CSIRO PUBLISHING

Marine and Freshwater Research, 2018, **69**, 82–94
https://doi.org/10.1071/MF16381

Regionalisation is key to establishing reference conditions for neotropical savanna streams

Isabela Martins[A,E], *Raphael Ligeiro*[B], *Robert M. Hughes*[C], *Diego R. Macedo*[D] *and Marcos Callisto*[A]

[A]Laboratório de Ecologia de Bentos, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Avenida Antônio Carlos 6627, CP 486, CEP 31270-901, Belo Horizonte, MG, Brazil.
[B]Laboratório de Ecologia e Conservação, Instituto de Ciências Biológicas, Universidade Federal do Pará, Rua Augusto Corrêa 01, CEP 66075-110, Belém, PA, Brazil.
[C]Amnis Opes Institute and Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, OR 97331-4501, USA.
[D]Departamento de Geografia, Instituto de Geociências, Universidade Federal de Minas Gerais, Avenida Antônio Carlos 6627, CEP 31270-901, Belo Horizonte, MG, Brazil.
[E]Corresponding author. Email: isa.bela57@hotmail.com

Abstract. Areas with minimal anthropogenic influences are frequently used as reference sites and represent the best ecological state available in a region. Streams in such conditions are necessary for evaluating the conservation status of aquatic ecosystems of a region and to monitor them, taking natural environmental variability into consideration. Therefore, the aim of the present study was to analyse whether hydrological units are reliable regional units for aggregating reference sites. To this end, reference sites were studied in three different landscape units of the same hydrological unit. The study tested the hypothesis that water quality, physical habitat structure and the composition and structure of macroinvertebrate assemblages will be more similar for sites in the same landscape unit than for sites located in different landscape units in the same hydrological unit. The study showed that taxonomic richness and composition of the macroinvertebrate assemblages were negatively affected by site slope and positively affected by the presence of leaf packs on the streambed. The three landscape units supported significantly different macroinvertebrate assemblages and indicator taxa. Therefore, a hydrological unit does not constitute a homogeneous entity in terms of environmental variables and biological composition if it incorporates high landscape heterogeneity. These results should improve and facilitate the selection of reference sites for biomonitoring programs and for managing tropical headwater streams.

Additional keywords: benthic macroinvertebrates, bioindicators, Brazil, ecoregions, headwater streams, landscape units, least-disturbed conditions.

Received 18 November 2016, accepted 23 May 2017, published online 1 August 2017

Introduction

Water is an essential resource for life, and rivers and streams are the major sources of water for many human uses globally. However, aquatic ecosystems are threatened by anthropogenic activities such as urbanisation, industrialisation and changes in land use and coverage, as well as dam construction (von Sperling 2012). A gradient of anthropogenic disturbances produces streams close to the natural state, some under intermediate conditions and others severely affected by human activities (Hughes *et al.* 1986; Davies and Jackson 2006; Ligeiro *et al.* 2013). Areas with minimal or low anthropogenic influences are known as reference sites and represent the best ecological state possible in a region (Hughes *et al.* 1986; Feio *et al.* 2007). Depending on the intensity and extent of human influences in the region being evaluated, it may be necessary to identify the least-disturbed sites available as opposed to minimally disturbed sites (Stoddard *et al.* 2006; Whittier *et al.* 2007; Herlihy *et al.* 2008). Therefore, among the different classifications of reference conditions, the most commonly used is the least-disturbed condition (LDC), which considers the least-altered biological, chemical and physical conditions taking into account the current state of the landscape (Hughes *et al.* 1986; Stoddard *et al.* 2006). Typically, minimally disturbed and least-disturbed reference sites occur in small headwater streams, which represent between 60 and 80% of total stream length in any hydrological unit or river basin (Benda *et al.* 2005).

Reference sites are required to evaluate the conservation status of aquatic ecosystems in a region and to monitor them, considering the natural environmental variability present in each region (US Environmental Protection Agency 2016). In

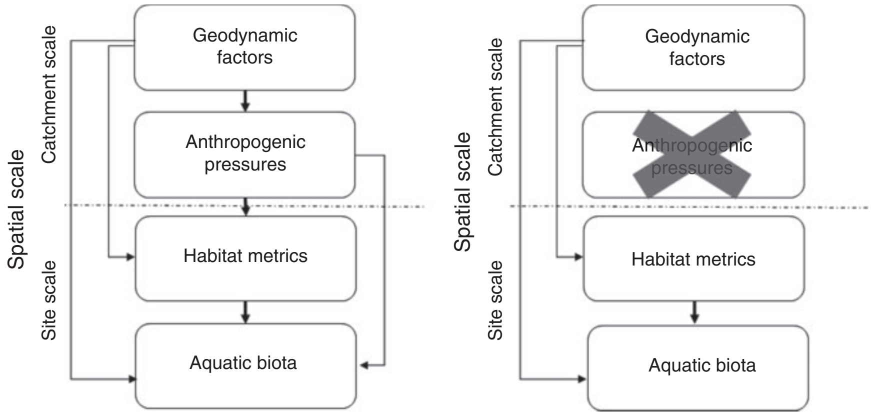

Fig. 1. In the absence of anthropic pressures, the geodynamic factors directly influence the metrics of local habitat, which is reflected in the structure and composition of the aquatic biota (adapted from Macedo *et al.* 2014).

several areas around the world, there are examples of large-scale biomonitoring programs that rely on the use of regional reference sites (Buss *et al.* 2015), including the Canadian Aquatic Biomonitoring Network (CABIN; Bailey *et al.* 2004) and the Australian biomonitoring program (AUSRIVAS; Davies *et al.* 2010). In Europe, the search for reliable reference sites is intensive and challenging (Nijboer *et al.* 2004; Sánchez-Montoya *et al.* 2009; Pardo *et al.* 2012). In the US, government agencies have developed multimetric indices and predictive models at regional and national scales by using regional reference conditions (US Environmental Protection Agency 2016).

In several nations, biomonitoring studies are conducted taking into consideration the importance of the regional distinctions when comparing sites (Buss *et al.* 2015). For example, Montgomery *et al.* (1995), Omernik (1995), Bailey (1980, 1995) and the US Environmental Protection Agency (2016) have argued that bioassessments must be calibrated by physiographic regions or ecoregions. An ecological region (or ecoregion) is a relatively homogeneous area that differs from other areas in its large-scale characteristics, including climate, geology, physiography, soil and vegetation types (Hughes *et al.* 1986). Ecoregions were developed to identify areas with similar geographical features that cause and reflect regional differences in ecosystem quality (Bailey 1995; Omernik 1995; Omernik and Griffith 2014). Because of their multivariate and multiscale nature, the delimitations of ecoregions are independent of the limits of hydrological units and river basins (Omernik and Bailey 1997; Omernik *et al.* 2017). However, in Brazil, hydrological units and river basins are used as templates for water resources management and ecological studies, in accordance with the Brazilian federal law number 9,433/1997, which establishes the National Water Resources Policy. The geodynamic factors that are used to define ecoregions, including climate, geology, physiography and soils (Omernik and Griffith 2014; Feio *et al.* 2015), drive land use and cover (Whittier *et al.* 2007; Macedo *et al.* 2014), and both affect habitat metrics (fluvial physical habitats and water chemistry; Allan 2004). Considering that all scales affect the composition and structure of local biological communities (Poff 1997; Elias *et al.* 2016), it is necessary to remove land use to evaluate natural influences on biota (Fig. 1). Therefore, the environmental and biological variability of reference sites in a region must be well characterised (Stoddard *et al.* 2006) to allow rigorous comparisons with other sites found in the same region (Bailey *et al.* 2004; Bowman and Somers 2005; Silva *et al.* 2017). In this context, biomonitoring programs and biological indicators that incorporate the effects of natural variability provide more precise and effective responses (Moya *et al.* 2007, 2011; Chen *et al.* 2014; Macedo *et al.* 2016; Pereira *et al.* 2016; US Environmental Protection Agency 2016).

Considering the importance of regionalisation for establishing reference sites, the aim of the present study was to evaluate whether hydrological units are valid regional units for this purpose. We studied reference sites sampled in different landscape units of a single hydrographic basin. We tested the hypothesis that the water quality, physical habitat structure and the composition and structure of macroinvertebrate assemblages in sites in the same landscape unit are more similar to each other than to those of sites located in different landscape units in the same hydrological unit. The degree of internal heterogeneity of hydrological units may indicate whether they can be regarded as efficient regional units in the selection of reference sites, with direct consequences for the management and biomonitoring of water bodies in tropical regions (Omernik *et al.* 2017).

Materials and methods

Study area

Headwater streams (first to third orders at 1 : 100 000 scale, according to Strahler 1957) in the Nova Ponte hydrologic unit inthe Araguari River Basin were sampled. The Araguari River Basin is located in southwestern Minas Gerais state (Brazil), within the Neotropical savanna (known as the Cerrado biome). The Araguari River Basin has an area of 21 856 km^2 and has been intensely altered by anthropogenic activities, leaving only remnants of the natural Cerrado vegetation (Fig. 2). Among these activities, deforestation, mining, irrigated agriculture (occupying ~50% of the basin area), livestock grazing and hydroelectric projects are prominent (Ligeiro *et al.* 2013). The headwaters of this basin are located in the plateaus of the

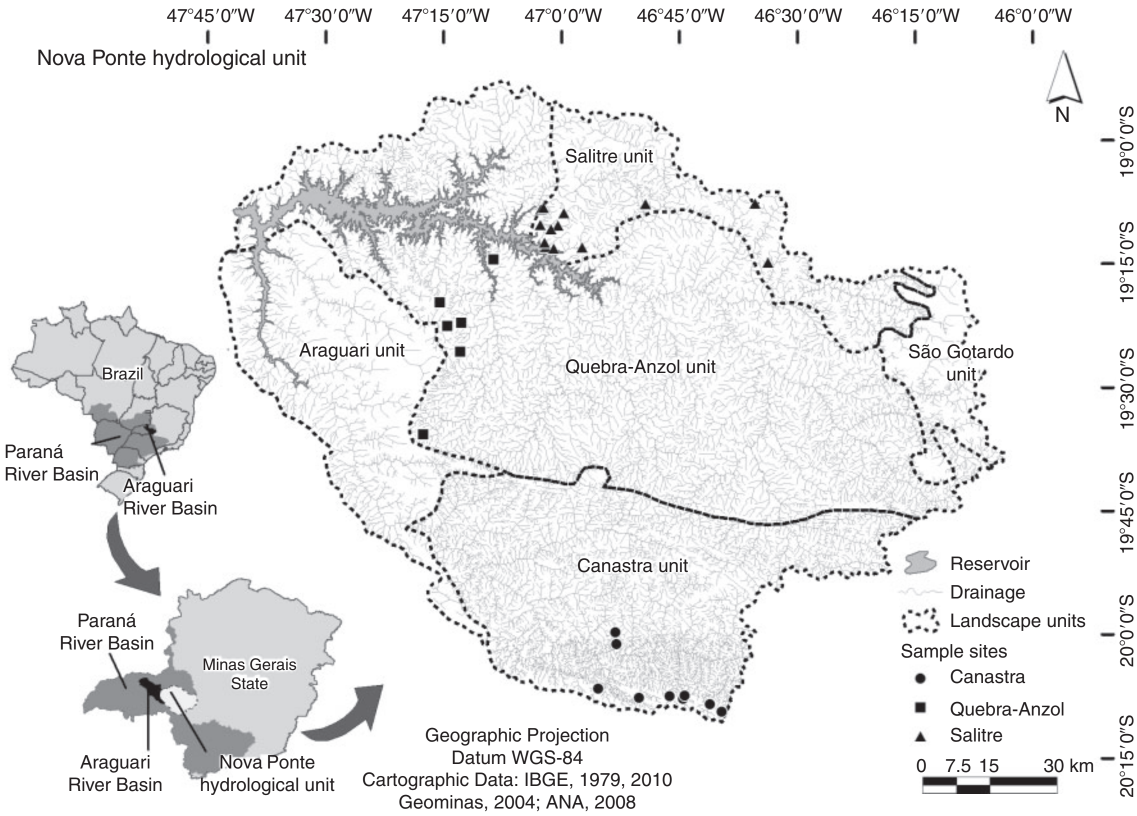

Fig. 2. Locations of the reference sites sampled in the Nova Ponte Hydrologic Unit. Circles, Canastra unit; rectangles, Quebra-Anzol unit; triangles, Salitre unit.

Canastra Mountain Range at an altitude of ~1440 m above sea level. Sixty locations were initially selected as potential reference sites based on the land cover interpretation of a combination of fine-resolution images (0.6- to 5-m spatial resolution; Google Earth images, see https://www.google.com/earth/, accessed April 2014), together with Landsat Thematic Mapper (TM) multispectral satellite images (Macedo *et al.* 2014). Of those 60 locations, 29 were selected after field reconnaissance and verification of the criteria suggested by Hughes *et al.* (1986) and Bailey *et al.* (2004), including minimal anthropogenic catchment disturbance, absence of a direct effect of anthropogenic changes at the sites and the presence of native riparian vegetation at the sites. Eight of the 29 sites are located in the Serra da Canastra National Park (see Table S3, available as Supplementary material to this paper).

Landscape units criteria

Landscape units were defined through map overlay of geodynamic variables by geographic information system (GIS) procedures. Annual temperature and precipitation data (~50 years climate baseline) were defined from the wordclim dataset (Hijmans *et al.* 2005). Geological data were extracted from the Brazilian geological map (1:250 000 scale; http://www.visualizador.inde.gov.br, accessed April 2014). Physiographic data were extracted from Shuttle Radar Topography Mission (SRTM) data (1 arc-sec; US Geological Survey 2015). Elevation was extracted directly from SRTM imagery, slope was calculated from the maximum rate of change in elevation in every grid cell and local relief was calculated using the Riley index (Riley *et al.* 1999), which determines the difference between the mean elevation value of a cell and the mean elevation of eight neighbouring cells. The information for these three SRTM-derived parameters were combined into a red, green and blue (RGB) additive colour model composition to identify physiographic boundaries. Geodynamic information was interpreted by GIS overlay and homogeneous areas were defined through screen digitising of homogeneous areas (see Fig. S1, available as Supplementary material to this paper). Five landscape units were identified: São Gotardo, Araguari, Salitre, Quebra-Anzol and Canastra. However, sites were only located in the latter three landscape units (Fig. 2). To confirm our classification, we ran a multidimensional scaling (MDS) ordination using the value of all geodynamic variables in each site catchment (after standardising variables and applying the Euclidian distance between pair of sites; Fig. S2).

Field data collection

Sites were sampled in April and May 2014, 13 of which were in the Salitre landscape unit, 6 in the Quebra-Anzol landscape unit and 10 in the Canastra landscape unit. Sites were 25 m long and subdivided into six equidistant transects (Agra 2014). Physical habitat characteristics were measured based on the protocol developed by the US Environmental Protection Agency (Peck

Table 1. Correlations between disturbance metrics (from Kaufmann *et al.* 1999) and biological and habitat variables
W1_HALL, riparian human disturbance index

Habitat metrics	Description	Percentage catchment agriculture	Percentage catchment pasture	W1_HALL
–	Richness	0.18	0.14	0.07
–	Abundance	−0.15	−0.23	−0.26
FLOW_2	Discharge (m^3 s^{-1})	0.15	−0.11	0.10
XVEL	Mean water velocity (m s^{-1})	0.10	−0.06	0.14
PCT_RA	Percentage rapids	0.07	0.34	−0.03
SEQ_FLO_1	Flow heterogeneity	−0.33	0.29	−0.21
XDEPTH_T	Thalweg mean depth (cm)	0.27	−0.19	−0.08
SDDEPTH_T	Thalweg s.d. of depth (cm)	0.09	−0.19	−0.17
XSLOPE_%	Mean channel slope (%)	−0.29	−0.24	−0.28
PCT_GC	Percentage substrate large gravel and cobble	0.16	−0.04	0.37
PCT_BIGR	Percentage sbstrate >16-mm diameter	−0.06	−0.30	−0.12
PCT_SA	Percentage substrate sand	0.08	0.12	0.02
XCDENMID	Canopy cover midstream (%)	0.13	0.21	−0.06
XC	Riparian woody canopy cover (%)	−0.02	0.18	−0.04
XM	Riparian woody mid-layer cover (%)	0.04	0.23	0.13
XPCAN	Percentage riparian canopy in site	−0.06	0.11	−0.02
XFC_ALG	Filamentous algae areal cover (%)	−0.19	−0.20	−0.15
XFC_LWD	Large wood areal cover (%)	−0.03	0.12	−0.09
XFC_LEB	Leaf pack areal cover (%)	−0.11	0.10	−0.04
NITRO	Total nitrogen (mg L^{-1})	−0.10	0.08	0.27
ALC	Total alkalinity (μEq L^{-1} CO_2)	−0.00	0.51	0.00
TDS	Total dissolved solids (mg L^{-1})	0.00	0.49	0.01

et al. 2006), adapted and validated for streams of the Brazilian Cerrado and mountaintop grasslands (Agra 2014; Callisto *et al.* 2014). The characteristics evaluated included channel slope, habitat type (riffle, glide, pool), substrate size, depth, embeddedness (percentage of substrate buried by sand and fines), bank full height and width, bank angle, riparian canopy coverage, thalweg depth, discharge, macroinvertebrate cover, riparian condition and human disturbance on the river bank and riparian zone.

At each site *in situ* measurements of temperature (°C), electrical conductivity (μS cm^{-1}), pH, total dissolved solids (mg L^{-1}), and turbidity (nephelometric turbidity units, NTU) were made. In the laboratory, dissolved oxygen (mg L^{-1}) was determined by the Winkler (1888) method and total alkalinity (μEq L^{-1} CO_2) was determined using the Gran method (Carmouze 1994). Total nitrogen (mg L^{-1}) and total phosphorus (μg L^{-1}) were measured according to the methods of Golterman *et al.* (1978) and Mackereth *et al.* (1978) respectively. Nitrite and nitrate concentrations were also measured (American Public Health Association 1998).

Macroinvertebrates were collected from all six transects using a D-frame kick-net (opening 30 cm, 500-μm mesh, area 0.09 m^2), following a systematic zig-zag pattern along the transects. The six samples collected were individually stored in plastic bags and fixed with 10% formalin. In the laboratory, the samples were washed on a 500 μm-mesh sieve and sorted on light boxes, and combined into a single sample for each site. Organisms were classified by Family using a stereoscopic microscope (32× magnification) and taxonomic keys (Pérez 1988; Merritt and Cummins 1996; Fernández and Domínguez 2001; Costa *et al.* 2006; Mugnai *et al.* 2010; Hamada *et al.* 2014). The organisms were stored in 70% ethanol and deposited in the Benthic Macroinvertebrates Reference Collection of the Institute of Biological Sciences, Universidade Federal de Minas Gerais (UFMG).

Data analyses

First, we ensured that anthropogenic disturbance gradients did not affect our interpretations of reference site stream habitats, water quality and biological characteristics. To this end, we used a Pearson correlation matrix between the response variables that would be evaluated later (taxonomic richness, abundance of individuals, physical habitat measures) *v.* a local disturbance metric (W1_Hall) representing total human impact in the riparian zone, and catchment anthropogenic disturbance (percentage agriculture, percentage grazing land). The biological and habitat variables did not correlate with any disturbance metric (Table 1), supporting no disturbance gradient acting on the reference sites used in the study.

Selection of habitat metrics

The physical habitat metrics were calculated according to Kaufmann *et al.* (1999). Metrics with a frequency of null values >80% were disregarded, as were those with a CV (s.d. ÷ mean) <0.2.

The physical habitat metrics were assembled into six groups: channel morphology, substrate, habitat type, riparian vegetation, macroinvertebrate cover and water quality. To assess collinearity among metrics, a Pearson correlation matrix was calculated between the metrics of each group. For highly correlated variables ($|r| > 0.80$), we chose those with the highest ecological relevance for the macroinvertebrate assemblages and those that are more intuitively understood (Little *et al.* 1999).

Finally, we performed a principal component analysis (PCA) for each block with the remaining metrics to determine which were responsible for generating the most variation among the sites. Metrics with loadings >0.7 on the first or on the second PCA axis were selected; in this way, metrics were selected that mostly contributed to distinguishing the sites. PCA was performed using standardised data (mean = 0, s.d. = 1) through a correlation matrix.

Comparison between habitats in the three landscape units

To verify whether the three landscape units differed with regard to physical habitat metrics, we initially performed one-way analyses of variance (ANOVA) with each metric selected. The ANOVA assumptions were tested using Shapiro's test (normality) and Levene's test (homogeneity of variances). We applied Bonferroni's correction to avoid inflating Type I statistical error. After correction, α was set to 0.0025. Subsequently, we conducted a PCA using all habitat metrics selected in the previous steps. We sought to verify whether the sites were grouped together in the first two generated axes according to the different landscape units, and which variables were responsible for those groupings.

Analysis of indicator taxa

Indicator taxa were analysed to identify which taxa were significantly associated with the three landscape units (Dufrene and Legendre 1997). The indicator value (*IndVal*) considers the relative abundance (specificity) and the relative frequency (fidelity) of each of the taxa in the groups defined *a priori*. The IndVal ranges from 0 to 100, where 100 represents occurrence and maximum relative abundance in all sites of a group, but absence and minimum relative abundance in sites of other groups. A 0 represents the opposite: occurrences and similar relative abundances in all sites in all groups (Dufrene and Legendre 1997). The equalised version of this index was used in order to account for the unequal number of sites between groups (De Cáceres and Legendre 2009). To test the significance of *IndVal*, the Monte Carlo test was used with 10 000 randomisations ($\alpha = 0.05$).

Comparison among assemblages in the three landscape units

One-way ANOVA was used to evaluate the taxonomic richness and abundance ($\log(x + 1)$) between the three landscape units. The ANOVA assumptions were tested as described above for landscape units. To evaluate whether the three landscape units differed in taxonomic composition of the macroinvertebrate assemblages, a permutational multivariate analysis of variance (PERMANOVA) was performed. A principal coordinate analysis (PCoA) was employed to visually assess whether taxa were grouped together in the first two axes as a function of the landscape units. The three analyses described above were performed on the Jaccard (taxa presence or absence) and Gower (taxa relative abundance; as modified by Anderson *et al.* 2006) dissimilarity measures.

Effects of habitat metrics on taxonomic richness and composition

To determine the metrics that best explained variation in taxonomic richness within the macroinvertebrate assemblages, multiple linear regressions (MLRs) were performed. Models were generated using the best-subsets procedure (Harrell 2001). The best models were chosen based on the corrected Akaike information criterion (AICc; Burnham and Anderson 2002). According to the parsimony principle, when choosing between models with an AICc variation (ΔAICc) $\leq$2, one should opt for the model with the fewest variables. A maximum number of three predictors, representing 10% of the total number of sites, was adopted to avoid inflating model explanation (Gotelli and Ellison 2004). To evaluate the metrics that best explained the variation in assemblage composition, the values obtained by the sites in the first axis of the two PCoAs (with the Jaccard and modified Gower dissimilarities) were inserted as dependent variables in the MLRs following the same steps. Each model was validated by spatial autocorrelation of MLR residuals (Diniz-Filho *et al.* 2003; Rangel *et al.* 2010). In addition, spatial autocorrelation for macroinvertebrate richness was evaluated by the Moran I-test (Anselin and Bera 1998) and assemblage composition was evaluated using the Mantel test (Legendre and Legendre 1998).

Results

Habitat comparison among landscape units

Twenty physical habitat and water quality metrics were selected for the subsequent analyses, with half differing significantly among the three landscape units of the Nova Ponte hydrologic unit (Table 2).

The first two axes retained for PCA interpretation explained 49% of stream physical habitat variability (Fig. 3; Table S1). The first axis explained 31.25% of the data variation and was positively affected by the percentage of substrates >16 mm in diameter, thalweg mean depth and the percentage of filamentous algae areal cover; it was negatively affected by the percentage of riparian woody mid-layer cover, the percentage of canopy cover midstream and total dissolved solids. The second axis explained 17.8% of the data variation and was positively affected by mean water velocity, discharge and the percentage of substrate composed of large gravel and cobble, and negatively affected by mean channel slope, flow heterogeneity and total nitrogen.

The three landscape units differed in site habitat characteristics. Salitre landscape unit sites had higher average velocities and discharges, and more coarse gravel. The Quebra-Anzol landscape unit sites had more rapids, flow type heterogeneity, canopy cover in the riparian zone and over the channel, and higher total dissolved solids and total alkalinity. The Canastra landscape unit sites had greater algal cover, average variation in thalweg depth, slope and coarse substrates. Conversely, the percentage of sand and mid-layer woody vegetation in the riparian zone was significantly lower in the Canastra landscape unit sites.

Benthic macroinvertebrates and indicator taxa

In all, 27 861 individuals and 70 families were identified. In the Salitre landscape unit sites, 8922 individuals and 61 taxa were found, compared with 3993 individuals and 54 taxa in the Quebra-Anzol unit sites and 14 946 organisms and 59 taxa in the

Canastra landscape unit sites (see Table S2). The most abundant families were Chironomidae (36% of total individuals), Simuliidae (14%), Elmidae (11%) and Baetidae (6%). Seventeen statistically significant indicator taxa were found for the three landscape units: four for the Salitre unit sites, seven for the Quebra-Anzol unit sites and six for the Canastra unit sites, all with *IndVal* ≥30 (Table 3).

Comparison between assemblages in the three landscape units

There were no significant differences in taxonomic richness (ANOVA, $F_{2,26} = 1.34$, $P = 0.28$) or abundance (ANOVA, $F_{2,26} = 2.10$, $P = 0.14$) between the three landscape units. However, the taxonomic composition of macroinvertebrates,

Table 2. Selected physical habitat and water quality metrics by landscape unit

Unless indicated otherwise, data are given as the mean ± s.d. Probabilities are significant at *, α < 0.0025

Metric	Salitre	Quebra-Anzol	Canastra	*F*-value	*P*-value
Water chemistry					
Alkalinity (µEq L^{-1} CO_2)	80.66 ± 65.67	479.15 ± 146.38	81.06 ± 101.42	39.02	<0.0001*
Total nitrogen (mg L^{-1})	0.04 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	1.25	0.30
Total dissolved solids (g L^{-1})	0.88 ± 0.55	2.07 ± 1.02	0.00 ± 0.00	23.49	<0.0001*
Channel morphology					
Mean channel slope (%)	0.01 ± 0.00	0.02 ± 0.01	0.07 ± 0,06	9.40	0.0008*
Thalweg mean depth (cm)	28.3 ± 16.71	16.56 ± 2.61	39.4 ± 22.84	3.21	0.05
Thalweg s.d. depth (cm)	9.21 ± 6.50	9.77 ± 2.90	18.90 ± 6.58	8.32	0.001*
Flow					
Percentage rapids	11.42 ± 12.74	26.14 ± 13.43	6.30 ± 9.05	5.48	0.01
Flow heterogeneity	0.12 ± 0.08	0.39 ± 0.12	0.24 ± 0.14	11.30	0.0002*
Discharge (m^3 s^{-1})	0.29 ± 0.33	0.02 ± 0.02	0.04 ± 0.07	4.60	0.02
Mean water velocity (m s^{-1})	0.40 + 0.19	0.12 ± 0.11	0.04 ± 0.02	18.75	<0.0001*
Bed substrate					
Percentage substrate sand	19.39 ± 19.22	33.33 ± 11.96	2.46 ± 4.39	9.22	0.0009*
Percentage substrate coarse gravel	17.73 ± 12.77	7.57 ± 5.33	9.84 ± 10.50	2.38	0.11
Percentage substrate >16-mm diameter	53.17 ± 22.87	7.57 ± 5.33	80.59 ± 28.30	8.69	0.0001*
Riparian vegetation					
Riparian woody canopy cover	3.52 ± 3.54	20.24 ± 14.59	8.70 ± 12.87	5.50	0.01
Riparian woody mid-layer cover	122.72 ± 20.04	147.43 ± 20.40	45.64 ± 44.09	26.48	<0.0001*
Canopy cover midstream	37.21 ± 9.89	49.50 ± 1.90	26.65 ± 20.12	5.33	0.01
Percentage riparian canopy in site	0.53 ± 0.34	0.93 ± 0.17	0.43 ± 0.45	3.63	0.04
Shelter					
Filamentous algae areal cover	0.45 ± 1.25	0.00 ± 0.00	3.04 ± 3,16	5.92	0.0007*
Leaf pack areal cover	6.50 ± 7.58	22.23 ± 13.33	9.25 ± 17.59	3.12	0.06
Large wood areal cover	10.33 ± 8.18	14.12 ± 7.11	7.79 ± 10.42	0.96	0.39

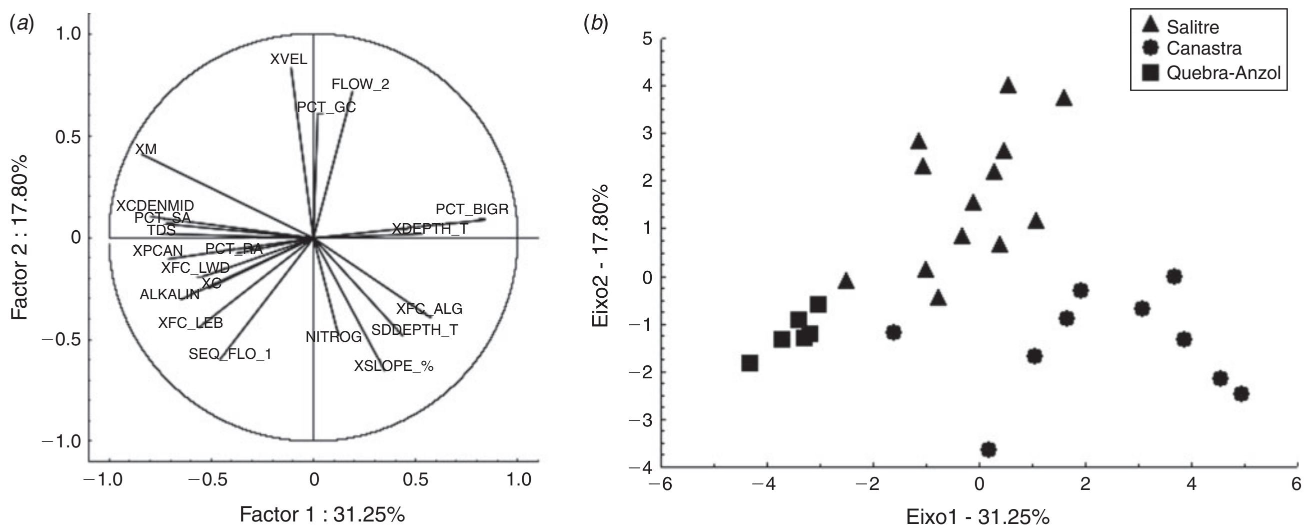

Fig. 3. (*a*) PCA performed with the physical habitat and water quality metrics. (*b*) The same PCA depicting sites by landscape units. Circles, Canastra unit; rectangles, Quebra-Anzol unit; triangles, Salitre unit.

considering only taxa presence, was significantly different among the three units (PERMANOVA (Jaccard), $F_{2,26} = 2.38$, $P < 0.001$; Fig. 4*a*). Pairwise comparison showed that the three types were different from each other. Similarly, taxonomic composition based on relative abundance was significantly different among the three units (PERMANOVA (Gower), $F_{1,27} = 2.35$, $P < 0.001$; Fig. 4*b*). Pairwise comparisons showed that the Quebra-Anzol and Salitre unit sites did not differ from each other, whereas the Canastra unit differed from both the others.

Effects of habitat metrics on assemblage richness and composition

The model that best explained the variation in macroinvertebrate taxonomic richness had two predictive metrics: average channel slope, which negatively affected richness, and leaf pack cover, which positively affected richness (Table 4). The multiple regression model considering the first PCoA axis (Jaccard) as the dependent variable contained the same two variables. The multiple regression model considering the first PCoA axis (Gower) as the dependent variable had only one variable, namely percentage of sand on the streambed, affecting the relative abundance of macroinvertebrate taxa (Table 4). The MLRs had no spatially autocorrelated residuals. Richness was not spatially autocorrelated either; however, assemblage composition showed autocorrelation through the Mantel test results (Tables S4–S6).

Discussion

The characterisation of reference sites in the Nova Ponte hydrologic unit indicates that there were differences among the three landscape units regarding physical habitat, water quality and the taxonomic composition of macroinvertebrate assemblages. Therefore, classification of the hydrological unit into landscape units enabled improved classification of the biotic and abiotic characteristics of the reference sites. This approach was also used in other studies (Sánchez-Montoya *et al.* 2009; Villamarín *et al.* 2013), in which the analysed streams had distinct morphological characteristics. The results of the present

Table 3. Indicator taxa by landscape unit

The indicator value (*IndVal*) considers the relative abundance (specificity) and the relative frequency (fidelity) of each of the taxa in the groups, defined *a priori*. *IndVal* ranges from 0 to 100, with higher values indicating better indication. Probabilities are significant at *, $P < 0.05$

Landscape unit	Family or Order	*IndVal*	*P*-value
Salitre	Corydalidae	55	0.01*
	Glossosomatidae	58	0.01*
	Odontoceridae	66	0.03*
	Psephenidae	73	0.003*
Quebra-Anzol	Veliidae	41	0.03*
	Aeshnidae	45	0.01*
	Dicteriadidae	45	0.01*
	Gerridae	50	0.01*
	Calamoceratidae	51	0.02*
	Gomphidae	53	0.02*
	Hirundinea	59	0.02*
Canastra	Notonectidae	30	0.04*
	Hydracarina	39	0.03*
	Chironomidae	53	0.04*
	Hydroptilidae	55	0.04*
	Pyralidae	68	0.02*
	Baetidae	80	0.03*

Table 4. Multiple linear regressions of benthic macroinvertebrate taxonomic richness and composition *v.* physical habitat and water quality

XSLOPE_%, mean channel slope; XFC_LEB, leaf pack areal cover; PCT_SA, percentage substrate sand. Probabilities are significant at *, $P < 0.05$

	F-value	*P*-value	R^2	Metric	β	β s.d.
Richness	3.66	0.04*	0.22	XSLOPE_%	−0.32	0.18
				XFC_LEB	0.43	0.18
Composition Jaccard	13.31	<0.0001*	0.50	XSLOPE_%	−0.57	0.14
				XFC_LEB	0.58	0.14
Composition Gower	5.53	0.03*	0.17	PCT_SA	0.41	0.18

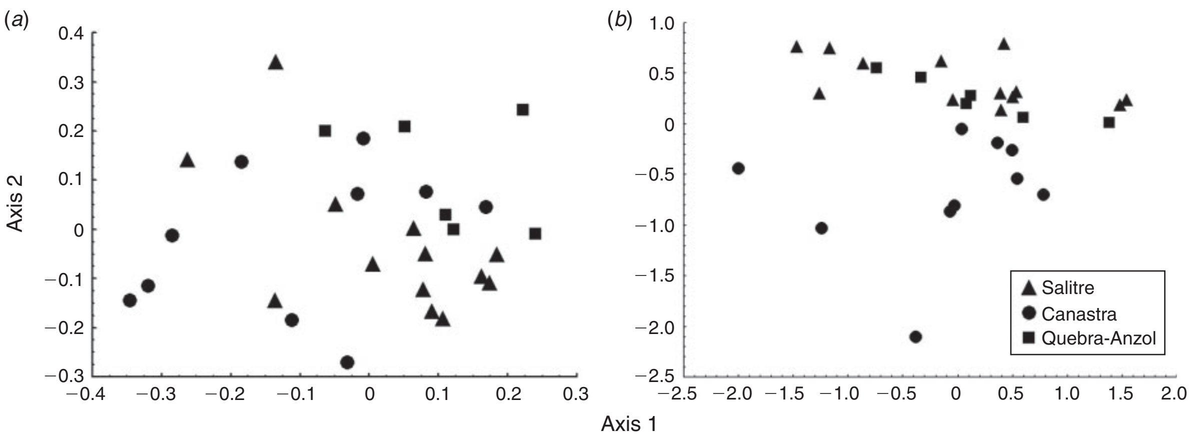

Fig. 4. PCoA indicating the similarity in taxonomic composition of the macroinvertebrate assemblages among sites in the three landscape units considering (*a*) Jaccard index (taxa presence or absence) and (*b*) Gower distance (taxa relative abundances).

study corroborate our initial hypothesis that a hydrological unit does not constitute an appropriate regional unit to define reference sites, if the internal heterogeneity in biological and environmental variables is high. This is the rationale for using ecoregions *v*. (or in addition to) basins and hydrologic units as geographic classification units, regardless of the spatial scale being analysed (Hughes *et al.* 1986; Bailey 1995; Omernik 1995; Omernik and Bailey 1997; Stoddard *et al.* 2008; Omernik and Griffith 2014; Omernik *et al.* 2017).

Environmental characterisation of the reference sites

In the Salitre landscape unit, located in the northern part of the basin, the flow velocities and discharges were higher, and consequently the predominant channel substrate was coarse gravel. In the Quebra-Anzol unit, the high frequency of rapids and several flow types were associated with high faunal diversity, as discussed by Barbour *et al.* (1999). Several other studies also reported a correlation between flow type and macroinvertebrate assemblage composition (e.g. Heino *et al.* 2007; Ferreira *et al.* 2014; Silva *et al.* 2014; Graça *et al.* 2015). In the Canastra unit, streams were deeper and steeper; Ribeiro and Freitas (2010) reported similar results from this landscape unit. The percentage of sand and understorey coverage of riparian vegetation were also significantly lower in the Canastra unit. In addition, in the Canastra Mountain Range of the Canastra unit, riparian vegetation was reduced and there was little allochthonous organic matter input; however, there was abundant light penetration, explaining the greater amount of algae in those sites. The riparian zone has a crucial role in protecting springs and headwater streams (Boyero *et al.* 2015). Therefore, streams in the Canastra Mountain Range with little riparian vegetation are particularly susceptible to anthropogenic alterations, becoming priority regions for conservation.

Effects of physical habitat on macroinvertebrate assemblages

Areas with similar environmental characteristics rarely correspond to the limits of watersheds or hydrological units (Omernik and Bailey 1997; Omernik *et al.* 2017). As seen in the present study, hydrological units are not homogeneous for abiotic characteristics and biotic assemblages. In our case, the same hydrological unit embraced landscape units with different macroinvertebrate assemblages. According to Omernik and Bailey (1997), factors such as physical habitat, water chemistry and biota are directly associated with aggregate factors operating at larger scales (climate, geology, physiography, soils and land cover characteristics, including vegetation). Environmental characteristics such as climate, geology and physiography vary spatially in any large hydrological unit, affecting the characteristics of local fluvial habitats, such as the presence and type of riparian vegetation, and the type and granulometry of streambed substrates (Petts 2000). These characteristics directly affect local macroinvertebrate assemblages (Reynoldson *et al.* 2001; Chessman 2004; Feio *et al.* 2009). In the present study, the large differences in habitats among the three landscape units affected the biological dissimilarity observed among them.

The variables that best explained macroinvertebrate assemblage richness were channel slope, percentage of leaf pack cover and percentage of streambed sand (Table 4). The geology and physiography of the region directly affects stream slope, which, in turn, determines current velocity and flow types, and consequently the types of habitats and shelters available for macroinvertebrates (Feio *et al.* 2015; Heino *et al.* 2015). The amount of sand present in streams is also associated with geology and soils, and usually negatively correlated with the richness and abundance of macroinvertebrates, because an increase in the amount of fine sediments reduces the area available for shelter and rearing (Peck *et al.* 2006; Bryce *et al.* 2010). Macedo *et al.* (2014) observed this negative correlation in two basins in the Brazilian Cerrado of Minas Gerais, including the one evaluated in the present study. However, Agra (2014) found opposite results with headwater streams in another Cerrado basin. A few biological groups are favoured by an increase in fine sediments, such as some Chironomidae and Coleoptera (de Castro Vasconcelos and Melo 2008), as well as taxa with integumentary respiration and those classified as collectors and filterers (Agra 2014). The percentage of leaf packs indicates the amount of leaves available in the streambed for use by macroinvertebrates as shelter, substrate and food (Ferreira *et al.* 2015). In forested headwater streams, there is low light penetration and autochthonous primary production is reduced (Vannote *et al.* 1980). Therefore, leaf debris, originating from the riparian vegetation, is the main energy source for these systems (Cummins *et al.* 1973; França *et al.* 2009; Gonçalves and Callisto 2013; Graça *et al.* 2015).

Macroinvertebrates associated with each landscape unit

Differences in landscape units were associated with differences in macroinvertebrates. Organisms that are indicators of good water quality, such as the Glossosomatidae and Odontoceridae, were found in the Salitre unit sites. Those organisms live in streams with clear and shaded waters (Pescador *et al.* 2004). Furthermore, Psephenidae, also an indicator in the Salitre unit, are typically found in streams with high-quality environmental conditions and high current velocities (Jerez and Moroni 2006; Hamada *et al.* 2014). In Quebra-Anzol unit sites, the more pronounced riparian vegetation and canopy coverage help explain the presence of the Calamoceratidae, because they depend on leaves for food and case construction (Moretti *et al.* 2009; Ferreira *et al.* 2015). Dicteriadidae, also indicators of the Quebra-Anzol unit, depend on forest cover, being susceptible to the fragmentation and reduction of riparian vegetation (Hamada *et al.* 2014). This is because these damselflies have limited dispersal ability (Corbet 1999), related to their dependence on ambient temperature to regulate body temperature (de Oliveira-Junior *et al.* 2015). Some Zygoptera, like Dicteriadidae, are indicators of preserved environments with high heterogeneity because they have smaller bodies and greater ecophysiological restrictions (de Oliveira-Junior *et al.* 2015). The Hydroptilidae show a preference for habitats with higher water velocities and discharge. Their larvae are also associated with algal substrates, typically found in the open canopies of Canastra unit streams (Pescador *et al.* 2004). It is no surprise that the Mantel test showed positive values for assemblage composition. The first geographic law states that 'everything is related to everything else, but near things are more related than distant things' (Tobler 1970). Therefore, sites located in the same landscape unit, closer to each other, have more similar physical habitat and water chemistry, which influence aquatic biota. However, the other spatial autocorrelation tests showed non-significant values. Like ecoregions, our landscape units identified areas with similarities in the combination of geodynamic factors that cause and reflect differences in instream habitats (Bailey 1995; Omernik 1995; Omernik and Griffith 2014).

Differences observed within the hydrological unit

In a hydrological unit and in its landscape units, geophysical factors affect the structure and composition of riparian zones, the predominant substrate and flow types, as well as potential nutrient inputs, thereby indirectly affecting the availability and quality of local habitats for aquatic communities (Allan 2004; Wang *et al.* 2008). Climate, hydrological regime and geology and physiography may determine the type of plants in the riparian vegetation, as well as sediment and flow characteristics (Cooper *et al.* 2003; Kaufmann and Hughes 2006), possibly explaining the differences in environmental metrics found among the three landscape units analysed in the present study. Similarly, ecoregions were found useful for distinguishing geographic patterns in stream assemblages of fish (Hughes *et al.* 1994; Abell *et al.* 2008; Pinto *et al.* 2009) and macroinvertebrates (Whittier *et al.* 1988; Feminella 2000; Rabeni and Doisy 2000; Stoddard *et al.* 2008). Nonetheless, the greater assemblage similarity between the Salitre and Quebra-Anzol units sites (Fig. 4*b*) is likely a consequence of the greater spatial proximity of those sites, as reported by Van Sickle and Hughes (2000). In global studies, Hawkins *et al.* (2000) and Heino *et al.* (2015) observed that the structure of aquatic insect assemblages have low predictability and that many environmental variables affect their structure and composition. Therefore, there is no global pattern in the environmental metrics that could explain the organisation of aquatic insect assemblages, which necessitates smaller-scale studies.

The results of the present study corroborate those of previous studies showing that streams in the same basin may have very distinct characteristics, governed by ecoregional differences (e.g. Whittier *et al.* 1988; Bailey *et al.* 2004; Sánchez-Montoya *et al.* 2007). In places where the limitations of basins are biologically relevant, considering both ecoregions and basins is required to establish regional reference sites that facilitate a clear understanding of the quality, integrity and health of the ecosystems and their components (Omernik and Bailey 1997). Even though the National Water Resources Policy in Brazil defines river basins as the political units for water management, we suggest, similar to what already takes place in other countries (Omernik and Bailey 1997), that smaller-scale patterns and characteristics also should be considered to better monitor the conservation status and trends of waterbodies (Wasson *et al.* 2002). For similar reasons, the metrics of biological indices are often calibrated by natural environmental gradients, and metrics based on guilds, traits, and richness (*v.* specific taxa) are increasingly used in biomonitoring and bioassessment programs (Moya *et al.* 2007; 2011; Chen *et al.* 2014; Pereira *et al.* 2016; US Environmental Protection Agency 2016).

Reference streams may indicate the structure and behaviour of communities and ecosystems under conditions closest to natural, including information on the dominant species, susceptible to pollution, potential species diversity and expected physical and chemical habitat conditions. In addition, an appropriate set of metrics representing each region is essential to the success of the environmental assessment (Muxika *et al.* 2007). Together, these characteristics may serve as parameters to be used as aims in future restoration programs of streams belonging to the same region (Hughes *et al.* 1986; Bouchard *et al.* 2016; Silva *et al.* 2017). The present study revealed that to define reference sites, the regional heterogeneity within hydrologic units and river basins must be considered, because those hydrologic units do not constitute homogeneous units in terms of environmental structure and biological composition. For future assessments of environmental quality in Cerrado basins, we recommend the use of a reference site approach incorporating local geographic features. Finally, we expect that the present study can be used as a baseline for understanding the effectiveness of reference sites when facing ongoing changes in land use, to support freshwater conservation in the Neotropical savanna biome and to implement effective water management practices. Such information is necessary for improving the conservation status of aquatic biota and for maintaining ecosystem services for humans.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

The authors acknowledge funding from the Companhia Energética de Minas Gerais, Pesquisa & Desenvolvimento, Agência Nacional de Energia Elétrica (GT-487), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG). Colleagues from the Universidade Federal de Minas Gerais (UFMG) helped with field work and sample processing. Isabela Martins received a M.Sc. scholarship from CNPq. Marcos Callisto was awarded research productivity grants from CNPq (number 303380/2015-2, 446155/2014-4) and a Minas Gerais research grant (FAPEMIG PPM-IX – 00525-15). Diego R. Macedo received a research grant from Pró-Reitoria de Pesquisa (PRPq)-UFMG (ADRC-05/2016) and a research project grant from CNPq (number 402907/2016-7). Robert M. Hughes received a Fulbright Brasil grant.

References

Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Balderas, S. C., Bussing, W., Stiassny, M. L. J., Skelton, P., Allen, G. R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Higgins, J. V., Heibel, T. J., Wikramanayake, E., Olson, D., Lopez, H. L., Reis, R. E., Lundberg, J. G., Perez, M. H. S., and Petry, P. (2008). Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *Bioscience* **58**, 403–414. doi:10.1641/B580507

Agra, J. U. M. (2014). Condições de referência em riachos de cabeceira tropicais. M.Sc. Thesis, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Allan, J. D. (2004). Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology and Systematics* **35**, 257–284. doi:10.1146/ANNUREV.ECOLSYS.35.120202.110122

American Public Health Association (1998). 'Standard Methods for the Examination of Water and Wastewater.' (APHA: Washington, DC, USA.)

Anderson, M. J., Ellinqsen, K. E., and McArdle, B. H. (2006). Multivariate dispersion as a measure of beta diversity. *Ecology Letters* **9**, 683–693. doi:10.1111/J.1461-0248.2006.00926.X

Anselin, L., and Bera, K. A. (1998). Spatial dependence in linear regression models with an introduction to spatial econometrics. In 'Handbook of Applied Economic Statistics'. (Eds A. Ullah and D. E. A. Giles.) pp. 237–290. (Marcel Dekker: New York, NY, USA.)

Bailey, R. G. (1980). Descriptions of the Ecoregions of the United States. Miscellaneous Publication number 1391, USDA Forest Service, Ogden, UT, USA. Available at Descriptions of the Ecoregions of the United States. Miscellaneous Publication number 1391 [Verified 27 July 2017].

Bailey, R. G. (1995). 'Ecosystem Geography.' (Springer-Verlag: New York, NY, USA.)

Bailey, R. C., Norris, R. H., and Reynoldson, T. B. (2004). 'Bioassessment of Freshwater Ecosystems Using the Reference Condition Approach.' (Kluwer Academic Publishers: Boston, MA, USA.)

Barbour, M. T., Gerritsen, J., Snyder, B. D., and Stribling, J. B. (1999). Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. EPA 841-B-99-002. Environmental Protection Agency, Washington, DC, USA.

Benda, L., Hassan, M. A., Church, M., and May, C. L. (2005). Geomorphology of steepland headwaters: the transition from hillslopes to channels. *Journal of the American Water Resources Association* **41**, 835–851. doi:10.1111/J.1752-1688.2005.TB04466.X

Bouchard, R. W., Jr, Niemela, S., Genet, J. A., Yoder, C. O., Sandberg, J., Chirhart, J. W., Feist, M., Lundeen, B., and Helwig, D. (2016). A novel approach for the development of tiered use biological criteria for rivers and streams in an ecologically diverse landscape. *Environmental Monitoring and Assessment* **188**, 196. doi:10.1007/S10661-016-5181-Y

Bowman, M. F., and Somers, K. M. (2005). Considerations when using the reference condition approach for bioassessment of freshwater ecosystems. *Water Quality Research Journal of Canada* **40**, 347–360.

Boyero, L., Pearson, R. G., Gessner, M. O., Dudgeon, D., Ramírez, A., Yule, C. M., Callisto, M., Pringle, C. M., Encalada, A. C., Arunachalam, M., Mathooko, J., Helson, J. E., Rincón, J., Bruder, A., Cornejo, A., Flecker, A. S., Mathuriau, C., M'Erimba, C., Gonçalves, J. F., Jr, Moretti, M., and Jinggut, T. (2015). Leaf-litter breakdown in tropical streams: is variability the norm? *Freshwater Science* **34**, 759–769. doi:10.1086/681093

Bryce, S. A., Lomnicky, G. A., and Kaufmann, P. R. (2010). Protecting sediment-sensitive aquatic species in mountain streams through the application of biologically based streambed sediment criteria. *Journal of the North American Benthological Society* **29**, 657–672. doi:10.1899/09-061.1

Burnham, K., and Anderson, D. (2002). 'Model Selection and Multimodel Inference: a Practical Information–Theoretical Approach.' (Springer: New York, NY, USA.)

Buss, D. F., Carlisle, D., Chon, T. S., Culp, J., Harding, J. S., Keizer-Vlek, H. E., Robinson, W. A., Strachan, S., Thirion, C., and Hughes, R. M. (2015). Stream biomonitoring using macroinvertebrates around the globe: a comparison of large-scale programs. *Environmental Monitoring and Assessment* **187**, 4132. doi:10.1007/S10661-014-4132-8

Callisto, M., Alves, C. B. M., Lopes, J. M., and Castro, M. A. (2014). 'Ecological Conditions in Hydropower Basins'. (Companhia Energética de Minas Gerais: Belo Horizonte, Brazil.)

Carmouze, J. P. (1994). 'O metabolismo dos ecossistemas aquáticos: fundamentos teóricos, métodos de estudo e análises químicas.' (Edgar Blücher Ltda/Fundação de Amparo à Pesquisa do Estado de São Paulo: São Paulo, Brazil.)

Chen, K., Hughes, R. M., Xu, S., Zhang, J., Cai, D., and Wang, B. (2014). Evaluating performance of macroinvertebrate-based adjusted and unadjusted multi-metric indices (MMI) using multi-season and multi-year samples. *Ecological Indicators* **36**, 142–151. doi:10.1016/J.ECOLIND.2013.07.006

Chessman, B. C. (2004). Bioassessment without reference sites: use of environmental filters to predict natural assemblages of river macroinvertebrates. *Journal of the North American Benthological Society* **23**, 599–615. doi:10.1899/0887-3593(2004)023<0599:BWRSUO>2.0.CO;2

Cooper, D. J., Andersen, D. C., and Chimner, R. A. (2003). Multiple pathways for woody plant establishment on floodplains at local and regional scales. *Journal of Ecology* **91**, 182–196. doi:10.1046/J.1365-2745.2003.00766.X

Corbet, P. S. (1999) 'Dragonflies: Behavior and Ecology of Odonata.' (Comstock Publishing Associates: Ithaca, NY, USA.)

Costa, C., Ide, S., and Simonka, C. E. (2006). 'Insetos Imaturos-Metamorfose e Identificacão.' (Holos: Ribeirao Preto, Brazil.)

Cummins, K. W., Peterson, R. C., Howard, F. O., Wuychek, J. C., and Holt, V. I. (1973). The utilization of leaf litter by stream detritivores. *Ecology* **54**, 336–345. doi:10.2307/1934341

Davies, S. P., and Jackson, S. K. (2006). The biological condition gradient: a descriptive model for interpreting change in aquatic ecosystems. *Ecological Applications* **16**, 1251–1266. doi:10.1890/1051-0761(2006)016[1251:TBCGAD]2.0.CO;2

Davies, P. E., Harris, J., Hillman, T., and Walker, K. (2010). The Sustainable Rivers Audit: assessing river ecosystem health in the Murray–Darling Basin, Australia. *Marine and Freshwater Research* **61**, 764–777. doi:10.1071/MF09043

De Cáceres, M., and Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. *Ecology* **90**, 3566–3574. doi:10.1890/08-1823.1

de Castro Vasconcelos, M., and Melo, A. S. (2008). An experimental test of the effects of inorganic sediment addition on benthic macroinvertebrates of a subtropical stream. *Hydrobiologia* **610**, 321–329. doi:10.1007/S10750-008-9447-4

de Oliveira-Junior, J. M. B., Shimano, Y., Gardner, T. A., Hughes, R. M., De Marco Júnior, P., and Juen, L. (2015). Neotropical dragonflies (Insecta: Odonata) as indicators of ecological condition of small streams in the eastern Amazon. *Austral Ecology* **40**, 733–744. doi:10.1111/AEC.12242

Diniz-Filho, J. A. F., Bini, L. M., and Hawkins, B. A. (2003). Spatial autocorrelation and red herrings in geographical ecology. *Global Ecology and Biogeography* **12**, 53–64. doi:10.1046/J.1466-822X.2003.00322.X

Dufrene, M., and Legendre, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* **67**, 345–366.

Elias, C. L., Calapez, A. R., Almeida, S. F. P., Chessman, B., Simões, N., and Feio, M. J. (2016). Predicting reference conditions for river bioassessment by incorporating boosted trees in the environmental filters method. *Ecological Indicators* **69**, 239–251. doi:10.1016/J.ECOLIND.2016.04.027

Feio, M. J., Reynoldson, T. B., Ferreira, V., and Graça, M. A. S. (2007). A predictive model for freshwater bioassessment (Mondego River, Portugal). *Hydrobiologia* **589**, 55–68. doi:10.1007/S10750-006-0720-0

Feio, M. J., Norris, R. H., Graça, M. A. S., and Nichols, S. (2009). Water quality assessment of Portuguese streams: regional or national predictive models? *Ecological Indicators* **9**, 791–806. doi:10.1016/J.ECOLIND.2008.09.012

Feio, M. J., Ferreira, W. R., Macedo, D. R., Eller, A. P., Alves, C. B. M., França, J. S., and Callisto, M. (2015). Defining and testing targets for the recovery of tropical streams based on macroinvertebrate communities and abiotic conditions. *River Research and Applications* **31**, 70–84. doi:10.1002/RRA.2716

Feminella, J. W. (2000). Correspondence between stream macroinvertebrate assemblages and 4 ecoregions of the southeastern USA. *Journal of the North American Benthological Society* **19**, 442–461. doi:10.2307/1468106

Fernández, H. R., and Domínguez, E. (2001). 'Guia para la Determinación de los Artrópodos Bentônicos Sudamericanos.' (Universidad Nacional de Tucumán: San Miguel de Tucumán, Argentina.)

Ferreira, W. R., Ligeiro, R., Hughes, R. M., Kaufmann, P. R., Oliveira, L. G., and Callisto, M. (2014). Importance of environmental factors on the richness and distribution of benthic macroinvertebrates in tropical headwater streams. *Freshwater Science* **33**, 860–871. doi:10.1086/676951

Ferreira, W. R., Ligeiro, R., Macedo, D. R., Hughes, R. M., Kaufmann, P. R., Oliveira, L. G., and Callisto, M. (2015). Is the diet of a typical shredder related to the physical habitat of headwater streams in the Brazilian Cerrado? *Annales de Limnologie* **51**, 115–127. doi:10.1051/LIMN/2015004

França, J. S., Gregório, R. S., De Paula, J. D., Gonçalves, J. F., Jr, Ferreira, F. A., and Callisto, M. (2009). Composition and dynamics of allochthonous organic matter inputs and benthic stock in a Brazilian stream. *Marine and Freshwater Research* **60**, 990–998. doi:10.1071/MF08247

Golterman, H. L., Clymo, R. S., and Ohmstad, M. A. M. (1978). 'Methods for Physical and Chemical Analysis of Freshwaters.' (Blackwell Scientific: London, UK.)

Gonçalves, J. F., Jr, and Callisto, M. (2013). Organic-matter dynamics in the riparian zone of a tropical headwater stream in Southern Brasil. *Aquatic Botany* **109**, 8–13. doi:10.1016/J.AQUABOT.2013.03.005

Gotelli, N. J., and Ellison, A. M. (2004). 'A Primer of Ecological Statistics.' (Sinauer Associates: Boston, MA, USA)

Graça, M. A. S., Ferreira, W. R., Firmiano, K., França, J., and Callisto, M. (2015). Macroinvertebrate identity, not diversity, is affected by substrate particle size in selected low order tropical streams. *Limnetica* **34**, 29–40.

Hamada, N., Nessimian, J. L., and Querino, R. B. (2014). 'Insetos Aquáticos na Amazônia Brasileira: Taxonomia, Biologia e Ecologia.' (Editora do INPA: Manaus, Brazil.)

Harrell, F. E. (2001). 'Regression Modeling Strategies: with Applications to Linear Models. Logistic Regression and Survival Analysis.' (Springer: New York, NY, USA.)

Hawkins, C. P., Norris, R. H., Gerritsen, J., Hughes, R. M., Jackson, S. K., Johnson, R. K., and Stevenson, R. J. (2000). Evaluation of the use of landscape classifications for the prediction of freshwater biota: synthesis and recommendations. *Journal of the North American Benthological Society* **19**, 541–556. doi:10.2307/1468113

Heino, J., Mykra, H., Kotanen, J., and Muotka, T. (2007). Ecological filters and variability in stream macroinvertebrate communities: do taxonomic and functional structure follow the same path? *Ecography* **30**, 217–230. doi:10.1111/J.0906-7590.2007.04894.X

Heino, J., Melo, A. S., Bini, L. M., Altermatt, F., Al-shami, S. A., Angeler, D. G., Bonada, N., Brand, C., Callisto, M., Cottenie, K., Dangles, O., Dudgeon, D., Encalada, A., Gothie, E., Gronroos, M., Hamada, N., Jacobsen, D., Landeiro, V. L., Ligeiro, R., Martins, R. T., Miserendino, M. L., Rawi, C. S. M., Rodrigues, M. E., Roque, F. O., Sandin, L., Schmera, D., Sgarbi, L. F., Simaika, J. P., Siqueira, T., Thompson, R. M., and Townsend, C. R. (2015). A comparative analysis reveals weak relationships between ecological factors and beta diversity of stream insect metacommunities at two spatial levels. *Ecology and Evolution* **5**, 1235–1248. doi:10.1002/ECE3.1439

Herlihy, A. T., Paulsen, S. G., Van Sickle, J., Stoddard, J. L., Hawkins, C. P., and Yuan, L. L. (2008). Striving for consistency in a national assessment: the challenges of applying a reference-condition approach at a continental scale. *Journal of the North American Benthological Society* **27**, 860–877. doi:10.1899/08-081.1

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., and Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**, 1965–1978. doi:10.1002/JOC.1276

Hughes, R. M., Larsen, D. P., and Omernik, J. M. (1986). Regional reference sites: a method for assessing stream potentials. *Environmental Management* **10**, 629–635. doi:10.1007/BF01866767

Hughes, R. M., Heiskary, S. A., Matthews, W. J., and Yoder, C. O. (1994). Use of ecoregions in biological monitoring. In 'Biological Monitoring of Freshwater Ecosystems'. (Ed. S. L. Loeb.) pp. 125–151. (Lewis: Chelsea, MI, USA.)

Jerez, V., and Moroni, J. (2006). Diversity of freshwater beetles of Chile. *Gayana* **70**, 72–81. [In Spanish].

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

Kaufmann, P. R., Levine, P., Robison, E. G., Seeliger, C., and Peck, D. V. (1999). Quantifying physical habitat in wadeable stream. EPA/620/R-99/003, US Environmental Protection Agency, Washington DC, USA.

Legendre, P., and Legendre, L. (1998). 'Numerical Ecology.' (Elsevier: Amsterdam, Netherlands.)

Ligeiro, R., Hughes, R. M., Kaufmann, P. R., Macedo, D. R., Firmiano, K. R., Ferreira, W. R., Oliveira, O., Melo, A. S., and Callisto, M. (2013). Defining quantitative stream disturbance gradients and the additive role of habitat variation to explain macroinvertebrate taxa richness. *Ecological Indicators* **25**, 45–57. doi:10.1016/J.ECOLIND.2012.09.004

Little, T. D., Lindenberger, U., and Nesselroade, J. R. (1999). On selecting indicators for multivariate measurement and modeling with latent variables: when 'good' indicators are bad and 'bad' indicators are good. *Psychological Methods* **4**, 192–211. doi:10.1037/1082-989X.4.2.192

Macedo, D. R., Hughes, R. M., Ligeiro, R., Ferreira, W. R., Castro, M. A., Junqueira, N. T., Oliveira, D. R., Firmiano, K. R., Kaufmann, P. R., Pompeu, P. S., and Callisto, M. (2014). The relative influence of catchment and site variables on fish and macroinvertebrate richness in Cerrado biome streams. *Landscape Ecology* **29**, 1001–1016. doi:10.1007/S10980-014-0036-9

Macedo, D. R., Hughes, R. M., Ferreira, W. R., Firmiano, K. R., Silva, D. R. O., Ligeiro, R., Kaufmann, P. R., and Callisto, M. (2016). Development of a benthic macroinvertebrate multimetric index (MMI) for neotropical savanna headwater streams. *Ecological Indicators* **64**, 132–141. doi:10.1016/J.ECOLIND.2015.12.019

Mackereth, F. J. H., Heron, J., and Talling, J. F. (1978). 'Water Analysis: Some Revised Methods for Limnologists.' (Freshwater Biological Association: Ambleside, UK.)

Merritt, R. W., and Cummins, K. W. (1996). 'An Introduction to the Aquatic Insects of North America.' (Kendall/Hunt Publishing: Dubuque, ID, USA)

Montgomery, D. R., Grant, G. E., and Sullivan, K. (1995). Watershed analysis as a framework for implementing ecosystem management. *Water Resources Bulletin* **31**, 369–386. doi:10.1111/J.1752-1688.1995.TB04026.X

Moretti, M. S., Loyola, R. D., Becker, B., and Callisto, M. (2009). Leaf abundance and phenolic concentrations codetermine the selection of case-building materials by *Phylloicus* sp. (Trichoptera, Calamoceratidae). *Hydrobiologia* **630**, 199–206. doi:10.1007/S10750-009-9792-Y

Moya, N., Tomanova, S., and Oberdorff, T. (2007). Initial development of a multi-metric index based on aquatic macroinvertebrates to assess streams condition in the Upper Isiboro-Sé cure Basin, Bolivian Amazon. *Hydrobiologia* **589**, 107–116. doi:10.1007/S10750-007-0725-3

Moya, N., Hughes, R. M., Dominguez, E., Gibon, F.-M., Goita, E., and Oberdorff, T. (2011). Macroinvertebrate-based multimetric predictive models for measuring the biotic condition of Bolivian streams. *Ecological Indicators* **11**, 840–847. doi:10.1016/J.ECOLIND.2010.10.012

Mugnai, R., Nessimian, J. L., and Baptista, D. F. (2010). 'Manual de Identificação de Macroinvertebrados Aquáticos do Estado do Rio de Janeiro.' (Technical Books Editora: Rio de Janeiro, Brazil.)

Muxika, I., Borja, A., and Bald, J. (2007). Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. *Marine Pollution Bulletin* **55**, 16–29. doi:10.1016/J.MARPOLBUL.2006.05.025

Nijboer, R. C., Johnson, R. K., Verdonschot, P. F. M., Sommerhäuser, M., and Buffagni, A. (2004). Establishing reference conditions for

European streams. *Hydrobiologia* **516**, 91–105. doi:10.1023/B:HYDR.0000025260.30930.F4

Omernik, J. M. 1995. Ecoregions: a spatial framework for environmental management. In 'Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making'. (Eds W. Davis and T. Simon.) pp. 49–62. (Lewis Publishers: Boca Raton, FL, USA.)

Omernik, J. M., and Bailey, R. G. (1997). Distinguishing between watersheds and ecoregions. *Journal of the American Water Resources Association* **33**, 935–949. doi:10.1111/J.1752-1688.1997.TB04115.X

Omernik, J. M., and Griffith, G. E. (2014). Ecoregions of the conterminous United States: evolution of a hierarchical spatial framework. *Environmental Management* **54**, 1249–1266. doi:10.1007/S00267-014-0364-1

Omernik, J. M., Griffith, G. E., Hughes, R. M., Weber, M. H., and Glover, J. B. (2017). How misapplication of the hydrologic unit framework diminishes the meaning of watersheds. *Environmental Management* **60**, 1–11. doi:10.1007/S00267-017-0854-Z

Pardo, I., Gómez-Rodríguez, C., Wasson, J. G., Owen, R., van de Bund, W., Kelly, M., Bennett, C., Birk, S., Buffagni, A., Erba, S., Mengin, N., Murray-Bligh, J., and Ofenböeck, G. (2012). The European reference condition concept: a scientific and technical approach to identify minimally-impacted river ecosystems. *The Science of the Total Environment* **420**, 33–42. doi:10.1016/J.SCITOTENV.2012.01.026

Peck, D. V., Herlihy, A. T., Hill, B. H., Hughes, R. M., Kaufmann, P. R., Klemm, D. J., Lazor-Chak, J. M., McCormick, F. H., Peterson, S. A., Ringold, P. L., Magee, T., and 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, US Environmental Protection Agency, Washington DC, USA.

Pereira, P. S., Souza, N. F., Baptista, D. F., Oliveira, J. L. M., and Buss, D. F. (2016). Incorporating natural variability in the bioassessment of stream condition in the Atlantic Forest biome, Brazil. *Ecological Indicators* **69**, 606–616. doi:10.1016/J.ECOLIND.2016.05.031

Pérez, G. R. (1988). 'Guía para el Estudio de los Macroinvertebrados Acuáticos del Departamento de Antioquia.' (Editorial Presencia Ltda: Bogotá, Colombia.)

Pescador, M. L., Rasmussen, A. K., and Harris, S. C. (2004). 'Identification Manual for the Caddisfly (Trichoptera) Larvae of Florida.' (Department of Environmental Protection: Tallahassee, FL, USA.)

Petts, G. E. (2000). A perspective on the abiotic processes sustaining the ecological integrity on running waters. *Hydrobiologia* **422/423**, 15–27. doi:10.1023/A:1017062032685

Pinto, B. C. T., Araujo, F. G., Rodriguez, V. D., and Hughes, R. M. (2009). Local and ecoregion effects on fish assemblage structure in tributaries of the Rio Paraíba do Sul, Brazil. *Freshwater Biology* **54**, 2600–2615. doi:10.1111/J.1365-2427.2009.02269.X

Poff, N. L. (1997). Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. *Journal of the North American Benthological Society* **16**, 391–409. doi:10.2307/1468026

Rabeni, C. F., and Doisy, K. R. (2000). Correspondence of stream benthic invertebrate assemblages to regional classifications in Missouri. *Journal of the North American Benthological Society* **19**, 419–428. doi:10.2307/1468104

Rangel, T. F. L. V. B., Diniz-Filho, J. A. F., and Bini, L. M. (2010). SAM: a comprehensive application for spatial analysis in macroecology. *Ecography* **33**, 46–50. doi:10.1111/J.1600-0587.2009.06299.X

Reynoldson, T. B., Rosenberg, D. M., and Resh, V. H. (2001). Comparison of models predicting invertebrate assemblages for biomonitoring in the Fraser River catchment, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1395–1410. doi:10.1139/F01-075

Ribeiro, K. T., and Freitas, L. (2010). Potential impacts of changes to Brazilian forest code in campos rupestres and campos de altitude. *Biota Neotropica* **10**, 239–246. doi:10.1590/S1676-06032010000400029

Riley, S. J., DeGloria, S. D., and Elliot, R. (1999). A terrain ruggedness index that quantifies topographic heterogeneity. *Intermountain Journal of Sciences* **5**, 23–27.

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., and Prat, N. (2007). Concordance between ecotypes and macroinvertebrate assemblages in Mediterranean streams. *Freshwater Biology* **52**, 2240–2255. doi:10.1111/J.1365-2427.2007.01826.X

Sánchez-Montoya, M. M., Vidal-Abarca, M. R., Puntí, T., Poquet, J. M., Prat, N., Rieradevall, M., Alba-Tercedor, J., Zamora-Muñoz, C., Toro, M., Robles, S., Álvarez, M., and Suárez, M. L. (2009). Defining criteria to select reference sites in Mediterranean streams. *Hydrobiologia* **619**, 39–54. doi:10.1007/S10750-008-9580-0

Silva, D. R. O., Ligeiro, R., Hughes, R. M., and Callisto, M. (2014). Visually determined stream mesohabitats influence benthic macroinvertebrate assessments in headwater streams. *Environmental Monitoring and Assessment* **186**, 5479–5488. doi:10.1007/S10661-014-3797-3

Silva, D. R. O., Herlihy, A. T., Hughes, R. M., and Callisto, M. (2017). An improved macroinvertebrate multimetric index for assessment of wadeable streams in the neotropical savanna, Brazil. *Ecological Indicators* **81**, 514–525. doi:10.1016/j.ecolind.2017.06.017

Stoddard, J. L., Larsen, D. P., Hawkins, C. P., Johnson, R. K., and Norris, R. H. (2006). Setting expectations for the ecological condition of streams: the concept of reference condition. *Ecological Applications* **16**, 1267–1276. doi:10.1890/1051-0761(2006)016[1267:SEFTEC]2.0.CO;2

Stoddard, J. L., Herlihy, A. T., Peck, D. V., Hughes, R. M., Whittier, T. R., and Tarquinio, E. (2008). A process for creating multi-metric indices for large-scale aquatic surveys. *Journal of the North American Benthological Society* **27**, 878–891. doi:10.1899/08-053.1

Strahler, A. N. (1957). Quantitative analysis of watershed geomorphology. *Transactions – American Geophysical Union* **38**, 913–920. doi:10.1029/TR038I006P00913

Tobler, A. W. R. (1970). A computer movie simulating urban growth in the Detroit region. *Economic Geography* **46**, 234–240. doi:10.2307/143141

US Environmental Protection Agency (2016). National rivers and streams assessment 2008–2009: a collaborative survey. EPA/841/R-16/007. (Office of Water and Office of Research and Development: Washington, DC, USA). Available at http://www.epa.gov/national-aquatic-resource-surveys/nrsa [Verified April 2017].

US Geological Survey (2015). SRTMGL1: NASA Shuttle Radar Topography Mission Global 1 arc second V003. (Land Processes Distributed Active Archive Center.) . doi:10.5067/MEASURES/SRTM/SRTMGL1.003

Van Sickle, J., and Hughes, R. M. (2000). Classification strengths of ecoregions, basins and geographic clusters for aquatic vertebrates in Oregon. *Journal of the North American Benthological Society* **19**, 370–384. doi:10.2307/1468101

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., and Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 130–137. doi:10.1139/F80-017

Villamarín, C., Rieradevall, M., Paul, M. J., Barbour, M. T., and Prat, N. (2013). A tool to assess the ecological condition of tropical high Andean streams in Ecuador and Peru: the IMEERA index. *Ecological Indicators* **29**, 79–92. doi:10.1016/J.ECOLIND.2012.12.006

von Sperling, E. (2012). Hydropower in Brazil: overview of positive and negative environmental aspects. *Energy Procedia* **18**, 110–118. doi:10.1016/J.EGYPRO.2012.05.023

Wang, L., Brenden, T., Seelbach, P., Cooper, A., Allan, D., Clark, R., Jr, and Wiley, R. (2008). Landscape based identification of human disturbance gradients and reference conditions for Michigan streams. *Environmental Monitoring and Assessment* **141**, 1–17. doi:10.1007/S10661-006-9510-4

Wasson, J. G., Barrera, S., Barrère, B., Binet, D., Collomb, D., Gonzales, I., Gourdin, F., Guyot, J. L. and Rocabado, G. (2002). Hydro-ecoregions of the Bolivian Amazon: a geographical framework for the functioning of river ecosystems. In 'The Ecohydrology of South American Rivers and Wetlands'. (Ed. M.E. McClain.) IAHS Special Publication number 6, pp. 69–91. (IAHS Press: Wallingford, UK.)

Whittier, T. R., Hughes, R. M., and Larsen, D. P. (1988). The correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon. *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 1264–1278. doi:10.1139/F88-149

Whittier, T. R., Stoddard, J. L., Larsen, D. P., and Herlihy, A. T. (2007). Selecting reference sites for stream biological assessments: best professional judgment or objective criteria. *Journal of the North American Benthological Society* **26**, 349–360. doi:10.1899/0887-3593(2007)26[349:SRSFSB]2.0.CO;2

Winkler, L. W. (1888). Die Bertimmung des im wasser gelosten Sauerstoffs. *Berichte der Deutschen Chemischen Gesellschaft* **21**, 2843–2854. doi:10.1002/CBER.188802102122