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Defining quantitative stream disturbance gradients and the additive role of habitat variation to explain macroinvertebrate taxa richness

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ABSTRACT

Most studies dealing with the use of ecological indicators and other applied ecological research rely on some definition or concept of what constitutes least-, intermediate- and most-disturbed condition. Currently, most rigorous methodologies designed to define those conditions are suited to large spatial extents (nations, ecoregions) and many sites (hundreds to thousands). The objective of this study was to describe a methodology to quantitatively define a disturbance gradient for 40 sites in each of two small southeastern Brazil river basins. The assessment of anthropogenic disturbance experienced by each site was based solely on measurements strictly related to the intensity and extent of anthropogenic pressures. We calculated two indices: one concerned site-scale pressures and the other catchment-scale pressures. We combined those two indices into a single integrated disturbance index (IDI) because disturbances operating at both scales affect stream biota. The local- and catchment-scale disturbance indices were weakly correlated in the two basins ($r = 0.21$ and 0.35) and both significantly ($p < 0.05$) reduced site EPT (insect orders Ephemeroptera, Plecoptera, Trichoptera) richness. The IDI also performed well in explaining EPT richness in the basin that presented the stronger disturbance gradient ($R^2 = 0.39$, $p < 0.001$). Natural habitat variability was assessed as a second source of variation in EPT richness. Stream size and microhabitats were the key habitat characteristics not related to disturbances that enhanced the explanation of EPT richness over that attributed to the IDI. In both basins the IDI plus habitat metrics together explained around 50% of EPT richness variation. In the basin with the weaker disturbance gradient, natural habitat explained more variation in EPT richness than did the IDI, a result that has implications for biomonitoring studies. We conclude that quantitatively defined disturbance gradients offer a reliable and comprehensive characterization of anthropogenic pressure that integrates data from different spatial scales.

1. Introduction

The development and maintenance of human societies rely on the conservation of freshwater resources and of the ecological services that streams and rivers provide (Karr, 1999). Monitoring the "ecosystem health" of streams (*sensu* Norris and Thoms, 1999) is a fundamental step for conscious and effective management of catchments (Boulton, 1999). Currently, biomonitoring is considered one of the most efficient ways to assess stream condition (Marchant et al., 2006). Macroinvertebrate assemblages are responsive to environmental condition and thus integrate physical, chemical and biological aspects of ecosystems. Accordingly, they are considered good biological indicators of stream ecological condition (Karr and Chu, 1999; Bonada et al., 2006; Hughes and Peck, 2008) and are extensively used in multimetric indices (MMIs) for such purposes (Reynoldson et al., 1997; Barbour et al., 1999; Klemm et al., 2003; Hering et al., 2006; Whittier et al., 2007a). The EPT assemblages (insect orders Ephemeroptera, Plecoptera and

Abbreviations: LDI, local disturbance index; CDI, catchment disturbance index; IDI, integrated disturbance index.

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Trichoptera), particularly, have proven effective ecological indicators of human disturbances (Rosenberg and Resh, 1993; Stoddard et al., 2008).

A goal of many biomonitoring approaches is to report how test sites deviate from the "undisturbed" (natural) condition in terms of the structure and/or composition of the assemblages they support. This is typically accomplished by designating "reference sites", that is, sites minimally affected by human activities and whose biological, physical and chemical features serve as reference condition for natural levels of patterns and processes (Hughes et al., 1986; Stoddard et al., 2006; Hawkins et al., 2010). A set of reference sites should be specific for a particular typology (e.g., altitude, stream size, and predominant substrate) and geographic domain (biome and ecoregion) because these are important natural drivers of stream characteristics, including their biota (Hughes et al., 1986, 1990; Gerritsen et al., 2000; Waite et al., 2000; Sánchez-Montoya et al., 2007). This framework has been established as the "reference condition approach" (RCA) (Bailey et al., 2004). In most cases it is not practical to seek sites that have truly undisturbed/minimally disturbed conditions because (1) human modifications are widespread in most landscapes worldwide, and (2) many places have been modified for hundreds (or even thousands) of years (Stoddard et al., 2006; Whittier et al., 2007b; Herlihy et al., 2008). Instead, sites in least-disturbed condition, i.e., the best set of sites available in a continuous gradient of disturbance, are typically used to represent "reference" conditions (Reynoldson et al., 1997; Stoddard et al., 2006; Yates and Bailey, 2010).

It is explicitly stated in the RCA that the reference condition should be chosen based strictly on criteria concerning the minimal exposure of the sites to human disturbances (Bailey et al., 2004). Although human disturbances affect stream biological and habitat attributes (Maddock, 1999), reference site selection should not be based on either because it is difficult to distinguish between effects from human disturbance and natural variation (Dovciak and Perry, 2002; Moreno et al., 2006). In fact, a key aspect of the RCA is that natural variability is intrinsic in ecosystems and that this variability must be accounted for by using models to understand the effects of human disturbance on assemblage structure of fish (Oberdorff et al., 2002; Tejerina-Garro et al., 2006; Pont et al., 2006, 2009) and macroinvertebrates (Clarke et al., 2003; Bailey et al., 2004; Hawkins et al., 2010; Moya et al., 2011).

A multitude of stressors have been identified and used as criteria for determining reference sites. As geographic information system (GIS) technology has become operationally simpler and widely available (King et al., 2005), disturbances identified at the catchment scale have been used for defining potential reference areas (Collier et al., 2007; Wang et al., 2008). However, human modifications acting at both large (catchment) and local (stream channel and riparian zone) scales should be investigated because pressures or stressors operating at both scales can impair the stream biota (Bryce et al., 1999; Whittier et al., 2007b; Hughes et al., 2010).

Increasingly, methods for defining and selecting reference sites are applied to large spatial extents (whole ecoregions, states, and countries), commonly involving hundreds or thousands of sites. The Environmental Protection Agency of the United States of America (US-EPA), in its national Wadeable Stream Assessment (WSA) program, screened a series of physical habitat and water quality data, setting thresholds for the selection of least-disturbed sites in different ecoregions (Herlihy et al., 2008). The same "filtering" approach was employed in regional assessments made by the same agency (Klemm et al., 2003; Whittier et al., 2007b). In a similar approach, a large set of criteria of human disturbances operating at both local and regional spatial scales were used to select least- and most-disturbed sites on European streams (Nijboer et al., 2004; Pont et al., 2006; Sánchez-Montoya et al., 2009).

However, methodologies employed at large spatial extents may be inappropriate for studies dealing with more restricted spatial extents and far fewer sites. First, for most ecosystems located in less studied regions of the world, such as in tropical developing countries, there is no reliable information about the physical and chemical thresholds that indicate substantial disturbance (Boyero et al., 2009). Second, the application of rigid filters to a small number of sites is likely to select too few sites, or none at all. Even in Europe, when hundreds of sites from 4 countries were analyzed, for many stream types it was not possible to find any single site that fulfilled all the criteria proposed for European reference conditions (Nijboer et al., 2004). Nevertheless, many monitoring initiatives are applied at more restricted geographic areas (small to medium-sized basins or sub-basins) and far fewer sites (dozens at best) (Baptista et al., 2007; Moreno et al., 2009; Oliveira et al., 2011; Suriano et al., 2011). To our knowledge, no systematic methodology has been proposed to clearly define disturbance conditions in those situations.

When working with few sites, instead of trying to allocate sites into 'boxed' categories from the onset of the project (e.g., least-, intermediate-, and most-disturbed sites), the use of a continuous disturbance gradient can be more advantageous for classifying the sites included in the study, enabling the definition a posteriori of the least-disturbed sites and the most-disturbed sites. This contrast is necessary for the development of MMIs (e.g., Stoddard et al., 2008; Oliveira et al., 2011). For instance, predictive models are first concerned with describing assemblage composition in reference conditions (Reynoldson et al., 1995), i.e., the "good tail" of a disturbance gradient. In addition to biomonitoring studies, any applied ecological research concerned with changes in patterns and processes associated with the intensity of human modifications will benefit from the use of a disturbance gradient.

In this study we present a methodology to quantitatively define disturbance gradients in two basins sampled with a relatively small number of sites (40 each), each basin including a range of sites from relatively undisturbed to greatly altered. To this end, we worked with two hypotheses. (1) Disturbances taking place at both local (stream sites) and catchment spatial scales reduce the EPT assemblage richness of the sites. (2) The proportion of variation in EPT richness associated with natural variability among site habitats will be greater in the basin with the weaker anthropogenic disturbance gradient.

2. Methods

2.1. Study area

We sampled streams in two basins of the Cerrado biome in the state of Minas Gerais, southeastern Brazil: Upper Araguari basin (in the Paraná river basin) and the Upper São Francisco basin (in the São Francisco river basin) (Fig. 1). Both study areas were demarcated upstream of the first big reservoir of each basin (Nova Ponte and Três Marias reservoirs, respectively). The Cerrado is the second-most extensive biome of the Neotropics (Wantzen, 2003), originally covering 20% of Brazilian territory, and one of the terrestrial biodiversity "hotspots" of the planet (Myers et al., 2000). It is also one of the most threatened due to ever-expanding pasture and agricultural activities (Wantzen et al., 2006). The Cerrado climate has two well defined annual seasons: a dry season from October to March, and a wet season from April to September, with 1200–1800 mm of precipitation per year. The vegetation is typically savannah-like, with denser forest formations along water courses and wet areas.

Most people living in the study areas dwell on farms and in small towns (up to 20,000 inhabitants), although a few small cities (up to 80,000 inhabitants) are present. The Upper Araguari has a well developed system of irrigated agriculture, encompassing mainly

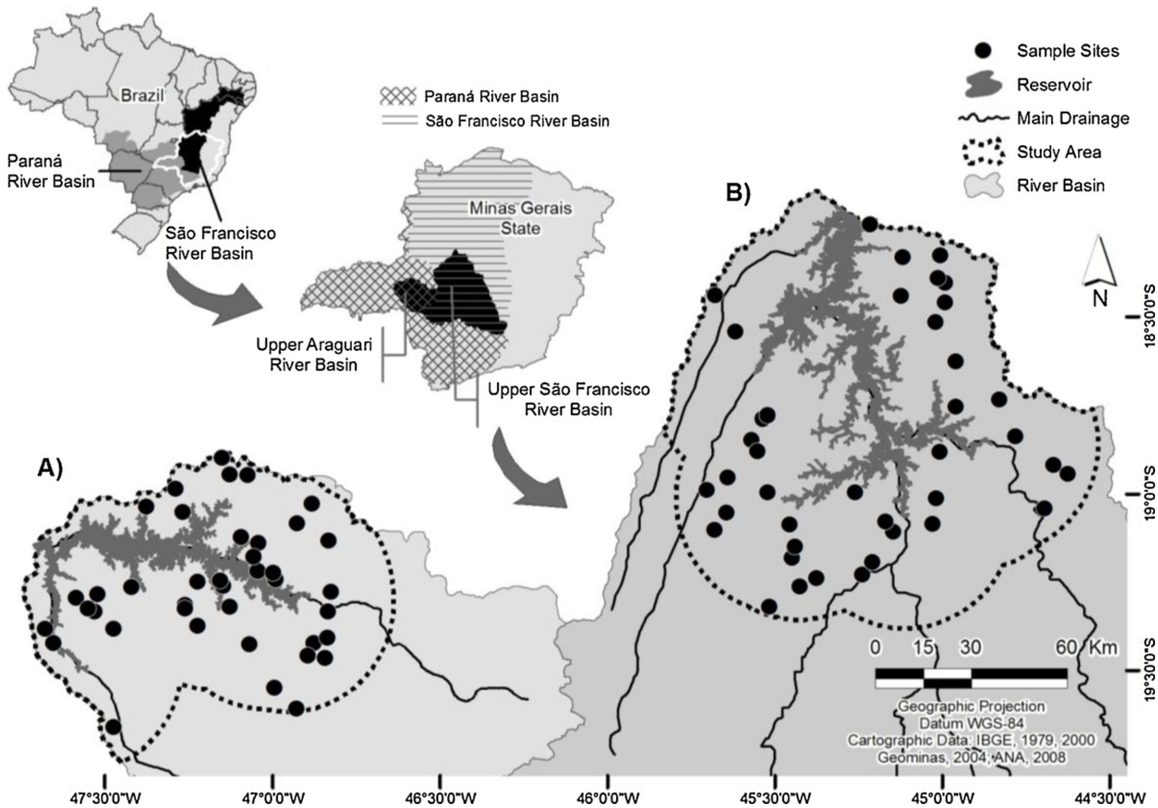

Fig. 1. Location of the basins and stream sites sampled. (A) Upper Araguari basin and (B) Upper São Francisco basin.

soy, coffee, corn, and sugar cane culture. Irrigated agriculture is less common in the Upper São Francisco, where pasture and small family farms predominate.

2.2. Site selection

Forty "wadeable" stream sites (that can be traversed by a person wading) ranging from 1st to 3rd order (*sensu* Strahler, 1957) were selected on 1:100,000 scale maps in each basin and sampled during the dry season. The site selection was performed through a probability-based design as described in Olsen and Peck (2008), the same procedure used by the US-EPA in the Environmental Monitoring and Assessment Program Western Pilot Study (EMAP-West, Stoddard et al., 2005) and its national Wadeable Stream Assessment (WSA, Paulsen et al., 2008). In this approach, a master sample frame (MS) is first established using a digitized drainage system map (1:100,000 scale), and then the sample sites are selected via a hierarchical, spatially weighted criteria (Stevens and Olsen, 2003). This procedure assures a balanced selection of sites across the range of stream orders and geographic location. The Upper Araguari sites were sampled in September 2009 and the Upper São Francisco sites were sampled in August/September 2010.

2.3. Site habitat measurements

The field physical habitat was measured as described in Peck et al. (2006). The site lengths were set at 40 times their mean wetted width, and a minimum of 150 m. Given their narrow widths, most sites were 150 m long. In each site, 11 equidistant cross-sectional transects were marked, defining 10 sections of the same length.

In each transect and along the sections, a large set of measurements were recorded, including site morphology (e.g., slope, sinuosity, wetted and bankfull width, depth, and incision height), habitat characteristics (e.g., substrate size and embeddedness, flow type, and large wood), riparian structure (e.g., mid-channel and margin shading, tree and herbaceous cover density) and human disturbance in the channel and riparian zone (e.g., presence of pasture, crops, pipes, and trash). Habitat metrics were then calculated following Kaufmann et al. (1999).

The following physical and chemical characteristics of the water column were also measured in the field for each site: pH, electrical conductivity, and total dissolved solids (TDS). Water samples were collected for further analysis in the laboratory, including dissolved oxygen, turbidity, total alkalinity, total nitrogen, and total phosphorus. Those analyses were conducted following APHA (1998).

The site nutrient concentrations of both basins were extremely low and not indicative of anthropogenic sources. In the Upper Araguari, the values were 0.06 ± 0.01 mg/L (mean ± SD) for total nitrogen and 0.03 ± 0.01 mg/L for total phosphorus. The concentrations in the Upper São Francisco were 0.08 ± 0.06 mg/L for total nitrogen and 0.02 ± 0.01 mg/L for total phosphorus.

2.4. Macroinvertebrate sampling and laboratory processing

The biological sampling also followed the protocol of Peck et al. (2006) and Hughes and Peck (2008). Eleven sample units were taken per stream site, one per transect, generating one composite sample for each site. Each sample unit was collected through use of a D-net (30 cm mouth width, 500 μm mesh), effectively sampling 1 m^2 of stream bottom area sampled per site. The sample units were obtained by following a systematic zigzag pattern along the sites to avoid bias in habitat selection. Immediately after collection, the composite samples were placed in individual plastic buckets and preserved with 10% formalin.

In the laboratory, the macroinvertebrates were sorted by eye, and the EPT individuals were identified to genus under a 100× magnification stereoscope microscope through use of taxonomic

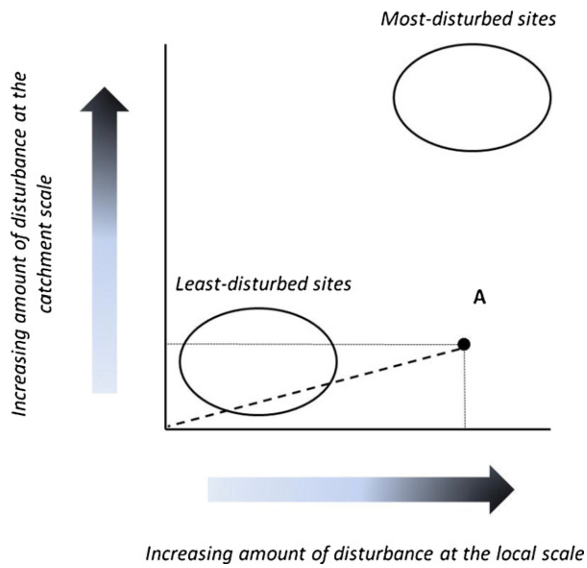

Fig. 2. Conceptual model of the disturbance plane whose axes represent the amount of disturbance observed at the local scale (in-stream and riparian zone) and at the catchment scale. The ideal least-disturbed sites would be those located closest to the origin of the axes, with few disturbances observed at both scales. The ideal most-disturbed sites would be those located in the opposite corner of the plane. A single measurement of disturbance can be the Euclidean distance (calculated through the Pythagorean theorem) between the location of the site in the plane and the origin of the axes (see the example in the figure with site "A"). For this purpose the axes values should be standardized at the same scale.

keys (Pérez, 1988; Fernández and Domínguez, 2001; Mugnai et al., 2010).

2.5. Data analyses

2.5.1. Calculation of the disturbance gradient

To describe the total exposure of the sites to human pressures, we developed two separate indices: one reflecting disturbances at the site scale and one reflecting disturbances at the catchment scale, both having their origins (0 values) representing the absence of evidence of disturbances. In each index, the higher the site value, the greater the intensity of human modifications observed for that site, i.e., the greater the deviation from the pristine condition at that spatial scale. Thus, we positioned each site in a 'disturbance bi-plane' constructed with the two disturbance indices as axes. The 'ideal' reference sites should be those lacking evidence of human modifications at both near/in-stream and catchment scales (concept of minimally disturbed condition; Stoddard et al., 2006). Typically, however, reference sites are those with the least disturbances among the sites available (concept of least-disturbed condition; Stoddard et al., 2006). Through this conceptual model, the least- and most-disturbed sites in a pool of sites can be visualized according to their positions in the disturbance plane, the least-disturbed sites being closer to the origin (lower left corner of the plane) and the most-disturbed sites being farthest from the origin (upper right corner of the plane) (Fig. 2).

For quantifying the local disturbance index (LDI) we used the metric *W1_hall*, calculated as described in Kaufmann et al. (1999), a measure commonly used in the US-EPA stream assessments. This metric summarizes the amount of evidence observed in-channel and in the riparian zone for 11 types of disturbances (buildings, channel revetment, pavement, roads, pipes, trash and landfill, parks and lawns, row crop agriculture, pasture, logging and mining) along the eleven transects demarked at the stream site. The values are weighted according to the proximity of the observation from the stream channel (Kaufmann et al., 1999).

We assessed watershed land uses for each site through use of manual image interpretation. Watersheds were extracted from the terrain model from the Shuttle Radar Topographic Mission – SRTM (USGS, 2005). We manually interpreted high resolution multispectral images in conjunction with the Landsat TM sensor using Spring software (Camara et al., 1996). The high-resolution images provided information about the shape and texture of the elements, and the Landsat images showed spectral response for different targets. Our mapping identified three human-influenced land uses (pasture, agriculture, and urban). The catchment percentages of each land use were estimated for each site.

The catchment disturbance index (CDI) was based on the human land uses in the catchments and was calculated following Rawer-Jost et al. (2004), according to the formula:

$$\text{catchment disturbance index (CDI)} = 4 \times \%\,\text{urban areas} + 2 \times \%\,\text{agricultural areas} + \%\,\text{pasture areas}$$

We evaluated the collinearity between local and catchment human disturbances in each basin through use of Pearson correlations between the LDI and the CDI values of the sites.

Because the local and the catchment disturbance indices do not share the same numerical scale, both were separately standardized to provide a similar scale in values. This transformation was necessary to reliably calculate an integrated disturbance index for each site, based on both the local and catchment indices (see below). The values of each index were divided by 75% of the maximum value that each can theoretically achieve. We did not use the maximum values of each index for these standardizations because those values are rarely achieved. Dividing by the maximum values would shrink greatly and unnecessarily the values in the standardized indices, shifting nearly all the sites very close to the origin of the disturbance plane.

The CDI values potentially range from 0 (no land use in the catchment) to 400 (entire catchment occupied by urban areas). So the values of this index were divided by 300. The LDI values (*W1_hall* metric) potentially range from 0 (no evidence of any type of disturbance in the channel or riparian zone) to 16.5 (all 11 types of disturbances observed inside the stream channel in all transects). But this theoretical upper value is highly unlikely because of spatial limitations and negative colinearities among the types of disturbance (listed above). The empirical maximum value of the *W1_hall* metric is around 7 (Kaufmann et al., 1999), so the values of this index were divided by 5.

To summarize the disturbances measured at both scales in a single index we calculated for each site an integrated disturbance index (IDI). It was measured as the Euclidian distance between the position of the site in the disturbance plane (axes standardized) to the origin of the plane (Fig. 2). This was performed through application of the Pythagorean theorem:

$$\text{integrated disturbance index (IDI)} = \left[\left(\frac{\text{LDI}}{5}\right)^2 + \left(\frac{\text{CDI}}{300}\right)^2\right]^{1/2}$$

The higher the IDI of a site, the more that site deviates from the 'origin', i.e., from the 'ideal' reference condition of no disturbance inside the stream channel, in the riparian zone, or in the catchment. Thus, we defined the disturbance gradient simply as the ascending ordination of the IDI's in a pool of sites. The steeper the disturbance gradient in a pool of sites, the greater the difference in ecological condition between the least- and most-disturbed sites in the pool.

2.5.2. EPT richness associations with the disturbance indices

To evaluate how EPT assemblages responded to the degree of human disturbances at both local and catchment scales, we

Table 1
Candidate site habitat metrics for explaining EPT richness variability in both studied basins.

Metric name	Metric code	Not significantly correlated		Not strongly correlated	
		With disturbances ($p > 0.05$)		Among each other ($r < 0.6$)	
		Upper Araguari	Upper São Francisco	Upper Araguari	Upper São Francisco
Mean width	xwidth		*		
Mean depth	xdepth	*			
Mean slope	xslope				
Mean bankfull width	XBKF_W				
Mean width × mean depth	XWXD	*	*	*	*
Mean (width/depth)	xwd_rat		*		*
Mean depth × mean slope	xdepth_xslope				
Bankfull (width/depth)	BKF_WDrat	*	*	*	*
Mean residual pool area	rp100	*			
Mean water volume/m^2	v1w_msq				
Riparian canopy (>5 m high) presence	xpcan				
Riparian canopy (>5 m high) cover	XC		*		
Total riparian cover (all vegetation layers)	xcmg	*	*	*	*
Total riparian woody cover	xcmgw				
Mean canopy density (mid-stream)	xcdenmid				
Natural cover in the stream (all)	xfc_nat		*		*
Natural cover provided by large wood	xfc_lwd	*		*	
Percentage of fast water	pct_fast	*	*	*	*
Percentage of fines (silt and clay)	pct_fn		*		
Percentage of sand + fines	pct_sfgf		*		
Percentage of cobble	pct_cb		*		*
Percentage of coarse substrate (>16 mm)	pct_bigr		*		
Log of mean substrate diameter	lsub_dmm		*		*
Mean substrate embeddedness	xembed				
Log of relative bed stability	LRBS				
pH	pH		*		*
Conductivity (μS/cm)	Cond				
Total dissolved solids (g/L)	TDS		*		*
Turbity (NTU)	Turb	*		*	
Dissolved oxygen (mg/L)	DO	*		*	
Alkalinity (mequiv./L)	Alk				

conducted multiple linear regressions between EPT richness and the standardized LDI and CDI of the sites for each basin. We also regressed EPT richness against the IDI to evaluate its performance relative to EPT richness variability.

2.5.3. Contribution of natural variability of site habitat characteristics to explaining the variation of EPT richness

Through the following methodology, we evaluated how much natural physical habitat variability added to the explanation of EPT richness provided by the disturbance gradient alone. The process was performed separately for each basin (Fig. 3).

We started with a set of 31 habitat metrics calculated from the raw field data (Table 1). With these metrics we aimed to represent key aspects of the habitats of the sites, such as morphology (e.g., mean wetted and bankfull width, mean depth, and mean slope), riparian condition (e.g., riparian vegetation extent and mean canopy cover), habitat heterogeneity (e.g., % fast water, % large substrates, % fine substrates, and mean substrate embeddedness) and water quality (e.g., dissolved oxygen, pH, and alkalinity). We obtained Pearson correlations between those metrics and all the disturbance descriptors we had available: the 3 land uses percentages, the 11 types of local site disturbances, the LDI, the CDI and the IDI. All metrics significantly correlated ($p < 0.05$) with any of the disturbance descriptors were disregarded for the next step of the analysis. In this way, we filtered all the habitat metrics that could be affected by human disturbances of any kind; the remaining metrics were considered as sources of natural variation in the sites. Next, a Pearson product–moment correlation matrix was calculated with all the metrics not correlated with disturbance evidence. The redundant metrics ($r > 0.6$) were removed and the choice of the metrics to be retained was based on ecological rationale.

Among the 31 initial habitat metrics, many were not significantly correlated with human disturbances (9 in the Upper Araguari and 15 in the Upper São Francisco, Table 1). In both basins, some of the remaining metrics were removed because of high colinearities ($r > 0.6$). In the Upper Araguari, mean depth and mean residual pool area were removed and mean wetted width × mean thalweg depth was kept, because we believe the latter metric best summarized the stream channel size. In the Upper São Francisco, mean wetted width was removed and mean wetted width × mean thalweg depth was kept (same reason as above) and riparian canopy cover was removed and total riparian cover, a more embracing metric, was kept. Percentage of coarse substrates (>16 mm), percentage of fines (<0.06 mm: silt and clay), percentage of sand + fines (<2.0 mm), and log of the geometric mean substrate diameter had high correlations. We chose to include log of mean substrate diameter because it best represented the predominant substrate sizes of the sites.

We used the reduced set of habitat metrics to perform a hierarchical multiple regression, forcing the entrance of the integrated disturbance index (IDI) in the first block and allowing, in the second block, a best-subsets multiple regression procedure search for the combinations of habitat metrics that best explained the remaining variability in EPT richness. The R^2 values were considered as criteria for the selection of the best models. We restricted the number of predictor variables in the final models to a total of 4 (10% of 40 sites) to avoid model over-fitting (Harrell, 2001; Tabachnick and Fidell, 2007). Thus, in addition to the IDI in the first block, three habitat metrics were allowed to enter in the second block. Hierarchical multiple regression is an efficient way to isolate the contribution of some factor in a regression model because residual regressions can lead to biased estimations of the parameters of the models (see Freckleton,

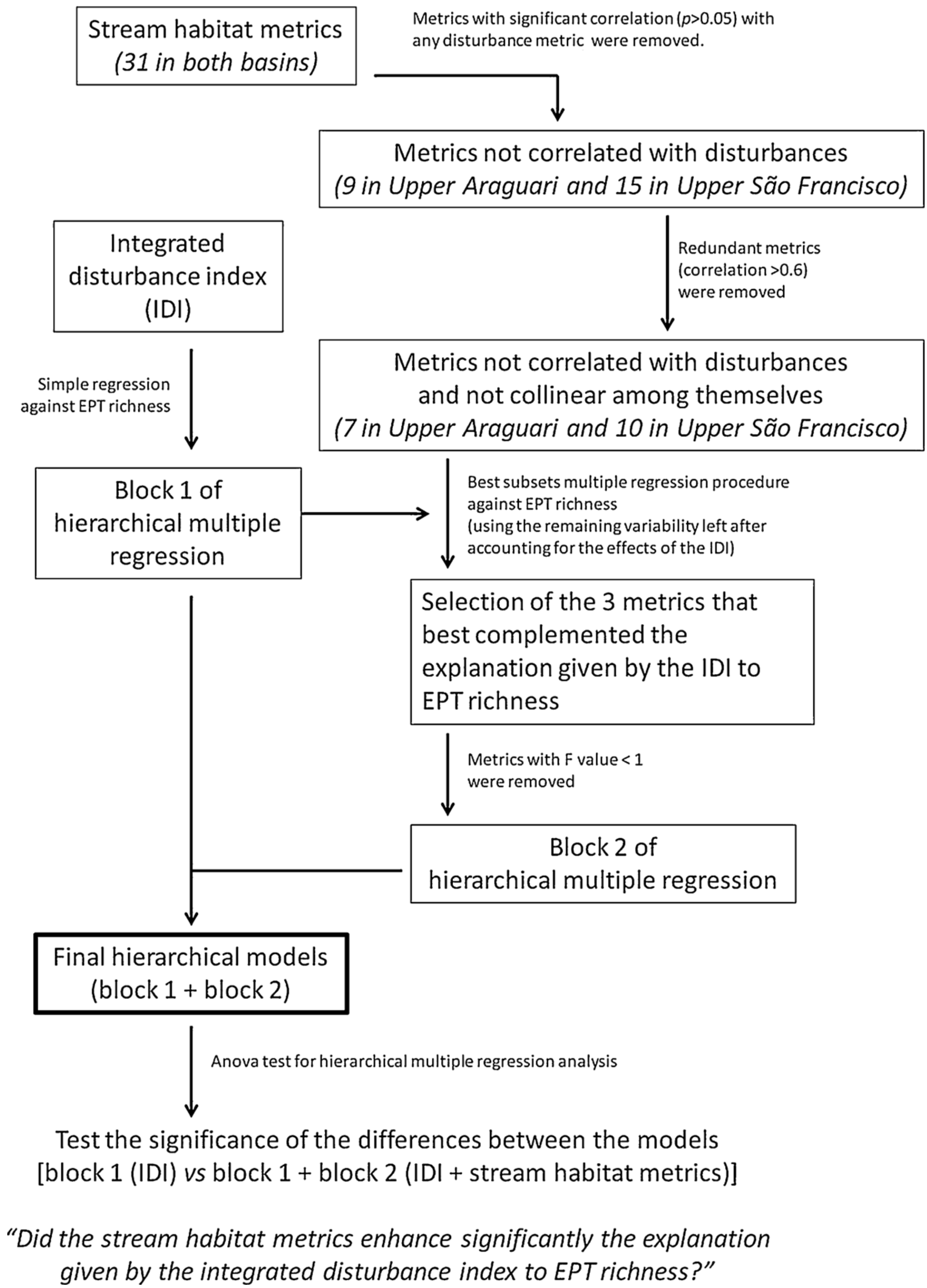

Fig. 3. Summary of the methodological design used to test statistically how site habitat metrics not subjected to human disturbances enhanced the explanation given by the integrated disturbance index (IDI) to EPT richness in each basin.

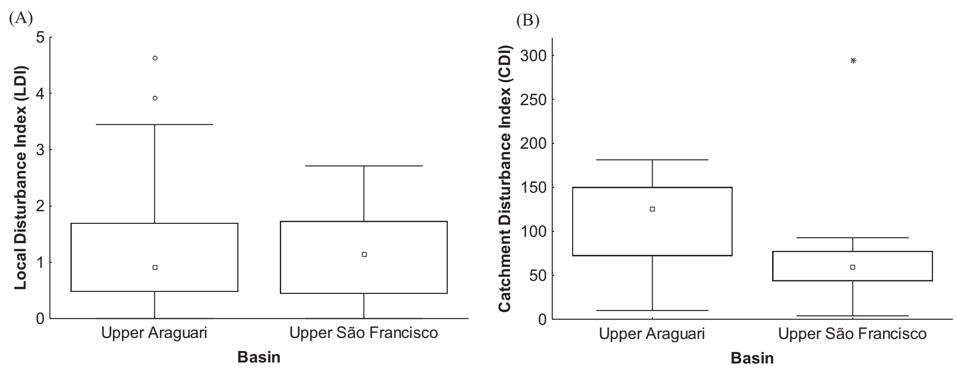

Fig. 4. Distribution (medians and quartiles) of the values of the (A) local disturbance index and of the (B) catchment disturbance index in each studied basin.

2002). Only predictor variables with individual *F*-values > 1 were allowed in the final models. The statistical significance of the hierarchical multiple regressions (block 1 vs block 1 + block 2) were tested through analysis of variance (ANOVA's). In this way we tested whether the habitat metrics contributed significantly to the explanation of EPT richness derived from the IDI for each basin.

3. Results

3.1. Local and catchment disturbance indices and the disturbance plane

The two basins had similar patterns in most LDI values (Fig. 4A), although the Upper Araguari basin had a few higher values, resulting from urban sites. On the other hand, the patterns of CDI values varied considerably between the basins, the Upper Araguari had higher CDI values than the Upper São Francisco (Fig. 4B). In the Upper São Francisco, only one site had a CDI value >100. The differing patterns are explained by the land use patterns in both basins (Fig. 5A–C). In the Upper Araguari we observed a higher proportion of agriculture in the catchments, whereas in the Upper São Francisco pasture predominated. Proportions of urban areas were low in both basins, most catchments having none. The Pearson correlations between the LDI and CDI scores were weak ($r = 0.21$ in the Upper Araguari and $r = 0.35$ in the Upper São Francisco).

In both basins few sites were located close to the origin on the disturbance plane (Fig. 6), but because of higher CDI values, more Upper Araguari sites were located farther from the origin. This distribution pattern is summarized by the different slopes of the disturbance gradients of the basins, showing the IDI values in ascending order (Fig. 7). In the Upper Araguari we observed a much wider range in site IDI values (i.e., more sites nearer and farther from the origin), indicating a much stronger disturbance gradient in that basin.

3.2. Description of the EPT assemblages

A total of 5463 EPT individuals (61 genera) were identified in Upper Araguari sites, and 15,133 EPT individuals (65 genera) were identified in Upper São Francisco sites. In both basins Ephemeroptera comprised the majority of the EPT genera (30 in the Upper Araguari and 35 in the Upper São Francisco) and number of organisms (3291 in the Upper Araguari and 12,529 in the Upper São Francisco). In the Upper Araguari, the most abundant genera were *Smicridea* (Trichoptera), and the Ephemeroptera *Thraulodes*, *Traverhyphes* and *Tricorythopsis*. Those four genera represented 43% of the EPT individuals collected in the Upper Araguari. In the Upper São Francisco, the most abundant genera were *Callibaetis*, *Cloeodes*, *Americabaetis*, *Caenis* and *Traverhyphes*, all Ephemeroptera. Those five genera represented 54% of the EPT individuals collected in the Upper São Francisco. Around 25% of the taxa identified in the Upper Araguari, and 20% of the taxa identified in the Upper São Francisco, can be considered rare taxa, with just 5 or fewer individuals identified across all sites of each basin.

3.3. EPT richness versus disturbance indices

The variation of EPT richness explained by the LDI and CDI together was much higher in the Upper Araguari ($R^2 = 0.40$) than in the Upper São Francisco ($R^2 = 0.18$) (Table 2). In both basins, EPT richness was significantly related to the CDI, but only in the Upper Araguari did the LDI contribute significantly to explain EPT richness variation (Table 2). The slope between LDI and EPT richness in the Upper São Francisco approached zero (Table 2). As expected, all significant relationships were negative. In the Upper Araguari, the IDI explained a moderate amount of EPT richness (Simple linear regression, $R^2 = 0.39$, $F_{(1,38)} = 24.6$, $p < 0.001$; Fig. 8A), nearly the same as the combined explanations given by the LDI and CDI in the multiple regression. In the Upper São Francisco, the IDI explained poorly, but significantly, EPT richness variation (simple linear regression, $R^2 = 0.11$, $F_{(1,38)} = 4.55$, $p = 0.039$; Fig. 8B).

Table 2
Multiple regression results for each basin with EPT richness as the response variable and the local disturbance index (LDI) and the catchment disturbance index (CDI) as predictor variables.

	F-Value (2,37)	*p*-Value	*R*-Square		Beta	Std. err. of beta	*t*(37)	*p*-Value
Upper Araguari	12.5	<0.001	0.403	Intercept			9.806	<0.001
				CDI	−0.450	0.130	−3.467	0.001
				LDI	−0.364	0.130	−2.802	0.008
Upper São Francisco	4.005	0.027	0.178	Intercept			8.820	<0.001
				CDI	−0.424	0.159	−2.671	0.011
				LDI	0.007	0.159	0.043	0.966

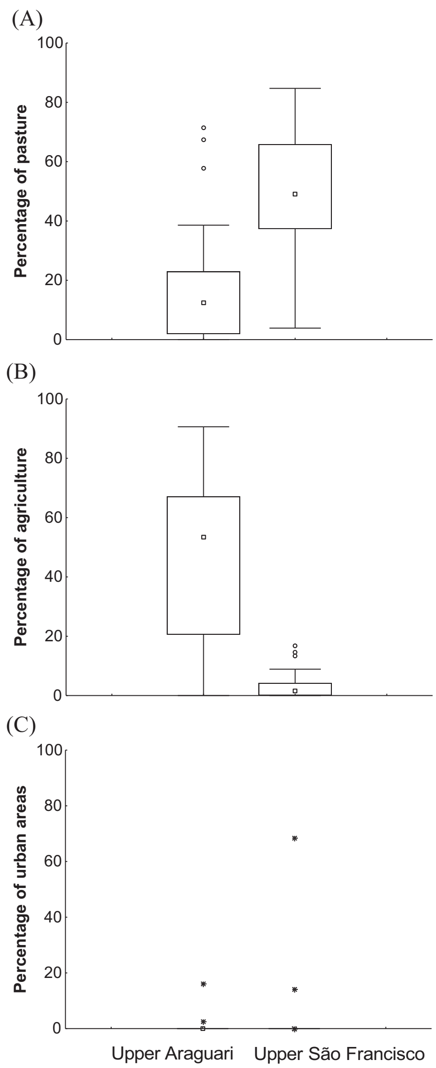

Fig. 5. Distribution (medians and quartiles) of the percentages of (A) pasture, (B) row crop agricultural and (C) urban areas in the catchments of the sites sampled in each basin.

3.4. Contribution of natural variability of habitat characteristics in explaining EPT richness

The hierarchical regressions informed how the explanations (R^2 values) given by the IDIs to EPT richness variations were increased by the addition of habitat metrics not related to human disturbances. In the Upper Araguari, the increment was low and just marginally significant (Table 3). In that basin, the R^2 value increased from 0.39 to 0.49, an increase of 0.1. On the other hand, in the Upper São Francisco the increment was much greater, the R^2 value rising from 0.11 to 0.50, an increment of 0.39. The amount of explanation given by the combined models (IDI + habitat metrics not correlated with disturbance) were similar in both basins, with R^2 values around 0.5, meaning that the final models explained only about half the variation.

The combined models generated from best-subsets multiple regressions had, in addition to the IDI, 2 habitat metrics in the Upper Araguari and 3 habitat metrics in the Upper São Francisco (all with F-values > 1, Table 3). In both basins, a site size metric (mean width × mean depth) was important in explaining EPT richness variation. In the Upper Araguari, another morphologic metric (bankfull width/depth) was incorporated in the model, whereas in the Upper São Francisco, microhabitat metrics (percent fast flows and log of mean substrate diameter) were included.

4. Discussion

4.1. Premises for comparisons between sites

It has been long recognized that some geographic (e.g., ecoregions) and non-geographic features (e.g., typologies) of stream sites exercise a strong influence on the composition and structure of their macroinvertebrate assemblages (Hughes, 1985, 1995; Gerritsen et al., 2000). Accordingly, it is important for the assigned reference sites and the test sites of a study to share these key biological drivers, allowing reliable comparisons between them (Herlihy et al., 2008). In the words of Gerritsen et al. (2000) it is important to "put like with like".

Gradual changes in the habitat template, in the available food resources, and in the biological assemblages naturally occur along the longitudinal gradient of lotic ecosystems (from spring to mouth), resulting mainly from downstream changes in their morphological dimensions, catchment areas and discharges (Vannote et al., 1980; Poole, 2002; Hughes et al., 2011). We reduced such sources of variation by selecting streams with similar morphological dimensions. All sites can be classified as small streams, close to the headwaters.

There is no geographic classification formally designed for Brazil that is comparable in detail to the ecoregion classifications of the USA (Omernik, 1995) or Europe (e.g., Gustafsson and Ahlén, 1996). However, the basins studied are in the same biome

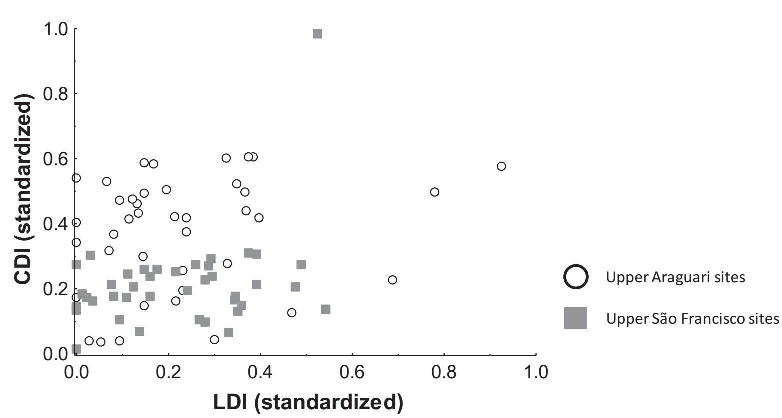

Fig. 6. Distribution of the sites of each basin in the disturbance plane, with Upper Araguari sites represented by open circles (○) and Upper São Francisco sites represented by filled boxes (■). The axes of the local disturbance index (LDI) and the catchment disturbance index (CDI) were standardized at the same scale (relative positions of the sites on each axis, and in the plane, were retained).

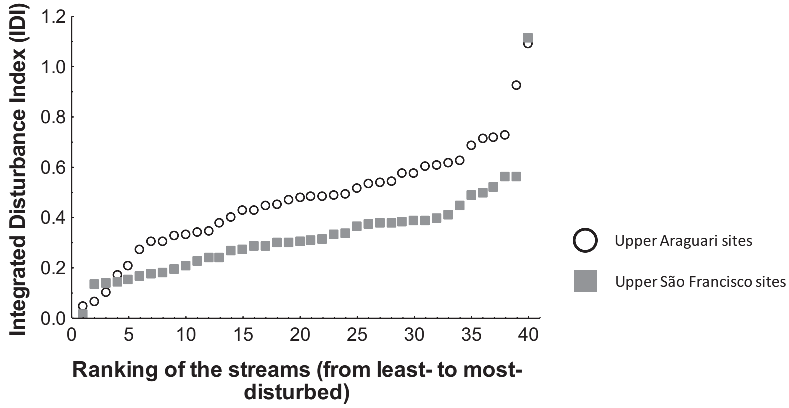

Fig. 7. Disturbance gradients in both basins, represented by ascending site values of the integrated disturbance index (IDI). Upper Araguari sites are represented by open circles (◯) and Upper São Francisco sites are represented by filled boxes (■).

(Cerrado) and in the same general terrestrial and aquatic ecoregions outlined by Olson et al. (2001), meaning that the sites share similar climatic, edaphic, vegetation, geological and biogeographic conditions (Olson et al., 2001; Wantzen, 2003). Moreover, the basins were analyzed separately, and their individual areas are much smaller than those of the US level IV ecoregions, the most detailed level of their classification. Thus, although lacking an official detailed classification, we consider all the sites in the same ecoregion.

4.2. The role of the disturbances measured at local and catchment spatial scales

As stated in the classical view of stream impairment, human disturbances operating at multiple scales can alter patterns and processes of the natural habitat, ultimately leading to modifications or impairment of biological assemblages (Karr, 1999; Norris and Thoms, 1999; Bryce et al., 1999; Feld and Hering, 2007). However, the exact mechanistic pathways among the origins of impairment, the habitat modifications, and the biological responses are not well known in most cases (Bedford and Preston, 1988; Karr, 1991). For this reason, rather than searching for all the individual sources of impairment, it is important to develop a group of disturbance metrics that can serve as general indicators of the total pressure to which an ecosystem may be subjected (Boulton, 1999).

Disturbances in the channel or riparian zone can impair the habitats and the biota (Bryce et al., 1999; Death and Joy, 2004; Kaufmann and Hughes, 2006). Because catchments drive the stream features in almost every aspect (Hynes, 1975; Wiens, 2002), human land uses are also usually linked with the ecological condition of streams (Bryce et al., 1999; Allan, 2004; Wang et al., 2008). Non-point sources in catchments commonly contribute excess sediments, nutrients and pollutants to streams and rivers (Allan and Castillo, 2007; Allan, 2004). Human activities in the catchment also influence the condition of stream riparian zones (Van Sickle et al., 2004; Sponseller et al., 2001; Miserendino et al., 2011). The ordering of "disturbance potential" used in this study (urban areas having more weight than row crop agriculture, which in turn has more weight than pasture), as well as the use of the whole catchment area as the "buffer" to estimate catchment human pressures, are corroborated by many previous studies (Sponseller et al., 2001; Mebane et al., 2003; Wang et al., 2008; Gucker et al., 2009; Trautwein et al., 2011). In our study, disturbances measured at local and catchment spatial scales both reduced EPT richness, corroborating our first hypothesis. In agreement with Kail et al. (2012), catchment disturbances had a greater effect than local disturbances in these basins. The latter were not even significantly related to macroinvertebrate richness in the Upper São Francisco sites.

Local disturbance was not correlated with catchment disturbance. This lack of association means that catchment land uses were not driving near or in-stream modifications, and what is observed at one scale can differ from what is observed at the other. For instance, in our study we observed catchments highly dominated by row crop agriculture but with undisturbed riparian vegetation and stream channels. Conversely, we also had catchments with mostly natural land cover but stream channels altered by livestock. Scenarios like these are likely to happen elsewhere (Nijboer et al., 2004). Consequently, relying on just one scale to describe the level of human pressure at a site can lead to misleading interpretations of biological responses (Bryce et al., 1999; Feld and Hering, 2007).

Table 3

Hierarchical multiple regression results contrasting the significance of the differences between the regression models in each basin. The first models (block 1) consisted of simple regressions with EPT richness as the response variable and the integrated disturbance index (IDI) as the predictor variable. The second models (block 1 + block 2) included as predictor variables the habitat metrics selected by the best subsets procedure as those which, together with the IDI, better explained EPT richness. Habitat metric codes are defined in Table 1.

Basin		*F*-Value	*p*-Value	*R*-Square	Metrics' mean beta values				ANOVA test for hierarchical regression analysis [block 1 vs (block 1 + block 2)]	
									F-Value	*p*-Value
Upper Araguari	Model 1	24.6	<0.001	0.393	IDI −0.627				3.194	0.053
	Model 2	11.28	<0.001	0.484	IDI −0.523	XWXD 0.227	BKF_WDrat 0.299			
Upper São Francisco	Model 1	4.548	0.04	0.107	IDI −0.327				9.144	<0.001
	Model 2	8.726	<0.001	0.499	IDI −0.134	XWXD 0.245	pct_fast 0.414	lsub_dmm 0.276		

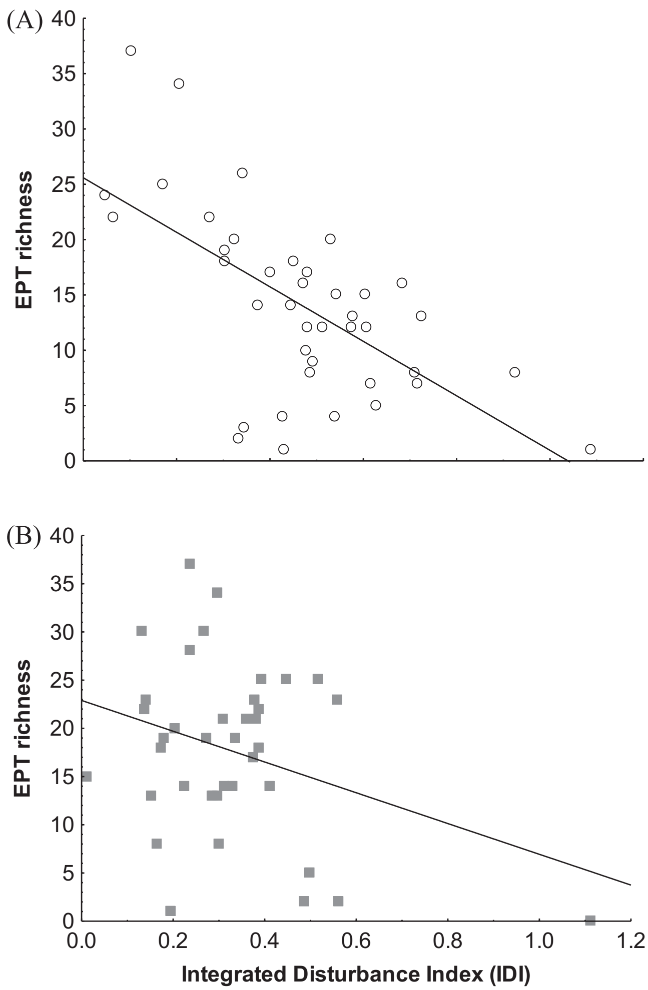

Fig. 8. Linear regressions between the integrated disturbance index (IDI) values and the EPT richness of the sites of (A) the Upper Araguari basin, represented by open circles (○), and (B) the Upper São Francisco basin, represented by filled boxes (■).

The integrated disturbance index (IDI) proved to be a useful and accurate univariate descriptor of the totality of disturbances measured at different spatial scales. It explained the variability in EPT richness better than separate local and catchment indices, and almost as well as when those two indices were separately included in multiple regression. The existence of a single index to summarize the overall ecological condition, although never perfect, is a quick and practical way to describe the condition of individual sites and the relative condition of a site in comparison to others (Bryce et al., 1999; Wang et al., 2008). This is necessary to set disturbance thresholds and to present to society and stakeholders an objective and simple measurement of site conditions (Hughes and Peck, 2008). The range and distribution of IDI values across a representative pool of sites can indicate the strength of the disturbance gradient in a region. The greater the range and evenness of the distribution of sites across that range, the greater the strength of the disturbance gradient (shown in the ascending ordinations of Fig. 7), and the greater the expected differences in ecological condition between the least- and the most-disturbed sites.

4.3. The role of natural habitat variation

The importance of natural stream habitat variation has been long recognized in stream ecology (Karr and Dudley, 1981; Allan and Castillo, 2007). Metrics related to hydromorphology (percentage of fast flows, mean wetted width × mean thalweg depth, bankfull width/depth, log of geometric mean substrate diameter), which were not related to human disturbances in these basins, helped explain EPT richness variability, apart from the effects that could be attributed solely to human influences. Those factors are commonly reported as important for structuring stream macroinvertebrate assemblages (Schmera and Erős, 2004; Brooks et al., 2005; LeCraw and Mackereth, 2010). Consistent with our second hypothesis, the relative and absolute contribution of the natural habitat was much more pronounced in the Upper São Francisco basin, which had a weaker disturbance gradient.

One conclusion emerging from our results is that if the anthropogenic disturbance gradient is not strong, the deleterious effect of human activities on assemblage richness will be mostly eclipsed by variation associated with stream habitat natural variability. In other words, the disturbance "signal" will be buried by habitat variation "noise" (Parsons and Norris, 1996; Gerth and Herlihy, 2006). As can be observed in the Upper São Francisco Basin (Fig. 8B), sites that were slightly more perturbed frequently had higher EPT richness than others that were slightly less perturbed. Many of these divergences in relation to what would be expected from the disturbance-only model were probably driven by differences in stream hydromorphology. In the Upper Araguari basin, which had a stronger disturbance gradient, those situations also occurred, but less frequently (Fig. 8A). A second conclusion is that the effort to control broad-scale drivers of biological assemblages through use of ecoregions and stream typologies does not eliminate the necessity to account for local habitat variability when comparing sites (Hughes et al., 1986; Waite et al., 2000; Pinto et al., 2009). Although we aimed to standardize the stream sizes, a size metric (mean width × mean depth) still explained significant differences in EPT richness. In addition, even neighboring sites may have highly dissimilar habitats and biological assemblages (Downes et al., 2000; Finn and Poff, 2005; Ligeiro et al., 2010), so that ecoregion standardization also is not enough.

The amount of EPT richness variability explained was similar in both basins (around 50%). This value can be considered high, given: (1) the intrinsic complexity and unpredictability of stream ecosystems and the difficulty of obtaining good models of them (Harris and Heathwaite, 2011), (2) the sources of variation not accounted for in this study, such as legacy effects (Allan, 2004) and conditions at upstream reaches (Kail and Hering, 2009) or at neighboring sites (Sanderson et al., 2005), and (3) the intrinsic unpredictability ("noise") related to seasonal and sampling variability (Kaufmann et al., 1999; Kaufmann and Hughes, 2006). We emphasize that the stream habitat contribution to richness explanation was analyzed in a very conservative way. To reliably determine the degree that natural habitat variability can add explanation at varying levels of disturbance strength, we dealt only with the habitat metrics not significantly correlated with any of the disturbance measurements we had available. In this regard, we even discarded metrics significantly but weakly correlated to disturbance (e.g., $r < 0.4$). Thus, we believe that habitat variability has a greater role in structuring macroinvertebrate assemblages than shown in our results, because those rejected habitat metrics that were related to human disturbances were also driven by natural variability to some degree (King et al., 2005).

4.4. Importance of the construction of a disturbance gradient

The explicit, quantitative determination of a disturbance gradient is more advantageous than a set of disturbance categories because distinct separations in ecological conditions should be rare in any group of sites (Whittier et al., 2007b; Herlihy et al., 2008). This is true for all sites we call reference, least-disturbed, most-disturbed, or impaired. Depending on the intensity and extent of human influences in the landscape, sometimes it is necessary to relax the stringency of the acceptance thresholds in order to find least-disturbed conditions (Stoddard et al., 2006; Whittier et al., 2007b; Herlihy et al., 2008). So it is important to recognize the relativity of terms like "least", or "most", when describing ecological condition (Stoddard et al., 2006). Absolute, "boxed" designations, although comfortable and operationally easier to handle, can lead to misunderstandings or erroneous comparisons among studies simply because the true ecological conditions of the sites along the disturbance gradient continuum were not explicitly stated.

Often the designations of reference and most-disturbed sites are made prior to sampling (Bailey et al., 2004). GIS data and techniques have been widely applied when screening for reference sites (Collier et al., 2007; Yates and Bailey, 2010) and field reconnaissance is strongly recommended (Hughes et al., 1986; Yates and Bailey, 2010). Yet, even in those cases we encourage researchers to quantitatively re-assess the disturbance gradient after field sampling to check the validity of any previous classifications and the exact quantitative difference in the conditions between the "reference" and "test" sites.

4.5. The benefits, scope and further possibilities of the proposed methodology

The disturbance plane conceived in this work, visually describing the intensity of human disturbances at both local and catchment scales, established an easy and intuitive way to describe the total amount of pressure at sites. The disturbance plane facilitates comparisons of site conditions in a more straightforward and specific manner, quantitatively positioning each site along a disturbance continuum, rather than assigning labels to the sites. When necessary, labels such as "minimally-", "least-" and "most-disturbed" can be assigned to sites based on quantitative data versus subjective decisions. Objective criteria and quantitative approaches to select reference sites have been proven more efficient for selecting the "best" sites (Whittier et al., 2007b), and the same may be true for selecting the "worst" ones.

Because only direct observations of human activities were used to describe anthropogenic pressure, further characterization of the chemical and physical habitat of the least- and most-disturbed sites can be made without incurring any conceptual circularity. As addressed before, metrics like dissolved nutrient concentrations, riparian cover and sediment sizes, although commonly associated with human modifications, are also subject to natural variability (King et al., 2005; Miserendino et al., 2011). For example, in this study no land use measurement or local modification was correlated with nutrient concentrations (total phosphorous and total nitrogen). Low nutrient concentrations are common in Cerrado streams because of naturally oligotrophic soils (Wantzen, 2003). In the Upper São Francisco, no evidence of disturbance was correlated with substrate sizes and riparian vegetation cover (Table 1). So, in accord with Bailey et al. (2004), natural patterns, not researchers' opinions, should be used to characterize reference condition attributes.

The proposed methodology was well suited for describing the disturbance gradient of the 40 sites we studied in each basin. When necessary, sites from different regions can be incorporated in the same disturbance plane (as shown in Fig. 6). We believe that this methodology is also applicable to larger datasets, although further research is needed to confirm this assumption and to compare outputs generated through other approaches.

Depending on researcher preferences and the amount of data available, local and/or catchment disturbance indices can be calculated in different ways, perhaps using different disturbance measurements. For instance, other commonly used metrics to characterize human pressure include human population density, livestock density, number of dwellings and road density (Wang et al., 2008; Brown et al., 2009). If one desires further changes in this methodology, more disturbance axes can be added to the model, perhaps representing factors considered key stressors in particular studies (e.g., dams and toxic substances). This will generate *n*-dimensional disturbance polygons, rather than the bi-dimensional disturbance plane presented in this work. Although such refinements erode the simplicity and visual appeal of the model, they could improve the accuracy of the integrated disturbance quantifications of the sites (Danz et al., 2007).

In our study, the IDI was a reliable univariate measurement of site disturbance status. The IDI is also a good tool for describing the disturbance gradient strength in a pool of sites, via the range and distribution of its values. So, rather than a standardized and rigid methodology, we offer a flexible and adaptive framework for characterizing and quantifying disturbance in many situations.

5. Conclusions

We showed through our results that a reliable and comprehensive characterization of human pressures on streams relies on the use of different tools and should integrate data from different spatial scales. In our study, local and catchment disturbances were not correlated, and both independently affected site EPT assemblages. The proposed methodology quantified the human pressure on sites without resorting to naturally varying habitat metrics. We demonstrated that the strength of the disturbance gradient influenced the degree to which natural habitat variability explained EPT richness variation, a finding that has important implications for biomonitoring studies. Thus, the use of quantitative disturbance gradients is essential for efficient use of ecological indicators and we advise researchers to define quantitatively the disturbance status of their study sites. In this study we presented a framework for doing so.

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References

Allan, J.D., 2004. Landscape and riverscapes: the influence of land use on river ecosystems. Annu. Rev. Ecol. Evol. Syst. 35, 257–284.

Allan, J.D., Castillo, M.M., 2007. Stream Ecology: Structure and Function of Running Waters, 2nd ed. Chapman and Hall, New York, NY.

American Public Health Association (APHA), 1998. In: Clesceri, L.S., Greenberg, A.E., Eaton, A.D. (Eds.), Standard Methods for the Examination of Water and Wastewater. , 20th ed. American Public Health Association, Washington, DC.

Bailey, R.C., Norris, R.H., Reynoldson, T.B., 2004. Bioassessment of Freshwater Ecosystems using the Reference Condition Approach. Kluwer Academic Publishers, Boston.

Baptista, D.F., Buss, D.F., Egler, M., Giovanelli, A., Silveira, M.P., Nessimian, J., 2007. A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil. Hydrobiologia 575, 83–94.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid Bioassessment Protocols for use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, 2nd ed. EPA 841-B-99-002, Office of Water, US Environmental Protection Agency, Washington, DC, p. 339.

Bedford, B., Preston, E., 1988. Developing the scientific basis for assessing cumulative effects of wetland loss and degradation on landscape functions: status, perspectives and prospects. Environ. Manage. 12, 751–771.

Bonada, N., Prat, N., Resh, V.H., Statzner, B., 2006. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annu. Rev. Entomol. 51, 495–523.

Boulton, A.J., 1999. An overview of river health assessment: philosophies, practice, problems and prognosis. Freshw. Biol. 41, 469–479.

Boyero, L., Ramirez, A., Dudgeon, D., Pearson, R.G., 2009. Are tropical streams really different? J. N. Am. Benthol. Soc. 28, 397–403.

Brooks, A.J., Haeusler, T., Reinfelds, I., Williams, S., 2005. Hydraulic microhabitats and the distribution of macroinvertebrate assemblages in riffles. Freshw. Biol. 50, 331–344.

Brown, L.R., Cuffney, T.F., Coles, J.F., Fitzpatrick, F., McMahon, G., Steuer, J., Bell, A.H., May, J.T., 2009. Urban streams across the USA: lessons learned from studies in 9 metropolitan areas. J. N. Am. Benthol. Soc. 28, 1051–1069.

Bryce, S.A., Larsen, D.P., Hughes, R.M., Kaufmann, P.R., 1999. Assessing relative risks to aquatic ecosystems: a Mid-Appalachian case study. J. Am. Water Res. Ass. 35, 23–36.

Camara, G., Cartaxo, R., Souza, M., Freitas, U.M., Garrido, J., 1996. SPRING: integrating remote sensing and GIS by object-oriented data modelling. J. Comput. Graph. 20, 395–403.

Clarke, R.T., Wright, J.F., Furse, M.T., 2003. RIVPACS models for predicting the expected macroinvertebrate fauna and assessing the ecological quality of rivers. Ecol. Model. 160, 219–233.

Collier, K.J., Haigh, A., Kelly, J., 2007. Coupling GIS and multivariate approaches to reference site selection for wadeable stream monitoring. Environ. Monit. Assess. 127, 29–45.

Danz, N.P., Niemi, G.J., Regal, R.R., Hollenhorst, T., Johnson, L.B., Hanowski, J., Axler, R., Ciborowski, J.J.H., Hrabik, T., Brady, V.J., Kelly, J.R., Brazner, J.C., Howe, R.W., Host, G.E., 2007. Integrated measures of anthropogenic stress in the U.S. Great Lakes basin. Environ. Manage. 39, 631–647.

Death, R.G., Joy, M.K., 2004. Invertebrate community structure in streams of the Manawatu–Wanganui region, New Zealand: the roles of catchment versus reach scale influences. Freshw. Biol. 49, 982–997.

Dovciak, A., Perry, J.A., 2002. In search of effective scales for stream management: does agroecoregion, watershed, or their intersection best explain the variance in stream macroinvertebrate communities? Environ. Manage. 30, 365–377.

Downes, B.J., Hindell, J.S., Bond, N.R., 2000. What's in a site? Variation in lotic macroinvertebrate density and diversity in a spatially replicated experiment. Aust. J. Ecol. 25, 128–139.

Feld, C.K., Hering, D., 2007. Community structure or function: effects of environmental stress on benthic macroinvertebrates at different spatial scales. Freshw. Biol. 52, 1380–1399.

Fernández, H.R., Domínguez, E., 2001. Guia para la determinación de los artrópodos bentônicos sudamericanos. Universidad Nacional de Tucumán, Tucumán.

Finn, D.S., Poff, N.L., 2005. Variability and convergence in benthic communities along the longitudinal gradients of four physically similar Rocky Mountain streams. Freshw. Biol. 50, 243–261.

Freckleton, R.P., 2002. On the misuse of residuals in ecology: regression of residuals vs. multiple regression. J. Anim. Ecol. 71, 542–545.

Gerth, W.J., Herlihy, A.T., 2006. Effect of sampling different habitat types in regional macroinvertebrate bioassessment surveys. J. N. Am. Benthol. Soc. 25, 501–512.

Gucker, B., Boechat, I.G., Giani, A., 2009. Impacts of agricultural land use on ecosystem structure and whole-stream metabolism of tropical Cerrado streams. Freshw. Biol. 54, 2069–2085.

Gustafsson, L., Ahlén, I., 1996. Geography of Plants and Animals. Almqvist and Wiksell International, Stockholm.

Gerritsen, J., Barbour, M.T., King, K., 2000. Apples, oranges, and ecoregions: on determining pattern in aquatic assemblages. J. N. Am. Benthol. Soc. 19, 487–496.

Harrell Jr., F.E., 2001. Regression Modeling Strategies, with Applications to Linear Models, Logistic Regression, and Survival Analysis. Springer-Verlag, New York.

Harris, G.P., Heathwaite, A.L., 2011. Why is achieving good ecological outcomes in rivers so difficult? Freshw. Biol, http://dx.doi.org/10.1111/j.1365-2427.2011.02640.x.

Hawkins, C.P., Olson, J.R., Hill, R.A., 2010. The reference condition: predicting benchmarks for ecological and water-quality assessments. J. N. Am. Benthol. Soc. 29, 312–343.

Hering, D., Feld, C.K., Moog, O., Ofenbock, T., 2006. Cook book for the development of a multimetric index for biological condition of aquatic ecosystems: experiences from the European AQEM and STAR projects and related initiatives. Hydrobiologia 566, 311–342.

Herlihy, A.T., Paulsen, S.G., Van Sickle, J., Stoddard, J.L., Hawkins, C.P., Yuan, L.L., 2008. Striving for consistency in a national assessment: the challenges of applying a reference-condition approach at a continental scale. J. N. Am. Benthol. Soc. 27, 860–877.

Hughes, R.M., 1985. Use of watershed characteristics to select control streams for estimating effects of metal mining wastes on extensively disturbed streams. Environ. Manage. 9, 253–262.

Hughes, R.M., Larsen, D.P., Omernik, J.M., 1986. Regional reference sites: a method for assessing stream potentials. Environ. Manage. 10, 629–635.

Hughes, R.M., Whittier, T.R., Rohm, C.M., Larsen, D.P., 1990. A regional framework for establishing recovery criteria. Environ. Manage. 14, 673–683.

Hughes, R.M., 1995. Defining acceptable biological status by comparing with reference conditions. In: Davis, W., Simon, T. (Eds.), Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL.

Hughes, R.M., Peck, D.V., 2008. Acquiring data for large aquatic resource surveys: the art of compromise among science, logistics, and reality. J. N. Am. Benthol. Soc. 27, 837–859.

Hughes, R.M., Herlihy, A.T., Kaufmann, P.R., 2010. An evaluation of qualitative indexes of physical habitat applied to agricultural streams in ten U.S. states. J. Am. Water Res. Assoc. 46, 792–806.

Hughes, R.M., Kaufmann, P.R., Weber, M.H., 2011. National and regional comparisons between Strahler order and stream size. J. N. Am. Benthol. Soc. 30, 103–121.

Hynes, H.B.N., 1975. The stream and its valley. Verh. Int. Verein. Theor. Ang. Limnol. 19, 1–15.

Kail, J., Hering, D., 2009. The influence of adjacent stream reaches on the local ecological status of Central European mountain streams. River Res. Appl. 25, 537–550.

Kail, J., Arleb, J., Jähnig, S.C., 2012. Limiting factors and thresholds for macroinvertebrate assemblages in European rivers: empirical evidence from three datasets on water quality, catchment urbanization, and river restoration. Ecol. Indic. 18, 63–72.

Karr, J.R., Dudley, D.R., 1981. Ecological perspective on water quality goals. Environ. Manage. 5, 55–68.

Karr, J.R., 1991. Biological integrity: a long-neglected aspect of water resource management. Ecol. Appl. 1, 66–84.

Karr, J.R., 1999. Defining and measuring river health. Freshw. Biol. 41, 221–234.

Karr, J.R., Chu, E.W., 1999. Restoring Life in Running Waters: Better Biological Monitoring. Island Press, Washington, DC.

Kaufmann, P.R., Levine, P., Robison, E.G., Seeliger, C., Peck, D.V., 1999. Quantifying Physical Habitat in Wadeable Streams. EPA/620/R-99/003. U.S. Environmental Protection Agency, Washington, DC.

Kaufmann, P.R., Hughes, R.M., 2006. Geomorphic and anthropogenic influences on fish and amphibians in Pacific Northwest coastal streams. In: Hughes, R.M., Wang, L., Seelbach, P.W. (Eds.), Landscape Influences on Stream Habitat and Biological Assemblages, Symposium 48. American Fisheries Society, pp. 429–455.

King, R.S., Baker, M.E., Whigham, D.F., Weller, D.E., Jordan, T.E., Kazyak, P.F., Hurd, M.K., 2005. Spatial considerations for linking watershed land cover to ecological indicators in streams. Ecol. Appl. 15, 137–152.

Klemm, D.J., Blocksom, K.A., Fulk, F.A., Herlihy, A.T., Hughes, R.M., Kaufmann, P.R., Peck, D.V., Stoddard, J.L., Thoeny, W.T., 2003. Development and evaluation of a macroinvertebrate biotic integrity index (MBII) for regionally assessing Mid-Atlantic Highlands streams. Environ. Manage. 31, 656–669.

LeCraw, R.M., Mackereth, R., 2010. Sources of small-scale variation in headwater stream invertebrate communities. Freshw. Biol. 55, 1219–1233.

Ligeiro, R., Melo, A.S., Callisto, M., 2010. Spatial scale and the diversity of macroinvertebrates in a neotropical catchment. Freshw. Biol. 55, 424–435.

Maddock, I., 1999. The importance of physical habitat assessment for evaluating river health. Freshw. Biol. 41, 373–391.

Marchant, R., Norris, R.H., Milligan, A., 2006. Evaluation and application of methods for biological assessment of streams: summary of papers. Hydrobiologia 572, 1–7.

Mebane, C.A., Maret, T.R., Hughes, R.M., 2003. An index of biological integrity (IBI) for Pacific Northwest rivers. Trans. Am. Fish. Soc. 132, 239–261.

Miserendino, M.L., Casaux, R., Archangelsky, M., Di Prinzio, C.Y., Brand, C., Kutschker, A.M., 2011. Assessing land-use effects on water quality, in-stream habitat, riparian ecosystems and biodiversity in Patagonian northwest streams. Sci. Total Environ. 409, 612–624.

Moreno, J.L., Navarro, C., Las Heras, J.D., 2006. Abiotic ecotypes in south-central Spanish rivers: reference conditions and pollution. Environ. Pollut. 143, 388–396.

Moreno, P., França, J.S., Ferreira, W.R., Paz, A.D., Monteiro, I., Callisto, M., 2009. Use of the BEAST model for biomonitoring water quality in a neotropical basin. Hydrobiologia 630, 231–242.

Moya, N., Hughes, R.M., Dominguez, E., Gibon, F.M., Goita, E., Oberdorff, T., 2011. Macroinvertebrate-based multimetric predictive models for measuring the biotic condition of Bolivian streams. Ecol. Indic. 11, 840–847.

Mugnai, R., Nessimian, J.L., Baptista, D.F., 2010. Manual de Identificação de Macroinvertebrados Aquáticos do Estado do Rio de Janeiro. Technical Books, Rio de Janeiro.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Nijboer, R.C., Johnson, R.K., Verdonschot, P.F.M., Sommerhäuser, M., Buffagni, A., 2004. Establishing reference conditions for European streams. Hydrobiologia 516, 93–107.

Norris, R.H., Thoms, M.C., 1999. What is river health? Freshw. Biol. 41, 197–211.

Oberdorff, T., Pont, D., Hugueny, B., Porcher, J.P., 2002. Development and validation of a fish-based index (FBI) for the assessment of "river health" in France. Freshw. Biol. 47, 1720–1735.

Oliveira, R.B.S., Mugnai, R., Castro, C.M., Baptista, D.F., Hughes, R.M., 2011. Towards a rapid bioassessment protocol for wadeable streams in Brazil: development of a multimetric index based on benthic macroinvertebrates. Ecol. Indic. 11, 1584–1593.

Olsen, A.R., Peck, D.V., 2008. Survey design and extent estimates for the Wadeable Streams Assessment. J. N. Am. Benthol. Soc. 27, 822–836.

Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'Amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P., Kassem, K.R., 2001. Terrestrial ecoregions of the world: a new map of life on Earth. Bioscience 51, 933–938.

Omernik, J.M., 1995. Ecoregions: a spatial framework for environmental management. In: Davis, W., Simon, T.P. (Eds.), Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishing, Boca Raton, FL.

Parsons, M., Norris, R.H., 1996. The effect of habitat specific sampling on biological assessment of water quality using a predictive model. Freshw. Biol. 36, 419–434.

Paulsen, S.G., Mayio, A., Peck, D.V., Stoddard, J.L., Tarquinio, E., Holdsworth, S.M., Van Sickle, J., Yuan, L.L., Hawkins, C.P., Herlihy, A.T., Kaufmann, P.R., Barbour, M.T., Larsen, D.P., Olsen, A.R., 2008. Condition of stream ecosystems in the US: an overview of the first national assessment. J. N. Am. Benthol. Soc. 27, 812–821.

Peck, D.V., Herlihy, A.T., Hill, B.H., Hughes, R.M., Kaufmann, P.R., Klemm, D.J., Lazorchak, J.M., McCormick, F.H., Peterson, S.A., Ringold, P.L., Magee, T., Cappaert, M.R., 2006. Environmental Monitoring and Assessment Program – Surface Waters Western Pilot Study: Field Operations Manual for Wadeable Streams. EPA 600/R-06/003. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

Pérez, G.R., 1988. Guía para el estudio de los macroinvertebrados acuáticos del Departamento de Antioquia. Editorial Presencia Ltda., Bogotá, Colombia.

Pinto, B.C.T., Araújo, F.G., Rodriguez, V.D., Hughes, R.M., 2009. Local and ecoregion effects on fish assemblage structure in tributaries of the Rio Paraíba do Sul, Brazil. Freshw. Biol. 54, 2600–2615.

Pont, D., Hugueny, B., Beier, U., Goffaux, D., Melcher, A., Noble, R., Rogers, C., Roset, N., Schmutz, S., 2006. Assessing river biotic condition at the continental scale: a European approach using functional metrics and fish assemblages. J. Appl. Ecol. 43, 70–80.

Pont, D., Hughes, R.M., Whittier, T.R., Schmutz, S., 2009. A predictive index of biotic integrity model for aquatic-vertebrate assemblages of western U.S. streams. Trans. Am. Fish. Soc. 138, 292–305.

Poole, G.C., 2002. Fluvial landscape ecology: addressing uniqueness within the river discontinuum. Freshw. Biol. 47, 641–660.

Rawer-Jost, C., Zenker, A., Böhmer, J., 2004. Reference conditions of German stream types analysed and revised with macroinvertebrate fauna. Limnologica 34, 390–397.

Reynoldson, T.B., Bailey, R.C., Day, K.E., Norris, R.H., 1995. Biological guidelines for freshwater sediment based on BEnthic Assessment of SedimenT (the BEAST) using a multivariate approach for predicting biological state. Aust. J. Ecol. 20, 198–219.

Reynoldson, T.B., Norris, R.H., Resh, V.H., Day, K.E., Rosenberg, D.M., 1997. The reference condition: a comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. J. N. Am. Benthol. Soc. 16, 833–852.

Rosenberg, D.M., Resh, V.H., 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, New York.

Sánchez-Montoya, M.M., Puntí, T., Suárez, M.L., Vidal-Abarca, M.R., Rieradevall, M., Poquet, J.M., Zamora-Munõz, C., Robles, S., Álvarez, M., Alba-Tercedor, J., Toro, M., Pujante, A., Munné, A., Prat, N., 2007. Concordance between ecotypes and macroinvertebrate assemblages in Mediterranean streams. Freshw. Biol. 52, 2240–2255.

Sánchez-Montoya, M.M., Vidal-Abarca, M.R., Puntí, T., Poquet, J.M., Prat, N., Rieradevall, M., Alba-Tercedor, J., Zamora-Munõz, C., Toro, M., Robles, S., Álvarez, M., Suárez, M.L., 2009. Defining criteria to select reference sites in Mediterranean streams. Hydrobiologia 619, 39–54.

Sanderson, R.A., Eyre, M.D., Rushton, S.P., 2005. The influence of stream invertebrate composition at neighbouring sites on local assemblage composition. Freshw. Biol. 50, 221–231.

Schmera, D., Erős, T., 2004. Effect of riverbed morphology, stream order and season on the structural and functional attributes of caddisfly assemblages. Ann. Limnol. Int. J. Limnol. 40, 193–200.

Sponseller, R.A., Benfield, E.F., Valett, H.M., 2001. Relationships between land use, spatial scale and stream macroinvertebrate communities. Freshw. Biol. 46, 1409–1424.

Stevens, D.L., Olsen, A.R., 2003. Variance estimation for spatially balanced samples of environmental resources. Environmetrics 14, 593–610.

Stoddard, J.L., Peck, D.V., Olsen, A.R., Paulsen, S.G., Van Sickle, J., Herlihy, A.T., Kaufmann, P.R., Hughes, R.M., Whittier, T.R., Lomnicky, G., Larsen, D.P., Peterson, S.A., Ringold, P.L., 2005. An Ecological Assessment of Western Streams and Rivers. U.S. Environmental Protection Agency, Oregon State University, and Dynamic Corporation, Corvallis, OR.

Stoddard, J., Larsen, D.P., Hawkins, C.P., Johnson, R.K., Norris, R.H., 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecol. Appl. 16, 1267–1276.

Stoddard, J.L., Herlihy, A.T., Peck, D.V., Hughes, R.M., Whittier, T.R., Tarquinio, E., 2008. A process for creating multi-metric indices for large-scale aquatic surveys. J. N. Am. Benthol. Soc. 27, 878–891.

Strahler, A.N., 1957. Quantitative analysis of watershed geomorphology. Trans. Am. Geophys. Union 38, 913–920.

Suriano, M.T., Fonseca-Gessner, A.A., Roque, F.O., Froehlich, C.G., 2011. Choice of macroinvertebrate metrics to evaluate stream conditions in Atlantic forest, Brazil. Environ. Monit. Assoc. 175, 87–101.

Tabachnick, B.G., Fidell, L.S., 2007. Using Multivariate Statistics, 5th ed. Pearson/Allyn & Bacon, Boston.

Tejerina-Garro, F.L., de Mérona, B., Oberdorff, T., Hugueny, B., 2006. A fish-based index of large river quality for French Guiana (South America): method and preliminary results. Aquat. Living Resour. 19, 31–46.

Trautwein, C., Schinegger, R., Schmutz, S., 2011. Cumulative effects of land use on fish metrics in different types of running waters in Austria. Aquat. Sci. 74, 329–341.

USGS (United States Geological Survey), 2005. Shuttle Radar Topography Mission – SRTM., http://www.srtm.usgs.gov

Van Sickle, J., Baker, J., Herlihy, A., Bayley, P., Gregory, S., Haggerty, P., Ashkenas, L., Li, J., 2004. Projecting the biological condition of streams under alternative scenarios of human land use. Ecol. Appl. 14, 368–380.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Cushing, C.E., 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37, 130–137.

Waite, I.R., Herlihy, A.T., Larsen, D.P., Klemm, D.J., 2000. Comparing strengths of geographic and nongeographic classifications of stream benthic macroinvertebrates in the Mid-Atlantic. J. N. Am. Benthol. Soc. 19, 429–441.

Wang, L., Brenden, T., Seelbach, P., Cooper, A., Allan, D., Clark Jr., R., Wiley, M., 2008. Landscape based identification of human disturbance gradients and reference conditions for Michigan streams. Environ. Monit. Assess. 141, 1–17.

Wantzen, K.M., 2003. Cerrado streams – characteristics of a threatened freshwater ecosystem type on the tertiary shields of South America. Amazoniana 17, 485–502.

Wantzen, K.M., Siqueira, A., Nunes Da Cunha, C., Sá, M.F.P., 2006. Stream–valley systems of the Brazilian cerrado: impact assessment and conservation scheme. Aquat. Cons.: Mar. Freshw. Ecosyst. 16, 713–732.

Whittier, T.R., Hughes, R.M., Stoddard, J.L., Lomnicky, G.A., Peck, D.V., Herlihy, A.T., 2007a. A structured approach for developing indices of biotic integrity: three examples from western USA streams and rivers. Trans. Am. Fish. Soc. 136, 718–735.

Whittier, T.R., Stoddard, J.L., Larsen, D.P., Herlihy, A.T., 2007b. Selecting reference sites for stream biological assessments: best professional judgment or objective criteria. J. N. Am. Benthol. Soc. 26, 349–360.

Wiens, J.A., 2002. Riverine landscapes: taking landscape ecology into the water. Freshw. Biol. 47, 501–515.

Yates, A.G., Bailey, R.C., 2010. Selecting objectively defined reference sites for stream bioassessment programs. Environ. Monit. Assess. 170, 129–140.