Importance of environmental factors for the richness and distribution of benthic macroinvertebrates in tropical headwater streams

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Abstract: An understanding of the interactions among local environmental factors (e.g., physical habitat and water quality) and aquatic assemblages is essential to conserve biodiversity in tropical and subtropical headwater streams. We evaluated the relative importance of multiple physical and chemical habitat variables that influence the richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT) assemblages in wadeable Brazilian Cerrado (savanna) streams. We sampled macroinvertebrate assemblages and quantified physical and chemical habitat in 79 randomly selected sites in 2 Cerrado basins in southeastern Brazil. The environmental variables selected by multiple regression models (MLRs) via corrected Akaike Information Criteria (*AICc*) contributed significantly to variation in EPT taxon richness. The variance explained by physical-habitat variables was slightly greater in the Upper São Francisco Basin (adjusted $R^2 = 0.53$) than in the Upper Araguari Basin (adjusted $R^2 = 0.46$), and both were greater than the variance explained by a combined basin model (adjusted $R^2 = 0.39$). Physical-habitat variables were more important than water-quality variables in structuring EPT genera in streams with catchments dominated by agriculture or pasture land uses. Regional models can be improved by incorporating basin-specific information to refine biological assessments and to provide better understanding of the interactions that maintain biodiversity in stream networks.

Key words: EPT assemblages, physical habitat, hydromorphology, stream conservation, macroinvertebrate bioindicators, Cerrado headwater streams

Agriculture, pasture, and riparian deforestation hinder stream conservation through their effects on in-stream habitat conditions (Dovciak and Perry 2002, Pinto et al. 2006, Egler et al. 2012). Physical-habitat structure and water quality have received attention recently as important elements of environmental quality and as agents structuring aquatic biotic assemblages (Karr and Dudley 1981, Sály et al. 2011, Ligeiro et al. 2013). Assessing and understanding the interactions among physical habitat features, water chemistry, and aquatic assemblages are essential to the conservation of headwater streams (Maddock 1999, Nerbonne and Vondracek 2001, Pinto et al. 2009). At the site scale, physical-habitat complexity (e.g., structural cover, substrates, and water flow) influences assemblage composition, richness, and temporal stability and ecological processes (Hughes et al. 2010, Kaufmann and Faustini 2012, Kovalenko et al. 2012).

Benthic macroinvertebrates often are used for biological assessments of environmental change in streams (Barbour et al. 1996, Lammert and Allan 1999, Bonada et al. 2006) because they show a continuum of responses to environmental variables (Rosenberg and Resh 1993, Maddock

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DOI: 10.1086/676951. Received 31 January 2013; Accepted 6 January 2014; Published online 14 May 2014.
Freshwater Science. 2014. 33(3):860–871. © 2014 by The Society for Freshwater Science.

1999, Dohet et al. 2008). These organisms are used often to assess water-body condition in spatially extensive biomonitoring programs (Hering et al. 2006, Paulsen et al. 2008, USEPA 2013) via the use of multimetric indices (Klemm et al. 2003, Baptista et al. 2007, Stoddard et al. 2008, Ferreira et al. 2011, Mugnai et al. 2011, Oliveira et al. 2011) and predictive models (Feio et al. 2009, Moya et al. 2011, Chen et al. 2014).

Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa are sensitive indicators of high-quality ecological conditions because of their low tolerance to stressors (Usseglio-Polatera et al. 2000, Callisto et al. 2001, Klemm et al. 2003, Ferreira et al. 2011). The EPT also are important in nutrient cycling (Righi-Cavallaro et al. 2010), processing of coarse organic matter (Graça et al. 2001, Boyero et al. 2011), and diets of vertebrates and invertebrates (Ferro and Sites 2007). Altered environmental conditions can adversely affect EPT taxon richness and composition. For example, removal of riparian vegetation may increase erosion, turbidity, water temperature, streambed sedimentation, and habitat loss (Chapman and Chapman 2002, Kaufmann et al. 2009). Increased fine sediments are detrimental to many EPT taxa (Bryce et al. 2010), and decreased wood and leaves reduce food and shelter for EPT assemblages (Melody and Richardson 2007).

The Cerrado biome (Brazilian tropical savanna) covers >2 million km^2, mostly inside Brazil, and is a priority hotspot for biodiversity conservation on a global scale because it supports many endemic species (Myers et al. 2000). In the past 60 y, >½ of the Cerrado has been deforested (Klink and Machado 2005, Wantzen et al. 2006), and large natural areas have been transformed into livestock pasture and croplands (Diniz-Filho et al. 2009). Replacement of native vegetation by pasture and intensive agriculture is associated with degradation of water quality, increased soil erosion, siltation of water bodies, and degradation of physical habitat (Dovciak and Perry 2002, Wantzen et al. 2006). Our objective was to evaluate the importance of site-scale physical-habitat and water-quality variables for EPT generic richness in Brazilian Cerrado streams. The goal is to use this information to manage headwater streams and their catchments. Adjacent basins in the same biome can differ in land use, ecological condition, geomorphology, and precipitation, so we hypothesized that 1) the 2 basins would differ in EPT assemblage structure and composition, 2) different sets of habitat metrics would explain EPT richness in each basin, and 3) a combined model would explain less variability than individual models.

METHODS

Study area

We studied seventy-nine 1st- to 3rd-order (at 1 : 100,000 scale) wadeable stream sites in the Upper São Francisco and Upper Araguari River Basins, Minas Gerais, southeastern Brazil (Fig. 1). Both basins are in the Cerrado biome at altitudes between 520 and 1300 m asl. The region experiences a distinct dry season from May to September (rainfall 10–55 mm/mo) and a distinct rainy season from October to April (rainfall 100–300 mm/mo). The total mean annual rainfall is ~1600 mm (Brasil 1992). In the Upper Araguari Basin, lithology is mostly metamorphic rock and land use is primarily agricultural (soy, coffee, corn, and sugar cane). In the Upper São Francisco Basin, the predominant lithology is sedimentary rocks and the land use is mostly pasture and small family farms (Ligeiro et al. 2013).

Site selection

We selected sites as described by Stevens and Olsen (2004) for the USA Wadeable Stream Assessment (Olsen and Peck 2008, USEPA 2013). Briefly, we drew randomly selected sites from a digital hydrographic map to produce a spatially balanced network of sites with a minimum distance of 1 km between sites. We sampled 40 sites in September 2009 in the Upper Araguari Basin and 39 sites in September 2010 in the Upper São Francisco Basin.

Physical habitat

At each site, we sampled a longitudinal distance equal to 40× the mean width, with a minimum distance of 150 m. We established 11 equidistant transects perpendicular to the longitudinal axis of the streams and defined 10 sections of the same length. In each transect and along the sections, we measured stream physical-habitat variables as described by Peck et al. (2006). At the 11 transects, we recorded channel dimensions (e.g., wetted and bankfull width and depth), bank angle, riparian vegetation (e.g., % cover of tree, understory, ground layer), presence and proximity of human disturbances (e.g., buildings, trash, land use), and presence of fish cover (e.g., undercut banks, trees and fallen branches, filamentous algae, aquatic macrophytes). We determined bed substrate (e.g., sand, gravel, boulder) at 100 points. In addition, we measured flow type (e.g., pools, riffles), thalweg depths, sinuosity, and channel slope between transects. We generated quantitative physical habitat metrics as described in Kaufmann et al. (1999), but calculated $\log_{10}$(relative bed stability) as in Kaufmann et al. (2009).

Water quality

We measured temperature, electrical conductivity, pH, turbidity, and total dissolved solids (TDS) in situ between 0900 and 1000 h using a multiparameter probe (650 MDS probe 6920; Yellow Springs Instruments, Yellow Springs, Ohio). In the laboratory, we measured the concentrations of total P (TP), total N (TN), and dissolved O_2 (DO) (APHA

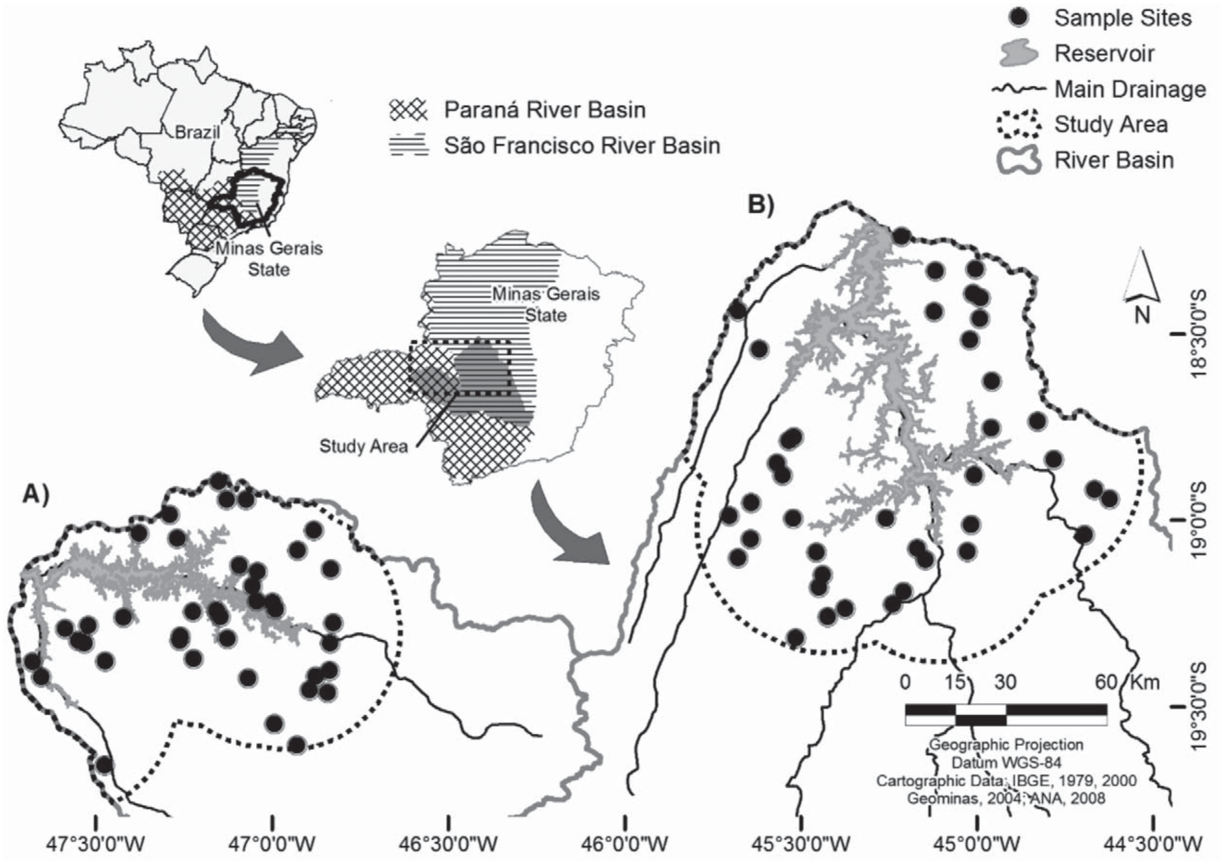

Figure 1. Study area and site locations in the Upper Araguari (A) and Upper São Francisco (B) Basins

2005) from a water sample taken from each site and retained in a chilled cooler.

Benthic macroinvertebrates

We collected benthic macroinvertebrates with a D-frame kick net (30-cm aperture, 500-μm mesh). We sampled 1 unit (0.09 m^2) per transect, totaling 1 m^2 of sampled area per site. The sampling followed a systematic zig-zag pattern (right, center, left) along the site. We preserved the composite samples with 4% formalin solution. In the Universidade Federal de Minas Gerais (UFMG) Benthic Ecology Laboratory, we washed the samples through a 500-μm sieve and sorted the organisms. We identified EPT individuals to genus with the aid of a stereomicroscope (80×) and taxonomic keys (Merritt and Cummins 1996, Wiggins 1996, Hamada and Couceiro 2003, Salles et al. 2004, Pés et al. 2005, Mugnai et al. 2010, Falcão et al. 2011).

Data analyses

To identify the most important metrics, we followed the procedures suggested by Marzin et al. (2012) and first separated 158 habitat metrics into 6 groups of metrics that described key stream-habitat characteristics (channel morphology, bed substrate, flow type, riparian vegetation, fish cover, and water quality). Within each group, we examined correlation matrices (Pearson product moment) to eliminate redundant metrics ($\geq|0.8|$ correlation) and retained the most ecologically meaningful ones. For instance, proportion of pools was highly correlated with proportion of trench pools (r = 0.94), so we chose proportion of pools because we consider it a more comprehensive metric describing slowly flowing habitat types. After this screening step, we retained a total of 83 metrics (15 in the channel-morphology group, 15 in the bed-substrate group, 6 in the flow-type group, 19 in the riparian-vegetation group, 23 in the fish-cover group, and 5 in the water-quality group).

In a 2nd metric screening step, we selected the metrics within each metric group that contributed most to the dispersion of the data in the multivariate space of a principal components analysis (PCA) and retained 29 metrics (Table 1). The 1st axis of each PCA (PCA1) represents the clearest univariate gradient formed by the habitat metrics in each group. The coordinate values of the metrics express the relative contribution of each metric to PCA1. For instance, PCA1 for the channel-morphology metric group in the Upper Araguari Basin represents a contrast between channel slope and channel size (depth and width) (Table 1).

Table 1. Mean (SD) values for physical-habitat metrics and physicochemical water variables and the factor scores on the 1st axis of the principal components analysis (PCA) ordination. * indicates significant *t*-test results, n/a = not assessed.

Variable group and name	Variable code	Comparison of basins			PCA 1st axis scores (% variation explained)	
		Upper Araguari Basin	Upper São Francisco Basin	*p*	Upper Araguari Basin	Upper São Francisco Basin
Channel morphology					(23.94%)	(30.73%)
Mean depth of cross-section (cm)	xdepth_s	20.63 (10.36)	27.77 (11.68)	0.005*	−0.61	−0.54
Mean wetted width (m)	xwidth	2.54 (1.08)	3.68 (2.44)	0.005*	−0.69	−0.89
Mean bankfull width (m)	xbkf_w	5.20 (1.74)	6.50 (3.61)	0.044*	−0.40	−0.85
Mean residual depth (cm)	rp100	10.27 (5.63)	23.72 (12.81)	<0.001*	−0.82	0.04
Reach mean water surface slope (%)	xslope	1.01 (0.62)	0.60 (0.59)	0.003*	0.45	−0.28
Channel sinuosity (m/m)	Sinu	3.53 (15.48)	1.05 (0.07)	0.314	0.04	0.54
Bed substrate					(32.09%)	(35.13%)
Mean embeddedness (channel and margin) (%)	xembed	64.08 (19.67)	58.38 (24.81)	0.259	0.56	0.79
SD of embeddedness in channel + margin (%)	vembed	35.77 (9.73)	35.63 (11.22)	0.955	−0.27	−0.48
$\log_{10}$ (relative bed stability)	lrbs	−2.52 (0.86)	−2.06 (1.33)	0.070	−0.78	−0.59
Size ($\log_{10}$[geometric mean diameter]) (mm)	lsub_dmm	−0.76 (0.87)	−0.64 (1.47)	0.645	−0.92	−0.96
Cobble (areal proportion with diameter 64–250 mm)	p_cb	0.05 (0.07)	0.06 (0.1)	0.618	−0.69	−0.45
Riparian					(25.32%)	(34.73%)
Mean mid-channel canopy density (%)	xcdenmid	77.06 (21.54)	73.03 (24.83)	0.440	0.70	−0.32
SD mid-channel canopy density (%)	vcdenmid	13.35 (10.50)	14.16 (8.59)	0.708	−0.57	0.21
Riparian vegetation cover (%)	xcmg	102.59 (30.81)	94.28 (43.19)	0.325	−0.31	−0.78
Flow type					(37.16%)	(49.21%)
Glide (proportion of reach)	p_gl	0.12 (0.17)	0.38 (0.31)	<0.001*	0.45	0.76
Pools (proportion of reach)	p_pool	0.28 (0.16)	0.52 (0.37)	<0.001*	0.61	0.86
Slow-water habitat (proportion glide + pool)	p_slow	0.40 (0.20)	0.89 (0.12)	<0.001*	0.88	−0.80
Shelter					(20.22%)	(29.32%)
Coarse litter (areal proportion)	p_bf	0.001 (0.01)	0.07 (0.15)	0.006*	n/a	−0.17
Large woody debris in channel (pieces/m^2)	clw_msq	0.07 (0.013)	0.05 (0.07)	0.296	−0.74	−0.77
Brush and small debris (areal % cover)	pct_xfc_brs	11.98 (15.57)	7.01 (9.49)	0.088	0.14	−0.81
Undercut banks (areal % cover)	pct_xfc_ucb	5.06 (5.41)	3.65 (5.28)	0.242	0.54	−0.40
Anthropogenic fish cover (areal % cover)	pct_xfc_ant	5.66 (17.02)	6.71 (11.82)	0.749	−0.39	0.22
Water quality					(31.54%)	(33.09%)
Dissolved O_2 (mg/L)	DO	7.47 (1.16)	7.67 (2.86)	0.694	0.62	−0.71
pH	pH	6.90 (0.46)	7.67 (0.49)	<0.001*	0.82	−0.12
Material in suspension (NTU)	Turbidity	7.56 (10.52)	8.22 (14.55)	0.816	−0.56	0.88
Total N (mg/L)	N−total	0.05 (0.01)	0.24 (0.98)	0.236	0.23	0.07
Electrical conductivity (μS/cm)	Cond.	23.2 (17.7)	76.1 (92.3)	<0.001*	–	–
Total dissolved solids (mg/L)	TDS	15.2 (11.8)	41.1 (33.5)	<0.001*	–	–
Water temperature	T°C	20.3 (1.8)	17.2 (1.8)	<0.001*	–	–

To identify the best predictor variables for explaining EPT generic richness, we ran a multiple linear regression (MLR) for each basin and for all 79 studied sites combined. The predictors were measured over a variety of numerical scales, so we mean-centered and standardized them before the analyses. We used the best-subsets procedure (Harrell 2001) for creating MLR models and included a maximum of 4 explanatory variables in the models for each individual basin and 8 explanatory variables in the model for the 2 basins combined to avoid model overfitting (Gotelli and Ellison 2004). We used the corrected Akaike Information Criteria (*AICc*) values to identify the best models (Burnham and Anderson 2002). The *AICc* is suitable for small data sets like ours. We also checked whether the models could be simplified, i.e., if the number of explanatory variables could be reduced in each case. When the difference between the *AICc* values of 2 models ($\Delta AICc$) is ≤ 2, the reduced model can be considered equivalent and preferable. We ran these analyses in Statistica for Windows (version 7; StatSoft, Tulsa, Oklahoma).

For each basin, we also used the PCA1 coordinate values as surrogate variables representing each of the dimensions of habitat quantified by the habitat groups (Table 1) in a canonical correspondence analysis (CCA) to assess EPT assemblage composition relative to the site environmental conditions. We ran the CCAs using only EPT genera with counts >10 individuals to avoid misinterpretation of the results. We $\sqrt{(x)}$-transformed invertebrate abundances because of the wide range in abundances. The CCAs were run in the *vegan* package (Oksanen et al. 2012) in R (version 2.15.1; R Project for Statistical Computing, Vienna, Austria).

We used metrical multidimensional scaling (MDS) ordination to evaluate dissimilarities in the taxonomic composition of benthic assemblages between the 2 basins. We used the altered Gower distance based on relative abundances, with $\log_2(x + 1)$-transformed data (Anderson et al. 2006) and Jaccard dissimilarity on presence/absence data. We used Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson 2001) with 10,000 simulations to test the observed difference. We also ran *t*-tests to detect significant differences ($p < 0.05$) in EPT richness and density, habitat, and water-quality metrics between the 2 basins. We ran the *t*-tests, MDS, and PERMANOVAs in R.

RESULTS

Physical and chemical variables

In general, sites in both basins had good quality water, with low values for TDS and N, and high values for DO. The largest differences in water quality between the 2 basins were observed for electrical conductivity ($t_{1,77} = -3.55$, $p < 0.001$), TDS ($t_{1,77} = -4.63$, $p < 0.001$), and water temperature ($t_{1,77} = 7.75$, $p < 0.001$). Sites in the Upper São Francisco Basin had higher mean electrical conductivity (76.1 μS/cm) and TDS (41.1 mg/L), whereas Upper Araguari sites had higher mean water temperature (20.3°C) (Table 1).

Site physical habitat differed between basins. The Upper Araguari sites had higher mean slopes and less slow-water habitat, whereas the Upper São Francisco sites had greater depths and larger cross-sectional widths. The highest mean proportion of cover (coarse litter) and flow types (proportion of glide, proportion of pools) occurred in the Upper São Francisco Basin (Table 1).

EPT assemblages

In the Upper Araguari Basin, we collected 5463 individuals distributed in 19 families and 61 genera, and in the Upper São Francisco Basin, we collected 15,133 individuals distributed in 20 families and 65 genera (Table 2). Total richness of EPT genera did not differ between basins. The Upper São Francisco Basin had fewer sites with EPT richness between 1 and 10 (15%) and more sites with taxon richness between 20 and 30 (36%). The Upper Araguari Basin had more sites (48%) with taxon richness between 10 and 20. Both basins had the same number of sites (5%) with taxon richness between 30 and 37 (Fig. 2A). Upper São Francisco sites supported higher EPT densities than Upper Araguari sites ($t_{1,7} = -3.54$, $p = 0.001$; Fig. 2B).

Based on the *AICc*, the best model selected for the Upper Araguari Basin had 3 predictor variables (adjusted $R^2 = 0.46$), and the best model for the Upper São Francisco Basin had 4 predictor variables (adjusted $R^2 = 0.53$) (Table 3). The best model selected for the combined basins had 4 predictor variables (adjusted $R^2 = 0.39$). The best models selected in both basins included stream bottom substrate (areal proportion of cobble, $\log_{10}$[relative bed stability]) and channel size (mean bankfull width, mean width) variables. Percent brush cover was important in the Upper Araguari Basin, and proportion of slow water and DO were important in the Upper São Francisco Basin. The combined model included size (mean bankfull width) and substrate (% cobble) variables, together with mean slope and SD of midchannel canopy density. We analyzed regression residuals to evaluate the validity of model assumptions.

The CCA axes accounted for little of the variability of EPT assemblages in both basins. In the Upper Araguari Basin, CCA axes explained only 21% (8 and 4% for axes 1 and 2, respectively; Fig. 3A) of the total variability. *Hagenulopsis*, *Camelobaetidius*, and *Varipes* abundances were correlated with the channel-morphology PCA1. The abundances of those organisms were higher in streams having lower mean depths, widths, and bankfull widths, and higher slopes and sinuosities. *Cloeodes* and *Polyplectropus* abundances were associated with the riparian PCA1, and *Chimarra*, *Leptohyphes*, and *Phylloicus* abundances were

Table 2. Ephemeroptera, Plecoptera, Trichoptera taxon list from sites of the Upper Araguari and Upper São Francisco Basins. Genera collected from only 1 basin are indicated in bold italic.

Upper Araguari Basin								Upper São Francisco Basin			
Ephemeroptera	*n*	Plecoptera	*n*	Trichoptera	*n*	Ephemeroptera	*n*	Plecoptera	*n*	Trichoptera	*n*
Americabaetis	211	*Anacroneuria*	288	*Alisotrichia*	1	*Americabaetis*	1761	*Anacroneuria*	210	*Alisotrichia*	3
Apobaetis	10	*Gripopteryx*	33	*Atopsyche*	5	*Apobaetis*	84	*Gripopteryx*	1	***Anchitrichia***	3
Askola	49	***Kempnyia***	57	*Austrotinodes*	9	*Askola*	16	***Macrogynoplax***	1	*Atopsyche*	48
Aturbina	26	***Paragrypopteryx***	278	*Barypenthus*	34	***Asthenopus***	56			*Austrotinodes*	5
Baetodes	87	***Tupiperla***	215	*Chimarra*	25	*Aturbina*	365			*Chimarra*	749
Caenis	116			***Grumicha***	2	*Baetodes*	190			***Cyrnellus***	27
Callibaetis	4			*Helicopsyche*	4	*Caenis*	1156			*Helicopsyche*	33
Camelobaetidius	19			*Hydroptila*	4	*Callibaetis*	1438			*Hydroptila*	145
Campylocia	9			***Itaura***	12	*Camelobaetidius*	84			*Leptonema*	41
Cloeodes	167			*Leptonema*	42	*Campsurus*	88			*Macronema*	86
Criptonympha	64			*Macronema*	12	*Campylocia*	31			*Macrostemum*	7
Farrodes	151			*Macrostemum*	9	*Cloeodes*	1117			*Marilia*	99
Hagenulopsis	33			*Marilia*	100	*Criptonympha*	33			*Metrichia*	71
Hydrosmilodon	2			*Metrichia*	4	*Farrodes*	757			*Mortoniella*	6
Hylister	1			*Mortoniella*	185	*Hagenulopsis*	38			*Nectopsyche*	80
Leptohyphes	71			*Nectopsyche*	81	***Hermanella***	32			*Neotrichia*	2
Massartella	37			*Neotrichia*	2	***Hexagenia***	6			*Ochrotrichia*	2
Miroculis	52			*Ochrotrichia*	3	*Hydrosmilodon*	353			*Oecetis*	48
Paracloeodes	64			*Oecetis*	22	***Latineosus***	20			*Oxyetira*	68
Rivudiva	1			*Oxyetira*	7	*Leptohyphes*	26			*Phylloicus*	44
Terpides	6			*Phylloicus*	53	*Massartella*	43			*Polycentropus*	116
Thraulodes	786			*Polycentropus*	3	*Miroculis*	317			*Polyplectropus*	95
Traverella	1			*Polyplectropus*	35	*Needhamella*	1			*Protoptila*	3
Traverhyphes	589			*Protoptila*	5	*Paracloeodes*	184			*Smicridea*	591
Tricorythodes	60			*Smicridea*	526	***Paramaka***	6			***Taraxitrichia***	1
Tricorythopsis	468			*Triplectides*	116	***Simothraulopsis***	40			*Triplectides*	18
Ulmeritoides	104					***Spiritiops***	8			***Wormaldia***	1
Varipes	15					*Terpides*	1				
Waltzoyphius	21					*Thraulodes*	631				
Zelusia	67					*Traverhyphes*	2729				
						Tricorythodes	200				
						Tricorythopsis	45				
						Ulmeritoides	267				
						Waltzoyphius	339				
						Zelusia	67				

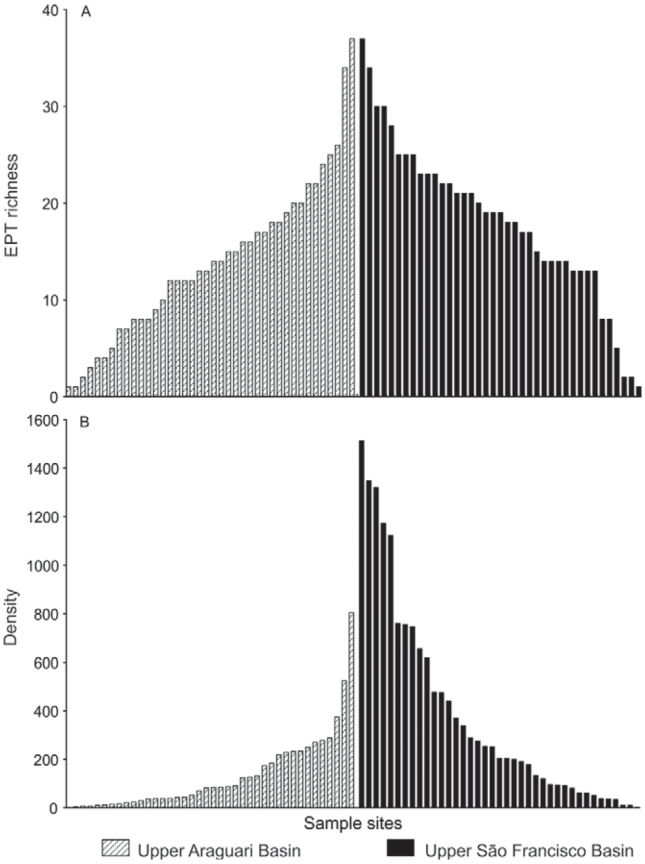

Figure 2. Distribution of Ephemeroptera, Plecoptera, and Trichoptera taxon richness (A) and densities of total individuals (ind/m^2) (B) at sites in the Upper Araguari Basin and Upper São Francisco Basin.

associated with the flow-type PCA1. *Itaura* and *Leptonema* abundances were associated with bed-substrate and water-quality PCA1. In the Upper São Francisco Basin, CCA axes explained 24% of the total variability in EPT assemblages (10 and 4% for axes 1 and 2, respectively; Fig. 3B). *Cyrnellus*, *Campsurus*, and *Macronema* abundances were associated with channel-morphology and water-quality PCA1s. These results indicated that those genera were associated with streams of smaller widths, depths, and slopes; lower DO concentrations; and higher sinuosities and turbidities. *Triplectides* and *Asthenopus* abundances were associated with the bed-substrate PCA1. *Hermanella*, *Camelobaetidius*, and *Leptohyphes* were associated with flow-type PCA1, and *Helicopsyche* and *Massartella* were associated with shelter PCA1. Considerable overlap in genera occurred between basins (Table 2), but we found no common correlations among abundances of individual genera and physical or chemical habitat predictors.

The MDS based on Jaccard's index indicated dissimilarity in the composition of EPT genera between basins (Fig. 4A). This separation was confirmed by the PERMANOVA test ($F_{1,77}$ = 9.91, p < 0.001). The MDS based on modified Gower distance (Fig. 4B) indicated greater overlap between basins. The difference between basins was confirmed by the PERMANOVA ($F_{1,77}$ = 7.99, p < 0.001).

DISCUSSION

Despite significantly greater EPT densities and fewer genus-depauperate sites in the Upper São Francisco Basin (Fig. 2A, B), the basins had similar total EPT generic richness. However, some genera differed between basins (Table 2), and the dissimilarity indices revealed differences between basins in the structure and composition of their EPT assemblages (Fig. 4A, B). The differences were confirmed in the MDS plots and PERMANOVA tests. Thus, we found support for hypothesis 1 (EPT assemblage structure and composition differ between basins).

In both basins individually and in the combined model, channel size, streambed substrate, and slope/flow metrics were significant predictors of EPT generic richness, as found by Klemm et al. (2003). However, the specific metrics explaining taxon richness in each of those categories differed slightly between basins: $\log_{10}$(relative bed stability) vs areal proportion of cobble, and mean width vs mean bankfull width for the Upper São Francisco Basin vs the Upper Araguari Basin, respectively. In addition, DO was an important predictor in the Upper São Francisco Basin and % brush cover was an important predictor in the Upper Araguari Basin. The explanatory variables were similar, but they differed enough that we accepted our 2nd hypothesis that different basins would yield different predictors of EPT generic richness. The combined model selected by the *AICc*, containing 4 predictor variables explained a little less of the EPT generic richness than the models of the individual basins according to the adjusted R^2 values, a result that supported our 3rd hypothesis. This result suggests that general regional models could be improved somewhat by incorporating basin-specific information and is in agreement with Stoddard et al. (2008) and USEPA (2013) who reached similar conclusions for national vs ecoregional models.

The importance of the many naturally varying metrics in our models supports the use of such variables or their surrogates in predictive models used for bioassessment. Failure to include natural variables at minimally disturbed sites when developing model expectations for macroinvertebrate assemblage condition in disturbed or test sites will lead to confounded or inaccurate biological assessments. Therefore, the effects of natural variability on various biological metrics are incorporated in predictive multimetric models to assess the effects of anthropogenic disturbances on assemblages (e.g., Pont et al. 2009, Moya et al. 2011, Marzin et al. 2012, 2013, Chen et al. 2014). Future studies should include these approaches and should combine field data with laboratory experiments (Woodward et al. 2012) and predictive modeling.

Table 3. Best subsets multiple linear regression (MLR) models of environmental variables explaining Ephemeroptera, Plecoptera, Trichoptera richness. *AICc* = Akaike's Information Criterion for small sample sizes, Δ*AICc* = difference in *AICc* between the full and reduced models, adj = adjusted. See Table 1 for full names of metrics.

Sites	Model	Metrics	β	β (SE)	*AICc*	*ΔAICc*
Upper Araguari Basin	4 variables	xbkf_w	0.40	0.13	271.65	1.16
	R^2 adj. = 0.51; $F_{4.38}$ = 11.19; $p < 0.001$	p_cb	0.43	0.12		
		pct_xfc_brs	0.29	0.12		
		xslope	−0.28	0.13		
	3 variables	xbkf_w	0.51	0.12	272.81	
	R^2 adj. = 0.46; $F_{4.38}$ = 12.19; $p < 0.001$	p_cb	0.32	0.12		
		pct_xfc_brs	0.23	0.19		
Upper São Francisco Basin	4 variables	p_slow	−0.64	0.14	256.04	–
	R^2 adj. = 0.53; $F_{4.39}$ = 13.15; $p < 0.001$	lrbs	0.33	0.11		
		xwidth	0.34	0.13		
		DO	−0.36	0.14		
Combined basins	8 variables	xbkf_w	0.44	0.10	530.932	0.005
	R^2 adj. = 0.44; $F_{8.79}$ = 8.69; $p < 0.001$	vcdenmid	−0.16	0.10		
		p_cb	0.21	0.10		
		xslope	−0.22	0.10		
		xembed	−0.17	0.10		
		p_pool	188.87	71.34		
		p_gl	168.18	63.50		
		p_slow	−180.64	68.20		
	4 variables	xbkf_w	0.53	0.10	530.927	
	R^2 adj. = 0.39; $F_{8.79}$ = 13.96; $p < 0.001$	xslope	−0.19	0.10		
		p_cb	0.26	0.10		
		vcdenmid	−0.20	0.10		

Our MLRs explained nearly ½ the variation in EPT generic richness. Marzin et al. (2013) used partial constrained redundancy analyses and found that reach-scale environmental variables explained only 11% of the variability in macroinvertebrate taxonomic composition of 301 French stream sites. These results may indicate the value of obtaining multiple quantitative physical-habitat measurements vs the qualitative observations commonly used in stream surveys (Barbour et al. 1999, Marzin et al. 2012a, b). Streams with minimally disturbed riparian forest contribute branches and large wood to channels, thereby increasing habitat complexity and habitats that favor increased abundance of macroinvertebrates (Kaufmann and Faustini 2012). Ligeiro et al. (2013) reported that the Upper São Francisco Basin experienced lower levels of agriculture and general anthropogenic disturbance than the Upper Araguari Basin. The small towns and agriculture in the Upper Araguari Basin may be affecting EPT taxa via increased erosion, stream sedimentation, and the resulting degradation of physical habitats and water quality. This, combined with physicochemical variables (e.g., electrical conductivity, TDS) and flow types (Table 1), may have affected site-level EPT richness and abundance.

Streambed sediment size is a major factor governing macroinvertebrate richness and abundance. Bryce et al. (2010) described the importance of sediment size for structuring benthic macroinvertebrate assemblages and recommended levels of fine sediments that would protect sediment-sensitive macroinvertebrate and fish taxa in mountain streams of the western USA. Duan et al. (2008) reported a positive relationship between benthic macroinvertebrates, pebbles, and cobbles, which are more stable substrates than sand and fine sediments.

Water quality and the relative occurrence of habitat types also affect macroinvertebrate assemblage composition. For example, *Hydroptila* (Trichoptera:Hydroptilidae) are found more frequently in slowly flowing waters and pools than in faster moving water (Dolédec et al. 2007). The distribution and composition of benthic assemblages also are affected by dissolved O_2 (DO) (Baptista et al. 2007, Ferreira et al. 2011) and conductivity (Kennedy et al. 2004, Pond 2010).

Our site-scale physical-habitat variables explained little of the distribution and abundance variability of EPT genera composition via CCAs. On the other hand, MLR models were better able to explain relationships between

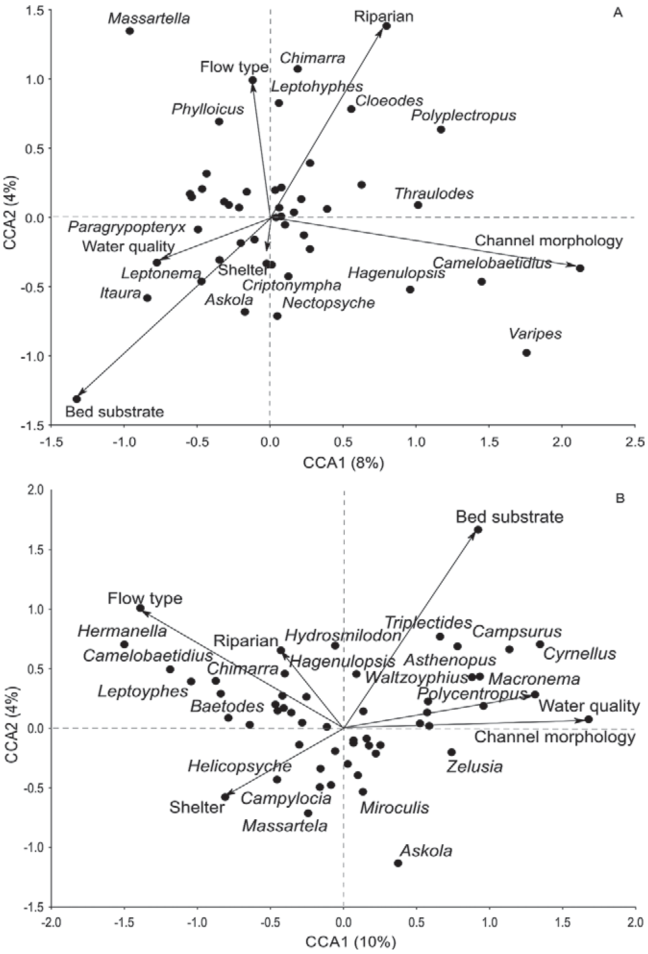

Figure 3. Canonical correspondence analysis (CCA) plots for Ephemeroptera, Plecoptera, Trichoptera genera and environmental variables at sites in the Upper Araguari Basin (A) and the Upper São Francisco Basin (B). The length and direction of the arrows indicate the strength of correlation of the environmental variable with the ordination axes.

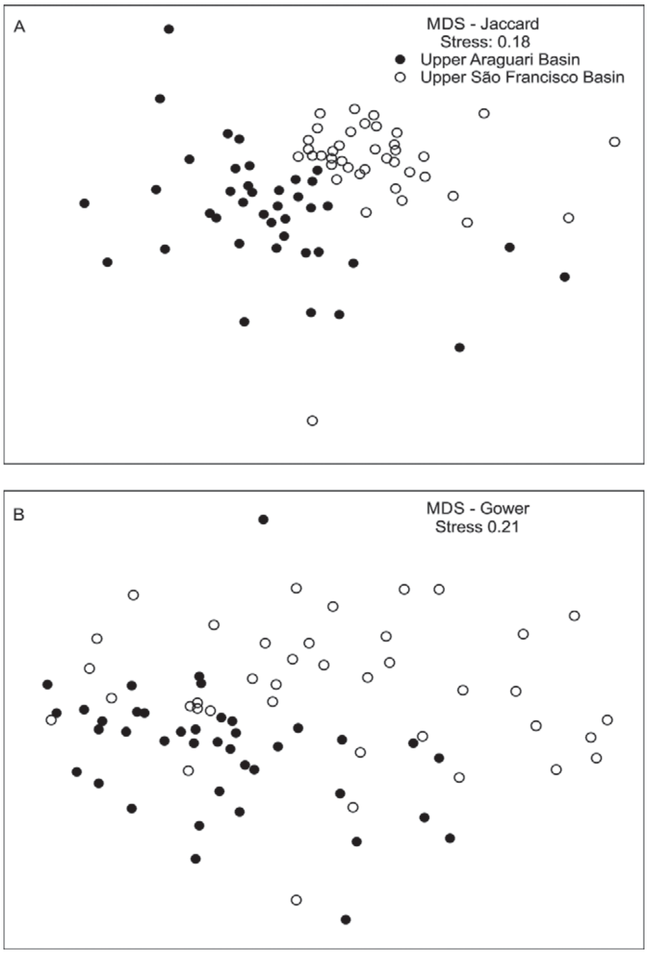

Figure 4. Dissimilarities of Upper Araguari Basin and Upper São Francisco Basin sites in metrical multidimensional scaling (MDS) ordinations of Jaccard (A) and altered Gower (B) dissimilarities.

taxonomic richness and habitat variables. We stress that MLR models are developed to analyze the variability of a single response variable (in our case, taxonomic richness), whereas CCA analyzes the variability in the composition of the whole assemblage. Explaining the variability of a single response variable is easier than explaining the variability of multiple species simultaneously, one reason for the differing performances of MLR models vs CCA. Regardless of which analysis explains the greater amount of variability, both offer important and complementary insights that help us understand assemblage structure.

In summary, we showed the importance of site-scale physical-habitat factors for understanding differences in EPT assemblage richness in headwater Cerrado streams. Because we used a randomized and spatially balanced survey design, we can confidently assume that the ecological data were representative of the region (Stevens and Olsen 2004). We think the information in our study may be useful for suggesting improved ecological assessment programs, watershed management practices, aquatic ecosystem rehabilitation measures, and protection strategies for aquatic biota of the Brazilian Cerrado, especially those that limit the amount of sand and fine sediments entering streams.

ACKNOWLEDGEMENTS

We thank the Peixe-Vivo Program of Companhia Energética de Minas Gerais, Pesquisa & Desenvolvimento/Agência Nacional de Energia Elétrica/Companhia Energética de Minas Gerais-P&D ANEEL/CEMIG GT-487, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa do Estado de Minas Gerais, and Fulbright-Brasil for financial support. MC received a research grant and a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq no. 471385/211-5 and 302960/2011-2) and research grant from the Fundação de Amparo da Pesquisa do

Estado de Minas Gerais (FAPEMIG PPM-00077/13). Tony Olsen, Marc Weber, and Phil Larsen of the Corvallis US Environmental Protection Agency Laboratory (Oregon, USA) kindly assisted in developing spatial sampling designs and selecting wadeable stream sample sites in our Brazilian basins. We thank Juliana França, Déborah Silva, and Ana Paula Eller for water-quality analyses; Carlos Bernardo Mascarenhas Alves for logistical support; colleagues from the Federal University of Minas Gerais Benthic Ecology Laboratory, Federal Center of Technological Education of Minas Gerais, Federal University of Lavras, and Pontifical Catholic University of Minas Gerais for help with field collections; and researchers at the US EPA Western Ecology Division, Corvallis, Oregon, for discussions during an October 2011 workshop. Our manuscript was improved based on reviews by Bob Ozretich and Michael Griffith of the US EPA and 2 anonymous referees. This manuscript has been subjected to review by the US EPA National Health and Environmental Effects Research Laboratory's Western Ecology Division and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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