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# Ecoregions and stream types help us understand ecological variability in Neotropical reference streams

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**Abstract.** Stream site classification is a fundamental step in defining reference conditions for freshwater bioassessments globally. Landscape regionalisation and stream typology approaches have both been used to determine classes that reduce inherent environmental variation and to show classes with ecological meaning. We evaluated the applicability of ecoregion and stream typology approaches in a river basin located between the Neotropical Savanna and the Atlantic Forest biomes in south-eastern Brazil. We delimited two ecoregions and assessed whether stream types within each ecoregion would further reduce environmental and macroinvertebrate assemblage variability. In addition, we determined indicator taxa for each of the reference condition classes. Our results confirm the importance of defining ecoregions *a priori* and for using a nested *a posteriori* stream typology approach for further explaining macroinvertebrate assemblage variation. Geology and natural vegetation physiognomy were the key ecoregion factors likely to influence macroinvertebrate assemblages, and stream dimension attributes best delimited meaningful stream types. Although stream classification is a very important step in any monitoring, management or restoration program, it has been widely neglected in many tropical regions. In this study we demonstrated how it can be conducted to determine macroinvertebrate assemblage potentials even with a relatively limited number of sites.

Additional keywords: benthic macroinvertebrates, Espinhaço Range, instream physical habitats, rupestrian grassland, tropical rainforest.

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# Introduction

Monitoring programs use minimally or least-disturbed reference sites to characterise the potential ecological status of freshwater ecosystems and to measure natural variability (Hughes *et al.* 1986). Subsequently, empirical models are developed based on natural environmental characteristics to predict the biota expected at test sites (Hawkins *et al.* 2010). Stream site classification is a fundamental step in defining reference condition (Hughes *et al.* 1986). Classes should cover most biological variability included in a group or in an area with relatively homogeneous ecosystem characteristics (Gerritsen *et al.* 2000), thereby reducing their inherent variations and making anthropogenic disturbance identification more efficient and accurate (Hawkins *et al.* 2010). Different approaches have been used to develop classification systems suitable for biological predictions in freshwater systems (e.g. Hughes *et al.* 1986; Snelder and Biggs 2002; Clarke *et al.* 2003). In a proper classification it is expected that the biological variability within the same class will be lower than that observed in the sum of two or more classes (Hughes *et al.* 1986). Because biological assemblages respond to environmental factors from large to small spatial scales (Frissell *et al.* 1986), hierarchical landscape regionalisation approaches (e.g. ecoregions) are commonly used by ecologists and environmental managers to classify stream sites. In the ecoregion approach, classes are delimited by overlaying natural landscape features (e.g. geomorphology, lithology, climate, land use) to identify large-scale ecosystem patterns (Omernik and



Fig. 1. Study area and reference sites occurring in two ecoregions. Stream types are nested in ecoregions.

Griffith 2014). Those ecoregions are relatively homogeneous, discrete and geographically dependent (Hughes *et al.* 1986). Therefore, inferences can be made about the expected inherent or natural biological conditions in each ecoregion and then used in bioassessments (Hughes *et al.* 1986).

However, classifications that consider only landscape features have shown weak strength to explain the observed total biological variation (Hawkins *et al.* 2000). To further refine ecoregion classification, several stream typology classifications have been proposed. In a general sense, stream typologies are identified through the combination of fluvial features and processes along spatial hierarchical frameworks (Turak and Koop 2008). In the present study, we considered an integrative approach involving geographic information system (GIS) and field measures (water quality, physical habitat structure) to identify stream types presumed to be ecologically relevant.

Although multiple classification methods have been discussed and tested in temperate ecosystems (e.g. North America, Europe, Australia), few studies in the tropics have used landscape regionalisation and stream typology approaches to delimit reference conditions and develop predictive models (e.g. Villamarín *et al.* 2013; Martins *et al.* 2018). In Brazil, the National Water Policy (Federal Law 9433/1997) considers only river basins as management units; however, studies indicate disadvantages of this practice (e.g. Omernik *et al.* 2017). Because tropical freshwater is recognised for high environmental and biological heterogeneity, it is also likely to exhibit heterogeneity within large river basins. Thus, a rigorous ecological approach to classify least-disturbed stream sites is necessary to establish reference standards and ecological targets in the tropics (Feio *et al.* 2015; Martins *et al.* 2018).

In this study, a river basin incorporating portions of two global biodiversity hot spots, namely the Neotropical Savanna and the Atlantic Forest biomes, provided an opportunity to evaluate the applicability of ecoregion and stream typology approaches. Our goal was to better understand how environmental variation from large to local scales determines macroinvertebrate assemblages under minimally disturbed conditions, thereby demonstrating the biological relevance of tropical stream classification. Because we expected that macroinvertebrate assemblages would differ between ecoregions defined *a priori*, we assessed whether identifying stream types *a posteriori* within each ecoregion would further reduce biological and environmental variability.

# Materials and methods

# Study area

The Upper Rio das Velhas Basin and the Espinhaço Range (UNESCO Biosphere Reserve) occur in south-eastern Brazil (Fig. 1). The region has rugged local relief (~1000–1500 m) and a dry winter highland climate (Cwb) according to Köppen's classification. The dry winters (June–August) have average temperatures ranging between 13.5 and 15.5°C, whereas the rainy summers (November–January) have temperatures ranging between 20 and 21°C. During the rainy season, mean precipitation varies between 1300 and 1900 mm. Our study area was inside the Andorinhas Environmental Protected Area

 Table 1. Mean (±s.d.) values of natural landscape attributes per ecoregion

Landscape attributes	Ecoregion units			
	Caraça Grassland	Velhas Rainforest		
Altitude (m)	$1131.82 \pm 120.07$	$1119.47 \pm 75.10$		
Drainage area (ha)	$26.40\pm21.15$	$112.15 \pm 145.62$		
Strahler order	1	1–3		
Vegetation cover (%)				
Rupestrian grassland	$72.94\pm20.71$	$0.00\pm0.00$		
Tropical rainforest	$24.50\pm20.86$	$96.48 \pm 6.46$		
Lithology (%)				
Schist	$45.40\pm37.06$	$75.07 \pm 32.88$		
Quartzite	$51.41\pm36.26$	$19.87\pm31.05$		

 $(\sim 18700 \text{ ha})$ , created in 1989 to protect the springs and head-waters of Rio das Velhas Basin, which are in minimally disturbed condition.

This study was approved by the Instituto Estadual de Florestas (IEF number 053/13) and by the Ministério do Meio Ambiente and Instituto Chico Mendes de Conservação da Biodiversidade (licence number 43630-2).

## Delimitation of ecoregions

Following an approach widely used by the US Environmental Protection Agency (EPA; Omernik and Griffith 2014), we used map overlays to subjectively analyse landscape patterns and determine ecoregion boundaries. To represent the broad landscape patterns, we combined images from elevation data provided by Shuttle Radar Topographic Mission (~30 m; NASA Jet Propulsion Laboratory 2013), land use and vegetation cover obtained through high-resolution satellite images (Quickbird; 0.6 m; images care of the Environmental Agency of Ouro Preto Municipality) and lithology types (Iron Quadrangle geologic map; 1:50000 scale). We identified two ecoregions: Velhas Rainforest and Caraça Grassland. Vegetation cover and lithology were the main landscape attributes varying substantially across the basin, and governed our *a priori* ecoregion classification (Table 1).

Caraça Grassland streams are dominated by quartzitic lithology and shallow soils, which are associated with rupestrian grassland (rocky outcrops). Despite the low nutritional levels in the soil, rupestrian grasslands have high plant species richness and high rates of endemism (Silveira *et al.* 2016). The vegetative forms are, for the most part, sclerophyllous and herbaceous, whereas others assume arbuscular forms (Silveira *et al.* 2016). Velhas Rainforest streams are dominated by schist lithology, with red and yellow oxisols (Monteiro and Bacellar 2014). The deeper soils support large and medium-sized trees that are semideciduous in the dry season.

#### Reference site selection

Within each of these ecoregions, we selected minimally disturbed reference sites on first- to third-order streams from a hydrographic map (1:50000 scale). First, we identified 52 minimally disturbed candidate sites from maps. Field reconnaissance campaigns were conducted to verify unmapped disturbances and evaluate the safety and accessibility conditions for field sampling. In total, 30 sites were selected, 19 in the Velhas Rainforest ecoregion and 11 in the Caraça Grassland ecoregion (see Table S1, available as Supplementary material to this paper). To further evaluate our site selection, we measured levels of anthropogenic disturbance (bare soil, unpaved roads, railroads, agriculture and artificial lakes) present in the catchment of each site and, through a Pearson correlation matrix, certified that none of those variables was significantly correlated with water quality, physical habitat structure or biological metrics at the 30 study sites.

# Site-scale data collection

Site-scale data were collected from each reference site during the dry season, between August and September 2013. Water temperature (°C), pH, total dissolved solids (TDS; mg L<sup>-1</sup>) and electrical conductivity ( $\mu$ S cm<sup>-1</sup>) were measured *in situ* using a multiprobe (650 MDS, model 6920, YIS Incorporated, Yellow Springs, OH, USA). Total nitrogen (mg L<sup>-1</sup>), orthophosphate ( $\mu$ g L<sup>-1</sup>), nitrite ( $\mu$ g L<sup>-1</sup>), nitrate ( $\mu$ g L<sup>-1</sup>) and dissolved oxygen (DO; mg L<sup>-1</sup>) were determined in the laboratory, following the procedures of the American Public Health Association (1998).

Physical habitat structure was assessed as described in the US EPA protocol (Peck *et al.* 2006). We adapted the protocol slightly to make it more suitable to very small streams (e.g. sample sites located <50 m from their source, channel width  $\sim50$  cm, depth  $\sim10$  cm; Fig. S1). The main changes were the reduced reach length (25 m) and number of stream sections (six) defined in each stream. Changes to the original protocol were based on results from studies using the US EPA protocol in other small Brazilian streams (e.g. Macedo *et al.* 2014), and have been successfully used in previous studies (e.g. Martins *et al.* 2018).

We calculated 177 physical habitat metrics (Kaufmann *et al.* 1999), which were divided into five categories (channel morphology, substrate, flow, canopy and shelter for biota). Physical habitat and water chemistry have been used to guide subsequent analytical procedures (Martins *et al.* 2018). Final analytical metrics were selected in three screening steps.

- We excluded metrics with values of 0 in more than 80% of sites, and those that had coefficients of variation (CVs) (s.d./mean) <0.2.</li>
- (2) We eliminated all but one of any strongly correlated metrics (|r| > 0.70), observed through Pearson correlation matrices made within each category of physical habitat (of the correlated metrics, we selected the one that is most recognised in the literature for explaining macroinvertebrate assemblage patterns; e.g. Macedo *et al.* 2014).
- (3) We then selected metrics with greater dispersion in multivariate space through principal component analyses (PCAs) for each physical habitat category. After these steps, we selected more intuitive or ecologically relevant metrics, obtaining a final set of 10 site-scale variables from which we determined stream types.

# Macroinvertebrate sampling

To measure the structure and composition of the macroinvertebrate assemblages at each stream site, we collected six subsamples of substrate along equidistant transects, using a D-frame kick net (mesh size, 500  $\mu$ m, aperture area 0.09 m<sup>2</sup>; Fig. S1). All individuals were identified to family level (except for Oligochaeta, Hydracarina, Bivalvia, Collembola and Hirudinea) through the use of a stereomicroscope (32×) with the help of taxonomic keys (chiefly Mugnai *et al.* 2010). The organisms were deposited in the Reference Collection of Benthic Macro-invertebrates of the Instituto de Ciências Biológicas (Universidade Federal de Minas Gerais).

# Data analysis

Our sampling unit corresponded to each stream site sampled. Site-scale environmental metrics were standardised (mean = 0, s.d. = 1) and then subjected to PCA to identify patterns of stream types within each ecoregion. Then, we analysed the variability of the structure and composition of the macroinvertebrate assemblages considering both classifications, namely ecoregion (defined *a priori*) and stream type (defined *a posteriori*).

We ran *t*-tests to compare taxonomic richness and abundance  $(\log(x + 1))$  between ecoregions and one-way analysis of variance (ANOVA) to compare between stream types, with  $\alpha = 0.05$  considered significant. We used post hoc Bonferroni criteria for multiple comparisons between stream type classes. The data distribution met the assumptions of homoscedasticity and normality of residuals (Gotelli and Ellison 2004). To test for differences in assemblage composition between ecoregions and between stream types, permutation multivariate analysis of variance (PERMANOVA) was performed using the Bray–Curtis dissimilarity index, considering transformed abundance data (log(x + 1)). We used non-metric multidimensional scaling (nMDS) ordination to plot the variability in macroinvertebrate assemblage composition for each classification.

To identify indicator taxa representing each ecoregion and type, we used indicator species analysis (Dufrene and Legendre 1997). This analysis calculates an indicator value (IndVal) for each taxon in each group based on its relative abundance and relative frequency in the samples. IndVal values range from 0 to 100, with values closest to 100 indicating the best indicator taxon. *P*-values indicate the significance of indication for each

taxon. This method has been used as an effective tool to establish biologically meaningful classification criteria (Dufrene and Legendre 1997). In our case, we subjected raw benthic composition data to our *a priori* ecoregion classification and to the stream types defined *a posteriori*.

All analyses were performed in R software (R Foundation for Statistical Computing, Vienna, Austria) using the VEGAN package (ver. 2.4–6, J. Oksanen, F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs and H. Wagner, see https://CRAN.R-project.org/package=vegan) for multivariate analyses and the INDICSPECIES package (Cáceres and Legendre 2009; see also https://cran.rproject.org/web/packages/indicspecies/indicspecies.pdf) for IndVal analyses.

#### Results

# Environmental characterisation of ecoregions and stream types

The first two PCA axes explained 46.5% of the variability in site-scale environmental data (Table 2). PC1 explained 25.8% of the data variation, determined primarily by the percentage of fast water (riffles, rapids), percentage of gravel, total nitrogen concentration and mean wetted width. The second axis explained 20.7% of the variation, determined primarily by TDS, DO and mean wetted depth.

Through PCA, three stream types were identified visually *a posteriori* within our ecoregion units (Fig. 2). In the Caraça Grassland, we classified streams into only one type (Type 1), whereas two stream types (Types 2 and 3) were identified within the Velhas Rainforest ecoregion (Fig. 1). Stream Type 1 had a greater wetted depth with moderate velocity and lower amounts of TDS and DO. Streams in the Velhas Rainforest were clearly separated on PC1 (Fig. 2). Type 2 streams were characterised by narrow channels, with shallow and slow waters, little coarse substrate and higher concentrations of total nitrogen. Type 3 streams had greater channel wetted width dominated by rapids and riffles, gravelly streambeds and lower total nitrogen concentrations (Fig. 2).

#### Table 2. Selected site-scale environmental variables

Loadings from the first and second principal component analysis axes (PC1 and PC2 respectively) are shown, with the mean  $\pm$  s.d. per stream type. TDS, total dissolved solids; DO, dissolved oxygen; TN, total nitrogen

Site-scale variable	Variable code	PC1 loading	PC2 loading	Caraça Grassland	Velhas F	ainforest
				Type 1	Type 2	Type 3
Mean depth (cm)	XDEPTH	-0.0106	0.4287	$18.47 \pm 8.53$	$10.85 \pm 5.11$	$13.65 \pm 4.14$
Mean width (m)	XWIDTH	-0.3626	0.1512	$1.02\pm0.28$	$0.7\pm0.24$	$1.35\pm0.44$
Embeddedness (channel + bankfull; %)	XEMBED	0.2956	-0.2499	$45.25\pm26.33$	$79.75 \pm 14.70$	$56.33 \pm 11.29$
Gravel (%)	PCT_GC	-0.4579	-0.2398	$4.35\pm8.18$	$1.91 \pm 3.99$	$16.14\pm8.19$
Fast water (%)	PCT_FAST	-0.548	-0.1445	$39.33 \pm 25.91$	$20.89 \pm 21.43$	$69.64\pm16.52$
Riparian canopy (>5 m high) cover	XC	-0.1717	-0.1984	$12.11\pm9.63$	$16.33\pm6.25$	$26.4 \pm 12.12$
Total shelter	XFC_ALL	0.2153	-0.107	$30.46 \pm 17.93$	$38.41 \pm 29.95$	$28.36 \pm 15.26$
TDS (g $L^{-1}$ )	TDS	0.084	-0.5806	$0.00\pm0.00$	$13.41 \pm 9.71$	$12.32\pm8.41$
$DO(mgL^{-1})$	DO	-0.0309	-0.5087	$8.29 \pm 0.99$	$10.16 \pm 1.57$	$10.55\pm2.01$
$TN (mg L^{-1})$	NTotal	0.4324	-0.0767	$0.06\pm0.02$	$0.09\pm0.02$	$0.05\pm0.02$

Ecological variability of reference streams

## Macroinvertebrate assemblage responses

We identified 18 669 benthic macroinvertebrates distributed in 16 orders and 58 families (Table S2). The Chironomidae (Diptera) was the most abundant family in both ecoregions. Considering the Caraça Grassland ecoregion Type 1 sites, Chironomidae represented 46.4% of total abundance, followed by Leptophlebidae (Ephemeroptera; 14.9%) and Elmidae



**Fig. 2.** Principal component analysis (PCA) performed with site-scale natural environmental variables. Confidence interval ellipses = 75%. DO, dissolved oxygen; NTotal, total nitrogen; PCT\_FAST, percentage of fast water; PCT\_GC, percentage gravel; TDS, total dissolved solids; XC, riparian canopy; XDEPTH, mean depth; XEMBED, embeddedness; XFC\_ALL, total shelter; XWIDTH, mean width.

(Coleoptera; 4.1%). In the Velhas Rainforest, Chironomidae represented 31.8% of total abundance, followed by Leptohyphidae (Ephemeroptera; 10.2%) and Leptophlebidae (4.7%). The most abundant families in Type 2 were Chironomidae (36.7%), Leptohyphidae (13.7%) and Elmidae (6.4%), compared with Chironomidae (21.2%), Leptophlebidae (10.8%) and Elmidae (10.1%) in Type 3.

There was no statistical significant difference in taxonomic richness (t = 1.97; P = 0.06) or abundance of individuals (t = 0.97; P = 0.34) between Caraça Grassland and Velhas Rainforest sites (Fig. 3). Conversely, ANOVA between *a posteriori* classes of stream types detected significant differences in both richness ( $F_{2,27} = 7.42$ ; P < 0.001) and abundance ( $F_{2,27} = 5.60$ ; P < 0.001). Small Velhas Rainforest sites (Type 2) had a greater mean richness and abundance than the other two types (Fig. 3). In addition, macroinvertebrate assemblage composition varied significantly between ecoregions (pseudo  $F_{2,27} = 4.75$ ;  $R^2 = 0.15$ ; P < 0.001) and stream types (pseudo  $F_{2,27} = 4.27$ ;  $R^2 = 0.24$ ; P < 0.001; Fig. 4).

The IndVal analyses revealed 11 significant indicator taxa for the ecoregions and 6 taxa for the stream types (Table 3). The Hyallelidae (Amphipoda) occurred only in the Velhas Rainforest, whereas the Psephenidae and Noteridae (Coleoptera) were found exclusively in the Caraça Grassland. No indicator taxon was identified for Type 3 streams.

#### Discussion

Identification of stream types within ecoregions offers a useful refinement to identify classes with ecological relevance (Turak



**Fig. 3.** Box plot showing macroinvertebrate (a, b) richness and  $(c, d) \log(x + 1)$  abundance for ecoregions (a, c) and stream types (b, d). Stream type classes exhibited significant differences after application of Bonferroni criteria, as indicated by different letters. The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range. Circles indicate outliers.



**Fig. 4.** Non-metric multidimensional scaling plot indicating the taxonomic composition of the macroinvertebrate assemblages among stream sites.

Class	Taxa	IndVal	P-value
Caraça Grassland	Helichopsychidae	85.2	0.003
	Psephenidae	73.9	0.001
	Notonectidae	66.3	0.004
	Noteridae	52.2	0.032
Velhas Rainforest	Leptohyphidae	94.5	0.001
	Gomphidae	85.1	0.004
	Calamoceratidae	84.3	0.016
	Bivalvia	84.2	0.005
	Leptoceridae	80.8	0.031
	Planaridae	76.6	0.036
	Hyallelidae	64.9	0.039
Type 1	Psephenidae	73.9	0.004
	Notonectidae	65.1	0.017
Type 2	Odontoceridae	88.8	0.001
	Ptylodactilidae	86.8	0.001
	Lutrochidae	79	0.036
	Hydrophilidae	74.7	0.019
Type 3	_	-	_

 Table 3. Indicator taxa by ecoregion and stream type

 IndVal, indicator value

and Koop 2008). The *a priori* delimitation of ecoregions in the basin reduced the environmental and biological variability of reference sites, as we initially expected and as has been found in other studies (e.g. Mykrä *et al.* 2004; Martins *et al.* 2018). In addition, the *a posteriori* ordination analysis yielded three meaningful stream types nested in the ecoregions, further reducing the ecological variability and providing additional explanation of differences in macroinvertebrate assemblage structure. Furthermore, our study provides indicator taxa of undisturbed tropical freshwater streams, which may be targets for future bioassessments.

# Regional and site-scale variables are tools for stream classification

Geology and vegetation physiognomy were effective criteria for defining ecoregions *a priori*. This is likely because they are among the regional-scale factors that vary most across the Upper Rio das Velhas Basin. These are also key environmental factors, acting directly on water chemistry, physical habitat and aquatic biota (Macedo *et al.* 2014). The quartz geology and steep slopes predominant in the 'Grupo Caraça' geological formation are linked to low TDS, greater water depths and the rocky bottoms observed in this ecoregion. Conversely, the greater TDS observed in the Velhas Rainforest ecoregion is associated with the predominant schist lithology, which is more susceptible to leaching.

Although site-scale variables did not separate Caraça Grassland sites, resulting in a single type (Type 1), Velhas Rainforest sites were separated according to channel morphology and stream power characteristics. Thus, we identified two stream types (Type 2 and 3) in this ecoregion. When substantial natural differences are detected within an ecoregion, sites should be treated as distinct reference sets (Hughes et al. 1986; Gerritsen et al. 2000). This a posteriori classification approach is in line with other studies that recommend stream type definition within ecoregions based on meaningful features (e.g. elevation, water quality, stream size, substrate; e.g. Mykrä et al. 2004; Turak and Koop 2008; Villamarín et al. 2013). Delineating stream types will clarify complex processes into categories that share common characteristics (Orr et al. 2008). In the present study, we named Type 2 and Type 3 as smaller and larger streams respectively to discuss further ecological issues and management implications.

Variation in vegetation cover may also explain the Type 1 outliers shown in the PCA (Fig. 2) and nMDS (Fig. 4). Patches of riparian trees, resembling woodlands, occur in rupestrian grasslands. Such clusters of small trees are associated with accumulations of soil in depressions of the terrain, which can support small arborescent islands amid the rupestrian grassland landscape (Silveira *et al.* 2016). Such variations are unlikely to be detected by remote sensing, which demonstrates the importance of field reconnaissance campaigns (Hughes *et al.* 1986; Orr *et al.* 2008; Turak and Koop 2008).

# Class and macroinvertebrate variability

Slightly smaller streams in the Velhas Rainforest (Type 2) supported more macroinvertebrate families than slightly larger streams (Type 3; Fig. 3). We believe that this pattern can be explained by the higher nitrogen concentration of the smaller streams, which, when scarce, is a limiting factor to whole-ecosystem metabolism (Dodds and Smith 2016). Tropical lotic systems tend to concentrate nutrients at low discharges, as in the Type 2 streams (Lewis 2008). In addition, small headwater streams provide sinuous pathways that hold many materials (e.g. wood, leaf packs and soil), thereby increasing nutrient retention and habitat heterogeneity for macroinvertebrate assemblages (Gooderham *et al.* 2007).

Stream size is a key driver of aquatic biodiversity (Vander Vorste *et al.* 2017). A recent review of 165 papers showed a positive relationship between stream size and aquatic biodiversity, but there is great inconsistency in the mechanisms that explain this relationship (Heino *et al.* 2005; Vander Vorste *et al.* 2017). In Neotropical ecosystems, Macedo *et al.* (2014) showed that variables related to stream size (wetted width, bank full width and wetted area) are positively correlated with

macroinvertebrate richness, and the same tendency was observed in temperate zones (Vander Vorste *et al.* 2017). Similarly, Ibañez *et al.* (2009) reported the same patterns for stream fishes of both tropical and temperate continents. Nonetheless, an inverse relationship was observed in the present study between stream size and aquatic biodiversity. This can be explained by the greater availability of shelter for macroinvertebrates and total nitrogen observed in these small streams (Fig. 2). Thus, we highlight the importance of stream size in protecting the biodiversity of stream networks.

Indicator taxa exhibited functional attributes that mirror the predominant physical habitat and water chemistry characteristics of each ecoregion. Our results corroborate the idea that different ecoregions have different ecological filters, defining distinct taxonomic and functional patterns within the whole basin (Heino et al. 2007). Leptohyphidae was one of the most abundant and frequent insect families in the Velhas Rainforest, as also observed by Graça et al. (2015) in that ecoregion. In general, in this ecoregion the predominant taxa have functional attributes that tolerate higher TDS and higher embeddedness, including burrowing behaviour (Gomphidae; Carvalho and Nessimian 1998), integumentary breathing, collector-gather habit (Leptohyphidae and Hyalellidae; Bouchard 2004; Shimano et al. 2013) and filter feeding (Bivalvia; Cummins et al. 2005). In contrast, the Caraça Grassland indicator taxa have traits sensitive to fine sediments, such as swimming habit (Notonectidade and Noteridae), attached behaviour (Psephenidae) and gill respiration (Helichopsychidae; Larsen et al. 2011).

IndVal calculations for each taxon are based on comparisons of relative abundances and frequencies between stream types (Dufrene and Legendre 1997). It was not possible to establish indicator taxa for stream Type 3, because the taxa that best discriminated Type 3 streams (Calopterigidae and Corydalidae) had low relative abundances and frequencies, or the relative abundances and frequencies did not differ significantly from another stream type (see Table S2).

#### Implications for freshwater management

In freshwater assessment and management, site-scale detail may be necessary for more effective site-scale assessment and management than that provided by landscape-scale characteristics (Hawkins *et al.* 2000). This is because biological indicators are sensitive to local environmental variation within large-scale landscapes (Ligeiro *et al.* 2010). Contrasting our results with current Brazilian water resources management policy, we reinforce the importance of including natural regional- and local-scale variations to define more effective management units within large river basins.

There is a continued discussion on the best method to predict ecological indicators. For example, much of what we ascribe as ecoregion effects may often simply be a result of the effect of the proximity among sites (Hawkins *et al.* 2000). However, in this study we showed that the ecoregion and stream types had different assemblage compositions, even considering sites very close to each other and near ecoregion boundaries (even <1 km; Fig. 1), demonstrating the sharp spatial discontinuities that can be generated purely due to environmental variation. In addition, Hawkins *et al.* (2010) and Moya *et al.* (2011) have shown that

streams are more likely to vary continuously across landscapes, meaning that variation may be better explained with predictive modelling than by stream types and ecoregions. Nonetheless, patterns evident in ecoregions and stream types provide useful management benchmarks for streams. Consequently, decisions regarding the most appropriate combination of methods for Neotropical stream research and management will depend on the costs, level of effort and accuracy required by environmental scientists, politicians and water resource managers (Hughes and Peck 2008; Hawkins *et al.* 2010).

The application of existing classification approaches (e.g. ecoregion, typologies) to tropical streams is scarcely developed and faces difficulties because of the lack of reference condition data. We are aware that this study is based on a modest number of reference sites compared with previous studies in temperate regions. In addition, it encompasses restricted portions of the Neotropical Savanna and Atlantic Forest biomes. The inclusion of more reference sites over a greater area would increase environmental variability, resulting in additional ecoregions and stream types. Finally, an evaluation of a separate set of test sites would have improved the rigor of this study. Unfortunately, we lacked a sufficiently robust set of test sites from both ecoregions and from the three stream typologies to do so. However, the study provides a reasonably simple and straightforward methodology that can be tested and applied to any dataset of least-disturbed streams, thereby improving ecologically meaningful stream classification.

The results of the present study indicate that delimitation of ecoregions is an important step for defining reference conditions, but the use of site-scale variables to classify stream types *a posteriori* further reduces the variability of environmental data and biological indicators. Finally, the reference stream indicator taxa may serve as explicit targets in stream restoration programs and provide baseline data for developing predictive models for Neotropical aquatic ecosystems.

# **Conflicts of interest**

The authors declare that they have no conflicts of interest.

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