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

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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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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., 2016; 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^2 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^2 (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, non-wadeable, inaccessible, access denied).

A probability survey like ours usually comprises sites across a wide

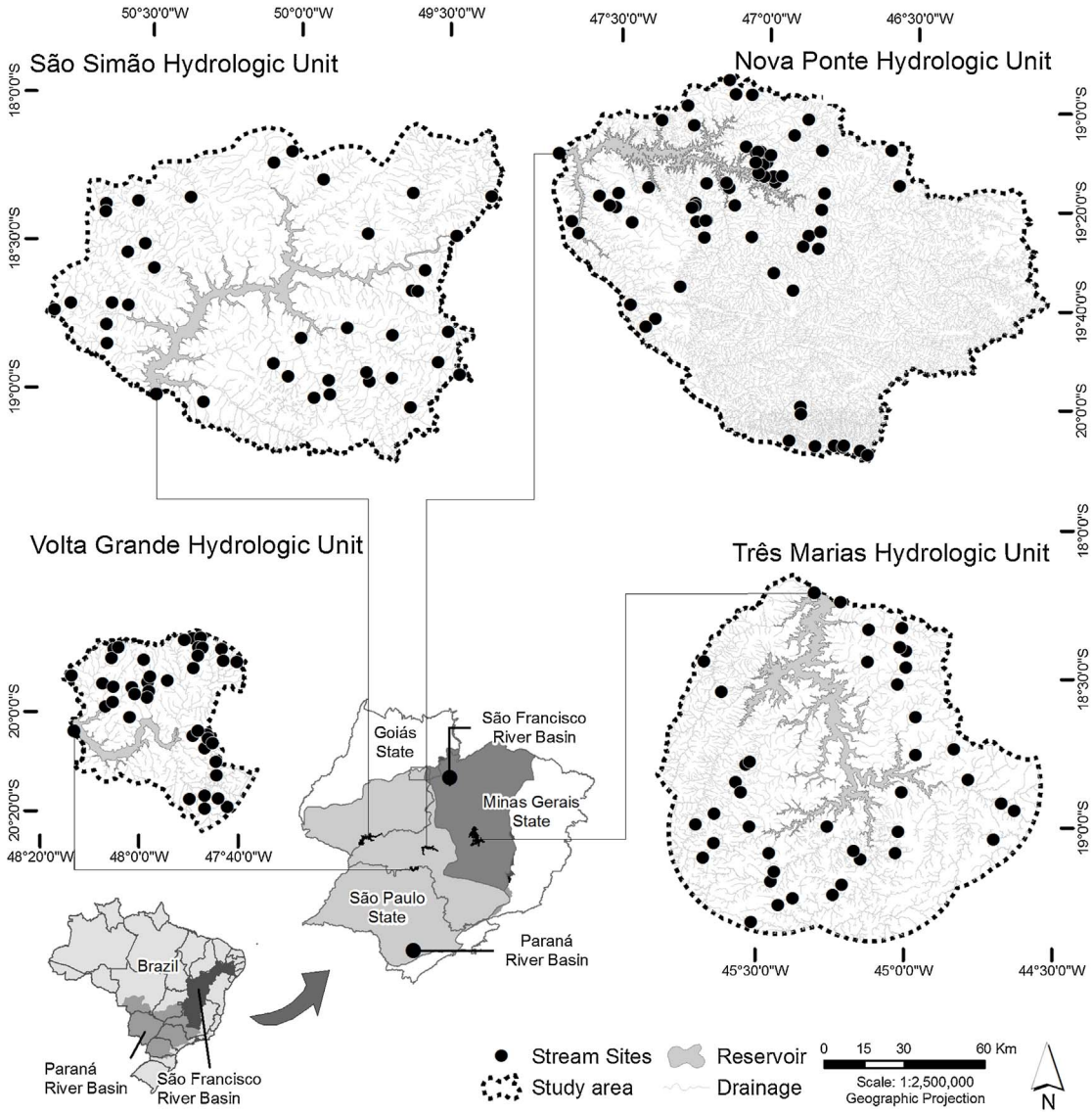

Fig 1. Distribution of sampled stream sites among four hydrologic units: Nova Ponte, Três Marias, Volta Grande, and São Simão.

range of intermediate disturbance condition, but is expected to have fewer sites in minimally disturbed or highly disturbed conditions (Herlihy et al., 2008; Stoddard et al., 2006). To guarantee a clearer disturbance gradient, we additionally hand-picked a number of sites likely to be in minimally disturbed condition (distant from urban areas, natural vegetation cover, no upstream dams or pollution sources), as well as a set of urban sites with highly altered physical and chemical conditions in each of the four hydrologic units, resulting in a total of 143 target random sites and 16 hand picked sites (~40 sites in each hydrologic unit). Because reference condition is a key component in developing a MMI, we also sampled an additional 31 hand-picked sites near or within protected areas in the Nova Ponte Basin (see Martins et al., 2017).

Each random sample site has a weight, calculated as the inverse of its selection probability, indicative of the length of stream it represents in the target population. These site weights were used to make estimates of regional condition from site data. Hand-picked sites have a weight of zero and are not used in estimating regional condition. Based on our sampling, there is an estimated 9432 km of target streams in the study area: 4515 km in Nova Ponte, 1641 km in Três Marias, 482 km in Volta Grande, and 2794 km in São Simão.

2.3. Benthic macroinvertebrate sampling

At each stream site, we set up a longitudinal sampling reach equal to 40 times the mean wetted width or a minimum of 150 m (Silva et al., 2014). Sample reaches had a mean depth of 35.4 cm (± 17.1) and mean width of 3.4 m (± 1.9). In each stream reach, we took six D-frame kick-net (500 μm mesh, 0.9 m^2 area) samples of the macroinvertebrate assemblage. The six samples were spaced at equal intervals along the sample reach with alternating left, center, and right cross-sectional positions, yielding a multi-habitat composite sample representative of natural patterns found in the stream reach and sensitive to environmental gradients (Gerth and Herlihy, 2006; Hughes and Peck, 2008; Li et al., 2014). Focusing on specific (target) or rare habitats can influence and overweight the final composite sample. Previous papers have found that for bioassessment purposes a systematic design is recommended (Gerth and Herlihy, 2006). The samples were fixed with 10% formalin, and taken to the laboratory. Macroinvertebrate samples were sorted and identified through use of a stereomicroscope (100× magnification) and taxonomic keys (Costa et al., 2006; Fernández and Domínguez, 2001; Merritt et al., 2008; Mugnai et al., 2010). All invertebrates were identified to the family level, except for non-insects, which were identified to either order or class levels (e.g., Oligochaeta, Bivalvia, Decapoda, Hirudinea).

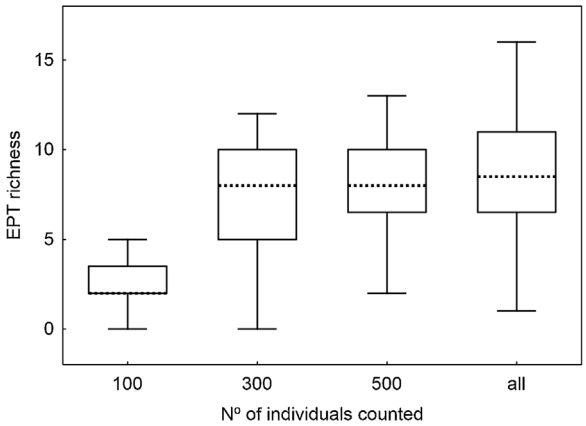

Fig. 2. Ephemeroptera, Plecoptera and Trichoptera (EPT) richness for fixed-counts of 100, 300, 500, and all individuals collected. Boxes represent the 25th and 75th percentiles, dotted lines within the boxes are medians, and whiskers are non-outlier ranges.

As taxa richness depends on the number of organisms counted, we wanted to standardize results to a fixed number of individuals in each sample (Larsen and Herlihy, 1998). We also sought to recommend a reliable number of individuals that can be used in future studies to save costs and processing time (Ligeiro et al., 2013a; Oliveira et al., 2011b). To test this, we calculated Ephemeroptera, Plecoptera and Trichoptera (EPT) richness using the full sample and fixed counts of 100, 300 and 500 individuals (Larsen and Herlihy, 1998) by just randomly picking the desired number of individuals from each sample. As expected, the total number of EPT taxa increased with increased number of individuals counted. By one-way ANOVA, counts of 300, 500, or all individuals collected did not differ significantly, but did differ from counts of 100 individuals (Fig. 2). Thus, 300 individuals counted was determined to be an appropriate sample size to assess ecological condition in savanna streams, which also limits costs and processing time without compromising ecological information. Other authors also have recommended counting 300 individuals for bioassessment purposes (Boonsoong et al., 2009; Larsen and Herlihy, 1998), although more samples and sample counts are recommended for accurate assemblage-structure comparisons (Li et al., 2014; Silva et al., 2016). Hereafter, all analyses were performed with the data set of 300 individuals counted or the entire sample when there were fewer than 300 individuals collected (less than 30% of samples).

2.4. Physical and chemical habitat measures

In each sample reach we recorded quantitative measures of physical habitat following Peck et al. (2006). Those measures describe stream channel morphology (e.g., slope, sinuosity, depth, wetted and bankfull widths, incision, bank angle), habitat features (substrate size, flow types, amount of wood in the channel), riparian structure (canopy cover, vegetation type), and human alterations in riparian zones (e.g., presence of buildings, pasture, crops, roads, trash). Following Kaufmann et al. (1999), we calculated metrics and indices combining those field measurements into a single value. For example, the riparian disturbance index (RDI) combines the various types of anthropogenic disturbance observations weighted by their proximity to the streambed. Similarly, relative bed stability is an anthropogenic sedimentation index calculated from the mean particle size measured in the field compared with the potential particle size in an undisturbed stream with the same stream power. More details on metric calculation are available in Kaufmann et al. (1999).

Water temperature, electrical conductivity, total dissolved solids, turbidity, and pH, were measured at each stream reach by use of portable equipment (YSI Model 650). A water sample was collected and transported to the laboratory for determining dissolved oxygen, total nitrogen, and total phosphorus (APHA, 1998).

2.5. Land use and cover

We determined the main land uses and cover in the catchment of each site through manual interpretation of fine resolution images from Google Earth (Google, 2016) and multispectral images from the Landsat satellite (see Macedo et al., 2014). Our evaluation resulted in four vegetation cover physiognomies (forested savanna, gramineous-woody savanna, park savanna, and palm swamp), being grouped into a single metric of natural cover and three anthropogenic land uses (urban, agriculture, and pasture). Following Ligeiro et al. (2013b) we calculated the integrated disturbance index (IDI), a combination of site- and catchment −scale measurements of anthropogenic pressures. The IDI is calculated by first measuring the riparian disturbance index (RDI; described above) and the catchment disturbance index (CDI). The latter is calculated by summing the% land uses, weighted by the potential of degradation that each one has in the aquatic ecosystem (CDI = 4 x % urban + 2 x % agriculture + % pasture). The IDI is the Euclidian distance between the site and the origin of the disturbance plane formed by the RDI and CDI. Therefore, the higher the IDI, the greater the disturbance on both scales. We also calculated other commonly used metrics to characterize anthropogenic disturbance, like population size, household and road densities, and distance from roads and cities (OpenStreetMaps®).

2.6. Site disturbance classification

We used a filtering procedure to identify least- and most-disturbed sites (Herlihy et al., 2008; Waite et al., 2000). The method is based on filtering all sites to previously established thresholds for physical habitat metrics, water-quality parameters, and land use. Because we were interested in achieving a minimum number of least-disturbed sites in each hydrologic unit, we developed specific thresholds for our nine parameters in each one (Table 1). If a site failed any one of the filters in Table 1 it was not considered to be least-disturbed. In a similar way, we defined most-disturbed sites using a similar filtering process. Any site that had any urban land use in the catchment, a riparian disturbance index > 2, or extreme values for water parameters (dissolved oxygen < 4.0 mg/L; total nitrogen > 0.2 mg/L or total phosphorus > 0.1 mg/L) was considered to be most-disturbed. Sites that did not match the least- or most-disturbed categories were classified as intermediate.

Table 1
Criteria for physical habitat structure, water quality, and land use for identifying least-disturbed sites. Sites that did not meet all criteria were excluded from the least-disturbed set. NP = Nova Ponte, TM = Três Marias, VG = Volta Grande, SS = São Simão. – Indicates the absence of the criterion in a hydrologic unit.

	Filter criteria	NP	TM	VG	SS
Physical Habitat	Riparian disturbance index	< 1	< 1	< 1	< 1
	% fine substrate	< 20	< 40	< 40	< 40
Water Quality	Dissolved oxygen (mg/L)	> 6.0	> 6.0	> 6.0	> 6.0
	pH	> 6; < 9	> 6; < 9	> 6; < 9	> 6; < 9
	Turbidity (NTU)	< 10	< 10	< 10	< 10
	Total nitrogen (mg/L)	< 0.2	< 0.2	< 0.2	< 0.2
	Total phosphorus (mg/L)	< 0.03	< 0.03	< 0.03	< 0.03
Land Use	% natural cover	> 40	> 40	–	–
	% urban	0	0	0	0

2.7. MMI development

2.7.1. Candidate metrics

We proposed *a priori* 114 metrics belonging to seven categories that describe aspects of taxonomic richness, taxonomic composition, diversity and dominance, tolerance, feeding group, mobility, and respiration. Those metrics were expected to have the potential to respond to anthropogenic impacts on macroinvertebrate assemblages and discriminate least- from most-disturbed sites (Karr and Chu, 1998; Tomanova et al., 2008).

Taxonomic richness is a common biodiversity measure and is defined as the number of taxa in a known area (Gotelli and Colwell, 2001). We calculated taxonomic richness at the family level representing the total assemblage (total taxonomic richness) and by subgroups of the macroinvertebrate assemblage (e.g., Ephemeroptera richness, EPT richness). Taxonomic composition was also expressed in terms of relative abundance of selected groups in terms of both percent of individuals (number of individuals in group/total number) and percent of total taxa richness (subgroup richness/total taxa richness). Groups consisted of both families and orders (e.g., % Diptera, % Chironomidae) or combined groups (e.g., % Chironomidae plus Oligochaeta, % EPT) in different taxonomic levels. Diversity and dominance metrics were calculated through use of popular indices (e.g., Shannon-Wiener Diversity Index, Simpson Diversity Index, and Margalef Diversity Index) and individual dominance measures (e.g., % of individuals in top 2 dominant taxa).

The tolerance metrics were based on taxa sensitivity to organic pollution, where we assigned values ranging from 1 (most tolerant) to 10 (most sensitive) for each taxon following the scores proposed by Junqueira et al. (2000) and additional sources when the information was not available for a specific taxon. We calculated individual metrics (e.g., % of sensitive taxa, super-tolerant taxa richness) and biological indices adapted to Brazil, such as the Biological Monitoring Working Party (BMWP, Junqueira et al., 2000), which is a sum of taxa tolerance scores in each site.

Metrics for richness, % of individuals, and% of taxa richness were also calculated for feeding groups, mobility, and respiration autecology following the taxa classifications of Tomanova et al. (2008) for neotropical streams and by additional sources in cases where the information was lacking. For each functional attribute (e.g. feeding group), we used fuzzy-coding to assign scores, based on the taxa affinity for each category (e.g. predators, collector-gatherer, scrapers), ranging from 0 (no affinity) to 3 (strong affinity). The advantage of this approach is that it accounts for the various types and levels of information available, the plasticity of a taxon, and its different life cycle stages (Chevenet et al., 1994). The fuzzy code scores were expressed as proportions in each category and the final percentage metrics were obtained by multiplying the proportion by the abundance of individuals. To obtain the richness and percent richness metrics we only considered the presence or absence of a category independently of the score (see Supplementary Material Table S1).

2.7.2. Metric screening

To increase comparability with studies from other continents, we followed the same metric screening steps used in other MMI development studies (Hering et al., 2006; Stoddard et al., 2008). Metrics that failed any of the set of screening criteria were removed from consideration in the final MMI. Initially, we performed a range test to eliminate metrics with very low variability (richness metrics with range < 5 and percentage metrics with range < 10% were dropped).

In the second screen, we assessed the influence of natural environmental variability on macroinvertebrate metrics. To do that, we tested the relationship of macroinvertebrate biological metrics to GIS-extracted environmental variables of altitude, elevation range, catchment slope, and catchment area obtained from Shuttle Radar Topographic Mission – SRTM (3 arc seconds; USGS, 2005) and catchment total annual rainfall (ANA, 2014) (see also Chen et al., 2014; Macedo et al., 2016; Pereira et al., 2016). We used multiple linear regression models (forward-stepwise) with our biological metrics dataset from least-disturbed sites against the predictor environmental variables, normalized by $\log_{10}$ when necessary and checked by Kolmogorov-Smirnov tests. For metrics where we obtained a significant ($p < 0.001$) relationship and the correlation coefficient (R^2) was greater than 0.3, we derived a natural gradient corrected metric by replacing the original metric with the residual of the metric based on the regression equation with the natural variable(s).

We screened our set of metrics for responsiveness by calculating *t*-tests comparing mean metric values of least- versus most-disturbed sites. We also measured the discriminance effect by calculating the quartile overlap (hereafter delta) obtained by subtracting the 25th percentile of least-disturbed sites from the 75th percentile of most-disturbed sites. We excluded metrics with t-values less than 3 and or deltas with interquartiles overlapping medians.

For the last screen, we quantified the stability of each metric to sampling variability by comparing the variance among sites (signal, S) to the variance between re-visits at the same sites (noise, N) (Kaufmann et al., 1999). The higher the signal-to-noise (S:N) ratio, the more stable the metric (Herlihy et al., 2008; Stoddard et al., 2008). Thresholds to eliminate metrics based on the S:N have varied among different studies with different indicator assemblages. Because our re-sampling visit occurred 4 years after the first sampling, we adopted a somewhat conservative approach, where we kept metrics with S:N > 1 or the highest possible values for categories of metrics in which we did not obtained S:N values > 1 (see Supplementary Material Table S2).

2.8. MMI calculation and selection

All metrics that passed the screening described above were considered for the MMI. Metrics were then distributed in the 7 categories that represented different structural and functional attributes of macroinvertebrate assemblages: taxonomic richness, taxonomic composition, diversity and dominance, tolerance, feeding habit, respiration, and mobility.

We used the continuous metric scoring method to calculate the MMI because of its better responsiveness and lower variability, also avoiding the subjectivity of a discrete scoring method (Blocksom, 2003; Hughes et al., 1998; Stoddard et al., 2008). Metrics were standardized to a 0–10 scale by interpolating metrics between floor and ceiling values. We assumed equal importance of metrics considering that previous studies did not find improved MMI performance by weighting metrics (Bellenger and Herlihy, 2010; Chen et al., 2017). For metrics that responded negatively to disturbance, we set the 95th percentile of the reference values as the ceiling and the 5th percentile of all sample values as the floor. In an opposite way, metrics that responded positively to disturbance received the 5th percentile of the reference values as the floor and the 95th percentile of all sites as the ceiling. This procedure is summarized below:

$$Positive\ \ metrics = 10^{*}\left(\frac{metric - floor}{ceiling - floor}\right)$$

$$Negative\ \ metrics\text{:}\ 10^{*}\left[1 - \left(\frac{metric - floor}{ceiling - floor}\right)\right]$$

Metric scores below/above the floor/ceiling were set to 0 or 10, respectively. We obtained the final MMI value by summing the seven metric 0–10 scaled values and standardizing to a 0–100 scale by multiplying by 100/70.

We ran an all subsets procedure to assemble all possible combinations of an MMI with 7 metrics (one from each category). For each possible MMI model combination, we obtained the S:N, *t*-test, delta, and maximum correlation among the 7 metrics.

The choice of the best MMI from the all subsets results was made first by screening out candidate MMIs that had S:N values > 2 and a correlation coefficient between any two metrics in the MMI < 0.7. The remaining candidate MMIs after this screen were evaluated for both delta and t-value. We picked the top 8 candidate MMIs with the highest delta and t-values. We regressed those MMIs against a series of anthropogenic stressors, and choose as the final MMI the one with the highest correlation coefficient to the greatest number of stressors.

2.9. MMI condition classification and stressors association

To evaluate the biological condition of savanna streams we established three categories representing different ecological quality levels. Thresholds for each class were obtained from the distribution of scores in the set of least-disturbed sites. Sites with an MMI score lower than the 5th percentile of the least-disturbed distribution were classified as "poor", scores between the 5th and 25th percentile were classified as "fair", and those higher than the 25th percentile were classified as "good". We inferred those results to the total target stream length in each hydrologic unit and for the entire target region.

We were also interested in identifying a stressor-response model that integrated multiple anthropogenic disturbances and best explained the distribution of MMI scores. To do that, we performed an all-subsets multiple regression approach with the MMI as a response variable and a list of candidate anthropogenic disturbances as predictive variables. When necessary, we log-transformed and checked normality (Kolmogorov-Smirnov tests) of the explanatory metrics and excluded redundant metrics with a Pearson correlation coefficient > 0.7. To choose the best model we used the corrected Akaike Information Criterion (AICc) weighted by importance of variables, which meant that we considered not only a non-overfitting model but also took into account the frequency in which the metric was present in all possible models (Burnham and Anderson, 2004; Sifneos et al., 2010). We additionally inspected for breakpoints in AICc weighted variable importance values to help determine variables to be included in the final regression model. All analyses were performed using SAS statistical software (SAS Institute, version 8.0, Cary, North Carolina) and Statistica software (StatSoft Inc., version 8.0).

3. Results

3.1. Biological data

We collected a total of 89 taxa, 65 in Nova Ponte, 63 in Três Marias, and 59 in both Volta Grande and São Simão. Diptera (56%), Ephemeroptera (15%), and Coleoptera (11%) were the most abundant groups, represented respectively at the family level, by the Chironomidae (46%), Baetidae (7%), and Elmidae (10%).

3.2. Site disturbance classification

After filtering all the sites using the criteria in Table 1, we identified a total of 53 least-disturbed sites, 95 intermediate sites and 42 most-disturbed sites. Of the total number of least-disturbed sites, 30 were hand-picked in Nova Ponte. Those sites were within or near protected areas in the Serra da Canastra National Park and Serra do Salitre region, whereas the others were distributed throughout the study area. Therefore, we adopted the "least-disturbed" term for regions where "minimally disturbed" sites did not exist because of intensive human exploitation of the land (Stoddard et al., 2006; Whittier et al., 2007). Although we recognize the lack of some important filters in our definition of least-disturbed (e.g., presence of contaminants), we followed rigorous criteria with 9 disturbance factors to obtain the set of least-disturbed sites (Table 1). Flexibility in some thresholds also allowed a minimum number of least-disturbed sites for each hydrologic unit and ensured that our MMI was representative of all four hydrologic units and could be extended to the neotropical savanna.

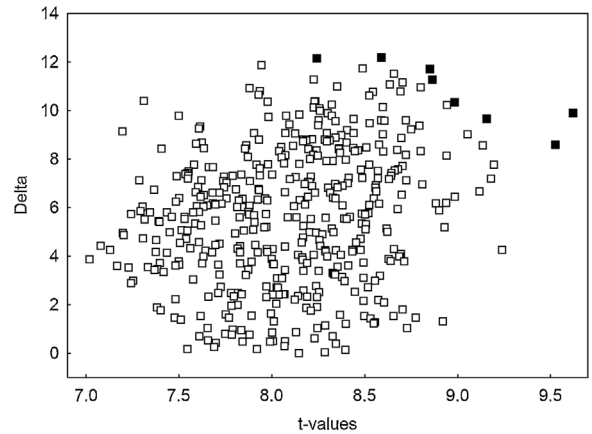

Fig. 3. All subsets results of MMI delta (disturbance interquartile overlap) and t-values for distinguishing least- from most-disturbed sites. Only models with an S:N > 2, maximum correlation < 0.7, and delta > 0 are shown. Dark rectangles represent the MMIs with the best balance between both values that were chosen for final consideration.

3.3. Metric selection and index development

We reduced our initial set of 114 metrics down to 35 by excluding 11 metrics that showed no variability, 28 metrics that failed to distinguish least- from most-disturbed sites in the responsiveness test (t-value < 3), 19 that failed the delta discriminance evaluation, and 21 metrics with low signal to noise (S:N < 1). We only found 3 metrics correlated with natural environmental variables: % of Diptera individuals (catchment area, altitude, catchment slope), BMWP index (altitude, catchment slope), and % of gilled individuals (catchment rainfall, catchment elevation range, catchment slope), which we adjusted based on the residuals of the models.

The final set of 35 metrics that were candidates for the MMI included 6 taxonomic richness metrics, 7 taxonomic composition metrics, 3 diversity and dominance metrics, 8 tolerance metrics, 5 feeding group metrics, 2 mobility metrics, and 4 respiration metrics. All possible combinations of metrics that included one from each group yielded 40,320 different MMI models. We picked eight models with the highest balance of delta and t values (Fig. 3), and chose the one with the best response to anthropogenic disturbances (Table 2). The final MMI metrics were Ephemeroptera richness, % Gastropoda individuals, Shannon-Wiener diversity index, % sensitive taxa richness, % scraper individuals, temporarily attached taxa richness, and gill respiration taxa richness.

The final MMI scores clearly separated the 25th percentile of least-disturbed sites from the 75th percentile of most-disturbed sites (Fig. 4). When we examined the MMI by hydrologic unit, we observed an interquartile overlap only for the Volta Grande hydrologic unit (Fig. 5), in which the least-disturbed sites were more disturbed by anthropogenic stressors than in the other hydrologic units.

3.4. Assessment of ecological status

The final MMI scores were strongly associated with anthropogenic disturbances ($r^2 = 0.41$) and the stressor model that best explained the MMI included the integrated disturbance index (IDI) score, % fines, log relative bed stability, total nitrogen, % urban land use, and distance from road (Table 3). The IDI was the metric that contributed most to explaining MMI scores and was present in all considered models (AICc weighted importance value of 1.0).

Because of our probabilistic survey design, we were able to infer our results to the total target stream length in the sampled area to assess ecological status (Fig. 6). The Nova Ponte hydrologic unit had the

Table 2
Pearson correlation coefficients between the final MMI and anthropogenic stressors.

Anthropogenic stressors	Correlation coefficient (r)	
IDI – Integrated Disturbance Index[a]	−0.52	**
Land use and cover		
% anthropogenic land use	−0.38	**
% urban	−0.36	**
% pasture	−0.09	n.s.
% agriculture	−0.13	n.s.
Catchment road density	−0.31	**
Distance from road	0.18	*
Distance from cities	0.11	n.s.
Physical habitat		
Riparian disturbance index[b]	−0.43	**
Mean embeddedness	−0.31	**
Log relative bed stability	0.45	**
Mean woody riparian vegetation	0.23	*
% fine sediment	−0.46	**
Chemical habitat		
Dissolved oxygen	−0.10	n.s.
Turbidity	−0.32	**
Conductivity	−0.29	**
Total dissolved solids	−0.22	*
Total nitrogen	−0.36	**

*p < 0.01, **p < 0.0001, n.s. not significant.
[a] Ligeiro et al. (2013b).
[b] Kaufmann et al. (1999).

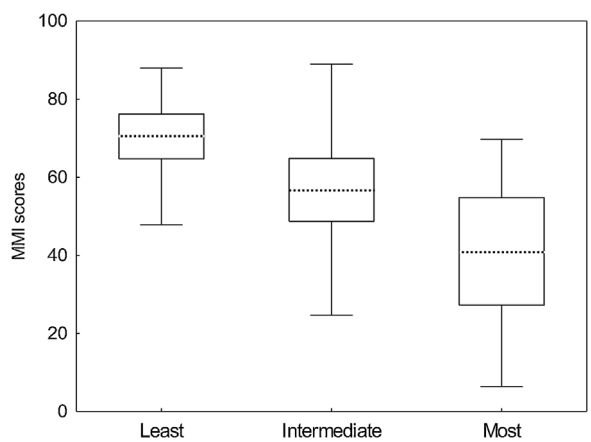

Fig. 4. Regional assessment for disturbance categories (ANOVA test, $F_{(2,187)} = 51.6$, $p < 0.0001$). Boxes represent the 25th and 75th percentiles, dotted lines within the boxes are medians, and whiskers are non-outlier ranges.

highest percent of stream length in good condition (over 50%), whereas Volta Grande had the highest percent of stream length in poor condition (35%). Our overall regional bioassessment estimated that 38% of the stream length sampled (3594 km) was in good condition, 35% (3275 km) in fair condition, and 27% (2546 km) in poor condition.

4. Discussion

4.1. Reference site and metric selection

We developed a macroinvertebrate MMI based on 7 metrics to assess the ecological condition of wadeable streams in the Brazilian neotropical savanna. The use of different hydrologic units allowed us to account for different sources of anthropogenic disturbances and natural environmental conditions. Therefore, the index should be applicable in other neotropical savanna streams and perhaps savanna globally. In addition, the methodological development of the MMI followed rigorous criteria, facilitating comparisons with other studies.

The building of an MMI is a stepwise process that begins with the

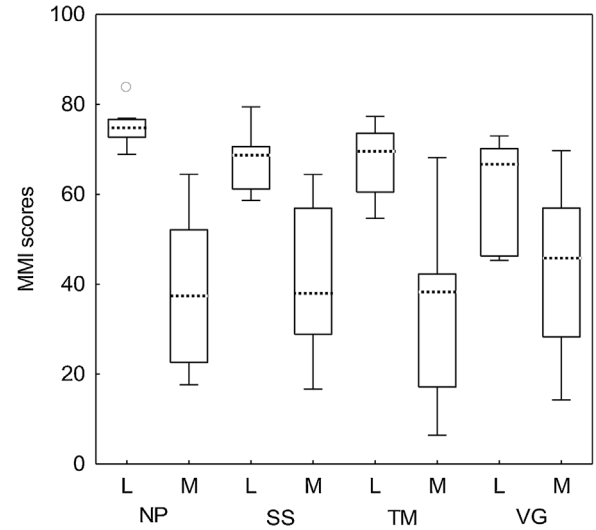

Fig. 5. MMI scores for disturbance categories in each of the four hydrologic units. Boxes represent the 25th and 75th percentiles, dotted lines within the boxes are medians, whiskers are non-outlier ranges, and outlier denoted by open circle. L = least-disturbed, M = most-disturbed, NP = Nova Ponte, SS = São Simão, TM = Três Marias, and VG = Volta Grande.

Table 3
Multiple linear regression model of predicted anthropogenic stressors explaining the final MMI ($F = 22.4_{6,181}$; AICc = 1500; adjusted $R^2 = 0.41$; $p < 0.0001$). Beta is the regression coefficient and Std. Err. is the standard error of the regression coefficient. The AICwi column indicates the stressor variable weighted by its AIC importance.

	AICwi	Beta	Std.Err.	*p* value
Intercept	1.00	73.4	2.83	< 0.0001
Integrated Disturbance Index (IDI)	1.00	−16.3	3.69	< 0.0001
% fines	0.99	−0.127	0.0543	0.021
Log relative bed stability	0.88	2.27	1.106	0.041
Total nitrogen (mg/L) (log x + 1)	0.79	−135	67.1	0.046
% urban	0.76	−0.217	0.112	0.054
Distance from road (km) (log x + 1)	0.71	0.000409	0.000231	0.079

definition of reference sites. An MMI is expected to be able to distinguish disturbed sites from a reference natural condition by means of the biological assemblage responses (Hughes et al., 1986; Ligeiro et al., 2013b; Stoddard et al., 2006). Because of this, the reference condition should describe sites minimally affected by anthropogenic activities and where physical, chemical, landscape, and biological features represent natural patterns and processes (reference) condition across a region (Ligeiro et al., 2013b; Stoddard et al., 2006; Whittier et al., 2007). However, minimally disturbed sites are rarely found in regions where human activities are long-term and widespread (Whittier et al., 2007). This is especially the case for the neotropical savanna, where agriculture and pasture activities are in continuous expansion (Hunke et al., 2015). Our selected reference sites were filtered for well defined criteria of water quality parameters, land use, and riparian disturbance and can be considered as least-disturbed sites (Herlihy et al., 2008; Whittier et al., 2007). Specific hydrologic units had their thresholds relaxed to allow the inclusion of sites in the best available condition for that unit. Still, in general, our set of least-disturbed sites described streams with low nutrient concentrations, minimal riparian disturbance, and no urbanization in the catchment. Although not representing an optimal scenario of natural condition (a true minimally-disturbed reference condition, sensu Stoddard et al., 2006), the proposed MMI succeeded in distinguishing least- from most-disturbed sites.

The proposed MMI based on 7 macroinvertebrate assemblage metrics represents different structural and functional assemblage attributes. This approach accounts for the various dimensions of biological systems, which facilitates its ability to reflect human disturbances in

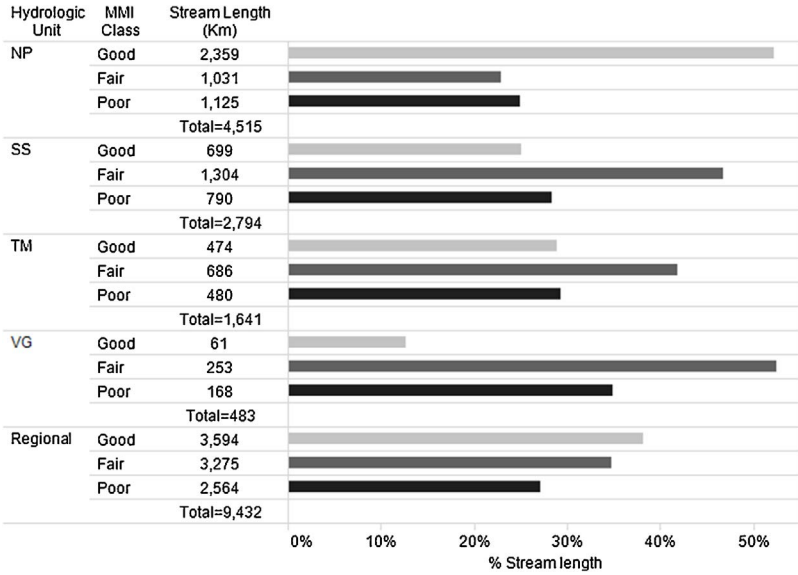

Fig. 6. Total and percent of stream length in MMI classes of biological condition (good, fair, and poor) in each hydrologic unit and for the regional assessment. NP = Nova Ponte, SS = São Simão, TM = Três Marias, and VG = Volta Grande.

aquatic ecosystems (Karr and Chu, 1998). The representativeness of several biological aspects in an integrated MMI is appropriate and recommended by many authors (Hering et al., 2006; Huang et al., 2015; Karr and Chu, 1998; Stoddard et al., 2008).

Considering the metrics included in our MMI, the number of Ephemeroptera families is a common richness metric also used in other MMIs (Bellucci et al., 2013; Mereta et al., 2013). This macroinvertebrate order has long been recognized as an important indicator of biological health because of its sensitivity to disturbance (Arimoro and Muller, 2010; Bauernfeind and Moog, 2000; Pond, 2010; Siegloch et al., 2014). Firmiano et al. (2017) found a clear decrease in specific Ephemeroptera taxa to multiple anthropogenic disturbances. Although some Ephemeroptera taxa are tolerant (e.g., some Baetidae and Caenidae), which can lead to a variable response to disturbance (Pereira et al., 2016), that seems more reflected in composition metrics than richness metrics. Percent Gastropoda individuals was the metric representing the composition category, increasing in abundance with increased disturbance. Those organisms are commonly associated with the increase in organic matter accompanying eutrophication processes (Verdonschot et al., 2012).

The Shannon-Wiener diversity index is a common diversity and dominance metric in bioassessment studies, including many indices developed for the neotropical region (Dedieu et al., 2016; Helson and Williams, 2013; Suriano et al., 2011; Touron-Poncet et al., 2014). It differs from taxonomic richness by including both a taxa richness component and an evenness component, and in our case it was not correlated with richness of Ephemeroptera families.

For the tolerance category, we selected the percent of taxa richness with pollutant tolerance values ≥ 7 (on a scale where 10 is the most sensitive taxa to pollution and 0 is the most tolerant). This category is especially important because, as opposed to diversity or richness metrics, it accounts for the specific responses of the different taxa to disturbance (Gabriels et al., 2010; Hilsenhoff, 1988; Whittier and Van Sickle, 2010).

The use of functional attributes of assemblage composition is also recommended for the development of MMIs (Moya et al., 2011; Saito et al., 2015), because of their ability to detect anthropogenic disturbances independently of taxonomic composition (Tomanova and Usseglio-Polatera, 2007). We evaluated 3 functional attributes to improve the robustness of our MMI approach. The richness of taxa that use gills for respiration is particularly sensitive to environmental stress because of the permeability of gills and their relation to dissolved oxygen concentrations (Chapman et al., 2004; Dolédec et al., 2006; Saito et al., 2015). Temporarily attached taxa richness was the metric selected in the mobility category; those organism often have adaptations (hooks, suction structures or fixed cases) to heterogeneous habitats, characterized by fast flows (Lamouroux et al., 2004). Such organisms are sensitive to the amounts of sand and fine sediments suspended in the water column and deposited on the streambed, which are typical products of excess catchment and streambank erosion (Bryce et al., 2010). Finally, in the feeding group category, % scraper individuals responded negatively to impairment. That metric is commonly associated with sediment and nutrient inputs to the streambed (Larsen et al., 2011).

Differences in the final selected metrics for the MMI compared with other neotropical studies can be attributed to biogeographic differences, anthropogenic impact levels and types, and differences in the methodological development of the MMI (Dedieu et al., 2016). Another important differences is the taxonomic resolution that the different studies used (Dedieu et al., 2016). A MMI can benefit from refined taxonomic resolution because of the greater sensitivity of certain genera (or species) in detecting multiple stressors and better discriminating differences in biological condition (Lakew and Moog, 2015; Touron-Poncet et al., 2014). Nonetheless, we developed an MMI at the family taxonomic level that can be reproduced at lower cost, less laboratory time, and by non-experts (Hilsenhoff, 1988; Ríos-Touma et al., 2014; Suriano et al., 2011) without compromising excellent index performance.

As highlighted by Macedo et al. (2016), who developed a preliminary MMI for one hydrologic unit in the neotropical savanna, further improvements were needed to select metrics. Indeed, evaluating metric stability (S:N test) helped us to identify metrics that varied among sites because of differences in stream condition rather than by sampling variation within a site (Chen et al., 2014; Stoddard et al., 2008). From the 4 metrics selected by Macedo et al. (2016) to build their best performing MMI, 3 of them failed in our responsiveness or discriminance test and had low signal-to-noise values in the screening step. Because of that, their index may perform well in the context it was developed but certainly would perform below expectations when applied elsewhere.

Finally, another important step in developing MMIs involves correctly distinguishing the effect of natural variation from covarying anthropogenic pressures. Recent papers suggested that correcting for the effect of natural variation on biological metrics increased MMI responsiveness and sensitivity to impairment, although responding similarly when compared with unadjusted models (Carvalho et al., 2017; Chen et al., 2014; Macedo et al., 2016; Pereira et al., 2016). Macedo et al. (2016) found that the adjusted MMI for savanna streams in a small

geographic area (~7500 km^2) performed better when compared with unadjusted models, and recommended adjustments for future studies especially in larger geographic areas, as we did in this study (~45,000 km^2). Nonetheless, although we corrected 3 metrics, none of them were retained in the final MMI. Besides, our final MMI was not related to any of the natural gradients we assessed ($r^2 < 0.06$), and was more strongly related to anthropogenic stressors. Because natural variation is important in larger geographic areas and can affect biological assemblages differently (Stoddard et al., 2008), we recommend that future studies evaluate that variation to avoid biased assessments and erroneous inferences (Chen et al., 2017, 2014).

4.2. Overall MMI assessment

The multiple linear regression model explained 41% of the variation in MMI scores through the combination of six explanatory variables describing land use, water parameters, and physical habitat structure. The Integrated Disturbance Index (IDI; Ligeiro et al., 2013b) was the explanatory variable that most contributed to the model explanation. That index aggregates in a single measure information about anthropogenic disturbance at both local and catchment scales (Ligeiro et al., 2013b). The IDI was used as an objective method to define least- and most-disturbed sites in other studies concerning the development of multimetric indices in the neotropics (Chen et al., 2017; Macedo et al., 2016; Terra et al., 2013) because of its ability to summarize multiple anthropogenic disturbances independently of biological measures (Ligeiro et al., 2013b). Other authors found the IDI an important explanatory variable for macroinvertebrate richness in streams (Firmiano et al., 2017) and reservoirs (Martins et al., 2015), reinforcing the ability of the IDI to reflect biological condition and corroborating our findings. Two sediment related variables were also in the model: log relative bed stability and% fine sediments. Although based on different concepts, both variables have been reported as strongly affecting macroinvertebrate assemblage composition, structure, and function (Bryce et al., 2010; Sutherland et al., 2012).

Nutrient enrichment of aquatic ecosystems is the main cause of water quality impairment worldwide (Woodward et al., 2012). Previous studies have demonstrated the association of watershed land use with the concentration of nutrients in aquatic ecosystems (Herlihy et al., 1998). Excess nutrients in water bodies result in a cascade of effects involving excessive primary production, habitat degradation, altered food sources, higher turbidity, and fish kills, among others (Herlihy and Sifneos, 2008; Wang et al., 2007). Although nutrient effects on aquatic communities can be variable (Heino et al., 2003), other authors have addressed the negative impacts of increased nitrogen on macroinvertebrate structure, corroborating our findings (USEPA, 2016; Wang et al., 2007; Yuan, 2010).

Both the urbanization and distance from road explanatory variables reflect changes to the natural land cover of the region, which can have substantial impacts on stream ecosystems (Roy et al., 2003; Walsh et al., 2007; Wang et al., 2012). Urbanization and roads affect the biota via multiple direct and indirect pathways by increasing impervious surface area, altering hydrology and sediment transportation, modifying channel morphology, increasing pollutant loads, and creating migration barriers (Hughes and Dunham, 2014; Leitão et al., 2017; Sterling et al., 2016).

Differences found in ecological conditions among the four hydrologic units can be associated with the different degrees and types of human impacts and the quality of the selected reference (or least-disturbed) sites (Hughes et al., 1986; Pont et al., 2009). These differences explain why Volta Grande showed an interquartile overlap between least- and most-disturbed sites (Fig. 5), had the highest percentage of stream length in poor condition, and had the lowest percentage of stream length in good condition (Fig. 6). Volta Grande is dominated by row crop agriculture (mean ~70% in site catchments) and it has the most intense urban use compared with Nova Ponte, São Simão, and Três Marias (Callisto et al., 2014; Ferreira et al., 2017), so it is likely that Volta Grande lacks streams with biological integrity. That said, our MMI scoring was subject to the varying thresholds used in each hydrologic unit to classify least-disturbed condition, and interpretations of results must account for that. MMI assessments based on a hydrologic unit perspective facilitate focusing on more local management practices. For example, Nova Ponte had the highest percentage of stream length in good condition of the four hydrologic units, which should guide efforts toward catchment protection. However, Volta Grande management efforts should focus on catchment rehabilitation and mitigation of human impacts.

Overall, our regional bioassessment estimated that 27% of the stream length was in poor condition. Jim & nez-Valencia et al. (2014) estimated that 62% of the stream length in the Guapiaçu-Macacu Basin (Rio de Janeiro state) was in poor condition. The major stressors there were site habitat degradation and riparian and catchment deforestation. In the conterminous U.S.A., Paulsen et al. (2008) reported that 28%, 52%, and 40% of stream length was in poor condition in the West, Eastern Highlands, and Plains and Lowlands aggregated ecoregions, respectively. They did not assess catchment disturbance, but determined that excess nutrients and fine streambed sediments were the most important stressors in all three regions.

4.3. Future perspectives

The MMI we developed accounted for many shortcomings in previous studies, allowing us to improve current methodologies in developing a cost-effective biological tool to assess stream condition. Our methodology is being effectively applied in another important Brazilian hydrographic basin assessment (the Pandeiros River Basin; FAPEMIG, 2015). Nonetheless, in Brazil, as in most South American countries, the lack of legislation for biological assessments hinders the application and development of MMIs. Minas Gerais state is an exception because it has a regulation recommending the use of biological indicators in the assessment of aquatic ecosystems (COPAM/CERH-MG/2008). Our MMI application not only demonstrates development of another MMI, it is also demonstrates how a state-wide and national biological assessment could be implemented in a cost-effective manner.

5. Summary and conclusions

We successfully developed a macroinvertebrate-based MMI capable of distinguishing least- from most-disturbed streams. The MMI responded to a variety of anthropogenic stressors describing land use, water quality, and physical habitat structure. Our MMI is an improvement over the preliminary MMI of Macedo et al. (2016) for several reasons. Compared with Macedo et al. (2016), we sampled 4 hydrologic units versus 1, 190 sites versus 40, 31 additional least-disturbed reference sites, and 40 revisit sites. In addition, we selected reference sites by a filtering process based largely on site abiotic conditions, evaluated 114 metrics versus 80, added a signal-noise screen of the candidate metrics, selected 7 versus 4 final metrics, and examined 8 candidate MMIs versus 4. Those differences resulted in our ability to conduct both regional and hydrologic unit assessments and develop a more rigorous MMI with only 1 metric in common with Macedo et al. (2016). Finally, we inferred our results from approximately 22 km of studied stream reaches to the whole population of over 9000 km of wadeable streams in the sample region.

To guarantee wide applicability of the MMI, we followed a probabilistic sampling design to select sites where we applied a standardized field sampling protocol (US-EPA, Peck et al., 2006) and established rigorous criteria for defining reference sites (Herlihy et al., 2008) and screening metrics (Hering et al., 2006; Stoddard et al., 2008). This resulted in a more robust and accurate tool for assessing ecological condition of neotropical savanna streams. We believe that our index can be applied in the four studied hydrologic units as well as in all neotropical

savanna streams. Furthermore, our approach is especially important to hydropower companies and environmental managers, because our study area comprises the catchments of four important hydroelectric dams in the states of Minas Gerais, São Paulo, and Goiás. Therefore, our MMI can be used for assessing the effects of management practices, land uses, and mitigation projects in those states (Macedo et al., 2016).

The constant threats to the savanna biome, its hydropower potential, and its high biological diversity, make effective conservation practices and sustainable management urgent (Callisto et al., 2014; Loyola and Bini, 2015). Our improved MMI is intended to support decision makers and scientists interested in: 1) assessing and diagnosing the stream-length condition of the entire neotropical savanna, 2) detecting potential areas for focused management and conservation practices, 3) identifying the major stressors altering biological condition, and 4) providing an ecological foundation for managing river basins, including those influenced by hydropower plants. In other words, we believe that our results provide a foundation for developing improved legal policies and monitoring programs for improving and assessing water resources comparable to those existing in the U.S.A., Europe, and Australia (Clean Water Act, Water Framework Directive, and Sustainable Rivers Audit, respectively).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2017.06.017.

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