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Development of a benthic macroinvertebrate multimetric index (MMI) for Neotropical Savanna headwater streams

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ABSTRACT

Assessing the ecological impacts of anthropogenic pressures is a key task in environmental management. Multimetric indices (MMIs), based on aquatic assemblage responses to anthropogenic pressures, have been used increasingly throughout the world. The MMI approach is a low-cost, rapid field method that produces an aquatic condition index that responds precisely to anthropogenic pressures, making it useful for conservation and environmental management. We developed four candidate MMIs based on benthic macroinvertebrate assemblages sampled at 40 randomly selected sites to assess the environmental condition of streams upstream of a hydroelectric power plant in the Brazilian Neotropical Savanna biome. Those MMIs were built from landscape-adjusted and unadjusted biological metrics as well as two alternative ways of choosing metrics. The alternative MMIs performances were tested by comparing their precision to distinguish least-disturbed areas, responsiveness to discriminate least- and most-disturbed areas, and sensitivity to anthropogenic pressures at catchment and local scales. The best performing MMI had landscape-adjusted metrics and was produced through use of principal component analysis for metric selection. It included 4 metrics: Ephemeroptera richness, average tolerance score per taxon, percentage of predator individuals, and percentage of Odonata individuals adjusted by elevation. This index discriminated well the anthropogenic pressures at local- and catchment-scales, and at both scales simultaneously, as indicated by an integrated disturbance index. Our methodological development included statistical criteria for identifying least- and most-disturbed sites, calibrating for natural landscape variability, and use of non-redundant metrics. Therefore, we expect it will provide a model for environmental assessment of water resources elsewhere in Brazil and in other nations.

1. Introduction

Aquatic ecosystems around the world have been highly altered by anthropogenic pressures, inducing alterations in energy and matter flows that compromise biotic viability (Hughes and Noss, 1992; Karr, 1998, 1999; Davies and Jackson, 2006). Several studies have shown that catchment-level anthropogenic pressures are reflected in local physical habitat simplification, water pollution, and degraded aquatic assemblage condition (Wang et al., 1997; Allan, 2004; Hughes et al., 2010). Multimetric indices (MMIs), built from multiple biological assemblage attributes, are very useful for evaluating anthropogenic pressures. The multimetric approach integrates responses of several assemblage components (e.g., richness, composition, trophic guilds, dominance) related to impacts caused by anthropogenic pressures in a simple, but accurate manner (Hughes et al., 1998; Karr, 1998; Hering et al., 2006; Stoddard et al., 2008; Ferreira et al., 2011; Helson and Williams, 2013). Furthermore, MMIs show an integrated response of multiple anthropogenic pressures at both catchment and site scales, and thereby avoid equivocal responses of single biological, physical or chemical measurements (Karr, 1999).

The index of biotic integrity (IBI) was the first MMI developed to evaluate biological responses to anthropogenic pressures for fish assemblages (Karr, 1998). Since then it has been applied

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successfully to benthic macroinvertebrate assemblages worldwide (e.g., Hering et al., 2006; Stoddard et al., 2008; Moya et al., 2011; Chen et al., 2014). MMIs are very useful ecological indices that are easily interpretable and rapidly developed (Karr, 1998). When correctly developed, the MMI approach is more cost effective than purely physical and chemical water monitoring (Hughes and Noss, 1992).

However, covariance of anthropogenic pressures with natural environmental gradients can confound our efforts to discern biologic responses to anthropogenic pressures (Klemm et al., 2003; Moya et al., 2011; Feld et al., 2016). To counteract the effects of this covariance, researchers model the influence of natural landscape variables on biological metrics by regressing potential controls (e.g., drainage area, slope, elevation and precipitation) against those metrics. The regression residuals are extracted to produce adjusted metric scores (e.g., Cao et al., 2007; Stoddard et al., 2008; Moya et al., 2011; Chen et al., 2014).

Another critical point in MMI development is the process for selecting among highly correlated metrics to avoid redundancy (Cao et al., 2007; Stoddard et al., 2008). Multivariate analysis has been found capable of revealing complex data structure and removing redundant metrics in a quantitative, non-subjective manner (O'Connor et al., 2000; Cao et al., 2007; Van Sickle, 2010). Principle Components Analysis (PCA) is a useful approach for selecting metrics wherein patterns of covariation and independent variation are identified to reduce the dimensions of the assemblage space and then the metric that is most strongly correlated with each significant PCA axis is selected for use in the index (O'Connor et al., 2000). For example, O'Connor et al. (2000) used PCA to select metrics for several lake assemblages (birds, fish, macroinvertebrates, zooplankton, diatoms).

Macroinvertebrate-based MMIs have been applied widely in developed countries since the 1980s (Ruaro and Gubiani, 2013). However, in neotropical biomes the number of applications has increased markedly in only the last 5 years in the Amazon Rainforest (e.g., Couceiro et al., 2012; Dedieu et al., 2015a), in the Atlantic Rainforest (Oliveira et al., 2011; Suriano et al., 2011), in the Tropical Andes (Moya et al., 2011; Villamarín et al., 2013), and in the Caribbean and Central America Rain Forest (Helson and Williams, 2013; Touron-Poncet et al., 2014). The Neotropical Savanna is the one of the largest neotropical biomes (Wantzen et al., 2006), a global biodiversity hotspot (Myers et al., 2000), and rapidly being deforested by anthropogenic activities (Ratter et al., 1997; Silva et al., 2006; Wantzen et al., 2006). However, few MMIs have been developed there (e.g., Ferreira et al., 2011), reinforcing the need for new studies. In addition, most neotropical MMIs were based on relatively few and convenient ad hoc sites, no landscape-corrected metrics, insufficient numbers of least-disturbed reference sites, metric selection to represent specific categories (e.g., richness, composition, behavior, tolerance, dominance), and inadequate quantitative assessments of chemical and physical habitat and biological conditions of the sites.

Our objective was to evaluate the biological condition of headwater streams in the Neotropical Savanna through use of randomized site selection, a standardized field protocol for sampling physical and chemical habitat and macroinvertebrates, and an MMI based on benthic macroinvertebrate assemblages. Empirical evidence suggests that gradients of anthropogenic pressure influence overall stream environmental condition, and in turn affect biotic condition. However, landscape factors that influence streams also co-vary with anthropogenic stressors. We therefore propose two methodological approaches based on two premises: (1) an MMI based on landscape-adjusted metrics will perform better than one based on unadjusted metrics; (2) metric selection based on significant PCA axes will produce a more sensitive MMI than one constructed in the more traditional manner of selecting metrics to fit specific categories.

2. Materials and methods

2.1. Study area

We conducted this study in the Upper Araguari Basin (located in the Neotropical Savanna biome and part of the Paraná River Basin) in Minas Gerais State, southern Brazil, in September 2009, during the dry season (Fig. 1). The sampled area was delimited around 7376 km^2 upstream of Nova Ponte Reservoir, the first of a sequence of hydroelectric reservoirs along the Paraná River system. There is a six-month dry season (April to September) followed by a six-month rainy season (October to March). The landscape consists of relatively flat, well-drained interfluves, with riparian forests along watercourses. Upland vegetation is typical xerophytic savanna, ranging from dense herbaceous fields, sparse shrubs and small tree cover, to occasional forests with 12–15 m high canopies (Ratter et al., 1997).

2.2. Site selection

We selected a spatially balanced sample of 40 sites using a randomized systematic procedure described by Olsen and Peck (2008) in the U.S. EPA Wadeable Stream Assessment. The spatially balanced selection algorithm was based on a Generalized Random Tessellation Stratified process (Stevens and Olsen, 2004). We targeted wadeable streams by excluding all tributaries of Strahler order >3 on a digital map (1:100,000 scale).

2.3. Data collection

2.3.1. Natural catchment-scale variables

We delineated catchments of each sampled site through use of the terrain model from Shuttle Radar Topographic Mission – SRTM (3 arc seconds; USGS, 2005). Their contributing drainage areas were calculated via GIS software. Mean catchment elevation was extracted directly from SRTM imagery, whereas mean catchment slope was calculated from the maximum rate of change in elevation in every grid cell, based on SRTM elevation rasters. Catchment total annual rainfall was calculated through use of time series data from the Brazilian National Water Agency (ANA, 2014) obtained at 14 stations within our study area. Each station had total annual rainfall records of >30 years, and those data were interpolated through use of ordinary kriging (Johnston et al., 2001). Then, the overlapping grid cell values (30 m raster resolution) of mean annual rainfall were transferred to each catchment.

2.3.2. Catchment-scale anthropogenic pressure variables

We assessed catchment land use and land cover for each site by screening digitized satellite images. We manually interpreted fine resolution (0.6–5.0 m) Google Earth images (Google, 2014) in conjunction with the September 2009 Landsat TM sensor, as described by Macedo et al. (2014). The fine resolution images provided information about the shape and texture of the elements, and the Landsat images showed specific spectral response for each land use or vegetation cover type. Our mapping identified three anthropogenic land uses (pasture, agriculture, urban) and four natural savanna cover types (woodland savanna, parkland savanna, grassy-woody savanna, wetland palm swamp). The catchment percentages of each land use and cover type were estimated for each site because preliminary analyses showed stronger correlations occurred between site conditions and catchment conditions than for riparian buffers of varying widths and distances from the sites.

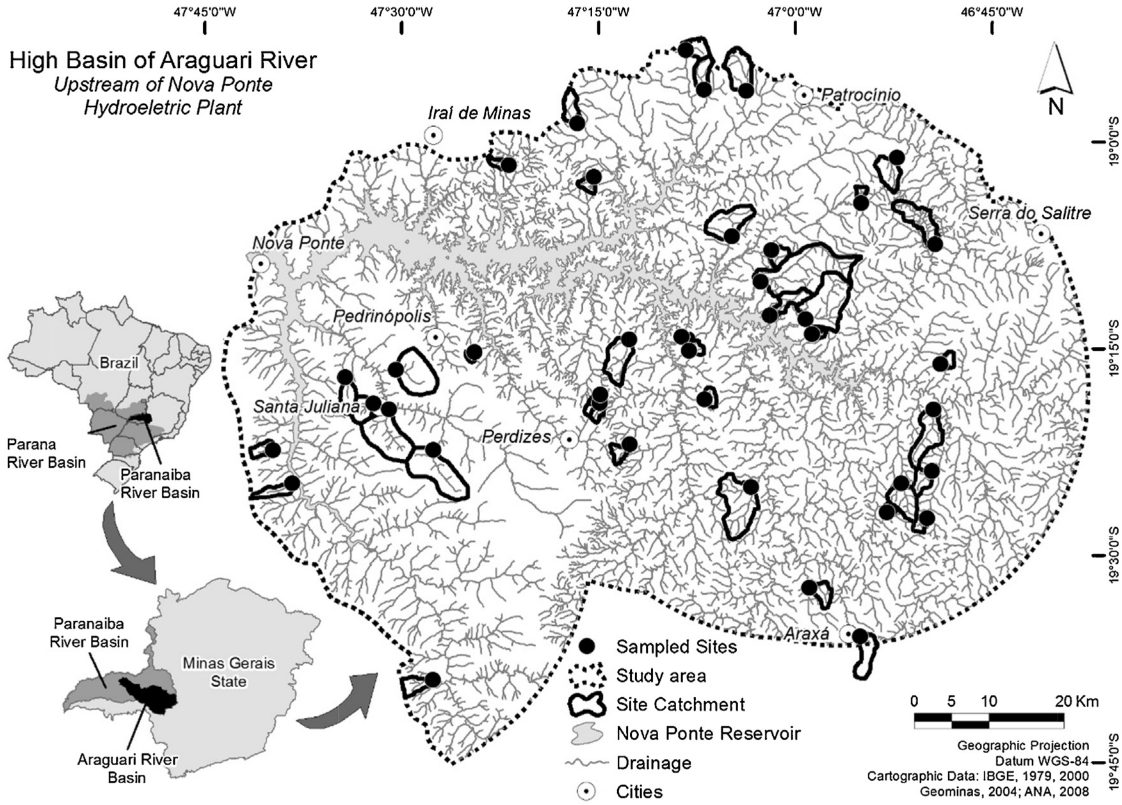

Fig. 1. Locations of sites and study area in Brazil and Minas Gerais State.

For a more comprehensive evaluation, we also calculated a catchment disturbance index (CDI) based on weighted land use in the catchment. That is, urban areas were weighted more highly than agricultural areas, which were weighted more highly than pasture areas (Ligeiro et al., 2013). Additionally, we calculated the density of households (number of houses/km^2) in each sampled catchment (house_den; Macedo et al., 2014) and the proximity to the study sites (latitude and longitude) of each household in the study area by using 2010 Brazilian Census data (IBGE, 2011).

2.3.3. Local-scale physical habitat and anthropogenic pressure variables

We measured local-scale anthropogenic pressures through physical habitat assessment and water column characteristics following Peck et al. (2006). The length of each stream site sampled was 40 times its mean wetted width, with a minimum length of 150 m. Each site was divided into 11 equally spaced transects and we quantified physical habitat characteristics (channel morphology, visual substrate size, riparian vegetation cover and structure). To quantify stream bank anthropogenic pressures, we calculated metrics describing the presence and proximity of human activities. The local disturbance status (local disturbance index, LDI, Ligeiro et al., 2013) of each site was calculated based on presence of trash, sewers, buildings, domestic animals, row crops, pasture, erosion and dams, as described in Kaufmann et al. (1999). We used the sum of riparian woody cover in three vegetation layers (Xcmgw) and mean substrate embeddedness (xembed; Kaufmann et al., 1999) to describe riparian vegetation cover complexity and the degree of streambed sedimentation by sand and fines (<2 mm diameter). We calculated Log-transformed Relative Bed Stability (LRBS), which is the $\log_{10}$ of the ratio of bed surface geometric mean particle diameter divided by estimated critical diameter at bankfull flow as described by Kaufmann et al. (2008). LRBS is an indicator of the anthropogenic increase or decrease of sediment supply to streams (Kaufmann et al., 2009). To characterize anthropogenic pressures on water quality we measured electrical conductivity, pH, and total dissolved solids (TDS) in situ with a multi-probe. In the laboratory, we determined dissolved oxygen, turbidity, total alkalinity, total nitrogen, and total phosphorus following APHA (2005) protocols.

2.3.4. Integrated-scale anthropogenic pressure variables

We analyzed anthropogenic pressures at both local (LDI) and catchment (CDI) scales, and both scales together in an Integrated Disturbance Index (IDI, Ligeiro et al., 2013). Each index measures how much a site deviates from the absence of anthropogenic pressures at each scale. The IDI of each site was calculated as the Euclidian distance from the position of the site in a disturbance plane formed by the CDI and the LDI to the origin of the plane (where both CDI and LDI = 0; Ligeiro et al., 2013).

2.3.5. Benthic macroinvertebrate sampling

We sampled benthic macroinvertebrate assemblages through use of D-frame kick nets (30 cm aperture, 500 mm mesh). Following a systematic zig-zag pattern along the site, eleven samples were taken per site (i.e., one per transect) then aggregated into one composite sample for each site, totaling 1 m^2 per site (Peck et al., 2006; Stoddard et al., 2008). The samples were fixed in the field with 10% formalin and taken to the laboratory in individual plastic containers. In the laboratory, the samples were washed, sorted, and all individuals were identified (mostly to family) with the aid of taxonomic keys (Róldan-Pérez, 1988; Fernández and Domínguez, 2001; Merritt et al., 2008; Mugnai et al., 2010). The specimens were cataloged and deposited in the Macroinvertebrate Reference Collection of the Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais.

2.4. MMI development

2.4.1. Identifying least- and most-disturbed sites

A first step in environmental quality assessments using biological assemblages is the choice of least-disturbed (reference) sites (Hughes et al., 2004; Whittier et al., 2007b). These are the areas where the biota are exposed to lower levels of anthropogenic pressures, and the physical, chemical, and landscape conditions make good ecological status likely (Stoddard et al., 2006; Hawkins et al., 2010a). The critical requirement for developing an effective MMI is to determine metrics that best discriminate least-disturbed sites from most-disturbed sites. The statistical difference in biological metric values between least- and most-disturbed sites is a measure of the effect of anthropogenic pressures on aquatic biota within the study area (Barbour et al., 1996; Karr, 1998).

We ranked sites in the Upper Araguari River Basin from low to high disturbance based on the IDI gradient. We then calculated the mean and standard deviation (SD) of the IDI distribution, and defined the least- and most-disturbed sites, respectively, as those deviating from the mean by +1SD and −1SD.

2.4.2. Candidate biological metrics

We examined 80 benthic macroinvertebrate assemblage metrics for possible inclusion in the MMI via a stepwise screening process (Appendix A, Fig. 2). The metrics were categorized in four groups: richness and composition (~46% of the metrics); biological and ecological traits (~25%); tolerance (~18%); and diversity and dominance (~10%).

Candidate richness and composition metrics included total taxa richness, order richness, and percentage of individuals in each taxon. The trait metrics included trophic groups and mobility (Gooderham and Tsyrlin, 2002; Cummins et al., 2005; Merritt et al., 2008; Tachet et al., 2000; Tomanova et al., 2008; Lunde and Resh, 2012; Appendix B). Tolerance metrics were based on taxa organic pollution tolerance scores taken from the Biological Monitoring Working Party (BMWP) system (adapting the scores of Alba-Tercedor, 1996; Junqueira and Campos, 1998; Roldán-Pérez, 2003; Lunde and Resh, 2012; Appendix B), and ratios between abundant and resistant families and orders (Baetidae/Ephemeroptera, Hydropsychidae/Trichoptera, Chironomidae/EPT, and Chironomidae/Diptera). Diversity and dominance metrics included Shannon, Evenness, Margalef, and Simpson indices and dominant taxa percentages.

2.4.3. Metric screening

Biological metrics with small range values, or with most values identical, are incapable of differentiating least-disturbed and most-impaired sites (Whittier et al., 2007a; Stoddard et al., 2008). Therefore, we eliminated metrics with insufficient range: richness metrics with range <5, percentage metrics with range <10%, and metrics with >95% of values equal to zero (Klemm et al., 2003). Next, we eliminated metrics that lacked a normal distribution, even after transformation (Kolmogorov–Smirnov test, $p > 0.05$).

Both anthropogenic pressures and natural landscape factors influence biological assemblages, producing co-varying responses (Moya et al., 2011; Chen et al., 2014; Macedo et al., 2014; Feld et al., 2016) thereby confounding biological responses to anthropogenic pressures. To reach our first objective, we conducted two separate analyses. In the first approach, we used regression to identify the potential influence of natural features on biological metrics. To do so, we regressed the biological metrics of least-disturbed sites with catchment area, mean catchment slope, elevation, and annual rainfall. Significant results ($r^2 > 0.75$, $p < 0.05$) were corrected by subtracting the regression-predicted metric values from each raw value to extract residual metric values (residual = observed value − predicted value) (Klemm et al., 2003; Cao et al., 2007; Stoddard et al., 2008; Chen et al., 2014). The residual metric values were those employed in subsequent MMI development. In the second approach, biological metrics were analyzed without landscape corrections. In both datasets, we tested metric capacity to discriminate least- and most-disturbed sites through significant *t*-test scores (corrected by Bonferroni criteria, $p < 0.05$). We identified redundant metrics through Pearson product-moment correlation and we retained no metrics with correlation coefficients >90. We compared correlated metrics and retained the one with the greatest *t*-score. For example, if metric "a" was correlated with metrics "b" and "c", we retained the metric with the greatest *t*-score, and discarded the others.

2.4.4. MMI options

We applied two approaches in each dataset to choose the final metrics to build four MMIs: MMI-1, MMI-2, MMI-3 and MMI-4. In the first approach, we used Principal Component Analysis (PCA) to identify significant PCA axes and, for each axis, we chose the metric with the largest Eigenvector value as suggested by O'Connor et al. (2000) for lake assemblages. We standardized all biologic metrics (x − mean/SD) and conducted the analysis by correlation matrix. In the second approach, we separated metrics into four categories (richness and composition; biological and ecological traits; tolerance; and diversity and dominance) and chose the metric with the greatest *t*-score from each category (Cao et al., 2007; Huang et al., 2014). Thus, we had four datasets to calculate MMIs: (a) MMI-1 with the most sensitive metric from each of four first significant PCA axes of the landscape-corrected metrics dataset; (b) MMI-2 with one metric representing each of four categories of the landscape-corrected metrics dataset; (c) MMI-3 with the most sensitive metric from each of four first significant PCA axis from the landscape-*uncorrected* dataset; (d) MMI-4 with one metric representing each of four categories from the landscape-*uncorrected* dataset.

2.4.5. Metric standardization

The selected metrics were standardized to the same scale (0–100) so that they could be combined in the MMI to equally influence the overall MMI response to anthropogenic pressures. Positive metrics (those with increases indicating better biotic condition) were divided by their 95th percentile and multiplied by 100. Values above 100 were considered super optimal, and were scored as 100. For negative metrics (those with decreases indicating better biotic condition), we subtracted the metric value from 100 and divided that result by 100 minus the 5th percentile, also multiplied by 100, thus becoming a positive metric. As above, values >100 were scored as 100. Each of the four MMIs was calculated as the average of its standardized biologic metrics.

2.4.6. Final MMI choice

To choose the best performing MMI we used three criteria: precision, responsiveness, and sensitivity to anthropogenic pressures at catchment and local scales (Hawkins et al., 2010b; Van Sickle, 2010; Chen et al., 2014). We calculated the standard deviation of each MMI in the least-disturbed areas to assess precision. We tested the responsiveness of each MMI by using *t*-tests to verify the degree to which each MMI discriminated the pre-determined least- and most-disturbed sites. To evaluate sensitivity, we ran stepwise-forward regression between each MMI and all local- and catchment-scale anthropogenic pressure variables to identify the best-fitted model. We chose the MMI with the best performance in all three tests. To further evaluation, we calculated the Pearson correlation with the local- and catchment-scale anthropogenic stressors.

After we chose the best option, three condition classes were established as suggested by Ganasan and Hughes (1998): poor (values <60% of the maximum observed index score), fair (values

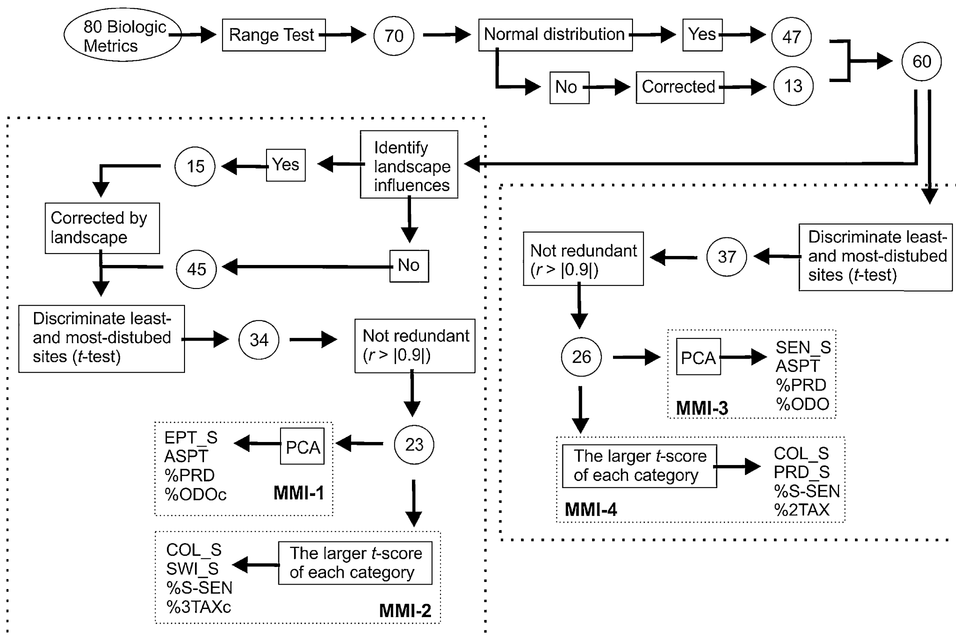

Fig. 2. Flow diagram showing the steps for metric screening and MMI development.

Table 1
Natural environmental gradients in the Upper Araguari River Basin.

Variable	Mean ± SD	Range
Basin drainage area (km^2)	10.74 ± 10.70	1.38 to 50.75
Mean basin elevation (m)	968 ± 43.5	895 to 1036
Mean basin slope (%)	8.23 ± 3.03	3.15 to 17.16
Total annual rainfall (mm/m^2)	1584 ± 116.2	1413 to 1869

between 60% and 80% of that score), and good (values >80% of the top MMI score).

3. Results

3.1. Natural landscape variables

Some natural landscape variables varied little among the sites. Elevation range was less than 100 m (895 to 1036 m) and total annual rainfall ranged from ~1400 mm to ~1800 mm (Table 1), which is typical of the Brazilian Neotropical Savanna biome. However, mean catchment slope ranged from <4% to ~17% and catchment areas ranged from <2 km^2 to 50 km^2.

3.2. Anthropogenic pressure variables

The most prevalent land use or land cover types in the study catchments were row crops (~47%) and pasture (~17%), with little urbanization and few houses. However, there were catchments with up to 94% natural cover (Table 2). Local anthropogenic pressures were grouped into physical habitat and water chemistry variables. Physical habitat stress was mostly from fine sediments, reflected in high mean embeddedness (>60%) and negative values of log-transformed relative bed stability. Such unstable streambeds indicate deposition of inputs of fine sediments from eroding uplands and banks that are large relative to sediment transport by these streams. On the other hand, several sites had riparian vegetation cover-complexity index values >60% and low values of local anthropogenic pressure (LDI). Almost all sites had good water quality, with dissolved oxygen concentrations near saturation, nutrient

Table 2
Anthropogenic pressures in the Upper Araguari River Basin.

Anthropogenic stressors	Mean ± SD	Range
Integrated Scale		
IDI – Integrated Disturbance Index	0.47 ± 0.21	0.04 to 1.08
Catchment Scale		
CDI – Catchment Disturbance Index	111.5 ± 52.72	10.02 to 181.24
%_Nat – % of natural cover	35.86 ± 25.31	7.08 to 94.15
%_Past. – % of pasture land use	16.55 ± 18.26	0 to 71.32
%_Agr – % of agricultural land use	46.56 ± 29.74	0 to 90.62
%_Urb – % of urban land use	0.48 ± 2.62	0 to 90.62
House_den – density of households (number of houses/km^2)	0.97 ± 0.94	0 to 3.49
Local Scale		
Xembed – mean embeddedness (%)	64.08 ± 19.67	22.18 to 95.64
LRBS – (Log$_{10}$) relative bed stability	−2.74 ± 0.81	−4.69 to +1.33
XCMGW – site mean for sum of canopy-, mid-, and ground-layer wood cover (% areal cover)	61.99 ± 29.06	12.95 to 130.11
LDI – Local Disturbance Index	1.18 ± 1.04	0 to 4.63
pH – negative log of hydrogen ion concentration	6.90 ± 0.46	5.54 to 8.02
Cond – electrical conductivity (μS/cm)	23.26 ± 17.72	5.0 to 99.0
TDS – total dissolved solids (mg/L)	15.17 ± 11.78	1.0 to 64.0
Turb – turbidity (NTU)	7.56 ± 10.5	0.94 to 61
DO – dissolved oxygen (mg/L)	7.47 ± 1.16	4 to 9.7
Nt – total nitrogen (mg/L)	0.06 ± 0.01	0.04 to 0.08
Pt – total phosphorus (mg/L)	0.03 ± 0.03	0.01 to 0.23

and turbidity values below the limits of Brazilian environmental law, and neutral pH (Table 2).

3.3. Reference condition sites

The IDI values revealed a strong anthropogenic pressure gradient across the sites (average 0.47; range 0.04 to 1.08; Table 2). We used the upper and lower SD values (0.21) to define most-disturbed

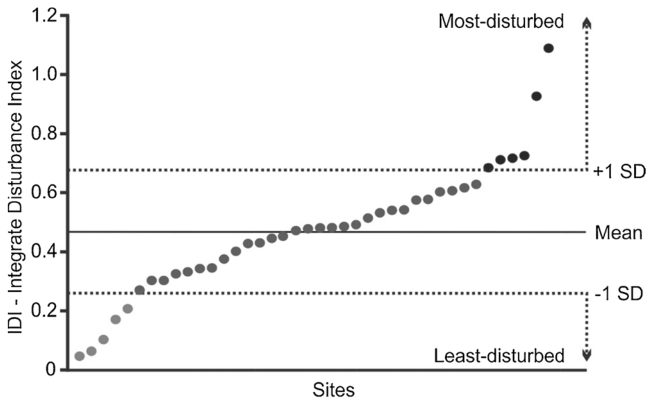

Fig. 3. Gradient of anthropogenic pressures indicated by the Integrated Disturbance Index.

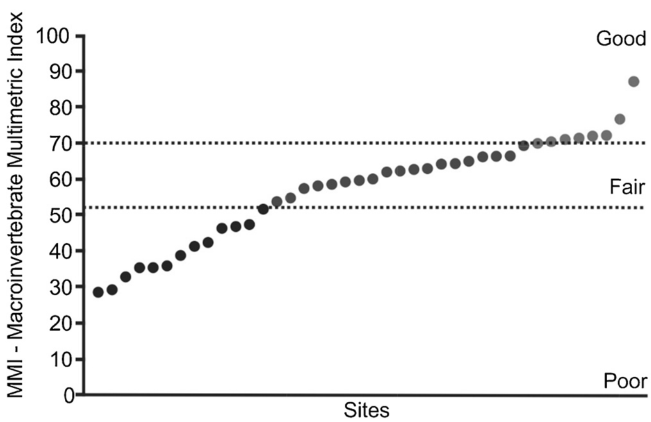

Fig. 4. Classification of final MMI scores. The upper fair boundary is 80% of the highest MMI score and the lower fair boundary is 60% of the highest MMI score.

and least-disturbed boundaries of 0.68 and 0.26, respectively. Five sites were considered least-disturbed and six sites were considered most-disturbed (Fig. 3).

3.4. MMI development

We identified 23,348 individuals composing 69 macroinvertebrate taxa. The most commonly occurring individuals were Chironomidae (39% of total number of individuals), Elmidade (11%), Simuliidae (10%), Leptophlebiidae (7%), and Leptohyphidae (6%) (Appendix B).

Seventy of the 80 candidate metrics passed the range test; of those, 47 were normally distributed and 13 others were successfully transformed. In the landscape adjusted approaches, 15 metrics needed adjustment for natural environmental gradient and 34 distinguished least- and most-disturbed sites. In the unadjusted approaches, 37 metrics distinguished least- and most-disturbed sites (Appendix C). All MMIs were built with four metrics (Fig. 2).

Among the four candidates, MMI-1 performed best. It was the most precise (having the lowest standard deviation in the predetermined least-disturbed areas), most responsive (having the highest *t*-score to discriminate least- and most-disturbed sites), and most sensitive to catchment- and site-scale variables (Table 3). Therefore, we chose MMI-1 for further applications. It scored 13 sites as poor, 19 as fair, and eight as good (Fig. 4).

3.5. Local- and catchment-scale MMI evaluation

As expected, MMI-1 scores were negatively correlated with local- and catchment-scale anthropogenic disturbances (absence of catchment natural cover, presence of pasture and urban areas, and higher catchment house density ($r = -0.34$ to -0.56, $p < 0.05$), including the IDI and its subcomponents CDI and LDI, which were used in metric screening (Table 4). Lower MMI values also were significantly ($p < 0.05$) negatively correlated with embeddedness ($r = -0.32$), and positively correlated with both bed stability ($r = 0.41$), and riparian vegetation woody cover complexity ($r = 0.66$). There were no significant correlations between MMI-1 and water quality variables.

4. Discussion

In this study, we assessed local- and catchment-scale anthropogenic pressure effects on stream macroinvertebrate assemblages in a typical tropical basin. To do so, we used an MMI developed by screening metrics for range and normality, calibrating metrics for natural environmental variables, and then using PCA to select non-redundant metrics. This sequence of analytical steps offers a framework for large-scale future environmental assessments in tropical biomes and ecoregions threatened by anthropogenic pressures and land use changes.

4.1. MMI effectiveness

In general, our MMI was effective for discriminating least- and most-disturbed sites, for evaluating anthropogenic pressures at local- and catchment-scales, and at both scales combined using an IDI (Ligeiro et al., 2013). A critical point in ecological assessment is defining reference and impaired sites (Hughes et al., 2004) before building an MMI, because the ability to distinguish least- and most disturbed sites defines success in its development (Fore et al., 1996; Karr, 1999). Currently, most MMI approaches have used subjective criteria to define least-disturbed areas, but the use of the IDI to define least-and most-disturbed sites introduces a quantitative and standardized method to assess the anthropogenic pressure gradient. Whittier et al. (2007b) and Herlihy et al. (2008) showed that objective, quantitative methods – rather than subjective hand-picked choices – improved reference site selection.

Ecological data typically contain extraneous variance (noise) that interferes with distinguishing conditions among sites in an area (signal). This extraneous variance is derived from several sources, including reading errors, measurement variation, and short-term temporal variation in sampling (Kaufmann et al., 1999; Chen and Jackson, 2000). By averaging disparate variables defining ecological condition, MMIs can have lower error variance than their sub-component metrics.

Confounding environmental natural gradients with anthropogenic pressures is another source of variance in multimetric indices, and it can be a major source of bias in ecological assessments (Cao et al., 2007; Chen et al., 2014; Moya et al., 2011; Stoddard et al., 2008). This is an important aspect, because other studies conducted in Neotropical Savanna streams indicated that natural landscape influences on macroinvertebrate assemblages (Macedo et al., 2014; Feio et al., 2015) and, in regional studies, can influence macroinvertebrate responses to a greater degree than anthropogenic pressures (Macedo et al., 2014; Feld et al., 2016). Our results showed that landscape-adjusted MMIs performed better than unadjusted MMIs (Table 3). Furthermore, there are no accurate ecoregion classifications of the Neotropical Savanna or other South American biomes, that could allow one to build a robust MMI for

Table 3
Results of the criteria for choosing the best MMI: precision (standard deviation-SD value of MMI at least-disturbed sites), responsiveness (*t*-test value of MMI between least- and most-disturbed sites), and sensitivity (r^2 of OLS regression among MMI and anthropogenic stressors). Best performing MMI for each criterion in bold.

Proposal MMIs	Precision	Responsiveness			Sensitivity			
	SD	*t*-score	*F*	*p*	r^2	*F*	Variables	*p*
MMI-1 (Adjusted, PCA)	**4.17**	**7.05**	3.7	<0.0001	**0.66**	35.63	XCMGW + %_NAT	<0.0001
MMI-2 (Adjusted, one each category)	9.84	6.21	2.4	<0.0001	0.65	34.25	XCMGW + %_NAT	<0.0001
MMI-3 (Unadjusted, PCA)	7.39	6.51	1.7	<0.0001	0.61	18.59	XCMGW + %_NAT – DO	<0.0001
MMI-4 (Unadjusted, one each category)	10.19	6.32	1.1	<0.0001	0.60	17.93	XCMGW + %_NAT – TDS	<0.0001

Table 4
Pearson correlations between MMI-1and anthropogenic stressors at integrated, catchment, and local scales.

Anthropogenic stressors	Pearson correlation with MMI-1
Integrated Scale	
IDI	−0.56***
Catchment Scale	
%_Nat	0.55***
CDI	−0.48**
House_den	−0.36*
%_Urb	−0.34*
%_Agr	−0.32*
%_Past	−0.16
Local Scale	
Xcmgw	0.66***
LRBS	0.41**
LDI	−0.40**
Xembed	−0.32**
Nt	−0.12
TDS	−0.10
Turb	−0.06
Pt	−0.03
DO	−0.01

*** $p < 0.001$.
** $p < 0.01$.
* $p < 0.05$.

large areas, such as was done for the United States (Paulsen et al., 2008; Stoddard et al., 2008).

An effective MMI should incorporate independent biological metrics to aid the evaluation of multiple anthropogenic pressures (Fore et al., 1996; Karr, 1998; Stoddard et al., 2008). Our MMI-1 included two composition metrics (% Ephemeroptera-Plecoptera-Trichoptera individuals, % Odonata individuals adjusted by elevation), a trophic metric (predator richness) and a tolerance metric (Average Tolerance Score Per Taxon – ATSPT). We developed our MMI-1 using metrics calculated at the macroinvertebrate family resolution because other taxonomic levels (species or genus) are unavailable for South America (Macedo et al., 2014; Dedieu et al., 2015b). However, Whittier and Van Sickle (2010) concluded that there was little difference between family and genus tolerances for western USA benthos in relation to integrated local- and catchment-scale anthropogenic pressures.

The metric selection approach can greatly influence MMI metric composition and yield a biased index (Chen et al., 2014; Vander Laan and Hawkins, 2014). The use of PCA for selecting biological metrics is preferable to taking a metric from each category (Table 3). Selecting the most representative metric (larger Eigenvector value) from significant PCA axes is a simpler and more powerful alternative, because it employs an objective process for choosing metrics. To our knowledge, this approach has rarely been used in a biomonitoring context. Because it performed well in a relatively small tropical basin, this approach also may be appropriate for assessing entire biomes or ecoregions. Nonetheless, our MMI metrics were similar to those selected in other MMI studies (Chen et al., 2014; Ferreira et al., 2011; Fore et al., 1996; Herbst and Silldorff, 2006; Huang et al., 2014; Kerans and Karr, 1994; Weigel et al., 2002).

Limitations of most MMIs included the absence of statistical criteria for identifying a priori least- and most-disturbed sites, failure to account for natural environmental variability, use of redundant metrics, and non-continuous metric scoring (Ruaro and Gubiani, 2013). Our MMI development avoided those limitations, but still was simple to build. The main drawback of our study is the relatively small sample size and single sampling visit, precluding the use of separate datasets for calibration and testing of the MMI, as recommended by others (Stoddard et al., 2008; Moya et al., 2011; Chen et al., 2014). This is a preliminary approach and future studies will improve it by using temporal sampling to assess temporal stability of metrics, and calibrating and testing additional sites in the Neotropical Savanna biome. Furthermore, the study area is relatively small (~7500 km^2) compared with those in some other MMI studies. Because natural landscape controls are likely to be even more important in larger regions, we believe that landscape adjustments would be even more advantageous for future MMI development in larger areas of the Neotropical Savanna or Brazil in general.

4.2. Influences of catchment and site scales on the MMI

Catchment anthropogenic pressures were moderately correlated with MMI-1 (Table 4). Presence of natural cover in a catchment is an important factor in aquatic environmental conservation, because it is inversely related to stream sediment load and pollutants (Aksoy and Kavvas, 2005). Furthermore, presence of natural cover is evidence of greater habitat complexity and, consequently, of higher biological condition scores (Sponseller et al., 2001; Hrodey et al., 2009). Others have reported high correlations between MMI scores and catchment land use and cover for forested areas (Sponseller et al., 2001; Shandas and Alberti, 2009), agricultural areas (Wang et al., 1997; Hrodey et al., 2009), and impervious (urban) areas (Morley and Karr, 2002; Wang et al., 2003).

At the local scale, MMI-1 responded largely to physical habitat variables (Table 4). In general, our study sites lacked poor water quality, but physical habitat disturbance, especially high embeddedness and bed instability, were common. These disturbances led to reduced physical habitat complexity and, consequently, poorer biotic conditions (Kaufmann et al., 2009; Bryce et al., 2010). However, the cover and structural complexity of woody riparian vegetation (xcmgw) was the strongest local positive influence on our MMI. This underscores the importance of intact riparian areas in moderating inputs of fine sediments, as the source of sediment fines is more related to catchment land use than to riparian cover itself (Macedo et al., 2014).

Site physical habitat characteristics are key determinants influencing the structure and composition of aquatic assemblages (Frissell et al., 1986; Allan, 2004). However, catchment land use and cover not only influence runoff and the rate of sediment inputs from erosion, but also influence riparian zones, and thereby affect streambed texture, channel morphology, and the availability of physical habitat (Wang et al., 1997; Allan, 2004; Kaufmann and Hughes, 2006; Herringshaw et al., 2011). Given that both local- and catchment-scale variables can influence macroinvertebrate assemblages, it is important to develop an IDI that incorporates both scales as a univariate descriptor of anthropogenic pressures (Ligeiro et al., 2013). In general, other approaches have tested their MMIs against individual metrics at catchment or local scales, but rarely both in an integrated manner. Our results show that several metrics responded to anthropogenic pressures at different scales, and our MMI responded to both assessed scales. Thus, this is an important tool to indicate catchment and local-scale anthropogenic pressures simultaneously.

4.3. Potential tools for Neotropical Savanna management

Currently, the Neotropical Savanna is one of the most threatened biomes in the world (Myers et al., 2000), suffering from replacement of natural vegetation with crops, pastures, and urbanization (Silva et al., 2006). Furthermore, most Brazilian hydropower facilities are located in this biome (Brazil, 2007), which increases the exploitation of such ecosystem services as fisheries production, water supply, and recreation. Anthropogenic pressures lead to exports of excess sediment and other pollutants downstream (Karr, 1998), threatening the operation and service life of reservoirs (Arias et al., 2011). Therefore, the MMI and IDI can be used in a broader sense to aid conservation of the Neotropical Savanna biome by measuring the effectiveness of reservoir basin rehabilitation and mitigation projects, as well as assessing and monitoring the effects of continued agricultural conversion and urbanization. In addition, Brazilian legislation establishes the catchment as a unit of analysis and management of water resources (Brazil, 1997). Therefore, catchment condition as expressed by MMI and IDI scores can be important tools for management and conservation.

Our study allowed us to evaluate biological and environmental conditions along an anthropogenic pressure gradient through use of a probabilistic survey design, standard sampling methods, and quantitative biological and environmental indicators. Similar approaches have been applied at the continental scale in the United States (Stoddard et al., 2008; USEPA, 2013), indicating great potential for implementation in a country with continental dimensions like Brazil. To fully implement this important and necessary tool, Brazil must first improve its legal framework. In the European Union and the United States the use of biological assemblages in environmental assessments is legally required (Ruaro and Gubiani, 2013), whereas in Brazil it is only optional (Brazil, 1997).

The availability of quantitative anthropogenic pressure indicators at both local and catchment scales aids comparisons between environmental conditions and pressures over a wide range of scales. For example, loss of biodiversity can be associated with these quantitative indicators of anthropogenic pressures at biome, ecoregional, national, or continental scales. Because these local scale, catchment scale, and integrated pressure indicators are easily calculated, and have been tested at the basin scale, we expect that they will provide a model for environmental assessment of water resources elsewhere in Brazil, as well as other nations.

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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.2015.12.019.

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