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# Incorporating natural variability in the bioassessment of stream condition in the Atlantic Forest biome, Brazil

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ABSTRACT

Most bioassessment programs in Brazil face difficulties when scaling up from small spatial scales because larger scales usually encompass great environmental variability. Covariance of anthropogenic pressures with natural environmental gradients can be a confounding factor in the evaluation of biologic responses to anthropogenic pressures. The objective of this study was to develop a multimetric index (MMI) with macroinvertebrates for two stream types and two ecoregions in the Atlantic Forest biome in Rio de Janeiro state, Brazil. We hypothesized that by using two approaches – (1) testing and adjusting metrics to landscape parameters, and (2) selecting metrics using a cluster analysis to avoid metrics redundancy – the final MMI would perform better than the traditional approach (unadjusted metrics, one metric representing each category). Four MMIs were thus developed: MMI-1 – adjusted MMI with metrics selected after cluster analysis); MMI-2 – adjusted MMI with one metric from each category; MMI-3 – unadjusted MMI with metrics selected after cluster analysis; MMI-4 – unadjusted MMI with one metric from each category. We used three decision criteria to assess MMI's performance: precision, responsiveness and sensitivity. In addition, we tested the MMI's by using an independent set of sites to validate the results. Although all MMIs performed well in the three criteria, adjusting metrics to natural variation increased MMI response and sensitivity to impairment. In addition, the selected MMI-2 was able to classify sites of two stream types and two ecoregions. The use of cluster analysis, however, did not avoid high redundancy between metrics of different branches. The MMI-4 had the poorest performance among all tested MMIs and it was not able to distinguish adequately reference and impaired sites from both ecoregions. We present some considerations on the use of metrics and on the development of MMI's in Brazil and elsewhere.



## 1. Introduction

Multimetric indices (MMIs) with macroinvertebrates are the most widely used approach for biological assessment and monitoring of aquatic ecosystems. This approach has been widely accepted because: (a) it is based on ecological concepts and processes, (b) it has the potential to assess ecological functions, and (c) it can discriminate human impacts (Helson and Williams, 2013; Mereta et al., 2013). However, one difficulty many bioassessment programs in Brazil face is scaling up from small spatial scales – from where most indices are developed (e.g. a river basin or hydrographic region) – to larger scales. Buss et al. (2015), analyzing 13 large-scale macroinvertebrate protocols from around the world discussed that limitations for scaling up may be associated with lack of logistics and funding, a reluctance to change established techniques or gear, or the fact that locally developed methods sometimes yield more accurate results than regionally applicable ones. Despite these difficulties, many countries succeeded in building widely applicable monitoring programs by using the same and/or compatible sampling protocols and by selecting metrics that are sensitive on large-scales (e.g. Stoddard et al., 2008; Moya et al., 2011; Jun et al., 2012).

Large-scale studies, however, may encompass great environmental variability. One approach to describe and account for variability in ecological studies is to classify areas in ecoregions. Ecoregions are usually defined as relatively homogeneous areas that have similar environmental conditions (Omernik, 1995). Ecoregions can be defined at different spatial scales, and aim to

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serve as a territory for investigation, assessment, management and monitoring of ecosystems, including the development of biological criteria and water quality standards (Kong et al., 2013). Some authors have found relationships between the macroinvertebrate fauna and environmental conditions at the ecoregional level (Barbour et al., 1996; Reynoldson et al., 1997). Other authors, however, have found macroinvertebrate fauna to be more strongly associated with micro/local scales, such as substrate and microhabitat (Gerth and Herlihy, 2006; Costa and Melo, 2008; Ligeiro et al., 2013). Acknowledging that, some countries incorporate local characteristics (stream types) in different biomonitoring protocols (AQEM, 2002; Buss et al., 2015). For example, New Zealand has different protocols for hard-bottomed and soft-bottomed streams (Stark et al., 2001). The authors argue that this separation is necessary because of significant differences in the structure and composition of aquatic communities among stream types, and thus different methods are required for sample collection and processing to be cost-effective. The definition of stream types allows the correct establishment of reference conditions that are comparable to the ecological status classifications within each group of rivers with similar characteristics (Stark et al., 2001).

Covariance of anthropogenic pressures with natural environmental gradients can be a confounding factor in the evaluation of biologic responses to anthropogenic pressures (Stoddard et al., 2008; Hawkins et al., 2010; Moya et al., 2011). One simple technique for normalizing metrics for natural gradients is to remove the stressor gradient from the data by focusing on reference-site data and to quantify the remaining correspondence between the metric value and the natural gradient (Stoddard et al., 2008). Recent studies aimed to test this and other alternative approaches for the development of MMIs (e.g. Chen et al., 2014; Macedo et al., 2016). The objective of this study was to develop a MMI for the Atlantic Forest biome in Rio de Janeiro state, Brazil. We hypothesized that by using two of these approaches – (1) testing and adjusting metrics to landscape parameters, and (2) selecting metrics using a cluster analysis to avoid metrics redundancy – the final MMI would perform better than the traditional approach (unadjusted metrics, one metric representing each category).

## 2. Materials and methods

### 2.1. Study area

The geomorphology of Rio de Janeiro state is composed of a group of coastal plains separated by hills and two mountain chains that run parallel to the ocean (Serra do Mar, ranging from altitudes 0–2000 ma s.l and Serra da Mantiqueira, ranging from 800 to 2500 m.a.s.l). In between the two mountain chains, lies the valley of the state's main river, Paraiba do Sul (at an altitude of around 800 ma s.l.). According to Alvares et al. (2013) 44% of Rio de Janeiro state's mid-lower portions is classified as tropical with a summer rainy season, with the most mountainous regions and plateaus classified as humid subtropical with hot summer, without dry season or with a dry winter. Temperatures oscillate between 15 °C and 28 °C and annual rainfall is around 1000–1500 mm. The Atlantic Forest biome, which originally covered virtually the entire region, now represents less than 12% of its original extent, and is mostly spread in the higher parts of the mountains and in remnants interspersed with agriculture and pasture (Ribeiro et al., 2011).

### 2.2. Sampled sites

We sampled 73 sites (once, during the dry season, in streams ranging from 1 st to 4th order, according to Strahler classification using 1:50,000 scale maps) representing two stream types, two ecoregions and three classes of impairment. Ecoregions were based on the classification of 'dominions' of RadamBrasil (Brasil, 1983). Stream types were classified as "transitional/sedimentary areas" (stream type 1) and "rocky substrates" (stream type 2; see below for details). We chose sites based on ad hoc indication and/or by previous knowledge of the area to represent sites classified as reference, intermediate or impaired. Sites classified as "reference" had "Optimal" or "Good" environmental condition according to the Habitat Assessment Protocol (HAP; see below for logic and measurement), absence of channelization and <25% upstream urban or industrial areas. Impaired stream reaches were classified as "Poor" condition according to HAP and >40% of upstream area affected by urban areas or intense farming or livestock grazing. Intermediate sites had characteristics between these two classes.

We sampled forty-nine sites in the sedimentary deposits ecoregion (SD). The SD ecoregion is located at the piedmont of Serra do Mar mountain range, with altitudes about 200 ma.s.l. and is a depositional zone formed by marine, lacustrine and fluvial sedimentation processes (Brasil, 1983). Being a transitional zone between erosive/depositional zones, sampled streams were divided into two predominant substrate types: reaches with >80% sand and clay ("transitional/sedimentary areas"; stream type (1) and reaches with >70% particles of gravel size or greater ("rocky substrates"; stream type (2). In this region, land use is dominated by patches of small-scale agriculture and livestock grazing, and minimally impacted areas are scarce. Reference areas in this ecoregion were classified as "least disturbed areas", according to the reference condition approach (RCA; Stoddard et al., 2006). Twenty-two sites of stream type 1 were sampled; of which six were reference, six intermediates and ten impaired. Twenty-seven sites of stream type 2 were sampled, of which fifteen were reference, three intermediate and nine impaired (Fig. 1).

We sampled twenty-four sites in the mountainous scarps ecoregion (MS). This ecoregion is located at higher altitude (from >200 ma.s.l. to around 1,800 ma.s.l) in a mountainous region with high slope and steep scarps. Streams in MS have a > 80% predominance of rocky substrates (stream type 2) – bedrocks in some reaches – with few patches of sand and formation of pools intertwined with riffles or runs. All sites were sampled within or near protected areas (conservation units), which were classified as "minimally disturbed areas" or "best attainable" (RCA; Stoddard et al., 2006). The latter occurred in areas outside conservation units, but had low to moderate impact by rural activities, and full or partial riparian vegetation and forest fragments.

### 2.3. Environmental and biological data

We sampled macroinvertebrates by using a kick-net sampler with mesh size of 500 μm. Twenty samples (around 20 $m^2$) were taken proportional to the substrates available in each site, following the multi-habitat method (Barbour et al., 1999). The percentage of available habitats was estimated by visual inspection. Substrates with less than 5% of the site area were not sampled. Samples were obtained from a site length of approximately 20 times the channel width. Samples were composited and conserved in the field in 80% ethanol and taken to the laboratory for further inspection. In the laboratory, samples were washed to remove coarse organic matter, such as leaves and twigs. The remaining material was placed in a sub-sampler (64 × 36 cm), divided into 24 quadrats, each measuring 10.5 × 8.5 cm (Patent application number PCT/BR2011/000144). Sub-sampling is a procedure widely used in formulating multimetric indices, to assure randomness of the procedure, making it less subject to inherent variations from changes in team members (Oliveira et al., 2011). Eight quadrats were chosen at random, following the procedures described in Oliveira