

# Are multiple multimetric indices effective for assessing ecological condition in tropical basins?



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## ABSTRACT

The quality and availability of water resources in tropical watersheds are threatened by increased multiple use demands by human populations. Therefore, there is a need for cost-effective ecological indicators of water body status and trends. Multimetric indices (MMIs), based on responses of biological assemblages to anthropogenic disturbances, are excellent examples of such indicators and they have been applied globally. However, creating new MMIs for each water body or study area requires considerable analytical effort and hinders our ability to make regional or global comparisons. Therefore, we tested the effectiveness of 17 published benthic macroinvertebrate MMIs for assessing the environmental quality of a tropical anthropogenically least-disturbed river basin in the Neotropical Savanna (Brazilian Cerrado) biome. We tested those MMIs through use of macroinvertebrate data sampled at 40 stream sites in the Pandeiros River basin, Brazil. Disturbances in the basin were related to local factors such as pasture, garbage, and cropland in stream riparian areas. Index performance was tested by comparing precision, bias, responsiveness and sensitivity to anthropogenic pressures and stressors. Ten indices performed satisfactorily in evaluating the environmental condition of the basin. Therefore, we do not recommend developing new benthic MMIs for rapid environmental quality assessments. On the other hand, we do recommend using standard data collection methods for evaluating conditions throughout the biome.

## 1. Introduction

The increasing demand for water uses by humans affects the quality and availability of water resources (Gangloff et al., 2016) and threatens global aquatic biodiversity (Reid et al., 2018). In some regions this becomes particularly important, as in the Brazilian Cerrado (Neotropical Savanna) biome. Although this biome contains important hydrographic basins, has high biodiversity, high endemism, and covers 2 million km<sup>2</sup>, it is one of the most threatened biomes in South America (Strassburg et al., 2017). Despite the great importance of the Cerrado as a biodiversity hotspot (Myers et al., 2000), its current protection is insufficient: public protected areas cover only 7.5% of the biome (Strassburg et al., 2017). The Cerrado biome has historically been neglected by the Brazilian government, resulting in its devastation through intense land use change, with only 20% remaining of its original natural area (Strassburg et al., 2017). Aquatic ecosystems in this biome are largely threatened by habitat fragmentation, sedimentation, flow regulation (dam construction), water pollution and biological

invasions (Callisto et al., 2019; Linares et al., 2017; Macedo et al., 2018; Reid et al., 2018; Sánchez-Bayo and Wyckhuys, 2019). Because of its great biological importance and current threats, ecological studies in the Cerrado are justified to ensure that management actions are effective and based on widely used and validated scientific studies and methodologies. Therefore, identifying the major anthropogenic changes in aquatic ecosystems and understanding how they affect biological conditions are important steps in the assessment of Cerrado environmental quality (Revenge et al., 2005).

Environmental quality assessment of tropical aquatic ecosystems is critically important for the management and conservation of water resources and for the protection of aquatic biodiversity (Sánchez-Bayo and Wyckhuys, 2019) for several reasons. The Tropics support over three quarters of global biodiversity (Barlow et al., 2018). In addition, the risk of biodiversity losses because of anthropogenic disturbances (e.g., urbanization, agriculture, pasture, mining, dams) is increasing in tropical regions (Dirzo et al., 2014). Studies in neotropical regions have become more frequent, but remain insufficient for understanding their

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biodiversity (Barlow et al., 2018; Hortal et al., 2015). This is especially important in freshwater ecosystems where there are higher rates of degradation and loss of species than in terrestrial or marine ecosystems (Reid et al., 2018).

Unlike assessments of water chemistry or physical habitat structure, biological assessments of water bodies are direct measures of biological condition that integrate both long- and short-term and small- and large-extent anthropogenic disturbances (Davies and Jackson, 2006; Hughes, 2019; Karr and Dudley, 1981). Assessments of water chemistry, physical habitat structure, and landscape or riverscape condition typically explain less than half the variability in biological condition (Hughes, 2019; USEPA, 2016) and are extremely sensitive to sampling effort and natural variability (Hughes, 2019). Unlike species richness or tolerance or diversity indices, multimetric indices (MMIs) integrate multiple biological attributes of aquatic macroinvertebrate assemblages (Hughes et al., 1998) and have been used to evaluate water body quality globally (Buss et al., 2015; Ruaro and Gubiani, 2013). MMIs are robust tools for assessing aquatic ecosystem status and trends (Buss et al., 2015; Ruaro and Gubiani, 2013; USEPA, 2016) because they can discriminate the effects of different types of anthropogenic pressures and stressors (Hering et al., 2006; Lunde and Resh, 2012; USEPA, 2016). Therefore, they are considered one of the best approaches for aquatic ecosystem biomonitoring and bioassessment (Bonada et al., 2006; Ruaro and Gubiani, 2013).

In Brazil, macroinvertebrates MMIs were developed in different biomes, including Amazonia (Couceiro et al., 2012), Cerrado (Ferreira et al., 2011; Macedo et al., 2016; Saito et al., 2015; Silva et al., 2017), Atlantic Forest (Baptista et al., 2007; Oliveira et al., 2011) and Pampas (Melo et al., 2015). However, this approach has not yet been standardized for evaluating tropical aquatic ecosystems nationally (Buss et al., 2015) despite their high biological diversity (Barlow et al., 2018). There are difficulties in extending this approach because there is no legal provision for its use at the national level, which would require defining and standardizing the tools used for this purpose (Macedo et al., 2016; Ruaro and Gubiani, 2013; Silva et al., 2017).

Despite the large number of MMIs available in the literature, they have not been evaluated for their efficacy and applicability in places other than those where they were developed (Ruaro and Gubiani, 2013; Silva et al., 2017). Although several researchers have tested alternative MMIs or metrics to arrive at the best final index based on their data sets, they did not test other MMIs developed in different places. Therefore, we carefully selected existing MMIs, calculated their metrics, and performed statistical tests to validate their reliability as measured by their precision, bias, responsiveness and sensitivity to anthropogenic pressures and stressors (Chen et al., 2019).

The maintenance or improvement of water quality, ecological health, and biodiversity are major societal goals. Therefore, the objective of this work was to test the applicability of seventeen existing MMIs to our study area, evaluate the metrics used, and conduct an environmental quality assessment in an environmentally protected area. We tested the hypothesis that those benthic MMIs are efficient in assessing environmental quality, regardless of where they were developed. We assumed that the indices were accurate, lacked a natural variability bias, and responded to anthropogenic disturbances, making them useful for evaluating environmental quality. To meet our objectives, we evaluated how anthropogenic disturbances affected water quality, physical habitat structure, and benthic macroinvertebrate assemblage MMIs.

## 2. Material and methods

### 2.1. Study area

The Pandeiros River basin is located in the Cerrado biome in northern Minas Gerais state of Brazil (Fig. 1). The basin area is

3,960 km<sup>2</sup> and the flooded areas (palm swamp, wetlands, and marginal lagoon complexes) of the Pandeiros River are priority areas for conservation in the biome (Drummond et al., 2005). They are designated of Special Biological Importance, being unique environments in an otherwise semi-arid region (Azevedo et al., 2009). The entire Pandeiros River Basin is an Environmental Protection Area, the largest Conservation Unit in the state of Minas Gerais (IEF - Instituto Estadual de Florestas, 2019).

The Pandeiros River is a strategic tributary on the left bank of the São Francisco River and of fundamental importance for the protection of that basin (Azevedo et al., 2009). The climate is semi-arid and the basin drains mostly sandy soils, but many Pandeiros tributaries are perennial, which makes the river network critically important annually and during long-term droughts (IEF, 2019). The basin also has a small hydropower dam, decommissioned since 2007 and slated for future removal, which is unprecedented so far in South America (Linares et al., 2018, 2020).

### 2.2. Selection of stream sites and sample sections

Forty stream sites were selected through use of a spatially balanced randomized survey design developed by the United States Environmental Protection Agency (Olsen and Peck, 2008) and also widely used for assessing other Brazilian Cerrado streams (Callisto et al., 2019, 2014; Macedo et al., 2018, 2016; Silva et al., 2017) and Atlantic Forest streams (Jiménez-Valencia et al., 2014; Terra et al., 2015). Such a design allows one to obtain an unbiased sample and to infer results from a relatively small number of sites to the entire stream population from which the sample was drawn, with known confidence intervals (Jiménez-Valencia et al., 2014; Silva et al., 2017; USEPA, 2016). This is not possible with ad hoc or systematic survey designs. After sorting the basin by Strahler (1957) stream orders, a balanced selection of stream sites was carried out for 3rd to 5th order streams on a 1:100,000 map with a minimum distance of 1 km between them. We sampled sites deemed wadeable, i.e., capable of being safely crossed by an adult with the water depth up to breast height (Kaufmann et al., 1999). We sampled sites during the dry season (April/June 2016) when water flow variations are relatively small, there is greater bed stability, habitats and microhabitats are more accessible, and macroinvertebrate abundances are high (Hughes and Peck, 2008; Melo and Froehlich, 2001).

### 2.3. Benthic macroinvertebrate sampling

Each stream site was 40 × its mean width, with a minimum length of 150 m. In each site, 11 transects (perpendicular to the stream) were marked, defining 10 sections where physical habitat was measured and benthic macroinvertebrates collected (Peck et al., 2006; USEPA, 2016). In each transect, marked "A" to "K", benthic macroinvertebrates were sampled, totaling 11 sub-samples per site and 440 sub-samples in total. A D-frame kick-net (500 µm mesh, 0.9 m<sup>2</sup> area) was used to sample benthic organisms. Each sample was placed in a plastic bag and fixed with 50 ml of formaldehyde. The samples were taken to the UFMG's (Universidade Federal de Minas Gerais) Benthos Ecology laboratory, where they were washed on a 500 µm mesh screen. The washed material was placed in clear trays on a light box and the invertebrates were identified through use of a stereoscopic microscope (32x) with identification keys (Fernández and Domínguez, 2001; Merritt and Cummins, 1996; Mugnai et al., 2010; Pérez, 1988). Identification was performed to family, except for Bivalvia, Hydracarina, Hirudinea, Nematoda, Collembola and Oligochaeta. All specimens were identified and deposited in the Reference Collection of Benthic Macroinvertebrates of ICB/UFMG (Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais).

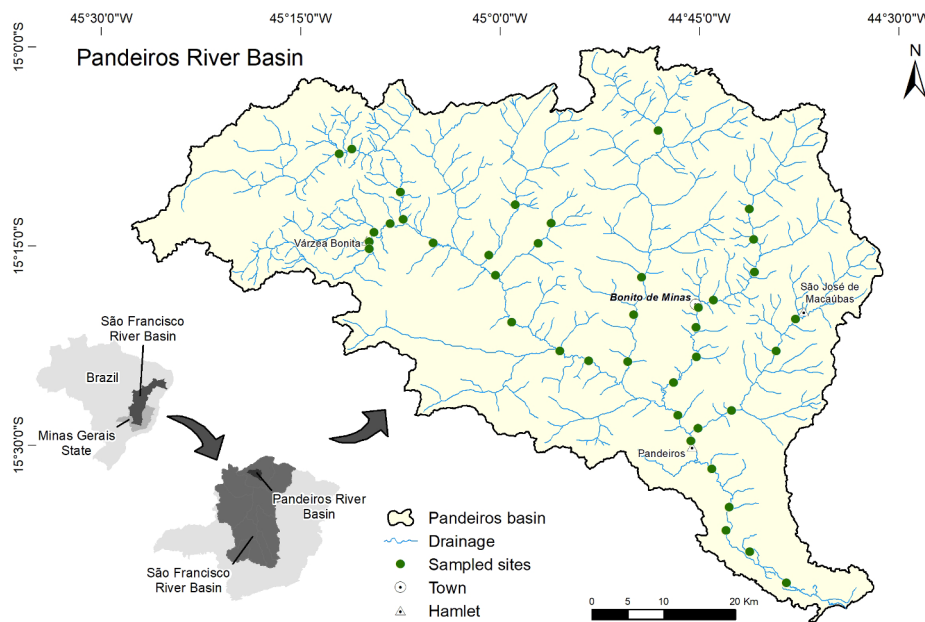


Fig. 1. Location of the sampling sites in the Pandeiros River basin.

## 2.4. Anthropogenic stressor and pressure metrics to test the MMIs

### 2.4.1. Water quality and physical habitat stressors

Following Peck et al. (2006), at each sampling site, water quality (temperature ( $^{\circ}\text{C}$ ), pH, and conductivity ( $\mu\text{S}/\text{cm}$ ) were determined through use of a multimeter (YSI Model 650). Water samples were taken to the laboratory and total solids (ppm), turbidity (UNT), dissolved oxygen (mg/L), total alkalinity (mEq/L  $\text{CO}_2$ ), total nitrogen (mg/L), total phosphorus (ug/L), and chlorophyll (ug/L) were determined via standard methods (APHA - American Public Health Association, 1998).

Physical habitat structure, such as hydrological, geomorphological, riparian vegetation, and anthropogenic impacts were evaluated through use of the US-EPA protocol (Peck et al., 2006; USEPA, 2016), adapted for use in the Cerrado biome and widely used in Cerrado stream assessments (Callisto et al., 2019, 2014; de Carvalho et al., 2017; de Castro et al., 2017; Macedo et al., 2018, 2016; Martins et al., 2018; Silva et al., 2017; Silveira et al., 2018). A series of measurements were performed on each transect and in each section between transects. Channel characteristics (e.g., wetted depth, height and width, bank-full height and width, incision height, margin slope, sinuosity, channel slope, etc.), habitat characteristics (e.g., substrate size and embeddedness, habitat types and complexity), riparian vegetation characteristics (e.g., shading of the bed and margins, density of plant strata, etc.) and human influences (e.g., presence of roads, trash, plantations, pastures, etc.) were measured. The physical habitat data were converted to metrics according to Kaufmann et al. (1999, 2009).

Estimates of anthropogenic impacts included the complexity of riparian vegetation and the degree of substrate sedimentation by sand and fines (clay and silt; Xembad), the presence and proximity of anthropogenic impacts in the riparian zone (W1\_Hall), and multilayer woody vegetation cover (Xcmgw) as described in Kaufmann et al. (1999). W1\_Hall summarizes the amount of evidence from eleven types of disturbances (walls/dikes/revetments; buildings; pavement; roads/railroads; pipes; landfills/trash; parks/lawns; row crops; pasture/range/hay fields; logging operations; mining activities) at each bank along the 11 transects at each site. The values were weighted according to their proximity to the stream (Kaufmann et al., 1999). In addition, we calculated the relative bed stability (LRBS), according to Kaufmann et al. (2009). Those physical habitat metrics were used in previous studies and proved effective for evaluating environmental quality in

Cerrado streams (Macedo et al., 2016; Silva et al., 2017).

### 2.4.2. Land use pressures

Evaluation of land use and cover was based on supervised classification and post-hoc evaluation of digital images, where classes were assigned to pixels of satellite images, creating homogeneous patterns to which different classes of land use and cover are associated (Hughes et al., 2019; Santos et al., 2017). The images used in this work were from the Landsat-8 satellite, sensor OLI, orbit scene 219/71 e 219/70, for the year 2016, made available by INPE (Instituto Nacional de Pesquisas Espaciais) (<http://www.dgi.inpe.br>).

## 2.5. Selection of multimetric indices and biological metrics calculation

We performed a bibliographic search in the Web of Science, Scopus, and SciELO, on the terms IBI (index of biotic integrity), multimetric index, and macroinvertebrates. We found 32 papers that included indices developed in different parts of the world, from the year 2002 to 2018. We selected 17 papers that described indices with potential to be applied in the Cerrado biome, considering the reproducibility of the index and whether the metrics used to elaborate each index could be calculated from our data. We discarded studies that included metrics not obtained in our study, such as insect genera and environmental quality indices specific to each region (e.g., Lunde and Resh, 2012; Melo et al., 2015; Mondy et al., 2012; Pond et al., 2013; Saito et al., 2015; Shi et al., 2017; Weigel and Dimick, 2011). Biological metrics based on the final metrics chosen for each index were calculated from Pandeiros data (Table 1, Supplementary Material - Table S1). Each index was calculated and applied as described by its authors, including their processes for defining floor and ceiling values, standardization and scoring, and assessment thresholds. We had only one exception to the authors' index development. If the original index metrics did not consider correction for natural variability (e.g., catchment area, channel slope, or climate data), we determined if that correction was needed for our data.

## 2.6. Data analyses

### 2.6.1. Classification and validation of least disturbed sites

Selection of reference sites is a first step in any MMI development (Hughes et al., 1986). In the Pandeiros River basin, we used the concept of least-disturbed sites (Martins et al., 2018; Stoddard et al., 2008),

**Table 1**  
Final indices evaluated.

Region	Biome	Authors
South America Brazil	Amazonian Forest	Couceiro et al. (2012)
	Atlantic Forest	Baptista et al. (2007)
		Oliveira et al. (2011)
	Cerrado	Ferreira et al. (2011)
		Macedo et al. (2016)
Chile	Mediterranean Shrub	Silva et al. (2017)
		Fierro et al. (2018)
Central America Panamá	Rain forest	Helson and Williams (2013)
North América USA	Temperate Broadleaf Forest	Klemm et al. (2003)
	Mediterranean Shrub	Ode et al. (2005)
Europe Belgium	Temperate Broadleaf Forest	Gabriels et al. (2010)
Africa Ethiopia	Savanna	Lakew and Moog (2015)
		Mereta et al. (2013)
Asia China	Rainforest	Chen et al. (2014)
		Li et al. (2010)
Vietnam	Rainforest	Nguyen et al. (2014)
South Korea	Temperate Broadleaf Forest	Jun et al. (2012)

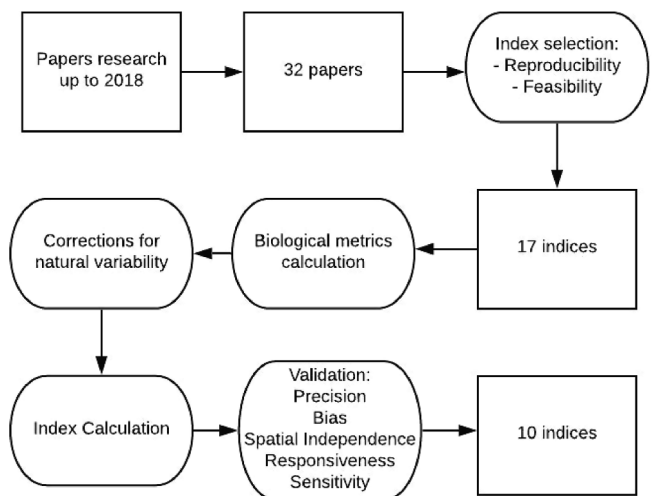
because no place on the planet can be considered totally pristine as a result of atmospheric contaminants and anthropogenic climate change (Hughes, 1995, 2019). Those sites are where the best biological, water quality and physical habitat conditions are found, considering the current state of the landscape (Stoddard et al., 2006). To calculate locations with least anthropogenic disturbance, we used the Integrated Disturbance Index (IDI; Ligeiro et al., 2013), which is calculated from local (LDI – Local Disturbance Index) and catchment (CDI – Catchment Disturbance Index) anthropogenic impacts. For evaluating the LDI, we used the metric W1\_Hall, which summarizes the amount of evidence observed in the channel and in the riparian zone. Those values are weighted according to the proximity of the observation from the stream channel, calculated as described in Kaufmann et al. (1999). The CDI was based on the % of human land uses in the catchment of each site, weighted by the potential of degradation that each has on the aquatic ecosystem ( $CDI = 4x \% \text{ urban} + 2x \% \text{ agriculture} + \% \text{ pasture}$ ; Ligeiro et al., 2013).

Through examination of an ordered IDI plot, stream sites with the lowest IDI values were considered least disturbed, and those with the highest IDI values were considered most-disturbed. This procedure has been used in a series of multimetric indices in South America (e.g., Chen et al., 2017; de Carvalho et al., 2017; Fierro et al., 2018; Macedo et al., 2016; Terra et al., 2013).

To test whether the macroinvertebrate assemblages responded to impacts and to validate sites as least-disturbed, a Mann-Whitney test (U-Test) was performed. To do so we used the biological metrics of total richness and richness and percentage of sensitive organisms (Ephemeroptera, Plecoptera and Trichoptera - EPT) versus the IDI, W1\_Hall, and the metrics comprising W1\_Hall in one-to-one analyses. To assess whether the disturbances affected water quality, we performed a Pearson correlation analysis between each of the nine water quality variables and the IDI, one by one. In addition, we used thresholds defined in the Brazilian national environmental law (CONAMA – Conselho Nacional do Meio Ambiente 357/2005) to evaluate water quality (Brasil, 2005).

### 2.6.2. Natural variability

We used multiple linear regression to assess the influence of natural variability (catchment area, elevation, slope, temperature and rainfall)

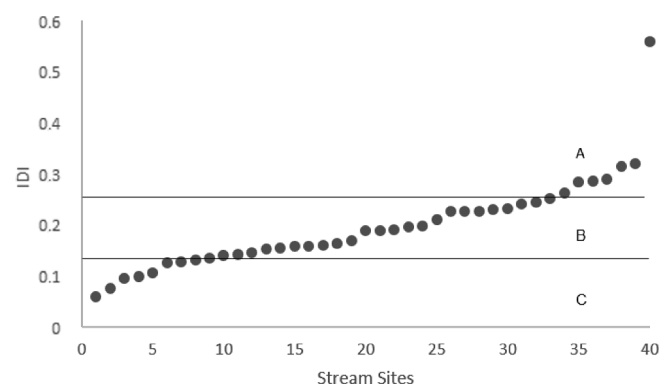


**Fig. 2.** Steps for index selection, metric calculation and validation.

on the biological metrics comprising each MMI. The analysis was performed only for the sites classified as least disturbed. Significant results ( $r^2 > 0.70$ ;  $p < 0.05$ ) were corrected by subtracting the predicted metric values obtained by regression from each raw value (residual value = observed – expected) (Cao et al., 2007; Chen et al., 2014; Klemm et al., 2003; Stoddard et al., 2008).

### 2.6.3. MMI performance tests

To verify if the indices calculated from our data were effective in evaluating Pandeiros sites, we conducted five statistical analyses (Figs. 2 and 3), as suggested in many MMI development procedures (e.g., Chen et al., 2014; Hering et al., 2006; Klemm et al., 2003; Macedo et al., 2016; McCormick et al., 2001; Ruaro and Gubiani, 2013; Silva et al., 2017; Stoddard et al., 2008). 1) *Precision* was assessed through use of the Coefficient of Variation (CV) based on the MMI scores calculated from the least-disturbed sites. The lower the CV, the more precise the MMI (Chen et al., 2014). 2) *Bias* was determined by evaluating the degree to which MMIs were influenced by natural variation. To do so we performed Pearson correlations between the MMI and the natural variables, one by one (Cao et al., 2007; Hawkins et al., 2010). 3) *Spatial independence* was evaluated by measuring the degree of spatial autocorrelation or the degree to which the MMI score at one site was influenced by that of a neighboring site (Anselin and Bera, 1998). In other words, this test assesses the degree to which an MMI could distinguish nearby sites. 4) *MMI responsiveness* assessed the degree to which the disturbance classes (good, fair, poor) were significantly



**Fig. 3.** Integrated Disturbance Index (IDI) values in stream sites in the Pandeiros River basin. IDI values range from 0 to 1, with 0 sites having the best environmental quality. Cut-off values for site classification: A) most-disturbed:  $IDI \geq 0.26$ ; B) intermediate:  $0.13 \leq IDI \leq 0.25$ ; C) least-disturbed:  $IDI \leq 0.12$ .



**Table 2**

Comparative values of index tests. (\*significant result, F = Failed, P = Passed).

	Precision	Bias	Space Independence			Responsiveness		Sensitivity			
MMIs	CV		Moran-I	p		Anova (p)	r <sup>2</sup>	p	Residual validation		
Klemm et al. (2003)	19.01	F	No	0.02	0.83	P	< 0.001*	P	0.28	0.004*	P
Ode et al. (2005)	7.25	P	No	−0.05	0.62	P	< 0.001*	P	0.27	0.016*	P
Baptista et al. (2007)	4.36	P	No	−0.09	0.38	P	< 0.001*	P	0.29	0.003*	P
Li et al. (2010)	0.93	P	No	−0.09	0.36	P	< 0.001*	P	0.32	0.007*	P
Gabriels et al. (2010)	53.08	F	No	−0.11	0.3	P	0.71	F	0.08	0.752	F
Oliveira et al. (2011)	24.53	F	No	−0.05	0.72	P	< 0.001*	P	0.38	0.001*	P
Ferreira et al. (2011)	6.32	P	No	−0.02	0.82	P	< 0.001*	P	0.20	0.030*	P
Couceiro et al. (2012)	11.14	P	No	−0.15	0.12	P	< 0.001*	P	0.17	0.006*	F
Jun et al. (2012)	10.39	P	No	−0.11	0.28	P	< 0.001*	P	0.30	0.012*	P
Mereta et al. (2013)	7.55	P	No	−0.02	0.88	P	< 0.001*	P	0.50	0.000*	F
Chen et al. (2014)	95.82	F	No	0.22	0.01*	F	–	F	0.31	0.002*	P
Nguyen et al. (2014)	7.00	P	No	−0.11	0.22	P	< 0.001*	P	0.24	0.010*	P
Helson and Williams (2013)	13.39	P	No	−0.09	0.37	P	< 0.001*	P	0.36	0.003*	P
Lakew and Moog (2015)	29.76	F	No	0.09	0.38	P	–	F	0.22	0.002*	F
Macedo et al. (2016)	8.10	P	No	−0.08	0.48	P	< 0.001*	P	0.38	0.001*	P
Silva et al. (2017)	8.96	P	No	−0.08	0.41	P	< 0.001*	P	0.25	0.004*	P
Fierro et al. (2018)	13.19	P	No	−0.1	0.35	P	< 0.001*	P	0.37	0.003*	P

different from each other. To do so, we performed analysis of variance (ANOVA) with Bonferroni correction to test for differences between the disturbance classes given for each index and their respective boxplots (Vander Laan and Hawkins, 2014). 5) *Index sensitivity* relative to the disturbance metrics was tested via multiple linear regressions between each index and the anthropogenic disturbance metrics (land use and cover, water quality and physical habitat variables; Macedo et al., 2016). We considered regression models significant that were Bonferroni corrected.

The normal distribution of each variable (land use and cover, physical habitats and water quality) was first determined using the Kolmogorov-Smirnov test; those that were not normally distributed were treated with square root arcsine (percentage data) or  $\log(x + 1)$  (other types of data) (Gotelli and Ellinson, 2013). Redundant variables were eliminated if correlated  $| > 0.70 |$ ; we retained the variable with the highest ecological relevance for the macroinvertebrate assemblages and those that are more intuitively understood (Little et al., 1999). The MMIs were validated through use of analyses of normality, homoscedasticity (Gotelli and Ellinson, 2013) and spatial autocorrelation of residuals (Anselin and Bera, 1998; Diniz-Filho et al., 2008). All MMIs that passed the five tests were considered valid for our data. To assess similarity of response between the MMIs, a Pearson correlation test was performed between the results of each of the validated indices, one by one.

### 3. Results

#### 3.1. Benthic macroinvertebrates

In total, 32,271 organisms and 82 taxa were identified. The most abundant families were Chironomidae (41%), Hydrobiidae (11%), Elmidae (7%) and Leptohyphidae (4.3%). Three non-native mollusk species were found: *Corbicula fluminea* (Corbiculidae), *Melanoides tuberculatus* (Thiaridae) and *Limnoperna fortunei* (Mytilidae).

#### 3.2. Anthropogenic pressure measures

The water quality parameters analyzed (Table S2) were within the limits established for Class 1 waters in Brazilian national legislation. Waters in this class can be used for human consumption after simple treatment, protection of aquatic communities, primary contact recreation, and irrigation of vegetables and fruits (Brasil, 2005). No correlation was observed between water quality parameters and the IDI as demonstrated by Pearson correlation analysis (Table S3). Only total

alkalinity was negatively correlated with the IDI ( $r^2 = -0.32$ ,  $p < 0.05$ ).

Riparian woody vegetation cover (Xcmgw) and substrate embeddedness (Xembed), were not significantly affected by anthropogenic impacts ( $p > 0.05$ ) and were not correlated with the IDI (Table S4). However, the presence and proximity of anthropogenic impacts in the riparian zone (W1\_Hall), which was a component of the IDI, was highly correlated with the IDI, indicating that IDI scores were driven by LDI (W1\_Hall) scores.

The evaluation of land use and cover (catchment) types showed a high proportion (65.08%) of natural savanna vegetation in the basin, followed by pasture (33.44%), agriculture (1.43%), and urban areas (0.02%).

#### 3.3. Least-disturbed sites selection

Based on the IDI, 7 stream sites were deemed highly altered, 26 sites were in intermediate condition, and 7 sites were considered least-disturbed (Fig. 3). The classification of least-disturbed sites (IDI scores) was validated through the U-test (least-disturbed sites versus most-disturbed sites:  $U = 0.00$ ;  $z$  adjusted =  $-3.09839$ ;  $p = 0.002$ ). EPT richness was significantly different between least- and most-disturbed sites ( $U = 10.00$ ;  $z$  adjusted =  $2.547529$ ;  $p = 0.012355$ ) and the organisms were significantly affected by catchment disturbance (IDI:  $U = 0.00$ ;  $z$  adjusted =  $-3.46410$ ;  $p = 0.001$ ), and local disturbance (w1\_Hall:  $u = 0.00$ ;  $z$  adjusted =  $-3.46410$ ;  $p = 0.000532$ ). The key local disturbance metrics were pasture (45%), litter/garbage in the channel or channel margins (20%), and riparian agriculture (10%).

#### 3.4. Multimetric indices

Of the 17 MMIs evaluated, seven failed one or more of our validation steps (Table 2). In the evaluation of precision, five of the 17 indices showed a Coefficient of Variation above 15% among reference sites. None of the indices indicated natural variability bias because there was no correlation between the MMIs and the natural variation metrics. Only one index (Chen et al., 2014) indicated spatial autocorrelation in the index scores. We believe this resulted from the absence of a metric that is sensitive to differences among least disturbed sites. One index (Gabriels et al., 2010) responded weakly to disturbances and was eliminated. The remaining indices showed significant differences between the different disturbance classes defined for each index. The indices of Chen et al. (2014) and Lakew and Moog (2015) did not classify stream sites into quality classes (good, intermediate, poor) according to

**Table 3**

The MMIs selected, including their regressions with various stressors and pressures.

Index	r <sup>2</sup>	Variables and $\beta$ (std) values			
Macedo et al. (2016)	0.38	Nitrogen: -0.32	Natural (%): 0.57	Alkalinity: 0.47	
Fierro et al. (2018)	0.37	Fines (%): 0.44	Pheophytin: 0.34	Urban (%): -0.32	
Helson and Williams (2013)	0.36	Turbidity: -0.38	Natural (%): 0.44	pH: 0.31	
Li et al. (2010)	0.32	Turbidity: -0.33	Natural (%): 0.34	Nitrogen: -0.34	
Jun et al. (2012)	0.30	Nitrogen: -0.40	Natural (%): 0.41	pH: 0.34	
Baptista et al. (2007)	0.29	Turbidity: -0.41	Natural (%): 0.33		
Ode et al. (2005)	0.27	Turbidity: -0.40	Natural (%): 0.36		
Silva et al. (2017)	0.25	Turbidity: -0.44			
Nguyen et al. (2014)	0.24	Turbidity: -0.29	Natural (%): 0.38		
Ferreira et al. (2011)	0.20	Natural (%): 0.42	pH: 0.38		

**Table 4**

Pearson's correlation between the final indices that were validated by the tests. Significant correlations are marked with \*.

	Fierro et al. (2018)	Macedo et al. (2016)	Jun et al. (2012)	Mereta et al. (2013)	Baptista et al. (2007)	Nguyen et al. (2014)	Li et al. (2010)	Ode et al. (2005)	Helson and Williams (2013)	Ferreira et al. (2011)
Fierro et al. (2018)	1.00									
Macedo et al. (2016)	-0.07	1.00								
Jun et al. (2012)	0.03	0.79*	1.00							
Mereta et al. (2013)	0.20	0.56*	0.62*	1.00						
Baptista et al. (2007)	-0.05	0.61*	0.83*	0.42*	1.00					
Nguyen et al. (2014)	-0.07	0.77*	0.75*	0.59*	0.75*	1.00				
Li et al. (2010)	-0.05	0.78*	0.90*	0.53*	0.87*	0.85*	1.00			
Ode et al. (2005)	-0.03	0.63*	0.60*	0.41*	0.69*	0.83*	0.72*	1.00		
Helson and Williams (2013)	-0.06	0.54*	0.78*	0.29	0.92*	0.70*	0.85*	0.62*	1.00	
Ferreira et al. (2011)	0.01	0.63*	0.86*	0.42*	0.87*	0.73*	0.85*	0.59*	0.90*	1.00
Silva et al. (2017)	-0.14	0.67*	0.84*	0.44*	0.86*	0.78*	0.95*	0.68*	0.90*	0.86*

the scales defined by the authors, so it was not possible to perform the variance tests (Supplementary Material – Fig. S1). Four indices (Couceiro et al., 2012; Gabriels et al., 2010; Lakew and Moog, 2015; Mereta et al., 2013) were insensitive to stressor or pressure variables because they violated normal and homoscedasticity assumptions and lacked residual normality. At the end of the five validation steps, ten of the 17 MMIs passed all tests and were correlated with various stressor and pressure variables (Table 3). Except for the Fierro et al. (2018) MMI, all the index results were strongly or moderately correlated with each other when calculated through use of the Pandeiros River data (Table 4).

#### 4. Discussion

From the total of seventeen indices tested, ten were considered effective for assessing the environmental condition of Pandeiros basin stream and river sites. That is, they were not influenced by natural variability, were spatiality independent, and had good responsiveness and sensitivity to identify anthropogenic disturbances. Those ten indices were effective in a basin with moderately altered environmental conditions (Callisto et al., 2019) and only local stressors, indicating that the indices were sensitive and responsive even to low disturbance gradients. The biological indicators (MMIs) and the disturbance indicators (IDI) indicated the mostly local (LDI, W1\_Hall) effects of riparian pasture, trash and agriculture.

Natural landcover predominates in the Pandeiros River basin, but locally significant anthropogenic impacts influenced environmental and biological quality at some sites (Fig. S2). We found that the basin has higher quality environmental conditions when compared to other studies using the IDI (e.g., Fierro et al., 2018; Ligeiro et al., 2013; Macedo et al., 2016; Silva et al., 2017; Terra et al., 2013), reflecting the relatively good ecological condition and low environmental fragility in this basin (Callisto et al., 2019). Such protected areas are critical to limit anthropogenic pressures and the effects of local stressors (Barlow et al., 2018). As observed, human impacts in the basin are evidenced at local

scales, that is, the protection status of the area is limiting large-extent anthropogenic pressures; however, it is not limiting local stressors. Other studies also have shown a decrease in biodiversity, even in protected areas (Hallmann et al., 2017; Sánchez-Bayo and Wyckhuys, 2019).

Benthic MMIs reflect anthropogenic disturbances, with decreasing scores as disturbance increases (Ruaro and Gubiani, 2013; Silva et al., 2017; Stoddard et al., 2008). We observed that natural variability among the evaluated sites was only relevant at the metric level, and after their correction, it was not relevant in the MMI scores that we calculated for the Pandeiros River basin, as reported in other studies (Fierro et al., 2018). Such corrections for natural variability increase the accuracy, responsiveness and sensitivity of MMIs, thereby improving their performance across large regions in China (Chen et al., 2019, 2014), Bolivia (Moya et al., 2011), the United States (Stoddard et al., 2008), and Brazil (de Carvalho et al., 2017; Macedo et al., 2016; Pereira et al., 2016; Silva et al., 2017).

Our validation steps offer useful insights for MMI development and testing. The indices were tested in an area covered by natural vegetation that has low to moderate human impact (Callisto et al., 2019), and this impact was evidenced only on a local scale. The indices tested under these conditions have some limitations as to their reproducibility; however, the ten indices that passed all validation stages proved to be extremely efficient for detecting even moderate and local impacts. In general, most indices were eliminated by lack of precision as indicated by high coefficients of variation in their reference areas, even when their metrics were corrected for natural variability. Therefore, we recommend considering reference area heterogeneity in an initial screening step (Martins et al., 2018) and correcting for it when appropriate (Fierro et al., 2018; Ruaro et al., 2020). Some indices were eliminated because of the lack of normality of their residuals, meaning that they had a tendency for greater predictive error at low or high MMI scores. Because scores at those extremes are often deemed most important in local and regional risk assessments (Paulsen et al., 2008; Silva et al., 2017), residual evaluation can be a critical validation step.

Except for the Lakew and Moog (2015) MMI, all indices that were eliminated were developed for tropical or temperate forests. However, other indices that were also developed in these biomes performed well in our analyses, suggesting that index construction or metric selection were more important factors than biome. Spatial autocorrelation was not a preponderant factor for index elimination, only one index was eliminated in this phase (Chen et al., 2014). The index elaborated by Chen et al. (2014) consists of 4 metrics that describe only richness and composition (Trichoptera taxa richness, Ephemeroptera and Plecoptera taxa richness, Total\_insect taxa richness, % Ephemeroptera, Plecoptera and Trichoptera individuals). Many authors recommend that indices be composed of metrics that represent the multiple biological aspects of an assemblage (Hering et al., 2006; Huang et al., 2015; Karr and Chu, 1999; Stoddard et al., 2008), such as richness, composition, diversity, dominance, tolerance, feeding groups, mobility and breathing types. An index composed of only four metrics focused on taxa richness is likely to be highly sensitive to natural taxa distributions, and therefore be spatially autocorrelated for purely natural reasons. The final MMI correlation analysis showed that the selected indices are moderately or highly correlated with each other, except for Fierro et al. (2018), presumably because that index is the only one that included a total macroinvertebrate density metric. Density and abundance metrics are not commonly used in MMIs because density and abundance vary greatly with location, season, collection method, and species counted (Aguar et al., 2015; Hughes et al., 1998).

The tolerance of benthic organisms does not vary significantly between different regions and different climates (Jacobsen et al., 2008), which in part justifies the applicability of several indices to our study area. Therefore, any of the ten MMIs validated in this study are likely to be effective in discriminating most-disturbed stream sites from least-disturbed sites or reference conditions because of the integrated response of biological assemblage metrics (Hughes et al., 1998; Stoddard et al., 2008). The MMIs developed from several macroinvertebrate metrics that represent different assemblage structural and functional characteristics have better performance than MMIs that fail to do so (Hering et al., 2006; Silva et al., 2017; Stoddard et al., 2008). Thus, several dimensions of biological systems are incorporated into a single index, which increases their ability to reflect anthropogenic disturbances in aquatic ecosystems (Karr and Chu, 1999).

Among the metrics selected to calculate MMIs used in this study, taxonomic richness and sensitivity/tolerance metrics stand out. Taxonomic richness reflects a key component of taxonomic diversity (Baptista et al., 2007) and MMI construction (Ruaro et al., 2020). The percentage and richness of Ephemeroptera, Plecoptera and Trichoptera (EPT) are widely used because these organisms are sensitive to various types of anthropogenic impacts (Ferreira et al., 2011; Firmiano et al., 2017; Klemm et al., 2003; Li et al., 2014; Macedo et al., 2016; Mereta et al., 2013; Pescador et al., 1995; Ruaro et al., 2020; Silva et al., 2017; Stoddard et al., 2008). Diversity indices, such as the Shannon-Wiener diversity index (Gabriels et al., 2010; Helson and Williams, 2013; Jun et al., 2012; Li et al., 2010; Oliveira et al., 2011; Silva et al., 2017) and the Margalef diversity index (Helson and Williams, 2013; Mereta et al., 2013; Nguyen et al., 2014) are also frequently used in MMIs. These indices integrate assemblage taxonomic richness and dominance or evenness. Functional attributes or traits are also commonly used in MMIs (Chen et al., 2019; Moya et al., 2011; Saito et al., 2015; Silva et al., 2017; Stoddard et al., 2008) because of their ability to detect anthropogenic disturbances independently of taxonomic composition (Tomanova and Usseglio-Polatera, 2007).

In the United States, Europe and Australia, there is widespread use of biological indicators to assess continental-scale aquatic conditions (Barbour et al., 1999; Davies et al., 2010; Hering et al., 2006; USEPA, 2016) because of legal statutes. However, in most South American countries, there are no such statutes, which is reflected in the small number of studies on the development and application of MMIs for evaluation in national or continental programs (Buss et al., 2015; Ruaro

and Gubiani, 2013). In Latin America, interest in developing and testing rapid assessment tools has increased over the past decade, but few studies have tested and standardized methods and indices that are central to the development of a systematic and effective national or continental biomonitoring program (Buss et al., 2015). However, multimetric indices can be used effectively to support environmental managers in national and continental water body monitoring programs (Hering et al., 2006; Moya et al., 2011; Pont et al., 2006; Ruaro and Gubiani, 2013; USEPA, 2016). Despite those international examples, Brazil still lacks a standardized national approach to evaluate and maintain the quality of its watersheds (Buss et al., 2015). Anthropogenic disturbances have become increasingly frequent in the Cerrado biome (Strassburg et al., 2017) and environmental catastrophes, such as mine tailings dam failures, have recently occurred (Silveira et al., 2019). Such chronic and acute pollution has considerably degraded the water quality of Cerrado river basins. Thus, it is necessary to apply fast and efficient tools, such as those developed in this study, for the monitoring and diagnosis of environmental quality in the Cerrado biome.

To be effective and used at national and continental levels, MMIs must use comparable metrics that represent key ecological parameters of aquatic assemblages (Ruaro et al., 2020; Ruaro and Gubiani, 2013; Stoddard et al., 2008). However, in many aquatic ecosystem studies, metrics were adapted for specific regional conditions and are difficult to compare globally (e.g., Pond et al., 2013). This lack of standardization hinders using MMIs in water resource management (Ruaro and Gubiani, 2013). Other challenges to wide use of MMIs include the lack of standardized sampling, ignorance of all factors that may influence aquatic assemblages, and determination of sensitive metrics that are applicable at regional and national spatial extents (Stoddard et al., 2008). Contrary to the implicit assumptions indicated by the many published MMIs (e.g., Ruaro and Gubiani, 2013), we found that several relatively simple existing indices, composed of a few metrics replicable in any region and easily calculated with a sufficiently robust data set, were effective for assessing stream sites at basin extents. However, this does not mean that any single MMI will suffice for all sites globally because we found several inappropriate MMIs for our study area. Nonetheless, we did find a set of metrics (taxa richness, diversity, sensitivity/tolerance, function) that should be considered for application and perhaps for moving towards a more standardized MMI that would facilitate national, continental, or global comparisons of site status and trends (Buss et al., 2015; Moya et al., 2011; Ruaro et al., 2020; Stoddard et al., 2008). Furthermore, the correlations among MMIs (Table 4) indicate that those MMIs were consistent for assessing water body condition at the sampling sites, meaning that any one of those indices might suffice for a rapid assessment of water body condition. The selection and development of such biological indicators is very important for decision making (Nöges et al., 2009). If used in conjunction with other water body assessment and forecasting tools (Alizadeh et al., 2018; Chen and Chau, 2016; Hughes, 2019; Olyaei et al., 2015; Shamshirband et al., 2019), they can be used effectively as a basis for protecting and rehabilitating degraded environments (Statzner and Bêche, 2010).

## 5. Conclusions

This study indicated that ten indices, originally developed in multiple continents, were effective in evaluating ecological conditions in the Pandeiros River basin. Therefore, it is not necessary to elaborate new benthic MMIs for environmental quality assessments in each neotropical river basin. Instead, we recommend developing standard sampling and processing methods so that published indices can be used in national extent evaluations. In addition, this study offers an approach for standardizing and using MMIs in future evaluations of environmental quality in other neotropical basins. Lastly, even in protected areas, we observed that local disturbances degraded biological



condition, indicating the importance of local actions for conserving and rehabilitating water resources in this and similar basins. As long as the particularities of our study area are observed, such as the presence of large areas of natural vegetation and mostly local anthropogenic impacts, our conclusions can be extended to other regions. In our case, there was a disturbance gradient, but at a local extent, because the study was conducted in a well-preserved area protected by national laws. For future applications of this metric testing approach, it would be useful to include more sampling sites, consider a wider diversity of river basins, and assess a stronger disturbance gradient in the Cerrado and multiple neotropical biomes by collaborating with other aquatic ecologists nationally and globally.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2019.105953>.

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