Science of the Total Environment 633 (2018) 179–188

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Assessing the extent and relative risk of aquatic stressors on stream macroinvertebrate assemblages in the neotropical savanna

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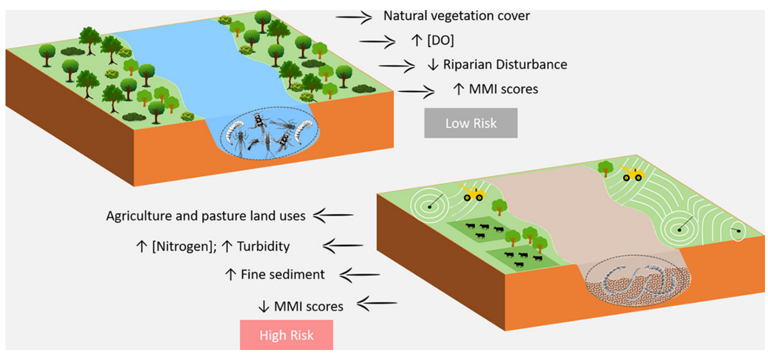
HIGHLIGHTS

- Aquatic ecosystems are among the most threatened worldwide.
- The extent and relative risks of stressors to biological condition were assessed.
- We used a probabilistic survey design to obtain estimates of relative risk and extent.
- Excess fine sediments posed the greatest risk to biological condition.
- To improve biological condition, management practice should consider both RR and RE.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 December 2017
Received in revised form 6 March 2018
Accepted 11 March 2018
Available online xxxx

Editor: Henner Hollert

Keywords:
Bioassessment
Probabilistic survey design
Land use
Physical habitat
Water quality
Cerrado

ABSTRACT

Freshwater ecosystems are among the most threatened by human activities, influencing losses of biodiversity. To efficiently address management practices to conserve and restore those ecosystems it is important to correctly identify and quantify the severity and magnitude of anthropogenic stressors degrading freshwater biota. In this study we assessed seven stressors describing poor water quality, physical habitat alteration, and land use by means of the relative risk (RR) and relative extent (RE) approach. The RR measures the co-occurrence probability of high stressor condition and poor biological condition. The RE measures the proportion of stream length in the region in high stressor condition. To obtain accurate estimations of RR and RE we used a probabilistic survey design to select a representative sample of perennial, wadeable and accessible streams within four hydrologic units in the neotropical savanna. Results were evaluated at two spatial scales: local – within each of the four hydrologic units, and regional – all four units combined. From 143 randomly selected sites we inferred our results to a target population of 9466 km of streams. Regionally, we found turbidity, % fine sediments and % agriculture as key stressors associated with poor biological condition. At the local scale, we also found that % pasture and total nitrogen were key stressors of biological condition, but their extent was relatively small. By evaluating both RR and RE we conclude that reducing excess sedimentation on streambeds should be the most effective means of improving biological condition over the region. That finding should guide decision makers and land managers to better focus their efforts and resources on improving biological condition of savanna streams.

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https://doi.org/10.1016/j.scitotenv.2018.03.127
0048-9697/

1. Introduction

Freshwater ecosystems are among the most threatened ones, facing a long history of exploitation of their resources to meet human-needs (Dudgeon, 2010; Dudgeon et al., 2006; Nieto et al., 2017; Revenga et al., 2005). Intense human pressures on these ecosystems from water pollution, sedimentation, habitat degradation, flow regulation, overfishing, and alien species invasion are among the main causes of biodiversity losses (Dudgeon, 2010; Vörösmarty et al., 2010). Because of the extents and intensities of such stressors, application of available environmental assessment methods are urgently need to improve the management, conservation, and rehabilitation of aquatic resources (Buss et al., 2015; Helson and Williams, 2013; Ruaro and Gubiani, 2013). In particular, it is essential to identify and focus on managing the major stressors directly or indirectly impairing freshwater ecosystems. In addition, it is critical to employ biologically based approaches for assessing stream condition because of the ability of biological indicators to integrate and detect multiple stressors in aquatic environments (Hughes et al., 2000; Karr, 1981; Moya et al., 2011).

In the United States (US), the relative risk (RR) and relative extent (RE) approaches have been used by the Environmental Protection Agency (EPA) to report on the regional and national condition of wadeable streams, boatable rivers, lakes, and wetlands (Angradi et al., 2011; Paulsen et al., 2008; USEPA, 2016a, 2016b, 2016c). The foundation of this approach is its ability to provide quantifiable associations between key stressors of concern and biological responses (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). RR describes the probability of good versus poor biological condition given the presence/absence of low versus high stressor condition. RE provides the magnitude of which the high stressor condition was found within a region. It should be noted that the RR and RE approaches are based on discrete measures (good and poor classes) rather than continuous variables. As such, they provide risk estimates that are easily interpreted and familiar to broad audiences (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). In addition, the RR and RE approaches help decision makers focus regulation, rehabilitation, and mitigation efforts on the stressors most strongly associated with poor biological condition (Hughes et al., 2000).

Accurate estimations of RR and RE can be obtained by randomly sampling sites (Van Sickle and Paulsen, 2008). The use of probabilistic survey designs is strongly recommended for site selection in regional stream condition assessments for several reasons (Dobbie and Negus, 2013; Herlihy et al., 2000; Olsen and Peck, 2008; Stevens and Olsen, 2004). 1) This design ensures representativeness over the surveyed region where physical, chemical and biological characteristics reflect ecological condition (Herlihy et al., 2008, 2000). 2) It is a cost-effective tool that allows confident and precise inferences to vast geographic areas and thousands of stream kilometers with a minimum number of sites (Paulsen et al., 2008). 3) Such a design allows statistical estimations over the stream length of the entire target population of interest, not only the sampled sites (Herlihy et al., 2000). 4) This randomized approach avoids biased conclusions inherent when convenience-based sampling site selection is used in ecological assessment studies (Dobbie et al., 2008; Dobbie and Negus, 2013; Jiménez-Valencia et al., 2014). 5) A probability site-selection design is far more cost-effective for regional ecological studies and assessments than a stratified-random design where the number of potential strata, and therefore the sampling costs, are typically very large and the statistical inferences are very complex (Stevens and Olsen, 2004).

A well-designed monitoring program provides reliable, credible, and valid inferences regarding environmental questions of concern (Dobbie and Negus, 2013; Paulsen et al., 2008). However, the practice is still neglected in Brazil and most other South American countries (Jiménez-Valencia et al., 2014; Macedo et al., 2014b), where biodiversity is high (Barlow et al., 2016; Brook et al., 2006; Myers et al., 2000) and widespread environmental changes are rapidly occurring (Barlow et al., 2016; Brook et al., 2006; Hernández et al., 2016; Vörösmarty et al., 2010). There is an urgent need to improve methods of selecting sites to achieve high quality data that support improved management practices to protect and rehabilitate streams. This is especially the case for the neotropical savanna, a highly threated biome, suffering from rapid natural cover replacement and pasture and crop expansion (Hunke et al., 2015; Ratter et al., 1997; Strassburg et al., 2017).

Therefore, the goal of our study was to evaluate the extent of stressors and their risk to biological condition. To achieve our objective, we used a probabilistic survey design to estimate total stream length of a target population of wadeable, perennial and accessible streams and estimate non-target situations of dry, inaccessible, non-wadeable streams and map errors in our frame length. We used a macroinvertebrate multimetric index developed for savanna streams as a measure of biological condition (Silva et al., 2017) and stressors describing physical habitat degradation, poor water quality, and land uses. We assessed results at two scales: local (within each of four hydrologic units) and regional (all four hydrologic units combined).

2. Methodology

2.1. Study area

We defined our sample frame as the stream network in the drainage area within 35 km upstream of the limits of four major hydropower reservoirs: Nova Ponte, Volta Grande, São Simão (Paraná River Basin) and Três Marias (São Francisco River Basin) (Fig. 1). Sampling occurred during the dry season in each hydrologic unit in subsequent years from 2009 to 2012. Dry season sampling facilitates data collection, and the more constant discharges during this time reduces the effect of flash floods. Also, available aquatic habitats are more distinct, macroinvertebrate assemblage structure is more stable, and crew safety hazards and road access difficulties are minimized during the dry season (Hughes and Peck, 2008; Plafkin et al., 1989). This geographic area covered a total of 45,180 km^2, with land uses and cover characterized by agricultural cash crops, charcoal production, grazing, and urbanization (Macedo et al., 2014a). Climate is described as humid tropical savanna, with a dry season from May to September, precipitation averaging from 800 to 2000 mm, and air temperature averaging between 18 and 28 °C (Ratter et al., 1997).

2.2. Survey design and sampling sites

Sites were selected based on a probabilistic survey design in each of the four areas (hereafter hydrologic units, sensu Ferreira et al., 2017; Firmiano et al., 2017; Seaber et al., 1987). This site selection procedure is based on the first one used by the US-EPA in the Environmental Monitoring and Assessment Program for the Mid-Atlantic Highlands Streams Assessment (Herlihy et al., 2000) and refined in subsequent regional and national stream monitoring programs (Olsen and Peck, 2008; Paulsen et al., 2008; Stoddard et al., 2005). The approach consists of the establishment of a sample frame in a 1:100,000 scale digitized map where we sorted sites by means of a randomized, systematic, spatially balanced criterion (Herlihy et al., 2000; Olsen and Peck, 2008; Stevens and Olsen, 2004).

Our survey design allowed us to obtain an even distribution of sites over the geographic location (Herlihy et al., 2000; Stevens and Olsen, 2004). Probability sampling provides reliable population estimates from a representative set of sample sites in the surveyed region. However, the distribution of sites from the probability sampling design mirrors that in the sampling frame; there will be lots of sample sites with commonly occurring (intermediate) conditions but rare conditions will have few if any sample sites depending on their rarity (Karr and Chu, 1999; Stoddard et al., 2008). As such, minimally disturbed sites (e.g., preserved areas) or highly degraded sites (e.g., urban areas) are rare in our sample, as our study area is highly dominated by agriculture and pasture (Callisto et al., 2014). Therefore, to ensure a gradient of

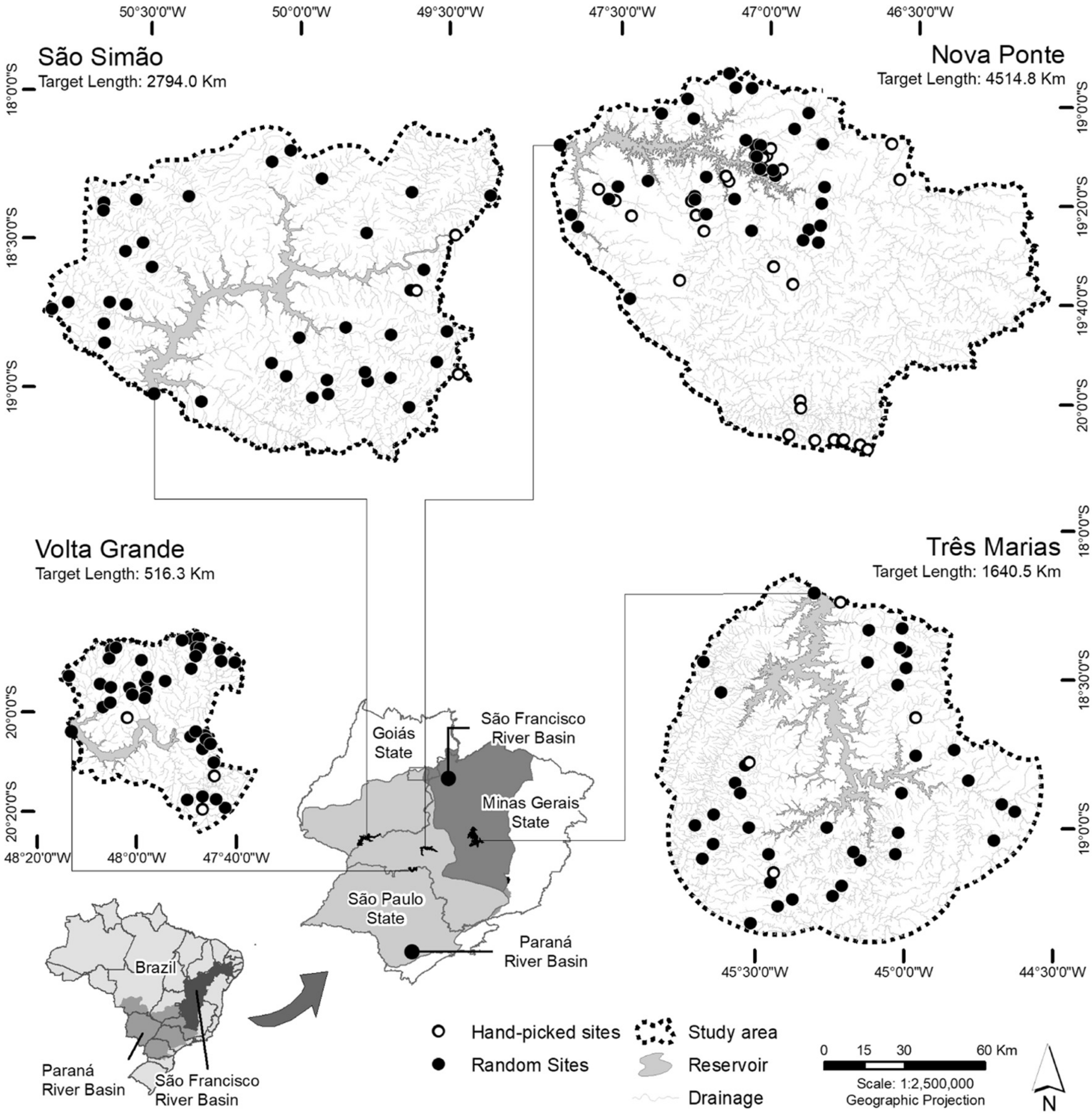

Fig. 1. Locations of random (●) and hand-picked (○) sites sampled in each of the four hydrologic units.

ecological conditions, we also hand-picked a set of sites with apparently minimal disturbance as well as urban sites with highly altered physical and chemical conditions.

We targeted a population of wadeable, perennial and accessible streams, of first- through -third order (sensu Strahler, 1957). For each hydrologic unit, we established a random set of potential sampling sites and an additional set of sites to use as substitutes where a site was non-target (e.g., dry, non-wadeable, access denied, map error). Each site received a weight proportional to the inverse of its selection probability, interpreted as the stream length it represents in the target population. These site weights were then used to estimate stream condition extents and relative risk. Hand-picked sites were not used to make stream length estimations because they have a weight equal to zero. But both sets of probabilistic and hand-picked sites were considered for the establishment of thresholds, if they passed the reference screening criteria (see below).

A field reconnaissance was necessary to confirm the set of sampling (or target) sites, account for situations where sites were not accessed for any reason (non-target sites), and to optimize field crew time (Macedo et al., 2014b).

At each site, we established a sampling reach equal to 40 times the mean wetted channel width or a minimum of 150 m around the randomly selected point or x-site (Peck et al., 2006). Macroinvertebrate assemblages were sampled with a kick-net sampler (500 μm mesh, 0.9 m^2 area). Samples were taken to the laboratory where the individuals were sorted and identified through use of a stereomicroscope (100× magnification) and taxonomic keys (Costa et al., 2006; Fernández and Domínguez, 2001; Merritt et al., 2008; Mugnai et al., 2010).

At the stream reach we obtained quantitative measures of physical habitat structure following a standardized field sampling protocol (Peck et al., 2006). Those measures describe channel morphology (e.g., slope, sinuosity, wetted and bankfull depth and width, incision, bank angle), habitat features (e.g., substrate size, flow types, amount of wood in the channel), riparian structure (e.g., canopy cover, vegetation type), and human alterations in the riparian zone zones (e.g., presence of buildings, pasture, crops, roads, trash). We then calculated metrics and indices combining the field measures into a single value. For example, we obtained the Riparian Disturbance Index (RDI) by combining the various types of anthropogenic disturbance observations weighted by their proximity to the streambed, varying from 0 (no evidence of disturbance in the riparian and channel zone) to 7 (the empirical maximum value for observing disturbance in the riparian and channel zone). Percent of fine sediments, for example, was obtained as the percentage of the streambed with substrate smaller than 0.6 mm (silt and clay substrates). More details on metric calculation are available in Kaufmann et al. (1999) (Table S1).

Water temperature, electrical conductivity, total dissolved solids, turbidity, and pH were measured once at each site by use of portable equipment (YSI Model 650). Dissolved oxygen concentrations were obtained with the Winkler titration method (APHA, 1998). Water samples were collected in a 0.5 L bottle and transported to the laboratory for determining total nitrogen (Kjeldahl digestion method; APHA, 1998) and total phosphorus (ascorbic acid method; APHA, 1998) (Table S1).

We characterized the different land uses (% natural cover, % agriculture, % pasture, % urban area) in the catchment of each site via satellite images provided by Landsat TM sensor and Google Earth fine resolution imageries (Google, 2016; Macedo et al., 2014a) (Table S1).

2.3. Establishment of stressor and biological indicator thresholds

We used the macroinvertebrate multimetric index (MMI) developed by Silva et al. (2017) as a measure of stream biological condition. To build the MMI they followed a standardized metric screening procedure that accounted for natural environmental variation, responsiveness and discriminance to disturbance, sampling variability and redundancy. The final MMI is composed of 7 metrics describing macroinvertebrate composition, richness, tolerance, diversity, feeding groups, mobility, and respiration type. As a step in the development of the MMI, Silva et al. (2017) established a set of least-disturbed sites by filtering all sites (both sets of random and hand-picked sites) to previously established parameters of water quality, physical habitat, and land use (Herlihy et al., 2008; Silva et al., 2017; Waite et al., 2000) using the same data set as in this study. Condition class thresholds for biological condition were established based on the distribution of MMI scores in the least-disturbed sites. MMI scores lower than the 5th percentile of the least-disturbed distribution were classified as "poor", scores between the 5th and 25th percentile were classified as "fair", and those higher than the 25th percentile were classified as "good" (Silva et al., 2017). The classes were used as thresholds to indicate quality boundaries for further analysis.

From a list of potential stressors indicating physical habitat alteration, water quality and land uses disturbances we selected: dissolved oxygen, turbidity, total nitrogen, % of fine sediments, riparian disturbance index, % agriculture and % pasture for RE and RR analysis. Thresholds for water quality parameters of dissolved oxygen and turbidity were based on Brazilian legislation (CONAMA 357, 2005), whereas for total nitrogen we used a more restricted value. Thresholds for physical habitat structure are usually based on regional distributions of observed values at least-disturbed sites. Using a similar approach as for biological condition, we defined sites with % of fine sediments greater than the 75th percentile of least-disturbed site distribution as in poor condition and sites with % of fines below the 75th percentile as good condition (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). For the riparian disturbance index (RDI) we used the same threshold value that Silva et al. (2017) used to screen reference sites is savanna streams, where sites with RDI < 1 were classified as good and RDI > 1 were classified as poor. We quantified % of agriculture and pasture in the catchment area upstream of each site and defined thresholds based on Silva et al. (2017), considering that each hydrological unit is strongly dominated by agriculture or pasture. Condition classes and thresholds are summarized in Table 1.

2.4. Assessing relative risk and extent

We used the relative risk approach (RR) to evaluate the severity of seven stressors in affecting biological condition (measured by the MMI) and the relative extent (RE) to evaluate the magnitude of the stressors (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). We obtained the RR by means of a contingency table whereby we addressed all possible situations of having good or poor macroinvertebrate MMI condition given high or low stressor condition. We excluded the MMI "fair" condition category to obtain a 2×2 table. By only considering the two extreme classes of "good" and "poor", we reduce the overlap in actual condition between sites assigned to the two classes (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). Instead of using number of sites counted in each situation, we used the estimated stream length in each category based on the site survey design weights.

The RR is a conditional probability representing the likelihood that poor MMI scores are associated with high stressor scores and is calculated as follows:

$$RR = \frac{\Pr\left(\mathrm{MMI}_p|\mathrm{S}_h\right)}{\Pr\left(\mathrm{MMI}_p|\mathrm{S}_l\right)}$$

where the numerator is the probability of finding poor biological conditions ($\mathrm{MMI_p}$) given high stressor scores ($\mathrm{S_h}$) and the denominator is the probability of finding poor biological condition given low stressor scores ($\mathrm{S_l}$) (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008).

In this formulation, a RR equal to 1 denotes the absence of association between the biological indicator and the stressor, i.e., poor MMI scores are equally likely to occur with both high and low stressor scores (Van Sickle et al., 2006). For a RR > 1, we interpret the value as how many times more likely poor MMI condition would occur given high stressor conditions relative to low stressor condition. We calculated 95% confidence intervals for RR estimations using the method of Van Sickle and Paulsen (2008), and considered the RR to be significant when the lower 95% confidence interval was >1.

Whereas the RR measures the severity of a stressor, the RE is a measure of its magnitude (Angradi et al., 2009; Van Sickle and Paulsen, 2008). The RE represents the length and proportion of streams with high stressor scores within a given study area. It is obtained as a sum of sampling weights of sites found with high stressor scores divided by the sum of all site weights (expressed in %). Combined, RR and RE provide an excellent overview of the stressor's dimensions and its association with biological condition (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). We also calculated the Pearson correlations between all stressors to assess stressor relationships and evaluate stressor independence. RR, RE, and confidence intervals were obtained using R statistical software (R Development Core Team, 2016) and the R package *spsurvey* (version 3.3, available from http://www.epa.gov/nheerl/arm).

Table 1
Thresholds of condition classes for macroinvertebrate MMI and stressor indicators.

Variable	Thresholds		Source
	Good/Low	Poor/High	
Biological condition			
MMI	>65	<47	25th/5th percentile of reference sites (Silva et al., 2017)
Stressor condition			
Land use			
% Agriculture	<60	≥60	Silva et al., 2017
% Pasture	<60	≥60	Silva et al., 2017
Physical habitat			
Riparian Disturbance Index	≤1	>1	Silva et al., 2017
% fines (silt/clay; <0.6 mm)	≤40	>40	75th percentile
Water quality			
DO (mg/L)	≥6	<6	CONAMA 2005
Turbidity (NTU)	≤20	>20	CONAMA 2005
N (mg/L)	≤0.2	>0.2	Silva et al., 2017

3. Results

We estimated a total map length of 24,417 km of streams belonging to first to third order in the sample frame. From this, 9466 km of streams (or 39% of the total frame length) were part of the target length, i.e., sampled sites, defined as perennial, accessible, wadeable, and with flowing water (Table 2). The difference was a result of a number of non-target sites that weren't sampled because they did not meet target criteria. The main reason for dropping a site was lack of water (dry streams), which accounted for 32% of the total frame length. The impossibility of access accounted for 17% of the total frame length. Non-wadeable streams accounted for 12% of the map frame length, map errors (where a stream was not found based on the geographic coordinates) were rare and accounted for only 0.7% of the mapped length. A total of 143 probabilistic sites were sampled and 176 sites were dropped. Detailed results on target and frame stream length for each hydrologic unit are shown in Table 2.

The Pearson product-moment correlations matrix did not reveal any strong correlation among stressors (Table 3). The highest correlation was between % pasture and % agriculture ($r = -0.52$). All other pairs of stressors showed estimated product-moment lower than 0.35. This suggests little confounding effects between stressors that represented different disturbance gradients, allowing us to interpret associations of stressors and biological condition independently in this region.

For the regional assessment, we found that turbidity, % fines, and % agriculture were the only stressors significantly associated with increased risk for poor macroinvertebrate MMI condition (relative risk values of 1.8–2). In terms of extent, agriculture was the most widespread stressor in the region (over 40% of stream length exceeded the agriculture threshold) followed by fine sediment (18% of total stream length). High turbidity was found in <6% of stream length. Although high riparian disturbance occurred in almost 55% of the stream length it did not represent a significant risk for biological condition (Fig. 2). Missing relative risk and relative extent values (Fig. 2) resulted from the lack of association between the stressor and the biological condition and that no sites in that study basin had stressor levels exceeding the established thresholds.

RR and RE estimations varied among the hydrologic units. Turbidity, % fines, and % pasture were the stressors representing risk to biological condition in NP and TM. In NP, the risk of finding poor MMI condition given high pasture (i.e., high stressor condition) was 3.6 times more likely to occur compared to a situation where pasture did not exceed the threshold (i.e., low stressor condition). Nonetheless, high percent of pasture only occurred in <5% of the total stream length. In TM, streams with excess fine sediments or pasture were 2 to 4 times more likely to have poor MMI condition than streams without. Those stressors were each present in ~23% of the TM stream length. In both NP and TM, although turbidity indicated a risk, it rarely occurred (<10% of total stream length). In SS and VG, excess nitrogen and turbidity represented a risk (1.3–2), but with very low occurrence (around 3% of the total stream length). Also, in both VG and SS, although agriculture land use is widespread (>80% of the estimated stream length in high stressor level), it was not significantly associated with poor biological condition. We observed that low dissolved oxygen did not have a significant relative risk in any hydrologic unit or in the regional assessment. Although we considered pH and total phosphorus as potential stressors to biological condition, very few or no sites had high stressor levels so it was not possible to calculate relative risks for them.

Considering the MMI weighted distributions, NP was the hydrologic unit in better ecological condition (Fig. 3). Total nitrogen, which posed a risk only in VG and SS, also showed similar medians in both hydrologic units. Similar medians were also found for turbidity among the four hydrologic units. Concerning land uses, we found TM as the hydrologic unit most influenced by pasture, whereas the others had highest means of agricultural land uses. Detailed weighted distributions of MMI and stressors scores are shown in Fig. 3.

Table 2
Estimated extent (km) of target and non-target stream sites for the regional assessment and for each hydrologic unit: Nova Ponte (NP), Três Marias (TM), Volta Grande (VG), and São Simão (SS), parentheses indicate the number of probability sites in each hydrologic unit. Numbers in bold are the percents of frame length.

Scale	Frame Length (km)	Target Length (km)	Non-target length			
			Dry (km)	Non-wadeable (km)	No access (km)	Map error (km)
Hydrologic unit						
NP (32)	11195	4514.8	3877.5	222	2581.2	0
		40.3%	**34.6%**	**2.0%**	**23.1%**	**0.0%**
TM (36)	5989	1640.5	2445.4	923.2	979.5	0
		27.4%	**40.8%**	**15.4%**	**16.4%**	**0.0%**
VG (38)	1528	516.3	163.8	457.2	234.7	156.1
		33.8%	**10.7%**	**29.9%**	**15.4%**	**10.2%**
SS (37)	5705	2794	1316.6	1230.8	341.4	22.6
		49.0%	**23.1%**	**21.6%**	**6.0%**	**0.4%**
Regional (143)	24417	9465.7	7803.2	2833.2	4136.8	178.7
		38.8%	**32.0%**	**11.6%**	**16.9%**	**0.7%**

4. Discussion

Although providing benefits in ecological assessments, the use of probabilistic survey designs in estimating stream lengths are in their early stages in the neotropics (Carvalho et al., 2017; Jiménez-Valencia et al., 2014; Macedo et al., 2014a). Jiménez-Valencia et al. (2014) conducted a risk assessment of tropical rainforest streams in a small Brazilian river basin and reinforced the importance to employ more probability-based surveys to improve the quality of regional assessments in tropical regions experiencing rapid land use conversion and dam construction. Under this perspective, our study represents a first attempt to use a probabilistic design to rigorously assess the relative risk and extent of stressors of biological condition in neotropical savanna streams.

From only 143 randomly sampled sites, we were able to infer results to a target population of 9466 km of wadeable, accessible and perennial streams representing 39% of the total frame length depicted on maps. A reconnaissance field trip prior to the sampling helped us properly account for non-target situations (61% of frame length). Regionally, map errors represented only 0.7% of the sample frame, mainly occurring in VG where the total length of target streams is relatively small. However, dry streams accounted for 32% of the sample frame, especially in first order streams. This situation is recurrent in other studies that normally adjust probability inclusions to account for the high % of expected dry first order streams (Herlihy et al., 2000; Jiménez-Valencia et al., 2014). Climate change (Marengo et al., 2012) and the commonly occurring severe droughts experienced in the Brazilian savanna (Oliveira et al., 2014), can also contribute to a high % of dry stream length. Another reason is the quality of Brazilian 1:100,000 topographic maps, because they were built with 1960's aerial photographs and through two methodological approaches by IBGE (Brazilian Statistic and Geographic Institute) and DSG (Brazilian Army Geographic Division), resulting in a heterogeneous hydrographic map (Guimarães et al., 2008). Although sampling in the wet season would result in a higher % of streams with flowing water, it would also threaten crew safety, reduce site access, and increase sampling variability (Hughes and Peck, 2008).

Two other conditions produced non-target streams. The non-wadeable streams in the regional sample frame (12%) were mainly identified as marshes, very deep streams or small impoundments, not representing our target criteria. A first contact with land owners secured permission for sampling in most situations, however, a number of sites were inaccessible mainly due to locked gates, and physical barriers (e.g., canyon streams) or absence of trails or roads. Olsen and Peck (2008) reported 11.5% of the frame length not sampled due to access denials in a probability survey of the conterminous US. Lesser (2001) also reported

Table 3
Pearson correlation coefficients among stressors in the study region.

	Riparian disturbance index	% Fines (silt + clay)	Turbidity (NTU)	Nitrogen (mg/L)	DO (mg/L)	% Pasture	% Agriculture
Riparian Disturbance Index	—						
% Fines (silt + clay)	0.28	—					
Turbidity (NTU)	0.18	0.28	—				
Nitrogen (mg/L)	0.25	0.26	0.35	—			
DO (mg/L)	−0.15	−0.15	−0.21	−0.01	—		
% Pasture	0.14	0.26	0.07	−0.03	−0.13	—	
% Agriculture	0.12	0.04	0.01	0.25	0.15	−0.52	—

a high % of denials in wetland assessments and suggested adjustments to reduce bias. Genet and Olsen (2008), assessing wetlands in Minnesota, USA, reported increased successful access to private properties by making personal contacts with land owners. In general, they all agreed that a first contact (mailing, in person) helps reduce this problem, thereby providing better estimations. That reinforces the importance of reconnaissance prior to sampling, which adds costs and time, but increases chances of successful access (Olsen and Peck, 2008). Another reason for the success of our access to private property is related to the research character of an academic institution, being totally dissociated from federal or state government agencies, which could represent an obstacle to access permission.

Identifying stressors that can properly represent a risk and their magnitudes is strongly desirable for assessment and management purposes. In our study, excess fine sediments posed the greatest risk to biological condition with a considerable extent, not only at the regional scale but also within specific hydrologic units. Paulsen et al. (2008) and Van Sickle et al. (2006) also found fine sediments a stressor of major concern for macroinvertebrates for both regional and national stream assessments in the US. Although finding a high percentage of stream length with excess sediment in the neotropical Atlantic Forest, Jiménez-Valencia et al. (2014) did not find significant risk to the biota (lower 95% confidence boundary < 1). Excess fine sediments in streambeds are regarded as one of the most important threats to ecological

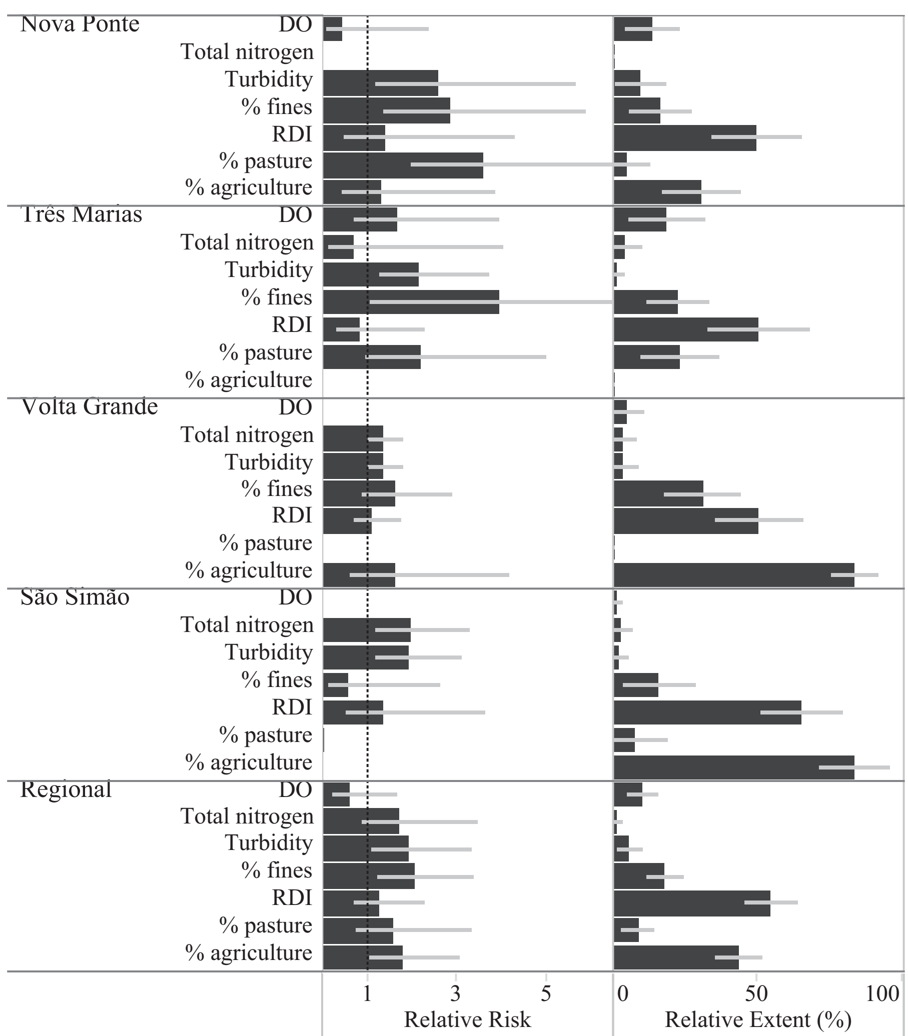

Fig. 2. Estimated relative risk (RR) for poor MMI condition given seven stressors and their relative extents (RE) in high levels of stress for each hydrologic unit and for the regional assessment (represented as % of total target stream length). Lines represents 95% confidence boundaries. RR below 1 indicates the absence of association. (DO = dissolved oxygen; RDI = Riparian Disturbance Index).

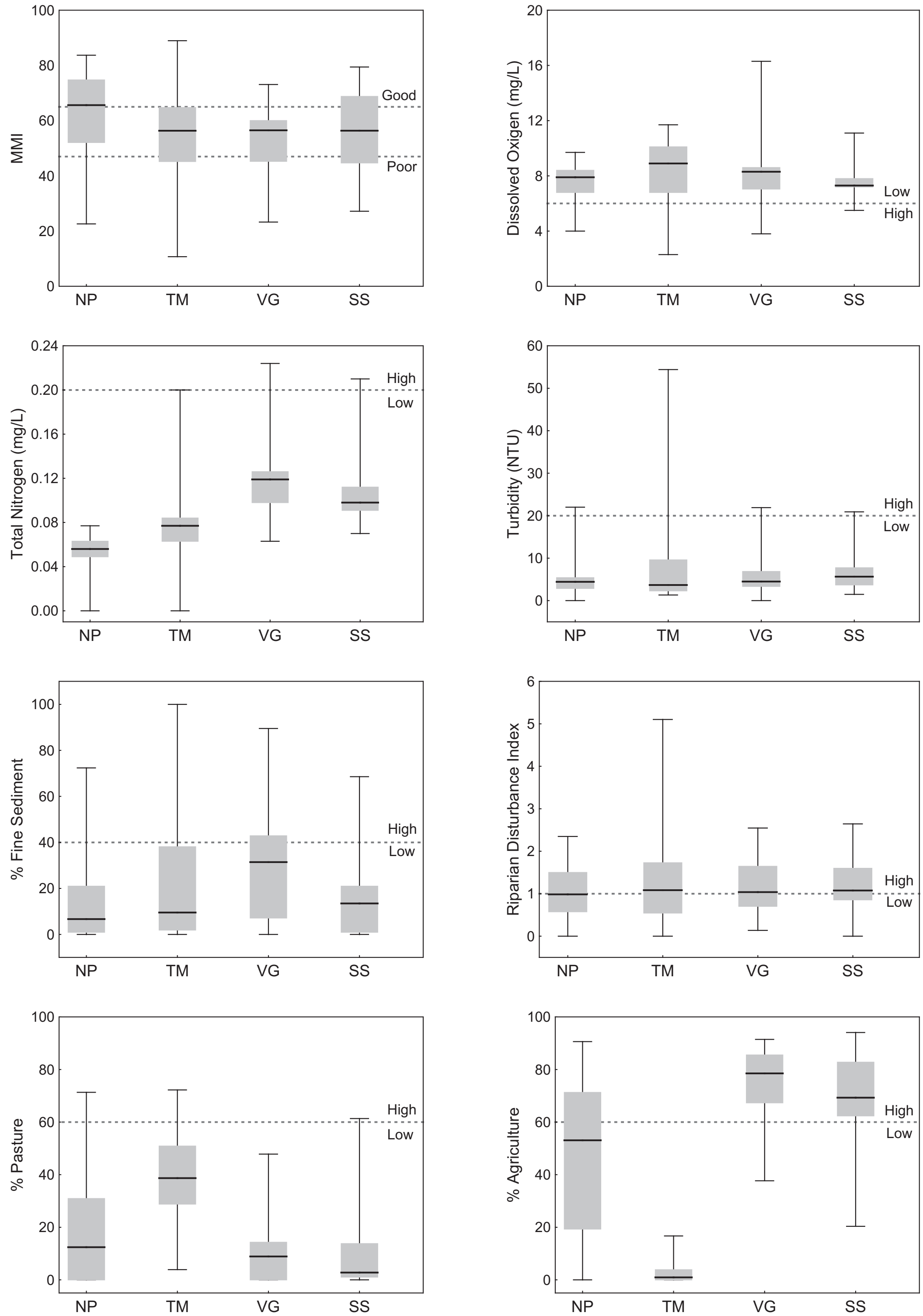

Fig. 3. Weighted box-plots for the MMI and seven selected stressors for each hydrologic unit: Nova Ponte (NP), Três Marias (TM), Volta Grande (VG), and São Simão (SS). Boxes represent the 25th and 75th percentiles, whiskers are minimum and maximum ranges, and lines within boxes are medians. Dotted lines represent threshold limits for condition classes (see Table 1).

integrity in lotic ecosystems (Bryce et al., 2010; Buendia et al., 2013; Wood et al., 2016; Wood and Armitage, 1997). It alters habitat availability and suitability for aquatic biota (Buendia et al., 2013), directly affecting macroinvertebrate assemblage structure, composition and function (Mathers and Wood, 2016; Wood and Armitage, 1997). The main causes for excess streambed sediment are associated with human activities that increase erosion and sediment delivery to streams, such as agricultural land use (Benoy et al., 2012; Jessup et al., 2014; Kaufmann et al., 2009; Kaufmann and Hughes, 2006). Nonetheless, many other natural factors can also be associated with the input of streambed sediments, such as bedrock geology, relief, channel slope, basin size, bank erosion, seasonality of rains, and tectonic activities (Chakrapani, 2005). Because of that, at regional scales it is difficult to measure direct relationships between land use and excess sediments, because of the co-varying effects of those natural characteristics and land uses and cover (Guerra et al., 2017; Kaufmann and Hughes, 2006; Leal et al., 2016; Lisle et al., 2015). We recommend further investigations concerning the causes of excess sediment affecting stream biota to minimize misleading interpretations.

Although agriculture land use is dispersed over most of the hydrologic units, it only showed significant risk as a stressor in the regional assessment (RR = 1.8). Agriculture, as well as other forms of land use, provides an indirect measure of one or more stressors affecting biological condition, rather than the direct measures generally preferred in the relative risk approach (Paulsen et al., 2008). Many authors have shown how agriculture strongly affects water quality and consequently aquatic biota (Lammert and Allan, 1999; Leitão et al., 2018; Riseng et al., 2011; Waite, 2014). In our study, we did not find a correlation between agriculture land use and water quality parameters (Table 3). We believe that the maintenance of a minimum wooded riparian zone as required by Brazilian Environmental Law (No. 12651/2012) may help reduce the direct impacts of agriculture by filtering contaminants (Sweeney and Newbold, 2014; Tanaka et al., 2016). That may partly explain the small extents in which we found high stressor condition for water quality parameters even in hydrological units dominated by agriculture.

High turbidity was a good indicator (high relative risk) of poor biological condition in the regional assessment and in all hydrologic units, but only occurred in a small proportion of stream length. That was similar to the high nitrogen concentrations found in VG and SS, which represented a high risk to biological condition but occurred in low extents. Both are indicators of anthropogenic activities leading to sedimentation and eutrophication.

The combined RR and RE approach provides guidance for management practices. Regional efforts toward reducing excess sediment inputs to streams would greatly improve biological condition based on both the severity and magnitude of that stressor. On the other hand, high turbidity and nitrogen were two high-risk stressors that were relatively uncommon throughout the region. If we consider that these stressors may result mostly from local sources of impact at specific sites, then they may be best managed at a more local focused scale, rather than through regional efforts. Many authors have also determined that extensive row-crop agriculture is detrimental to stream biota (Allan, 2004; Wang et al., 2006). The impacts from agricultural land uses operate through multiple pathways and at several spatial scales (Leitão et al., 2018). Given the continued growth of agricultural land use in Brazil and throughout the tropics, those results for the risk of high levels of agricultural land use should be seen as an important alert for further studies in the neotropical savanna to identify its effects on stream biota and to mitigate its impacts.

Van Sickle et al. (2006) attempted to correctly interpret RR and RE estimations in the assessment of stream condition. The relative risk and extent approach provide associational data rather than causality relations between stressors and biological condition. Therefore, management practices based on this approach can be strengthened by conceptual models and BACI (before-after-control-impact) studies that elucidate causal mechanisms of ecosystem impairment. Another important concern is to keep in mind that “high” and “low” condition classes are based on established thresholds for the different stressors and the obtained estimates of RR and RE must necessarily be constrained by them. Water parameter thresholds were based on Brazilian Federal Legislation (CONAMA 357/2005), but do not account for natural variability (Jiménez-Valencia et al., 2014) or intensity of human activities across different regions (Paulsen et al., 2008). Firmiano et al. (2017) reported that the legislation thresholds are much higher than the turnover thresholds of several mayfly genera, suggesting that the legislation should set benchmarks considering biological information to avoid the loss of sensitive taxa. Future studies should consider the inclusion of other stressors that we did not consider because of their lack of responses in our study. Dissolved oxygen did not represent a risk to biological condition and total phosphorus and pH were omitted because in general they did not exceed thresholds. The % of urban land use, although recognized as a potential stressor to biological condition (Pompeu et al., 2005; Yeakley, 2014) was not found in significant amounts at the sites (<2% of the hydrologic units, see Table S1, Supplementary Material). However, it, as well as mining (Daniel et al., 2015; Hughes et al., 2016) and intensively farmed land (Allan, 2004; Paulsen et al., 2008) are likely important stressors in this region.

5. Summary and conclusions

The probabilistic survey design allowed us to estimate the total stream length of a target population of wadeable, accessible and perennial streams and account for non-target situations. This design provided a consistent base from which we estimated relative risks and extents to assess ecological condition in neotropical savanna streams at both hydrologic unit and regional perspectives. This approach facilitates 1) identification of the major stressors associated with poor biological condition; 2) evaluation of the magnitude of the stressors, and 3) provision of guidelines to improve stream condition by focusing management efforts on specific targets when a stressor poses a risk but is not widespread or at large scales when stressors represent regional risks. It represents an important ecological tool, considering the context in which this study was developed – the savanna biome – a biodiversity hotspot highly endangered by rapid natural cover replacement and pasture and crop expansion.

Overall, our results should assist decision makers and managers interested in improving the ecological condition of savanna streams. In particular, we recommend mitigating the sources of excessive sediments, which could lead to marked improvements in the biological condition of neotropical savanna streams and reduce the erosion and deterioration of agricultural soils. Future studies in the neotropical region would benefit from: 1) the use of probabilistic designs for unbiased site selection; 2) the establishment of thresholds representative of the study region, and 3) the assessment of other potential stressors associated with poor biological condition.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.03.127.

Acknowledgements

We acknowledge funding from the Peixe-Vivo Program of Companhia Energética de Minas Gerais, Pesquisa & Desenvolvimento/Agência Nacional de Energia Elétrica/Companhia Energética de Minas Gerais - P&D ANEEL/CEMIG (GT-487), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG). We are especially grateful to Jeannie Sifneos who helped with statistical analysis. D.R.O.S. received a Ph.D. scholarship from CNPq (no. 158971/2013-2) and from CAPES Foundation (no. 99999.006833/2015-02) during a visiting scholar period at Oregon State University. M.C. was awarded research productivity (CNPq no. 303380/2015-2), research project (CNPq no. 446155/2014-4), and Minas Gerais research (FAPEMIG PPM-IX – 00525-15) grants. R.M.H. received a Fulbright Brazil grant. D.R.M. received a research grant from Pró-Reitoria de Pesquisa (PRPq)-UFMG (ADRC-05/2016) and a research project grant from CNPq (no. 402907/2016-7).

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