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Multimetric index for assessing ecological condition of running waters in the upper reaches of the Piabanha-Paquequer-Preto Basin, Rio de Janeiro, Brazil

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ABSTRACT. The objective of this study was to develop a rapid multimetric index using benthic macrofauna as bioindicators of ecological conditions for the upper reaches of the Piabanha-Paquequer-Preto Basin located in the state of Rio de Janeiro, Brazil. A total of 33698 macroinvertebrates were collected in 27 sites. Benthic macroinvertebrates were sampled using the multi-habitat method that consists in sampling 20 m^2 of substrate collected in approximate proportion to the representation of all major habitat types in the reach. A subsampling procedure was used. The main steps followed to develop and test the index were: exclusion of unsuitable metrics using box-whisker plots, considering the degree of overlapping among interquartile limits (1°-3°) and confirmed by Mann-Whitney U test ($p \leqslant 0.05$) using six reference rivers and six impaired rivers. From all the candidate potential metrics, 36 were considered suitable. After identifying redundant metrics, through Spearman correlation analysis, and considering practical application criteria, six core metrics were selected to compose the Piabanha-Paquequer-Preto Multimetric Index (PPPMI) using the continuous method: Family richness, Shannon-Wiener family diversity, EPT family richness, %Diptera, %MOLD (Mollusca + Diptera) and %Collectors. The sensitivity of the index was tested in 15 rivers using a Principal Components Analysis (PCA) of the six environmental variables. The first axis of the PCA was highly correlated to the PPPMI scores ($r = 0.703$, $p < 0.001$). The PPPMI responded to a set of environmental variables associated to a gradient of human disturbance affecting the ecological condition of the waterbodies. This indicates that the PPPMI is an effective tool for biological monitoring and decision making in the hydrographic region of the Piabanha-Paquequer-Preto rivers.

KEY WORDS. Biomonitoring; conservation; river ecosystems; macroinvertebrates.

Beginning in the 1970's, several countries around the world began to defend a policy which stated that the primary objective of surface water assessments should be to understand their ecological condition. This policy was based on the idea that water quality should not be measured only through its physical-chemical attributes, but also through measurements that indicate the effects that non-natural disturbances have over the biological structure and ecosystem function of aquatic systems.

The ecosystems under study should have the appropriate conditions to maintain their aquatic populations. Therefore the aquatic biota is the fundamental objective of biological assessments (Barbour *et al.* 1999). In general, biological assessments are based on the comparison of attributes (composition, structure, function and richness/diversity) of the biological communities found in healthy rivers, also known as reference or minimally impaired rivers, with those present in impaired rivers (Chessman *et al.* 2006).

The tools most commonly used for biological monitoring programs are Indexes of Biotic Integrity (or Multimetric Indexes) and Predictive Models. Both approaches use biological assemblages of diverse taxonomic groups. The most frequently used groups are macroinvertebrates, fish and algae (Griffith *et al.* 2005). The logic behind the indexes is to use a set of reaches from reference rivers as a pattern, in order to compare their ecological condition. When the difference between the reference communities and the communities being assessed is high, the ecological condition of the sites under evaluation is considered impaired. Karr & Dudley (1981) argue that biotic integrity represents "The ability to support and maintain a balanced, integrated adaptive assemblage of organisms having species composition, diversity, and functional organization comparable of that of natural habitat of the region".

Several developed countries, such as United States of America, Canada, Australia and New Zealand have invested on the development of models and biological indicators (Plafkin *et al.* 1989, Wright 1995, Reynoldson *et al.* 1995, Barbour *et al.* 1996, 1999, Bonada *et al.* 2006). Likewise, the European Union (European Commission 2000) pioneered two projects: AQEM and STAR, meant to develop multimetric indexes based on benthic macroinvertebrate fauna (Buffagni *et al.* 2004, Böhmer *et al.* 2004, Hering *et al.* 2004, Pinto *et al.* 2004, Vlek *et al.* 2004, Furse *et al.* 2006).

In the last two decades, multimetric indexes built to assess the impact of anthropogenic disturbances on fauna have used different spatial scales to estimate specific or multiple impacts. In the USA, many studies found an important relationship between macroinvertebrate fauna and macro scale environmental conditions (ecoregions) (Barbour *et al.* 1996, Karr & Chu 1997, Waite *et al.* 2000, Mykrä *et al.* 2004, Frimpong *et al.* 2005, Munn *et al.* 2009). McCormick *et al.* (2001) created one index for multiple ecoregions. Other studies suggest, however, that it might be necessary to create different indexes of biotic integrity for bioregions located within a single ecoregion (Fore *et al.* 1994, Maxted *et al.* 2000). The review of the first national assessment of stream ecosystems in the USA presented by Paulsen *et al.* (2008) indicates that it is feasable to use multimetric indexes in large scale monitoring programs in order to assess the ecological condition of rivers.

In Brazil, monitoring programs of aquatic ecosystems carried out by public authorities usually are restricted to physical-chemical analyses of water (Braga *et al.* 2002). These evaluations might be unsuitable since they most likely do not assess the ecological condition of surface waters. However, some Brazilian research institutions have recently developed biological monitoring tools in response to the extreme impairment of aquatic ecosystems in Brazil. Most of these initiatives have used benthic macroinvertebrates (Junqueira & Campos 1998, Junqueira *et al.* 2000, Baptista *et al.* 2007, Mugnai *et al.* 2008, Monteiro *et al.* 2008, Moreno *et al.* 2009).

In all regions of the Atlantic Rain Forest, aquatic ecosystems have suffered severe man-induced stress and are threatened by pollution and loss of riparian vegetation. This generates physical disturbances that directly impact the ecological condition of the aquatic ecosystems. The objective of this work was to develop a rapid index of biotic condition using the macroinvertebrate fauna for small and mid-sized rivers from the Piabanha-Paquequer-Preto hydrographic region following the methodologies used in Europe (Hering *et al.* 2006) and the USA (Stoddard *et al.* 2008).

MATERIAL AND METHODS

Study area

The region drained by the Piabanha, Paquequer and Preto rivers covers an area of 2075 km². Its population is estimated to be 590,000. The vegetation of the region is Atlantic Rain Forest. Its headwaters are located in the Serra dos Órgãos region, within the central portion of the Serra do Mar in the state of Rio de Janeiro. The region supplies water to 10 municipalities (Areal, Carmo, Paraíba do Sul, Paty do Alferes, Petrópolis, São José do Vale do Rio Preto, Sapucaia, Sumidouro, Três Rios and Teresópolis). The headwater streams are usually in steep slopes and include wadeable riffles and pools. The substrate size ranges from silt to large boulders. The lower reaches of the rivers, close to the Paraíba do Sul river, are non-wadeable.

The most preserved forested areas are located in the high mountainous zone of the basin. Within the valleys, in the mid-sized reaches located at altitudes below 850 m a.s.l, the land is used for agriculture and urban development, which increases water pollution caused by sewage discharge, riparian vegetation loss, physical habitat modification and nonpoint source pollution caused by use of pesticides and fertilizers (Moreira *et al.* 2002). The region includes several conservation units (Serra dos Órgãos National Park, Três Picos State Park, Araras Biological Reserve) that supply water to this hydrographic region (Bergallo *et al.* 2009).

Study sites and sampling design

The benthic macrofauna was sampled during the dry season (August 2007) in 27 sites from the Piabanha, Paquequer and Preto sub-basins (Fig. 1). This set of sites was selected according to three criteria: (i) minimally impaired areas (reference; eight rivers); (ii) fair or slightly impaired areas (intermediate; 10 rivers) and (iii) severely impaired areas (nine rivers). Sites were located at 800-1100 m a.s.l as no reference sites were found in lower areas. The headwaters of the hydrographic region are located within the Serra dos Órgãos (SO). The SO region covers an area of approximately 12,904 km², with 200 km² protected by the Serra dos Órgãos National Park (22°26'58"S, 42°59'08"W). The climate is tropical super humid, with an annual average temperature of 18°C and annual precipitation between 1250 and 1500 mm. To characterize the stream-typology used in this study, the criteria adopted include catchment area between 10-100 km² and granite geology.

An *a priori* classification of river conditions was carried out using an Index of Habitat Integrity (IHI) (Barbour *et al.* 1999). The percent of forested area upstream from the sampling locations was calculated using a digital map of the watershed. For the river basin, a site was considered "minimally impaired" when it met all of the following criteria: water pH 6-8; dissolved oxygen ⩾ 4 mg/l; maximum urbanized basin area of 20% and ⩾ 75% of forested upstream area; riparian vegetation width ⩾ 15 m; no visible channelization, and a Excellent or Very Good classification according to the IHI (Barbour *et al.* 1999). For the "impaired" condition, any of the following *a priori* conditions should be met: deforestation of the upstream area ⩾ 75%, and a Poor classification according to the IHI index.

Environmental and microbiological data

The following environmental variables were determined in the field, using a YSI550A analyzer: dissolved oxygen (DO), pH, electric conductivity. Other physical-chemical water variables were measured with a HATCH SR 2500 analyzer: total ammonia, nitrate, nitrite, chlorides, and total alkalinity. Water samples were preserved in sterile plastic bags (whirl-pak) and the analyses conducted through the Colibert-Hach method in order to measure fecal and total coliforms. The percentage of sand in the reach under study was based on the number of samples (m²) that contained sand out of the total sampled habitat area (20 m²) (Tab. I).

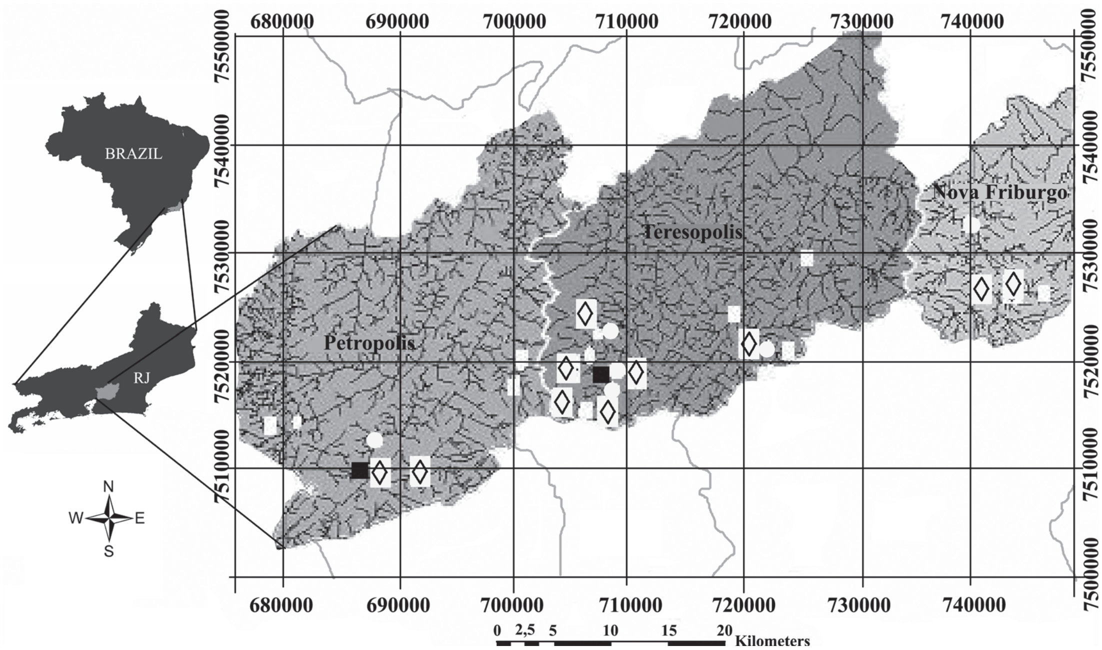

Figure 1. Map of Brazil and the study site in state of Rio de Janeiro. Light grey areas indicate limits of the Piabanha-Paquequer-Preto rivers watershed. White solid points indicate the macroinvertebrate assemblage sampling sites. Reference areas (white squares), impaired areas (circles) and intermediate areas (diamonds). Municipality (black squares).

Table I. Environmental and physical-chemical variables measured at sites in the Serra dos Órgãos region,Rio de Janeiro, Brazil (Median, SD). * Indicates variables used during the analysis of the environmental PCA for the sensibility test of the Piabanha-Paquequer-Preto Multimetric Index (PPPMI). All sites (reference, impaired and test) have the same stream typology: size classes (based on catchment area- 10 -100 km^2), geology of the catchment area constituted of granite and altitude between 700-1200 m a.s.l.

Environmental and physical-chemical variables	Sites used for PPPMI development		Sites used for PPPMI testing
	References (n = 06)	Impaired (n = 06)	Streams test (n = 15)
Water temperature (°C)	16.67 ± 1.55	21.00 ± 1.76	17.62 ± 1.58
pH	7.16 ± 0.63	7.15 ± 0.27	7.13 ± 0.26
Dissolved Oxygen (mg/l)	7.57 ± 1.20	2.95 ± 2.15	7.7 ± 1.6*
Conductivity (µS/cm)	15.49 ± 3.30	127.86 ± 69.90	40.0 ± 19.83
Nitrate + Nitrite (mg/l)	1.28 ± 0.67	2.37 ± 1.32	1.37 ± 0.22
Ammonian (mg/l)	0.02 ± 0.01	2.09 ± 0.60	0.90 ± 0.61
Chlorides (mg/l)	15.65 ± 2.97	21.13 ± 10.60	15.0 ± 5.0
Alkalinity total (mg/l)	39.27 ± 15.16	87.22 ± 88.84	34.1 ± 16.1*
Altitude (m a.s.l.)	968.3 ± 105.91	871.67 ± 68.82	974.55 ± 61.45
Stream order (max-min)	4 – 2	4 – 2	4 – 2
Mean width (m)	9.33 ± 3.03	7.58 ± 3.75	8.27 ± 3.85
Upstream area forested (%)	98.67 ± 1.97	2.50 ± 1.97	40 ± 25
Riparian width (m)	100.0 ± 0.00	2.17 ± 1.72	39.45 ± 7.02*
Sand (%)	12.50 ± 2.74	76.67 ± 22.29	40 ± 34*
Fecal Coliform (NCP)	21.00 ± 16.63	633.33 ± 280.48	441 ± 376*
IHI Index	16.74 ± 0.55	1.57 ± 1.72	10.3 ± 5.36*

Sampling, sorting and identification of organisms

Benthic fauna was sampled using a kick-net sampler (30 x 30 cm), with 500-micron mesh netting. Samples were obtained from a reach length of approximately 20 times the channel width. The multi-habitat approach was used for sampling, where the substrates were sampled in an approximate proportion to the representation of all major habitat types within the reach. In each reach, 20 substrate samples were taken and combined into one composite sample. Each sample represented 1 m^2 of substrate (Hering *et al.* 2003).

The samples were preserved in 80% ethanol and then sorted in the laboratory. After removal of coarse material, the remaining composite sample was mixed together and sub-sampled in a tray sub-divided in 24 units (10 x 10 cm), from which six units were randomly selected to compose a single sub-sample. The effectiveness of the sub-sampling process had been previously tested (Oliveira *et al.* 2011). The sub-sample was then sorted to remove all benthic macroinvertebrates, which were identified to genus level except for those only identified to family level, namely Diptera, Lepidoptera and Hemiptera. Other non-insect groups were identified to higher taxonomic levels. The specimens collected and identified were stored in the Laboratório de Avaliação e Promoção da Saúde Ambiental/FIOCRUZ.

Data analysis

The individual metrics must reflect the stress caused by specific impacts and the addition of all metrics composing the index should respond to cumulative disturbance (Karr & Chu 1999). Suter (1993) addresses a risk of biological information loss when using a multimetric method without doing a robust evaluation, calibration and statistical analysis. Accordingly, data was analyzed in three stages: (a) selection of the core metrics to compose the index; (b) building of the multimetric index and (c) test of the index.

Selection of core metrics

Twelve sites (six reference and six impaired sites) were used to select the core metrics for the multimetric index, where the following Richness and Diversity metrics were calculated: Total Richness; Dominance (D); Shannon Diversity (H); Simpson Diversity (1-D); Evenness (e^H/S); Menhinick Index; Margalef Index; Equitability (J Index); Fisher Alpha Index; Berger-Parker Index; Family Richness; Plecoptera Family Richness; Ephemeroptera Family Richness; Trichoptera Family Richness; EPT Family Richness; Shannon-Wiener Family Diversity; Margalef Family Diversity; Simpson Family Diversity; Plecoptera Genera Richness; Ephemeroptera Genera Richness; Trichoptera Genera Richness; EPT Genera Richness.

Likewise, the following Composition metrics (%Coleoptera; %Ephemeroptera; %Plecoptera; %Diptera; %Trichoptera; %Odonata; %EPT), Tolerance metrics (MOLD – Molusca + Diptera – richness; %MOLD; BMWP-CETEC; BMWP-ASPT; IBE-IOC; proportion Baetidae/Ephemeroptera; Hydropsychidae/Trichoptera; %Chironomidae), and Trophic metrics (%Scraper – periphyton consumers; %Shredder – leaf consumers; %Collector – feed on suspense and deposited fine particulate organic matter; %Predator; %Filterer – feed on drifting fine particulate organic matter – were also calculated.

The selection of core metrics for the multimetric index (MI) was conducted in four steps: 1) analyses of metrics that discriminate between "reference" sites and impaired sites was achieved using box-whisker plots, considering the degree of overlap among interquartile limits (1°-3°) along with the direction and intensity of the response as the impact increased; 2) statistical significance of metric values differences between reference and impaired sites using a Mann-Whitney U test (those metrics with p values ≤ 0.05 were considered suitable. This test was used to confirm the sensitivity of metrics to impairment); 3) identification of redundancy among suitable metrics using a Spearman correlation analysis; 4) selection of metrics considering key operational criteria to employ the index, e.g. coarse taxonomic resolution level required to apply the metric.

Multimetric Index construction and validation

The Continuous Scoring Method suggested by Frey (1977) was used to score or standardize the metrics values. Blocksom (2003) recommended it as the best option because it increases the sensitivity and stability of the resulting index. This method was originally used by Minns *et al.* (1994) in the littoral zone of the Great Lakes. Ganasan & Hughes (1998) and Klemm *et al.* (2003) used it in stream ecosystems. The scoring method uses the distribution of values from each set of reference or impaired rivers. The following descriptors were used: minimum and maximum values, and first and third quartile. The scoring procedure is completed using these distribution metrics and two formulae (see below), to determine whether metric values increase or decrease when degradation increases.

The procedure used to apply the index was achieved in five steps: 1) computing all six core metrics; 2) scoring the metrics using the formula (a) for metrics that decrease with increasing impairment and formula (b) for metrics that increase with increasing impairment; 3) applying a simple interpolation to adjust values to a range between 0-10 (negative values must be considered zero and the highest value must be considered 10); 4) multiplying the values of each one of the metrics times 1.6 (individual weights) in order to obtain values ranging from 0 to 100; 5) in this last step the values of each metric are summed to obtain a final score for the Piabanha-Paquequer-Preto Multimetric Index (PPPMI). This final PPPMI value that results from the application of the index in each test site falls into one of five quality categories, indicating the ecological integrity of the assessed location.

Formule (a)

$$\text{Standardized metric} = \frac{(\text{Metric result} - 25^{th}\text{ percentile of impaired sites})}{(75^{th}\text{ percentile of reference sites} - 25^{th}\text{ percentile of impaired sites})} \times 10$$

Formule (b)

$$\text{Standardized metric} = \frac{(\text{Metric result} = 75^{th} \text{ percentile of impaired sites})}{(25^{th} \text{ percentile of reference sites} - 75^{th} \text{ percentile of impaired sites})} \times 10$$

The formulas above follow the continuous scoring method to score metrics, using distribution values from metrics in reference and impaired sites. The procedures are different for metrics with values that increase or decrease as degradation increases (KLEMM *et al.* 2003).

Test of the index

A validation test of the PPPMI sensitivity was conducted in 15 sites located in the upper reaches of the Piabanha-Paquequer-Preto basin, judged to be representative of the degradation gradient. These sites were not used to build the index. The test was performed using a Principal Component Analysis (PCA) of environmental variables (%DO, conductivity, total alkalinity, width of riparian area, fecal coliforms, IHI index). The data matrix was standardized by applying $(x_i - \bar{x})/\sigma$, where x_i is the observed value, $\bar{x}$ is the mean and σ is the standard deviation. The assessment of the index was done using a Pearson correlation between the scores of sites in PCA axis 1 and the PPPMI scores. Analyses were done using BioEstat 2.0 (AYRES *et al.* 2000).

RESULTS

Benthic fauna

Ninety one benthic macroinvertebrate Operational Taxonomic Units (OTU) were found in the rivers from the Piabanha-Paquequer-Preto hydrographic region. The highest OTU richness belonged to the group of aquatic insects, with nine insect orders composed of 49 families and 43 identified genera (some taxa were only identified to family level) (Appendix).

Of the 42 metrics evaluated in this study, 36 were considered sensitive to differences between the reference and the impaired areas. There was no overlap among the Box-and-Whisker plots interquartile limits (1°-3°) of these metrics (Fig. 2), which was confirmed by the Mann-Whitney U-test. The six metrics considered unsuitable were: %Odonata, MOLD (Mollusca + Diptera) richness, Baetidae/Ephemeroptera, Hydropsychidae/Trichoptera, Chironomidae abundance and equitability. The next stage was to test metrics redundancy.

Eight pairs of metrics that were significantly correlated (Spearman correlation, $r > 0.75$, $p < 0.05$) were considered redundant. All were chosen giving priority to those metrics that facilitate the final application of the index. Of the six metrics that compose the PPPMI (Family Richness, Family Shannon-Wiener Diversity, EPT Family Richness, %Diptera, %MOLD – Mollusca + Diptera and %Collectors), three describe assemblage structure, two describe tolerance and one describes function. For each metric, upper and lower thresholds were set using reference and impaired sites distribution values (Tab. II). The scaling for the different classifications of ecological condition that resulted from the scoring process was divided in five classes of environmental stream quality: Severely Impaired (values ranging from 0-20), Impaired (20-40), Fair (40-60), Good (60-80) and Excellent (80-100).

Validation Test of the PPPMI

The first PCA axis was highly correlated with PPPMI scores ($r = 0.703$, $p < 0.001$) indicating a significant response to a gradient disturbance (Fig. 3).

DISCUSSION

This study aims to serve as an example for the development of multimetric indices for other watersheds and to help the managers of the Serra do Mar region to assess regional impacts and disturbances within first to fifth Strahler order wade-

Table III. Expected responses with increased impairment and metric value thresholds for the six metrics selected to integrate the Piabanha-Paquequer-Preto Multimetric Index.

PPPMI metrics	Expected response with impairment	Metrics values of upper and lower limits
Family Richness	Decrease	(Upper) 75% – ref. = 29
		(Lower) 25% – impaired = 3
Family Shannon-wiener Diversity	Decrease	(Upper) 75% – ref. = 2.2
		(Lower) 25% – impaired = 0.2
EPT Family Richness	Decrease	(Upper) 75% – ref. = 12
		(Lower) 25% – impaired = 0
Percentage of Diptera	Increase	(Upper) 25% – ref. = 26.3
		(Lower) 75% – impaired = 96.5
Percentage of MOLD (Mollusca + Diptera)	Increase	(Upper) 25% – ref. = 11.8
		(Lower) 75% – impaired = 96.0
Percentage of Collectors	Increase	(Upper) 25% – ref. = 20.0
		(Lower) 75% – impaired = 98.0

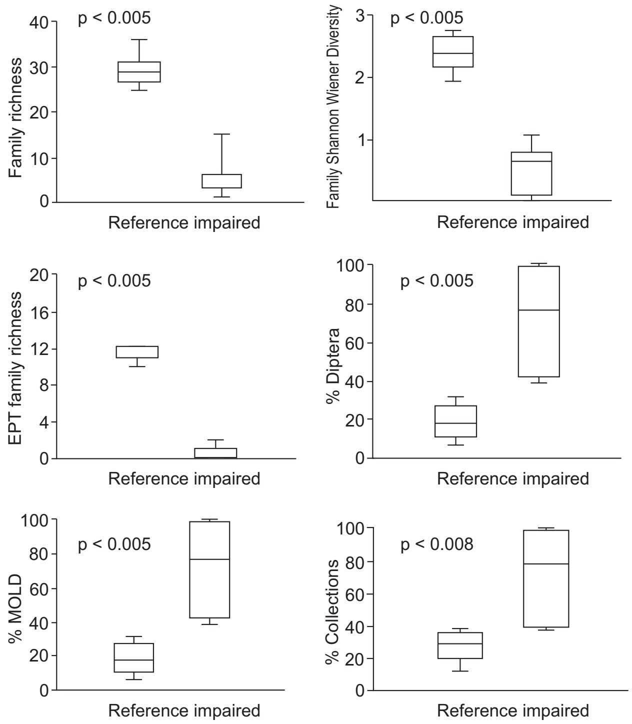

Figure 2. Comparison of distributions of each metric composing the PPPMI index. In the Box-&-Whisker plots, center lines represent the median, boxes represent the first and third quartiles, and bars represent the maximum and minimum. P-values were obtained using Mann-Whitney U tests.

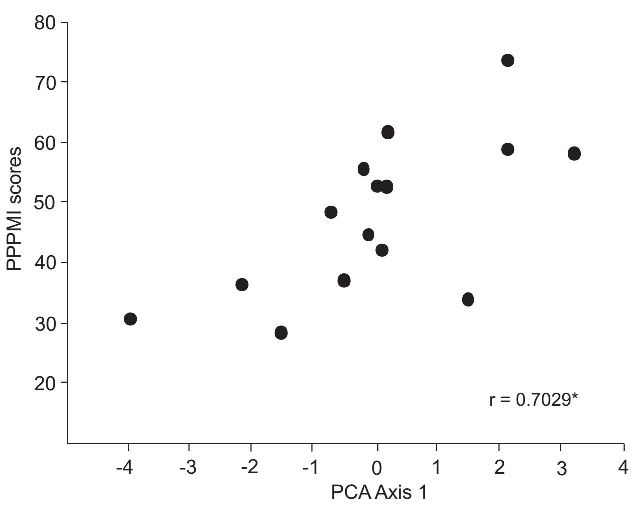

Figure 3. Scores obtained for 15 test-sites using the Piabanha-Paquequer-Preto Multimetric Index (PPPMI) as a function of PCA axis 1 performed with environmental parameters. These 15 locations were not used to build the index.

able streams, using specific regional features. This paper reinforces the idea that multimetric indices can be built at a watershed scale (KING & RICHARDSON 2003).

In this study, twenty seven sites were sampled, which is a representative number of sites for rivers that belong to a typology defined by having a catchment area between 10-100 km^2, granite geology and altitude ranging between 700-1200 m a.s.l,, within the Paquequer, Piabanha and Preto Basin. In the European Union, multimetric indexes in smaller scales were built considering the concept of river typology which usually involves a smaller number of sites. In the 30 river typologies studied in the European Union, the mean sample size used was 15.23 sites (HERING *et al.* 2003).

The core metrics of the PPPMI respond to different impacts. In general, the metrics that decrease with impact, for example, diversity indexes do not require adaptation to different geographical areas (BRADLEY 2008). So, the inclusion of the EPT richness and the Shannon-Wiener Index metrics in the PPPMI was considered useful given its feasibility and also because these are good measures that respond to structural changes and a clear response to the impaired gradient in the macroinvertebrate assemblages (SURIANO *et al.* 2011).

The other metric that decreases with impact (EPT – Ephemeroptera + Plecoptera + Trichoptera – Richness to family level) includes the Ephemeroptera and Plecoptera, which are sensitive to low oxygen levels and substrate changes (GERHARDT *et al.* 2004). This metric also includes the Trichoptera, which include some groups that are tolerant of heavy metals and low oxygen levels (BRADLEY 2008). ARIAS *et al.* (2007) also found some sites, in the study area, which are impaired due to habitat loss and high pesticide contamination, where some genera of Trichoptera (*Nectopsyche* Mueller, 1879) and Ephemeroptera (*Americabaetis* Kluge, 1992) were tolerant to these impacts. However, even though studies show that some groups of EPT are tolerant, it is worth using the EPT richness to family level, once it aggregates more sensitive groups than tolerant ones and also because the family identification level used is easily achieved when compared to species and genus level. In addition, other studies showed that the number of EPT families decreased at sites with increasing impact due to disturbance (agricultural, pastures, and urban sites), possibly because of a higher nutrient concentrations (SANDIN & JOHNSON 2000, HEPP & SANTOS 2009).

The metrics which increase with impact (%Diptera, %MOLD – Mollusca + Diptera, %Colletor), respond positively to conditions associated to organic pollution caused by untreated wastewater effluents in urban areas and to the increase of suspended organic particles in rural areas. The metric %Diptera was one of the best indicators of organic pollution in this study area (BAPTISTA *et al.* 2007). HEPP & SANTOS (2009) also found that %Diptera tended to increase when degradation increased, while richness, diversity, evenness, and EPT families decreased with the impact of degradation. The metric %Collector was useful to discriminate multiple impacts and organic pollution in other rivers within the Serra dos Órgãos, southeastern Brazil (Silveira *et al.* 2005, Baptista *et al.* 2007, Buss & Sales 2007). The core metric %MOLD was chosen as the number of Diptera and Mollusca individuals tend to increase in the assemblages in response to impacts such as increased fine sediments and high organic pollution. This phenomenon has been shown to occur in several regions in Brazil (Giovanelli *et al.* 2005, Hepp & Santos 2009).

Diversity and richness metrics of the PPPMI required identification of specimens only to family level. Metrics at the genus level were found to be valid and sensitive. However, they were not chosen due to the limited taxonomic knowledge and additional work to make identifications. Of the metrics used, only %Collector requires some taxa to be identified to genus level, particularly Trichoptera. Even so, at higher levels of taxonomic identification, the PPPMI responded appropriately to generalized measurements of disturbance, representing a wide variety of combined stressors, being a suitable and efficient tool to detect environmental impacts.

The good index performance reinforces its potential use to assess ecological quality of water in river reaches. Future studies will indicate if the index can be applied in sites located at altitudes lower than 800 m (a.s.l) belonging to the same typology for which it was developed

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Appendix. List of taxa collected in the Piabanha-Paquequer-Preto hydrographic region, state of Rio de Janeiro, Brazil, to compose the PPPMI.

Ephemeroptera
- Baetidae
  - *Baetodes* Needham & Murphi, 1924
  - *Cloeodes* Traver, 1938
  - *Americabaetis* Kluge, 1992
- Euthyplociidae
  - *Campylocia* Needham & Murphi, 1924
- Caenidae
  - *Caenis* Stephens, 1835
- Leptohyphidae
  - *Leptohyphes* Eaton, 1882
  - *Tricorhytodes* Ulmer, 1920
  - *Thricorythopsis* Traver, 1958
- Leptophlebiidae
  - *Askola* Peters, 1969
  - *Farrodes* Peters, 1971
  - *Massartela* Lestage, 1930
  - *Hylister* Dominguez & Flower, 1989
  - *Miroculis* Edmunds, 1963
  - *Thraulodes* Ulmer, 1920
  - *Hermanella* Needham & Murphy, 1924
  - *Ulmeritoides* Traver, 1959

Odonata
- Aeshnidae
  - *Staurophlebia* Brauer, 1865
- Calopterygidae
  - *Haeterina* Hagen & Selys, 1853
- Libellulidae
  - *Erythrodiplax* Brauer, 1868
- Megapodagrionidae
  - *Heteragrion* Selys, 1862
- Protoneuridae
  - *Peristicta* Hagen & Selys, 1860

Plecoptera
- Gripopterygidae
  - *Gripopteryx* Pictet, 1841
  - *Paragripopteyx* Enderlei, 1909
  - *Guaranyperla* Froehlich, 2001
- Perlidae
  - *Anacroneuria* Klapálek, 1909
  - *Kempnyia* Klapálek, 1916
  - *Tupiperla* Froehlich, 2001
  - *Macrogynoplax* Enderlei, 1909

Hemiptera
- Helotrephidae (nd)
- Mesovellidae (nd)
- Naucoridae (nd)
- Notonectidae (nd)
- Pleidae (nd)
- Vellidae (nd)
- Megaloptera
- Corydalidae
  - *Corydalus* Lastreille, 1802

Trichoptera
- Calamoceratidae
  - *Phylloicus* Müller, 1880
- Hydrobiosidae
  - *Atopsyche* Banks, 1905
- Helicopsychidae
  - *Helicopsyche* Siebold, 1856
- Hydroptilidae
  - *Ochrotrichia* Mosely, 1934
- Hydroptilidae (nd)
- Hydropsychidae
  - *Blepharopus* Kolenati, 1859
  - *Leptonema* Guérin, 1843
  - *Macronema* Pictet, 1836
  - *Smicridea* McLachlan, 1871
  - *Synoestropsis* Ulmer, 1905
- Leptoceridae
  - *Grumichella* Müller, 1879
  - *Nectopsyche* Müller, 1879
  - *Triplectides* Kolanati, 1859
  - *Oecetis* McLachlan, 1877
- Odontoceridae
  - *Barypenthus* Burmeister, 1839
  - *Marilia* Müller, 1880
- Polycentropodidae
  - *Cyrnellus* Banks, 1903
- Sericostomatidae
  - *Grumicha* Mueller, 1879
- Xiphocentronidae
  - *Xiphocentron* Brauer, 1870

Lepidoptera (nd)

Coleoptera
- Dytiscidae (nd)
- Dryopidae (nd)
- Elmidae
  - *Heterelmis* Sharp, 1882
  - *Hexacylloepus* Hinton, 1940
- Elmidae
  - *Hexanchorus* Sharp, 1882
  - *Macrelmis* Motschulsky, 1859
  - *Microcylloepus* Hinton, 1935
  - *Neoelmis* Musgrave, 1935
  - *Phanocerus* Sharp, 1882
  - *Promoresia* S&erson, 1954
  - *Xenelmis* Hinton, 1936
- Hydrophilidae (nd)
- Lutrochidae (nd)
- Psephenidae (nd)
- Staphilinidae (nd)

Diptera
- Ceratopogonidae (nd)
- Chironomidae (nd)
- Culicidae (nd)
- Dixidae (nd)
- Ephydridae (nd)
- Empididae (nd)
- Psychodidae (nd)
- Simuliidae (nd)
- Stratiomyidae (nd)
- Syrphidae (nd)
- Tabanidae (nd)
- Tipulidae (nd)

Crustacea
- Decapoda (nd)

Acarina (nd)

Annelida
- Hirudinea (nd)
- Oligochaeta (nd)

Chelicerata(nd)

Mollusca
- Ancilidae (nd)
- Physidae (nd)
- Planorbidae (nd)