



Do wider riparian zones alter benthic macroinvertebrate assemblages' diversity and taxonomic composition in neotropical headwater streams?

Zonas ripárias mais largas alteram a diversidade e composição taxonômica de assembleias de macroinvertebrados bentônicos em riachos de cabeceira neotropicais?

Marden S. Linares^{1*} , Livia B. dos Santos² , Marcos Callisto¹  and Jean C. Santos² 

¹Laboratório de Ecologia de Bentos, Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais – UFMG, Av. Antônio Carlos, 6627, CEP 31270-901, Pampulha, Belo Horizonte, MG, Brasil

²Departamento de Ecologia, Universidade Federal de Sergipe – UFS, Campus São Cristóvão, Cidade Universitária Prof. José Aloísio de Campos, CEP 49100-000, São Cristóvão, SE, Brasil

*e-mail: mslx@hotmail.com; mslinares@ufmg.br

Cite as: Linares, M.S. et al. Do wider riparian zones alter benthic macroinvertebrate assemblages' diversity and taxonomic composition in neotropical headwater streams? *Acta Limnologica Brasiliensia*, 2021, vol. 33, e22.

Abstract: Aim: The maintenance and condition of riparian vegetation are important factors for conserving headwater streams and their species diversity. Thus, variations in the width of a riparian zone can have dramatic effects in the structure and functioning of the adjacent freshwater ecosystem. In this study, we aimed to determine if increased riparian zone width changed the benthic assemblages' structure (diversity, taxonomic and functional composition) in headwater streams. **Methods:** We tested two predictions: (1) increased riparian zone width will change the diversity and taxonomic composition of benthic macroinvertebrate assemblages because narrow riparian zones do not buffer the anthropogenic impacts from the surrounding landscape; (2) wider riparian zones will change benthic macroinvertebrate assemblages' functional structure, due to changes to energetic input and quality. To test the first prediction, we assessed the benthic macroinvertebrate assemblages' taxonomic composition, richness and Shannon-Wiener diversity index. To test the second prediction, we assessed functional feeding groups (FFG) and metrics based on their proportion. **Results:** Our results showed that our first prediction was not corroborated, because taxonomic structure and diversity did not show significant variation with increased riparian zone width. Our second prediction was partially corroborated, because there were significant alterations in the functional structure of benthic macroinvertebrate assemblages between the narrowest riparian zone width (30 m) and the others two (50 and 100 m). **Conclusions:** Our results suggest that, contrary to the Brazilian Federal Law 12651/2012, 30-m wide riparian zones are insufficient to protect headwater stream ecosystem functioning.

Keywords: benthic macroinvertebrates; functional feeding groups; functional metrics; bioindicators; ecological processes.

Resumo: Objetivo: A conservação da vegetação ripária é um fator importante para a conservação de riachos de cabeceira e sua biodiversidade. Assim, variações na largura da zona ripária podem ter efeitos dramáticos na estrutura e funcionamento de ecossistemas lóticos adjacentes. Nesse estudo objetivamos determinar se o aumento da largura da zona ripária altera a estrutura de assembleias bentônicas (diversidade taxonômica e funcional e composição taxonômica) em riachos de cabeceira. **Métodos:**



Testamos duas predições: (1) o aumento da largura da zona ripária altera a diversidade e a composição taxonômica de assembleias de macroinvertebrados bentônicos, porque as zonas ripárias mais estreitas não protegem as pressões antrópicas da paisagem circundante; (2) zonas ripárias mais largas alteram a estrutura funcional de assembleias de macroinvertebrados bentônicos, devido a mudanças na entrada de energia nos sistemas. Para testar a primeira predição, avaliamos a composição taxonômica de assembleias de macroinvertebrados bentônicos, a riqueza taxonômica e o índice de diversidade de Shannon-Wiener. Para testar a segunda predição, avaliamos grupos funcionais de alimentação e métricas baseadas em sua proporção. **Resultados:** Nossos resultados revelaram que a primeira predição não foi corroborada, pois a estrutura taxonômica e a diversidade não apresentaram variação significativa com o aumento da largura de zona ripária. Nossa segunda predição foi parcialmente corroborada, pois houve alterações significativas na estrutura funcional de assembleias de macroinvertebrados bentônicos entre a largura da zona ribeirinha mais estreita (30 m) e as duas outras (50 e 100 m). **Conclusões:** Nossos resultados sugerem que, ao contrário da Lei Federal 12651/2012, as zonas ribeirinhas de 30 m de largura são insuficientes para proteger o funcionamento de ecossistemas de córregos de cabeceira.

Palavras-chave: macroinvertebrados bentônicos; grupos funcionais de alimentação; métricas funcionais; bioindicadores; processos ecológicos.

1. Introduction

Headwater streams represent the majority, circa 80%, of the total stream length in river basins (MacDonald & Coe, 2007). These ecosystems usually harbor high biodiversity and are tightly linked to the surrounding terrestrial ecosystems, forming a riparian stream meta-ecosystem (Callisto et al., 2019; Osakpolor et al., 2021). The condition of riparian ecosystems are very important factors for conserving headwater streams because they act as buffers between streams and anthropogenic disturbances in the surrounding terrestrial landscape (Gregory et al., 2007; Tonkin, 2014). Riparian vegetation is also one of the main factors influencing ecosystem structure and functioning in headwater streams (Rezende et al., 2016). This is because it regulates temperature and controls solar energy availability to stream ecosystem metabolism while providing allochthonous detritus as an alternative energy source (Linares et al., 2018; Santos et al., 2019). Thus, variations in the width of riparian zones can have dramatic effects on the structure (taxonomic and functional) of adjacent lotic ecosystems (Milner & Gloyne-Phillips, 2005; Rios & Bailey, 2006; Stanford et al., 2020).

Riparian vegetation width is an essential parameter for biodiversity conservation policies (Dala-Corte et al., 2020). In Brazil, Federal Law 12651/2012 states that, for streams up to 10m wide, at least 30m of riparian vegetation width must be protected in all biomes. In spite of this, there is little scientific support that this minimum riparian zone width is adequate to maintain the biodiversity of the associated stream ecosystems (Brito et al., 2020; Leal et al., 2018; Luke et al., 2019; Metzger, 2010).

Among the many taxa that comprise lotic ecosystem biodiversity, benthic macroinvertebrates

are one of the most widely used as bioindicators, because of their ability to respond predictably to modifications in freshwater environments (Bonada et al., 2006). The structure of benthic macroinvertebrate assemblages strongly correlates with ecological condition at local and regional spatial extents, and can provide information on the effect of anthropogenic disturbances on lotic ecosystems (Firmiano et al., 2021, 2017; Libório and Tanaka, 2016).

Taxonomic assessments, however, may not always sufficiently indicate changes in ecosystem functioning (Benke, 1993; Benke & Huryn, 2010). Differences in habitat are often reflected mainly in ecosystem processes and may not be perceived through taxonomic indicators (Aguiar et al., 2015). However, direct measurements of ecosystem functioning are difficult for natural assemblages because they require intensive field sampling efforts (Dolbeth et al., 2012). Therefore, proxy metrics based on functional feeding groups may reflect ecosystem functioning (Cummins et al., 2005; Linares et al., 2019).

In this study, we aimed to determine the degree to which increased riparian zone width changed the structure (diversity, taxonomic and functional composition) of headwater stream ecosystems. To do so, we compared 30m, 50m and 100m wide riparian zones. We tested two predictions. (1) Increased riparian zone width will change the diversity and taxonomic composition of benthic macroinvertebrate assemblages because narrow riparian zones do not buffer the anthropogenic impacts from the surrounding landscape. We predicted that sites with greater riparian zone widths would have taxonomic richness, Shannon diversity index and taxonomic

composition significantly different from sites with lower riparian zone width; (2) Wider riparian zones will change benthic macroinvertebrate assemblages' functional structure, due to changes to energetic input and quality. We predicted that the scores of the five functional feeding group metrics would be significantly different in streams with wider riparian zones than in those with narrower zones.

2. Material and Methods

2.1. Study area

We conducted this study in the Grande River basin, inside the Rio Uberaba Environmental Protection Area, Minas Gerais state, southeastern Brazil. This area is a biodiversity reserve but allows sustainable land uses, mainly pastures and cultivations of coffee, soy and sugarcane (Mauro et al., 2016). We selected nine stream sites among the streams (Figure 1), divided into three categories based on the width of the riparian zone: three sites with an approximately 30m riparian zone width, three with an approximately 50m riparian zone width and three with an approximately 100m riparian zone width. All sampling sites were chosen to have similar physical habitat characteristics: no direct human disturbances (reference condition)

within the streams, predominance of riffle habitat, sandy substrate and similar amounts of organic matter, water with similar temperature, conductivity, turbidity and total dissolved solids (Table 1). To guarantee that the conditions were similar between sampling sites, we ran a series of preliminary analyses comparing the physical and chemical variables (Figures 2-6). Since we did not find any significant differences on these variables, we can attribute a large proportion of the variation observed in the benthic macroinvertebrate assemblages to differences in the riparian zone width. For establishing riparian zone width, we used satellite images gathered in the Google Earth software, and then verified the measurements in situ by using a measuring tape. The measurements were taken in the same area where the benthic macroinvertebrate samplings were taken. The sampling campaigns were made in both rainy (December/2015) and dry (June/2016) seasons, pooled together to sample the full extent of the sampling sites seasonal variability.

2.2. Benthic macroinvertebrate sampling & processing

In each of the nine stream sites, benthic macroinvertebrates were collected using a D-net

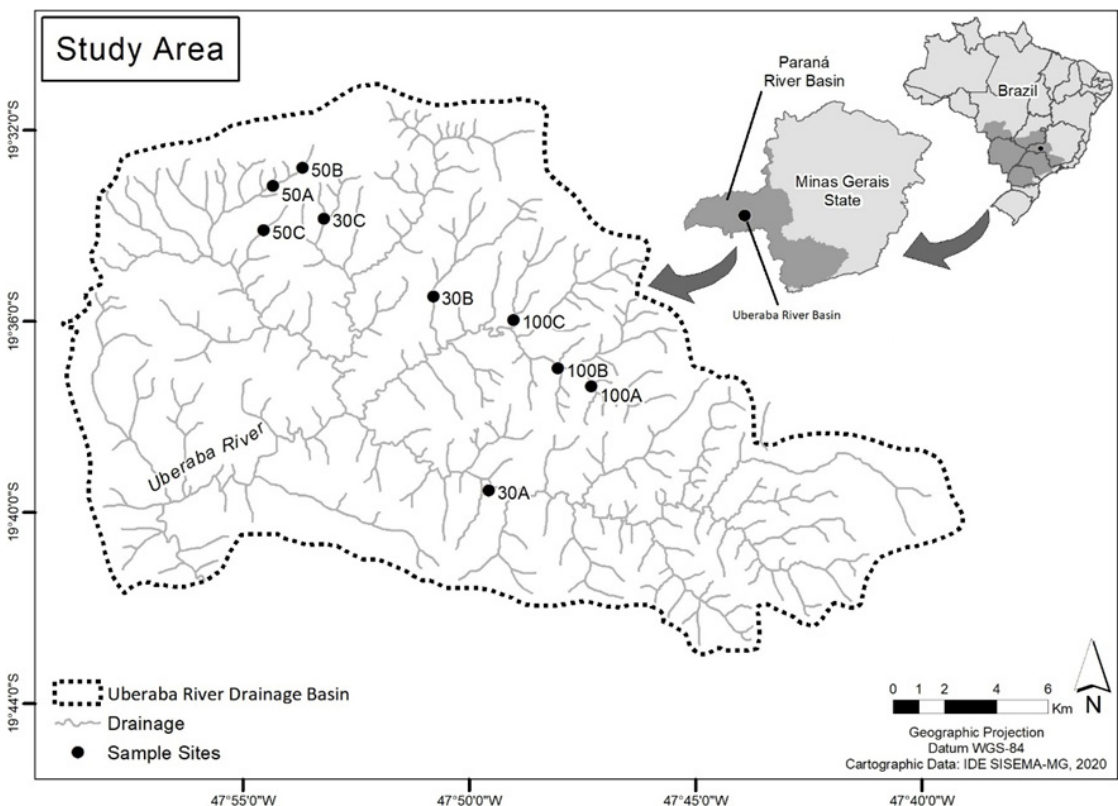


Figure 1. Location of the sampling sites in the Uberaba River drainage basin, a tributary of the Rio Grande basin.

Table 1. Measured physical habitat and water quality metrics.

Stream Site	Width	Season	Sediment	Water Temperature	Sediment Organic Matter	Conductivity	Total Dissolved Solids	Turbidity
30A	30m	Rainy	Sandy	22.9	0.6	91	45	20.4
30B	30m	Rainy	Sandy	23.49	0.1	140	69	10
30C	30m	Rainy	Sandy	22.8	0.1	87	43	12.1
50A	50m	Rainy	Sandy	23.66	0.1	3	1	4.54
50B	50m	Rainy	Sandy	23.39	0.1	143	71	9.73
50C	50m	Rainy	Sandy	23.2	1.3	2	1	2.78
100A	100m	Rainy	Sandy	24.2	0.8	1	1	2.2
100B	100m	Rainy	Sandy	23.6	0.1	113	56	11.3
100C	100m	Rainy	Sandy	22.63	2	198	99	2.41
30A	30m	Dry	Sandy	15.74	0.4	74	37	4.92
30B	30m	Dry	Sandy	17.61	1.3	91	45	5.17
30C	30m	Dry	Sandy	17.8	0.3	54	27	11.5
50A	50m	Dry	Sandy	16.8	0.5	2	1	5.1
50B	50m	Dry	Sandy	17.44	0.3	91	45	4.9
50C	50m	Dry	Sandy	19.1	0.8	2	1	5.24
100A	100m	Dry	Sandy	18.5	0.5	1	1	1.57
100B	100m	Dry	Sandy	17.9	0.4	73	36	7.1
100C	100m	Dry	Sandy	17.7	0.7	168	84	1.5

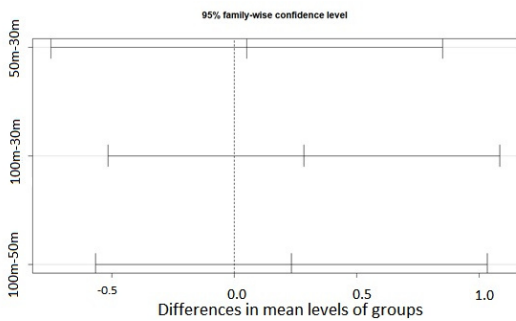


Figure 2. Post-hoc Tukey HSD test results of Organic Matter percentage in the sediment between riparian zone width categories.

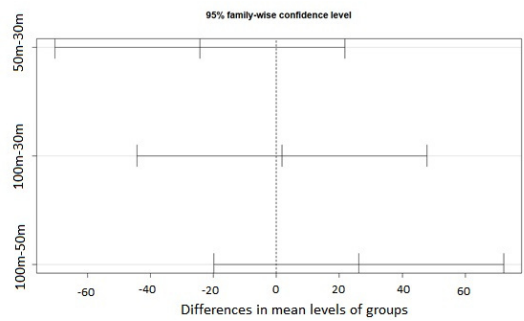


Figure 4. Post-hoc Tukey HSD test results of Total Dissolved Solids (mg/l) in the water column between riparian zone width categories.

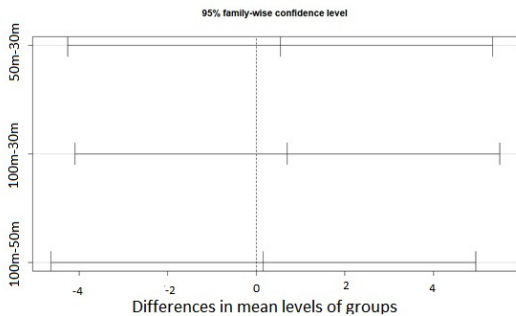


Figure 3. Post-hoc Tukey HSD test results of Water Temperature (°C) between riparian zone width categories.

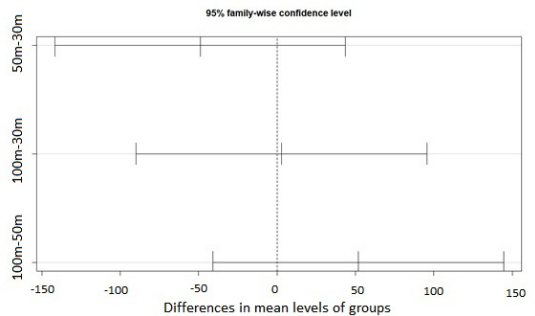


Figure 5. Post-hoc Tukey HSD test results of Conductivity (µS/cm) in the water column between riparian zone width categories.

sampler (250 µm mesh) in a small area (1m²) for two minutes, with a single sampling at each sampling site (Callisto et al., 2021). The samples were stored in plastic bags, fixed with 70% alcohol

and transported to the laboratory. In the laboratory, the samples were washed over a sieve (250 µm mesh). The invertebrates retained in the sieve were

placed in plastic jars and fixed with 70% ethanol. The specimens were identified to family (Insecta), class (Mollusca) or subclass (Annelida) levels using Mugnai et al. (2010). That level of taxonomic resolution requires markedly less laboratory time without compromising the performance of the tested indices (Heino et al., 2018; Silva et al., 2017; Whittier & van Sickle, 2010).

2.3. Functional feeding group metrics

To test if wider riparian zones will change benthic macroinvertebrate assemblages' functional structure, we assessed functional feeding groups (FFG) and a series of metrics derived from them (Cummins et al., 2005; Table 2). For that, the taxa were classified into one of the five FFG: gathering-collectors, filtering-collectors, shredders, scrapers, or

predators. These groups are defined by the way they acquire their main food source: gathering FPOM deposited in the sediment (gathering-collectors); filtering FPOM on the water column (filtering-collectors); shredding CPOM (shredders); scraping periphyton on hard surfaces (scrapers); preying on other animals (predators) (Cummins et al., 2005; Merritt et al., 2008; Ramírez & Gutiérrez-Fonseca, 2014).

We then calculated five metrics based on the functional feeding groups that serve as proxies for ecosystem functioning (Cummins et al., 2005). 1) The autotrophy to heterotrophy ratio was calculated as $\frac{\text{scrapers}}{\text{shredders} + \text{collectors}}$; 2) A surrogate for the amount of coarse organic matter (CPOM) versus fine particulate organic matter (FPOM) was calculated as $\frac{\text{shredders}}{\text{collectors}}$; 3) A surrogate for the amount of fine organic sediment (FPOM) transported in the water column from that in the bed sediment was calculated as $\frac{\text{filtering collectors}}{\text{gathering collectors}}$; 4) The substrate stability index was estimated as $\frac{\text{scrapers} + \text{filtering collectors}}{\text{shredders} + \text{gathering collectors}}$; 5) The top-down control index was calculated as $\frac{\text{predators}}{\text{all other FFG}}$.

2.4. Data analyses

Due the spatial proximity of stream sites, we ran Moran's I tests for spatial autocorrelation

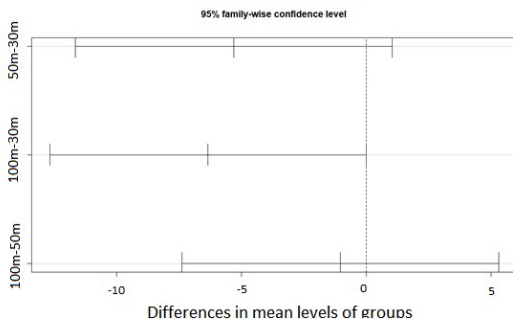


Figure 6. Post-hoc Tukey HSD test results of Turbidity (NTU) in the water column between riparian zone width categories.

Table 2. Functional Feeding Groups (FFG) metrics.

Stream Site	Width	Season	Production/ Respiration	CPOM/ FPOM	TFPOM/ BFPOM	Stable_Channel	TOP-DOWN-CONTROL	Richness	Shannon
30A	30m	Rainy	0	0.020134	0.295652	0.288136	0.111842	21	2.433422
30B	30m	Rainy	0	0.008658	0.03125	0.030973	0.090129	20	1.745826
30C	30m	Rainy	0	0	0.108374	0.108374	0.088889	18	1.583867
50A	50m	Rainy	0	0.009259	0.018868	0.018692	0.211009	21	1.653316
50B	50m	Rainy	0	0.01487	0.030651	0.030189	0.062271	23	1.865904
50C	50m	Rainy	0	0.005128	0.010363	0.010309	1.270408	23	1.73321
100A	100m	Rainy	0	0.028169	0.025271	0.024561	0.212329	21	1.928693
100B	100m	Rainy	0	0	0.060241	0.060241	0.102273	15	1.756201
100C	100m	Rainy	0.002681	0.006748	0.037815	0.040334	0.086898	32	2.071032
30A	30m	Dry	0.001464	0.005891	0.014948	0.016345	0.046784	22	0.855798
30B	30m	Dry	0	0.045375	0.273333	0.258403	0.100167	23	2.030372
30C	30m	Dry	0	0.003436	0.217573	0.216667	0.054795	21	1.22075
50A	50m	Dry	0	0.001328	0.00534	0.005333	0.070292	19	0.653139
50B	50m	Dry	0	0	0.060729	0.060729	0.072519	16	1.28028
50C	50m	Dry	0	0.003802	0.003817	0.003802	0.42803	22	1.591055
100A	100m	Dry	0	0	0	0	0.395833	9	1.568822
100B	100m	Dry	0.004739	0.009569	0.066327	0.070707	0.141509	16	1.65779
100C	100m	Dry	0.004283	0.248663	0.005376	0.008602	0.164179	23	1.954744

(Lecocq et al., 2019) as preliminary tests for taxa richness and Shannon-Wiener diversity index. Spatial autocorrelation was not significant for both indices. For these analyses we used the *ape* package (Paradis & Schliep, 2019) in R. In order to sample the full extent of the sampling sites seasonal variability we pooled together the samples taken during the rainy and dry seasons, resulting in six samples (three for each season) for each riparian zone width category.

To test if increased riparian zone width changed the taxonomic composition of benthic macroinvertebrate assemblages, we used the total abundance of each taxon for each sample to run a PERMANOVA pairwise contrasts analysis with Bray-Curtis distance (Anderson, 2017). To further compare the taxonomic structure of the benthic macroinvertebrate assemblages, we calculated taxonomic richness and Shannon-Wiener diversity (Table 2). We then ran a two-way ANOVA with fixed (riparian zone width) and random (season) factors to determine whether richness and Shannon-Wiener diversity were correlated to the riparian zone width. To test if wider riparian zones will change benthic macroinvertebrate assemblages' functional structure, ran a two-way ANOVA with fixed (riparian zone width) and random (season) factors to determine whether the functional feeding group metrics were correlated to the riparian zone width. As a complementary test for those variables that showed significant correlation with riparian zone width, we ran a one-way ANOVA followed by a post-hoc Tukey HSD, to determine if they differed significantly between riparian zone width categories. All statistical analyses were performed in R (R Development Core Team, 2017), using the "FSA", "labdsv" and "vegan" packages.

3. Results

We collected a total of 7,258 specimens, divided among 58 taxa (Table 3). Regarding taxonomic composition, we did not find significant differences between the three riparian width categories for the taxonomic composition (F value for the whole model: 1.0466; Table 4), nor any significant correlation between taxonomic richness or Shannon-Wiener diversity and riparian zone width (Table 5). Only two of the five functional feeding group metrics showed significant correlation with riparian zone width, TFPOM/BFPOM (filterers/gatherers) and substrate stability index (scrapers and filterers/shredders and gatherers) (Table 6). The complementary tests showed that the 30m

sites had significantly higher TFPOM/BFPOM (filterers/gatherers) than the 50m ($p = 0.0158$) and 100m ($p = 0.0262$) sites, but there was no significant difference between the 50m and 100m sites ($p = 0.9644$). The substrate stability index (scrapers and filterers/shredders and gatherers) showed the same pattern, with the 30m sites showing higher values than 50m ($p = 0.0149$) and 100m ($p = 0.0273$) sites, but no significant difference between those two ($p = 0.9494$). The autotrophy to heterotrophy index showed values of zero (Table 2) in most of the samples, as the functional feeding group Scrapers did not have sampled individuals on most of the sampling sites.

4. Discussion

Our first prediction, that wider riparian zone would change the taxonomic composition of benthic macroinvertebrate assemblages, was not corroborated, because Shannon-Wiener diversity, taxonomic richness and taxonomic composition showed no significant correlation with riparian zone width. This lack of significant correlation may be explained by the good ecological status of the sites (Santos et al., 2019). Anthropogenic disturbances are some of the most important determinants of the structure of benthic macroinvertebrate assemblages, acting as environmental filters in local and regional scales (Castro et al., 2017; Firmiano et al., 2021). Similar results were found when comparing reference condition streams with open and shaded canopies, where no significant difference in taxonomic indices was found (Linares et al., 2018).

Our second prediction, wider riparian zones will change benthic macroinvertebrate assemblages' functional structure, was corroborated by only two of five metrics, and only between 30m riparian widths versus 50m and 100m widths. The higher densities of filtering-collectors compared with burrowing collectors in the 30m riparian zones versus wider riparian zones suggest that there is higher local input of fine particulate organic matter from terrestrial ecosystems in these sites, compared to those with wider riparian zones, which can be the responsible for the higher, but not significantly so, turbidity values in the 30m sites (Table 1 and Figures 2 to 6). Higher levels of fine inorganic sediment are correlated with anthropogenic impacts in the riparian zone (Fierro et al., 2017; Firmiano et al., 2017) and the basin (Martins et al., 2021; Silva et al., 2017), such as agriculture and pasture. Our results suggest that a 30m wide riparian zone, as dictated by Brazilian

Table 3. Benthic macroinvertebrate abundance.

Stream Site	30A		30B		30C		50A		50B		50C		100A		100B		100C		
	30m	Rainy	30m	Rainy	30m	Rainy	50m	Rainy	50m	Rainy	50m	Dry	100m	Dry	100m	Dry	100m	Dry	
Width																			
Season																			
Collembola	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Ostracoda	0	5	2	0	0	2	1	0	0	3	14	0	0	0	0	0	0	0	0
Chironomidae	25	119	145	140	146	91	169	146	51	306	595	408	698	33	68	235			
Ceratopogonidae	0	0	4	16	40	7	94	40	0	10	9	8	41	9	7	16			
Empididae	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	2			
Tabanidae	0	0	0	0	0	2	0	0	0	0	0	4	0	0	0	0			
Simuliidae	23	1	1	0	6	4	1	6	1	1	1	93	0	0	11				
Phoridae	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0				
Dolichopodidae	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0				
Stratiomyidae	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0				
Culicidae	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0				
Oligoneuridae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0				
Baetidae	36	42	2	0	38	13	0	38	17	32	19	2	1	1	5	12	1	3	
Leptophlebiidae	11	3	4	0	10	2	0	10	1	102	7	2	3	1	12	0	0	34	
Caenidae	1	0	1	0	0	0	0	0	0	15	6	112	0	3	0	1	0	15	
Leptophlebiidae	21	0	1	2	0	3	17	0	1	146	2	22	0	0	10	16	4	5	
Hydropsychidae	9	0	16	0	1	1	0	1	0	5	1	1	8	0	10	0	1	1	
Leptoceridae	0	2	0	1	1	0	2	1	0	2	0	0	0	0	0	10	0	60	
Hydroptilidae	0	0	0	0	0	2	0	5	0	1	0	1	0	0	0	0	0	0	
Ecnomidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	
Glossosomatidae	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	
Helicopsychidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Calamoceratidae	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	1	0	0	
Philopotamidae	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
Odontoceridae	3	0	0	2	3	1	1	3	0	2	4	0	1	0	0	2	0	93	
Polycentropodidae	0	0	0	0	0	0	0	0	1	1	6	0	0	0	0	0	0	0	
Hydrobiosidae	0	0	0	1	0	0	1	0	0	1	0	1	0	0	2	9	0	14	
Perlidae	4	0	1	0	1	0	1	1	2	1	0	0	0	0	5	1	0	5	
Libellulidae	0	1	0	0	1	1	1	1	0	9	0	0	2	0	0	2	0	0	
Calopterygidae	0	1	0	0	4	1	3	4	1	20	0	0	0	0	0	1	0	1	
Aeshnidae	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	

Table 3. Continued...

Stream Site	30A	30B	30C	50A	50B	50C	100A	100B	100C	30A	30B	30C	50A	50B	50C	100A	100B	100C
Dictyrididae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Cordiuliidae	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coenagrionidae	1	0	0	0	0	6	2	0	1	12	1	0	0	0	0	0	0	3
Gomphidae	3	5	14	8	1	8	0	0	1	1	1	1	0	1	1	1	0	0
Perllestidae	0	2	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Protoneturidae	0	4	0	1	1	7	4	0	0	1	3	2	1	0	1	0	0	0
Megapodagrionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Gyrinidae	0	0	0	1	0	1	4	0	0	0	0	2	1	0	1	0	0	0
Dytiscidae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Hydrophilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Noteridae	0	0	0	0	0	1	0	0	1	2	0	0	0	0	0	0	0	0
Elmidae	9	4	7	5	8	0	6	5	17	37	150	61	12	15	1	5	111	15
Lutrochidae	1	0	1	1	0	1	2	3	0	0	1	0	0	0	0	0	0	3
Scirtidae	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Staphylinidae	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Pleidae	0	0	0	0	0	0	0	0	0	4	14	0	0	0	0	0	0	0
Naucoridae	0	4	0	1	0	0	0	0	0	0	3	0	0	0	0	0	0	0
Mesovelidae	1	0	0	0	1	2	0	0	1	0	0	0	0	1	0	0	0	0
Belostomatidae	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	0
Pyralidae	0	2	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0
Hydracarina	5	3	0	8	3	12	5	5	11	2	5	10	5	4	6	9	6	35
Oligochaeta	11	5	17	58	67	0	70	6	92	3	0	5	30	4	21	7	11	4
Hirudinea	0	0	0	7	0	112	0	0	5	0	0	1	1	0	0	0	0	0
Nematoda	1	48	26	4	77	4	6	2	2	0	0	0	1	0	3	1	0	1
Bivalvia	1	1	2	2	1	0	0	0	6	0	5	0	4	0	0	0	0	0
Planorbidae	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Hydrobiidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table 4. PERMANOVA pairwise contrasts comparing the taxonomic composition of benthic macroinvertebrate assemblages.

Pairs	F Model	R ²	p
30m vs 50m	1.055	0.095	0.346
30m vs 100m	0.814	0.075	0.592
50m vs 100m	1.319	0.117	0.212

Table 5. Two-way ANOVA with fixed (riparian zone width) and random (season) factors for Shannon-Wiener diversity index and Richness of benthic macroinvertebrate assemblages.

Variable	Riparian zone width (fixed factor)		Season (random factor)	
	F Model	p	F Model	p
Shannon-Wiener Diversity	1.45	0.268	6.48	0.023
Richness	0.17	0.846	1.22	0.287

Table 6. Two-way ANOVA with fixed (riparian zone width) and random (season) factors for benthic macroinvertebrate functional feeding group (FFG) variable scores.

Variable	Riparian zone width (fixed factor)		Season (random factor)	
	F Model	p	F Model	p
Top Down Control	1.86	0.192	0.24	0.632
Channel Stability	4.83	0.025	0.12	0.736
Transported/Sediment FPOM	4.32	0.035	0.20	0.662
CPOM/FPOM	1.80	0.202	0.01	0.904
Autothrophy/Heterotrophy	1.43	0.173	0.73	0.478

Federal Law Number 12,651/2012, may not suffice as a buffer against anthropogenic impacts on lotic ecosystems. We suggest that future studies verify if this pattern can be generalized for streams in the neotropical savanna and other Brazilian biomes, as it can have big implications in the conservation and management of lotic ecosystems.

Our results also indicate that streams with 30m riparian zone widths appear to have greater substrate stability than those with wider riparian zones, as indicated by greater abundance of scrapers and filterers versus shredders and gatherers in 30m widths than in wider riparian zones. Greater riparian zone development is linked to lower substrate stability, due to sites with less stable soils and less hard substrates next to the surface, such as embedded rocks, allow for more development of the riparian zone (Fanny et al., 2013; Galetti et al., 2020; Milner & Gloyne-Phillips, 2005). Low level disturbances are an important factor in maintaining the biodiversity in lotic ecosystems, suggesting that wider riparian zones may be better in maintaining the biodiversity in these ecosystems (Death & Winterbourn, 1995; Zimmermann & Death, 2002).

These results suggest that the 30m minimum riparian zone width established by the Brazilian environmental law (Federal Law Number 12,651/2012) may be inadequate for maintaining the ecosystem functioning of stream ecosystems. Previous studies in Brazil suggested that a ~50m wide riparian zone is more adequate for protecting and maintaining the environmental health of lotic ecosystems (Dala-Corte et al., 2020; Metzger, 2010). This highlights the necessity for guidelines based on biological data for managing riparian buffers in tropical areas (Luke et al., 2019) and support environmental policy to conserve the freshwater biodiversity and ecosystem structure.

We did not find any significant difference between stream sites with 50m and 100m wide riparian zone. This result corroborates with those found in a more extensive study about the effects of riparian zone in tropical streams, that suggested that 50m is a better threshold to protecting the biodiversity of lotic ecosystems (Dala-Corte et al., 2020).

5. Conclusion

Our results suggest that, contrary to what is determined by the Brazilian environmental law

(Federal Law Number 12,651/2012), 30m width riparian zones may be inadequate for protecting the biodiversity of headwater stream ecosystems. Streams with 30m riparian zone width showed significant differences in two of five functional metrics when compared to those with wider riparian zones. It provides technical data for lawmakers and environmental managers, suggesting that wider riparian zones are necessary for maintaining ecosystem biodiversity structure and consequently their ecosystem services (e.g., nutrient retention, avoid siltation, ecological corridor for many terrestrial plant species, semi-aquatic and fly animal species). Our results also highlight the necessity of studies using indicators based on ecosystem structure to support policy decisions and biodiversity conservation efforts in the riparian meta-ecosystems (Callisto et al., 2019).

Future studies should focus on the application of these methods in other regions of the tropical zone, to test the universality of these results. We also suggest assessing the efficiency of riparian zone buffers against different kinds of anthropogenic pressures and that this scientific data be used to modernize legislation and environmental management as needed.

Acknowledgements

This research was supported by the Programa de Pesquisa e Desenvolvimento of Companhia Energética de Minas Gerais (CEMIG) P&D Aneel-Cemig GT-599 (MSL received a post-doc scholarship from this Project); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; research productivity grant 304060/2020-8 to MC and 312752/2018-0 to JCS), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance Code 001, Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, PPM 00104/18 to MC). The authors thank Diego Macedo for help with the map and Robert M. Hughes for a draft manuscript review. The authors thank Duratex S.A. for providing the access to the study area. All methods are compliant with the current Brazilian laws for ethical science.

References

- AGUIAR, A.C.F., GÜCKER, B., BRAUNS, M., HILLE, S. and BOËCHAT, I.G. Benthic invertebrate density, biomass, and instantaneous secondary production along a fifth-order human-impacted tropical river. *Environmental Science and Pollution Research International*, 2015, 22(13), 9864-9876. <http://dx.doi.org/10.1007/s11356-015-4170-y>. PMID:25647497.
- ANDERSON, M.J. Permutational Multivariate Analysis of Variance (PERMANOVA). In: N. BALAKRISHNAN, T. COLTON, B. EVERITT, W. PIEGORSCH, F. RUGGERI and J. TEUGELS, eds. *Wiley StatsRef: statistics reference online*. Chichester: John Wiley & Sons, 2017, pp. 1-15. <http://dx.doi.org/10.1002/9781118445112.stat07841>.
- BENKE, A.C. and HURY, A.D. Benthic invertebrate production: facilitating answers to ecological riddles in freshwater ecosystems. *Journal of the North American Benthological Society*, 2010, 29(1), 264-285. <http://dx.doi.org/10.1899/08-075.1>.
- BENKE, A.C. Concepts and patterns of invertebrate production in running waters. *Internationale Vereinigung für Theoretische und Angewandte Limnologie: Verhandlungen*, 1993, 25(1), 15-38. <http://dx.doi.org/10.1080/03680770.1992.11900056>.
- BONADA, N., PRAT, N., RESH, V.H. and STATZNER, B. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. *Annual Review of Entomology*, 2006, 51(1), 495-523. <http://dx.doi.org/10.1146/annurev.ento.51.110104.151124>. PMID:16332221.
- BRITO, J.G., ROQUE, F.O., MARTINS, R.T., NESSIMIAN, J.L., OLIVEIRA, V.C., HUGHES, R.M., DE PAULA, F.R., FERRAZ, S.F.B. and HAMADA, N. Small forest losses degrade stream macroinvertebrate assemblages in the eastern Brazilian Amazon. *Biological Conservation*, 2020, 241, 108263. <http://dx.doi.org/10.1016/j.biocon.2019.108263>.
- CALLISTO, M., MUGNAI, R., CASTRO, D. and LINARES, M. Sampling methods for aquatic insects. In: J.C. SANTOS and G.W. FERNANDES, eds. *Measuring arthropod biodiversity: a handbook of sampling methods*. 1st ed. New York: Springer, 2021. http://dx.doi.org/10.1007/978-3-030-53226-0_20.
- CALLISTO, M., SOLAR, R., SILVEIRA, F.A.O., SAITO, V.S., HUGHES, R.M., FERNANDES, G.W., GONÇALVES-JÚNIOR, J.F., LEITÃO, R.P., MASSARA, R.L., MACEDO, D.R., NEVES, F.S. and ALVES, C.B.M. A Humboldtian approach to mountain conservation and freshwater ecosystem services. *Frontiers in Environmental Science*, 2019, 7, 195. <http://dx.doi.org/10.3389/fenvs.2019.00195>.
- CASTRO, D.M.P., DOLÈDEC, S. and CALLISTO, M. Landscape variables influence taxonomic and trait composition of insect assemblages in Neotropical savanna streams. *Freshwater Biology*, 2017, 62(8), 1472-1486. <http://dx.doi.org/10.1111/fwb.12961>.
- CUMMINS, K.W., MERRITT, R.W. and ANDRADE, P.C.N. The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil. *Studies on Neotropical*

- Fauna and Environment*, 2005, 40(1), 69-89. <http://dx.doi.org/10.1080/01650520400025720>.
- DALA-CORTE, R.B., MELO, A.S., SIQUEIRA, T., BINI, L.M., MARTINS, R.T., CUNICO, A.M., PES, A.M., MAGALHÃES, A.L.B., GODOY, B.S., LEAL, C.G., MONTEIRO-JÚNIOR, C.S., STENERT, C., CASTRO, D.M.P., MACEDO, D.R., LIMA-JUNIOR, D.P., GUBIANI, É.A., MASSARIOL, F.C., TERESA, F.B., BECKER, F.G., SOUZA, F.N., VALENTE-NETO, F., SOUZA, F.L., SALLES, F.F., BREJÃO, G.L., BRITO, J.G., VITULE, J.R.S., SIMIÃO-FERREIRA, J., DIAS-SILVA, K., ALBUQUERQUE, L., JUEN, L., MALTCHIK, L., CASATTI, L., MONTAG, L., RODRIGUES, M.E., CALLISTO, M., NOGUEIRA, M.A.M., SANTOS, M.R., HAMADA, N., PAMPLIN, P.A.Z., POMPEU, P.S., LEITÃO, R.P., RUARO, R., MARIANO, R., COUCEIRO, S.R.M., ABILHOA, V., OLIVEIRA, V.C., SHIMANO, Y., MORETTO, Y., SÚAREZ, Y.R. and ROQUE, F.O. Thresholds of freshwater biodiversity in response to riparian vegetation loss in the Neotropical region. *Journal of Applied Ecology*, 2020, 57(7), 1391-1402. <http://dx.doi.org/10.1111/1365-2664.13657>.
- DEATH, R.G. and WINTERBOURN, M.J. Diversity patterns in stream benthic invertebrate communities: the influence of habitat stability. *Ecology*, 1995, 76(5), 1446-1460. <http://dx.doi.org/10.2307/1938147>.
- DOLBETH, M., CUSSON, M., SOUSA, R. and PARDAL, M. Secondary production as a tool for better understanding of aquatic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences*, 2012, 69(7), 1230-1253. <http://dx.doi.org/10.1139/f2012-050>.
- FANNY, C., VIRGINIE, A., JEAN-FRANÇOIS, F., JONATHAN, B., MARIE-CLAUDE, R. and SIMON, D. Benthic indicators of sediment quality associated with run-of-river reservoirs. *Hydrobiologia*, 2013, 703(1), 149-164. <http://dx.doi.org/10.1007/s10750-012-1355-y>.
- FIERRO, P., BERTRÁN, C., TAPIA, J., HAUENSTEIN, E., PEÑA-CORTÉS, F., VERGARA, C., CERNA, C. and VARGAS-CHACOFF, L. Effects of local land-use on riparian vegetation, water quality, and the functional organization of macroinvertebrate assemblages. *The Science of the Total Environment*, 2017, 609, 724-734. <http://dx.doi.org/10.1016/j.scitotenv.2017.07.197>. PMID:28763669.
- FIRMIANO, K.R., CASTRO, D.M.P., LINARES, M.S. and CALLISTO, M. Functional responses of aquatic invertebrates to anthropogenic stressors in riparian zones of Neotropical savanna streams. *The Science of the Total Environment*, 2021, 753, 141865. <http://dx.doi.org/10.1016/j.scitotenv.2020.141865>. PMID:32891996.
- FIRMIANO, K.R., LIGEIRO, R., MACEDO, D.R., JUEN, L., HUGHES, R.M. and CALLISTO, M. Mayfly bioindicator thresholds for several anthropogenic disturbances in neotropical savanna streams. *Ecological Indicators*, 2017, 74, 276-284. <http://dx.doi.org/10.1016/j.ecolind.2016.11.033>.
- GALETI, G., CAPITANIO, B.M. and BALDISSERA, R. Variation of benthic macroinvertebrate communities in streams of three landscapes of south Brazilian grasslands. *Revista de Biología Tropical*, 2020, 68(1), 108-121. <http://dx.doi.org/10.15517/rbt.v68i1.37652>.
- GREGORY, S.V., SWANSON, F.J., MCKEE, W.A. and CUMMINS, K.W. An ecosystem perspective of Riparian zones Focus on links between land and water. *Bioscience*, 2007, 41(8), 540-551. <http://dx.doi.org/10.2307/1311607>.
- HEINO, J., MELO, A.S., JYRKÄNKALLIO-MIKKOLA, J., PETSCH, D.K., SAITO, V.S., TOLONEN, K.T., BINI, L.M., LANDEIRO, V.L., SILVA, T.S.F., PAJUNEN, V., SOININEN, J. and SIQUEIRA, T. Subtropical streams harbour higher genus richness and lower abundance of insects compared to boreal streams, but scale matters. *Journal of Biogeography*, 2018, 45(9), 1983-1993. <http://dx.doi.org/10.1111/jbi.13400>.
- LEAL, C.G., BARLOW, J., GARDNER, T.A., HUGHES, R.M., LEITÃO, R.P., MACNALLY, R., KAUFMANN, P.R., FERAZ, S.F.B., ZUANON, J., DE PAULA, F.R., FERREIRA, J., THOMSON, J.R., LENNOX, G.D., DARY, E.P., RÖPKE, C.P. and POMPEU, P.S. Is environmental legislation conserving tropical stream faunas? A large-scale assessment of local, riparian and catchment-scale influences on Amazonian fish. *Journal of Applied Ecology*, 2018, 55(3), 1312-1326. <http://dx.doi.org/10.1111/1365-2664.13028>. PMID:32831394.
- LECOCQ, T., HARPKE, A., RASMONT, P. and SCHWEIGER, O. Integrating intraspecific differentiation in species distribution models: Consequences on projections of current and future climatically suitable areas of species. *Diversity & Distributions*, 2019, 25(7), 1088-1100. <http://dx.doi.org/10.1111/ddi.12916>.
- LIBÓRIO, R.A. and TANAKA, M.O. Does environmental disturbance also influence within-stream beta diversity of macroinvertebrate assemblages in tropical streams? *Studies on Neotropical Fauna and Environment*, 2016, 51(3), 206-214. <http://dx.doi.org/10.1080/01650521.2016.1237801>.
- LINARES, M.S., ASSIS, W., CASTRO SOLAR, R.R., LEITÃO, R.P., HUGHES, R.M. and CALLISTO, M. Small hydropower dam alters the taxonomic composition of benthic macroinvertebrate assemblages in a neotropical river. *River Research and Applications*, 2019, 35(6), 725-735. <http://dx.doi.org/10.1002/rra.3442>.

- LINARES, M.S., CALLISTO, M. and MARQUES, J.C. Compliance of secondary production and exergy as indicators of benthic macroinvertebrates assemblages' response to canopy cover conditions in Neotropical headwater streams. *The Science of the Total Environment*, 2018, 613–614, 1543-1550. <http://dx.doi.org/10.1016/j.scitotenv.2017.08.282>. PMID:28882459.
- LUKE, S.H., SLADE, E.M., GRAY, C.L., ANNAMMALA, K.V., DREWER, J., WILLIAMSON, J., AGAMA, A.L., ATIONG, M., MITCHELL, S.L., VAIRAPPAN, C.S. and STRUEBIG, M.J. Riparian buffers in tropical agriculture: Scientific support, effectiveness and directions for policy. *Journal of Applied Ecology*, 2019, 56(1), 85-92. <http://dx.doi.org/10.1111/1365-2664.13280>.
- MACDONALD, L.H. and COE, D. Influence of headwater streams on downstream reaches in forested areas. *Forest Science*, 2007, 53, 148-168. <http://dx.doi.org/10.1093/forestscience/53.2.148>.
- MARTINS, I., MACEDO, D.R., HUGHES, R.M. and CALLISTO, M. Major risks to aquatic biotic condition in a Neotropical Savanna River basin. *River Research and Applications*, 2021, 37(6), 858-868. <http://dx.doi.org/10.1002/rra.3801>.
- MAURO, M.L., CASTRO, K.J., CAMPOS, I.C., RODRIGUES, N.U.A. and VALERA, C.A. Challenges in the zoning limitation of environmental protection area of Uberaba River (Uberaba/MG). *Ambiência*, 2016, 12, 851-858. <http://dx.doi.org/10.5935/ambiencia.2016.Especial.10>.
- MERRITT, R.W., CUMMINS, K. and BERG, M. *An introduction to the aquatic insects of North America*. 4th ed. Dubuque: Kendall/Hunt Publishing, 2008.
- METZGER, J.P. O código florestal tem base científica? *Natureza & Conservação*, 2010, 8(1), 92-99. <http://dx.doi.org/10.4322/natcon.00801017>.
- MILNER, A.M. and GLOYNE-PHILLIPS, I.T. The role of riparian vegetation and woody debris in the development of macroinvertebrate assemblages in streams. *River Research and Applications*, 2005, 21(4), 403-420. <http://dx.doi.org/10.1002/rra.815>.
- MUGNAI, R., NESSIMIAN, J.L. and BAPTISTA, D.F. *Manual de identificação de macroinvertebrados aquáticos do Estado do Rio de Janeiro*. Rio de Janeiro: Technical Books, 2010.
- OSAKPOLOR, S.E., KATTWINKEL, M., SCHIRMEL, J. and FECKLER, A. Mini-review of process-based food web models and their application in aquatic-terrestrial meta-ecosystems. *Ecological Modelling*, 2021, 458, 109710. <https://doi.org/10.1016/j.ecolmodel.2021.109710>.
- PARADIS, E. and SCHLIEP, K. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, 2019, 35(3), 526-528. <http://dx.doi.org/10.1093/bioinformatics/bty633>. PMID:30016406.
- R DEVELOPMENT CORE TEAM. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing, 2017.
- RAMÍREZ, A. and GUTIÉRREZ-FONSECA, P. FFG of aquatic insect families in Latin America: a critical analysis and review of existing literature. *Revista de Biología Tropical*, 2014, 62(Suppl 2), 155-167. <http://dx.doi.org/10.15517/rbt.v62i0.15785>. PMID:25189076.
- REZENDE, R.S., GRAÇA, M.A., SANTOS, A.M., MEDEIROS, A.O., SANTOS, P.F., NUNES, Y.R. and GONÇALVES JÚNIOR, J.F. Organic matter dynamics in a tropical gallery forest in a grassland landscape. *Biotropica*, 2016, 48, 301-310. <http://dx.doi.org/10.1111/btp.12308>.
- RIOS, S.L. and BAILEY, R.C. Relationship between riparian vegetation and stream benthic communities at three spatial scales. *Hydrobiologia*, 2006, 553(1), 153-160. <http://dx.doi.org/10.1007/s10750-005-0868-z>.
- SANTOS, G.M., LINARES, M.S., CALLISTO, M. and MARQUES, J.C. Two tropical biodiversity hotspots, two different pathways for energy. *Ecological Indicators*, 2019, 106, 105495. <http://dx.doi.org/10.1016/j.ecolind.2019.105495>.
- SILVA, D.R.O., HERLIHY, A.T., HUGHES, R.M. and CALLISTO, M. An improved macroinvertebrate multimetric index for the assessment of Wadeable streams in the neotropical savanna. *Ecological Indicators*, 2017, 81, 514-525. <http://dx.doi.org/10.1016/j.ecolind.2017.06.017>.
- STANFORD, B., HOLL, K.D., HERBST, D.B. and ZAVALETA, E. In-stream habitat and macroinvertebrate responses to riparian corridor length in rangeland streams. *Restoration Ecology*, 2020, 28(1), 173-184. <http://dx.doi.org/10.1111/rec.13029>.
- TONKIN, J.D. Drivers of macroinvertebrate community structure in unmodified streams. *PeerJ*, 2014, 2, e465. <http://dx.doi.org/10.7717/peerj.465>. PMID:25024926.
- WHITTIER, T.R. and VAN SICKLE, J. Macroinvertebrate tolerance values and an assemblage tolerance index (ATI) for western USA streams and rivers. *Journal of the North American Benthological Society*, 2010, 29(3), 852-866. <http://dx.doi.org/10.1899/09-160.1>.
- ZIMMERMANN, E.M. and DEATH, R.G. Effect of substrate stability and canopy cover on stream invertebrate communities. *New Zealand Journal of Marine and Freshwater Research*, 2002, 36(3), 537-545. <http://dx.doi.org/10.1080/00288330.2002.9517109>.

Received: 26 May 2021

Accepted: 01 September 2021

Associate Editor: Victor Satoru Saito.