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Rapid Bioassessment Protocols using benthic macroinvertebrates in Brazil: evaluation of taxonomic sufficiency

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Abstract. Rapid Bioassessment Protocols (RBPs) have been widely used to assess the ecological health of aquatic ecosystems. Specific aims of RBPs for wadeable streams are to indicate the ecological condition of a stream using low-cost protocols to allow long-term and widespread routine monitoring. Our study was part of an ongoing effort to test and standardize a protocol using benthic macroinvertebrates as indicators of the water quality of wadeable streams in southeast Brazil. One of the most controversial issues during RBP development is deciding the taxonomic resolution that should be used. We evaluated how well genus-, family-, and order-level taxonomic resolution detected a gradient of impairment. All 3 taxonomic resolutions statistically discriminated reference, intermediately impaired, and impaired sites based on assemblage structure, water-quality classification, and biotic index responses. Analysis at the genus level was more effective than analysis at other levels of taxonomic resolution for discriminating sites that varied in degradation conditions, especially when considering biotic index responses, but the lack of comprehensive taxonomic keys and information about the ecology of those genera hinder their widespread use in bioassessments. On the other hand, analyses at the order level had lower discriminating power to separate reference sites from intermediately impaired sites when considering biotic index responses. Analyses at the family level gave results similar to results at the genus level, and we support its use in a RBP program for this region, at least until better keys and autoecological knowledge are available.

Key words: biomonitoring, freshwater ecology, wadeable streams, neotropical region, evaluation of methods.

The issue of taxonomic resolution has long been a topic of interest in benthological studies, and the increasing use of benthic invertebrates to assess water quality has heightened the importance of understanding the tradeoffs associated with different levels of taxonomic resolution. In the past few years, benthic ecologists have been debating to what level macroinvertebrates should be identified, and the most logical answer to that question is that it depends on the particular aims of the study.

Rapid Bioassessment Protocols (RBPs) have been used in many countries to assess biological water quality and ecological health of aquatic ecosystems (Wright et al. 1984, Plafkin et al. 1989, Chessman 1995, AQEM 2002). RBPs for wadeable streams should be efficient, effective, low cost, and easy to use (Resh and Jackson 1993) to allow long-term and widespread monitoring and assessment. Adoption of procedures must consider costs and assure data quality.

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Benefits of species-level information in bioassessment programs have been discussed by several authors (e.g., Resh and McElravy 1993, Lenat and Resh 2001), but others question if this level of detail is needed to determine whether an ecosystem deviates from the reference condition. The purpose of an RBP is not to describe the macroinvertebrate assemblage, but rather to identify potential impacts or differences from the reference condition (Bailey et al. 2001). Several authors have shown that macroinvertebrate assemblage variation changes little in response to changing the level of taxonomic resolution of the analysis from genus to order (Furse et al. 1984, Wright et al. 1995, Marchant et al. 1995, Bowman and Bailey 1997), and some have even suggested that higher taxonomic levels might be more appropriate than the species level because species show a greater response to natural environmental variation, and these differential responses can contribute to noise and mask the effects of human activity (Warwick 1993, Bailey et al. 2001).

One of the main advantages of using coarse taxonomic resolution is that it could offer a substantial time and cost savings over identification to species. Ferraro and Cole (1995) estimated that the cost savings of phylum-, class-, order-, and genus-level identification compared to species-level identification were 95, 80, 55, and 23%, respectively. Clearly, a considerable opportunity exists to reduce the resources needed for identification by adopting coarser taxonomic levels in RBPs, but some questions remain. What, if any, are the losses in ability to discriminate the level of health or impact of a system? Is there significant redundancy between different taxonomic resolution levels or does the use of a coarser taxonomic level result in a meaningful loss of ecological information?

Our study was part of an effort to test and standardize RBP methods using macroinvertebrates as bioindicators of water quality of wadeable streams in southeast Brazil. Other studies were conducted to evaluate sampling procedures and mesh sizes (Buss and Borges 2008), to test subsampling methods, and to develop multimetric indices to assess biological condition (e.g., Buss 2001, Silveira et al. 2005, Baptista et al. 2007).

The concept of *taxonomic sufficiency* (Ellis 1985) is defined as the necessary taxonomic resolution to satisfy the objectives of a study. Our aim was to determine the sufficient taxonomic level (genus, family, or order) to detect a gradient of environmental impairment conditions in 2 wadeable stream basins in southeast Brazil.

Methods

We used data from 3 previous studies representing a significant sampling effort in 2 river basins from the same ecoregion. Two studies were done in the Guapimirim River basin (Buss 2001, Giovanelli 2005) and one in the São Lourenço River basin (Egler 2002). Buss (2001) and Giovanelli (2005) aimed to evaluate the effects of organic effluents and urbanization on the macroinvertebrate fauna, whereas Egler (2002) aimed to evaluate the effects of pesticide use and deforestation. We used the following criteria to choose these particular data sets: 1) all streams were 3^{rd} or 4^{th} order; 2) all streams were about the same altitude (0–240 m asl for the Guapimirim basin, and 820–1000 m asl for the São Lourenço basin), minimizing the effect of altitude in each data set; 3) the same sampling method was used for all samples (3 replicates each from litter in riffle areas, litter in pool areas, sediment and stones; Surber sampler with 900-cm^2 area and 125-μm-mesh size); 4) streams were sampled in a wet season, an intermediate season, and a dry season; and 5) each data set represented a gradient of environmental conditions, including reference, intermediate, and impaired sites. For both river basins, a site was considered as in reference condition if it met all of the following criteria: water pH = 6 to 8, dissolved $O_2 \geq 5$ mg/L, land use with $\leq$20% of the basin area urbanized and $\geq$75% of the upstream basin area forested, width of the riparian buffer $\geq$15 m, no visible channelization, and "optimal" environmental integrity according to the Habitat Assessment Field Data Sheet for High Gradient Streams (habitat assessment index; Barbour et al. 1999). A site was considered as in impaired condition if any of the following a priori conditions were met: deforestation $\geq$75% of the upstream area, and a "poor" classification according to the habitat assessment index.

Data for the Guapimirim River basin consisted of 396 benthic samples collected at 11 sites (5 reference, 4 intermediate, and 2 impaired sites). The São Lourenço River basin was represented by 216 samples collected at 6 sites (3 reference, one deforested, and 2 impaired by pesticide use). Water-chemistry and environmental variables for reference, intermediate, and impaired sites are shown in Table 1.

All macroinvertebrates were identified to the lowest possible taxonomic level with the available taxonomic keys (Ephemeroptera: Da Silva et al. 2003, Salles et al. 2004; Odonata: Belle 1992, Carvalho 1989, Carvalho and Calil 2000, Carvalho et al. 2002; Plecoptera: Olifiers et al. 2004; Hemiptera: Nieser and De Melo 1997; Trichoptera: Angrisano 1995, Wiggins 1996; Coleoptera: Passos et al. 2007; other groups: Merritt and Cummins 1996). In Brazil, there is insufficient information to identify organisms to species, and in some cases, even information on genus-level taxonomy is sparse and incomplete. Organisms of the insect orders Ephemeroptera, Odonata, Plecoptera, Hemiptera, Megaloptera, and Trichoptera, and all mollusks were identified to genus. With the exception of some rare taxa, most Coleoptera also were identified to genus. Diptera were identified to family, except Chironomidae, which were classified to subfamily. Hirudinea and Oligochaeta were represented by few individuals and were identified only to class. Thus, in our study, "lowest taxonomic level possible" refers to genus, and most groups were identified to genus level.

Influence of taxonomic level on assemblage structure

First, we analyzed whether the relationships among sampling sites remained the same when different taxonomic levels were used in a way similar to that of Bowman and Bailey (1997). First, the Bray–Curtis

Table 1. Mean (minimum–maximum) values of environmental and physical-chemical variables at sites sampled in the Guapimirim and São Lourenço River basins, Rio de Janeiro State, Brazil.

Variable	Reference sites (n = 24)	Intermediate sites (n = 15)	Impaired sites (n = 12)
Altitude (m asl)	280 (160–600)	160 (60–300)	70 (40–120)
Stream discharge (m^3/s)	1.4 (0.7–1.9)	1.8 (0.5–2.3)	2.2 (0.6–2.9)
pH	6.9 (6.2–7.4)	7.0 (6.3–7.4)	6.8 (6.3–7.4)
Alkalinity (mg/L)	7.3 (10.0–16.0)	15.2 (7.3–30.0)	18.6 (9.1–42.0)
Hardness (mg/L)	12.3 (9.5–18.0)	16.9 (10.0–26.0)	24.4 (14.5–45.3)
Conductivity (μS/cm)	20.6 (8.5–34.0)	23.9 (9.0–45.0)	61.2 (40.0–100.0)
Dissolved O_2 (mg/L)	8.7 (7.5–10.5)	8.3 (7.2–10.0)	6.9 (3.5–8.6)
NH_3-N (mg/L)	0.09 (0–0.20)	0.07 (0–0.20)	1.23 (0.43–2.88)
NO_3-N (μg/L)	0.36 (0–1.30)	1.30 (0.02–3.50)	1.12 (0.17–3.50)
PO_4-P (mg/L)	0.02 (0–0.06)	0.11 (0–0.51)	0.21 (0.04–0.43)
Cl^- (mg/L)	5.9 (0.9–11.7)	3.7 (0.9–8.7)	7.2 (2.4–10.7)
Total coliforms	250 (80–750)	2100 (500–4600)	6000 (800–15,000)
Fecal coliforms	40 (0–150)	287 (0–400)	635 (0–1100)
Predominant stream bottom	Boulder + cobble	Pebble + sand	Sand + silt/clay
Habitat Assessment Index	17.6 (16–19)	11.4 (8–15)	4.1 (0–5)

index of dissimilarity was calculated for each pair of sampling sites/occasion, using taxon abundances as descriptors. A matrix of dissimilarity among sites/occasions was generated for each taxonomic level. Then, the Mantel's matrix comparison test (Mantel 1967) was used to measure the correlation between the 3 matrices (genus vs family, genus vs order, family vs order).

Influence of taxonomic level on water-quality classes

The first step indicated if use of different taxonomic levels affected the relationships among site samples, based on assemblage structure. This information is valuable, but this approach might be of limited use for establishing efficient RBP tools if the results are disconnected from information on the ability of a given taxonomic level to distinguish environmental impairment (e.g., 2 dissimilarity matrices could be correlated but indicate different levels of impairment). Thus, an ordination technique (nonmetric multidimensional scaling [nMDS]) was used to determine whether macroinvertebrate assemblages at each taxonomic level could clearly discriminate reference sites from impaired sites. The analysis was based on Bray–Curtis dissimilarity, and 95% confidence ellipses were constructed around reference plots. Prior to the tests, all data were $\log_{10}(x + 1)$ transformed to reduce the influence of the most common taxa. Ordinations were done with PAST (version 1.68; Hammer et al. 2001).

Influence of taxonomic level on biotic indices responses

The performance of 3 biological indices was compared to investigate further the ability of each taxonomic resolution to detect environmental impairment. The Serra dos Órgãos Multimetric Index at genus-level (SOMI) was developed and tested for the study ecoregion in Brazil (Baptista et al. 2007); the Biological Monitoring Working Party (BMWP) index is a family-level index used worldwide (Wright et al. 1984); and the Índice Biológico para Voluntários (IBVol), is an order-level index created for use by volunteers in this ecoregion (Buss 2008).

One-way analyses of variance (ANOVAs) with Tukey's Honestly Significant Difference (HSD) post hoc comparisons were used to test the ability of each index (taxonomic resolutions) to differentiate reference, intermediate, and impaired sites. The significance level was corrected by the Bonferroni equation for all pairwise tests (e.g., differences in SOMI scores between reference and intermediate sites, reference and impaired sites, and intermediate and impaired sites). The data were normally distributed, and no transformation was needed (Shapiro–Wilk test). A taxonomic level was considered sufficient if the index was capable of statistically discriminating among sites in these 3 environmental conditions.

Results

A total of 276,494 benthic macroinvertebrates were included in our study. The Guapimirim River basin was represented by 146,308 organisms from 11 orders, 54 families, and 98 genera. The São Lourenço River basin was represented by 130,186 organisms from 10 orders, 50 families, and 94 genera.

Influence of taxonomic level on assemblage structure

For both river basins, the assemblage structure inferred by the genus-level identifications remained consistent when genera were aggregated to higher taxonomic levels. For both river basins, all pairs of matrices (genus vs family, genus vs order, family vs order) were highly correlated (>0.90) according to the Mantel test.

Influence of taxonomic level on biological condition classes

Stress values were low (0.08–0.14) for all 2-dimensional ordinations. In general, nMDS ordinations separated reference and impaired sites regardless of taxonomic level. For the Guapimirim basin, all impaired sites were clearly separated from reference and intermediate sites, regardless of taxonomic level. In the ordinations at genus level, 6 intermediately impaired sites fell inside the 95% confidence ellipse built around reference-site data (Fig. 1A), whereas at higher taxonomic levels those sites were outside the confidence ellipses (Fig. 1B, C). This result might have occurred because some collector, scraper, and filterer genera typical of transitional areas (e.g., *Baetodes*, *Camelobaetidius*, *Leptohyphes*, *Smicridea*) were present with relatively high abundance at both reference and intermediately impaired sites, but their respective families (including other genera belonging to these families) were more associated with intermediate sites. For the São Lourenço basin, intermediate sites fell inside reference data confidence ellipses regardless of taxonomic level (Fig. 2A–C). In this case, reference and intermediate sites were environmentally different, but the distance between reference and intermediate sites (deforested and no intensive agriculture) from forest fragments were <500 m (Egler 2002). As a result, richness numbers were similar (54–61 genera in reference sites, 38–60 genera in intermediate sites), and many groups typically found in forested reference sites (e.g., *Phylloicus*, *Triplectides*, *Psephenus*) also were found with low abundance in these intermediately disturbed sites. At genus and family level, impaired sites were clearly separated from reference confidence limits (Fig. 2A, B), but at the order level, 1 site fell inside the reference confidence limits (Fig. 2C).

Influence of taxonomic level on biotic indices responses

Biotic indices calculated for the 3 taxonomic levels were capable of discriminating environmental conditions (reference, intermediate, and impaired sites) for both river basins (Figs 3A–C, 4A–C). All 1-way ANOVAs were highly significant ($p < 0.001$). Tukey's HSD post hoc comparison discriminated significantly all pairs of data ($p < 0.05$), except that IBVol (order level) did not separate reference and intermediate sites in the Guapimirim basin ($p = 0.058$; Fig. 3C).

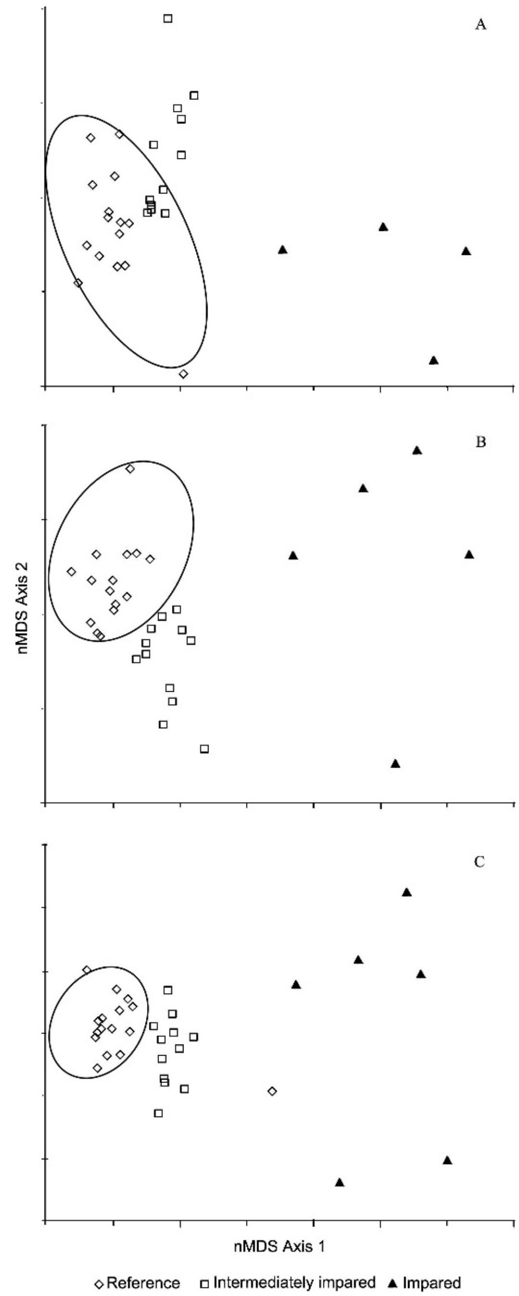

FIG. 1. Axis 1 and 2 of the nonmetric multidimensional scaling (nMDS) ordination of macroinvertebrate samples collected at the Guapimirim River basin and identified at genus (A), family (B), and order (C) taxonomic levels. Ellipses represent 95% confidence constructed around reference sites.

Discussion

Species-level identification is not a practical goal for most RBPs because of limited time and resources for sampling and identification, lack of species' autecological information, and lack of taxonomic expertise

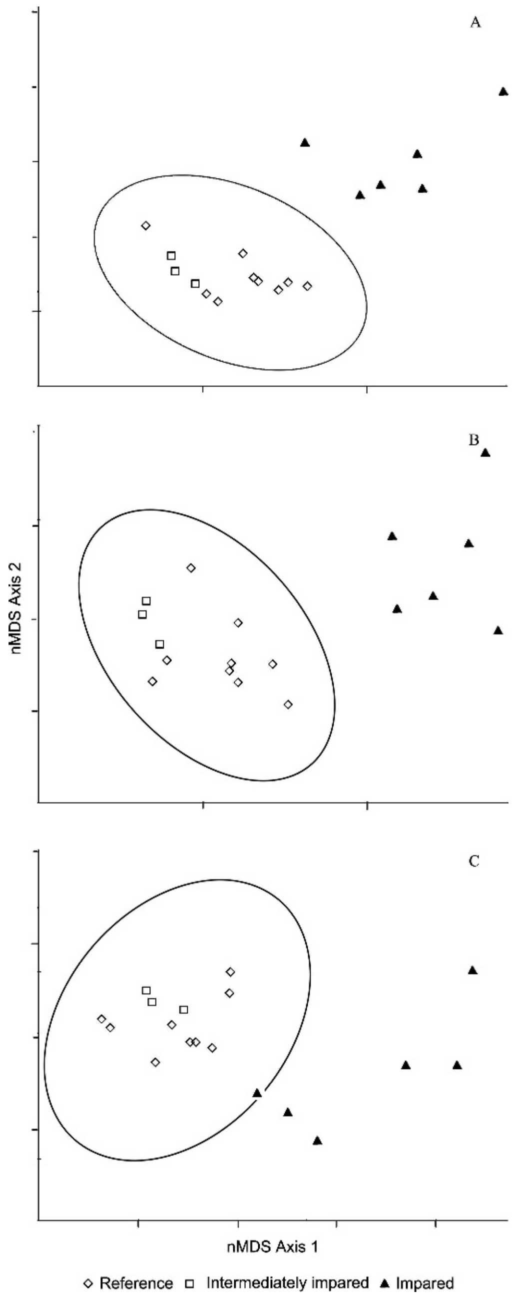

FIG. 2. Axis 1 and 2 of the nonmetric multidimensional scaling (nMDS) ordination of macroinvertebrate samples collected at the São Lourenço River basin and identified at genus (A), family (B), and order (C) taxonomic levels. Ellipses represent 95% confidence constructed around reference sites.

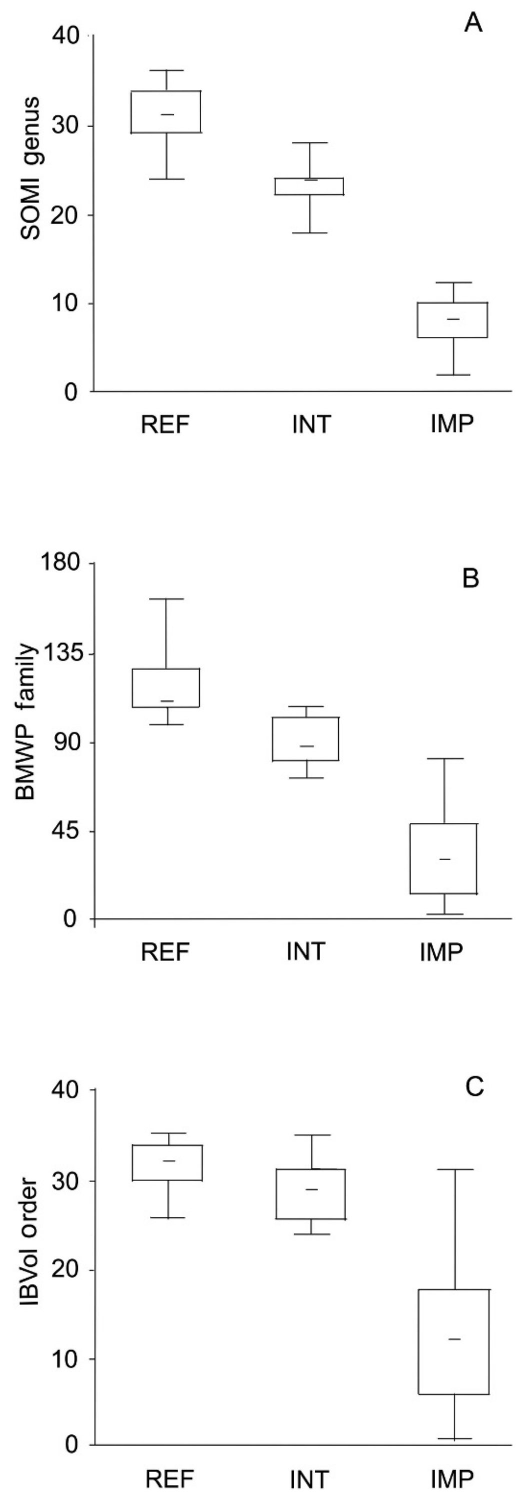

FIG. 3. Box-and-whisker plots of site scores for reference (REF), intermediately impaired (INT), and impaired (IMP) sites in the Guapimirim River basin. Scores were obtained with biotic indices calculated at genus (Serra dos Órgãos Multimetric Index [SOMI]) (A), family (Biological Monitoring working Party [BMWP]) (B), and order (Índice Biótico para Voluntários [IBVol]) (C) levels. Central lines in boxes represent medians, ends of boxes represent quartiles, whiskers represent the range.

and lack of taxonomic keys to all groups (Resh and McElravy 1993, Schmidt-Kloiber and Nijboer 2004). Thus, the most common identification pattern in aquatic bioassessment is a mosaic ranging from species to class because of difficulties in achieving a uniform level of taxonomic resolution for all aquatic macroinvertebrates groups (Cranston 1990). The problem is that the choice of the level taxonomic resolution to use often is made on a purely pragmatic basis (Ellis 1985). Heterogeneous identification levels permit inclusion of the entire assemblage while extracting the maximum amount of information with limited resources. However, the chosen taxonomic resolution depends on the purpose of the study and the experience of researchers and not on any ecological principle (Bouchard et al. 2005).

In Brazil, identification of benthic macroinvertebrates at the species level is not yet possible because of insufficient taxonomic knowledge and lack of comprehensive taxonomic keys. A large number of species and genera are described each year, but they are restricted to few regions. Also, insect taxonomy is based on adults, and correlation of adults with the immature forms is hindered without rearing studies.

Several authors have argued that the degree to which species assemblage patterns are conserved when taxonomic detail is reduced depends on overall richness, and less detailed data suffice where diversity is low (Ferraro and Cole 1992, Marshall et al. 2006, Jones 2008). Based on multivariate analysis of assemblage structure in our study, all 3 levels of taxonomic resolutions (genus, family, and order) were capable of discriminating reference, intermediate, and impaired sites in both river basins, even though our assemblages had high diversity. Multivariate analyses are powerful tools to reveal differences in structure and composition of biological assemblages among sites (Bowman and Bailey 1997, Bailey et al. 2001), but for relevance to bioassessment, information should be focused on specific taxa (i.e., the principles of bioindicator species and creation of indices). In our study, biotic index responses were most useful for assessing this information. As in the multivariate approach, we found that indices calculated at all 3 taxonomic levels were able to discriminate among site condition classes. However, the index calculated from data at order-level resolution had less power to separate reference from intermediate sites than did the indices calculated from data at family- and genus-level resolution.

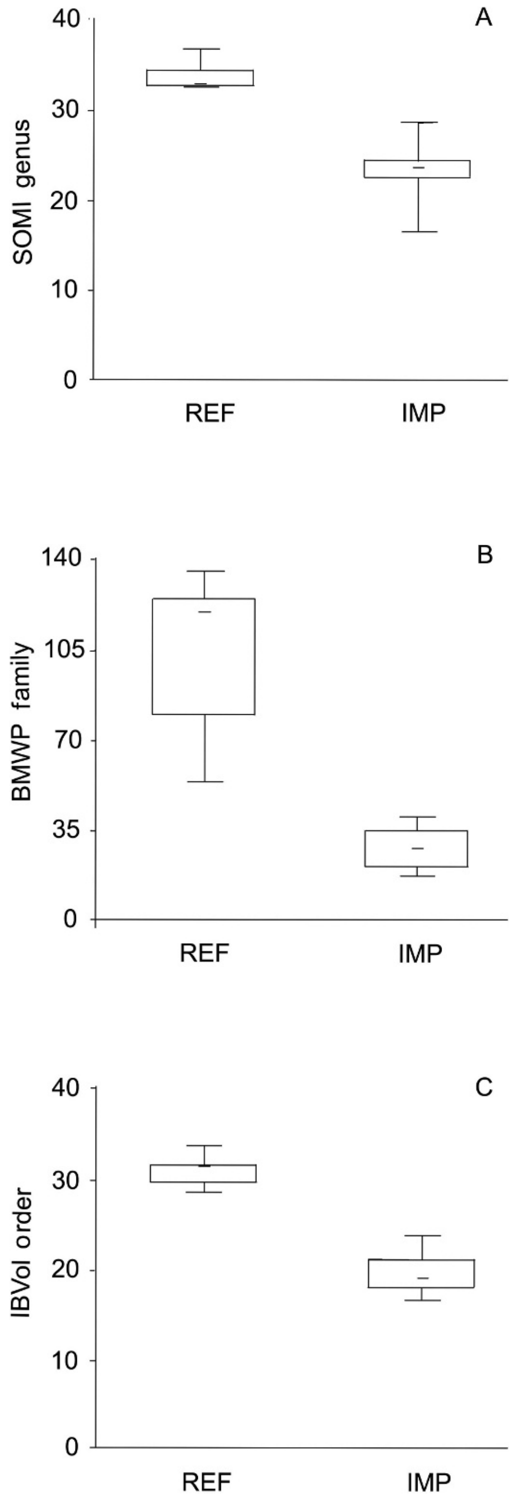

FIG. 4. Box-and-whisker plots of site scores for reference (REF) and impaired (IMP) sites in the São Lourenço River basin. Scores were obtained with biotic indices calculated at genus (Serra dos Órgãos Multimetric Index [SOMI]) (A), family (Biological Monitoring working Party [BMWP]) (B), and order (Índice Biótico para Voluntários [IBVol]) (C) levels. Central lines in boxes represent

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medians, ends of boxes represent quartiles, whiskers represent the range.

In general, our results were consistent with those of other studies in which results based on family or higher taxonomic resolution agreed with results based on finer taxonomic resolution (Chessman 1995, Zamora-Muñoz and Alba-Tercedor 1996, Bowman and Bailey 1997, Bailey et al. 2001, Reece et al. 2001, Waite et al. 2004, Marshall et al. 2006). In contrast, some studies have shown that the ability to detect impairment decreases as taxonomic resolution becomes coarser until only gross pollution can be identified. Species-based models were able to detect subtle impacts of nonpoint-source effects of logging in streams in California that family-based models were unable to detect (Hawkins et al. 2000). Ephemeroptera, Plecoptera, Trichoptera (EPT) family diversity and abundance indices strongly underestimated differences among acidic and reference streams compared with genus and species indices (Guerold 2000), and Guerold (2000) stated that the use of family-level identification would lead to erroneous interpretations. Many authors have speculated that coarser taxonomic resolution might be adequate for bioassessment in large geographic areas, whereas studies in smaller areas would require finer taxonomic resolution (Waite et al. 2004, Marshall et al. 2006). In our study, measured impairment was fairly mild and was more related to habitat alteration than to industrial/chemical effluents. Moreover, the most widely separated sites were ~11 km apart in Guapimirim basin and ~9 km apart in São Lourenço basin, and all sites were similar in altitude within each basin. Nevertheless, indices at all 3 levels of taxonomic resolution were able to distinguish sites in different environmental conditions. Another study in a small basin in southeast Brazil found similar results (Melo 2005).

Other arguments in favor of use of species-level taxonomic resolution are that the species level is the only one able to detect subtle impacts because species exhibit a wide range of ecological characteristics and tolerances to a variety of disturbances (Nijboer and Schmidt-Kloiber 2004), and when genus-level resolution is used, these more subtle and specific impacts might be missed because the loss of species can be masked by replacement by more tolerant congeners. This pattern was clearly shown by Buss and Salles (2007) in a 4-km reach of the Guapimirim River. They found the genus *Americabaetis* along the whole impairment gradient, but more detailed analysis showed that *Americabaetis labiosus* was replaced by *Americabaetis alphus* as sewage effluents increased. This finding could be considered contradictory to the results in our study, but we think they should be seen as complementary. Bioassessments require only sufficient information to distinguish sites impaired by human activities from those in their natural or near-natural state (Jones 2008). The disagreement lies in determining the *optimal* amount of detail.

We see 2 ways to help resolve the issue of taxonomic resolution relative to RBP objectives: 1) more studies on pollution ecology are needed to understand better the effects of anthropogenic stresses and species' responses based on their biology and ecology, and 2) increased knowledge of species' life histories and development are needed to improve taxonomic keys and assessment tools, especially in developing countries where industrial activities are growing. Species- or genus-level identifications are needed for comprehensive national ecological surveys in which rigorous chemical and physical-habitat data are collected consistently along with the biota at all sites. Such surveys will help provide the autecological data needed to improve understanding of species/genus responses to natural gradients and anthropogenic stressors (Lenat and Resh 2001, Whittier et al. 2007, Hughes and Peck 2008).

We reaffirm that the *sufficient* taxonomic level depends on study objectives. Therefore, we agree with Lenat and Resh (2001) and Bailey et al. (2001) who suggested family-level identifications for situations where resources (money, time, or expertise) are limited, so that resources can be used for improving sampling methods and increasing replication (Marshall et al. 2006, Jones 2008). Studies focused on detection of gross among-site differences, studies in areas of known low diversity, and studies using multimetric and multivariate techniques probably can use coarser taxonomic resolutions. Several authors have suggested that family-level resolution could be used to distinguish between unimpaired, moderately impaired, and severely impaired systems (Plafkin et al. 1989, Hewlett 2000, Lenat and Resh 2001, Waite et al. 2004). Coarser taxonomic levels also might be used in volunteer programs to raise public awareness about stream and river quality (Buss 2008). However, studies dealing with conservation, life histories, and indicator groups, and those looking at specific types of perturbation are likely to require genus or species-level identifications. Also, coarse taxonomic resolution is not an option in some situations, such as when evaluation of known indicator species for a particular stressor could greatly reduce the cost of impact assessment (Bowman and Bailey 1997).

In conclusion, in our study, the 3 levels of taxonomic resolution were sufficient to discriminate sites according to their degree of impairment, but genus-level resolution was more effective for showing differences among environmental conditions in both river basins, especially when considering biotic index responses. However, this taxonomic level is not currently a feasible option to be implemented as a routine bioassessment program in Brazil. Order-level taxonomic resolution presents low costs and is easily applied, but our results showed lower discrimination of reference sites from intermediately impaired sites when considering biotic index responses. Of the 3 taxonomic resolutions tested, the family level is feasible for use over a wide area and many ecosystems, probably with few local/regional adjustments. Our results showed that family-level resolution provided similar discriminatory power to genus-level resolution. Moreover, family-level taxonomic resolution is being used in other countries, so our decision to use this level would allow data and methods to be compared, tested, and even shared among countries.

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