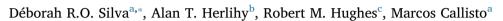
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**Original Articles** 

# An improved macroinvertebrate multimetric index for the assessment of wadeable streams in the neotropical savanna



<sup>a</sup> Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Laboratório de Ecologia de Bentos, Av. Antônio Carlos 6627, CP

486, CEP 30161-970, Belo Horizonte, Minas Gerais, Brazil

<sup>b</sup> Oregon State University, Department of Fisheries & Wildlife, 104 Nash Hall 97331-3803, Corvallis, OR, USA

<sup>c</sup> Amnis Opes Institute and Oregon State University, Department of Fisheries & Wildlife, 104 Nash Hall 97331-3803, Corvallis, OR, USA

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## ABSTRACT

Multimetric indices (MMIs) have been successfully used to assess ecological conditions in freshwater ecosystems worldwide, and provide an important management tool especially in countries where biological indicators are fostered by environmental regulations. Nonetheless, for the neotropics, the few published papers are limited to small local scales and lack standardized sampling protocols. To fill the gaps left by previous studies, we propose a stream MMI that reflects anthropogenic impacts by using macroinvertebrate assemblage metrics from a data set of 190 sites collected from four hydrologic units in the Paraná and São Francisco River Basins, southeastern Brazil. Sites were selected through use of a probabilistic survey design allowing us to infer ecological condition to the total of 9432 kilometers of wadeable streams in the target population in the four hydrologic units. We used a filtering process to determine the least- and most-disturbed sites based on their water quality, physical habitat structure, and land use. To develop the MMI, we followed a stepwise procedure to screen our initial set of biological metrics for influence of natural variation, responsiveness and discriminance to disturbances, sampling variability, and redundancy. The final MMI is the sum of 7 scaled assemblage metrics describing different aspects of macroinvertebrate assemblage characteristics: Ephemeroptera richness, % Gastropoda individuals, Shannon-Wiener diversity index, % sensitive taxa richness, % scraper individuals, temporarily attached taxa richness, and gill respiration taxa richness. The MMI clearly distinguished the least-disturbed sites from the most-disturbed sites and showed a significant negative response to anthropogenic stressors. Of the total length of wadeable streams in the study area, 38%, 35%, and 27% were classified by the MMI as being in good, fair, and poor condition, respectively. By reducing the subjectivity of site selection, rigorously selecting the set of reference sites, and following a standardized metric screening method, we developed a robust MMI to assess and monitor ecological condition in neotropical savanna streams. This improved MMI provides an effective ecological tool to guide decision makers and managers in developing and implementing improved, cost-effective environmental policies, regulations, and monitoring of those systems.

#### 1. Introduction

High quality and abundant water resources are directly associated with the integrity of biological communities inhabiting aquatic ecosystems (Dudgeon et al., 2006). Sustainable management and use of water resources provide multiple benefits and services to humans (Grizzetti et al., 2016; Vörösmarty et al., 2010). However, despite providing essential goods, freshwater ecosystems are among the most threatened by human pressures worldwide (Dudgeon et al., 2006). The intense demand for water by constantly growing human populations and economies results in widespread degradation of freshwaters (Abell et al., 2008; Limburg et al., 2011), as a result of habitat loss, water pollution, invasive species, overharvesting, and flow modification (Abell et al., 2008; Dudgeon et al., 2006; Revenga et al., 2005). Given this scenario, assessing ecological condition of aquatic ecosystems is critical for addressing efficient management practices to protect and rehabilitate integrity and ecosystem services (Balderas et al., 2016; Revenga et al., 2005).

Some of the most recognized ecological tools to monitor and manage freshwater ecosystems are multimetric indices (MMIs). In this

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<sup>\*</sup> Corresponding author. Present address: Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Laboratório de Ecologia de Bentos, Av. Antônio Carlos 6627, CP 486, CEP 30161-970, Belo Horizonte, Minas Gerais, Brazil.

E-mail addresses: deborah.ufmg@gmail.com (D.R.O. Silva), alan.herlihy@oregonstate.edu (A.T. Herlihy), hughes.bob@amnisopes.com (R.M. Hughes), callistom@ufmg.br (M. Callisto).

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approach, a combination of metrics representing assemblage attributes (e.g., composition, structure, function) are combined into a single measure (index) capable of reflecting multiple anthropogenic disturbances (Helson and Williams, 2013; Karr, 1999). First proposed for freshwater fish assemblages (Karr, 1981) and later adapted for other assemblages and ecosystem types, the plasticity of the MMI approach is based on a robust theoretical foundation (Karr, 1981). Over the years, the methodological process for developing an MMI has experienced a series of improvements aimed at increasing its applicability (Nazeer et al., 2016). Key improvements included the definition and selection of reference sites (Elias et al., 2016; Herlihy et al., 2008; Hughes et al., 1986: Ligeiro et al., 2013b: Stoddard et al., 2006: Whittier et al., 2007). rigorous statistical metric screening (Hering et al., 2006; Stoddard et al., 2008; Whittier et al., 2007), calibration for natural variance (Cao et al., 2007; Chen et al., 2017, 2014; Moya et al., 2011; Pereira et al., 2016), continuous MMI scoring criteria (Blocksom, 2003; Hughes et al., 1998), probabilistic sampling designs (Herlihy et al., 2000; Hughes and Peck, 2008), and national applicability (Moya et al., 2011; Paulsen et al., 2008; Stoddard et al., 2008).

It is desirable for MMIs to be applicable for large spatial scales (Hughes and Peck, 2008; Stoddard et al., 2008). Nonetheless, an MMI must be modified to account for regional differences (Dedieu et al., 2016; Klemm et al., 2003; Stoddard et al., 2008). In the U.S.A., specific MMIs were developed to account for well-established differences among regions (i.e. ecoregions, Omernik, 1987), subregions (Barbour and Gerritsen, 1996; Maxted et al., 2000), or aggregate ecoregions (Stoddard et al., 2008). In Europe, approaches for MMI development differ among countries and regions, considering its heterogeneous environments and political particularities (Hering et al., 2006; Mondy et al., 2012). Nonetheless, both the U.S.A. and Europe have legal statutes that support the use of biotic indicators to assess integrity at continental scales (Barbour et al., 1999; Bonada et al., 2006; Dedieu et al., 2016).

In contrast, neotropical countries lack specific legislation or guidelines for biological assessment, which is reflected by relatively few studies concerning the development and application of MMIs compared to the U.S.A. and Europe, where biotic and abiotic databases are well developed (Ruaro and Gubiani, 2013).

Despite many structural and political challenges, macroinvertebrate MMIs for neotropical regions have been successfully developed (Baptista et al., 2007; Dedieu et al., 2016; Helson and Williams, 2013; Macedo et al., 2016; Moya et al., 2011; Oliveira et al., 2011a; Pereira et al., 2016). For Brazil, there is a trend to develop macroinvertebrate MMIs for different regions (or biomes) such as the Atlantic Forest (Baptista et al., 2013, 2007; Oliveira et al., 2011a; Pereira et al., 2015, Suriano et al., 2011), Amazon (Couceiro et al., 2012), and more recently the savanna (Macedo et al., 2016). However, because they involve multiple academic institutions and lack a standardized methodology, those MMIs were developed using different methods, making it difficult to integrate information and compare results nationally (Buss et al., 2015).

The Brazilian neotropical savanna (sensu, "cerrado biome"), had an original natural cover area of approximately 2 million km<sup>2</sup> which has been strongly reduced as a result of pasture and monoculture expansion (Hunke et al., 2015). The second largest biome in Brazil, the savanna is considered a hotspot for biodiversity conservation strategies (Myers et al., 2000). It harbors many important large rivers and its network of headwater streams contain a large diversity of species and ecosystem services (Strassburg et al., 2017). However, stream and river ecological integrity is at risk because recent legislation has reduced the minimum required riparian buffer width (from 30 to 5–15 m, Brasil, 2012; see also Brancalion et al., 2016). Clearly there is a need to implement better ecological tools to assess stream condition (Buss et al., 2015; Moya et al., 2011).

A recent effort in the development of a preliminary macroinvertebrate MMI for savanna streams was proposed by Macedo et al. (2016), but it was developed for a single basin and based on few sites and few reference sites. As such, the index does not encompass enough variability to be applicable across the savanna biome.

To improve the development of an MMI in the neotropical savanna we: 1) extended the sampling area to four hydrologic units; 2) increased the number of least-disturbed reference sites for model development; 3) evaluated metric sampling variability by re-sampling sites; and 4) standardized the laboratory counting effort across samples. Thus, our approach embraced a greater variability and a wider range of anthropogenic impacts at multiple scales (e.g., agriculture, urbanization, nutrients, sedimentation). In that way, we not only filled gaps left by previous studies, but also provided the foundation and guidelines for developing and applying the MMI in other regions. Additionally, we used a probabilistic survey design to select the sampled sites, which allowed us to infer results to the total length of wadeable streams in the sampled area (Herlihy et al., 2000; Olsen and Peck, 2008). We also evaluated stream condition throughout each of the four different hydrologic units, and developed a regional neotropical savanna assessment. Following rigorous metric screening criteria, our objective was to develop a robust macroinvertebrate MMI for neotropical savanna streams, assess biological integrity, and relate the MMI scores to environmental disturbances.

## 2. Methods

#### 2.1. Study area

The study area comprised the upstream portion of 2 important river basins in the neotropical Brazilian savanna draining into four hydropower reservoirs: Nova Ponte, Volta Grande, São Simão (Paraná River Basin) and Três Marias (São Francisco River Basin). It covers a total geographic area of 45,180 km<sup>2</sup> (Fig. 1). We sampled sites once in each area (hereafter: hydrologic units, sensu Ferreira et al., 2017; Firmiano et al., 2017; Seaber et al., 1987), during the dry season in 2009-2012. The dry season is preferable to other seasons for sampling because it facilitates habitat distinction, the more constant discharges reduce natural flow variability, macroinvertebrate assemblage structure is more stable, and crew safety hazards and road access difficulties are minimized (Hughes and Peck, 2008; Melo and Froehlich, 2001; Plafkin et al., 1989). We re-sampled the Nova Ponte sites in 2013 to assess interannual sampling variability within the same season (Kaufmann et al., 1999). Also, an additional set of hand-picked reference sites (see below) were sampled in preserved areas of the Nova Ponte hydrologic unit in 2014.

The regional climate in the study area is humid tropical savanna, with a well-defined dry season from May to September (Hunke et al., 2015). Average precipitation ranges from 800 to 2000 mm, and average annual temperature ranges between 18 and 28 °C (Ratter et al., 1997). The savanna vegetation consists of dispersed trees and shrubs, small palms, and grass (Quesada et al., 2008) with heterogeneous gallery forests along watercourses (Urbanetz et al., 2013). The major land uses are agricultural cash crops, charcoal production, grazing, and urbanization (Macedo et al., 2014; Ratter et al., 1997).

#### 2.2. Survey design

Sites were selected through use of a randomized, systematic, spatially balanced sample design (Herlihy et al., 2000; Stevens and Olsen, 2004). We targeted a population of wadeable streams with access and flowing water at the time of sampling, defined as first to third order (Strahler, 1957), on 1:100,000 scale maps, located within an area 35 linear km upstream from the limits of the reservoirs. A random set of primary and alternate sites were selected to account for the fact that a number of primary random sites were non-target (e.g., dry, nonwadeable, inaccessible, access denied).

A probability survey like ours usually comprises sites across a wide

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