



## Incorporating costs, thresholds and spatial extents for selecting stream bioindicators in an ecotone between two Brazilian biodiversity hotspots

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### ABSTRACT

Biomonitoring is critical for characterizing and monitoring status, spatial patterns, and long-term trends in the ecological condition of freshwater ecosystems. The selection of cost-effective bioindicators is a critical step in establishing such monitoring programs. Key indicator considerations are a reliable response to anthropogenic disturbances, a high benefit-cost-ratio and sensitivity at multiple spatial extents. We evaluated non-linear responses of Ephemeroptera, Plecoptera and Trichoptera (EPT) and fish to the effects of native vegetation loss within buffers of 100 m and 1000 m and assessed the sampling and processing costs involved for each assemblage. We sampled 37 neotropical stream sites in the Formoso River network, a karstic region of the Bodoquena Plateau, midwest Brazil, lying in the ecotone between the Cerrado and Atlantic Forest biological hotspots. We used TITAN (threshold indicator taxa analysis) to identify six indicator taxa, four EPT genera and two fish species. The four EPT genera had low negative thresholds to native vegetation loss, whereas the two fish species had positive thresholds. Thresholds were lower for the 100 m buffers than the 1000 m buffers for EPT. The most sensitive taxon (*Macronema*, Trichoptera) had a threshold of 0% native vegetation loss in the 100 m buffers and nearly 40% in the 1000 m buffers. For taxa richness, we found no non-linear response to the effects of native vegetation loss for buffer extent nor assemblage. The total cost for EPT biomonitoring was US\$ 3,616; whereas for fish, the total was US\$ 1,901. Although fish were less expensive than EPT, they did not respond negatively to native vegetation loss and their positive threshold started at 48%, a level of vegetation loss that was highly disruptive of EPT. Therefore, we do not recommend using fish to monitor the effects of native vegetation loss on headwater streams in the Bodoquena Plateau. Although EPT monitoring costs 52% more than fish assemblage monitoring, it detected earlier impacts of the effects of native vegetation loss on stream biota, especially in the 100 m buffer. Therefore, EPT are more cost-effective early warning indicators for monitoring the effects of native vegetation loss in Bodoquena Plateau headwaters.

### 1. Introduction

Freshwater ecosystems are speciose systems and provide multiple

ecosystem services (Dudgeon et al., 2006). However, they are experiencing declines in biodiversity and ecosystem services, mainly because of hydromorphological modifications, species invasions, and water

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pollution driven by natural resource exploitation (Allan, 2004; Dudgeon et al., 2006). Therefore, conservation and monitoring practices are crucial, highly recommended (Lindenmayer et al., 2012), and part of international agreements, such as the Aichi Targets, the 2030 United Nations Agenda for Sustainable Development, and the Post-2020 Global Biodiversity Framework (Navarro et al., 2017; CBD, 2020).

Biomonitoring programs are crucial for characterizing and evaluating status and trends in the ecological condition of freshwater ecosystems, including the presence, extent, and severity of biological impacts, as well as conservation and management decisions (Rosenberg and Resh, 1993; Lindenmayer et al., 2012). Those impacts are measured by bioindicators and their rigorous selection is a critical step for implementing biomonitoring programs (Bonada et al., 2006; Caro, 2010; Lindenmayer et al., 2012). The selection of a bioindicator needs to fulfill at least three key criteria (rationale, implementation, and performance) to guarantee a reliable response to anthropogenic disturbances and reasonable cost-effectiveness (Hughes, 1993; Bonada et al., 2006).

Understanding how a potential indicator responds to anthropogenic impacts is the first step in selecting a bioindicator (rationale) (Bonada et al., 2006; Gardner et al., 2008). Most studies in freshwater systems use linear relationships to understand how a potential indicator is affected by predictor variables. Linear models can predict changes in a response variable based on change in levels of a predictor variable (e.g., loss of native riparian vegetation), which can guide decision-making (Mac Nally, 2000). But assuming linear relationships impedes determination of levels of anthropogenic impacts that cause disproportional effects on the indicators. For example, if a given indicator measure (e.g., species richness) is linearly affected by loss of native riparian vegetation, the establishment of a legal width of native riparian vegetation that protects most biodiversity is unclear. However, a growing number of studies have demonstrated non-linear effects of riparian vegetation on biodiversity, constituting ecological thresholds (Roque et al., 2018; Brito et al., 2020; Dala-Corte et al., 2020). These thresholds arise from the disproportional change in the response variable caused by a small change in a predictor variable. Such thresholds are useful for setting regulatory limits and defining conservation actions involving social choices and negotiating values and goals in conservation, rehabilitation and management initiatives (Suding and Hobbs, 2009). Although some assemblage studies have identified thresholds in freshwater systems (Brejão et al., 2018; Roque et al., 2018; Brito et al., 2020; Dala-Corte et al., 2020), that information has not yet been incorporated into bioindicator selection.

Given the dramatic rate of vegetation loss and its consequences for biodiversity in Neotropical areas (Ribeiro et al., 2009; Strassburg et al., 2017), the measurement of riparian vegetation loss via remote sensing is *per se* an important source of information for ecosystem monitoring and decision making. However, such a pressure indicator is only weakly suitable for monitoring aquatic biota, which must be assessed by on-the-ground surveys (Schmeller et al., 2015). Some studies have shown low associations between aquatic biodiversity and landscape metrics, such as forest cover (Heino et al., 2008; Roque et al., 2010). This low association may result from other co-occurring pressures and stressors that are not captured by remote sensing, such as pollution, selective logging, fire, edge effects, migration barriers, channel morphology, and delayed species extirpations (Dudgeon et al., 2006; Kuussaari et al., 2009). Poor associations may also result from the use of insensitive predictor or response indicators as well as poor study designs or statistical analyses (Hughes, 1993; Brito et al., 2020). In addition, efforts to evaluate management interventions in riparian forests require *in situ* biological data across a gradient of riparian forest loss. Multi-scale remote sensing, landscape variables and biological and habitat data from *in situ* monitoring systems are all needed to understand how aquatic biological indicators respond to anthropogenic pressures and how direct and indirect indicators can be used in complementary ways for decision making (Herlihy et al., 2020).

Bioindicators must also be cost-effective to be implemented in monitoring programs (implementation; Hughes, 1993; Bonada et al., 2006; Caro, 2010; Gardner et al., 2008). Several interconnected requirements need to be considered to calculate costs of obtaining biodiversity information, including the time spent on sampling, sorting, identification and the availability of technical knowledge (Hughes, 1993; Gardner et al., 2008). These requirements can be used to estimate the monetary costs and the cost-effectiveness of a bioindicator (Gardner et al., 2008; Valente-Neto et al., 2018b). In this sense, a good bioindicator should have (i) low cost for both fieldwork and sample processing, (ii) straightforward sampling protocols, and (iii) low cost for taxa identification (Hughes, 1993; Gardner et al., 2008; Caro, 2010; Valente-Neto et al., 2018b). However, the monetary costs involved in sampling and processing bioindicators in freshwater systems remains incipient and varies among institutions (Valente-Neto et al., 2018b).

Different species are influenced by, and perceive the environment at different spatial and temporal extents (Levin, 1992). Many bioindicator selection studies are biased towards the assessment of local site extent responses, i.e., a limited area close to the sampling site. The use of local site extent is interesting in the biomonitoring context because it is directly related to local impacts on riparian vegetation, which provides organic substrates, such as leaves and wood, and bank stability to streams. However, stream and river functioning is also affected by fluxes of energy and matter from terrestrial ecosystems over large spatial and temporal extents (Hughes et al., 2019). These fluxes carry nutrients, toxics and sediments from entire catchments to sites, increasing stream sedimentation, contamination, channel erosion, and flood intensity and frequency (Von Schiller et al., 2008). In this context, evaluation of multiple spatial extents provides a better assessment of assemblage responses to anthropogenic impacts (Roque et al., 2010; Herlihy et al., 2020). This is important because assemblages that respond to impacts at different spatial extents can be selected to indicate multiple anthropogenic impacts (performance).

In this paper, we address three dimensions in the selection process of neotropical stream condition indicators: ecological threshold identification (rationale), response extent (performance) and monetary costs (implementation). Our aim is to provide a framework that can be applied in different biomonitoring contexts, particularly those experiencing limited funding. We used as a system model the karstic region of the Bodoquena Plateau, midwest Brazil, because this region is composed of three characteristics that make it a priority for conservation and biomonitoring studies. i) Streams have high levels of biodiversity and many endemic species (Sabino and Andrade, 2003; Koroiva et al., 2017). ii) The region lies in an ecotone between two biodiversity hotspots, Cerrado and Atlantic Forest (Myers et al., 2000). iii) The region is an important Brazilian ecotourism area (Sabino and Andrade, 2003).

Therefore, we evaluated EPT and fish assemblage responses to the effects of native riparian vegetation loss at different spatial extents to determine the thresholds at which that loss resulted in loss or increase of indicator species. EPT and fish are recognized as key indicators of stream condition, because they have predictable responses to habitat loss gradients (Valente-Neto et al., 2018b; Herlihy et al., 2020). In general, we expected to identify different threshold levels for EPT and fish. We predicted that EPT would be more sensitive than fish to native vegetation loss mainly at small spatial extents because they are highly responsive to substrate quality and more directly dependent on the riparian zones for feeding, refuge and dispersal (Valente-Neto et al., 2018b; Brito et al., 2020; but see Herlihy et al., 2020). Furthermore, we expected that species that depend on conditions or resources provided by the riparian forest (e.g. wood and leaves) would show synchronous threshold response to native vegetation loss. We evaluated the costs to collect, process and identify each assemblage sampled. Then, we present a user-friendly framework to facilitate dialogue among scientists, decision makers, and other stakeholders interested in biomonitoring by visualizing trade-offs among rationale, spatial extent, and cost for selecting bioindicators.

## 2. Methods

### 2.1. Study area and land use

The study was conducted between July and October 2016 (dry season) in the Formoso River network, part of the Upper Paraguay River basin. The Formoso River network is located in the central region of Bonito municipality, Mato Grosso do Sul state, Brazil. The Formoso River basin is a mosaic of two Brazilian biomes and biodiversity hotspots: the Cerrado and Atlantic Forest (Myers et al., 2000). These Brazilian hotspots are well known for their high biodiversity and anthropogenic pressures, mainly urbanization and agrobusiness (Myers et al., 2000; Ribeiro et al., 2009; Strassburg et al., 2017). The landscape of the study area is fragmented by anthropogenic activities, mostly pasture and agriculture, and most land conversion occurred in the 1970 s and 1980 s. The study area comprises remnants of native vegetation embedded in a matrix dominated by livestock pastures and soya, maize and sugar cane monocultures. We selected 37 wadeable stream sites (Fig. 1) in the Formoso River network according to their accessibility and along a near-zero to 100% buffer vegetation loss gradient (Appendix Fig. A.1). The sites reflected that matrix of native vegetation remnants embedded in a pasture and monoculture matrix. Overall, sites were small to medium sized streams (width: mean = 3.88 m, range = 1.27–10.95 m; depth: mean = 0.62 m, range = 0.16–3.79 m), and their waters were characterized by relatively low temperatures (mean: 21.80 °C; range: 17.37–25.50 °C), very low conductivity (mean: 0.36  $\mu\text{S}/\text{cm}$ ; range 0.03–0.62  $\mu\text{S}/\text{cm}$ ), mostly high levels of dissolved oxygen (mean: 7.30 mg/L; range 1.06–17.7 mg/L) and low turbidity (mean: 7.59 FNU; range 0.50–46.50 FNU). Streams within the study area are characterized by low productivity (total phosphorous: 0.09–0.176  $\mu\text{g}/\text{L}$ ; total nitrogen: 0.85–1.87  $\mu\text{g}/\text{L}$ ) (Corrêa et al., 2019).

### 2.2. Gradient of native vegetation loss

Around each site, we established two circular buffers of 100 and 1000 m in diameter and used them to measure the percentage of native vegetation both upstream and downstream of the site (Appendix Fig. A.1). We chose the proportion of native vegetation loss because it is a landscape measure that is associated with biodiversity distribution, occurrence and persistence, extinction probability, and assemblage structure (Fahrig, 2013). To calculate the proportion of native

vegetation loss, we used a land use cover map provided by MapBiomass (Souza et al., 2020) taken in 2016 at 30 m resolution.

### 2.3. Collection of biological data

We used the multihabitat approach to sample EPT (Barbour et al., 1996). We first selected a 50 m stream section and visually estimated the amounts of rock outcrops, cobble, gravel, sand, mud silt, organic matter, wood, aquatic vegetation, leaf litter, and roots. We used a D-frame net (0.5 mm mesh, 30 cm wide) and employed a kick-sampling method from downstream to upstream. Each site sample was composed of 20 sub-samples proportionally distributed among substrate types to account for micro-habitat biodiversity (Ligeiro et al., 2010). Each sub-sample measured 1 m long and 30 cm wide (net width) (covering 0.3 m<sup>2</sup>) and the entire sample covered 6 m<sup>2</sup> of stream bottom. The samples were preserved in 4% formalin, and all individuals were sorted in our laboratory. All EPT were identified to genus by using taxonomic keys (Hamada et al., 2018).

For fish, we followed Anjos and Zuanon (2007) and Casatti et al. (2013) and used a 100 m stream section that was isolated both up- and downstream with block nets (5 mm mesh) prior to sampling. Fish were sampled by 2 collectors using a seine (1.5 × 2 m, 2-mm mesh) and dip nets (0.5 × 0.8 m, 2-mm mesh) for one hour (equal effort). This method provides reliable sampling of expected richness for small streams (more than 90% of expected richness) (Casatti et al., 2013; Carvalho et al., 2017). Fish were fixed in 10% formalin for 24 h, then transferred to 70% ethanol and identified to species through use of taxonomic keys (Britski et al., 2007). All materials were deposited in the zoological collection of the Universidade Federal de Mato Grosso do Sul (ZUFMS). Because fish were not found at all sites, we could only use the results from the 25 sites where fish were collected; however, the gradient of vegetation loss was maintained in those 25 sites (Appendix Fig. A.1).

We realize that rare fish and macroinvertebrate species may be missed at these levels of sampling effort (Li et al., 2001; Terra et al., 2013; Silva et al., 2016; Hughes et al., 2021). However, rare species are omitted from threshold analyses to minimize random errors and because they provide too little information along forest loss gradients to determine thresholds (Arscott et al., 2006; Baker and King, 2010).

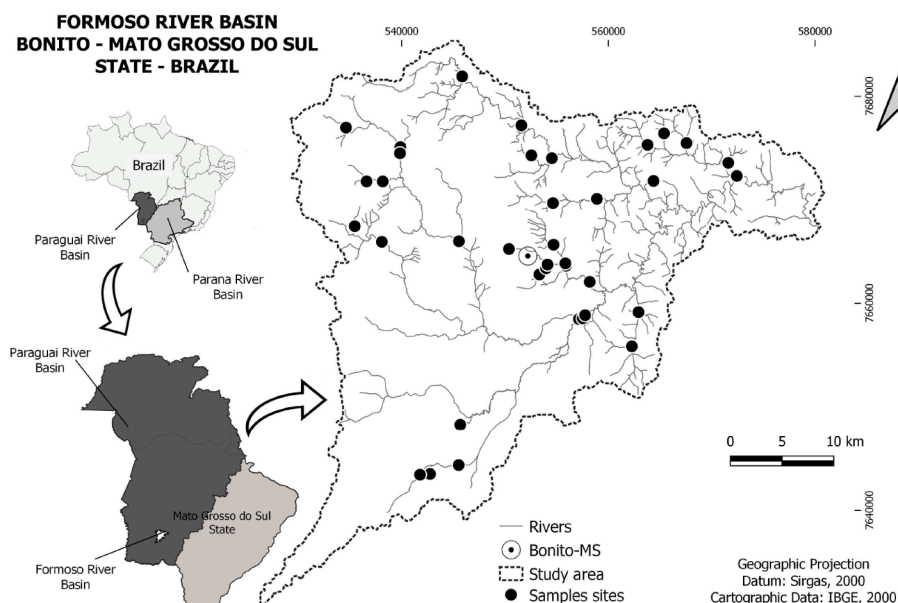


Fig. 1. Location of the 37 sites in the Formoso River basin. Note, the stream courses do not indicate intermittent reaches.

#### 2.4. Assessing EPT and fish response to vegetation loss

To determine the EPT and fish taxa with the best response to the effects of native vegetation loss at different spatial extents, we used Threshold Indicator Taxa Analysis (TITAN) (Baker and King, 2010) for each buffer size. This analysis allowed us to detect non-linear responses of taxa to an environmental gradient (native vegetation loss) as abrupt changes in taxa distribution along specific points of the gradient and it can show points of synchronized taxa changes (thresholds) as evidence for an assemblage thresholds.

TITAN uses indicator species analysis (IndVal) (Dufrêne and Legendre, 1997) for combining abundance and occurrence to describe associations between taxa and groups of samples. TITAN defines candidate change points along a gradient of an environmental variable and measures the IndVal for each taxon below and above each candidate change point, retaining the greater value (Baker and King, 2010). Then, it identifies for each species the maximum IndVal across all candidate change points, i.e. the greatest change in taxon abundance and frequency within the observed sample, retaining both IndVal and observed change point (possible threshold). This procedure is repeated by reshuffling the same sample abundances across the environmental gradient to estimate the frequency of IndVals greater than the observed maximum IndVal, and the mean and standard deviation of IndVals. The observed IndVals are then standardized as z-scores using mean and standard deviation of permuted IndVals. These z-scores can indicate negative (z-) and positive (z+) distribution changes in response to the environmental gradient changes. As a standardized effect, the z-score for each species can be summed (sum z) and used to detect change points for assemblage composition.

TITAN employs a bootstrap resampling technique for the entire analysis described above to estimate confidence limits for sum z- and sum z+ and taxon-specific change points and to estimate two important diagnostic indices for each taxon: purity and reliability. Purity evaluates the consistency in the response direction as the proportion of times (bootstraps) where the group assignment matches the observed assignment. Reliability is the proportion of times in which the maximum IndVal is less than a user-defined p value and it evaluates the frequency of strong response magnitude.

To perform the TITAN analysis, we only used taxa that had more than three occurrences. We used 1000 repetitions and 1000 bootstraps and, we set purity and reliability at 0.80. That value means we were more inclusive in indicator selection to reduce the potential of Type II errors (failure to detect an effect when it really exists; Underwood, 1997). We recognize that this decision means that a possible indicator may not be highly consistent in its response (purity) to the gradient and does not present high frequency of strong response magnitude (reliability). To inspect our results, we plotted cumulative threshold frequencies for those taxa that decrease or increase in abundance along the gradient of native vegetation loss, which are targets in the biomonitoring context. We used the TITAN2 package in R (Baker et al., 2019).

We also tested if the taxa richness of each assemblage responded non-linearly to the effects of native vegetation loss at the two buffer sizes. To test this relationship, we employed a null model, a generalized linear model (GLM), and a segmented linear regression (segmented model) and performed a model selection procedure. The null model assessed the absence of effect of native vegetation loss on richness and is suitable to verify if the other models (GLM and segmented regression analysis) were better than would be expected by chance. GLM (Poisson distribution, link = log) assumes a linear relationship between native vegetation loss and richness. Segmented regression analysis assumes a breakpoint or threshold in the relationship between native vegetation loss and richness, indicating an abrupt loss of taxa (Muggeo, 2003). Segmented regression analysis splits explanatory variables into two or more linear regressions to locate points where the linear relationships change. Estimating thresholds or breakpoints is accomplished by using different starting points and identifying regressions with the highest R<sup>2</sup> values

(Muggeo, 2003). We compared the null model, the GLM, and the segmented model by using the Akaike Information Criterion corrected for small sample sizes (AICc), the difference between the best model (lowest AICc), and each model ( $\Delta$ AICc) and Akaike weights (wAICc) (Burnham and Anderson, 2002). Models with  $\Delta$ AICc < 2.0 are considered to have substantial support, and can be considered equally plausible (Burnham and Anderson, 2002). We reported only the best model results. Segmented models were run with the segmented R package (Muggeo, 2008) and model comparisons were made with the bblme R package (Bolker and R Development Core Team, 2020).

#### 2.5. Monitoring costs of each assemblage

We estimated the collection time for each EPT taxon as 60 min and 120 min for fish based on the time consumed by four people in the field. For EPT, the sorting time was estimated by the average time spent to sort all individuals of one site (i.e., 180 min). Fish did not need to be sorted in the laboratory; thus, this step was not considered in the calculation of total time. We quantified the time spent to identify all organisms of each taxon (i.e., time spent in the identification) because some taxa are more difficult to identify than others. Finally, the total time spent for each group was the sum of the time spent in collecting, sorting, and identifying. To estimate the monetary cost of monitoring each assemblage, we considered the amount of time required for each taxon to be sampled, sorted, and identified. This total time spent for each taxon was multiplied by the wage per working hour of a biologist from the Regional Biology Council (CRBio-1 – R\$60 in 2018) and converted it to US dollars (US\$15.30; exchange rate = 3.92, 26 December 2018 according to the Brazil Central Bank).

#### 2.6. Evaluation of cost-effectiveness

We considered the assemblage having the best cost-effectiveness as the one with the lowest monetary cost, selected by TITAN as z- species, prioritizing taxa with the lowest observed threshold value (observed value of the predictor that resulted in the maximum indicator z score), and congruence in its response in the 100 m and 1000 m buffers. To facilitate the visualization of potential trade-offs between these dimensions, we plotted each taxon observed threshold value for each spatial extent and its cost.

### 3. Results

#### 3.1. Aquatic insect and fish assemblages

We collected 1282 specimens belonging to 39 genera of EPT, of which the most abundant were *Americabaetis*, *Farrodes*, *Traverhyphes* and *Ulmeritoides* (Ephemeroptera) and *Triplectides* (Trichoptera). On average, each site sample contained only 35 individuals (ranging from 5 to 150) and six genera (ranging from 3 to 17). Retaining only taxa with  $\geq 3$  occurrences, the EPT data was composed of 1220 individuals and 24 taxa. We collected 3766 fish specimens and 40 species. The most abundant were *Odontostilbe pequiira*, *Moenkhausia bonita*, *Astyanax lacustris*, *Piabarchus analis*, *Astyanax lineatus*, *Astyanax* sp., *Corydoras aeneus*, *Characidium zebra* and *Otocinclus vittatus*. Removing species < 3 occurrences left a fish dataset with 21 species and 3473 individuals.

#### 3.2. EPT assemblage response to native vegetation loss

Of the 24 EPT genera analyzed, six were selected by TITAN to indicate reliable change to the effects of vegetation loss in the 100 m buffers. *Macronema* and *Miroculis* responded negatively (z-) to vegetation loss, whereas *Americabaetis*, *Caenis*, *Thraulodes* and *Traverhyphes* responded positively (z+). *Macronema* and *Miroculis* had very high purity (0.98 & 0.97), but slightly lower reliability (0.90 & 0.85). *Americabaetis* had very high purity (0.98) and reliability (0.95). *Caenis*, *Thraulodes* and

*Traverhyphes* had high to relatively high purity (0.93 to 0.87), but lower reliability (0.86 to 0.85) (Table 1). The cumulative threshold frequency of genera that negatively responded to the effects of native vegetation loss showed a synchronic change at 11.5% (5th-95th percentiles: 0–16%) of vegetation loss (Fig. 2; Tables 1 and 2). The null model provided the best fit for EPT richness in the 100 m buffer (AICc = 208.08, intercept = 1.73; Table 3).

In the 1000 m buffers, we determined that the same two EPT genera (*Macronema* and *Miroculis*) decreased in abundance as vegetation loss increased, and no genera increased with increased vegetation loss. At this spatial extent, the two genera were more consistent indicators based on both purity (0.95 to 0.99) and reliability (0.93 to 0.96) (Table 1); however, synchronous change did not occur until 46% (5th-95th percentiles: 16–60%) of vegetation loss. This is a higher threshold value and less synchronic response compared to those z- species in the 100 m buffers (Fig. 2; Table 1 and 2). The GLM model provided the best fit for total EPT richness in the 1000 m buffer (AICc = 207.37; intercept = 2.01; slope = -0.004; Table 3), but the difference between the GLM and the null model was small (i.e., both models are equally plausible).

### 3.3. Fish assemblage response to native vegetation loss

For fish assemblages, *Astyanax lacustris* was selected as a z + species by TITAN in the 100 m buffers as an indicator of assemblage composition change. This species increased in abundance with native vegetation loss and had moderately high purity (0.89) and reliability (0.81) (Table 1). Its change threshold was 48% (5th-95th percentiles: 0–58%) of native vegetation loss (Fig. 2; Table 1 and 2). The GLM model provided the best fit for total fish richness in the 100 m buffer (AICc = 116.60; intercept = 1.80; slope = 0.004; Table 3), but both the GLM and null models models were equally plausible.

In the 1000 m buffers, *Corydoras aeneus* was selected as a z + species by TITAN and it had very high purity (0.98) but much lower reliability (0.81) (Table 1). The *C. aeneus* change threshold was 65% (5th-95th percentiles: 65–79%) of native vegetation loss (Fig. 2; Table 1 and 2). The null model provided the best fit for fish richness in the 1000 m buffer (AICc = 116.98; intercept = 1.91; Table 3).

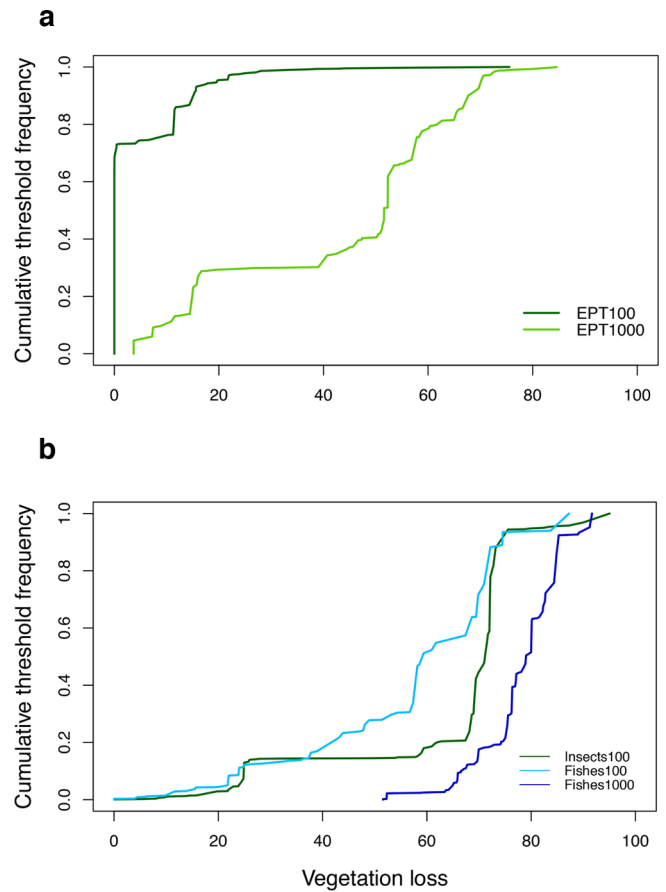
### 3.4. Biomonitoring costs for EPT and fish

The total cost for EPT biomonitoring (all genera) was US\$ 3,616 (R\$ 14,175), with an average cost per species of US\$ 94. The process of obtaining both the field and lab processing information for EPT averaged 6 h per site. The EPT genera requiring the most time were *Traverhyphes* (8 h), *Americabaetis*, *Farrodes*, *Thraulodes*, *Tripletides*, and *Callibaetis* (all with 7 h), consequently, these are the genera with the highest cost (Appendix Table A.1).

**Table 1**

Taxa selected by Threshold Indicator Taxa Analysis (TITAN) for EPT and fish assemblages depicting observed thresholds (Obs thresh; observed value of the predictor that resulted in the maximum indicator z score), frequency of occurrence (Freq), IndVal value and z-score. 5%, 10%, 50%, 90% and 95% indicate the change point quantiles among bootstrap replicates. Purity represents the consistency in the response direction as the proportion of times (bootstraps) the group assignment matched observed assignment. Reliability (Reliab.) is the proportion of times in which the maximum IndVal is < a p value and it evaluates the frequency of strong response magnitude. The sign of response (Resp) denotes taxa that increase in abundance (+) and those that decrease in abundance (-) with native vegetation loss.

Buffer	Taxa	Obs thresh	Freq	IndVal	zscore	5%	10%	50%	90%	95%	Purity	Reliab.	Resp	
<b>EPT</b>														
100 m	<i>Americabaetis</i>	69.38	17	72.72	3.13	15.58	15.84	26.79	69.38	69.38	0.98	0.95	+	
	<i>Caenis</i>	72.18	10	68.37	3.89	0.00	5.02	71.73	73.2	73.2	0.93	0.85	+	
	<i>Macronema</i>	0.00	8	36.99	2.82	0.00	0.00	0.03	15.39	15.58	0.98	0.90	-	
	<i>Miroculis</i>	11.51	8	33.92	2.76	0.00	0.00	10.3	28	28	0.97	0.85	-	
	<i>Thraulodes</i>	72.18	6	68.86	6.15	0.00	0.00	72.18	73.2	75.55	0.87	0.86	+	
1000 m	<i>Traverhyphes</i>	69.38	13	66.21	3.5	0.00	19.53	69.38	71.73	72.75	0.90	0.85	+	
	<i>Macronema</i>	40.71	8	61.75	5.23	16.01	16.66	40.71	60.13	68.07	0.95	0.92	-	
	<i>Miroculis</i>	52.31	8	55.24	5.13	44.93	46.61	52.31	54.42	65.39	0.99	0.96	-	
	<b>Fish</b>													
	100 m	<i>Astyanax lacustris</i>	48.12	19	79.08	2.68	4.42	19.72	48.12	57.35	57.35	0.89	0.81	+
1000 m	<i>Corydoras aeneus</i>	64.95	12	63.37	2.16	54.45	55.54	65.2	79.03	84.84	0.95	0.8	+	



**Fig. 2.** Cumulative threshold frequency for EPT and fish assemblages in the 100 m and 1000 m buffers. Plot a) shows all taxa that decreased in abundance with native vegetation loss (z-). Note that TITAN selected no z- fish species in the 100 or 1000 m buffers. Plot b) depicts all taxa that increased in abundance with native vegetation loss (z+). Note that TITAN selected no z+ EPT genera in the 1000 m buffer.

For fish, the total cost for all species was US\$ 1,901 (R\$ 7,451) and the average cost per species was US\$ 48, requiring an average of 3 h per site. The species requiring the most time were *Odontostilbe pequirá*, *Moenkhausia bonita*, *Astyanax lacustris*, *A. lineatus* and *Piabarchus analis* (6, 4, 4, 4, 4 h, respectively). (Appendix Table A.2).

**Table 2**

Observed change point (cp) defined by the the filtered sum(z) (fsumz- and fsumz + ), and selected quantiles (0.05–0.95) of the change points determined by resampling the observed data. Fsumz uses only those taxa that are determined to be pure and reliable indicators. For EPT at 1000 m no genera positively responded to the effects of native vegetation loss (z + ), so there is no fsumz + line. For fish at 100 m and 1000 m, no species negatively responded to the effects of native vegetation loss (z-), so there is no fsumz- line.

	Buffer	Taxa	cp	0.05	0.1	0.5	0.9	0.95
fsumz-	100 m	EPT	11.51	0	0	11.51	15.39	15.58
fsumz+	100 m	EPT	69.38	24.82	33.54	69.38	72.18	72.75
fsumz-	1000 m	EPT	46.27	16.01	16.66	50.33	53.40	60.13
fsumz+	100 m	Fish	48.12	0.00	22.84	48.12	57.35	58.56
fsumz+	1000 m	Fish	64.95	54.45	55.53	64.95	75.19	79.02

**Table 3**

Model selection assessing the response of EPT or fish taxa richness to the effects of native vegetation loss at two spatial extents (100 m and 1000 m buffer). Model indicates the model evaluated (null model, GLM with Poisson distribution, or segmented linear regression); AICc corresponds to Akaike information criteria corrected to small samples; dAICc is the difference between AICc from the best model; df is the degrees of freedom; weight is information criteria weight; intercept, slope, slope after threshold (only for segmented models), and breakpoint (the last two are only for segmented models) are coefficients of models fitted. se = standard error; ci = confidence interval. The column "slope" for segmented models is the slope before breakpoint. The best models were those with  $\Delta AICc < 2.0$  and greater values of wAICc.

Model	AICc	dAICc	df	Weight	Intercept (se)	Slope (se)	Slope after threshold (se)	Breakpoint (ci)
EPT 100m								
GLM	210.09	2.00	2	0.23	1.69 (0.09)	0.001 (0.002)	-	-
Segmented	210.92	2.80	4	0.15	1.84 (0.11)	-4.58 (9.08)	4.58 (9.08)	0.084 (-0.25-0.42)
<b>Null</b>	<b>208.08</b>	<b>0.00</b>	<b>1</b>	<b>0.62</b>	<b>1.73</b>	-	-	-
EPT 1000m								
<b>GLM</b>	<b>207.37</b>	<b>0.00</b>	<b>2</b>	<b>0.59</b>	<b>2.01 (0.17)</b>	<b>-0.004 (0.002)</b>	-	-
Segmented	-	-	-	-	-	-	-	Not converged
Null	208.08	0.70	1	0.41	1.73 (0.07)	-	-	-
Fish 100m								
<b>GLM</b>	<b>116.60</b>	<b>0.00</b>	<b>2</b>	<b>0.47</b>	<b>1.80 (0.11)</b>	<b>0.004 (0.002)</b>	-	-
Segmented	118.97	2.37	4	0.14	1.90	-0.005 (0.008)	0.01 (0.01)	38.8 (-2.09-79.68)
Null	116.98	0.38	1	0.39	1.91 (0.07)	-	-	-
Fish 1000m								
GLM	119.04	2.1	2	0.24	1.76 (0.29)	0.002 (0.004)	-	-
Segmented	121.51	4.5	4	0.07	1.95 (0.31)	-0.001 (0.005)	0.04 (0.02)	83.48 (71.32-95.64)
<b>Null</b>	<b>116.98</b>	<b>0.00</b>	<b>1</b>	<b>0.07</b>	<b>1.91 (0.07)</b>	-	-	-

### 3.5. Evaluation of cost-effectiveness

Although fish assemblage data cost US\$ 1,715 less to obtain than EPT data, no fish species indicated a negative change point to native vegetation loss at both buffer extents. The EPT yielded negative threshold indicators for the effects of native vegetation loss in both buffer sizes and their observed threshold was most sensitive in the 100 m buffer. Considering these results, EPT should be chosen to indicate negative responses to the effects of native vegetation loss (Fig. 3).

EPT cost-effectiveness indicated that *Macronema* (Trichoptera) was the best single genus for use in biomonitoring (Fig. 3a) for three reasons. i) It had low to intermediate cost compared to other EPT genera (US\$ 45). ii) It was the most sensitive genus at both buffer extents (0% and 40% of native vegetation loss at 100 and 1000 m extents, respectively). iii) It was selected by TITAN in both buffer sizes.

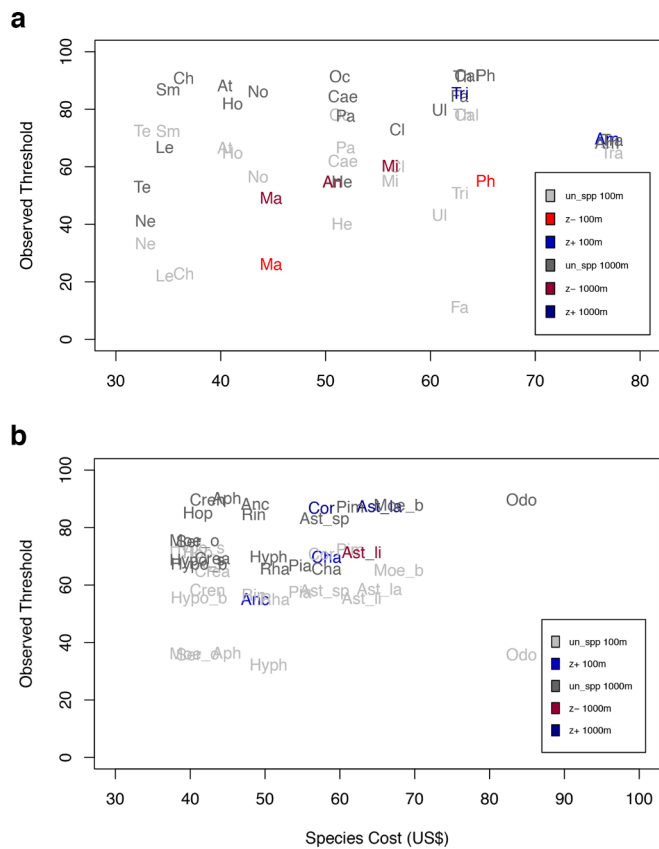
## 4. Discussion

The EPT were sensitive to the effects of native vegetation loss as we hypothesized and in agreement with the Neotropical extent of non-linear responses of aquatic invertebrates to native vegetation loss (Dala-Corte et al., 2020). Indeed, we found that this pattern was true for both 100 m and 1000 m buffers, reinforcing the use of EPT as early-warning aquatic bioindicators of the effects of native vegetation loss (Rosenberg and Resh, 1993; Valente-Neto et al., 2018b; Dala-Corte

et al., 2020). We found support for congruent responses of taxa to native vegetation loss for EPT, although few genera were representative of the thresholds detected. We also showed that EPT taxa richness failed to capture non-linear responses to native vegetation loss, as did Rodrigues et al. (2016) and Roque et al. (2018). We found that sampling, processing, and identifying EPT was US\$ 1,715 (about 52%) more expensive than fish, but this greater cost was compensated by its negative response and high sensitivity to native vegetation loss at both buffer extents.

### 4.1. Indicator selection: Ecological threshold and spatial extent

A growing number of studies show that the relationship between biodiversity and the gradient of anthropogenic impacts (particularly land use) can be non-linear (Rodrigues et al., 2016; Brito et al., 2020; Dala-Corte et al., 2020). In this study, as a first step for selecting a high-performance indicator, we identified potential taxa of insects and fish that showed threshold response to native vegetation loss. We identified 10 taxa, eight EPT and two fish, as potential indicators of the effects of native vegetation loss for the study area in the different buffer extents assessed. Among them, two EPT genera declined with native vegetation loss at both buffer sizes, supporting their value as indicator taxa. The most sensitive taxon to vegetation loss (*Macronema*) had a change point at 0% of native vegetation loss in the 100 m buffer. These results corroborate previous studies that showed that the EPT were highly susceptible to the



**Fig. 3.** Cost-effectiveness of each taxon of a) EPT and b) fish. Grey and dark grey represent those species not selected as reliable indicators (either z + or z-) in 100 m and 1000 m buffers, respectively. Red and dark red depict reliable z- species in 100 m and 1000 m buffers, respectively. Blue and dark blue depict reliable z + species in 100 m and 1000 m buffers, respectively. The codes for each species are listed in Appendix Table A.3 and A.4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effects of native vegetation loss, such as increased temperature, increased conductivity, reduced habitat heterogeneity and decreased flow, all of which affect organism distribution in freshwater systems (Valente-Neto et al., 2018b; Brito et al., 2020).

Thresholds were lower in the 100 m than the 1000 m buffers for EPT, agreeing with Dala-Corte et al. (2020). They found that thresholds for EPTOD (EPT plus Odonata and Diptera) averaged below 40% for aquatic invertebrates and below 50% for fish. Our results indicated a higher sensitivity of EPT, because we found a threshold for EPT around 11% in 100 m buffers and 46% in 1000 m buffers. Aquatic insects have a varied response to native vegetation loss, including the very low forest-loss threshold found in Amazonian streams (Brito et al., 2020), similar to our findings in the Cerrado-Atlantic Forest ecotone streams in the 100 m buffer, and higher values (Dala-Corte et al., 2020), as we found in the 1000 m buffer. This varied response to native vegetation loss may indicate context dependence linked to ecoregion or biome, despite recent evidence for the Neotropics that showed no difference among Brazilian biomes regarding EPTOD responses to native vegetation loss (Dala-Corte et al., 2020). However, Herlihy et al. (2020) also reported ecoregional differences in fish and macroinvertebrate responses to anthropogenic stressors in USA streams. Another explanation for this varied response is that various non-exclusive mechanisms can explain threshold patterns to the native vegetation loss gradient. In freshwater systems, changes in native vegetation may affect different aspects of ecosystem functioning, such as excessive input of sediments, reduced quality and quantity of organic substrates, increased light, and trophic

cascade effects (Allan, 2004). Furthermore, co-occurring stressors such as agricultural chemicals, stream-road crossings and introduced non-native species can affect fish and macroinvertebrate assemblages differently (Hughes et al., 2019). All these effects may result in synchronic loss of species. In this way, it is difficult to isolate a single cause because our predictor variable (native vegetation loss) is a proxy of several factors that operate simultaneously. However, we believe that species responses are probably most strongly associated with the loss of site-specific conditions created by changes in the most immediate riparian forest, such as environmental heterogeneity and substrate quality for feeding, reproduction, and input of high quality allochthonous resource for the aquatic fauna (Roque et al., 2010; Valente-Neto et al., 2018b; Brito et al., 2020). Such changes can cause abrupt shifts in the environmental conditions of several species all at once.

For fish, the expectation of a congruent threshold was not supported by our results, because no species negatively responded to native vegetation loss. Some fish datasets assessed by Dala-Corte et al. (2020) also did not indicate congruent thresholds. However, our results differ from an assessment of non-linear responses of fish to native vegetation loss in Amazonian streams that showed a synchronic negative change in composition at 43% and <5% of riparian vegetation loss and watershed deforestation, respectively (Brejão et al., 2018). This difference between our results may be partly explained by the higher species pool they evaluated (84 species) compared to the 21 species we collected. Also, karstic systems, such as our study area, are challenging for assessing fish biodiversity responses to the effects of native vegetation loss because some streams in the region have hyporheic connections between surface and ground water (Corrêa et al., 2019). This naturally fragments streams, hampering fish dispersion. Such conditions decouple the effects of native vegetation loss from instream biodiversity responses and partially explain the absence of fish from 12 sites in our study and the absence of fish species that negatively responded to the effects of vegetation loss.

In accordance to our expectations, two EPT genera (*Macronema* and *Microculis*) that depend on conditions and resources provided by riparian forest showed synchronous thresholds to the effects of native vegetation loss. Mayflies and caddisflies from Leptophlebiidae and Hydropsychidae, such as *Macronema* and *Microculis*, have tracheal gills for gas exchange and, overall, require high dissolved oxygen. *Macronema* larvae inhabit stream riffles, and they are basically collector-filterers that depend on good local conditions and feed on diatoms, green algae and insects (Oliveira and Froehlich, 1996). *Microculis* were reported to be sensitive to changes in local conditions in Cerrado streams (Firmiano et al., 2017), and they are scrapers (i.e. organisms that exploit the autochthonous periphyton and other particles adhered to substrate) (Brasil et al., 2014). Streams with high percentages of native vegetation have more organic substrates where *Microculis* can feed. In this way, the selected EPT genera may indicate both good water quality and availability of organic substrate.

Unlike the EPT, the two indicator fishes (*Corydoras aeneus*, *Astyanx lacustris*) are generally tolerant species, capable of living in a wide range of physical habitat types, water body sizes, and water quality conditions. Both are omnivorous and can thrive on a wide range of food types and food sizes. *C. aeneus* is a bottom feeder and phytophilic spawner with near-internal fertilization (Kohda et al., 1995; Huysentruyt and Adriaens, 2005). Because of its tolerances and traits, it is widely distributed throughout South America east of the Andes (Burgess, 1989) and is a commonly used aquarium species (Huysentruyt and Adriaens, 2005; Axelrod, 2006). *A. lacustris* is one of the most widely distributed South American fish species, with multiple local morphological adaptations that were recently synonymized into a single species (Lucena and Soares, 2016). It is commonly used as a bait fish because of its capacity to persist in poor holding conditions (Súarez et al., 2017). Although it commonly occurs in or near aquatic macrophytes (Súarez et al., 2017), it also consumes allochthonous plant debris (Viana et al., 2013). All these functional traits of both species are advantageous for persistence in

disturbed environments. Also, reduced riparian vegetation cover markedly stimulates the growth of aquatic macrophytes because of the increased sunlight incidence (Allan and Castillo, 2007), further improving habitat conditions for *C. aeneus* and *A. lacustris*.

The number of aquatic insects affected by the effects of native vegetation loss in a region is likely related to many evolutionary and ecological factors, including the number of regional species pool, historical legacy, extinction debts, degree of dependence of forest condition and resources, and landscape permeability. Methodological aspects, such as taxonomic resolution and sampling effort, also determine the number of taxa identified. Therefore, the fact we detected fewer insects that negatively responded to the effects of native vegetation loss than those reported by other researchers (e.g. Brito et al., 2020; Dala-Corte et al., 2020) should not be interpreted as evidence that vegetation loss does not have severe consequences on aquatic biodiversity in the study region. For example, Brito et al. (2020) found 24 taxa as indicators of the effects of forest loss in Amazonian streams when sampling site lengths were 75 m. We found that only two insect genera negatively responded to native vegetation loss. We believe that this result may be attributed to the low EPT species pool in the study region and the many rare species in our dataset that were removed from the analyses. We used 24 EPT taxa and 21 fish species, whereas Brito et al. (2020) analyzed 112 taxa. Also, the results of Dala-Corte et al. (2020) support this idea, because they found that only 15–25% of the taxa in the species pool demonstrated threshold responses, which overlaps our range (14–16%).

Nonetheless, one might argue that greater sampling effort for both fish and EPT at all the sites would have produced more individuals and thus more taxa, at least some of which might not have been deemed rare (occurring at < 3 sites). For example, Terra et al. (2013) determined that Atlantic Forest stream sites must be longer than 40 channel widths, or 400–500 individuals must be collected, to produce reasonably accurate fish species richness estimates. Similarly, Silva et al. (2016) reported that sites must be longer than 40 channel widths for determining site EPT richness in Cerrado streams. In the USA, Li et al. (2001) found constant increases in macroinvertebrate richness even after 50 subsamples were taken from each Oregon headwater stream site. Kanno et al. (2009) and Hughes et al. (2021) determined that fish species that were both rare and spatially discontinuous required greater levels of sampling effort for detection. In summary, both sampling effort and the number and distribution of uncommon taxa affect the accuracy and precision of site taxa richness estimates, and therefore, the taxa pool available for data analyses.

*Macronema* and *Miroculis* negatively responded within the same range of native vegetation loss at both buffer extents (100 m: *Macronema* = 0%, *Miroculis* = 11%; 1000 m: *Macronema* = 40%, *Miroculis* = 52%). Are both important in a monitoring program? We believe that it is important to monitor all taxa with high indicator potential for the effects of native vegetation loss to reduce the risk of false absences and minimize misinterpretations. That is because some genera in a given area are naturally uncommon or rare and not always determined by the amount of native vegetation. For example, stochastic events, such as flash floods, can remove organisms from a suitable patch (Flecker and Feifarek, 1994; Valente-Neto et al., 2018a), leading to unexpected absences. In this context, the use of only *Macronema* (the most sensitive taxa at both buffer extents) is not advisable, because it increases the chance of false absences. Maintaining some indicator redundancy may increase identification costs, a clear trade-off that needs to be calculated when considering cost-effectiveness. However, as Landres et al. (1988) argued, the use of one or a few indicator taxa is problematic for at least four ecological reasons. 1) Taxa useful for assessing the impacts of a single pressure or stressor may be inappropriate for assessing different or multiple anthropogenic pressures and stressors. 2) There are multiple statistical and sampling problems with assessing a single taxon or few taxa if they have low densities or tend to vary spatially and temporally for purely natural reasons. 3) There are interpretative problems if indicator taxa vary along co-varying natural and anthropogenic gradients.

4) It is unrealistic to assume that an indicator taxon that is ideal for one area is appropriate or even present in another area.

#### 4.2. Incorporating monetary costs when selecting indicators

Evaluating cost-effectiveness relationships is rather challenging in tropical systems because there is insufficient knowledge of all the costs involved in sampling and identifying many assemblages (Gardner et al., 2008). Furthermore, few studies have quantitatively evaluated the response of different aquatic taxa to pressures or assessed the time and costs of biomonitoring (Dala-Corte et al., 2020). We provide here the first attempt to value EPT and fish in a Brazilian stream biomonitoring context.

Our results showed that the inclusion of the monetary dimension is a complementary and critical step for indicator selection, which can lead to reduced costs for conservation and biomonitoring (Gardner et al., 2008). Under a scenario with few financial constraints, decision makers should monitor multiple aquatic groups to have a more complete assessment of biodiversity responses to native vegetation loss or other pressures and stressors (Hughes, 1993; Herlihy et al., 2020). Nevertheless, this may be unrealistic because of paucity of funds, time, and expert constraints. Fish may be less expensive than EPT, however, they did not negatively respond to native vegetation loss and their positive response started at 48%, a value of native vegetation loss that would be disruptive of EPT. Therefore, using fish to monitor the effects of native vegetation loss in the Bodoquena Plateau is not advisable. Although, using EPT raises costs by 52% more than for fish, they can detect early impacts at both buffer extents.

In conclusion, we recommend that bioindicator selection should be based on taxa sensitiveness to environmental gradients at multiple spatial extents, that have clear impact thresholds, and that have low costs to sample and process. Our results pointed to EPT as early warning indicators of the effects of native vegetation loss on stream biota, which has been widely reported by others (Lenat and Penrose, 1996; Stoddard et al., 2008). Our novelty is that by including the monetary dimension, we demonstrate that they have high cost-effectiveness compared to fish, including early responses at two spatial extents. We believe that biomonitoring programs in tropical systems should consider the cost-effectiveness of potential indicators, prioritizing greater sensitivity to impacts over slightly lower costs. However, because a good deal of the monitoring costs involve transportation to and from the sites, we recommend collecting both fish and macroinvertebrates during the same visit (Hughes and Peck, 2008). We further recommend rigorously assessing assemblage sampling effort and sampling protocols before implementing large biomonitoring and bioassessment programs (Buss et al., 2015).

#### CRedit authorship contribution statement

**Francisco Valente-Neto:** Conceptualization, Data curation, Formal analysis, Writing - original draft, Visualization, Writing - review & editing. **Bruno Téllez Martínez:** Conceptualization, Data curation, Methodology, Writing - review & editing. **Robert M. Hughes:** Supervision, Writing - review & editing. **Anderson Ferreira:** Methodology, Writing - review & editing. **Francisco Severo-Neto:** . **Franco Leandro de Souza:** Funding acquisition, Writing - review & editing. **Rodolfo Portela Souza:** Funding acquisition, Methodology, Writing - review & editing. **Suzana Cunha Escarpinati:** Methodology, Writing - review & editing. **Fabio de Oliveira Roque:** Conceptualization, Writing - original draft, Visualization, Funding acquisition, Supervision.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



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## Appendix A. Supplementary data

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