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PRIMARY RESEARCH PAPER

A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil

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Abstract This study describes the application of a protocol for biological assessment of water quality at first to third order streams at Serra dos Órgãos, an area covered by Atlantic Forest in Rio de Janeiro State, Brazil. Major impacts in the region are domestic effluents and deforestation. Our main objective is to establish biocriteria for the establishment of the Serra dos Órgãos Multimetric Index (SOMI) based on benthic macroinvertebrates. We used data from previous studies, sampled by experienced biologists, from 1999 through 2002. The benthic macroinvertebrate community was sampled in 12 reference sites and seven impaired sites in three river basins: Guapimirim, Macaé and Grande, all from the same bioregion. From the 22 tested metrics, 6 were included in the SOMI (% Diptera, % Coleoptera, Family Taxa, EPT Taxa, BMWP-CETEC and % Shredders). Scores (5, 3 or 1) were developed for these metrics to allow for aggregation into the index. Seven intermediately impaired sites were used for evaluating the applicability of the multimetric index. We concluded that the SOMI is a robust easy-to-apply tool for biomonitoring programs in the Serra dos Órgãos region, south-east Brazil.

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Introduction

Atlantic Forest remnants at Rio de Janeiro State, south-east Brazil, are under strong anthropogenic pressure, with high risk of losing the remaining non-disturbed aquatic ecosystems. Major impacts are domestic effluents that are thrown *in natura* in streams and rivers but also deforestation, unplanned urbanization and agricultural activities. Currently, programs of aquatic ecosystem monitoring carried out by Brazilian public authorities consider only water chemical analyses, which are inadequate tools to assess the ecological aspects of stream ecosystems (Rosenberg & Resh, 1993).

Since the last decade, research institutions and Brazilian environment protection agencies have been developing studies to use of benthic macroinvertebrate communities to assess environmental condition of streams and rivers (Araújo et al., 1998; Junqueira & Campo, 1998; Junqueira et al., 2000; Buss, 2001; Silveira et al., 2005).

Biological monitoring of aquatic ecosystem programs began to be used after the Saprobic System developed by Kolkwitz & Marsson (1908, 1909), which established the conceptual basis for biomonitoring methods. At the end of the 1980s, most biotic indices were based in score systems, such as the Biotic Condition Index (Winget & Mangun, 1979), Biological Monitoring Working Party score system (BMWP; Armitage et al., 1983), Indice Biotico Esteso (IBE; Ghetti, 1997) and the Family Biotic Index (Hilsenhoff, 1987, 1988) (see Metcalfe, 1989; Reynoldson & Metcalfe-Smith, 1992; Rosenberg & Resh, 1993).

Since then, new approaches on biomonitoring have been developed. The United Kingdom, Australia and Canada have been using in their biomonitoring programs an approach based on *a posteriori* site classification (RIVPACS—Clarke et al., 2003; AusRivAs—Wright, 1995; Sloane & Norris, 2003; and BEAST—Reynoldson et al., 1995, respectively). In the United States, researchers have been developing multimetric indices using fish, periphyton and macroinvertebrate communities based on *a priori* site classification (Plafkin et al., 1989; Barbour et al., 1995, 1996; Davis et al., 1996; Gibson et al., 1996; Yuan & Norton, 2003). Both types of bioassessment methods are based on the establishment of reference conditions at unimpaired sites and data comparison with test-impaired sites.

Recently, the European Community announced its determination for the development of multimetric indices in a routine biomonitoring program, the AQEM program (Böhmer et al., 2004; Buffagni et al., 2004; Hering et al., 2004; Ofenböck et al., 2004; Vlek et al., 2004), following the directives established by the European Community Water Framework Directive (European Commission, 2000).

In contrast, such studies are still incipient in most South American countries. In Brazil, the first multimetric index using benthic macroinvertebrates was developed by Buss (2001) and recently other studies were developed (Egler, 2002; Silveira et al., 2005). These studies, however, considered one main river basin, and indices could be applied only locally. In this paper we aim to develop a multimetric index for assessing the biological integrity of streams and rivers in a broader scale, comprising the mountainous region of Serra dos Órgãos, an area of Atlantic Forest remnants, one of the most diverse and threatened ecosystems in the world.

Methods

Study area

Serra do Mar is a mountainous region extending through 1,000 km at Rio de Janeiro and other States in south-east Brazil. The core section of Serra do Mar at Rio de Janeiro State is known as Serra dos Órgãos, covering an area of 12,904 km^2. In this region, mean annual rainfall averages 2.5 m. The rainy season occurs from November to February (more than 250 mm/month), and a dry season occurs from June to September (less than 100 mm/month). Throughout the other months, rainfall remains within this range.

The high anthropogenic pressure over the aquatic ecosystems in this region is a consequence of the ever-increasing population and urban sprawl of the metropolitan area of Rio de Janeiro city (more than 8 million people). Atlantic Forest covers the remaining areas, especially at high altitudes.

Field and laboratory procedures

The benthic macroinvertebrate community was sampled in 12 reference sites and seven impaired sites in three river basins: Guapimirim—Lat 22°29′92″ S Long 42°58′73″ W, Macaé—Lat 22°21′00″ S Long 42°27′00″ W and Grande—Lat 22°21′08″ S Long 42°37′79″, all from the same bioregion (Araújo, 2004).

Reference sites were defined as streams minimally disturbed, obeying to the following *a priori* physical-chemical water and environmental conditions: DO (Dissolved Oxygen) ≥ 6 mg/l; pH between 6 and 8; area of urbanized land ≤20% of total upstream drainage basin; forested area ≥25% of total upstream drainage basin; riparian zone ≥15 m; no visible sign of channeling; ''excellent'' or ''very good'' classification according to the RCE index (Petersen, 1992). Major impacts at impaired sites were the removal of riparian vegetation and alterations of physical characteristics of streams. For the ''impaired'' condition, the following *a priori* conditions should be met: deforestation of ≥75% of the upstream area; silting in riffle mesohabitats ≥50%; ''poor'' classification according to the RCE index (Petersen, 1992).

At each site, three pseudo-replicates of each of the four main substrate types (sediment, stones, litter in riffle areas, and litter in pool areas) were taken using a Surber sampler (0.09 m^2 area, 125 μm mesh size). The 12 samples were then pooled, representing a single sample for each site. Each site was sampled three times: at the end of the rainy season (March–June), during the dry season (July–October) and during the rainy season (November–February). Thus, in total, 36 composite samples were taken in reference sites and 21 samples in impaired sites. All sites were at about the same altitude and were of first to third orders (Table 1).

Samples were fixed in ethanol 80% in the field and fully processed in the laboratory. Macroinvertebrates were sorted using a stereoscopic microscope (magnification 10×) and most were identified to genus taxonomic level (only Diptera and Lepidoptera were identified to family level), using the available taxonomic keys (Odonata—De Marmels, 1990; Nieser & de Melo, 1997; Plecoptera—Froehlich, 1984; Dorvillé & Froehlich, 1999; Ephemeroptera—Dominguez et al., 1992; Trichoptera—Angrisano, 1995; Wiggins, 1996; for other taxa—Merrit & Cummins, 1996) and/or the aid of specialists. Macroinvertebrates were assigned to Functional Feeding Groups based on the literature (Angrisano, 1995; Merritt & Cummins, 1996), the aid of specialists and personal observations.

Data analysis

Metrics selection

Twenty-two metrics were examined in order to establish the multimetric index. A careful selection of the metrics was made aiming to assure that various aspects of the macroinvertebrate communities were assessed (measures of richness, composition, tolerance and trophic status), in order to represent different responses, thus increasing the ecological information included in the index (Resh & Jackson, 1993; Kerans & Karr, 1994; Barbour et al., 1995, 1996, 1999) (Table 2).

Test 1—metrics sensitivity

The sensitivity of each metric was judged according to the degree of interquartile overlap in Box-and-Whisker plots, according to Barbour et al. (1996). Box-and-Whisker plots allow the visualization of metrics range variation between reference and impaired sites. Metrics were judged to have one of five sensitivity values: a sensitivity score 3 was awarded if no overlap existed in interquartile range; a sensitivity score 2 if there was some overlap in interquartile range but both

Table 1 Hydromorphological and hydrochemical characterization of reference and impaired sites at Serra dos Órgãos region

	Reference sites Mean (Max–Min)	Degraded sites Mean (Max–Min)
Catchment area (km^2)	7.57 (1.7–18.3)	17.16 (1.5–48.8)
Water discharge (m^3 s^{-1})	0.80 (0.57–1.89)	1.23 (0.11–2.45)
Stream width (m)	7.71 (4.0–15.6)	8.70 (4.0–13.3)
PH	6.5 (6–7)	6.5 (5–7.5)
Dissolved oxygen (mg l^{-1})	8.5 (7.0–9.6)	5.75 (4.9–6.9)
NO_3-N (μg l^{-1})	0.053 (0.176–0.004)	0.073 (0.230–0.01)
Predominant substrates	Gravel, Cobble, Riffle and pool litter	Cobble, Pebble, Sand, Silt
RCE index integrity class (score)	Very Good–Excellent (160–300)	Poor (23–44)

Table 2 Candidate metrics to integrate the multimetric index and their predicted responses to impairment

Metrics	Predicted response	
Composition measures		
EPT %	Decrease	Composition measures provide information on relative contribution (relative abundance) of each bioindicator group in relation to the total fauna.
Plecoptera %	Decrease	
Ephemeroptera %	Decrease	
Trichoptera %	Decrease	
Coleoptera %	Decrease	
Odonata %	Increase	
Diptera %	Increase	
Richness measures		
Total Taxa	Decrease	High richness numbers are related to good environmental health, suggesting that niche, habitat availability and food resources are adequate for sustaining a large number of species.
Family Taxa	Decrease	
Ephemeroptera Taxa	Decrease	
Plecoptera Taxa	Decrease	
Trichoptera Taxa	Decrease	
EPT Taxa	Decrease	
Tolerance measures		
BMWP-CETEC*	Decrease	Indicate the degree of sensitivity of a taxa or individual species to one or more types of disturbance.
IBE-SORJ	Decrease	
EPT/Chironomidae	Decrease	
Baetidae/Ephemeroptera	Increase	
Trophic measures		
Collector %	Variable	The biological trophic measurements evaluated is likely to reflect the relative abundance of representatives of organisms adopting the following different classes of feeding strategies.
Filterer %	Decrease	
Shredder %	Decrease	
Scraper %	Decrease	
Predator %	Variable	

medians were outside the interquartile range overlap; a sensitivity score 1 if there was moderate overlap of interquartile range but one median was outside the interquartile range overlap; a sensitivity score 0a if one range was completely overlapping the other interquartile range but one median was outside the interquartile range overlap; and a sensitivity score 0b if both medians were inside interquartile range overlap (Fig. 1). A metric was considered sensitive when comparison between Box-and-Whisker plots of reference and impaired sites awarded a sensitivity score 3 and results were confirmed by the Mann–Whitney *U*-test.

Test 2—metrics redundancy

A Spearman correlation test was performed with pairs of metrics considered sensitive according to test 1. This test was performed in order to simplify the index, reduce costs of analyses, and avoid redundant information. In case of high correlation (Spearman $r > 0.75$, $p < 0.05$), one or more redundant metrics were excluded in order to have only one metric representing that information in the index.

Additionally, for the selected metrics in the two testing steps, seasonal variation was assessed through comparison of metric numbers between the three sampling periods (end of rainy season, dry season and middle of rainy season) with data from reference sites, by means of Box-and-Whisker plots and the Kruskal–Wallis test.

Development of the Serra dos Órgãos Multimetric Index (SOMI)

Metrics considered sensitive in the two testing steps and that were seasonally stable were chosen to integrated the multimetric index. However, the use of acceptance criteria requires standardization of the different numeric scales. This was made in order to allow for their aggregation in a single index, as proposed by Karr et al. (1986), Karr (1991), Barbour et al. (1996) and Fore et al.

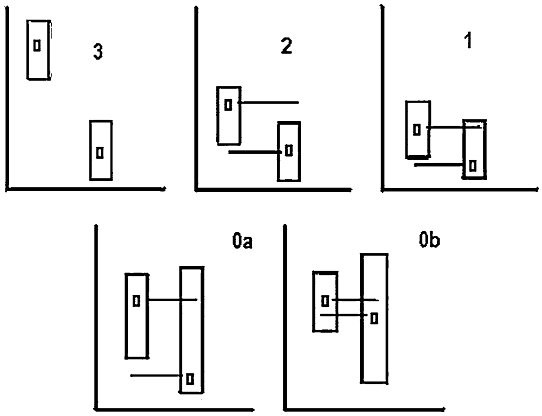


Fig. 1 Evaluation of metrics sensitivity, according to Barbour et al. (1996). Small squares represent median numbers and boxes represent inter-quartile ranges (25–75% percentiles)

(1996). The range of metric values was divided into three possible scores for each metric. For metrics expected to have decreasing numbers with increasing pollution or disturbance, numbers above the lower quartile (25%) of the reference distribution was awarded the score 5. On the other hand, for metrics expected to have increasing numbers in response to disturbance, each value lower than the upper quartile (75%) of the reference distribution was awarded the score 5. Therefore, the appropriate quartile was used as a threshold depending on the kind of response to degradation. A score 5 represents that sample is a part of the reference population, a score 3 indicates an intermediate condition and the score 1 indicates the greatest deviation from the expected numbers for reference sites (Fig. 2).

Therefore, a table with the Minimum, 25%, 50%, 75% and Maximum numbers of each metric at reference sites was used as the threshold for separating score ranges for the establishment of the multimetric index.

SOMI testing at intermediately impaired sites

After the determination of the SOMI, seven intermediately impaired sites were used for evaluating the applicability of the index. These seven sites were sampled at the same three periods than the reference and impaired sites, thus performing 21 samples. To verify the efficacy of the index, SOMI scores were calculated for each sample, and our hypothesis was that they should be classified as intermediate class sites (Regular or Good classes).

Results

Sensitivity tests

Box-and-Whisker plots were used to determine if a metric was sensitive, i.e. if it could be used to discriminate between reference and impaired sites. From the 22 metrics evaluated in this study, 14 were considered sensitive according to this test, with a sensitivity score 3 between reference and impaired sites, and statistically different according to the Mann–Whitney *U*-test ($p < 0.05$; Fig. 3; Table 3).

The following step was to test for metrics redundancy. According to the Spearman test, the

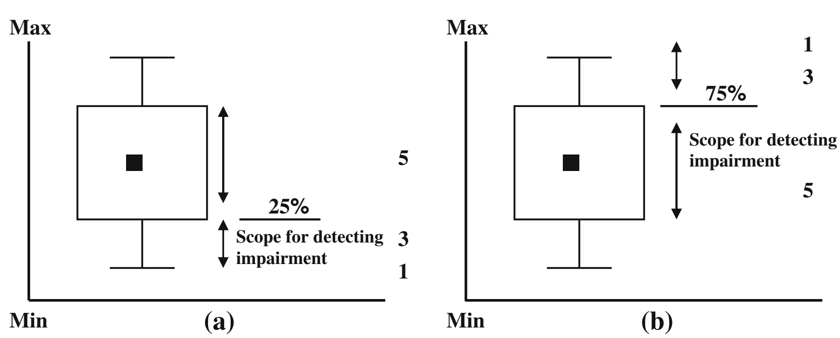
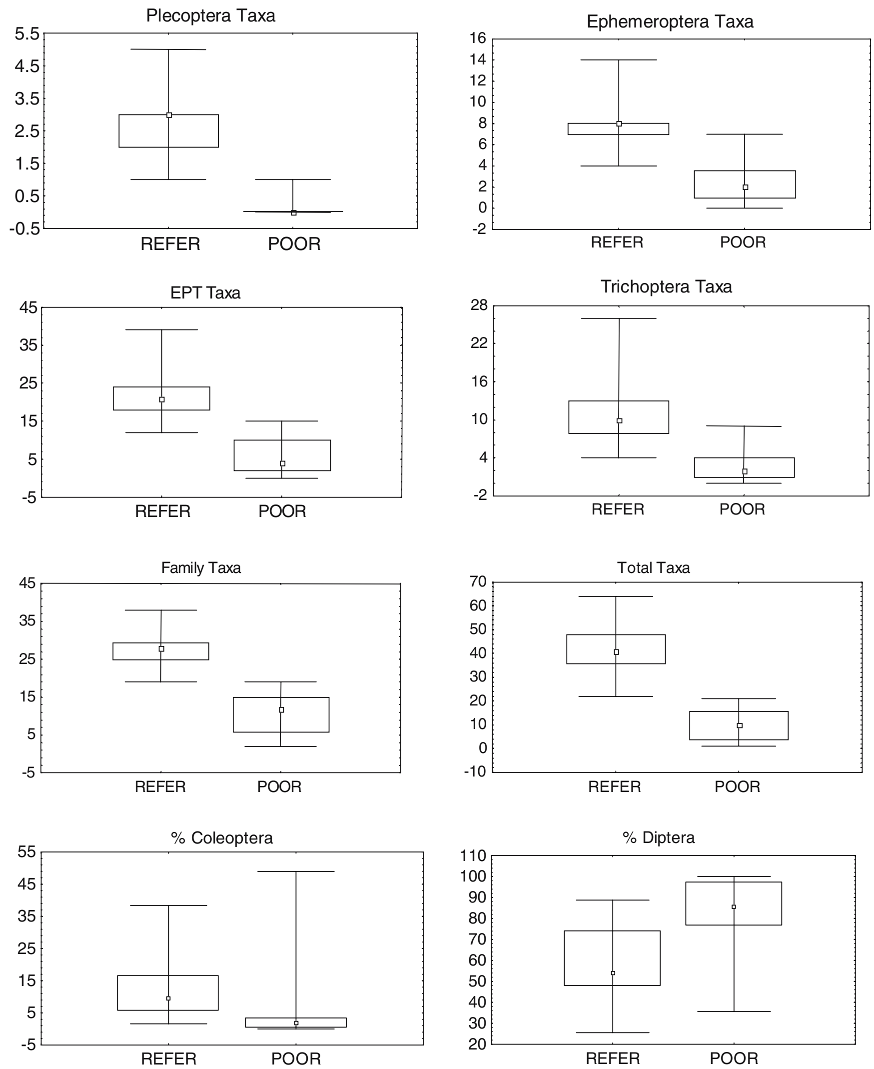


Fig. 2 Scoring criteria for metrics: (**a**) metrics expected to have decreasing numbers in response to impairment; (**b**) metrics expected to have increasing numbers in response to impairment. Small squares represent median numbers, boxes represent inter-quartile ranges (25–75% percentiles), according to Barbour et al. (1996)

Fig. 3 Box-and-Whisker plots of each of the 14 metrics sensitive to discriminate between reference (REFER) and impaired (POOR) sites, and two examples of metrics not sensitive to sites conditions (% Filterers and % Odonata). Small squares represent median numbers, boxes represent inter-quartile ranges (25–75% percentiles) and range bars show maximum and minimum of non-outliers numbers

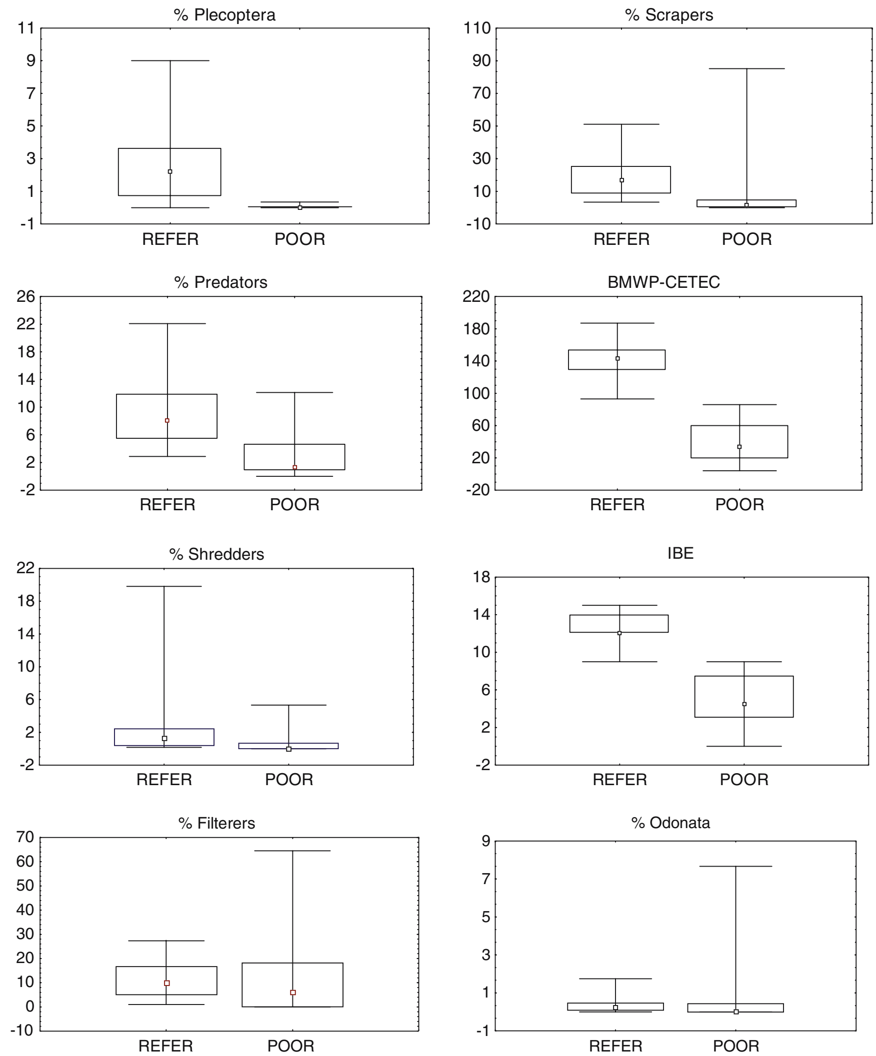


only pairs of data that were significantly correlated ($r > 0.75$; $p < 0.05$) were BMWP-CETEC vs. IBE and EPT Taxa vs. Trichoptera Taxa. We selected the BMWP-CETEC metric instead of the IBE because it is a more commonly used metric in biomonitoring programs, and works at Family taxonomic level, therefore easier to calculate. Also, we opted or the use of the EPT Taxa metric instead of Ephemeroptera Taxa, Plecoptera Taxa and Trichoptera Taxa because although all three metrics were sensitive, the combined index was a more robust measure to discriminate between reference and impaired sites.

After these two testing steps, 10 of the 22 metrics were sensitive enough to integrate the multimetric index. However, in order to make the index more operational, we decided to reduce the number of metrics, excluding the ones that were more difficult/costly to sample or calculate. For example, the metric Family Taxa was preferred in front of Total Taxa in order to simplify the index and reduce taxonomic identification costs.

From the three trophic metrics considered sensitive, we chose only the % Shredders to integrate the multimetric index because organisms are usually larger and easier to identify. Also, metrics % Filterer and % Collector had smaller differences between reference and impaired sites, and were excluded from the index.

Fig. 3 continued



Considering the measures of community composition, three from the six metrics were sensitive. Although the % Plecoptera was a sensitive metric to discriminate reference from impaired sites, it was excluded because its abundance at reference sites were relatively low, being more susceptible to sampling errors in routine biomonitoring. We opted to include both % Coleoptera and % Diptera because they represented different responses to degradation: the first decreased with degradation and the latter increased its numbers in impaired sites.

Therefore, six metrics representing the four categories of the macroinvertebrate fauna were selected for integrating the multimetric index: two Composition metrics (% Diptera and % Coleoptera); two Richness metrics (Family Taxa and EPT Taxa); one Tolerance metric (BMWP-CETEC) and one Trophic metric (% Shredders).

After this selection, we performed a test to verify if numbers of the six selected metrics were stable seasonally. According to the Kruskal–Wallis test, four metrics were stable seasonally (Fig. 4). For the BMWP-CETEC and Family Taxa metrics seasonal variation was found between the autumn season (end of rainy season) and the other two sampling periods. However, since these differences were not sufficient to change metrics sensitivity, we included both in the index.

Table 3 Responses of metric comparison between reference and impaired sites

Metrics	Test 1	U	p-level	Validation
Composition measures				
EPT %	1	92	0.13822	–
Plecoptera %	3	15	0.00000	Valid
Ephemeroptera %	1	25	0.25310	–
Trichoptera %	1	52	0.4214	–
Coleoptera %	3	156	0.000006	Valid
Odonata %	0a	122	0.18524	–
Diptera %	3	92	0.000005	Valid
Richness measures				
Total Taxa	3	0.00	0.000001	Valid
Family Taxa	3	0.00	0.000001	Valid
Ephemeroptera Taxa	3	34.5	0.000001	Valid
Plecoptera Taxa	3	1.50	0.000001	Valid
Trichoptera Taxa	3	29.5	0.000001	Valid
EPT Taxa #	3	5.50	0.000001	Valid
Tolerance measures				
BMWP-CETEC*	3	1.00	0.000001	Valid
IBE-SORJ	3	1.00	0.000001	Valid
EPT/Chironomidae	0b	25	0.6543	–
Baetidae/Ephemeroptera	1	22	0.5326	–
Trophic measures				
Collector %	0a	88	0.4758	–
Filterer %	1	82	0.3787	–
Shredder %	3	82	0.0420	Valid
Scraper %	3	72	0.00001	Valid
Predator %	3	99	0.000004	Valid

Test 1 column refers to scores for each metric based on Box-and-Whisker plots observation and results of the Mann–Whitney *U*-test (*U* values and *p*-level)

Development of SOMI

The value for the appropriate quartile of each of the six selected metrics at reference areas was used as a threshold for separating the maximum possible score from lower scores (Table 4). Using the metric scores in Table 4, SOMI was calculated by aggregating the scores of the six metrics. The range of possible scores for the SOMI was determined by the minimum and the maximum scores for the metrics, from 6 to 30. The SOMI range was then quadrisected to provide the four ordinal ratings of assessment: category Very Good which pertained to the desired reference biological condition, Good and Regular to identify those not meeting the unimpaired condition, and Poor (Table 5).

SOMI testing at intermediately impaired sites

From the 21 samples collected in intermediately impaired sites, 18 (85.7%) were classified as Good and Regular, according to the SOMI. Therefore, the index was sensitive enough to detect not only reference and impaired sites, but also intermediate environmental conditions.

Discussion

All the possible source of errors must be minimized during the establishment of a multimetric index in order to obtain an ideal accuracy and an adequate reproducibility through time (Smith et al., 2005). The natural seasonal variability, associated to sampling problems and dubious interpretations of physical-chemical data, may become additional source of errors in an accurate classification of reference sites (Barbour et al., 1996). Usually, more consideration is granted to the spatial variability, but studies on metrics seasonal variability have received little or no consideration in the establishment of multimetric indices. In this study, all biological sampling were made by the same research team and a low seasonal variability was verified for most metrics at

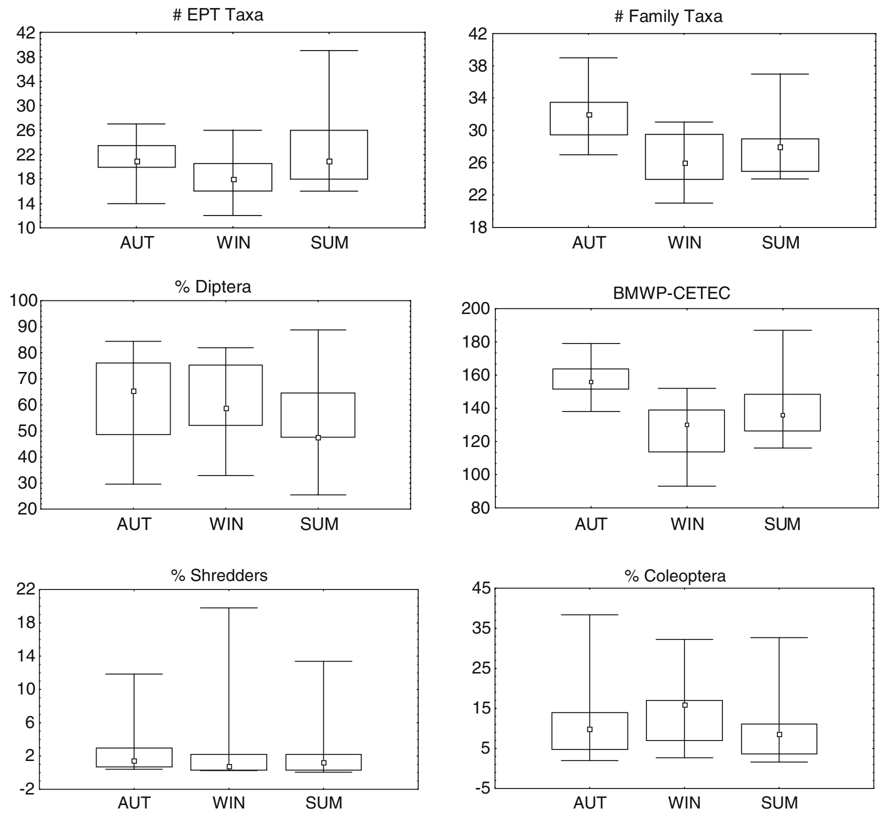


Fig. 4 Box-and-Whisker plots of the three sampling periods (AUT, Autumn—end of rainy season; WIN, Winter—dry season; SUM, Summer—rainy season) of each of the six selected metrics to integrate the SOMI

reference sites. Other studies corroborate this seasonal stability of the macroinvertebrate fauna composition in these streams (Baptista et al., 2001; Buss et al., 2002; Egler, 2002). We believe that this is a way of minimizing errors related to sampling and biological sorting in biomonitoring programs.

Our decision to deal with the complex biological patterns through the use of descriptive statistics and simple graphical analyses of the metric percentiles proved adequate. It supports the arguments for the applicability and robustness of this methodology in the establishment of biological criteria to discriminate between reference and impaired sites (Barbour et al., 1996; Maxted et al., 2000; Weigel, 2002; Ofenböck et al., 2004). However, some authors indicate the need for improvement of the methodology for data analysis, bioevaluation and biomonitoring, using macroinvertebrates (Barbour et al., 1995; Gerritsen, 1995; Smith et al., 2005).

The six selected metrics to integrate the SOMI represent different responses to anthropogenic impairment. The %Diptera usually show increasing numbers at impaired sites and may be considered as an indicator of silting in streams (Rosenberg & Resh, 1993; DeShon, 1995; Relyea et al., 2000). By its turn, the %Coleoptera metric is often associated to the increase in primary production, because many Coleoptera species in neotropical region feed on algae (Barbee, 2005). The EPT Taxa metric is one of the most commonly used metric in biomonitoring programs (Rosenberg & Resh, 1993). In this study, we opted to include this metric in the index instead of the three metrics that compose it (Ephemeroptera Taxa, Plecoptera Taxa and Trichoptera Taxa), in order to simplify the index calculation. Other studies in this bioregion showed that some algae-feeding Ephemeroptera species may increase its numbers in river basins influenced by the use of fertilizers in crops (Egler, 2002). Simi-

Table 4 Score thresholds for each of the six metrics integrating SOMI

Biotic measure /reference	Statistics					Score		
	Min	25%	50%	75%	Max	5	3	1
Diptera %	25	50	63	74	89	≤73	≥74 < 88	>89
Coleoptera %	4	12	20	34	57	≥12	11–8	< 8
Family Taxa #	19	25	32	32	34	≥25	24–20	< 19
EPT Taxa #	11	17	20	23	31	≥17	16–11	< 11
BMWP-CETEC	100	130	150	160	210	≥130	129–100	< 100
Shredder %	0.2	0.5	1.3	3.0	11	≥0.6	0.5–0.2	< 0.2

Table 5 Ordinal scale for the SOMI

Class	Poor	Regular	Good	Very Good
Scores	6 7 8 9 10 11 **12**	**13** 14 15 16 17 **18**	**19** 20 21 22 23 **24**	**25** 26 27 28 29 30

Bold numbers represent scores that may require sampling replication to confirm the determination of stream biological class

lar responses may occur in relation to increased organic pollution, benefiting less sensitive families, such as Baetidae and Leptohyphidae (Buss et al., 2002). According to the literature, the order Plecoptera is considered highly sensitive to environmental degradation (Fore et al., 1996; Maxted et al., 2000), but in this study the genus *Anacroneuria* Klapálek, 1909 (Perlidae) was an exception (see Electronic supplementary material). Biotic indices are useful to assess organic pollution. The BMWP-CETEC metric was sensitive in this study, but further studies are needed to adapt the BMWP for Atlantic Forest streams in Brazil. Metric % Shredder was included in SOMI because it is an indirect way to assess riparian vegetation quality (Bryan & Wilhm, 1990).

Selected metrics contributed to make the SOMI an easy-to-apply tool. Most aquatic insects must be identified only up to the family taxonomic level, with exception for the orders Ephemeroptera, Plecoptera and Trichoptera and the Shredders taxa, which must be identified to the genus level.

The sensitivity testing of SOMI indicated that it is a tool with enough sensitivity to correctly classify sites with intermediate degree of impairment. However, we verified that the SOMI tended to overestimate water quality condition when applied to sites with modified physiographic characteristics (such as local deforestation or low/moderate silting), but that are near to unimpaired forested areas (with no clear organic or chemical pollutants inflows). This is an indicative of the need of integrated tools, including water chemical analysis, environmental integrity assessment and biological information, as suggested by Buss (2001) for this region.

In conclusion, our results support the recommendation of SOMI as a useful tool for monitoring the biological quality of streams in Serra dos Órgãos, Rio de Janeiro state, Brazil.

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