 52,367 inhabitants (IBGE 2016). The region presents tropical climate (Cfa), with average temperatures between 12°C and 28°C. The predominant vegetation cover consists of the Cerrado biome, but also contains transition forests of Atlantic Forest. The soil is predominantly dark red latosols (IMASUL 2011).

Sampling was carried out in December 2015 in the streams Tarumã, Touro and Cumandaí, of the Amambai River Basin, Upper Paraná River. In general, these three streams can be characterized by having a moderate current velocity (about 0.6 m/s), shallow depth (between 1 and 1.5m), pH between 5 and 7 and temperature ranging between 24 and 30°C. A total of thirteen sampling sites were defined along the streams (Figure 1) from which three samples of biological material were obtained (on the right and left margins and in the center of the watercourse) from each site. The sampling sites were chosen according to the different levels of environmental integrity along the tributaries.

The sampled sites of Tarumã stream are within an anthropic landscape with the absence of native gallery forest and the predominance of herbaceous vegetation comprising C4 plants (Cyperaceae, Poaceae). The three sampling sites upstream the confluence with Touro stream are located in the least impacted places; the three downstream this confluence receives residues from the urban area, which makes them more impacted (Figure 1).

Touro stream is highly anthropized, with numerous sandbanks, domestic sewage discharge, and remnant of sparse ciliary forest. The predominant vegetation consists of opportunistic species of degraded environments with fast development (*Cyperus compressus* L., *Melinis minutiflora* P. Beauv., *Ricinus communis* L.).

The Cumandaí stream passes through degraded areas with organic pollution, extensive erosion, sedimentation and sediment flow. The vegetation comprises shrubby trees established in

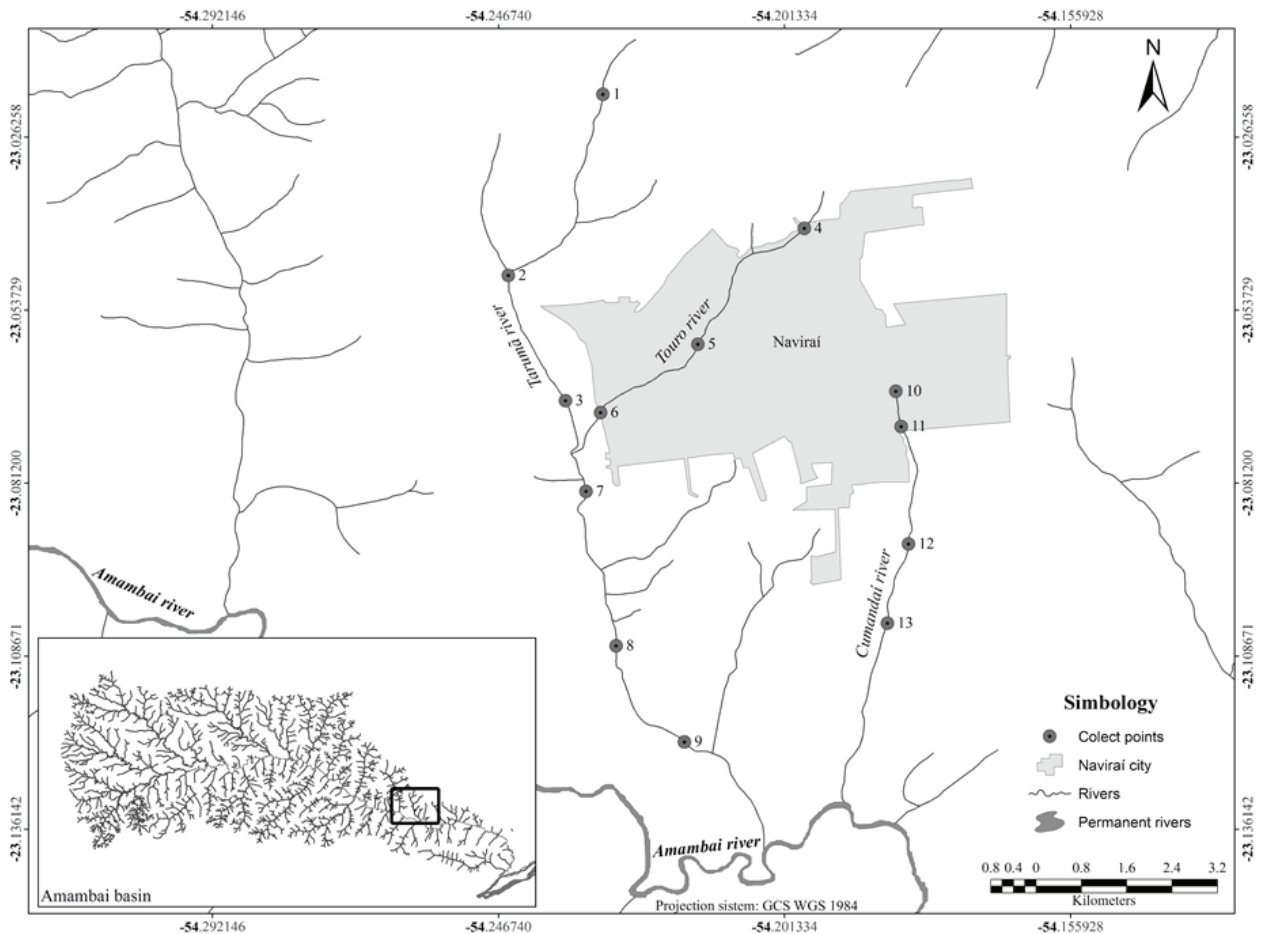


Figure 1. Location of the sampling sites in Tarumã, Touro and Cumandaí streams, Amambai River Basin, Upper Paraná River, Brazil.

deeper soil, with a predominance of opportunistic species indicative of degraded environments, formerly cited.

Data collection and analysis

A set of limnological variables was measured in the field (in the center of the watercourse) with a multiparameter probe Horiba U53®: conductivity ($\mu\text{S cm}^{-1}$), dissolved oxygen (mg L^{-1}), temperature ($^{\circ}\text{C}$), turbidity (NTU) and pH. Stream depth (m) and width (m) were measured with a measuring tape (in triplicate) and the water velocity (m s^{-1}), also in triplicate, was measured with a Global Water Fluxometer FP 101. For analysis of the environmental parameters, a Principal Component Analysis (PCA) – with the data of the physical-chemical variables and the data averages for depth, width and velocity – was performed with the basic purpose of synthesizing data, eliminating overlap between variables and allowing the selection of the most representative

form of the data from linear combinations of the original variables (Manly & Alberto 2017). In this way, the ordering of sites indicated which ones were most impacted and which places were less impacted.

A surber sampler (900cm^2 of area and mesh of $250\mu\text{m}$) was used to sample the macroinvertebrates. The collector was placed upstream so that the material revolved with a spoon – was concentrated in the collecting mesh. The material obtained was packed in plastic bags and fixed with 4% formalin.

In the laboratory, the collected biological material was stained with rose bengal (12mg L^{-1}) to facilitate visualization of the macroinvertebrates. Subsequently, the material was washed, screened and identified under a stereoscopic magnifying glass. The identification of the orders and families was made using Silveira (2004), Mugnai *et al.* (2010) and Hamada *et al.* (2014).

After identification, the biotic index BMWP

(Biological Monitoring Working Party), modified and adapted by the Environmental Institute of Paraná State (IAP 2003), was calculated for each site. This is a qualitative index that considers only the presence/absence of benthic macroinvertebrate families. For each family listed in the index is assigned a value of 1 to 10, according to their tolerance or sensitivity to organic pollutants, with 1 being the most tolerant to impact and 10 the most sensitive.

A Canonical Correlation Analysis (CCA) was performed with the abiotic and biotic data with the objective of finding a linear combination between the two sets of variables so that the correlation between them is maximized. For the set of environmental variables, the scores of the first two main components obtained in the Principal Component Analysis were used in order to eliminate any redundancy existing among the original variables, according to one of the assumptions of CCA (Manly & Alberto 2017). For the biotic data was used the abundance of the biological groups that occurred in at least three sampled sites. The statistical analyses (PCA and CCA) were performed with Statistica 13.2 software (Tibco 2017).

RESULTS

The first two axes of the PCA explained 62.55% of the total variance of the environmental data (Table 1). The highest correlations of the first principal component (PC1) were for the variables conductivity, pH and width, all with positive correlations. For the second principal component (PC2) the variables O₂ (positive correlation) and depth (negative) were significant (Table 2). The sites 5, 6, 7, 8, 9 and 13 showed higher values of conductivity (besides pH and width), forming a group of more impacted sites on the right side of the first axis. Regarding to PC2, sites 1, 2, 3, 8 and 9 present lower values of O₂ and higher values of depth. This axis, however, explained about half of the variability explained by PC1 (Figure 2).

A total of 3,031 individuals were collected, distributed among four phyla, with Arthropoda being the most abundant (Table 3). The class Insecta had the greatest representation, mainly Diptera (30.29% of the total number of individuals) and Ephemeroptera (16.20%). Among Diptera, the family Chironomidae was the most abundant, being present at all the sampling sites (Table 3). The class Oligochaeta (phylum Annelida) represented 28.93% of the individuals collected and was absent only at sampling site 7 (Table 3).

Table 1. Environmental data recorded in the sampling sites in Touro, Tarumã and Cumandaí streams, Upper Paraná River Basin, Brazil.

Sites	O ₂ (mg L ⁻¹)	Turbidity (NUT)	Conductivity (µS cm ⁻¹)	pH	Temperature (°C)	Width (m)	Depth (m)	Velocity (m s ⁻¹)
1	5.46	28	14	5.46	26.87	2.0	1.15	0.48
2	5.51	23	18	4.96	27.86	2.0	2.53	0.48
3	6.37	540	18	6.03	27.76	2.0	2.58	0.66
4	6.09	124	54	5.88	24.20	1.0	0.99	0.29
5	6.66	216	134	6.37	25.64	5.0	0.43	0.81
6	6.10	223	171	7.37	28.64	10.0	0.65	0.58
7	6.31	122	79	6.78	30.25	7.0	0.37	0.92
8	3.41	201	251	7.20	28.00	7.0	1.45	0.87
9	4.62	241	215	7.02	27.80	3.5	1.30	0.65
10	7.66	258	96	6.61	25.38	2.0	0.35	0.20
11	7.31	0	85	6.28	25.86	2.0	0.80	0.63
12	6.48	146	68	6.09	24.00	2.0	1.15	0.67
13	7.13	898	56	6.71	26.16	2.5	0.93	0.96

The BMWP biotic index calculated for water quality classification based on the taxa sampled indicated that most sites were in bad conditions (Figure 3). For Tarumã stream, the sites from its source to river mouth were classified from class IV (doubtful – moderate effect of pollution) to class VII (heavily polluted – strongly altered system). For Touro and Cumandaí streams, the index indicated class VI (very polluted) for the upstream sites and class VII (heavily polluted) for the downstream ones (Figure 3).

In CCA was used the abundance of the biological groups that occurred in at least three sampled sites: orders Diptera, Collembola, Coleoptera, Odonata, Ephemeroptera and Trichoptera of the class Insecta, as well as the

Table 2. Correlations between eight environmental variables and the first two principal components produced in the analysis of the variables from Upper Paraná River Basin, Brazil. Correlations modules greater than 0.75 (in bold) were considered significant.

Environmental variables	PC 1	PC 2
O ₂	-0.4662	0.7575
Turbidity	0.1258	0.3622
Conductivity	0.8309	0.0039
pH	0.8636	0.3999
Temperature	0.6206	-0.4577
Width	0.8907	-0.0084
Depth	-0.3039	-0.7893
Velocity	0.6050	0.0174

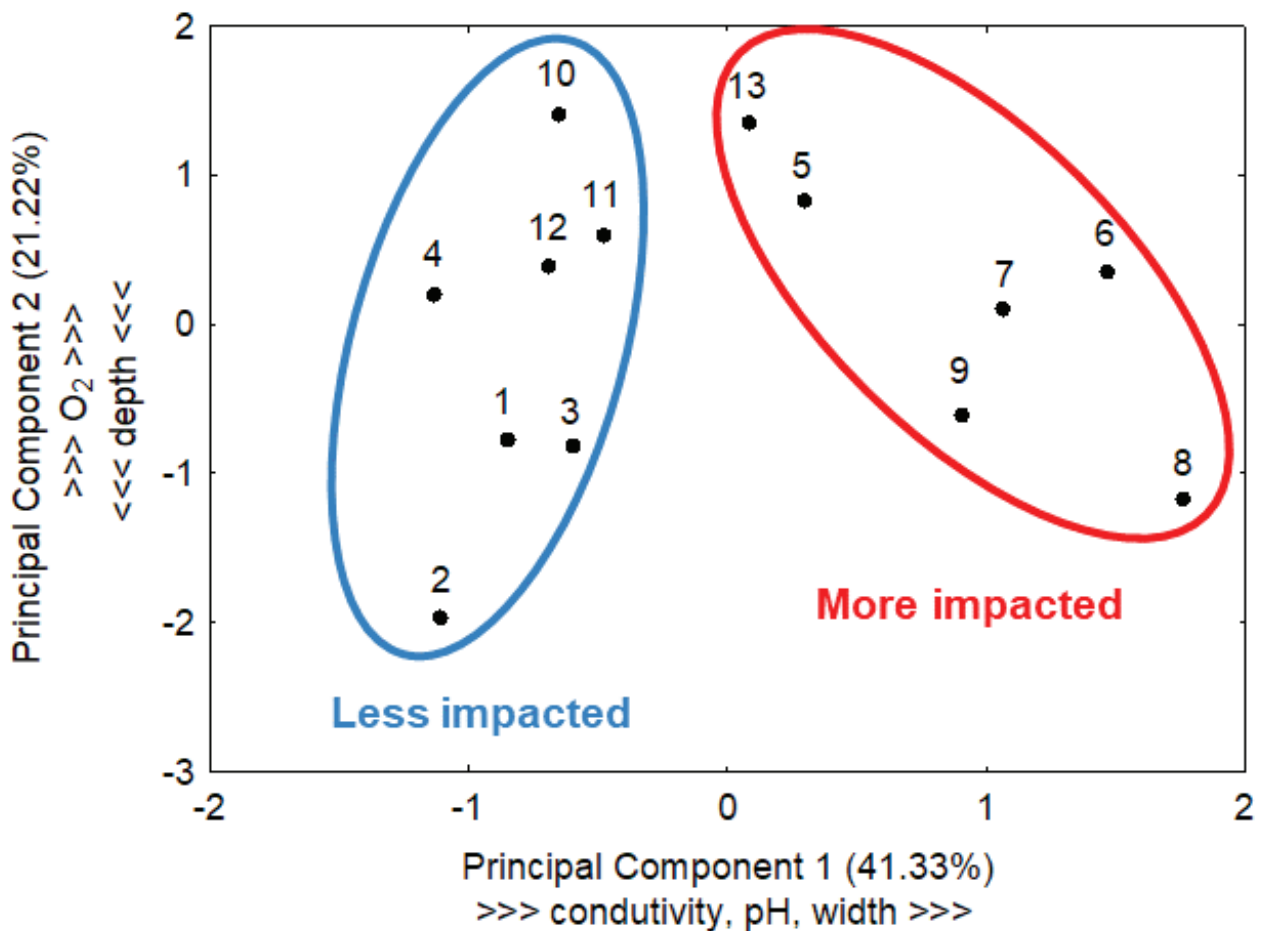


Figure 2. Distribution of the scores of each sampled site at the Upper Paraná River Basin, Brazil, in the plan formed by the first two principal components axis.

Table 3. Benthic invertebrates sampled in Touro, Tarumã and Cumandaí streams, Upper Paraná River Basin, Brazil, with absolute (N) and relative (%) abundance values and occurrence on the 13 sites sampled.

Phylum/Class	Order	Family	N	%	Occurrence
Arthropoda/Insecta	Diptera	Chironomidae	795	26.23	1 - 13
Arthropoda/Insecta	Diptera	Ceratopogonidae	11	0.36	1 - 3, 10 - 12
Arthropoda/Insecta	Diptera	Simuliidae	89	2.94	1 - 3, 5
Arthropoda/Insecta	Diptera	Empididae	6	0.20	1
Arthropoda/Insecta	Diptera	Tipulidae	3	0.10	1, 2
Arthropoda/Insecta	Diptera	Psychodidae	10	0.33	2, 6, 10, 11
Arthropoda/Insecta	Diptera	Stratiomidae	3	0.10	6, 10, 11
Arthropoda/Insecta	Diptera	Culicidae	1	0.03	7
Arthropoda/Insecta	Coleoptera	Elmidae	26	0.86	1, 2, 4, 10, 11
Arthropoda/Insecta	Lepdoptera	Pyralidae	6	0.20	1, 10
Arthropoda/Insecta	Hemiptera	Belostomatidae	1	0.03	1
Arthropoda/Insecta	Odonata	Dicteriadiidae	2	0.07	2
Arthropoda/Insecta	Odonata	Calopterigidae	14	0.46	2, 3
Arthropoda/Insecta	Odonata	Perilestidae	2	0.07	2
Arthropoda/Insecta	Odonata	Libelulidae	7	0.23	1, 2
Arthropoda/Insecta	Odonata	Aeshnidae	3	0.10	2
Arthropoda/Insecta	Ephemeroptera	Baetidae	443	14.62	1, 3, 4
Arthropoda/Insecta	Ephemeroptera	Leptohyphidae	28	0.92	1
Arthropoda/Insecta	Ephemeroptera	Leptophlebiidae	20	0.66	2, 4
Arthropoda/Insecta	Trichoptera	Hydropsychidae	16	0.53	1, 3, 4
Arthropoda/Insecta	Plecoptera	Perlidae	6	0.20	1, 3
Arthropoda/Insecta	Collembola		173	5.71	2, 3, 6, 8 - 13
Annelida/Oligochaeta			877	28.93	1 - 7, 9 - 13
Annelida/Hirudinea			231	7.62	2 - 5
Mollusca/Gastropoda			3	0.10	10
Mollusca/Bivalvia			218	7.19	2
Nematoda			37	1.22	2, 3, 9, 11, 13
Total	9	21	3031	100%	-

phyla Annelida and Nematoda. Only the first root showed a significant correlation between the abiotic and biotic variables (Canonical R = 0.98; df = 16; p = 0.02). The variables conductivity, pH and width (which comprise PC1) were positively correlated with the abundance of Diptera and Nematoda. The PC2 (positive for O₂ and negative for depth) was positively correlated with the abundance of Trichoptera (Figure 4).

DISCUSSION

The evaluation of the streams located in the surroundings of the urban perimeter of the

municipality of Naviraí, Mato Grosso do Sul state, Brazil, allowed to classify these sampled sites in less impacted and more impacted, since none of the sampling sites was pristine. The electrical conductivity of water is important for detecting pollution sources, enabling to check the direct and indirect influence of land use and activities developed in the hydrographic basins, as the discharge of effluents can be related to an increase on conductivity in the watercourse (Viana *et al.* 2013). In the studied streams, this variable was also important to separate the sites in “less impacted” and “more impacted”.

Insects of the order Diptera (manly

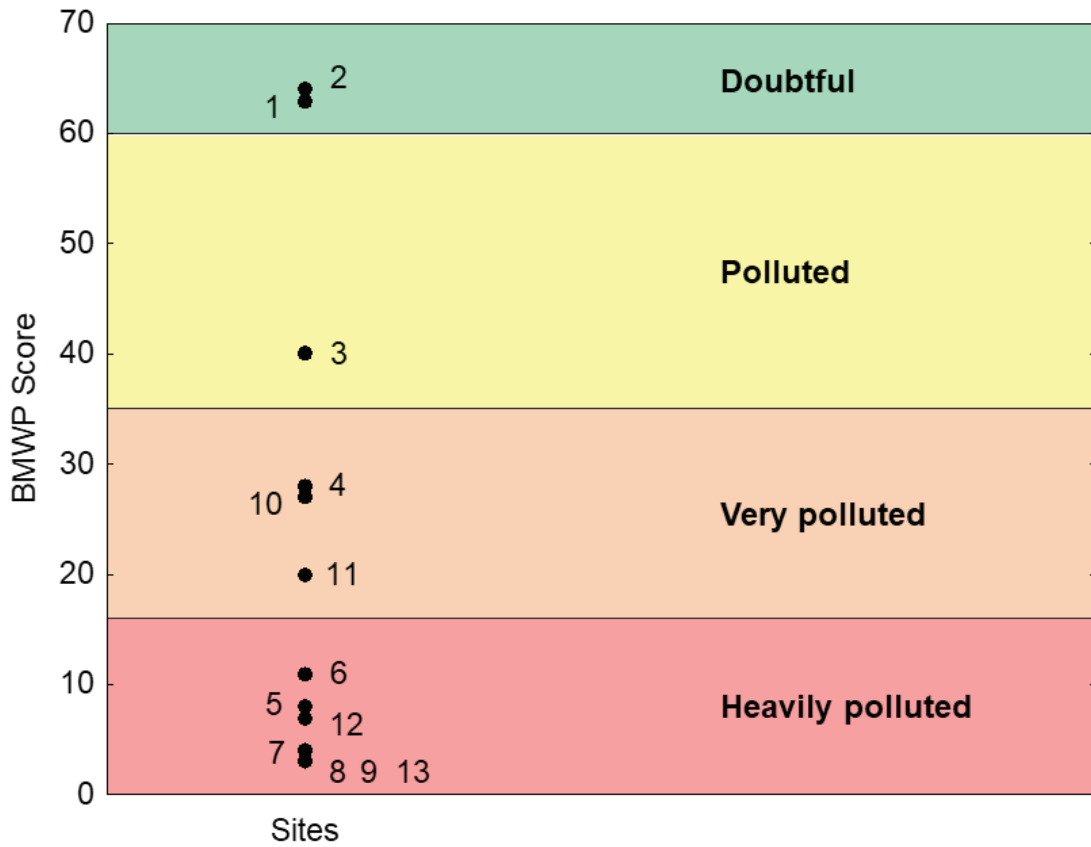


Figure 3. BMWP scores and category of quality for the sampling sites of Touro, Tarumã and Cumandaí streams, Upper Paraná River Basin, Brazil.

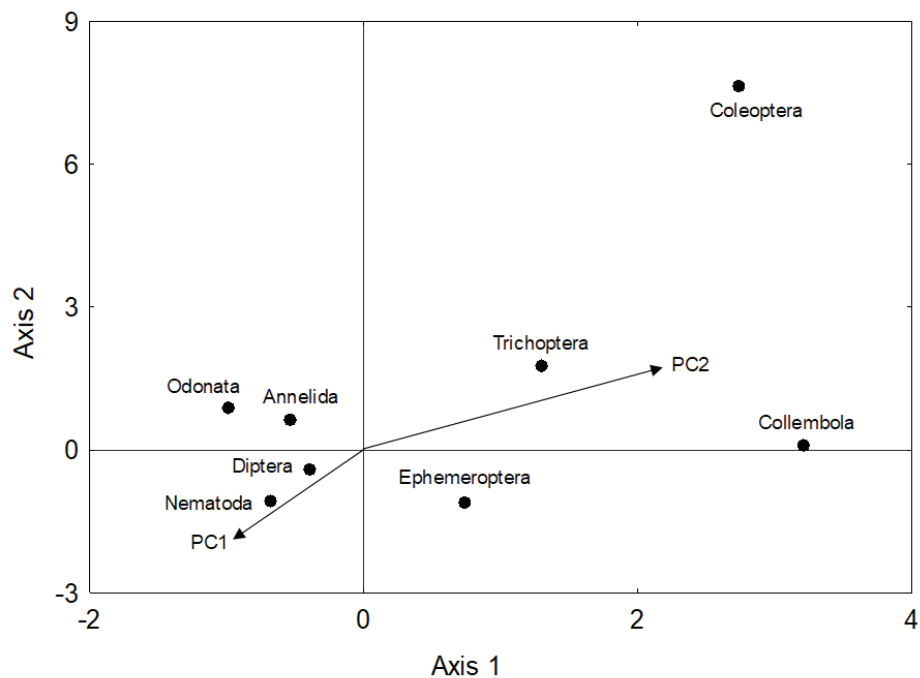


Figure 4. Scatterplot of Canonical Correspondence Analysis between the more representative taxa of benthic macroinvertebrates and the principal components obtained from the environmental data in the Upper Paraná River Basin, Brazil.

Chironomidae) and annelids of the class Oligochaeta were predominant at all the sampling sites. This pattern was expected since these groups are generally more tolerant to more disturbed aquatic ecosystems (Mugnai *et al.* 2010). Queiroz *et al.* (2008), Bem *et al.* (2015) and Flor & Souto (2016) also found these groups to be dominant in altered environments. The occurrence of chironomids at all of the sampling sites may be related to their feeding, since most consume fine particulate organic matter (Kikuchi & Uieda 2005), which is an abundant food resource in environments impacted by domestic effluents. In addition, Diptera larvae occupy all types of aquatic environments due to several mechanisms used for respiration including gills, aerial or a combination of both (Sterz *et al.* 2011). According to Trivinho-Strixino (2014), these larvae are apneustic, and can breathe oxygen dissolved in the water through the surface of the body because they have hemoglobin that allows them to store more oxygen, thus allowing them to inhabit environments with low oxygen concentrations.

Annelids of the classes Oligochaeta and Hirudinea also have resistance to changes in the aquatic environment and are able to survive in environments with low oxygen concentration and feed on organic matter, algae and bacteria that grow in these places (Bis & Kosmala 2010). Mugnai *et al.* (2010) found that Hirudinea is abundant in streams polluted with domestic waste, but with running water that provides oxygenation. In fact, although the sites where Hirudinea was found (sites 2, 3, 4 and 5) are classified in different categories by the BMWP index, all these sites presented dissolved oxygen concentrations considered satisfactory to biota, between 5.5 and 6.7 mg L⁻¹. The class Oligochaeta occurs where there is moderate current, shallow depth and higher concentration of organic matter (Carvalho & Uieda 2004), conditions found on the majority of sites sampled, which explains the high representation of this class in our study.

Ephemeroptera, Plecoptera and Trichoptera, together referred as EPT, are the orders of insects considered most sensitive to changes in the aquatic environment (Mugnai *et al.* 2010). In our samplings, we observed low representation of these groups, corroborating the fact that all of the sampling sites have some degree of environmental impact. It was observed that the sampling sites where EPT (Baetidae, Leptohyphidae, Leptophlebiidae,

Hydropsychidae and Perlidae) were found are located in Tarumã upstream to its confluence with Touro (sites 1, 2 and 3) and at the source of Touro (site 4), before the stream crosses the urban region. These sites are less impacted and have conditions favoring the establishment of these groups, although they are also moderately degraded. Therefore, these groups were effective at differentiating moderate from strongly altered aquatic environments.

Among EPT, Ephemeroptera was the most frequent, proving to be a dominant group among the other communities of sensitive invertebrates. This fact may be related to the characteristics of the less impacted sampling sites (headwaters and flooded low land associated with gallery forest) since, according to Copatti *et al.* (2010), this group is dominant in rock substrate, because it is composed of scraper organisms, filters and collectors. Plecoptera was found only at sampling sites 1 and 3, represented by the family Perlidae, and its abundance was positively correlated with high concentration of oxygen, characteristic of less impacted sites. The low abundance of Plecoptera can be related to the fact that the studied streams have their courses near and within the urban zone, limiting the occurrence of these individuals in greater abundance since they have preference for clean flowing water. Trichoptera also exhibited relatively low abundance since they also live preferentially in well-oxygenated environments, with clean flowing water (Mugnai *et al.* 2010).

In a study on the Correntoso River, in the Pantanal of Mato Grosso do Sul, Silva *et al.* (2011) evaluated the community structure of aquatic insects that are indicators of environmental quality using three biotic indicators (BMWP, BMWP-ASP and IBF), and found that the index BMWP was the one that best represented the studied environmental conditions.

In our results, the sampling sites classified as doubtful quality by the application of the BMWP index were locations where organisms sensitive to changes in the aquatic environment were found, as well as groups tolerant to environmental degradation. The locations classified as polluted to heavily polluted, in turn, are environments in which only tolerant groups were found. In fact, the results obtained in ordering the sites by the environmental variables analysis in a PCA were very close to the classification obtained by the application of the

BMWP Index. The only divergence was in relation to site 12, which was grouped to the less impacted sites by the environmental variables and was classified as heavily polluted by BMWP Index. These results demonstrate that, in the system studied, the BMWP Index proved to be efficient in discriminating the most impacted and least impacted places.

In conclusion, there was a spatial variation in the composition of macroinvertebrates in the sampled streams in response to variation of environmental quality. The groups indicative of good environmental quality, such as Ephemeroptera, Plecoptera and Trichoptera, exhibited relatively low abundances and only occurred on less impacted sites. Tolerant organisms, mainly chironomids, were found in high abundance and distributed in all streams, including the most impacted sites.

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